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STUDIES ON SOME TREMATODE PARASITES ASSOCIATED WITH SALT MARSHES OF SOUTHEASTERN NEW HAMPSHIRE

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38161	Ascocotyle leighi Burton, 1956
38169	Phagicola longicollis Kuntz and Chandler, 1956
36790	Phagicola lageniformis Chandler, 1941
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SECTION I.

INTRODUCTION

Numerous references dealing with the life histories of trematodes involving animal hosts from marine and fresh-water habitats are found in American literature. Among these references, there are reports of trematodes from intermediate and definitive hosts found in brackish waters. However, only three reports of complete trematode life histories from the salt marsh habitats of this country were found. These life cycles were all reported by Stunkard (1958-60).

Chandler (1941) reported the occurrence of Echinochasmus schwartzi Price, 1931 and Phagicola lageniformis Chandler from muskrats trapped in slightly brackish to brackish meadow-like marshes of southeastern Texas. Hutton and Sogandares-Bernal (1959) studied trematode parasites encysted in Florida mullets from various bays, bayous, rivers, and creeks of the coastal areas of Florida. Sogandares-Bernal and Bridgman (1960) reported on heterophyid trematodes encysted in littoral poeciliid and cyprinodont fishes from brackish Lake Pontchartrain, Louisiana. Stunkard and Uzmann (1955) found that Fundulus heteroclitus, a species which commonly comes up into brackish water, served as the second intermediate host of Ascocotyle (Phagicola) diminuta. They were able to obtain the sexual stages (adults) of this trematode in laboratory-reared rats, mice, hamsters, sea

gulls, and a night heron.

In Europe, Rothschild (1941c) studied growth and trematode infections of Peringia ulvae in pools of the Tamar Saltings, Plymouth, England. Rothschild thought Peringia ulvae was unique among marine and brackish water species since larval forms were found which belonged to such well known trematode families as Heterophyidae, Echinostomatidae, Microphallidae and Notocotylidae. In this situation trematode life history studies involved all the main groups of animals found in the vicinity and demonstrated the importance of larval trematodes in the local ecology.

Rothschild's work was from an estuarian situation. However, her finding with respect to larval forms in Peringia ulvae and the assignment of these larval forms to the above mentioned families parallels my findings in various species of the Hydrobiidae collected from depressions in the salt marshes of southeastern New Hampshire.

Rothschild (1941c) reported that the heterophyid cercariae from Peringia ulvae were of the pleurolophocerca type and was able to experimentally infect gobies (a species of fish commonly found in pools with the snails). The cercariae encysted as metacercariae in the skin of the fish (including the surface of the eye and fins). The infected gobies were fed to laboratory-reared chickens, ducks, Herring Gulls, Black-headed Gulls and one Redshank after exposure to the cercariae, ranging from a few hours up to

3 months after infection, but she was not able to recover the adults of this trematode.

From studies on dissected metacercariae, Rothschild stated that the testes had not sufficiently developed to make an assignment with confidence to a special group. However, she found that the intestine of the metacercariae was of the Haplorchis type and the position of the reproductive organs was similar to that of the Haplorchiinae.

Rothschild (1941c) reported on the life history of the notocotylid cercariae parasitizing Peringia ulvae. She found that six species of these larvae were represented and that three belonged to the Monostomi sub-group and the other three to the Yenchingensis sub-group. The Monostomi sub-group was more plentiful, but all of her efforts at rearing the adult worms failed. However, she found that two species of the Yenchingensis sub-group developed in the intestinal caeca of ducks, into flukes of the genus Paramonostomum.

I studied the larval trematodes of Hydrobia salsa collected from depressions near Johnson Creek in Durham, New Hampshire; Hydrobia minuta collected from an isolated depression near Great Bay in South Newington, New Hampshire; and Amnicola sp. collected from depressions, near Hampton, New Hampshire.

The family Hydrobiidae is medically important since some of its members serve as the first intermediate host of Schistosoma japonica. No record on the study of the

larval trematode fauna of the genus Hydrobia was available from this country until 1956. In 1956 Dr. Bullock of the department of Zoology at the University of New Hampshire sent several hundred Hydrobia minuta, collected from the isolated depression near Great Bay in South Newington, New Hampshire to the American Museum of Natural History for study and identification of the two types of cercariae that were emerging from them. Dr. Stunkard identified one of these cercariae as a microphallid and the other as a heterophyid. Dissection of the snails yielded a third trematode species, an encysted microphallid. Working with the encysted microphallid, Stunkard (1958) worked out the morphology and life history of Levinseniella minuta, a trematode reaching maturity in scaups and other diving ducks.

Stunkard (1960) described Notocotylus minutus, a species which encysted as metacercariae on the shell and opercula of Hydrobia minuta as well as the empty shells of Gemma gemma. Metacercariae were fed to laboratory-reared gulls (Larus argentatus) and to eider ducks (Somateria mollissima) and the adults were recovered from the eider duck. The Hydrobia were collected near Boothbay Harbor, Maine.

Stunkard (1960) described Himasthla compacta obtained from laboratory-reared gulls (L. argentatus) that were fed Mya arenaria which had been experimentally infected with cercariae from Hydrobia minuta collected in Sagadahoc Bay, near, Boothbay Harbor, Maine. Stunkard

stated that the incidence of infection in H. minuta was low since only one snail in 500 was infected. He also stated that cercariae emerged from only one-half of the infected snails. Stunkard maintained that Hydrobia minuta harbored at least 6 species of cercariae, but did not state to what special groups they belonged.

On July 6, 1959, I started to study the growth, morphology and development of the heterophyid cercariae that were being passed by Hydrobia minuta, a common snail found in the Great Bay area. At the time this problem was started, only the life cycle of Levinseniella minuta had been reported by Stunkard (1958) where these snails were involved as first or second intermediate hosts for trematodes. It was decided that I should work with Hydrobia salsa from the depression near Johnson Creek in Durham, New Hampshire. My work was to include descriptions of the cercariae, seasonal variations, asexual development in the snail, and the complete life histories of two of the cercariae (heterophyid and allocreadiid).

During the summer of 1960, I started to work on the larval trematode fauna of Hydrobia minuta. The success that I had with the life cycles of the heterophyid the allocreadiid and the occurrence of an echinostome trematode which encysted as metacercariae in the gills of Fundulus heteroclitus motivated my interest in Hydrobia minuta in South Newington. My objective was to determine if the families Heterophyidae, Microphallidae,

Allocreadiidae, and Notocotylidae, found in Hydrobia salsa were also present in Hydrobia minuta. Since H. minuta was found in an ecologically different habitat and was a different hydrobiid species, I was interested in determining whether or not the families (named above) included the same trematode species. I was interested also in finding out if the echinostome cercariae were emerging from H. minuta though they had not been seen in H. salsa. It was suspected that these snails might be passing echinostome cercariae, but perhaps there was a very low incidence of infection. The reasoning behind such thinking was that only a few adult echinostomes were obtained in feeding experiments where the gills of Fundulus were collected from the habitats of these snails and fed to laboratory-reared animals. It was believed that if the echinostome cercariae were found in hydrobiid snails, infections would be greater in Hydrobia minuta. This idea was formulated for two reasons. (1) Hydrobia minuta was found in an isolated depression which had lost all previous outlets with water that it might have had and (2) the isolated ditch was only affected by spring tides.

Two obvious differences in the larval trematode fauna of the two snails were the complete absence of the allocreadiid cercariae and the abundance of the notocotylid cercariae in Hydrobia minuta. The notocotylid cercariae from the H. minuta encysted on the shell and opercula of the snail and were quite prevalent from the middle of July to November. The notocotylid cercaria from Hydrobia salsa

was being passed spontaneously and was quite abundant in snails that had been isolated in vials from August to December. However, notocotylid cercariae have been observed on the opercula and shells of Hydrobia salsa not isolated in vials and crushed in the laboratory (October and November, 1960).

Several attempts were made to infect white mice with the notocotylid metacercariae from Hydrobia minuta during the summer of 1960 without success. Six months later Stunkard (1960) published the life cycle of Notocotylus minutus, a species which encysted as metacercariae on the opercula and shells of Hydrobia minuta. Stunkard (1960) also published the life cycle of an echinostome (see the above) for which Hydrobia minuta served as the first intermediate host.

Life cycle work on the allocreadiid cercariae from Hydrobia salsa and the conspicuous absence of this form from Hydrobia minuta suggested that this might be due to differences in salinity and ecology rather than snail species. This led me to marshes near the Hampton River in Hampton, New Hampshire which is more of an estuarian situation with lower salinity than what was encountered at habitats in Durham and South Newington, New Hampshire. The allocreadiid cercariae were found quite abundantly in Amnicola sp., a hydrobiid species that is largely restricted to fresh water. Fundulus were also collected from this area and were found to be highly infected with Echinochasmus magnovatum (an echinostome). This trematode had often

occurred in small numbers as a result of feeding the gills of Fundulus to laboratory-reared animals for the recovery of the adults of Ascocotyle (Phagicola) diminuta. However, in Fundulus collected from Hampton and fed to laboratory-reared animals more specimens of Echinochasmus and few specimens of Ascocotyle were recovered.

This paper reports the morphology and life history of Ascocotyle (Phagicola) diminuta, a trematode belonging to the "so called" Ascocotyle-Phagicola-Parascocotyle complex. Not one complete life cycle of an Ascocotyle complex trematode has ever been worked out. Only as recently as 1955, Stunkard and Uzmann became the first to demonstrate the second intermediate host of Ascocotyle (Phagicola) diminuta, but were not able to find the first intermediate host.

The first intermediate host has been found to be Hydrobia salsa, a brackish water snail collected from depressions in the salt marshes near Johnson Creek in Durham, New Hampshire. The gills of various species and varieties of poeciliids have been experimentally infected with the heterophyid cercariae from Hydrobia salsa and have been fed to laboratory-reared day old chicks, a white rat, and mice after the metacercariae had been allowed to mature for three weeks. Worms have been recovered from the various experimental hosts after 2 to 10 days of development.

Studies have also been made on the life cycle of Ascocotyle tenuicollis Price, 1935, a species restricted to the conus arteriosus (metacercariae) of Fundulus heteroclitus

collected from depressions near Johnson Creek in Durham, New Hampshire. This species has been found in the conus arteriosus of Fundulus heteroclitus from all of the study areas. However, only metacercariae from Fundulus collected in Durham were fed to experimental hosts. Morphological studies have been made on worms recovered from 5 day-old chicks after 1 to 4 days of development. This is the first report of Fundulus heteroclitus (Poeciliidae) serving as the second intermediate host of Ascocotyle tenuicollis. Therefore, this is a new host record and a new locality record.

Previous reports of other species of Ascocotyle with metacercariae restricted to the conus arteriosus of fish have been made by other authors. Burton (1956) described Ascocotyle leighi from Mollienisia latipinna (sailfin molly: Poeciliidae) in Florida. Sogandares-Bernal and Bridgman (1960) found Ascocotyle leighi in sailfin mollies taken from Lake Pontchartrain, Louisiana.

This paper also reports the finding of the second intermediate host (Fundulus heteroclitus) of Echinochasmus magnovatum (Stunkard and Haviland, 1924) Price, 1931, one of the three species of this genus described from this country. Its morphology and development in the final host from 6 to 30 days was studied. The species is redescribed from biometric studies on 40 specimens. Other observations have also been made on uterine egg counts and the hatching of the miracidium in various solutions.

It is hoped that this dissertation will stimulate interest in ecological observation on the trematode fauna

found in animals that often visit salt marshes i. e. rails, black ducks, blue-winged teals, bitterns, sparrows, soras, sandpipers, plovers, wrens, herons and egrets, muskrats, meadow mice, otters, mink, raccoon, and opossums. Such wildlife frequent the salt marshes of the Atlantic coast (McAtee, 1941). A major study should be made of all the gastropods and invertebrates found in the salt marshes as well as critical feeding experiments involving the small fishes that often come up into marshes e.g. cyprinodonts and poeciliids.

Such a study might throw light on variations that occur in trematodes or problems of speciation in trematodes that utilize varieties of birds and mammals as final hosts.

SECTION II.

LITERATURE REVIEW

Family HETEROPHYIDAE Odhner, 1914

Syn. Coenogonimidae Nicoll, 1907; Cotylogonimidae Nicoll, 1907; Stictodoridae Poche, 1926

Looss (1899) erected the genera Coenogonimus, Tocotrema, Ascocotyle, and Centrocestus under the subfamily Coenogoniminae. He designated C. heterophyes as the type of the genus Coenogonimus. Earlier, Lühe (1899) had used the species heterophyes as type of his new genus Cotylogonimus. Luhe also placed in a new genus (Cryptocotyle) the species which Looss had placed in the genus Tocotrema. Pratt (1902) placed Cotylogonimus and Cryptocotyle in the subfamily Cotylogoniminae. Looss (1902) reported that Cotylogonimus was a synonym of Heterophyes Cobbold (1866). Since both Coenogonimus and Cotylogonimus were synonyms of Heterophyes, the subfamilies Coenogoniminae and Cotylogoniminae were invalidated.

Odhner (1914) proposed the name Heterophyidae to replace the incorrect names Cotylogonimidae and Coenogonimidae. Odhner included the following genera in the family: Heterophyes, Tocotrema (synonym of Cryptocotyle), Scaphanocephalus, Centrocestus, Ascocotyle, Pygidiopsis, and Apophallus. Odhner made no attempt to split these groups into subfamilies. Ransom (1920) was the first to attempt to bring all the known species together. He gave a new and modified diagnosis of the

family and a key to the valid genera and species. Ransom thought that Scaphanocephalus should be excluded from the family and that Metagonimus Katsurada (1913), Paracoenogonimus Katsurada (1914) (possibly a synonym of Cryptocotyle), and the new genus Cotylophallus should be added.

Nicoll (1923) extended the family Heterophyidae Odhner, 1914, to contain the subfamilies Microphallinae Ward, 1901; and Gymnophallinae Odhner, 1905. He placed the following genera in the subfamily Cryptocotylinae Luhe, 1909: Cryptocotyle Luhe, 1899 (Tocotrema Looss, 1899), Scaphanocephalus Jagerskiold, 1903; Apophallus Luhe, 1909; Ascocotyle Looss, 1899; and Galactosomum Looss, 1899. Some of these genera were originally placed under the family Heterophyidae (see the above).

Ciurea (1924) was the first to divide the family into subfamilies. He erected the subfamilies Heterophyinae, Metagoniminae, Centrocestinae, Apophallinae, and Cryptocotylinae. These subfamilies were separated on the basis of the structure of the terminal portion of the genital ducts. Stunkard and Haviland (1924) suggested that Ciurea's paper of 1924 had gone to press before the appearance of Nicoll's revision since no comment was made in his paper conerning the inclusion of Microphallinae and Gymnophallinae in the family.

Stunkard and Haviland (1924) affirmed that the remaining genera, after the exclusion of Paracoenogonimus and the reinstatement of Scaphanocephalus should be

arranged in the five subfamilies: Heterophyinae, Metagoniminae, Centrocestinae, Apophallinae, and Cryptocotylinae. All of these subfamilies were based upon details of development, position of the ventral and genital suckers, and terminal portions of the genital organs. Stunkard and Haviland (1924) felt that Ciurea's arrangement might prove valid though further data were necessary before it could be unconditionally accepted. These authors did not think the differences between the subfamilies of Ciurea were great enough to separate groups of subfamily rank and did not correspond to the differences between the subfamilies of Nicoll.

Faust and Nishigori (1924) added Monorchitreminae as the sixth subfamily. According to Witenberg (1929) the work of Poche (1926) listed additional genera and families. Poche (1926) summarized all the existing knowledge on the Heterophyidae up to 1926. Witenberg's (1929) monographic study of the Heterophyidae contributed a great deal to our understanding of the taxonomy of this group. He redefined the family as the following:

> Small and very small forms. Pseudodermis covered with scale-like spines. The body is usually divided into two parts, one anterior flattened, free from genitalia and more motile than the posterior part which is oval or round in cross-section and contains the genital apparatus. The oral sucker may be provided with all or a part of the following structures: A contractile dorsal lip-like appendage, a posterior funnel-shaped appendage and rows of circumoral spines.
>
> Prepharynx and oesophagus vary in different genera and species. Pharynx always present. Intestinal caeca simple, of varying length. Ventral sucker, except in the genus Heterophyes, reduced and included in the modified genital sinus ('ventro-genital sac') or even

absent.

The reproductive organs, except the vitellaria in some genera, are grouped in the posterior part of the body behind the level of the genital aperture which is generally situated near the middle of the body. Testes, two or one, globular or lobed: their situation varies in different genera. The cirrus pouch is absent. The seminal receptacle is voluminous and may be divided into several parts by constrictions. The terminal portion of the seminal vesicle may form a separate vesicle-shaped organ which is usually provided with chitinised walls; the term 'expulsor' is proposed for this structure (in Heterophyes, Tocotrema, Diorchitrema, etc.). Ovary globular or slightly lobed and, except in Adleria, is situated in front of the testes. Mehlis' gland present. Seminal receptacle well developed. Laurer's canal usually reduced. The vitellaria are usually reduced. The vitellaria are usually situated near the lateral or dorsal surface of the body and the degree of their development varies in different species. The uterus in most cases does not proceed anteriorly to the genital aperture. The latter, except in Heterophyes, opens on the inner wall of the ventro-genital sac, which is situated on the middle line or moved towards the lateral border of the body. Near the genital aperture a more or less developed gonotyl is often present. Eggs usually numerous with thick shell 18 to 37u long. Excretory vesicle usually Y-shaped; the length of the stem varies in different genera and it is either straight, S-shaped or divided into branches which may re-unite (as in Scaphanocephalus); the branches may be long, short or entirely absent (as in Galactosomum).

Adults parasitise the intestines of mammals, birds, and rarely fish (Haplorchis). Metacercariae encysted in fish. Cercariae, as far as is known, develop in operculated molluscs.

Type genus:--Heterophyes Cobbold, 1866.

On the basis of his new family definition, Witenberg (1929) excluded the following subfamilies and genera which had been included in the family by Nicoll (1909) Poche (1926):

1. The genera united in the subfamily Microphallinae Ward (1901) were excluded because they lacked a seminal receptacle and possessed a cirrus pouch.

2. The species united in the subfamily Gymnophallinae Odhner (1905) were excluded from the family because they lacked a seminal receptacle.

3. The genus Sigmapera Nicoll, though it greatly resembled the Heterophyidae was rejected because of its well developed cirrus pouch.

4. The genus Nanophyetus Chapin (in Hall, 1927) was excluded because of the presence of a well developed cirrus pouch and the presence of a seminal receptacle.

5. Since the genera Euryhelmis Pouche, 1926, and Taphrogonymus Cohn (1904) were based on insufficient descriptions of their representatives there were insufficient reasons for including them in the Heterophyidae.

6. The genera Parabascus Looss (1907) and Cryptotrema Ozaki, 1926, were also excluded.

7. Paracoenogonimus Katsurada (1914) appeared to be a synonym of the genus Prohemistomum Odhner (1914) or Cyathocotyle Muhling (1896) which never belong to the Heterophyidae.

8. Opisthometra Poche (1926) was transferred to Acanthochasmidae.

9. Witenberg (1929) included Stictodora Looss (1899), as a member of the Heterophyidae. He stated that Poche had created an unnecessary family (Stictodoridae).

Witenberg (1929) believed many of the genera and subfamilies which he excluded from the family would be assigned to the superfamily Opisthorchoidea after further investigations. He pointed out that since certain genera

had been excluded, the remainder should be distributed in subfamilies according to the method defined by Ciurea, i.e., according to the details of the structure of the genital pore. However, he suggested that the extent of its use as a taxonomic character should only be valid for generic characters, since the Heterophyidae, in contrast to other trematode families, varied considerably in the structure and position of the genital pore.

Witenberg (1929) reported that the danger of Ciurea's method was that one would be able to create almost as many subfamilies as there were genera. He concluded that not the most changeable, but the most constant features should be taken as a basis for division into subfamilies. Witenberg set up the following complex of features found useful for characterizing a subfamily:

(1) The shape of the anterior part of the body (dilated or not)

(2) The presence or absence of conspicuous spines around the oral aperture.

(3) The number of testes and their position in relation to the ovary (in front or behind it).

The distribution of vitellaria was to be utilized to distinguish tribes.

Witenberg (1929) listed the following combinations of characters for distinguishing genera:

(1) The arrangement of the genital glands.

(2) The structure and position of the ventro-genital sac.

(3) The additional structures of the oral apparatus.

(4) The distribution of the vitellaria where division into tribes is not indicated.

On the basis of the above scheme, Witenberg distributed all of the genera of the Heterophyidae among the following five subfamilies: Heterophyinae Ciurea (1924), Centrocestinae Looss (1899), Cercarioidinae Witenberg (1929), Haplorchinae Pratt (1902), and Adleriinae Witenberg (1929). Witenberg utilized the following key for separating these subfamilies:

A. Testes two:

(1) the anterior part of the body very dilated .....Cercarioidinae

(2) the anterior part of the body not dilated
(a) circumoral spines present .....Centrocestinae
(b) circumoral spines absent .....Heterophyinae

B. One testis:

(1) ovary in front of the testis..Haplorchinae

(2) ovary behind the testis.....Asleriinae

Witenberg (1929) argued that every subfamily could be divided into two tribes, according to the distribution of the vitellaria as in some genera the vitellaria are confined to the region behind the level of the ovary, in others they are extended anteriorly beyond the genital aperture.

Earlier, Faust (1929) had erected the superfamily Opisthorchoidea for the family Opisthorchidae. He also erected the superfamily Heterophyoidea for the family Heterophyidae and asserted that further information would warrant the inclusion of Lecithodendriidae Odhner, 1910, Microphallinae Ward, 1907, and Gymnophallinae Odhner, 1905.

Faust separated these two superfamilies by the miracidium. He claimed that the miracidium was bilaterally symmetrical in the Heterophyoidea and asymmetrical in the Opisthorchoidea. The cercariae in both groups were similar, but those of the Opisthorchoidea lacked the spinose armature of the Heterophyoidea.

Witenberg (1929) pointed to the similar morphology of heterophyids and opisthorchid cercariae and adults. He also noted that these groups had similar life cycles. Using this as evidence of close relationship, Witenberg erected the superfamily Opisthorchoidea which included the Opisthorchidae and the Heterophyidae. Vaz (1932) agreed with Witenberg's superfamily.

The family Heterophyidae as constituted by Witenberg (1929) was to consist of trematodes which developed to maturity in fish-eating vertebrates. However, Mueller and Van Cleave expanded the family to include genera which were parasitic in fishes.

Witenberg (1929) was aware of the possibility of heterophyids occurring in fishes. With reference to the genus Haplorchis, Witenberg (1929) made the following statement.

> Two species of the Haplorchis are known. H. caharinus (Looss, 1896 and H. pumikio (Looss, 1896). It is noteworthy that the first is the only species of Heterophyidae found in the adult stage as a parasite of fish. This circumstance leads to the supposition that H. caharinus may belong to quite another family.

