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Euryhelmis cotti n. sp. (Trematoda: Heterophyidae) with Observations on Its Life Cycle

Michael Joseph Simon
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AN ABSTRACT OF THE THESIS OF Michael Joseph Simon for the Master of Science in Biology presented March 31, 1972.

Title: Euryhelms Cotti N. Sp. (Trematoda: Heterophyidae) with Observations on Its Life Cycle.

APPROVED BY MEMBERS OF THE THESIS COMMITTEE:

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Richard Forbes

Fish of the genus Cottus were found infected with heterophyid metacercariae. Laboratory animals were infected with the metacercariae, and adult heterophyid trematodes were recovered. These flukes were found to represent an undescribed species of the genus Euryhelms. Various streams in the Willamette Valley and coastal areas were sampled for infected Cottus sp. Snails of the genera Oxytrema and Fluminicola were collected. Several possible definitive hosts were examined. A partial review of the subfamily Apophallinae and a complete review of the genus Euryhelms are presented. Euryhelms cotti n. sp. is placed in the subfamily Apophallinae, and its life cycle is partially described.

EURYHELMIS COTTI N. SP. (TREMATODA: HETEROPHYIDAE)

WITH OBSERVATIONS ON ITS LIFE CYCLE

by

MICHAEL JOSEPH SIMON

A thesis submitted in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE
in
BIOLOGY

Portland State University
1972

TO THE OFFICE OF GRADUATE STUDIES

The members of the Committee approve the thesis of
Michael Joseph Simon presented March 31, 1972.


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INTRODUCTION

An examination of sculpins of the genus Cottus, collected in the Nehalem River near Vernonia, Oregon, revealed the presence of many metacercariae of several different types of trematodes. Metacercariae of heterophyid were found among these. The systematic position and life cycle of which have been investigated with results presented in this study.

The heterophyid under consideration has been found to represent a new species of the genus Euryhelms Poche, 1926. At the present time, five species have been described for the genus as follows: Euryhelms squamula (Rudolphi, 1819), Euryhelms monorchis Ameel, 1938 Euryhelms pacificus Senger and Macy, 1952; Euryhelms pyriformis Webster and Wolfgang, 1956 and Euryhelms costaricensis Brenes and Arroyo, 1960. The adults have typically been found in the small intestine of mustelids and the metacercariae in amphibians. The complete life cycles have been described for Euryhelms squamula (Anderson and Pratt, 1965), Euryhelms monorchis (Ameel, 1938), and partially for Euryhelms pacificus (Senger and Macy, 1952). The life cycles of the species belonging to the genus Euryhelms differ from the typical heterophyid in that the metacercariae are generally found in amphibians and not fish. (See Table I.)

The members of the family Heterophyidae are small distomes or monostomes, generally not more than 3 mm in length, and are oval or pyriform in shape. The anterior end is more motile and less bulky

than the more posterior portion which generally contains the reproductive organs of the adult. The cuticle is spiny or scaly, either over the entire body or at least anteriorly. The ceca are long, and a cirrus pouch is absent. A gonotyl is present in the genital atrium. The acetabulum may be reduced and incorporated into the wall of the genital atrium and is lacking in some genera. Adults of the family show a very low host specificity. Most species are able to develop to maturity in the small intestine of a variety of warm blooded animals. As an example, Haplorchis taichui Nishigori has been described from cattle egrets (Africa, 1938), sea gulls (Witenberg, 1929), ducklings (Hsu, 1951), cats and dogs (Kuntz and Chandler, 1956), Vulpes vulpes (Kuntz and Chandler, 1956), and man (Africa, DeLeon and Garcia, 1940).

The life cycles of the members are very similar. The adults inhabit the small intestine of mammals and birds that eat fish and amphibians. The eggs are operculate and may contain early cleavage stages or miracidia. The eggs are ingested by operculate snails in which one generation of sporocyst, one or two generations of rediae, and pleurolophocercus cercariae develop. The cercariae generally have oral spines; however, the cercariae of Euryhelms monorchis (Ameel, 1938) is an exception in that the cercariae lack oral spines. The cercariae leave the snail host and generally encyst in either fresh water or marine fish. Species of Euryhelms Poche, 1926 have been shown generally to encyst in amphibians (Ameel, 1938; Senger and Macy, 1952; and Anderson and Pratt, 1956) as does Metagonimoides oregonensis Price, 1931 (Burns and Pratt, 1953).

This family is of particular interest because of its frequent occurrence in the intestine of man. Stunkard and Willey (1929) thought that every heterophyid was a potential human parasite. Although such a statement is probably an overgeneralization, members of the family are frequently found in man, often causing serious complications (Africa, Garcia and DeLeon, 1935). The ova appear to be picked up by the lymph or blood and carried to the heart, brain, spinal cord and liver. Martin (1958) found four heterophyid species that constitute a possible health hazard to man in Hawaii. Laird (1961) found what were assumed to be heterophyid eggs in fecal concentrates from four percent of Tokelauans examined. Apophallus ventrus Ransom, has been reported from man in Canada (Cameron, 1937). Serious cases of Distomiasis in western countries are reduced because fish are rarely eaten raw intentionally. It is in the oriental areas, where improperly cooked or raw fish is customarily eaten that heterophyids present a definite health problem.

TABLE I

HETEROPHYID LIFE CYCLES

<u>Species</u>	<u>Definitive Hosts</u>	<u>Snail Host</u>	<u>Second Intermediate Hosts</u>	<u>Source</u>
<u>Euryhelms squamula</u>	<u>Mustela putoris</u> <u>Mustela nivalis</u> <u>Lutreola lutreola</u> <u>Lutreola vison</u> <u>Vulpes vulpes</u> <u>Mustela vison</u> <u>Procyon lotor</u> experimental in hamster	<u>Bythinella</u> <u>hemphilli</u>	<u>Rana temporaria</u> <u>Rana esculenta</u> <u>Triturus cristatus</u> <u>Rana pipiens</u> <u>Rana aurora</u> <u>Rana cascadae</u> <u>Ascaphus truei</u>	Anderson and Pratt, (1965) Parker, (1950) Senger and Macy, (1952) Senger and Neiland, (1955) Baer, (1931) Babero and Shepperson, (1956)
<u>Euryhelms monorchis</u>	mink	<u>Pomatiopsis</u> <u>lapidaria</u>	<u>Rana clamitans</u> experimental in <u>Rana pipiens</u> and <u>Rana palustris</u>	Amel, (1938)
<u>Euryhelms pacificus</u>	mink muskrat experimental in white rat, golden hamster, <u>Peromyscus</u> <u>maniculatus</u>	-	<u>Dicamptodon ensatus</u>	Senger and Macy, (1952)

<u>Species</u>	<u>Definitive Hosts</u>	<u>Snail Host</u>	<u>Second Intermediate Hosts</u>	<u>Source</u>
<u>Apophallus</u> <u>muehlingi</u>	<u>Larus ridibundus</u>	-	-	Ransom, (1920)
<u>Apophallus</u> <u>lerouxii</u>	red skunk	-	-	Rayski and Fahmy, (1962)
<u>Apophallus</u> <u>crami</u>	California gull	-	-	Price, (1931)
<u>Pricetrema</u> <u>zalophi</u>	sea lion	-	-	Price, (1930)
<u>Apophallus</u> <u>eccentricus</u>	dogs	-	-	Africa and Garcia, (1935)
<u>Phagicola</u> <u>lageniformis</u>	muskrat experimental in chicks	-	<u>Fundulus pallidus</u>	Martin, (1953)
<u>Ascocotyle</u> <u>mcintoshii</u>	<u>Guara alba</u>	-	-	Price, (1936)

<u>Species</u>	<u>Definitive Hosts</u>	<u>Snail Host</u>	<u>Second Intermediate Hosts</u>	<u>Source</u>
<u>Euryhelminis costaricensis</u>	<u>Mustela frenata costaricensis</u>	-	-	Brenes and Arroyo, (1960)
<u>Euryhelminis pyriformis</u>	<u>Mephitis mephitis</u>	-	-	Webster and Wolfgang, (1956)
<u>Euryhelminis cotti</u>	Experimental in mink, white rats, white mouse, hamster, chicks	-	fresh water fish <u>Cottus rothreus</u> <u>Cottus sp</u>	-
<u>Apophallus ventrus</u>	<u>Arenherodias herodias</u> cats, dogs, raccoon, blue heron, gulls	<u>Goniobasis livescens</u>	fresh water fish Garpike, carp, catfish, suckers, small mouth bass	Cameron, (1931)
<u>Apophallus brevis</u>	<u>Gavia immer</u> <u>Larus delawarensis</u> experimental in domestic cats	<u>Ammicola limosa</u>	fresh water fish trout	Ransom, (1920) Lyster (1939)

<u>Species</u>	<u>Definitive Hosts</u>	<u>Snail Host</u>	<u>Second Intermediate Hosts</u>	<u>Source</u>
<u>Strictodora tridactyla</u>	experimental in chicks	<u>Pironella conica</u>	<u>Aphanius fasciatus</u>	Martin, (1955)
<u>Metagonimoides oregonensis</u>	raccoon experimental in hamsters	<u>Goniobasis sp</u>	Frogs	Price, (1931) Burns and Pratt, (1953) Lang and Gleason, (1967)
<u>Parasitictodora handcocki</u>	-	<u>Cerithidea californica</u>	<u>Gillichthys mirabilis cooper</u> <u>Fundulus parvipinnus</u> <u>parvipinnus</u>	Martin, (1950)

METHODS

COLLECTING FISH AND SNAILS

The most successful method for obtaining fish of the genus Cottus was found to be sweeping a Needham rake across the bottom of a stream. The fish inhabit the bottom of the stream, often near the bank, frequently under rocks, and in grass over-hanging the bank. Rocks were overturned, and the Needham rake was swept through the vicinity. In grassy over-hangs, the rake was swept through the submerged grass, often with excellent results. Snails were obtained either by sweeping the rake across the substrate or picking them out by hand. All collecting was conducted in shallow water. The collector most often wearing hip boots. Collecting was most successful prior to winter flooding.

TRANSPORTING FISH AND SNAILS FROM THE FIELD TO THE LABORATORY

The fish and snails were placed in plastic cake pans half filled with stream water with Otaba (Pemble Laboratories, River Falls, Wisconsin) added for aeration. The cake pans were covered and placed in styrofoam containers to which crushed ice had been added. This method of transporting fish and snails from the field to the laboratory proved to be successful since the mortality rate was very low.

MAINTAINING ANIMALS IN THE LABORATORY

The fish were placed in aerated aquaria and partially submerged in a soft drink cooler, which maintained the temperature at 8° centigrade. Water in the aquaria was changed about once a week. The water

in the aquaria was obtained by allowing tap water to stand at room temperature for several days, then cooling it to 8° centigrade before replacing the original water.

Fish were maintained in this way for at least a month and were not fed during this period.

