AN ABSTRACT OF THE THESIS OF

GLENN ARTHUR ANDERSON for the Ph. D. in Zoology
(Name) (Degree) (Major)

Date thesis is presented April 24, 1964

Title DIGENETIC TREMATODES OF ASCAPHUS TRUEI IN WESTERN OREGON

Redacted for Privacy

Abstract approved
(Major professor)

The Tailed Frog Ascaphus truei, the only species in the family Ascaphidae found outside New Zealand, is a common inhabitant of the mountain streams of the Northwestern United States and British Columbia. The anatomical peculiarities of this primitive anuran have been investigated, but no literature is available on the digenetic trematodes it harbors.

In Western Oregon, Ascaphus was found to serve as a definitive host for two digenetic trematodes which live in the small intestine, and as a second intermediate host for two digenetic trematodes which live as encysted metacercariae in the subcutaneous connective tissue.

Tetracheilos ascaphi Anderson and Pratt, 1964, is a trematode in the family Allocreadiidae, with a life cycle somewhat similar to several species of Crepidostomum. The adult lives in the anterior

portion of the small intestine of Ascaphus. Eggs pass with the feces, and the clam Pisidium idahoense becomes infected with the miracidia. The miracidia develop into sporocysts on the gonads and liver. The cercariae emerging from the sporocysts enter the caddisfly Rhyacophila grandis and encyst to develop into metacercariae. Ascaphus eat adult Rhyacophila and the excysting metacercariae become adult in the intestine of the frog.

Euryhelmis squamula Rudolphi, 1819, (Heterophyidae) is found as encysted metacercariae in the subcutaneous connective tissue of Ascaphus. Metacercariae eaten by golden hamsters become mature in 70 to 92 hours, and eggs pass out with the feces of the host. In the wild, the snail Bythinella hemphilli is infected by the miracidia hatching from the egg. The lophocercous cercariae emerge from the snail and penetrate the frog's skin. Metacercariae become infective in 44 days or less in Rana aurora.

Euryhelmis pacificus Senger and Macy, 1952, is also found encysted under the skin of Ascaphus. This trematode has been reported from the salamander Dicamptodon ensatus, and from Mustela vison and Ondatra zibethica in Oregon. It matures in 55 to 72 hours, and lives for more than eight months in the small intestine of the golden hamster. The first intermediate host and early larval stages are unknown.

Cephalouterina dicamptodoni Senger and Macy, 1953,

(Lecithodendriidae) originally found in the small intestine of Dicamptodon ensatus, also lives in the small intestine of Ascaphus. Metacercariae are found encysted in the Salmonfly Acroneuria californica which is eaten by Ascaphus. The excysted flukes mature in the frog's intestine and eggs are passed with the feces. The first intermediate host and the early larval stages are unknown.

DIGENETIC TREMATODES OF ASCAPHUS TRUEI IN WESTERN OREGON

by

GLENN ARTHUR ANDERSON

A THESIS

submitted to

OREGON STATE UNIVERSITY

in partial fulfillment of
the requirements for the
degree of

DOCTOR OF PHILOSOPHY

June 1964

APPROVED:

Redacted for Privacy

Professor of Zoology

In Charge of Major

Redacted for Privacy

Chairman of Zoology Department

Redacted for Privacy

Dean of Graduate School

Date thesis is presented April 24, 1964

Typed by Nancy Kerley

ACKNOWLEDGEMENTS

I wish to express my appreciation to the many individuals who helped me during this study. Among these were Dr. Clyde Senger of Western Washington State College who presented me with several specimens from his collection, and Drs. Rodrigo Brenes and Otto Jiménez-Quirós of the University of Costa Rica for the loan of a specimen; Dr. Joseph Morrison of the U. S. National Museum who identified the molluscs; Dr. G. B. Wiggens of the Royal Ontario Museum in Toronto who identified the caddisfly; Stanley Jewett of the U. S. Fish and Wildlife Service in Portland who identified the stonefly, and John Belton of Oregon State University who aided on many collection trips and assisted with the photomicrography.

Recognition is also due several staff members of the U. S. National Museum for the loan of several trematode specimens from the Helminthological Collection.

My thanks also to the many members of the Department of Zoology at Oregon State University who supplied assistance, and to Dr. Ivan Pratt, my major professor, to whom I am especially indebted for his advice and encouragement.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

DIGENETIC TREMATODES OF ASCAPHUS TRUEI IN WESTERN OREGON

PART I

Ascaphus truei Stejneger, 1899

The Tailed Frog, Ascaphus truei Stejneger, 1899, also known as the Bell Toad, is a small anuran which inhabits the mountain streams of the northwestern United States and the southwestern portion of British Columbia. Ascaphus truei (family Ascaphidae) is a voiceless, primitive frog characterized by the absence of eardrums, the presence of two pairs of free, bony ribs, a nonprotrusible tongue, small lungs and a tail-like projection of the body of the male which functions as a copulatory organ (37). The family Ascaphidae consists of two genera, Liopelma, with three species found only in New Zealand, and Ascaphus, with one species.

The adults and tadpoles of Ascaphus are found in streams from near sea level to elevations of about 3500 feet, and appear to be confined to small, shallow streams in which the maximum temperature does not exceed 16° C (26). These streams are enclosed by forest, and the overhanging canopy of trees and brush shades the water from excessive exposure to direct sunlight. The streams are also characterized by having beds composed primarily of rocks and boulders and a relatively rapid water movement. The tadpoles, which lack external gills, are found clinging to rocks in fast water

by means of their large, sucker-like mouths. During the day adults conceal themselves under rocks in the stream or in moist crevices along the edges. They emerge after dark and can readily be observed and collected with the aid of a light. Most of the frogs are observed on moss-covered rocks which are partially submerged along the edges of the stream. In the course of this study very few Ascaphus were observed or collected from exposed stretches of the stream where moss was absent.

Adult Ascaphus feed mostly on aquatic insects during the warmer, drier months of the year when they are confined to the streams. Remains of larvae, nymphs and adults of aquatic insects, as well as elytra of terrestrial beetles, were found in the stomach contents of the frogs during this study. Frogs in the water were seldom observed swimming, but were often seen crawling along the bottom--a more advantageous means of locomotion in the swift current. This trait, and the quantities of insect larvae and nymphs in the diet, make it appear that Ascaphus does a considerable amount of feeding underwater.

PART II

The Life Cycle of Tetracheilos ascaphi Anderson and Pratt, 1964

Tetracheilos ascaphi (Allocreadiidae, Tetracheilosinae) is a digenetic trematode which inhabits the small intestine of Ascaphus truei in Western Oregon (3). It shares some of the anatomical characteristics of the Bunoderinae and the Crepidostominae; it differs from both in other morphological characteristics, and appears to lie in a position between these two subfamilies of the Allocreadiidae.

The life cycle of Tetracheilos differs markedly from that of Bunodera, but is similar to the life cycles which have been described for four species of Crepidostominae. The life cycle of Bunodera luciopercae Müller, 1776, (Bunoderinae) has been completely solved. This allocreadiid has been found in the intestine of 11 genera of fresh water fishes in Europe and North America, according to Yamaguti (41, p. 131). In 1873 Linstow (21) reported the cercariae as developing in a sporocyst in the snail Paludina impura and encysting in the same host. Wright, in 1884 (40), and Linton, in 1892 (22), reported the metacercariae in crayfish.

The life cycle of Crepidostomum farionis Müller, 1784, was the first cycle completely solved in this subfamily. In 1915 Cooper (10, p. 194, 196) discovered the metacercaria encysted in the mayfly

Hexagenia and in the crayfish Cambarus propinquus. Brown, in 1927 (8, p. 88), reported finding the cercariae in the small, fresh-water clams Pisidium amnicum and Sphaerium corneum. He found the metacercariae in the mayfly Ephemera danica, and the adults in the intestine, pyloric ceca and gall bladder of trout and grayling. Crawford (11, p. 6) confirmed this.

In 1931 Hopkins (16) found Crepidostomum isostomum Hopkins in a perch in Illinois, and in 1934 he reported (18, p. 35-37) that the oculate xiphidiocercariae developed in Sphaerium and metacercariae encysted in mayfly nymphs. The life cycle of Crepidostomum cooperi Hopkins, 1931, also was described by Hopkins (17; 18, p. 18-29). The adults were found in 12 species of naturally infected fish. The cercariae developed in the clam Musculium transversum, entered aquatic insects, and developed into metacercariae. Yamaguti (41, p. 131-132) listed three genera of fishes as definitive hosts of Crepidostomum isostomum and 19 genera of fishes as definitive hosts of Crepidostomum cooperi.

Crepidostomum cornutum (Osborn, 1903) Stafford, 1904, was described from black bass taken from Lake Chautauqua, New York. Stafford (36) recovered the adults from catfish and metacercariae from crayfish. In 1937 Ameel (1) discovered the cercariae of this fluke in Sphaerium and Henderson (15) recovered the cercariae from Musculium transversum. Yamaguti (41, p. 131) cites the recovery

of metacercariae from crayfish by several investigators, and lists 13 genera of fishes as definitive hosts.

Although normally an intestinal parasite of fresh-water fishes, Crepidostomum has been reported from the bats, Vespertilio noctuo and Vespertilio lascopterus, by Braun (5), from the salamander Necturus maculatus by Stafford (36, p. 490), and from the frog Rana hecksheri by Parker (27, p. 39). Pratt and McCauley (30, p. 14-16) listed only fishes as definitive hosts of Crepidostomum in Oregon.

METHODS AND MATERIALS

Ascaphus truei were collected in mountain streams of Western Oregon at elevations between 2200 and 3000 feet during the late spring and throughout the summer. Some of the frogs were examined for endoparasites within one or two days of collection and the remainder were kept alive at approximately 10° C. These were examined at intervals of up to two months after collection.

Juvenile and adult Tetracheilos ascaphi recovered from the intestines of the frogs were placed in small watch glasses and stored in a refrigerator at 5° C for intervals of one to several days in order to relax them and to recover eggs.

Caddisflies (Rhyacophila grandis Banks, 1911) were collected in their cases in both the larval and pupal stages and stored in a refrigerator at 5° C until examined. Encysted metacercariae taken from the caddisflies were fed to young Rana aurora and to adult Rana pipiens and Hyla regilla, but Tetracheilos was not found at the time of examination. Metacercarial cysts fed to Ascaphus, held for two months without feeding, developed into adult Tetracheilos lacking eggs.

A fine-screened sieve was employed in collecting Pisidium idahoense from the fine sand and mud of the stream bottoms. These clams were brought into the laboratory where they were held in petri

dishes in a refrigerator at 5° C. The clams were opened with jewelers' forceps and sporocysts were removed from the gonads and liver. Unopened clams were stored in petri dishes which were examined at intervals to collect emerging cercariae.

Mayfly nymphs taken from Berry Creek, Benton County, Oregon, were experimentally infected by placing them in small watch glasses and introducing large numbers of cercariae into the water maintained at 5° C.

Eggs were treated in several ways in attempts to induce them to hatch. Some were placed in watch glasses containing stream water and kept for intervals of up to six weeks at room temperature, at 10° C and at 5° C. Some were fed to clams, and the others were treated with trypsin or pepsin or a combination of the two. None of these methods was successful and the miracidium was not obtained.

Specimens prepared for mounting were fixed in Lavdowsky's AFA Solution. Adults and metacercariae were stained with Celestine blue, Delafield's hematoxylin, Meyer's carmalum and Semichon's carmine. Sporocysts were stained with Semichon's carmine. All stages were studied alive, both unstained and using aqueous solutions of neutral red and methylene blue.

Photomicrographs were taken with Kodachrome and Panatomic X film using Edixa Reflex and Olympus cameras mounted on compound microscopes. Photographs and a Leitz projector and

microprojector were used in making the drawings.

All measurements are in millimeters. In all measurements the average is given first and the range in measurements follows in parentheses.

LIFE HISTORY RESUMÉ

The life cycle of Tetracheilos ascaphi involves three hosts--a mollusc, an insect and a frog--which inhabit the cold, shaded mountain streams of Western Oregon.

Sporocysts develop in the tiny fresh-water clam Pisidum idahoense. Cercariae leave the clam and enter the water, swimming about until they encounter a caddisfly larva. They penetrate the exoskeleton, make their way into the haemocoel, encyst, and undergo development into metacercariae. The only naturally infected caddisfly encountered in the course of this study was Rhyacophila grandis.

Emerging adult caddisflies are eaten by Ascaphus truei, and the flukes mature in the anterior half of the small intestine. The flukes mature slowly, and on becoming adult, deposit eggs which are evacuated with the feces of the host.

EGG

The egg of Tetracheilos ascaphi is pale, golden-brown when laid. It is smoothly oval showing no sign of an operculum or any distinctive markings (Figure 1). The size of 25 eggs deposited in water was 0.056 (0.051-0.061) long by 0.037 (0.031-0.042) in diameter. Twenty-five eggs in the distal end of the uteri of five mature flukes, fixed, stained and mounted in Canada balsam, measured 0.055 (0.051-0.061) long by 0.029 (0.027-0.032) wide.

Attempts to hatch eggs were unsuccessful and no miracidia were observed. Eggs ruptured by applying pressure to the cover slip released several yolk cells but nothing recognizable as a developing miracidium. One egg of Crepidostomum farionis hatched after 34 days in water (18, p. 13) and Hopkins reported (18, p. 22) eggs of Crepidostomum cooperi hatching after being kept in water at room temperature about ten days. The ciliated miracidia of these two species of Crepidostomum exhibit two pigmented eyespots as do the cercariae. Since the cercariae of Tetracheilos have two eyespots, Tetracheilos miracidia also may have this characteristic.