Mueller and Van Cleave (1932) stated that they had not studied specimens of Haplorchis and found the descriptions and drawings in the literature inadequate to serve as a

basis for reaching any conclusion concerning this species. They showed that Vietosoma, Acetodextra, Allacanthochasmus, Neochasmus, Cryptogonimus, Caecincola, and Centrovarium were seven trematode genera which regularly occur in fish. They thought that these genera showed characteristics which united them with the Heterophyidae. Mueller and Van Cleave (1932) stated that there was no possibility that these heterophyid genera were avian or mammalian parasites accidentally misplaced in fishes. Their extensive faunal surveys failed to yield any evidence that members of the genera ever occurred in either birds or mammals.

Mueller and Van Cleave (1932) indicated that they were aware of Witenberg's (1929) remark which drew attention to the fact that Heterophyidae were not the only trematodes with complicated genital sinus. But other trematode groups such as Microphallus, Hemiuridae, Azygiidae possessed a complicated genital sinus. However, Mueller and Van Cleave stated that they had made a thorough investigation of the genital apparatus of Microphallus and several species of Azygiidae. They found no difficulty in sharply differentiating between the copulatory modifications and genital sinus of these forms and the ventro-genital complex of heterophyids. Mueller and Van Cleave thought the statement of Witenberg ("Heterophyid trematodes are not the only trematodes with complicated genital sinuses") should be interpreted in the broadest manner, since their genera from fish had genitalia that were intimate in agreement with the distinctive plan of organization found in the

Heterophyidae. Mueller and Van Cleave accepted Witenberg's formulation of characters and redefined the family as the following:

> Small to very small trematodes with the body covered with scale-like spines and frequently with a crown of circum-oral spines. Pharynx always present. Body usually divided into a motile anterior, flattened region devoid of genitalia, and a posterior part containing the genital organs. Ventral sucker usually reduced and intimately associated with the genital pore. Genital ducts usually opening into a common genital sinus which frequently contains a copulatory organ known as a gonotyl. Genital pore either median or lateral in position. Ovary and testes highly variable in shape, the ovary almost always anterior to the testes. Cirrus pouch lacking. Seminal receptacle voluminous. Uterus usually not extending anterior to the genital pore. Parasitic in mammals, birds and fishes.

Mueller and Van Cleave (1932) asserted that the diversity of form and organization manifested in the family was an expression of evolutionary progress rather than accidental convergence or parallelism. They thought the extent to which evolution had led to diversification was clearly demonstrated by the following specific instances of variable conditions in the family:

1. Circum-oral spines either present or lacking
2. Either one or two testes present
3. Ovary either pre-or post-testicular and ranging in form from spheroidal to follicular
4. Uterine loop may be either wholly pre-testicular or extend to the extremity of the body.
5. Ventro-genital sac varies widely in position as well as in the extent of development or suppression of its component parts.

These workers were of the opinion that the degree of relationship within the family Heterophyidae is not clearly shown by the condition of the gonotyl. They based their evidence on the fact that in a single genus (Parascocotyle)

two different conditions occur. For example, in P. italica, there is a single, small, oval gonotyl anterior to the ventral sucker, while in P. longa there were two widely separate gonotyls located anterior and lateral to the ventral sucker.

The family as constituted by Witenberg (1929) included five subfamilies, but as emended and extended by Van Cleave and Mueller (1932) (the family composition was as follows:)

1. Heterophyinae Ciurea, 1924
2. Centrocestinae Looss, 1899
3. Haplorchinae Pratt, 1902
4. Cercarioidinae Witenberg, 1929
5. Adleriellinae Witenberg, 1930
6. Neochasminae Van Cleave and Mueller, 1932

Mueller and Van Cleave found by extending the host list to include fishes as definitive hosts, five of their seven genera from fishes fitted into the subfamily Heterophyinae. Since extending the boundaries of the subfamily, the necessity of creating a new subfamily for Acetodextra and another for Vietosoma was avoided. These workers noted that Cryptogoniminae Osborn, (1903) though never previously assigned as a subfamily under the Heterophyidae, is based upon a concept which falls within the Heterophyinae, except for the fact that its members are from fishes.

According to the new concept of Mueller and Van Cleave (1932) the Heterophyinae, also, included Vietosoma, Acetodextra, Cryptogonimus, Caecincola, and Centrovarium. The genera Neochasmus and Allacanthochasmus could not be allocated in existing subfamilies of the Heterophyidae and these workers proposed the subfamily Neochasminae for

these genera.

Faust (1932) included Heterophyidae Odhner (1914), Microphallidae Viana (1924), and Lecithodendriidae Odhner (1910) in his superfamily Heterophyoidea. His superfamily Opisthorchoidea contained only the family Opisthorchidae Luhe (1901). Faust indicated, in his diagnosis of the superfamilies, the importance of the symmetry and asymmetry of the miracidia as superfamily characters. He apparently considered flame cell patterns as a character of superfamily value, since he pointed to the fact that the fundamental pattern for the Heterophyoidea was 2(1+1)+(1+1) and for the Opisthorchoidea was 2(2+2+2+2+2+2).

In 1933 Ciurea agreed to the Opisthorchoidea as set up by Faust (1929), but proposed a revision of the Heterophyoidea. Ciurea recognized Heterophyidae Odhner (1914) Cryptogonimidae Ciurea (1933) and Microphallidae Viana (1924), but rejected Lecithodendriidae Odhner (1910) because of the position of the vitelline glands. He included the following subfamilies in the Heterophyidae: Heterophyinae Ciurea (1924), Metagoniminae Ciurea (1924), Apophallinae Ciurea (1924), Centrocestinae Looss (1899), Cryptocotylinae Luhe (1909), and Sigmaperinae Poche (1926). Witenberg had already rejected Sigmapera Nicoll (1918), the type genus of the subfamily, because of a well developed cirrus pouch. Ciurea distinguished his newly proposed Cryptogonimidae from the Heterophyidae by the larger post-testicular uterus. He assigned the following subfamilies to the Cryptogonimidae Osborn (1903): Neochasminae Mueller

and Van Cleave (1932), Galactosominae Ciurea (1933), Haplorchiinae Looss (1899), and Adleriellinae Witenberg (1930). Ciurea thought that Microphallidae Viana (1924) should include the subfamilies Microphallinae Ward (1901), Maritrema Nicoll (1907), and Gymnophallinae Odhner (1905).

Rothschild (1937) reviewed the life histories and larval stages of the Microphallidae and pointed to the resemblance of the cercariae of members of this family to those of the Plagiorchiidae, both having Xiphidiocercariae. The Microphallidae, Lecithodendriidae and Dicrocoeliidae should be included in the superfamily Plagiorchioidea Dollfus.

Vogel (1934) agreed with Witenberg (1929) in including the Heterophyidae and Opisthorchidae in the same superfamily. However, he proposed for them the new superfamily name, Opisthorchoidea. His abolition of the superfamily Heterophyoidea Faust was based on the fact that the cercariae in both families were of the same type.

Price (1940) reviewed the life histories of heterophyid and opisthorchid trematodes and all were found to have cercariae sufficiently similar in type to indicate close relationship. These cercariae were found to belong to the Pleurolophocerca and Parapleurolophocerca groups established by Sewell (1922). They developed in rediae which were provided with short intestinal ceca without collar or locomotor appendages.

Price reported that the following life histories had been described for trematodes having cercariae of this type:

Stamnosoma formosanum Nishigori (=Centrocestus formosanus (Nishigori)), by Nishigori (1924a); Monorchotrema taihokui Nishigori (=Haplorchis pumilo (Looss)), by Nishigori (1924b) and Faust and Nishigori (1926); M. taichui Nishigori (=H. taichui (Nishigori)), by Nishigori (1924a) and Faust and Nishigori (1926); Clonorchis sinensis (Cobbold) by Faust and Khaw (1927) and Yamaguti (1935); Cercaria floridensis McCoy (=Acanthostomum floridensis (McCoy)), by McCoy (1929); Stamnosoma armatum (Tanabe) (=Centrocestus armatus (Tanabe)), by Takahashi (1929a) and Yamaguti (1938); Metagonimus yokogawai (Katsurada), by Takahashi (1929b) and Yamaguti (1933); M. takahashii (Suzuki), by Takahashi (1929b); Exorchis major Hasegawa (=Pseudexorchis major (Hasegawa)), by Takahashi (1929b); Cryptocotyle lingua (Creplin), by Stunkard (1930); Kasraini Khalil (=Haplorchis pleurolophocerca (Sonsino)), by Khalil (1932); Opisthorchis felineus (Rivolta) (=O. tenuicollis (Rudolphi)), by Vogel (1934); Metagonimoides (') oregonensis Price, by Ingles (1935); Apophallus venustus (Ransom), by Cameron (1937); Heterophyes heterophyes (Siebold), by Khalil (1937); Metorchis intermedius Heinemann, by Heinemann (1937); Cryptocotyle jefuns (Nicoll), by Rothschild (1938a); Cercaria coronanda Rothschild (=Acanthostomum coronandum (Rothschild)), by Rothschild (1938b); Euryhelmis monorchis Ameel, by Ameel (1938); and Caecincola parvulus Marshall and Gilbert, by Lundahl (1939).

Price (1940) gave an analysis of cercaria characters and indicated that they showed sufficient similarities as to indicate a single superfamily. All had eye spots, except the cercaria of Euryhelmis monorchis; all had rudimentary acetabula; all were apparently provided with oral spines; and all except species of Centrocestus were provided with tail fin-folds. Price (1940) suggested that most species possessed dorsoventral tail fin-folds (ventral only in Metagonimoides sp. Ingles, 1935) except the species of Haplorchis. The fin-folds in Haplorchis are lateral and has been regarded by Rothschild (1938b) as possible family significance. Rothschild (1938b) stated that all parapleurolophocerca cercariae belong to

the genus Haplorchis.

Price (1940) reported that the cercariae of three species of Haplorchis for which the adults were known, the lateral type fin-fold correlated with the posterior extent of penetration glands. He maintained that the cercariae of Haplorchis pumilio, H. pleurolophocerca possessed penetration glands that were lineal in arrangement and extended to the posterior part of the cercarial body, and the gland ducts were not grouped in bundles as in the other species. He implied that this condition was not found in other parapleurolophocercous cercariae. Therefore, Price suggested that when other species of the genus Haplorchis are known, the combination of the arrangement of the penetration gland duct may have taxonomic significance.

Price stated that since the other cercarial characters showed such wide variation, hardly little more than specific value could be assigned to them. He reported that even the excretory system, though regarded by several investigators as having great taxonomic significance, shows great variation. Price (1940) reported variation in the excretory bladder from sac-like to Y-shape with intermediate shapes. Illustrating his point of view, regarding variation in the highly acclaimed excretory system as a taxonomic vehicle, he cited the following evidence:

> The collecting duct pattern is in general of the "stenostoma" type but in Cercaria coronanda (Acanthostomidae) and in the cercaria of Centrocestus armatus (Heterophyidae) it is of the "mesostoma" type. The flame cell pattern varies from 2((5)+(5+5+5+5)) in the cercaria of Opisthorchis tenuicollis (Vogel, 1934) and 2((3)+(3+3+3+3+3)) in the adult of Opisthorchis

pedicellata (Verma, 1927) in the Opisthorchiinae (Opisthorchiidae); to 2((2+2)+(2+2)) in Cercaria coronanda (Acanthostomidae) (Rothschild, 1938b), 2((2+2)+(2+2)) in Caecinola parvulus (Cryptogonimidae) (Lundahl, 1939), 2((3+3)+(3+3)) in Heterophyes heterophyes (Heterophyinae; Heterophyidae) (Looss, 1894), 2(2+2+3+2) in Euryhelmis monorchis (Apophallinae; Heterophyidae) (Ameel, 1938), 2((2+3)+(3+2+3)) in the metacercaria of Apophallus donicus (Apophallinae) (Hall, 1935), and 2((3+7+7)+(7+7+7)) in the metacercaria of Cryptocotyle lingua (Cryptocotylinae; Heterophyidae) (Stunkard, 1929). The flame cell patterns of the other species of Opisthorchioidea are not known, and in view of the above it appears unwise to attempt to base major groups on this character. This is especially true, since in the case of Pseudamphistomum truncatum (Metorchiinae; Opisthorchiidae) the collecting duct pattern, as figured by Dollfus (1936), suggests that the anterior and posterior groups of flame cells are equal in number instead of unequal as in Opisthorchis.

Price (1940) also believed that adult characters as well as cercarial character gave evidence of superfamily relationships. On the basis of adult characters, the Acanthostomatidae and Cryptogonimidae were included with the Heterophyidae and Opisthorchiidae in the single superfamily, the Opisthorchioidea. All four of these families agreed in lacking a cirrus pouch, in possessing seminal receptacle, and in the fusion of the terminal parts of the male and female ducts into an hermaphrodite duct. In two of the families (Heterophyidae and Cryptogonimidae) a gonotyl or genital sucker was present, however, traces of a gonotyl were found in the immature stages of members of the Opisthorchiidae and Acanthostomatidae. For an example, Rothschild (1938b) reported a gonotyl-like structure in the metacercaria of Cercaria coronanda (Acanthostomidae).

Subfamily CENTROCESTINAE Looss, 1898
(Ascocotylinae Yamaguti, 1958)

Looss (1899) established the genus Ascocotyle for Distomum coleostomum Looss, 1896, a species described from the oecum and large intestine of the pelican in Egypt. Looss (1899) also described A. minuta from the small intestine of dogs and cats and included it as a second species in the newly erected genus. In the 62 years following Looss' initial work, numerous species have been added to this genus, namely: A. italica Alessandrini, 1906; A. angrense Travassos, 1916; A. longa Ransom, 1920; A. nana Ransom, 1920; A. diminuta Stunkard and Haviland, 1924; A. angeloi Travassos, 1928; A. felippei Travassos, 1928; A. ascolona Witenberg, 1928; A. arnaldoi Travassos, 1928; A. megalocephala (Price, 1932) Price, 1935; A. puertoricensis (Price, 1932) Price, 1935; A. tenuicollis Price, 1935; A. intermedius (Srivastava, 1935) Price, 1936; A. mcintoshi Price, 1936; and A. leighi Burton, 1956.

Faust (1920) described a new genus and species, Phagicola pithecophagicola, from the intestine of the monkey-eating eagle (Pithecophaga jefferyi) of the Phillipine Islands. He erected the subfamily Phagicolinae to include this species. However, in describing this species, Faust (1920) failed to notice the posterior oral appendage and gonotyls which would have placed his specimens in the genus Ascocotyle Looss, 1899.

Stunkard and Haviland (1924) contended that the morphological differences between A. coleostoma and

A. minuta were such that they doubted whether they could be included in a natural genus. These differences were listed as the following:

Ascocotyle coleostoma

1. Double row of oral spines.
2. Esophagus absent.
3. Ceca entirely preacetabular.
4. Acetabulum some distance posterior to bifurcation of digestive tract.
5. Coils of uterus extend across the body anterior to the genital pore.
6. Vitellaria entirely pretesticular, extend forward anterior to the genital pore.
7. Habitat: cecum and large intestine of birds

Ascocotyle minuta

1. Single row of oral spines.
2. Esophagus present.
3. Ceca extend postacetabular.
4. Acetabulum near the bifurcation of digestive tract.
5. Uterus does not cross the body anterior to the genital pore.
6. Vitellaria partially post-testicular, entirely postovarian, do not extend forward one-third of the distance to the genital pore.
7. Habitat: small intestine of mammals and possibly of bird, Ardea.

Stunkard and Haviland (1924) suggested that though the species descriptions of Travassos (1916) were brief and somewhat indefinite, there was substantial agreement with A. coleostoma, and both were parasitic in birds. The other described species were from mammals and appeared similar to A. minuta. They pointed to the fact that the latter group constituted a distinct section of the genus for which Parascocotyle was proposed with A. minuta as the type species. These authors described trematodes from the intestine of wild rats, collected at the Cleason Point dump near New York by the City board of Health, as Ascocotyle

(Parascocotyle) diminuta; placing it in the subgenus Parascocotyle. In defense of their action, the following argument was given:

> The specimens from the rat agree more closely with A. minuta than any other known form and in certain respects the likeness is striking. Many organs agree in size with those of A. minuta, but the worms themselves are much smaller, hardly more than half the size of A. minuta, and the suckers, ovary and testes are relatively much larger. The eggs on the other hand, are smaller. The discovery of additional material may supply specimens intermediate in these respects and demonstrate the identity of these worms and A. minuta, but at present such identity appears hardly probable and we describe them as new species.

Faust and Nishigori (1926), in their paper dealing with the life cycles of two new species of Heterophyidae stated: "In 1920 one of us (Faust) described a new species from the intestine of the monkey-eating eagle under the name Phagicola pithecophagicola, a fluke which on restudy has been found to belong to the genus Ascocotyle and should, therefore, be designated as Ascocotyle pithecophagicola." Thus, the genus Phagicola and subfamily Phagicolinae were invalidated.

Witenberg (1929) interpreted the subgenus Parascocotyle Stunkard and Haviland, 1924 as a genus. He observed that members of the genus Ascocotyle possessed two rows of circumoral spines and several coils of the uterus were situated in front of the genital aperture, while the genus (should be subgenus) Parascocotyle exibited only one row of circumoral spines, and the coils of the uterus confined to the region behind the genital aperture. Witenberg found that in Ascocotyle, the vitellaria extended

in front of the ventral sucker, while in Parascocotyle they passed beyond the level of the ovary. Although Witenberg (1929) recognized the distinctive nature of the genus (subgenus) Parascocotyle of Stunkard and Haviland, 1924 as being fully justified, he thought the creation of the species Ascocotyle (Parascocotyle) diminuta was unwarranted. A. diminuta was, therefore, a synonym of A. minuta. Witenberg (1929) believed that the differences on which A. (Parascocotyle) diminuta was erected could be attributed to age of fixation and were not of specific value. However, he transferred A. minuta Looss, 1899; A. italica Alessandrini, 1906; A. longa Ransom, 1920; A. nana Ransom, 1920; Ascocotyle pithecophagicola Faust, 1926 (synonymy Phagicola pithecophagicola Faust, 1920); and Parascocotyle ascolonga Witenberg, 1928 to the genus (subgenus) Parascocotyle Stunkard and Haviland, 1924. Witenberg (1929) included P. pithecophagicola (Faust, 1920) Faust, 1926 in the genus (subgenus) Parascocotyle though pointing to its insufficient description and apparent need for restudy before its position or validity could be determined. His key to the species of Parascocotyle Stunkard and Haviland, 1924 was as follows:

Key to the species of Parascocotyle Stunkard and Haviland, 1924 (after Witenberg, 1929)

A. The ceca reach only up to the level of ventral sucker:

(1) adequately described species.....
.....P. minuta Looss, 1899
(2) insufficiently described species.....
.....P. pithecophagicola Faust, 1920

B. The ceca reach the ovary or more posteriorly:

(1) the vitellaria compact:
  (a) the appendix of the oral sucker reaches the pharynx....P. ascolonga Witenberg, 1929
  (b) the appendix of the oral sucker half as the prepharynx....P. italica Alessandrini, 1906
(2) Vitellaria divided into follicles:
  (a) the uterine coils entangled; one muscular papilla in front of the genital aperture.....P. nana Ransom, 1920
  (b) the uterine coils have a transverse direction; there are two muscular papillae in front of the genital aperture.....P. longa Ransom, 1920

Witenberg (1930) restudied the type specimens of P. pithecophagicola Faust, 1920. He was not able to add anything to the original description of Faust. He suggested that only a detailed study of new specimens of the species would determine if Parascocotyle and Phagicola were both valid genera.

Travassos (1930), as reported by Stunkard and Uzmann, 1955, accepted the two subgenera of Stunkard and Haviland. However, Parascocotyle was suppressed as a synonym of Phagicola and all previously described species were placed in the genus Ascocotyle. Travassos arranged them in the two subgenera, Ascocotyle and Phagicola. Faust's species of 1920 and 1926 was listed as Ascocotyle (Phagicola) pithecophagicola, while Stunkard and Haviland's species was listed as Ascocotyle (Phagicola) diminuta.

Price (1932b) studied the type specimens of P. pithecophagicola which had been allocated to the genus (subgenus) Parascocotyle by Witenberg (1929). Price found that these specimens possessed (1) a posterior oral projection, which extended to the pharynx, (2) two gonotyls in

the genital sinus, and (3) a globular seminal receptacle median to the ovary.

The first and second findings of Price (1932b) were overlooked by Faust (1920) and no doubt prompted him to create a new genus and subfamily. Price thought that his newly found structures in P. pithecophagicola Faust, 1920 indicated the apparent synonymy of Phagicola and Parascocotyle. In accordance with the law of priority, Phagicola was re-established as the valid name. Price (1932b) reported that Phagicola differed sufficiently from Ascocotyle to warrant generic rank, and referred the following species to the genus Phagicola: Phagicola pithecophagicola (Faust, 1920) Faust, 1926; P. minuta Looss, 1899; P. ascolonga Witenberg, 1929; P. longa Ransom, 1920; P. arnaldoi Travassos, 1928; P. italica Alessandrini, 1906; P. piriforme Blanc and Hedin, 1913; P. angrense Travassos, 1916; P. nana Ransom, 1920; P. diminuta Stunkard and Haviland, 1924; and P. angeloi Travassos, 1928.

Price (1932c) described Ascocotyle megalocephala and Ascocotyle puertoricensis from the intestine of Butorides sp. Phagicola diminuta Stunkard and Haviland, 1924 was also reported from the same host. Price (1933a) studied the original specimen of Ascocotyle plana Linton, 1928 and solved the "riddle" connected with this species. Previously Witenberg (1928) had considered this species as a synonym of Pygidiopsis genata (Looss, 1896), while Travassos (1930) asserted that it was a synonym of Ascocotyle (Phagicola) angrense Travassos, 1916, a species from various herons of

South America. Price (1933b) transferred these specimens to the genus Pygidiopsis as P. plana Linton, 1928. He reasoned that the absence of the posterior prolongation of the oral sucker (frequently referred to as an "appendix"), and the general body organization warranted this change.

Price (1933c) extended the host record for the genus Phagicola. He had several specimens of Phagicola nana Ransom 1920 at his disposal, originally reported as Ascocotyle nana from the Alaskan fox (Vulpes lagopus). These same worms had been also collected from a booby (Sula bassona) in 1893 by Dr. Albert Hassal. These specimens possessed oral coronets with a complete anterior row of 16 spines and an incomplete posterior dorsal row of 3 to 4 spines. Specimens of Phagicola longa, originally described by Ransom from an Alaskan fox (Vulpes lagopus), were compared with P. longa reported from the dog, cat, and a Persian wolf by Witenberg and, also, with P. longa found in the intestine of a pelican (species not determined) by Price, 1933b. Price (1933b) found that specimens of P. longa Ransom, 1920 corresponded with the description given by Witenberg, but differed from that of the anomalous type specimen of Ransom. Ransom's material showed only two vitelline follicles on each side of the body compared to 5 in the normal condition. Price emended the specific description.

Ciurea (1933) obtained specimens identical to Parascocotyle longa (Ransom, 1920) Witenberg, 1929 through feeding the gills and superficial muscles of Mugil capito

from the Black Sea to dogs and common cormorant, Phalocrocorax carbo. Morphological studies revealed that the acetabulum was located within the genital sinus and were described as Metacocotyle witenbergi. Price (1935) described Ascocotyle tenuicollis as a new species and gave better descriptions and figures of Ascocotyle megalcephala Price, 1932, and Phagicola pithecophagicola Faust, 1920. He declared that the location of the acetabulum within the genital sinus is common to all members of Phagicola and Ascocotyle. Price (1932) did not agree with Travassos (1930) that Phagicola should be considered as a subgenus of Ascocotyle. He argued as follows:

> Members of the genus Ascocotyle have two rows of spines in the oral coronet, the cuticle is entirely covered with spines; the uterus extend anterior to the genital aperture; and the vitellaria extend anterior to the level of the ovary. Members of the genus Phagicola have only a single row of spines in the oral coronet; the cuticular spines are absent at the posterior end of the body; the uterus does not extend anterior to the genital aperture; and the vitellaria are confined to the post-ovarian region of the body.

