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Title: STUDIES ON SOME DIGENETIC TREMATODES FROM RITNER CREEK, POLK COUNTY, OREGON

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Stuart E. Knapp

Seven species of fresh water fishes belonging to three different families and two species of snails from Ritner Creek, Polk County, Oregon were surveyed for adult and larval trematodes. A total of seven species of trematodes representing four families was recovered from the intestine of the fishes. Of these, at least two were undescribed new species. The trematodes were described and their taxonomy discussed.

The stream snails, Oxytrema silicula and Flumenicola virens, were found to harbor at least 17 apparent species of larval trematodes. Five of these were described from the snails for the first time. A check-list of cercariae known to infect O. silicula and F. virens in the Pacific Northwest was presented.

Life histories of five of the trematodes encountered during the surveys were studied. The experimental life cycle of Apophallus donicus Skrjabin and Lindtrop, 1919 was completed. The cercaria, of

the pleurolophocercous group, was found in F. virens, while the metacercaria encysted in a number of fresh water fishes. The life cycle of Plagioporus siliculus Sinitsin, 1931 was demonstrated for the first time. The cotylomicrocercous cercaria developed in sporocyst in O. silicula and penetrated crayfish, Pacifastacus lenuisculus to encyst in the abdominal muscles. The experimental definitive host used was the rainbow trout.

A single adult Echinochasmus milvi Yamaguti, 1939 was recovered from a duck fed echinostome cysts found naturally in the gills of blackside dace, Rhinichthys osculus nubilus and redside shiner, Richardsoni balteatus hydrophlox. This constituted the third record of the trematode in the Pacific Northwest. The cercaria of E. milvi was believed to be Cercaria gorgonocephala Ward, 1916, which Martin (1968) redescribed from O. silicula. Two other macrocercous cercariae--one aggregating but albino while the other non-aggregating--were also found in O. silicula. The body of these cercariae was identical to that of C. gorgonocephala. The taxonomic relation of the three cercariae was discussed.

The partial life cycle of a new monorchiid trematode from the torrent sculpin, Cottus rhotheus, was presented. The natural secondary intermediate host was found to be lampreys, both brook (Lampetra richardsoni) and Pacific (L. tridentata). The first intermediate host is still unknown.

The partial life cycle of another new trematode, an Echinochasmus sp., was also reported. The adult was obtained from a duck experimentally fed small cysts in the gills of blackside dace. The first intermediate host is unknown.

Studies on Some Digenetic Trematodes from Ritner Creek, Polk County, Oregon

by

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Chairman of Department of Zoology

Redacted for privacy

Dean of Graduate School

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Typed by Mary Jo Stratton for Daniel Tin-Hung Law

Science can account for many things in the world; it may some day account for all that which the world of phenomena actually is. But why anything at all is, or exists, science knows not, precisely because it cannot even ask the question.

—E. Gilson

To Him

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STUDIES ON SOME DIGENETIC TREMATODES FROM RITNER CREEK, POLK COUNTY, OREGON

GENERAL INTRODUCTION

As a result of several extensive general surveys and numerous reports of individual parasites, the helminth parasite fauna of fresh water fishes from the eastern and central parts of North America is fairly well known (Bangham and Adams, 1954; Hoffman, 1967). The same, however, cannot be said of the situation in the western part, especially the coastal states, of the continent. Except for the two relatively extensive studies, one in British Columbia (Bangham and Adams, 1954) and the other in northern California (Haderlie, 1953), the rest of the reports to date were concerned mostly with local surveys of individual fish species (Dunlap, 1951; Griffith, 1953; Fritts, 1955; Harms, 1959; Hugghins, 1959; Alexander, 1960; Jones and Hammond, 1960; Fox, 1962; Colley and Olson, 1963; Edwards and Nahhas, 1968; Miller and Olson, 1973). In Oregon, surprisingly few surveys have been done, with the possible exception of Alexander (1960), whose study was concerned only with the parasite fauna of trouts.

Our knowledge of fresh water larval trematodes in the Pacific Northwest is even more meager. Isolated records of cercariae as reported in life cycle studies of trematodes are by no means lacking,

but actual surveys for cercariae have been few. From North Colorado, Acholonu (1968) reported a total of 26 species of cercariae from 10 different molluscs. Davis (1958) surveyed two gastropod species from the Columbia Basin of Washington, and noted six separate cercariae. Other surveys included Miller (1952) from San Juan Island, Washington, and Burns (1961b), who described six virgulate xiphidiocercariae from Oregon.

To augment our limited knowledge of fresh water fish parasites and larval trematodes, particularly those of Oregon, the present study was undertaken to gain additional information on adult trematode fauna from some common stream fishes in the mid-Willamette Valley. A survey of the two dominant snails, Oxytrema silicula and Flumenicola virens from Ritner Creek, Oregon was undertaken to determine the prevalence and diversity of larval trematode infections in these two gastropod species. The several life cycle studies included in the present work were directly or indirectly the outgrowths of the surveys of both fresh water fishes and snails in Ritner Creek.

The following dissertation thus consists of three parts: (1) the survey of fresh water fishes for adult intestinal trematodes; (2) the survey of O. silicula and F. virens for larval trematodes; and (3) life cycle studies on five different species of trematodes encountered during the surveys.

PART I. SURVEY OF FRESH WATER FISHES FOR INTESTINAL TREMATODES

Introduction

For the present study, Ritner Creek, Polk County, Oregon, a small tributary of the Luckiamute River was chosen because of its proximity to the campus of Oregon State University. Ritner Creek is situated approximately two miles north of the small town of King's Valley which lies 19 miles northwest of Corvallis, Oregon. Highway 223 traverses Ritner Creek as the latter merges with the Luckiamute River and flows eastward toward the direction of the Willamette River.

Materials and Methods

Fresh water fishes of seven species belonging to three separate families were collected from Ritner Creek during the months of July to October, 1974. The fishes were caught by dip nets, seines and electrofishing. The fishes were taken to the laboratory and kept at $10^{\circ}C$ and killed as soon as possible. It was discovered that most fishes were able to survive for several months at $10^{\circ}C$. With few exceptions, most fishes were examined alive. The present study is concerned only with digestive tract trematodes. As soon as a fish was pithed, its abdomen was opened and its digestive tract removed. The parts were examined for larger parasites. Then the mucosa was

scraped and the entire digestive tract or segments of it vigorously shaken in water. The sediment was then examined under a dissecting microscope for smaller trematodes.

All of the trematodes were fixed and killed in AFA (Alcohol-Formalin-Acetic Acid) under slight coverslip pressure. Since most of the trematodes encountered were very small forms, a pipette or a camel hair brush was used to transfer the flukes during fixation, storage and subsequent staining. The trematodes were stored, after being fixed in AFA overnight, in 70% alcohol in small vials labeled with the name of the host, the date and the organ from which the parasites were removed. The trematodes were then later stained with Borax or Semichon's carmine, cleared in xylene and mounted in Canada Balsam.

All host fishes were identified by the author with the help of the Keys to Oregon Freshwater Fishes by Bond (1973).

All measurements of trematodes are in millimeters unless indicated otherwise. Sucker ratios are transverse diameters of oral sucker:acetabulum, with the oral sucker as 1. Specimens prepared in this study are in my personal collection. A paratype of each species represented, however, will be deposited in the personal collection of Dr. Stuart E. Knapp, major professor of the investigator.

Results

A total of 77 fishes from Ritner Creek, Polk County, Oregon was examined for intestinal trematodes. These fishes represented three families and seven different species. A list of fishes examined can be found in Table 1. The number of fish per species examined and the incidence of trematode infection in the fishes is outlined in Table 2.

Of all fishes examined, 49.4% were infected with at least one species of trematode. The salmonids, in general, had a very high incidence of infection. Of the 23 salmonids examined, 21 (91.3%) were infected. Cottus perplexus, reticulate sculpin, was relatively free from intestinal trematodes, whereas 52.3% of Cottus rhotheus, torrent sculpin, were infected. Multiple parasitism among the salmonids and C. rhotheus was also high. Salmo clarki clarki, coastal cutthroat trout, for example, was found to harbor five different trematodes; O. kisutch, coho salmon, three and C. rhotheus, four. It was not uncommon to find at least two different trematodes parasitizing the same fish host.

The cyprinids, on the other hand, were remarkably free from intestinal flukes. Of the four species examined (Rhinichthys osculus, speckled dace; R. osculus nubilus, blackside dace; R. cataractae dulcis, longnose dace; and Richardsoni balteatus hydrophlox, redside shiner), only two species of trematodes were recovered from

Table 1. Host-parasite list of fresh water fishes from Ritner Creek, Polk County, Oregon. New host records are marked with an asterisk.

Fish Host:

Family SALMONIDAE

Salmo clarki clarki, Richardsoni, coastal cutthroat trout

Parasites:

Family HEMIURIDAE

Deropegus aspina (Ingles, 1936) McCauley and Pratt, 1961

Family OPECOELIDAE

Plagioporus siliculus Sinitsin, 1931

*Plagioporus sp. n. sp.

Family ALLOCREADIIDAE

Crepidostomum farionis (Mueller, 1784) Braun, 1900

Unknown immature trematode

Oncorhynchus kisutch (Walbaum), coho salmon

Parasites:

Family HEMIURIDAE

Deropegus aspina (Ingles, 1936) McCauley and Pratt, 1961

Family OPECOELIDAE

*Plagioporus siliculus Sinitsin, 1931

Unknown immature trematode

Family COTTIDAE

Cottus rhotheus (Smith), torrent sculpin

Parasites:

Family HEMIURIDAE

*Deropegus aspina (Ingles, 1936) McCauley and Pratt, 1961

Family MONORCHIIDAE

*A new monorchiid n. gen., n. sp.

Family OPECOELIDAE

*Plagioporus siliculus Sinitsin, 1931

*Podocotyle virens (Sinitsin, 1931) Yamaguti, 1971

Cottus perplexus Gilbert and Evermann, reticulate sculpin

Parasite:

Family OPECOELIDAE

*Podocotyle virens (Sinitsin, 1931) Yamaguti, 1971

Family CYPRINIDAE

Rhinichthys osculus (Girard), speckled dace

Parasites:

Family HEMIURIDAE

*Deropegus aspina (Ingles, 1936) McCauley and Pratt, 1961

Family OPECOELIDAE

*Plagioporus siliculus Sinitsin, 1931

(Continued on next page)

Table 1. (Continued)

Fish Host:

Family CYPRINIDAE (continued)

Rhinichthys osculus nubilus (Girard), blackside dace

Parasite:

An unknown allocreadiid n. gen., n. sp. (?)

Rhinichthys cataractae dulcis (Girard), longnose dace

Parasite:

None

Richardsoni balteatus hydrophlox (Cope), redside shiner

Parasite:

None

Table 2. Rates of intestinal trematode infection in the various fish species examined.

Fish Host	No. Examined	No. Infected	% Infected
Family: SALMONIDAE			
Salmo clarki clarki	8	7	87.5
Oncorhynchus kisutch	15	14	93.3
Family: COTTIDAE			
Cottus rhotheus	21	11	52.3
Cottus perplexus	6	1	16.6
Family: CYPRINIDAE			
Rhinichthys osculus	11	4	36.3
Rhinichthys osculus nubilus	3	1	33.3
Rhinichthys cataractae dulcis	4	0	0
Richardsoni balteatus hydrophlox	9	0	0
Total	77	38	49.4

R. osculus, one unknown allocreadid from R. osculus nubilus, and none from R. cataractae dulcis and Richardsoni balteatus hydrophlox. The incidence of infection was 0% for R. balteatus hydrophlox and R. cataractae dulcis, 33.3% for R. osculus nubilus and 36.3% for R. osculus.

A total of seven species of trematodes was recovered from the fishes examined. They belong to four separate families. A list of these trematodes is also outlined in Table 1.

In Table 3 is listed the incidence of trematode infection for each species of fish. The numbers in parentheses refer to immature flukes recovered during examination of the fishes. Fifteen immature specimens of an unidentified fluke (Figure 1) were removed independently from the intestine of one 13 cm female cutthroat trout (S. clarki clarki) and one 8.4 cm coho salmon (O. kisutch). Since no mature specimens were available, it was impossible to verify its identity.

A host-parasite list has been compiled for the present study (Table 1). New host records are marked with an asterisk.

Trematodes from Fishes

Family HEMIURIDAE

Deropegus aspina (Ingles, 1936) McCauley and Pratt, 1961

(Figure 2)

This species was first described by Ingles (1936) as

Table 3. Incidence of adult intestinal trematodes in fishes from Ritner Creek, Oregon. Numbers indicate trematodes recovered from the fishes. Those in parentheses refer to immature forms.

Fish Host	Family: Hemiuridea *Deropegus aspina*	Family: Monorchiidae A New Monorchiid n. gen., n. sp.	Family: Opecoelidae *Plagiosporus siliculus*	*Plagiosporus* sp. n. sp.	*Podocotyle virens*	Family: Allocreadiidae *Crepidostomum farionis*	Unknown allocreadiid n. gen., n. sp. (?)
Family: SALMONIDAE							
Salmo clarki clarki	112(28)	0	1(5)	14(35)	0	1(1)	0
Oncorhynchus kisutch	94(35)	0	16(28)	0	0	0	0
Family: COTTIDAE							
Cottus rhotheus	25(0)	10(28)	2(0)	0	2(3)	0	0
Cottus perplexus	0	0	0	0	1	0	0
Family: CYPRINIDAE							
Rhinichthys osculus	1(2)	0	0(8)	0	0	0	0
R. osculus nubilus	0	0	0	0	0	0	1(1)
R. cataractae dulcis	0	0	0	0	0	0	0
Richardsoni balteatus hydrophlox	0	0	0	0	0	0	0

Halipegus aspina from the stomach of Rana boylei Baird from Butte County, California. Rankin (1944) and Skrjabin and Guschanskaja (1955) contended that H. aspina resembled Halipegus but did not think it conformed to the genus in all aspects. McCauley and Pratt (1961), after examining 125 specimens of the fluke from Salmo clarki clarki, S. gairdneri, Oncorhynchus kisutch, and O. tshawytscha from Benton County, Oregon, and after studying Ingle's holotype and one paratype, thought that the species from fishes was identical to Ingle's fluke from the frog. Since it resembled both Derogenes and Halipegus but differed from each significantly, they proposed the new genus Deropegus.

This fluke was found in the stomach of Salmo clarki clarki, Oncorhynchus kisutch, Rhinichthys osculus and Cottus rhotheus, the last two being new host records for the parasite. A total of 232 mature and 65 immature specimens of D. aspina was collected from the infected fishes.

This species has been found commonly in fresh water salmonid fishes of western Oregon. Aside from the four fish hosts mentioned by McCauley and Pratt (1961), Haderlie (1953) reported an immature Derogenes sp. in the intestine of O. kisutch from Humboldt County, California, which was identified as Deropegus aspina by McCauley and Pratt (1961). The Derogenes sp. of Shaw (1947) from the stomach and intestine of cutthroat in Oregon was probably also D. aspina.

Frogs are probably as common as hosts for D. aspina as are fresh water fishes. Since Ingle's collection of the flukes from the stomach of yellow-legged frogs (Rana boylei, Baird), Wotton and Powell (1964) recovered 22 more specimens from the same species of frogs in Shasta and Butte Counties of northern California. The creation of the genus Parahalipegus by Wotton and Powell (1964) for Deropenes aspina (Ingle, 1936), however, should be suppressed since McCauley and Pratt (1961) had previously proposed Deropegus for the species.

Family MONORCHIIDAE

A new monorchiid n. gen., n. sp.

(Figures 3-9)

Definitive Host: Cottus rhotheus (Smith), torrent sculpin.

Intermediate Host: Lampetra richardsoni Vladykov and Follett, western brook lamprey, and L. (Entosphenus) tridentata (Gairdner), Pacific lamprey; first unknown.

Habitats: Intestine of definitive host; pericardium, kidneys and liver of intermediate host.

Location: Ritner Creek, Polk County, Oregon.

Frequency: 7 of 21 hosts examined.

Description (based on four specimens, averages in parentheses): Body fusiform, heavily spined, very small; length 0.64 to 0.80 (0.74); width at acetabular level 0.218 to 0.268 (0.239). Anterior end bluntly

Figure 1. Unidentified immature fluke from the small intestine of a female cutthroat trout, Salmo clarki clarki.

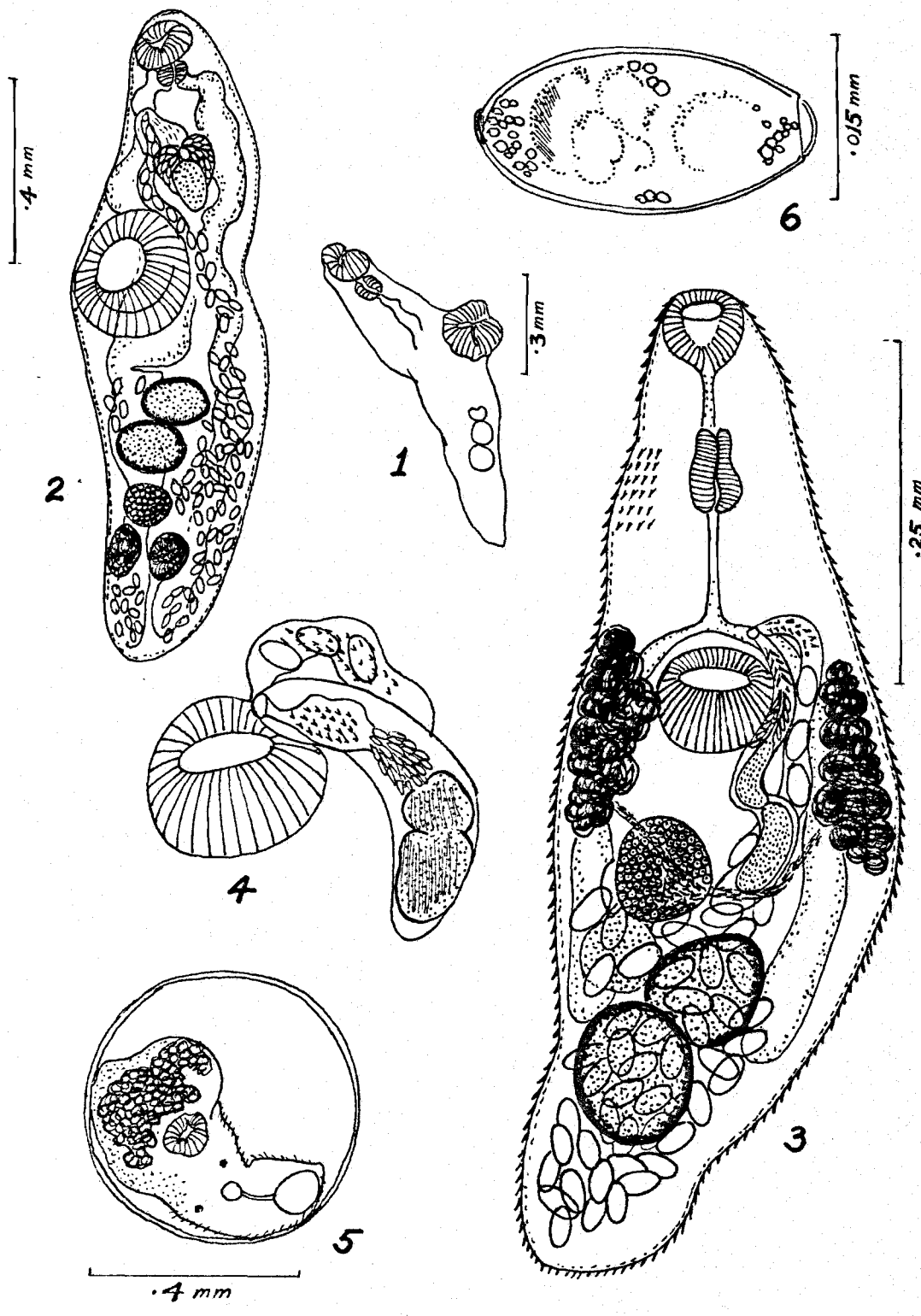
Figure 2. Adult Deropegus aspina (Ingles, 1936) McCauley and Pratt, 1961. Ventral view.

Figure 3. Adult of the new monorchiid trematode n. gen., n. sp. from the intestine of torrent sculpin, Cottus rhotheus.

Figure 4. Terminal reproductive organs of the new monorchiid. Note spiny terminal organ and cirrus.

Figure 5. Cyst of the new monorchiid found naturally embedded in the pericardium, liver and kidneys of lampreys.

Figure 6. Egg of the new monorchiid.


.4 mm
2
.3 mm
1
.015 mm
6
.25 mm
3
4
5
.4 mm

round and broader than tapering posterior end. Oral sucker subterminal, nearly round, 0.059 to 0.082 (0.072) wide by 0.056 to 0.086 (0.07) long; mouth opening ventrally. Pre-pharynx relatively long, 0.028 to 0.056 (0.044). Pharynx pear-shaped, large, 0.037 to 0.05 (0.045) wide by 0.063 to 0.084 (0.078) long. Esophagus long; cecal bifurcation immediately anterior to acetabulum which lies medially at posterior end of anterior half of body. Intestinal ceca wide, reaching level of anterior testis. Acetabulum size medium, slightly wider than long, measuring 0.084 to 0.10 (0.091) wide by 0.071 to 0.099 (0.086) long. Cirrus pouch long, extending from anterior testis to level of cecal bifurcation. Genital pore opens submedially on left. Seminal vesicles bipartite; cirrus spined. Terminal organ present and heavily spined. Ovary large, ovoid, with broader posterior end, situated submedially to right, 0.065 to 0.087 (0.077) wide by 0.082 to 0.106 (0.090) long. Anterior testis, 0.076 to 0.100 (0.087) wide by 0.069 to 0.106 (0.089) long, lies posterior to the ovary on the left and separated from it by some loops of uterus, is ovoid with anterior narrower portion. Posterior testis median, ovoid, larger than and touching anterior testis, 0.100 to 0.100 (0.100) wide by 0.078 to 0.115 (0.089) long. Seminal receptacle spherical, 0.046 to 0.059 (0.053) in diameter, lies opposite to anterior testis and immediately behind the ovary. Uterus occupies entire area behind the ovary. Eggs operculate, medium in size, ovoid and yellowish, measure 0.015 to 0.016 (0.0157)

wide by 0.032 to 0.035 (0.0336) long. Vitellaria weakly developed, in bunches lateral and slightly posterior to acetabulum.

Discussion: In classifying the new monorchiid trematode n. gen., n. sp., the use of Skrjabin's (1964) as well as Yamaguti's (1971) keys allows one to arrive at subfamily Monorchinae. The new genus resembles Monorcheides Odhner, 1905 in the position of the vitellaria and the presence of the double testes. However, it differs from the latter in having tandem or slightly oblique testes, a much longer cirrus pouch and an oval to round ovary. Physochoerus Poche, 1925, the validity of which as a genus was accepted by Skrjabin (1964) but rejected by Yamaguti (1971) because of inadequate original description and lack of figures, resembles the new genus in the number of testes, but differs from it in the extent of the vitellaria, the position of the testes and the unarmed terminal organ (Yamaguti, 1953). Table 4 shows a comparison of generic characteristics between the new genus and the two closely related genera, Monorcheides and Physochoerus.

Generic Diagnosis of the New Monorchiid: Very small, heavily spined monorchiids with fusiform body. Oral sucker and acetabulum nearly round with the latter being larger and pre-equatorial. Pre-pharynx relatively long, pharynx pear-shaped, long esophagus, ceca reaching level of anterior testis. Genital pore pre-acetabular, sub-median to the left. Cirrus pouch on left, long and reaching area between ovary and anterior testis. Cirrus spined. Seminal vesicle

Table 4. Comparison of generic characteristics of the new genus (Family: Monorchiidae) with genera Monorcheides and Physochoerus.

	New Genus	Monorcheides	Physochoerus
No. of testes	2	2	2
Symmetry of testes	tandem or slightly oblique	opposite	?
Location of testes	hindbody	hindbody	midbody
Cirrus pouch	large, reaching anterior testis	small, not posterior to acetabulum	?
Ovary morphology	round to oval	deeply lobed	?
Vitellaria	lateral to acetabulum	lateral to acetabulum	in dorsal anterior half of body reaching pharynx
Genital pore	submedian	median	?

bipartite. Terminal organ spined. Ovary spherical to ovoid, submedian to the right. Testes contiguous, spherical to ovoid, tandem or slightly oblique in posterior part of body. Uterus occupies area posterior to ovary. Vitellaria paired bunches of follicles, one on each side of acetabulum. Eggs operculate, medium in size, often filling body posterior to the ovary.

Family OPECOELIDAE

Plagioporus siliculus Sinitsin, 1931

(Figures 10, 11a-c)

Since the original description of Plagioporus siliculus from the intestine of Salmo clarki clarki (Sinitsin, 1931), the same parasite has not been reported from Oregon or from the entire Pacific Northwest. Pratt and McCauley (1961) made no mention of other records in their Trematodes of the Pacific Northwest: An Annotated Catalog. Hoffman (1967) was likewise unaware of further record of the fluke. Alexander (1960), in his survey of Oregon trouts, however, did mention the recovery of "a few small allocreadid trematodes" from rainbow trout and cutthroat trout of different hatcheries in Oregon. Unfortunately species identification of the flukes was not made, although he inferred that these flukes might be identical to the ones described by Haderlie (1953) from the Klamath River, Siskiyou County, California. Haderlie (1953) described five Plagioporus sp. from the intestine of one of 91 rainbow trouts, Salmo gairdneri, but was

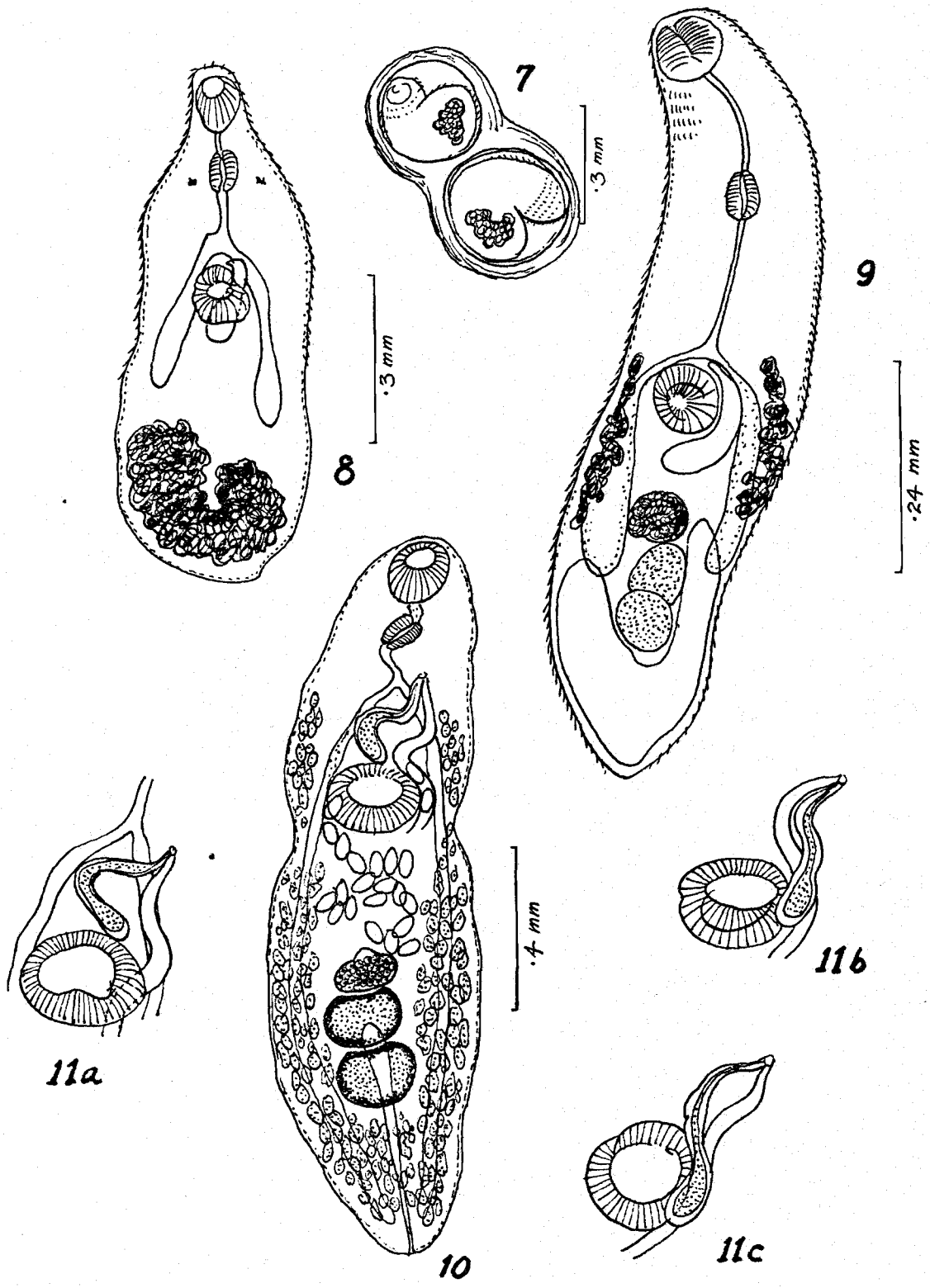
Figure 7. Compound cyst of the new monorchiid.

Figure 8. A young, freshly excysted metacercaria of the new monorchiid.

Figure 9. Older excysted metacercaria of the new monorchiid. Note advanced gonadal development and emptied excretory vesicle.

Figure 10. Adult Plagioporus siliculus Sinitsin, 1931, from the intestine of Oncorhynchus kisutch.

Figure 11a-c. Extent of cirrus pouch in P. siliculus.


7
.3 mm
8
.3 mm
9
.24 mm
10
.4 mm
11a
11b
11c

uncommitted as to the species identity of the flukes. A careful study of his description and diagrams of Plagioporus sp. shows it to have a remarkable resemblance to P. siliculus: the relative size, sucker ratio, "trilobed ovary," testes pressed together, and "often flattened on one side. . . ." The only exception, however, seems to be in the extent of the cirrus pouch. Sinitsin's type specimen depicts an oblique cirrus pouch "with its bottom not quite reaching the anterior margin of the acetabulum" (Sinitsin, 1931). In Haderlie's specimen, however, the posterior extent of the cirrus pouch is described as near to the posterior margin of the acetabulum (Haderlie, 1953). In carefully analyzing the length and extent of the cirrus pouches in my specimens, I have also noticed an obvious pattern of variation (Figures 11a-c). The cirrus pouches in three out of seven stained and mounted mature specimens occupy a position wholly in front of the acetabulum. Those of the remaining specimens, however, may extend beyond the anterior margin of the acetabulum. In the face of these variations, it thus becomes doubtful if the extent of the cirrus pouch, as described by Sinitsin (1931) does constitute naturally a dependable criterion for species differentiation. Haderlie's Plagioporus sp. as well as Alexander's Plagioporus sp. might very well be identical to P. siliculus.

In the present study, P. siliculus was found in the intestine of Salmo clarki clarki, Oncorhynchus kisutch, Cottus rhotheus and

Rhinichthys osculus (Table 3). A total of 19 mature and 41 immature specimens was recovered. A study of the stained and mounted specimens shows them to possess all the major characteristics of P. siliculus as described by Sinitsin in 1931. The measurements of seven specimens of mature P. siliculus are as follows: length 1.80 to 3.24 (2.55); width 0.40 to 0.66 (0.55); oral sucker 0.104 to 0.28 (0.21) wide by 0.11 to 0.25 (0.20) long; pharynx 0.05 to 0.12 (0.09) wide by 0.07 to 0.16 (0.12) long; acetabulum 0.20 to 0.32 (0.27) wide by 0.20 to 0.30 (0.25) long; ovary 0.16 to 0.30 (0.20) wide by 0.10 to 0.22 (0.15) long; anterior testis 0.17 to 0.28 (0.22) wide by 0.15 to 0.24 (0.19) long; posterior testis 0.18 to 0.27 (0.21) wide by 0.15 to 0.34 (0.23) long.

Incidence of P. siliculus infection among the fishes is listed in Table 3. Only immature specimens were recovered from R. osculus. However, these specimens did resemble the immature flukes of P. siliculus. Oncorhynchus kisutch, C. rhotheus and R. osculus are new host records for the trematode.

Plagioporus (Plagioporus) sp. n. sp.

(Figures 12, 13)

Host: Salmo clarki clarki, Richardson, coastal cutthroat trout.

Habitat: Intestine.

Location: Ritner Creek, Polk County, Oregon.

Frequency: 14 mature and 35 immature specimens from 4 of 8 hosts examined.

Description: Body elongate, slightly constricted posterior to acetabulum, frequently broadest at region of testes, 0.35 to 0.58 (0.45) wide by 1.62 to 2.43 (2.04) long; anterior end slightly more attenuated than posterior. Oral sucker terminal, nearly round, 0.168 to 0.22 (0.18) wide by 0.152 to 0.200 (0.18) long, anterior margin slightly broader than posterior margin with mouth opening ventrally. Pre-pharynx very short. Pharynx elongate, 0.08 to 0.12 (0.09) wide by 0.10 to 0.144 (0.12) long. Esophagus long, reaching at least halfway between pharynx and anterior margin of acetabulum. Intestinal ceca ending near posterior end of body. Genital pore submedian, opening to left of cecal bifurcation or anterior to it. Cirrus may become protruded, nonspinous. Cirrus pouch extending posterior to acetabulum. Acetabulum pre-equatorial, broader than long, 0.24 to 0.34 (0.29) wide by 0.224 to 0.28 (0.25) long and larger than oral sucker. Sucker ratio, 1:1.2 to 1.8. Ovary lobed, pretesticular, submedian and to right, 0.12 to 0.228 (0.16) wide by 0.08 to 0.156 (0.13) long. Testes lobed (Figure 13), tandem and contiguous, situated in middle of posterior half of body; anterior testis 0.16 to 0.30 (0.23) wide by 0.112 to 0.188 (0.16) long; posterior testis 0.168 to 0.348 (0.23) wide by 0.148 to 0.304 (0.20) long. Vitelline follicles large, extending from level of cecal bifurcation to posterior end of the

body. Uterus between ovary and acetabulum and of few coils. Metraterm long, to left of cirrus pouch. Eggs 0.32 to 0.40 (0.37) wide by 0.63 to 0.75 (0.69) long, yellowish-brown.