SPOROCYST

The pea clam, Pisidium idahoense, serves as the first intermediate host for Tetracheilos ascaphi. The sporocyst stage was observed only in naturally infected clams, since no experimental infections were tried. Of 178 clams examined, Tetracheilos sporocysts were found in 20, or 11.2 percent. The greatest number of sporocysts found in one clam was 18; the average number of sporocysts per clam for the remainder of the infected clams was 3.53.

The sporocysts in the infected Pisidium were attached to the gonads and liver of the host and varied greatly in size. Those containing immature cercariae were quite small, while the largest, containing mostly mature cercariae, were often several times as large. Fifteen sporocysts measured 1.122 (0.323-2.025) long by 0.366 (0.141-0.753) wide. The average number of germ balls or cercariae in one sporocyst was not calculated due to the large number present in the largest of the sporocysts. Figure 22 shows germ balls and cercariae from one sporocyst in various stages of development.

Hopkins (18, p. 22-23) found 67.6 percent of the clams Musculium in Illinois harbored larval stages of Crepidostomum cooperi, but found the larvae in only one specimen of an unidentified species of Pisidium. However, he found no sporocyst stages--only the rediae, in all stages of development. A few of the rediae were

distinctive in having two eyespots--a characteristic that is not evident in the sporocysts of Tetracheilos (Figure 2).

CERCARIA

The cercaria of Tetracheilos ascaphi is a spineless, tailed ophthalmoxiphidocercaria. The average body length is about 0. 35 at rest and the average width approximately 0. 15. However, these cercariae are very active and even when greatly flattened under cover slip pressure they continue to creep along by extending and contracting their bodies and by use of the oral and ventral suckers. These activities can vary the body length from about 0. 2 to more than 0. 5. The tail, 0. 271 (0. 255-0. 292), is somewhat shorter than the body. It is finless, moderately contractile, and tapers to a blunt point (Figure 3). The oral and ventral suckers are nearly equal in size; the ventral sucker is more nearly round. The oral sucker measures 0. 115 (0. 092-0. 134) by 0. 113 (0. 098-0. 127) and the acetabulum 0. 115 (0. 099-0. 126) by 0. 116 (0. 101-0. 129). The ventral sucker is located in the posterior one-half of the body, but its relative position varies as the cercaria extends or contracts. The ventral sucker is edged with a ring of small swellings which are most obvious when it is attached to something. Hopkins (18, p. 24), observing this in Crepidostomum cooperi, also noted the presence of these protuberances on the oral sucker--a characteristic not present in Tetracheilos.

The two prominent eyespots are located near the dorsal

surface posterior to the oral sucker, anterior to the pharynx and approximately equidistant between the esophagus and the lateral edges of the body. Under low magnification these appear as two solid masses of pigment, but under high magnification each mass is seen to be composed of numerous small, irregular, dark granules. The stylet, 0.015 (0.014-0.016) long, has a narrow point just anterior to two lateral protuberances (Figure 4). It lies in the anterior end of the body with the enlarged posterior portion buried in the dorsal surface of the oral sucker. Six cystogenous glands lie in the lateral fields between the acetabulum and the pharynx with ducts extending forward to pass dorsolateral to the oral sucker.

From the oral sucker a long, narrow prepharynx extends posteriorly to its juncture with the pharynx. The prominent oval pharynx is followed by a short esophagus which opens into the intestinal ceca, which extend posterolaterally from the bifurcation to a level slightly caudad of the anterior edge of the acetabulum.

The excretory bladder is generally tubular, extending forward from a position near the posterior extremity. The position of the anterior end of the bladder with relation to the acetabulum varies greatly with the activity of the cercaria. At the posterior end the bladder opens to the outside through a duct ending in a terminal pore near the base of the tail. Four excretory tubes open into the anterior end of the excretory bladder. The posterior pair extend laterally away from each other,

and then recurve posteriorly along the lateral margins to end near the posterior extremity of the body. The anterior pair also extend laterally away from each other, and then curve forward, winding back and forth in the lateral fields, to extend anteriorly into the region of the oral sucker.

The total flame cell number has not been determined; the number observed varied from one specimen to another. The flame cell pattern has only been delineated where a flame cell could be paired with one observed in the opposite side of the body (Figure 3). Hopkins (18, p. 58-59) reported that the most common flame cell pattern in the Allocreadiidae is 2[(2+2+2) + (2+2+2)]. Flame cells located in the adults of Tetracheilos (Figure 8) indicate that this is probably the pattern that will be found in the cercariae.

The cercaria assumed a characteristic pose while attempting to penetrate the exoskeleton of a host (Figure 5). Cercariae were observed in this attitude on many aquatic insect larvae. They moved about with a creeping motion by use of the suckers on the substrate, including the surfaces of watch glasses and specimen slides, but they adopted this position only when on a potential host.

METACERCARIA

The metacercariae are found in the haemocoel of Rhyacophila grandis. The cysts are often not attached to any of the host tissue and flow out with the body fluids when the insects are opened. This larval stage approaches adult form (see Figures 7 and 8). There is a great range in size, depending on the degree of development. Five cysts removed from four mayfly nymphs, tentatively identified as Heptagenia sp., and one unidentified ephemerid nymph, four days after they were experimentally infected with cercariae, measured 0. 193 (0. 178-0. 209) in diameter. Fifteen cysts from larvae and nymphs of Rhyacophila grandis measured 0. 557 (0. 285-0. 738) in diameter.

Of 19 Rhyacophila larvae and pupae collected, Tetracheilos ascaphi metacercariae were found in 13 or 68. 3 percent. The greatest number of cysts found in one individual was ten; the average number for an individual specimen was 6. 5.

The cyst is composed of a transparent membrane which sometimes has a coat of yellow-brown substance which peels off easily. The metacercaria is coiled up inside the cyst and can be seen through the cyst membrane. Rupture of the cysts can be effected by placing them in water on a microscope slide and applying light pressure to the cover slip.

The metacercariae were relatively inactive when removed from

the cyst and measure 1. 273 (0. 707-2. 216) long and 0. 287 (0. 239-0. 490) wide. The oral sucker measured 0. 174 (0. 112-0. 272) long by 0. 197 (0. 123-0. 328) wide and the ventral sucker was 0. 134 (0. 093-0. 191) in diameter. The prepharynx had become greatly reduced in length and the pharynx was located at the posterior margin of the oral sucker. The esophagus had lengthened and the intestinal crura extended along the lateral fields to a point near the posterior extremity. The excretory bladder was greatly enlarged, filled with minute spherical droplets, and extended from the posterior end forward approximately half the length of the metacercaria. The four excretory tubes opening into the bladder followed the same course as in the cercaria, but because of the extension of the excretory bladder, the proximal ends of the excretory tubes had moved cephalad (Figure 7). Anlagen of the gonads were evident in the more nearly mature metacercariae. The testes were located in the posterior one-third of the body, placed diagonally on either side of the excretory bladder. The ovary was situated near the middle of the body at the anterolateral edge of the bladder and on the same side of the bladder as the posterior testis.

Cysts removed from the experimentally infected Heptagenia after four days released undeveloped metacercariae when ruptured. Two of these measured 0. 302 (0. 294-0. 310) long by 0. 143 (0. 141-0. 145) wide. The stylet had been lost and the eyespots were less

prominent (Figure 6). The eyespots were completely lacking in the more nearly mature metacercariae.

ADULT

The adult of Tetracheilos ascaphi was found in the anterior portion of the small intestine of Ascaphus truei during the course of this study. One hundred thirty-nine frogs were collected in mountain streams in the Cascade Mountains and the Coast Range of Western Oregon during the summers of 1962 and 1963, and examined for parasites. Ninety-nine of these frogs were examined shortly after collection and 161 adult and sub-adult specimens of Tetracheilos were recovered. Sixty-seven of the 99 frogs were infected--an incidence of 67.6 percent--and from one to 13 flukes were found in each of the infected frogs. The percentage of frogs infected ranged from 50 percent in one June collection to 84.4 percent in a collection made in August. Frogs examined after being kept in a cold room for one month at 10° C still had an infection incidence of 11.8 percent, and those examined at the end of two months retained an incidence of infection of 8.3 percent. In these last two instances the flukes were found only in the posterior portion of the small intestine, and in the large intestine.

The adults have an elongate, flattened body, which lacks spines. Twenty-five mature adults, from which all measurements were taken, were 4.05 (2.94-6.24) long and 0.77 (0.59-0.97) wide (Figure 8); the widest portion of the body is usually posterior to the

acetabulum. The ventroterminal mouth aperture is located in the oral sucker, which is subterminal, equipped with two anterolateral and two posterolateral lobes, and is 0.357 (0.217-0.449) long by 0.456 (0.287-0.581) wide at the level of the anterolateral lobes. The well-developed pharynx, 0.147 (0.101-0.177) long by 0.149 (0.116-0.197) wide, is located at the posterior margin of the oral sucker.

The moderately short esophagus extends from the pharynx to a point about one-half the distance between the pharynx and the cirrus pouch. The intestinal ceca extend nearly to the posterior extremity in the lateral fields. The acetabulum, situated slightly forward of the middle of the body, is 0.343 (0.257-0.424) in length and 0.353 (0.252-0.419) in width. A single ovary, 0.265 (0.202-0.333) long by 0.278 (0.202-0.359), is located on either the right or the left side between the acetabulum and the anterior testis, and always on the opposite side of the body from the anterior testis. Two ovoid testes are arranged diagonally to each other, on opposite sides of the medially placed excretory bladder. The anterior testis is 0.422 (0.318-0.545) long and 0.367 (0.242-0.444) wide, and the slightly larger posterior testis is 0.452 (0.353-0.565) by 0.378 (0.267-0.480). The oval to claviform cirrus pouch, 0.434 (0.303-0.566) long and 0.252 (0.126-0.328) wide, is situated anterior to the ventral sucker. Posterior to the ventral sucker and medial to the ovary is the seminal receptacle, 0.253 (0.214-0.313) long by 0.122 (0.101-0.143) wide.

Laurer's canal runs transversely in the intercecal field between the ovary and the anterior testis, passing ventral to the vasa efferentia and dorsal to the uterus. The uterus occupies the intercecal field to the posterior extremity and then continues forward, passing between the testes in the ventral half of the worm. It runs along the median line, passes dorsal to the ventral sucker and opens into the genital pore anterior to the ventral sucker (Figure 9). The numerous eggs measure 0. 056 (0. 051-0. 061) by 0. 037 (0. 031-0. 042). Extensive vitellaria (Figure 24) surround the ceca from the posterior extremity and continue forward to near the level of the pharynx. A tubular excretory vesicle runs from a point just posterior to Laurer's canal, between the testes, and empties through a terminal excretory pore. Excretory tubules arise in the anterior and posterior ends of the body and run more or less parallel to the intestinal ceca toward the middle of the body. The two tubules on each side unite into a common tube which empties into the excretory bladder near its anterior end. Twenty flame cells have been located in the adult fluke. It is possible that more are present, but the extensive vitellaria make it difficult to observe the flame cells.

Adults are very motile when alive and show a considerable variation in shape and size as they move about. Taking accurate measurement of living adults is therefore nearly impossible. Even when fixed they show some variation in shape (see Figures 23, 24

and 25).

Ascaphus starved for two months were experimentally fed metacercarial cysts from Rhyacophila, and Tetracheilos were recovered from the anterior region of the small intestine. The frogs were examined at intervals, but the Tetracheilos were still lacking eggs 13 days after the infection. This possibly may be attributed to the lack of available food in the intestines of the starved frogs.

MOLLUSCAN HOST

The first intermediate host of Tetracheilos ascaphi is Pisidium idahoense, a small clam, whichinhabits streams of the Pacific Northwest. It is found in a muddy, to slightly sandy, substrate in shallow, vegetation-choked streams, and in the quiet eddies and back waters of large streams.

Only sporocysts located on the gonads and liver were found in these clams. Since the sporocysts contain cercariae, there apparently is no redial stage. Attempts to infect the clams by feeding eggs did not succeed as the eggs were passed through the digestive tract without hatching, and inspection of the digestive tract revealed unhatched eggs throughout the intestine. Considering Hopkin's report (18, p. 22) that Crepidostomum cooperi eggs hatched in water, and that the clams would, because of their feeding habits, be more likely to become infected by swimming miracidia, it is probable that the eggs of Tetracheilos also hatch in the water and not after ingestion by the clams.

INSECT HOSTS

Rhyacophila grandis was the only insect found to be serving as secondary intermediate host for Tetracheilos ascaphi in the wild. This caddisfly constructs a case of large sand granules on the underside of rocks in the sections of the stream where the water is relatively fast. The pupa case of this insect is slightly translucent and green in color--Rhyacophila was the only caddisfly collected in this study with such a pupal case.

Although evidence indicates that Ascaphus feeds under water part of the time, Rhyacophila must be taken after it emerges from its pupal case, since it is doubtful that the frogs can break open the hard, sandy case.

Several other species of caddisflies, as well as numerous other aquatic insects, were examined for metacercariae of Tetracheilos without success. However, 16 mayfly nymphs obtained from Berry Creek, Benton County, were exposed to cercariae and five of these became infected. Four of these ephemerids were tentatively identified as Heptagenia sp.; the fifth individual was not identified.