Srivastava (1935) described Ascocotyle intermedius as a new species from the Indian Fishing Eagle, Haliaeetus leucoryphus. He assigned this species to the subgenus (Phagicola) Travassos, 1930 on the basis of the length of the esophagus, the intestinal ceca, and the extent of the uterus. He indicated that his species resembled the subgenus (Ascocotyle) Travassos, 1930 in the arrangement of the oral spines and extent of the vitellaria. But it differed in the presence of a fairly large esophagus, long ceca which extended far behind the acetabulum, and the

uterus which never extends in front of the genital sinus; features in which it resembles A. (Phagicola). However, it differed from the species of the subgenus A. (Phagicola) Travassos, 1930 in the enormous development and extent of the vitellaria and double crown of oral spines. Srivastava concluded that A. intermedius differed from all species of the genus in the number of oral spines (28-30), larger extent of the vitellaria, and the size of the eggs.

Srivastava maintained that the genus Phagicola as constituted by Price (1932) differed from Ascocotyle only by the presence of an esophagus, the length of the intestinal ceca which extended posteriorly beyond the acetabulum, the postacetabular position of the vitellaria, and the extent of the uterus which never extends beyond the ventro-genital sinus. He felt that his species connected the two genera in regard to the extent of the vitellaria. Srivastava argued that only the remaining important differences between the two genera were the extent of the intestinal ceca and the uterus. He argued further that the extent of the intestinal ceca could not be considered of generic importance since all the gradations in their length exist between such forms as Phagicola minuta and P. arnaldoi. He also contended that the extent of the uterus alone was not of sufficient justification for maintaining two distinct genera. Although Srivastava (1935) was in agreement with Travassos (1930) that the genus Phagicola should be reduced to the rank of a subgenus, he found it necessary to modify the diagnosis of Ascocotyle

as given by Travassos (1930). Srivastava's treatment of the genera Ascocotyle Looss, 1899, and Phagicola Faust, 1920 is shown below in the following set of keys:

Key to the Subgenera of Ascocotyle Looss, 1899 (after Srivastava)

1. Vitellaria extending in front of acetabulum; Uterus extending in front of ventro-genital sinus; Oesophagus almost absent...Ascocotyle (Ascocotyle).
2. Vitellaria post-acetabular, except in A. intermedius; Uterus confined behind ventro-genital sinus; Oesophagus well developed.....Ascocotyle (Phagicola).

Key to the Species of the Subgenus Ascocotyle (Ascocotyle) (after Srivastava)

1. Vitellaria extending from the level of pharynx to center of acetabulum.....Ascocotyle (Ascocotyle) megalocephala.
   Vitellaria confined between ends of ceca and body.....2
2. Vitellaria pretesticular.....Ascocotyle (Ascocotyle) coleostomum.
   Vitellaria extending into testicular region.....3
3. Oral spines 36 in number.....A. (Ascocotyle) felippei.
   Oral spines 32 in number.....A. (Ascocotyle) puertoricensis.

Key to the Species of the Subgenus Ascocotyle (Phagicola) (after Srivastava)

1. Vitellaria extending from the hinder end up to the level of pharynx.....A. (Phagicola) intermedius.
   Vitellaria post-acetabular.....2
2. Oral spines in double row.....3
   Oral spines in single row.....4
3. Oral spines in double row on the dorsal side and in single row on the ventral...A. (Phagicola) nana.
   Oral spines in double row on both the surfaces.....A. (Phagicola) angeloi.
4. Genital pore situated at intestinal bifurcation.....A. (Phagicola) pithecophagicola.
   Genital pore situated behind intestinal bifurcation.....5
5. Intestinal ceca not reaching ovary.....6
   Intestinal ceca reaching or extending beyond ovary.....8

6. Oral sucker distinctly larger than acetabulum.....
.....A. (Phagicola) angrense.
Suckers about equal in size.....7
7. Oral spines 16 in number....A. (Phagicola) diminuta.
Oral spines 19 (rarely 20 or 18 in number.....
.....A. (Phagicola) minuta.
8. Vitellaria follicular.....9
Vitellaria composed of compact masses.....10
9. Vitellaria composed of 2-8 follicles on each side-
Eggs 0.0016-0.018 x 0.0001 in size.....
.....A. (Phagicola) longa.
Vitellaria composed of 9-12 follicles on each side-
Eggs 0.02-0.024 x 0.01-0.012 in size.....
.....A. (Phagicola) arnaldoi.
10. Vitellaria lateral and post-ovarian; Oral
appendage and prepharynx equal in length.....
.....A. (Phagicola) ascolonga.
Vitellaria lateral extending up to or beyond ovary;
Oral appendage half the length of prepharynx.....
.....A. (Phagicola) italica.

Price (1935) pointed to the inter-generic variability of the characters used by Srivastava (1935) as justification for placing A. intermedius in the Phagicola group, and assigned the species to the genus Ascocotyle. It was the opinion of Price (1935) that Metascocotyle witenbergi Ciurea, 1933, type species, was a synonym of Phagicola longa (Ransom, 1920) Price, 1932. Although Price (1935) thought that most of the characters used by Srivastava were inter-generic, the extent of the uterus as a differentiating character in his separation of genera was dropped in 1936. This action was taken since A. intermedius Srivastava, 1935 was an exception to this character. However, Price (1936) refuted the arrangement of Srivastava and argued as follows:

> The species comprising the Ascocotyle-Phagicola complex fall quite distinctly into 2 categories one group, Ascocotyle, having 2 rows of spines in the oral coronet, body completely spined and vitellaria extending anterior to the level of the ovary, and the other group, Phagicola, having a single row of spines in the oral coronet, the body incompletely spined (spines absent on

posterior portion of body) and vitellaria confined to the postovarian region. In view of these facts that in each of these groups there are at least 3 correlated characters the writer regards Ascocotyle and Phagicola as better established genera than some of the other genera of heterophyids, as well as many genera of other families, the validity of which rests largely upon a single character which in many cases is decidedly variable.

Price (1936) re-affirmed that species of the Ascocotyle-Phagicola complex should be grouped separately. He recognized both Ascocotyle and Phagicola as valid genera on the basis of the following:

Ascocotyle

1. Two rows of spines in oral coronet.
2. Cuticle entirely spinous.
3. Uterus extending beyond level of genital aperture.
4. Vitellaria extending into preovarian region.

Phagicola

1. Single row of spines in oral coronet.
2. Cuticle spines absent in posterior region of body.
3. Uterus not extending beyond level of genital aperture.
4. Vitellaria confined to postovarian region.

Travassos' (in Burton, 1958) publication of 1928 gave descriptions of P. angeloi and P. arnaldoi which were in contradiction to the characters used by Price (arrangement of oral spines and the distribution of the cuticular spines) to separate Phagicola and Ascocotyle. Price (1936) had maintained that the genus Phagicola is characterized by a single row of oral spines and a posterior body devoid of cuticular spines. P. angeloi had two complete rows of spines in the oral coronet. Both P. angeloi and P. arnaldoi had spinous cuticles, even though the spines on the anterior region of the body were determined to be slightly longer.

Lal (1939) reported varability in the extent of the esophagus and intestinal ceca within the complex and thought that the groups should be reduced to a single group.

Chandler (1941) described Phagicola lageniformis as a new species from the intestine of muskrats of southeastern Texas. He reported that this species had 18 spines on the oral coronet. Of these spines, 16 were in a single circle while the other two spines were situated more posteriorly on the dorsal side. Chandler thought his species resembled P. nana Ransom, 1920 and P. angrense Travassos, 1916 in the number and arrangement of the oral spines. It differed from P. nana in shape of body, size of oral diverticulum, and size of spines. It was different from P. angrense in shape of body and in length of the pharyngeal region, which in P. angrense is very short, resulting in the oral diverticulum reaching beyond the pharynx.

Stunkard and Uzmann (1955) redescribed Ascocotyle (Phagicola) diminuta and found in the oral coronet, a single row of 16 spines and a second row of two dorsal accessory spines. They expressed their view as the following:

> It appears, therefore, that in these species a second row is represented by a few persistent spines. If these spines are actually members of a second incomplete row of smaller spines, and in species of Ascocotyle the spines of the second row are smaller than those in the anterior row, the distinction between Ascocotyle and Phagicola rest on the extent of body spination and of the vitellaria. Moreover, the figure of Ascocotyle puertoricensis, published by Price (1935), show that the vitellaria extend a short distance anterior to the ovary and do not reach the level of the genital aperture. Decision on the taxonomic state of Phagicola should await more complete information, especially on

the developmental stages of its members.

Burton (1956) described Ascocotyle leighi, a species that encysts as metacercariae in the conus arteriosus of Mollienisia latipinna LeSueur. He found only 12 out of 341 Mollienisia latipinna from southern Florida devoid of infection. Burton was not able to find the natural definitive host. His description was based on adults recovered from day-old unfed chicks which were infected with the metacercariae. This species differed from closely resembling species such as A. tenuicollis Price, 1935 and A. puertoricensis Price, 1935 which possessed 32 spines in their oral coronets (16 in each of two rows), while A. leighi had 48 to 52 spines in the oral coronet (24 to 26 in each of two rows). Burton also pointed out other differences (e.g.-the species of Price (1935) were characterized by seminal vesicles which taper anteriorly from a bulb-like expansion). The vesicle in A. leighi was in a transverse plane and tapered medially toward the ovary.

Robinson (1956) described Phagicola macrostomus and Phagicola byrdi from the turkey vulture. Robinson claimed that Phagicola macrostomus exibited an oral sucker diameter that was one fifth of the body length, with no dorsal anterior prolongation, and possessed 18 thick, blunt-pointed oral spines evenly spaced in a single row around the oral opening. He declared that this species differed from other species of Phagicola from the Western Hemisphere by the larger size of the oral sucker. The oral spines were either half as long or two to four times as long as other

species and the anterior half of the body was much broader.

Robinson (1956) reported that P. byrdi differed from other species of Phagicola in that it had a very long recurved oral diverticular, an acetabulum asymmetrically placed, but associated with a prominant gonotyl. The oral sucker was only one tenth of the body length, with 16 oral spines in a single row, spaced around the oral opening. Robinson believed the turkey vulture was an unusual host since phagicolids are usually found in fish-eating mammals.

Kuntz and Chandler (1956), working with trematodes from Egypt, described Phagicola longicollis from the cat. This species was thought closely related to P. longa, but differed in several details. In comparison with P. longa, the body is much longer (because of the increased length of the slender, neck-like portion anterior to the genital pore), the pharynx is situated a greater distance from the anterior end, the ventral sucker instead of only slightly over half of the body length from the anterior end is as much as 70 per cent, suckers are smaller, armed with 14 or sometimes 15 spines as compared to the 16 of P. longa, the ceca are short (ending anterior to the ovary instead of reaching the level of the testes. The principle characters which this species have in common with P. longa are the structures of the gonotyls, the transverse folds of the uterus and the follicular character of the vitellaria.

Hutton and Sogandares-Bernal (1958) had occasion to study many specimens of Phagicola longicollis Kuntz and Chandler, 1956. They found variation in the number of oral

spines. Their findings indicated that this species had a range in the number of oral spines from 14 to 17. Therefore the species description was expanded to include forms with 14 to 17 spines in the oral coronet.

Hutton and Sogandares-Bernal (1958) described P. inglei, a species closely related to P. longa and P. longicollis. This worm was described from one specimen among P. longicollis and P. longa sent to these authors by Dr. A. C. Chandler. Hutton and Sogandares-Bernal found that P. inglei exhibited an oral appendage which was almost in contact with the pharynx, while it is only half way to the pharynx in other species. The pharynx of P. inglei is located less than one third of the body length from the anterior end, but approaches the midbody length in P. longa. The vitellaria in P. inglei extended only to the anterior border of the ovary while they were restricted behind the ovary of P. longa. P. inglei possessed 19 very heavy crown spines which are hooked at the tips as compared with the 15 to 18 lighter, straight spines in P. longa. Finally, P. inglei has an esophagus proportionately about 3 times longer, the eggs twice the length and diameter of those of P. longa.

Burton (1958) reviewed the taxonomy of the genera Ascocotyle Looss, 1899 and Phagicola Faust, 1920. He was not in agreement with Srivastava's publication of 1935 which reduced Ascocotyle and Phagicola to subgenera and revised the diagnosis of Ascocotyle to contain species once included in Phagicola. As reported above, Srivastava

(1935) described Ascocotyle intermedius and placed it in the subgenus Phagicola; basing his argument on facts such as the vitellaria extended beyond the ovary to the level of the pharynx, long esophagus, long intestinal ceca, and that the uterine coils were confined posterior to the genital sinus.

Burton (1958) thought Srivastava's statement to the effect that A. intermedius connected the genera of Price (1935) with reference to the extent of the vitellaria was vague. He pointed out that since the vitellaria in A. intermedius extended beyond the ovary to the posterior level of the pharynx, it should be placed in Price's (1935) revision of Ascocotyle (s. str.). Burton (1958) felt that since Srivastava's description of A. intermedius listed the esophagus as short, this did not correspond to the long esophagus criteron by which he attempted to show that his species belonged to the subgenus Phagicola. Burton suggested that the other characters set up by Srivastava should be invalidated since A. mcintoshi Price, 1935 possessed long intestinal ceca terminating in the region of the testes and several other species of Ascocotyle had the greater part of the uterus confined to the region posterior to the genital sinus.

Burton (1958) was not entirely in agreement with the arrangement of Price (1936). He felt that P. nana (Ransom, 1920) Price, 1932, and P. longeniformes (Chandler, 1941) should be considered intermediate in regards to the oral spines criteron of Price (1936), since P. nana was described

as having oral spines arranged dorsally in a double row and ventrally in a single row while P. longeniformes possessed a single complete circle of spines with two spines separated more posteriorly on the dorsal surface.

Burton argued further that Travassos' description of P. angeloi Travassos, 1928 and P. arnaldoi Travassos, 1928 indicated spinous cuticles, even though the spines on the anterior region of the body were determined to be slightly longer. Burton affirmed that in view of these exceptions, the arrangement of oral spines and the distribution of cuticular spines could no longer be considered as valid generic characters, and only the extent of the vitellaria should be left to separate genera.

Burton (1958) agreed that there were variations in the characters mentioned by Lal (1939) as there were variations in the arrangement of the oral spines, the distinction of cuticular spines, and the extent of the uterus. Burton maintained that none of the aforementioned could be used to justify separation of the two distinct genera that make up the complex. He felt that further revision would fail to solve the problem, but rather cause greater confusion. However, he stated that of the four differentiating characters used by Price (1936) in separating the genera Ascocotyle and Phagicola, only the extent of the vitellaria should remain valid until such time that new analysis, unquestionably, warrantsrevision. He gave the following key to the species of the genus Ascocotyle and Phagicola; stating that in Ascocotyle the vitellaria extended anteriorly beyond

the level of the ovary, whereas in Phagicola the vitellaria are restricted to the postovarian region:

A Key to the North and South American Species of the Genus Phagicola (after Burton 1958)

1. (2) Uterine coils confined to postacetabular region.....3
2. (1) Uterus with a few coils anterior to acetabulum.....P. angrense (Travassos, 1916) Price, 1932
3. (4) Totality of spines in oral coronet in single complete circle.....9
4. (3) Totality of spines in oral coronet not in single complete circle.....5
5. (6) Oral coronet with 16 to 20 spines.....7
6. (5) Oral coronet with more than 20 spines (two rows; 14 in each).....P. angeloi (Travassos, 1928) Price, 1932
7. (8) Oral coronet with 16 spines in a single complete circle with 2 spines situated more posteriorly on dorsal side.....P. longeniformis Chandler, 1941
8. (7) Oral coronet with 16 to 20 spines situated in a double and ventrally in a single row.....P. nana (Ransom, 1920) Price, 1932
9. (10) Intestinal ceca terminating near posterior margin of acetabulum.....11
10. (9) Intestinal ceca extending beyond posterior margin of acetabulum.....13
11. (12) Oral coronet with 18 to 20 spines.....P. minuta (Looss, 1899) Price, 1932
12. (11) Oral coronet with less than 18 spines (about 16.....P. diminuta (Stunkard and Haviland 1924) Price, 1932
13. (14) Oral coronet with 16 spines.....15
14. (13) Oral coronet with 18 spines.....P. macrostomus Robinson, 1956
15. (16) Gonotyl bipartite.....17
16. (15) Gonotyl single.....P. byrdi Robinson 1956
17. (15) Cuticula entirely spinous; vitelline follicles 9-12 in each lateral field.....P. arnaldoi (Travassos, 1928) Price, 1932
18. (17) Cuticula spinous on anterior body region only; vitelline follicles 2-6 in each lateral field.....P. longa (Ransom, 1920) Price, 1932

A Key to the Genus Ascocotyle (after Burton, 1958)

1. (2) Body pyriform.....3
2. (1) Body shaped like a tall beaker.....A. megalocephala Price, 1935
3. (4) Vitellaria restricted to postpharyngeal region.....5
4. (3) Vitellaria extending anteriorly to posterior level of pharynx.....A. intermedius (Srivastava, 1935) Price, 1936
5. (6) Intestinal ceca terminating anterior to testes.....7
6. (5) Intestinal ceca extending posteriorly to anterior margin of testes.....A. mcintoshi Price, 1936
7. (8) Oral coronet with less than 48 total spines.....9
8. (7) Oral coronet with 48 to 52 total spines (24-26 in each of two rows).....A. leighi Burton, 1956
9. (10) Oral coronet with 32 total spines (16 in each of two rows).....11
10. (9) Oral coronet with 36 total spines (18 in each of two rows).....A. filippei Travassos, 1928
11. (12) Uterine coils extending into post-testicular region.....13
12. (11) Uterine coils confined to pretesticular region.....A. coleostoma Looss, 1899
13. (14) Vitellaria extending anteriorly to posterior level of acetabulum. Apex of posterior oral projection lying 1/3 to 1/2 distance between oral aperture and pharynx.....A. puertoricensis Price, 1935
14. (13) Vitellaria extending anteriorly to level of genital opening. Apex of posterior oral projection lying more than 1/2 distance between oral aperture and pharynx.....A. tenuicollis Price, 1935

When Hutton and Sogandares-Bernal (1958) described P. inglei, they maintained that it had been the practice of other workers (Chandler, 1941; Kuntz and Chandler, 1956; and Robinson, 1956) to describe several species under the generic name of Phagicola, therefore separating this genus from Ascocotyle. They thought that evidently these workers separated Phagicola from Ascocotyle on the basis of the following:

Phagicola

1. Single row or an incomplete second row of oral spines.
2. Body incompletely spined.
3. Vitellaria not extending forward beyond the level of the ovary.

Ascocotyle

1. Two complete rows of oral spines.
2. Body completely spined.
3. Vitellaria extending anterior to the level of the ovary.

Hutton and Sogandares-Bernal stated that they chose to follow the same practice of separating Phagicola and Ascocotyle, at least until the taxonomic status of Phagicola is accurately determined.

Later, that same year (1958), in another publication Hutton and Sogandares-Bernal decided to recognize Ascocotyle Looss, 1899; Phagicola Faust, 1920; and Parascocotyle Stunkard and Haviland, 1924 as valid genera. They separated these genera in a newly constructed key which is as follows:

1. Vitellaria extending as far forward as acetabulum; with two complete rows of oral spines..... .....Ascocotyle, sensu stricto
1.- Vitellaria extending forward only to ovary, never beyond; never with two complete rows of oral spines .....Ascocotyle, sensu stricto
2. With a single complete row of oral spines..... .....Phagicola, sensu stricto
2.- With a single complete row of oral spines and an incomplete second row of 2 to 4 accessory spines... .....Parascocotyle, sensu stricto

On the basis of their newly constructed key, Hutton and Sogandares-Bernal included the following species in the genus Ascocotyle Looss, 1899: A. angeloi Travassos, 1928; A. coleostoma (Looss, 1896) Looss, 1899; A. filippei Travassos, 1928; A. intermedius Srivastava, 1935; A. leighi

Burton, 1956; A. mcintoshi Price, 1936; A. megalocephala Price, 1932; A. puertoricensis Price, 1932; and A. tenuicollis Price, 1935.

Hutton and Sogandares-Bernal included the following species in the genus Phagicola Faust, 1920: P. arnaldoi (Travassos, 1928) Price, 1932; P. ascolonga (Witenberg, 1929) Price, 1932; P. byrdi Robinson, 1958; P. italica (Alessandrini, 1906) Price, 1932; P. longa (Ransom, 1920) Price, 1932; P. longicollis Kuntz and Chandler, 1956; P. macrostomum Robinson, 1956; P. minuta (Looss, 1899) Price, 1932; P. piriforme (Blanc and Hedin, 1913) Price, 1932; and P. pithecophagicola Faust, 1920 (type species).

Hutton and Sogandares-Bernal (1958) included the following species in the genus Parascocotyle Stunkard and Haviland, 1924: P. angrense Travassos, 1916) n. comb.; P. diminuta Stunkard and Haviland, 1924 (type species); P. langeniformis (Chandler, 1941) n. comb.; and P. nana (Ransom, 1920) n. comb. These authors reported that Burton (1958) errored in placing P. diminuta in his key to the North and South American species of the genus Phagicola and was evidently unaware of Stunkard and Uzmann's (1955) redescription of Ascocotyle (Phagicola) diminuta (see the above).

Hutton and Sogandares-Bernal (1959) reported that they had overlooked the species, Ascocotyle (Phagicola) angeloi Travassos, 1928, which although having vitellaria extending to the level of the ovary, has two rows of oral spines. Therefore, they revised their key of 1958 as the following:

the genus (Pseudascocotyle) was closely related to Ascocotyle Looss, 1899; Phagicola Faust, 1920; and Parascocotyle Stunkard and Haviland, 1924 though it differed by possessing a gonotyl perforated by the uterus and in lacking oral spines. They thought that their genus was most closely related to Phagicola and Parascocotyle, since the vitellaria, like these genera, extended to the level of the ovary.

Sogandares-Bernal and Bridgman (1960) stated that the lack of oral spines in their species was not an artifact since they were also absent in the metacercariae. They also reported that the cuticular spines in their species, unlike Ascocotyle, Phagicola, and Parascocotyle which begin a short distance posterior crown spines, left a bare zone which extended almost to the oral aperture.