Discussion: This species was found in the intestine of coastal cutthroat trout, Salmo clarki clarki, Richardson and in no other fishes examined. Of the eight fish dissected, four were infected. The numbers varied from nine mature and no immature individuals to two mature and 19 immature individuals. A total of 14 mature and 35 immature flukes was recovered (Table 3).

The position of the genital pore, the extent of the vitellaria, the tandem testes and the extent of the ceca place this fluke in the subgenus Plagioporus of the genus Plagioporus (Yamaguti, 1971). The use of Skrjabin's (1964) key leads to Plagioporus acanthogobii, Yamaguti, 1952. It resembles P. acanthogobii in sucker ratio, lobed testes and ovary, and size of ova, but differs from it in general body shape, longer esophagus, shape and extent of cirrus pouch, and the anterior position of the vitellaria. The new species also differs from the other two known members of the subgenus with lobed testes: P. lobata Yamaguti, 1934 and P. polymiyiae Yamaguti, 1970. The former has a larger sucker ratio of 1:1.8 to 2.36 as compared to the new species' 1:1.2 to 1.8. The latter has a more anterior acetabulum and a cirrus pouch which does not extend posterior to the anterior margin of the acetabulum.

Manter (1954) recognized two subgenera, Plagioporus and Caudotestis in Plagioporus. Species of Caudotestis differ from those of Plagioporus chiefly in possessing ceca that do not extend posterior to the testes. The subgenus Caudotestis was first named by Isaitschikow in 1928. Yamaguti (1934) raised it to generic rank while Miller (1941) and Prudhoe (1945) did not recognize it as a genus. Yamaguti later (1951, 1971) changed it to a subgenus. While the extent of intestinal ceca is a small one for generic differentiation, Manter (1954) felt that it nevertheless served a practical function of splitting up an otherwise large genus, and Caudotestis does characterize a number of species mostly from fresh water fishes.

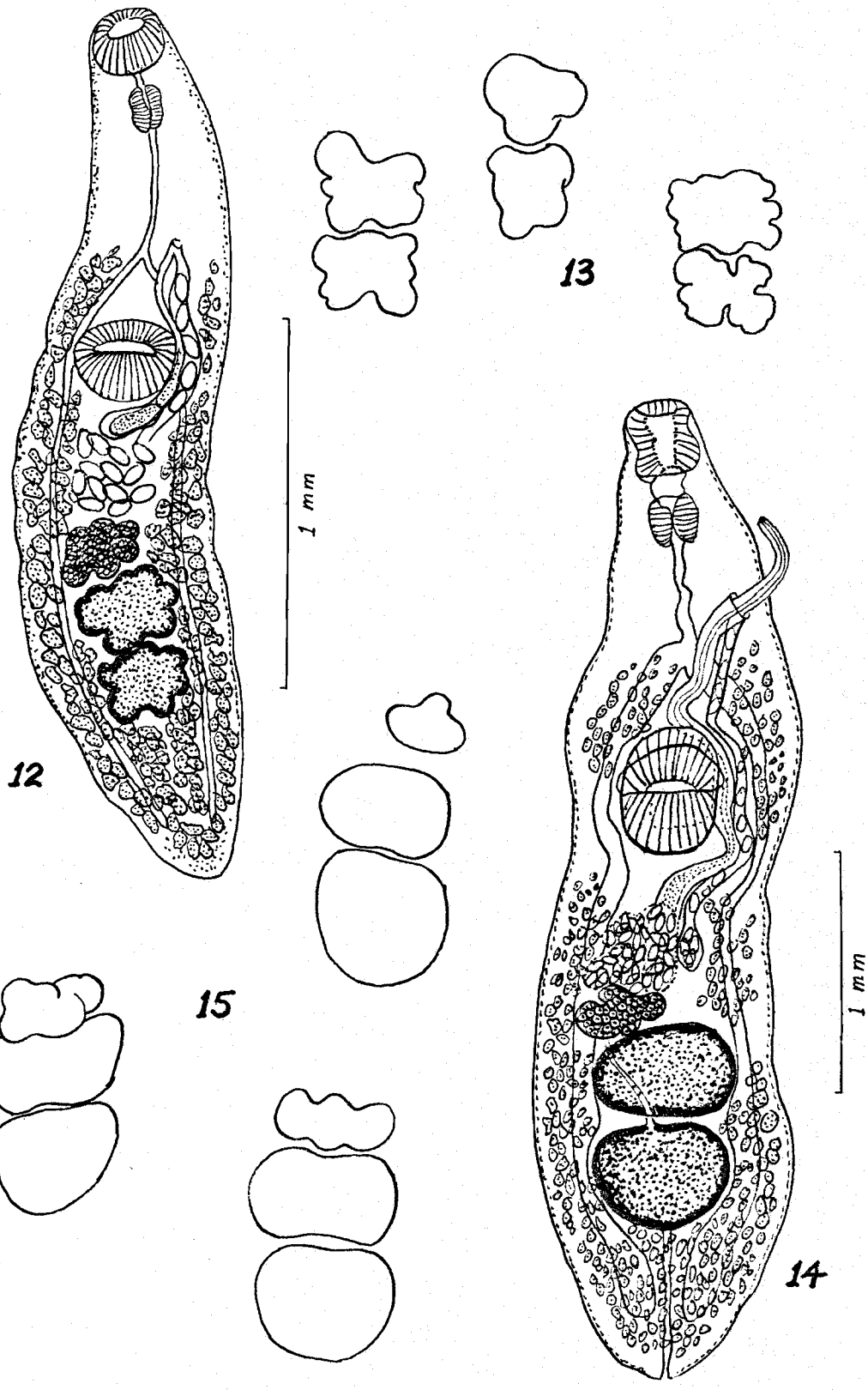
Podocotyle shawi, McIntosh, 1939 (Figures 14, 15), which resembles the new species, is almost twice as large, has larger eggs and round to ovoid testes. It has been the subject of much discussion in the past (Pratt and McCauley, 1961; Aldrich, 1960). Yamaguti (1953) placed it in the subgenus Peracreadium, Nicoll, 1909, of the genus Allocreadium, Looss, 1900 and, in 1958, changed it to Cainocreadium only to have it transferred again, and this time, back to Peracreadium (Yamaguti, 1971). Aldrich (1960) did not think the parasite should be placed in Cainocreadium by the fact that the genital pore in P. shawi is on the left, as is characteristic for Podocotyle and not median as is characteristic for Cainocreadium. I find myself in agreement with Aldrich (1960). For a similar

Figure 12. Adult Plagioporus sp. n. sp. from the intestine of Salmo clarki clarki.

Figure 13. Testis morphology of Plagioporus sp.

Figure 14. Adult Plagioporus shawi n. comb. Ventral view. Redrawn from McIntosh (1939).

Figure 15. Ovary and testis morphology of P. shawi.


12
1 mm
13
15
14
1 mm

reason, however, P. shawi cannot be placed in the genus Peracreadium either, because a median genital pore plus a round ovary are characteristic of that genus. Manter (1947), noting that P. shawi has a very long cirrus pouch that reaches to the ovary, a spined cirrus, and vitellaria that extends to the intestinal fork, argued that it could not belong to Podocotyle but did not suggest a relocation. Aldrich (1960), however, felt that the arguments used by Manter (1947) were not valid. Although McIntosh (1939) described P. shawi as having a spined cirrus he did not show any spines in his illustrations of the new species, and Aldrich (1960) and Pratt and McCauley (1961) failed to observe spines on the cirrus of any of the specimens they examined. The elongate cirrus pouch, according to Aldrich (1960), is quite common in Podocotyle and the anterior extent of the vitellaria is not uncommon throughout the genus Podocotyle. Consequently, he felt that the parasite should remain in its original genus until further work was done.

A closer study of P. shawi and careful comparison of this fluke with Miller's (1940) redescription of Plagioporus serotinus Stafford, 1904, the type species, however, shows an even more obvious similarity of the parasite to the genus Plagioporus. The close similarity of Podocotyle to Plagioporus Stafford, 1904, has been noted previously by Manter (1947, 1954). Since, as pointed out by Pritchard (1966), most authors have accepted the absence of vitellaria in the forebody

as an important character of Podocotyle and all species of Plagioporus have vitellaria anterior to the acetabulum (the only two exceptions, P. lepomis and P. virens were transferred to Allopodocotyle by Pritchard (1966) and again to Podocotyle by Yamaguti (1971)), P. shawi, because of the presence of its vitellaria in the pre-acetabular region, should be removed from the genus Podocotyle. The anterior vitellaria, tandem testes and sinistral genital pore of P. shawi all match the generic description of Plagioporus serotinus. Although P. serotinus has small sac-like excretory vesicle and unlobed ovary, as contrasted to the lobed ovary of P. shawi, a few recognized species of Plagioporus also have a lobed ovary (P. choerodonis Yamaguti, 1934; P. lobata Yamaguti, 1934; and P. fusiformis Price, 1934b). It appears that P. shawi should be more appropriately placed in the genus Plagioporus and, since its intestinal ceca reach beyond the posterior testis, as is characteristic of sub-genus Plagioporus, it thus becomes Plagioporus (Plagioporus) shawi (McIntosh, 1939) n. comb. Table 5 shows the chief comparative differences among the genera Plagioporus, Podocotyle, Cainocreadium and Peracreadium. These diagnostic characteristics are also matched with those of Plagioporus shawi on the left.

Table 5. Chief comparative differences among the genera Plagioporus, Podocotyle, Cainocreadium and Peracreadium.

P. shawi[1]	Diagnostic Characteristics[2]	Plagioporus[3]	Podocotyle[2]	Cainocreadium[2]	Peracreadium[2]
4.1	size (mm)	1.6 to 7.3	1.5 to 4.5	2 to 10	1 to 3
elongated, flattened	shape	elongated, flattened	elongated, subcylindrical	elongated, flattened	elongated, ovate, not much flattened
1/2 to 1/3	length of neck	1/2 to 1/3	1/7 to 1/3	1/2	1/3
lateral	genital pore	lateral	lateral	median	median
reaching to ovary	cirrus pouch	may or may not extend beyond acetabulum	more or less beyond acetabulum	not posterior to acetabulum	as far back as ovary
lobed	ovary	round or lobed	trilobed	trilobed	round
tandem, round to oval	testes	tandem or oblique, round or oval	round or oval	round	transverse oval
reaching cecal bifurcation	vitellaria	well anterior to acetabulum	not anterior of acetabulum	reaching anterior of acetabulum	reaching anterior of acetabulum
short to long	esophagus	short	short	short	short
no filaments	ova	no filaments	no filaments	no filaments	no filaments
.078 by .055	size of ova	?	.07 to .095 by .04 to .05	.07 to .10 by .04 to .06	.07 to .10 by .03 to .06
	type	P. seratinus	P. atomon	C. labracis	P. genu

[1] McIntosh (1939)

[2] Based on Nicoll (1909)

[3] Skrjabin (1964), Manter (1947), Yamaguti (1971)

Podocotyle virens (Sinitsin, 1931) Yamaguti, 1971

Synonyms: Plagioporus virens Sinitsin, 1931

Allopodocotyle virens Pritchard, 1966

(Figure 16)

One mature specimen of P. virens was found in the intestine of one of six Cottus perplexus from Ritner Creek. In a 6 cm Cottus rhotheus, one mature and one immature P. virens were found in the intestine; whereas in the gall-bladder of the same fish, two additional immature P. virens were found. In the gall-bladder of another C. rhotheus, a fully-mature P. virens was also recovered. Podocotyle virens was collected from no other fishes in the study. Apparently the trematode is not very common and relatively host specific. Both the gall-bladder and the intestine are suitable habitats for the development of the fluke.

This species has been reported only once previously. Sinitsin (1931) reported Plagioporus virens from the intestine of a Cottus sp. from Siuslaw River, near Mapleton, Oregon but reported as hosts of the parasite, the various "fresh-water fishes." Pritchard (1966) revised the genus Podocotyle and split it into five different genera. She transferred Plagioporus virens to genus Allopodocotyle. Schell (1970) agreed with Pritchard (1966) and considered Allopodocotyle a valid genus. However, Yamaguti (1971) relegated the five genera of Pritchard (1966) into the status of subgenera and merged Allopodocotyle

with Podocotyle. Thus, Allopodocotyle virens, according to Yamaguti (1971) becomes Podocotyle virens. This species does not belong to the genus Plagioporus since its vitelline follicles are restricted to the area posterior to the acetabulum. Although its cirrus pouch is short and thus differs from that of the type specimen of Podocotyle (Dujardin, 1845), many species of Podocotyle also have a short cirrus pouch. In view of the above observations, I am in agreement with Yamaguti (1971) that Plagioporus virens should be transferred to Podocotyle virens.

The three mature specimens recovered from C. rhotheus and C. perplexus resemble in all major aspects the type specimen of Sinitsin (1931). Their average sizes, however, are considerably smaller than the latter's. Since the measurements of the type specimen by Sinitsin (1931) were not averages and could very well represent those of a larger specimen, the difference in size alone does not warrant the status of a separate species. These flukes, therefore, are considered as identical to Podocotyle virens.

Pritchard (1966), who restudied the paratypes of P. virens, found at least two apparent discrepancies between Sinitsin's description of the type and the slides. Two of three paratypes of P. virens have a cirrus pouch extending to mid-acetabulum whereas the cirrus pouch was described by Sinitsin as wholly in front of the acetabulum. Eggs of P. virens are 0.072 to 0.086 by 0.042 to 0.056 as contrasted

to 0.058 to 0.042 as originally reported. Two of my three mature specimens have a cirrus pouch entirely in front of the acetabulum and the measurements of the eggs in my specimens agree more closely with the measurements of Pritchard (1966) than those of Sinitsin's (1931). In comparing Plagioporus siliculus and Podocotyle virens, which reflect a high degree of resemblance, a characteristic was found which seems to have apparent diagnostic value. This is the ratio of the longitudinal diameter of the acetabulum to the distance between the posterior margin of the acetabulum and the anterior extremity of the ovary. The ratio is 1:1.7 in P. siliculus and 1:2.9 in P. virens.

Family ALLOCREADIIDAE

Crepidostomum farionis (Mueller, 1784) Braun, 1900

(Figure 17)

Synonyms: Fasciola farionis Mueller, 1784
F. truttae Froelich, 1789
Distoma laureatum Zeder, 1800
Fasciola laureata (Zeder, 1800) Normann, 1840
Crossodera laureata (Zeder, 1800) Cobbold, 1860
Distoma farionis (Mueller, 1784) Blanchard, 1890
Crepidostomum laureatum (Zeder, 1800) Braun, 1900
Stephanophialia transmarina Nicoll, 1909
S. laureata (Zeder, 1800) Nicoll, 1909
S. farionis (Mueller, 1784) Faust, 1918
S. vitelloba Faust, 1918

Crepidostomum ussuruense Layman, 1930

C. vitellobum (Faust, 1918) Hopkins, 1931

The parasite is very common in salmonids of North America. It has been reported in Oregon (Alexander, 1960; Pratt and McCauley, 1961), Wyoming (Bangham, 1951), British Columbia (Bangham and Adams, 1954), Montana (Fox, 1962), California (Haderlie, 1953), Utah (Jones and Hammond, 1960), Wisconsin (Pearse, 1924), Minnesota (Warren, 1952) and Illinois (Fritts, 1959). The same parasite has also been reported from Europe: in Czechoslovakia (Dyk, 1958), England (Awachie, 1968), and Spain (Cordero del Campillo and Martinez Fernandez, 1971). There is at least one record of C. farionis from South America (Manter, 1962).

Hoffman (1967) mentioned the following genera of fresh water fishes as definitive hosts for C. farionis in North America: Coregonus, Cristovomer, Etheostoma, Lepomis, Leachichthys, Lota, Notropis, Oncorhynchus, Perca, Prosopium, Salmo, Salvelinus and Thymallus.

In the present survey, C. farionis was found only in coastal cutthroat trout, Salmo clarki clarki. One single adult C. farionis was recovered from the intestine of a 13 cm female fish. Another immature C. farionis was obtained from the stomach of a 7.8 cm S. clarki clarki. According to Alexander (1960), the most common site for C. farionis in S. clarki clarki is the gall-bladder. The presence of

this immature fluke in the stomach probably represents a freshly excysted specimen.

The incidence of C. farionis infection is exceptionally low in this study. Among the salmonids, only two out of eight S. clarki clarki and none out of 15 O. kisutch examined were parasitized. The life cycle of C. farionis requires the presence of the fingernail clam, Pisidium sp. (Crawford, 1943). Although Pisidium was not included in my survey for larval trematodes, its presence has not been noted in Ritner Creek. Its absence or probable presence in small numbers in the area where the fishes were sampled could have contributed to the low reported incidence of the infection.

Measurements of the only adult C. farionis collected in this survey are: length 3.17; width 0.71; oral sucker wider than long, 0.29 by 0.26; no pre-pharynx; pharynx 0.184 to 0.16; acetabulum wider than long, 0.38 by 0.36; ovary longer than wide, 0.27 by 0.16; anterior testis longer than wide, 0.19 by 0.16; posterior testis longer than wide, 0.24 to 0.16.

Allocreadiid n. gen., n. sp. (?)

(Figures 18-21)

Host: Rhinichthys osculus nubilus (Girard), blackside dace.

Habitat: Intestine.

Location: Ritner Creek, Polk County, Oregon.

Frequency: 1 of 3 hosts examined.

Figure 16. Adult Podocotyle virens (Sinitsin, 1931) Yamaguti, 1971.

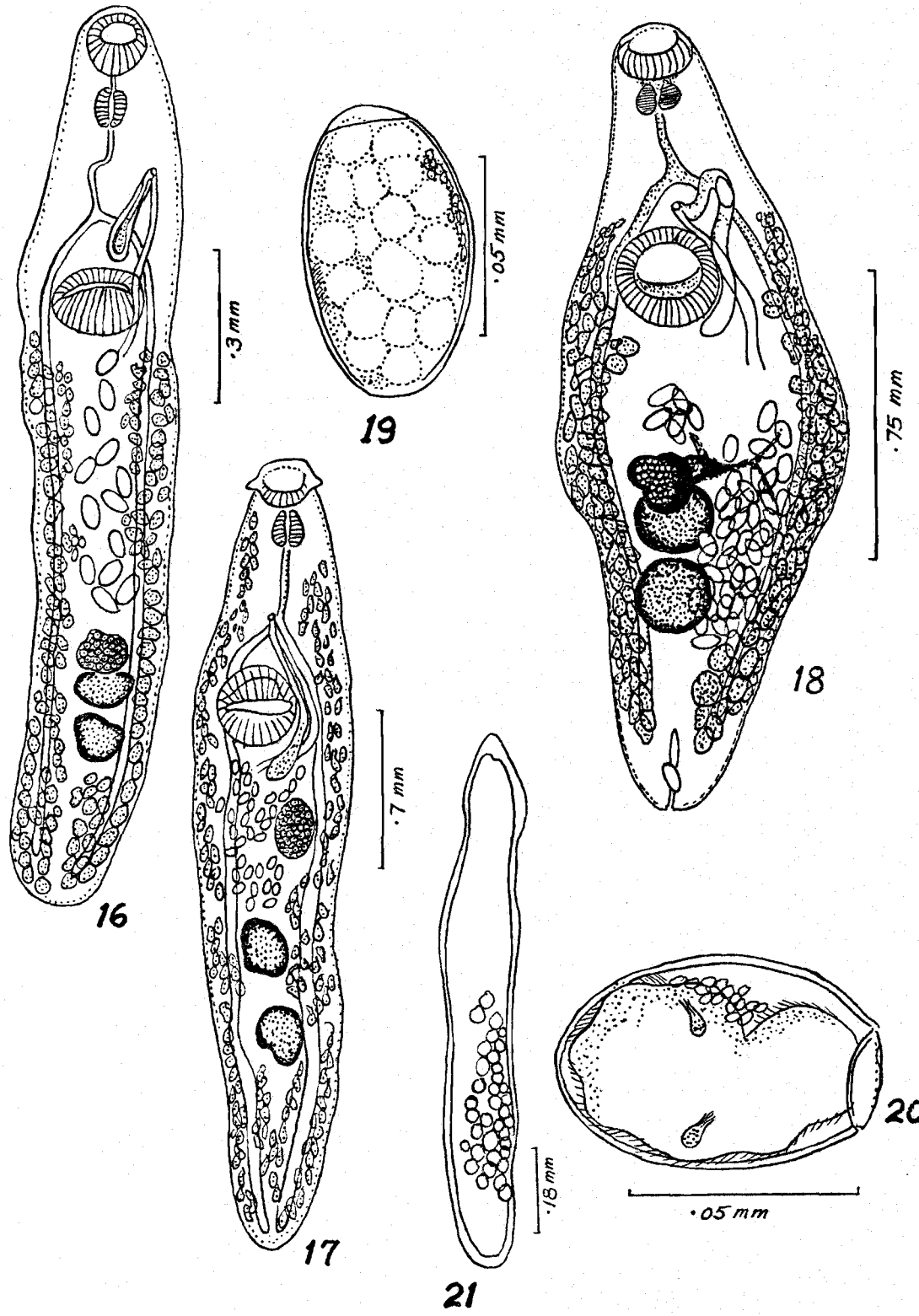
Figure 17. Adult Crepidostomum farionis (Mueller, 1784) Braun, 1900.

Figure 18. Adult allocreadiid from the intestine of Rhinichthys osculus nubilus, blackside dace.

Figure 19. Egg of the unknown allocreadiid trematode.

Figure 20. Egg of the unknown allocreadiid trematode with fully-developed miracidium.

Figure 21. Sporocyst found in F. virens exposed to miracidia of the unknown allocreadiid trematode.


.3 mm
16
.05 mm
19
.75 mm
18
.7 mm
17
.18 mm
21
20
.05 mm

Description (based on a single mature specimen): Body fusiform, nonspinous, with more tapering posterior end, 2.18 long by 0.77 wide. Body widest in area between ovary and acetabulum. Oral sucker subterminal, 0.228 wide by 0.16 long, with mouth opening ventrally. Pre-pharynx very short or non-existent. Pharynx large, wider than long, 0.108 by 0.068. Esophagus relatively long, measuring 0.172. Bifurcation of ceca occurs about halfway between posterior end of pharynx and anterior margin of acetabulum. Acetabulum spherical, larger than oral sucker, 0.32 diameter, situated at posterior end of anterior extremity of body.

Ovary lobed, 0.192 wide by 0.168 long, submedian and on the right overlapping anterior testis which is round measuring 0.168 in diameter. Posterior testis follows anterior testis but not pressing on each other; also round and of relatively the same size, 0.168 diameter. Excretory vesicle short and I-shaped. Uterus occupies area between acetabulum and posterior aspect of posterior testis. Most eggs in area to left of gonads. Vitellaria well-developed, occupying areas lateral to ceca and overlapping them, reaching to anterior margin of the acetabulum. Cirrus pouch present, on the left of acetabulum. Genital pore median or slightly submedian, immediately in front of acetabulum. Eggs medium in size, many, yellowish-brown in color, operculate and measuring 0.088 long by 0.048 wide.

Notes on Life Cycle: One mature and one immature specimen of this fluke were collected from the intestine of a 6 cm blackside dace, Rhinichthys osculus nubilus. This represents the only trematode parasite recovered from a total of three fish examined. On exposure to de-chlorinated water in a dish, the mature fluke immediately shed its eggs. Under room temperature and in de-chlorinated water, the eggs hatched out in 12 days (Figures 19, 20). Five young and presumably uninfected Flumenicola virens and two Oxytrema silicula were then exposed to the hatched miracidia. The two Oxytrema died shortly but the Flumenicola survived. Twenty-seven days post-exposure, one Flumenicola was cracked and many immature but very active sporocysts were found in the gonads, digestive gland and the rectal sinus. These sporocysts contained only germ balls and resembled those that produce cotylomicrocercous cercariae (Figure 21). In the subsequent period of 50 days, the remaining Flumenicola were isolated repeatedly and finally cracked to determine infectivity. None of them were found to be infected. Since these snails were not checked for prior infection, the one instance of infected snail provides no clue as to the identity of the adult trematode.

Discussion: This trematode has all the characteristics of the family Allocreadiidae but apparently does not belong to any existing genera. Schell's (1970) key puts my parasite in the genus Allocreadium Looss, 1900. However, this specimen obviously differs

from Allocreadium in its posterior extent of the uterus. In the latter, the uterus is located between the anterior testis and the acetabulum, but the uterus of my specimen extends at least to the rear margin of the posterior testis and may probably reach the extremity of the body. It also resembles genus Urorchis Ozaki, 1927 in the subfamily Urorchiinae Yamaguti, 1958 (Yamaguti, 1971) in most aspects but differs from it in having longer ceca and a median genital pore.

Since only one mature specimen of the parasite was available for study, its taxonomic status must await further clarification.

PART II. SURVEY OF LARVAL TREMATODES FROM THE TWO DOMINANT GASTROPOD SPECIES, *OXYTREMA SILICULA* AND *FLUMENICOLA VIRENS* FROM RITNER CREEK

Introduction

This study was designed to augment our meager knowledge of the larval trematodes of Oregon. Since the two snails under study, *Oxytrema silicula* and *Flumenicola virens* (Plates 1, 2), harbor a variety of trematodes, it was hoped that a survey of these snails would add to our understanding of the digenetic trematode fauna in Oregon. Incidence of their infections was investigated, and a check-list of cercariae known to infect them was compiled.

Materials and Methods

A total of 3, 333 snails of the two species was collected from Ritner Creek, Polk County, Oregon from July, 1974 to October, 1974. Two stations, approximately two miles apart on Ritner Creek, were chosen for the collection of the snails. Station I was in Ritner Creek Park, about two miles upstream from the intersection of Highway 223 and the creek, while station II was immediately under the bridge on Highway 223 and close to the junction where Ritner Creek merges with the Luckiamute River. Both *Oxytrema* and *Flumenicola* were found at each of the two stations.

In the laboratory, snails were isolated individually or in twos or threes in small stender dishes for at least 24 hours. Then the dishes were examined with the dissecting microscope for shed cercariae. Snails found in dishes with cercariae were isolated again individually to determine the infectivity of each snail. All snails were subsequently cracked and examined for infection and the incidence of infections tabulated. Actively shedding snails were kept in a 10°C-cold room as a subsequent source of cercariae for the studies of shedding cycles, longevity, swimming behavior and tropism of the cercariae.

To study the periodicity of emergence of cercariae, snails were separately placed in small stender dishes. They were then exposed to alternating periods of 12 hours light from 8 a.m. to 8 p.m. and 12 hours darkness from 8 p.m. to 8 a.m. The experiments were done at room temperature (20-22°C). The condition of darkness was simulated by transferring the snails into a box at 8 p.m. every evening. Cercarial counts were made at the end of each 12 hour period. The snails were transferred to another set of stender dishes, and the number of shed cercariae was then determined by pouring the water into a graduated Petri-dish and counting the number of cercariae observed under the dissecting microscope. De-chlorinated water was used in all the experiments and the snails were fed with fresh, green lettuce.

For microscopic study of the larval trematodes, wet mounts were made with 0.6% saline solution. Vital stains--neutral red and methylene blue--were used in determining the various internal structures of the larvae. However, cercariae were best studied alive and unstained under slight pressure from a coverslip. Larval trematodes were fixed in 10% formalin under slight coverslip pressure. Measurements, in millimeters, were made either on preserved specimens or on live specimens under coverslip pressure.

Results

A total of 1,328 Oxytrema and 2,005 Flumenicola was collected of which 34.44% of Oxytrema and 52.32% of Flumenicola were found to be infected with a variety of trematode larvae. The incidence of infections of the two snails is outlined in Table 6, and a list of cercariae encountered during the survey is presented in Table 7. This includes at least 17 apparent species. Oxytrema were most heavily infected with the cercariae of Nanophyetus salmincola (21.61%), next were the virgulate cercariae which constituted 8.28% of all infections. It is believed that up to five virgulate cercariae exist in Oxytrema (Burns, 1961b). But, because of their small size and high degree of similarity, no attempt was made to differentiate between them. Sixteen Oxytrema were infected with the cercaria of Metagonimoides oregonensis and another 16 with the cercaria of

Table 6. Incidence of trematode infections of O. silicula and F. virens from Ritner Creek. NAM = Non-aggregating macrocercous cercaria; AA = Aggregating-albino cercaria.

	Station I						Station II						Total	%
	July		August		September		August		September		October			
	no. infected	%	no. infected	%	no. infected	%	no. infected	%	no. infected	%	no. infected	%		
Snail Host: Oxytrema silicula														
Uninfected	10	71.43	0	0	15	26.30	330	78.76	319	63.17	198	75.29	872	65.66
C. of Nanophyetus salmincola	2	14.29	55	78.57	26	45.60	46	10.98	120	23.76	38	14.45	287	21.61
Virgulate c.	1	7.14	12	17.14	14	24.56	19	4.53	44	8.71	20	7.60	110	8.28
C. of Metagonimoides oregonensis	0	0	3	4.29	2	3.50	4	0.95	4	0.79	3	1.14	16	1.20
C. of Plagioporus siliculus	0	0	0	0	0	0	8	1.90	7	1.39	1	0.38	16	1.20
Cystophorous c.	0	0	0	0	0	0	4	0.95	2	0.40	1	0.38	7	0.53
Cercaria gorgonocephala	0	0	0	0	0	0	1	0.24	2	0.40	1	0.38	4	0.30
NAM c.	0	0	0	0	0	0	2	0.48	1	0.20	0	0	3	0.23
AA c.	0	0	0	0	0	0	1	0.24	1	0.20	0	0	2	0.15
C. of Levinseniella minuta	0	0	0	0	0	0	4	0.95	2	0.40	1	0.38	7	0.53
Brevifurcate-apharyngeate c.	0	0	0	0	0	0	0	0	2[2]	0.40	0	0	2	0.15
Double-infection	1[1]	7.14	0	0	0	0	0	0	1[3]	0.20	0	0	2	0.15
													1328	100

(Continued on next page)

Table 6. (Continued)

	Station I								Station II						Total	%
	July		August		September		October		August		September		October			
	no. infected	%	no. infected	%	no. infected	%	no. infected	%	no. infected	%	no. infected	%	no. infected	%		
Snail Host: Flumenicola virens																
Uninfected	168	60.43	119	56.13	33	66	177	52.99	138	49.64	129	35.05	192	39.59	956	47.68
C. of Podocotyle virens	77	27.70	33	15.57	3	6	79	23.65	18	6.47	13	3.53	16	3.30	239	11.92
Cercaria X	21	7.55	27	12.74	3	6	30	8.98	11	3.96	3	0.82	7	1.44	102	5.09
C. of Astacatrematula macrocotyla	0	0	1	0.47	0	0	1	1.20	4	1.44	0	0	2	0.41	11	0.55
C. of Sphaeridiotrema spinoacetabulum	2	0.72	0	0	2	4.9	6	1.8	0	0	4	1.09	2	0.41	16	0.80
C. of Apophallus donicus	2	0.72	19	8.96	5	10	29	8.68	79	28.41	191	51.90	213	43.92	538	26.83
Virgulate c.	7	2.52	8	3.77	4	8	7	2.10	23	8.27	27	7.34	50	10.31	126	6.28
Cysts ?	1	0.36	0	0	0	0	0	0	0	0	0	0	0	0	1	0.05
Chaetomicrocercous c.	0	0	5	2.36	0	0	2	0.6	0	0	1	0.27	1	0.21	9	0.45
C. of Lissorchis heterorchis	0	0	0	0	0	0	0	0	3	1.08	0	0	1	0.21	4	0.20
Double-infection	0	0	0	0	0	0	0	0	2[4,5]	0.72	0	0	1[6]	0.21	3	0.15
Total															2005	100

[1] Virgulate/ psilostome

[2] Double-infection; one with M. oregonensis; one with N. salmincola

[3] Virgulate/N. salmincola

[4] Virgulate/ chaetomicrocercous

[5] Virgulate/P. virens

[6] Virgulate/L. heterorchis

Table 7. Cercariae encountered in the survey of Oxytrema silicula and Flumenicola virens from Ritner Creek. Species of virgulate cercariae not differentiated. O. s. = O. silicula; F. v. = F. virens.

Cercarial Type	Snail Hosts		Station	
	O. s.	F. v.	I	II
Microcercous				
C. of Nanophyetus salmincola	X		X	X
C. of Plagioporus siliculus	X			X
C. of Podocotyle virens		X	X	X
C. of Lissorchis heterorchis		X		X
Chaetomicrocercous c.		X	X	X
Pleurolophocercous				
C. of Metagonimoides oregonensis	X		X	X
C. of Apophallus donicus		X	X	X
Psilostome				
C. of Astacatrematula macrocotyla		X	X	X
C. of Sphaeridiotrema spinoacetabulum		X	X	X
Virgulate	X	X	X	X
Zygocercous				
C. gorgonocephala (= C. of Echinochasmus milvi)	X			X
Aggregating-albino c.	X			X
Macrocercous				
Non-aggregating macrocercous c.	X			X
Furcocercous				
Brevifurcate-apharyngeate c.	X			X
Cystophorus c.	X			X
Cercariaeum				
C. of Levinseniella minuta	X			X
Miscellaneous				
Cercaria X		X	X	X

Plagioporus siliculus. Of the two stations, station II under the bridge was found to yield Oxytrema infected with a greater diversity of cercariae (Table 6). Besides the virgulate cercariae, Oxytrema from station II harbored a total of 10 different species of cercariae whereas station I had only three. One Oxytrema from station I and three from station II were found to contain double-infections. One interesting feature in the double-infections is that: two of the four instances involved a virgulate cercaria. The two combinations were: virgulate/psilostome and virgulate/Nanophyetus salmincola. The only two cases of brevifurcate-apharyngeate cercaria infections in Oxytrema were also involved in double-infections: one with Metagonimoides oregonensis and the other with Nanophyetus salmincola. It is likely that some cercariae are by nature less host specific and do not render their hosts refractive to further infection by other cercariae.