DISCUSSION

The adult Tetracheilos differs anatomically from the apparent nearest related forms in the Allocreadiidae in that the six muscular lobes, present on the anterior margin of the oral sucker in Crepidostomum and Bunodera, are lacking in this genus. The cirrus pouch does not overlap the acetabulum, and the uterus extends to the posterior extremity rather than being pretesticular. In this latter respect it is similar to Bunodera, which also has the uterus extending posterior to the testes. Hopkins (18, p. 26, 31, 35, 37 and 42) described metacercariae or young adults of five species of Crepidostomum which had eyespots. Megalogonia and two species of Bunodera (18, p. 54 and 56) retain prominent eyespots as young adults. In this respect Tetracheilos differs from these other members of the Allocreadiidae in that it loses its eyespots in the metacercarial stage.

The life cycle of Tetracheilos is quite different from that of Bunodera, which involves a snail, with encystment in the snail or a crayfish, and a fish as a definitive host. Its life cycle is similar to that of Crepidostomum, for both have a small, fresh-water clam as the first intermediate host and an aquatic insect as the second. The second intermediate host of Crepidostomum is usually a mayfly rather than a caddisfly, but it has been shown experimentally in this study that Tetracheilos will also encyst in a mayfly.

Compared anatomically it appears that Tetracheilos should be placed taxonomically between the Crepidostominae and the Bunoderinae, and perhaps closer to the Bunoderinae. However, a comparison of the morphological development and the life cycles indicates a closer affinity to the Crepidostominae.

SUMMARY

Sporocysts of Tetracheilos develop on the gonads and liver of the small fresh-water clam Pisidium idahoense. The oculate xiphidiocercariae leave the sporocyst, pass out of the clam and swim about until coming in contact with the caddisfly Rhyacophila grandis. The cercariae penetrate the exoskeleton of the caddisfly and encyst in the haemocoel where they develop into metacercariae. Ascaphus truei feed on the emerging caddisflies and become infected when the encysted trematodes attach themselves to the lining of the small intestine. The mature flukes remain in the small intestine except when the host is subjected to a prolonged period of starvation. When this occurs, the flukes are gradually displaced posteriorly and those that remain are in the large intestine.

Nymphs of the mayfly Heptagenia sp. were experimentally infected with Tetracheilos cercariae and encysted metacercariae were recovered from them. Metacercarial cysts from Rhyacophila grandis were fed to young Ascaphus and subadult Tetracheilos were removed from the host 13 days later.

Table II, on page 74, contains a resumé in tabular form.

PART III

The Life Cycle of Euryhelmis squamula (Rudolphi, 1819) Poche, 1926

The life history of Euryhelmis squamula (Rudolphi, 1819) Poche, 1926, has never been completely described. This trematode is in the family Heterophyidae, subfamily Euryhelminae. The Euryhelminae contains one genus, Euryhelmis, having five species.

Euryhelmis squamula, the type genus for this subfamily, was described by Rudolphi (32, p. 103, 309) in 1819 under the name Distomum squamula from Mustela putorius, the polecat. In 1824 Bremser (6) found this trematode in the intestine of the polecat and published the first illustration of it. Dujardin (14, p. 406), in 1845, reported Euryhelmis (=Distomum) squamula from the intestines of two polecats and renamed it Eurysoma squamula, a name later rejected as being preoccupied. In his Systema Helminthum, published in 1851, Diesing (13, p. 321) changed Distomum squamula to Monostomum squamula.

The metacercariae were found in 1867 by Zeller (42) encysted under the skin of the brown grassfrog (Rana temporaria). He removed the metacercariae from the cysts and recognized them as the larval stage of Rudolphi's Distomum squamula from Rudolphi's description and Bremser's illustrations but did not attempt experimental

infection of a mammal. His illustration (42, Plate XIII) is clearly recognizable as the metacercaria of Euryhelmis squamula.

Poche (29, p. 150), in 1926, proposed Euryhelmis as the genus name for Distomum squamulum of Rudolphi and placed it in the family Heterophyidae, but Witenburg (39, p. 137) in his "Studies on the trematode family Heterophyidae", in 1929, claimed Euryhelmis should not be included in this family since the descriptions of the genus were insufficient to place it in the Heterophyidae.

Baer (4) found several hundred specimens of Euryhelmis squamula in the intestine of a polecat in 1931, and redescribed this genus adequately to place it in the family Heterophyidae, subfamily Heterophyinae. He reported it had been known for a long time that the metacercariae of Euryhelmis squamula encysted under the skin of brown frogs and toads and that he had found cysts of the metacercariae in the crested salamander. Cysts from the skin of Rana esculenta were fed to a young cat and adult worms were recovered from the small intestine; however, attempts to infect experimentally rats and water snakes (Tropidonotus natrix and Tropidonotus viperinus) failed in experiments by Joyeux, Baer and Carrére (19) in 1934.

McIntosh (24) reported the first occurrence of Euryhelmis in the United States when he fed cysts from Rana pipiens, collected near Alexandria, Virginia in 1936, to cats and recovered mature flukes from the small intestine after five days. Although his was the first

published report of the presence of Euryhelmis squamula in North America, he stated that there were specimens from mink in Maryland and Minnesota in the Helminthological Collection of the United States National Museum.

A racoon, Procyon lotor, collected by Parker (28) near Durham, North Carolina in 1949, had "several hundred" mature Euryhelmis squamula in the duodenum, and was the first naturally infected, non-mustelid host recorded in North America.

Euryhelmis was removed from the Heterophyinae in 1950 and placed in the newly proposed Euryhelminae by Morozov (25).

Senger and Macy (33) and Senger and Neiland (35) found Euryhelmis squamula in the small intestine of mink in Western Oregon in 1951, and Rana aurora and Rana cascadae were found to harbor metacercariae which were tentatively identified as those of Euryhelmis squamula.

The only life cycle of a species of Euryhelmis was worked out by Ameel (2) in 1938. Euryhelmis monorchis Ameel, 1938, was recovered from the small intestines of mink in Michigan, Ohio and Wisconsin over the course of several years, but was largely ignored until it was also recovered from white rats fed cysts taken from green frogs (Rana clamitans) which had been exposed experimentally to cercariae.

The cercariae of Euryhelmis monorchis are lophocercous and

spinose, as is typical of heterophyid cercariae, but they lack the characteristic eyespots (31). The first intermediate host is an operculate snail (Pomatiopsis lapidaria). The second intermediate host is Rana clamitans. Ameel succeeded in experimentally infecting Rana pipiens and Rana palustria. The definitive host is the mink (Mustela vison), and Ameel experimentally infected white rats and a cat (2).

The third species, Euryhelmis pacificus, was described in 1952 by Senger and Macy (33) from the small intestine of mink (Mustela vison) and muskrat (Odontra zibethica) in Oregon. The metacercariae of this species were found encysted in the striated muscles of the Pacific Giant Salamander, Dicamptodon ensatus.

In 1956 Webster and Wolfgang (38) described Euryhelmis pyriformis from one specimen from the skunk, Mephitis mephitis, in Quebec.

The most recently discovered species is Euryhelmis costaricensis from the weasel Mustela frenata costaricensis. This was described in 1960 by Brenes, Arroyo and Jiménez-Quirós from six specimens collected in Cartago, Costa Rica (7).

METHODS AND MATERIALS

Snails infected with the larvae of Euryhelmis were collected in a small stream entering the south fork of the McKenzie River in the Cascade Mountains in Oregon. Collections were made in the summer and autumn of 1963. The snails were brought into the laboratory and placed in groups of 50 in culture dishes containing stream water taken from the same stream. These dishes then were placed in a refrigerator at 5° C.

Young Rana aurora raised in the laboratory were placed in clear stream water in covered culture dishes and cercariae emerging from the snails were transferred into the culture dishes containing these frogs. Metacercariae were recovered from cysts under the skin of the frog.

Mature metacercariae removed from subcutaneous tissues of Ascaphus truei were fed to the Golden Hamster, Cricetus auratus, and to the White-Footed Deer Mouse, Peromyscus maniculatus. Flukes in various stages of development were recovered from these experimental hosts after different periods of infection.

Adult flukes were relaxed by placing them in small watch glasses containing aerated tap water and these dishes in turn were placed in a refrigerator at 5° C for a period of from 12 to 24 hours. On removal they were flattened under light cover slip pressure, fixed

in Lavdowsky's AFA Solution and stained with Celestine blue or Semichon's carmine. Metacercariae were not relaxed but were otherwise prepared in the same way as the adult flukes. Cercariae and metacercariae were studied in the living condition with the aid of aqueous solutions of neutral red and methylene blue.

Many of the drawings were made with the aid of photomicrographs and a Leitz microprojector.

All measurements are in millimeters. In all measurements the mean size is given first, followed by the range in parentheses.

LIFE HISTORY RESUMÉ

The life history of Euryhelmis squamula in the free state includes at least one operculate aquatic snail as a first intermediate host. Five anurans and one urodele have been identified as second intermediate hosts, and seven mammals as definitive hosts.

Cercariae in the McKenzie River emerge from the snail Bythinella hemphilli Pilsbry into the stream. They swim by rapid movement of their tails and rest at intervals on the bottom. They penetrate the frog's skin and form cysts in the subcutaneous connective tissues. The presence of the cysts is discernable to the naked eye because they appear as small swollen areas. Metacercariae have been found in the urodele Triton cristata and the batrachians Rana temporaria, Rana esculenta and Bufo in Europe, and in Ascaphus truei and Rana pipiens in the United States.

The amphibians infected by the metacercariae are eaten by carnivores, following which adult flukes mature rapidly in the small intestine. In Europe the definitive hosts are Mustela putorius, Mustela nivalis, Lutreola lutreola, Lutreola vison and Vulpes vulpes. In the United States the definitive hosts are Mustela vison and Procyon lotor.

EGG

The eggs of Euryhelmis squamula are golden-brown when deposited. They are moderately thick-shelled, operculated, and have a pronounced knob at the end opposite the operculum (Figure 10). The size of ten eggs deposited by the flukes held in aerated tap water for 24 hours was 0.030 (0.028-0.032) by 0.014 (0.013-0.015). Ten eggs in the uterus of a fluke, four days old, fixed in Lavdowsky's AFA Solution and stained in Celestine blue, were 0.029 (0.027-0.031) by 0.013 (0.012-0.014), and ten eggs from a fluke 269 days old prepared as above measured 0.032 (0.029-0.034) 0.012 (0.011-0.014).

Baer (4) gave the first egg measurement recorded as 0.034 long by 0.015 in diameter. Callot (9) gave the egg size in living flukes as 0.030-0.032 long by 0.014-0.016 wide. This range of measurements agrees closely with mine. Additional egg measurements are given in Table I.

Several techniques were used in trying to hatch eggs. Some were placed in dishes of water in the refrigerator at 5° C, others were left in a temperature-controlled room at 10° C. Eggs were fed to clams and snails, and other eggs were placed in solutions of pepsin and trypsin. All attempts to hatch eggs failed.

The operculum seemed securely attached and could not be removed by applying pressure, which always ruptured the egg first.

At the time the egg leaves the uterus it contains several cells, which when expressed from the ruptured egg, appear to be yolk cell.

Golden hamsters were used in feeding experiments conducted to determine the time needed for the flukes to attain an egg-bearing adult stage.

Flukes removed from hamsters at 50 hours contained no eggs. At 70 hours most of the flukes had from a few to many eggs, and at 92 hours eggs were present in all the flukes and were numerous in most of them. This compares closely with the findings of Ameel (2) for the development of Euryhelmis monorchis.

Senger and Macy (33) found Euryhelmis pacificus maturing at about 55 hours in white rats and about 72 hours in hamsters.

CERCARIA

The operculate, aquatic snail (Bythinella hemphilli Pilsbry) harbors the cercaria of Euryhelmis squamula in Western Oregon. Because of the small size of the snail--three millimeters--and the low incidence of infection with Euryhelmis squamula, no sporocysts or rediae were observed in Bythinella. Ameel (2) found that Euryhelmis monorchis had a redia stage in the snail Pomatiopsis lapidaria, and one can assume that a redia of Euryhelmis squamula is probably to be found in Bythinella. Only 15 cercariae of Euryhelmis were recovered from approximately 800 snails in August 1963. These were placed in culture dishes containing laboratory-raised young Rana aurora in attempts to infect these frogs experimentally.

In November 1963 approximately 1100 Bythinella were collected and placed in culture dishes containing stream water. Only seven cercariae of Euryhelmis were obtained from this November collection of snails.

The cercaria of Euryhelmis squamula is lophocercous and spinose. The tail fin extends the posterior two-thirds of the length of the tail along the dorsal surface, around the tip and up the ventral surface of the posterior one-third of the tail (Figure 11). The cercaria is a vigorous swimmer, but occasionally ceases its movements

and sinks to the bottom. In its inactive state it generally assumes a position in which the anterior portion of the body is arched (Figure 13). The body surface is covered with minute spines--a characteristic of this genus. The acetabulum is much smaller than the oral sucker and is quite inconspicuous in the living, unstained cercaria.

No digestive tract is observable. Ameel (2) noted the presence of a cluster of cells between the oral sucker and the penetration glands of Euryhelmis monorchis, which he thought might be interpreted as the site of the pharynx, but he discerned no other evidence of a digestive tract.

Twelve nearly spherical penetration glands are present in the posterior half of the body. Ducts from these glands lead anteriorly in a mass to the anterior sucker. The genital primordium is a large mass of cells lying dorsal to the acetabulum just posterior to the penetration glands. The excretory bladder is large, generally broader than long, and flattened on the anterior end when full. It becomes more nearly spherical when partially emptied. The excretory duct from the bladder ends dorsally and slightly posterior to the base of the tail.

Excretory tubules emptying into the bladder and flame cells were not observed. Ameel (2) did not observe excretory tubules but located 18 flame cells in Euryhelmis monorchis cercariae with a pattern of 2(2+2+3+2).