Snails were kept in plastic cake pans which were placed either in the soft drink cooler or in a cold room that was 3° centigrade. The snails remained alive for at least a month.

OBTAINING METACERCARIAE

The fish were keyed to species, and each species was then examined for cysts. When it was observed that several species contained the heterophyid metacercariae, the species were no longer separated. The fish were killed by pithing, weighed and then ground with a meat grinder. The ground fish were then placed in a digestive solution (see appendix) and allowed to digest for about one hour at 37° centigrade with gentle agitation. The digest was then strained and diluted with 0.9 percent saline. Diluted digest was allowed to set for twenty minutes, then the supernatant was slowly poured off and more 0.9 percent saline was added. After allowing fifteen minutes for settling, material from the bottom of the beaker was pipetted into a Petri dish. The cysts were then observed under a dissecting microscope. The cysts were separated according to morphological type using an eye dropper and placed in Stender dishes containing 0.9 percent saline solution.

INFECTING LABORATORY ANIMALS

Laboratory animals were infected either by force feeding

concentrated suspensions of metacercariae or by feeding them fish collected from an area of known infection. The latter method was used in work utilizing mink (Mustela vison) because of various problems in handling these animals. Force feeding was employed with all other animals. Mammal feeding was done in one of two ways, either feeding cysts to a conscious animal using an eyedropper or by rendering the animal semiconscious with ether or chloroform and infecting them by means of a syringe and tubing. (For a detailed account of the procedures used in infecting animals see the appendix.)

After infection the animals were kept in cages with a water supply but were not fed. Infected animals were generally kept a maximum of four days. Animals were killed with chloroform or ether, and the intestine was removed immediately and placed in 0.9 percent saline solution. The intestine was cut into approximately 4 centimeter lengths and placed in Petri dishes with 0.9 percent saline and then slit open. The linear arrangement of the removed intestine was maintained by numbering the dishes. The Petri dishes were then covered and allowed to stand for approximately thirty minutes giving any flukes present time to move from the villi of the intestine to the bottom of the Petri dish. The intestine was then scraped and teased apart to remove any trematodes that had not moved from the villi within the thirty minute period.

Trematodes were transferred with an eyedropper to Stender dishes containing 0.9 percent saline. The flukes were then studied alive under the compound microscope; various details of the anatomy were observed, and measurements were taken. The flukes were then fixed in Gilson's fluid, flattened under cover slip pressure and stained

with either Ehrlich's acid hematoxylin or carmine stains. Material to be sectioned was not flattened, but merely fixed in Gilson's fluid. A detailed account of the various procedures involved in fixing and staining will be found in the appendix.

EXCYSTATION OF METACERCARIAE

Metacercariae obtained after pepsin digestion were excysted either mechanically or chemically. The nature of the cyst wall and the small size of the organisms dealt with in this research dictated the use of enzymatic treatment. The in vitro excystation techniques employed were after those of Macy, Berntzen and Benz (1968). Details of some of the mechanical and chemical procedures available will be found in the appendix.

After excystation, the metacercariae were placed in 0.9 percent saline and studied alive. They were then fixed and stained by the same procedures as were the adults. Excysted metacercariae were maintained living for several days in 0.9 percent saline at a temperature of 18° centigrade.

EXAMINING SNAILS FOR CERCARIAE

There are several methods commonly employed in attempting to find the cercarial form. One may attempt to find the cercariae either by cracking snails or by allowing the cercariae to be shed naturally from the snail. The first method entailed crushing the snails by striking them with a light hammer, and picking away the shell fragments with dissecting needles. The shell-free tissue was placed in 0.75 percent saline and observed under a dissecting microscope. The hepatic tissue was teased apart to expose any sporocysts, rediae,

and cercariae which might be present. The second method employed natural shedding of cercariae from the snails. Snails were placed in Petri dishes, four snails per dish. The dishes were filled about one-half full with water and partially covered to retard evaporation of water, and the snails were allowed to stand at approximately 25^o centigrade for twenty-four hours. The dishes were then examined for cercariae under a dissecting microscope.

RESULTS

Fish of the genus Cottus were collected from various streams in Northwest Oregon. (See Table II.) When metacercariae obtained from the fish were ingested by laboratory animals, adult heterophyids were obtained. Examination of a number of these flukes indicated that they represented an undescribed species of the genus Euryhelms Poche, 1926. All measurements are in microns unless otherwise stated. The average is followed by the extremes in parentheses.

DESCRIPTION OF ADULT EURYHELMIS COTTI

Euryhelms cotti n. sp. (Figures 1,2,3 and 4)

Specific diagnosis: Euryhelms: Body thin, leaflike, spinose overall excepting extreme posterior; pyriform or elongate. Length 419 (270 to 550) width 222 (150 to 290). Oral sucker either terminal or subterminal 39 (32.5 to 42.5) long by 45.65 (37.5 to 55.0) in diameter. Pharynx spherical, 26.25 (25 to 30) in diameter connected to the oral sucker by a short but definite prepharynx 8 (7 to 9) in length very apparent in living material but often not evident in fixed material. Esophagus slender, bifurcating anterior to the acetabulum; length from the posterior aspect of the pharynx to the cecal bifurcation 91.25 (62.5 to 140). The ceca extend obliquely towards the sides and then follow the contour of the body, terminating at the extreme posterior of the animal, being somewhat enlarged at their posterior ends. Acetabulum 28.95 (25 to 29) in diameter situated slightly pre-equatorial. A slight but definite constriction in the body wall at the level

of the acetabulum. Testes ovoid, opposite or oblique in the posterior part of the body; the right testis generally located slightly posterior to the left. Right testis 97.0 (76 to 152.5) wide, 67.5 (55 to 80) antero-posterior axis. Seminal vesicle sac-like, dorsal to uterus and posterior and to the right of acetabulum. Cirrus not evident. Genital atrium located immediately anterior to the acetabulum and overhung by a bilobed gonotyl. Gonotyl appears as a bilobed fold of tissue rather than a sucker. Uterus consisting of three to four loops confined between intestinal ceca, testes, and acetabulum, opens into the genital atrium. Ovary, located on right side and anterior to right testis, somewhat club-shaped 56.25 (37.5 to 67.5) wide; 44.1 (40 to 50) long. Seminal receptacle, located between right testis and ovary, spherical or club-shaped. Neither Laurer's canal nor Mehlis' gland were observed. Vitelline follicles numerous, follicular, confined laterally, extending from the level of the acetabulum to the posterior end. Eggs operculated, with polar thickenings, 32.5 (30 to 37) by 20.5 (16 to 22.5). Excretory bladder, Y or T-shaped, extending forward from the posterior end of the body between the testes and bifurcating immediately anterior to them.

Hosts: (experimental) Mustela vison, white rat, white mouse, golden hamster, chicks.

Habitat: small intestine

Locality: Northwest Oregon, U.S.A.

ENCYSTED METACERCARIAE

The metacercariae are found in Cottus rhotheus and several unidentified species of the genus Cottus. Infection in these fish

is common. The metacercariae are found encysted in the somatic musculature of the fish. The cysts are ovoid, 198.15 by 145.00. Although numerous in pepsin digest, the cysts are difficult to locate in fresh material. The cyst wall is tough, approximately 7 thick, and composed of three distinct layers, an outer opaque layer approximately 3 thick, a middle clear layer about 2 thick, and a dark innermost layer 2 thick. The metacercaria within is doubled on itself, and yellowish granules are numerous. Numerous yellowish granules are typical in the metacercarial cysts of heterophyids. The metacercariae within the capsule are generally active; the excretory bladder is typically filled with black droplets. The oral and ventral suckers are apparent as is the gonotyl, and the cuticle is distinctly spinose.

EXCYSTED METACERCARIAE

Excysted animals are very active and have comparatively well-developed reproductive organs; the testes are ovoid, and the ovary is club-shaped to spherical. The vitelline glands are evident but not fully developed. The testes are not yet producing sperm. Stained and mounted specimens average 222.5 long by 142.25. The oral sucker is 40.45 (37.5 to 45.0) in diameter. The pharynx averages 22 in diameter and is connected to the oral sucker by a definite prepharynx 8.75 in length. The intestinal ceca are present. The acetabulum averages 26.15 in diameter. The gonotyl also is present.

CERCARIAE

The cercaria and snail host for Euryhalmis cotti have not been described. Snails of the genera Oxytrema and Fluminicola were

collected from the same localities as were the fish. Snails were examined for cercariae by dissection and shedding. The results were negative. Approximately 1,000 snails were examined.

DEFINITIVE HOSTS IN NATURE

Efforts to locate animals naturally infected with Euryhelminis cotti were negative. One raccoon, Procyon lotor, a road kill was examined. The animal was found along the Sunset Highway several miles west of Beaverton, Oregon. Four sea gulls (Larus sp.) were collected from a land fill operation one-half mile north of Oregon City, Oregon. The birds were shot with a 12 gauge shot gun and taken immediately to the laboratory for examination.

RESULTS OF EXPERIMENTAL INFECTIONS

The animals experimentally infected with metacercariae have been noted previously. The fluke appears to be very adaptive in regard to maturing to the adult condition in a variety of warm blooded vertebrates and therefore has a low specificity relative to definitive hosts. In all cases where experimental infections were attempted, egg-producing adult flukes were recovered. The time required for maturation to the adult from metacercaria is relatively short as egg-producing adults were obtained from an experimentally infected hamster 17 hours after infecting this animal with metacercariae. These results may have been influenced slightly by previous treatment in the pepsin digest. This treatment has been shown to have an effect on the cyst wall of Sphaeridiotrema globulus (Macy, Berntzen, and Benz, 1967). The effect of the pretreatment is probably

insignificant since the metacercariae are relatively well-developed as was observed in excysted metacercariae. Animals infected were routinely killed after three or four days, and in all cases, mature flukes were recovered.

In comparison to body size, the eggs produced by Euryhelminis cotti are extremely large. Also, at any given time, the number of eggs present in an adult did not exceed twenty. The average number of eggs per adult is about sixteen. The number of eggs found in these flukes is very small in comparison to the number found in most adult heterophyids.