Of 2,005 Flumenicola virens collected, 1,049 or 52.32% were found to be infected. The highest rate of infection belonged to that of the cercaria of Apophallus donicus, which constituted 26.83%. Second was the cercaria of Podocotyle virens (11.92%). Virgulate cercariae were found in 6.28% of Flumenicola while Cercaria X was found in 5.09% of the snails. The rest of the infections, involving at least five different species of cercariae, had below 1% of incidence each. Three double-infections were noted and in all three, a

virgulate cercaria was involved in the combination (Table 6). This further supports my idea that some cercariae tend to be less host specific than others.

In F. virens, the cercaria of Lissorchis heterorchis and all double-infections were found only in station II. Comparing the infection incidences in the two stations, the cercaria of Apophallus donicus was found in much greater number in station II while station I had more Flumenicola infected with the cercaria of Podocotyle virens and Cercaria X (Table 6).

Description and Notes of Cercariae from O. silicula and F. virens

Microcercous Cercariae

Cercaria of Nanophyetus salmincola (Chapin)

Host: Oxytrema silicula.

Incidence of Infection: 287 of 1,328 snails, or 21.61%.

Locality: Stations I and II, Ritner Creek, Polk County, Oregon.

In both stations in Ritner Creek, 21.61% of Oxytrema collected were found to be infected with the cercaria of Nanophyetus salmincola. This constitutes the highest rate of infection among all known cercariae infecting the snail in this locality. The cercaria of N. salmincola, the "Salmon-poisoning" fluke, was first described by

Sinitsin (1930). Bennington and Pratt (1960), however, provided the first adequate description and diagram of the cercaria.

Cercaria (Figure 22): It is a xiphidiomicrocercous cercaria, measuring 0.31 to 0.47 by 0.03 to 0.15, spinous with a bluntly conical tail which also bears fine hair-like spines. Oral sucker, pharynx and acetabulum are all prominent. Penetration glands are in four clusters of four cells each. A large excretory vesicle shares the post-acetabular region with a mucus gland which empties near the base of the tail.

The cercaria does not swim but elongates and contracts rapidly and crawls on the substrate. Law (1969) studied the migration pathway and emergence of the cercaria. The majority of mature cercariae made use of the venous system to arrive at the ctenidial leaflets and actively emerged at the tips. Light was found to enhance cercarial emergence and the optimal pH and temperature for the emergence of N. salmincola cercariae were found to be pH 7.6 and approximately 22°C respectively.

Sporocyst and Redia (Figures 23-25): Inside the molluscan host, the parasite develops through a sporocyst and a redial stage and emerges as a cercaria. For descriptions and the biology of these larval stages, see Bennington and Pratt (1960), Law (1969), and Carter (1973).

The life history of N. salmincola has been extensively studied (Bennington and Pratt, 1960; Carter, 1973). The cercariae penetrate a variety of fresh water fishes and encyst as metacercariae. Definitive hosts are fish-eating mammals. The epizootiology of "Salmon-poisoning" disease has recently been reviewed by Millemann and Knapp (1970a, b) and Knapp and Millemann (1970), and the etiologic agent, Neorickettsia helminthoeca, was successfully cultured by Noonan (1973).

Cercaria of Plagioporus siliculus Sinitsin, 1931

Host: Oxytrema silicula.

Incidence of Infection: 16 of 1,328 snails, or 1.2%.

Locality: Station II, Ritner Creek, Polk County, Oregon.

The cercaria of Plagioporus siliculus was first described by Sinitsin (1931). It develops in sporocysts in O. silicula and penetrates crayfish to encyst as a metacercaria in the abdominal muscles. The definitive hosts are "species of fresh water fishes" including Salmo clarki clarki, coastal cutthroat trout (Sinitsin, 1931), Oncorhynchus kisutch, coho salmon, Rhinichthys osculus, speckled dace, and Cottus rhotheus, torrent sculpin (Part I, this study). Pratt and McCauley (1961) and Crandell (1963) failed to observe penetration of crayfish by the cercaria of P. siliculus as claimed by Sinitsin (1931), but such penetration was observed by this author. The first laboratory-demonstrated life cycle of P. siliculus is presented in Part III of this study.

Sinitsin (1931) described the cercaria, sporocyst, metacercaria and adult of P. siliculus and discussed some aspects of the behavior of the cercaria. The following observations on the larval stages are intended to supplement those of Sinitsin's.

Cercaria (Figures 26-28): Sinitsin's work should be referred to for detailed descriptions of the cercaria which belongs to the cotylomicrocercous group. The cercaria of P. siliculus does not swim but stays on the bottom of the container. It may crawl about using only the oral sucker and the tail (Figure 27). While attaching to the substrate by the tail, the whole body is extended forward until the oral sucker touches the substrate. Then the tail is lifted up and moves forward with the contraction of the body. The cercaria can also move by "inch-worming" with its oral sucker and acetabulum while the entire posterior portion of the body remains contracted (Figure 28). At times, the cercaria may stand on its tail and wave about. This behavior has been described by Sinitsin (1931).

The cercaria did not show particular preference for either light or dark, although shedding observations with infected snails for five days indicated a nocturnal pattern of cercarial emergence. At least four times as many cercariae emerged during the night (Table 8). Cercaria in room temperature (20-22^{o}C) lived for two to three days. Most died within 45 hours of shedding although some managed to live for 51 hours.

Figure 22. Cercaria of Nanophyetus salmincola. Redrawn from Bennington and Pratt (1960).

Figure 23. Sporocyst of N. salmincola. Redrawn from Carter (1973).

Figure 24. Immature redia of N. salmincola. Redrawn from Bennington and Pratt (1960).

Figure 25. Mature redia of N. salmincola. Redrawn from Bennington and Pratt (1960).

Figure 26. Cercaria of Plagioporus siliculus. Redrawn from Sinitsin (1931).

Figure 27. Locomotion of the cercaria of P. siliculus.

Figure 28. The "inch-worming" movement of the cercaria of P. siliculus.

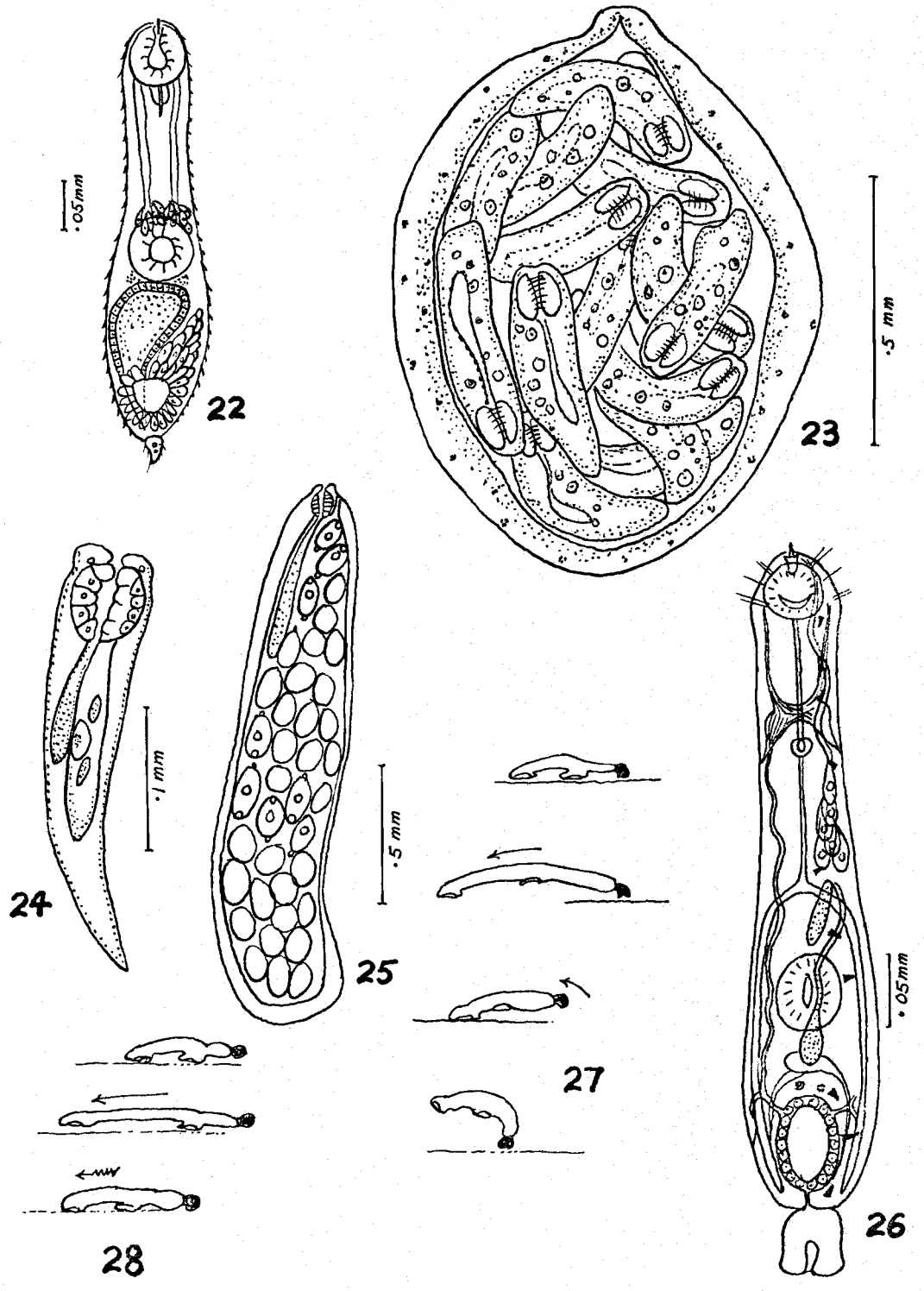

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Table 8. Emergence patterns of some cercariae encountered in the survey of O. silicula and F. virens from Ritner Creek. D = dark; L = light; NAM c. = Non-aggregating macrocercous cercaria.

		C. of Podocotyle virens				Average	Cercaria X			Average	C. of Apophallus donicus				Average	NAM c.		Average	C. of Plagio-porus siliculus		Average	Chaetomicro-cercous c.
		1	2	3	4		1	2	3		1	2	3	4		1	2		1	2		
8 pm-8 am	D	18	20	8	37	21	0	0	26	9	605	17	1	225	212	2	0	1	0	14	7	.67
8 am-8 pm	L	11	143	0	30	46	172	322	170	221	1325	0	703	928	738	23	109	66	16	3	10	31
8 pm-8 am	D	43	10	0	48	25	0	0	16	5	526	12	1	1	135	0	3	2	68	372	220	23
8 am-8 pm	L	42	106	3	65	54	179	169	60	136	1707	526	154	755	786	74	86	80	47	98	73	27
8 pm-8 am	D	30	10	3	98	35	1	0	5	2	142	224	2	0	92	0	10	5	121	714	418	20
8 am-8 pm	L	51	177	7	28	66	156	313	350	273	1724	dead	162	561	816	39	74	57	8	93	51	16
8 pm-8 am	D	6	4	2	41	13	0	0	49	16	222		2	6	77	1	4	3	68	512	290	26
8 am-8 pm	L	45	139	5	0	47	102	181	383	222	1181		17	353	517	29	74	52	100	47	74	18
8 pm-8 am	D															5	1	3	404	619	512	27
8 am-8 pm	L															20	68	44	109	88	99	4

Sporocyst (Plate 3; Figures 29, 30): Long and sac-like, yellowish in color, 1.08 to 2.32 (1.485) long by 0.24 to 0.28 (0.255) wide. Sporocysts were found embedded in the digestive gland with the gonad completely destroyed in heavier infections, and in sinuses, especially the rectal sinus. The sporocysts of P. siliculus were less active compared to those of P. virens in F. virens but were much more numerous. Mature cercariae could be seen moving inside sporocysts. Although the average number of mature cercariae per sporocyst was not determined, a large sporocyst might contain 20 or more cercariae. Only one generation of sporocysts was observed during the course of this study.

Cercaria of Podocotyle virens (Sinitsin, 1931) Yamaguti, 1971

Host: Flumenicola virens.

Incidence of Infection: 239 of 2,005 snails, or 11.92%.

Locality: Stations I and II, Ritner Creek, Polk County, Oregon.

The cercaria of Podocotyle virens resembles the cercaria of Plagioporus siliculus (Figure 31). Both are cotylom icrocercous cercariae. The first report and description of cercaria P. virens was by Sinitsin (1931), who found the cercaria in "simple, sporocyst-like worm" in the liver of F. virens. The cercaria can be differentiated from that of P. siliculus by the absence of bristles on the anterior end, smaller stylet and smaller size. Its biology, according

Plate 1. Oxytrema silicula.

Plate 2. Flumenicola virens.

Plate 3. Cracked O. silicula showing sporocysts of Plagioporus siliculus in the gonad and digestive gland of the snail.

to Sinitsin, is drastically different from that of the cercaria of P. siliculus. Instead of attacking crayfish, the cercaria penetrates F. virens, its original snail host, and encysts in it. If the snail happens to be infected with any kind of trematode larvae, the cercaria exhibits a predilection to encyst inside the larvae. However, the accuracy of Sinitsin's description of this unique behavior is questionable. In examining 239 P. virens infected snails, I did not notice the encystment of the cercaria inside the snail as Sinitisin claimed. Nor did I notice any metacercariae in the sporocysts of infected F. virens. Sinitsin claimed 90% of F. virens from the Willamette River were infected with the cysts of P. virens. It is probable that he had confused the metacercariae of another species with that of P. virens. Dobrovolny (1939), in discussing the behavior of the cotylomicrocercous cercariae, also doubted the accuracy of Sinitsin's observation. He thought it unlikely that the cercaria of P. virens should show a predilection for sporocysts. In his opinion, Sinitsin was possibly "dealing with two species and failed to distinguish the metacercariae of P. virens from those of a species which has a development similar to P. sinitsini" (Dobrovolny, 1939).

Sinitsin's description of the metacercaria of P. virens was inadequate, although his description of the adult from a "Cottus sp." was accurate enough to allow verification. In my survey of fish trematodes from Ritner Creek, adult P. virens were collected from

Cottus rhotheus, torrent sculpin, and C. perplexus, reticulate sculpin (see Part I, this study). Since Sinitsin did not prove the life cycle by experiments, the validity of the life cycle of P. virens as described by him remains to be verified.

Cercaria (Figures 31, 32a-l): The cercaria of P. virens develops inside the sporocyst as a germ ball which eventually elongates (Figures 32 a-l). The tail apparently differentiates rather early in the development of the cercaria. Next to develop is the ventral sucker and the oral sucker. It seems that as the larva grows, the pharynx is pushed backward and the pre-pharynx lengthens. This coincides with the rapid development of the oral sucker and the anterior part of the body. For description of shed cercaria, refer to Sinitsin (1931).

The behavior of the cercaria is as described by Sinitsin and is very similar to the cercaria of P. siliculus. Besides the usual "standing-on-tail-and-wagging-to-and-fro" and crawling behavior, I have also noticed an interesting "flip-flop" pattern which is not practiced by the cercaria of P. siliculus (Figure 34). The cercaria stands on its tail and slowly bends ventrally until the oral sucker touches the substrate. Then the cercaria lets go of its tail and contracts its body in the direction of the oral sucker. Then suddenly the oral sucker is lifted from the substrate and the entire body "flips over" in a somersault. The tail finally lands on the substrate and the

cercaria stands erect as it was before. The acetabulum is not used in this mode of locomotion.

The cercaria of P. virens did not show preference either for light or dark and, in contrast to the cercaria of P. siliculus, showed a diffused pattern of emergence. Although the average numbers of cercariae shed by four snails showed a distinct diurnal pattern, analysis of individual patterns (Table 8) gave conflicting results. Cercariae of P. virens in room temperature (20-22°C) lived for four to five days. Most, however, died within 85 hours of shedding.

The cercaria did not encyst in F. virens.

Sporocyst (Figure 33): When alive, sporocysts of P. virens are elongate, whitish-yellow, covered with very fine spines, sac-like and capable of great extension and contraction. The size of the sporocyst, 1.68 to 2.32 (1.9) long by 0.32 to 0.36 (0.34) wide, is large compared to the size of the snail host. Sporocysts were found between the tunica propria and the digestive gland with the gonad completely destroyed in most cases; they were also found most frequently in the rectal sinus of the snail. Only one generation of sporocysts was observed during the course of this study. No redia was observed. Porter (1970) was probably mistaken when he referred to the effects of parasitism by the "rediae" of P. virens on F. virens. All Podocotyle develop in sporocysts in their molluscan hosts.

Cercaria of Lissorchis heterorchis Krygier and Macy, 1969

Host: Flumenicola virens.

Incidence of Infection: 4 of 2,005 snails, or 0.2%.

Locality: Station II, Ritner Creek, Polk County, Oregon.

The adult Lissorchis heterorchis (Trematoda: Lissorchiidae) was first described by Krygier and Macy (1969) from the sucker Catostomus macrocheilus Girard in western Oregon. The life cycle, however, was not completed until 1972 when Onyejekwe discovered the cercaria of L. heterorchis in F. virens. Three percent of Flumenicola snails from the Tualatin River, Portland, Oregon were found to be infected by this cercaria (Onyejekwe, 1972). The same cercaria was found in only 0.2% of 2,005 F. virens examined from Ritner Creek. Its descriptions in general matched those of Onyejekwe's. The life cycle of L. heterorchis, according to Onyejekwe (1972) is as follows: the eggs are swallowed by F. virens and develop into rediae that give rise to lissorchiid cercariae. The cercariae encyst either in planarians or an annelid as metacercariae which, when eaten by a sucker, develop into adult L. heterorchis.

Although I am convinced that the trematode Onyejekwe studied is identical to the one I am reporting here, his descriptions of the cercaria as well as the redia differ from mine in several aspects. Since the materials available to me were limited, my observations on the cercaria and redia as reported below must be considered as

preliminary. It is further hoped that my observations may supplement those of Onyejekwe's.

Cercaria (Figures 35-37): The cercaria is distinctly microcercous and bears little resemblance to the cercaria of Lissorchis mutabile, as was erroneously asserted by Onyejekwe (1972). The body of 10 relaxed cercariae of L. heterorchis under coverslip pressure measured 0.448 to 0.508 (0.48) long by 0.176 to 0.236 (0.2) wide. Cuticular spines are absent from the body except at the posterior end. The cuticle of the body instead is covered with a regular pattern of small tubercles (Figure 36). The tail is ball-shaped and covered with spines. A typical fixed cercaria is elongate, spindle-shaped, with the broadest portion of the body situated just anterior to the acetabulum. The oral sucker is nearly round, 0.076 to 0.084 (0.08) in diameter and subterminal with mouth opening ventrally. Pre-pharynx is relatively long; pharynx is ovoid and situated closer to the oral sucker than the acetabulum. The acetabulum is nearly round, slightly pre-equatorial, 0.084 to 0.092 (0.088) in diameter. The excretory vesicle is thick-walled and typically S-shaped, occupying the posterior one-third of the body. Two excretory tubules, one on either side of the body, reach forward from the excretory vesicle to the level of the pharynx. The flame cell pattern was not worked out due to the heavy granulation of the body. Onyejekwe (1972) assigned a flame cell formula of 2[(2+2+2+2) + (2+2+2+2)] to the

Figures 29-30. Sporocysts of P. siliculus.

Figure 31. Cercaria of Podocotyle virens. Redrawn from Sinitsin (1931).

Figure 32a-l. Developmental stages of the cercaria of P. virens.

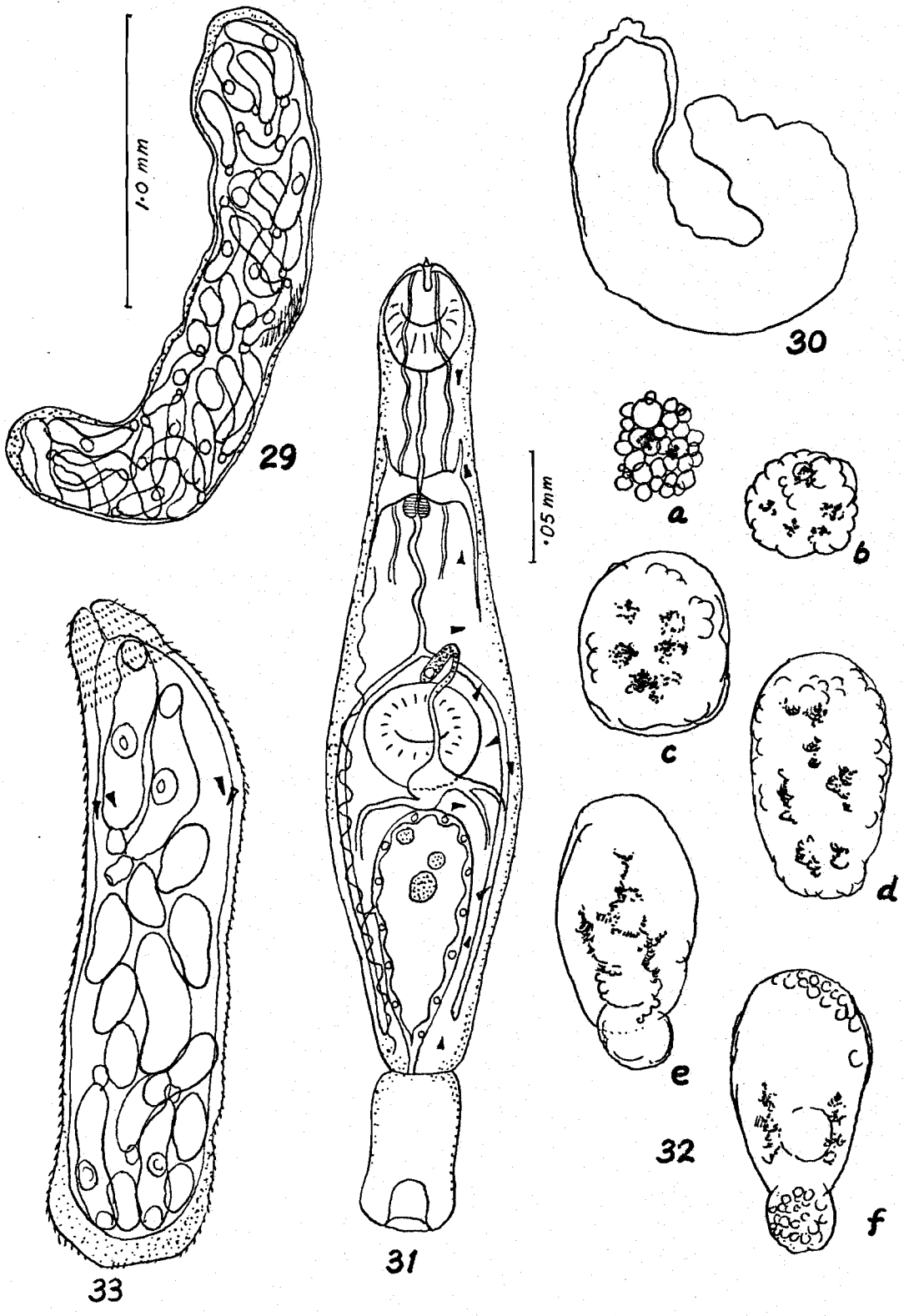
Figure 33. Sporocyst of P. virens. Note spinous body.

Figure 34. Locomotion of the cercaria of P. virens.

Figure 35. Cercaria of Lissorchis heterorchis.

Figure 36. Regular pattern of small tubercles on the body of the cercaria of L. heterorchis.

Figure 37. Cercaria of L. heterorchis contracted into a ball on the bottom of the dish.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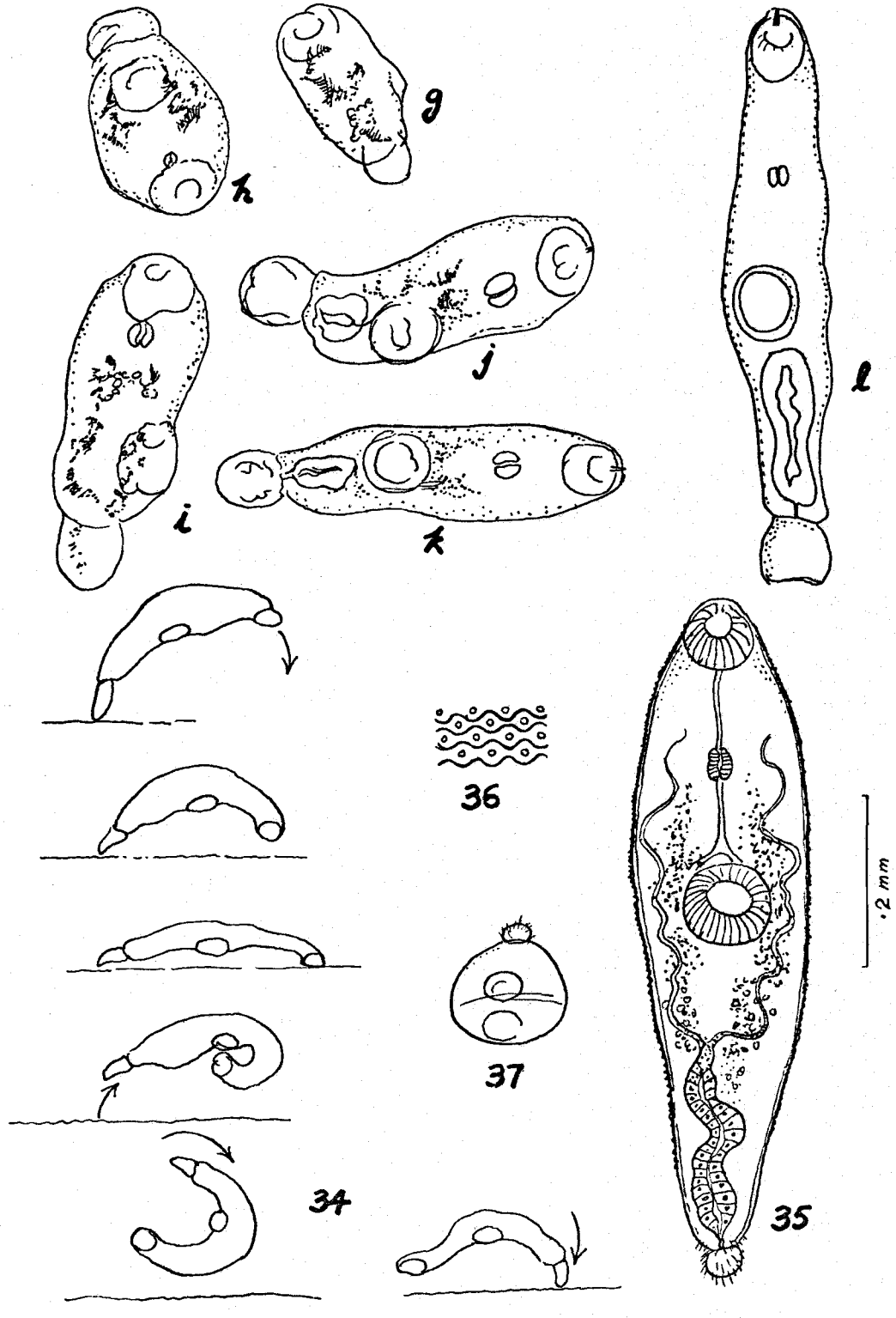
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cercaria. His figure of the cercaria, however, presented a total of 32 flame cells on each side of the body. This latter pattern is identical to the one in the cercaria of L. mutabile but is in direct variance with his own formula.

The cercaria of L. heterorchis is essentially a bottom crawler and moves by a typical worm-like movement: the anterior part of the body is extended. The oral sucker attaches to the substrate and the anterior body is contracted to pull the posterior part forward. The cercaria spends much of the time contracted into a ball on the bottom of the dish (Figure 37). The tail of the cercaria is shed quite readily. At room temperature (20-22°C), cercaria of L. heterorchis could survive for two to three days. At the end of two days most cercariae shed their tails and became very immobile. All died by 80 hours.

Redia (Figures 38, 39): The rediae of L. heterorchis were found in the gonad and digestive gland of the snail. When alive, the rediae are greyish-white in direct light. They are plump or sausage-shaped and are either straight or curved. A typical redia has a distinct pharynx at the anterior body which tapers to a blunt end. The posterior end is broader and round. The body is heavily granulated. A long intestine, filled with brown concretions, reaches posteriorly from the pharynx, occupying at least three-fourths of the body. The birth pore is situated anterior to the pharynx and on one side. A

mature redia may contain from one to five full-grown crecariae in its body. Ten AFA-fixed rediae measured 0.88 to 1.12 (1.02) long by 0.24 to 0.36 (0.26) wide. The pharynx measured 0.056 to 0.076 (0.067) by 0.06 to 0.076 (0.066). Only one generation of rediae was observed during the course of this study.

It is apparent that the systematic position of the genus Lissorchis is quite uncertain (Wallace, 1941; Smith, 1968). Triganodistomum mutabile (Cort, 1918; Wallace, 1941) was synonymized with Lissorchis by Smith (1968). The genus Triganodistomum, however, has been placed variously in families Plagiorchiidae (Simer, 1929) and Allocreadiidae (Van Cleave and Mueller, 1932). Magath (1917) suggested putting Lissorchis with the family Plagiorchiidae but it was Poche (1925) who established the family Lissorchiidae with Lissorchis Magath as the type genus. The systematic uncertainty of Lissorchis is further underscored by the diversed life cycle patterns and larval morphologies represented in the members of the genus. Of the three life cycles in the genus completed to date, three separate groups of cercariae are involved. The cercaria of L. mutabile is a tailless cercariaeum of the "mutabile group," while those of L. fairporti Magath, 1917 and L. heterorchis Krygier and Macy, 1969 are xiphidiocercaria and microcercous cercaria respectively. While both L. mutabile and L. heterorchis use planarians and commensal chaetogasters as secondary hosts (Wallace,

1941; Smith, 1968; Onyejekwe, 1972), the cercariae of L. fairporti penetrate and encyst in chironomid larvae instead (Magath, 1917).

Chaetomicrocercous Cercaria

Host: Flumenicola virens.

Incidence of Infection: 9 of 2,005 snails, or 0.45%.

Locality: Stations I and II, Ritner Creek, Polk County, Oregon.

This cercaria is reported here for the first time from Flumenicola virens. It resembles superficially the cercaria of Lissorchis heterorchis but differs from it in being heavily spinous and armed with a very small stylet.

Cercaria (Figures 40-42): Body spinous throughout, 0.38 to 0.59 (0.45) long by 0.128 to 0.15 (0.137) wide, elongate, tapering broadly at both ends. Oral sucker wider than long, 0.068 to 0.081 (0.073) by 0.054 to 0.076 (0.065), opens subterminally and ventrally. The stylet small, 0.003 by 0.003, lies embedded in dorsal lip of the oral sucker. Pre-pharynx present; pharynx half-way between oral sucker and acetabulum. The acetabulum, 0.058 to 0.072 (0.065) long by 0.063 to 0.077 (0.069) wide, median, equatorial, slightly smaller than oral sucker. Body heavily granulated obscuring internal structures and flame cell pattern. Tail small, capable of contraction tapering at posterior end; long hairs occupy basal portion and tip of the tail (Figure 42). Excretory bladder thick-walled, long, lined with

a single layer of tall epithelial cells. When contracted, excretory bladder occupies most of posterior body and folds into a convoluted sac-like structure.

The cercaria moves slowly on the substrate. Its creeping movement resembles that of the cercaria of Lissorchis heterorchis. The body is capable of great extension and contraction. At rest, the cercaria assumes the shape of a top (Figure 43). The tail is shed quite readily, especially under coverslip pressure.

The cercaria could live for three days at room temperature (20-22°C). They became inactive, rounded up and stayed on the bottom at the end of two days. When dead at 71 hours, the cercarial body was extended and spindle-shaped.

Infected Flumenicola shed only a few chaetomicrocercous cercariae (Table 8). A single infected snail, isolated for five consecutive days, shed an average of 52 cercariae per day. The shedding pattern, however, is difficult to interpret.

Redia (Figures 44, 45): One generation of rediae was observed in this study. They were found in the gonad and digestive gland of the snail. The mature rediae are plump and sausage-shaped, resembling those of Lissorchis heterorchis. Ten fixed specimens measured 0.663 to 1.48 (1.065) long and 0.234 to 0.312 (0.285) wide. The pharynx, 0.068 in diameter, is anterior. The birth pore opens anteriorly and on one side of the pharynx.

Figure 38. Young redia of L. heterorchis.

Figure 39. Older, mature redia of L. heterorchis.

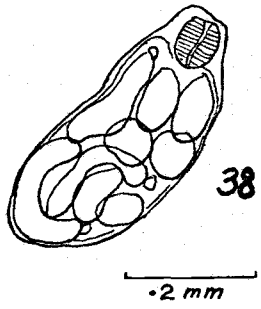
Figure 40. Chaetomicrocercous cercaria from F. virens. Ventral view.

Figure 41. Chaetomicrocercous cercaria. Lateral view.

Figure 42. Tail of chaetomicrocercous cercaria.

Figure 43. Chaetomicrocercous cercaria assuming the shape of a top.

