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LIFE CYCLE INVOLVING RANA AURORA

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A fluke found in the intestine of the red-legged frog, Rana aurora Baird and Girard, was identified as Brachycoelium lynchi Ingles 1936. The life cycle of this fluke was studied and found to be of the lecithodendriid type rather than brachycoeliid. Instead of having one intermediate host, as is the case with the brachycoeliids, this fluke developed through a typical three host lecithodendriid life cycle. The adults shed eggs which passed from Rana aurora with the feces. Rediae developed in seven percent of the Bythinella hemphilli Pilsbry snails which inhabited the streams at high elevations on Marys Peak, Benton County, Oregon. Small numbers of microcercous xiphidiocercariae were shed from the snails and penetrated aquatic insect larvae. Acroneuria californica (Banks) was the primary second intermediate host and harbored small (0.240 mm) and large (0.50 mm) metacercarial cysts in the

fat body, some of which were progenetic. The two sizes of metacercariae were probably due to the downstream migration of the insect nymphs through the area in which the infected snails were found. Also periodic cercarial shedding and the long nymphal life of the insect may have contributed to this condition. When these metacercariae were fed to uninfected frogs the adult worms were recovered from the intestine. Only Rana aurora collected in the foothills of Marys Peak harbored natural infections of this fluke.

This study indicated that this fluke was taxonomically between the lecithodendriid genera Prosthodendrium Dollfus 1931, and Pycnoporus Looss 1899. It had characters of both genera but differed markedly from them; therefore, a new genus, Prosthopycoides, which will be published at a later date, is proposed for this fluke, the new combination being Prosthopycoides lynchi (Ingles 1936).

A PROGENETIC TREMATODE (LECITHODENDRIIDAE) LIFE CYCLE INVOLVING RANA AURORA

by

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TABLE OF CONTENTS

A PROGENETIC TREMATODE (LECITHODENDRIIDAE) LIFE CYCLE INVOLVING RANA AURORA

INTRODUCTION

In 1936 Ingles described a digenetic trematode from the red-legged frog Rana aurora Baird and Girard 1852, in California. He gave this fluke the name Brachycoelium lynchi. Manter (1938) considered this worm to be a doubtful species of Brachycoelium and suggested that it was probably a member of the genus Lecithodendrium Looss 1896, due to its far anterior vitellaria, its pre-acetabular, pre-ovarian testes and its V-shaped excretory vesicle. Rankin (1938), following Manter's suggestion, and after a careful study of the description and figure, transferred B. lynchi to Lecithodendrium due to the presence of the following characteristics: V-shaped excretory bladder, small egg size, and the position of the ovary posterior to the testes. Cheng (1958) studied the 17 specimens in the United States National Museum Helminthological Collection and concluded that Rankin (1938) was correct in transferring this species to another genus. However, while recognizing the similarity, he doubted whether Lecithodendrium was the correct genus due to the apparent lack of the characteristic prostate mass in any of the specimens. Yamaguti (1958) retained this species in Brachycoelium and noted that Rankin (1938) had transferred it to Lecithodendrium. Dubois (1960 a and b) did not mention this fluke in his revision of

the lecithodendriids.

The life histories of trematodes in the family Lecithodendriidae have not been studied extensively. Most of the definitive hosts for species of Lecithodendrium, Pycnoporus Looss 1899, and Prosthodendrium Dollfus 1931 are mammals, especially Chiroptera, or in one case, a reptile. Lühe (1909) partially reported the life cycle of Prosthodendrium ascidia (Van Beneden 1873) Dollfus 1931, as encysting in the larvae of Chironomus plumosus L. (Diptera) in spherical, thin walled cysts 0.15 mm in diameter, up to fifteen individuals in a host. The stylet of the cercaria remaining in the cyst was 0.31 mm long, with a short, sword shaped point which appeared to be inserted in a broad handle. The same juveniles also occurred encysted in Ephemera (Ephemeroptera) and Perla (Plecoptera) larvae.

Skrjabin (1915) found sexually mature specimens of Prosthodendrium chilostomum (Mehlis 1831) Dollfus 1931, which contained eggs in the uterus, freely motile in the body cavity of a single adult caddis fly (Phryganea sp.) and concluded that this trematode attained sexual maturity in its intermediate host. Brown (1933) recognized this as a case of progenesis. He reported the life cycle as involving long tailed xiphidiocercariae which penetrated the second intermediate host, larval Phryganea grandis (Trichoptera), where they lost their tails and fed as motile metacercariae. During later

pupation, after about eight months of free motility, the worms encysted in the thoracic muscles of the hosts which, after emergence, were ingested by Chiroptera. Brown found two sizes of metacercariae in P. grandis larvae during October and November, none of which was progenetic.

Hall (1957) discovered a progenetic lecithodendriid trematode free in the body cavity of damselfly naiads in Indiana. He considered this trematode to be closely related to the genus Prosthodendrium.

The life cycles of two other lecithodendriids, Allassogonoporus vespertilionis Macy 1940, and Acanthatrium oregonense Macy 1939, were described by Knight and Pratt (1955). In the former cycle sporocysts were found to develop in Flumenicola virens (Lea), and metacercarial cysts were found in Limnephilus sp. caddis flies. The latter utilized Oxytrema silicula (Gould) as the first intermediate host and Limnophilus as the second host. Allassogonoporus and Acanthatrium metacercariae were found simultaneously in the caddis fly larvae; however, those of Allassogonoporus were encysted whereas those of Acanthatrium were not. Both of these trematodes are parasites of bats.

Burns (1961) redescribed the cercariae of Allassogonoporus vespertilionis and Acanthatrium oregonense and reported on their penetration, encystment and development in the caddis fly larvae.

Hall (1959) discussed the life cycles that were known for the family Lecithodendriidae and considered all the known cercariae of this family to be of the xiphidiovirgulate type.

The life cycle of another lecithodendriid, Cephalophallus obscurus, as described by Macy and Moore (1954) developed sporocysts in Flumenicola virens and metacercariae in the abdominal muscles of crayfish. The adults developed experimentally in the intestine of mink, Mustela vison.

After determining the life cycle of the present trematode and comparing the adult morphology and life cycle with that of other lecithodendriid species, I propose to erect a new genus Prosthopycoides, to accommodate it. This new combination is Prosthopycoides lynchi (Ingles 1936). The reasons for this are included in the following sections of this thesis. The name Prosthopycoides lynchi will be used for this fluke throughout the remainder of this thesis; however, this name is not official since it has not yet been published.