The following measurements were taken of five living cercariae under slight cover slip pressure: body, 0. 146 (0. 122-0. 195) by 0. 068 (0. 061-0. 085); tail, 0. 211 (0. 195-0. 229) by 0. 024 (0. 022-0. 029) at the base; diameter of oral sucker 0. 033 (0. 022-0. 044) and diameter of acetabulum 0. 018 (0. 015-0. 022).

METACERCARIA

The cercariae penetrate the skin of frogs exposed to them and encyst in the subcutaneous connective tissue. The presence of the cysts is discernible by the small swollen areas, particularly on the ventral surfaces of the legs and on the toes, since the skin on the dorsal surface of Ascaphus is normally quite rough. These cysts are most abundant on the hind legs, but are found on all parts of the body in heavily infected frogs. The cyst is composed of a thin inner membrane and a thick, tough, often pigmented, outer layer of host tissue.

Thirty-eight of 43 Ascaphus examined (88.4 percent) harbored metacercarial cysts of Euryhelmis. The infection ranged from one cyst per frog to 73. These 38 frogs harbored a total of 897 cysts--an average of 23.6 metacercariae per frog.

Three cysts removed from an experimentally infected frog (Rana aurora) 44 days after infection measured 0.412 (0.363-0.461) in diameter. One of these metacercariae removed from the cyst and studied alive under slight cover slip pressure measured as follows: body 0.588 long by 0.599 wide; oral sucker 0.051 in diameter; pharynx 0.026 in diameter and ventral sucker 0.044 in diameter. This specimen had well developed gonads, seminal receptacle and uterus (Figure 14). This compares with Ameel's findings (2) that cysts of 45-day metacercariae of Euryhelmis monorchis measured

0.36 (0.32-0.45). He stated that these cysts had attained maximum size and that the metacercariae were infective.

A metacercaria of unknown age taken from Ascaphus, when excysted, measured 0.531 in length by 0.520 in width. The oral sucker was 0.071 in diameter, the pharynx 0.045 and the ventral sucker 0.067. This metacercaria was smaller than the 44-day metacercaria from the experimental frog and its uterus was not evident (Figure 12).

In body shape the metacercaria closely resembles the adult fluke. The infective metacercaria is only slightly smaller than the adult flukes recovered from the golden hamster. Like the cercaria, the metacercaria is covered with minute spines. Both oral and ventral suckers are muscular. The oral sucker is subterminal in the living metacercaria, but often assumes a terminal position in fixed and flattened specimens. Both suckers are nearly round. The diameter of the ventral sucker is about three-fourths that of the oral sucker.

The muscular pharynx, slightly longer than broad, is located immediately posterior to the oral sucker with no evidence of a prepharynx. A short esophagus, extending caudad from the pharynx, bifurcates into two ceca and each branch extends to near the posterior end of the body, passing lateral to the reproductive organs and excretory bladder.

The excretory system consists of a relatively large Y-shaped

bladder extending forward in the posterior third of the body. Two excretory ducts empty into the anterolateral branches of the excretory bladder. Each duct bifurcates--one branch continuing anteriorly along the lateral margin to unite with another duct at the level of the cecal bifurcation. The other branch continues medial to the cecum and unites with a duct from the other side of the metacercaria just anterior to the acetabulum. Other branches extend forward to the level of the pharynx (Figures 12 and 14). The bladder is emptied through a small duct which terminates medially on the posterior end.

Baer (4) observed a well developed reproductive system in the metacercariae, and this was evident in the specimens from Rana aurora and Ascaphus. There are two testes present in the posterior portion of the body, one on each side of the excretory bladder. The seminal receptacle is located just anterior to the right or left horn of the excretory bladder and the single ovary is just anterior to the seminal receptacle. The uterus extends from the medial end of the ovary, winds back and forth across the intercecal field and then passes lateral to the acetabulum on the side of the body opposite the ovary, and ends at the genital pore just anterior to the acetabulum.

ADULT

Baer, 1931 (4) has given the first adequate description of the adult Euryhelmis squamula. His description was based on "... several hundred specimens of this small trematode in the intestine of a polecat..." (4, p. 328). His description (translated) is as follows:

> The form of these small worms is very characteristic, for they are always broader than long. The average measurements based on the numerous specimens have given us a total length of 0.6 mm to 1.0 mm and a maximum width of 1.4 mm to 1.9 mm. The body is clearly leaflike and very transparent. All of the anterior region is covered by very delicate minuscule spines. The oral sucker has a diameter of 0.11 mm to 0.12 mm and a length of 0.8 mm; its opening directed anteriorly. There is a very short prepharynx in general invisible on whole mounts. The pharynx is nearly spherical; it measures 0.05 mm long by 0.06 mm to 0.07 mm wide. The esophagus is well developed and of quite variable length. The two diverticuli of the digestive tract are strongly recurved and reach nearly to the posterior extremity of the worm. The ventral sucker, little smaller than the oral sucker, is located at the middle of the body and measures 0.11 mm to 0.12 mm by 0.07 mm to 0.09 mm. The two testes are situated on each side of the median line in the posterior region of the worm. They are spherical or elongate, most often cut into a certain number of lobes. It would seem, moreover, that they become lobate only in the adult individuals, for in the metacercariae and the juvenile individuals, the testes are spherical. The deferent canals empty into a voluminous seminal vesicle folded upon itself and presenting several constrictions. This seminal vesicle is located, in most cases, to the right of the ventral sucker. A short ejaculatory canal empties directly into the genital atrium at the side of the uterus. The genital atrium is situated immediately in front of the ventral sucker; its structure is very peculiar and presents some arrangements

> not heretofore noticed in heterophyids. The genital sucker is absent, but a portion of it persists in the form of a small muscular lobe which is located above the genital pore. In certain cases, when the lobe is folded forward, it can completely close the genital atrium. The ovary is found in the right half of the worm, in front of the testis on the same side. It is most often pyriform. The seminal receptacle is enormous, elongate, and is found between the ovary and the right testis. The shell gland is well developed. Laurer's canal is directed obliquely forward and extends anteriorly to empty into the oviduct. The vitelline glands are very extensive and clearly separated into two lateral groups. The follicles reach the bifurcation of the intestine. One sometimes notices an anomaly in which the follicles of one side are much more developed than those of the other. The two vitelline ducts unite into a small vitelline reservoir which precedes the oviduct. The uterus is fairly long and describes, in general, three principal loops which are located between the testes and the ventral sucker. In the adult worms, the uterus is situated essentially in the middle of the left side of the body. This disposition can be seen even with the naked eye in the living animal, for the dark spot formed by the mature uterus stands out clearly, against a milky white background, from the rest of the worm. The eggs are 34 microns long and 15 microns in diameter. They are operculated and have a small projection at the posterior pole.

In his description of Euryhelmis squamula Dawes (12, p. 399) adds that the worm is quadrilateral. The measurements he gave for this worm differ slightly from those reported by Baer. Measurements of specimens reported by different investigators, and measurements I have made of specimens loaned to me and of those I have collected, are given in Table I. The adult is pictured in Figures 15 and 26.

TABLE I

Measurements of Euryhelmis squamula

Source	Length	Width	Oral Sucker	Pharynx	Acetabulum	Egg Length	Egg Width
This Study	0.677-0.808	0.616-0.808	0.076-0.088	0.054-0.061	0.073-0.081	0.027-0.032	0.013-0.015
Baer (4)	0.6-1.0	1.4-1.9	0.11-0.12	0.06-0.07	0.07-0.09	0.034	0.015
Callott (9)	0.95-1.2	1.45-1.50				0.030-0.032	0.014-0.016
Dawes (12)	0.6	1.45	0.07			0.028-0.032	0.012-0.014
Dujardin (14)	1.12	1.50-1.66	0.107	0.05	0.103	0.031-0.035	
Luhe (23)	0.6	1.45	0.07				
Parker[a]	0.705-0.946	0.860-0.999	0.090	0.056-0.066	0.102	0.029-0.034	
Senger[b]	0.880-1.105	0.935-1.00	0.092-0.097	0.035	0.066-0.082	0.029-0.031	
Maryland[c]	0.616-0.672	0.660-0.695	0.077	0.046-0.051	0.075-0.090	0.030-0.034	

[a]From three specimens (No. 46460) loaned to me from the Helminthological Collection of the U. S. National Museum, Washington 25, D. C. Collected by M. V. Parker in North Carolina (28).

[b]From two specimens given to me by C. M. Senger (33).

[c]From two specimens (No. 45095) loaned to me from the Helminthological Collection of the U. S. National Museum, Washington 25, D. C.

MOLLUSCAN HOST

The tiny, operculate snail Bythinella hemphilli is the only snail found to be infected with the larvae of Euryhelmis squamula. This snail was identified by Morrison[1] who stated that this is probably one of the least known members of the family Hydrobiidae in North America.

In August 1963 approximately 800 snails were collected at Frissel's Crossing on the south fork of the McKenzie River in Lane County, Oregon. From these, 15 emerged Euryhelmis cercariae were collected and placed with young Rana aurora. A collection of 1100 snails in November 1963, from the same location, yielded only seven cercariae of Euryhelmis. Attempts to recover sporocysts or rediae from these snails were unsuccessful.

While the incidence of infection was low in the snails at the times they were collected, the high percentage of frogs harboring cysts of Euryhelmis metacercariae indicates that the peak period of infection in the snails may be during some other part of the year.

---

[1]Joseph E. P. Morrison, Associate Director to Curator, Division of Mollusks, United States National Museum, Washington 25, D. C. (Personal letter, dated August 16, 1963).

AMPHIBIAN HOSTS

In the course of this study I found metacercariae of Euryhelmis squamula encysted subcutaneously in Ascaphus truei. Senger and Neiland (35) found the metacercariae in Rana aurora and Rana cascadae in Oregon. In Virginia, McIntosh (24) found metacercariae in Rana pipiens.

In Europe, Zeller (42) found the metacercariae encysted in Rana temporaria and published the first report and illustration of this larval stage. Baer (4) reported its presence in toads and the crested salamander (Triton cristata) and in the tadpoles and young of Rana esculenta.

MAMMALIAN HOSTS

In 1819 Rudlophi recovered the first specimens of the adult of Euryhelmis squamula from the polecat Mustela putorius. It has been reported from several mammals by various investigators. Yamaguti (41, p. 875) listed Mustela putorius, Mustela nivalis, Lutreola lutreola, Lutreola vison and Vulpes vulpes as definitive hosts for Euryhelmis squamula in Europe. In the United States Parker (28) found this worm in Procyon lotor in North Carolina; Senger and Macy (33) and Senger and Neiland (35) found it in Mustela vison from the upper Sandy River in Oregon.

Cats have been experimentally infected by Joyeux, Baer and Carrére (19) in Europe and by McIntosh (24) in the United States. Additionally, golden hamsters and deer mice (Peromyscus maniculatus) have been experimentally infected in the course of this study.

DISCUSSION

The adult form of Euryhelmis squamula has been known for nearly 150 years and the metacercarial stage was first identified nearly 100 years ago. Seven naturally infected definitive hosts have been identified and seven secondary intermediate, amphibian hosts are known.

Although five of the seven mammalian hosts are mustelids, this appears to be more a reflection on their feeding habits than on the host specificity of the adult stage of the worm. The host specificity of the metacercaria seems to be even less marked than that of the adult, in that six different anurans and an urodele act as hosts for this intermediate form. There is some evidence of a metacercarial host preference exhibited in Western Oregon to the extent that Senger and Macy (33) examined 53 Dicamptodon ensatus, which can be found in the same habitat as Ascaphus truei, without finding any metacercariae of Euryhelmis squamula. In the course of this study I have examined five of these salamanders collected in the same areas as the Ascaphus, without discovering any metacercariae of Euryhelmis squamula. Thus, it seems that the normal host for the metacercaria is probably an anuran.

In my studies of this trematode I found only three molluscs in the streams frequented by Ascaphus. A small fresh-water clam and

a planorbid snail did not harbor any sporocysts or rediae of Euryhelmis--only the hydrobiid snail Bythinella hemphilli was the primary intermediate host.

The frequency and degree of infection reported from amphibians by Zeller (42) and Joyeux, Baer and Carrére (19) indicate the probable abundance of a molluscan host in those parts of Europe where Euryhelmis occurs. Observations made in the course of this study indicate that the snail host may have been overlooked because of its small size. Fully mature Bythinella do not attain a length of three millimeters and scarcely exceed a diameter of one millimeter, so it is almost impossible to collect these snails without the use of a sieve having a mesh opening of one millimeter or less.

Since Bythinella, the primary intermediate host of Euryhelmis squamula, and Pomatiopsis, the first intermediate host of Euryhelmis monorchis, are both members of the family Hydrobiidae, there is a good possibility that the European host of Euryhelmis squamula may also be a hydrobiid snail.

Comparison of the measurements of adult Euryhelmis squamula (Table I) indicates a difference between the European and American specimens. This difference is to be noted also in the description of the adult ". . . they are always broader than long. . ." (4, p. 328). The American form tends to be more nearly square than the European strain--the largest and broadest of the American worms

being nearly a millimeter narrower than the largest European worms. This discrepancy is not evident in a comparison of body lengths.

SUMMARY

Cercariae of Euryhelmis squamula develop in the snail Bythinella hemphilli in Oregon. The emerging lophocercous cercariae swim until coming in contact with an amphibian. They burrow through the skin of the amphibian and form a cyst in the subcutaneous tissues.

In Oregon the encysted metacercariae have been found in the anurans Ascaphus truei, Rana aurora and Rana cascadae. Rana pipiens is a secondary intermediate host in Virginia, and in Europe Rana temporaria, Rana esculata, Triton cristata and a Bufo serve as hosts for the metacercariae.