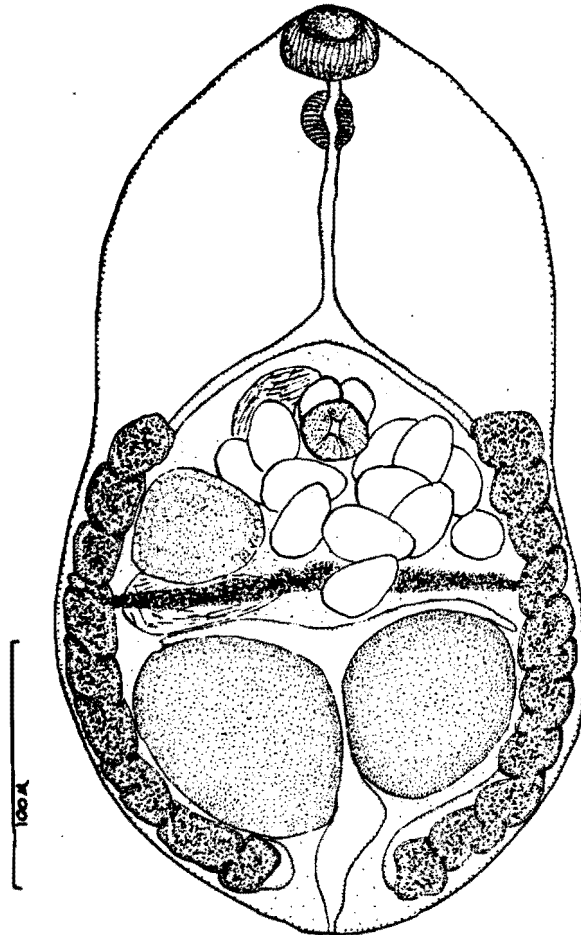


Figure 1. Euryhalmis cotti n. sp. ventral view. Adult specimen from the small intestine of an experimentally infected hamster; three days post infection. (original)

TABLE II
 OREGON STREAMS SAMPLED FOR COTTUS SPECIES
 INFECTED WITH METACERCARIAE
 OF EURYHELMIS COTTI

<u>Stream</u>	<u>Locality</u>	<u>Infection</u>
Clackamas River	Estacada	+
Crystal Springs	Portland	-
Mill Creek	Colton	+
Mill Creek	Turner	+
Molalla River	Molalla	+
Nate Creek	Colton	+
Nehalem River	Vernonia	+
Tillamook River	Coast	+
Wilson River	Coast	+

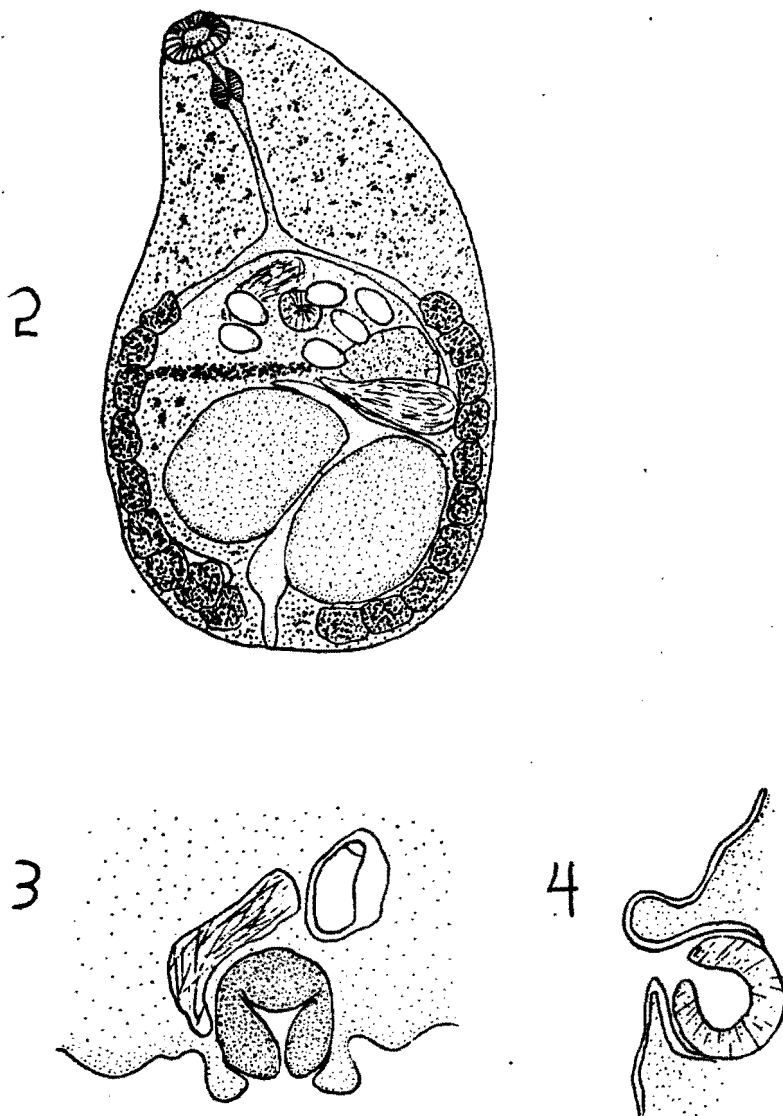


Figure 2. *Euryhalmis cotti* n. sp. dorsal view. Specimen from the small intestine of a hamster, 17 hours post infection. (original)

Figure 3. *Euryhalmis cotti* n. sp., transverse section through the gonotyl and ventral sucker. (original)

Figure 4. *Euryhalmis cotti* n. sp., longitudinal section through the gonotyl and ventral sucker. (original)

A PARTIAL REVIEW OF THE SUBFAMILY APOPHALLINAE CIUREA, 1924

DIAGNOSIS OF THE SUBFAMILY APOPHALLINAE

Since Euryhelms, to which the new species belongs, is included in the subfamily Apophallinae, a review of this group is presented.

Subfamily diagnosis: Heterophyidae: Body elongate to pyriform or elliptical. Oral sucker rather small, exceptionally large. Esophagus long, ceca terminating at or near posterior extremity. Acetabulum enclosed in genital atrium, may be large or small. Testes diagonal, occasionally juxtaposed, near posterior extremity. Seminal vesicle winding or divided. Genital pore median in middle third of body, sometimes more anterior. Ovary submedian, pretesticular; receptaculum seminis present. Uterus anterior to testes. Vitellaria extending in lateral fields almost whole length or greater part of intestine. Excretory vesicle Y-shaped. Parasites of birds and mammals.

KEY TO GENERA

- 1a Prepharynx short, length being less than esophageal length ---- 2
- 1b Prepharynx longer than esophagus ---- Pricetrema
- 2a Body much elongated, esophagus long, excretory vesicle Y-shaped, stem long and sigmoid, passing between the testes ---- Apophallus
- 2b Body quadrilateral or pyriform, esophagus short, excretory vesicle Y or T-shaped, short stem passing between testes ---- Euryhelms

DIAGNOSIS OF THE GENUS APOPHALLUS

Apophallus Lube 1909 (Figure 5)

Generic diagnosis: Heterophyidae; Apophallinae; prepharynx present but much shorter than the long esophagus. Bifurcation of intestine nearer to ventral sucker than to oral sucker. Intestinal ceca extend into the posterior end of the body well behind the testes. Ventral sucker median, about midway of the body, opening to the exterior through the genital pore. The genital sinus, which opens to the exterior through the genital pore, and in which the vas deferens and vagina terminate, is situated immediately in front of the ventral sucker. Well-developed seminal vesicle behind the ventral sucker. Testes globular or oval, in posterior third of body, the right testis usually obliquely behind the left, but the two may be side by side at the same level. Seminal receptacle in front of and to the right of the left (anterior) testis. Ovary globular, on the right side of the median line, in front of the seminal receptacle. Vitellaria extend forward to about the level of the ventral sucker, may be limited to the lateral fields in this region, but may extend inward to the median line in front of the ventral sucker. Posteriorly the lobules of the vitellaria are numerous behind and between the testes, and often are present on the dorsal and ventral sides of the testes. Transverse vitelline ducts located in the neighborhood of the boundary between the ovarian and testicular zones. Uterus disposed in a few loops in the median field, none in front of the genital pore.

DESCRIPTION OF APOPHALLUS MUEHLINGI

Type species: Apophallus muehlingi (Jagerskiold, 1899) Lube, 1909

Specific diagnosis: Apophallus: Length, 1,200 to 1,600; width, 190 to 230. Body much elongated, constricted near the middle in the region

of the ventral sucker. Cutaneous scales, 2.9 long. Oral sucker, 54 in diameter. Prepharynx well-developed (about as long as the diameter of the oral sucker according to Muehling's illustration). Pharynx, 37 in diameter. Testes globular, near the posterior end of the body; right testis obliquely behind the left. Ventral sucker of about the same size as the oral sucker. Genital sinus median, immediately in front of the ventral sucker. Seminal vesicle well-developed, S-shaped, in median line behind the ventral sucker. Ovary globular or pyriform, on right side of body between testes and sucker. Vitellaria do not extend anteriorly beyond the level of the ventral sucker and do not encroach upon the median field in this neighborhood. Posteriorly they are numerous in the median field behind the testes, between them, in front of them, and on the dorsal sides of the testes. Uterus relatively short, containing only a few eggs, which are brownish in color, 32.4 long by 18 wide.

Host: Larus ridibundus

Habitat: Intestine

Locality: East Prussia

DIAGNOSIS OF THE GENUS PRICETREMA

Pricetrema Ciurea, 1933 (Figure 6)

Generic diagnosis: Heterophyidae, Apophallinae; body elongated pyriform, spinose. Oral sucker subterminal, comparatively large. Prepharynx distinct. Esophagus shorter than prepharynx, ceca extending to near posterior extremity. Acetabulum smaller than oral sucker, just post-equatorial, opening into genital atrium. Testes situated side by side in posterior fourth of body. Seminal

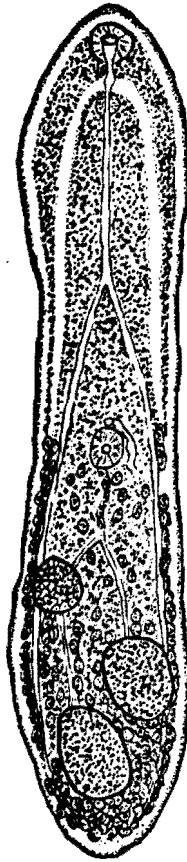


Figure 5. Apophallus muehlingi, ventral view of adult.
(redrawn from Ransom, 1920)

vesicle C-shaped, very voluminous, encircling acetabulum. Two gonotyls present, one on each side of genital pore. Ovary submedian, anterior to right testis. Receptaculum seminis posterior and dorsal to ovary. Vitellaria consisting of large, closely packed follicles, extending from level of pharynx to anterior end of testes, extensive dorsally, but confluent ventrally near intestinal bifurcation only. Uterine coils confined to intercecal field between testes and genital pore; eggs small. Intestinal parasites of marine mammals.

DESCRIPTION OF PRICETREMA ZALOPHI

Price (1932) incorrectly placed the species in the genus Apophallus. Ciurea (1933) erected the genus Pricetrema for the species. The following specific diagnosis is from Price (1932).