METHODS AND MATERIALS

Collections of adult Rana aurora frogs and other amphibians were made mainly in the vicinity of Marys Peak, Benton County, Oregon. Amphibians were also collected from various other places in western Oregon. These animals were examined for internal parasitic infections and the results were recorded. The flukes that were found were either observed alive or were fixed under slight coverslip pressure in Lavdowsky's or Gilson's fluids. The specimens were then either stained in Delafield's Hematoxylin or treated with dioxane, embedded in paraffin and sectioned. Some specimens were sectioned in a cryostat and fixed in neutral formalin. These sections were then stained with Delafield's Hematoxylin. Collections of aquatic insects were made from streams in the vicinity of Marys Peak and examined for the metacercarial stage. A small aquatic dip net was used to collect the insect larvae. The net was set firmly into the substratum and the gravel and rocks in front of the net were agitated; the current then swept any insects into the net. The insects were transported to the laboratory in plastic bags where they were held in aerated plastic containers at 15° C until used. These insects were dissected and the large metacercariae removed for study and use in experimental infection studies. The insects were then macerated in a Waring Blendor and the smaller metacercariae were counted and

removed for experimental infection studies. Collections of the molluscs in these streams revealed only a Pisidium sp. (Musculidae) clam and a very small snail, Bythinella hemphili Pilsbry. These molluscs were collected by washing silt through coarse and fine mesh sieves (1.397 mm and 0.351 mm openings). The material washed through the coarse sieve and collected in the finer one was washed into plastic "zipper" bags and transported back to the laboratory. It was placed in white enamel pans, illuminated with a lamp and the clams and snails were removed with forceps. These molluscs were then isolated in individual stacking dishes containing charcoal filtered water. They were kept at 5° C for up to one month and observed for cercarial shedding. Only the snails were found to be infected with Prosthopycoides lynchi; these shed the cercariae in small numbers. These cercariae were observed and measured alive, used in experimental infection studies, or fixed, stained with Delafield's Hematoxylin and mounted in picolyte. In order to find the redia stage, snails were dissected with watchmakers forceps. The rediae were removed from the snail tissue, observed and measured alive, or the entire infected snail was removed from the shell, fixed in Bouin's fluid, embedded, sectioned and stained with Harris Hematoxylin and Eosin. Some of the rediae were removed and fixed in AFA, stained with Delafield's Hematoxylin and mounted in picolyte.

Experimental infections of the second intermediate and definitive hosts were carried out. Several amphibians known to be free of infections of Prosthopycoides lynchi were used as experimental definitive hosts. Rana aurora and Rana cascadae Slater 1939, raised from eggs in the laboratory were used, as were Ambystoma gracile (Baird 1859), Ambystoma macrodactylum Baird 1849, and Hyla regilla Baird and Girard 1852. These three latter animals, not raised in the laboratory, were collected from areas considered to be free of P. lynchi infections. This was established by examining several individuals collected at the same time and place as those used as experimental hosts and found to be negative for P. lynchi infections. In addition, these animals were all collected at least 15 miles and at a lower altitude from the nearest place where insects infected with P. lynchi were found. These experimental hosts were fed metacercariae from naturally infected Acroneuria californica (Banks, 1905) collected on Marys Peak and in Woods Creek, northwest of Philomath, Benton County, Oregon. This was done by dissecting out the cysts, collecting them in a finely drawn pipette, inserting it into the throat of the host and expelling the cysts. After waiting for up to a month for the flukes to develop, the hosts were sacrificed and the intestines examined for flukes. Acroneuria californica nymphs collected in Oak Creek, near Corvallis, Oregon, were used as experimental hosts. They were found to be free of

Prosthopycoides lynchi cysts by macerating several in the blender. These stonefly nymphs were placed in stacking dishes with the cercariae shed from the snail, Bythinella hemphilli, and the penetration of the insect observed under a dissecting microscope. After seven to sixteen days of metacercarial development, these hosts were dissected and macerated to find the small metacercariae.

All of the measurements of the cercariae and rediae were taken from living specimens. The metacercariae and adults were measured in both the living and fixed conditions. All measurements are in millimeters unless otherwise indicated. The average measurement is listed first with the size ranges following in parentheses.

All drawings were made with the aid of a Wild drawing apparatus.

LIFE CYCLE RESUMÉ

The adult flukes which inhabit the small intestine of the frog host release the eggs into the lumen of the intestine. The eggs pass from the frog with the fecal material and, if they are deposited in a stream, are probably ingested by the tiny snail, Bythinella hemphilli. The redia develops in contact with the digestive gland tissue of the snail and produces the microcercous xiphidiocercariae in small numbers. These cercariae, upon release from the snail, adhere to the substratum by means of a mucous secretion, elevate the anterior end and wait for a passing host. The second intermediate host, the stonefly Acroneuria californica, acquires these parasites by passing over them and sweeping them up with the thoracic gills. The cercariae penetrate and encyst to form metacercariae which continue to develop to infectivity and further to develop eggs progenetically. When these metacercariae are ingested by the frogs, they excyst and continue their development to sexual maturity. The incidence of infection when feeding small cysts experimentally was quite low. The life cycle is illustrated diagrammatically in Figure 12.

ADULT

In 1936, Ingles described this worm from the intestine of Rana aurora in California. His description was incomplete and his figure of the worm was inadequate. Therefore, a comparison of Ingles' description and the present data was included. Ingles' description from his 1936 paper was as follows:

> Body oval-shaped; length between 0. 65 mm and 1. 00 mm; width between 0. 37 mm and 0. 51mm. Cuticle very thin with no spines on mounted material. Oral sucker subterminal, large, from 0. 15 mm to 0. 23 mm across its long diameter. Acetabulum measures from 0. 12 mm to 0. 18 mm in diameter; located in posterior half of body. Ratio of oral sucker to acetabulum between 3:2 and 5:4. Pharynx globular; prepharyx short. Esophagus almost non-existent. Ceca very short, anterior to all genital organs, but posterior to vitellaria. Ovary on either side of acetabulum and anterior or posterior to it; pyriform, 0. 06 mm to 0. 13 mm across its greatest diameter. Seminal receptacle and Mehlis' gland posterior to ovary. Uterus occupies all of region posterior to genitalia. Genital pore just anterior to acetabulum. Vitellaria lateral in two grape-like clusters at anterior end. Eggs oval, 25μ to 10 μ. Testes symmetrically placed anterior to ovary and seminal receptacle; diameter 0. 09 mm to 0. 10 mm. Cirrus sac falciform, 0. 10 mm to 0. 15 mm in length. Seminal vesicle large. Excretory system V-shaped; crura end just posterior to level of acetabulum.

The following description was made from stained, slide mounted material by the present author.

The adults (Figs. 2 and 5) were small, oval, slightly flattened flukes measuring 1.045 (0.682 - 1.370) in length and 0.609 (0.342 - 0.782) in width. The cuticle was thin and equipped with short spines over the entire body. The subterminal oral sucker was large and muscular, bearing short spines on its inner surface. It was 0.261 (0.184 - 0.332) long and 0.268 (0.202 - 0.326) wide. The acetabulum was a large, muscular organ located medially in the third quarter of the body. It likewise had spines on its inner surface and measured 0.196 (0.158 - 0.230) long and 0.221 (0.168 - 0.270) wide. The pharynx followed a short prepharynx and was a globular structure 0.043 (0.033 - 0.051) long and 0.063 (0.061 - 0.066) wide, surrounded by small peripharyngeal glands. The esophagus extended posteriorly from the pharynx to the ceca, and was short, 0.041 (0.028 - 0.059) long by 0.051 wide. The intestinal ceca were short and extended laterally in fixed specimens. They were anterior to the gonads and posterior to the vitellaria. The ovary was between the testes and either on the right or left of the acetabulum, and usually projected anterior to it. The ovary was elongate, oval, 0.183 (0.142 - 0.209) long and 0.120 (0.112 - 0.127) wide. The Mehlis' gland, 0.054 (0.051 - 0.056) long and 0.125 (0.112 - 0.137) wide, was located in the same region as the ovary and the seminal receptacle. These three structures were all attached at the same point (Fig. 4) and apparently were variable in their positioning