Sogandares-Bernal and Bridgman accepted the subfamily Ascocotylinae Yamaguti, 1958 for the Ascocotyle-Phagicola Pseudascocotyle. They included the following artificial key to separate these genera:

1. Oral sucker, with one or more circlet (s) of spines; vitellaria extending either to level of ovary or to acetabulum.....2
   Oral sucker lacking spines; vitellaria extending to level of ovary.....Pseudascocotyle,
2. Oral sucker with two complete circlets of spines; vitellaria usually extending to level of acetabulum .....Ascocotyle, sensu stricto
   Oral sucker never with two complete circlets of spines; vitellaria never extending to acetabulum... 3
3. Oral sucker with a single complete circlet of spines and an incomplete accessory dorsal row of 2 to 4 spines; vitellaria extending to level of ovary.....Parascocotyle, sensu stricto

Family Echinostomidae Looss, 1902

Subfamily Echinochasminae Odhner, 1910

Genus Echinochasmus Odhner, 1909

Dietz (1909) erected the genus Echinochasmus for E. coaxatus Dietz, 1909; E. eurycporus Looss, 1896; and E. beleocephalus Linston, 1873. The following year Odhner (1910) emended the genus and established the subfamily Echinochasminae. Odhner included E. liliputanus Looss, 1896; E. africanus Stiles, 1901; and E. bursicola Creplin, 1830. Dietz (1910) listed E. oligacanthus Dietz, 1910, and E. perfoliatus Ratz, 1908 as members of the genus Echinochasmus. The 20 years following Dietz' work, saw the annexation of E. prosthonitellatus by Nicoll (1914; E. tenuicollis by Johnston (1917); E. amphibolus by Kaltan (1922); E. botauri by Baer (1922); E. elongatus by Miki (1923); E. cornaus by Bhalerao (1926) E. hortense Ghoto by Asada (1926); E. japonicus by Tanabe (1926) and E. dietzeni by Isaichikov (1927).

Luhe (1909) set up the genus Episthmium with E. africanus Stiles, 1901 as the type species and included E. bursicola Creplin, 1830 as an additional species. Luhe's principle character, which separated his genus (Episthmium) from Echinochasmus was that the vitellaria extended beyond the acetabulum. In Episthmium the vitellaria extended anteriorly as far as the pharynx and united in the median line. The vitellaria in Echinochasmus rarely extend as far forward as the anterior margin of the

acetabulum. Odhner (1910) did not recognize Luhe's genus and surpressed it as a synonym of Echinochasmus. However, Travassos (1923) recognized the genus Episthmium as valid and included E. proximun and E. ascari as two new species. Bhalerao (1926) subscribed to Odhner's opinion that the extension of the vitellaria was not a very good point of difference. Price (1931) felt that the distribution of the vitellaria was a character of generic value and recognized Episthmium Luhe, 1909 as a valid genus. Price believed that the following species should be allocated to the genus Episthmium Luhe, 1909: E. africanus Stiles, 1901; E. bursicola Creplin, 1830; E. prosthonitellatus Nicoll, 1914; E. proximum Travassos, 1923; E. ascari Travassos, 1923; and E. cornaus, Bhalerao, 1926. Price (1931) contended that on the basis of the extent and distribution of the vitelline follicles, these species formed a recognizable group.

Odhner (1910) had proposed the genus Heterechinostomum with H. mordax Looss, 1899, as the type species. Stunkard and Haviland (1924) added the second species, H. magnovatum. Odhner's (1910) principle character in separating Heterechinostomum from Echinochasmus was the cirrus pouch. He maintained that the cirrus pouch was almost entirely or completely atrophied in Echinochasmus while it was rather weakly developed in Heterechinostomum. Price (1931) thought that it was not possible for one to differentiate between entirely or completely atrophied and rather weakly developed with sufficient certainly to separate the two

genera. Therefore, he regarded Heterechinostomum as a synonym of Echinochasmus. Thus, H. mordax Looss, 1899; and H. magnovatum. Stunkard and Haviland, 1924; became Echinochasmus mordax Looss, 1899 and Echinochasmus magnovatum Stunkard and Haviland, 1924.

Price (1931) suggested that Echinochasmus tenuicollis Johnston, 1917 should not be included in the genus Echinochasmus. He thought the distribution of collar spines and the deeply lobed condition of the testes indicated that it could not be a species of Echinochasmus, but should be placed in the genus Paryphostomum Dietz. Thus, E. tenuicollis Johnston, 1917 became P. tenuicollis Johnston, 1917.

Price transferred Monilifer pitangi Lutz to the genus Echinochasmus on the basis of the distribution of the vitellaria. Prior to this, Bhalerao (1926) had recognized that this species should not be retained in the genus Monilifer (=Stephanoprora) but did not make the new combination with the generic and specific name.

Price (1931) reviewed the genus Echinochasmus and included the following species in the genus: E. coaxatus Dietz, 1909; E. euryporus Looss, 1896; E. beleocephalus Linston, 1873; E. liliputanus Looss, 1896; E. amphibolus Kaltan, 1922; E. boturi Baer, 1923; E. mordax Looss, 1899; E. magnovatus Stunkard and Haviland, 1924; E. hortense Goto (in Asada); E. japonicus Tanabe, 1926; E. dietzeni Isaisehikov, 1927; E. pitangi Lutz, 1924 and E. schwartzi Price, 1931.

Price (1931) described E. schwartzi and reported that it differed from all the species of Echinochasmus except E. oligacanthus, E. mordax and E. pitangi on the basis of collar spines. He stated that E. schwartzi differed from E. oligacanthus in the size and arrangement of the collar spines and in the comparative size of suckers. According to Price, E. schwartzi possessed collar spines that were distinctly smaller than those of E. oligacanthus; the row of spines is interrupted dorsally by a space as wide as the oral sucker, while in E. oligacanthus the dorsal interruption of spines was very slight. Price (1931) reported that the size ratio of oral sucker to the acetabulum was 1:2 in E. schwartzi and more than 1:4 in E. oligacanthus. He affirmed that E. schwartzi differed from E. mordax by its shorter anterior body length and by the position of the cirrus pouch. In E. mordax the cirrus pouch was largely preacetabular, while in E. schwartzi the posterior end of the cirrus pouch almost reach the posterior border of the acetabulum. The collar spines and eggs of E. mordax were considerably larger in proportion to the size than those of E. schwartzi. Price stated that it was difficult to distinguish this species from E. pitangi due to the meager description of the worm by Lutz (1924). He contended that with the exception of the length (2.4 to 3.4 mm) the characters given for E. pitangi might fit any species of the genus. However, Price believed that his species could be distinguished from E. pitangi by the more posterior position of the testes in

E. pitangi and the size of the eggs as compared with the ovary. In E. pitangi the eggs are larger than the diameter of the ovary and about two thirds as wide, while in E. schwartzi the eggs are not as large in comparison with the size of the ovary.

Beaver (1941) described Echinochasmus donaldsoni as a new species. He was able to complete the life cycle by experimentally infecting guppies (Lebistes reticulatus), perch (Perca flavescens), mollies (Mollienisia latipinna), and bluegills (Helioperca incisor) with the cercariae which were emerging from Amnicola limnosa and A. lustrica collected from Hook point on Douglas Lake and from a pond in Wilderness, Park, Michigan. Metacercariae were fed to a pigeon and the adult worm was recovered after 7 days. Beaver found that wild pied-billed grebes (Podilymbus podiceps) were naturally infected with this worm.

Beaver (1941) reported that there were 27 species which had been allocated to the genus Echinochasmus Dietz, 1909. He stated that the following species should be added to Price's list of 1931: Echinochasmus novalichensis Tubangui, 1932; E. rugosus and E. rediduplicatus Yamaguti, 1933; E. ruficallis Ishii, 1935; E. ruficapensis and E. bagulai Verna, 1935; E. narayani Mudaliar, 1938; E. gorsakii, E. milvi and E. tobi Yamaguti, 1939; and E. reinovarus and E. megavitellus Lal (1939). Beaver thought that E. reniovarus should not be placed in the genus Echinochasmus since it had vitellaria which extended anteriorly into the preacetabular region. Therefore, it was

placed in the genus Episthmium Luhe, 1909. The species is now correctly named Episthmium reniovarus Lal, 1939.

Beaver (1941) contended that the described species of Echinochasmus formed four sub-groups based on the numbers of collar spines. He found that four species, all of which were Japanese, had 28 collar spines; 15 species had 24; 6 species had 22; and E. dietzevi Issaitschikoff, 1927 and E. donaldsoni Beaver, 1941 had 10 collar spines. Beaver (1941) failed to include Echinochasmus magnovatum (Stunkard and Haviland, 1924) Price, 1931 among the 10 collar spined species of Echinochasmus.

Most of the research work on echinochasmid species has been done by investigators of other countries. Kurisu (1931) reported the finding of a new cercariae, Echinochasmus grandis, in Japanese snails (Melania) and its life history. He found that the cercariae developed in Semisulcospina, and encysted in tadpoles. The metacercariae were fed to white rats and puppies after they had been allowed to develop for two weeks in the intermediate host (tadpoles). The adults were recovered after two weeks of development in experimental definitive hosts.

Witenberg (1932), working in Palestine, found the metacercariae of Echinochasmus liliputanus encysted in fresh water fishes of the genera Tilapia and Nemachilus. The metacercariae were fed to cats and dogs. The eggs of the adult worms were found in the stools of these animals two weeks later. Witenberg has also found Echinochasmus mordax in dogs. He reported that specimens

from dogs were considerably smaller than those originally described by Looss (1899) from the pelican.

Verma (1935) studied the Indian species of the genus Echinochasmus and described two new species of Echinochasmus; E. bagulai and E. reficapensis from Indian Birds. He also described an allied genus and species Episthochasmus caninum, a common parasite of Calcutta street dogs. In that same year Ishii (1935) described Echinochasmus ruficallis as a new species from the little grebe. Mudaliar (1938) described Echinochasmus naragani as a new species from Milvus migrans gouinda. He differentiated this species from other Indian species by smaller size, range of yolk glands and notched testes. Yamaguti (1939) described Echinochasmus tobi, E. milvi and E. gorsakii was able to experimentally obtain Echinochasmus novalichesensis from piscine hosts. Lal (1939) described Echinochasmus megavitollus and E. reniovarus from birds of Indian. Prudhoe (1944) showed that the genus Allechinostomum Odhner was synonymous with Echinochasmus and E. famelicus Odhner new combination redescribed.

Johnston and Simpson (1944) worked out the life cycle of Echinochasmus pelecani from Australian host. These authors described this small echinochasmid from the intestine of Pelecanus conspicillatus. They claimed that the melanid snail, Plotiopsis tate, was infected with a cercaria which when exposed to fresh water fish (Oryzias latipes and Gambusia affinis encysted in the gills. These metacercariae had all of the characters of the adults of E. peleconi.

However, they fail to obtain the adults after feeding experiments involving pigeons and rats.

Komiya (1951) reported the finding of the cercaria of Echinochasmus perfoliatus Ratz. Komiya described redia and cercariae from Bythinia striatula in the Shanghai area. He found that the cercariae differed from that of E. perfoliatus var. japonicus described by Muto in several ways; namely: (1) the absence of head spines, (2) the absence of gut, and (3) presence of a row of spines on the acetabulum. Komiya claimed that the cercaria of E. perfoliatus resembled that of the American species, E. donaldsoni, but differed in having a row of acetabular spines, in the absence of a gut, and in the structure of the cystogenous glands. Komiya (1951) was able to infect a small Pseudorasbora parva with the cercariae. He claimed that the metacercariae resembled those of E. perfoliatus.

Yamaguti (1951) traced the developmental history of Echinochasmus japonicus. He found that the rediae and cercariae of E. japonicus developed in Bulimus striatulus japonicus. He was able to infect the gills of Pseudorasbora parva (gold-fish) and Rana ragosa (tadpoles). He was able to obtain the adult worms from a duck after two weeks of development in this host. He found Nycticorax nycticorax from the Bina Sea naturally infected as well as Milvus migrans lineatus. He claimed that these hosts were infected by feeding upon naturally infected gold fish.

Rao (1951), working in Canada, described Echinochasmus

cohensi from Larus argentatus. It had 22 collar spines with the corner spines more posterior than the other. He thought E. cohensi was like E. milvi but the testes lie one behind the other, with their long axis parallel to that of the body. He reported that the vitellaria did not extend so far anteriorly and the cephalic spines were larger, measuring 42-48u.

Gupta (1953) described Echinochasmus antigonus from the Sarus Crane, Antigone antigone Linn. He stated that his species was characterized by an extremely elongated body and longitudinally elongated testes 0.462 - 0.76 mm. a part. He differentiated this species from E. gorsaki by the pharynx being smaller than the oral sucker and by the 24 collar spines. The collar spines measured 0.065 - 0.041 mm. at their base and lie in a single row broken dorsally. He concluded that this species differed from related species in that the vitellaria do not reach the ventral sucker.

Chatterji (1954), also working in India, described Echinochasmus canai from a pariah dog at Allahabad. He claimed that this species was very near E. schwartzi Price, 1931. It differed in that the smooth ovary was on the right side of the median line. It also differed by the presence of a genital sucker. The ratio of the oral and ventral sucker was 1:3 while in E. schwartzi it was 1:4. His species had a well developed cirrus sac and numerous esophageal glands.

Vigueras (1954) studied the helminth fauna of Cuba

and redescribed Echinochasmus megatyphlus. Shigin (1956) working with hosts in Russia, described E. colymbi from Colymbus cristatus. He agreed with Boshkirova (1941) that Monilifer should be included in Echinochasmus as a subgenus. However, he felt that Episthmium Lühe, 1909 should be retained as an independent genus. He argued that the characteristic location of Episthmium in the cloaca, the bursa fabricii and, occasionally in the posterior intestine of birds warranted this action. He argued further that Episthmium was different from other echinochasmid species by its very well developed adhesive apparatus and cuticular spines, and the well developed vitellaria which not only reached beyond the anterior border of the ventral sucker but also filled the median area anterior to it. Shigin transferred Episthochasmus to Episthmium as a second subgenus to the type and gave a diagnosis for Echinochasmus and Episthmium with keys to their subgenera. Shigin differentiated E. colymbi from the six species in the subgenus Episthmium by its well developed collar with large spines and the measurement of its body and organs.

Shakbtakhtinskaya (1956) studied the helminth fauna of 1,044 aquatic birds representing 43 species from various areas of Azerbaijan, USSR. He described Echinochasmus matevassiani from Colymbus cristatus. Bronzini (1956) reported the occurrence of Echinochasmus perfoliatus, (for the first time) in stray cats in Rome.

SECTION III.

DISCUSSION OF FIELD LOCATION

> Maritime salt marshes occur in many parts of the world and locally may be called by other names such as saltings (Norfolk), merseland (Scotland), salt stepe, and so forth. They comprise areas of land bordering on the sea, more or less covered with vegetation, and subject to periodic inundations by tide. They originate as bare mud- or sandflats which, as they become higher, are colonized by algae and flowering plants, the species involved varying in different parts of the world. The plants are enabled to occupy this habitat by virtue of their tolerance of the special conditions obtaining in salt marshes. The advent of the plants on the bare flats promotes further growth in height of the land which, as this "rising" takes place, results in changes in the environment so that different species can enter the area. Salt marshes, therefore, may extend vertically from about mean sea level up to the extreme upper limit of the tides, where they will abut on normal land vegetation or else grade into fresh-water swamp.
>
> V. J. Chapman, 1960

Chapman (1960) has studied the salt marshes of the world and claimed that they generally conform to a definite geomorphological pattern though differing in physiography. His overall survey of marshes indicated that they fall into a number of distinct groups, which are based on types of vegetation and substratum. Chapman designated the maritime salt marshes on the western side of the Atlantic ranging from south-eastern Canada southward to Florida and Louisiana as the Eastern North American group. Of his nine groups of world-wide maritime salt marshes, the type I worked in is listed as number four and is subdivided into the following subgroups: (a) Fundy type which are characteric of the Bay of Fundy and adjacent part of the Canadian coast; (b) the

New England type which stretches from Maine to New Jersey and includes those of Long Island; and (c) the Coastal plain types which extend from south of New Jersey to Florida and Louisiana.

According to Chapman (1960) the above subgroups of marshes differ in origin and substratum. For example, the Fundy type is formed in front of soft coastal cliffs with rivers and tidal erosion providing a perpetual source of silt. The silt is usually reddish in color, extremely fine, and forms a compact firm substratum. In contrast to the Fundy type, the New England type has developed in front of hard-rock cliffs and relatively little silt is available from marine sources or rivers. In fact, they are largely built up of marine peat. Chapman stated that the Coastal plain type of marshes, like the Fundy type, have developed in front of soft-rock cliffs and the supply of silt is abundant. But, the soil differs from the Fundy type in that it is gray in color instead of red and does not form a firm compacted substratum.

Johnson (1925) gave an excellent account of the geological structure of New England type tidal-marshes. Knight (1934) has also studied marshes of New England and concluded that they were land forms which developed in conjunction with a shoreline of post glacial progressive submergence. He outlined the following stages associated with progressive submergence for the New Haven, Connecticut Region:

A. During early submergence, a bay-mouth bar develops between two headlands, formed of detritus from those headlands. A sheltered lagoon forms behind the bar.

B. Submergence continues and a part of the bordering upland and freshwater swamp is destroyed. The lagoon fills with silt to a certain level; then builds up with Spartina alterniflora (=S. stricta), then builds up to a hightide level with Spartina patens. (The so-called Shaler Marsh). The bar moves landward.

C. Submergence continues and more of the upland and freshwater swamp are destroyed by encroaching tidal-marsh. The lower silt and Spartina-alterniflora-peat horizon remains unchanged; Spartina-patens-peat layer thickens. Bar moves landward.

D. Submergence continues and more of the upland and freshwater swamp are destroyed. The Spartina-patens layer becomes thicker. The bar moves farther inland.

E. Submergence continues and more of the upland and freshwater swamp are destroyed. The patens layer becomes thicker. The bar moves so far inland that it now overlies freshwater-peat and upland remains at the bottom of the marsh; no silt or alterniflora layers remain. (The so-called Mudge-Davis Marsh).

F. The original bar is completely removed by erosion leaving the tidal-marsh unprotected and itself exposed to erosion. The eroded front is composed of patens peat, overlying freshwater peat and upland remains.

Miller and Egler (1950) studied vegetation of the Wequetequock-Pawcatuck tidal-marshes and gave the following general description of marshes on the New England Coast:

> The tidal-marsh of the New England coast is a distinctive and easily recognized land-form. It is a flat meadow at or below the level of the highest tides, originally bounded abruptly on the landward side by scrubby and forested uplands and by freshwater swamp and marsh, and equally abruptly on the seaward side either by a bay-mouth sandbar, or by an escarpment of 0.5-1.0 meters leading to a muddy tidal flat. The marsh substratum is generally a fibrous peat, mixed with more or less silt or sand, and is considered to be mainly an organic accumulation in valley mouths and behind off-shore bars.

V. J. Chapman (1960) points out that the two primary physiographic features of maritime salt marshes are the

creek system and the salt pan. It is granted by various workers that depressions or pans occur within marshes and some are formed in conjunction with creek systems. However, it appears that investigators are not in agreement as to the classification or origin of depressions or pans that have not developed along creeks (Penfound and Hathaway, 1938; Taylor, 1938; Chapman, 1940a; Miller and Egler, 1950).

Chapman's (1960) conception of a creek pan is that a depression is produced when vegetation grows across a creek and dams up any portion of it, or when lateral erosion causes blocks of soil to fall into the creek and dam it up, or when channels become dammed by vegetation growing across a creek. Consequently, effective drainage no longer takes place above the dam and water remains standing in the area after flooding tides.

Chapman (1960) is of the opinion that the majority of pans typical to salt marshes might be categorized as primary pans. The term implies that this type of pan is contemporaneous with the development of marshes. He believes that these pans are formed as a result of irregular plant colonization which takes place in the early stages of marsh development. Chapman suggested that irregular plant colonization leads to bare areas surrounded by vegetation. He maintained that these bare areas, as the marsh rise in level, loses any outlet with water that it may previously have had. However, during spring tides, and for some time thereafter, the pan will retain water.

Other types of pans occurring in marshes may be

derived from the creek or primary pan. Chapman (1960) postulated that such pans may be formed by vegetation slowly growing across a pan and dividing it up. This type of pan has been designated as subdivision pans. Chapman stated that pans of this type usually occur in marshes where one or more plant species propagate vegetatively, e.g. maritime grasses and rushes.

Since workers are not in agreement as to the origin or classification of the various pans that occur in marshes, I am simply calling the sites (where I have collected Hydrobia and Fundulus) "Depressions." Similar liberties were taken by Maul (1958) in reading his paper (Ecological observation of Pools in Salt Marshes of New Jersey) at the Salt Marsh Conference held at Sapelo Island, Georgia. No objection was made of his use of the term "pool" in his description of what was obviously a particular kind of depression.

Several depressions in the marshes of Southeastern New Hampshire were chosen as collection sites. The locations were at Durham, South Newington, and Hampton, New Hampshire.

The marsh in Durham is intersected by Johnson Creek which empties into Little Bay via the Oyster River. There are several depressions adjacent to the creek. At low tide, (depending upon the time of the day), these depressions have been found to contain 6 - 12 inches of water. At high tide the creek is filled to the brim and overflows into the depressions and general marsh area. Since all vegetation and depressions are completely covered by water at high

tide, at least once a day, the area was designated in a "low marsh" (Davis, 1957). At low tide the depression is nearly covered with a mat of green algae (Maul, 1958 found the algae in New Jersey to be primarly Cladophora expansa). This depression might very well correspond to V. J. Chapman's description of a creek pan.

The animals collected here were Fundulus heteroclitus and Hydrobia salsa. Hydrobia salsa was the predominate snail in the depression though some specimens of Nassa obsoleta were also in the depression. Other invertebrates observed were shrimps, amphipods, copepods, archiannelids and polychaetes depending on the time of the year. This type of association might be called a "Fundulus-Hydrobia Biotic Community."

The depression studied in the marshes at South Newington is adjacent to Great Bay. It is completely isolated and has no connection with water outlets or drainage ditches. The general area is not subjected to the daily floods of tidal waters. Since these marshes are only submerged by spring tides, they are designated as a "high marsh" (Davis, 1957).

This depression contained 2 to 3 feet of water. The bottom of the depression, like the one at Johnson Creek, was covered with a mat of green algae. The substratum is composed of an oozy, foul-smelling muck. This depression fits the description of the primary pan of Chapman (1960) or the "pothole" of Miller and Egler (1950) though the two workers are not in agreement with the names for these

depressions.

The animals collected here were Fundulus heteroclitus and Hydrobia minuta. The predominate snail in the depression was Hydrobia minuta. A few specimens of Nassa obsoleta were present. Apparently, the animals get trapped in the depression during submergence at the time of the spring thaw. Other animals observed were oligochaetes, alleocoels, archiannelids and amphipods.

The marshes at Hampton are adjacent to Hampton River. The river empties directly into the Atlantic Ocean. There are many depressions in the area and these are separated from each other by only a few feet. The bottom of these depressions contain a thick peat layer (one to two feet). The bottoms are unlike the oozy, foul, muck condition of those at Johnson Creek or South Newington. Amnicola and Fundulus were collected from these depressions. This might be described as a Fundulus-Amnicola Biotic Community.

Numerous muskrats have been observed in the marshes at Hampton. During the months of January and February twenty-six muskrat lodges were counted.

SECTION IV.

MATERIALS AND METHODS

The mollusks which served as the first intermediate host in this study were collected from three different depressions in the salt marshes of southeastern New Hampshire. Hydrobia salsa was collected from the depressions near Johnson Creek at Durham, New Hampshire while Hydrobia minuta was collected from an isolated depression near Great Bay at South Newington, New Hampshire. Amnicola sp. was collected near the Hampton River at Hampton, New Hampshire.