Type species: Pricetrema zalophi (Price, 1932) Ciurea, 1933
 Specific diagnosis: Pricetrema: Body elongated pyriform in shape, 435 by 215 to 263 wide at the level of the ovary. The cuticle is beset with small scalelike spines, 4 long by 2 wide, arranged in alternating transverse rows. Oral sucker slightly subterminal in position, 60 to 75 in diameter. Prepharynx 30 to 33 long; pharynx ovoid to spherical in shape, 29 to 33 wide; esophagus 18 long; intestinal ceca relatively wide and extending to near the posterior end of the body; their blind ends being hidden by the testes. The acetabulum is circular, 52 to 60 in diameter, situated from 235 to 259 from the anterior end of the body and enclosed in the shallow genital sinus. The genital ducts open into the anterior part of the sinus, and two elliptical gonotyls are present, one on each side of the genital aperture. The seminal vesicle is voluminous, more or less C-shaped

and lying to the right of the acetabulum; there is a sharp constriction of the vesicle near the level of the posterior margin of the acetabulum which divides it into an anterior pyriform part and a posterior globular part. The testes are somewhat triangular in outline, 81 to 96 by 81 to 110, and are situated side by side in the posterior fourth of the body. The ovary is more or less triangular in outline, 55 to 75 by 67 to 92, situated a short distance cephalad of the right testis. The seminal receptacle is spherical, 44 in diameter, and situated dorsal to the ovary and right testis. The vitellaria consists of large, closely packed follicles, which extend from the level of the acetabulum to the level of the anterior margin of the testes; the follicles are distributed over the entire dorsal surface but ventrally they are chiefly lateral except near the intestinal bifurcation where they form a distinct band across the body. The uterus consists of a few loops confined to the intercecal field between the anterior margin of the testes and the genital aperture. The eggs are 33 long by 18 wide, golden yellow, and slightly pyriform in shape.

Host: Zalophus californianus

Habitat: small intestine

Locality: North America (United States National Zoological Park, Washington, D.C.)

DIAGNOSIS OF THE GENUS EURYHELMIS

Euryhelmis Poche, 1926

Generic diagnosis: Heterophyiidae, Apophallinae; body small, flattened, leaflike. Excretory bladder Y or T-shaped. Testes, one transitory

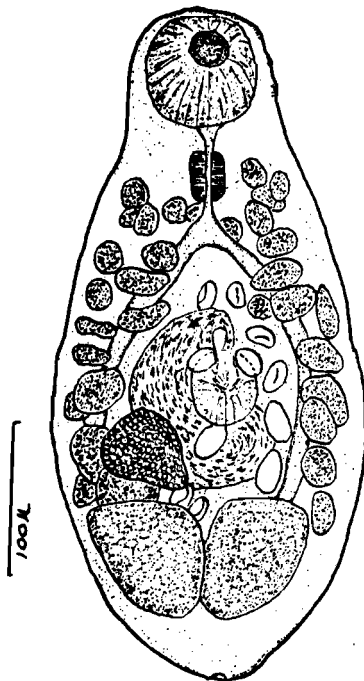


Figure 6. Pricetrema zalophi, ventral view of adult.
(redrawn from Price, 1932)

or two, spherical or lobate, in the posterior half of the body. Cirrus and cirrus pouch absent. Seminal vesicle present or absent. Uterus with only an ascending limb, relatively short, confined between the intestinal ceca. Vitelline follicles numerous, mainly lateral, extending from near the intestinal bifurcation to the posterior region of the body. Oral sucker, acetabulum, pharynx, and esophagus present. Prepharynx present or absent. Intestinal ceca long, extending to the posterior extremity of the body. Genital atrium immediately anterior to the acetabulum, overhung by a bilobed, fold-like gonotyl. Eggs operculated, with or without slight polar thickening.

KEY TO SPECIES OF EURYHELMIS

- 1a Body quadrilateral, wider than long or width and length nearly equal, oral sucker smaller than acetabulum ----- 2
- 1b Body not as above, generally pyriform, oral sucker larger than acetabulum ----- 4
- 2a Two testes present ----- 3
- 2b Right testis present, left absent, seminal receptacle often reduced or absent, egg size 29 x 14 --- E. monorchis
- 3a Vitellaria dendritic, uterus mainly in left portion of body, eggs 34 x 15 ----- E. squamula
- 3b Vitellaria follicular, uterus lies in both left and right portions of the body, eggs 29 x 16 ----- E. costaricensis
- 4a Two testes present ----- 5

- 4b Right testis present only, vitellaria dendritic
 anterior to acetabulum follicular posterior,
 eggs 28-34 x 18-23 ----- E. pyriformis
- 5a Vitellaria extend to level of intestinal bifurcation,
 eggs 20-34 x 10-17 ----- E. pacificus
- 5b Vitellaria follicular, extend to level of acetabulum,
 definite constriction in body wall at level of
 acetabulum, eggs 32.5 x 20.5 ----- E. cotti n.sp.

DESCRIPTION OF EURYHELMIS SQUAMULA

Type species: Euryhelms squamula (Rudolphi, 1819) Poche, 1926

Specific diagnosis: Euryhelms. Body wider than long, spinose, Length 600 to 1,000, width 1,400 to 1,900. Oral sucker 70 in diameter. Prepharynx nearly spherical, 50 by 60. Esophagus well-developed, length variable. The esophagus bifurcates anterior to the acetabulum, and the ceca follow the contour of the body to the posterior. Ventral sucker 100, in the middle of the body. The genital atrium opens immediately anterior to the acetabulum and is overhung by a genital papillae. Testes are lobed or globular, lie on either side of the median at the posterior of the body. Seminal vesicle is convoluted upon itself, confined to the right half of the body and opens via a short ejaculatory duct into the genital pore. No copulatory organ. Uterus consisting of three or four loops, confined mainly to the left side of the body, opens into genital atrium to the left of the ejaculatory canal. Ovary club-shaped, lies on right side, anterior to right testis. Seminal receptacle club-shaped, lies between right testis and ovary. Laurer's canal present. Vitelline follicles dendritic, extend

from intestinal bifurcation to posterior of body, following the ceca. Mehlis' gland present. Eggs operculated, with polar thickening 34×15 . Hosts: Mustela putorius L., Mustela nivalis L., Lutreola lutreola Wagner, Lutreola vison Schreber and Vulpes vulpes L. in Europe (Baer, 1931); Mustela vison Schriber, Procyon lotor, experimental house cat (McIntosh, 1936), and golden hamster (Anderson and Pratt, 1965) in U.S.A.

Habitat: small intestine

Life cycle: Anderson and Pratt (1965) described the life cycle for Euryhelmis squamula in Oregon. Prior to then a number of definitive and secondary intermediate hosts were known. The first intermediate host in Oregon was the operculate snail Bythinilla hemphilli Pilsbry.

The following is a description of the cercariae from Anderson and Pratt (1965). Lophocercous, spinose. Tail fin attached to posterior two-thirds of dorsal surface of tail, around tip and up ventral surface of posterior one-third of tail. Vigorous swimmer, body spinous. Oral sucker ringed with two rows of minute spines. Acetabulum smaller than oral sucker. Digestive tract not apparent. Twelve spherical penetration glands in posterior half of body, ducts leading in mass to oral sucker. Genital primordium present but undifferentiated. Excretory bladder large. Excretory duct from bladder ends dorsally, slightly posterior to base of tail. Flame cell formula $2(2+2+3+2)$. Measurements of living cercaria body length $146(122 \text{ to } 195)$, width $68(61 \text{ to } 85)$, tail $211(195 \text{ to } 229)$ long by $24(22 \text{ to } 29)$ at base, oral sucker $33(22 \text{ to } 44)$ and ventral sucker $18(15 \text{ to } 22)$.

Rediae are elongate, colorless, simple, smooth, active. Gut

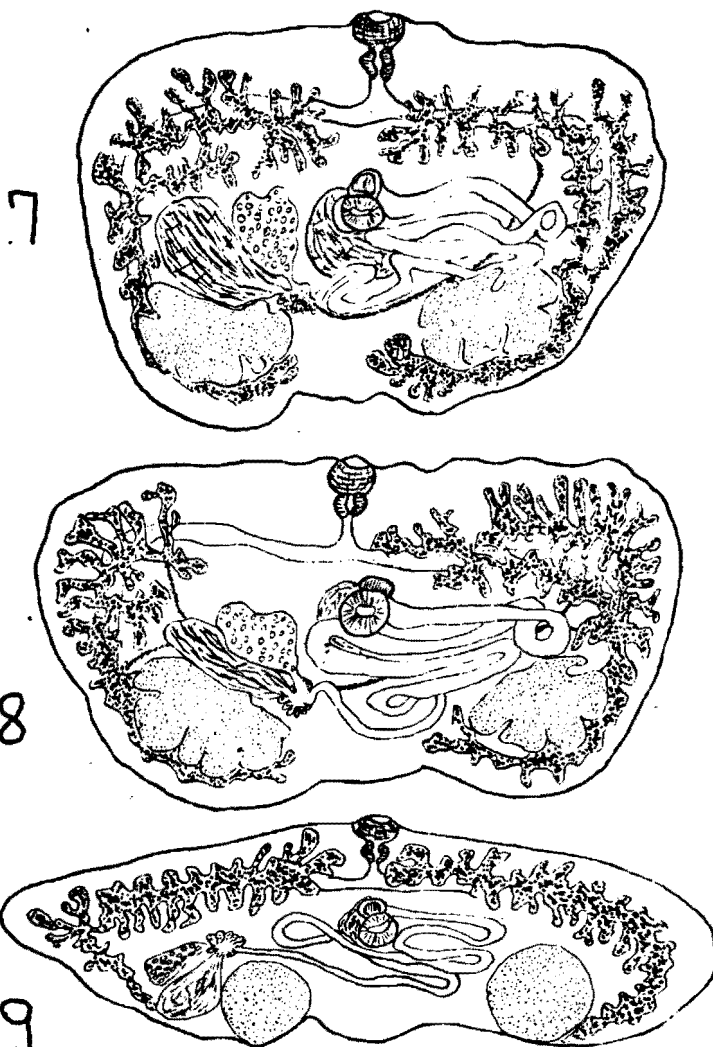


Figure 7. *Euryhalmis squamula*, ventral view of typical adult.
 Figure 8. *Euryhalmis squamula*, ventral view. Adult fluke showing atrophy of vitellaria on the right side of the animal.
 Figure 9. *Euryhalmis squamula* metacercaria
 (Figures 7, 8 and 9 redrawn from Baer, 1931)

small, indistinct. Length 674 (434 to 788), width 151 (131 to 172), pharynx 37 (32 to 41).

