TAXONOMY AND BIOLOGY OF LARVAL TREMATODES

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INTRODUCTION

For the past fifty years trematologists have been studying the classification and biology of larval trematodes, particularly cercariae. Their investigations have served to increase the number of described cercariae and to provide a new approach to trematode classification. Furthermore, such studies have pointed out biological relationships that were found to be useful in classification, trematode control and in completion of life cycles.

The earliest cercarial descriptions dealt only with the most obvious anatomical characteristics, and this would seem natural since the early investigators had little knowledge of the vast number of trematodes in existence and had microscopes which were inferior to our present-day models. As the number of described species increased so also did the complexity of cercarial descriptions. Increasing importance was attached to the less obvious characters such as caudal bodies, tail fin membranes, behavior, etc. As a result of this constant increase in complexity we now have a situation in which there are numerous cercarial descriptions with varying degrees of completeness. Beside this, to add to the confusion, many investigators do not agree to the number and arrangement of anatomical features in certain specific cercariae. In this connection it is interesting to note that disagreement arises mainly in those species which have received the most study; that is, the economically important ones such as those of the human schistosomes. Those species about which there is no disagreement as to anatomical features are usually the ones that have been studied by only one or two investigators.

With the increase in number of named and described cercarial forms, workers began to classify them into groups and sub-groups. These groupings served to facilitate cercarial classification, but they were not given Family or Order standing and were mainly for convenience. When a sufficient number of life cycle studies had been completed, a careful comparison of cercarial and adult features of the same species showed that in many the cercarial characteristics differed widely from those of the adult. This situation caused many subsequent investigators to stop and reconsider the entire question of trematode classification. They reasoned that closer relationship of species might be shown in similar cercariae rather than in marita stages, because of adaptive modifications in the latter. The tendency, therefore, among modern taxonomists is to consider both the larval and adult stages in a system of classification.

Biological investigations of cercariae, which are comparatively recent, have perhaps been the most important aspects of cercarial study, since many of our present-day control measures in preventing trematode infections are based on such studies. With studies concerned with completing life cycles of trematodes which involve a careful consideration of larval stages, control methods were made possible by combating the intermediate hosts involved in the cycle. These intermediate hosts are snails which have been controlled by placing copper sulfate in their aquatic habitat. Such a procedure kills the snails but at the same time renders the habitat unsuitable for many aquatic organisms including vertebrates. In countries where snail control is necessary to prevent trematode infection in the human population (China, Egypt, etc.) or domestic animals, or both, the destruction of all aquatic organisms is far from desirable. In these areas the human population depends upon rough fish as a major source of protein food and the rough fish in turn depend upon aquatic organisms for their growth. Consequently, because such mass destruction is not desirable these situations call for a more intensive study of the biology of cercariae and related larval stages in order to devise more specific measures of control.

After due consideration the writer decided to select a problem for his doctorate thesis which would acquaint him with the taxonomic and biological relationships of larval trematodes. The study was designed to give the investigator an opportunity to become familiar with the taxonomic features of as many cercariae as possible. In this way a sound comprehension of the majority of morphological features of cercariae used in classification and their relative values in a system of taxonomy could be determined. It was felt that a chance to record and compare the biological features of a number of similar cercariae would be valuable in determining minute anatomical and behavior differences that might otherwise be overlooked as a basis for differentiation. Furthermore, the study was so approached as to give the investigator the opportunity to complete cycles from the cercaria to the marita whenever possible. A comprehensive investigation of this nature was considered to be necessary as an introduction to the major field of trematology and would serve as a basis for future study of the physiology and biology of larval trematodes. As a major objective it was determined that the number and kind of known cercariae in the region could be ascertained and that new ones to be described would be encountered.

The ordinary objectives were as follows:

1. To determine the species present in the Stillwater region.

2. To secure data on the behavior and host-parasite relations of the cercarial and subsequent stages in the life cycle of the various species.

3. To compare the resistance of infected and uninfected snails to environmental conditions.

4. To determine whether there were seasonal and other fluctuations of parasitism in the snails.

5. To describe new species.

6. To consider new material in relation to taxonomy.

7. To determine the potential effect of the trematodes on the domestic and wild animals of the region.

REVIEW OF LITERATURE

In 1737 a Dutch microscopist, Swammerdam, first noticed cercariae escaping from dissected snails. He termed these objectives "worms" and made a series of drawings which undoubtedly depicts cercariae. In 1773 Müller also noticed similar forms and placed them in a newly created genus Cercaria. Subsequently Eichorn (1781) and Herman (1783) described cercariae, placing them in the genus Vibrio.

During the early part of the nineteenth century workers had begun to observe various larval forms of the trematode life cycle without realizing their significance or relationships to other organisms. Zeder (1800), apparently, was the first to record the hatching and escape of ciliated larvae from trematode eggs; Nitzsch (1807) observed encapsulation of cercariae in water and described this phenomenon as a curious form of death; Bojanus (1818) observed the emergence of cercariae from rediae; von Baer (1827) described development of cercariae within rediae from "germ granules"; Mehlis (1831) reported emergence of ciliated larvae from eggs of Monostomum flavum; and von Siebold (1835) observed rediae within the ciliated miracidium. By 1842 it was generally accepted that there were such things as complicated life cycles in parasites and that these curious organisms were stages in such a cycle. At this time Steenstrup advanced his theory of metagenesis and applied it to the reproductive processes of trematodes. He thought that the rediae nursed the cercariae and were, in turn, dependent on the cercariae for their existence. The cercariae were regarded as "trying to return to the snail in order to pupate because they had lost their tails," and he considered the mature fluke to live in the snail, which became infected through the cercariae. Von Siebold immediately suggested that the mature fluke existed in birds and not in snails, regardless of the kind of cercaria.

Between 1842 and 1855 other important contributions were made by various workers. Carus (1849) observed rediae developing within rediae; Müller (1850) discovered Trichocercous cercariae; Leuckart (1852) suggested the relationship between encysted cercariae on gills and adult flukes in the intestine of fishes; and Filippi's "Memoires" (1854) and Diesing's "Revision der Cercarieen" (1855) were published. In 1855 La Valette de St. George in his "Symbolae ad Trematodum Evolutionis Historiam" described the first feeding experiment, wherein he established mature flukes in birds by feeding them encysted cercariae from aquatic snails. Following La Valette's discovery, Wagener (1857) observed the penetration of miracidia into snails and the subsequent development of these larvae into rediae.

By 1857 it had been established that miracidia hatched from eggs and penetrated snails to develop into rediae that liberated cercariae. Furthermore, it was determined that cercariae encysted and that if these were eaten by a suitable host they developed into mature flukes. There remained now only one thing in order to prove such a complicated cycle; that of completing one in its entirety under laboratory conditions. And Thomas and Leuckart, in October, 1882, working independently, did just this and both described the life cycle of _Fasciola hepatica_, a liver fluke that occurs in wild and domestic animals and man. These classical studies gave the needed impetus for later investigations and established the cercaria as an important part of the trematode life cycle.

Studies of cercariae until early in the twentieth century had been confined primarily to taxonomic studies which appeared in the literature from time to time. Subsequent to Thomas' and Leuckart's independent discoveries in 1882 von Linstow and Looss were the main contributors to this line of endeavor. In 1909, Lühe made the first contribution to cercarial classification. He classified them as lopho-cercariae, gasterostome cercariae, monostome cercariae, amphistome cercariae and distome cercariae (eight subgroups in the latter). Lophocercariae were species possessing a longitudinal undulating membrane; gasterostome cercariae were species having a simple sac-like intestine with the oral aperature on the middle of the ventral surface of the body; monostome cercariae were species without a ventral sucker; amphistome cercariae were species with a terminal ventral sucker; and distome cercariae were those species with both an oral and ventral sucker, the latter being well forward of the termination of the body. The distome group was further divided into 8 subgroups on the basis of the tail characteristics. Following Lühe's classification, Lebour (1911) formulated a scheme whereby the importance of tail characteristics was emphasized to a lesser extent and the distome cercariae were divided into two main groups, depending on whether they developed in sporocysts or rediae.

Until 1914, only 12 cercariae had been reported from North America. Cort (1914) added 14 new species to this list. This was followed by Faust (1917), who contributed enough cercarial descriptions to bring the total to 81. Following these historic investigations many cercarial and life history studies have been published in the United States.

Although numerous cercarial descriptions have appeared in the literature, the primary objective since the time of Lühe has been to establish a natural system of classification for cercariae as well as for adult forms. Using Lühe's system as a basis of classification, various investigators tried to determine cercarial relationships that would enable them to distinguish subgroups under Lühe's major groupings and at the same time show cercarial relationships to the established system of adult trematode classification. Cort (1917) made a study of 5 furcocercous cercariae and advanced the idea that the furcocercous group of cercariae could be divided into subgroups on the basis of the characteristics of the excretory system. He even went so far as to state that furcocercous cercariae could be placed in the correct Family of their maritas on the basis of such characteristics. Faust (1919) believed likewise and stated that "when once the number and disposition of flame cells has been established for a particular group (referring to a group under the adult system of classification) it will be possible to predict the flame-cell formulae of the larvae in that group."

By 1922 sufficient information had been accumulated to permit Sewell in that year to modify Lühe's classification. By making use of taxonomic characteristics deemed important by Lühe and other workers, Sewell was able to form subgroupings of cercariae, whereas Lühe for lack of information had to be content with solitary but characteristics species. Sewell's classification also served to further Cort's 1917 contention as to the importance of the excretory system. Dubois (1929) founded the idea of dual origin of the Digenea, that is to say that digenetic trematodes originated from two basic stocks. He followed the lead of Sewell, who recognized in his group of monostome cercariae two types of excretory system which received the names "Stenostoma" and "Mesostoma," and applied this criterion to all cercariae. The stenostoma type was characterized by having paired excretory tubules that extended to the level of the oral sucker, formed reverse loops, and progressed posteriad to bifurcate in the latter half of the body. The mesostoma type was characterized by having paired excretory tubules that extended to the midbody level where they bifurcated to give rise to anterior and posterior tubules. Dubois found an additional character to be associated with these two types and stated that stenostoma cercariae developed in rediae whereas mesostoma cercariae developed in sporocysts. The former type was found to include the amphistome, monostome, echinostome, and gymnocephalus cercariae, and the latter included the xiphidiocercariae and furcocercariae. Although it was generally agreed at the time that they type of excretory system served to distinguish the larger subgroups, evidence was accumulating to indicate that differences could not be demonstrated between smaller ones. Cort and Brooks (1928) found that variation in the excretory system might occur in individual cercariae of the same species (_C. douglasi_) due to differences in division of the primary flame cells. They also found that the flame cell pattern in closely related forms, _C. laruei_ and _C. flexicauda_, might be exactly the same. They concluded from this investigation that flame cell patterns had been overemphasized in classification. Later work, Hussey (1941) and Kuntz (1950), served to establish major group differences in excretory systems, but failed to show distinctions in minor groups, such as at the Family level and lower.

Attention, meanwhile, was being focused on the value of other cercarial structures in a system of natural classification. Allison (1943) and Stunkard (1946) pointed out that the cercarial tail was not a reliable index of relationship, since the larval group Cercariaeum, restricted by Lühe (1909) to tailless distome cercariae, was comprised of forms in the Families Cyclocoelidae, Zoogonidae, Brachylaemidae, Lissorchiidae, and Eucotylidae, groups all with divergent characteristics within themselves. The discovery by Goodchild (1943) of both stylet and non-stylet cercariae in the same Genus caused him to doubt the taxonomic value of the stylet. Stunkard (1946) discovered further differences within groups and stated that "an acetabulum may appear in the cercaria stage and continue its development; it may appear in the cercaria and fail to develop further in certain cyclocoelids and eucotylids; it may not appear until later as in the metacercaria stage of certain microphallids, heterophyids, and opisthorchids; or it may fail to develop as in the so-called monostomes."

From these investigations it can be pointed out that the presence or absence of certain characteristics in cercariae does not necessarily indicate the taxonomic position of cercariae in relation to the scheme of adult trematode taxonomy. It is entirely possible, therefore, that two closely related species in the adult system of classification might have very different cercariae that are unlike their respective adults or the converse situation might prevail. This condition has brought taxonomists to the conclusion that in order to form the correct natural system of trematode classification it is necessary to evaluate and include characteristics of both larval and adult stages. In the words of Stunkard (1946), "the natural system of classification of the digenetic trematodes must utilize information afforded by the study of both larval and adult stages and every feature must be evaluated with the greatest care."

Within the past 20 years attention has been focused by many investigators, including Kuntz, Beaver, and Wesenberg-Lund, on the incompleteness and inaccuracy of cercarial descriptions. As an example of incompleteness Cort (1914) in describing Cercaria trivolvis failed to include the cephalic spination and tail fin membrane in detail. Beaver (1937) called attention to this when he re-examined the type specimen of Cercaria trivolvis submitted by Cort in 1914.

From the standpoint of inaccuracies a careful study of existing cercarial descriptions, especially those which have received the most attention such as the echinostome and human schistosome forms, reveals that discrepancies exist in observations made by different workers on the same cercaria, and in some cases interpretations differ. Examples of these are cited.

The cercaria of Echinoparyphium recurvatum, a widely distributed echinostome, was described by Harper (1929) as having a descending excretory tubule that does not bifurcate; Wesenberg-Lund (1934), however, in a study of this species, describes a bifurcation of the excretory tubule in the region of the acetabulum. Sewell (1922) placed the cercaria of Echinostoma revolutum, another echinostome, in the Echinata group, one in which cercariae have no fin-fold on the tail. Beaver (1939), on the other hand, stated that this cercaria has a fin-fold and therefore should be removed from the Echinata group.

Miyairi and Suzuki (Vide Kuntz, 1950) described the cercaria of Schistosoma japonicum as having 5 pairs of flame cells. Miyagawa (fide Cort, 1919) studied the same cercaria but noted only the main excretory tubules. Cort (1919) also studied this form and reported 4 pairs of flame cells. Similar confusion holds true for Schistosoma mansoni and Schistosoma haematobium. Khalil (1922) reported 3 pairs of flame cells in the cercaria of Schistosoma mansoni while Manson-Bahr and Fairley (920) reported 4 pairs. The cercaria of Schistosoma haematobium was described by Archibald and Marshall (1932) as having 4 pairs of flame cells while Blacklock and Thompson (1924) and Bettencourt and de Silva (1922) described it as having only 3 pairs.

In addition to these discrepancies, the morphological feature called the "Island of Cort" has been unduly emphasized. Cort (1917) in discussing the detailed structure of the cercaria of Schistosomatium douthitti described a small structure in its caudal excretory tube. Faust (1919) gave it the name of "Island of Cort." Subsequent cercarial descriptions by many investigators included reference to the "Island of Cort." Kuntz (1950) in an extensive study of the fork-tailed cercariae found this morphological feature in only a very few of the many schistosome cercariae that he studied. He stated that "its presence is so uncommon that the structure is considered to be a somewhat anomalous condition resulting from incomplete fusion of the excretory tubes in the tail."

With the advance of systemic knowledge of cercariae many workers turned to investigations concerned with the ecology, activity and physiology of cercariae.

Ecological studies were started in those regions where larval and adult trematodes had been worked extensively. Manson-Bahr and Fairley (1920) were the first to record seasonal incidence of cercariae. Their studies were concerned with schistosome cercariae in Egypt, and included results from one year's investigation. Miller and Northup (1926) were the first to publish on a study of this nature in the United States in which they recorded the seasonal infections of the snail, Nassa obsoleta, with larval trematodes. McCoy (1928) followed with a two-year seasonal survey of infections of *Planorbis trivolvis*. Wesenberg-Lund (1934) made an exhaustive study of larval trematodes of Danish fresh-water molluscs from which he concluded that there were no special trematode faunae that characterized lakes, small lakes and ponds, nor ponds of a dystrophic or entrophic nature. He found that there was a greater percentage of infected snails in ponds and that the incidence was not predictable from one year to the next.

Perhaps the most notable workers in this country on the ecology of cercariae have been Cort and his co-workers. From 1937 to 1941 they have studied the trematode infections of the snails, *Stagnicola emarginata* (Sowerby), *Helisoma campanulatum smithii* (Baker), and *Physa parkeri* Currier collected from Douglas Lake, Michigan during the summer months. In addition to recording the kinds of infection for each species, they (Cort, McMullen, and Brackett, 1937; Cort, McMullen, and Brackett, 1939; and Cort, Oliver, and McMullen, 1941) have correlated infection with the age of the snail, noted cases of multiple infection, and completed the life cycles of the snail hosts. Additional workers in the United States have been Rankin (1939), who examined *Pseudosuccinia columella* Say from a small pond in western Massachusetts each month for a year for trematode infections, and Byrd and Reiber (1940), who examined numerous specimens of *Helisoma trivolvis* for infection.

Cercarial activity has been studied mainly in conjuction with taxonomic, life history, and ecological investigations. Practically all modern studies covering any one or more of these phases deal in some degree with the characteristic motility, tropisms, emergence, and encystment of the organism. These aspects of study are necessary since some forms can be distinguished not only on the basis of morphology but also on the basis of their other characteristics. Cort and Brackett (1937) found that four strigeid cercariae could be distinguished on the basis of activity alone, and this character has been used more in the strigeids as a basis of distinction than in any other group of trematodes.

As with all other branches of biological science where taxonomic aspects have been extensively studied, many recent cercarial investigations are concerned with physiology and quantitative aspects. This attitude of study originates in the potential driving force in investigation of biological science, namely, the transfer from morphological to physiological studies. This transfer took place originally in the relatively simple organisms, bacteria. With the discovery by Koch that bacteria could be cultured on artificial media, the entire field of bacteriology became almost immediately a field of physiological rather than morphological study. As a result, a knowledge of biochemistry, mathematics, and biophysics are prime prerequisites for research in bacteriology at the present time. With the growth of bacteriology as a physiological science many new methods and instruments have been developed for the study of these organisms. These methods and instruments have in turn stimulated the interests of those scientists, namely parasitologists, protozoologists, and botanists, interested in organisms that have a greater degree of complexity. Consequently, we see now a growing tendency of parasitologists to undertake physiological studies. As examples, Geiman at Harvard has managed to maintain malarial parasites through several cycles in artificial media; Taliaferro, Culbertson, Larsh, and others have studied the immune mechanism produced in hosts by animal parasites; Chandler and co-workers have been investigating the use of radio-isotopes in the study of parasites; and Ackert, Chandler and Larsh have been investigating the effects of vitamins and hormones on cestodes.

In the field of trematology studies concerned with physiological aspects also have been undertaken. Davis (1936) has extracted a histolytic ferment from cercariae which will adversely affect frog skin. Brackett and Beckman (1942) inoculated chick embryos with Schistosome cercarial material and succeeded in demonstrating a cercariacidal substance in the blood. Rees (1934) mentioned an investigator who, in his studies of Halipegus occidualis, noted sex reversal in the snail, Peringia ulvae, due to extensive larval trematode infections. Krull (unpublished manuscript) has recorded the effects of environmental changes on the various larval stages in a trematode life cycle. Although little has been done regarding quantitative aspects Krull (1941) has recorded the number of cercariae of Fasciola hepatica from a snail infected with only one miracidium.

In this review of literature the problems associated with the development of cercarial studies have been traced. The discovery by Thomas and Leuckart of the first trematode life cycle focused attention on the importance of cercariae. Taxonomic studies became numerous and often resulted in erroneous as well as important contributions in the field of cercarial knowledge. Attempts to classify cercariae improved as more and better cercarial descriptions appeared in the literature. With an increase in the number of known trematode life cycles taxonomic comparisons between adult and larval forms were made possible. This comparison made many taxonomists think that in order to find the natural system of trematode classification the larval as well as adult forms would have to be considered. Along with taxonomic studies, results of investigations on the biology of cercariae were reported. All of these studies served as aids for those who were studying taxonomy, control, and life cycles. At present time more and more interest is being shown in the study of the physiology of parasites, and the information from such work should be valuable in understanding host-parasite relationships.

METHODS AND MATERIALS

Certain procedures had to be followed to determine the taxonomy and biology of larval trematodes. These procedures included the collecting of snails; examination of snails for pre-carcarial, cercarial, and post-cercarial stages; identification of cercariae; preparation of camera lucida drawings and measurement data; and the determination of cercarial relationships to their hosts and environmental conditions.

Species of snails in the genera Helisoma and Physa were collected from aquatic habitats surrounding Stillwater, Oklahoma. Habitats from which collections were made were chosen to represent a wide variety of environmental situations. They were selected also on the basis of their availability for different vertebrates. Those areas surrounded by trees, shrubs, and rocks were generally considered to be preferred by mammals, reptiles, and amphibians; whereas those located in open grassland were considered to be more for birds.

Collections were made from 14 habitats; 2 large named lakes, Lake Carl Blackwell and Boomer Lake; 3 smaller unnamed lakes, Thomas Lake, the "Old Country Club" Lake, and Fair Park Lake; 6 unnamed farm impoundments, Bliss's Pond, Venn's Pond, College pond, Boomer Lake spillway, pond, and 2 additional ponds located near Highway 40 between Stillwater and the boundary of Eden Township; 1 natural pond located near Lost Creek on Highway 33 east of Perkin's Corner; and 2 sites on Stillwater Creek, one immediately below the Lake Carl Blackwell dam and the other where the creek corsses Highway 51 about one mile west of Stillwater. Bodies of water that yielded infected snails were considered as primary collecting areas. These were checked periodically during the fall, winter, and spring months in order to determine the seasonal fluctuation in the incidence of infection in the snails. The primary collecting areas are briefly described.