The Hydrobia salsa and Hydrobia minuta were collected with a fine mesh net by making a number of sweeps along the surface of the mud and weeds of the habitat. They were brought back to the laboratory in one gallon plastic containers.

Snails were isolated from the mud and debris by several methods. The first method used was to cover the one gallon plastic container and leave them in the "cold room" (Aquarium Room 55°F). The snails would come to the surface of the water and adhere to the container in a complete ring above and below the surface line. The snails were then skimmed off and placed in dilute sea water (1/3-1/2 full strength). Another method was to dip some of the mud and debris directly into a plastic container of dilute sea water (one half strength) and stir vigorously by hand. The contents were decanted through a standard Tyler Screen Scale (32 meshes to the inch with 0.5 mm openings). All of the

debris and a few snails were slowly decanted through the screen. Most of the snails settled to the bottom of the container and could be transferred directly to clean dilute sea water. The few snails that were decanted with the debris were picked up with forceps. Later, it was found that snails often come up from the bottom of the depressions and settle in the surface mat of algae that often covered the depressions near Johnson Creek in Durham, and the depressions near Great Bay in South Newington. These snails could be isolated in the field by taking several handfuls of algae and stirring it vigorously in a half gallon of water. The snails would fall out of the algae and settle to the bottom of the container.

The Amnicola collected in Hampton were isolated on the field by shaking weeds, leaves, and other objects into a half gallon of water. The snails were dislodged and settled to the bottom of the container.

Approximately 30 to 50 snails were placed in each of 30 vials and covered with dilute sea water. The water was checked several times a day for cercariae. Vials containing snails that were passing cercariae were broken down into groups of 10 snails for each vial. Snails passing cercariae in the second group were broken down into a third group which contain 5 snails to a vial. Snails in the third group, if passing cercariae, were broken down into a fourth group which contained 2 snails to a vial, and these down to the infected snails. This method was abandoned after the first

summer since many of the snails died during the process of isolation. It was observed that only the larger snails (II age class) were infected and they were isolated in groups of 10 to a vial. This method was found superior since the infected individual could be isolated in a relative short period of time.

Infected snails were isolated for morphological studies on the cercariae and eventually cracked to observe developmental stages of the parasites. After considerable isolation, half of the uninfected individuals (those not passing cercariae) were cracked to see if developmental stages of the parasites were present. One-fourth of the other half were used for experimental infections. The other fourth was used as a final control.

Rediae stages were removed from the digestive glands of the snails by the use of a dissecting needle or otherwise left in Ringers solution and allowed to leave the digestive gland. Rediae were fixed in corrosive sublimate by placing them on a slide in a drop of saline, covering with a cover-glass, placing corrosive sublimate at one edge, and drawing it under the cover-glass by means of filter paper placed at the opposite edge.

Morphological features of the living cercariae were studied both with and without neutral red as an intra-vitam stain. Flame cell patterns were studied under cover-glass pressure and the oil immersion objective of the microscope. Description and measurements of the cercariae are based on cercariae that emerged spontaneously from the snail and were

killed in hot sea water according to the method of Cable (1956).

A reserve supply of snails was isolated in large finger bowls and maintained in the "cold room" (55°F). They were fed a weekly diet of boiled spinach. This method was abandoned because it was found time consuming. A better method was to stack the finger bowls containing the snails on top of each other and taking care to include some of the debris, algae, and organic content from their natural habitat. Snails kept in this manner were not fed and survived for over eight months in the laboratory.

In cases where attempts were made to study the biology of the snail, only one hundred to two hundred snails of the larger size or II age class were allotted to each finger bowl. Under these circumstances snail deaths were kept at a minimum.

In cracked snails, males were separated from female snails by the ovarian mass (containing the maturing ova) of the female, and the relatively large, fleshy, penis of the male. The male penis was located on the dorsal side and to the right (between the head and mantle) forming the vertex of an angle with the two tentacles.

During the routine cracking of snails and observations for developmental stages of parasites, it was difficult to determine if males were more often infected than females and vice versa. None of the developmental stages of parasites were ever found in females which contained developing ova in the ovarian mass. But developmental stages of the

parasite were often found in snails without male organs and without ovarian masses. Males that were either infected or uninfected could be easily spotted by the presence of the penis. A tentative hypothesis regarding infection in male and female snails is that if male and female snails occur equally in number in the population, male snails are more often infected (at least during breeding seasons) since developing stages of the parasite were never observed in reproducing female snails.

The second intermediate host was Fundulus heteroclitus. They were collected with a minnow trap by placing a slice of bread in the trap and lowering it into the depressions in the study area. They were kept in the "cold room" in a tank of circulating sea water supplied with a continuous stream of oxygen.

The experimental second intermediate hosts used were marble mollies, red swordtails, green tuxedo swordtail, red platies, red wagtail platies, and red tuxedo platies.

According to Axelrod and Schultz (1955) the marble molly is a hybrid resulting from the cross between the green sail-fin molly, Mollienesia latipinna, and the black sail-fin molly, Mollienesia sphenops. They reported that the red swordtail and the green tuxedo swordtail are both hybrids resulting from the cross between the green swordtail, Xiphophorus helleri Heckel, and the red platy, Xiphophorus maculatus Gunther. Axelrod and Schultz (1955) declared that the red wagtail platy, and the red tuxedo platy were all color variation of the red platy, Xiphophorus maculatus Gunther.

Various investigators are not in agreement in the classification of aquarium fishes and fishes which are wild. Both Innes (1951) and Axelrod and Schultz (1955) place the egg-laying tooth-carps including Fundulus in the family Cyprinodontidae, a family which embraces top minnows. They placed the live-bearing tooth-carps in the family Poeciliidae. Innes (1951) placed both groups in the order Cyprindontes (Cyprindontiformes: American Fisheries Society 1960). Bigelow and Schroeder (1953) placed Fundulus in the family Poeciliidae. However, the American Fisheries Society, Special Publication (No. 2, 1960) like Innes (1951) and Axelrod and Schultz (1955) placed both the egg-laying tooth-carps and the live-bearing tooth-carps in the order Cyprinodontiformes; placing the killifish (egg-laying tooth-carp) in the family Cryprindontidae and the live-bearing tooth-carp in the family Poeciliidae.

The experimental second intermediate hosts were closely related to Fundulus heteroclitus in that they were tooth-carps and belonged to the family Poeciliidae. They differed from Fundulus in that they were live-bearers while Fundulus is an egg layer. The experimental second intermediate hosts were gradually introduced to sea water to a concentration of half full strength over a period of two weeks and maintained at room temperature. It was only necessary to bring them up to half-strength of sea water since this represented the average salinity in the depression where the first intermediate host and Fundulus were taken during July and August.

The experimental second intermediate hosts were infected by placing them in a plastic gallon bucket which contained 3,000 cc of dilute sea water (one-half strength) and approximately 1/16 of a gallon of Hydrobia salsa. The fish were exposed to cercariae passed by the snails for a period of 4-6 hours. The water containing these animals were aeriated every other hour. Care was taken to see that cercariae were still present in the "exposure bucket" by drawing some of the water off with a pipette and examined in a Syracuse watch glass under the wide-binocular microscope.

The experimental tropical fish were purchased from the Ashmont Tropical Aquarium in Merrimack, Massachusetts. The owner (Lionel A. Lambert) stated that he ordered all fish from Tampa, Florida where they were laboratory bred. The fish were bought on three separate occasions in lots of one dozen. On each occasion several fish died before they could be brought to the laboratory. These fish were used as a control and were carefully examined (gills and conus arteriosus) for metacercariae. Of the seven control fish examined, none contained metacercariae.

Studies were made on the growth of the metacercariae (Ascocotyle (Phagicola) diminuta) from the gill arches of experimentally infected fishes. Measurements of the length and width were taken of 10 to 20 metacercariae from the gill arches after one day, three days, 14 days, 16 days, 18 days, and 30 days of encystment. These measurements were compared with measurements on the metacercariae from wild Fundulus heteroclitus that were taken from depressions in the study

area. The wild Fundulus were kept in the Aquarium Room for eight months, two months, and two weeks for purposes of determining if there were changes in the growth rate of metacercariae. From such studies an estimation was made on the average length and width which the experimental metacercariae had to reach before becoming infective for experimental definitive hosts. This was carried out by feeding gill arches containing metacercariae which had reached the estimated size limit of infection to the various experimental definitive hosts.

Further morphological studies were made on two weeks to four week old metacercariae. They were removed from the gill arches by allowing a gill arch to remain in Ringer's solution (consisting of the balance variety of salts plus dextrose) for a week. After a week, the gill filaments had started to break down. The metacercariae were removed from the decaying filaments by gentle agitation with a dissecting needle. Although the filaments decayed at a relatively fast rate, the metacercariae were still alive and moving around within the cyst. Next the metacercariae were transferred to clean Ringer's solution where they were allowed to remain for another week. On the second week they were removed from the partially decayed cyst wall with fine pointed insect needles. These experimental metacercariae were compared with metacercariae previously removed from wild Fundulus by the same technique employed for experimentally infected fishes.

Some excystment of metacercariae from the gills of

wild Fundulus was carried out by the method of Macy and Moore (1954) employing weak concentrations of sodium hydroxide. However, excysted metacercariae obtained by the method of Macy and Moore (1954) were not used for comparison studies since the method could not be refined and all specimens obtained were dead. It was not advisable to use such specimens for critical morphological studies.

The experimental definitive hosts used were white mice, white rats, and chicks. All of the white mice used were laboratory reared from 12 mice purchased by the Zoology Department during the Summer of 1959. All 7 to 10 day old chicks were hatched in an incubator by the Poultry Department. Two groups of one-day old chicks (10 in each group) were hatched out in the incubator of the Zoology Department from eggs obtained from the Bacteriology Department. A third group of 20 (one-day old) chicks was hatched in the incubator at the Poultry Department.

The white rats used were acquired from Dr. W. L. Bullock. All white mice and white rats were fed gill sections from fish. The animals were first separated one to a cage and were given no food in a 24 hour period. The animals were given water in a Syracuse watch glass placed on the floor of the cage. After the allotted 24 hours gills from one or several fish (see tables on feeding experiments) were placed in the Syracuse watch glass along with a little water. This method was followed with both gills from fish that were infected in nature and those from fish infected in the laboratory.

Chicks were fed differently. They were fed all or part of the gills from one fish by holding their mouths open and placing the gills in the back of their mouths with forceps. They were given water from pipettes or otherwise release and allowed to drink from syracuse watch glasses.

All chicks and mice were killed in 1,000 ml. beakers containing a layer of cotton on the bottom which was saturated with chloroform. These animals were placed in the beakers and covered with a flat object. White rats were killed by lowering a paste board box, containing cotton saturated with chloroform, over the cage.

All animals were examined for parasites by taking the intestine out and placing them in a saline solution. The small intestine was cut into two inch sections, placed in the bottom of a petri dish. The sections of the small intestine were laid open with scissors. After the extraction of obvious helminths, the organ was scraped and contents poured into a small jar. The contents from 2 inch sections of the intestine were concentrated by the shaking technique of Looss.

All specimens of Ascocotyle (Phagicola) diminuta were placed in syracuse watch glasses, containing Ringer's solution and placed in the refrigerator for 6 or 12 hours. The Ringer's solution was drawn off with a pipette and replaced with hot Bouins. Specimens of Echinochasmus magnovatum were treated in the same manner, but many specimens died in the unextended condition. Better results were obtained when the echinostomes were relaxed by adding a

small amount of menthol to the Ringer's solution which contained them. Some of the echinostomes were fixed on a slide under cover-glass pressure in the same manner as rediae were fixed (see the above) but with Bouin's fixative instead of corrosive sublimate.

All whole mounts were stained in Grenacher's alcoholic borax-carmine used by the Lynch's precipitation method. All sectioned material were stained in Ehrlich's hematoxylin and conterstained in eosin.

The millimeter was used as the unit of measurement.

SECTION V.

Morphology and Life History of Ascocotyle (Phagicola) diminuta (Stunkard and Haviland, 1924) Stunkard and Uzmann, 1955

A. Observation and Experiments

Fundulus heteroclitus are trapped, during low tide, in the depressions of salt marshes, bisected by Johnson Creek, near Durham, N. H. They were often seen swimming along the bottom of depressions which contain one to two feet of water (depending upon the time of the day). Fundulus heteroclitus collected from these sites had metacercariae of Ascocotyle (Phagicola) diminuta on their gills. The metacercariae were of various sizes and stages of development, including many that had recently encysted.

Numerous brackish water snails, Hydrobia salsa Pilsbry, were found in depressions; burrowed in the subtratum, adhering to the surface mat of algae or were among vegetation along the edges. Hydrobia salsa collected from these depressions and isolated in small vials, passed heterophyid cercariae of the pleurolophocercous type.

During the summer of 1959, killifish were collected and kept in the laboratory for over a month to allow all metacercariae to grow to maturity. Several of these fish were placed in a small aquarium containing diluted sea water (1/2 full strength) and a large number of snails (Hydrobia salsa) collected from the same depression.

A second group of fish, serving as the control, was

maintained in another aquarium without snails. The experiment was allowed to run for three weeks. After the allotted time, the gills of the fish were examined. Fish from the experimental aquarium had large numbers of metacercariae on their gills. Nearly all of the space on the gill filaments were occupied by metacercariae. Many of the metacercariae had recently encysted and had eye spots, no oral spines, poorly developed excretory vesicles (no granules on the inside and very small), and were unfolded inside their thin-walled cyst. Some of the cercariae had encysted on mucus secreted by the gills and were in the same stage of development. Their small size and unfolded condition inside the cyst indicated that they had recently encysted. Older metacercariae were larger, had scattered eye spot pigment, oral spines, prominent excretory vesicles (large with granules on the inside), and the worms were folded in their cysts.

Fish from the control aquarium did not possess the heavy infection of metacercariae on their gills as did those from the experimental aquarium. All metacercariae had reached the advanced stages of development. All worms had scattered eye spot pigment, oral crown spines, prominent excretory vesicle, and were folded inside of their cyst walls. The above experiment was repeated twice in 1959 and once during the summer of 1960 with the same results. These experiments indicated that the heterophyid cercariae (pleurolophocercus type) from Hydrobia salsa encysted on the gills of Fundulus heteroclitus and agreed substantially

with older metacercariae described as those of Ascocotyle (Phagicola) diminuta by Stunkard and Uzmann, 1955.

Killifish collected directly from Johnson Creek (stream) were also infected. Therefore infections were picked up while Fundulus heteroclitus were trapped in depressions (where the brackish water snail, Hydrobia salsa, are abundant) all along the creek. Since many of these animals come up into waters of low salinity to spawn many of them were probably infected while they were quite young. This, perhaps, could account for the fact that Fundulus heteroclitus without metacercariae on their gills are seldom found.

Killifish collected from the depression in South Newington were more heavily infected. The degree of infection in fish collected from this site came closer to the heavy infection obtained in experimental aquarium than those collected from Johnson Creek.

Fundulus collected from Crommet Creek in Durham, N. H. also were infected and the degree of infection was about the same magnitude as those collected from Johnson Creek.

Fundulus heteroclitus collected from depressions near Meadow Pond in the vicinity of the Hampton River at Hampton, N. H. where the salinity was much lower had only slight infections. The snail most often encounted was Amnicola sp., a member of the Hydrobiidae, but restricted to fresh water. These snails did not pass heterophyid cercariae.

The incidence of cercarial infection in snails were determined by crushing 100 to 300 snails (Hydrobia salsa) each month from Johnson Creek; when the second snail species (Hydrobia minuta) was examined from South Newington, 200 each were examined. It was found that only the larger snails (probably of the II age class) were infected. However, in selecting snails for crushing no attempt was made to separate larger snails from smaller snails. Therefore, the distribution of larval trematodes in the Hydrobiidae, shown in Table I, is based on random sampling. Although several families (Heterophyidae, Microphallidae, Allocreadiidae, and Notocotylidae) were present in these snails, no double infections were encounted in 2,881 snails.

The average level of infection (for all families) in H. salsa was from 1 to 4 percent. A peak of 5.4 was reached during the latter part of July and gradually tapering off during the months that followed. The incidence of infection in H. minuta was higher. There was an increase in infection from the latter part of July with a peak of 21.5 percent near the end of September. Infections with all parasites dropped to 10.6 percent in early November (see Table I).

Measurements of heterophyid metacercariae on the gills of wild Fundulus heteroclitus from all study areas indicated that there were considerable variations in both length and width. A second set of experiments with wild Fundulus was set up to determine (1) if the variations in size of metacercariae on their gills would reach uniformity after a long period of time and (2) if not, was the

TABLE I. DISTRIBUTION OF LARVAL TREMATODES IN THE HYDROBIIDAE OF SOUTHEASTERN NEW HAMPSHIRE

Date	Locality	Snail	Examined	Infected	Percent of Infected	Family
4/24/60	J. Creek	H. salsa	102	1	0.9	Allocreadiidae
6/17/60	J. Creek	H. salsa	310	6	1.9	Heterophyidae
7/21/60	J. Creek	H. salsa	164	3	1.8	Allocreadiidae
				1	0.6	Microphallidae
				5	3	Heterophyidae
7/24/60	S. N. Creek	H. minuta	321	5	1.5	Heterophyidae
				37	11.5	Microphallidae
				1	0.3	Notocotylidae
8/27/60	J. Creek	H. salsa	360	8	2.2	Heterophyidae
				2	0.5	Microphallidae
				5	1.1	Notocotylidae
				3	0.8	Allocreadiidae
8/27/60	S. N. Creek	H. minuta	300	16	5.0	Heterophyidae
				26	8.6	Microphallidae
				6	2.0	Notocotylidae
9/29/60	J. Creek	H. salsa	200	3	1.5	Heterophyidae
				2	1.0	Microphallidae
				1	0.5	Allocreadiidae

TABLE I. (Continued)

Date	Locality	Snail	Examined	Infected	Percent of Infected	Family
9/29/60	S. N. Creek	H. minuta	200	8	4.0	Heterophyidae
				33	16.0	Microphallidae
				3	1.5	Notocotylidae
11/11/60	J. Creek	H. salsa	300	4	1.3	Notocotylidae
				2	6	Heterophyidae
11/11/60	S. N. Creek	H. minuta	300	3	1.0	Notocotylidae
				4	1.3	Heterophyidae
				25	8.3	Microphallidae
11/12/60	H. Creek	Amnicola sp.	87	3	3.0	Allocreadiidae
12/8/60	H. Creek	Amnicola sp.	32	0	---	----------
12/10/60	Freeze					
4/3/61	S. N. Creek	H. minuta	150	0	---	----------
4/3/61	J. Creek	H. salsa	55	1	1.8	Allocreadiidae
			12881 Total			

variation in metacercarial size significant. Fish from South Newington were from standing water and remained in the depression with infected snails (Hydrobia minuta) for at least a year. This time period was estimated on the grounds that the depression was completely covered with water during the spring thaw; at which time fish had a chance to leave the depression. Fish from Johnson and Crommet Creek were in and out of the depressions at least once a day. Therefore there was no way of estimating the age of metacercariae.

Measurements were made on metacercariae from the gills of Fundulus heteroclitus taken from Johnson Creek and South Newington after three weeks isolation in the cold room (55°F). The three week time period was for the purpose of allowing metacercariae that had recently encysted to grow to the infective size. All metacercariae measured would represent the theoretical size that any experimental metacercariae would have to reach before becoming infective for experimental definitive host (white mice, white rats and day-old chicks). Fish from Crommet Creek were kept in the cold room for eight months before measuring metacercariae in order to determine (1) if there would be less variation and (2) did metacercarial size approach those from standing water.

The table below shows that the average measurements for metacercariae on the gills of fish from Johnson and Crommet Creek are nearly the same though the latter had been kept in the cold room for eight months. The number of metacercariae per gill arch is much greater in fish from

standing water than those from running water. It appeared that metacercariae stopped growing in the "cold room" after they reach the infective size. The average measurements of metacercariae from South Newington differ from those of both Johnson and Crommet Creek.

Table II. Comparison of Average Measurements of Metacercariae on Gills of Wild Fundulus heteroclitus

Metacercariae per Gill Arch	Range of Size	Mean	Coefficient of Variation	Location of Depression
12	(L) 0.11 - 0.21	0.16	19%	J. Creek (running water)
	(W) 0.07 - 0.14	0.10	23%	
42	(L) 0.12 - 0.25	0.19	17%	S. Newington (standing water)
	(W) 0.08 - 0.17	0.12	17%	
23	(L) 0.11 - 0.22	0.16	17%	C. Creek (running water)
	(W) 0.07 - 0.15	0.10	21%	

The graphs in Figures 1, 2, and 3 represent every metacercariae that was measured (length and width). The degree of variation from Johnson and Crommet Creek are considerable but comparable to each other as being representative of a running water habitat. The graph for South Newington shows less variation in length and width of metacercariae than those from Johnson and Crommet Creek. The graph for South Newington also indicated that the width of metacercariae approaches a more uniform size than for length.

FIGURE 1. METACERCARIAE FROM THE GILLS OF WILD Fundulus heteroclitus (JOHNSON CREEK AT DURHAM, N. H.).

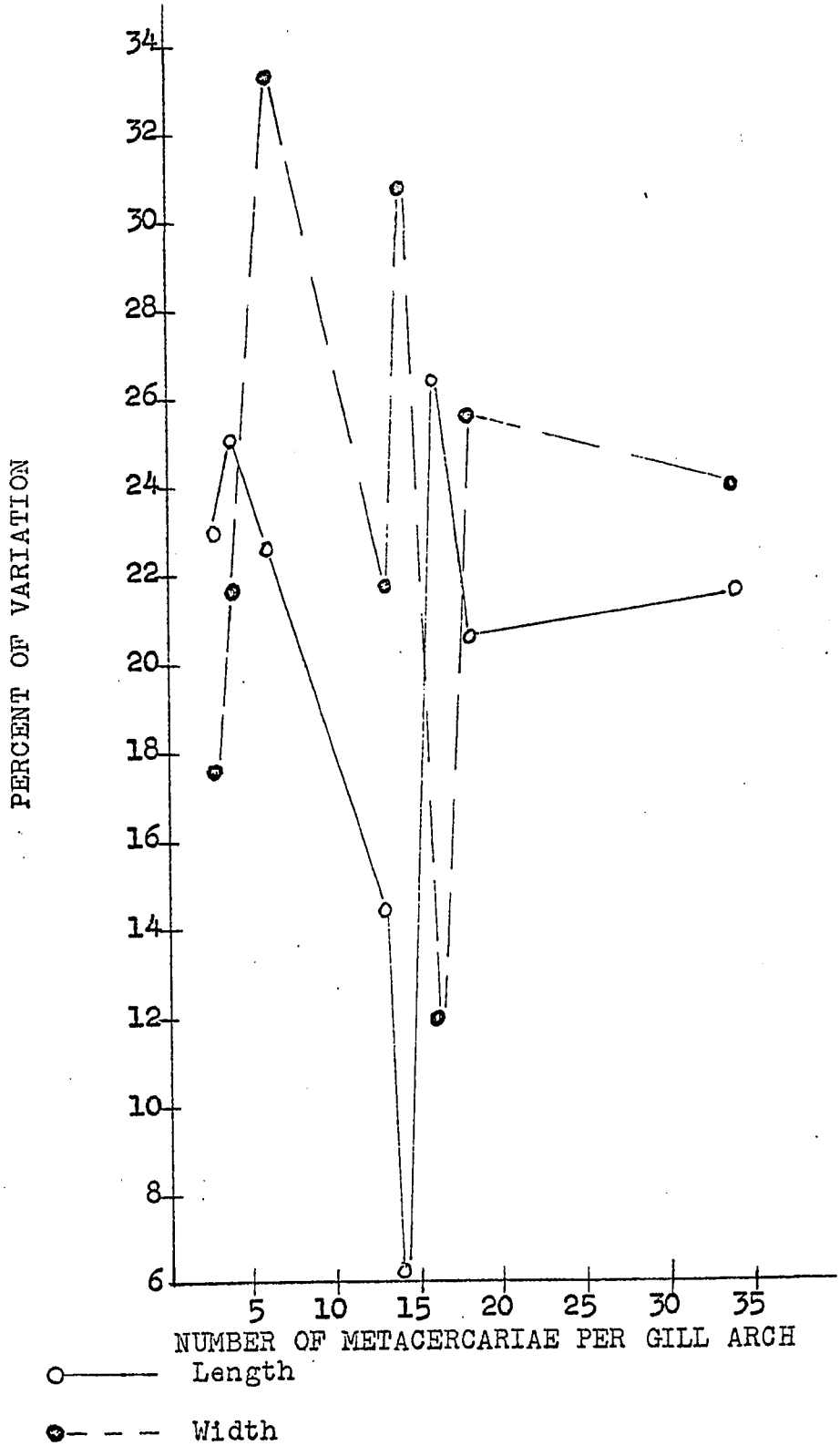


FIGURE 2. METACERCARIAE FROM THE GILLS OF WILD Fundulus heteroclitus (CROMMET CREEK AT DURHAM, N. H.)