The metacercariae were first found in Europe (Zeller, 1867) encysted under the skin of the brown grass frog, Rana temporaria, and were later reported from Rana esculenta, Tritura cristatus and toads (Baer, 1931). In the United States the metacercariae of Euryhalmis squamula have been found in Rana pipiens in Virginia (McIntosh, 1936), in Rana aurora, Rana cascadae (Senger and Macy, 1952), and Ascaphus truei (Anderson and Pratt, 1965) in Oregon. Anderson and Pratt (1965) note that Euryhalmis squamula has a body length and width ratio that is nearly equal. It appears that this is general for material in the United States. Measurements from European material show the width to be greater than the length. (Figures 7, 8 and 9)

DESCRIPTION OF EURYHELMIS MONORCHIS

Euryhalmis monorchis Ameal, 1932 (Figures 10, 11, 12, 13 and 14)
 Specific diagnosis: Euryhalmis. Body thin, leaflike, transparent, usually much broader than long, spinose. Length, 440 (390 to 460); greatest width, 610 (520 to 690). Oral sucker 41 (32 to 48 in antero-posterior, 59 (48 to 67.2) in transverse diameter. Ventral sucker 60 (40 to 64) in antero-posterior, 69 (62 to 76) in transverse diameter. Gonotyl ("genital sucker") about 32 by 48. Pharynx large, spherical, impinging on oral sucker, 33 (32 to 40) by 35 (32 to 43). Esophagus slender, bifurcating close to ventral sucker. Intestinal ceca extending diagonally to sides, then to posterior region of body. Testis one, small, spherical, on right side near posterior end of body.

Seminal vesicle absent. Ovary dextral, elliptical or lobate. Seminal receptacle large, between ovary and excretory bladder. Uterus usually consisting of three loops confined to region limited by ventral sucker, excretory bladder, ovary and left limb of intestine. Laurer's canal present. Mehlis' gland well-developed. Vitelline follicles numerous, largely lateral, extending from stem of excretory bladder to bifurcation of intestine. Eggs operculate, undeveloped when laid, 29 (25-30) by 14 (12-14). Excretory bladder Y-shaped, large and conspicuous.

Hosts: Primary, Mustela vison Schreber and, experimentally, white rat and domestic cat; first intermediate, operculate snail, Pomatiopsis lapidaria Say; second intermediate, Rana clamitans Latreille and, experimentally, R. pipiens Shreber and R. palustris Le Conte.

Habitat: small intestine of primary host

Locality: the United States - Wisconsin, Ohio (Napoleon) and Michigan (Jackson, Ann Arbor, Flushing and Whitehall).

Cercariae are lophocercous, spinose. Total length, 360 (330 to 390); body length, 110 (100 to 120); body width, 40; tail length 250 (230 to 270). Oral sucker, 22 (20 to 24) in diameter. Approximately 12 penetration glands in third fourth of body. Ducts in a central mass anterior, bifurcating posterior to the oral sucker. Excretory bladder is large and V-shaped. Genital primordium lies between the excretory bladder and penetration glands. Ventral sucker is small, weakly developed, and ventral to genital primordium. Mother redia gives rise to daughter redia. The rediae are simple, without appendages, rugose to smooth and are active. A birth pore is present.

Length 670 (530 to 850); width 130 (120 to 150). Pharynx 32 (32 to 33) in diameter. Gut small and clear, 20 (14 to 28) by 20 (16 to 24).

Adults were recovered from the small intestine of naturally infected mink and of laboratory infected cats and rats. Specimens from rats were found to be smaller than those from mink. Mature adults containing as many as 52 eggs were obtained three and one-half days post infection from rats. The flukes were located in the duodenum at this time. After sixteen days post-infection, the majority of flukes were observed in the jejunum.

One characteristic feature of the adult worm is the transitory nature of the single testis and associated structures. The majority of the specimens observed lacked the male genital system entirely. Only 2.7 percent of the Euryhelmis monorchis observed possessed a testis which was always on the same side of the body as is the ovary. Ameel (1932) postulated that the testis is present for only a brief period in the life of the adult, degenerating after the production and discharge of sperm.

The first intermediate host is the operculate snail, Pomatiopsis lapidaria. Cercariae were present in 5.1 percent of the snails collected near Ann Arbor, Michigan (Ameel, 1932). They are active swimmers, the periods of activity alternating with periods of rest when the cercaria sinks to the bottom in a characteristic flexed position. The cercariae of Euryhelmis monorchis differ from other heterophyid cercariae in that they lack eyespots and a row of double spines around the oral aperture.

The tail of the cercaria is several times longer than the body. The finfold extends from near the base of the tail dorsally to the

tip and is present on the posterior third, ventrally. The tip of the tail is observed to be very contractile and in continuous motion in an active cercaria.

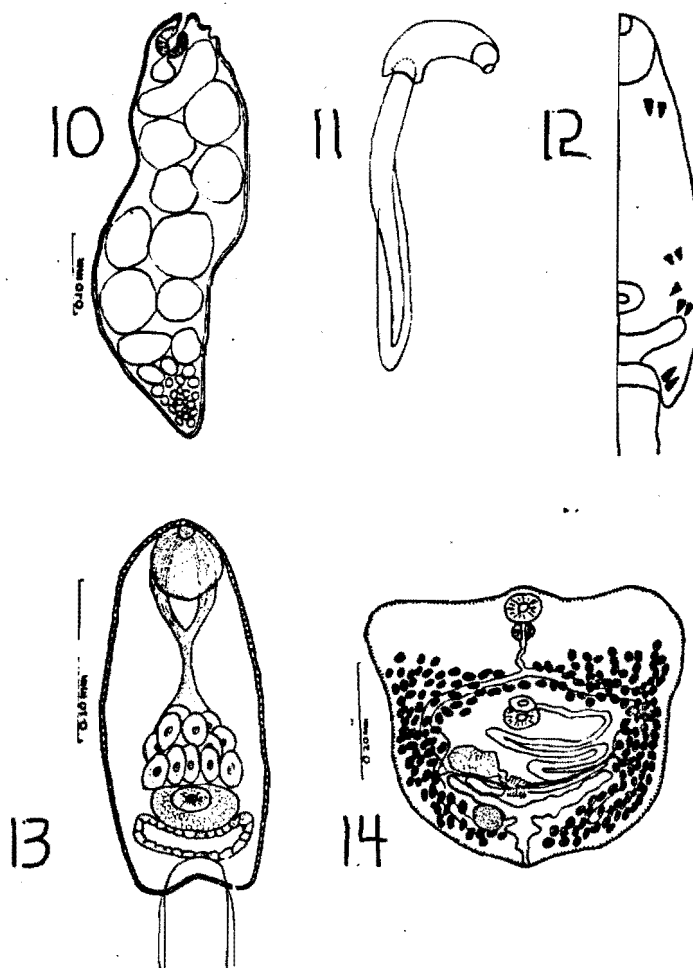
Numerous cystogenous glands are present. The genital primordium appears as a mass immediately posterior to the penetration glands. The ventral sucker is weakly developed. The excretory vesicle is large and conspicuous. Excretory tubules were not traced but nine flame cells per side were observed with an apparent pattern of $2(2+2+3+2)$.

The cercariae penetrate frogs and tadpoles, and a blister is formed at the point of entry. Blisters were observed over the entire body surface of tadpoles, but they were confined largely to the limbs and the lateral and ventral areas of adults that were experimentally exposed to cercariae. The authors failed to find infected tadpoles in nature, but 66 percent of Rana clamitans collected from endemic areas were infected with the metacercariae. Rana pipiens and Rana palustris from the same area were negative although these species were readily infected in the laboratory.

Metacercariae are located in the subcutaneous connective tissue. The cyst consists of a thin inner wall apparently secreted by the cercariae and a thick pigmented layer of host origin. Metacercariae are infective forty-five days post infection. Ninety-one and three tenths percent of the metacercariae have a single testis present; 8.7 percent are lacking the male reproductive structures.

DESCRIPTION OF EURYHELMIS PACIFICUS

Euryhelms pacificus Senger and Macy, 1952 (Figures 15, 16, 17, 18 and 19)



- Figure 10. Redia of Euryhelminis monorchis
Figure 11. Cercaria of Euryhelminis monorchis
Figure 12. Flame cell pattern of Euryhelminis monorchis
Figure 13. Cercaria of Euryhelminis monorchis showing penetration glands.
Figure 14. Euryhelminis monorchis, ventral view of a typical adult.
 (Figures 10 through 14 redrawn from Ameel, 1938)

Specific diagnosis: Euryhelmis. Body thin, leaflike, transparent, spinose overall, pyriform or elongate. Length 660 to 1040; width 340 to 680. Oral sucker either terminal or subterminal 35 to 87 long by 49 to 90 in diameter. Pharynx large, spherical, 35 to 59 in diameter, connected to the oral sucker by a short but definite prepharynx from 4 to 100 but averaging 39. 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 35 to 62 in diameter, situated slightly pre-equatorial. Two testes ovoid or lobed, opposite or oblique in the posterior region of the body. Right testis usually more flattened antero-posterior and wider transversely than left testis. Right testis 180 to 320 by 100 to 160. Left testis 150 to 260 by 140 to 180. 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 14 to 50. Uterus consisting of three or four loops confined between intestinal ceca, testes and acetabulum, opens into genital atrium to the left of ejaculatory canal. Ovary, located on right side anterior to right testis, generally club-shaped, 100 to 250 by 70 to 140. Seminal receptacle located between right testis and ovary, spherical or club-shaped 70 to 230 by 60 to 160. Laurer's

canal originates as a medial elongation of the seminal receptacle and after some coiling opens on the median dorsal surface somewhat posterior to ootype 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, 20 to 34 by 10 to 17 in preserved material; 14 by 31 in fresh material. Excretory bladder Y or T-shaped, extending forward from the posterior end of the body between the testis and bifurcating immediately anterior to them.

Hosts: Mustela vision Schreber, Ondatra zibethica Linnaeus and (experimental) white rat, golden hamster and the field mouse Peromyscus maniculatus Wagner; adults without eggs in Sorex bendirii palmeri.

Habitat: small intestine

Locality: Oregon, U.S.A.

Adults are found in Mustela vision, Sorex bendirii palmeri, and Ondatra zibethica. Experimentally the flukes developed in the white laboratory rat, golden hamster and the deer mouse Peromyscus maniculatus. The flukes found in the shrew were devoid of eggs, and the development of eggs in the deer mouse was poor. The absence or poor development of eggs is suggestive of an incompatibility between parasite and host.

It was observed that the flukes required forty-eight hours to reach maturity in the white rat and seventy-two hours in the golden hamster. The adult flukes inhabit the duodenum of the host.