Figures 44-45. Rediae of chaetomicrocercous cercaria.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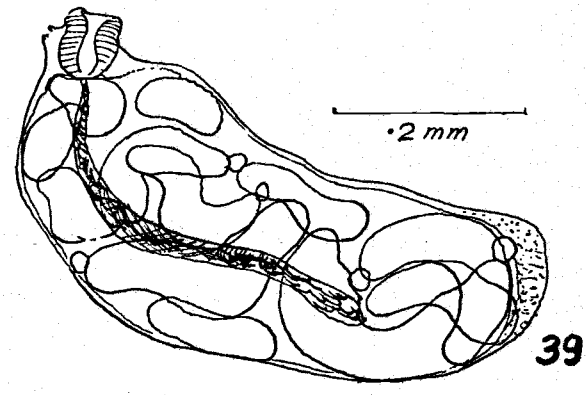
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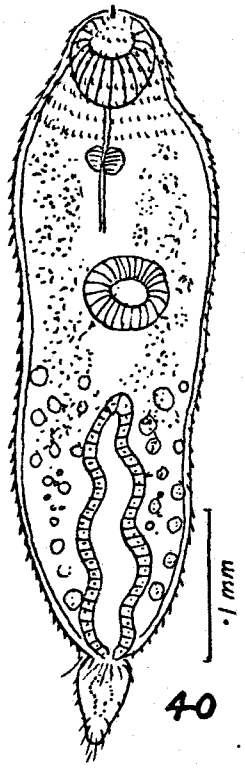
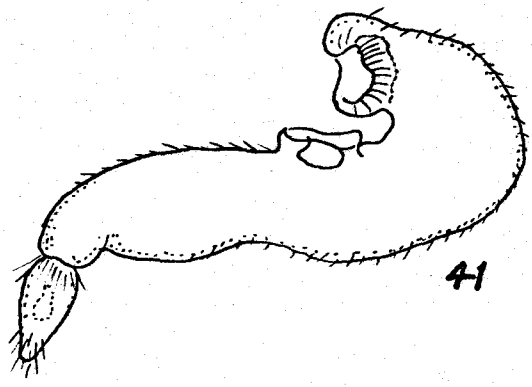
39


·1 mm
40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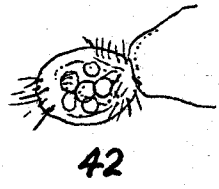
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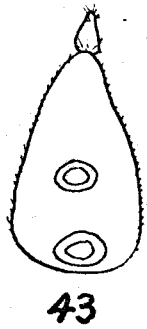
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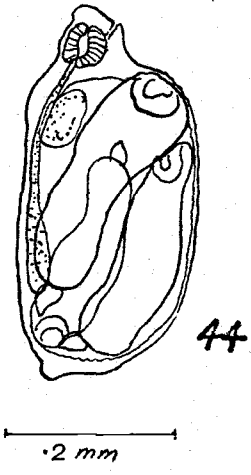
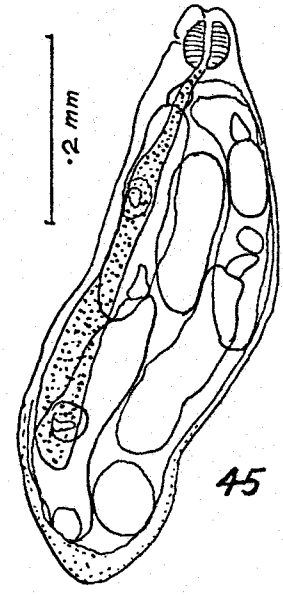

·2 mm
44


·2 mm
45

Many mature rediae and cercariae were observed in the rectal sinus, although no cercariae were seen to emerge from the rectum.

Pleurolophocercous Cercariae

Cercaria of Metagonimoides oregonensis Price, 1931

Host: Oxytrema silicula.

Incidence of Infection: 16 of 1,328 snails, or 1.2%.

Locality: Stations I and II, Ritner Creek, Polk County, Oregon.

Price (1931c) first described the adult heterophyid Metagonimoides oregonensis from the intestine of the raccoon, Procyon lotor pacifica Merriam. The life cycle was implicated by Ingles (1935), who briefly described the larval stages in the snail Goniobasis migrina (Lea). According to him, the cercariae of M. oregonensis developed directly into metacercariae within the redia. The complete life cycle of M. oregonensis was first reported by Burns and Pratt in 1953.

Cercaria (Figures 46, 47): The cercaria of M. oregonensis belongs to the pleurolophocercous group established by Sewell (1922). A detailed description of the cercaria may be found in Burns and Pratt (1953). The body of the cercaria is extremely contractile, spined anteriorly and has a pair of eyespots. The oral sucker opens subterminally. A long pre-pharynx is evident, although the small pharynx is inconspicuous. Penetration glands, 14 to 16 in number, occupy the central region of the body. The excretory vesicle is large,

thick-walled, and often deeply creased and folded upon itself. The tail is twice as long as the body, has a ventral fin-fold and is set in a pocket at the posterior end of the body.

The cercaria is a very active swimmer. It moves up and down vigorously for short periods followed by rest. When at rest, the cercaria typically assumes an upside-down position in the form of the letter J.

According to Burns and Pratt (1953), the cercariae of M. oregonensis readily penetrate frogs and tadpoles exposed to them. Adult Rana pipiens, R. aurora aurora, and tadpoles of R. catesbiana were seen penetrated by the cercariae.

Redia (Figure 48): Mature and immature rediae of M. oregonensis were found in the gonad and digestive gland of infected Oxytrema. Only one generation of rediae was observed in this study, although Burns and Pratt (1953) suspected the existence of either a mother redia or a sporocyst stage in the cycle. A typical mature redia is sausage-shaped, has a pharynx and a birth pore, and contains germ balls, immature cercariae, mature cercariae and mature metacercariae. Although infected Oxytrema isolated in finger bowls shed only cercariae, the mature metacercariae in full-grown rediae were shown to be infective to hamsters fed the digestive gland of snails infected with M. oregonensis rediae (Burns and Pratt, 1953).

Metacercaria (Figures 49, 50): Infective metacercariae of M. oregonensis taken from the redia resemble in most respects the adult fluke. They differ, however, from the adult in having a relatively large, Y-shaped excretory bladder which is filled with highly refractive bodies. Metacercariae identical to those in the rediae are found naturally encysting in striated muscles of frogs, Rana aurora aurora (Burns and Pratt, 1953) (Figure 50). Feeding experiments with hamsters using either metacercariae in the rediae or encysted metacercariae from frogs exposed to the cercariae of M. oregonensis resulted both in adult flukes of M. oregonensis (Burns and Pratt, 1953). The life cycle of M. oregonensis is thus unique in having metacercariae which are equally infective in the rediae of the snail or in the frog. Since both snails and frogs are known to be in the diet of the raccoon, the definitive host may become infected through eating either of the two animals. This undoubtedly represents a very successful adaptation on the part of the parasite.

Cercaria of Apophallus donicus Skrjabin and Lindtrop, 1919

Host: Flumenicola virens.

Incidence of Infection: 538 of 2,005 snails, or 26.83%.

Locality: Stations I and II, Ritner Creek, Polk County, Oregon.

The adult Apophallus donicus was first described by Skrjabin and Lindtrop in 1919 from Russia. Shaw reported the trematode from

an Oregon gull in 1947 (Shaw, 1947). The life cycle, however, was not known until 1974 when Niemi and Macy, and I independently completed the cycle (Niemi and Macy, 1974; Part III, this study).

Cercaria (Figures 51-53; 54a-e): The cercaria of A. donicus has been described in detail by Neimi and Macy. It belongs to the pleurolophocercous group and resembles the cercaria of Metagonimoides oregonensis in both morphology and behavior. The cercaria of A. donicus, however, uses Flumenicola virens as a snail host, and its tail has a continuous dorsal and ventral fin (Figure 52). It has 14 penetration glands in contrast to 14 to 16 in the cercaria of M. oregonensis. The oral sucker is equipped with protrusible lips which are armed with at least two rows of small spines (Figure 53).

The cercaria is positively phototropic and very active. It swims by lashing its tail and dashes up and down in sudden bursts of movement. When at rest, the body is suspended motionless in the form of a letter J.

The shedding pattern of cercaria A. donicus is distinctly diurnal. Many more cercariae were shed in the day (8 a.m. to 8 p.m.) than at night (Table 8). Cercariae emerged actively from the rectum. In de-chlorinated tap water at room temperature (20-22°C), they survived for four to five days although most died at the end of 85 hours.

Redia (Figures 55, 56): Only one generation of rediae was observed in the course of my study, although Niemi and Macy (1974)

Figures 46-47. Cercariae of Metagonimoides oregonensis. Redrawn from Burns and Pratt (1953).

Figure 48. Redia of M. oregonensis. Redrawn from Burns and Pratt (1953). Note both metacercariae and cercariae inside redia.

Figure 49. Infective metacercaria of M. oregonensis taken from the redia. Redrawn from Burns and Pratt (1953).

Figure 50. Metacercarial cyst of M. oregonensis in striated muscles of frogs. Redrawn from Burns and Pratt (1953).

Figures 51-52. Cercaria of Apophallus donicus. Redrawn from Niemi and Macy (1974).

Figure 53. Protrusible lips of the cercaria of A. donicus.

Figure 54a-e. Developmental stages of the cercaria of A. donicus.

48
·45mm
50
·1mm
46
·07 mm
47
·2 mm
49
·1mm
51
·1 mm
52
·1mm
53
54
a
b
c
d
e

noted a mother and a daughter generation. Mature second generation rediae measured 0.56 to 1.04 (0.822) long by 0.12 to 0.16 (0.155) wide. The pharynx measured 0.04 to 0.048 (0.044) long by 0.036 to 0.048 (0.04) wide. A birth pore was seen immediately behind the pharynx and on one side. Rediae were found in the gonad and the digestive gland of the snail. A typical full-grown redia contains germ balls and immature cercariae (Figure 56). No mature and active cercaria was observed inside the rediae, although many active cercariae were seen between the tunica propria and the digestive gland of the infected snails. Apparently immature cercariae were expelled from the rediae and completed their maturation in the sinuses of the snail, after which they might migrate to the rectal sinus and actively emerged through the rectum.

Cercariae of A. donicus penetrated a variety of fresh water fishes and encysted under the skin resulting in the blackspots of fishes. For a discussion of the life cycle and taxonomy of A. donicus, see Part III in this study.

Psilostome Cercariae

Cercaria of Astacatrematula macrocotyla Macy and Bell, 1968

Host: Flumenicola virens.

Incidence of Infection: 11 of 2,005 snails, or 0.55%.

Locality: Stations I and II, Ritner Creek, Polk County, Oregon.

The psilostome, Astacatrematula macrocotyla, was first described by Macy and Bell in 1968. The life cycle involves Flumenicola virens, crayfish Astacus (Pacifastacus) trowbridgii and the chick (experimental definitive host).

Redia (Figures 57, 58): Rediae of A. macrocotyla were found in the gonad and digestive gland of Flumenicola. They are sac-like, with two ventral lobes at the posterior end. A long gut extends two-thirds the length of the body and is filled with yellowish pigments. Only one generation of rediae was observed in this study.

Cercaria (Figure 59): Cercariae of A. macrocotyla resemble those of echinostomes but lack the collar spines. They are active swimmers, and move by whipping their tails in the form of a figure 8. According to Macy and Bell (1968), the cercariae are positively phototropic with diurnal pattern of emergence. Cercariae of A. macrocotyla encyst readily on the bottom of the container, but in nature, they often encyst on the substrate and the external surfaces of the crayfish, Astacus (Pacifastacus) trowbridgii.

For a detailed description of the cercaria, refer to Macy and Bell (1968).

Metacercaria (Figure 60): Metacercarial cysts of A. macrocotyla are medium in size and ovoid, 0.137 to 0.145 by 0.98 to 1.12. Oral sucker, pharynx and acetabulum are clearly visible. The unique feature of the cyst is in the excretory bladder, which is sac-like with

arms extending to the level of the pharynx and filled with refractile granules (Figure 60).

This trematode has been found only on crayfish in a few localities in Tillamook and Columbia Counties, Oregon, and its incidence of infection appeared to fluctuate widely (Macy and Bell, 1968). The presence of this trematode in Ritner Creek, Oregon, thus constitutes a new locality record.

For further discussion of the life cycle stages of the trematode, refer to Macy and Bell (1968).

Cercaria of Sphaeridiotrema spinoacetabulum Burns, 1961

Host: Flumenicola virens.

Incidence of Infection: 16 of 2,005 snails, or 0.8%.

Locality: Stations I and II, Ritner Creek, Polk County, Oregon.

Burns (1961a) first described Sphaeridiotrema spinoacetabulum as a new species from the ceca of ducks experimentally fed cysts in F. virens collected from Shot Pouch Creek, Lincoln County, Oregon. Except for the presence of cuticular spines around the acetabulum and differences in the cercarial morphology, this trematode is similar to S. globulum, another species in the genus. The adults are found in the ceca of domestic ducks.

Redia and Cercaria (Figures 61-63): The rediae of S. spinoacetabulum are very similar to those of Astacatrematula macrocotyla

(Figures 61, 62). Burns was unable to find any sporocysts or second generation rediae in the infected snails (Burns, 1961a). Cercariae of S. spinoacetabulum resemble those of A. macrocotyla except for the absence of refractile granules in the excretory tubules (Figure 63). They emerge from the rectal opening of infected snails and re-enter the snails through the mantle edge to encyst between the mantle and the shell (Burns, 1961a). Cysts of S. spinoacetabulum are much larger than those of A. macrocotyla. They measured 0.162 by 0.168 to 0.2 by 0.212 (Plate 4).

For further descriptions of the life cycle stages of S. spinoacetabulum and comparison of S. spinoacetabulum and S. globulum, refer to Burns (1961a).

This is the second report of the cercaria of S. spinoacetabulum in Oregon and constitutes a new locality record for the parasite.

Virgulate Cercariae

Host: Oxytrema silicula and Flumenicola virens.

Incidence of Infection: 110 of 1,328 Oxytrema, or 8.28%; 126 of 2,005 Flumenicola, or 6.28%.

Locality: Stations I and II, Ritner Creek, Polk County, Oregon.

A total of 236 out of 3,333 Oxytrema and Flumenicola was found to be infected with several different species of virgulate cercariae. These are small xiphidiocercariae with a bilobed or pyriform virgula

Figure 55. Mother redia of A. donicus. Redrawn from Niemi and Macy (1974).

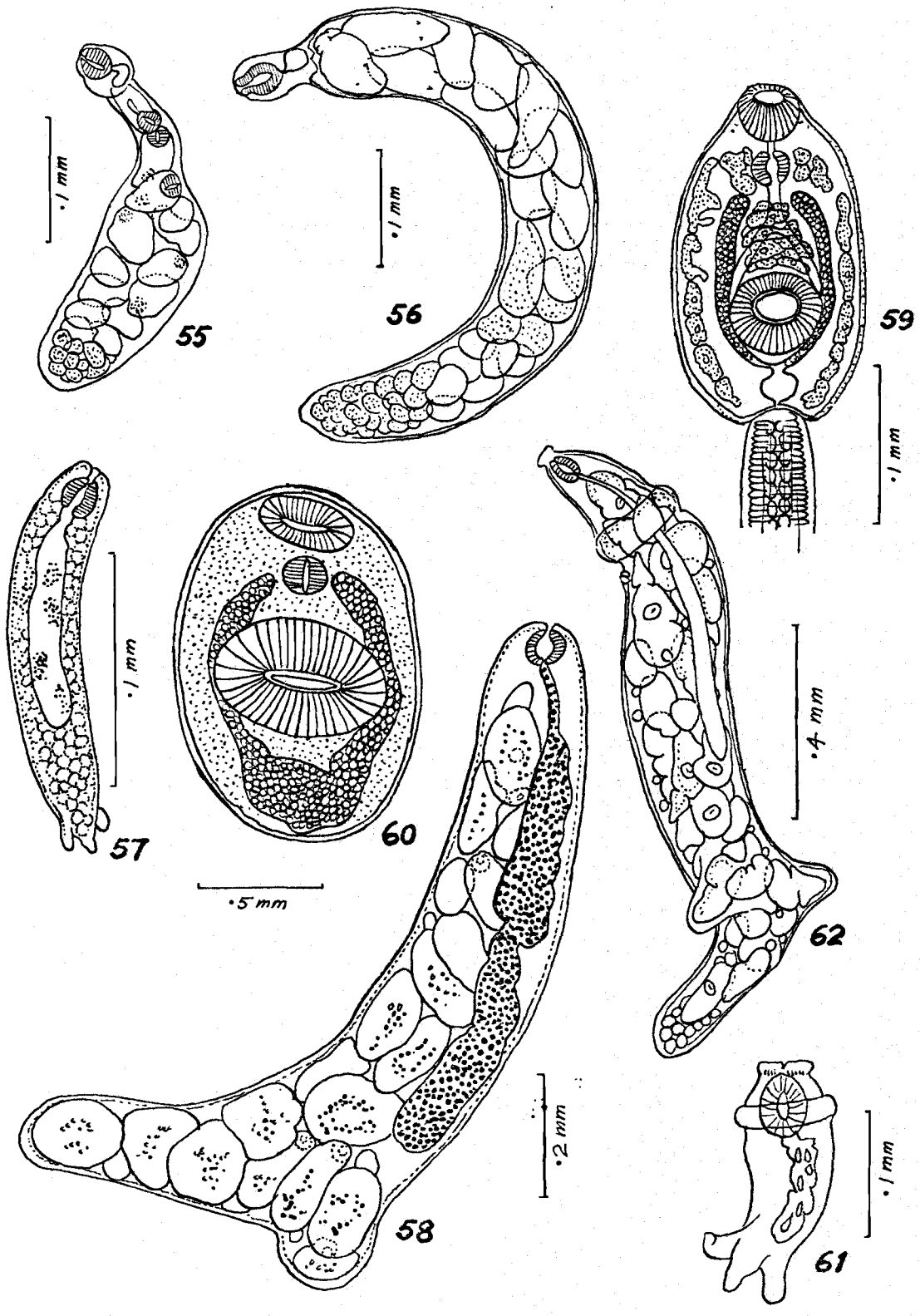
Figure 56. Daughter redia of A. donicus. Redrawn from Niemi and Macy (1974).

Figures 57-58. Immature and mature rediae of Astacatrematula macrocotyla. Redrawn from Macy and Bell (1968).

Figure 59. Cercaria of A. macrocotyla. Redrawn from Macy and Bell (1968).

Figure 60. Metacercarial cyst of A. macrocotyla. Redrawn from Macy and Bell (1968).

Figures 61-62. Immature and mature rediae of Spaeridiotrema spinoacetabulum. Redrawn from Burns (1961a).


55
·1 mm
56
·1 mm
59
·1 mm
57
·1 mm
60
·5 mm
62
·4 mm
58
·2 mm
61
·1 mm

organ located in the region of the oral sucker. The tail is simple and is usually shorter than the body. The virgulate cercariae develop typically in sporocysts in operculate snails and are the cercariae of lecithodendriid trematodes. The virgula organ is believed to function as the storage organ of mucoid secretions which aid in the attachment of the cercaria to the substrate (Schell, 1970). A key to the species of virgulate cercariae appears in Hall (1960).

At least five different species of virgulate cercariae have been described from Oxytrema and three species from Flumenicola (Knight and Pratt, 1955; Burns, 1961b). Since these cercariae were all superficially similar in appearance, no attempt was made in the present study to differentiate between them. Of the eight species, life histories of Allassogonoporus vespertilionis Macy, Acanthatrium oregonense Macy, and Cephalophallus obscurus Macy and Moore have been determined (Knight and Pratt, 1955; Macy and Moore, 1954). Four of the remaining virgulate cercariae were described and the last mentioned by Burns in 1961. The known life cycles of the three Oregon species (A. vespertilionis, A. oregonense, and C. obscurus) all involve a fresh water snail. The secondary intermediate hosts are either insect larvae or crayfish, and the definitive hosts are bats or minks (Knight and Pratt, 1955; Macy and Moore, 1954). Table 9 lists the eight virgulate cercariae found in Oxytrema and Flumenicola from the Pacific Northwest.

Table 9. Virgulate cercariae found in O. silicula and F. virens in the Pacific Northwest. O. s. = O. silicula; F. v. = F. virens.

Cercaria	Snail Host	Secondary Host	Definitive Host	Reference
C. of Acanthatrium oregonense	O. s.	caddis fly larvae	bats	Knight and Pratt (1955)
C. gyrinoides	O. s.	unknown, but observed to penetrate caddis fly larvae	unknown	Burns (1961b)
C. postguttata	O. s.	unknown	unknown	Burns (1961b)
C. guttata	O. s.	unknown	unknown	Burns (1961b)
C. perpusilla	O. s.	unknown	unknown	Burns (1961b)
C. of Allassogonoporus vespertilionis	F. v.	caddis fly and stone fly larvae	bats; hamster (experimental)	Knight and Pratt (1955); Burns (1961b)
C. of Cephalophallus obscurus	F. v.	crayfish	mink	Macy and Moore (1954)
C. of Burns	F. v.	unknown	unknown	Burns (1961b)

Zygocercous Cercariae

Cercaria gorgonocephala (Ward, 1916) Martin, 1968

Host: Oxytrema silicula.

Incidence of Infection: 4 of 1,328 snails, or 0.3%.

Locality: Station II, Ritner Creek, Polk County, Oregon.

Cercaria gorgonocephala was first described by Ward (1916) from Lake Erie. Williams (1931), who studied the cercaria, believed the molluscan host to be Goniobasis. Martin later found the same cercaria in O. silicula collected from Alsea River in Oregon and redescribed it (Martin, 1968). However, prior to Martin's redescription of the cercaria, McCauley and Pratt (1960) had observed "an undescribed zygocercous cercaria" in O. silicula in Oregon and Washington and had successfully completed its life cycle. The adult, which matured in the hamster and the duck, was shown to be Echinochasmus milvi Yamaguti, 1939. The undescribed zygocercous cercaria of McCauley and Pratt is believed to be identical to C. gorgonocephala redescribed by Martin from O. silicula. Recently, Dronen (1973) reported finding C. gorgonocephala in Goniobasis livescens from Michigan, and preliminary experiments indicated that it might be the cercaria of an Echinochasmus species. It thus appears that the cercaria of E. milvi (= C. gorgonocephala) in North America may use two separate snails as first intermediate hosts and that its distribution may range from the Great Lakes region westward to the Pacific Northwest (Martin, 1968).

Cercaria (Figures 64, 65a-g): The Oregon species of C. gorgonocephala appears as aggregates of up to 50 individuals. Each cercaria consists of a rather thick and granular body and a large tail. The oral sucker is somewhat smaller than the acetabulum, and has two rows of lappets embedded in its dorsal lip. The acetabulum has an inner ring of six papillae surrounding the opening of the ventral sucker. Outside of this is another ring of 32 to 38 lappets. Each side of the body is filled with heavily granulated glands. The excretory bladder is thin-walled, and is composed of two chambers. The anterior chamber occupies the posterior part of the cercarial body while the posterior chamber occupies the proximal portion of the tail. The tail is large and divided into a proximal main-tail region and a distal and narrow, rat-tail region. The rat-tail region is believed to contain some adhesive material which enables the cercariae to contact one another to form clusters or aggregates after leaving the snail. The tails of all cercariae contain a rusty-colored pigment (Plate 5). For detailed description of the morphology and behavior of the cercaria, refer to Martin (1968). In Ritner Creek, only 0.3% of Oxytrema were found to be infected with C. gorgonocephala.

Redia (Figure 66): Only one generation of rediae was seen in this study, although Martin reported two redial generations from Oxytrema. A typical redia has an anterior collar and a pair of appendages near the posterior end of the body. A relatively long gut

Figure 63. Cercaria of S. spinoacetabulum. Redrawn from Burns (1961a).

Figure 64. Cercaria gorgonocephala. Redrawn from Martin (1968).

Figure 65a-g. Developmental stages of Cercaria gorgonocephala.

Figure 66. Redia of C. gorgonocephala.

63
.08 mm
e
f
65
g
d
c
a
b
66
.2 mm
64
.06 mm

filled with brown pigments occupies at least half the length of the body. The birth pore was seen next to the pharynx.

Shedding observations on C. gorgonocephala can be found in Part III of this study.

Aggregating-Albino Cercaria (AA)

Host: Oxytrema silicula.

Incidence of Infection: 2 of 1, 328 snails, or 0. 15%.

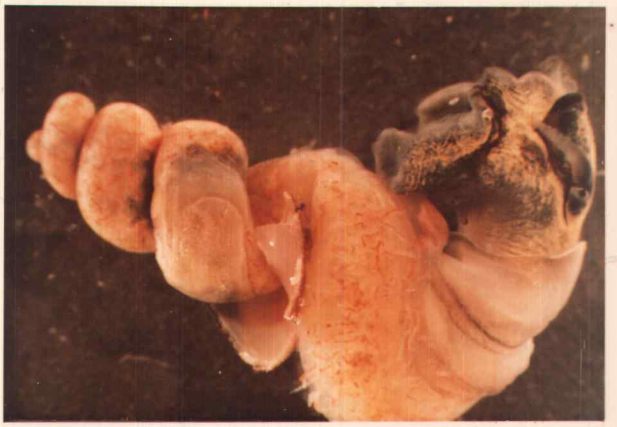
Locality: Station II, Ritner Creek, Polk County, Oregon.

During the survey of Oxytrema for larval trematodes, a second species of zygocercous cercaria was found. This cercaria is identical to C. gorgonocephala in all aspects except for the absence of pigments in the tail (Plates 6, 7). The cercariae aggregated into clusters of 30 to 40 individuals, and were produced in rediae identical in size and morphology to those of C. gorgonocephala. This albino form was also previously noticed by McCauley and Pratt (McCauley, personal communications). It is believed that both C. gorgonocephala and the aggregating-albino cercaria belong to the same species and that they may represent conditions of polymorphism in the trematode (see Part III of this study). For shedding observations of the aggregating-albino cercaria, see Part III of this study.

Plate 4. Cracked F. virens showing metacercarial cysts of Sphaeridiotrema spinoacetabulum.

Plate 5. Cracked O. silicula showing rediae and cercariae of Cercaria gorgonocephala in the gonad, digestive and reproductive glands of the snail. Note pigmented tails of the cercariae.

Plate 6. Aggregating-albino cercaria (AA) from O. silicula. From AFA-fixed specimens.



Macrocercous Cercaria

Non-Aggregating Macrocercous Cercaria (NAM)

Host: Oxytrema silicula.

Incidence of Infection: 3 of 1,328 snails, or 0.23%.

Locality: Station II, Ritner Creek, Polk County, Oregon.

Besides harboring Cercaria gorgonocephala and the aggregating-albino cercaria, as discussed briefly before, O. silicula from Ritner Creek was also found to produce another macrocercous cercaria which looked identical to C. gorgonocephala. This cercaria, however, did not form aggregates. The same cercaria was also previously noticed by McCauley and Pratt (McCauley, personal communications). Dronen (1973), who reported C. gorgonocephala from Goniobasis livescens in Michigan, likewise observed a non-aggregating macrocercous cercaria from the same snail. This cercaria, which was assigned the name Cercaria macrocauda by Dronen, was identical to C. gorgonocephala. Thus, in Ritner Creek, Oxytrema serves as hosts for three identical macrocercous cercariae, two of which are aggregating (zygocercous) forms while the remaining one is solitary. Life cycle observations on the three cercariae have indicated a strong likelihood that they may be of the same species, and that they may represent polymorphic conditions of the cercaria of Echinochasmus milvi (see life cycle studies and Table 15 in Part III of this study and Dronen, 1973).

Cercaria (Plate 8; Figures 67-69): The cercarial body of the non-aggregating macrocercous cercaria (NAM) is identical to that of C. gorgonocephala. For descriptions refer to Martin (1968) and the section under C. gorgonocephala in this part of the dissertation.

The tail of the cercaria, however, does not have the narrow, rat-tail region. Rusty-colored pigments were found in the tails of many of the shed cercariae. The tail is muscular and capable of great expansions and contractions and shows a typical undulating S-shaped movement.

Oxytrema infected with the NAM cercaria consistently shed the same non-aggregating cercariae, although the pigments in the tail grew lighter and lighter toward the later part of fall and the end of the shedding season (see Part III of this study).

The shedding pattern of two infected Oxytrema was clearly diurnal. Less than five cercariae were shed each day during the evening hours from 8 p.m. to 8 a.m. (Table 8). The cercariae were positively phototropic.

Furcocercous Cercaria

Brevifurcate-Apharyngeate Cercaria

Host: Oxytrema silicula.

Incidence of Infection: 2 of 1,328 snails, or 0.15%.

Locality: Station II, Ritner Creek, Polk County, Oregon.

Figure 67. Cercarial body of the non-aggregating macrocercous cercaria (NAM). Ventral view.

Figure 68. NAM cercaria. Note the absence of the narrow, rat-tail region.

Figure 69. NAM cercaria. Lateral view.

Figures 70-71. Immature and mature rediae of NAM cercaria.

Figure 72. Young metacercarial cyst of NAM cercaria in the gills of blackside dace.

67
.06 mm
68
.2 mm
69
70
.08 mm
71
.24 mm
72
.07 mm

This is the first report of a brevifurcate-apharyngeate cercaria from O. silicula. Two Oxytrema collected from Ritner Creek were found to be infected with the cercaria. In both snails, the cercaria was found in a double-infection with another species of trematode (Table 6). Because of the limited amount of materials available for study, the following descriptions of the cercaria and the sporocyst must be regarded only as preliminary. Until more materials are available for further study, no attempt will be made to establish it as a new species.

Cercaria (Figures 73, 74): Body elongate, 0.24 to 0.296 (0.279) long and 0.068 to 0.076 (0.073) wide. Tail stem 0.443 long; furcae 0.14 to 0.2 (0.168) long by 0.012 wide. Head organ occupies anterior one-fourth of body. Ventral sucker round, well-developed, 0.032 to 0.036 (0.034) in diameter lying at anterior margin of last quarter of body. Body uniformly spined. Two eyespots present. Four or more pairs of penetration glands occupy positions lateral to ventral sucker. Large ducts lead from glands to head organ. Esophagus long, reaching middle of body. Pharynx absent. Excretory vesicle large, thin-walled. Flame cell pattern not traced.

Sporocyst (Figures 75, 76): Sporocysts were found in the gonad and digestive gland of the snail. They are long, slender and stringy forms interwoven through the snail's tissues. They vary in size up to 2.6. The body wall is thin. A birth pore could be found at the

Figures 73-74. Brevifurcate-apharyngeate cercaria from O. silicula.

Figure 75. Sporocyst of brevifurcate-apharyngeate cercaria.

Figure 76. Active brevifurcate-apharyngeate cercaria emerging from the sporocyst.

.07 mm
73
.24 mm
74
.4 mm
75
76

narrower end. Active cercariae were seen to emerge through the birth pore (Figure 76).

Both snails infected with the brevifurcate-apharyngeate cercariae were collected from Ritner Creek late in the fall. No second generation of sporocysts was found. Further observations on the shedding and other behavioral aspects of the cercaria were not made due to the limited supply of live materials.

According to Schell (1970), the brevifurcate-apharyngeate cercariae are produced by trematodes of the families Schistosomatidae and Spirorchidae, the blood flukes of reptiles, birds and mammals. Many of these cercariae are capable of producing dermatitis in man. McFarlane and Macy (1946), Macy (1952), Macy and Moore (1953), and Macy, Moore and Price (1955) have studied dermatitis-producing schistosomes in the Pacific Northwest. Oxytrema, however, has not been incriminated as the snail host of any of the known dermatitis-producing forms.

Cystophorous Cercaria

Host: Oxytrema silicula.

Incidence of Infection: 7 of 1,328 snails, or 0.53%.

Locality: Station II, Ritner Creek, Polk County, Oregon.

This cercaria was first reported by McCauley and Pratt (1957) from O. silicula in Benton County, Oregon. They believed it to be the first record of a cystophorous cercaria from Oregon. In my

study, none of the seven infected snails was actively shedding cercariae when they were cracked. The following description of the cercaria, therefore, is based on unemerged and probably immature forms.

Cercaria (Figures 77, 78): The body of the cercaria is elongate and small, reaching a length of 0.2 and a width of 0.037. It could not be contracted into the tail but remained partially protruded from it. The attachment of the body to the tail was rather strong, and was not broken readily except under coverslip pressure. The oral sucker is subterminal with mouth opening ventrally. It measured 0.022 by 0.025. A small pharynx was seen posterior to the oral sucker. The body is heavily granulated, so that the flame cell pattern was not discernible. The acetabulum, measuring 0.027 to 0.03, is situated in the middle of the body. A large, saccular excretory vesicle lined with epithelial cells, occupies the posterior quarter of the body. The tail of the cercaria is plump, thick-walled and club-shaped. The convex surface is heavily lobed. At the posterior end of the tail is the excretory appendage. Originating from the base of the excretory appendage is a compound structure resembling the letter H and attached to it on its side by way of a stalk. The four arms of the "H" are of varying sizes, but may reach a length of three to four times the size of the cercaria. These ribbon-like structures may be homologous to the "epithelial streamers" of Thomas (1939). No

pyriform organ (Thomas, 1939) was seen at the base of the excretory appendage. The excretory tube, or the excretory projection of Thomas (1939), which is normally coiled within the tail, may be everted at a point directly opposite to the excretory appendage. It measured about three to four times the length of the cercaria.

Redia (Figure 79): No sporocyst or redia containing rediae was found in the course of this study. The rediae of this species vary considerably in size, ranging from 1.28 to 3.6 (2.39) in length and 0.32 to 0.56 (0.43) in width. The anterior end is broadly round while the posterior end is pointed. The pharynx is small and ovoid. A short gut extends about one-fourth the total length of the redia and is filled with concretions. No birth pore was observed. Cercariae and developing germ balls fill the cavity of the redia. Rediae freshly removed from the snail are greyish-white.

Cystophorous cercariae are produced by trematodes of the family Hemiuridae. These cercariae have been reviewed by Cort and Nichols (1920), Hunninen and Cable (1943), Dollfus (1950) and Ching (1959). More than 50 cystophorous cercariae have been reported, although only a few of the hemiurid life cycles have been determined. At least nine different hemiurids are known to parasitize fresh water fishes in Washington and Oregon (Pratt and McCauley, 1961), of which one, Deropegus aspina, was recovered from fishes in Ritner Creek (see Part I of this study). The cystophorous cercaria

described here from O. silicula may very well represent the larval form of one of these undetermined hemiurid life cycles in the Pacific Northwest.