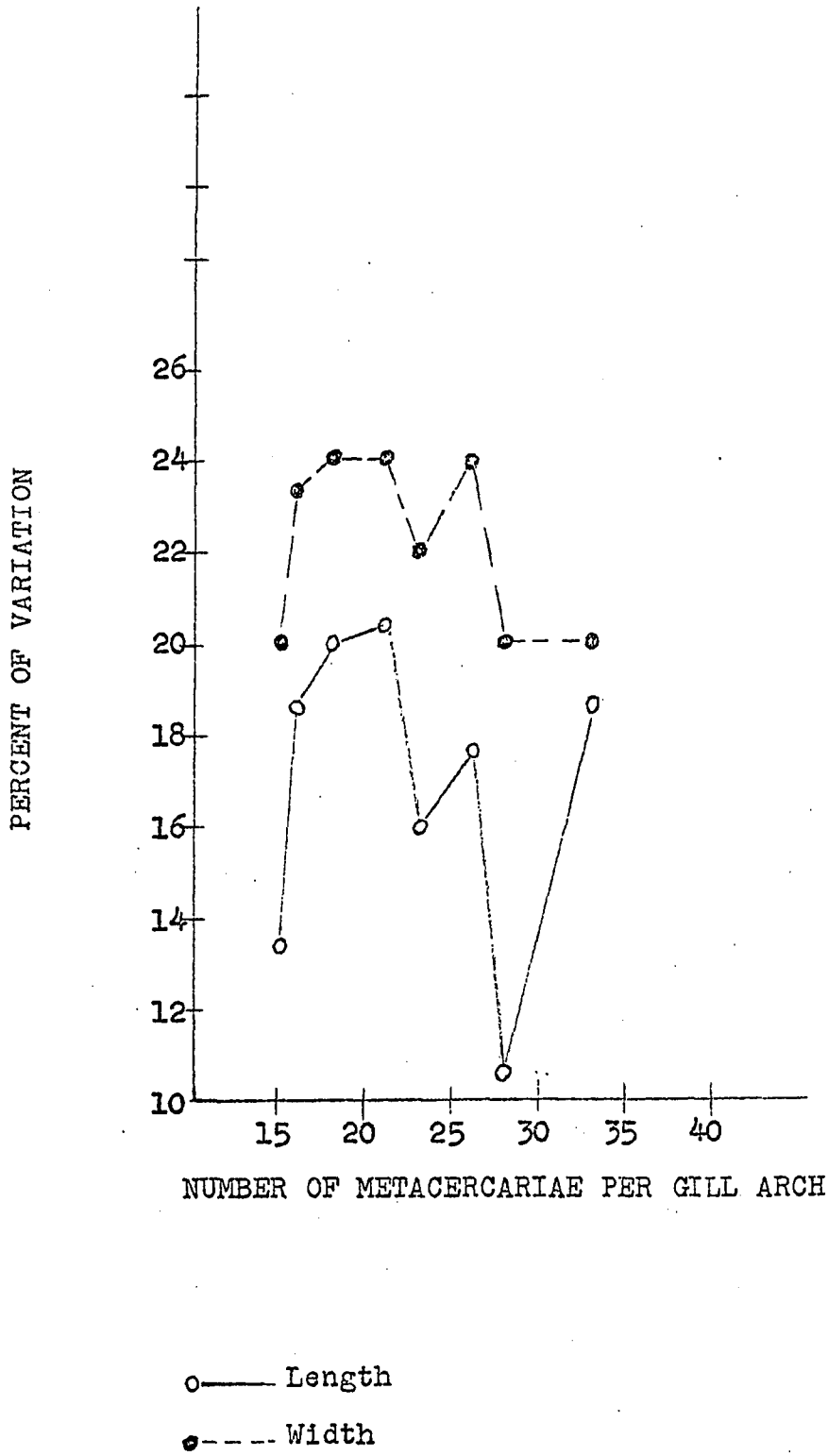
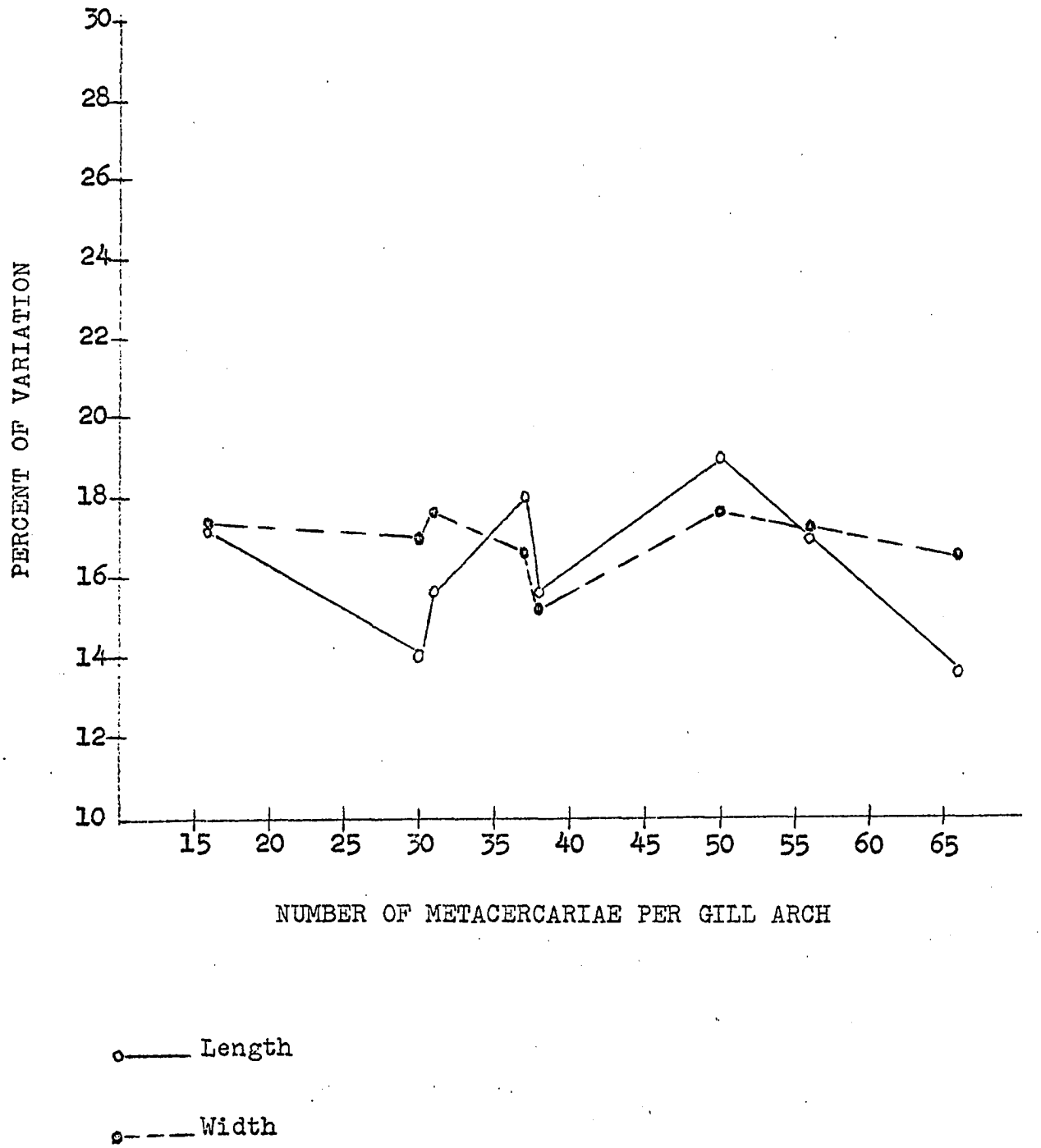


FIGURE 3. METACERCARIAE FROM THE GILLS OF WILD Fundulus heteroclitus (SOUTH NEWINGTON, N. H.).



This may be explained by the way metacercariae position themselves on gill filaments (their longer axis parallel to the longer axis of the gill filament). Therefore, they had more room to grow in length and less room for width.

The variation in sizes of metacercariae from different habitats studied led to critical feeding experiments which involved fish from the various localities and experimental definitive host (white mice and chicks). These experiments indicated that the same worms were involved. Worms were recovered from experimental host after allowing them to remain for different time periods. Appendix I shows the results of the feeding experiments and the remark column describes the method of feeding, length of experiment, and developmental status of worms after recovery.

The results of these feeding experiments agree and augment those of other workers. Some metacercariae of Ascocotyle (Phagicola) diminuta were able to grow to maturity in mice three days after the infective feeding, while others required as long as eight days. The worms appeared to mature at different sizes. All metacercariae were able to grow to maturity after only 4 days in day-old chicks. Mature worms grew to larger sizes in chicks than in mice. Infections were tried with 7 day-old chicks that had been on a diet of chicken feed; no worms were recovered. In all experiments with chicks, where worms were recovered, day-old chicks were used. The usual method of infection was to place infected gills in the back of their mouths with forceps (followed by a pipette of water) while their feathers were still wet.

Susceptibility to infection in day-old chicks and the degree of refractivity in older chicks lend support to the opinion of other workers (Sogandares-Bernal and Bridgman, 1960) that new born chicks are more susceptible to trematode infections than older chicks.

The earlier experiments (see the above) with wild Fundulus heteroclitus indicated that the heterophyid larvae (pleurolophocercous type) passed by Hydrobia salsa Pilsbry were encysting on the gills as metacercariae of Ascocotyle (Phagicola) diminuta. For more conclusive evidence, tropical fish which were laboratory-reared were used. The fish of choice was Fundulus chrysotus, a species that has been laboratory-reared. However, the only fish that were in stock were marble mollies, red swordtails, green tuxedo swordtails, red platies, red wagtail platies, and red tuxedo platies.

Cercariae encysted as metacercariae on the gills of all tropical fish used. Better infections were obtained with the red swordtail and their varieties and green tuxedo swordtails than with the marble molly. The marble molly appeared to have had a low degree of susceptibility to infection and had only a few metacercariae on their gills. There is no evidence that this was due to manner of infection since all mollies were exposed to cercariae along with other fish and in the same "exposure bucket."

Measurements were taken of 10 to 20 experimental metacercariae after 2 to 4 weeks of development. Some measurements were taken of metacercariae at 1 to 3 days

while studying cyst formation. Cercariae are swept into the gill chamber of fish and attached to gill filaments. They get a hold on the gill filament by the use of the special arrangement of spines around the mouth (spinose armature). They have been observed a few hours after attachment and were found lying against the gill filament with the spinose armature embedded and tail thrown off. At the end of the first day, a thin wall surrounds the cercariae, apparently secreted by the cystogenous glands. The worms actively moved inside the thin walled cyst. The eye spot pigments were still intact as were in the cercariae. No oral spines could be seen at this point in development and the excretory vesicle appeared in about the same stage of development as was in the cercariae.

Feeding experiments were carried out with these young metacercariae. A white mouse was examined five days later and found negative. It is generally known that metacercariae require two weeks or more in the second intermediate host before they are able to grow to maturity in definitive host. Therefore, negative results were expected, but there might have been a chance of these larval stages completing their development in the muscosa of the mouse. Serial sections of the intestine yielded further negative results.

After two weeks of development in tropical fish, metacercariae had increased their length by a factor of nearly $2\frac{1}{2}$, and their width almost by a factor of 2 (see Table III). The worms were folded inside the cyst walls, excretory vesicle well developed (characteristic Y-shape and

TABLE III. COMPARISON OF MEASUREMENTS OF EXPERIMENTAL METACERCARIAE ON THE GILLS OF EXPERIMENTALLY INFECTED TROPICAL FISH. (FIRST LINE OF FIGURES FOR EACH HOST REPRESENTS LENGTH WHILE THE SECOND REPRESENTS WIDTH).

No. of Metacercariae	Days of Dev.	Range	Mean	Coefficient of Variation	Host
17	1	0.06 - 0.09 0.05 - 0.06	0.07 0.06	14.3% 2.8%	Green Tuxedo Swordtail
18	1	0.06 - 0.08 0.05 - 0.08	0.07 0.06	14.2% 3.3%	Brick Red Swordtail
18	1	0.06 - 0.09 0.05 - 0.08	0.07 0.06	14.2% 16.6%	Red Wagtail Platy
20	1	0.05 - 0.08 0.03 - 0.06	0.06 0.05	16.6% 20%	Red Tuxedo Platy
15	3	0.05 - 0.11 0.03 - 0.06	0.08 0.05	21.2% 20%	Red Swordtail
20	14	0.11 - 0.16 0.06 - 0.11	0.15 0.09	8.6% 15.6%	Green Tuxedo Swordtail
15	16	0.14 - 0.17 0.08 - 0.11	0.16 0.09	6.6% 11.1%	Red Platy
10	18	0.09 - 0.17 0.06 - 0.11	0.15 0.09	14.6% 15.5%	Green Tuxedo Swordtail

TABLE III. (Continued)

No. of Meta-cercariae	Days of Dev.	Range	Mean	Coefficient of Variation	Host
15	18	0.14 - 0.17 0.08 - 0.09	0.15 0.09	6.6% 1.9%	Brick Red Swordtail
15	19	0.13 - 0.16 0.08 - 0.11	0.15 0.09	8.6% 2.4%	Green Tuxedo Swordtail
15	20	0.13 - 0.16 0.08 - 0.09	0.14 0.08	10% 2.5%	Green Tuxedo Swordtail
15	21	0.11 - 0.17 0.08 - 0.09	0.16 0.09	12.5% 1.5%	Marble Molly
15	21	0.13 - 0.19 0.08 - 0.11	0.16 0.10	8.7% 9.1%	Green Tuxedo Swordtail
15	28	0.11 - 0.19 0.06 - 0.11	0.15 0.08	11.3% 12.5%	Marble Molly
15	30	0.08 - 0.17 0.05 - 0.11	0.15 0.09	1.4% 18.8%	Red Swordtail
15	33	0.13 - 0.16 0.08 - 0.11	0.15 0.09	6.6% 3.3%	Red Swordtail

granules on the inside), oral spines visible, and eye spot pigments were dispersed. The average length was 0.15 and the width 0.09. These dimensions changed only slightly after 4 weeks or more (see Table III). The average length and width of metacercariae appear to be constant in all tropical fish after 2 weeks of development (except changes in size ranges). Many metacercariae were taken out of their cysts (from the green tuxedo swordtail) after three weeks of development. Whole mounts of experimental metacercariae are comparable with metacercariae from wild Fundulus. The only difference was size which was expected since they were younger.

All metacercariae on the gills of tropical fish do not develop beyond the third day. Some measurements were taken of 18 day old metacercariae from a green tuxedo swordtail that had not grown beyond those at 1 to 3 days of development. All of these small metacercariae were dead.

Feeding experiments with various types of tropical fish containing 3 to 26 day old metacercariae were conducted with 8 laboratory-reared white mice. The mice were killed at intervals of 1 to 4 days with negative results (Tables IV and V). Day old chicks were fed gills from tropical fish containing 16 to 20 day old metacercariae. Six chicks were killed 1 to 4 days later and infections were obtained in half of the chicks (Tables VI and VII). Both positive and negative results were obtained with chicks that had been fed 16 day old metacercariae from a green tuxedo swordtail. The few worms recovered after only 2 days of development in

TABLE IV. RESULTS OF FEEDING *Ascocotyle* (*Phagicola*) *diminuta* METACERCARIAE FROM EXPERIMENTALLY INFECTED RED SWORDTAILS TO WHITE MICE.

Experimental Animal	*A.* (*P.*) *diminuta*	Remarks
		All mice in this table were fed all of the metacercariae on the gills of experimentally infected Red Swordtails (*Xiphophorus maculatus* Gunther), their varieties, and hybrids. All metacercariae were from one to three days old.
Mouse No. 1	Negative	Mouse No. 1 was fed gills from one Red Swordtail. All metacercariae were three days old and very active. Eye spots were still intact and there was no evidence of oral spines. The mouse was killed and examined on the fourth day.
Mouse No. 2		Mouse No. 2 was fed two infective feedings of gills (containing one day old metacercariae) from two Red Tuxedo Platies. Most of the cercariae had formed very thin-walled cysts around themselves and were quite active. The mouse was killed and examined on the second day.

TABLE IV. (Continued)

Experimental Animal	A. (P.) diminuta	Remarks
Mouse No. 3	Negative	Mouse No. 3 was fed one infective feeding of gills from two Red Wagtail Platies (containing two day old metacercariae). The mouse was killed and examined on the third day.
Mouse No. 4	"	Mouse No. 4 and No. 5 were fed the gills (containing three day old metacercariae) of a Red Wagtail Platy in one infective feeding. The animals were killed and examined the next day.
Mouse No. 5		

TABLE V. RESULTS OF FEEDING *A. (P.) diminuta* METACERCARIAE FROM EXPERIMENTALLY INFECTED GREEN TUXEDO SWORDTAILS TO WHITE MICE.

Experimental Animal	*A. (P.) diminuta*	Remarks*
Mouse No. 6	Negative	Mouse No. 6 and Mouse No. 7 were given one-half of the gills (containing eighteen day old metacercariae) from one Green Tuxedo Swordtail (*Xiphophorus helleri* Heckel) in one infective feeding. The animals were killed on the third day.
Mouse No. 7	"	
Mouse No. 8	"	Mouse No. 8 was given one infective feeding of gills (containing twenty-six day old metacercariae) from the marble molly (a hybrid resulting from the cross between the Green Sail-fin Molly and the Black Sail-fin Molly). The animal was killed and examined on the third day.

* The metacercariae fed to mouse No. 8 were few in number. They measured 0.14 - 0.16 mm. in length and 0.06 - 0.08 mm. in width.

TABLE VI. RESULTS OF FEEDING *Ascocotyle (Phagicola) diminuta* METACERCARIAE FROM EXPERIMENTALLY INFECTED GREEN TUXEDO SWORDTAILS TO DAY-OLD CHICKS.

Experimental Animal	*A. (P.) diminuta*	Remarks*
Chick No. 1	3	Chicks No. 1, 2, 3, and 4 were fed two gill arches each from a Green Tuxedo Swordtail. All metacercariae were twenty-one days old.
Chick No. 2	Negative	
Chick No. 3	"	
Chick No. 4	"	
		Chick No. 1 was killed and examined on the second day.
		Chicks No. 2, 3, and 4 were killed and examined on the fourth day.

* All chicks in this table were one day old. They were given their first infective feeding of gill arches while their feathers were still moist. The chicks were given no other food, except water.

TABLE VII. RESULTS OF FEEDING *Ascocotyle (Phagicola) diminuta* METACERCARIAE FROM EXPERIMENTALLY INFECTED RED SWORDTAILS AND GREEN TUXEDO SWORDTAILS TO DAY-OLD CHICKS AND A WHITE RAT.

Experimental Animal	*A. (P.) diminuta*	Remarks
Chick No. 5	10	Chicks No. 5 and No. 6 were fed four gill arches each (containing 18 day old metacercariae) from the Brick Red Swordtail (Xiphophorus maculatus Gunther). These animals were killed and examined on the fourth day.
Chick No. 6	3	
Rat No. 1	63	A white rat was fed six whole fish (four Brick Red Swordtails and two Green Tuxedo Swordtails) all of which contained 18 to 20 day old metacercariae. The rat was killed 6 days later.

the chick and the failure to recover worms after the fourth day could be due to the limited amount of infective material that was available at the time of the feeding. Table VII lends support to this contention since infective material used in feedings was doubled and a maximum of 13 worms were recovered after four days of development (Table VII).

The feeding experiment with a white rat yielded better results (Table VII). The rat was fed two fish for 3 days, a total of six whole fish (4 brick red swordtails and 2 green tuxedo swordtails), all of which contained 18 to 20 day old metacercariae. The rat was killed six days later and most of the worms recovered were immature with a few mature specimens. It is the opinion of the writer that the mature specimens were three to four days old and the immature specimens were about two days old.

B. Description of Stages (all measurements are in millimeters and the figures in parentheses are averages)

The adult description given below does not pretend to be a redescription of an already adequately described Ascocotyle (Phagicola) diminuta Stunkard and Uzmann, 1955 It is described from day-old chicks, mainly, because they have not been described from experimental bird hosts. Further, when Stunkard and Uzmann (1955) redescribed and figured this species from a white rat, they reported it was refractive in chicks. Another reason for describing this species is that its complete life history, from the cercaria to the adult, has been worked out in our laboratory at the University of New Hampshire. Heretofore, the larval stages

from mollucian hosts have not been linked with adult trematodes belonging to the Ascocotyle Complex.

Adult

Diagnosis: Body triangular or flask shaped, 0.26 to 0.32 (0.29) long; 0.10 to 0.13 (0.11) wide at level of ovary. Cuticle covered with small scale-like spines arranged in alterating transverse rows that extend from the back of the oral sucker to the posterior end of the body; leaving a gap (without spines) which spans across the excretory pore and about half the distance of the testes. Oral sucker 0.03 to 0.04 in diameter, terminal, oval to conical, and surrounded by a single row of 16 spines and an incomplete second row of 2 spines.

The oral sucker is equipped with a funnel-like oral appendage (0.03 in length) which hangs in back of the prepharynx. The slender prepharynx is continuous with the cavity of the oral sucker and is in front of the oral appendage. The prepharynx is about as long as or may be shorter than the oral appendage (depending upon the degree of contraction of fore body). The pharynx is oval in shape, measures up to 0.03, and is located near the bifurcation of the intestine. The pharynx is followed by a short esophagus which has a varying length up to 0.03 or is sometimes equally as long as the prepharynx.