The adult flukes have been found in Mustela vison in Oregon and in Procyon lotor in North Carolina. In Europe Mustela mustela, Mustela putorius, Lutreola lutreola, Lutreola vison and Vulpes vulpes serve as definitive hosts. In this study flukes were grown to maturity in the golden hamster Cricetus auratus and in the deer mouse Peromyscus maniculatus.

A young Rana aurora experimentally infected with cercariae developed cysts containing fully developed metacercariae in 44 days. Cysts from Ascaphus truei produced mature adults in the anterior one-third of the small intestines of the golden hamsters and deer mice. Eggs were observed in the uterus after 70 hours. Eggs deposited in

culture dishes contained only yolk cells and attempts to hatch the eggs failed.

The definitive hosts, carnivorous mammals, become infected by eating frogs and salamanders infected with the metacercariae.

A tabular resumé of the life cycle of Euryhelmis squamula is recorded in Table II, page 74.

PART IV

Notes on the Life Cycle of Euryhelmis pacificus Senger and Macy, 1952

Adults of Euryhelmis pacificus Senger and Macy, 1952, were found by Senger and Macy (33) and Senger and Neiland (35) in the small intestine of the mink, Mustela vison Schreber, the muskrat, Ondatra zibethica Linnaeus, and the water shrew, Sorex bendirii palmeri, near Portland, Oregon.

Senger and Macy (33) reported finding the metacercariae of Euryhelmis pacificus encysted in the Pacific Giant Salamander Dicamptodon ensatus Eschscholtz. In this animal, the only species of amphibian they found serving as a second intermediate host for Euryhelmis pacificus, the cysts were located in the striated muscles. Cysts containing the metacercariae were excised from the muscle tissue and fed to white rats, golden hamsters and a deer mouse. When examined later, at different periods, all of these animals were found to be infected with Euryhelmis pacificus.

METHODS AND MATERIALS

Cysts containing metacercariae were taken from the subcutaneous connective tissue of Ascaphus truei and fed to a golden hamster. Three adult Euryhelmis pacificus were recovered from the small intestine of this animal after 269 days. After fixing in Lavdowsky's AFA Solution, two of the three trematodes were stained with Semichon's carmine and the third was stained in Celestine blue.

Photographs were taken with an Edixa Reflex camera and an Olympus camera mounted on compound microscopes, using Kodachrome and Panatomic X film. Figure 18 was drawn with the aid of a Leitz microprojector. All measurements are in millimeters; the average size is followed by the size range in parentheses.

EGG

The moderately thick-shelled, operculate egg has a pronounced, blunt projection at the end opposite the operculum. The operculum is simple, lacking the double-capped appearance of the egg of Euryhelmis squamula (see Figures 10 and 16).

Senger and Macy (33) found eggs in preserved specimens measured 0. 020 to 0. 034 in length by 0. 010 to 0. 017 in width, and eggs in live specimens measured 0. 031 long and 0. 014 wide.

In this study I found that the eggs in the three preserved, mature specimens collected from the golden hamster were 0. 031 (0. 029-0. 032) long by 0. 013 (0. 012-0. 015) in diameter.

METACERCARIA

Encysted metacercariae were situated in the striated muscles of Dicamptodon ensatus, none being found in the subcutaneous tissue, according to Senger and Macy (33). In Ascaphus truei, however, all metacercarial cysts were found in the subcutaneous connective tissue.

Senger and Macy (33) found that approximately 90 percent of the salamanders collected from eight different areas in the Cascade Mountains harbored metacercariae of Euryhelmis pacificus, while the salamanders they examined from the Oregon Coast Range were not infected. The average number of metacercariae found in one salamander was about 50, with a variation of from five or six cysts per individual salamander up to several hundred cysts. The cysts ranged in size from 0. 15 to 0. 29 and were surrounded by a capsule. They reported that the metacercariae were quite active when removed from the cysts and possessed well developed reproductive organs (Figure 17). The measurements given by these authors were: length 0. 680, width 0. 350; oral sucker 0. 061 to 0. 071; diameter of pharynx 0. 039; length of prepharynx 0. 024; diameter of acetabulum 0. 049.

ADULT

Adult Euryhelmis pacificus are harbored in the small intestine of mink (Mustela vison) and muskrat (Ondatra zibethica) in the wild. Senger and Macy (33) and Senger and Neiland (35) reported two of 11 mink, one of 34 muskrats and one water shrew from Oregon infected with this trematode.

Experimentally, Senger and Macy (33) fed the metacercariae to white rats, golden hamsters and a deer mouse, and recovered adult Euryhelmis pacificus from their intestines.

The description of the adult, as given by Senger and Macy (33), follows:

> Body thin, leaflike, transparent, spinose over-all, pyriform or elongate. Length 0.66 to 1.04 mm; width 0.34 to 0.68 mm. Oral sucker appearing either terminal or subterminal 0.035 to 0.087 mm long by 0.049 to 0.090 mm in diameter. Pharynx large, spherical, 0.035 to 0.059 mm in diameter, connected to the oral sucker by a short but definite prepharynx from 0.004 to 0.100 mm but averaging 0.039 mm. Esophagus slender, bifurcating anterior to the acetabulum. The intestinal ceca extend obliquely to the sides, then follow the contour of the body to the posterior end where they almost touch in some cases. Ventral sucker 0.035-0.062 mm in diameter, situated slightly pre-equatorially. Two testes ovoid or lobed, opposite or oblique in the posterior region of the body. Right testis usually more flattened anterio-posteriorly and wider transversely than left testis. Right testis 0.18-0.32 mm by 0.10-0.16 mm. Left testis 0.15-0.26 mm by 0.14-0.18 mm. Large sac-like seminal vesicle dorsal to uterus and posterior and to the right of acetabulum, constricted into a spherical posterior chamber and an elongate anterior chamber, being connected to genital atrium by a short ejaculatory

> canal. No copulatory organ. Genital atrium located immediately anterior to the acetabulum and overhung by a bilobed gonotyl. Gonotyl which appears to be a fold of tissue rather than a sucker, averages 0.050-0.014 mm. Uterus consisting of three or four loops confined between intestinal ceca, testes, and acetabulum, opens into genital atrium to the left of the ejaculatory canal. Ovary, located on right side anterior to right testis, generally club-shaped, 0.10 to 0.25 mm by 0.07 to 0.14 mm. Seminal receptacle, located between right testis and ovary, spherical or club-shaped 0.07 to 0.23 mm by 0.06 to 0.16 mm. Laurer's canal originates as a medial elongation of seminal receptacle and, after some coiling, opens on the median dorsal surface somewhat posterior to the oötype region. Mehlis' gland well developed, located to the left of ovary. Vitelline follicles numerous, confined laterally, extending from near the bifurcation of the intestinal ceca to the posterior end. Eggs operculated, 0.020 to 0.034 mm by 0.010 to 0.017 mm in preserved material, 0.031 to 0.014 mm in fresh material. Excretory bladder Y or T-shaped, extending forward from the posterior end of the body between the testes and bifurcating immediately anterior to them.

The authors found 3.7 percent of the 293 specimens of Eurytrema pacificus they examined were mirror images of the typical form, 0.3 percent had only one testis and 1.3 percent exhibited a partial degeneration of posterior vitellaria.

Figure 18 shows details of the adult anatomy and Figure 27 is a photograph of one of the three specimens collected from the small intestine of a golden hamster experimentally infected in the course of this study.

HOSTS

Euryhelmis pacificus adults were found to be harbored in the small intestines of two mink, a muskrat and a water shrew from Western Oregon. Experimental infections were introduced into white rats, golden hamsters and a deer mouse. Senger and Macy (33) observed that Euryhelmis pacificus matured after about 55 hours in the intestines of the rats and were mature 72 hours after infection in the golden hamsters. The infections in the rats were reported to last for about eight months. The golden hamster experimentally infected in this study died 269 days after being fed about 200 metacercarial cysts taken from Ascaphus truei; three of the 19 trematodes removed from the small intestine were mature Euryhelmis pacificus.

Metacercariae are found occurring naturally in the skeletal tissues of Dicamptodon ensatus and in the subcutaneous connective tissue of Ascaphus truei. The molluscan host of Euryhelmis pacificus remains unknown.

DISCUSSION

The mammalian hosts reported for Euryhelmis pacificus indicate that this fluke has a moderately wide range of tolerance for definitive hosts. It might be expected to be found in a number of carnivores, in addition to mink, if one considers that Ascaphus and Dicamptodon must be preyed upon by other animals. The fact that Euryhelmis squamula is found in a diversity of carnivorous mammals lends credence to this opinion.

The muskrat, while apparently an acceptable host, can be considered an infrequent, accidental host because of its food habits. Senger and Macy's statement (33) of finding over 100 Euryhelmis pacificus in only one of the 34 muskrats examined by them lends support to this opinion. The water shrew examined by these authors contained four flukes devoid of eggs, and from this they concluded that the water shrew is not a satisfactory definitive host. Such may be the case, but it also is possible that the infection had occurred only a short time previously and these four flukes had not matured sufficiently to produce eggs.

It is possible that other amphibians besides Ascaphus and Dicamptodon may serve as hosts to the metacercarial stage of this fluke. These two amphibians are the only amphibians usually encountered in the high mountain streams of Oregon. However, the

muskrat examined by Senger and Macy was trapped on the Reed College campus in Portland, and this may indicate the presence of other amphibian hosts in the life cycle of Euryhelmis pacificus.

SUMMARY

The life cycle of Euryhelmis pacificus is incompletely known. No molluscan host has been found and information is lacking on the early stages of development of this trematode.

Cercariae encyst in the striated muscles of Dicamptodon ensatus and in the subcutaneous connective tissue of Ascaphus truei.

Mammalian hosts, feeding on these amphibians in the wild, ingest the cysts containing the metacercariae. The excysted metacercariae attach themselves to the lining of the small intestine and become mature. The mink, the muskrat and the water shrew are the only naturally infected mammal hosts so far reported; the white rat, golden hamster and deer mouse have been infected experimentally. Flukes mature in the experimental animals in 55 to 72 hours after excysting and live for periods in excess of eight months in the intestines of the hamsters.

Refer to Table II, page 74, for a tabular resumé.

PART V

Notes on the Life Cycle of Cephalouterina dicamptodoni Senger and Macy, 1953

Cephalouterina dicamptodoni was described by Senger and Macy (34) from the small intestine of the Pacific Giant Salamander Dicamptodon ensatus. Adult flukes were recovered from about half of some 50 larval and adult salamanders collected in Western Oregon in 1950 and 1951. Lehman (20), in 1954, also reported finding Cephalouterina in Dicamptodon.

The authors placed this new genus in the subfamily Pleurogenetinae of the family Lecithodendriidae, although, as they stated at that time, the placement to subfamily was difficult since the excretory pattern and the life history remained unknown. In 1956 Yamaguti (41) transferred Cephalouterina from Pleurogenetinae to the subfamily Cryptotropinae.

METHODS AND MATERIALS

Adult flukes removed from the intestine of Ascaphus were stored in small watch glasses in a refrigerator for one or two days at 5° C in order to relax them. Cysts containing metacercariae were removed from the infected stonefly nymphs and excysted by placing them on slides and applying light pressure to the cover slips.

Both adults and metacercariae were fixed in Lavdowsky's AFA Solution and stained in Semichon's carmine, Meyer's carmalum and Celestine blue.

Photomicrographs were made with an Edixa Reflex camera and an Olympus camera mounted on compound microscopes. Measurements, taken from both living and preserved specimens, are given in millimeters; the average is followed by the range in size in parentheses.

EGG

Cephalouterina produces a pale brown, elongate, operculate egg, which bears a blunt projection on the abopercular end (Figure 20). It somewhat resembles the eggs of both Euryhelmis pacificus and Euryhelmis squamula, but has its greatest diameter near the mid-line instead of near the point.

Senger and Macy (34) gave measurements for the egg as follows: length 0. 041 (0. 038-0. 050) and diameter 0. 020 (0. 017-0. 022). Ten eggs in the uteri of preserved Cephalouterina recovered from Ascaphus measured 0. 052 (0. 049-0. 054) long and 0. 019(0. 018-0. 022) wide.

No eggs were observed to hatch, and the micracidium is unknown.

METACERCARIA

Cysts containing metacercariae were found in the nymphs of the stonefly Acroneuria californica Banks, 1905. These cysts, measuring 0.352 (0.312-0.417) in diameter, were situated mostly in the femoral and coxal regions of the legs and in the musculature of the thorax. Because of the semitranslucent nature of the ventral surfaces of the appendages and the thorax, many of the cysts were visible through the integument when the insects were examined under a dissecting microscope.

Excysted metacercariae appear more nearly oval than the adults, but otherwise show a strong resemblance (see Figures 19 and 21). The body is spinose overall, with the anterior spines being longest. The spines toward the posterior end of the body become progressively shorter and weaker. Six mounted specimens measure 0.514 (0.444-0.596) long by 0.276 (0.242-0.293) wide. The subterminal oral sucker measures 0.099 (0.093-0.105) in diameter; the ventral sucker, measuring 0.107 (0.093-0.117) in diameter, is located very near the equatorial line. A very short prepharynx is followed by a nearly spherical muscular pharynx of 0.057 (0.049-0.066) which connects to a thick esophagus of moderate length. The two intestinal ceca bifurcate about midway between the pharynx and the acetabulum, continue posterolaterally and end at a level near the

posterior edge of the acetabulum.