Cercariaeum

Cercaria of Levinseniella minuta Stunkard, 1958

Host: Oxytrema silicula.

Incidence of Infection: 7 of 1,328 snails, or 0.53%.

Locality: Station II, Ritner Creek, Polk County, Oregon.

The cercaria of Levinseniella minuta was originally described by Stunkard (1958) from snails Hydrobia minuta and Amnicola limosa from New Hampshire and Cape Cod, although the adult trematodes were first reported by Price (1934a) from scaup ducks in the West Indies. Burns (1963) found 15% of 300 Oxytrema silicula infected with the larval stages of this parasite in Benton County, Oregon. This report thus constitutes the second record of L. minuta in the Pacific Northwest. The life cycle of L. minuta has been adequately treated by Stunkard (1958) and Burns (1963).

Cercaria (Figure 80): As noted by Stunkard (1958) and Burns (1963), the cercariae, which are tailless, do not emerge from the snail but instead encyst as early embryos and remain encysted throughout their development to metacercariae (Burns, 1963). As a result, infections of the snail can only be detected by cracking the

shells. Naturally infected Oxytrema were packed with thousands of metacercariae so that much of the tissues of the body had become atrophied (Plate 9).

Sporocyst (Figure 81): Sporocysts of L. minuta were not studied. According to Stunkard (1958), there are two sporocyst generations. Small sporocysts are almost spherical, whereas older ones are ovoid to elongate. Mature sporocysts produce only few cercaria, seldom more than three.

Metacercaria (Figures 82-84): A typical metacercarial cyst is ovoid, 0.12 long by 0.1 to 0.104 (0.1) wide (Figure 82). The cyst wall measured 0.006 thick, which, on exposure to de-chlorinated water, slowly swelled to 0.028 thick (Figure 83). The encysted metacercaria lies folded on its ventral surface with the two posterior testes and vitelline follicles clearly shown. The excysted metacercaria closely resembles the adult (Figure 84). The body is ovoid to slightly triangular, 0.26 long by 0.156 wide. The oral sucker is round, 0.036 in diameter, with mouth opening subterminally. The pharynx measured 0.019 in diameter. No pre-pharynx was evident. A long esophagus leads from the pharynx to the midbody, terminating in two ceca which may reach the posterior margin of the acetabulum. The acetabulum is round, 0.03 in diameter and smaller than the oral sucker. The ovary is to the right and on the same level of the acetabulum. Two large testes are situated laterally in the posterior

Plate 7. Cracked O. silicula showing rediae and cercariae of the aggregating-albino cercaria in the gonad, digestive and reproductive glands of the snail. Note absence of pigments in tails of cercariae.

Plate 8. Non-aggregating macrocercous cercaria (NAM) from O. silicula.

Plate 9. Cracked O. silicula showing thousands of cysts of Levinseniella minuta in the snail. Note the atrophied tissues of the snail.

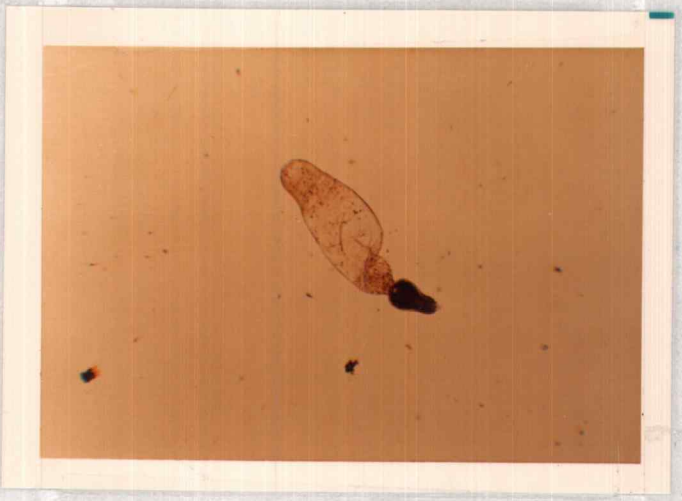


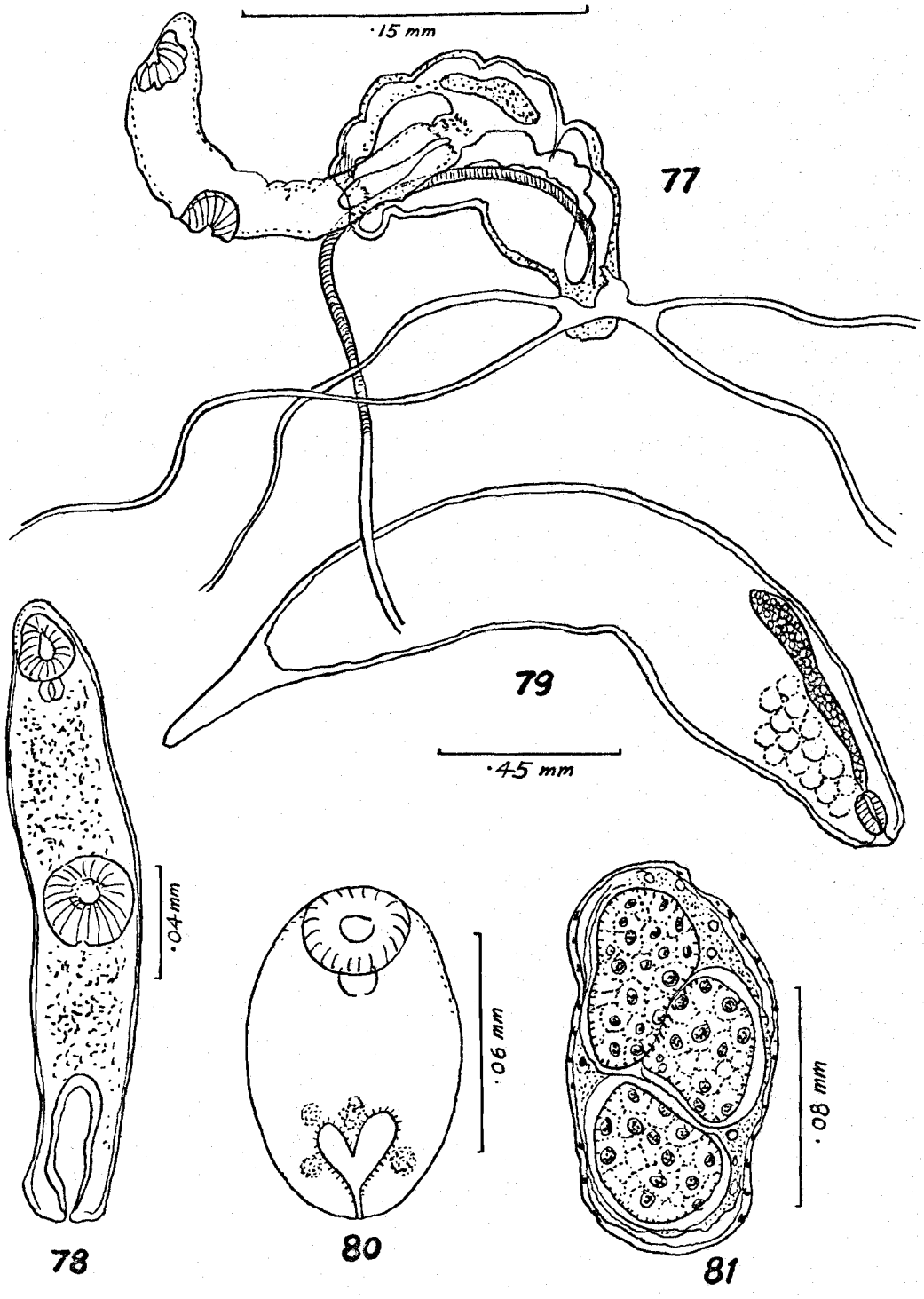
Figure 77. Cystophorous cercaria from O. silicula.

Figure 78. Cercarial body of the cystophorous cercaria.

Figure 79. Redia of the cystophorous cercaria.

Figure 80. Cercaria of Levinseniella minuta. Redrawn from Stunkard (1958).

Figure 81. Sporocyst of L. minuta. Redrawn from Stunkard (1958).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81

portion of the body. Several vitelline follicles were seen posterior to the testes. The genital atrium occupies a position opposite to the ovary with the club-shaped copulatory organ everted in some specimens.

The metacercariae, when fed to hamsters and mice, matured in the small intestine in 24 to 96 hours (Stunkard, 1958; Burns, 1963), and domestic ducks from Woods Creek near Philomath, Benton County, Oregon have been found to harbor large numbers of adults of L. minuta (Burns, 1963).

Miscellaneous Cercaria

Cercaria X

Host: Flumenicola virens.

Incidence of Infection: 102 of 2,005 snails, or 5.09%.

Locality: Stations I and II, Ritner Creek, Polk County, Oregon.

This cercaria is reported here for the first time from F. virens and is tentatively assigned the name Cercaria X. It does not resemble any of the existing major group of cercariae. The cercariae develop in small, whitish rediae in the gonad and digestive gland of the snail. The following description of the cercaria is based on eight AFA-fixed specimens prepared under slight pressure of the coverslip.

Cercaria (Figures 85-87; 88a-h): Body elongate, 0.208 to 0.28 (0.236) long by 0.112 to 0.164 (0.138) wide. Oral sucker subterminal,

nearly round, 0.03 to 0.04 (0.036) long by 0.024 to 0.035 (0.03) wide, with mouth opening ventrally; lips eversible. At least three rows of hooks, five to six in each row, project from ventral lip. Rest of oral sucker armed with concentric rings of fine cuticular spines. Body of cercaria nonspinous. Acetabulum nearly round, smaller than oral sucker, situated pre-equatorially in middle of body, 0.035 to 0.042 (0.039) long and 0.027 to 0.035 (0.031) wide. Eight to nine pairs of penetration glands occupy anterio-lateral positions of acetabulum. Two eyespots half-way between oral sucker and penetration glands. A large, V-shaped and thick-walled excretory vesicle lies just anterior to tail socket. Reproductive anlagen behind acetabulum. Pharynx, esophagus, and ceca not clearly visible; and flame cell pattern not discernible. Tail simple, slender, slightly shorter than body, 0.176 to 0.248 (0.214) long, 0.036 to 0.06 (0.044) wide with rows of globules extending nearly its full length.

The cercaria is an active swimmer. Its swimming activities consist of intermittent vigorous whipping of its tail while hanging its body downward (Figure 87). This lifts the cercaria in an upward direction. This is suddenly followed by the cessation of all movements, and the cercaria sinks slowly in the water. The cercaria was frequently found creeping on the bottom of the dish, although it is also capable of side-ways motions by the whipping of its tail.

Cercaria X is positively phototropic.

Three actively shedding Flumenicola showed a distinct diurnal pattern of cercarial emergence. The maximum average number of cercariae shed during the night was 16 while as many as 273 might be shed during the day (Table 8). Cercaria X seems to be rather short-lived. Most cercariae kept in de-chlorinated water at room temperature (20-22°C) died within 45 hours and all were dead at the end of 65 hours.

Redia (Figures 89, 90): No sporocyst and only one generation of rediae were found. The rediae are small, whitish and sac-like structures embedded in the digestive gland and gonad of the snail. They measured 0.32 to 0.62 (0.463) long to 0.1 to 0.128 (0.115) wide. The tails of immature rediae are pointed whereas those of mature rediae are rod-like. The pharynx is nearly round, 0.036 to 0.044 (o.039) by 0.028 to 0.04 (0.035). No birth pore or gut was observed. Rediae were never found to contain active and mature cercariae although in large sized rediae, cercarial embryos with tail buds and differentiating acetabula and oral suckers were common. This was true even in snails actively shedding cercariae. When cracked, Flumenicola virens infected with Cercaria X, however, always released large numbers of mature and immature cercariae. Dissection of the rectal sinus also revealed many immature cercariae with tails and eyespots. It is believed that immature cercariae emerge or are extruded from the rediae and complete their maturation in the sinuses of the snail.

Figure 82. Cyst of L. minuta.

Figure 83. Cyst of L. minuta on exposure to de-chlorinated water.

Figure 84. Excysted metacercaria of L. minuta.

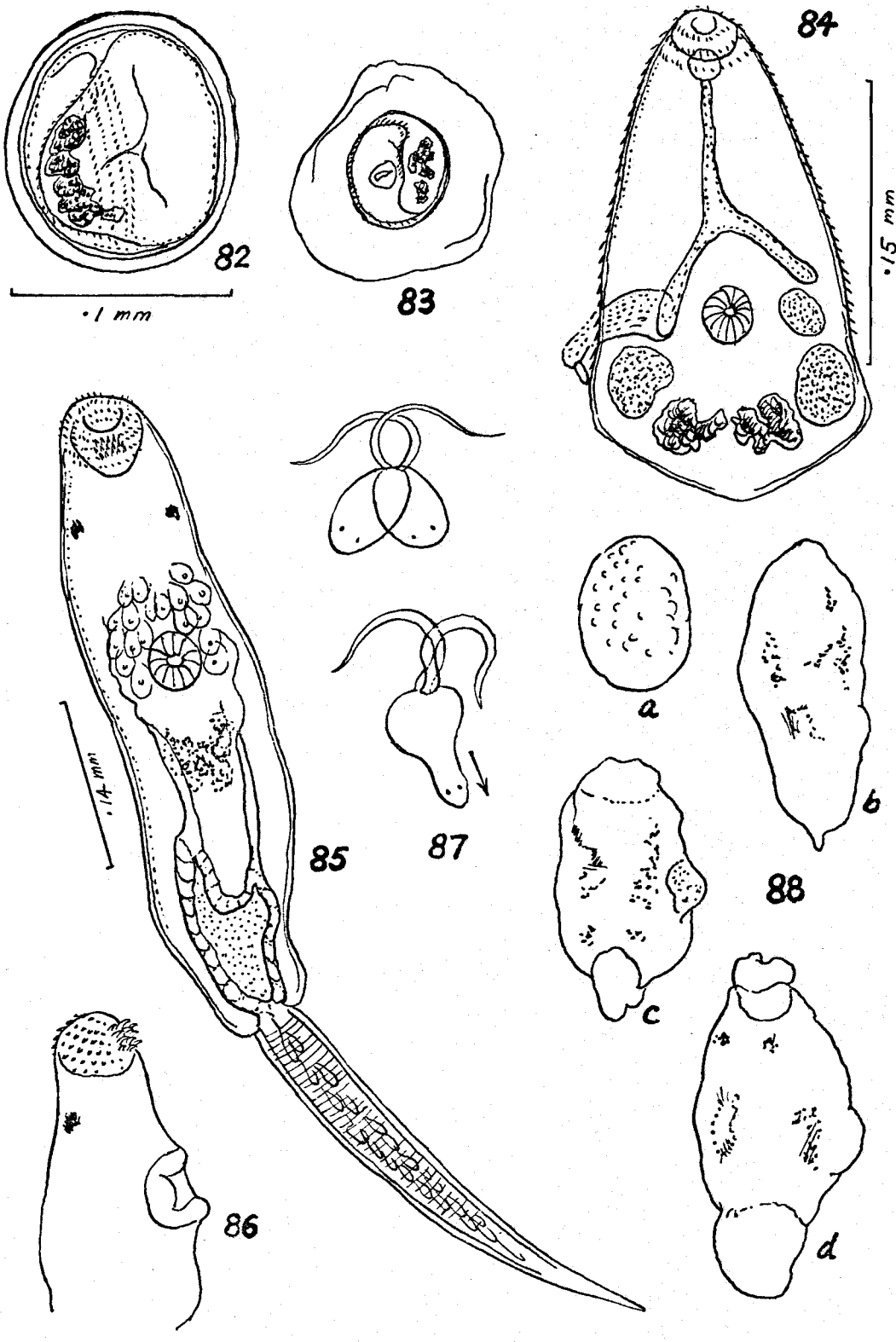
Figure 85. Cercaria X. Ventral view.

Figure 86. C. X. Lateral view.

Figure 87. Locomotion of C. X.

Figure 88a-h. Developmental stages of C. X.

Figures 89-90. Rediae of C. X.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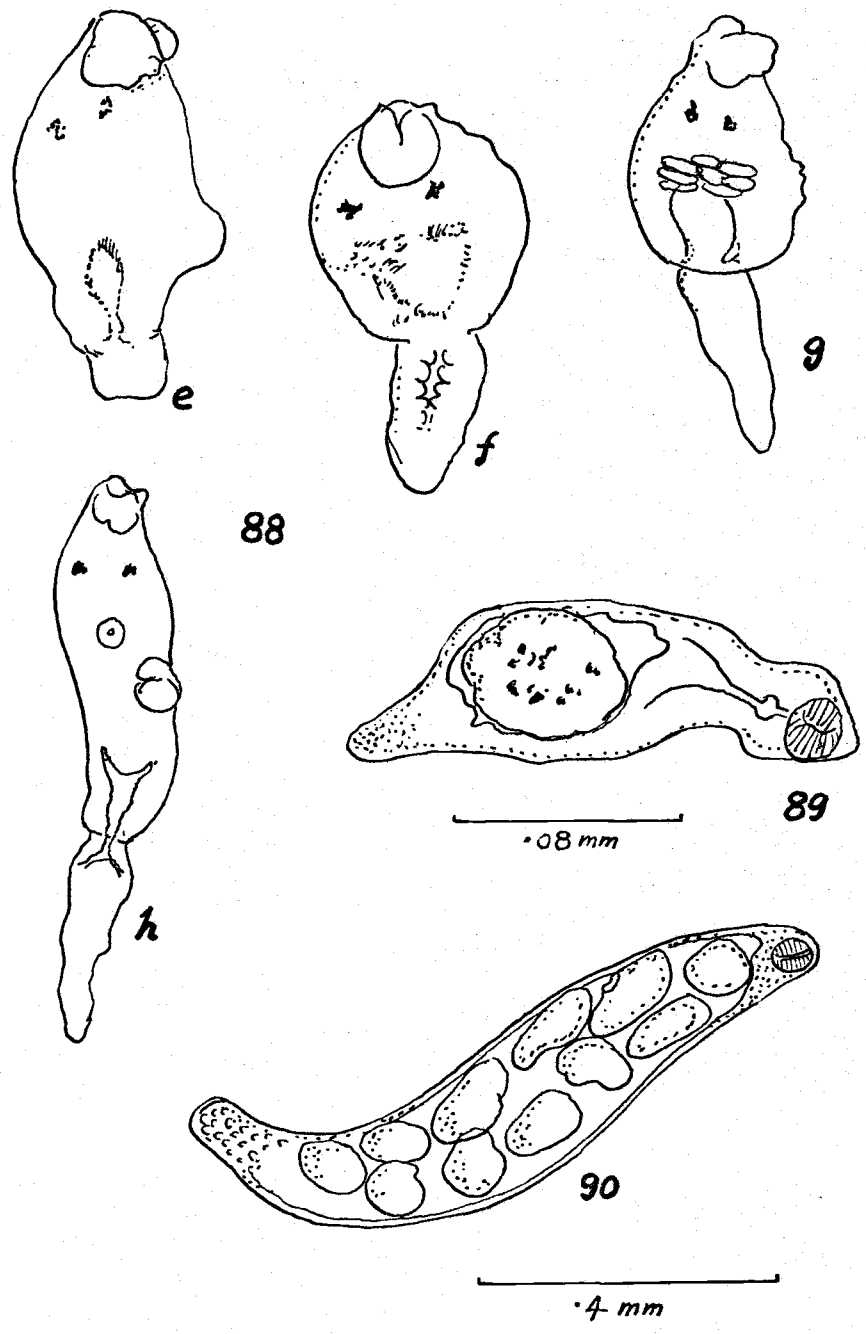
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Attempts were made to study the life history of this cercaria. Cercaria X did not penetrate any of the aquatic insect larvae and five species of fresh water fishes exposed to them (see Part III of this study). Nor did it encyst on the substrate. The life cycle of this trematode remains to be determined.

Check-list of Cercariae Infecting Oxytrema silicula and Flumenicola virens in the Pacific Northwest

A literature search was made to compile a list of cercariae known to infect O. silicula and F. virens in the Pacific Northwest. To date, a total of 16 different species of cercariae has been reported from O. silicula and 14 from F. virens. Of these, 18 life cycles have been determined. Table 10 shows a check-list of these cercariae from the two snail hosts in the Pacific Northwest.

Table 10. Check-list of cercariae infecting O. silicula and F. virens in the Pacific Northwest. O. s. = O. silicula; F. v. = F. virens.

Cercarial type	Host	Locality	Reference
Cercariaeum			
C. of Levinseniella minuta[1]	O. s.	Benton County, Oregon	Burns (1963)
		Polk County, Oregon	This study
C. of Palaeorchis problematicus[1]	F. v.	Marion County, Oregon	Macy and Berntzen (1970); Macy and English (in press)
Cystophorous c.	O. s.	Benton County, Oregon	McCauley and Pratt (1957)
		Polk County, Oregon	This study
Furcocercous			
Brevifurcate-apharyngeate c.[2]	O. s.	Polk County, Oregon	This study
Macrocercous			
Non-aggregating macrocercous c.[2]	O. s.	Polk County, Oregon	This study
Megalurus			
C. of Philophthalum megalurus[1]	O. s.	Clackamas County, Oregon	McMillen and Macy (1972)
Microcercous			
Chaetomicrocercous c.[2]	F. v.	Polk County, Oregon	This study
Cotylomicrocercous c.[1]	F. v.	Alsea River, Oregon	Crandell (1963)
C. of Lissorchis heterorchis[1]	F. v.	Portland, Oregon	Onyejekwe (1972)
C. of Nanophyetus salmincola[1]	O. s.	Washington, Oregon and California	Many references; Bennington and Pratt (1960)
C. of Plagioporus siliculus[1]	O. s.	Benton County, Oregon	Sinitsin (1931)
		Polk County, Oregon	This study
C. of Podocotyle virens[1]	F. v.	Benton County, Oregon	Sinitsin (1931)
		Polk County, Oregon	This study
Monostome			
C. of Notocotylus imbricatus[1]	O. s.	Oregon	Dikmans (1945); Pratt and McCauley (1961)

(Continued on next page)

Table 10. (Continued)

Cercarial type	Host	Locality	Reference
Pleurolophocercous			
C. of Apophallus donicus[1]	F. v.	Portland, Salem & Turner, Oregon	Niemi and Macy (1974)
		Polk County, Oregon	This study
C. of Metagonimoides oregonensis[1]	O. s.	Benton County, Oregon	Burns and Pratt (1953)
		Polk County, Oregon	This study
Psilostome			
C. of Astacatrematula macrocotyla[1]	F. v.	Tillamook and Nehalem Rivers, Oregon	Macy and Bell (1968)
		Polk County, Oregon	This study
C. of Sphaeridiotrema globulum[1]	F. v.	Portland, Oregon	Macy and Ford (1964)
C. of S. spinoacetabulum[1]	F. v.	Lincoln County, Oregon	Burns (1961a)
		Polk County, Oregon	This study
Virgulate			
C. of Acanthatrium oregonense[1]	O. s.	Lincoln County, Oregon	Burns (1961b)
		Benton and Lincoln Counties, Oregon	Knight and Pratt (1955)
C. of Allassogonoporus vespertilionis[1]	F. v.	Benton and Lincoln Counties, Oregon	Knight and Pratt (1955)
		Lincoln County, Oregon	Burns (1961b)
C. of Cephalophallus obscurus[1]	F. v.	Multnomah County, Oregon	Macy and Moore (1954)
C. guttata	O. s.	Lincoln County, Oregon	Burns (1961b)
C. gyrinoides	O. s.	Lincoln County, Oregon	Burns (1961b)
C. perpusilla	O. s.	Lincoln County, Oregon	Burns (1961b)
C. postguttata	O. s.	Lincoln County, Oregon	Burns (1961b)
Virgulate c. of Burns	F. v.	Lincoln County, Oregon	Burns (1961b)
Zygocercous			
C. gorgonocephala[1]	O. s.	Alsea River, Oregon	Martin (1968)
		Polk County, Oregon	This study
Aggregating-albino c.[2]	O. s.	Polk County, Oregon	This study
Miscellaneous			
Cercaria X[2]	F. v.	Polk County, Oregon	This study
Cercaria of Sinitsin	F. v.	Benton County, Oregon	Sinitsin (1931)

[1]Life cycles completed.

[2]First described in this study.

PART III. LIFE CYCLE STUDIES

The life cycles of five different species of trematodes encountered during the surveys of fresh water fishes and gastropods in Ritner Creek were studied. The results are summarized in Table 11.

The Experimental Life Cycle of *Apophallus donicus* (Skrjabin and Lindtrop, 1919) Price, 1931

In 1919, Skrjabin and Lindtrop created the genus *Rossicotrema* for the species *R. donicus* that they described from dogs and cats in Russia. Price (1931a) synonymized this genus with *Apophallus* Lühe, 1909 and recognized four separate species: *A. muhlingi* (type), *A. crami* Price, 1931, *A. donicus* (including *A. venustus* and *A. similis*), and *A. brevis*. Since then at least six other species of *Apophallus* have been described: *A. major* (Szidat, 1924), *A. americanus* (Van Cleave and Mueller, 1932), *A. bacalloti* (Balozet and Callot, 1939), *A. imperator* (Lyster, 1940), *A. itascensis* (Warren, 1953) and *A. lerouxi* (Rayski and Fahmy, 1962). The two species of *Apophallus* reported previously from Oregon are *A. crami* (Price, 1931a) from *Larus californicus* in Klamath Falls, Oregon, and *A. donicus* from gulls in Oregon (Shaw, 1947).

Table 11. Summary of results in the life cycle studies of five species of trematodes encountered during the surveys of fresh water fishes and gastropods in Ritner Creek. Refer to text for scientific names of secondary and definitive hosts.

Trematode	First Intermediate Host	Second Intermediate Host	Definitive Host
Apophallus donicus (Fam. Heterophyidae)	Flumenicola virens; oculate, pleurolophocercous cercaria develops in redia	coho salmon and rainbow trout (exptl.); metacercariae encyst under skin causing black spots	hamster (exptl.); adults recovered from small intestine in eight days
Plagioporus siliculus (Fam. Opecoelidae)	Oxytrema silicula; cotylo-microcercous cercaria develops in sporocyst	crayfish (nat. and exptl.); meta-cercariae encyst in abdominal muscles	rainbow trout (exptl.); adults recovered from small intestine in 34 or more days
A new monorchiid n. gen., n. sp. (Fam, Monorchiidae)	unknown	brook and Pacific lampreys (nat.); metacercariae encyst in pericardium, liver and kidneys of fish	torrent sculpin (exptl. and nat.); adults recovered from small intestine in 26 or more days
Echinochasmus milvi (Fam. Echinostomatidae)		blackside dace and redside shiner (nat.); echinostome cysts found embedded in gills	duck (exptl.); adult recovered from small intestine in eight days
	Oxytrema silicula; non-aggregating macrocercous cercaria (NAM) develops in redia. Cercarial body identical to Cercaria gorgonocephala	blackside dace (exptl.); cercariae encyst in gills	feeding experiments were done with ducks, hamsters, the rat and the chick, but no adults were recovered
Echinochasmus sp. n. sp.	unknown	blackside dace (nat.); meta-cercariae encyst in gills	duck (exptl.); adults recovered from small intestine in seven days

Life cycle studies of Apophallus thus far include those of A. venustus (Cameron, 1937), A. imperator (Lyster, 1940), A. bacalloti (Timon-David, 1963) and A. muhlingi (Odening, 1970).

The life cycle of A. donicus in Oregon was recently completed for the first time by Niemi (1973) and Niemi and Macy (1974). The experimental cycle of the same species was also independently completed by me in the summer and fall of 1974. A personal communication with Dr. Macy (of Portland State University) convinced both him and me that the flukes that we independently worked on were identical. While Niemi and Macy (1974) used both natural and experimentally infected fish hosts in determining the life cycle, I used only laboratory-exposed secondary intermediate hosts. The first intermediate host in Oregon is the stream snail, Flumenicola virens. The fish hosts are Rhinichthys osculus nubilus (Girard), blackside dace; Richardsoni balteatus hydrophlox (Richardson), redside shiner; Catostomas macrocheilus Girard, sucker; Ptychocheilus oregonensis, Richardson, squawfish; Salmo gairdneri, Richardson, rainbow trout and Oncorhynchus kisutch (Walbaum), coho salmon. The experimental definitive hosts used in Niemi and Macy's (1974) studies as well as mine included white rats, gerbils, golden hamsters, kittens and chicks.

The following description of the life cycle stages and biology of A. donicus represents work completed in my laboratory from July through November, 1974.

Materials and Methods

Snails, Flumenicola virens, for the present study were collected by hand from Ritner Creek, Polk County, Oregon from July to November, 1974. They were kept either at room temperature (20-22°C) for immediate use or maintained in a cold room at 10°C for prolonged observations. At room temperature, the snails were kept in plastic trays supplied with flowing de-chlorinated water and aerated with air-stones. In the cold room, however, no aeration was used as most snails were able to survive for one to two months in an uncrowded condition and at low temperature.

Hatchery-raised rainbow and coho fingerlings were used as experimental fish hosts. These fishes were kept in a cold room at 15-17°C and under continuous flow of de-chlorinated water. They were fed regularly with fish feed purchased from a local store.

Uninfected fish, 5 to 6 cm long used in the infection studies, were isolated singly in small dishes and gradually brought to room temperature. Pleurolophocercous cercariae of A. donicus collected from isolated infected F. virens were pipetted into each of the dishes to affect penetration of the fish hosts. The exposed fish were then returned to the cold room.

Metacercariae from infected fish were collected by first pithing the fish and then using one of the following methods: 1) the sides of the

fish were scraped with a scalpel to remove the scales. The scales and the underlying cysts were then shaken vigorously in water and the separated cysts pipetted out with a medicine dropper; 2) whole, or part of a fish was blended with about 150 ml of de-chlorinated water in a Waring blender for 1 to 1-1/2 minutes at top speed. The mixture was allowed to settle and the liquid decanted. The sediment was then poured into a Petri-dish for examination. Free, encysted metacercariae could then be collected by pipetting with a medicine dropper.

Results

Cercaria and Redia

The cercaria is pleurolophocercous, oculate and very active. The molluscan host in Oregon is F. virens. For detailed descriptions of the cercarial morphology and its behavior, as well as infection incidence in the snail host, refer to Part II in this study. Redial description and biology can also be found in Part II.

Infection of Fish by Cercariae

Cercariae of A. donicus readily penetrated many different species of fishes. These included brook and Pacific lampreys (Lampetra richardsoni and L. tridentata), reticulate sculpin (Cottus perplexus), speckled dace (Rhinichthys osculus), rainbow trout (Salmo gairdneri) and coho salmon (Oncorhynchus kisutch). Hatchery-raised

fishes of the last two species were used in the infection experiments in the laboratory. The procedure involved isolating individual fish in small glass dishes and introducing several hundred cercariae into each of the dishes. Exposure time was from 15 to 30 minutes. Upon introduction of the cercariae the fish began a series of erratic movements. Once contact with the host was made, the cercaria immediately crept underneath the scale and attached its oral sucker to the skin of the fish. The tail was shed momentarily and the body became almost totally motionless except for a few boring motions. Penetration was accomplished in 3 to 15 minutes. The tail and fins of the fish were likewise attacked by the cercariae. At the end of the exposure, fish were returned to the cold room; and the dishes were checked for remaining cercariae. As a rule, host-contact was made by the cercariae within a very short time, and usually 10 to 15 minutes were sufficient to effect entry into the host. Contrary to the findings of Niemi and Macy (1974), coho salmon and rainbow trout exposed to several hundred cercariae each did not suffer from an unusually high mortality rate. According to Niemi and Macy, when attacked simultaneously by as few as 35 cercariae, 6-cm salmons were killed. In my experiments, all exposed fish, except one that died on the 6th day, managed to survive for a month or longer, or until killed after 30 or more days. One infected fish actually lived for over six months. With lamprey ammocoetes, however, fatality was a result of cercarial

penetration. Two of five small brook lampreys (L. richardsoni), 3.8 cm and 3.4 cm long, exposed to 300-400 cercariae of A. donicus independently, died of mass hemorrhage. Both fish reacted violently to the introduction of the cercariae and within 15 minutes local hemorrhages all over the body, especially around opercular regions, were evident. The first fish (3.8 cm) died the next day, and the second died two days later. The vertebral column was twisted dorso-ventrally (Figure 91). Apparently, death of the fish was due to rupturing of blood vessels. On dissection of the fish, many young A. donicus cysts were found embedded loosely in the body musculature. The cysts had no cyst walls but were covered only with a thin membrane (Figure 92). Such cysts measured 0.16 by 0.144 and were oculate.

Metacercaria

Freshly encysted cercariae were ovoid, still oculate, with a thin membrane and had no cyst walls. Those removed from coho salmon 34 days post-infection tended to be more round and surrounded by a layer of dark piments (blackspot disease) (Plate 10). The eye-spots had disappeared. Measurements of free, encysted metacercariae at 34 days (based on ten cysts) were: 0.128 to 0.208 (0.179) long by 0.104 to 0.140 (0.124) wide, covered by a thin fibrous wall of 0.007 thick (Figure 93). Excysted infective metacercariae were quite

active (Figure 94). One such excysted metacercaria measured 0.399 long by 0.150 wide, spinous in anterior three-fourths of the body. Oral sucker wider than long, 0.06 by 0.05. Pharynx elongate, 0.019 wide by 0.028 long. Acetabulum nearly round, 0.035 wide by 0.030 long. Esophagus long; cecal bifurcation at half-way of body. Ceca reaching almost the extremity of the body. Excretory vesicle was V- or T-shaped and filled with concretions. Young and mature metacercariae up to 38 days old were found underneath scales or just beneath the fascia of the fish. Many of them could be collected easily by simply scraping the sides of the fish with a scalpel. A rainbow trout six months post-infection, however, yielded few metacercariae when the sides of the fish were scraped, so that the blending method was used to obtain the cysts. These older metacercariae were found to encyst deeper in the tissue but apparently not embedded in the musculature as many of the cysts were quite prominent on the surface of the body. In lampreys, the cercariae were found to migrate deep into the body, rupturing blood vessels as they went and encyst finally in the muscles of the fish.