Boomer Lake was the largest primary area from which infected snails were collected. This lake is surrounded by grassland and provides excellent feeding grounds for waterfowl. Collections of snails were made from the east shore, which was littered with dead vegetation containing numerous snails.

Thomas Lake was the next largest primary collecting area. This lake resembled Boomer Lake in that it was surrounded by grassland. Snails were collected from a beach that was approximately 40 feet long and located in the southwest corner of the lake near the dam. The beach had a mud bottom with little vegetation, which was covered by shallow water, rarely over a foot in depth.

"Old Country Club" Lake was another, situated in a heavily wooded depression. The collecting area was along a beach and was covered with abundant vegetation, especially lily pads and decaying leaves. The water depth did not differ appreciably from that of the Thomas Lake area.

The two farm ponds selected for primary collecting areas differed considerably. Bliss's Pond was located in a ravine or depression and was surrounded on three sides by trees, shrubs, or rocks. The collecting site was restricted to an area of the north shore which was open and without trees, shrubs, or rocks and provided the best snail habitat. The area from which the snails were collected had a mud bottom with little submerged vegetation but considerable emerging vegetation (cat tails). Venn's Pond was located in a pasture where horses grazed during the entire year. With the exception of grass and a single tree the surrounding area was devoid of vegetation. The pond had a mud bottom and very few aquatic plants.

An additional pond selected for snail collecting was located below the Boomer Lake spillway in a depression or ravine which received water from the lake through a drainage pipe. This pond was surrounded by trees and shrubs and contained numerous decaying leaves, as well as algae and other aquatic vegetation. Due to its source of water the writer believes that fluctuation in the water level was probably quite frequent.

An area of Stillwater Creek located immediately below the dam of Lake Carl Blackwell also was selected for snail collecting. Collections were made from an area of the creek 50 feet long. The slowly flowing water in the creek bed contained dead leaves and much aquatic vegetation which also aided in retarding the flow.

Snails brought into the laboratory were placed individually in 50 cc glass containers filled with tap water which had stood overnight in order to allow time for the escape of the residual chlorine. Maintenance procedures consisted of changing water when it began to become cloudy and in feeding the snails occasionally by introducing wilted lettuce leaves. The latter was done when it was particularly desirable to increase longevity beyond several weeks, or when it was thought that feeding might induce cercarial emission. The snails were maintained in the laboratory from one to two weeks in order to determine whether they were infected. Snails from which cercariae escaped were maintained until studies of these larvae were completed, after which time the mollusks were dissected and the pre-cercarial stages studied. When sufficient time had elapsed the snails from which cercariae had not escaped were examined by dissection to determine the presence or absence of immature infections. Examination procedures consisted of making mounts in water of the digestive gland, mantle, kidney, and at times the entire snail. These parts were examined under the microscope and the trematode larvae were studied in infected snails.

The cercariae that escaped from a snail were poured off with the water into a Syracuse watch glass periodically. Cercariae to be examined were transferred from the watch glass by means of a capillary pipette to a Bureau of Plant Industry watch glass in order to concentrate them. This was done by drawing off the excess water with the capillary pipette. These concentrations of cercariae were used in making slide mounts. A drop in which the cercariae were concentrated was then transferred to a glass slide and a cover slip added. The slide was examined under various magnifications. This preliminary examination was to determine the type of cercaria; such as a stylet, echinostome, fork-tailed, amphistome and others. Following this preliminary procedure cercariae were generally examined in a Nembutal-water mount. This mount was prepared by adding a small quantity of Nembutal, an amount that would adhere to the end of a small sized dissecting probe, to a drop of water, containing cercariae, on a slide. Cercariae mounted in this manner were relaxed and became inactive but remained viable for at least a half hour. This procedure permitted a more detailed examination for spination, flame cell patterns, and the position of other internal structures. This method was exceedingly valuable in the study of stylet and echinostome cercariae.

Another effective method of examination was found in the use of the egg albumin mount described by Krull (1934). This method consisted of adding a small amount of either thin or thick egg albumin to a Bureau of Plant Industry watch glass in which a number of cercariae had been concentrated in the smallest amount of water possible. The cercariae and albumin were mixed together by stirring with a dissecting needle, after which the mixture was transferred to a slide and covered. Before allowing albumin to dry around the slip it was necessary to draw out from under the cover slip on the slide a small portion of the albumin by means of a dissecting needle. This procedure prevented the accumulation of albumin on top of the cover slip as it dried around the slip. In approximately 30 minutes the thin albumin mounts settled enough to flatten out the cercariae, but thick albumin mounts required several hours. Since the cercariae were viable for at least four hours in egg albumin, long periods of study were possible. This method was particularly desirable when forked-tailed cercariae were being examined.

It was necessary to employ intra vitam stains in order to observe the extent of the ceca, position and shape of the genital primordia, and to determine the number of penetration glands in the cercariae. These stains were prepared in aqueous solution. A small quantity, that amount which would adhere to a tooth-pick, was added to a water or an albumin mount prior to the addition of the cover slip. Of the three stains used (neutral red, nile blue sulfate, and malachite green), neutral red proved to be the most effective to determine cercarial features, and particularly the ceca and the genital primordia.

Another method applicable only to the study of echinostome forms was a clearing procedure in which glycerin was used as the clearing agent. To make the preparations cercariae were concentrated in water, fixed in hot 5% formalin and transferred to a container filled with a glycerin-alcohol solution (5 cc. of glycerin to 100 cc. of 70% alcohol). This container was allowed to stand without a cover until the alcohol had evaporated leaving the cercariae in glycerin. Slides of the cercariae were made and examined when it was convenient. This method permitted the observer to make accurate counts of the cephalic spines, which was difficult or impossible in other procedures. The addition of methylene blue to the mount prior to the addition of the cover slip helped at times to further accentuate the spines.

After the morphology of a cercaria had been determined by the methods described, camera lucida drawings and measurements were made. Cercariae were mounted in Nembutal-water solution, which produced complete relaxation, and all drawings of living cercariae were made at a magnification of either 100X or 430X. Completed drawings were photographed, and the photographs were reduced for inclusion in the thesis manuscript.

Measurements also were made of cercariae fixed in hot 5% formalin. To make these preparations the cercariae were concentrated in a Bureau of Plant Industry watch glass prior to fixation. The cercariae were fixed by adding hot 5% formalin to the container. The cercariae were mounted in the fluid temporarily and measured by using an ocular micrometer.

In certain instances additional measurements were made of specimens heat-fixed on slides in water. This permitted a comparison of the cercariae studied by the writer with those described by other workers, who measured their specimens after heat fixation in water rather than in formalin.

Measurements were made of the body length and width, tail length and width, stylet length and width, and the diameters of the oral sucker, pharynx, and acetabulum. In certain instances the diameter of eye spots was measured. All such measurements were needed as an aid in identifying cercariae.

In addition to cercarial descriptions other phenomena such as activity and periodicity, as well as the post-cercarial stages were described.

To observe activity cercariae were transferred to a Bureau of Plant Industry watch glass and both macroscopical and microscopical examinations were made. Characteristics of cercariae in motion and at rest were studied and recorded. Responses to light were demonstrated by placing cercariae in a watch glass in direct light, covering half of the container with a black shield and observing the position of cercarial concentrations. Characteristics of periodic escape of cercariae from snails were studied by changing the water hourly, whether day or night, and determining the absence or presence and number of cercariae in each change.

In some cases experiments were undertaken to verify parts of life cycles that had been established, or to determine parts of new ones. Only cercarial or post-cercarial stages were used in the experiments. Possible intermediate hosts, such as fish, tadpoles, snails and others, were exposed by putting them in containers with cercariae for several hours. The exposed hosts were maintained a number of days to allow any metacercariae to develop. The hosts were examined for metacercariae and any that were recovered were fed to possible hosts including chickens, mice, and turtles, since they were the only animals readily available. They were usually examined in one or two weeks to determine if they were hosts for a particular fluke. Any trematodes recovered were processed and identified. In some instances cercariae encysted in containers and such encysted metacercariae were fed to possible definitive hosts.

ANALYSIS OF DATA

During this investigation 20 species of cercariae were encountered; 2 amphistome cercariae, 3 echinostome cercariae, 8 furcocercous cercariae, and 7 xiphidiocercariae. The individual species of cercariae are described and discussed in this section of the thesis under these 4 major groups. Each major group is prefaced by an introduction. The cercariae are presented in a certain order so that those condisered first are the ones for which the life cycle is known, next are those species previously described in literature and completely redescribed in this study, then the species previously described in literature but only partially redescribed in this investigation, and finally those species new to science. In addition to these there are the species of questionable identity, for which there are incomplete data.

For each cercarial species considered there are included the description, a taxonomic comparison with related cercarial species; a review of the outstanding taxonomic features of the species; a record of the percentage of snails infected during the seasons; notes on the pre-cercarial stage in the snail; a record of the time of cercarial emergence from the snail; a description of swimming and resting activities of the cercaria; notes on the encystment characteristics; known life cycle data; and a discussion of the economic importance.

AMPHISTOME CERCARIAE

Cercariae in the Amphistome group are characterized by having an acetabulum at or near the posterior end of the body, and by the presence of highly refractile granules within the main excretory ducts.

An attempt to divide this group into subgroups was first made by Cort (1915). He recognized two distinct types which he placed in the sub-families Diplodiscinae Cohn, 1904 and Paramphistominae Fischoeder, 1901. The former sub-family was distinguished from the latter on the basis of the presence of retro-dorsal pharyngeal pouches, a circum-esophageal sphincter, and a large transverse connection between the two main trunks of the excretory system. Sewell (1922) divided the amphistome cercariae into two types, "Pigmentata" and Diplocotylea." The pigmentate type consisted of those cercariae deeply pigmented, with the oral sucker larger than the acetabulum, and without pharyngeal pouches. The diplocotyle type included the cercariae that are less deeply pigmented, especially in the posterior region; have numerous cystogenous glands which appear as rounded or pyriform cells containing oval or rod-like granules; have an acetabulum much larger than the oral sucker; and have pharyngeal pouches. Beaver (1929) pointed out that Cort's distinction was erroneous since the diagnostic characteristics of the present sub-families of the Amphistomidae are largely restricted to characters that are not present in the larval forms.

The scheme proposed by Sweell (1922); namely, the division of amphistomes into types "Pigmentata" and "Diplocotylea" on the basis of characteristics cited, is thought by most investigators to be the only satisfactory method of classification at the present time.

The life cycle of the amphistome is relatively simple. After the cercariae have left the first intermediate host, they seek a suitable surface on which to encyste. After the encystment they are infective and must be eaten by a definitive host to complete the cycle. Since the cycle involves only one intermediate host very little importance, in the way of pathology, has been associated with cercariae.

Two amphistomes, cercaria of Allassostoma parvum and Cercaria S, were encountered in this investigation, and the latter is new. Both of these cercariae are described.

Cercaria of Allassostoma parvum Stunkard, 1916:
Paramphistomatidae
(Plate I, figure 2)

Specific Diagnosis:

Amphistome cercaria of Diplocotylea type. Body large, elongate pyriform, 0.730 to 1.051 mm (average, 0.890 mm) long by 0.525 to 0.627 mm (average, 0.561 mm) wide. Tail longer than body, 1.343 to 1.606 mm (average, 1.498 mm) long by 0.102 to 0.146 mm (average, 0.116 mm) wide. Pigmentation dense in anterior region and diminishing in amount posteriorly until negligible at region midway of body. Eye spots amorphous consisting of blotches of pigment, 41 to 44 u (average, 43 u) long by 24 to 38 u (average, 29 u) wide, with thin lens directed anteriorly. Cystogenous particles buscuit-shaped and distributed throughout body exclusive of oral sucker and acetabulum. Non-pigmented oral sucker, 138 to 190 u (average, 164 u) long by 103 to 179 u (average, 140 u) wide, with pair of posterio-lateral pouches, 38 to 44 u long by 34 to 38 u wide. Mouth with fringed border, opening into a relatively long pre-pharynx. So-called pharynx an inconspicuous internal muscular thickening of digestive tube, 44 to 86 u (average, 67 u) long. Digestive tube bifurcates at level of first fourth of body length to form ceca extending to level of anterior part of excretory bladder. Acetabulum terminal and larger than oral sucker, 207 to 321 u (average, 258 u) long by 276 to 321 u (average, 283 u) wide. Excretory bladder bulbous with posterior median excretory pore. Bladder receives on either side a common duct, each proceeds anterio-lateral to respective cecum in irregular convolutions which never encircle cecum, but lie alternately over and under it in a series of loops. Excretory granules present and in lineal series only in the section of duct associated with cecum. In region below oral sucker and midway between eye spot and pharynx each common duct turns posteriad, each bifurcates immediately posterio-lateral to its respective eye spot, forming a posterior lateral and posterior median canal. Each posterior median canal descends in irregular convolutions under cecum to bifurcate at mid-body level forming a median and lateral branch. Each median branch turns mesad and terminates in at least one capillary. Each lateral branch crosses posterior lateral canal, turns mesad and descends to region of excretory bladder where it terminates in at least one capillary which receives approximately 4 smaller capillaries. Each posterior-lateral canal descends near lateral margin of cecum to region opposite excretory bladder where it becomes greatly convoluted until anterior margin of acetabulum is reached, where it again straightens and bifurcates to form 2 branches in the posterior third of acetabulum. The anterior branch connects with the one from the opposite side, while the posterior one terminates, apparently, in fine capillaries. Caudal excretory canal extends into distal region of tail and bifurcates to form 2 bladder-like structures. Flame cells were observed in regions of oral sucker, inner margin of ceca, and at acetabulum; exact pattern not determined. Genital primordia represented by 3 masses forming triangle, 2 testes, oblique, just below bifurcation of cecum and ovary in posterior half of body. Host: Helisoma trivolvis.

Discussion:

This cercaria agrees with the description of the cercaria of Allassostoma parvu by Beaver (1929). Details of the excretory ducts, digestive system, genital organs, eye spots, and body pigmentation were identical. The writer was unable to determine as complete a flame cell pattern as that described by Beaver. Additional details have been observed in the excretory system and it has been shown that the ducts never encircle the cecum but that the loops lay alternately over and under it. Beaver (1929) considered this form to be identical with Cercaria inhabilis Cort, 1914 and Cercaria convoluta Faust, 1919.

Cercariae of Allassostoma parvum were recovered from snails, Helisoma trivolvis, collected from 2 habitats, Bliss's Pond and Venn's Pond. Collections made from Bliss's Pond on November 16, 1950 yielded 4 infected snails (8.6 per cent) of 46. Of the 19 snails collected from the same place on February 18, 1951 none was infected; and of 13 snails collected from this locality on April 9, 1951 only one (7.0 per cent) was infected. Snails were collected from Venn's Pond on March 7, 1951 and April 22, 1951. Of the 42 collected on March 7 only one (2.3 per cent) was infected and in the collection of 14 snails on April 22 two (14.2 per cent) were infected. It appears that the number of infected snails decreases during the winter months and increases during the spring months.

These cercariae develop in rediae in the digestive gland of the snail. These rediae measure 0.803 to 1.095 mm in length and 0.189 to 0.233 mm in width. They are characterized by a pair of locomotor appendages and a gut filled with material varying in color from light yellow in the smaller forms to dark brown in the larger ones. Each contains 1 or 2 developed cercariae. Beaver (1929) distinguished between a redia and a mother redia, but these harbored only mother rediae.

Cercariae generally emerge from the snail during the early morning hours prior to 8:00 A. M. Cercariae are very active, swim almost continuously, and consequently have very few rest periods. While swimming they keep the body in a contracted state with the anterior end bent ventrally and extended posteriorly. Movement is accomplished by the actively lashing tail. Cercariae in motion generally move with the tail forward. They occasionally relax in mid-water, drop to the bottom of the container, and test the substratum with the oral sucker. After a brief period they again start swimming. Encystment occurs a short time after emergence from the snail. The cercaria selects a spot on the container wall which is usually near the water surface, secretes cyctogenous material, and encysts. Tails are dropped during encystment and they continue to move actively in the water for several hours. All cercariae that emerged during the morning were encysted by 1 P. M. If the cercariae were placed in a dish of shallow water they would not encyst until the water had evaporated, and the encysted metacercariae remained viable for 3 hours after evaporation.

Beaver (1929) fed some of these metacercariae to snapping turtles (Chelydra serpentina) and bullfrogs (Rana catesbiana) and recovered adults of Allassostoma parvum from both hosts subsequently.

Cercaria S
(Plate I, figure 1)

Specific Diagnosis:

Amphistome cercaria of Diplocotylea type. Body elongate pyriform, 0.423 to 0.511 mm (average, 0.459 mm) long by 0.202 to 0.394 mm (average, 0.331 mm) wide. Tail longer than body, 0.600 to 0.934 mm (average, 0.836 mm) long by 0.116 to 0.131 mm (average, 0.123 mm) wide. Sensory spines distributed over body surface extending from region of mouth to level immediately anterior to acetabulum. Pigmentation scarce and confined to region around eye spots. Eye spots definite in outline, 50 ^{u} long by 21^{u} wide, with lenses directed laterally. Cystogenous particles biscuit-shaped, sparsely distributed in clumps over body. Oral sucker 166^{u} long by 114^{u} wide, with pair of posterio-lateral pouches. Mouth opening into a relatively long pre-pharynx. Pharynx a conspicuous muscular structure, 53 to 69^{u} (average, 61^{u}) long by 34 to 38^{u} (average, 37^{u}) wide. Digestive tube bifurcates at level of first third of body length to form ceca extending to level of anterior portion of excretory bladder. Acetabulum terminal and larger than oral sucker, 224 to 280^{u} (average, 248^{u}) by 128 to 159^{u} (average, 149^{u}). Excretory bladder bulbous with posterior median excretory pore. Bladder receives on either side a common duct, each of which procedes laterally. Ventral to its respective cecum each turns and continues anteriorly and parallel to it in irregular convolutions. At level midway of body each common duct turns mesad and forms a loop on ventral surface of cecum. Excretory granules present only in this ventral loop. Each duct continues dorsally and proceeds to region immediately lateral to eye spot where each turns posteriad. Each descending duct gives rise to small lateral branch midway between eye spot and cecum. Each duct bifurcates at level of common duct containing granules to form a median and lateral tubule, both of which continue posteriorly some distance apart and rejoin at level of posterior end of ceca. Lateral branch gives rise between junction points to a medial vessel running posteriorly and terminating in 2 capillaries. Duct continues medio-posteriad on either side of body and apparently terminates near excretory bladder. Excretory bladder surrounded by anastomosing excretory ducts from unknown sources. One of the ducts penetrates acetabulum giving rise to a medial branch that terminates in 2 capillaries and a posterior branch that terminates in 3 capillaries. Caudal excretory duct extends from region of excretory pore to level of lower one-fifth of tail to terminate in 2 branches. Flame cells not observed. Genital primordia represented by 3 masses, 2 testes just below bifurcation of intestine and ovary in region midway of body. Host: *Helisoma trivolvis*.

Discussion:

Cercaria S can be distinguished from all other amphistome cercariae on the basis of the arrangement of its excretory tubules. It closely resembles the cercaria of *Diplodiscus temperatus* in that both have the same general arrangement of genital primordia, excretory granules, and eye spots. *Diplodiscus temperatus* differs, however, in arrangement of excretory tubules, absence of sensory spines on body, and in having a tail shorter than the body. *Cercaria missouriensis*, another species closely related to Cercaria S, differs essentially in the arrangement of excretory tubules and size, *C. missouriensis* being larger.

Cercaria S was recovered from one of 13 *Helisoma trivolvis* (7.6 per cent) taken from Venn's Pond on March 7, 1951. Another collection of 10 snails from this pond on April 22, 1951 was negative.

The cercariae develop in rediae which have one pair of locomotor appendages. The rediae measure 0.919 to 1.810 mm (average, 1.389 mm) in length by 0.248 to 0.949 mm (average, 0.505 mm) in width. Each contains a conspicuous light brown gut and usually not more than 1 or 2 developed cercariae.

Cercariae emerge from the snail during the day, especially between 1:00 and 3:00 P. M. They are rapid, energetic swimmers and prefer to congregate on the side of the container nearest the light. When swimming, the anterior end of the cercaria is bent ventrally and posteriorly, and movement is accomplished by the actively lashing tail. The bent body appears to sway back and forth on an axis located just above the tail. This swaying action often suggests the wing motion in the flight of butterflies. Resting periods for the cercariae are very infrequent, and the cercariae seem to be intent on only one thing, which is to find a surface suitable for encystment. The majority seem to prefer to encyst on the container near the water surface. They attach, lose their tails, secrete cystogenous material, and encyst. The tails become detached and continue to move actively in the container for several hours. A careful examination of snail excreta showed that the snails habitually ate the metacercariae which passed through the intestinal tract intact. If the cercariae were placed in a dish of shallow water, many of the cercariae encysted as soon as evaporation stopped their swimming activity.