around this point in fixed material. The seminal receptacle was usually directly posterior to the acetabulum. It was elongate, oval, filled with sperm in mature specimens and measured 0. 185 (0. 148 - 0. 214) long by 0. 150 (0. 122 - 0. 194) wide. The uterus was long and coiled, filled the area posterior to the acetabulum with additional lateral anterior loops that reached up to the posterior level of the vitellaria, and had median coils that extended between the testes and intestinal cecum; the descending loops were dorsal to the ascending loops (Fig. 5). The eggs filled the uterus in mature specimens and were elongate, 0. 035 (0. 032 - 0. 039) by 0. 017 (0. 013 - 0. 018), and had a definite opercular ridge. The vitellaria consisted of clusters of follicles lateral to the oral sucker and anterior to the intestinal ceca. The vitelline clusters measured 0. 246 (0. 204 - 0. 311) by 0. 166 (0. 127 - 0. 204). There was a prominent vitelline duct extending posteriorly from each cluster of vitelline glands. These ducts extended back and emptied into a small vitelline reservoir (Fig. 4). The oval testes were transversely apposed and were lateral to the anterior border of the acetabulum, with the cirrus pouch and ovary in between. The left testis measured 0. 142 (0. 137 - 0. 148) long by 0. 143 (0. 122 - 0. 163) wide; the right measured 0. 155 (0. 143 - 0. 178) long by 0. 170 (0. 133 - 0. 199) wide. The vasa efferentia were short and entered the cirrus pouch separately. The cirrus pouch was large, 0. 279 (0. 240 - 0. 332) long by 0. 100

(0.092 - 0.110) wide, and was usually curled into a "U" shape; the end bearing the prostate mass was directed posteriorly. The large, distinct, coiled seminal vesicle filled the posterior half of the cirrus pouch. The seminal vesicle mass measured about 0.110 long by 0.050 wide, and was filled with sperm in mature specimens. The anterior half of the cirrus pouch was filled with the prostate mass which was composed of large gland cells and measured about 0.100 long and 0.080 wide. The anterior end of the cirrus pouch was eversible and since it lacked the characteristic structures of a true cirrus, it was considered to be a pseudo-cirrus (Fig. 3). The genital pore was median and directly anterior to the acetabulum. The excretory bladder was V-shaped and measured about 0.460 from its anterior extremity to the posterior end of the body. The crura reached almost to the posterior level of the testes and were about 0.066 wide. The excretory pore was median at the posterior extremity of the body.

A comparison of the two descriptions indicated several differences between that of Ingles and the present observations. Ingles described the cuticle as "thin with no spines on mounted material." Spines were visible on all of the live specimens observed and on many of the mounted specimens. The spines were always quite evident on the metacercariae, alive and mounted. Ingles described the ovary as being on "... either side of (the) acetabulum and

anterior or posterior to it." The positions of the female reproductive organs were variable because the ovary, seminal receptacle, and Mehlis' gland were all attached at the same point (Fig. 4) and were directed at various angles in variously prepared specimens. The ovary was found to be in the same anterior-posterior plane as the acetabulum, on either side, but never completely posterior to it. The seminal receptacle was usually posterior to the ovary as stated in Ingles' paper, but at times it was at about the same level. The Mehlis' gland was found to be in between the ovary and seminal receptacle, usually directed anteriorly and at a 90^{o} angle from a line drawn through the long axis of the ovary and the seminal receptacle. Ingles described the uterus as occupying "...all of (the) region posterior to (the) genitalia...." but did not mention the prominent lateral uterine loops that extended anteriorly up to the posterior level of the vitellaria. Ingles' description of the cirrus sac did not include mention of the very prominent prostate cell mass; the enclosed seminal vesicle was described simply as "large." The prominent, eversible "pseudo-cirrus" was not mentioned by Ingles. The type and paratype specimens (no. 8933) of Brachycoelium lynchi deposited in the USNM Helminthological Collection were obtained for comparison. The specimens had apparently deteriorated since originally prepared by Ingles and much of the detail described by him could not be observed; however, spines were observable on

several specimens and the cirrus pouch showed a division, with the seminal vesicle visible and the anterior portion solidly stained. None of the specimens contained eggs in the uterus, the extent of which was not observable.

A comparison of measurements taken from Ingles' description and the present data, presented in Table I, indicated that the flukes described by Ingles were closer in size to the metacercariae of this study. Therefore, the specimens examined by Ingles had probably not been in the frog long enough to attain maximal development.

TABLE I

Measurements of Prosthopycoides lynchi

	Ingles (1936) Data	This Study	
	Adult	Adult	Metacercaria
Length	0.65 - 1.00	0.68 - 1.37	0.63 - 1.10
Width	0.37 - 0.51	0.34 - 0.78	0.41 - 0.65
Oral Sucker	0.15 - 0.23	0.18 - 0.33	0.17 - 0.31
Acetabulum	0.12 - 0.18	0.16 - 0.27	0.14 - 0.23
Pharynx	"Globular"	0.03 - 0.07	0.03 - 0.06
Esophagus	"Short"	0.03 - 0.06	
Ovary	0.06 - 0.13	0.11 - 0.21	0.06 - 0.12
Seminal Receptacle	"Present"	0.12 - 0.21	0.06 - 0.11
Mehlis' Gland	"Present"	0.05 - 0.14	
Eggs, length	0.025	0.03 - 0.04	
Testes	0.09 - 0.10	0.12 - 0.20	0.08 - 0.15
Cirrus Pouch	0.10 - 0.15	0.24 - 0.33	0.21 - 0.31

EGG

The eggs of Prosthopycoides lynchi were very numerous and packed in the uterus which was coiled in the posterior part of the body of the adult with loops that extended laterally up to the posterior level of the vitellaria. The eggs recently released from the ovary had very thin transparent shells and descended posteriorly in dorsal loops of the uterus, while those with thicker, hardened, yellowed shells were found in the ventral, anteriorly directed, loops of the uterus. They were oblong, bluntly oval, and operculated with a definite opercular ridge (Fig. 8). They were yellowish brown in color when mature, and measured 0.035 (0.032 - 0.039) by 0.017 (0.013 - 0.018) in mounted specimens. No eggs were observed to hatch and the miracidium was not seen except within the eggs. In sectioned adults the miracidia were observed in the eggs and appeared elongated with a slightly pointed anterior end.(Fig. 8).

In progenetic metacercariae taken from Acroneuria californica nymphs the eggs recently released from the ootype region contained two cells from the ovary and from three to nine yolk cells.