Metacercariae of A. donicus did not seem to increase in size with prolonged development in the fish host. Metacercariae taken from an infected rainbow trout six months after exposure appeared to be only slightly broader than those at 34 days. The average measurements of the older cysts taken from the rainbow trout were 0.164

to 0.196 (0.173) long by 0.132 to 0.152 (0.141) wide. The older cysts, however, were covered by a more substantial fibrous wall, measuring 0.008 to 0.02 (0.016) thick.

Laboratory Infection of Definitive Hosts

Metacercariae became infective in about 34 days at 15-17°C. Three different laboratory animals, a chick, a duckling and a golden hamster, were used in the infection experiments. On September 18, 1974, a young chick was fed 31 cysts from a coho salmon exposed to cercariae of A. donicus 38 days previously. Six days later, the chick was killed but no fluke was recovered from the small and large intestine and the ceca. On September 25, 1974, a duckling was fed 90 cysts 34 days old from another coho salmon. Two hundred cysts from the same fish were likewise stomach-fed to a young golden hamster. On October 2, 1974, both the duckling and the hamster were killed. No fluke was found in the intestine of the duckling while a total of 13 mature trematodes identified as Apophallus donicus was recovered from the small intestine of the hamster. On recovery, all 13 A. donicus were mature and possessed eggs. No attempt was made to determine the first appearance of eggs in the feces. In using white rats, gerbils, golden hamsters, cats, chickens and even man, Niemi and Macy (1974) found that all birds and mammals successfully became infected. In my experiment with the chick, its failure to

become infected could have been due to the small number of cysts used (31 cysts). However, ducklings, which were not used by Niemi and Macy as experimental hosts, could very well be naturally refractive to the infection of A. donicus metacercariae.

Adult (Figure 95)

Adult A. donicus from my experiments are considerably larger than those described by Niemi and Macy (1974). The following measurements are based on seven stained and mounted mature specimens recovered from a golden hamster fed infective cysts from laboratory-exposed fish: body ovoid to pyriform, 0.50 to 0.82 (0.65) long by 0.28 to 0.43 (0.36) wide under slight coverslip pressure. Spines cover the anterior three-fourths of body. Oral sucker subterminal, nearly round, 0.06 to 0.072 (0.065) wide by 0.052 to 0.064 (0.06) long with mouth opening ventrally. Pre-pharynx very short or almost non-existent. Pharynx slightly elongate, 0.02 to 0.036 (0.028) wide by 0.028 to 0.036 (0.030) long. Esophagus 0.048 to 0.1 (0.074) long; cecal bifurcation pre-acetabular and about half-way between pharynx and acetabulum. Ceca reaching almost to posterior end of body. Acetabulum median, pre-equatorial, smaller than oral sucker, 0.04 to 0.044 (0.042) wide by 0.036 to 0.048 (0.04) long. Seminal vesicle plumply bipartite, situated transversely and posterior to acetabulum. Cirrus pouch not present. Gonotyle anterior to

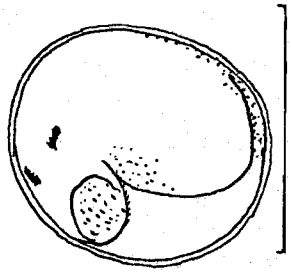
Figure 91. Dead brook lamprey ammocoete exposed 24 hours previously to cercariae of A. donicus.

Figure 92. Young A. donicus cyst in body musculature of lamprey ammocoete.

Figure 93. Cyst of A. donicus removed from a coho salmon 34 days post-infection. Note absence of eyespots.

Figure 94. Excysted infective metacercaria of A. donicus, 34 days old.

Figure 95. Adult A. donicus recovered from the small intestine of a hamster fed infective cysts eight days previously.



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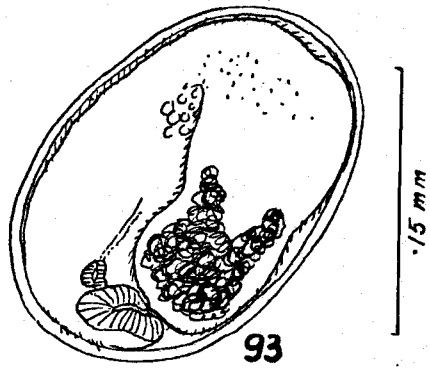

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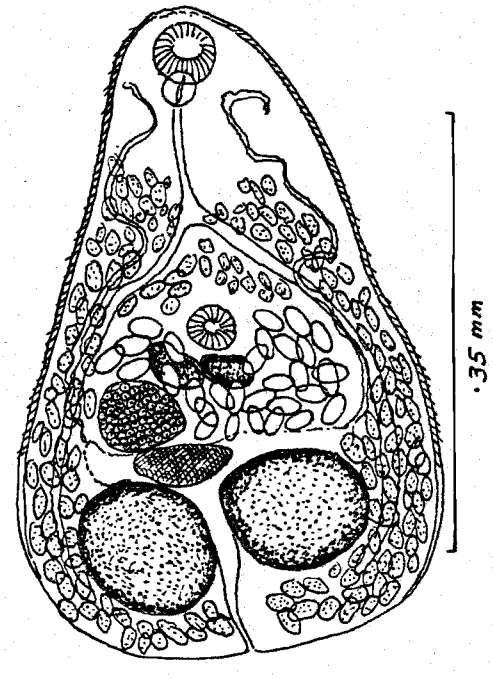
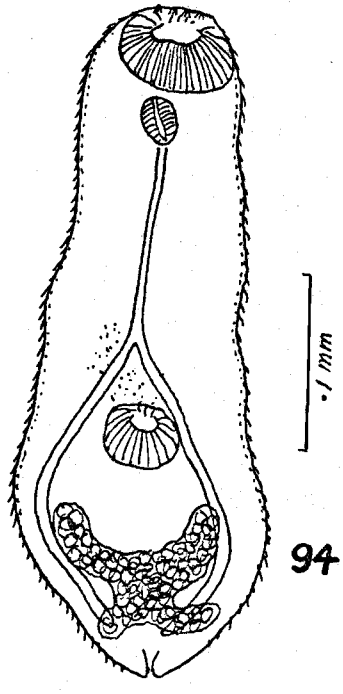


95



94

acetabulum. Ovary medium in size, ovoid, 0.052 to 0.1 (0.073) wide by 0.048 to 0.128 (0.099) long and submedian on the right. Seminal receptacle transversely elongate, posterior to the ovary and touching it. Testes relatively large and slightly oblique; left testis usually more anterior to the right, ovoid, 0.092 to 0.148 (0.114) wide by 0.12 to 0.172 (0.144) long; right testis posterior to seminal receptacle, ovoid, 0.096 to 0.148 (0.114) wide by 0.104 to 0.168 (0.131) long. Vitellaria well-developed; follicles of various sizes, occupying areas lateral and overlapping ceca and extending slightly anterior to cecal bifurcation. Uterus confined to mid-body region. Excretory vesicle T- or Y-shaped, occupying area between and in front of testes. Eggs ovoid, operculated, 0.019 to 0.021 (0.019) wide by 0.030 to 0.034 (0.031) long, and yellowish-brown.

Disussion

The taxonomy of the genus *Apophallus* and its related genus *Rossicotrema* is confusing. Price (1931a), Ciurea (1933) and Cameron (1936) dealt considerably with the problem, and recently Niemi (1973) and Niemi and Macy (1974) offered another view. Based primarily on the shape of the body and the extent of the vitellaria, Price (1931a) combined genera *Rossicotrema* and *Cotylophallus* into *Apophallus* and synonymized *A. venustus* and *A. similis* with *A. donicus*, and recognized *A. muhlingi*, *A. breyis* and *A. crami* as distinct species (see

Price's key in Table 12, and Figures 96-102). Ciurea (1933) and Cameron (1936), however, disagreed. The former regarded A. brevis, A. donicus, A. venustus and A. similis as distinct species and referred the latter three to the genus Rossicotrema. Cameron (1936), while agreeing with Price that Rossicotrema should be synonymized with Apophallus, disagreed with him in the synonymity of A. venustus and A. similis with A. donicus. On the basis of his restudy of A. mühlingi and the specimens of A. donicus provided him by Professor Ciurea, he suggested the following division in accordance to the extent of the vitellaria:

Group 1: which includes A. mühlingi, A. donicus and A. brevis. The vitellaria of all three do not reach level of the acetabulum.

Group 2: which includes A. venustus and A. similis, has vitellaria reaching to the level of esophageal bifurcation.

Accordingly, he agreed with Price in regard to A. similis as a synonym of A. venustus, but not in regard to A. venustus as a synonym of A. donicus. Thus far, much of the confusion concerning the synonymity of A. donicus and A. venustus seems to have stemmed from one or more of the following problems:

1. Some authors have placed great emphasis on subtle and often highly variable characteristics, such as the body shape, size and the extent of the vitellaria which differ often among individuals of the same species.

Table 12. Key to species of Apophallus by Price (1931a).

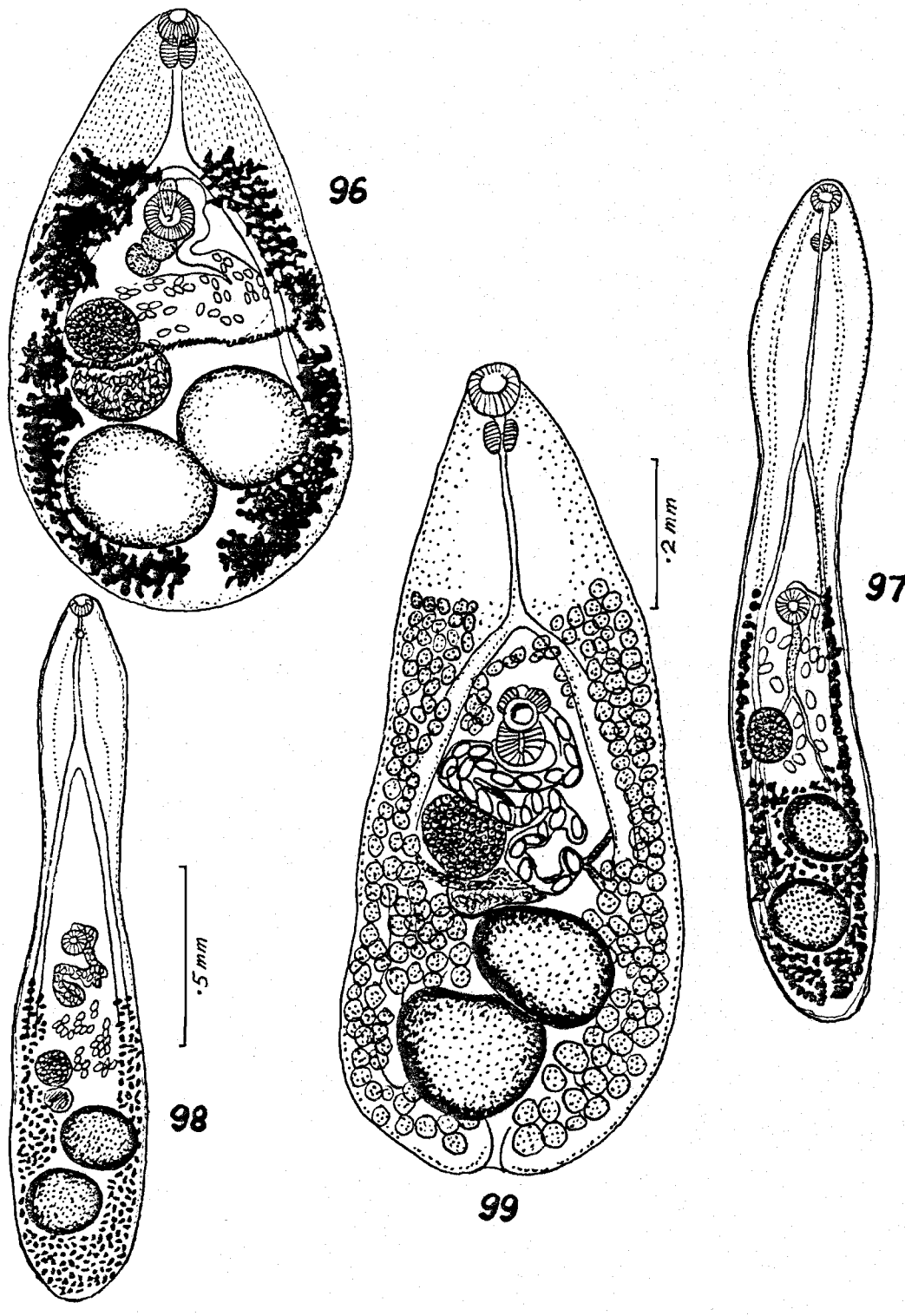
1 (3)	Body elongate, with more or less distinct constriction between acetabulum and bifurcation of intestine	2
2	Vitellaria extend to level of acetabulum; intestinal bifurcation about one-third of body length from anterior end	A. mühlingi
	Vitellaria do not extend anteriorly as far as acetabulum; intestinal bifurcation about one-fifth of body length from anterior end	A. crami
3	Body ovoid in shape; vitellaria extend to level of intestinal bifurcation or slightly beyond . . .	A. donicus (including venustus and similis)
	Body elongated pyriform in shape; vitellaria extend only slightly beyond acetabulum	A. brevis

Figure 96. A. donicus. Ventral view. Redrawn from Skrjabin (1964).

Figure 97. A. mühlingi. Ventral view. Redrawn from Skrjabin (1964).

Figure 98. A. crami. Ventral view. Redrawn from Price (1931a).

Figure 99. A. venustus. Ventral view. Redrawn from Ransom (1921).


96
97
98
99
.2 mm
.5 mm

2. Some authors, in treating the taxonomic problem of the genus, have unfortunately resorted to using secondary sources instead of referring to the original materials.
3. Lack of agreement as to what constitute generic or specific criteria.

With the generic problem, for example, Ciurea (1933) attached great importance to the shape of the body, the development of the metacercaria, and the position of the testes as valuable generic characters. Price (1931a), on the other hand, considered the genital sinus and its accessory structures as most important. Most authors to date, moreover, seem to have reached a concensus: Price (1931a), Cameron (1936) and Yamaguti (1971) agree that Rossicotrema should be synonymized with Apophallus.

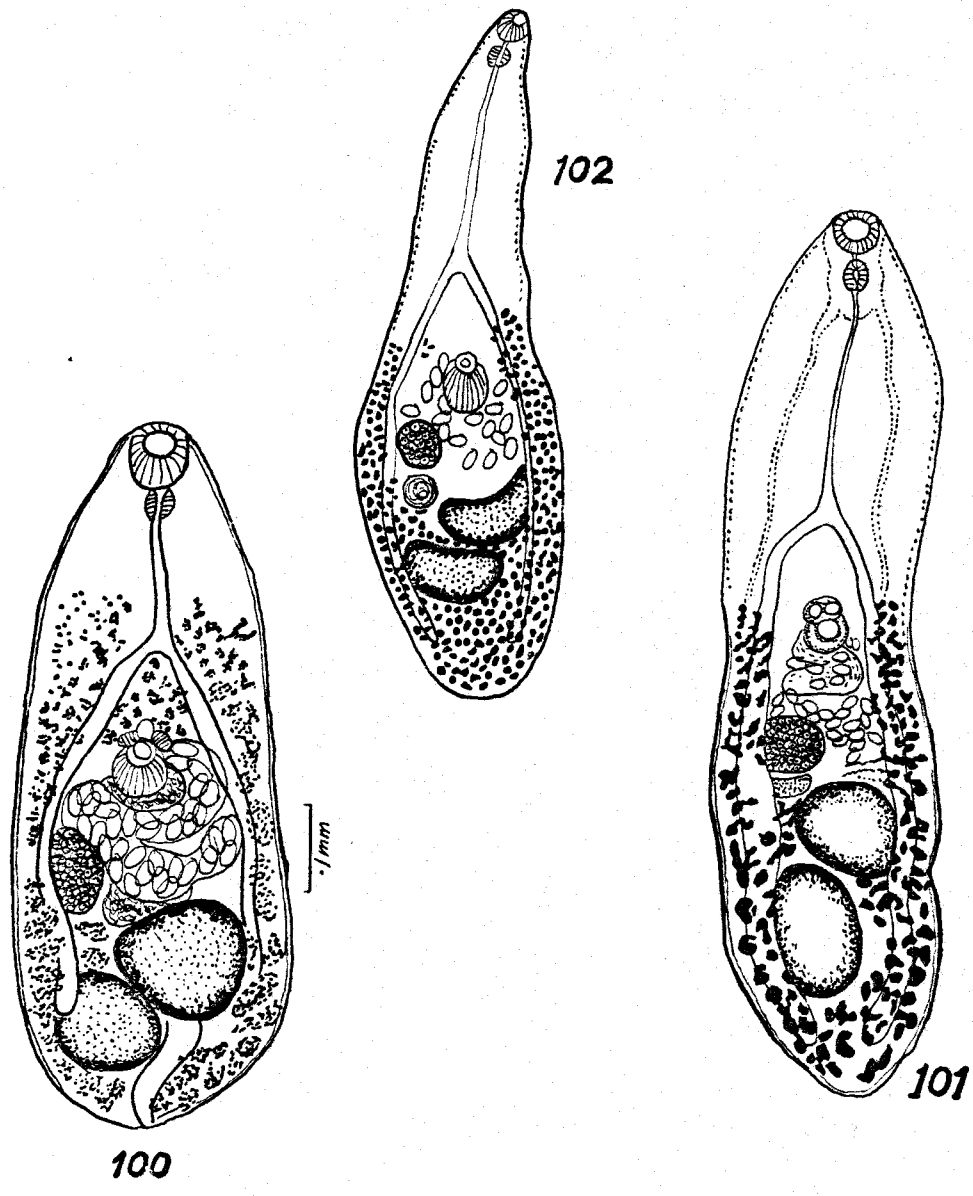
The problem of differentiating between the closely related species of Apophallus, however, remains confusing. The focal point of debate appears to center in the specific characters of A. donicus. As mentioned briefly before, Price (1931a) thought that A. donicus should be synonymized with A. venustus. His decision was apparently based on the original description of A. donicus by Skrjabin and Lindtrop (1919) who depicted a pyriform heterophyid with vitellaria reaching "the level of the transition of the esophagus into the intestine" (Figure 96; see also key to the genera of the subfamily Apophallina Ciurea, 1924, Skrjabin, 1952). Thus, on the basis of the

extent of vitellaria, Price separated A. muhlingi and A. crami from A. donicus and synonymized A. venustus and A. similis with the latter (Figures 97-100). Cameron (1936), on the other hand, argued for the separation of A. venustus from A. donicus. His description of A. donicus was based totally on the drawings as well as specimens of "A. donicus" sent to him by Ciurea. The "A. donicus" that he described showed the vitellaria terminating about the level of the acetabulum (Figure 101). On this and other more variable bases (such as position and shape of testes), Cameron considered A. venustus a distinct species from A. donicus. My objection to Cameron's argument, as was also pointed out by Niemi (1973), is in the source of materials he used for his comparative study. Ciurea's drawings and specimens were obviously in variance with the original descriptions and diagrams of Skrjabin and Lindtrop. Even the key Ciurea used in dividing A. venustus, A. similis and A. donicum (donicus) was in direct contradiction to the original description of Skrjabin (Ciurea, 1933). It is probable that either Ciurea was working with a totally different species from A. donicus, or that A. donicus is, by nature, variable in the extent of the vitellaria. If the latter is true, the extent of the vitellaria may not be a dependable criterion in differentiating between A. donicus and A. venustus, and consequently, A. venustus, A. similis and even A. brevis (Figure 102) would have to be considered as synonymous to A. donicus. Apparently, this position was taken by Niemi and Macy (1974).

Figure 100. A. similis. Ventral view. Redrawn from Ransom (1921).

Figure 101. A. donicus. Ventral view. Redrawn from Ciurea (1934).

Figure 102. A. brevis. Ventral view. Redrawn from Ransom (1921).


102
101
100
.1mm

The adult specimens of A. donicus in my collection agree in all major characteristics with those described by Niemi (1973) from Oregon and with the original description by Skrjabin and Lindtrop (1919). The size of Niemi's as well as my specimens, however, is considerably smaller than the type. As with Niemi's specimens, the vitellaria in all of my ten stained and mounted A. donicus reach slightly anterior to the level of cecal bifurcation. This appears to be a rather consistent characteristic, at least in the Oregon species of A. donicus. Until more work is done to clarify the variability of vitellaria in the European A. donicus, I agree with Price (1931a) in maintaining the synonymity of A. donicus, A. similis and A. venustus, but consider A. mühlingi, A. brevis, and A. crami as distinct species.

Of all the Apophallus life cycles completed, that of A. venustus, which is now considered to be synonymous with A. donicus, differs from the latter in several aspects. Niemi and Macy (1974) have discussed some of these differences. The redia of A. venustus (Cameron, 1937) has a much longer gut and contains fewer cercariae; the cercaria has 16 instead of 14 penetration glands and the snail host is Goniobasis instead of Flumenicola. The life cycle of A. mühlingi (Odening, 1970), which is considered to be a distinct species from A. donicus, uses a hydrobiid snail, Lithoglyphus naticoides, as first intermediate host. The metacercaria is found in several species of cyprinids and matures in dogs, cats and gulls. The redial and

cercarial stages of A. mühlingi and A. donicus are similar except that the body of the cercaria of A. mühlingi bears many hair-like projections.

One interesting observation by Niemi and Macy (1974) deserves mentioning. Niemi infected himself with metacercariae of A. donicus and recovered eggs in his feces between the eighth and the 23rd days. Most heterophyids have little definitive host specificity; A. donicus infected fish in the Pacific Northwest may become potential sources of human infection.

Life Cycle of Plagioporus siliculus Sinitsin, 1931

The life cycle of Plagioporus siliculus was first implicated by Sinitsin in 1931, who found the adults in cutthroat trout, Salmo clarki clarki, and the metacercariae in the muscles of crayfish "Potamobius sp." According to him, the cercaria developed in Oxytrema silicula and was of the cotylomicrocercous group. Sinitsin described the cercaria, the metacercaria and the adult but did not verify the life cycle with feeding experiments. However, Pratt and McCauley (1961) and Crandell (1963) failed to observe the penetration of cercaria P. siliculus into crayfish as claimed by Sinitsin.

During the surveys for fish trematodes and cercariae from Ritner Creek, the adults of P. siliculus were encountered in a number of fresh water fishes (see Part I of this study), and the cercaria was

found in the stream snail O. silicula. Exposure of the cercariae to crayfish Pacifastacus lenuisculus confirmed the observations of Sinitsin (1931), and feeding of metacercariae from the crayfish to rainbow trout resulted in immature and mature P. siliculus. The following thus represents the first experimentally demonstrated life cycle of Plagioporus siliculus.

Materials and Methods

Snails, crayfish, and fish definitive hosts were handled in the same way as described previously. Flowing de-chlorinated water at 15-17°C was used to keep the animals during the course of the experiments. The fish were starved for a few days before being fed infected crayfish.

Results

Cercaria and First Intermediate Host

The cotylomicrocercous cercaria was found in O. silicula. For description and biology of the cercaria and other intramolluscan stages see Part II in this study.

Infection of Crayfish

Local crayfish, Pacifastacus lenuisculus from Ritner Creek,

were found to be infected with the metacercariae of P. siliculus. The cysts were found in the abdominal muscles of the crayfish. On August 14, 1974, a small P. lenuisculus, about 2 cm long, was exposed to about 150 P. siliculus cercariae in a stender dish. As described by Sinitsin (1931), the cercariae stood on their tails on the bottom of the dish and wagged to and fro "as if sensing for something around." As soon as the crayfish was introduced, the movement of its appendages appeared to cause much agitation among the cercariae, which soon adhered themselves to the distal portion of the legs and began to crawl laboriously toward the abdomen of the crayfish. In another crayfish pinned ventral side up to a paraffin dish, cercariae of P. siliculus were observed to migrate toward the ventral portion of the abdomen. One cercaria was seen to lodge at the edge of the sternite, remain motionless for a while and then begin a characteristic "poking" motion at the mid-lateral portion of the sternite. The cercaria perched with its ventral sucker on the exoskeleton and "poked" around as if looking for a soft spot to penetrate. Then all of a sudden, the oral sucker was seen inside the abdomen and the cercaria began to crawl underneath the sternite. The entire process of penetration took less than five minutes to complete (Figure 103). Once underneath the sternite, cercariae were seen to wander about. Encystment did not seem to commence immediately after penetration. Many unencysted and free-moving metacercariae could still be found

among the abdominal muscles 24 hours post-exposure. The one-day old unencysted metacercaria looked almost identical to the cercaria of P. siliculus but was tailless.

Metacercaria

Metacercariae of P. siliculus were found naturally infecting crayfish (P. lenuisculus) in Ritner Creek. Of six crayfish sampled randomly, the numbers of cysts were: 5, 11, 2, 24, 8 and 14 respectively. The size of the cysts appeared to depend on the size of the crayfish. Cysts taken from two small P. lenuisculus, about 2 cm long, averaged 0.25 by 0.212 and 0.233 by 0.192, whereas those taken from crayfish of a larger size group (5 cm and above) averaged 0.351 by 0.327. The smallest cyst obtained from naturally infected crayfish measured 0.188 by 0.168 while the largest measured 0.52 by 0.512. This clearly lies within the range reported by Sinitsin (1931). A typical P. siliculus cyst is round to slightly ovoid, has a thin membrane and is surrounded by a fibrous cyst wall (Figure 104). The metacercaria in the cyst typically lies folded on its ventral side. The oral sucker, acetabulum and excretory vesicle were clearly visible.

An excysted young metacercaria, one-day old, is elongate, spindle-shaped and measured 0.54 by 0.14. The oral sucker is almost round, 0.064 by 0.06 and the acetabulum 0.072 by 0.068. The pharynx, 0.024 by 0.028, lies half-way between the oral sucker and the

acetabulum. The excretory vesicle is characteristic of P. siliculus, reaching way in front toward the acetabulum. Penetration glands and the stylet are still evident (Figure 105).

The excysted older metacercaria looks identical to that described by Sinitsin (Figure 106). Sinitsin, in his study of the metacercaria of P. siliculus, reported an interesting phenomenon. He noticed a gradual "metamorphosis" of the metacercaria within the cyst as it grew: the pre-acetabulum area becoming much shorter so that the pharynx nearly disappeared. My observations also seemed to verify this, for in a freshly encysted metacercaria the pre-pharynx is almost as long as the esophagus, whereas in the older metacercariae--those from a 0.268 diameter-cyst and a 0.432 by 0.416 cyst--there is a gradual shortening of the pre-pharynx, so that finally in the metacercaria of a 0.44 diameter-cyst, the pharynx almost touches the oral sucker (Figure 107a-c).

The stylet, which was present in the younger cysts, disappeared as the cysts grew. A study of 89 live cysts showed that the stylet began to disappear about the sixth day post-infection and by the 19th day, almost all had disappeared. The smallest cyst at which the stylet disappeared was about 0.254 by 0.184.

Infection of Definitive Host

Sinitsin (1931) found adult P. siliculus in the intestine of

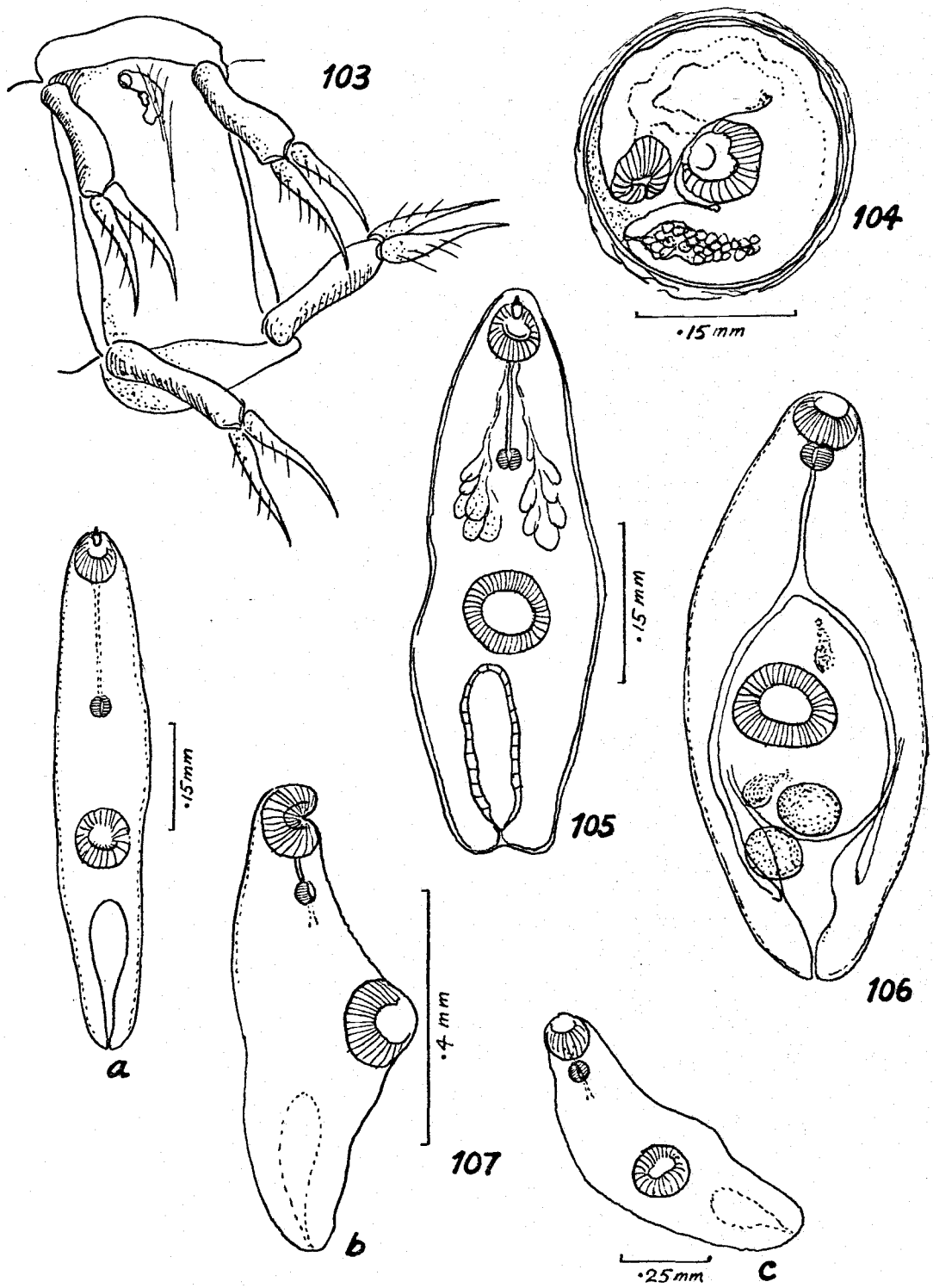
Figure 103. Cercaria of P. siliculus underneath the sternite of crayfish, Pacifastacus lenuisculus.

Figure 104. A typical P. siliculus cyst in the abdominal muscles of crayfish.

Figure 105. Excysted young metacercaria of P. siliculus (one day old).

Figure 106. Excysted older metacercaria of P. siliculus.

Figure 107a-c. "Metamorphosis" of the metacercaria of P. siliculus. As the metacercaria grows, the stylet disappears and the pre-pharynx shortens.


103
104
.15 mm
105
.15 mm
106
.15 mm
a
.4 mm
107
b
.25 mm
c

S. clarki clarki. My survey of Ritner Creek showed that O. kisutch, C. rhotheus, and R. osculus were also natural definitive hosts of the parasite. For the feeding experiments in the laboratory, however, only rainbow trouts, Salmo gairdneri, were used. A total of four hatchery-raised rainbow fingerlings was used. Two fish were fed infected crayfish exposed to cercariae of P. siliculus 38 days previously. When killed six days later, three immature P. siliculus were recovered from the first fish and one from the second. A third fish was killed 20 days after it had been fed infected crayfish harboring 36-days old cysts and had been kept at 15-17°C. In its intestine, only one immature P. siliculus was found. The last rainbow trout was killed 34 days after having been fed 36-days old cysts. A total of 41 P. siliculus was found in the intestine. Of these, one had two well-formed eggs in its uterus, four had a few developing eggs, and the rest had no eggs but possessed well-developed gonads.

Although the above evidence is not conclusive, my observations suggest that 36-days old metacercariae of P. siliculus may require more than 34 days in the fish (at 15-17°C) to mature as adults. The cysts found naturally in crayfish are unquestionably those of P. siliculus, and the crayfish (Pacifastacus lenuisculus) is shown to be the secondary host by observations of cercarial penetration.

Discussion

Although Sinitsin (1931) implicated the cutthroat trout and crayfish in the life cycle of P. siliculus, this report represents the first laboratory-demonstrated life cycle of the parasite. In Oregon, cercariae of P. siliculus are found in O. silicula. The secondary host is Pacifastacus lenuisculus (Astacidae), not "Potamobius sp." as reported by Sinitsin. Metacercariae in the abdominal muscles of the crayfish, when eaten by S. clarki clarki, O. kisutch, C. rhotheus and R. osculus (above, natural hosts), and S. gairdneri (experimental host, this study), develop into adults in the intestine in about 34 days at 15-17°C.