Three species of adult amphistomes, Diplodiscus temperatus in frogs, Allassostoma parvum in frogs and turtles, and Pseudodiscus zibethicus in muskrats, are known to occur in the vicinity of Stillwater. The life cycles of these forms, with the exception of Pseudodiscus zibethicus, have been determined. Since Cercaria S was a new form and the life cycle of _P_. _zibethicus_, a common muskrat fluke in this locality, was unknown, metacercariae of Cercaria S were fed to laboratory mice to determine whether they could be infected. One mouse was examined one week later and was negative for trematodes.

ECHINOSTOME CERCARIAE

Before describing the specific echinostome cercariae it is desirable to discuss briefly their classification, type of life cycle in which they are involved, and the relative importance of the larval stages in the cycle.

The echinostome cercariae are generally characterized by the presence of one or two rows of priminent spines which extend across the anterior dorsal surface of the body, turn ventral at the lateral margins, and progress for a short distance on the ventral surface. These spines are sometimes called "collar" spines since they partially encircle a pair of prominent shoulders which are found near the anterior end of the cercaria. Such spines are not present in a few echinostome cercariae, but these have other anatomical echinostome characteristics. The excretory system is characterized by widely dilated ascending tubules, which are filled with excretory granules, and extend from the excretory bladder to the vicinity of the oral sucker where they constrict, turn and progress posteriad.

Since 1909 attempts have been made to classify larval echinostomes into sub-groups on the basis of various characteristics. Lühe (1909) was the first to classify larval echinostomes. He distinguished two main larval types on the basis of the length of the gut in the rediae. The cercariae of these two types were further divided on the basis of the presence or absence of a fin fold on the tail. Sewell (1922) devised a more extensive classification of these cercariae in which he formed three groups; Echinatoides, Coronata, and Echinata. The Echinatoides group consisted of species characterized by having a transparent fin fold on the tail, ceca extending to posterior region of the body, angle spines of the cephalic collar slightly larger than the rest, and the main paired excretory tubules bifurcating into anterior and posterior branches at the level of the acetabulum. The Coronata group consisted of cercariae that were characterized by having cephalic spines of the same size and no fin fold on the tail. The Echinata group of cercariae was similar to the Echinatoides Group with the exception that the paired excretory tubules did not bifurcate at the level of the acetabulum as in the Echinatoides group. Faust (1924) attempted to separate echinostome cercariae into ten sub-groups on the basis of the excretory system. Miller (1936) remarked on the fallacy of such a scheme by pointing out that many of the known species of echinostome cercariae were described too inadequately for definite assignment to groups on the basis of the excretory system. Byrd and Reiber (1940) concurred with Miller.

Sewell's scheme, consisting of the groups Echinatoides, Coronata, and Echinata, is employed in classification of echinostome cercariae at the present time.

Echinostome cercariae develop in rediae that parasitize the digestive gland of the first intermediate host snail. When the cercariae have completed development and escaped from the snail they seek a second intermediate host which may be any one of a number of invertebrates or even a fish. The definitive host becomes infected by ingesting an infected second intermediate host.

The economic importance of larval echinostomes is relatively insignificant. They do, however, adversely affect snail populations since digestive gland destruction is associated with the numerous rediae resulting from echinostome infections in snails and death ensues sooner or later. Rees (1934) found that the physiological injury of the rediae to the snail was more important than the mechanical injury. He concluded that the part of the snail invaded by rediae ceases to function properly and occupies itself mainly in attempting to remove the toxic excretory products produced by the rediae, which leads to partial or complete atrophy of the tissue involved. The effects on second intermediate hosts have not been determined.

Three echinostome cercariae were encountered in the vicinity of Stillwater. Echinostoma revolutum, and Echinoparyphium recurvatum, and Petasiger nitidus are described, the latter being incomplete.

Cercaria of Echinoparyphium recurvatum (Von Linstow, 1873)
Lühe, 1909: Echinostomatidae
(Plate II, figures 6, 7)

Specific Diagnosis:

Echinostome cercaria of Echinata group. Body elongate oval, with conspicuous head collar, 0.269 to 0.342 mm (average, 0.307 mm) long by 0.155 to 0.190 mm (average, 0.171 mm) wide. Tail, 0.380 to 0.519 (average, 0.447 mm) long by 0.044 to 0.058 mm (average, 0.052 mm) wide. Forty-five collar spines, 10 on each side and shoulder, and 25 in 2 rows on dorsal surface. Oral row of spines shorter than those of aboral row. Inconspicuous spines in irregular rows cover dorsal and ventral surfaces of body from level of oral sucker to level of acetabulum. Granular cystogenous glands abundant over body. Oral sucker 41 to 51^{u} (average, 46^{u}) in length by 27 to 44^{u} (average, 39^{u}) in width. Mouth opening into relatively short pre-pharynx, followed by muscular pharynx 24^{u} in length by 17^{u} in width. Esophagus relatively long, bifurcating immediately above acetabulum, ceca extend to level midway of excretory bladder. Acetabulum muscular, 48 to 55^{u} (average, 51^{u}) long by 55 to 62^{u} (average, 58^{u}) wide, located in posterior half of body. Penetration glands not observed. Gland ducts open in region of oral sucker; 2 pairs of 3 each, situated laterally, open on anterio-lateral margin of oral sucker. Excretory bladder bulbous with small anterior chamber receiving on either side an excretory duct, which extends to anterior part of body. These ducts have large diameters anterior to bladder and contain excretory granules from level of pharynx to level of anterior border of acetabulum; each narrows anteriorly and forms a reverse loop in region of pharynx, descends to vicinity of excretory bladder, and constricts to form another ascending tubule. Twenty-one flame cells on either side of body, exact pattern not determined. Excretory canal extends from bladder into tail, bifurcating at first fifth of tail to form 2 canals which terminate at their respective lateral margins. Host: *Physa gyrina*.

Discussion:

The morphology of my specimens compared favorably with the description of the cercaria of *Echinoparyphium recurvatum* by Harper (1929). Number and arrangement of collar spines, arrangement of excretory ducts, and ralative size of suckers were identical. The writer's specimens differed only in measurements, Harper's form being slightly smaller, and in the number of flame cells, the writer's form had 12 more flame cells per side. These were considered to be the cercaria of *E. recurvatum*, because they were recovered from snails in close association with other snails naturally infected with the metacercariae of *E. recurvatum*. A closely related cercaria is that of *Echinoparyphium flexum* described by McCoy (1928). These cercariae differed from *E. flexum* primarily in the arrangement of excretory ducts. McCoy's form has a descending duct that bifurcates in the region of the acetabulum whereas the descending duct in these does not bifurcate.

The position of this cercaria and its adult form in classification is rather confused.

McCoy (1928), in his study of the cercaria of *E. flexum*, noted a bifurcation of the paired descending excretory tubules in the region of the acetabulum. Because of this bifurcation he placed the form in Sewell's Coronata group than includes echinostome cercariae with this type of excretory system. Harper (1929) studied the larval forms of *E. recurvatum*, which are similar to the larval forms of *E. flexum*, and noted no bifurcation of the paired descending excretory tubules. This meant that the cercaria of E. recurvatum belonged in Sewell's Echinata group. Wesenberg-Lund (1934) also studied the larval forms of E. recurvatum and according to Dawes (1946) placed this form in the group Coronata because of a bifurcation in the excretory tubule. The writer has examined the cercaria of E. recurvatum and has found no bifurcation of the excretory duct and therefore proposes that the cercaria remain in Sewell's group Echinata.

Von Linstow (1873) first described the adult E. recurvatum from ducks in Europe. Linton (1892) described a similar form in the United States and named it E. flexum. A comparison of these two descriptions shows that E. flexum is possibly a synonym of E. recurvatum. If this is true, McCoy's cercaria of E. flexum must be the same as the cercaria of E. recurvatum.

Cercariae of Echinoparyphium recurvatum were recovered from snails, Physa gyrina, taken from Thomas Lake. Six snail collections were made at this lake and all snails were taken from a beach approximately 50 feet in length. The number of specimens infected with E. recurvatum in relation to the total snails recovered on each collection day is as follows: September 24, 1950, 14 of 50 (28.0 per cent); October 15, 1950, 6 of 20 (30.0 per cent); December 14, 1950, 5 of 15 (33.3 per cent); January 21, 1951, none of 13; March 8, 1951, none of 10; and May 6, 1951, 2 of 23 (8.6 per cent).

These incidence figures indicate that there was a drastic reduction in the number of infected snails subsequent to December 14, 1950 and that this decreased incidence persisted for at least 2 months followed by a gradual increase beginning during May. A possible interpretation based on these figures would be that infected snails were weakened by infection and therefore died during the colder months of the year. Cort, Oliver, and McMullen (1941) found that a similar phenomenon occurred when they studied E. recurvatum infection of Physa parkeri at Douglas Lake, Michigan. In their investigations they found that 52 per cent of the snails were infected on July 11, 57 per cent on July 27, and only 19 per cent on September 3. They interpreted this marked reduction in incidence as being due to the lethal effect of the larval stages of E. recurvatum on Physa parkeri. The results in this investigation seem to substantiate the correctness of this statement.

Cercariae of Echinoparyphium recurvatum develop in rediae which are located in the digestive gland of the snail, Physa gryina. The rediae measure 0.400 to 1.160 mm in length and 0.184 to 0.484 mm in width. They are characterized by having a definite head collar, a gut varying from colorless in the smaller forms to dark brown in the larger ones, body with orange pigmentation, and a pair of appendages.

Cercariae emerge from the snail prior to 8:00 A. M. They are strong active swimmers but move in a lumbering fashion. While swimming, the body is contracted and the anterior end is bent ventro-posteriorly, giving the cercaria a U-shaped appearance. Forward movement is accomplished by means of the actively lashing tail. Rest periods are quite frequent and increase in number as the cercariae grow older. The cercariae swim to the bottom of the container periodically, where they usually remain in a contracted position with the tail actively lashing for a minute or two, after which they stretch out, roll over several times, and proceed to crawl caterpillar fashion along the substratum. Crawling may continue for some time before swimming is resumed.

Metacercariae were found encysted in snails of two species, Physa gyrina and Helisoma trivolvis. In nature, cysts are found in the kidney of the snail and as many as 295 cycts have been taken from a snail.

In the laboratory, if cercariae shed in the morning were permitted to remain in containers with snails, all would have penetrated a host by 8:00 P. M. If snails in the laboratory were repeatedly subjected to large numbers of cercariae, cysts occurred in the mantle and foot as well as the kidney. In many of the snails exposed to numerous cercariae, metacercariae were found in the foot and mantle when the kidney contained far less metacercariae than would be found under conditions in nature, where encystment apparently occurred only in the kidney.

Often concurrent infections were found in snails parasitized with E. recurvatum. Mesocercariae and metacercariae of a strigeid were often localized in the digestive gland of an infected snail. Larval stages of an acanthocephalid at times also were found attached to the foot, mantle, or various glands in such infected snails.

Cysts dissected from snails, which were found to be infected at the time of their collection, were fed to laboratory mice and one-day old chicks. One week after feeding, the mice and chicks were examined and mature adult flukes were found in the small intestines of both hosts. Specimens recovered from chicks were morphologically similar to the description of Echinoparyphium recurvatum by von Linstow (1873). Those recovered from the mice were considerably smaller but, except for their size, agree with the description.

Echinoparyphium recurvatum, like Echinostoma revolutum, has a wide distribution and lacks host specificity. The species has been recorded from Europe, Japan, Formosa, Philippine Islands, and the United States.

It is known to infect domestic fowl, ducks, pigeons, and muskrats. In the United States this species has been recorded by Cort, Oliver, and McMullen (1941) in muskrats of Michigan; by Annereaux (1940) in domestic turkeys of California; and by Self and Bouchard (1950) in wild turkeys of the Wichita Mountains Wildlife Refuge, Oklahoma. This is the first report of the mouse as an experimental host. In regard to pathology associated with this species, Mönnig (1941) states that infected fowl at autopsy have a marked enteritis with swelling of the mucosa and an increase in the mucous content of the bowel. Consequently, it may be of some economic importance.

Cercaria of Echinostoma revolutum (Froelich, 1802)
Looss, 1899; Echinostomatidae
(Plate II, figures 3, 4, 5)

Specific Diagnosis:

Echinostome cercaria of Echinata group. Body elongate oval with conspicuous head collar, 0.283 to 0.384 mm (average, 0.338 mm) long by 0.183 to 0.221 mm (average, 0.196 mm) wide. Tail approximately seven-eighths length of body, 0.352 to 0.415 mm (average, 0.375 mm) long by 0.038 to 0.048 mm (average, 0.039 mm) wide with distinct terminal projection because fin fold, causing bend in tail, extends along distal portion to near end. Thirty-seven head collar spines, 5 on each shoulder, 6 on each side, and 15 in 2 irregular rows on dorsal surface. Inconspicuous spines or cuticular markings cover dorsal and most of ventral surface of body. Stiff spines, probably sensory spines, equally spaced, project at right angles from body on lateral margins, extending from anterior end to level of posterior margin of acetabulum. Granular cystogenous glands abundant throughout body. Oral sucker 44 to 55^u (average, 50^u) in diameter. Mouth subterminal, pre-pharynx short followed by muscular pharynx 20 to 31^u (average, 27^u) in diameter. Esophagus relatively long, bifurcating immediately above acetabulum, ceca extend to level of posterior margin of excretory bladder. Acetabulum muscular, 58^u in diameter, located in posterior half of body. Penetration glands indistinct, approximately 6 on each side of esophagus and in linear series. Gland ducts open in region of oral sucker; 3 pairs distinguishable at level of pre-pharynx, curve dorsally over oral sucker to empty at its anterior margin; three pairs, situated laterally, open on its anterio-lateral margin. Excretory bladder bulbous with small anterior chamber receiving on either side an excretory duct. These ducts have large diameters anterior to bladder and contain excretory granules from level of pharynx to level of anterior border of acetabulum; each narrows anteriorly and forms a reverse loop in region of pharynx; descends to level of excretory bladder; constricts and turns to form another ascending tubule which receives capillaries from 6 series of 3 flame cells each, distributed length of body. Caudal excretory canal extends into tail, bifurcating at level of first fifth to form pair of diverging canals which terminate at their respective lateral margins. Host: Helisoma trivolvis.

Discussion:

The morphology of specimens agree with the description of the cercaria of Echinostoma revolutum by Beaver (1937). The characteristics which distinguish it from all other echinostome cercariae are the arrangement of the 37 head collar spines, the 12 conspicuous ducts opening in the region of the oral sucker, and the presence of a fin fold on the tail. Beaver determined that Cercaria helvetica XXIV Dubois and Cercaria trivolvis Cort were synonyms of Echinostoma revolutum.

Cercariae of Echinostoma revolutum were recovered from snails, Helisoma trivolvis, collected from 2 habitats, Thomas Lake and Stillwater Creek below Lake Carl Blackwell. Five specimens of Helisoma trivolvis were collected from Thomas Lake on March 8, 1951 and only 1 of the 5 (20 per cent) was infected. Collections from Stillwater Creek on November 3, 1950 yielded 12 of 39 snails infected (30.7 per cent); those on December 26, 1950 yielded 7 of 14 snails infected (50.0 per cent); those on February 6, 1951 yielded 8 of 14 snails infected (57.1 per cent); and those on April 1, 1951 yielded 15 of 21 snails infected (71.4 per cent). Although the percentage of infected snails increased from November until

April, cercariae did not emerge as readily from the snails in the late winter and spring months as they did during the fall and early winter months. Infected snails collected during November and December shed cercariae within 3 days after capture whereas those collected during February and April required from 2 to 3 weeks before cercariae appeared. This failure to emerge may be due to any of a number of factors, chief among which could be the low water temperature in late winter and spring. Rankin (1939) found that snails, Pseudosuccinea columella, collected in Massachusetts reached their peak of infection with Echinostoma revolutum during the month of January and that snails did not shed cercariae in any numbers until they had been in the laboratory at least 9 days. He concluded that temperature was a modifying, but not the determining factor in this phenomenon.

Cercariae of Echinostoma revolutum develop in rediae located in the digestive gland of the snail. These rediae measure 0.166 to 2.0 mm in length by 0.034 to 0.3 mm in width. They are characterized by having a collar that encircles the redia near its anterior end, a gut that varies from colorless in the smaller forms to deep orange in the older ones, and a pair of appendages. Beaver (1937) pointed out that size of rediae is widely variable and that mother and daughter rediae can be distinguished. The writer agrees with Beaver's statement concerning the size variation of radiae but mother and daughter rediae could not be distinguished.

Cercariae emerge from the snail during the daylight hours but there seemed to be no particular hours during which they preferred to emerge. When the cercariae swim they contract the body until the acetabulum appears as a sphere within an actively vibrating sphere. The posterior border of this sphere often appears to have clear alae-like structures attached to it, which disappear when swimming ceases. The swimming cercaria seem to sway back and forth on an axis located near the acetabulum and the tail propels it forward by actively lashing in a figure eight pattern. At times the swimming cercaria will appear to halt in midwater, with the tail still lashing, to extend the anterior end of the body several times, after which it continues its normal swimming activity. Resting periods are rather infrequent. They are initiated when the cercaria stops in midwater and drops to the bottom. It is inactive for several seconds, after which it proceeds to crawl caterpillar fashion over the substratum using the oral and ventral suckers.

Encystment may occur in water, snail slime, or in an intermediate host. This is the first time that encystment of this species has been observed outside of an intermediate host. This was discovered by preparing 3 containers; one was filled with water, another with a mixture of snail slime and water, and a third with water and grass blades. Cercariae were added to each of these and the containers were covered and examined 3 days later. A few viable, encysted metacercariae were recovered from each of the three receptacles. In several instances where infected snails were retained in a container for several weeks, metacercariae were recovered from the snail castings. Whether the cercariae had encysted in the castings or the snails had eaten metacercariae that encysted in the water was not determined. These conditions of encystment might increase chances of infection of the definitive host.

Encystment probably occurs normally in intermediate hosts. According to Beaver (1937) snails, clams, planarians, frogs, tadpoles, and catfish can serve as intermediate hosts for <u>Echinostoma revolutum</u>. In this investigation only snails of the genera Helisoma and Physa were verified as intermediate hosts of E. revolutum. In areas where the cercariae were common snails were infected with echinostome metacercariae. Many infections were observed in snails from Stillwater Creek just below Lake Carl Blackwell. The encysted metacercariae were confined to the kidney of the snail and as many as 488 were collected from the kidney of a specimen of Physa gyrina.

The life cycle of this species was verified from the cercaria to the adult under laboratory conditions. Laboratory reared specimens of a Physa were subjected in a container to cercariae for a period of 4 hours, and the snails were retained for a period of 3 days subsequently to allow for the development of metacercariae. Metacercariae were recovered by dissection and fed to 2 one-day old chicks. The chicks were examined in two weeks and mature specimens of Echinostoma revolutum were recovered from the cecum and the terminal parts of the small intestine of both.

Echinostoma revolutum is probably unique among trematodes since it lacks specificity and has almost a world-wide distribution. It is known to mature in 32 species of birds and 9 species of mammals, including man, and it has been found in every geographical region of the world with the exception of South Africa and Central China (Beaver, 1937). This is the first time that the species has been reported from Oklahoma. Its economic importance seems to be limited. Biester and Schwarte (1948) showed experimentally that this fluke was pathogenic to pigeons. In their experiments they observed that pigeons with light infections were not seriously affected whereas those with heavy infections, 621 to 5000 flukes, had severe enteritis followed by hemorrhagic diarrhea, emaciation, and finally died. It seems reasonable to assume that if this parasite affects chickens in a similar way it could be of negative economic importance in chickens. Areas of serious infections would probably be restricted to regions where water levels are changed in irrigation ditches. Chickens usually pick up all small organisms, including fish, when such areas are exposed. It is known that chickens acquire infections of other pathogenic flukes under similar conditions. The few localities of the world where this species is considered to be of medical importance are characterized by having native populations which are in the habit of eating raw fresh water mussels. Formosa has an incidence of 3 to 6 per cent in the native population. Five cases have been reported from Mexico and a few from Java (Rodriques, 1940).

Cercaria of <u>Petasiger</u> <u>nitidus</u> Linton, 1928: Echinostomatidae

<u>Specific Diagnosis</u>:

A large-tailed echinostome cercaria. Body ovate, 0.174 mm long by 0.076 mm wide. Rail many times larger than body. Head collar with 19 spines in irregular single row. Minute spines distributed over entire body, more conspicuous anteriorly. Cystogenous material consists of irregularly placed rectangular bars with longitudinal striations, distributed in most of body posterior to pharynx. Oral sucker 27 to 34^u (average, 29^u) in diameter. Mouth opening into short pre-pharynx, pharynx muscular, 6 by 8^u, and always contains brownish-orange pigment. Esophagus relatively long, bifurcating immediately above acetabulum, ceca extend to level of excretory bladder. Acetabulum, 27 to 34^u (average, 31^u) in diameter. Excretory bladder bulbous with an anterior and posterior chamber. Posterior chamber in proximal portion of tail. Anterior chamber in distal portion of body and receives on either side an ascending excretory duct. Ducts increase in diameter anterior to acetabulum and contain excretory granules from level of anterior border of acetabulum to level of pharynx where each narrows, forms a reverse loop, and descends posteriad. Flame cell pattern 2(3 + 3 + 3). Host: <u>Helisoma trivolvis</u>.