The esophagus opens up into the bifurcated intestine which continues toward the posterior, terminating just behind the posterior border of the acetabulum. The acetabulum is located just below the bifurcation of the intestine. It

measures up to 0.04 in diameter, is median in position, and situated in the posterior half of the body. The gonotyl is small, elongated transversely, in front, and slightly to the right of the acetabulum.

The testes are round to oval in shape, measuring 0.04 in diameter, symmetrically arranged, opposite each other, slightly wider than long, and situated near the posterior margin of the body. The ovary is small, round to oval; measuring up to 0.03 in diameter and is located above the testes near the center of body, but in some instances it is displaced slightly above the right testis. The seminal receptacle is small, oval and one third of the size of the ovary and is either immediately below the ovary or to the right of the ovary. The seminal vesicle is elongated transversely across the body, above and to the left of the ovary. Its wider bulb-like posterior margin is towards the ovary while its narrow anterior end extends toward the right and forward in the direction of the acetabulum.

The space between the ovary and acetabulum is filled with several coils of the uterus. The uterine coils start just below and to the right of the ovary where it passes posterior, for a short distance, toward the testes and loops forward; crossing the ovary and testes, zig-zags several times in the space between the ovary and acetabulum, and passes forward to the genital pore.

Hosts: First intermediate, Hydrobia salsa Pilsbry; experimental second intermediate, tropical fish (mollies, swordtails, and platies); experimental definitive, day-old chicks and white rats.

Location: Gill filaments of experimental tropical fish and small intestine of experimental definitive hosts.

Locality: First intermediate host, Hydrobia salsa Pilsbry, in salt marshes of southeastern N. H., near Johnson Creek in Durham, N. H.

Metacercaria

The method of infection, encystment, and subsequent developmental changes were ascertained from tropical fish that were experimentally infected in the laboratory. The weak swimming ability of the cercariae and their habits of settling on the bottom of dishes (landing on their dorsal sides with their long tails upward and motionless) after brief intervals of swimming, suggested that they were carried passively into the gill chamber, where they attach to the gill filaments.

Fish examined after 2 to 3 hours of exposure had large numbers of cercariae in the gill chamber. Many were trapped in mucus secreted by the gills. Some cercariae appeared to be trying to free themselves while others had already lost their tails. Those that had thrown the tail off were lying flat against the gill filament with the spinose aramature buried into the gill filament. After 24 hours, a thin primary cyst wall enclosed the cercariae. After 1 to 3 days the body of the metacercariae did not differ appreciably from that of the cercariae.

After 14 days the body of the metacercariae had grown in length and width and had other important changes: (1) body folded ventrally; (2) oral crown of spines well developed; (3) oral appendages distinctly visible;

(4) eye spots dispersed into scattered pigment near the pharynx; (5) intestinal ceca well developed and contained discernible cecal platelets; (6) excretory vesicle well developed and had acquired characteristic Y-shape; and (7) the cyst wall had thickened and included host tissue. After 21 days, the size of the cysts had not grown much beyond what they were at 14 days, but the morphology of the metacercariae was more pronounced though there appeared to be little, if any, change in growth of the metacercariae.

By comparing experimental metacercariae (see Table III) on the gills of tropical fish with those on the gills of wild Fundulus and experimental evidence from feeding experiments with day-old chicks, it was observed that experimental metacercariae reach the size of wild metacercariae 14 days after encystment but become infective for definitive host after 21 days or a few days earlier (18 days).

Diagnosis. Fairly large and elliptical in shape; wild metacercariae from running water measured 0.11 to 0.22 (0.16) in length and those from standing water 0.12 to 0.25 (0.19) while 33 day old metacercariae from experimentally infected tropical fish measure 0.13 to 0.16 (0.15) in length. The width of metacercariae is about 2/3 of the length. Metacercariae from wild Fundulus taken from running water measured 0.07 to 0.14 (0.10) in width, those from standing water 0.08 to 0.17 (0.12), and those from 33 day old metacercariae from experimentally infected tropical fish 0.08 to 0.11 (0.09) in width. The position of the metacercariae is such that the long axis of the metacercariae is parallel

to the long axis of the gill filament. Older metacercariae are folded ventrally so that the tail and head ends are nearly even. The excretory vesicle in the hind end of the body is large and has a characteristic V-shape. It appears to be filled with little solid bodies. The cyst wall of the metacercariae seemed to thicken as the metacercariae grew older. Part of this thickness is due to the primary and secondary cyst walls of the metacercariae and some is due to the connective tissue reaction of the host's tissue. Other features which distinguish the metacercariae are the oral crown of visible spines, oral appendage, pharynx, intestinal ceca, and V-shaped excretory vesicle.

Cercaria

Since all rediae contain only incompletely developed cercariae, it appears that the cercariae leave the rediae before completing their development. Other cercariae (Cryptocotyle lingua) of the family Heterophyidae go to the interlobular spaces of the digestive gland where they complete their development (Stunkard, 1930). The cercariae of Ascocotyle (Phagicola) diminuta follow this same pattern. Normally emerged cercariae, under slight cover slip pressure, measure 0.06 to 0.08 (0.08) in length and 0.05 to 0.07 (0.06) in width. The tail measures 0.11 to 0.14 (0.13) in length and 0.03 width. The oral sucker measures up to 0.02 in diameter. Fixed and stained cercariae measured 0.05 to 0.07 in length and 0.04 to 0.05 in width. The eye spots measure 0.01 in diameter and the oral sucker measures 0.02 in diameter. The tail is 0.05 to 0.08 in length and 0.01 width.

The body proper of the cercariae is pear-shaped. The oral sucker is terminal and is included in a chamber and modified as a protrusible organ (spinose aramature) with anteriorly directed spines used for penetrating the second intermediate host.

In some cercariae it appears that the oral sucker may be prolonged into a funnel-like appendage. This structure cannot be found in live cercariae, but may be detected in fixed and stained cercariae. There is no trace of a pharynx, esophagus, or intestine. Immediately below and lateral to the oral sucker there are two eye spots that measure up to 0.02 in diameter. Below the eyes there are seven pair of cephalic glands. The position of the cephalic glands differ from most heterophyid cercariae, i.e., they do not form a solid mass in the center of the body. Each gland is separate and pyriform in shape. There are two diagonal rows on each side of the body; three glands in the upper row and four in the lower rows. This can be seen best when the cercaria is motionless. When the body is in a stretched position, the glands appear to be in a single row. Each gland has a separate duct which passes up to the posterior margin of the oral sucker. There are two median group of ducts and two lateral groups; total 14 in all. The ducts pass forward and all open at the anterior margin of oral sucker near the spinose aramature.

There is a large oval mass of cells in the center and posterior third of the body. This material apparently gives rise to the reproductive structure and is called the

"genital primordial." Immediately below the "genital primordial" is a bow-tie shaped excretory bladder. The walls that make up the bladder are cellular and appear to be of cuboidal epithelium.

The excretory canals lead from the anterior lateral margin of the bladder and continues forward and laterally up to the center of the body. The excretory canal could not be traced beyond this point. There are five flame cells on each side of the body. There is one flame cell above the eye spot and another below the eye spot. There are two flame cells slightly above and lateral to the bladder. There is another flame cell near the tail stem in the hindmost part of the body. The flame cell formula is 2 (2+2)+(1).

The tail is powerful, without tail fin-folds, and is longer than the body of the cercaria. It is attached on the ventral side of the posterior end of the body; fits into a groove and attached to a tail stem. The tail stem is T-shaped and is located just beneath the excretory bladder.

The cercaria swam only at brief intervals and then settled on the bottom of the dish. They swim on their backs with the body folded and their tails lashing violently in an upward position. They usually settle to the bottom of the dish, tails straight upward and motionless. They gather on the lighted side of the dish. When disturbed, they start to swim again but only for a short interval.

Redia

Two types of rediae were found in the digestive glands of snails: (1) a small one, 0.3 to 0.4, without germ balls; and (2) a large one, up to 0.65, with germ balls. The germ balls occupied the posterior end of the rediae while developing cercariae were situated in the anterior end. Since no rediae were observed with other rediae developing within them, the two types of rediae were considered to be of the same generation. However, the larger type was mature and the smaller type was immature. This statement is based on an experiment where a lot of 10 snails was experimentally infected with worm eggs. Upon examination, eight weeks later, five were found with rediae; all of which were small type.

Cercariae developing in rediae were in various stages; some had short, stumpy, tails and eye spot pigment; and others had only eye spot pigment. As they mature enough to leave the rediae, they move to the peripheries and migrate forward to the birthpore where they exist. All cercariae within rediae had either a short, stumpy, tail bud or none at all. Therefore, it was apparent that they completed their development after leaving the rediae (probably in the interlobular areas of the digestive gland).

Some rediae were attached individually to host tissue while others were found in the interlobular areas of the digestive gland; attached, end to end, in a ring or "chain formation." A more thorough examination of rediae in "chain formation" revealed small pieces of digestive gland

separating individual rediae.

Diagnosis: Small, colorless, sausage-shaped bodies, 0.3 to 0.65 (0.5) long; up to 0.2 wide. Body wall smooth, thin, without locomotor appendages. Oral sucker small and opens into a relatively small and inconspicuous intestine.

It is difficult to say, with certainty, that there is a gut. At times, there appeared to be a small, short, expanded, gut that was pushed toward the oral sucker by developing cercariae. Then, there were times when the gut could not be seen at all.

C. Discussion

Systematic relations of the cercaria

The cercariae of Ascocotyle (Phagicola) diminuta (Stunkard and Haviland, 1924) Stunkard and Uzmann, 1955 is similar to the cercariae of Centrocestus armatus (Tanabe, 1922 Yamaguti, 1938 and closely related to Cercariae Indicae III (Pleurolophocerca group) described by Swell (1922). It is similar to the former in several features: (1) the tail fits into a groove at the hind-end of the body, from which the tail is mounted ventrally; (2) arrangement of penetration glands (two diagonal rows on each side of the midline of the body); and (3) absence of tail fin-folds. It is more closely related to the latter and has the following similar features: (1) shape of the body; (2) position and attachment of the tail; (3) absence of tail fin-folds; (4) arrangement, shape, and number of penetration glands (two diagonal rows; three pyriform-shaped glands in the first row and four in the

second); (5) number of flame cells (five on each side of the body); (6) position and number of cystogenous glands (eight groups on each side of the body; (7) cellular excretory vesicle (walls of bladder composed of simple cuboidal epithelium) that has a butterfly or bow-tie shape; and (8) absence of intestinal ceca.

The cercariae of Ascocotyle (Phagicola) diminuta differs from Cercariae Indicae III by having (1) the oral sucker prolonged into a small funnel-like oral appendage and (2) not having a prepharynx and a rudimentary pharynx. Though my material was carefully studied, including observations on hundreds of fixed and stained cercariae as well as live specimens, no traces of a prepharynx or pharynx could be detected. The similaries of these two cercariae, A. (P.) diminuta and Cercariae Indicae III, suggest that the latter might be the cercaria of an Ascocotyle Complex species.

Life Cycle

The heterophyid cercariae shed by Hydrobia salsa Pilsbry (a brackish water snail from an isolated depression in salt marshes near Johnson Creek in Durham, New Hampshire) are those of Ascocotyle (Phagicola) diminuta (Stunkard and Haviland, 1924) Stunkard and Uzmann, 1955. These cercariae have been exposed to tropical fish (marble mollies, red swordtails, green tuxedo swordtails, red wagtail platies, and red tuxedo platies) and found to encyst on gill filaments as metacercariae. After 18 to 21 days of development in the experimental second intermediate host (tropical fish), they were infective for experimental definitive host (white rats

and day-old chicks). Worms that agree with those described as Ascocotyle (Phagicola) diminuta (Stunkard and Haviland, 1924) Stunkard and Uzmann, 1955 were recovered after 1 to 4 days of development in these animals. The morphological characters were consistent with those described and figured by Stunkard and Uzmann, 1955 (see Table VIII). There was never any deviation in the morphological characters so as to appear similar to other described species belonging to the Ascocotyle Complex. Therefore, the species Ascocotyle (Phagicola) diminuta Stunkard and Uzmann, 1955 is valid until such time that the life history of Ascocotyle minuta Looss, 1899 is worked out and proved otherwise. The species name Ascocotyle (Phagicola) diminuta is retained, following Stunkard and Uzmann (1955), though Sogandares-Bernal and Lunsden (1963) synonymized it with Ascocotyle angrense Travassos, 1916. The subfamily Ascocotylinae Yamaguti (1958) is accepted rather than Centrocestinae Looss, 1898 because of differences in the larvae of Ascocotyle (P.) diminuta and Centrocestus armatus (Tanabe, 1922) Yamaguti, 1938.

Taxonomy

The taxonomy of heterophyid worms belonging to the subfamily Ascocotylinae has been under considerable discussion. Opinions differ as to what genera constitute the subfamily and separation of questionable species within questionable genera. The first member of the subfamily was figured and described (Distomum coleostoma from a pelican in Egypt) by Looss (1896) though in his revision of the genus Distomum he created the genus Ascocotyle to receive it

TABLE VIII. COMPARISON OF SIZE RANGES OF *Ascocotyle* (*Phagicola*) *diminuta* FROM NATURAL AND EXPERIMENTAL DEFINITIVE HOSTS.

Characters	*A. diminuta* from wild rats Stunkard and Haviland, 1924	*A. diminuta* from white rats Stunkard and Uzmann, 1955	*A. diminuta* from day-old chicks (Scott) a	*A. diminuta* from white rats (Scott) b	*A. diminuta* from day-old chicks (Scott c
Body length	0.25 - 0.3	0.2 - 0.44	0.28 - 0.40	0.13 - 0.23	0.26 - 0.36
Body width	0.08 - 0.1	0.11 - 0.24	0.12 - 0.17	0.07 - 0.17	0.10 - 0.13
Oral sucker	0.03 - 0.04	0.02 - 0.04	0.03 - 0.05	0.02 - 0.04	0.03 - 0.05
Pharynx	0.02	0.01 - 0.02	0.03 - 0.04	0.02 - 0.03	0.02 - 0.03
Acetabulum	0.03 - 0.04	0.03 - 0.04	0.04 - 0.05	0.02 - 0.04	0.03 - 0.04
Testis length	0.02 - 0.03	0.03 - 0.05	0.04 - 0.06	0.03 - 0.05	0.03 - 0.04
Testis width	0.01 - 0.02	0.07	0.04 - 0.07	0.03 - 0.08	0.03 - 0.05
Ovary length	not given	0.03	0.03 - 0.05	0.01 - 0.04	0.02 - 0.03
Ovary width	0.02 - 0.03	0.04	0.03 - 0.06	0.02 - 0.04	0.02 - 0.03
Spines					
Ant. row	16	16	16	16	16
Post. row	0	2	2	2	2

a. From natural infected *Fundulus* and experimentally infected day-old chicks.
b. From experimentally infected tropical fish and experimentally infected white rats.
c. From experimentally infected tropical fish and experimentally infected day-old chicks.

and added the second species (A. minuta, from the small intestine of dogs and cats of Egypt). It seem that Looss' conception of the genus Ascocotyle centered around the following: (1) mouth surrounded by a crown of spines; (2) oral sucker with elongated posterior cecum (dorsal to prepharynx); (3) long prepharynx; (4) pharynx near bifurcation of the intestine; (5) vitellaria not extending anterior of region of genital pore; (6) ovary globular or oval (on right of midline); (7) seminal receptacle in front of testes and behind ovary; (8) testes globular or oval, side by side, near posterior end of body. If one examines the figures for Looss' type species (Ascocotyle coleostoma) and his second species (A. minuta), stricking differences are noted between the two species. The differences noted, however, must be assumed as differences that Looss considered to be only of a specific value since he did not place them in different genera or subgenera. He made no issue of the fact that A. coleostoma had two rows of spines around the mouth whereas A. minuta had only one row of spines or that A. coleostoma had a dorsal triangular lip while A. minuta had a rounded dorsal lip though these features are obvious in his figures. Neither was an issue made of the vitellaria extending to region of genital pore in A. coleostoma and it being preovarian in A. minuta or that the pharynx was near the bifurcation of the intestine in A. coleostoma and further away in A. minuta. Again, these differences are clearly shown in his figures.

It is of interest to note that nearly every described

species of Ascocotyle Looss up until 1935 has resembled A. minuta Looss, 1899 rather than the type species, A. coleostoma Looss, 1896. The main morphological characteristic which have linked them to A. minuta are oral spination and distribution of vitellaria (single crown of spines and vitellaria restricted to ovarian testicular zone). Stunkard and Haviland (1924) made an issue of the difference between A. minuta and A. coleostoma. They listed the differences (see page 28) in tabular form and erected the subgenus, Parascocotyle to include all described species of Ascocotyle up to 1924 with the exception of Ascocotyle angrense Travassos, 1916 which species they thought conformed to the definition of A. coleostoma and therefore not a part of the new subgenus. They described an American form from wild rats as Ascocotyle (Parascocotyle) diminuta. The American species differed from A. minuta Looss, the type species of the subgenus Parascocotyle, only in regard to size of the body and sex organs. Faust (1920) had described a species from a monkey-eating eagle (Pithecophaga jefferyi) as Phagicola pithecophagicola which species resembled Stunkard and Uzmann's A. (P.) diminuta in that there was a single crown of spines and vitellaria restricted to ovarian testicular zone. Faust in 1926 admitted that he made an error in creating a new genus and subfamily (Phagicola and Phagicolinae) to receive his species from the monkey-eating eagle and therefore his species should be placed in the genus Ascocotyle. Witenberg (1929) noted that Parascocotyle Stunkard and Haviland, 1924 had one row of circumoral spines,

uterine coils behind genital pore, and vitellaria behind the level of the ovary while Ascocotyle had two rows of circumoral spines, coils of uterus in front of genital pore, and vitellaria extends in front of acetabulum. The subgenus Parascocotyle was raised to generic status and all species of Ascocotyle (Parascocotyle) were transferred to Parascocotyle Stunkard and Haviland, 1924. However, Witenberg (1929) maintained that Ascocotyle (P.) diminuta was a synonym of A. minuta Looss.

Travassos (1930) surpressed Parascocotyle as a synonym of Phagicola Faust, 1920. He placed all of the species which Witenberg (1929) had assigned to Parascocotyle in the genus Ascocotyle and subgenus Phagicola. For example, Parascocotyle diminuta (Stunkard and Haviland, 1924) Witenberg, 1929 became Ascocotyle (Phagicola) diminuta. Srivastava (1935) recognized the genus Ascocotyle Looss and stated that there should be two subgenera, Ascocotyle (Ascocotyle) and Ascocotyle (Phagicola).

Price (1935) described A. puertoricensis from Butorides sp. in Puerto Rico (Mayagues) and A. tenuicollis from Botaurus lentiginosus at College Station, Texas. These species resembled A. coleostoma in rows of circumoral spines and forward distribution of the vitellaria. Price's description of these two species clearly linked them to the type species, A. coleostoma, of the genus Ascocotyle Looss, 1899. Price redescribed Phagicola pithecophagicola Faust, 1920 (synonyms Ascocotyle pithecophagicola Faust and Nishigori, 1926; Parascocotyle pithecophagicola (Faust, 1920)

Witenberg, 1929; Ascocotyle (Phagicola) pithecophagicola (Faust, 1920) Travassos, 1930) and argued for the generic rank of both Ascocotyle and Phagicola. He did not question the validity of Parascocotyle as a genus or subgenus, but stated it was a synonym of Phagicola and on the basis of the law of priority Phagicola was considered as the correct name.

Stunkard and Uzmann (1955) redescribed Ascocotyle (P.) diminuta. They found a second row of spines represented by two small spines. Criteria, heretofore, used were two rows of oral spines and vitellaria extend to acetabulum for Ascocotyle and for Phagicola one row of spines and vitellaria restricted to ovarian testicular zone. These authors pointed out that A. puertoricensis had two rows of spines and the vitellaria only extend a short distance anterior of the ovary. They believed that the decision on the taxonomic state of Phagicola should be postponed until the developmental stages of its members were known. They named their species Ascocotyle (Phagicola) diminuta. Burton (1956), Robinson (1956), and Kuntz and Chandler (1956) described new species and apparently followed the taxonomic scheme of Price (1935). Burton, 1958 recognized Ascocotyle Looss and Phagicola Faust, 1920 as valid genera and gave a key to separate the species of North and South America.

Hutton and Sogandares-Bernal (1958), in one paper, recognized Ascocotyle and Phagicola as separate genera and in a second paper of the same year they recognized Ascocotyle, Phagicola, and Parascocotyle as separate genera. They

outlined a key to separate these genera. Sogandares-Bernal and Bridgman (1960) recognized three genera and erected a new one under the name of Pseudoascocotyle. Sogandares-Bernal and Lumsden (1963) recognized one genus, Ascocotyle Looss, 1899. In this genus they recognized the subgenera Ascocotyle Travassos, 1930; Leighia Sogandares-Bernal, 1963; and Phagicola Faust, 1920. These authors regarded Ascocotyle nana Ransom, 1920; A. diminuta Stunkard and Haviland, 1924; and Phagicola lageniformis Chandler, 1941 as synonyms of Ascocotyle angrense Travassos, 1916.

No doubt Ransom's report of A. nana having spines arranged in a double crown of 16 to 20 spines and a ventral crown gave them the notion that A. nana had accessory spines. I have studied Ransom's specimen which was badly cytolyzed and from my own experiences with thousands of specimens of this genus (obtained through feeding experiments), it appears that Ransom's specimen was dead for a considerable time before it was fixed. Further, the oral spination of the type specimen cannot be made out with certainty so as to identify them with those of A. (P.) diminuta. However, in spite of the poor condition of A. nana Ransom, 1920, one can make out the extremely small oral cecum and the long intestinal ceca which extends to the testes. These two differences make it impossible to identify A. nana with A. (Phagicola) diminuta. The only difference between Phagicola lageniformis Chandler, 1941 and A. (P.) diminuta Stunkard and Uzmann, 1955 is size. Therefore, Chandler's species is a synonym of A. (P.) diminuta (Stunkard and

Haviland, 1924) Stunkard and Uzmann, 1955 in conformity with Sogandares-Bernal and Lumsden (1963). As for synonymizing A. diminuta with A. angrense Travassos, 1916 which was improperly described to the point that Stunkard and Haviland, 1924 linked it with the type specimen, Ascocotyle coleostoma Looss, 1896, I thoroughly disagree. I would be more inclined to synonymize A. diminuta with A. minuta Looss (as did Witenberg, 1929) since the only difference between them is size and continent. Price (1936) bridged the gap between continents when he synonymized P. longa Ransom, 1920 from the United States (Washington, D. C.) with Metascocotyle witenbergi from Palestine and Raumania.

Conclusion

No species of Ascocotyle, as far as known, has been reported from the west coast of the United States. All have been reported from the east coast; from New Hampshire to Texas. The geographic distribution of species of Ascocotyle parallels that of the Eastern North America group of salt marshes; from south-east Canada southward to Florida and Louisiana (Chapman, 1960).