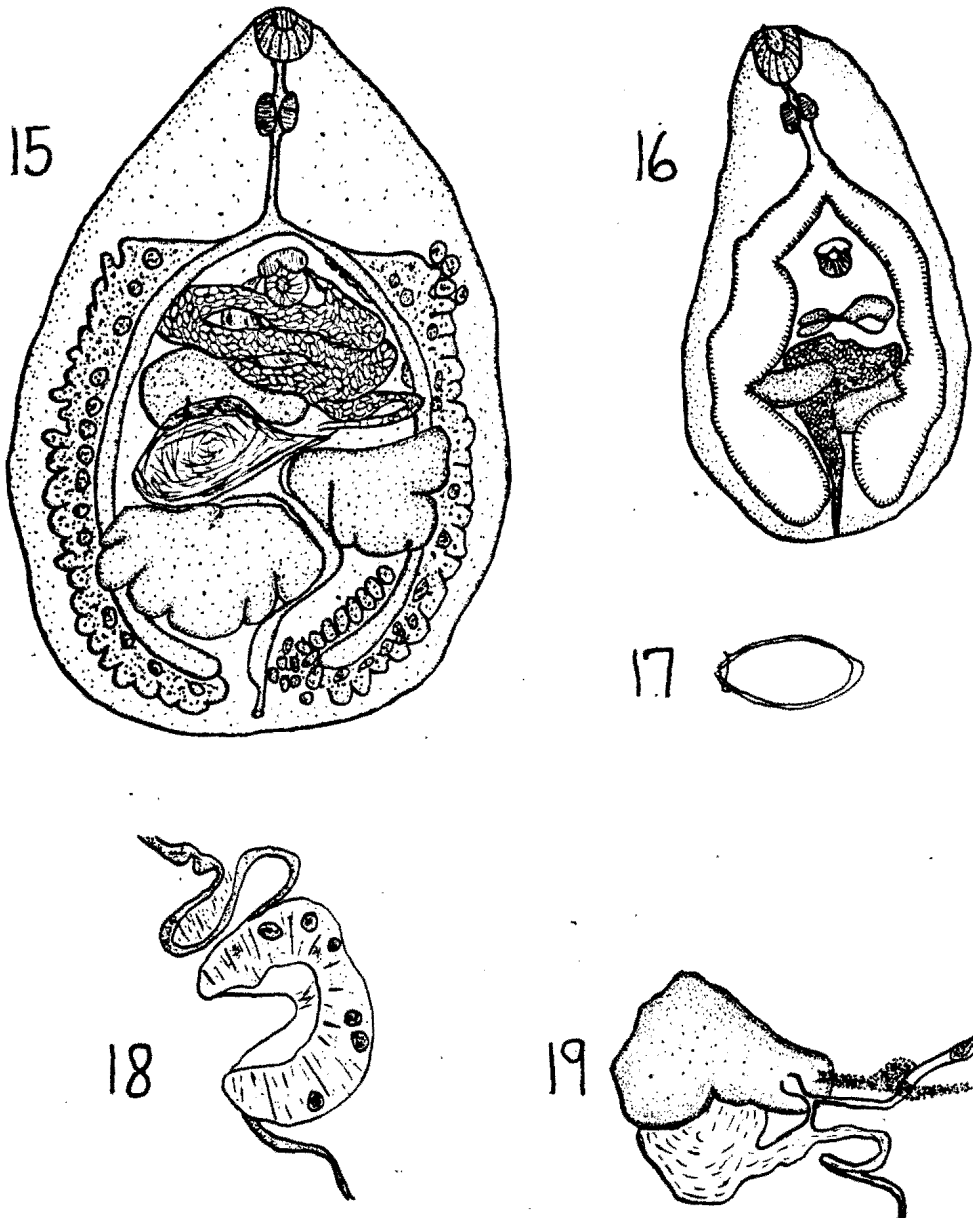


Figure 15. *Euryhelmsis pacificus* adult, ventral view.
Figure 16. Excysted metacercaria of *Euryhelmsis pacificus*.
Figure 17. Egg of *Euryhelmsis pacificus*.
Figure 18. Ventral sucker and gonotyl of *Euryhelmsis pacificus*, longitudinal section.
Figure 19. Ootype region of *Euryhelmsis pacificus*.
 (Figures 15 through 19 redrawn from Senger and Macy, 1952)

The snail intermediate host and consequently the cercariae for Euryhelms pacificus are not known. The metacercariae are reported only in the Pacific Giant Salamander, Dicamptodon ensatus in Oregon. The larval form of this amphibian is abundant in many of rocky streams of the Coast and Cascade Ranges in Oregon. Incidence of infection of the salamanders examined was found to be approximately 90 percent. The number of metacercariae per animal varies from five or six to several hundred with an average being approximately fifty cysts per animal. The cysts are located in the striated muscle of the host and not in the subcutaneous connective tissue where, in frogs, the cysts of Euryhelms monorchis, and Euryhelms squamula are found. It appears there is no preference for encystment as the metacercariae are rather evenly distributed throughout the tissue. The cysts are surrounded by a yellowish loose connective tissue capsule of host origin.

Excysted metacercariae are active, and the reproductive organs are well-developed. The intestinal ceca of many of the excysted metacercariae are packed with discrete bodies of uncertain nature. The excretory bladder contains discrete droplets, presumably of respiratory by-products. The gonads and genital ducts are easily seen in living metacercariae; testes are spherical and not producing sperm; the ovaries are oval or club-shaped; the seminal receptacle is spherical in shape and is lined with cilia as were the oviduct, Laurer's canal, and the duct leading to the ootype. The cilia beat actively. The primordia of the uterus and seminal vesicle appear to be devoid of cilia.

The life cycle of Euryhelms pacificus is completed when the infected salamander, Dicamptodon ensatus is eaten by a suitable host.

The natural host in nature for this fluke is mink with the muskrat being apparently a suitable but probably not uncommon one.

DESCRIPTION OF EURYHELMIS PYRIFORMIS

Euryhelmis pyriformis Webster and Wolfgang, 1956 (Figure 20)

Specific diagnosis: Euryhelmis. Body thin, leaflike, transparent, pyriform or elongate, spinose anterior, lacking posterior. Length 2,250; width 570 at the level of the acetabulum. Oral sucker weakly muscular and terminal 100 in diameter. Pharynx 50 by 70 and is contiguous with oral sucker. Esophagus 600 branches 250 from acetabulum into two ceca which terminate a short distance behind the testis. Acetabulum 90 transverse diameter and is depressed within a large papilliform gonotyl 230 transverse diameter. The gonotyl appears to surround the sucker and folds over the genital pore immediately anterior to acetabulum. Single testis large lying near the left cecum in posterior of body. The seminal vesicle is club-shaped, bends medially beneath the left edge of the gonotyl, and terminates in common with short ejaculatory duct, at the genital pore. Ovary is triangular and lies lateral and slightly posterior to seminal vesicle, broad at posterior becoming narrow at anterior; along the left side it follows the contour of the ceca. Seminal receptacle, 150 in transverse diameter, contiguous with, and posterior to, the ovary. It opens in common with the oviduct and common yolk duct into the ootype. There is no distinct Mehlis' gland, and Laurer's canal was not observed. Uterus consists of three compact and transverse coils. Eggs 28 to 34 by 18 to 23 and have a distinct operculum. Vitelline glands of two morphological types: dendritic

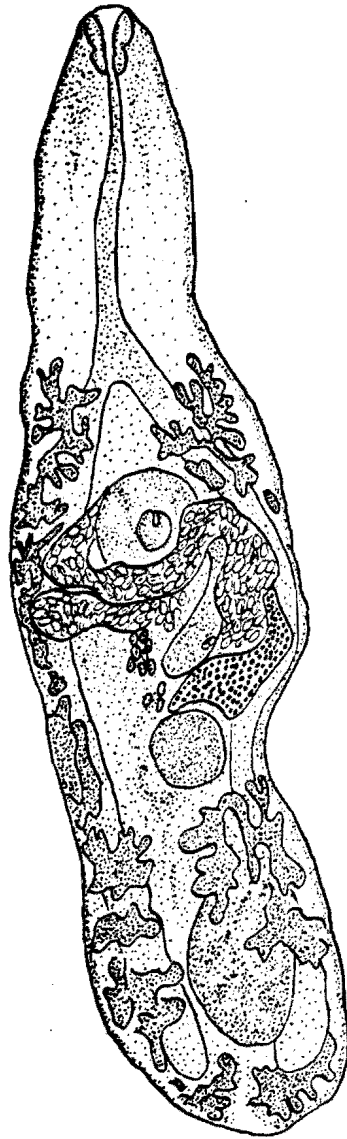


Figure 20. Euryhalmis pyriformis, dorsal view of adult.
(redrawn from Webster and Wolfgang, 1956)

anterior to acetabulum and follicular in the posterior of the fluke. They are confined to the ceca and extend from the end of the esophagus to the posterior of the body. Excretory bladder Y-shaped, terminating in a papilliform salience.

Host: Mephitis mephitis

Habitat: small intestine

Locality: Ste Anne de Bellevue, Province of Quebec

DESCRIPTION OF EURYHELMIS COSTARICENSIS

Euryhelmis costaricensis Brenes and Arroyo, 1960 (Figure 21)

Specific diagnosis: Euryhelmis. Body thin, quadrangular, transparent spinose. Length from 1,074 to 1,281; width 716 to 854. Oral sucker subterminal 73 to 80 by 53 to 82. Pharynx 64 by 48. Esophagus 115 to 161 long, 11 to 13 in width. The intestinal ceca extend obliquely to the sides, then follow the contour of the body to the posterior. Ventral sucker slightly pre-equatorial, length 96 to 101; width 96 to 110. Gonotyl situated immediately anterior to the acetabulum 73 by 52. Testes lobed, situated in the posterior of the body, bounded by the ceca, the right testis being located more posterior than the left. Right testis 277 transversely, 193 antero-posterior. Left testis 230 transversely, 184 antero-posterior. Ovary anterior to the right testes, elongated transversely, 29 by 230. Uterus confined between intestinal ceca, acetabulum and testes, opens into the genital atrium. Neither Mehlis' gland nor Laurer's canal were observed. Vitelline glands follicular, mostly extracecal but may be cecal and intercecal, extend from the cecal bifurcation to the posterior of the body. Seminal receptacle located between right testes and ovary, transversely

elongated, 184 by 298. Eggs small operculated, 29 by 16.

Host: Mustela frenata costaricensis

Habitat: small intestine

Locality: El Alto, Tres Rios, Provincia de Cartago.