The excretory bladder is in the form of a voluminous Y which occupies a large portion of the posterior half of the metacercaria. It empties through a short duct terminating at the posterior mid-line. Two excretory tubes, opening into the anterolateral ends of the Y-shaped bladder, continue anteriorly and laterally and pass just posterior to the ends of the intestinal ceca, where they each branch. One excretory tubule branching from each of these laterally-directed excretory tubes runs anteriorly and the other tubule runs posteriorly near the lateral margins of the body. The two somewhat oval testes are situated, one on each side of the body, lateral to the bladder and within the loops formed by the excretory tubes and the posterior excretory tubules (Figure 19).

ADULT

The type specimens of Cephalouterina dicamptodoni were described in 1953 by Senger and Macy (34) from the small intestine of Dicamptodon ensatus. In their description of the adult, which follows, all measurements given are in millimeters with averages in parentheses.

> Body ellipsoidal, 0.96-1.60 (1.34) long by 0.45-0.75 (0.57) wide. Cuticula spinose overall. Oral sucker subterminal, 0.16-0.20 (0.18) long and 0.18-0.22 (0.20) wide; prepharynx short. Pharynx 0.06-0.09 in diameter, esophagus about 0.18 long. Intestinal ceca short and expanded, ending at about mid-acetabular level. Ventral sucker 0.18-0.28 (0.22) in diameter, slightly anterior to mid-line of body. Testes opposite, just within posterior half of body and measuring 0.20-0.35 (0.28) long and 0.11-0.18 (0.15) wide. Cirrus sac on left of mid-line, 0.30-0.45 long by 0.04-0.07 wide, and extending anteriorly from near the intestinal bifurcation to the level of the oral sucker. Seminal vesicle bipartite, with small anterior portion somewhat resembling a pars prostatica, remainder sinous and 0.10-0.18 in length and 0.04-0.06 wide. A few prostate cells surround the cirrus. Genital pore dorsal and laterial, to left side of oral sucker. Genital atrium a shallow depression. Ovary anterior and median to right testis, reaching to about equator of acetabulum; varying in shape but usually ellipsoidal, measuring 0.12-0.25 (0.17) long and 0.09-0.14 (0.11) wide. Seminal receptacle between testes, 0.08-0.13 long by 0.02-0.06 wide, and constricted with Laurer's canal leaving smaller portion as a sinous tube opening dorsally. Vitellaria somewhat variable in extent generally with a compact mass of follicles on each side anterior to intestinal ceca and more scattered lateral ones posterior to ceca and extending to a point alongside or even posterior to testes. Follicles usually meet dorsally anterior to ventral sucker. From oötype region between the testes, the uterus passes anteriorly to left side of

acetabulum and has a few loose coils anterior to each intestinal cecum. Eggs not numerous, measuring 0.038-0.050 (0.041) by 0.017-0.022 (0.020); thin shelled, light brown, with prominent operculum and short polar spine. Excretory vesicle Y-shaped, with long, unbranched stem bifurcating between testes to form short arms which taper to narrow main excretory tubules passing anteriorly and laterally around intestinal ceca.

In this study 48 adult Cephalouterina were recovered from 139 Ascaphus examined. Measurements of the adults taken from Ascaphus were as follows: body length 1.172 (1.040-1.636), width 0.584 (0.455-0.838); oral sucker 0.165 (0.110-0.232) in diameter; pharynx 0.099 (0.073-0.132) in diameter and ventral sucker diameter 0.189 (0.146-0.227). Testes 0.346 (0.263-0.434) long by 0.237 (0.161-0.313) wide and ovary 0.175 (0.131-0.222) long and 0.152 (0.131-0.172) wide (Figure 21).

Figure 28 is a photomicrograph of a living specimen mounted in water and Figure 29 is a photomicrograph of a different specimen stained and mounted.

HOSTS

Cephalouterina adults live in the small intestine of Dicamptodon and Ascaphus. Senger and Macy (34) found this fluke infecting about 50 percent of the salamanders they examined in Western Oregon, but did not indicate the number of flukes found or the number harbored in any one salamander. Lehman (20) also reported this trematode from Dicamptodon in the Willamette Valley, but failed to give any data on incidence of infection.

Twenty-six specimens of Ascaphus, out of a total of 139 examined, were found to harbor 48 adult Cephalouterina. The incidence of infection was 15.4 percent and the infected frogs had from one to five flukes in the small intestine.

I found metacercariae in the nymphs of the California Salmonfly, Acroneuria californica. Eight of the eighteen examined harbored a total of ten metacercariae, with a maximum of two cysts in any one individual, for a 44.4 percent incidence of infection. No other aquatic insects were found to serve as hosts for this metacercaria.

A young, laboratory-raised Rana aurora was fed two cysts from Acroneuria. Two subadult Cephalouterina were recovered from the small intestine of this experimental host four days after infection.

The molluscan host remains unknown.

DISCUSSION

In placing this trematode in the subfamily Pleurogenetinae, Senger and Macy (34) pointed out that this was done tentatively on the basis of a consideration of the length of the intestinal ceca, the location of the gonads and the structure of the oötype region. Yamaguti (41) placed this fluke in the subfamily Cryptotropinae with Cryptotropa (the only genus previously in this taxon) without any explanation why he did so. However, when the characteristics of these two genera are compared to the characteristics given for the Pleurogenetinae, one observes that the uterus lies mostly anterior to the acetabulum and extends up into the neck region in both Cephalouterina and Cryptotropa, while in the Pleurogenetinae the uterus is situated mostly behind the acetabulum.

If one considers the life habits of Dicamptodon, it would appear that most of the infections with Cephalouterina must occur when these salamanders prey on the nymphal stage of the stoneflies, for these salamanders are frequently neotenic. Even those that do metamorphose seldom leave the water except during the wet, cold season of the year when the stoneflies would be least likely to emerge as adults.

SUMMARY

Cercariae, penetrating the integument of the stonefly Acroneuria californica, form a cyst in the muscles of the legs and thorax, and develop into metacercariae. The amphibian hosts, Ascaphus and Dicamptodon, feed on the stoneflies, and the flukes, excysting in the digestive tract, attach themselves to the intestinal mucosa and become mature.

The molluscan host and the early larval stages of this trematode are not known.

Refer to Table II on the next page for a tabular resumé.

TABLE II

	Tetracheilos ascaphi	Euryhelmis squamula	Euryhelmis pacificus	Cephalouterina dicamptodoni
Definitive Host	Ascaphus truei small intestine (Anderson).	Mustela vison, racoon in U. S. A. In Europe: Lutreola, 2 sp., Mustela, 2 sp. and fox. (exp: Hamster, white rat, Peromyscus, cat).	Mustela vison, Ondatra zibethica, Sorex bendirii palmeri--no eggs (Senger and Macy).	Dicamptodon ensatus intestine (Senger and Macy); Ascaphus truei small intestine (Anderson).
Egg	Passed in feces of Ascaphus. Operculate? (Anderson).	Operculate, blunt spine on opposite end, passed in feces.	Operculate, blunt spine on opposite end, passed in feces.	Operculate, blunt spine on opposite end, passed in feces.
Miracidium	Not seen.	Not seen.	Not seen.	Not seen.
Sporocyst	In Pisidium idahoense. Contained cercariae (Anderson).	Not seen.	Not seen.	Not seen.
Cercaria	Oculate, xiphidiocercariae freed into water, swim, crawl. Enter Rhyacophila grandis (Anderson).	Lophocercous, spinose, free swimming from Bythinella hemphilli (Anderson).	Not identified. Probably in Bythinella hemphilli.	Not identified. Probably in Bythinella hemphilli.
Metacercaria	In haemocoel of Rhyacophila. Caddisfly adults eaten by Ascaphus. Considerably longer than 4 days to mature in Caddisfly (Anderson).	Spinose, quadrangular. Encysted in subcutaneous C. T. of Ascaphus. Become infective in Rana aurora in 44 days (Anderson).	Spinose, pyriform. Encysted in muscles of Dicamptodon (Senger and Macy). Subcut. C. T. of Ascaphus (Anderson).	In muscles of upper legs and thorax of Salmonfly Acroneuria californica (Anderson).
Adult	Longer than 13 days to mature (Anderson).	Spinose, quadrangular. Senger and Macy--72 hours, hamster, white rat. McIntosh--5 days in cat. Anderson--some in 70 hours, all in 92 hours, in hamster.	Spinose, pyriform. Senger and Macy--white rats, golden hamsters, deer mouse, 55-72 hours. Anderson--golden hamster still infected at 269 days.	Experimentally in Rana aurora--4 days, no eggs, probably 10 days or more to mature (Anderson).

BIBLIOGRAPHY

1. Ameel, Donald J. The life history of Crepidostomum cornutum (Osborn). Journal of Parasitology 23:218-220. 1937.

2. _____. The morphology and life cycle of Euryhelmis monorchis n. sp. (Trematoda) from the mink. Journal of Parasitology 24:219-224. 1938.

3. Anderson, Glenn A. and Ivan Pratt. A new trematode, Tetracheilos ascaphi gen. n. and sp. n. (Allocreadiidae) from the tailed frog, Ascaphus truei. (Manuscript in preparation)

4. Baer, Jean G. Quelques helminthes rares ou peu connus du putois. Revue Suisse de Zoologie 38:328-334. 1931.

5. Braun, M. Einige Bemerkungen über die Fascioliden der Chiroptera. Zoologischer Anzeiger 23:387-391. 1900.

6. Bremser, J. G. Icones helminthum systema Rudolphi entozooligicum illustrantes. In: Ernst Zeller's Ueber das encystierte vorkommem von Distomum squamula Rud. im braunen grasfrosch. Zeitschrifte fur Wissenschaftliche Zoologie 17:215-220. 1867.

7. Brenes, Rodrigo R., Guido Arroyo and Otto Jiménez-Quirós. Helmintos de la Rupública de Costa Rica XVIII. Una nueva especie de Euryhelmis (Trematoda: Heterophyidae), parásito de Mustela frenata costaricensis. Revista de Biologia Tropical 8:247-251. 1960.

8. Brown, F. J. On Crepidostomum farionis O. F. Müll. (=Stephanophiala laureata Zeder), a distome parasite of the trout and grayling. I. The life history. Parasitology 19: 86-99. 1927.

9. Callot, J. Materiaux pour servir a la fauna des distomes de France. Annales de Parasitologie Humaine et Comparée 21: 119-201. 1946.

10. Cooper, A. R. Trematodes from marine and fresh-water fishes, including one species of ectoparasitic turbellarian. Transactions of the Royal Society of Canada ser. 3, 9 (Sect IV): 181-205. 1915.

11. Crawford, Wiley W. Colorado trematode studies. I A further contribution to the life history of Crepidostomum farionis (Müller). Journal of Parasitology 29:379-384. 1943.

12. Dawes, Ben. The Trematoda. Cambridge, University Press, 1946. 644 p.

13. Diesing, C. M. Systema helminthum. Berlin, Wilhelmum Braumuller, 1850. 591 p. Reprinted by Hafner Publishing Company, New York. 1960.

14. Dujardin, M. Félix. Histoire naturelle des helminthes ou vers intestinaux. Paris, Librairie Encylopedique de Roret, 1845. 654 p.

15. Henderson, Harold E. The cercaria of Crepidostomum cornutum (Osborn). Transactions of the American Microscopical Society 57:165-172. 1938.

16. Hopkins, Sewell H. Studies on Crepidostomum. I Crepidostomum isostomum n. sp. Journal of Parasitology 17: 145-150. 1931.

17. _____. Studies on Crepidostomum. II. The "Crepidostomum laureatum" of A R. Cooper. Journal of Parasitology 18:79-81. 1931.

18. _____. The papillose Allocreadiidae. Illinois Biological Monographs 13(2):1-80. 1934.

19. Joyeux, Charles E., Jean G. Baer and Pierre Carrére. Recherches sur le cycle evolutif d'Euryhelmis squamula. Comptes Rendus des Seances de l'Academie des Sciences. Paris 199:1067-1068. 1934.

20. Lehman, Donald L. Some helminths of West Coast urodeles. Journal of Parasitology 40:231. 1954.

21. Linstow, O. von. Über die Entwicklungsgeschichte des Distomum nodulosum Zeder. Archiv für Naturgeschichte 39:1-7. 1873.

22. Linton, Edwin. Notice of trematode parasites in the crayfish. American Naturalist 26:69-70. 1892.

23. Lühe, Max. Parasitische Plattwürmer. I. Trematoden. Süsswasserfauna Deutschland. Heft 17. Jena, Gustav Fischer, 1909. 215 p.

24. McIntosh, Allen. The occurrence of Euryhelmis squamula (Rud., 1819) in the United States. Journal of Parasitology 22:536. 1936.

25. Morozov, F. N. Phylogeny of trematodes of the subfamily Heterophyoidea. (Russian text). Akademiiâ Nauk SSSR. Doklady 74:645-648. 1950.

26. Noble, G. K. and Phillips G. Putnam. Observations on the life history of Ascaphus truei Stejneger. Copeia, 1931 p. 97-101.

27. Parker, Malcolm V. The trematode parasites from a collection of amphibians and reptiles. Journal of the Tennessee Academy of Science 16:27-44. 1941.

28. _____. Euryhelmis squamula (Rudolphi, 1819) reported from a racoon. Journal of Parasitology 36:89. 1950.

29. Poche, F. Das system der Platodaria. Archive für Naturgeschichte, Abteilung A, 91(2-3):1-436. 1925.

30. Pratt, Ivan and James E. McCauley. Trematodes of the Pacific Northwest, an annotated catalog. Corvallis, Oregon State University Press, 1961. 113 p.

31. Price, Emmett W. A review of the trematode superfamily Opisthorchioidea. Proceedings of the Helminthological Society of Washington 7:1-12. 1940.