REDIA

The rediae of Prosthopycoides lynchi were found infecting the snail Bythinella hemphilli in small numbers. The largest number of rediae found in any one snail was thirteen, the smallest number was four; six was the average number found. The rediae were elongate, bluntly rounded, and sac-like, measuring 0.492 (0.204 - 0.792) in length and 0.138 (0.065 - 0.175) in width (Fig. 7). The distinct, terminal oral opening was surrounded by an elevated lip-like region which measured 0.058 (0.049 - 0.065) in diameter. The oral opening led directly to the large muscular pharynx which was 0.050 (0.036 - 0.059) long and 0.052 (0.044 - 0.060) wide. The pharynx in turn emptied into the short, blind intestinal cecum which measured 0.085 (0.031 - 0.132) in length, 0.057 (0.041 - 0.088) in width, and was always, when visible at all, filled with material that had the same yellowish brown color as the cells of the glandular area surrounding the rediae. The birth pore was located at the anterior end of the redia at the level of the pharynx on the side opposite the intestinal cecum. The interior of the redia was filled with germ balls and cercariae in various stages of development. The germ balls ranged in size from those of two or three cells up to that of mature cercariae. The germ balls began as spherical cell masses which gradually elongated, thickened, and developed

into the mature cercariae. The largest number of germ balls found in any one redia was 21; there was one redia with no germ balls (Fig. 9); the average number was 12. There were never more than three mature cercariae showing active movement when dissected from the redia. Usually when dissecting snails, several active cercariae were found outside the rediae in the snail tissues. No rediae were found to contain daughter rediae and no sporocysts were observed.

CERCARIA

The cercariae of Prosthopycoides lynchi were microcercous xiphidiocercariae (Figs. 6 and 10). They were able to extend from 0.243 to 0.40. The width varied greatly with the state of contraction, and measured 0.072 (0.051 - 0.091). In live specimens the oral sucker was large, measuring 0.047 (0.041 - 0.052) by 0.040 (0.034 - 0.049) with a subterminal mouth, above which was located the large, simple stylet (Fig. 11) measuring 0.025 (0.021 - 0.028) by 0.005. On each side of the stylet were the openings of the four penetration gland ducts which were 0.005 in diameter and ran forward from the clusters of six glands located on either side of the acetabulum. The acetabulum was round, 0.028 (0.023 - 0.031) by 0.029 (0.023 - 0.038) and located near the center of the body. The pharynx, directly posterior to the oral sucker, was small and pyriform. No intestinal ceca were observed. The excretory vesicle measured 0.093 (0.060 - 0.150) from its anterior tip to the posterior end of cercarial body and was prominently V-shaped to nearly cardiform with thick cellular walls, reaching the posterior level of the acetabulum when the cercaria was at rest. A large globular mucous gland was observed ventral to the excretory vesicle. It measured 0.047 (0.039 - 0.057) long by 0.045 (0.026 - 0.063) wide and was 0.047 (0.044 - 0.049) dorso-ventrally. The

tail was very short with tiny hairlike spines at its bluntly pointed terminus. It measured 0.018 (0.013 - 0.026) by 0.014 (0.010 - 0.016). A small pore which appeared to open directly into an empty space (Fig. 10) was seen on the ventral surface near the tail.

The free cercariae were very active and attached themselves firmly to the substratum by their mucous secretions. They raised their bodies to make contact with passing objects. When a stonefly nymph was placed in a dish with them they adhered to the ventral surface of the insect and attached to the thorax at the base of the gills. They appeared to be swept up by the branched, finger-like gills of Acroneuria californica and Arctopsyche sp. The paddle-like, lateral gills of the ephemerids did not pick them up as well. Acroneuria californica seem to be especially suitable for picking up these cercariae because of the nature of their gills and also because of their peculiar method of aerating the gills by doing "push-ups." This would perhaps explain why the largest concentrations of metacercariae were found in A. californica, Arctopsyche and Rhyacophila sp. The cercariae were observed to penetrate at the base of the gills and did not seem to exhibit any sort of responsive behavior when coming in contact with the insects as has been observed occasionally with other cercariae.

The number of Prosthopycoides lynchi cercariae shed by nine snails were counted during April and May. The average

number of cercariae shed daily by each of the nine snails was three. Seven cercariae was the most shed by any one snail over a 24 hour period; some would shed none. The snails were kept in a refrigerator in total darkness except for the time it took to examine them daily. The effect of changes in the photoperiod was not studied. However, snails kept for a month in the refrigerator subsequently shed about the same number of cercariae as those examined immediately upon removal from their natural environment.

METACERCARIA

Metacercariae of Prosthopycoides lynchi were found by examining the naturally infected aquatic insect larvae in the streams originating on Marys Peak and descending the east slope. The stonefly, Acroneuria californica, was found to be the most common host for these metacercariae.

Two distinct size ranges of metacercariae were found in A. californica, all the other hosts yielded only the smaller sized ones. The small metacercarial cysts measured 0.168 (0.137 - 0.204) in diameter while the larger cysts measured 0.583 (0.440 - 0.660) in diameter. Both the large and small cysts were found in the thoracic and abdominal fat deposits of the A. californica nymphs. The small cysts were also found in other parts of the body. Because the metacercariae were found in two size ranges, measurements for each will be given. The smaller metacercariae were much less developed than the large ones and most of the organs were not well enough developed to measure in the living state. The small metacercariae were bluntly lanceolate to oval and were 0.295 (0.225 - 0.377) long and 0.140 (0.098 - 0.163) wide. The oral sucker was proportionately large and muscular, 0.064 (0.049 - 0.078) in diameter, and retained remnants of the stylet in some specimens. The pharynx was oval and measured 0.020 (0.018 - 0.023) long and 0.022

(0. 018 - 0. 029) wide. The gut was not well defined in most specimens. The acetabulum was nearly round and measured 0. 052 (0. 038 - 0. 080) in diameter. The ovary, testes and cirrus pouch were not visible in the living specimens examined. The V-shaped excretory bladder was always visible in living specimens and measured 0. 092 (0. 072 - 0. 117) from the anterior tip of the crura to the posterior end of the body of the fluke. The crura were spread 0. 059 (0. 033 - 0. 071) apart at their anterior tips.

The large metacercariae (Fig. 1) were found to be very well developed, compared to the small forms, to the extent of producing eggs progenetically. They were oval, rounded anteriorly, and usually somewhat bluntly pointed posteriorly. They were as large as the adults in some cases and measured 0. 876 (0. 625 - 1. 100) in length by 0. 522 (0. 407 - 0. 650) in width. The cuticle was equipped with short spines. The oral sucker was muscular, sub-terminal and measured 0. 245 (0. 173 - 0. 306) long by 0. 262 (0. 219 - 0. 306) wide. Directly posterior to the oral sucker was the short, wide pharynx which measured 0. 045 (0. 031 - 0. 060) long by 0. 060 (0. 050 0. 066) wide. The esophagus was short and surrounded with darkly staining cells. The intestinal ceca were short, with thick walls and were directed laterally from the mid line of the body. The acetabulum was slightly smaller than the oral sucker and measured 0. 194 (0. 143 - 0. 230) in length by 0. 199 (0. 115 - 0. 234) in width,

and was located medially in the posterior half of the body. Small, short spines lined the inner wall of the acetabulum and oral sucker. The testes were usually slightly more anterior to the acetabulum than in the adults and apposed each other laterally. The right testis measured 0.121 (0.091 - 0.138) long by 0.101 (0.087 - 0.122) wide, the left 0.120 (0.083 - 0.153) by 0.096 (0.071 - 0.117). The vasa efferentia were short and entered the posterior end of the cirrus pouch separately. The cirrus pouch was large and was usually not as coiled as in the adults. It was 0.316 (0.214 - 0.382) long by 0.090 (0.066 - 0.105) wide. The anterior half was filled with the well developed prostate mass, the remainder with the coiled, sperm-filled seminal vesicle. The short, eversible pseudo-cirrus was seen on several specimens. The cirrus pouch was located directly anterior to the acetabulum, directed either anteriorly or posteriorly from the genital pore in fixed specimens. The genital pore was median, directly anterior to the acetabulum. The female reproductive organs were found on either side of the acetabulum. The well developed ovary was usually between the cirrus pouch and the testes and on the same level with them. It measured 0.101 (0.074 - 0.122) long by 0.075 (0.066 - 0.083) wide. The oviduct extended from the ovary to the ootype region, where the vitelline ducts, seminal receptacle, uterus and Mehlis' gland all joined together. The seminal receptacle was a prominent, rounded structure