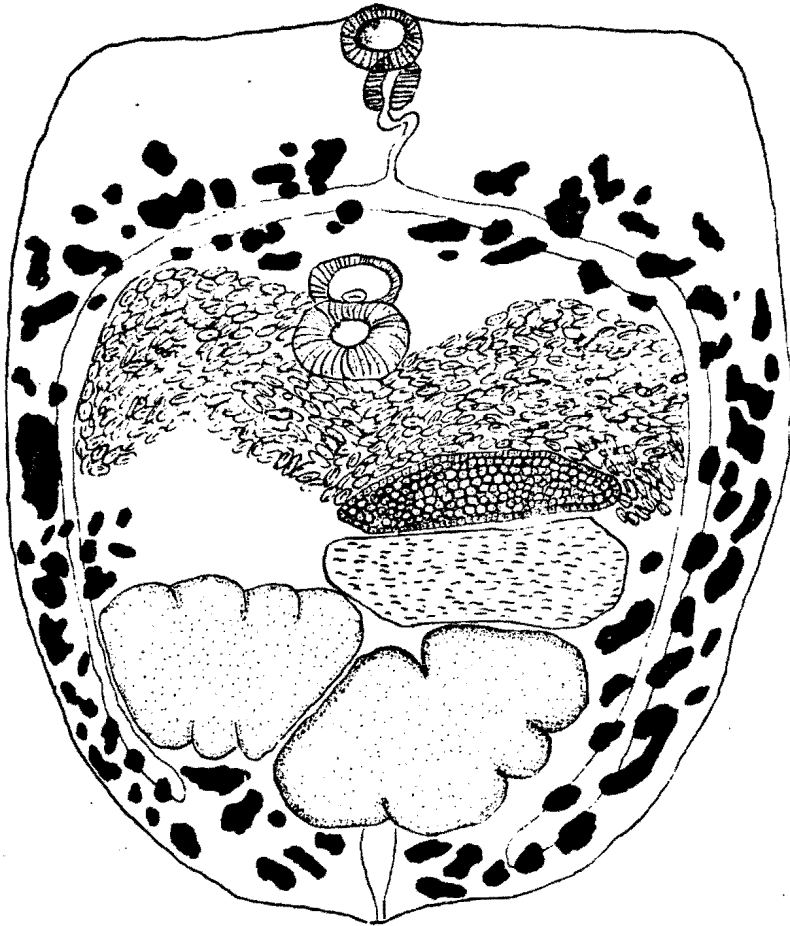


Figure 21. Euryhalmis costaricensis, dorsal view of adult.
(redrawn from Brenes and Arroyo, 1960)

TABLE III

COMPARATIVE MEASUREMENTS OF SOME MEMBERS OF
THE SUBFAMILY APOPHALLINAE CIUREA 1924
(MEASURED IN MICRONS)

	<u>Euryhelms</u> <u>squamula</u>	<u>Euryhelms</u> <u>monorchis</u>	<u>Euryhelms</u> <u>pacificus</u>	<u>Euryhelms</u> <u>pyriformis</u>
Body length	600-1000	440(390-460)	660-1040	2250
Body width	1400-1900	610(520-690)	340-680	570
Oral Sucker diameter	70	59(48-67)	49-90	100
Pharynx	50-60	33 x 35	35-59	50 x 70
Pre-Pharynx			39(4-100)	
Esophagus				600
Ventral Sucker	100	60(40-64)	35-62	90
Right Testis length			180-320	
width			100-160	
Left Testis length		absent	150-260	absent
width			140-180	
Gonotyl		32 x 48		
Ovary length			100-250	
width			70-140	
Seminal Receptacle	club-shaped		70-230 x 60-160	150
Egg	30-32 x 14-16	25-30 x 12-14	20-34 x 10-17	28-34 x 18-23

TABLE III (continued)

	<u>Euryhelmis costaricensis</u>	<u>Euryhelmis cotti</u>	<u>Pricetrema zalophi</u>
Body length	1074-1281	419	435
Body width	716-854	222	215-263
Oral Sucker diameter	53-82	45.65	60-75
Pharynx		26.25 x 26	29-33
Pre-Pharynx		8	30-33
Esophagus		91.25	18
Ventral Sucker	96-110	28.95	52-60
Right Testis length	277	97	81-96
width	193	67.50	81-110
Left Testis length	230	79.7	81-96
width	184	66.5	81-110
Gonotyl	73 x 52		bilobed
Ovary length	29	56	55-75
width	230	44	67-92
Seminal Receptacle	184 x 298		44
Egg	29 x 16	32.5 x 20.5	33 x 18

TABLE III (continued)

	<u>Apophallus donicus</u>	<u>Apophallus meuhlingi</u>	<u>Apophallus crami</u>
Body length	500-1140	1200-1600	1500-1900
Body width	200-390	190-230	279-341
Oral Sucker diameter	65-85	54	45-60
Pharynx	30-44	37	30
Pre-Pharynx	short	short	short
Esophagus	135-265		337
Ventral Sucker	45.58 x 48.60		52-62
Right Testis length	80-200	globular	150-160
width	60-200		187-210
Left Testis length	80-200	globular	180-187
width	60-200		180-187
Gonotyl	bilobed		bilobed
Ovary length	65-140	globular	75-97
width	40-120		
Seminal Receptacle	35-90 x 60-130		50-67 x 60-82
Egg	30-35 x 16-20	32.4 x 18	33 x 25

TABLE III (continued)

	<u>Apophallus</u> <u>lerouxi</u>	<u>Apophallus</u> <u>ventrus</u>	<u>Apophallus</u> <u>brevis</u>
Body length	850-930	950-1150	600-900
Body width	168-172	250-550	130-260
Oral Sucker diameter	24-26	60	30-50
Pharynx	16-18		20-28
Pre-Pharynx	31-36		20
Esophagus	123-144		
Ventral Sucker	33-37	60	35-55
Right Testis length	74-90	150-200	45-120
width	74-90	150-200	45-115
Left Testis length	70-82	150-200	45-120
width	70-82	150-200	45-115
Gonotyl		bilobed	
Ovary length	74	150	60-90
width	74	100	50-80
Seminal Receptacle			40-80
Egg	29 x 17	26-32 x 18-22	36-40 x 16-22

DISCUSSION

Euryhelmsis cotti resembles Euryhelmsis pacificus more than it does the other members of the genus. It is, however, easily differentiated from Euryhelmsis pacificus on the basis of its smaller body size and the nature and extension of the vitellaria. The vitellaria in Euryhelmsis cotti extends from the level of the acetabulum to the posterior of the body; whereas, the vitellaria in Euryhelmsis pacificus extends from the intestinal bifurcation to the posterior of the body. Euryhelmsis cotti differs from all the members of the genus whose life cycles have been partially or fully described in that Euryhelmsis cotti utilizes a fish for the second intermediate host. Amphibians serve as the second intermediate host for Euryhelmsis squamula, Euryhelmsis monorchis and Euryhelmsis pacificus. (See Table II.) Another notable difference is the size of the eggs in relation to body size, and the small number of eggs found in Euryhelmsis cotti. The eggs of Euryhelmsis cotti are largest in relation to the body of the fluke than are the eggs of any other species of the genus.

The genus Euryhelmsis was proposed by Poche, 1926 for Distomum squamula (Rudolphi 1819) which was poorly described. A complete description of Euryhelmsis squamula was published by Baer (1931) and Collot (1946) who gave measurements of the adult. Ameel (1938) described Euryhelmsis monorchis with a report on its life cycle. Ameel placed the genus Euryhelmsis in the subfamily Heterophyinae. Price (1940) reviewed the superfamily Opistorchioidea, placing the genus

Euryhelmis in the subfamily Apophallinae Ciurea, 1924 of the family Heterophyidae. Price's conception of the subfamily was based on the following characteristics: (1) acetabulum relatively well-developed, enclosed in a small muscular genital sinus, (2) genital aperture pre-acetabular, and (3) gonotyl single or double, papillae-like.

Morozov (1950) erected the subfamily Euryhelminae for the genus Euryhelmis based on Euryhelmis squamula and Euryhelmis monorchis. He used the following characteristics as basis for the subfamily: (1) a single lobed papilla-form gonotyl, located in front of the acetabulum, (2) vitellaria occupying all the lateral space from the pharynx to the posterior of the fluke, (3) body wider than long, and (4) uterus typically with three loops. Senger and Macy (1952) described Euryhelmis pacificus with notes on its life cycle and amended the genus. They were in agreement with Price (1940) that the genus should be placed in the subfamily Apophallinae. Webster and Wolfgang (1956) described Euryhelmis pyriformis from a single specimen, and they agreed with Price (1940) and Senger and Macy (1952) that the genus Euryhelmis showed affinities with the Apophallinae. Yamaguti (1958) amended Euryhelminae to Euryhelminthinae using the following characteristics: (1) Body wider than long, (2) vitellaria extensively developed extending the entire length of the ceca, and (3) a T-shaped excretory vesicle. Brenes and Arroyo (1960) described Euryhelmis costaricensis and suggested a revision of the genus. They were in agreement with Morozov (1950), supporting the view that the genus Euryhelmis should be assigned to the subfamily Euryhelminae Morozov, 1950. Euryhelmis cotti is described in this paper. The author is in accord with those placing the genus in Apophallinae.

Morozov (1950), Yamaguti (1958) and Brenes and Arroyo (1960) have used characters that are generic and/or specific as having sub-family significance. On the basis of these characters, they argue in favor of the erection of a subfamily to contain the genus Euryhelmsis. Yamaguti (1958) and Brenes and Arroyo (1960) acknowledge the species pacificus and pyriformis as being properly placed generically but then appear to ignore them at the subfamily level.

CONCLUSION

A new species of the genus Euryhelmsis is described in this paper. Its life cycle was partially determined, and data on the distribution of the species in Northwest Oregon is presented. A partial review of the subfamily Apophallinae is presented with a complete review of the genus Euryhelmsis. Original keys were prepared for the genera of the subfamily Apophallinae and for all the described species of Euryhelmsis.

The author is in accord with Price (1940), Senger and Macy (1952), and Webster and Wolfgang (1960) that the genus Euryhelmsis is properly placed in the subfamily Apophallinae Ciurea 1926.

The argument that the genus Euryhelmsis should not be included with the Apophallinae is considered invalid. The descriptions of Euryhelmsis pacificus, pyriformis and cotti clearly indicate a close relationship with the other genera of the subfamily.

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APPENDIX A
EXPERIMENTAL INFECTION OF LABORATORY ANIMALS:
SOLUTIONS AND TECHNIQUES

EARLE'S SALT SOLUTION

<u>Component</u>	<u>gm/liter</u>
NaCl	6.800
KCl	0.400
$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$	0.125
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.200
Glucose	1.000
CaCl_2 (anhydrous)	0.200
Phenol Red	0.010
NaHCO_3	2.200

Stock Solution 20 (x)

Prepare one liter by multiplying the quantities by 20 and combining all of the ingredients except the NaHCO_3 . Dispense into 50 ml containers and freeze.

Working Solution

Dilute 50 ml of stock solution to 900 ml, add 20 ml 5 percent sodium bicarbonate in distilled water and dilute to one liter.

0.9 PERCENT SALINE

<u>Component</u>	<u>Quantity</u>
NaCl	9.0 gm
Distilled water	1000.0 ml

PEPSIN SOLUTION (0.5 PERCENT SOLUTION)

<u>Component</u>	<u>Quantity</u>
Pepsin 1: 10,000	5 gm
Concentrated HCl	5 ml
0.9 percent saline	995 ml

The pepsin is added to the acid saline solution, and the solution is allowed to reach a temperature of 37°C with gentle agitation on a magnetic stirrer for one hour (vigorous agitation would denature the enzyme). Freshly ground fish is then added to the pepsin solution, one gram ground fish per 20 mls pepsin solution. The mixture is maintained at 37°C with gentle agitation for one hour.

TRYPsin SOLUTION (1 PERCENT SOLUTION)

Trypsin ----- 0.1 gm

Earle's salt solution -----10.0 ml

Add 5 percent NaHCO₃ to the above solution to obtain the desired pH.