<u>Discussion</u>:

Drawings and a complete description of the cercaria of <u>Petasiger nitidus</u> are not included in the thesis because the cercaria was encountered only once during the investigation.

A specimen of <u>Helisoma</u> <u>trivolvis</u> collected November 3, 1950 from Bliss's Pond yielded large-tailed echinostome cercariae which were identified as those of Petasiger nitidus described by Beaver (1939). The specimens agreed with Beaver's description, including measurements, flame cell pattern, collar spination, appearance of cystogenous material, and the presence of a characteristic and conspicuous brownish-orange pigmentation in the pharynx.

The cercariae emerge from the snail in the early morning hours prior to 8 A. M. Soon after emergence they assume a vertical position suspended in the water. This position is maintained by means of the constant action of the tail which undulates in a figure S. Occasionally the tail will stop undulating. When this occurs the cercaria sinks gradually to the bottom where it may remain motionless for a short time and then resumes its swimming activity.

They may be seen without the aid of a microscope and either in the swimming or resting position suggest a fish lure.

On November 6, 1950 a small fish, Lepomis cyanellus, was put in an aquarium containing numerous cercariae. The fish struck at the cercariae and proceeded to eat them, but their tails were eliminated through the opercular openings of the fish. It was exposed to cercariae overnight and retained for examination. The fish was examined November 13, 1950 and encysted echinstome metacercariae were recovered from the wall of the esophagus. The oblong cysts had relatively thick hyaline walls. The encysted metacercariae were fed to a day old chick. The chick was examined 12 days later and was negative for trematodes.

Five specimens of Gambusia affinus were exposed to cercariae November 7, 1950. The 5 fish were examined 20 days later and encysted metacercariae were recovered from the wall of the esophagus in all specimens. The cysts recovered from the gambusia were surrounded by more infiltrated tissue than those recovered from the lepomis. The encysted metacercariae were fed to a 2-day old chick. It was examined 7 days later and was found negative for trematodes.

These experiments show that *Lepomis cyanellus* and *Gambusia affinis* may serve as second intermediate hosts of this fluke.

Beaver (1939) was the first to complete this life cycle experimentally. He exposed fishes of the genera *Ameiurus*, *Notropis*, *Umbra*, *Lepomis*, *Ambloplites*, *Perca*, and *Lebistes* to cercariae. Following exposure he examined the fish and found metacercariae encysted in the walls of the mouth and esophagus. He fed metacercariae less than 5 days old to pigeons, mice, a rat, a cat, and a duck; and metacercariae 7 to 11 days old to canaries. All of the animals were examined 12 days after they had been subjected to infection and all except the canaries were negative for trematodes. The specimens found in the canaries were identified as *Petasiger nitidus*. Beaver believed that the failure to establish infection in the other animals was due to immaturity of the metacercariae.

Further study of this species would be profitable to establish more intermediate hosts and to find the natural definitive host in this locality since it is not known.

FURCOCERCOUS CERCARIAE

Forked-tailed or furcocercous cercariae are those that have a tail which consists of a stem and two distal furcae or appendages.

Before describing the specific furcocercous cercariae it is desirable to discuss briefly their classification, type of life cycle, and the relative importance of their larval stages.

Lühe (1909) was the first to attempt a classification of furcocercous cercariae; he worked with the ten European species known at that time. The cercariae of these species were subdivided on the basis of presence or absence of a ventral sucker into the Monostome and Furcocercous groups. The last group was subdivided on the basis of presence or absence of eye spots, degree of delimitation between tail stem and furcae, and whether the cercariae developed in sporocysts or rediae. Leiper (1915), while investigating schistosome cercariae in Egypt, discovered that the schistosome forms lacked a pharynx which was present in the other furcocercous cercariae and divided them on this basis. Cort (1917) compared six furcocercous cercariae and divided them into three groups, I, II, and III. Groups I and II were those forms without a pharynx and with the furcae definitely delimited from the tail stem and less than one-half its length. These two groups were placed in the family Schistosomatidae. Group III differed from the other two in having a pharynx and longer furcae not constricted from the tail stem. Sewell (1922) modified this classification by combining Cort's first two groups and dividing his third into two. Sewell limited his first group to cercariae which were brevifurcate apharyngeal. It was divided into two subgroups on the basis of the presence or absence of hollow piercing spines and fin folds on the furcae. These were further subdivided on the basis of the complexity of the excretory system. Sewell's second group included the brevifurcate pharyngeal forms which were subdivided into three subgroups on the basis of the details of the excretory system. His third group consisted of the longifurcate pharyngeal monostomes. Faust (1924) presented an elaborate system of classification that was based on the excretory system. Each ultimate group in classification consisted of those cercariae whose excretory pattern could be arrived at by the interpretation of a basic group formula. Since in many cases the excretory systems of cercariae are poorly described such a classification is considered impractical. Miller (1926) formulated the classification scheme which is used by most workers at the present time. Following Leiper's scheme of classification, he separated furcocercous cercariae into two groups on the basis of the presence or absence of a pharynx (pharyngeal or apharyngeal). Each of these groups he divided into two subgroups on the basis of furcae, calling them either brevifurcate of longifurcate. The brevifurcate forms had furcae that were less than one-half as long as the tail stem while the longifurcate forms had furcae as long or longer than the tail stem. These four groups were further subdivided into distome and monostome types, on the basis of the presence or absence of an acetabulum.

Forked-tail cercariae and their subsequent larval stages are considered to be important from the standpoint of damage or pathology produced in man and in domestic and wild animals. The detrimental effects may be produced by either the activity or mere presence of the organism in the host or both.

The forked-tailed cercaria leaves the first intermediate host snail and either penetrates a second intermediate host or a definitive host. Those cercariae which penetrate the definitive host directly have no true metacercarial stage. They are termed blood flukes and live in the blood stream. In certain instances cercariae of species which normally mature in birds and lower mammals may penetrate the skin of man and cause a severe allergic reaction called "swimmer's itch." The presence of such forms in a lake makes the area undesirable for swimming and other types of recreation. Those cercariae that penetrate a second intermediate host have several possibilities as to continued development. The cercaria may develop into a mesocercaria (agamodistomum) and later become a metacercaria in that same host or in a third intermediate host. Other cercariae develop directly into metacercariae. These various stages are considered to be of economic importance since some may render fish and domestic animals non-edible, some may cause extensive pathology in intermediate hosts, some may be lethal to intermediate or definitive hosts and some may possibly serve as means for the entrance of other pathogens.

In many instances mesocercariae and metacercariae of specific species cause intensive injury to their intermediate hosts. Cercariae of certain species that have birds as definitive hosts may penetrate the skin and muscles of fish to form metacercariae. Fish with such infections are called "grubby" and are considered to be non-edible. In cases where fish fry are exposed to numerous cercariae of these types, infections may be fatal (Krull, 1934). Cercariae named Cercaria flexicaudum, C. laruei and C. emarginatae penetrate fish and develop in the lens of the eye into metacercariae which feed on tissue of the retina (Cort and Brooks, 1928; Oliver and Cort, 1942), or they may be associated with a condition called "popeye." Of unusual interest is the fact that pigs are intermediate hosts of larval trematodes (mesocercariae). Duncker (1881) (fide Bosma, 1934) described Agamodistomum suis from the muscle of pigs slaughtered in Saxony. Stiles (1900) (fide Bosma, 1934) reported a similar condition in pigs in the United States where unidentified mesocercariae were found. Such an infection would probably mean a condemned carcass. Metacercariae of Alaria mustelae in the lungs of mice, mink, and raccoons have been responsible for a marked atrophy of the alveoli (Bosma, 1934). Metacercariae of Diplostomum flexicaudum if located near the central nervous system of the frog may cause paralysis or if located in the lungs may bring about a fatal hemorrhage (Davis, 1936).

There is some evidence to support the idea that these larval trematodes either carry viruses or provide means of entrance for viruses or other pathogens by their extensive migration and tissue destruction. Odlaug (1940) found that carcinomas in frog kidneys were often associated with the mesocercariae of Alaria intermedia. He consulted a pathologist, who thought that the tumors were due to viruses and that it was possible that either the mesocercaria carried the virus into the kidney or provided the means for its entrance by tissue destruction. Stabler and Pennypacker (1939) found that an unidentified metacercaria provided the avenue for the migration of a harmless intestine-dwelling Trichomonas augusta to the liver of a frog.

Eight furcocercous cercariae were encountered in the vicinity of Stillwater and these are described. Four species, Cercaria flexicorpa, Cercaria wardi, Cercaria Q, and Cercaria T were completely described; whereas four species, Cercaria stoni, cercaria of Posthodiplostomum minimum, cercaria of Clinostomum sp., and Cercaria A were incompletely described. Cercaria Q and Cercaria T are new species.

Cercaria of Posthodiplostomum minimum (MacCallum)
Ferguson, 1938.

Specific Diagnosis:

An apharyngeal, longifurcate, monostome cercaria. Body, 0.170 to 0.184 mm long in water, shorter than tail stem, and characterized by muscular annulations which permit expansion and contraction of length, resembling action of bellows. Tail stem 0.230 mm long in water. Furcae p.225 mm long in water. At least 6 transverse rows of body spines extending from anterior end to region midway of penetration organ. Minute spines on furcae. Penetration organ protrusible. Digestive tract not observed. Three pairs of penetration glands located mostly in posterior half of body. Gland ducts turn medially and run anterial between eye spots to empty in region of anterior margin of penetration organ. Pigmented eyespots midway of body. Presence of lens not determined. Excretory system composed of small bulbous posterior chamber extending partly into tail stem and a similar anterior chamber located entirely in body. Anterior chamber receives on either side a collecting tubule which extends laterally for a short distance and then turns anteriad. At least 10 pairs of flame cells, 2 of which are located in tail stem. Caudal excretory canal extends from posterior excretory chamber. Genital primordia consisting of 2 masses, one small circular mass in region of penetration glands and one larger elongated oval mass immediately anterior to excretory vesicles. At least 4 pairs of caudal bodies in tail stem. Host: Physa gyrina.

Discussion:

This cercaria was encountered on two occasions early in the investigation and a detailed description was not completed. The morphology of of my specimens agrees with the description of Cercaria multicellulata Miller, 1926 which was shown by Ferguson (1938), to be the cercaria of Postodiplostomum minimum (MacCallum). The distinguishing characteristics of this cercaria are the absence of a digestive tract; the presence of 3 pairs of penetration glands; and the relative sizes of body, tail stem, and furcae, the latter being longer than the body.

In a collection from Venn's Pond on September 21, 1950 one of 9 (11 per cent) Physa gyrina was infected and from Boomer Lake on November 27, 1950 one of 15 (6.6 per cent) was infected. There were no infections in collections from Venn's Pond on March 7 and 10 and April 22, 1951. Collections from Boomer Lake on February 12 and March 18, 1951 were negative. These data would indicate that the cercariae of P. minimum are present in the late fall months.

Development of the cercariae occurs in thread-like sporocysts located in the liver of the snail. These sporocysts appear as a continuous intertwining mass.

The cercariae emerge from the snail prior to 8 A. M. Their activity is characterized by brief periods of highly active swimming followed by longer rest periods. When swimming they move in a rotational path with the tail forward. Movement is accomplished by the cercaria constantly bending from side to side at the juncture of the body and tail stem. Rest periods are initiated by the cercaria suddenly stopping in water, spreading the furcae, and permitting the body to swing gently downward until body and tail stem are perpendicular to the water surface, but with the anterior end directed towards the bottom and furcae parallel with the water surface. In this position it remains perfectly stationary until it begins to drift towards the bottom, at which time it recommences swimming activity.

The life cycle of this species has been thoroughly investigated by Hunter and Ferguson. Hunter (1936) was the first to discover that Cercaria multicellulata penetrated a fish, Eupomotis gibbosus, to form the metacercaria previously described by Agersborg and Neascus vancleavei. This metacercaria developed in the liver of the fish in 14 to 17 days after cercarial penetration. A continuous exposure of the fish to cercariae for a period of from 24 to 36 hours resulted in a liver literally "riddled by parasites." Ferguson (1936) found that the metacercaria, Neascus vancleavei, developed to maturity in the intestine of chickens within 36 hours after feeding and was eliminated in about 2 weeks. Ferguson (1938) showed by morphological study and experimentation that Cercaria multicellulata and Posthodiplostomum vancleavei (formerly Neascus vancleavei) are developmental stages of Posthodiplostomum minimum, a parasite of herons. He found that eggs of the parasite hatched to produce miracidia in about 3 weeks. These miracidia were used in infection experiments with Physa gyrina and Physa integra to produce the characteristic strigeid sporocysts and cercariae. The cercariae were capable of developing into neasci in 20 species of fish.

Posthodiplostomum minimum and its larval stages are important from several standpoints. The cercariae penetrate a large variety of fish and may encyst in the liver in numbers sufficiently large to impair function. This species is admirably adapted for use in the laboratory to illustrate the strigeid life cycle. Of all the parasite life cycles, the development of the strigeid is the most difficult to comprehend due to its complexity. In the case of P. minimum you have a relatively easy cycle to handle in which cercariae can be developed to metacercariae in from 14 to 17 days in at least 20 species of fish and these neasci can be expected to reach maturity in 36 hours after being fed to chickens. Such a demonstration, using readily available experimental animals such as fish and chickens, would be vary helpful in the laboratory.

Cercaria flexicorpa Collins, 1935
(Plate IV, figure 11)

Specific Diagnosis:

A pharyngeal, longifurcate, monostome cercaria. Body 0.107 to 0.134 mm (average, 0.118 mm) long by 0.031 to 0.048 mm (average, 0.038 mm) wide. Tail stem longer than body, 0.173 to 0.207 mm (average, 0.191 mm) long by 0.027 to 0.038 mm (average, 0.030 mm) wide. Furcae 0.159 to 0.193 mm (average, 0.179 mm) long by 0.012 mm wide. At least 13 transverse rows of spines extending from mouth to posterior margin of penetration organ. Penetration organ, 31^{u} long by 20^{u} wide. Mouth opening into relatively long pre-pharynx. Pharynx non-muscular, 6 to 10^{u} (average, 9^{u}) in diameter. Esophagus ending in a blind sac immediately posterior to pharynx. Three pairs of penetration glands located mostly in posterior half of body. Gland ducts terminate in region of mouth. Two pairs of accessory gland structures adjacent to gland duct openings. Excretory system composed of bulbous posterior chamber, bipartite anterior vesicle, and caudal excretory tubule extending into furcae. Each portion of anterior vesicle receives terminally a collecting tubule which divides in region of penetration glands into ascending and descending branches. Flame cell pattern not determined, at least 5 flame cells anterior to, and 6 posterior to collecting tubule bifurcation, 2 of which are in tail stem. Genital primordia in 2 locations, one small mass in mid-region of body and one large mass immediately anterior to excretory vesicle. Host: Helisoma trivolvis.

Discussion:

The morphology of specimens agrees with the description of Cercaria flexicorpa Collins, 1935. The distinguishing characteristics of this cercaria are the accessory gland structures, the genital primordia, the absence of acetabulum and ceca, and the presence of 3 pairs of penetration glands.

Cercariae were recovered from 1 of 14 (7 per cent) specimens of Helisoma trivolvis collected from Venn's Pond on April 22, 1951. Forty two of these snails collected from this same locality on March 7, 1951 were negative.

This cercaria develops in thread-like sporocysts which appear as a continuous intertwining mass within the liver of the snail. Emergence of C. flexicorpa was not studied since cercariae were recovered after the snail was crushed.

When swimming, the cercaria moves in a rotational path, with the tail forward, toward the water surface or area of more intense light. In the progression there is a continual lateral flexing of the cercaria in one plane, first to one side and then to the other at the juncture of the body and tail stem. The resting position is preferred to swimming. This is assumed when the cercaria stops in mid-water, spreads its furcae wide apart, and the body swings to a vertical position with the head down. The body is then flexed and the anterior end is drawn towards the tail, giving the cercaria the appearance of a fish hook. This position is characteristic of at least 3 furcocercous cercariae, C. flexicorpa, C. hamata, and C. bessiae.

Hobgood (1938) found that Cercaria flexicorpa penetrated the sunfish, Lepomis cyanellus, in which the metacercaria developed in a black pigmented cycle in either the skin, muscle, or connective tissue. This black pigmentation of the cyst is responsible for the name, "Black Grub," and is found in many fish in the vicinity of Stillwater. Pigmentation of this type is undesirable in fish for aesthetic reasons.

Cercaria wardi Miller, 1923
(Plate III, figure 8)

Specific Diagnosis:

A apharyngeal, brevifurcate distome cercaria. Body pear shaped, 0.214 to 0.262 mm (average, 0.255 mm) long by 0.065 to 0.093 mm (average, 0.077 mm) wide. Tail stem longer than body and attached to it near ventral surface, 0.419 to 0.570 mm (average, 0.515 mm) long by 0.027 to 0.069 mm (average, 0.053 mm) wide. Furcae finned, 0.141 to 0.176 mm (average, 0.159 mm) long by 0.027 to 0.038 mm (average, 0.031 mm) wide. Fine spines cover entire body with exception of oral sucker. Eye spots composed of brownish-black granules arranged to form a cup with open side lateral. Oral sucker 72 to 79^u (average, 76^u) long. Mouth opening into long esophagus which turns ventrad and bifurcates in region immediately posterior to eye spots, dilated ceca extend to level midway of body. Acetabulum muscular and protrusible, 19^u in diameter, located in posterior half of body. At least 4 pairs of penetration glands situated lateral to acetabulum. Gland ducts open in region of anterior margin of oral sucker. Posterior mucin gland densely granular and located in posterior fourth of body. Excretory bladder small, receiving on either side an ascending excretory tubule which bifurcates in region midway between acetabulum and genital primordium to form anterior and posterior branches. Anterior branch receives capillaries from 5 flame cells whereas posterior branch receives capillaries from 3 flame cells, one of which is in tail stem. Caudal excretory duct bifurcates and extends into furcae. Genital primordium appears as oval, centrally located mass in posterior fourth of body. Nervous primodrium consists of a broad band extending across the cercaria between posterior margin of oral sucker and eye spots. Host: Helisoma trivolvis.

Discussion:

The cercariae agree essentially with the description of cercaria wardi except for the number of penetration glands. Miller (1923) described 6 pairs of glands for this species, Miller (1936) described only 5 pairs, and in my specimens there were only 4 pairs. These discrepancies indicate one of two things, either the penetration gland cells are difficult to distinguish as separate entities or there are three closely related species having different numbers. Until life cycle studies prove otherwise it is preferred to consider all three to be the same species. The distinguishing features of Cercaria wardi are its large size, the presence of fins on the furcae, the large posterior mucin gland, the length of the ceca, the pigmented eye spots with the lenses directed laterally, and the presence of from 4 to 6 pairs of penetration glands.

This cercaria was recovered from Helisoma trivolvis collected from a length of Stillwater Creek located below the Lake Carl Blackwell dam. One of 14 (7 per cent) was infected in a collection December 26, 1950 and 3 of 21 (14 per cent) were infected in one on April 1, 1951. Collections of 39 snails on November 3, 1950 and 14 on February 6, 1951 were negative.

Cercariae of this species develop in sporocysts of varying length and diameter in the digestive gland of the snail. The diameter of these sporocysts may vary from 20 to 109^{u}, the length may reach 1.6 mm., and 5 to 6 cercariae develop in each sporocyst.

Emergence occurs during the morning hours. The cercaria is an active swimmer that moves toward the water surface or area of more intense light. While swimming the cercaria advances with the anterior end forward, and motion is accomplished by actively extending and closing the furcae in a scissor-like fashion. In the resting position the organism is bent into a "horse shoe" shape with the anterior and posterior ends directed up. In this position it gradually sinks to the bottom of the container where it remains flexed for several minutes dorsal side up.

No experiments to determine other life cycle stages were undertaken with this cercaria. However, since it is a schistosome cercaria, attempts were made to determine whether it could cause schistosome dermatitis and these were failures.

Cercaria stoni Brooks, 1943

Specific Diagnosis:

A pharyngeal, longifurcate distome cercaria. Body small, 0.152 to 0.193 mm (average, 0.170 mm) long by 0.058 to 0.072 mm (average, 0.062 mm) wide. Tail stem 0.185 mm long by 0.034 mm wide. Furcae longer than body, 0.198 to 0.231 mm (average, 0.214 mm) long by 0.034 mm wide. Irregular rows of spines extend from mouth to cover body surface down to first one-third of penetration organ. Sensory spines evenly distributed over surface of tail stem. Eye spots non-pigmented, located in mid-region of body lateral to penetration glands. Penetration organ 37^u long by 31^u wide. Mouth opening into short pre-pharynx. Pharynx muscular, 17^u in diameter. Esophagus relatively long bifurcating in region immediately anterior to acetabulum, ceca extend to region of excretory bladder. Acetabulum 38^u in diameter, with at least 3 rows of concentric spines on internal margin. Two pairs of penetration glands located immediately anterior to acetabulum. Parallel gland ducts empty in region of mouth. Bulbous excretory bladder receiving on either side an ascending excretory tubule. At least 9 pairs of flame cells present of which 2 pairs are in tail stem. Host: Physa gyrina.