Most of Sinitsin's observations are confirmed in this study with one possible exception: contrary to his report, I have failed to observe eggs in the cysts of P. siliculus. Although gonadal development may reach an advanced stage in the older metacercariae, progenesis does not seem to occur. As demonstrated by feeding experiments in this study, a great majority of metacercariae that were 36 days old in the crayfish did not reach gravid stage even after 34 days of development in the fish. Had progenesis been a common phenomenon in P. siliculus, I would have noticed it in my observations, and a greater percentage of metacercariae would have become mature and gravid in a much shorter time.

Although the family Opecoelidae has more cases of progenetic metacercariae than other trematode families (Buttner, 1950), Plagioporus sinitsini, which is closely related to P. siliculus, requires 15-30 days to mature in the gall-bladder of its definitive host (Dobrovolny, 1939).

Partial Life Cycle of the New Monorchiid Trematode n. gen., n. sp.

A new monorchiid trematode from the intestine of the torrent sculpin, Cottus rhotheus, was described in Part I of this study. Of the 21 fish examined from Ritner Creek seven were infected. Metacercariae of the new monorchiid were found to encyst in the pericardium, liver and kidneys of Lampetra richardsoni, brook lamprey as well as L. tridentata, Pacific lamprey. Attempts were made to identify the cercaria and molluscan intermediate host of the parasite and to obtain mature individuals by feeding C. rhotheus with metacercariae from naturally infected secondary hosts.

Materials and Methods

Snails, Oxytrema silicula and Flumenicola virens, sources of prospective cercariae for the life cycle study, were obtained from the same location where the definitive hosts were collected. Cercariae were obtained by isolating snails singly in small stender dishes

for one to two days and then examining them for shed cercariae. Insect larvae (including caddis fly, stonefly and beetle) and various fishes from Ritner Creek were exposed to different cercariae in the search for a secondary intermediate host. The procedure of exposure involved the introduction of a large number of cercariae into a small dish in which the fish or the insect larvae were kept. Penetration was observed through a dissecting microscope. In feeding C. rhotheus with metacercariae from lampreys, the fish were starved for about four to five days and then fed infected liver or portions of infected lamprey. Infected C. rhotheus were kept at two different temperatures: 10°C and 15-17°C for various periods of time.

Results

Cercaria and First Intermediate Host

The cercaria and the first intermediate host are still unknown. Upon discovering the adult trematode, various attempts were made in search of the secondary as well as the first intermediate hosts. On August 1, 1974, two brook lamprey ammocoetes about 10-13 cm long were captured from Ritner Creek. Dissection of both revealed many large cysts in the pericardium, liver and kidneys (Plate 11). Excysted metacercariae of these cysts appeared identical to the immature monorchiids recovered previously from the sculpin,

C. rhotheus. Many of the younger excysted metacercariae showed the presence of a pair of eyes and a spinous body (Figure 8). On the basis of these observations, intensive attempts were carried out in search of the cercaria. Of the 20 or more cercariae infesting O. silicula and F. virens (see Part II in this study) in Ritner Creek, only three common oculate cercariae were found. Of these, one, a pleurolophocercous cercaria from O. silicula, was found to be the cercaria of Metagonimoides oregonensis whose life cycle had already been described and reported by Burns and Pratt (1953). Of the remaining two oculate cercariae from F. virens, the first was apparently an undescribed species henceforth known as Cercaria X (see Part II for description and biology), while the second, also a pleurolophocercous cercaria, was subsequently proven to be the cercaria of Apophallus donicus.

To identify the cercaria of the new monorchiid, the following insect larvae and fishes were exposed to Cercaria X and the pleurolophocercous cercaria. Cercaria X did not penetrate any of the insect larvae exposed to them. These included two limnephilid larvae (?), two stonefly larvae (Acroneuria ?) and a beetle larva. Nor did it penetrate any of the following fishes: C. rhotheus, the torrent sculpin; R. osculus nubilus, blackside dace; C. perplexus, reticulate sculpin; and L. richardsoni, brook lamprey.

The pleurolophocercous cercaria from F. virens, however, were

found to penetrate a variety of fishes. These included R. osculus nubilus, blackside dace; C. perplexus, reticulate sculpin; R. osculus, speckled dace; Oncorhynchus kisutch, coho salmon; Salmo gairdneri, rainbow trout and L. richardsoni, brook lamprey. In the last species, as mentioned in the life cycle study of Apophallus donicus, simultaneous penetration of lamprey ammocoetes by large numbers of cercariae resulted in mass hemorrhage and death of the fish. One C. rhotheus was also exposed to the pleurolophocercous cercaria but no penetration was observed.

Since Cercaria X failed to penetrate L. richardsoni, brook lamprey, the possibility of its being the cercaria of the new monorchiid is excluded. Although the pleurolophocercous cercaria successfully penetrated lampreys, the metacercariae recovered differed from those of the new monorchiid naturally infecting lampreys in both morphology and location of encystment. Any possible link of this cercaria to the life cycle of the new monorchiid was subsequently eliminated when it was proven to be the cercaria of Apophallus donicus.

Metacercaria

Metacercariae removed from the pericardium, liver and kidneys of L. richardsoni or L. tridentata, brook and Pacific lampreys, are of medium to large size, round or slightly ovoid and yellowish in color (Figure 5). Measurements based on 10 cysts are: length 0.36

to 0.408 (0.386), width 0.352 to 0.408 (0.383). The cyst wall averages 0.016 thick. In younger cysts, two distinct eyespots each composed of groups of pigment granules were found posterior to the pharynx, one on either side of the midline, while in the larger, and therefore, presumably older cysts, the eyespots were absent. The maximum size reached by the largest cysts is 0.408 by 0.388 to 0.408. The metacercaria typically lies folded on its ventral surface in the cyst. The oral sucker, pharynx, pre-pharynx, esophagus, acetabulum and the excretory vesicle, which is often filled with dark concretions, are all clearly visible. The anterior half of the body is spinous. It is not uncommon for two cysts to share a common fibrous wall, thus forming compound cysts (Figure 7). A young, freshly excysted metacercaria is elongate, oculate, and shows various degrees of development of the gonads and cirrus pouch (Figure 8). An old excysted metacercaria, however, is eyeless, and looks in all ways identical to the mature monorchiid except, of course, for the absence of eggs in the uterus. While the excretory vesicle of the younger cysts is filled with dark granules, the excretory vesicle of older cysts may become quickly emptied as they excyst. In older metacercariae spines are found all over the body (Figure 9).

Secondary Intermediate Host

Both brook and Pacific lampreys were found to be secondary

hosts for the parasite. Most of the lampreys used in the experiments were collected from Ritner Creek, although a few were taken from Rock Creek in Benton County. All brook and Pacific lampreys from Ritner Creek were found to be infected. The single brook lamprey from Rock Creek was likewise found to harbor cysts, although only three out of five Pacific lampreys from Rock Creek were infected. An attempt was made to determine the intensity of infection in both lampreys but this was later abandoned. The cyst wall was of such consistency that blending of lampreys at low speed for one minute ruptured all cysts and thus made their recovery impossible. Most lampreys, especially brook, were rather heavily infected. This rendered removal of cysts an extremely tedious task. Pepsin digestion was not used.

Infection of Definitive Host

In order to demonstrate the latter part of the life cycle, it was decided that uninfected C. rhotheus, the natural definitive host, should be fed metacercariae from both brook and Pacific lampreys, and then killed to recover the mature flukes. Since no laboratory-bred fish were available, it was necessary to use wild fish. The following precaution was taken, however, to ensure that these fish used in the feeding experiments were not naturally infected. Twenty C. rhotheus collected from Ritner Creek were dissected. The results showed that

all fish below 5 cm long were uninfected, and that only one out of 11 below 9 cm was infected. Consequently, only C. rhotheus under 5 cm would be used in the experiments. On August 22, 1974, a previously starved C. rhotheus about 5 cm long was fed an undetermined number of cysts from a brook lamprey. The fish was then returned to the cold room at 10°C. Six days later, the fish was killed and 14 immature but very active monorchiids were recovered from the intestine of the fish. The absence of mature flukes would seem to indicate that the infection was the result of the recent exposure, and that six days of development at 10°C might not be sufficient for the maturation of the fluke. On August 27, 1974, two more C. rhotheus, measuring 4 cm and 4.5 cm, were fed with cysts from brook lampreys. A 3-cm lamprey fed to the small C. rhotheus was devoured head first almost instantaneously. These fish, after being fed, were kept in 10°C. At the end of 14 days, the small fish was killed and 30 immature monorchiids were recovered from its intestine. Twenty-six days after feeding, the second fish was also killed, and nine immature monorchiids were found. Thus the parasite probably required more than 26 days at 10°C to become mature. On September 11, 1974, therefore, another C. rhotheus was fed with cysts from a Pacific lamprey and kept this time at 15-17°C. Twenty-six days later when the fish was killed, a single mature monorchiid was found in the intestine. This fluke had only eight eggs in its uterus which might be indicative of its recent

maturity. A repeat of the last experiment was unsuccessful: another C. rhotheus killed 32 days after being fed cysts from a Pacific lamprey did not become infected.

Cysts from a Pacific lamprey were also fed to a 7.4 cm starved C. perplexus. Eighteen days later, when the fish was killed, three immature monorchiids were found in the intestine. C. perplexus had not been found naturally infected with the trematode before but this observation would seem to indicate that it could become a potential host for the parasite.

The above observations plus the fact that mature metacercariae from lampreys looked identical to young flukes recovered from C. rhotheus would warrant the following conclusions:

1. The monorchiid naturally makes use of lampreys as secondary intermediate hosts.
2. It matures in the intestine of the torrent sculpin, Cottus rhotheus.
3. Maturation of the trematode requires more than 26 days at 10°C.
4. At 15-17°C it may require 26 or more days to become fully mature.
5. Cottus perplexus is a potential host for the parasite.

Discussion

Life cycles of only a few species in the Family Monorchiidae

have been reported. Stunkard (1959) reviewed the life history studies on members of the subfamily Asymphylodorinae Szidat, 1943. The tailless cercariae of the cercariaeum group of Asymphylodora amnicola (Stunkard, 1959) either encyst in uninfected Amnicola limosa, or develop directly to sexually mature adults in the snail. The adult stage is found in perch, killifish, small-mouthed bass or pumpkinseed. Life cycle of A. japonicum is similar to the above, but the cercariaeum encysts in the redia in Bulimus and the adult is found in Cyprinus carpio and Rhodeus sp. (Yamaguti, 1938; Kuyama, 1938). Recently, Macy and English (1975) described the life cycle of Palaeorchis problematicus Macy and Berntzen, 1970 , the cercariaeum of which, after emerging from Flumenicola virens, re-encysts in the snail as a metacercaria. The natural definitive host, in this case, is the squawfish, Ptychocheilus oregonensis Richardson. The fourth life cycle known in this subfamily--that of A. macrostoma Ozaki, 1925--is unique in its use of fish as secondary hosts. Metacercariae of A. macrostoma encyst in peribuccal connective tissues or gill arches of Chaenogobius macrognathos, Gnathopogon elongatus, and Cobitis bivae, while the adult parasites are found in a number of different fresh water fishes (Yamaguti, 1934, 1938). In the subfamily Lasiotocinae Yamaguti, 1958, the two life cycles that are known--Proctotrematoides pisodontophides Yamaguti, 1938 and Postmonorchis donacis Young, 1953--typically make use of pelecypods as secondary hosts (Hoshina, 1951; Young, 1953).

In 1964, DeMartini and Pratt described the life cycle of Telolecithus pugetensis (of subfamily Telolecithinae). The brevifurcate cercaria, like cercariae of most other known life cycles in the family, encysts in one of five bivalve molluscs. The definitive hosts are a number of marine fishes.

In subfamily Monorchinae Odhner, 1911, to which the new monorchiid belongs, the only known life history is that of Monorcheides cumingiae Martin, 1938. The life cycle, again, follows the typical pattern. Two species of clams, Cumingia and Tellina, serve as secondary intermediate hosts, while the adults are found in "eels and flounders" (Martin, 1940).

The life cycle of the new monorchiid reported herein is unique in using lampreys instead of the usual molluscs as secondary intermediate hosts. The only other known member in the family utilizing fish as secondary hosts is Asymphylodora macrostoma. The metacercariae of my monorchiid encyst in the pericardium, liver and kidneys of the host; whereas those of the former are found in the peribuccal connective tissues or gill arches of the fish. My monorchiid also resembles Monorcheides cumingiae in probably having an oculate cercaria, but differs in the nature of the secondary host.

As observed by Schell (1970), at least three different kinds of cercariae are represented in the family Monorchiidae. Although the majority of known life cycles involve molluscs as secondary

intermediate hosts, exceptions are nonetheless in existence (Yamaguti, 1971). While Monorchiidae is a relatively small family, by far the greatest problem in the study of its taxonomy may yet be the lack of understanding of its life cycles. The controversy raised over the life cycle studies of genus Asymphylodora alone in the past century (Stunkard, 1959) is indicative of the much needed work in this area.

Notes on the Life Cycle of Echinochasmus milvi and the Identity of Cercaria gorgonocephala in Oregon

As was mentioned in Part II of this study, a survey of Oxytrema silicula from Ritner Creek yielded a species of zygocercous cercaria identical to Cercaria gorgonocephala, which was originally described by Ward (1916) from Lake Erie, and later redescribed by Martin in 1968. In 1973, Dronen reported C. gorgonocephala in Goniobasis livescens from Michigan. The same species is also believed to be identical to the "undescribed zygocercous or rattenkönig cercaria" mentioned in an abstract by McCauley and Pratt (1960), in which they claimed the completion of the first life cycle of a zygocercous cercaria. The adults, identified as Echinochasmus milvi Yamaguti, 1939, were recovered experimentally from the duck and the hamster. Two other macrocercous cercariae were also found in the same snail in Ritner Creek. These two "species" of cercaria--an aggregating

but albino cercaria and a non-aggregating macrocercous cercaria--had a body that was identical to the one in Cercaria gorgonocephala. Both McCauley and Pratt had observed all three of these cercariae or "forms" previously (McCauley, unpublished communications), and suggested that the aggregating rusty-colored, tailed form (= Cercaria gorgonocephala) and the non-aggregating form were developmental stages of the same species, and that the former represented an immature stage. No explanation, however, was given to the existence of the aggregating-albino form.

In view of the occurrence of these three different forms that represented an apparent case of polymorphism in larval trematodes, intensive attempts were made: 1) to determine if the three forms actually belonged to different developmental stages of the same cercaria. This was done by isolating infected snails and observing them for prolonged periods of time. 2) To complete the life cycle using the non-aggregating form with the hope of demonstrating the identity and relationship between the aggregating and non-aggregating cercariae. Additional feeding experiments were also done which resulted in the verification of the existence of E. milvi in Oregon as reported by McCauley and Pratt (1960) and Uzmann and Hayduk (1964).

Materials and Methods

Oxytrema silicula infected with the three cercariae were collected

by hand from Ritner Creek in Polk County, Oregon. They were kept in aerated de-chlorinated water at room temperature (20-22°C) for observations, and were fed fresh green lettuce. Infection in snails was determined by the isolation method as described previously in this study, and those infected with any one of the three cercariae in question were isolated in medium-sized finger bowls for cercaria-shedding observations. O. silicula thus isolated lived from a few days to as long as 4-1/2 months, long after cercarial shedding had ceased.

Very small, uninfected blackside dace (Rhinichthys osculus nubilus) were collected by net from a small spring feeding into Ritner Creek underneath the bridge at Highway 223. Ten of these were dissected and their gills were determined to be free from echinostome infection.

Blackside dace and redside shiner, naturally infected with an echinostome cyst which was subsequently shown to be that of E. milvi, were collected also by net from Ritner Creek. Experimental definitive hosts used in the experiments--hamsters, chicks, ducks and rats--were all laboratory or farm-bred. Infection of definitive hosts was done by stomach-feeding either free encysted metacercariae or infected whole gills to the animals. Metacercariae were collected by removing gills from the fish and shaking them in a small vial together with a few glass beads. Free encysted metacercariae were then collected with a fine-tipped pipette.

Results

Recovery of Adult E. milvi from a Duck Fed Echinostome Cysts in Blackside Dace (R. osculus nubilus) and Redside Shiner (Richardsoni balteatus hydrophlox)

Large-sized blackside dace and redside shiner from Ritner Creek were found to be naturally infected with a large echinostome cyst. The cysts, found loosely embedded in the gills, are thin-walled, ovoid, 0.136 to 0.164 (0.151) by 0.092 to 0.1 (0.105) wide, with definite collar of spines in the larger cysts. The metacercaria lies folded on its ventral surface. The collar, oral sucker, pharynx and acetabulum can easily be seen from the outside (Figure 108). Simultaneously infecting the gills of many blackside dace were the echinostome cysts of an Echinochasmus species (see Part III, p. 185 in this study). The two, however, can be easily separated by size. Cysts of the Echinochasmus species are much smaller, contain two characteristic groups of refractile excretory granules, and encyst on the filaments of the gills.

On September 12, 1974, a medium-sized blackside dace and a redside shiner were killed. One hundred and five cysts recovered from their gills were fed to a muscovy duckling. Eight days later, the duckling was killed and in its lower small intestine a single mature and gravid adult trematode was found. This fluke was later identified

as E. milvi (Figure 109), the description of which is as follows: Body plump, widest at posterior half of body; tapering in region of the neck. Body 1.2 long by 0.35 wide. Head collar with 22 spines interrupted dorsal to the oral sucker. Oral sucker slightly longer than wide, 0.072 long by 0.064 wide. Pre-pharynx very short, 0.028. Pharynx ovoid to elongate, 0.052 wide by 0.076 long. Acetabulum nearly round, pre-equatorial, 0.14 wide by 0.144 long. Cirrus pouch plump and large, submedian on the left and overlapping acetabulum dorsally, 0.104 wide by 0.22 long. Seminal vesicle bipartite, almost filling entire cirrus pouch. Ovary immediately posterior to acetabulum, median or slightly to left, ovoid, more broad than long, 0.088 wide by 0.64 long. Testes tandem, contiguous, ovoid to spherical; anterior testis, 0.14 wide by 0.14 long; posterior testis, 0.14 wide by 0.132 long. Vitellaria extending to extracecal fields from the posterior margin of acetabulum to the end of the body, intruding into post-testicular area. Cuticular spines cover anterior body to level of anterior testis. A single egg lies left to ovary, is ovoid, yellowish-brown, 0.056 wide by 0.84 long.

My single specimen of E. milvi resembles the original description of Yamaguti (1939) in all major particulars with the exception that the sizes of acetabulum, cirrus pouch and testes in my specimen are all proportionally larger than those of the holotype. The egg size, however, is identical.

E. milvi was first described from Milvus migrans lineatus (Gray) in Japan by Yamaguti in 1939. McCauley and Pratt (1960) recovered the same from hamsters and ducks experimentally fed metacercariae in the gills of sticklebacks and guppies exposed to "an undescribed zygocercous or rattenkönig cercaria" found in O. silicula. Although the detailed life cycle studies of the parasite in Oregon were not published subsequently, it nevertheless represented not only the first record of E. milvi in North America, but also the completion of the first zygocercous cercaria life cycle in history. The "undescribed zygocercous cercaria" of McCauley and Pratt (1960) is now believed to be identical to Cercaria gorgonocephala (Ward, 1916), redescribed by Martin in 1968 (this study and personal communications with McCauley). Martin, who published the redescription of the cercaria, was apparently unaware of McCauley and Pratt's report at the time of his writing. In 1964, Uzmann and Hayduk, in Washington, reported the finding of E. milvi in sibling male albino rats fed echinostome cysts from naturally infected rainbow trout, Salmo gairdneri. Their finding constituted the first report of E. milvi metacercariae in wild fish from North America. My recovery of E. milvi, as reported in this study, therefore becomes the third record of the parasite in the Pacific Northwest. The life cycle of E. milvi in North America, as it appears to date, consists of the following: the snail intermediate host in Oregon and Washington is O. silicula. Cercariae (= Cercaria

gorgonocephala) of the zygocercous group develop in rediae and emerge in clusters which are swallowed by a number of fresh water fishes including rainbow trout, blackside dace, redside shiner (natural hosts) and sticklebacks and guppies (experimental hosts). The cysts, embedding loosely on gills of fish, when fed to experimental hosts of hamsters, ducks, and rats, mature in the small intestine as E. milvi. The natural definitive host of the parasite in North America is still unknown.

Since Cercaria gorgonocephala is also found in the Great Lakes region (Ward, 1916; Williams, 1931; Martin, 1968; Dronen, 1973), one might postulate that the range of E. milvi in North America may extend from Lake Erie westward all the way to the Pacific coast. As suggested by Williams (1931) and demonstrated later by Dronen (1973), the life cycle of C. gorgonocephala, and therefore, E. milvi, in the Great Lakes region involves the pleurocerid snail, Goniobasis livescens, which is a relative of O. silicula on the west coast.

Prolonged Observations of the Three Cercariae in Isolation

A total of six infected O. silicula was isolated in medium-sized finger bowls for prolonged periods of observation. Two of these snails each shed either Cercaria gorgonocephala (CG), the non-aggregating macrocercous cercaria (NAM) or the aggregating-albino

cercaria (AA). The purpose of such observations was to determine if the form of cercaria shed by any snail would be consistent with prolonged periods of growth and maturation. One would predict, if CG and the NAM forms actually represented two developmental stages of the same cercaria, that there would be a gradual shift in the shedding of one form to the next. Of all six snails, A to F, which were actively shedding cercariae at the beginning of the observations, snail D died on the fourth day, whereas snail C lived for almost 4-1/2 months and long after the shedding of cercariae had ceased. The shedding observations of these snails are recorded in Table 13.

Infection Studies with the Non-Aggregating Macrocercous (NAM) Cercaria from Oxytrema silicula

Since McCauley and Pratt's (1960) recovery of E. milvi involved the use of cysts from fish exposed to the aggregating-rusty-colored-tailed cercaria (C. gorgonocephala), a decision was made in the course of my study to use the non-aggregating macrocercous cercaria instead. The hypothesis was that if both C. gorgonocephala and the non-aggregating macrocercous cercaria represented different stages in the same species, feeding experiments using cysts from the latter should produce also E. milvi. Thus, during the period from September 4, 1974 to November 19, 1974, a major series of experiments was carried out. These experiments involved the following

Table 13. Shedding observations on Cercaria gorgonocephala (CG), the non-aggregating macrocercous cercaria (NAM), and the aggregating-albino cercaria (AA). Observations were made from August, 1974 to January, 1975.

Snail A
(CG shedder)

8-30 a single CG shed but no clusters, proximal part of tail heavily pigmented

8-31 no cercaria shed

9-4 two clusters of CG shed; proximal part of tail with light pigments

9-5 a few clusters of CG shed; proximal part of tail with light pigments

9-6 7-8 clusters of CG shed; pigments same as 9-5

9-7 snail dies; no cercaria shed

Concluding Remarks:

1. For 8 days from 8-30 to 9-7, snail A shed same form (CG) persistently.
2. Tail pigments became lighter toward the end of observation.
3. Tail pigments were found in the proximal part of the tail.

Snail B
(CG shedder)

9-3 observe cercaria migrate to tip of ctenidial leaflet and emerge; only clusters are shed; tail pigments on distal portion of tail

9-4 many clusters of CG shed; pigments dark in distal portion of tail

9-5 at least 12 clusters of CG shed

9-6 shed CG; tail same as before

9-7 at least 15 clusters shed; pigments lighter

9-9 6 clusters of CG shed; pigments very light, becoming light brown instead of rusty

9-10 at least 20 clusters shed, but pigments turn dark again

9-12 many clusters shed; tail pigments light brown, darker than 9-10

9-13 same as 9-12

9-15 same

9-20 same; 20 or more clusters shed

9-28 13 or more clusters shed; tail pigments darker but not as dark as 9-3; cercariae all dead within 24 hours

9-30 snail dies

Concluding Remarks:

1. Snail B shed persistently the same kind of cercaria (CG) for 27 days until death.
2. In contrast to snail A, pigments were found in distal portion of tail.
3. Tail pigment intensity tended to fluctuate, but did become lighter at the end.
4. Cercariae emerged at tip of ctenidial leaflets; clusters are probably formed inside the gill chamber of the snail before emerging as rosettes.

Table 13. (Continued)

Snail C
(NAM shedder)

8-30 NAM cercariae of both white and rusty-colored tails are shed; pigments in distal portion of tail

9-4 same as 8-30

9-5 NAM cercariae of both white and pigmented-tailed individuals are shed; many cercariae shed

9-6 same as 9-5

9-7 same as 9-6

9-8 same; but rusty-tailed cercariae seem to be decreasing in number

9-9 NAM cercariae of both kinds; rusty-tailed cercariae are few in number, and pigments are lighter

9-10 NAM of both kinds; pigments become greenish-brown; pigmented-tailed cercariae very few

9-11 same; but only 10-15 pigmented-tailed cercariae shed; pigments very light

9-12 same

9-13 same; only 4-5 pigmented-tailed cercariae; pigments light greenish-yellow

9-15 pigments light greenish-yellow, only a few; the rest are white-tailed

9-16 pigments more green than yellow

9-20 many cercariae shed; pigmented-tailed individuals still very light greenish-yellow but appear to be many more in number

9-22 pigments become light muddy-green; many more white-tailed cercariae

9-28 pigments very light, can hardly distinguish them from white-tailed individuals

10-4 14 out of 108 cercariae are pigmented; pigments very light green

10-17 still shedding NAM cercariae; pigments very light

10-22 pigments remain very light; number of cercariae greatly reduced

(Continued on next page)

Snail D
(NAM shedder)

9-3 many NAM cercariae shed; of both white and rusty-tailed individuals

9-4 same

9-5 same

9-6 many NAM cercariae shed; of both kinds

9-7 no cercaria shed; snail dies

Concluding Remarks:

1. For 5 consecutive days, snail D shed persistently NAM cercariae of both kinds.
2. Pigments did not become lighter but snail survived in laboratory for only 5 days.

Table 13. (Continued)

10-30 very few cercariae shed; snail still alive

11-15 no cercaria shed; snail still alive

11-30 no cercaria shed; snail still alive

12-10 same

1-13 no cercaria shed; snail finally dies

Concluding Remarks:

1. For two months and until shedding finally stopped, snail C persistently shed the same form of cercariae--NAM.
2. The pigmented-tailed cercariae gradually diminished in number; the pigments also became lighter with time.
3. Pigments were found on the distal portion of tail.
4. Shedding of cercariae ceased in late October.

Snail E
(AA shedder)

9-3 observe cercaria migrate to tip of ctenidial leaflet but do not observe emergence; many clusters of white-tailed cercariae shed

9-4 many clusters of AA cercariae shed

9-5 a few individual white-tailed cercariae are shed; many AA cercariae

9-6 5 clusters of AA cercariae shed

9-7 at least 7 clusters of same

9-8 at least 10 clusters; all white

9-9 18 or more clusters of same

9-10 39 clusters of same

9-11 over 35 clusters of same

9-15 more than 30 clusters of pure white cercariae plus 1 cluster with light brown tails; several other clusters also show shades of yellowish-brown tails

9-16 20 or more pure white clusters; pigments disappear

9-20 20 or more pure white clusters

9-28 13 clusters of pure white cercariae; 10 still alive at end of 24 hours

(Continued on next page)

Snail F
(AA shedder)

9-9 many AA clusters shed

9-11 4 AA clusters shed

9-12 many pure white clusters shed

9-13 8 or more pure white clusters shed

9-15 15 or more pure white clusters shed

9-16 all white clusters

9-17 snail dies suddenly

Concluding Remarks:

1. For 18 days since 9-9, snail F persistently shed AA cercariae until its death.
2. No pigments were found in tails.

Table 13. (Continued)

10-6 3 clusters of pure white cercariae

10-11 no cercaria shed; snail still alive

10-17 same

10-22 same; snail very active

10-30 no cercaria shed; snail still alive

11-15 same

12-6 no cercaria shed; snail finally dies

Concluding Remarks:

1. Since 9-3 and until shedding finally stopped, snail E shed persistently AA cercariae except on 9-15 when a few clusters containing some light pigments in the tails were found.
2. Shedding of cercariae ceased in the middle of October.

aspects:

1. Exposure of non-aggregating macrocercous cercaria from O. siliculus to various species of fresh water fishes to determine the potential secondary hosts for the parasite.
2. Exposure of uninfected small blackside dace to the same cercaria for the collection of infective cysts to be used in subsequent feeding experiments.
3. Time-sequence study of the growth and development of collar spines in the metacercariae.
4. Feeding of cysts of various ages to hamsters, ducks, chicks and rats to recover adults and to determine the length of time the cysts take to become infective in the fish.

For purposes of clarity, the results from these experiments will be reported below under separate categories.

Potential Fish Hosts for the Non-Aggregating Macrocercous Cercaria. From September 4 to 6, 1974, fishes of three different species were exposed to the non-aggregating macrocercous cercaria. In the gills of a C. rhotheus killed one hour post-exposure, many tailless cercariae identical to the non-aggregating macrocercous cercaria were found among the gill filaments of the fish. These tailless cercariae were very active, but no cysts were found. However, of the several speckled dace and eight blackside dace exposed similarly, many freshly encysted metacercariae were found among

gill filaments one to two hours after exposure (Figure 110). Two of these newly encysted metacercariae were mechanically excysted and their identity with the non-aggregating macrocercous cercariae was verified by microscopic examination. Description and measurements of these two young and collar spineless metacercariae are as follows: Body smooth, oval to elongate, 0.224 to 0.332 long by 0.164 to 0.176 wide; oral sucker subterminal, with lappets showing clearly on its dorsal lip, slightly wider than long, 0.059 to 0.061 wide by 0.052 to 0.054 long. Pre-pharynx short; pharynx pyriform, 0.026 wide by 0.030 long. Acetabulum in middle of posterior half of body, round or nearly round, 0.057 to 0.059 wide by 0.054 to 0.056 long. Obvious excretory bladder posterior to the acetabulum. Rod-like contents of cystogenous glands fill each side of body from level of pharynx to that of the posterior margin of the acetabulum. Collar and collar spines absent (Figure 111).

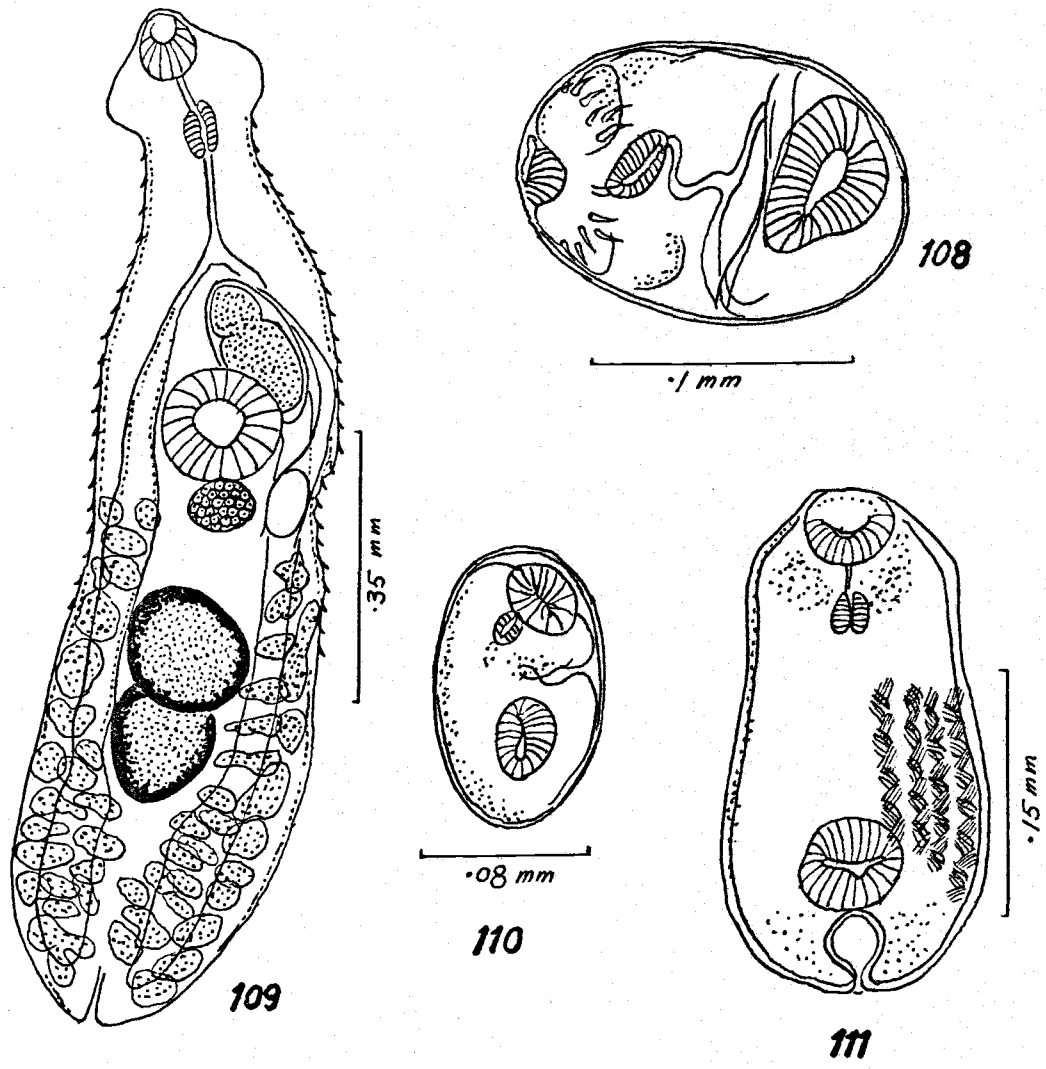
On September 25, 1974, a 5-cm C. perplexus was exposed to several hundred non-aggregating macrocercous cercariae. Twenty-four hours later, at least 20 new cysts were found in the gills. This observation suggests that a wide variety of fish could become potential fish hosts for the cercaria. This apparent lack of host specificity is not uncommon among echinostomes. Factors determining the infectibility of a fish appear to be more of an ecological than a genetic nature.