containing a cluster of yolk cells and measured 0. 084 (0. 061 - 0. 112) in diameter. This organ did not contain sperm. The seminal receptacle of the adult contained yolk cells in some specimens. The uterus was coiled down into the posterior part of the body with lateral loops extending up the sides as in the adults. Some of these progenetic individuals contained as many eggs as mature adults. The vitellaria were, as in the adult, clustered lateral to the oral sucker in masses which measured 0. 158 (0. 117 - 0. 204) long by 0. 149 (0. 066 - 0. 199) wide. A vitelline duct descended posteriorly from each cluster of yolk cells. The excretory bladder was large, V-shaped, and measured 0. 353 (0. 224 - 0. 510) from the anterior tip of its crura to the posterior tip of the body where the excretory pore was located. In the cysts the bladder was visible and filled with opaque granules.

MOLLUSCAN HOST

Bythinella hemphilli Pilsbry is a very small operculate snail of the family Hydrobiidae. It was found to be the only first intermediate host for Prosthopycoides lynchi although a pea clam (Pisidium sp.) was collected in the same streams. Collections were made in fast streams at about 2500 feet elevation (lower elevations yielded no snails) on the east slope of Marys Peak in western Oregon. The snails preferred sandy to slightly muddy areas along the edges of the streams. Snails collected from February to May had a low incidence of infection. Of the 353 snails examined for infections during this period, 26 were infected with P. lynchi, about seven percent. A collection of 128 snails in February yielded a five percent infection; in April 63 had an infection of three percent while 12 percent of 162 snails collected in May were infected.

INSECT HOSTS

The stonefly, Acroneuria californica (Banks 1905) was found to be the main host for the metacercariae of Prosthopycoides lynchi. It was the only species that was found to harbor the larger metacercariae. In addition, the following insects were found to harbor the small metacercariae: Trichoptera--Arctopsyche sp. and Rhyacophila sp.; Plecoptera--Pteronarcys sp., Peltoperla brevis Banks 1907, and Peltoperla campanula Jewett 1954; Ephemeroptera--Ironodes californicus (Banks 1910).

Acroneuria californica is a large stonefly which emerges from mid-April to late June (Jewett, 1959). The females fly upstream and lay their eggs from which the nymphs hatch and begin their larval development. Apparently the nymphs either migrate or are washed downstream during their development. This hypothesis seemed to be substantiated by the fact that nymphs at the upper elevation where Bythinella hemphilla were found harbored only small metacercariae of P. lynchi. The larger nymphs at the lower limit of the vertical range of B. hemphilli contained both large and small metacercariae while those below this level, at about 300 feet elevation, either had large metacercariae or none. More investigations need to be carried out to further substantiate this hypothesis,

especially since the extremely high water in the streams during the winter of 1964 may have upset the normal conditions.

The development of A. californica from the egg to the adult stage apparently takes more than one year. Therefore, they are ideal hosts for P. lynchi due to the length of time necessary for the development of the metacercariae to infectivity. The trichopterans, Arctopsyche sp. and Rhyacophila sp., were found to be heavily infected with small metacercariae but no large ones were found. All the other insects examined had comparatively few cysts except one large Pteronarcys sp. which had a heavy infection of small metacercariae.

AMPHIBIAN HOSTS

In naturally infected animals only Rana aurora Baird and Girard 1852 was found to be infected with adult Prosthopycoides lynchi. Of the twenty-one R. aurora examined from various localities in western Oregon only two were found to harbor these flukes. Both infected frogs were collected in the foothills on the east side of Marys Peak, a rather limited area.

Rana aurora is a large frog that breeds in temporary and permanent ponds. The frogs inhabit marshy areas and quiet pools along streams. They probably become infected when they ingest the adult A. californica bearing infective metacercariae.

Other amphibia collected in areas where infected A. californica occur and examined for P. lynchi but found without infections were: nineteen Rhyacotriton olympicus (Gaige 1917), five Dicamptodon ensatus (Eschscholtz 1833), six Plethodon vehiculum (Cooper 1860), two Ensatina eschscholtzi Gray 1850, one Aneides ferreus (Cope 1869), and one Ascaphus truei Stejneger 1899. Rhyacotriton olympicus and Dicamptodon ensatus were found to contain the remains of A. californica in their stomachs in areas where nearly 95 percent of these stoneflies were infected with P. lynchi metacercariae; yet upon examination, they contained no P. lynchi. Dicamptodon ensatus is the definitive host for Cephalouterina dicamptodoni

Senger and Macy 1953 (Lecithodendriidae) which also develops through A. californica (Anderson,1964). Both the metacercariae of this fluke and those of P. lynchi were often found in the same nymph. No experimental feedings were carried out to attempt an artificial infection of R. olympicus or D. ensatus. Apparently P. lynchi is quite host specific in the adult stage, but only ecologically specific for cercarial penetration.

EXPERIMENTAL INFECTION STUDIES

Both small (less than 0.204) and large (greater than 0.440) metacercarial cysts were used in feeding experiments involving several different uninfected amphibians. Ambystoma gracile, the northwest salamander, was trapped in ponds about 15 miles east of the closest point where Acroneuria californica were infected with Prosthopycoides lynchi. Eight were examined for previous infection and found negative for P. lynchi infections. Five were fed from five to forty small P. lynchi metacercariae from A. californica collected on Marys Peak at 2500 feet elevation. Three of the five were later found to contain immature, but recognizable P. lynchi (two of them had one, the other four) in the small intestine. Other experimental hosts fed an average of twenty small metacercariae and later found to be negative were : one Hyla regilla, two Ambystoma macrodactyllum, one Rana aurora, and one Rana cascadae.

Large P. lynchi were fed to one Ambystoma gracile and two Rana aurora. The A. gracile was fed one cyst and found negative after twelve days. The first R. aurora was fed five large metacercariae and thirteen days later four immature adults were recovered. The second R. aurora was fed twenty-one metacercariae from the fat body of A. californica nymphs collected at about 300 feet elevation and twenty-six days later only one immature P. lynchi adult

was recovered from the small intestine; however, five well developed but dead P. lynchi adults with eggs were recovered from the rectum. The infection was probably lost because the frogs had been without food for about a month previous to this experiment. The limited amount of data obtained suggested that the small metacercariae were not as infective as the larger ones.