Discussion:

A complete description cannot be given since only one infected snail was encountered.

These cercariae agree with the description of Cercaria stoni Brooks, 1943. The distinguishing features of this cercaria are its small size, non-pigmented eye spots, ceca extending to the region of the excretory bladder, 2 pairs of flame cells in the tail stem, and 2 pairs of penetration glands. All of these features are easily distinguished with the exception of the ceca, which require special staining and then are hard to distinguish. Brooks collected cercariae from *Lymnaea stagnalis jugularis* whereas these specimens were collected from *Physa gyrina*.

Cercariae were recovered from one of 12 (7 per cent) *Physa gyrina* collected from Thomas Lake on January 21, 1951. Collections of 50 snails on September 24, 1950; 20 on October 16, 1950; 15 on December 14, 1950; 10 on March 9, 1951; and 15 on May 1, 1951, were negative.

Cercariae develop in sporocysts in the liver of the snail. These sporocysts were numerous and appeared as a continuous thread-like structure intertwined in the liver tissue. The cercariae were secured by crushing the snail.

Swimming activity and resting positions were characteristic of furcocercous cercariae. The cercaria swims with the tail forward in a rotational path toward the water surface or area of greater light intensity. Motion is accomplished by cercaria actively bending from side to side at the juncture of the body and tail stem. In the resting position the cercaria hangs perpendicular in the water with furcae spread. In this position the cercaria gradually sinks, but before reaching the bottom, recommences swimming activity.

Cercaria Q
(Plate IV, figure 10)

Specific Diagnosis:

A pharyngeal, longifurcate distome cercaria. Body 0.121 to 0.176 mm (average, 0.141 mm) long by 0.041 to 0.062 mm (average 0.053 mm) wide. Tail stem longer than body, 0.190 to 0.207 mm (average, 0.193 mm) long by 0.034 to 0.041 mm (average, 0.037 mm) wide. Furcae 0.183 to 0.200 mm (average, 0.187 mm) long by 0.013 to 0.020 mm (average, 0.017 mm) wide. Spines numerous at anterior end, gradually diminishing in number until negligible in region immediately posterior to pharynx. Penetration organ 27 to 34^u (average, 29^u) long by 27^u wide. Mouth opening into a relatively short pre-pharynx. Pharynx muscular 13 by 10^u in diameter. Esophagus relatively long, bifurcating in region immediately anterior to mid-region of body, ceca extend to level midway between acetabulum and excretory bladder. Acetabulum muscular, 24^u in diameter, mostly in posterior third of body and having at least 2 rows of concentric spines on its internal margin. Two pairs of penetration glands located lateral and slightly anterior to acetabulum. Gland ducts terminating in region of mouth. Excretory bladder bulbous, receiving on either side in an enlarged atrium an ascending excretory tubule which bifurcates in region of acetabulum to form an ascending and descending branch, each of which receive capillaries from 3 flame cells, 2 (3 + 3). Excretory bladder receives posteriorly a caudal excretory canal which extends from furcae. Genital primordium located midway between acetabulum and excretory bladder. Tail stem with 5 pairs of caudal bodies. Host: *Physa gyrina*.

Discussion:

The morphology of cercaria T does not agree with any description of cercariae, but is similar to Cercaria furcicauda Faust, 1919. Cercaria Q differs from C. furcicauda in that the furcae are longer in relation to the tail stem, the acetabulum and penetration organ are smaller, and the host is Physa gyrina instead of Anculosa carinata.

The distinguishing features of Cercaria Q are body spines extending from the anterior end to the region immediately below the pharynx, ceca extending to the region midway between the acetabulum and excretory bladder, 2 pairs of penetration glands, and its small size.

This cercaria was encountered in one of 9 Physa gyrina taken from a pond immediately below the Boomer Lake spillway on February 24, 1951. Collections of 44 snails on February 28, 1951 and 90 on March 5, 1951 were negative.

Emergence of cercariae from the snail was not observed since the cercariae were secured by crushing the snail. Cercariae develop in thread-like sporocysts located in the liver of the snail. The cercariae behaved in much the same manner as most furcocercous cercariae. Activity was characterized by short periods of active swimming followed by longer rest periods. When swimming, the cercaria moved with tail forward and motion was accomplished by the cercaria actively bending from side to side at the juncture of the body and tail stem. The cercaria moved in a rotational path towards the water surface or area of more intense light. Near the water surface it would stop, extend its furcae, and the anterior end would swing down pivoted at the furcae. In a vertical position with anterior end down it would slowly start to sink. During this time it would bend the body and draw the anterior end towards the tail and then straighten out, and this activity was repeated several times before swimming was recommenced. This latter activity was quite characteristic and served to distinguish Cercaria Q from all other furcocercous cercariae encountered in this investigation.

Cercaria T

Specific Diagnosis:

A pharyngeal, longifurcate, distome cercaria. Body 0.193 to 0.228 mm (average, 0.216 mm) long by 0.027 to 0.031 mm (average, 0.028 mm) wide. Tail stem 0.200 to 0.217 mm (average, 0.207 mm) long by 0.027 to 0.034 mm (average 0.028 mm) wide. Furcae 0.190 to 0.200 mm (average, 0.192 mm) long by 0.010 to 0.020 mm (average, 0.014 mm) wide. Nine conspicuous pre-oral spines. At least 11 transverse rows of body spines extending from mouth to midway to oral sucker. Sensory spines distribted over tail stem. Penetration organ 41 to 51^{u} (average, 45^{u}) long by 13 to 17^{u} (average, 16^{u}) wide. Mouth opening into relatively short pre-pharynx. Pharynx non-muscular, 10^{u} in diameter. Esophagus long, bifurcating midway of body, ceca appear segmented into 3 parts and extend to region immediately anterior to genital primordium. Acetabulum 17 to 20^{u} (average, 19^{u}) in diameter with one row of at least 30 concentric spines on its internal margin. Penetration glands 2 pairs, anterior and slightly lateral to acetabulum. Excretory bladder bulbous, receiving on either side in an enlarged atrium an ascending excretory tubule which bifurcates in region of acetabulum into anterior and posterior branches. Anterior branch receives capillaries from 4 flame cells whereas posterior branch receives capillaries from 5 flame cells, the latter 2 of which are in the tail stem. Excretory bladder receives posteriorly a caudal canal which extends from external margin of proximal one-fifth of furcae. Genital primordium immediately anterior to excretory bladder. Tail stem with 5 pairs of caudal bodies. Host: Physa gyrina;

Discussion:

The morphology of cercaria T does not agree with the description of any species but resembles Cercaria tenuis Miller, 1923. It differs from C. tenuis in having 9 instead of 7 pairs of flame cells and in having a smaller acetabulum.

Its distinguishing features are 9 pairs of flame cells, 2 of which are in the tail stem; the cecae segmented into 3 parts; and the presence of 2 penetration glands. It was difficult to determine the number of caudal bodies in the tail stem. Repeated examinations of cercariae mounted in water showed that there were at least 6 caudal bodies. If a cover-slip was added to a mount, the number of caudal bodies was undeterminable; and if cercariae were mounted in egg albumen, all traces of caudal bodies disappeared within one and one-half hours. These facts would indicate that there were limitations in the use of caudal bodies as a differentiate character in cercarial classification and identification. Any structure so delicate that it has to be examined in an actively moving cercaria without the aid of even a cover-slip can only lead to "taxonomic confusion."

Cercariae of this species were recovered from one of 43 (2.3 per cent) Physa gyrina collected from Stillwater Creek below Lake Carl Blackwell dam on April 1, 1951. Collections of 23 snails on November 3, 1950; 11 on December 26, 1950; and 19 on February 6, 1951 were negative.

The cercariae develop in long filamentous sporocysts located in the liver of the snail. These sporocysts are thin walled structures measuring 65 to 69^{u} in diameter. Pigmentation occurs throughout the sporocyst wall in the form of dark brown scattered pigment areas.

Emergence of the cercariae from the snail occurs prior to 8 A. M. After emergence their activity is characterized by brief periods of highly active swimming followed by longer rest periods. Swimming activity of any species but resembles _Cercaria tenuis_ Miller, 1923. It differs from _C. tenuis_ in having 9 instead of 7 pairs of flame cells and in having a smaller acetabulum.

Its distinguishing features are 9 pairs of flame cells, 2 of which are in the tail stem; the cecae segmented into 3 parts; and the presence of 2 penetration glands. It was difficult to determine the number of caudal bodies in the tail stem. Repeated examinations of cercariae mounted in water showed that there were at least 6 caudal bodies. If a cover-slip was added to a mount, the number of caudal bodies was undeterminable; and if cercariae were mounted in egg albumen, all traces of caudal bodies disappeared within one and one-half hours. These facts would indicate that there were limitations in the use of caudal bodies as a differentiate character in cercarial classification and identification. Any structure so delicate that it has to be examined in an actively moving cercaria without the aid of even a cover-slip can only lead to "taxonomic confusion."

Cercariae of this species were recovered from one of 43 (2.3 per cent) _Physa gyrina_ collected from Stillwater Creek below Lake Carl Blackwell dam on April 1, 1951. Collections of 23 snails on November 3, 1950; 11 on December 26, 1950; and 19 on February 6, 1951 were negative.

The cercariae develop in long filamentous sporocysts located in the liver of the snail. These sporocysts are thin walled structures measuring 65 to 69^{u} in diameter. Pigmentation occurs throughout the sporocyst wall in the form of dark brown scattered pigment areas.

Emergence of the cercariae from the snail occurs prior to 8 A. M. After emergence their activity is characterized by brief periods of highly active swimming followed by longer rest periods. Swimming activity is similar to that of all strigeid cercariae. Resting periods, however, are quite characteristic and serve to distinguish this species from the other strigeids encountered in the investigation. The cercariae rest suspended in water with furcae spread apart, but the distinguishing feature about this position is that they may rest either parallel, perpendicular, or diagonal to the surface of the water, while others are perpendicular. When these are perpendicular either end may be up. In the resting position the body of the cercaria is always slightly curved ventrally.

Cercaria of Clinostomum sp. Possibly Clinostomum marginatum (Rud., 1819) or Cercaria Whitentoni Croft, 1933

Specific Diagnosis:

An apharyngeal, brevifurcate monostome cercaria. Body pear-shaped with dorsal fin, 0.110 to 0.121 mm (average, 0.119 mm) long. Tail stem longer than body, 0.276 to 0.290 mm (average, 0.283 mm) long by 0.035 to 0.040 mm (average, 0.038 mm) wide. Furcae shorter than body with terminal fin-like projections, 0.100 to 0.121 mm (average, 0.110 mm) long by 0.020 mm wide. Body spines in irregular transverse rows extending from anterior end to region midway of penetration organ. Pigmented eye spots immediately anterior to mid-region of body with lens directed laterally. Penetration organ protrusible, 20^{u} long. Mouth opening into long esophagus that turns dorsad in region immediately anterior to eye spots and terminates in a large oval primitive gut located midway of body. At least 2 penetration glands in region of penetration organ. Gland ducts ascending anteriorly from mid-region of body but no gland cells observed. Excretory system consisting of inconspicuous excretory bladder receiving on either side ascending tubules that bufurcate in mid-region of body to form ascending and descending branches. Five pairs of flame cells present, 4 pairs in body and one pair in tail stem. Host: Helisoma trivolvis.

Discussion:

The morphology of these specimens resembles cercariae described by Croft (1933) and Krull (1934). Croft's description of Cercaria Whitentoni was incomplete and only the most obvious morphological characteristics are described. The extent of the esophagus, presence of gut, number and distribution of glands, details of the flame cell pattern, and presence of tail fins were not recorded in his description, Krull

(1934) made a more complete description of a cercaria that apparently agrees with the superficial description of C. whitentoni. This cercaria was described as a cercaria of a Clinostomum sp. possibly C. marginatum and the description included complete details as to the number and distribution of penetration glands, flame cell pattern, digestive system, and presence of tail fins or terminal projections on the furcae.

Due to the fact that only 10 cercariae of this species were recovered throughout the course of this investigation, a complete species description was impossible. However, free hand drawings and measurements were made of these specimens and a number of morphological features studied. These features agreed with the description of C. whitentoni and also to some extent with Krull's cercaria. Study of the 10 specimens showed that there were penetration glands and that there is a good possibility that the distribution and number of glands are similar to those of Krull's cercaria. Also terminal fin projections on the furcae and a primitive gut were observed, both of which agree with Krull's description.

Cercariae of this species were collected from 2 of 19 (10 per cent) Helisoma trivolvis collected from Bliss's Pond on February 18, 1951. Collections of two snails on January 8, 1951 and 13 on April 9, 1951 were negative.

Development of cercariae occurred in long filamentous sporocysts located in the digestive gland of the snail.

Emergence of this species was not observed. When swimming, the cercaria moves towards the water surface or area of more intense light. The anterior end is forward and motion is accomplished by the cercariae actively extending and closing the furcae in a scissor-like fashion. In the vicinity of the water surface the cercaria stops, and the body and tail are curled towards each other giving the cercaria a "horse shoe" shaped appearance. The cercaria then slowly sinks to the bottom of the container where it may remain for a short while before renewing activity.

The subsequent stages in development and the economic importance of this species were not studied. However, since this cercaria appears to be a *Clinostomum sp*. it may have considerable economic importance. The cercaria of *C. marginatum* is known to produce a disease in fish called "yellow grub." This disease is produced by the cercariae which penetrate the fish and form large yellow metacercariae in the muscles. The presence of these metacercariae in fish are of considerable economic importance since they render the fish undesirable for consumption.

Cercaria A

Specific Diagnosis:

A pharyngeal, longifurcate distome cercaria. Body 0.145 to 0.166 mm (average, 0.153 mm) long by 0.058 to 0.069 mm (average, 0.062 mm) wide. Tail stem 0.121 to 0.155 mm (average, 0.147 mm) long by 0.056 to 0.066 mm (average, 0.061 mm) wide. Furcae 0.114 to 0.166 mm (average, 0.142 mm) long by 0.010 to 0.019 mm (average, 0.013 mm) wide. Irregular rows of spines over body surface from anterior end to posterior margin of anterior two-thirds of penetrating organ. Sensory spines evenly distributed over tail stem. Unpigmented eye spots lateral to an on same level as bifurcation of esophagus. Penetrating organ 27^u in diameter. Mouth opening into relatively short pre-pharynx. Pharynx non-muscular, 13^u in diameter. Relatively long esophagus bifurcating immediately anterior to acetabulum, ceca extend to region immediately anterior to excretory bladder. Acetabulum 31^u in diameter, with at least 2 rows of concentric spines on internal margin. Two pairs of penetration glands anterior and slightly lateral to acetabulum. Bulbous excretory bladder receiving on either side in an enlarged atrium an ascending excretory tubule that bifurcates in region of acetabulum to form an ascending and descending branch. Six pairs of flame cells in body, 3 pairs anterior to acetabulum and 3 pairs posterior to acetabulum. Flame cell pattern in tail consistently asymmetrical. When cercaria viewed ventrally 2 flame cells are found to left of median line and one to right. Excretory bladder receives posteriorly a caudal excretory canal which extends from furcae. Host: Physa gyrina.

Discussion:

This cercaria was encountered on 3 occasions early in the investigation. It does not agree with any of the described species. The main difference is in the asymmetrical flame cell pattern. Cercaria A most closely resembles C. marcianae Cort and Brooks, 1928 and the cercaria of Alaria intermedia Odlaug, 1940. The features that distinguish it from C. marcianae are the asymmetrical flame cell pattern in the tail stem and a longer body length. It is distinguished from the cercaria of A. intermedia on the basis of the shorter body and tail stem, longer furcae, and the asymmetrical flame cell pattern.

The asymmetrical flame cell pattern observed in this species was a consistent characteristic noted in every cercaria studied. This pattern consists of 8 flame cells on one side, 6 in the body and 2 in the tail stem, and 7 on the other side, with 6 in the body and one in the tail stem. The side with 7 flame cells was always on the left as you faced the ventral surface.

Flame cell patterns were thoroughly investigated by Cort and Brooks (1928). They found that the development of the excretory system in trematodes was characterized by division of flame cells to form new flame cells and that this flame cell division rate might differ among those in a given cercaria.

Cercaria A was collected from snails taken from Venn's Pond, Thomas Lake, and Bliss's Pond. One of 9 specimens taken from Venn's Pond on September 21, 1950 was infected. Collections of 13 on March 7, 1951 and ten on April 22, 1951 were negative. One of 15 (6.6 per cent) Physa collected from Thomas Lake on December 14, 1950 was infected. Collections of 20 on October 16, 1950; 50 on September 24, 1950; 13 on January 21, 1951; 10 on March 9, 1951; and 15 on May 1, 1951 were negative. One of 23 (4 percent) Physa collected from Bliss's Pond on November 3, 1950 was infected. Collections of 13 on January 8, 1951; 32 on February 18, 1951; and 60 on April 9, 1951 were negative. These collection data indicate that Cercaria A is widely distributed in the vicinity of Stillwater and that it is prevalent during the late fall and early winter months.

These cercariae develop in thread-like sporocysts which appear as a continuous intertwining mass within the liver of the snail. Cercariae emerge from the snail prior to 8 A. M. After emergence their activity is aimilar to that of most furcocercous cercariae. There are short periods of active swimming followed by longer rest periods.

XIPHIDIOCERCARIAE

Before describing the specific xiphidiocercariae it is desirable to discuss briefly their classification, type of life cycle in which they are involved, and the relative importance of the larval stages in the cycle.

The xiphidiocercariae or stylet forms are characterized by the presence of a stylet on the anterior margin in the oral sucker near the mouth. This stylet possesses a single sharp point and sometimes a bulb-like swelling. Stylets are found also in other cercaria such as in the Microcercous or Cystocercous groups. These stylets, however, differ considerably in morphology from the one described.

Diesing (1855) defined the xiphidiocercariae as "Slender-tailed distome cercariae with a boring spine on the rounded anterior end, eyes lacking, develop in sporocysts; encystment in a second intermediate host." He divided this group into four subgroups; Microcotylae, which included small cercariae less than 0.2 mm long with the ventral sucker situated behind the mid-body and smaller than the oral sucker; Virgulae, that included spinous forms in which the ventral sucker is smaller than the oral, the tail lacks a fin fold, and a characteristic "virgula" organ is present which consists of two pyriform sacs near the oral sucker; Ornatae, included those cercariae with a fin fold on the tail; and Armatae, which included forms in which tail and body were of about equal length, suckers of equal size, and a Y-shaped excretory vesicle. Lebour (1911) created the group Spelotrema, which included the stylet cercariae that develop in sporocysts.

Cort (1915) created the Poladenous group which was similar to Lühe's Armatae group. Sewell (1922) erected three sub-groups; Cellulosa, Vesiculosa, and Pusilla, under Lühe's Microcotyle. Cercariae in the subgroup Cellulosa were characterized by having a simple excretory system and only two pairs of penetration glands; those in the Vesiculosa were distinguished on the basis of a pair of anterior penetration glands and three pairs of posterolateral glands; and the Pusilla group contained the small forms having a slender tail, three or four pairs of penetration glands; an undeveloped gut, small ventral sucker, and a bicornuate excretory vesicle. He further subdivided Lühe's Ornatae group by erecting a subgroup Prima which included those forms of moderate size, with tail shorter than body, and with a 2 + 6 + 1 flame cell pattern. Sewell later subdivided the Armatae into the Polyadenous Cort, 1914 and the Daswan subgroups. This grouping is not discussed further since later investigators found it to be invalid (Miller, 1935). Faust (1924) differentiated a subgroup, the Hemilophura, from the group Ornatae on the basis of the excretory system. He considered this subgroup to be made up of forms with the $2[(3 + 3) + (3 + 3 + 3)]$ flame cell formula. McCoy (1928) further divided the Hemilophura by finding a form classified by Faust (1924) as a Hemilophura to have a flame cell formula of $2[(3 + 3 + 3) + (3 + 3 + 3)]$.

The present status of xiphidiocercarial classification is greatly confused due to the numerous subdivisions made by various investigators, many of which have later proved to be invalid. Since cercarial classification on the basis of groups and subgroups is considered as a matter of convenience without much real taxonomic value in trematode classification, the writer has grouped the cercariae found in this investigation according to the simpler and early scheme devised by Lühe, namely, the division of xiphidiocercariae into the four groups; Microcotylae, Virgulae, Ornatae, and Armatae.

After stylet cercariae have left their first intermediate host they seek the second intermediate host which, according to McMullen (1937), may be either an insect, snail, or tadpole. In this host the cercariae encyst, become infective metacercariae, and if eaten by the proper definitive host they develop to maturity. Since the pathology of xiphidiocercariae and subsequent stages has not been investigated to any great extent, it is impossible to discuss their economic importance.