Figure 108. Cyst of E. milvi found loosely encysting in the gills of blackside dace and redside shiner.

Figure 109. Adult E. milvi Yamaguti, 1939.

Figure 110. Young cyst removed from the gills of blackside dace exposed to NAM cercariae two hours previously.

Figure 111. Excysted young metacercaria taken from the gills of a blackside dace exposed to NAM cercariae two hours previously.



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Growth and Spine Development in Metacercaria. Very small blackside dace below 2.5 cm long collected from a small spring feeding into Ritner Creek were found to be free of any echinostome cysts in the gills. At least 20 of these fish were exposed to the cercaria and kept separately at 15-17°C and 20-22°C for different periods of time. One infected dace, maintained at 20-22°C and killed on the third day post-infection, showed collar spines in 26% of the cysts recovered (Table 14). The cysts were 0.124 to 0.18 (0.152) long by 0.104 to 0.144 (0.124) wide. Another fish killed on the sixth day showed collar spines in 54% of the cysts. The percentage of collar-spined cysts increased rapidly through the eighth day, and at the 20th day, 100% of the cysts bore collar spines (Table 14). The growth, as measured in size, of the cysts, however, is difficult to interpret. It appears that as the cysts develop, there is a tendency for them to become less and less ovoid. One thing is certain, however, there is little or no increase in size of the cysts with time. Collar-spined cysts at 20th day compared favorably in both size and organ development with those of C. gorgonocephala obtained from natural infections.

One conclusion that can be drawn from the above observations is that, collar spines, which are absent in the cercariae, begin to develop shortly after encystation and that, in 20 days or earlier, most cysts develop the collar and collar spines which are most typical of echinostome cysts. This observation is of significance especially

Table 14. Growth and development of collar spines in the cysts of the NAM cercaria from O. silicula.

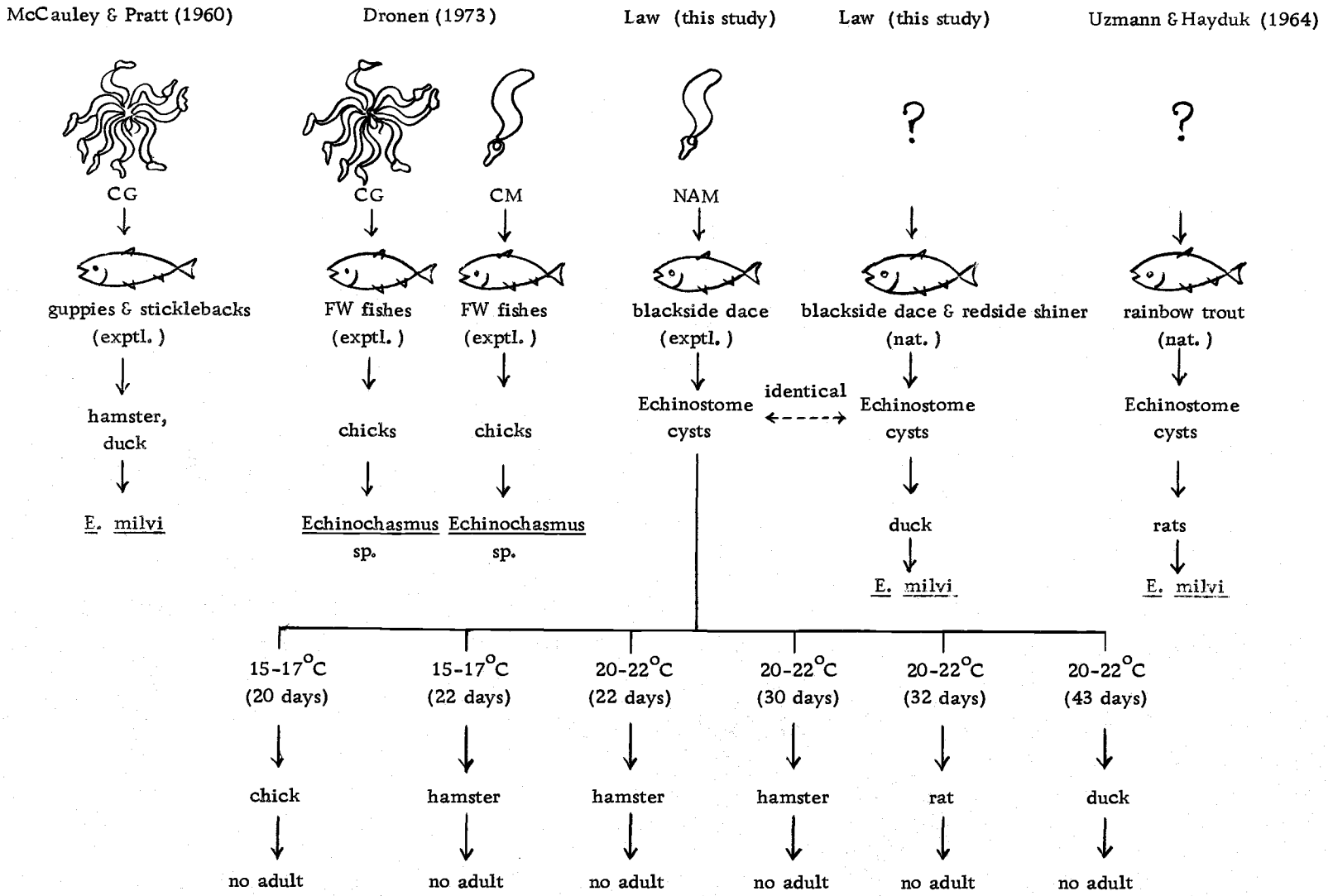
	3rd Day	6th Day	8th Day	20th Day
No. cysts recovered	15	11	8	28
% of spined cysts	26	54	75	100
Length	0.124 to 0.18 (0.152)	0.136 to 0.168 (0.15)	0.124 to 0.152 (0.144)	0.116 to 0.156 (0.137)
Width	0.104 to 0.144 (0.124)	0.08 to 0.124 (0.106)	0.10 to 0.12 (0.11)	0.096 to 0.128 (0.111)

when one recalls the characterization Lühe (1909) gives to the echinostome cercariae. Lühe subdivided leptocercous cercariae into three groups: echinostome cercariae, with a collar and collar spines; xiphidiocercariae, bearing a stylet; and gymnocephalous cercariae, which are unarmed. As noted by Nasir, Diaz and Hamana S. (1969), Lühe's grouping is extremely unnatural. There are many cases of echinostomes in which the cercaria is without a head collar or collar spines. These structures, however, invariably appear in the metacercarial and adult stages. Echinochasmus donaldsoni Beaver, 1941 is a better known example. Other recent examples include: E. zubedakhaname (Nasir and Diaz, 1968), Cercaria udoi and C. paraudoi (Nasir, Diaz and Hamana S., 1969). To quote the latter authors (1969), "had it not been for the knowledge of subsequent cyclic forms, these cercariae, which are true echinostomes, could have been erroneously considered as gymnocephalids."

Feeding Experiments with Various Laboratory Hosts. Echinostome cysts from the non-aggregating macrocercous cercaria in blackside dace maintained at 15-17°C or 20-22°C for various periods of time were fed to different laboratory animals. The results are represented in Table 15.

On September 28, 1974, 150 20-day-old cysts at 15-17°C were fed to a chick. When killed ten days later, no flukes were found in the intestine. Two days later, 45 22-day-old cysts at 15-17°C were

Table 15. Summary of feeding experiments involving the cysts of Cercaria gorgonocephala and the non-aggregating macrocercous cercaria. Other previous related experiments are also included. CG = C. gorgonocephala; CM = C. macrocauda; NAM = non-aggregating macrocercous cercaria; ? = Identity of cercaria unknown.


McCauley & Pratt (1960)
Dronen (1973)
Law (this study)
Law (this study)
Uzmann & Hayduk (1964)
CG
CG
CM
NAM
?
?
guppies & sticklebacks
(exptl.)
FW fishes
(exptl.)
FW fishes
(exptl.)
blackside dace
(exptl.)
blackside dace & redside shiner
(nat.)
rainbow trout
(nat.)
hamster,
duck
chicks
chicks
Echinostome
cysts
identical
Echinostome
cysts
Echinostome
cysts
E. milvi
Echinochasmus
sp.
Echinochasmus
sp.
duck
rats
E. milvi
E. milvi
15-17°C
(20 days)
15-17°C
(22 days)
20-22°C
(22 days)
20-22°C
(30 days)
20-22°C
(32 days)
20-22°C
(43 days)
chick
hamster
hamster
hamster
rat
duck
no adult
no adult
no adult
no adult
no adult
no adult

fed to a hamster, but no flukes were recovered seven days later. Over 90% of these cysts in fish maintained at 15-17°C for 22 days had collar spines when fed to the animals. Apparently they were yet uninfective.

From there on, cysts from fish maintained at 20-22°C for various periods of time were used in the infection experiments. However, the results of these experiments were all negative (Table 15).

The following conclusions are drawn from the above observations:

1. Cysts from non-aggregating macrocercous cercaria in fish maintained at 15-17°C are not infective for the chick and the hamster even after 22 days of development in the fish.
2. Cysts in fish maintained at a higher temperature (20-22°C) are still uninfective to the hamster, the duck and the rat after as long as 43 days in the fish.

Discussion and Conclusions

In analyzing the observations and results presented previously the following conclusions may seem appropriate:

1. The existence of E. milvi in Oregon was confirmed by feeding cysts in blackside dace and redside shiner to a duck. In addition to the rainbow trout (Uzmann and Hayduk, 1964), the last two fishes were also found to be natural hosts of the parasite in Oregon (see also Table 15).

2. Although the non-aggregating macrocercous (NAM) cercaria from O. silicula successfully encysted in the gills of blackside dace and developed collar spines in the metacercarial stage, to date feeding experiments with ducks, hamsters, the chick and the rat failed to recover the adult trematode.

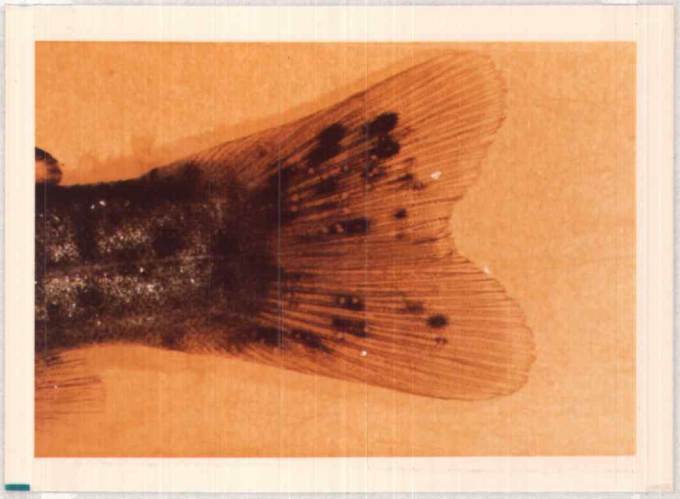
3. Isolated observations of the shedding of the three cercariae--CG, NAM and AA--from O. silicula revealed that the snails persistently shed the same form of cercariae, even for as long as 4-1/2 months. It thus appears that the three forms cannot be the different stages in the development of the same cercaria. A more logical explanation is that the three probably represent polymorphic conditions within the same species of trematode. Since pigments appear in the tails of individuals of both CG and NAM forms, and both tend to become light in prolonged captivity (Plate 12), one may speculate that the pigmentation of the tail is probably connected with the diet of the snails in nature. In captivity, the snails may be deprived of a certain food item which is responsible for the accumulation of excretory pigments in the tail.

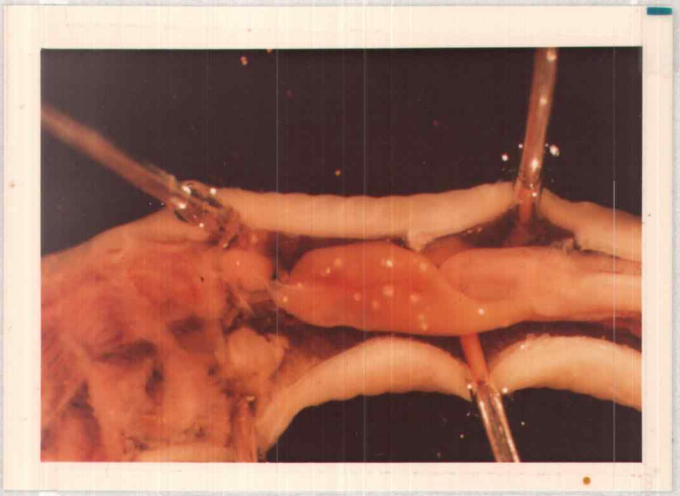
The AA cerceria probably represents a mutant form which has lost the capacity to assimilate the particular diet in question. Consequently, the tail is white instead of pigmented. While I am convinced that the three cercariae are of the same species, i.e. E. milvi,

Plate 10. Blackspot disease of rainbow trout. The fish was exposed to cercariae of Apophallus donicus. Note cysts on tail and fins of the fish.

Plate 11. Cysts of the new monorchiid in the liver of brook lamprey, Lampetra richardsoni.

Plate 12. Cracked O. silicula showing rediae and cercariae of Cercaria gorgonocephala in the gonad, digestive and reproductive glands of the snail. Note the reduced intensity of tail pigments in the cercariae. The snail had been in captivity for 27 days. Compare with the intensity of pigments in Plate 5.





the failure to complete the life cycle with the NAM form--which would provide the much needed evidence--still remains a puzzle. Nasir, Diaz and Hamana S. (1969), likewise, failed to recover the adults of Cercaria udoi and C. paraudoi, two obvious echinostomes which developed, much like mine, collar-spined metacercariae in fresh water fishes. It might be that the cercariae that I used in the experiments were uninfective to begin with, since they were taken from late fall or the later part of the shedding season.

Dronen's (1973) experiments with C. gorgonocephala and C. macrocauda appear to lend support to my theory of polymorphism in C. gorgonocephala. As mentioned in Part II of this study, Dronen described a non-aggregating macrocercous cercaria (which he assigned the name C. macrocauda) from the same snail which harbored C. gorgonocephala. This cercaria was identical to the latter but was solitary. Preliminary experiments with both resulted in the recovery of echinostome adults of the genus Echinochasmus. Dronen's C. macrocauda is apparently identical to the NAM cercaria described in this study.

Polymorphism is, of course, not uncommon among invertebrates. Representatives of the ectoprocts, cnidarians and insects are well-known for their polymorphic individuals (Hyman, 1940, 1959; Gardiner, 1972). Even in parasitic protozoans, the trypanosomes are notoriously polymorphic (Hyman, 1940). Yet, polymorphism in

trematodes has not been reported. While I am cautious in making any unwarranted assertions, I am excited about the prospects as well as problems raised in this study. Evidently, more work is needed in order to solve the enigma of the life cycle of E. milvi and the identity of the three cercarial "forms."

Partial Life Cycle of Echinochasmus sp. n. sp.

In the course of my survey for fresh water fish trematodes from Ritner Creek, small blackside dace (R. osculus nubilus) about 4 cm long were collected and used in the exposure studies with the non-aggregating macrocercous cercaria (see previous life cycle report). While examining the gills of these fish for freshly encysted metacercariae, I noticed some small ovoid and glassy cysts in the gills of the fish. These cysts resembled those of gymnocephalids superficially, but on closer observation, were found to be echinostome cysts. Feeding experiments with these cysts resulted in the recovery of a new species of Echinochasmus in the intestine of muscovy ducklings. The first intermediate host and the other larval stages of the trematode remain unknown. The following consists of the description of this new Echinochasmus and some observations on its partially completed life cycle.

Materials and Methods

Small blackside dace were collected from Ritner Creek, Polk County, Oregon, under the bridge at Highway 223. Since these fish were found to contain, besides cysts of the new Echinochasmus, also those of Echinochasmus milvi in their gills, special precaution had to be taken to remove unwanted cysts before the gills could be used in feeding experiments. The gills were put together with about 150 ml of de-chlorinated water in a Waring blender and blended at low speed for about one minute. This method, however, resulted in the isolation of only very few cysts from the gills. It was then decided that whole gills would be used in the feeding experiments. Gills were dissected from the fish, and cysts of E. milvi, which are of considerable larger size, were removed painstakingly one at a time from the gills by dissecting needles. The whole gills were then force-fed to ducklings with a regular medicine dropper. The two muscovy ducklings used in the experiments were purchased locally and were uninfected.

Results

Metacercaria and Secondary Intermediate Host

Metacercarial cysts of the new Echinochasmus were found naturally infecting gills of blackside dace (R. osculus nubilus). Ten

of these small, ovoid and glassy cysts measured 0.077 to 0.84 (0.08) long by 0.049 to 0.07 (0.057) wide. The cyst wall is thin, about 0.001. Two almost parallel masses of refractile excretory granules fill the excretory tubules. The metacercaria typically lies folded on its ventral surface with its head at one end of the cyst. The collar spines are small but nonetheless visible with high magnification. The oral sucker, pharynx, and acetabulum can often be seen at higher magnification (Figure 112). Most cysts are found tightly encysting along gill filaments and are surrounded by fibrous connective tissues which are clearly of host origin (Figure 113).

Only blackside dace were found to be infected by these metacercariae, although no systematic survey for the metacercariae was made of other fish in the area.

Because of the smallness of the cysts, no attempt was made to excyst the metacercaria either mechanically or by pepsin digestion. Consequently, the excysted metacercaria was not studied.

Infection of Definitive Host

On September 9, 1974, about 40 cysts pooled from several infected blackside dace were force-fed to each of two muscovy ducklings. The infected gills were taken up with a little water into a medicine dropper and then pipetted into the esophagus of the birds. On September 16, 1974, seven days after feeding, one of the two ducklings

was killed and 11 mature Echinochasmus sp. were recovered from the small intestine. These worms contained few but large, broadly ovoid eggs. The number of eggs varied from one to four. The second duckling was also killed the next day, and six flukes were found in its small intestine. Four of these, however, had no eggs in the uterus; one had two and the remaining one had four.

Thus, a total of 17 adults or sub-adults was recovered from 80 cysts administered to the ducklings.

Adult (Figures 114, 115)

Family: Echinostomatidae
Subfamily: Echinochasminae

Measurements from four mature, stained and mounted specimens. Mature worms small, elongate, 0.77 to 1.05 (0.78) long by 0.16 to 0.21 (0.193) wide, tapering both at neck and posterior end. Body widest posterior to the testes or anterior to acetabulum. Oral sucker small, almost round, 0.044 to 0.06 (0.05) wide by 0.044 to 0.068 (0.058) long, terminal with mouth opening ventral-anteriorly. Pre-pharynx very short or almost non-existent; pharynx ovoid, relatively large, 0.032 to 0.056 (0.041) wide by 0.056 to 0.076 (0.66) long. Collar armed with a crown of 20 spines interrupted dorsally. Esophagus long, 0.136 to 0.196 (0.171) in length. Intestinal ceca extending to posterior border of body. Acetabulum almost round, 0.076 to 0.088 (0.081) wide by 0.08 to 0.096 (0.085) long, in middle

of body or nearly so. In four of five specimens it is slightly submedian to left. Cirrus pouch ovoid, dorsal to acetabulum, submedian to right; large, plump, extending beyond mid-level of acetabulum, 0.052 to 0.06 (0.06) wide by 0.1 to 0.14 (0.123) long. Seminal vesicle bipartite, voluminous. Ovary entire, ovoid, pretesticular, submedian to right, broader than long. Testes large, tandem, transversely elongate; anterior testis, 0.124 to 0.144 (0.134) wide by 0.064 to 0.092 (0.077) long; posterior testis contiguous with anterior testis, 0.104 to 0.124 (0.116) wide by 0.076 to 0.088 (0.081) long. Vitelline follicles extend from level just behind acetabulum to posterior end; posterior to the testes, vitellaria form rather solid fields, almost meeting at midline. Eggs (Figure 116) few, large, 0.074 to 0.077 (0.075) by 0.05 to 0.057 (0.056) occupying area between acetabulum and posterior testis. Cuticular spines cover anterior part of body to level of posterior testis.

Host: Muscovy ducklings (experimental definitive); Rhinichthys osculus nubilus, blackside dace (natural secondary intermediate).

Habitats: Adult in small intestine of duckling; metacercaria in gills of blackside dace.

Location: Secondary host in Ritner Creek, Polk County, Oregon.

Discussion

This adult clearly belongs to genus Echinochasmus in the

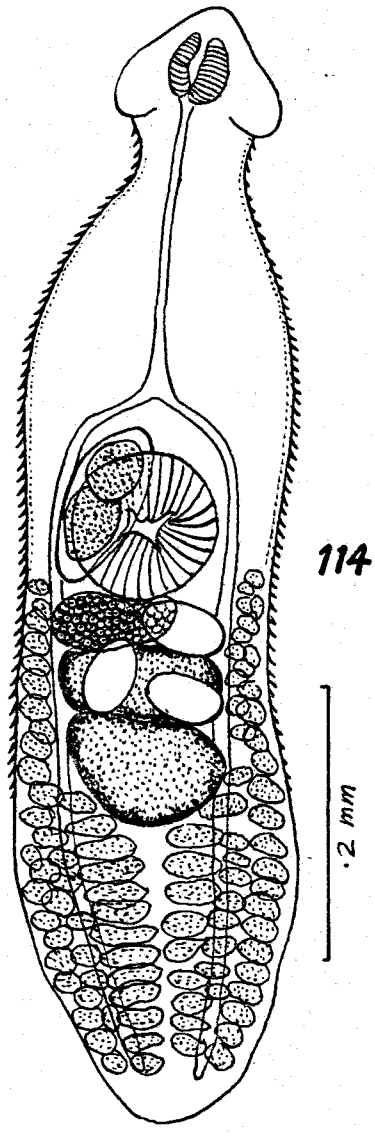
Figure 112. Cyst of Echinochasmus sp. n. sp. naturally infecting gills of blackside dace.

Figure 113. Cysts of Echinochasmus sp. encysting among gill filaments of blackside dace.

Figure 114. Adult Echinochasmus sp. recovered from ducklings fed small echinostome cysts from the gills of blackside dace.

Figure 115. Collar and spine pattern of adult Echinochasmus sp.

Figure 116. The egg of Echinochasmus sp.



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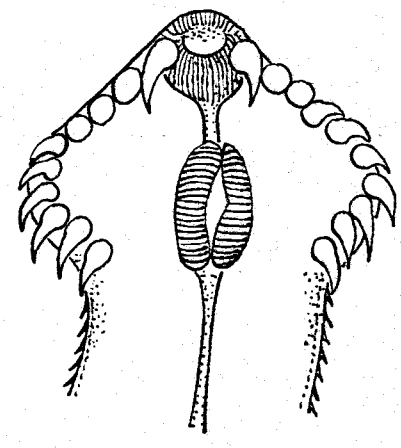

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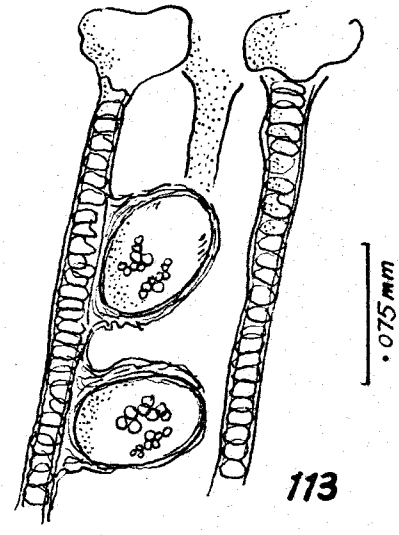

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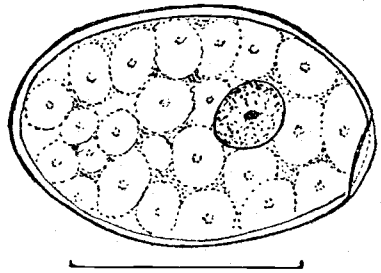

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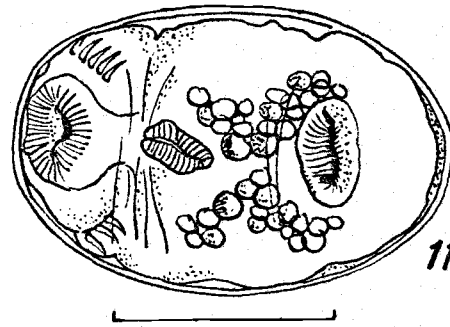


112

subfamily Echinochasminae. In 1909, Dietz created the genus Echinochasmus, and in 1931, Price reviewed the genus and listed the known species (Price, 1931b). Since then, many more new species have been reported. Of the approximately 40 known species in genus Echinochasmus Dietz, 1909, sub-groups may be recognized based on the number of collar spines (Beaver, 1941). Until the time of Nasir and Diaz (1968), four known Echinochasmus species with 20 collar spines were recorded. These species, namely: E. dietzevi Issaitchikoff, 1927; E. magrovatum (Stunkard and Haviland, 1924) Price, 1931; E. donaldsoni Beaver, 1941 and E. zubedakhaname Nasir and Diaz, 1968, however, may be further separated according to the pattern of angle spines, relative size of various organs, size and location of the cirrus pouch, and the extent of vitellaria both anteriorly and in the post-testicular area (Figures 117-121).

The new species is similar to all of the above in possessing a single row of 20 definite collar spines interrupted dorsally. It also resembles E. dietzevi and E. magrovatum but differs from E. donaldsoni in the extent of vitellaria in the post-testicular region. In E. donaldsoni, the vitellaria of the two sides are joined by isthmuses along both of the dorsal and ventral sides, while those of the former three form solid fields that almost meet at the midline. Although E. zubedakhaname also has solid fields of vitelline follicles in the post-testicular area, it differs from the other four by the anterior

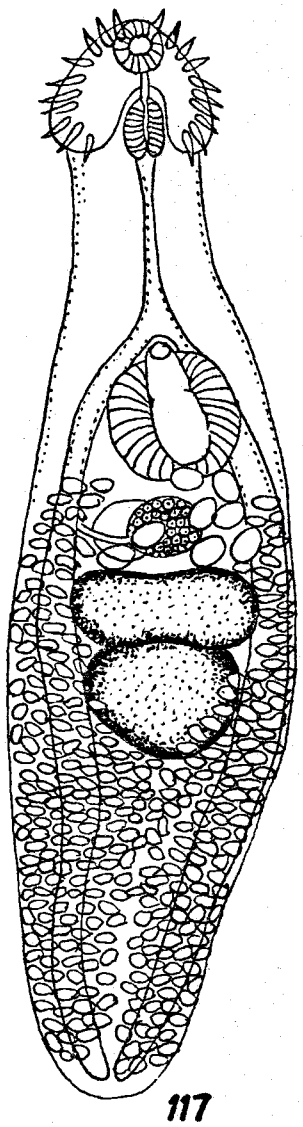
Figure 117. Adult E. dietzevi Issaitschikoff, 1927. Redrawn from Skrjabin (1927).

Figure 118. Adult E. zubedakhaname Nasir and Diaz, 1968. Redrawn from Nasir and Diaz (1968).

Figure 119. Adult E. donaldsoni Beaver, 1941. Redrawn from Beaver (1941).

Figure 120. Adult E. magrovatum Stunkard and Haviland, 1924. Redrawn from Stunkard and Haviland (1924).

Figure 121. Head crown of adult E. magrovatum. Redrawn from Stunkard and Haviland (1924).



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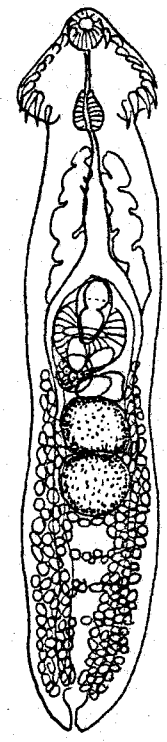

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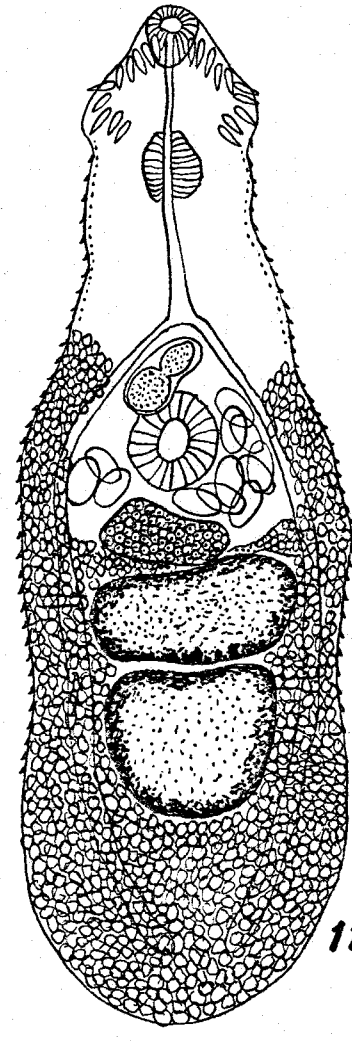

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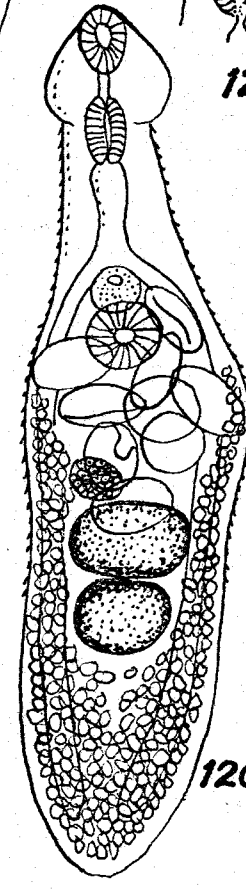

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extent of its vitellaria which reach anterior to the acetabulum. The new species can be differentiated from E. dietzevi by the much smaller body size in proportion to egg size, the position and size of cirrus pouch, and can further be differentiated from E. magrovatum by its larger testes and a well-formed cirrus pouch which extends to the posterior margin of the acetabulum. Nasir and Diaz (1968) stressed the "angle spines" pattern in these closely related forms and suggested that they might be thus separated. I disagree with them regarding the validity of the angle spines pattern as a useful diagnostic character. The patterns do not appear to be stable enough, and the problem of deciding which and how many of these spines may be legitimately considered as angle spines is no simple matter at all. The eggs of all five species are very similar in shape and size, although the new species and E. magrovatum are probably the smallest. Consequently, the eggs appear to be unproportionally large in these two species. Mature worms of all five contain few eggs in the uterus, although E. dietzevi and E. zubedakhaname may contain as many as eight to 12. For a comparison of the major diagnostic characteristics of the five species, see Table 16 and Figures 117-121.

Of the life cycles of all five, only those of E. donaldsoni and E. zubedakhaname are known in their entirety, although that of the new species, reported here, has been partially completed. The first intermediate hosts of the first two species are fresh water molluscs,

Table 16. Comparison of the major diagnostic characteristics of the five closely related species in the genus Echinochasmus. V. S. = ventral sucker.

Diagnostic Characteristic	Echinochasmus sp. n. sp.	E. donaldsoni	E. dietzevi	E. magrovatum	E. zubedakhaname
Length	0.77 to 1.05	0.88 to 1.84	1.13 to 1.47	0.8 to 1.0	0.74 to 1.25
Width	0.16 to 0.21	0.256 to 0.32	0.396 to 0.55	0.13 to 0.23	0.22 to 0.385
Anterior testis	0.064 to 0.144 by 0.064 to 0.092	0.12 by 0.154		0.05 to 0.09 in diameter	0.11 to 0.168 by 0.18 to 0.279
Posterior testis	0.104 to 0.124 by 0.076 to 0.088	0.118 by 0.146		0.05 to 0.09 in diameter	0.153 to 0.189 by 0.105 to 0.231
Cirrus sac	well-formed, projecting almost to posterior of V. S.	like Echinochasmus sp.	like Echinochasmus sp.	rudimentary, not behind center of V. S.	not beyond mid V. S.
Vitellaria in post testicular area	solid fields	isthmuses	solid fields	solid fields	solid fields
Vitellaria anterior extent	posterior to V. S.	like Echinochasmus sp.	like Echonochasmus sp.	like Echinochasmus sp.	anterior to V. S.
Egg size	0.074 to 0.077 by 0.055 to 0.057	0.072 to 0.076 by 0.05 to 0.054	0.67 to 0.079 by 0.046 to 0.058	0.07 to 0.08 by 0.06 to 0.065	0.042 to 0.067 by 0.032 to 0.04
life cycle	1st int. host unknown; 2nd host: in gills of R. osculus; adults in ducklings (exptl.)	1st int. host: Amnicola limosa & A. lustrica; 2nd host: gills of FW fish; adults in pied-billed grebe (nat.) & pigeons (exptl.)	1st int. host: unknown; 2nd host: unknown; adults in Podiceps sp. (nat.)	1st int. host: unknown; 2nd host: unknown; adults in rats, Rattus norvegicus	1st int. host: snail Pomacea glauca; 2nd host: Lebistes sp.; adults in F. pica (nat.) & chickens & pigeons (exptl.)

and the secondary hosts are typically fresh water fishes. The cercaria of E. donaldsoni encysts on gills of various fishes including Lebistes reticulatus in contrast with the cercaria of E. zubedakhaname which, in both the natural and experimental fish hosts, encysts in the intestinal mesentries of L. reticulatus. The new species is similar to E. donaldsoni in that its metacercariae are found also encysting on the gills of fresh water fish.

In four of the five species mentioned, birds are found to be definitive hosts. Adults of E. donaldsoni are found in intestine of the pied-billed grebe (Podilymbus podiceps), while those of E. zubedakhaname are found in the small intestine of Fluvicola pica (natural infection) and chickens and pigeons (experimental infection).

The adults of the new species were obtained from the small intestine of ducklings (experimental infection). The natural definitive host for the fluke is still undetermined.

However, the adults of E. magrovatum were not found in birds, but instead, in the intestine of rats, Rattus norvegicus, frequenting the water fronts of New York City.

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