Experimental infections were also carried out with the cercaria and the first intermediate host. The cercariae from Bythinella hemphilli were placed in stacking dishes with small Acroneuria californica nymphs which were found to be free of these cysts by previous examination. They were observed under the dissecting microscope and the cercarial penetration was witnessed. The cercariae elevated their anterior ends and when touched by the insect, usually by its gills, would adhere and begin "inch-worm" movements over the body. Eventually a site of penetration would be selected on the soft membranes at the base of the gills and the cercariae would bore into the host. The process took at least ten minutes after penetration was begun. Tailless cercariae were found within the body of the insect a few minutes after penetration. In one experiment seven small metacercarial cysts, like those naturally occurring, were recovered from a small A. californica seven days after exposure to the cercariae. The cysts measured 0. 11 in diameter and the excysted worms were about 0. 260 long. They retained part of the

stylet; the bladder was V-shaped and contained no excretory granules. Another A. californica exposed to three Prosthopycoides lynchi cercariae contained three small metacercariae sixteen days later. They appeared the same as in the previous experiment except that the stylet was smaller. Due to the small number of cercariae obtained from the snails, only enough experimental penetration studies were carried out to establish that this cercaria produced the same metacercariae as found in the naturally infected insect host.

DISCUSSION

The taxonomic status of the proposed Prosthopycoides lynchi has been unsettled up to now due to the fact that its life cycle was not known. This life cycle study indicated a closer affinity to the lecithodendriids than to the brachycoeliids. The life cycle of Brachycoelium, as indicated for B. obesum Nicoll 1914 by Cheng (1960c), utilized only one intermediate host, a terrestrial gastropod. The cercariae developed into free metacercariae within the molluscs, which were then ingested by the definitive host. The lecithodendriids, on the other hand, usually were harbored by aquatic insects as second intermediate hosts as shown by Allassogonoporus vespertilionis Macy 1940 which developed through the snail Flumenicola and the insect Limnephilus; the latter was then ingested by the definitive host (Knight and Pratt 1955). Another example of the lecithodendriid three-host cycle was Prosthodendrium ascidia (Van Beneden 1873) where the cercariae developed in Lymnaea stagnalis and encysted in Ephemera and Perla larvae or the larvae of Chironomus plumosus (von Linstow 1884; Lühe 1909).

The present life cycle study indicated that Manter (1938) was correct in removing this species from the genus Brachycoelium and that Rankin (1938) was also correct in assigning it to the family Lecithodendriidae. As suggested by Cheng (1958), re-examination

of the adult morphology indicated that Lecithodendrium was not the correct genus, but not for the reason that he proposed, namely "the apparent lack of the characteristic prostate mass." The specimens taken by the author from Rana aurora showed a prostate mass, but revealed other characters that removed it from the genus Lecithodendrium, the main one being the position of the vitellaria anterior to the ceca and testes, whereas the vitellaria were posterior to the testes in Lecithodendrium.

The morphology of the adults indicated a close affinity to the genera Prosthodendrium and Pycnoporus; Prosthopycoides lynchi appeared to be between them. It fitted many of the characteristics of both Prosthodendrium and Pycnoporus. It differed from Prosthodendrium by having a prominent pseudo-cirrus, spines on the cuticle, lateral anterior uterine loops, testes within the uterine zone and no genital sinus. Prosthopycoides lynchi differed from Pycnoporus by being oval instead of elongate, having a much shorter esophagus, having the ovary in the same zone as the testes instead of acetabular or post-acetabular and having the vitellaria pre-cecal instead of between the testes and ceca. The comparisons of the three genera are summarized in Table II. Since it would make Prosthodendrium and Pycnoporus nearly synonymous if either one was amended to accept P. lynchi, the present author proposes to erect a new genus, Prosthopycoides.

TABLE II

Comparison of Pycnoporus, Prosthodendrium, and Prosthopycoides

Morphological Condition	Pycnoporus	Prosthodendrium	Prosthopycoides
Oval body	elongate	P*	P
Spines on body	P	absent	P
Short esophagus	long	P	P
Acetabulum in mid-third of body	P	P	in third quarter
Ovary sub-median in zone of testes	post-acetabular	P	P
Vitellaria between testes and ceca	P	in some species	always pre-cecal
Short intestinal ceca	P	P	P
Uterus filling all of hindbody with coils up through the vitellaria	P	in hindbody only	lateral loops up to vitellaria only
Excretory bladder V-shaped	P	P	P
Testes in the uterine zone, laterally apposed	P	outside uterine zone, oblique	P
Pre-acetabular cirrus pouch	P	P	P
Pseudo-cirrus	P	absent	P
Genital sinus	absent	P	P

*P - indicates structure or condition present

Dubois[1] examined several of the author's specimens and concurred in the proposal to erect a new genus to accommodate this species.

The life cycle of P. lynchi was similar to that of Prosthodendrium ascidia and Prosthodendrium chilostomum. Basically all three involved three hosts: a mollusc, an aquatic insect, and a vertebrate. The occurrence of progenetic metacercariae as reported for P. chilostomum by Skrjabin (1915) and by Hall (1959) for an unidentified species closely related to Prosthodendrium, compared with a similar condition in Prosthopycoides lynchi. However, both of the former flukes were free in the body cavity of the insect host while those of P. lynchi were encysted. Many of the metacercariae found in large (12 - 31 mm body length) Acroneuria californica nymphs were progenetic; however, as was the case with those reported by Skrjabin (1915), it was not known if the eggs were fertile. It was probable that they were not, since the seminal receptacle did not contain sperm. It is possible that P. lynchi cercariae were shed periodically as was reported by Brown (1933) for Prosthodendrium chilostomum; however, cercariae were shed in small numbers by Bythinella hemphilli in the laboratory while kept at 5° C

[1] Dr. Georges Dubois, Gymnase Cantonal, Neuchâtel, Switzerland. Personal letter dated April 12, 1965.

from January through June. The large number of metacercariae in the Acroneuria californica population indicated the possibility of a period of greater cercarial shedding than was observed. Snails were collected in late June, the time of maximal shedding reported by Brown (1933) for Prosthodendrium chilostomum; however, no increase in shedding was observed. It was possible that a combination of periodic cercarial shedding, downstream movement of the A. californica nymphs, and the length of their larval development was what produced the two distinct size ranges of metacercariae. At high elevations (2500 feet) only small metacercariae were found, at middle elevations both large and small were found while at lower elevations only large metacercariae were seen.

Brown (1933) also found two sizes of Prosthodendrium chilostomum metacercariae in larval Phryganea grandis collected during October and November, none of which was progenetic. Ten percent of these were about 0.165 mm long and retained the stylet and part of the salivary gland system. The remaining ninety percent were about 0.30 mm long. Brown concluded that the former type had penetrated the host very recently and that there was a "swarm" of cercariae released by the molluscan hosts in October and November; however, since ninety percent of the metacercariae were nearly twice as long as this first group, he suggested there must have been an earlier and probably "maximal" swarming. By comparing

growth rates of the metacercariae and the size range found in the hosts, Brown concluded that the maximal swarming occurred in July, followed by a prolonged period of intermittent and decreasing emergence until November, when it fell off completely. He found no stylet-bearing metacercariae after November.