Seven different xiphidiocercariae were encountered in this investigation. Six of these, the cercaria of Alloglossidium corti, cercaria of Dasymetra conferta, cercaria of Pneumatophilus variabilis, cercaria of Tetrapapillatrema concavocorpa, Cercaria J, and Cercaria R are described. One species, Cercaria ramonae, is incompletely described. The species, Cercaria J and Cercaria R, are new.

Cercaria of Alloglossidium corti (Lamont): Plagiorchiidae
(Plate VI, figure 17)

Xiphidiocercaria of Armata group. Body small, elongate oval, 0.162 to 0.214 mm (average, 0.208 mm) long by 0.079 to 0.093 mm (average, 0.085 mm) wide. Tail more than one-half body length, 0.110 to 0.145 mm (average, 0.132 mm) long by 0.017 to 0.020 mm (average, 0.018 mm) wide. Spines distributed over entire body in regular rows. Stylet, 20 by 3^u, with abrupt large anterior shoulders and slight posterior enlargement. Oral sucker 38 to 44^u (average, 41^u) in diameter. Mouth opening into a relatively long pre-pharynx. Pharynx muscular, 17^u long by 13^u wide. Short esophagus bifurcating immediately above acetabulum, ceca extend to bulbous part of bladder. Acetabulum smaller than oral sucker, 31^u in diameter, mostly in posterior half of body and having several rows of spines on internal margin. Penetration gland cells, 4 pairs, located anterior and lateral to acetabulum, opening at anterior margin of oral sucker in vicinity of stylet. Two or 3 conspicuous accessory gland structures on either side of stylet. Excretory bladder with bulbous posterior chamber and "T"-shaped anterior vesicle which receives subterminally on either side a common duct formed by anterior and posterior collecting tubules. Four groups of 3 flame cells each per side $2[(3 + 3) + (3 + 3)]$. Tail receptacle with pair of lateral sinuses, each containing at least 8 spines. Host: Helisoma trivolvis.

Discussion:

The morphology of these specimens agrees with the description of the cercaria of Alloglossidium corti by Crawford (1937). The distinguishing characteristics of this species are its small size, the presence of 4 pairs of penetration glands, the extent of the excretory bladder, and the characteristic shoulders of the stylet. McCoy (1928) was the first to describe this cercaria; he believed that the adult was a form that he called Plagiorchis ameiurensis. Crawford (1937) showed that Plagiorchis ameiurensis was a synonym of Alloglossidium corti (Lamont).

Cercariae of this species were recovered from snails collected from Bliss's Pond. Two of 21 (9.5 per cent) snails collected February 18, 1951 and 4 of 13 (30.7 per cent) snails collected April 9, 1951 were infected. Collections of 23 on November 3, 1950 and 13 on January 8, 1951 were negative. These data indicate the cercariae are probably scarce during the winter months.

The cercariae develop in elongated sporocysts which are threaded throughout the liver tissue. These sporocysts are greatly enlarged at irregular intervals and these enlargements may contain as many as 17 cercariae.

Cercariae emerge from the snail during the evening hours. Emergence begins at about 6:30 P. M. and continues until after midnight. The cercariae are less active than most xiphidiocercariae. When they swim, the anterior end of the body is bent ventrally and drawn posteriorly and the tail is actively lashed in a figure eight pattern. Resting periods are frequent, at which time the cercariae drop to the bottom, rest for a few minutes on their dorsal surface, then turn over and crawl measuring worm fashion across the bottom of the container. They prefer resting and crawling to swimming, and the reason is clear when the second intermediate hosts are considered.

McCoy (1928) and Crawford (1937) were the first to study the life cycle. They found that the cercariae encysted in the branchial basket of dragon fly naids and the muscles of crayfish. When the metacercariae were fed to *Ameriurus natalis* the adult trematode, *A. corti*, developed in the intestine.

Cercaria of Dasymetra conferta Nicoll, 1911: Plagiorchiidae
(Plate V, figure 13)

Specific Diagnosis:

Xiphidiocercaria of Armata group. Body elongate oval, 0.266 to 0.335 mm (average, 0.303 mm) long by 0.121 to 0.145 mm (average, 0.135 mm) wide. Tail approximately three-fourths length of body, 0.224 to 0.256 mm (average, 0.245 mm) long by 0.027 to 0.038 mm (average, 0.033 mm) wide at base. Spines in regular rows cover entire body, larger at anterior end. Stylet needle-shaped with thickened sides and basal plug, 20^u by 6^u. Diameter of oral sucker 51^u. Mouth opening into a short prepharynx. Pharynx muscular, 17 to 24^u (average, 20^u) in diameter. Relatively long esophagus bifurcating midway between pharynx and acetabulum, ceca extend to level midway of excretory bladder. Acetabulum muscular, 51 to 58^u (average, 53^u) in diameter, mostly in posterior third of body and covered with fine spines directed internally. Penetration gland cells 8 or 9 pairs, coarsely granular, no nuclei visible, located immediately above and to side of acetabulum, opening at anterior margin of oral sucker in vicinity of stylet. Excretory bladder consists of bulbous posterior chamber and "T"-shaped anterior visicle, which receives subterminally a common duct on either side formed by an anterior and posterior collecting tubule. Flame cell pattern 6 groups of 3 each per side, $2[(3 + 3) + (3 + 3) + (3 + 3)]$ or $(2 \times 6 \times 3)$. Tail receptacle with a pair of lateral sinuses, each containing at least 8 spines on the posterior margin. Host: Physa gyrina.

Discussion:

The morphology of these specimens agrees with the description of the cercaria of Dasymetra conferta McCoy, 1928. The distinguishing characteristics of this cercaria are the long ceca and the presence of a contracting excretory bladder, the 2 arms of which may extend beyond the anterior border of the ventral sucker. Aside from these two features it is difficult to distinguish the cercaria of D. conferta from other species within the subfamily Reniferinae. McCoy (1928), after considerable study of this group, concluded that the extent of ceca and characteristics of the excretory bladder were about the only features which could be used to distinguish the various cercariae of the group.

Cercariae were recovered from one of 43 snails (2.3 per cent) taken from Stillwater Creek below Lake Carl Blackwell on April 1, 1951. Collections of 23 on November 13, 1950; 11 on December 26, 1950; and 19 on February 6, 1951 were negative. Although Dunham (1950), in his investigation of helminths from Natrix sipendon captured in the vicinity of Stillwater, was able to find a high percentage of snakes infected with this trematode, the writer was unable to find a similar high infection among snails. This difference may be due to seasonal fluctuations in snail infectivity, the writer having made collections only in the fall, winter, and spring months whereas infection may take place during the summer months.

Cercariae developed in long sac-like sporocysts varying in length from 153 to 499^{u}. These sporocysts often contained much orange pigment. In this connection it was noted that the more developed the sporocyst the deeper and more intense was the pigmentation.

Cercariae did not emerge from the infected snail until the fifth day after collection. The cercariae emerged during the morning hours prior to 11 A. M. The cercariae were very active. Their swimming position was typical of most xiphidiocercariae. The anterior end was bent ventrally and drawn posteriorly while the tail was kept actively lashing in a figure eight pattern. This position resembles a partially opened jack knife when viewed laterally. If the water surface were disturbed, the cercaria would roll up immediately into a compact ball, with the anterior end forming the core, and sink to the bottom of the container. On the bottom it would rest several moments on its side and then roll over to rest on its dorsal surface with the tail directed towards the water surface. This "disturbance effect" seemed to be characteristic for the species, since no other cercaria encountered showed the same response.

McCoy (1928) was the first to show that this was the cercaria of Dasymetra conferta. He removed the adult flukes from the water snake, Natrix sipedon. From these flukes he recovered eggs which eventually hatched and liberated miracidia. Snails, Physa gyrina, were exposed to the miracidia and infections established. Subsequent developmental stages were studied and the cercaria described. McCoy did not establish the second intermediate host but assumed that it was the tadpole.

Cercaria of Pneumatophilus variabilis: Plagiorchiidae
(Plate V, figure 12)

Specific Diagnosis:

Xiphidiocercaria of Armata group. Body elongate oval, 0.269 to 0.335 mm (average, 0.301 mm) long by 0.117 to 0.186 mm (average, 0.156 mm) wide. Tail slightly longer than body 0.276 to 0.342 mm (average, 0.318 mm) long by 0.027 to 0.041 mm (average, 0.032 mm) wide. Spines distributed over entire body. Stylet 24^{u} long with slight shoulder enlargements and basal plug. Oral sucker 51^{u} in diameter. Mouth opening into a short pre-pharynx. Pharynx muscular, 19^{u} in diameter. Esophagus bifurcates immediately above acetabulum, ceca extend to level midway of body. Acetabulum mostly in posterior half of body, 55^{u} in diameter. Eight pairs of penetration glands, without visible nuclei, anterior and lateral to acetabulum. Gland ducts terminate in region of stylet. Excretory bladder consists of bulbous posterior chamber and "V"-shaped anterior vesicle whose cornua extend to region immediately anterior of acetabulum. Each cornu receives subterminally a common duct resulting from fusion of anterior and posterior collecting tubules. Flame cell pattern 6 groups pf 3 each per side, $2[(3 + 3) + (3 + 3) + (3 + 3)]$. Tail receptacle with pair of lateral sinuses. Host: Physa gyrina.

Discussion:

The morphology of these specimens agrees with the description of the cercaria of Pneumatophilus variabilis described by McCoy (1928). The distinguishing features of this cercaria are the short ceca and the extension of the contractile excretory bladder to the level of the anterior margin of the acetabulum. These features distinguish it from the other cecariae in the subfamily Reniferinae.

This species was recovered from one of 60 (1.6 per cent) Physa gyrina collected from Bliss's Pond on April 9, 1951. Collections of 23 snails on November 3, 1950; 13 on January 8, 1951; and 32 on February 18, 1951 were negative. As in the case of Dasymetra conferta, it is obvious that there must be a high incidence of snail infections at some season other than the ones in which collections were made because Dunham (1950), found P. variabilis quite abundant in snakes in the Stillwater area.

Cercariae develop in sac-like sporocysts located in the liver of the snail. These sporocysts are slightly granular in appearance but were not observed to contain orange pigmentation.

Cercariae did not emerge from the snail until the third day after capture. Emergence occurred only during the morning hours from 7:30 A. M. until noon. The cercariae were very active and would distribute themselves rather uniformly throughout the container. Resting periods were infrequent. The swimming position was characterized by the cercaria turning the anterior end ventrally and posteriorly so that it was parallel to the posterior end. The tail was actively lashed in a figure eight pattern and the cercaria would move in a jerky rotational path in the direction opposite the two ends. In order to rest, the cercaria would stop, maintain its contracted state, and gradually sink to the bottom of the container. Here it would remain for a short time while resting on its dorsal surface with the tail held perpendicular to the body and directed towards the water surface. In this position it was always slightly curved ventrally.

Encystment of this species occurred in egg albumen. When mounted in this material on a slide the cercaria would loose its tail in from 15 to 30 minutes. The body would then contract into an oval form and cystogenous material would be extruded from the body surface. The cercaria would continually move within the cystogenous material and appeared to be forming a cyst. Within 15 minutes after the tail was dropped the cercaria would be confined a loose pliable cyst. About one-half hour later the cyst would harden and the cercaria would be quiescent. Contraction of the excretory vesicle and the continued operation of the flame cells was observed within the encysted metacercaria.

McCoy (1928) recovered eggs of this species from the water snake, *Natrix sipedon*. These eggs were maintained in the laboratory and eventually they hatched, liberating the miracidia. Snails, *Physa gyrina*, were exposed to the miracidia and infections established. McCoy studied the two subsequent sporocyst stages and the cercaria. He did not, however, establish the identity of the second intermediate host.

Cercaria of Tetrapapillatrema concavocorpa (Sizemore, 1936)
Ralph, 1938: Plagiorchiidae
(Plate V, figure 13)

Specific Diagnosis:

Xiphidiocercaria of Armata group. Body large, elongate oval, 0.256 to 0.294 mm (average, 0.264 mm) long by 0.096 to 0.138 mm (average, 0.114 mm) wide. Tail over one-half body length, 0.186 to 0.197 mm (average, 0.191 mm) long by 0.024 to 0.031 mm (average, 0.028 mm) wide. Spines cover entire body, diminishing in number posteriorly. Stylet, 25 by 4^u, with anterior enlargement near end followed by abrupt shoulders. Posterior end terminating by a plug. Oral sucker 55^u long by 48^u wide. Mouth opening into long prepharynx. Pharynx muscular, 17^u in diameter. Long esophagus bifurcating immediately above acetabulum, ceca extend to posterior margin of first two-thirds of acetabulum. Acetabulum muscular, 49^u in diameter, mostly in posterior half of body and having at least papillae on inner margin. Penetration gland cells, 8 pairs located anterior and lateral to acetabulum. At least 4 pairs of gland ducts progress anteriorly, opening at anterior margin of oral sucker in vicinity of stylet. Excretory bladder with bulbous posterior chamber and "T"-shaped anterior vesicle which receives subterminally on either side a common duct formed by anterior and posterior collecting tubules. Flame cell pattern, 6 groups of 3 each per side, $2[(3 + 3) + (3 + 3) + (3 + 3)]$. Excretory canal extends from bladder into proximal third of tail. Primordia of nervous system extend on either side from level of oral sucker to region immediately posterior to excretory bladder. Commissure, connecting lateral extensions, located between oral sucker and pharynx. Tail receptacle with pair of lateral sinuses, each containing at least 8 spines. Host: Helisoma trivolvis.

Discussion:

The morphology of these specimens agrees with the description of Cercaria concavocorpa. Certain additional characteristics not noted by Sizemore were the nervous primordia and papillae on the ventral sucker.

The most striking feature of this cercaria are the nervous primordia which appear to be more developed than in any other cercaria studied. Sizemore (1936) and Ralph (1938) noted the wide, pronounced Commissure extending across the anterior portion of the body between the oral sucker and pharynx but failed to note any extensive anterior or posterior trunks. The writer on several occasions observed long descending lateral trunks on either side which extended almost to the posterior end of the body and short anterior trunks which apparently terminate on the dorsal surface of the oral sucker. As far as can be determined nerve trunks of this nature have only been described once before by Rees (1934) in Cercaria patellae.

The distinguishing characteristics of this cercaria are the shape of the stylet, extent of the ceca 8 pairs of penetration glands, and the nervous primordia.

Cercariae of T. concavocorpa were recovered from one of 21 (4.7 per cent) Helisoma trivolvis collected from Stillwater Creek below the Lake Carl Blackwell dam on April 1, 1951. Collections of 39 snails on November 3, 1950; 20 on December 26, 1950; and 14 on February 6, 1951 were negative.

Development of cercariae occurs in sac-like, colorless sporocysts in the liver of the snail. Cercariae emerged from the snail after it had been maintained in the laboratory for 4 days. Emergence commenced more or less daily at 8 A. M. and terminated at 4 P. M. In the morning very few cercariae were given off whereas in the afternoon, especially, at 3 P. M., the greatest numbers emerged. No more than 10 cercariae ever emerged in a day.

The cercariae swim towards the water surface or area of more intense light. The swimming position is typical of xiphidiocercariae. The tail is actively lashed and the cercaria moves in a jerky rotational path in the direction opposite its two ends, which are together in swimming position. When the cercaria is near the water surface it flattens out and sinks gradually. It remains on the bottom of the container for several minutes during which time it is resting on its dorsal surface with the body slightly curled ventrally and the tail directed towards the water surface.

Ralph (1938) completed this life cycle using tadpoles as the second intermediate host and snapping turtles as the definitive host. Ralph obtained the tadpoles that he used from natural habitats instead of from a laboratory reared stock. As a result, his tadpoles were infected with several different types of metacercariae, and it is questionable whether the infections resulted from the metacercariae that were fed experimentally.

Cercaria ramonae McCoy, 1928

Specific Diagnosis:

Xiphidiocercaria of Armata group. Body elongate oval, 0.232 to 0.294 mm (average, 0.248 mm) long by 0.197 to 0.228 mm (average, 0.213 mm) wide. Tail longer than body, 0.418 to 0.439 mm (average, 0.432 mm) long by 0.038 to 0.045 mm (average, 0.041 mm) wide. Spines cover entire body. Stylet needle-shaped with thickened sides and basal plug. Oral sucker 67^u in diameter. Mouth opening into a short prepharynx. Pharynx muscular, 24^u in diameter. Relatively long esophagus, bifurcating immediately above acetabulum, ceca extend to level midway of excretory bladder. Acetabulum muscular, 86^u in diameter, mostly in posterior half of body. Penetration gland cells 8 pairs, located lateral and slightly anterior to acetabulum, opening at anterior margin of oral sucker in region of stylet. Excretory bladder consists of bulbous posterior chamber and an anterior vesicle whose cornua at times encircle the acetabulum to meet medially. Anterior vesicle receives subterminally a common duct on either side formed by an anterior and posterior collecting tubule. Flame cell pattern 6 groups of 3 each per side, $2[(3 + 3) + (3 + 3) + (3 + 3)]$. Tail receptacle with pair of lateral sinuses. Host: Physa gyrina.

Discussion:

This cercaria was the first one encountered in the investigation. The morphology of these specimens agrees with the description of Cercaria ramonae McCoy, 1928. The distinguishing characteristics of this cercaria are the presence of a contracting excretory bladder, whose anterior cornua at times encircle the acetabulum to meet medially, and the extension of the ceca to the level of the posterior excretory chamber.

The life cycle of C. ramonae has not been completed but cercarial characteristics indicate that the species is probably closely related to species in the subfamily Reniferinae.

Cercariae were recovered from 3 of 50 (6 per cent) Physa gyrina collected from Thomas Lake on September 24, 1950 and from one of 20 (5 per cent) collected on October 16, 1950. Collections of 15 snails on December 14, 1950; 13 on January 21, 1951; 10 on March 9, 1951; and 15 on May 1, 1951 were negative.

Development of cercariae occurs in sac-like sporocysts within the liver of the snail. The walls of these sporocysts are generally clear with an occasional patch of scattered black pigment. The sporocysts contain few cercariae.

On several occasions a condition of hyperparasitism was observed. Unidentified strigeid neasci were found inhabiting these sporocysts, and appeared to be thriving.

Cercariae emerge from the snail during the morning hours from daylight until about 10:30 A. M. They are very active swimmers and resting periods are infrequent. When swimming they adopt the characteristic position of most xiphidiocercariae. The tail is actively lashed in a figure eight pattern and the cercaria moves in a jerky rotational path in the direction opposite the two ends, which are together in the swimming position. Occasionally, while swimming, the anterior end will be extended causing the body of the cercaria to become elongated. Occasionally they creep, measuring worm fashion, along the bottom of the container.

Encystment of this cercaria would occur in egg albumen but not in methocellulose of the same consistency or in water. In water mounts the cercariae repidly flattened out and died. In the methocellulose mounts cercariae remained active but no encystment occurred. In the egg albumen mounts all cercariae encysted within one-half hour. Cercariae were subjected to these media in Bureau of Plant Industry Watch Glasses and all of those in the albumen encysted in 45 minutes while the others failed to encyst in two hours. Cercariae were put in a thin film of water which was allowed to evaporate slowly but the cercariae did not encyst. These results indicate that the presence of some substance, possibly protein, in the egg albumen was essential for encystment.

Cercaria J
(Plate VI, figure 16)

Specific Diagnosis:

Xiphidiocercaria of Armata group. Body elongate oval, 0.224 to 0.259 mm (average, 0.243 mm) long by 0.100 to 0.114 mm (average, 0.109 mm)wide. Tail approximately seven-eights length of body, 0.190 to 0.224 mm (average, 0.209 mm) long by 0.024 to 0.027 mm wide at base. Spines cover entire body, those in region of oral sucker larger and increasing in size from oral to fifth aboral row and then decreasing in size to level of pharynx, from which extremely fine spines, arranged in parallel rows, continue to posterior end of body. Stylet, 24^{u} long by 10^{u} wide, with shoulders and basal plug. Cystogenous glands conspicuous and distributed throughout body. Oral sucker 50^{u} in diameter. Mouth opening into a pre-pharynx longer than pharynx. Pharynx muscular, 17^{u} in diameter. Esophagus bifurcates midway between pharynx and acetabulum, ceca extend to level of bulbous posterior chamber of excretory bladder. Acetabulum muscular, 46 to 49^{u} in diameter, mostly in posterior third of body and having at least 6 rows of spines on its internal margin. Six pairs of penetration gland cells anterior and lateral to acetabulum, their ducts terminating in region of stylet. Excretory bladder consists of bulbous posterior chamber and v-shaped anterior vesicle whose cornua each receive subterminally a common duct resulting from fusion of anterior and posterior collecting tubules. Flame cell pattern 6 groups of 3 cells each per side, $2[(3 + 3) + (3 + 3) + (3 + 3)]$. Genital primordium immediately anterior and posterior of acetabulum. Tail receptable with pair of lateral sinuses, each containing spines on the posterior margin. Host: <u>Physa gyrina</u>.