32. Rudolphi, Carolo A. Entozoorum synopsis cui accedunt mantissa duplex et indices locupletissimi. Berolini, Sumtibus Augusti Rucker, 1819. 811 p.

33. Senger, Clyde M and Ralph W. Macy. Helminths of Northwest mammals. Part III. The description of Euryhelmis pacificus n. sp., and notes on its life cycle. Journal of Parasitology 38:481-486. 1952.

34. _____. A new digenetic trematode (Cephalouterina dicamptodoni n. g , n. sp., Pleurogenetinae) from the Pacific giant salamander. Journal of Parasitology 39:353-355. 1953.

35. Senger, Clyde M. and Kenneth A. Neiland. Helminth parasites of some fur bearers of Oregon. Journal of Parasitology 41: 637-638. 1955.

36. Stafford, J. Trematodes from Canadian fishes. I. Zoologischer Anzeiger 27:481-495. 1904.

37. Stejneger, Leonhard. Description of a new genus and species of discoglossoid toad from North America. Proceedings of the United States National Museum 21:899-901. 1899.

38. Webster, Gloria A. and R. W. Wolfgang. Alaria canadensis sp. nov. and Euryhelmis pyriformis sp. nov. from the skunk Mephitis mephitis in Quebec. Canadian Journal of Zoology 34:595-601. 1956.

39. Witenberg, G. Studies on the trematode family Heterophyidae. Annals of Tropical Medicine and Parasitology 23:131-239. 1929.

40. Wright, R. Ramsey. Trematode parasite in American crayfish. American Naturalist 18:429-430. 1884.

41. Yamaguti, Satyu. Systema helminthum. Part 1 and 2. The digenetic trematodes of vertebrates. Vol. 1. New York, Interscience Publishers, Inc., 1958. 1575 p.

42. Zeller, Ernst. Ueber das encystierte vorkommen von Distomum squamula Rud. im braunen grasfrosch. Zeitschrifte für Wissenschaftliche Zoologie 17:215-220. 1867.

APPENDIX

Figure 1. Egg of Tetracheilos ascaphi.

Figure 2. Sporocyst and cercariae of Tetracheilos.

Figure 3. Ventral aspect of Tetracheilos cercaria.

Figure 4. Stylet from Tetracheilos cercaria.

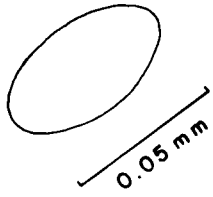

FIG. 1

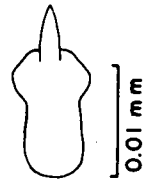

FIG. 4

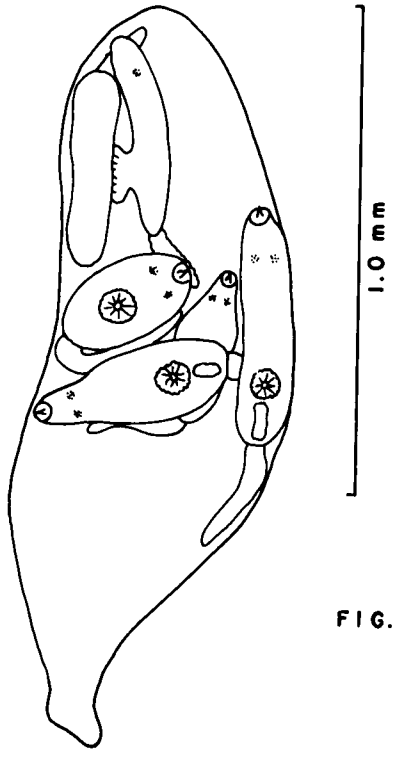

FIG. 2

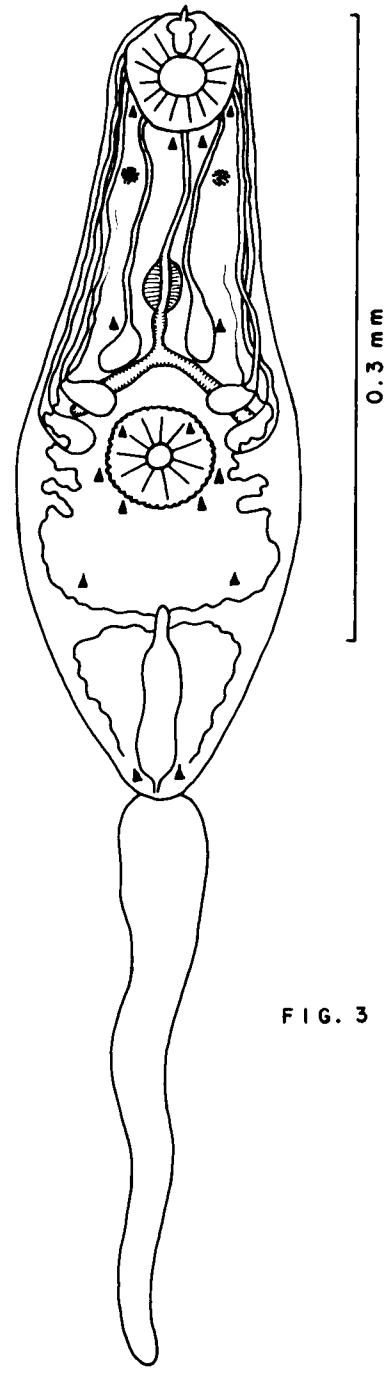

FIG. 3

Figure 5. Cercaria of Tetracheilos ascaphi in position assumed for penetration of insect exoskeleton.

Figure 6. Four-day metacercaria of Tetracheilos from a mayfly.

Figure 7. Infective metacercaria of Tetracheilos from the caddisfly Rhyacophila grandis.

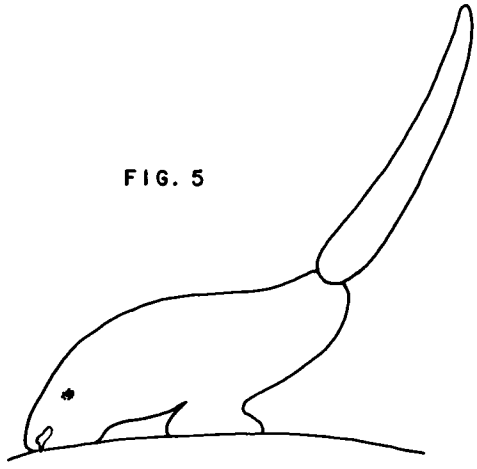

FIG. 5

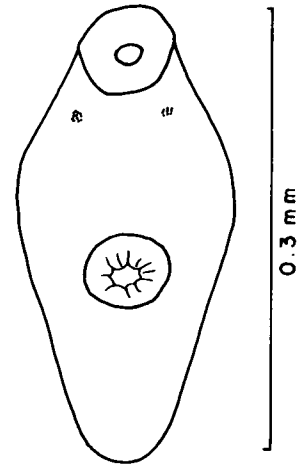

FIG. 6

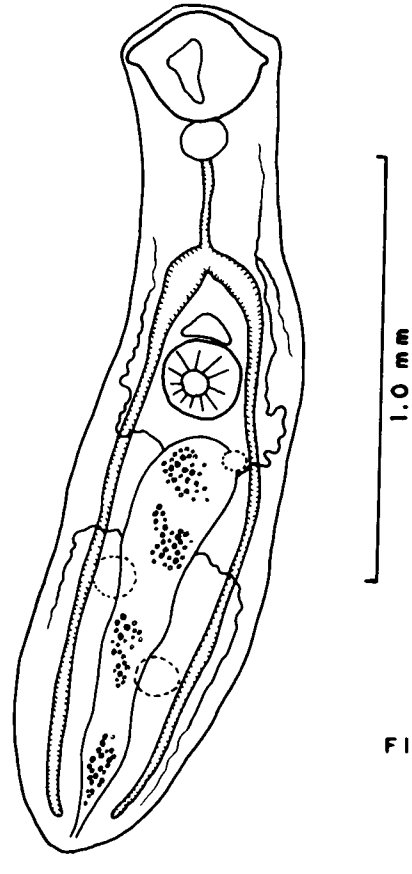

FIG. 7

Figure 8. Ventral aspect of adult Tetracheilos ascaphi.

Figure 9. Right, lateral aspect of adult Tetracheilos.

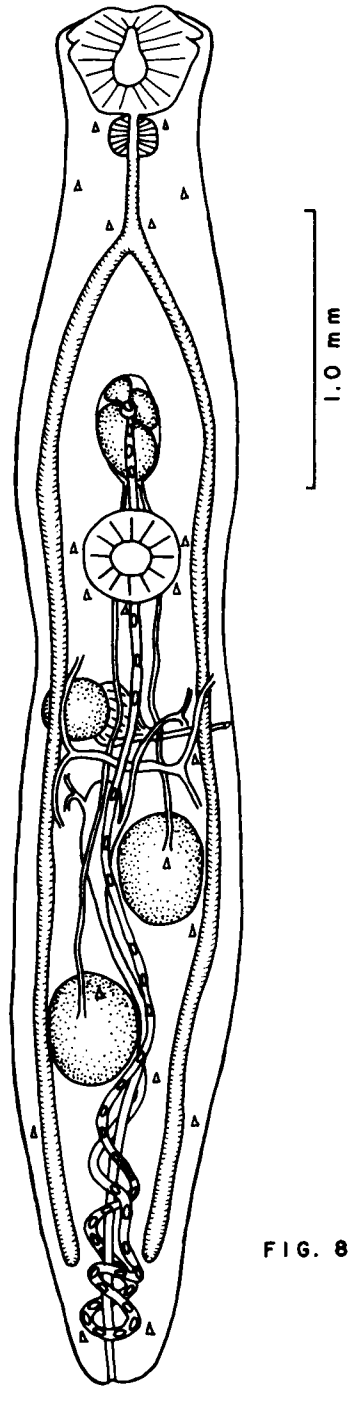

FIG. 8

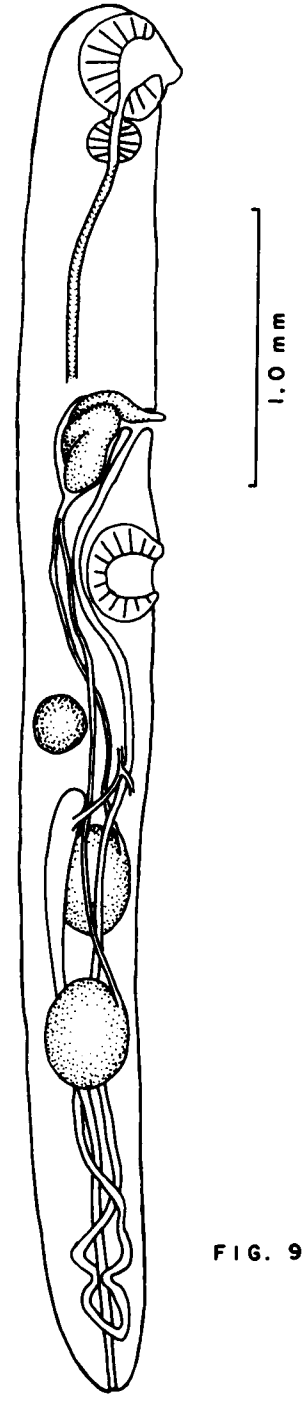

FIG. 9

Figure 10. Egg of Euryhelmis squamula.

Figure 11. Ventral aspect of cercaria of Euryhelmis squamula.

Figure 12. Ventral aspect of Euryhelmis squamula metacercaria removed from a subcutaneous cyst in Ascaphus truei.

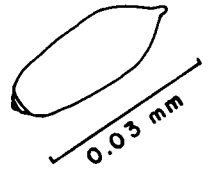

FIG. 10

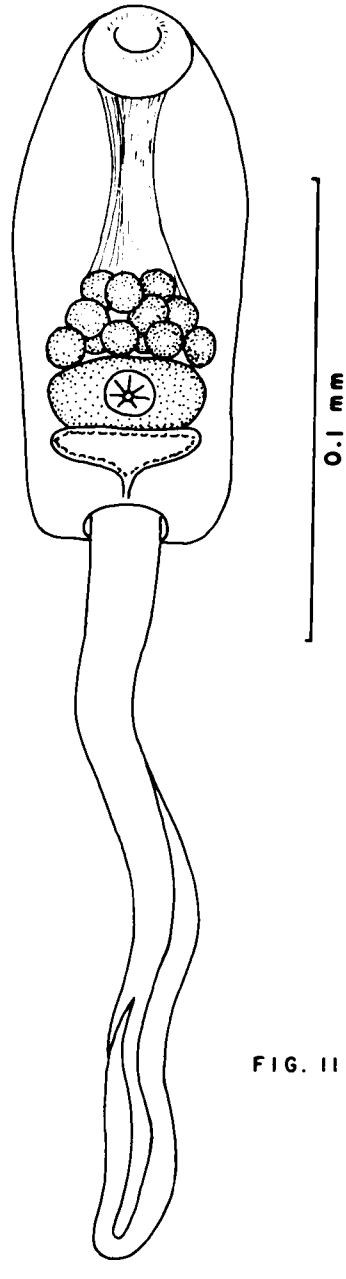

FIG. 11

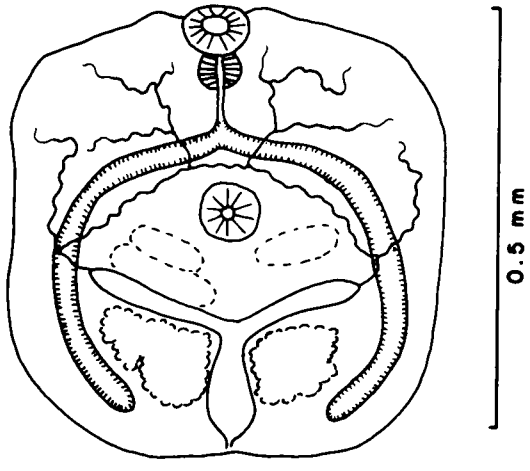

FIG. 12

Figure 13. Lateral aspect of cercaria of Euryhelmis squamula.