It seems more probable, in the case of P. lynchi, that the two sizes of metacercariae can be explained by two facts about Acroneuria californica. The insects spend more than one season in the streams as nymphs during which time they presumably migrate down from high elevations through the area where Bythinella hemphilli are shedding cercariae. Those with only large metacercariae at the lower elevations may have passed rapidly through the B. hemphilli zone. Those with two sizes of cysts may have spent considerable time in the snail zone acquiring cercariae during widely separated periods of cercarial shedding. The two distinct sizes of metacercariae suggest seasonal shedding; however, no evidence was found that cercariae were shed in "swarms" as suggested by Brown (1933). The nymphs harboring only small metacercariae probably had migrated into the snail zone quite recently since the metacercariae at times contained remnants of the cercarial stylet.

It is probable that a complete size range of metacercariae from the smallest to the progenetic ones would be found if collections were made over the entire nymphal life of A. californica.

Prosthopycoides lynchi metacercariae did not exhibit the free, motile condition of Prosthodendrium chilostomum (Brown, 1933) but rather encysted within seven days; however, one experimentally infected A. californica nymph was found to contain six dead, unencysted metacercariae which, after 37 days infective time, had increased in body length from that of the cercaria (0.24) to about 0.9 mm.

The feeding experiments with both the small and the large metacercariae proved that they were both of the same species.

SUMMARY

The taxonomic status of Lecithodendrium lynchi (Ingles 1936) Rankin 1938 was reviewed.

The morphology of the adult fluke was studied and compared with Ingles' (1936) original description. It was found that this fluke did not belong in the genus Lecithodendrium, as suggested by Rankin (1938), but rather was taxonomically between the genera Prosthodendrium and Pycnoporus. Therefore a new genus, Prosthopycoides was proposed to accommodate it, the proposed new combination being Prosthopycoides lynchi (Ingles 1936).

The life cycle of this fluke was studied and found to be a typical three host lecithodendriid type. The rediae developed in seven percent of the Bythinella hemphilli snails collected at about 2500 feet elevation on Marys Peak, Benton County, Oregon. The microcercous xiphidiocercariae left the snails in small numbers (an average of three per day) and penetrated aquatic insect larvae, mainly Acroneuria californica stoneflies.

Experimentally the cercariae were observed to penetrate uninfected A. californica at the base of the branched thoracic gills. They encysted within seven days.

Metacercariae, some of which were progenetic, were found encysted in the fat body of naturally infected A. californica. These

developed into adult Prosthopycoides lynchi when fed to uninfected Rana aurora frogs. The smaller metacercariae were less infective than the larger progenetic ones.

Acroneuria californica was the only insect that harbored large progenetic metacercariae; however, in addition, the following were found to contain the small metacercariae: Trichoptera--Arctopsyche sp. and Rhyacophila sp.; Plecoptera--Pteronarcys sp., Peltoperla brevis and Peltoperla campanula; Ephemeroptera--Ironodes californicus.

Adult P. lynchi were found in only two of the twenty naturally infected R. aurora examined during this study. Nineteen Rhyacotriton olympicus and five Dicamptodon ensatus salamanders that prey on A. californica in areas where 95 percent of these insects were infected with P. lynchi metacercariae, were found to be free of these flukes.

This study suggested that Prosthopycoides lynchi was quite host specific in the adult stage while cercarial penetration was probably limited only by the mechanical ability of the insects to pick them up from the substratum.

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APPENDIX

Figure 1. Ventral aspect of the Prosthopycoides lynchi progenetic metacercaria.

Figure 2. Ventral aspect of a mature P. lynchi adult.

Figure 3. Detail of the cirrus pouch of P. lynchi showing the eversible pseudo-cirrus.

Figure 4. Detail of the P. lynchi ootype region.

Figure 5. Semidiagrammatic drawing of a P. lynchi adult showing the extent of the uterine coiling.

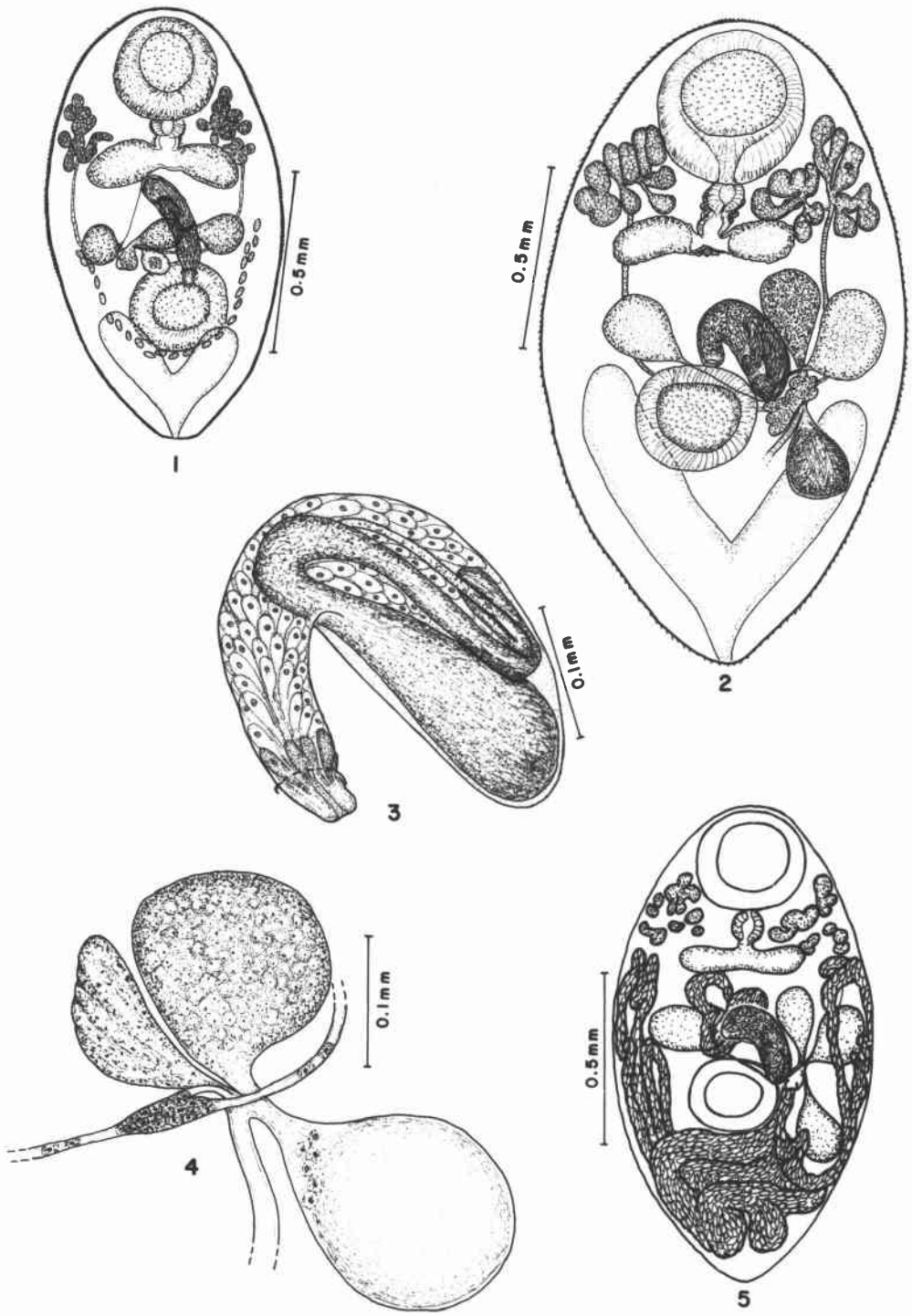
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Figure 6. Dorsal aspect of the cercaria of Prosthopycoides lynchi.