Discussion:

The morphology of these specimens does not agree with the morphology of any previously described cercaria. It is similar to *Cercaria isocotylea* Cort, 1914 and the cercaria of *Cerorchis medius* (Stunkard, 1916) described by McMullen (1934). The former differs from my species in that the digestive tract is not developed. The latter differs in the number of penetration glands, *C. medius* having 10 pairs whereas Cercaria J has only 6 pairs. The distinguishing characteristics of the new species are the arrangement of spines in the vicinity of the oral sucker, the presence of 6 pairs of penetration glands, the extent of the digestive tract, and the shape of the stylet which is asymmetrical when viewed laterally.

Cercariae were recovered from a crushed specimen of *Physa gyrina* collected from Bliss's Pond on February 3, 1951. The total number of snails collected at that time was 14, making an incidence of infection of 7 per cent. Collections from Bliss's Pond of 23 snails on November 3, 1950; 13 on January 8, 1951; and 60 on April 9, 1951 were negative.

The cercariae develop in connecting sac-like sporocysts. They have the characteristic swimming position of xiphidiocercariae. One swimming feature of Cercaria J appeared to be characteristic and would serve to distinguish it from all others. The posterior end was consistently narrower than the anterior end during swimming, but in the other xiphidiocercariae the posterior end is as wide if not wider than the anterior end. Resting periods were frequent. The cercaria in its contracted swimming position, would drop to the bottom of the container where it would remain contracted for a short time, then stretch out and crawl caterpillar fashion along the bottom.

Discussion:

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During the examination of the crushed snail many cercariae were noted to penetrate the mantle of the snail and to encyst. Several of these encysted forms were studied and the presence of the characteristic anterior spination and stylet were noted. Examination of snail mantles taken from 13 of the snails collected on January 8, 1951; 15 collected on February 18, 1951; and of 36 collected on April 9, 1951 revealed an incidence of infection for metacercariae comparable to the incidence of Cercaria J, 53 per cent for January 8, 66 per cent for February 18, and negative snails for April 9. From this information it is apparent that Cercaria J or a cercaria closely resembling it is quite abundant during a certain part of the year. From this information it is also apparent that there was a drastic reduction of metacercariae-infected snails between February and April.

Cercaria R
(Plate VI, figure 15)

Specific Diagnosis:

Xiphidiocercaria of Armata group. Body small, elongate oval, 0.063 to 0.068 mm (average, 0.066 mm) long by 0.043 to 0.047 mm (average, 0.044 mm) wide. Tail approximately one-half length of body, 0.037 mm long by 0.011 mm wide. Spines distributed evenly over entire body. Large cystogenous glands distributed throughout body. Stylet asymmetrical with one side thicker than the other, 5^u long by 1.5^u wide. Oral sucker 16^u in diameter. Mouth opening into a relatively long prepharynx. Pharynx muscular, 6^u in diameter. Esophagus bifurcates at level of posterior margin of first one-third of body. ceca extend to region of posterior chamber of excretory bladder. Acetabulum muscular, 18^u in diameter, mostly in posterior half of body. At least 4 pairs of penetration glands anterior and lateral to acetabulum, their ducts terminating in region of stylet. Excretory bladder consists of bulbous posterior bladder and "T"-shaped anterior vesicle whose cornua receive terminally a common duct resulting from fusion of anterior and posterior collecting tubules. Genital primordium extending from immediately anterior to posterior end of acetabulum. Tail receptacle with pair of lateral sinuses, each containing spines. Host: Helisoma trivolvis.

Discussion:

The morphology of these specimens does not agree with any previously described cercaria. It most closely resembles the cercaria of Plagiorchis muris described by McMullen (1937). P. muris differs only in that it has 6 pairs of penetration glands instead of 4 as in the case of Cercaria R. Cercaria R is characterized by its small size, the presence of refractile globules in the parenchyma, at least 4 pairs of penetration glands, and the extent of the ceca.

This cercaria was recovered from Helisoma trivolvis collected from the "Old Country Club Lake" on March 16, 1951 and only one of 30 specimens was infected (3 per cent).

Cercariae develop in clear sac-like sporocysts. These sporocysts average 1.416 mm in length by 0.189 mm in width.

Emergence of cercariae occurred in the morning hours prior to 8 A. M. The cercariae swim at infrequent intervals, preferring to rest suspended in the water most of the time. Their swimming position was typical of most xiphidiocercariae. The resting position was characterized by the cercaria maintaining its contracted swimming state but ceasing tail activity. In this position the cercaria remained suspended in water.

Encystment of this species was noted to occur in snails. For this reason and also because the life cycle of P. muris, a closely related form, was known, experiments were performed to determine the life cycle of Cercaria R.

Four laboratory reared snails were exposed to cercariae for a 12 hour period. After exposure these snails were maintained in the laboratory for one week, after which they were examined and found to be infected with metacercariae. The infected snails were fed to 3 laboratory reared mice, the experimental mammal used by McMullen (1937) for the P. muris cycle. Two days following feeding one mouse was examined and was negative for infection. The remaining mice were examined two weeks later and were negative for infection.

SUMMARY AND CONCLUSIONS

General Discussion

The investigation concerning cercariae found in the vicinity of Stillwater, Oklahoma has resulted in a study of 20 species. These cercariae have been classified, described and notes relative to biology recorded. The biological data include seasonal occurrence, descriptions and behavior of precercarial stages within the snail, time of emergence, swimming and other activities of cercariae, encystment of cercariae, life cycle experiments, and notes on economic importance. Five new species have been described.

The study of larval trematodes was confined to two intermediate host snail species, _Physa gyrina_ and _Helisoma trivolvis_. Periodic collections revealed that amphistome infections were present during the fall and spring months; echinostome and furcocercous infections during the fall, winter, and spring months; and xiphidiocercariae infections during the fall, winter, and spring months. April was the peak month for infections.

All of the cercariae encountered in this investigation developed in the liver tissue of the snail host. Development occurred in either sporocysts or rediae. Cercariae of the groups, Furcocercous and Xiphidiocercariae, developed in sporocysts whereas those of the groups, Echinostome and Amphistome, developed in rediae. With few exceptions, the Xiphidiocercariae developed clear sac-like sporocysts. The furcocercous cercariae, in general, developed in narrow continuous sporocysts. The rediae of the amphistome and echinostome cercariae were very similar.

Both were heavily pigmented, active organisms with locomotor appendages. The rediae of echinostome cercariae had a definite head collar whereas the amphistome cercariae did not.

The time of cercarial emergence from the snail varied considerably within the four groups. Each of the four groups included at least one cercaria that emerged during the morning hours, and in the Amphistome and Xiphidiocercariae groups there were cercariae that emerged throughout the day. One emerged between 6:30 P. M. and midnight. Others emerged regularly, but for a comparatively short period sometime during the day.

Behavior of cercariae was studied in order to determine its value in identification. Interesting differences in swimming were observed but they were of little value for economic purposes, except in a few specific cases. The activity was so similar in most cercariae that it could not be used to even make group determinations.

The amphistome cercariae swim almost constantly. While swimming they keep the body in a contracted state with the anterior end bent ventrally and drawn posteriorly. Movement is accomplished by the actively lashing tail. The swimming position of the echinostome cercariae is very similar to that of the amphistomes. The anterior end is bent ventrally and drawn posteriorly, and the actively lashing tail propels the cercaria in the direction opposite its two ends. Xiphidiocercariae have the same swimming position. In fact it is impossible to distinguish between the echinostome cercariae and xiphidiocercariae when they are swimming since they are both about the same size. The furcocercous cercariae, on the other hand, can be more easily distinguished from the other groups. They move in a jerky rotational path, generally towards the water surface. The lashing tail moves forward and motion is aided by the cercaria actively bending from side to side at the juncture of the body and tail stem. There is less uniformity in the behavior of furcocercous cercariae and many of them swim without being flexed, with the tail forward. The relative size of body and tail seems to produce the differences in swimming, behavior and sometimes this is specific. Resting attitudes and positions will usually identify cercariae of this group. These positions are many times characteristic for a species. Most of these cercariae rest suspended in water in a vertical position with the anterior end directed towards the bottom of the container. When other types of cercariae rest they are usually on the substratum.

The furcocercous cercariae showed pronounced intra-group differences in behavior. Five of the 8 cercariae described had distinctive resting positions. The resting positions of Cercaria flexicorpa and Cercaria Q were characterized by the cercariae, while in their inverted positions, drawing the anterior end of the body towards the posterior, giving the larva a "fish hook" shape. Cercaria flexicorpa would maintain this position while resting; whereas Cercaria Q, would repeatedly draw the anterior end towards the posterior end and then straighten out while resting. The schistosome, Cercaria wardi, and the cercaria of a Clinostomum sp. had resting positions quite different from those of the other furcocercous cercariae. Instead of the cercariae resting suspending in an inverted position like the others, these two would draw the body towards the tail, thus giving the cercaria a "horseshoe" shape. Cercaria T was another species with a distinctive resting position. It would rest either parallel, perpendicular, or diagonal to the surface of the water with the anterior end either up or down.

Encystment characteristics were used as aids in distinguishing between closely related cercariae. The two amphistome cercariae, Allassostoma parvum and Cercaria S, showed one difference. When cercariae of the two species were placed in a small quantity of water that was allowed to evaporate, only Cercaria S would encyst. Differences were observed in two of the three echinostome cercariae. The cercaria of E. revolutum encysted in water, snail slime, and snails; E. recurvatum encysted in snails but not in water and snail slime.

These observations show the value of ecological studies as aids in taxonomy.

This study indicates that the life cycle of a number of cercariae is not known and there are representatives in all of the groups. Those for which cycles are not known are one of two amphistomes, seven of eight furcocercous cercariae, and three of seven Xiphidiocercariae. This shows the necessity of doing life cycle work before the full economic importance of the parasites can be ascertained. Some of those for which the cycle is known are of importance in a negative way. Two species, E. revolutum and E. recurvatum, are known to cause disease in domestic and wild fowl. The two echinostomes are capable of infecting chickens, ducks, turkeys, and other birds. E. recurvatum has been found to cause marked enteritis in domestic turkeys (Annereaux, 1940) and has been recorded from wild turkeys of the Wichita Mountains Wildlife Refuge (Self and Bouchard, 1950). Perhaps the most important ones economically are those species that inhabit the muscles of fish in the metacercarial stage. The cercariae of species that cause yellow grub disease and black grub disease and render the fish undesirable for eating occur in the Stillwater area. Cercaria flexicorpa causes black grub in fish, and cercaria of Clinostomum sp. causes yellow grub. The cercariae of closely related species are known to be very destructive to small fish, and those that do not succumb may be stunted or deformed.

This study has shown that there is a large number of unsolved trematode problems in the vicinity of Stillwater, Oklahoma. The finding of five new cercariae presents five cycles for solution and there are inadequate biological data for most of the trematodes for which the cycle is known. The few data on seasonal fluctuations of infections in snails leaves many questions unanswered concerning the cause. The fact that cercariae which normally encyst in hosts could be induced to encyst in egg albumen suggests a problem concerned with the encystment in other types of protein, and development of metacercariae in vitro in such media. Experimental studies with definitive hosts are certainly necessary to secure more specific data on the effects of the parasites on the host.

A study has been made of the trematodes, with particular emphasis on the cercariae, that infect the snails, Helisoma trivolvis and Physa gyrina in the region of Stillwater, Oklahoma.

On the basis of cercariae twenty species of trematodes were identified, of which five are new. Uniform descriptions of cercariae have been given for the known species, and new descriptive material was supplied in several. The cercariae of the five new species have been described as Cercaria S, Cercaria T, Cercaria Q, Cercaria J and Cercaria R.

Cercariae of the following species were recovered from the snails: Allassostoma parvum, Echinostoma revolutum, Echinoparyphium recurvatum, Petasiger nitidus, Dasymetra conferta, Alloglossidium corti, Pneumatophilus variabilis, Tetrapapillatrema concavocorpa, Posthodiplostomum minimum, Cercaria flexicorpa, C. wardi, C. ramonae, C. stoni, and Clinostomum sp., possibly C. Whitentoni.

Points of dispute in the anatomy of the cercaria of Echinoparyphium recurvatum and Cercaria wardi involving the excretory system in the former and penetration glands in the latter, were settled.

The following trematode species were reported for the first time from Oklahoma: Petasiger nitidus, Posthodiplostomum minimum, Alloglossidium corti, Cercaria wardi and C. stoni.

The intramolluscan stages of the new species have been described.

The behavior of the cercaria of all species has been described, and discussed with relation to its use as an aid in differentiating species.

Notes concerning abundance and seasonal occurrence of cercariae in snails have been related.

The cercaria of Echinostoma revolutum, which normally encysts in a second intermediate host, was observed to encyst in snail slime.

Cercaria ramonae and the cercaria of P. variabilis, which normally encyst in an intermediate host, were observed to encyst in egg albumen.

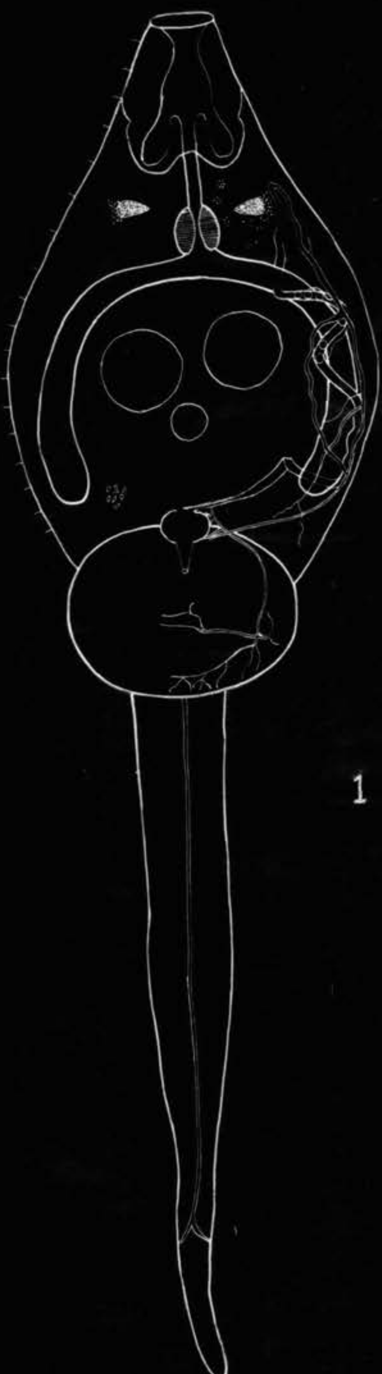
The fish, Gambusia affinus, was shown experimentally to be a new second intermediate host of Petasiger nitidus

The white mouse has been demonstrated experimentally to be a new definitive host of Echinoparyphium recurvatum.