Metacercarial cysts removed from the pepsin digest are added to the trypsin solution. One may observe the excystment under either a dissecting or compound microscope.

Other techniques of excystation are available: (1) One may mechanically disrupt the cyst wall with needles or other suitable devices, and (2) it has been shown that Paragonimus westermani metacercariae excyst after two hours when placed in warm 0.5 percent to 1.0 percent sodium bicarbonate. Macy (1952) excysted the metacercariae of Cephalophallus obsurus by placing them in 0.1 to 0.5 molar sodium hydroxide.

INFECTION OF LABORATORY ANIMALS

If the experimenter is not experienced in the procedures explained below, it would be an excellent idea to carry them out using distilled water only on a practice animal. This will enable one to become somewhat proficient without the possible waste of cysts. This is suggested because of the difficulty in obtaining metacercarial cysts in some cases.

Infection by pipette

This method is fast and relatively easy. The advantages of this technique are: (1) limited material needed and (2) no danger of killing the animal by over-anesthetizing (anesthetic not used). The disadvantages are: (1) animal may "spit" out the cyst (2) it is generally ineffective in giving large numbers of cysts at one feeding, and (3) the handler may be bitten by the animal being fed.

The cysts are first concentrated in the bottom of a Stender dish or a concave slide. They are then washed in distilled water to remove the salt (saline may cause the animal to regurgitate). Next, the animal to be infected is grasped behind the neck and held gently but firmly with one hand, the other hand being used to handle the pipette (care must be made in selecting a pipette that has no sharp edges). The concentrated cysts are sucked into the pipette and then the pipette is placed about mid-way into the mouth of the animal (the mouth is generally open as the animal is attempting to nibble the finger tips of the handler), and a drop of the infective suspension is placed in the animal's oral cavity. Care must be taken at this point to make sure that the animal swallows before one

introduces another drop of fluid, if the animal has not had time to swallow, the newly introduced material will dribble down his chin and be wasted. After the administration of the metacercariae, it is desirable to give the animal a few drops of fresh water to wash down material that may be remaining in the mouth.

Infection by Stomach Tube

Use a stomach tube fitted to a needle and attached to a 2.5 ml syringe. For rats, hamsters, and mice, Tygon tubing 15 cm long and 1 mm wide is used. When feeding mice, the amount of fluid should not exceed 1 ml. When introducing the stomach tube into the esophagus, immediately withdraw the tube if the animal begins to choke or exhibits difficulty in breathing. These symptoms indicate that the tube is in the trachea. If pressure is encountered when the fluid is expelled, do not force the fluid through the tube as this is indicative of being in the trachea. Withdraw the tube and try again.

ANESTHETIZING SMALL MAMMALS

When infecting small laboratory animals, it is often convenient to lightly anesthetize them prior to infection to facilitate handling. Place the animal in a jar containing cotton soaked with chloroform or ether. Watch the animal closely, and remove it as soon as it becomes disoriented and sleepy. It is important not to allow the animal to remain in the jar too long as (especially mice and hamsters) they are rapidly affected by the anesthetic. When infecting chicks it is advised not to use an anesthetic as they are extremely susceptible to chloroform and ether.

APPENDIX B
FIXATION AND STAINING: SOLUTIONS AND TECHNIQUES

CARMINE STAINS

Grenacher's Alcoholic Borax Carmine

Carmine ----- 3 gm

Borax (Sodium tetraborate)----- 4 gm

Distilled water ----- 100 ml

Boil until the carmine is dissolved then add:

70 percent methanol or ethanol --- 100 ml

Allow the solution to stand at room temperature for several days then filter it.

MAYER'S PARACARMINE

Carminic acid ----- 1.0 gm

Aluminum chloride ----- 0.5 gm

Calcium chloride ----- 4.0 gm

Alcohol, 70 percent ----- 100.0 ml

Heat the mixture until all the ingredients are dissolved, then filter.

SEMICHON'S CARMINE

Mix equal parts glacial acetic acid and distilled water. Add powdered carmine until a saturated solution is obtained. Place this solution in a water bath, maintaining the temperature at 95 to 100° centigrade for fifteen minutes. Cool and let settle. Decant

and filter the supernatant.

EHRlich'S ACID HEMATOXYLIN

Water -----	100 ml
95 percent ethanol -----	100 ml
Hematoxylin -----	2 gm
Glycerine -----	100 ml
Glacial acetic acid -----	10 ml
Ammonia alum -----	20 gm

Dissolve the hematoxylin in alcohol, then add acid, water and glycerine in the aforementioned sequence. Mix well, add the alum (aluminum ammonium sulfate). Allow the solution to ripen in the sunlight for about two months, opening the bottle occasionally.

EOSIN (STOCK)

Eosin powder -----	2 gm
95 percent methanol -----	200 ml

Dilute 1:10 in 95 percent methanol before using.

FAST GREEN

Fast green solution (1 percent)--	1 ml
95 percent ethanol -----	49 ml

GILSON'S FIXATIVE

Distilled water -----	220 ml
70 percent ethanol -----	25 ml
80 percent nitric acid -----	4 ml
Glacial acetic acid -----	1 ml
Mercuric chloride -----	5 gm

TECHNIQUES OF FIXATION AND STAININGWhole Mounts

Place one fluke on a glass slide in a drop or two of 0.9 percent saline. Place a cover slide over the animal and observe under a dissecting microscope. When it appears that the animal is somewhat relaxed, place a piece of lens paper against the air water interface simultaneously placing a drop of Gilson's on the opposite side of the cover slip. Capillary action will draw off the saline replacing it with Gilson's. Movement will cease, and the animal will take on a dark color, when this occurs remove the lens paper and let the slide set for fifteen minutes. Place a drop of Gilson's near the cover slip, floating it then lift the cover slip off the fluke. Next float the animal from the surface of the slide. This can be done by holding the cover slip at about 85° to the surface of the slide and gently nudging the fluke until it no longer adheres to the slide, then squirt fixative on it, washing it from the slide into a Stender dish containing Gilson's. Then proceed as follows:

- | | |
|------------------------------|-----------------------|
| (1) Gilson's ----- | 12 hours to overnight |
| (2) 35 percent ethanol ----- | 30 minutes each |
| (3) 50 percent ethanol ----- | 30 minutes each |
| (4) 70 percent ethanol ----- | 30 minutes each |
| (5) 70 percent ethanol ----- | 2 hours to overnight |
| (6) 70 percent ethanol ----- | 30 minutes each |
| (7) 70 percent ethanol ----- | 30 minutes each |
| (8) 50 percent ethanol ----- | 30 minutes each |
| (9) 35 percent ethanol ----- | 30 minutes each |

(10) Ehrlich's Hematoxylin -----	overnight
(11) 35 percent ethanol -----	30 minutes
(12) 50 percent ethanol -----	30 minutes
(13) 70 percent ethanol -----	30 minutes
(14) destain * -----	-
(15) 80 percent ethanol -----	30 minutes
(16) 95 percent ethanol -----	30 minutes
(17) counterstain (fast green) -----	10 to 20 seconds
(18) 95 percent ethanol -----	15 minutes
(19) 100 percent isopropyl -----	60 minutes
(20) xylene -----	30 minutes

Mount in balsam, place slides in oven with the temperature maintained at about 37 to 50° centigrade for several days to one week. If one wishes to employ a carmine stain, place the animals at step (7) in the carmine stain and allow them to remain overnight, then wash in 70 percent ethanol and destain and go to step (15) following the flow sheet to its conclusion.

* Place one to several flukes in Stender dish containing 70 percent ethanol, then add a drop of strong destain, observing the fluke(s) under a dissecting microscope. When the stain appears to be of the desired intensity, remove the flukes from the solution, placing them in fresh 70 percent ethanol.

Sections

Flukes to be sectioned are not flattened. Place living flukes in a Stender dish containing Gilson's fixative and allow them to remain in the fixative overnight, then dehydrate as follows:

- (1) 30 percent ethanol ----- 30 minutes
- (2) 50 percent ethanol ----- 30 minutes
- (3) 70 percent ethanol ----- 30 minutes
- (4) 70 percent ethanol + iodine ----- overnight
- (5) 70 percent ethanol ----- 30 minutes
- (6) 70 percent ethanol ----- 30 minutes
- (7) 80 percent ethanol ----- 30 minutes
- (8) 95 percent ethanol ----- 30 minutes

(tint small flukes by adding small amount of eosin to the 95%)

- (9) 100 percent isopropyl ----- at least 2 hours

Then proceed with dealcoholization (keep containers tightly covered)

- (1) 1 part toluene, 3 parts 100 percent isopropyl alcohol-- 30 minutes
- (2) 1 part toluene, 1 part 100 percent isopropyl alcohol -- 30 minutes
- (3) 3 parts toluene, 1 part 100 percent isopropyl alcohol - 30 minutes
- (4) 100 percent toluene ----- 2-3 hours

(Change to fresh toluene once in this period).

The next step is to infiltrate with paraffin. For material that is to be microtomed into sections of 12 micra or less, hard paraffin is used. The melting point of hard paraffin is 56° centigrade. The oven is maintained at 57° centigrade. Keep a supply of melted paraffin in the oven. Preliminary infiltration is as follows:

- (1) Remove toluene, cover the material with warm mixture of 1 part melted paraffin and 3 parts toluene. Leave this uncovered in oven for 30 minutes.
- (2) Place flukes in 1 part paraffin and 1 part toluene for 30 minutes.
- (3) 3 parts paraffin, 1 part toluene for 30 minutes.

(4) Place flukes in pure paraffin, changing to fresh paraffin every 20 minutes for 3 changes.

(5) Embed and cut ribbon.

(6) Afix to slide.

Treat slides as follows:

- | | |
|---|--------------------------|
| (1) Xylene ----- | 2 minutes |
| (2) Xylene + 100 percent isopropyl alcohol -- | 2 minutes |
| (3) 100 percent isopropyl alcohol ----- | 2 minutes |
| (4) 95 percent ethanol ----- | 2 minutes |
| (5) 80 percent ethanol ----- | 2 minutes |
| (6) 70 percent ethanol ----- | 2 minutes |
| (7) 50 percent ethanol ----- | 2 minutes |
| (8) 35 percent ethanol ----- | 2 minutes |
| (9) Water ----- | 2 minutes |
| (10) Stain with iron hematoxylin ----- | approximately 20 minutes |
| (11) Water ----- | 10 minutes |
| (12) 35 percent ethanol ----- | 2 minutes |
| (13) 50 percent ethanol ----- | 2 minutes |
| (14) 70 percent ethanol ----- | 2 minutes |
| (15) 80 percent ethanol ----- | 2 minutes |
| (16) 95 percent ethanol ----- | 2 minutes |
| (17) Eosin ----- | approximately 30 seconds |
| (18) 100 percent isopropyl alcohol ----- | 2 minutes |
| (19) 100 percent isopropyl alcohol+xylene --- | 2 minutes |
| (20) Xylene ----- | 2 minutes |

Mount in balsam.