Figure 14. Forty-four-day Euryhelmis squamula metacercaria from an experimentally infected Rana aurora.

Figure 15. Ventral aspect of an adult Euryhelmis squamula from an experimentally infected golden hamster.

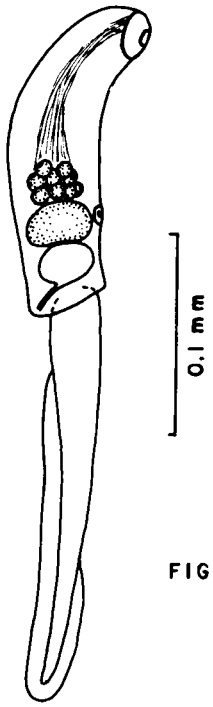

FIG. 13

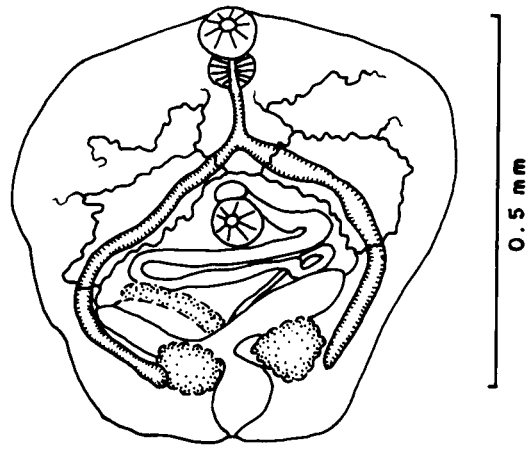

FIG. 14

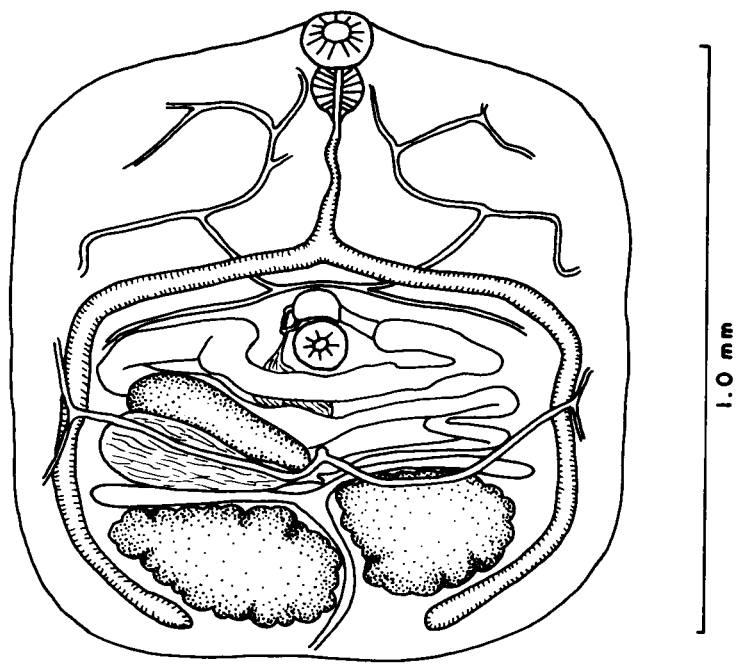

FIG. 15

Figure 16. Egg of Euryhelmis pacificus.

Figure 17. Metacercaria of Euryhelmis pacificus. Drawing from Senger and Macy (33).

Figure 18. Ventral aspect of 269-day adult Euryhelmis pacificus from an experimentally infected hamster.

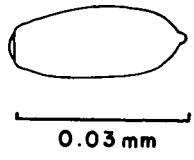

FIG. 16

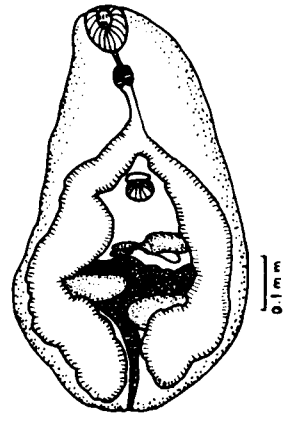

FIG. 17

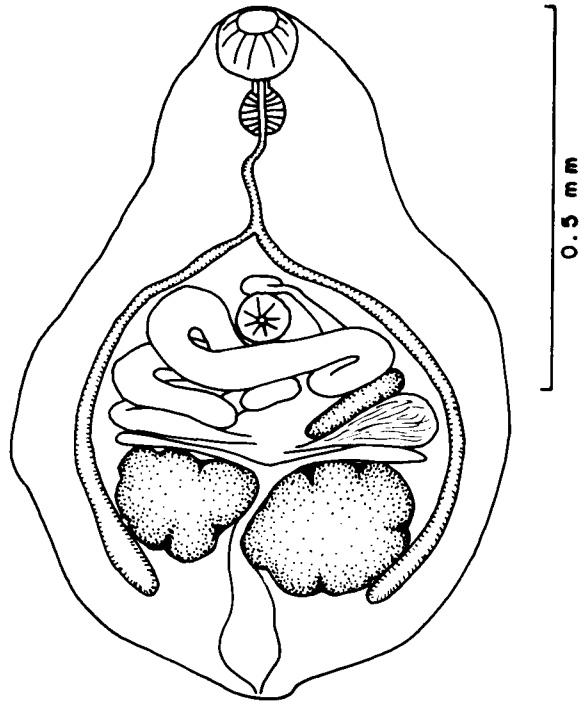

FIG. 18

Figure 19. Metacercaria of Cephalouterina dicamptodoni from the Salmonfly Acroneuria californica.

Figure 20. Egg of Cephalouterina.

Figure 21. Ventral aspect of adult Cephalouterina from intestine of Ascaphus truei.

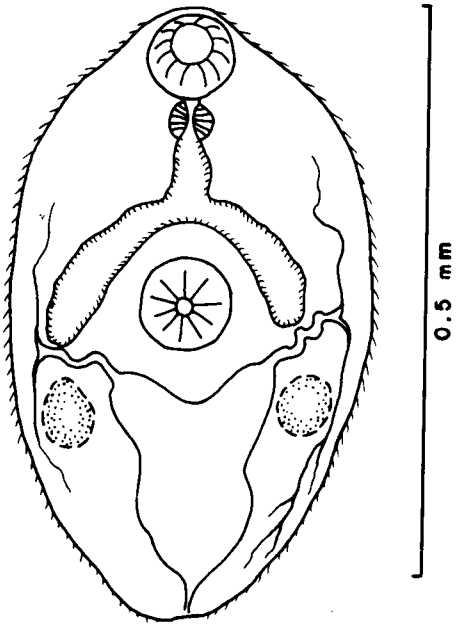

FIG. 19

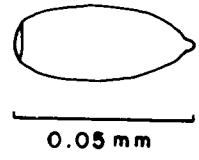

FIG. 20

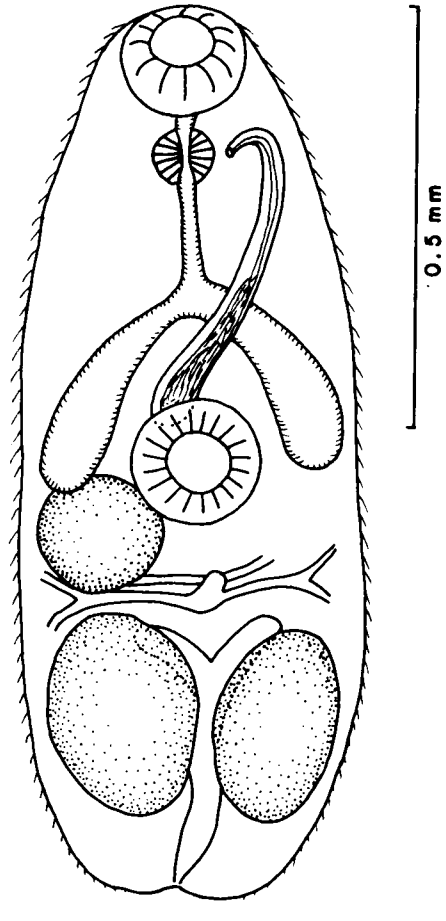

FIG. 21

Figure 22. Cercariae and germ balls from a sporocyst of Tetracheilos ascaphi. X 72.

Figure 23. Photomicrograph of a living Tetracheilos, showing the extensibility of the neck.

Figure 24. Ventral aspect of a stained and mounted adult Tetracheilos. X 19.

Figure 25. Dorsal aspect of a stained and mounted adult Tetracheilos. X 24.

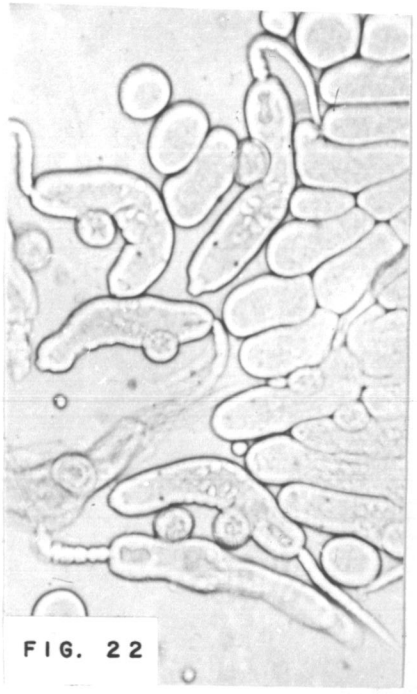

FIG. 22

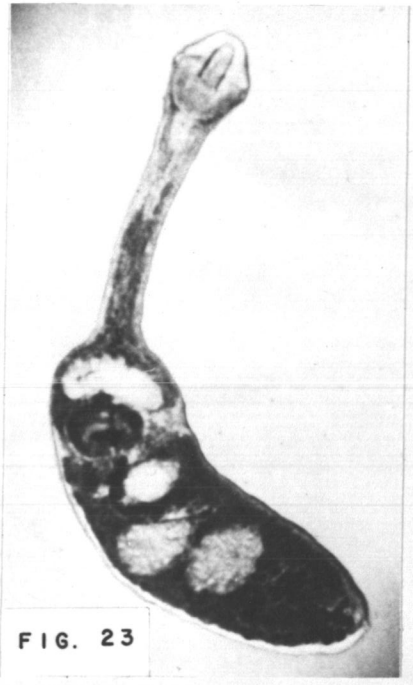

FIG. 23

FIG. 24

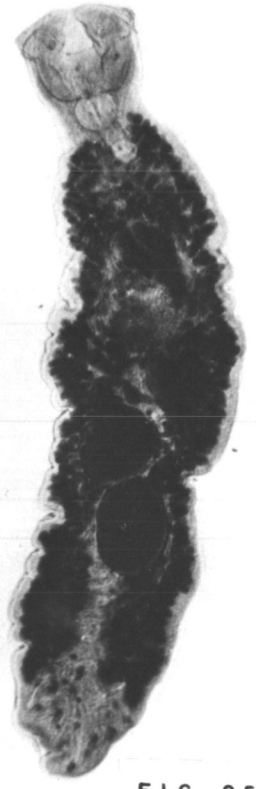

FIG. 25

Figure 26. Ventral aspect of a stained and mounted 269-day Euryhelmis squamula from a golden hamster. X 67.

Figure 27. Dorsal aspect of a stained and mounted 269-day Euryhelmis pacificus from a golden hamster. X 79.

Figure 28. Photomicrograph of a live, adult Cephalouterina dicamptodoni from the intestine of Ascaphus truei. X 72.

Figure 29. Photomicrograph of a stained and mounted adult Cephalouterina from Ascaphus. X 78.

FIG. 26

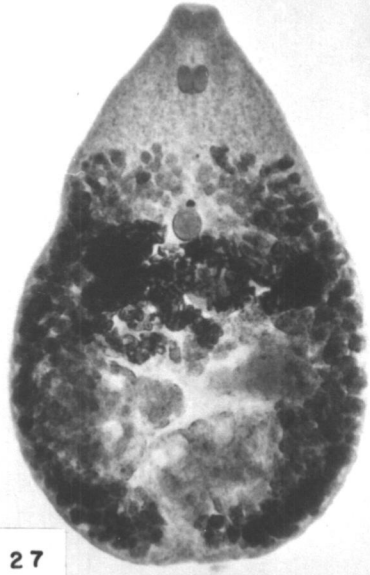

FIG. 27

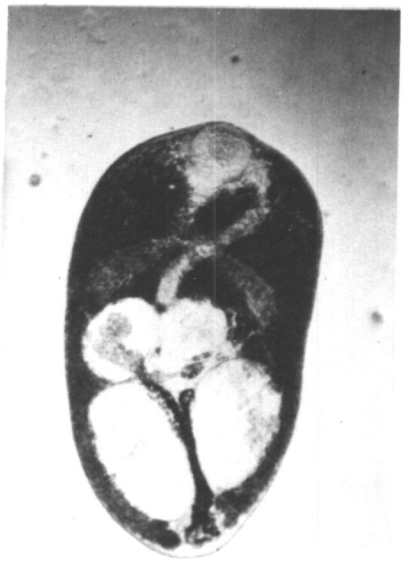

FIG. 28

FIG. 29