P L A T E S

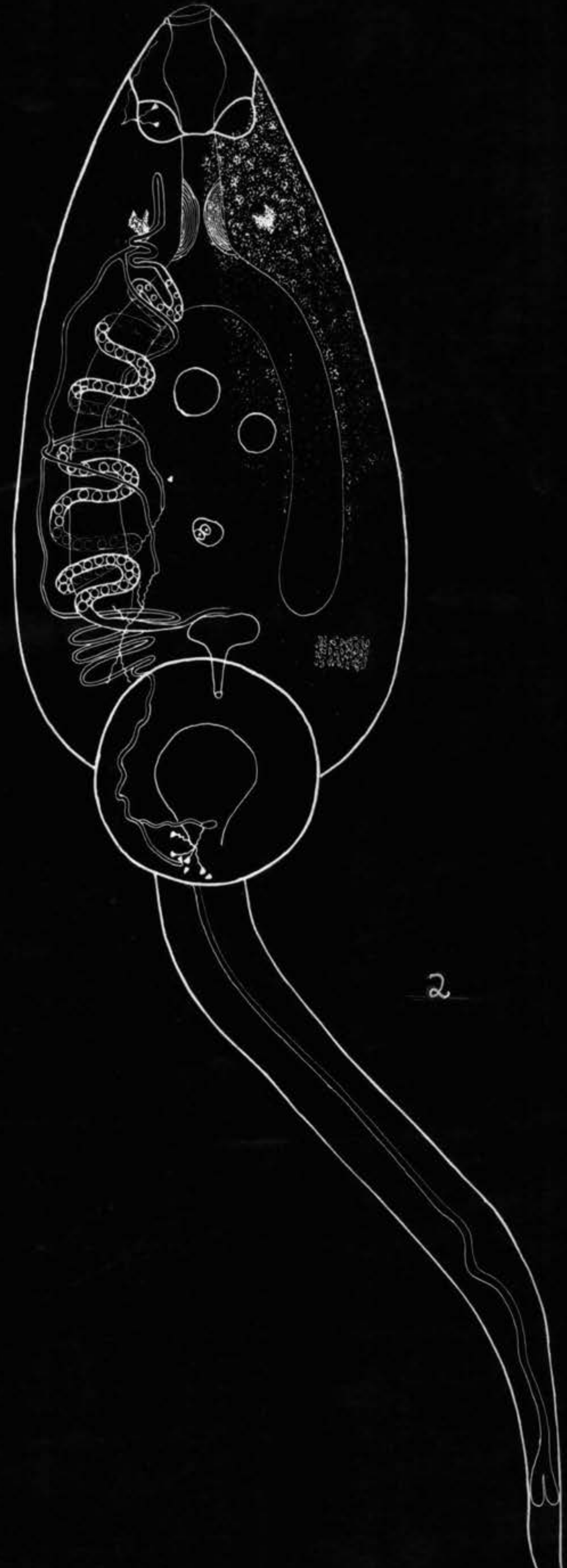
1. Cercaria S

2. Cercaria of <u>Allassostoma parvum</u>



1

146 μ



2

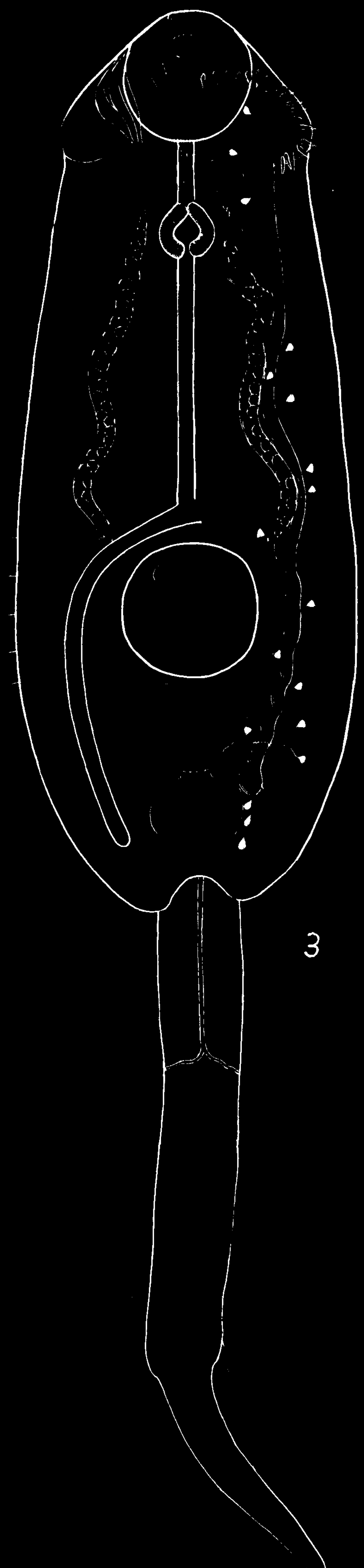
3. Cercaria of *Echinostoma revolutum*

4. Arrangement of collar spines for *E. revolutum*

5. Tail fin of *E. revolutum*

6. Cercaria of *Echinoparyphium recurvatum*

7. Arrangement of collar spines for *E. recurvatum*

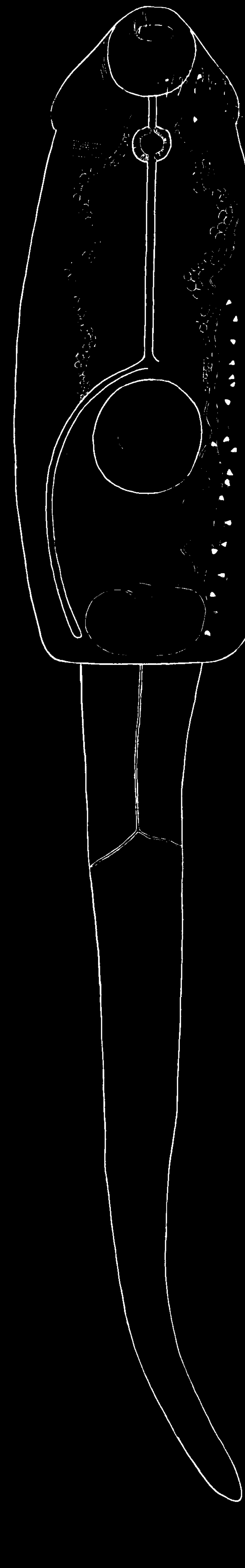


3

4

34.6 μ

5

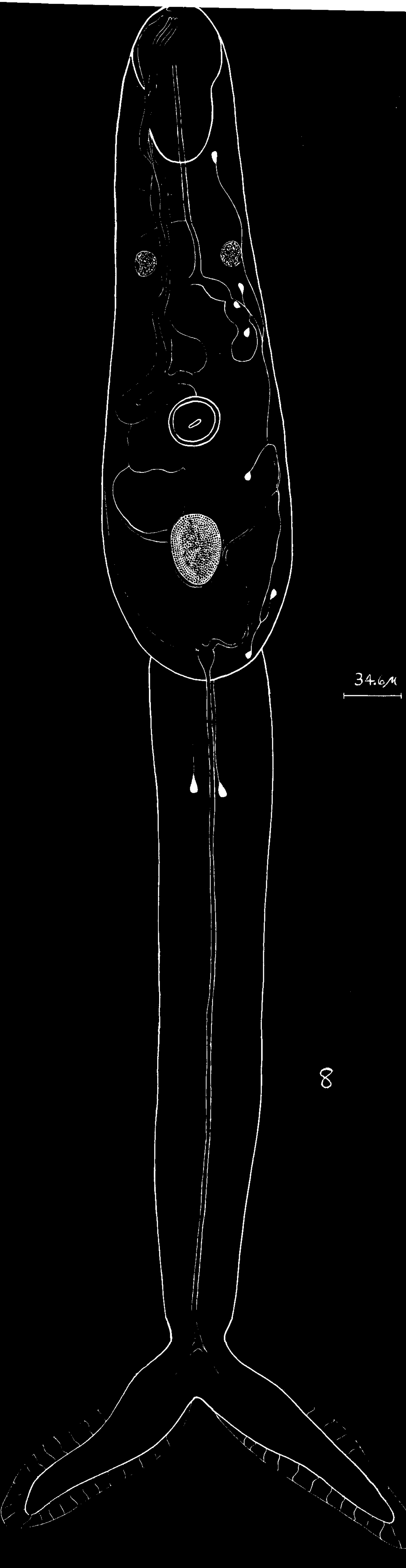


6

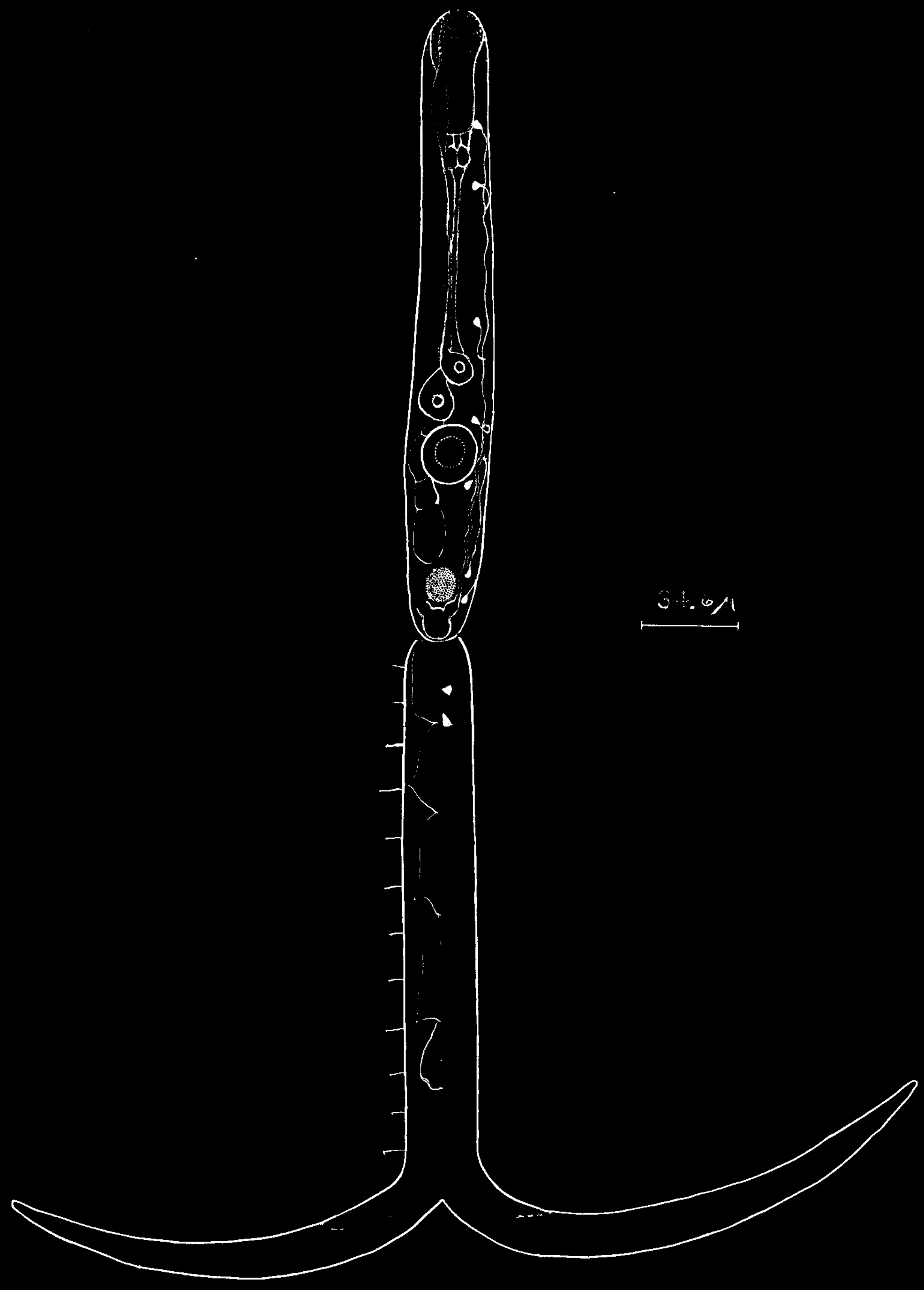
7

8. *Cercaria wardi*

9. Cercaria T



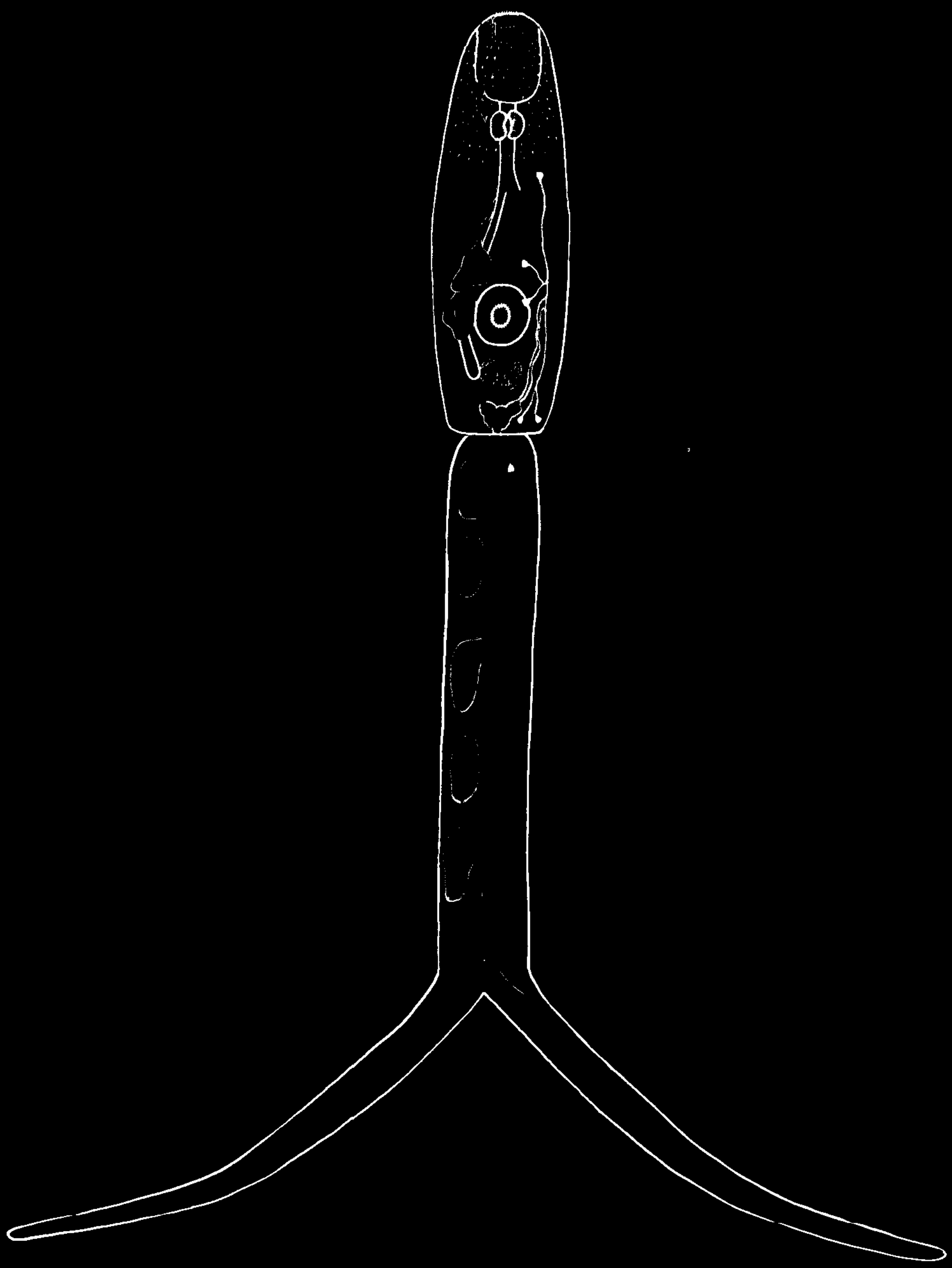
8



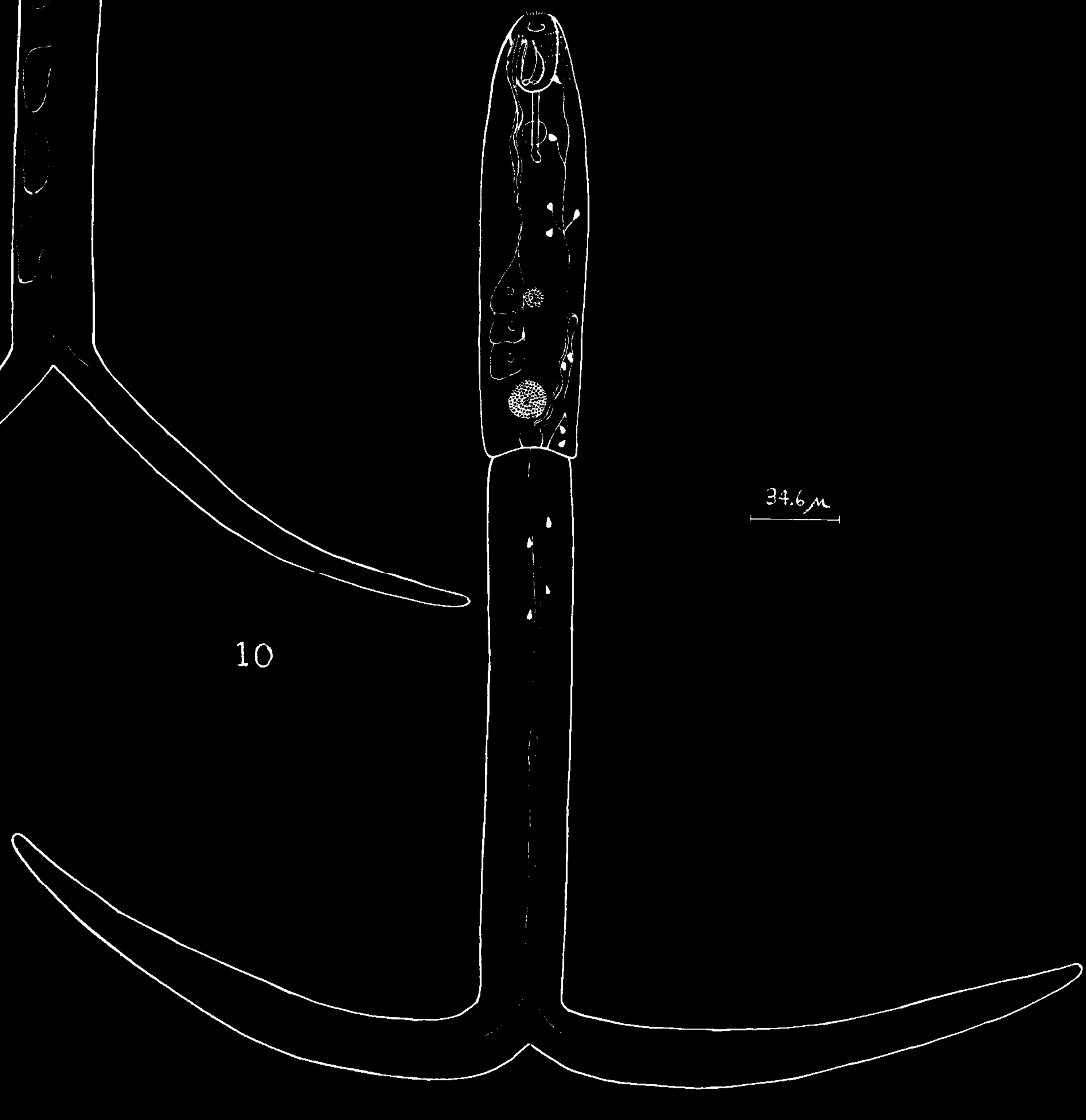
9

10. Cercaria Q

11. Cercaria flexicorpa



10



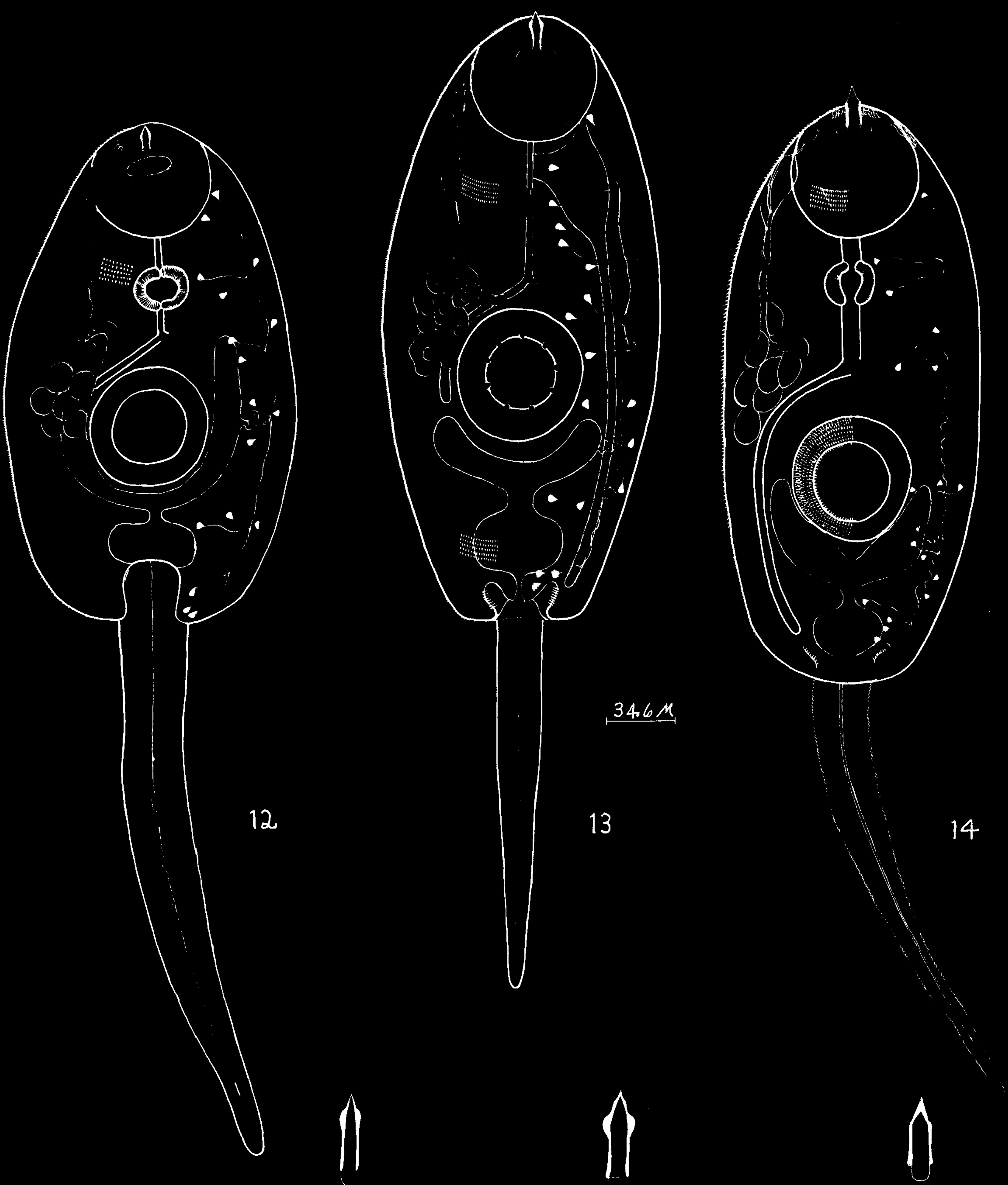
$34.6\ \mu$

11

12. Cercaria of <u>Pneumatophilus variabilis</u>

13. Cercaria of <u>Tetrapapillatrema</u>

14. Cercaria of <u>Dasymetra conferta</u>

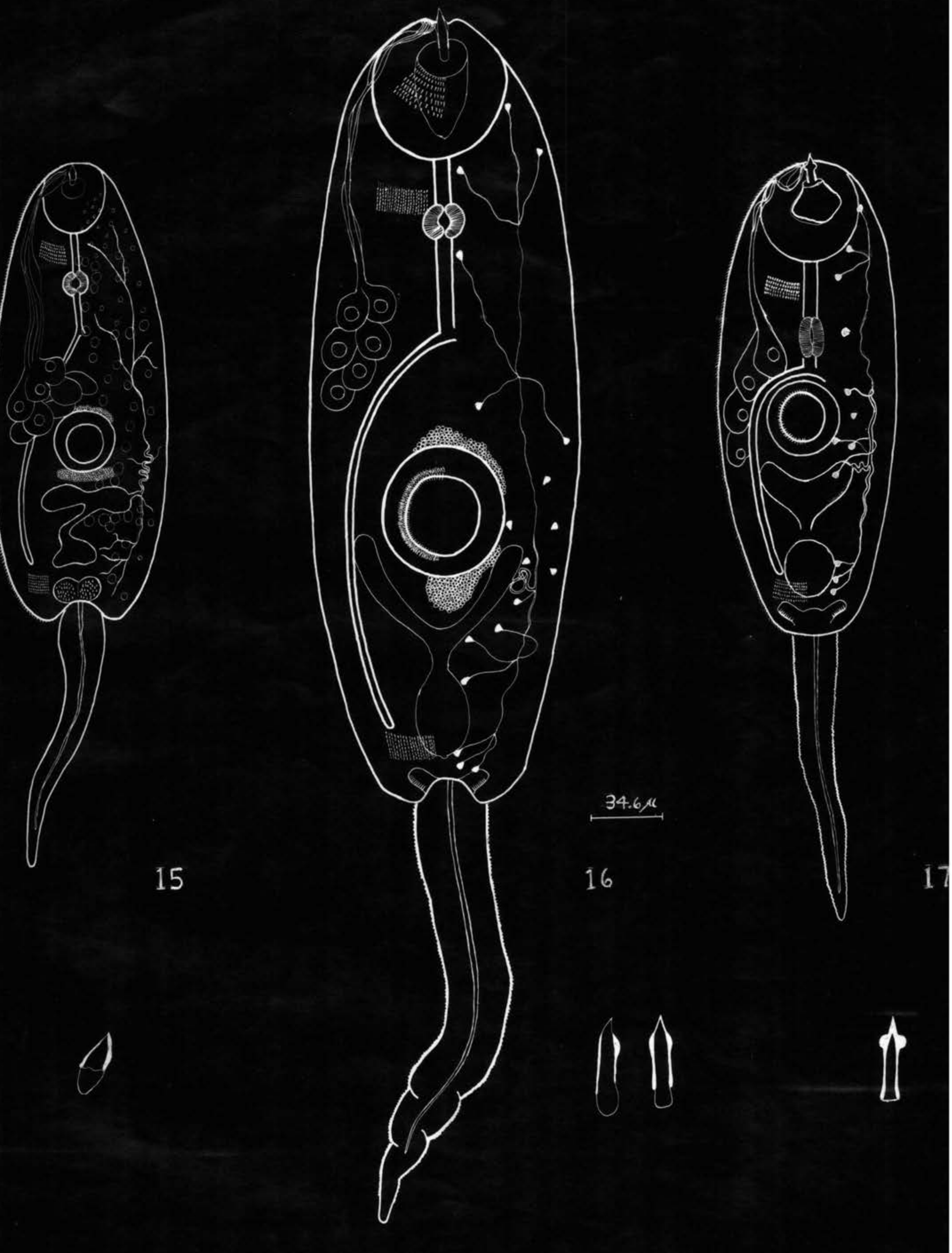


12 13 14

15. Cercaria R

16. Cercaria J

17. Cercaria of <u>Alloglossidium corti</u>


34.6 µ
15
16
17

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