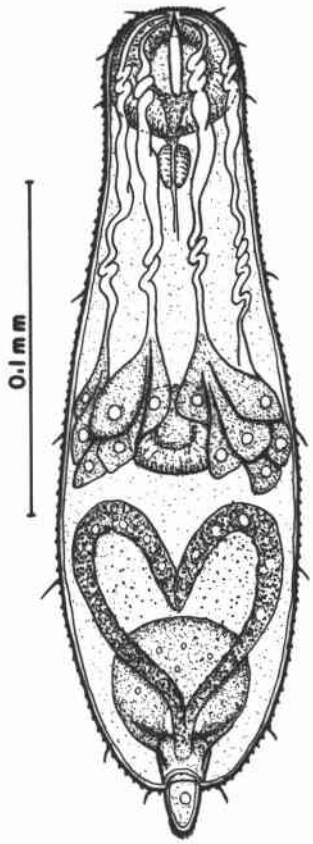
Figure 7. Large P. lynchi redia from Bythinella hemphilli.

Figure 8. Prosthopycoides lynchi egg showing the enclosed miracidium.

Figure 9. Small P. lynchi redia lacking germ balls.

Figure 10. Lateral aspect of the P. lynchi cercaria.

Figure 11. Detail of the P. lynchi cercarial stylet.



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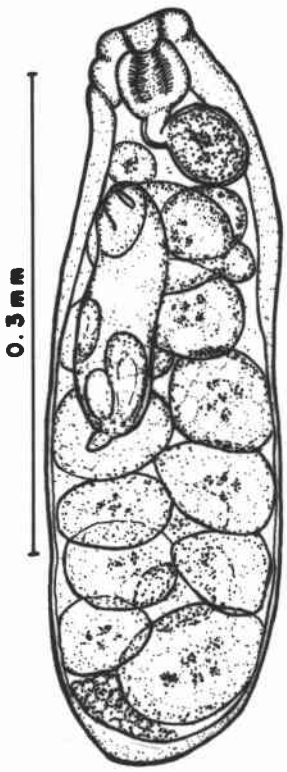

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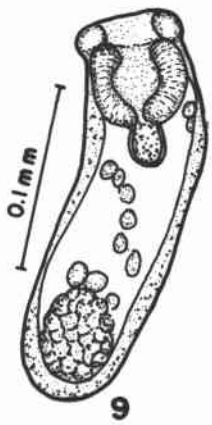

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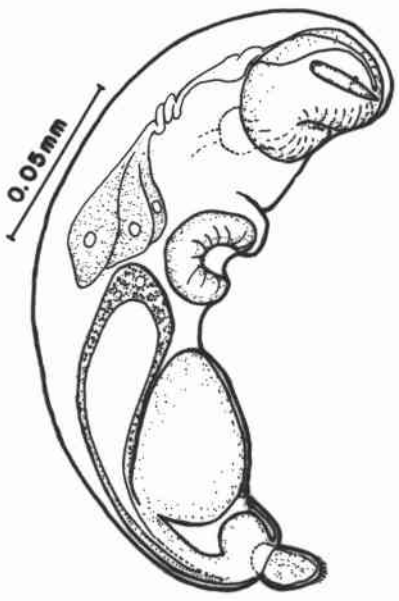
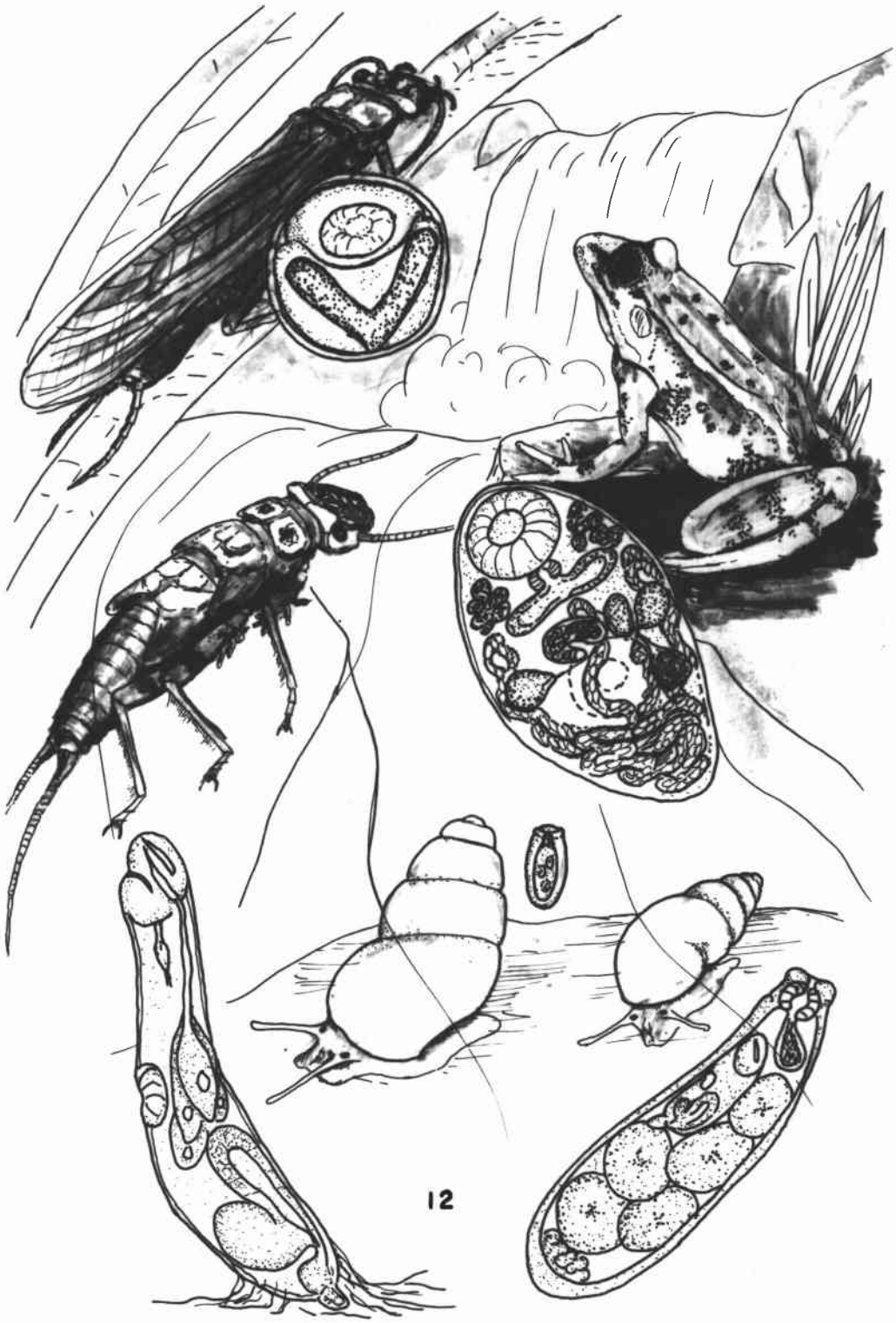


10

Figure 12. Diagrammatic representation of the life cyle of Prosthopycoides lynchi.


12