The discovery of the first intermediate host, Hydrobia salsa Pilsbry, from depressions in the New England sub-group of salt marshes (in southeastern N. H.), and the completion of the life cycle of A. (P.) diminuta with parasite free poeciliids (closely related to Fundulus heteroclitus), lead me to conclude: (1) that the solution of life cycles for all species of Ascocotyle from the United States lie in the salt marshes of the Eastern North America

group (where brackish water gastropods are abundant); (2) that Fundulus heteroclitus (ranges from the Gulf of St. Lawrence to Texas - Bigelow and Schroeder, 1953) plays a large role in the life cycle though the role may be divided with closely related species; and (3) only the solution of life cycles can correctly determine or synonymize species from the United States.

It is apparent from a study of the range of the first intermediate hosts, Hydrobia salsa and H. minuta, that they are not the only snails involved with Ascocotyle Complex species. Johnson (1934) reported that the range of Hydrobia minuta was from Labrador to New Jersey and that of H. salsa was from Rowley, Massachusetts to New Jersey. Clench (1938) extended the range of H. salsa to New Hampshire. Therefore, the combined ranges of H. salsa and H. minuta extend from Labrador to New Jersey.

In view of the above evidence, I submit the following: (1) Hydrobia minuta and H. salsa are the first intermediate hosts for Ascocotyle (P.) diminuta associated with the New England sub-group of marshes (from Maine to New Jersey) where these snails are found in depressions along with fish in associations called "Fundulus-Hydrobia Biotic Communities"; and (2) since south of New Jersey does not include the range of the above snail species, other snails, perhaps closely related to hydrobiids, are involved and probably form "Fundulus-snail-? Biotic Communities" in depressions of the Coastal Plain sub-group of marshes (south of New Jersey to Florida to Louisiana).

SECTION VI.

THE SEXUAL CYCLE OF ASCOCOTYLE TENUICOLLIS PRICE, 1935

A. Observation and Experiments

The conus arteriosus and ventricle of the heart of Fundulus heteroclitus collected from the three study areas (described above) were found infected with metacercariae. The infection was restricted to the lumen of the conus arteriosus and walls of the ventricle of the heart. The cyst is oval in shape, thick-walled, and contain large amounts of oil droplets (appear to be a fatty substance). The morphological characters of the encysted worm were obscured by the oil droplets and their identity as an Ascocotyle Complex species could not be determined until removed from the cyst.

The incidence of infection was determined from Fundulus heteroclitus collected from Johnson's Creek. A total of 57 fish, ranging in sizes between 4 and 7 cm., was carefully examined. All fish were infected with 3 to 22 metacercariae (with an average of 15 metacercariae per fish heart). Fish from the other study areas were routinely checked and had about the same level of infection. However, only fish from Johnson's Creek were used in feeding experiments.

15 to 20 fish hearts (containing many metacercariae) were fed to each of three white mice. The animals were killed and examined for helminths at intervals of 2, 4, and 6

days after the infective feedings. All mice were negative for helminths. The experiment was repeated for a second time with the same results. The negative results obtained with mammals indicated that this particular heterophyid trematode might be better suited to bird hosts. Therefore, the experiment was repeated with day-old chicks. Large numbers of worms were recovered after one to 3 days of development. Worms were recovered from 3 out of 6 chicks used in the experiment. Table IX describes the manner in which the experiment was carried out and lists the number of worms recovered. No worms were recovered after the third day of infection.

These worms have been carefully studied and found to agree with Ascocotyle tenuicollis Price, 1935 in many respects, but also differ in that there is a variation in the number of spines and the worms are of a smaller size. These differences are not considered as specific and the worm is redescribed below.

B. Redescription of Ascocotyle tenuicollis Price, 1935

Diagnosis: Sexually mature 3 day old worms from day-old chicks are pyriform in shape, 0.34 to 0.43 (0.38) in length and 0.08 to 0.17 (0.11) in width. The anterior portion of the body (oral sucker to level of intestinal bifurcation) is narrow than the rest of the body. The body starts to widens at the level of the intestinal bifurcation and is widest at the level of the ovary. The width of the body tapers off past the ovary and is rounded at the hind-

end of the body. The cuticula is covered with small, scale-like, spines that start posterior to oral sucker and continue to the posterior end of the body. The oral aperture is terminal, provided with a triangular lip, and is surrounded by a double coronet of 32 to 36 spines. There are 16 to 18 spines in the first row and 16 to 18 spines in the second row. The oral sucker is oval to conical and measures 0.03 to 0.05 (0.04) in diameter. The oral sucker is prolongated into a funnel-like oral appendage (oral cecum) that hangs in back of the slender prepharynx. The oral appendage has a varying length which depends upon the degree of contraction of the fore body. The oral aperture is joined by a long slender prepharynx. The pharynx measures 0.03 to 0.05 (0.04) in diameter and is nearly as large as the oral sucker. Cercaria eye spots (dispersed pigment) are still visible and are located lateral to the pharynx on each side of the body. The esophagus follows the pharynx and is very short, but not visible in the majority of observations. The spacious, expanded, bow-tie, or butterfly shaped intestinal ceca are clearly visible below the pharynx. The branches of the intestine do not extend to the posterior border of the acetabulum, but are located quite a distance above the acetabulum. The acetabulum is located below the intestine. It is round to oval in shape and measures 0.02 to 0.05 (0.04) in diameter. The genital opening is anterior and slightly to the left of the acetabulum. The genital opening is surrounded by a sucker-like structure. The globular seminal vesicle is large and located in the

center of the body or to the right and above the right testis. The ovary is on the left side of the body (above the left testis) and measures 0.03 to 0.06 (0.04) in diameter. Testes opposite each other globular in shape, measures 0.04 to 0.07 (0.05) in diameter, and tend to be forward in position; a good distance from the posterior border of the body. The vitellaria is lateral, extends from posterior border of the acetabulum to anterior border of the testis. The uterine coil passes posterior from the ovary, passes the right testis, loops in back of the testes, and then passes forward across the left testis; continues to loop back toward ovary and across to the seminal vesicle, and forward toward the genital pore.

Hosts: Second intermediate, Fundulus heteroclitus. Experimental definitive, day-old chicks

Location: In lumen of conus arteriosus of the second intermediate host and attached to walls of ventricle; and small intestine of experimentally infected day-old chicks.

Locality: Salt marshes of southeastern New Hampshire, near Johnson's Creek in Durham; near Great Bay in South Newington; and near the Hampton River in Hampton, New Hampshire.

C. Discussion

Burton (1956) found metacercariae in the conus arteriosus of Mollienisia latipinna LeSueur from southern Florida. Although he did not find the natural definitive host, adults were recovered from day-old unfed chicks that were experimentally infected with the metacercariae. The adult worms were described as Ascocotyle leighi, a new species. Burton found the conus arteriosus of Gambusia

TABLE IX. RESULTS OF FEEDING HETEROPHYID *Ascocotyle tenuicollis* METACERCARIAE FOUND IN THE CONUS ARTERIOSUS OF *Fundulus heteroclitus* FROM JOHNSON CREEK TO LABORATORY-REARED ANIMALS.

Experimental Animal	*A. tenuicollis*	Remarks*
Chick No. 3	183	Chick No. 3 was given 8 fish hearts in one infective feeding and killed two days later. The worms recovered were both mature and immature. Mature specimens had only a few eggs in the uterus.
Chick No. 4	170	Chick No. 4 was fed a total of 15 fish hearts in two infective feedings and killed on the third day. Worms recovered were both mature and immature. Mature worms had few eggs in the uterus.
Chick No. 5	Negative	Chick No. 5 was fed only 2 hearts and killed eighteen hours later. The purpose of this experiment was to locate the point where metacercariae excysted, but success was not attained.
Chick No. 6	"	Chick No. 6 was fed 16 hearts over a period of four days and killed on the fourth day.

TABLE IV. (Continued)

Experimental Animal	A. tenuicollis	Remarks*
Chick No. 7	25	Chick No. 7 was fed the same as chick No. 6 and killed on the same day. Twenty-five metacercariae were recovered from the gizzard. No worms were found in the intestines.

* All chicks in this table were one day old. They were given their first infective feeding of fish hearts while their feathers were still moist. The chicks were given no other food except water along with additional infective feedings.

affinis holbrooki (Giard) was similarly infected though a different species of Ascocotyle was involved. He believed this species to be Ascocotyle tenuicollis Price, 1935 because of the similarity of general morphology and number of spines in the oral coronet (16 spines in each of two rows). In differentiating between the two types of metacercariae, he reported that the metacercariae of A. leighi were small and spherical while those of A. tenuicollis were large and oval. He noted differences in the degree and incidence of infection in the two fish hosts: (1) Gambusia were infected with smaller numbers of metacercariae (usually less then 10); (2) Mollienisia were infected with larger numbers of metacercariae (30 to 35); (3) 23 of 53 Gambusia were infected; and (4) 329 Mollienisia were infected.

Burton (1956) maintained that Ascocotyle leighi closely resembled A. puertoricensis, and A. tenuicollis. An important difference was that A. leighi had 48 to 52 spines in the oral coronet (24 to 26 in each of two rows) while both A. puertoricensis and A. tenuicollis had 32 spines in their oral coronets (16 in each of two rows). Other differences were found in their seminal vesicles; for example, the seminal vesicle of A. leighi was in a transverse plane and tapered medially toward the ovary. The seminal vesicle of A. puertoricensis and A. tenuicollis tapered anteriorly from a bulb-like expansion.

For a time, I considered the metacercariae found in the lumen of the conus arteriosus of Fundulus heteroclitus to be those of Ascocotyle leighi Burton, 1956.

However, critical observations proved them to be different from Ascocotyle leighi. This was especially noticeable in regard to the number of spines in the oral coronet and the rather forward position of the testes. Worms from experimentally infected day-old chicks had (32-36) spines in their oral coronets (16 to 18 spines in each of two rows). In all worms the testes did not occupy the extreme hind-end of the body (near the posterior border) as other Ascocotyle Complex species, but tended to be shifted forward, quite a distance from the hind-end of the body. The only species of Ascocotyle with the testes in this position was Ascocotyle tenuicollis Price, 1935, a specimen taken from Botaurus lentiginosus collected at College Station, Texas, in November, 1921. New Hampshire specimens of Ascocotyle tenuicollis, Price, 1935, obtained experimentally from day-old chicks after 3 days of development were both mature and immature. Measurements of 12 mature specimens agree more with measurements of Ascocotyle puertoricensis than Ascocotyle tenuicollis (see Table X), but agree substantially with A. tenuicollis in regards to extent of the vitellaria (anterior border of acetabulum to half the length of the testis) and anterior position of the testes. The smaller size ranges for N. H. specimens of Ascocotyle tenuicollis are attributed to the length of time that they were allowed to develop in the experimental host. Further, it is not known how many specimens Price (1935) measured to determine the size range listed. Since some trematodes grow for as long as they live, size ranges are of importance only when a

TABLE X. A COMPARISON OF MORPHOLOGICAL CHARACTERS OF SOME *Ascocotyle* COMPLEX SPECIES WITH 16 TO 18 SPINES IN THE ORAL CORONETS.

Characters	*Ascocotyle puertoricensis* Price, 1932 a	*Ascocotyle coleostoma* Looss, 1899 b	*Ascocotyle felippei* Travassos, 1929 c	*Ascocotyle tenuicollis* Price, 1935 d	*Ascocotyle tenuicollis* Scott, e
Body length	0.26 - 0.46	0.7 - 0.8	0.5	0.57 - 0.76	0.34 - 0.43
Body width	0.17 - 0.20	0.25	0.16	0.22 - 0.23	0.08 - 0.17
O. sucker diameter	*	0.09	0.05	*	0.03 - 0.05
Prepharynx length	0.12	*	0.01	0.22 - 0.24	*
pharynx length	0.04 - 0.05	0.06	0.04	0.03 - 0.05	0.03 - 0.05
Acet. diameter	0.04	*	0.05	0.04 - 0.06	0.02 - 0.05
Ovary width	0.03 - 0.04	0.06	0.04	0.06	0.02 - 0.03
Testes length	0.04 - 0.05	0.07	0.05	0.04 - 0.08	0.05 - 0.07
Testes width	*	*	*	*	*
Spines					
Ant. row	16	16	18	16	16 - 18
Post row	16	16	18	16	16 - 18

a. From natural infected *Butorides sp.*
b. From an Egyptian pelican (species not known).
c. From *Ardetta erythromelos.*
d. From *Botaurus lentiginosus.*
e. From experimental day-old chicks (3 days development).
* Not given

significant number of worms are measured, and average figures are given. Sogandares-Bernal and Lumsden (1963) expressed the view that the body length for Ascocotyle angrense was dependent upon the size and age of metacercariae previous to ingestion by the definitive host and upon recency of infection.

New Hampshire specimens of Ascocotyle tenuicollis have a variation in the number of spines in the oral coronet (16 to 18 spines in each of two rows). The difference between this species, A. puertoricensis, A. coleostoma, and A. felippei is indeed small. The size ranges for body length, body width, and other morphological characters for A. puertoricensis Price, 1932 overlap those listed for New Hampshire specimens of A. tenuicollis. This is in contrast to A. tenuicollis Price which is decidely larger than A. puertoricensis Price, 1932. The vitellaria in A. puertoricensis extends from a distance below the posterior border of the acetabulum to the posterior border of the testes while in A. tenuicollis (N. H.), the vitellaria extends from the anterior border of the acetabulum to half the distance of the testes. In view of these findings, based on observation on over 100 fixed and stained specimens, the difference between A. tenuicollis (from N. H.) and A. puertoricensis is not size, but only the forward position of the testes in A. tenuicollis and slight variation in the vitellaria.

A. tenuicollis (N.H.) differ from A. coleostoma in size (the former smaller than the latter), position of the

testes, and slight variation in the vitellaria. Specimens from New Hampshire are smaller with testes forward in position. A. coleostoma is larger with testes near posterior border of the worms. The vitellaria in A. tenuicollis (N. H.) extend from the anterior border of the acetabulum to half the distance of the testes while in A. coleostoma, the vitellaria extend from the genital pore (slightly in front of the acetabulum) to the posterior border of the seminal receptacle and is a short distance above the anterior border of the testes (determine from figures of Looss, 1907). Therefore, the decided difference between A. coleostoma and A. tenuicollis Price, 1935 is not size, but position of the testes. The view is expressed that the only appreciable difference in A. puertoricensis and A. coleostoma is size and that A. puertoricensis may be small A. coleostoma. Price stated that the difference between A. tenuicollis Price, 1935 and A. felippei is that the latter had 36 crown spines (18 in each of two rows) as opposed to 32 spines of the former. A. felippei is not available for study; and this is unfortunate since A. tenuicollis (N. H.) has 32 to 36 crown spines. There appear to be no difference between these two species with regards to oral spination. They are alike in regard to distribution of vitellaria; lateral in groups of small follicles extending from acetabulum to the middle of testicular zone. The position of the testes in A. felippei is not known.

A. tenuicollis (from N. H.) was allowed to develop in day-old chicks for 3 days in order to see how they

compared with Ascocotyle leighi. Both species are from the conus arteriosus of fish; the former utilizes Fundulus heteroclitus and the latter uses Mollienisia latipinna as second intermediate host. The main differences are as followers: (1) A. tenuicollis has 36 spines (16 to 18 in the first row and 16 to 18 in the second row) while A. leighi has 48 to 52 spines (24 to 26 in each of two rows); (2) testes near the posterior margin of the body in A. leighi while in A. tenuicollis testes tend to be more anterior in position; and (3) A. leighi utilizes Mollienisia latipinna as second intermediate host while A. tenuicollis utilizes Gambusia affinis, Mollienisia latipinna, and Fundulus heteroclitus. Burton (1956) reported that metacercaria of A. leighi, since small numbers of these cysts were frequently recovered from Mollienisia along with those of A. leighi. However, it is important to note that he examined only 53 Gambusia compared to over 300 Mollienisia. Therefore, it is obvious, Burton's statement in regard to host specificity of these metacercariae would have more weight provided more Gambusia had been examined. Further, it is not known how serious an attempt was made to determine how frequent A. tenuicollis and A. leighi metacercariae occurred together in Mollienisia latipinna.

SECTION VII.

THE SEXUAL CYCLE OF ECHINOCHASMUS MAGNOVATUM (STUNKARD AND HAVILAND, 1924) PRICE, 1931.

A. Observation and Experiments

In many of the feeding experiments where laboratory-reared white mice were fed metacercariae on the gills of Fundulus heteroclitus, two worms, Ascocotyle (Phagicola) diminuta and Echinochasmus magnovatum were recovered (see Table XXI to XXII). All Fundulus collected from various salt marshes in southeastern New Hampshire such as those near Johnson's Creek in Durham; near Great Bay in South Newington; and those near the Hampton River in Hampton have been found to be infected. Fundulus collected from Duxbury, Massachusetts were also infected with the two types of metacercariae. It is doubted that this is a local problem.

The occurrence of the second worm (echinostome) in feeding experiments was first observed July 10, 1959, during the initial stages of this study. However, when reported to my advisor, Dr. W. L. Bullock, it was found that he had observed the occurrence of the echinostome in feeding experiments with white rats and Fundulus collected from South Newington, New Hampshire. However, no attempt was made to key the second worm (echinostome) to genus.

During the course of the study I often made brief notes as to the occurrence of "echinostome" in feeding experiments. Tables XXI to XXII show the occurrence of the echinostome in feeding experiments involving white mice and

day-old chicks; their state of development in these animals is also described in the remark column.

My attention was not seriously drawn to this worm until November 1960 when Fundulus were collected from marshes near the Hampton River and subsequently used in feeding experiments. At the termination of an experiment where a white mouse had been fed the gills from three Fundulus and allowed 18 days of development, 1,192 echinostomes were recovered. The availability of these specimens in such numbers prompted me to key them to genus. It was found that these worms agreed very closely to Echinochasmus magnovatum (Stunkard and Haviland, 1924) Price, 1931. The worm is redescribed below from biometric studies on 18 day old specimens from the mouse.

Further experiments with Fundulus from Hampton marshes were possible through the efforts of Mr. B. E. Barrett who collected fish through ice on December 8, 1960. Growth of these worms in white mice and chicks were observed by allowing them varying periods of development (6 to 30 days) in these animals. Over 3,000 worms at various stages of development were recovered.

Specimens of Echinochasmus magnovatum from the mouse after 6 days of development are all immature and quite small, while those from the chick are larger and mature after only four days of development. After 10 days of development in the mouse, some specimens are able to reach maturity and have from one to three eggs in the uterus. After 13 days of development in the mouse nearly all specimens of Echinochasmus

reach maturity and have two to six eggs in the uterus. After 18 days of development in the mouse, all worms reach maturity and have many eggs in the uterus. Specimens after 20, 22, and 30 days in the mouse are somewhat larger than 18 day old specimens.

Although Echinochasmus magnovatum was recovered from the intestine of experimental animals, a great deal of difficulty was encounted in distinguishing the metacercariae. Echinostome metacercariae on the gills of Fundulus heteroclitus collected from marsh areas near Johnson Creek in Durham, N. H.; and these near Great Bay in South Newington, N. H. were scanty, while those of the heterophyid were numerous. Therefore, even under careful observations, they were often overlooked.

Since mature echinostomes were so much larger than mature heterophyids, I had assumed that the metacercariae of the echinostome should be larger than that of the heterophyid. Metacercariae were removed, as described above, from the gill arches (by allowing them to remain in Ringer's solution, for a week). Large metacercariae were separated from the smaller ones and maintained in different containers (Syracuse watch glass). They were opened with dissecting needles after they had remained in Ringer's solution for an additional week. It should be pointed out that as metacercariae from the gills of Fundulus heteroclitus collected near Johnson's Creek and near Great Bay were being observed at the same time, only a sample of 25 metacercariae of each size and from each collecting area were opened; totaling 100 metacercariae. All

of the metacercariae opened turned out to be those of the heterophyid rather than the echinostome. The experiment was repeated the second time with the same results.

As the negative results, in part, could have been associated with the size of the sample, the experiment was modified to include more metacercariae. The same technique was employed in removing the metacercariae and allowing them to remain in Ringer's solution for at least two weeks. However, after the second week they were expose to a weak solution of NaOH (.02M), a method of Macy and Moore (1954), whereby the cyst wall breaks down and the worms excyst. The worms were placed in hot Bouin's fixative as they excysted, but many were already dead, and many of the metacercariae did not excyst. All of the excysted metacercariae were those of the heterophyid rather than those of the echinostome. Nevertheless the echinostomes though small in number, were still turning up in feeding experiments involving the same lot of Fundulus collected from the same area.

Later it was found, through feeding experiments, that Fundulus heteroclitus collected near the Hampton River at Hampton, N. H. were heavily infected during the months of November and December. Nearly all of the metacercariae on their gills are those of Echinochasmus magnovatum. They differ from those of Ascocotyle in shape, in size, manner in which they position themselves on the gill filaments or branchiae, and the shape of excretory vesicle and their ducts.

Adult echinostomes yielded large numbers of eggs. Eggs were obtained by placing the worms in petri dishes

containing Ringer's solution and keeping them at room temperature for one to two hours. Most of this work was done during the winter and room temperature ranged between 60°F and 76°F. Eggs are oval in shape and are yellowish brown in color. They measure 0.07 to 0.09 (0.08) in length and 0.05 to 0.06 (0.05) in width. Eggs were separated into 4 lots with 24 eggs in each lot, and transferred to petri dishes containing tap water for hatching experiments. After 10 days in tap water, most of the eggs had formed miracidia which were actively moving inside the egg membrane. By the 12th day some miracidia hatched and nearly all miracidia had hatched by the 14th day. After 16 days miracidia were hatched from all eggs under observation.

The experiment on miracidia was continued during the summer.. It was found that it was better to let the worms die in Ringer's in the refrigerator. They die in the extended condition after a week in the refrigerator. The bodies of these worms were teased apart with disecting needles and the eggs released in petri dishes. This method provided opportunity for uterine egg counts. Careful counts on 125 worms revealed that there is a range of 3 to 49 eggs in the uterus and an average of 16 eggs per worm. None of the eggs showed signs of segmentation while in the uterus. Eggs were washed in several changes of Ringer's and allowed to develop in this solution. After seven days in Ringer's, most of the eggs had developed miracidia which were actively moving around in their egg membranes and appeared ready to hatch. On the 8th day few of the eggs hatched while most

hatched on the 9th day and by the 10th day all of the eggs had hatched.

It appears that eggs hatch much faster in Ringer's (0.85%) than in tap water. Since the first experiment was conducted in the winter, it is not possible to tell whether the faster rate of miracidia hatching during the summer months could be due to temperature or salinity.

Some miracidia were observed under the microscope in the live condition. They are oval in shape; measuring 0.054 in length and 0.032 in width. One pair of flame cells were observed in the hind-end of the body.

B. Description of Stages (all measurements are in millimeters and the figures in parentheses are averages)

Adult

Diagnosis. Sexually mature 18 day old worms from the mouse measure 0.71 to 1.55 (0.98) in length and 0.21 to 0.41 (0.31) in width. The body widens at the level of the acetabulum and is widest at the level of the anterior testes; taper some what and are more or less rounded at the hind-end. Anterior neck portions above the acetabulum is narrower than the rest of the body and widens in the area of the subterminal oral sucker due to a conspicuous anterior collar which surrounds the oral opening. The anterior collar measures 0.13 to 0.21 (0.16) in width and bears a single row of spines which are dorsally interrupted at the anterior border of the oral sucker. Oral spines are 20 to 22 in number. Starting at the point of the dorsal interruption, spines may be divided into 4 groups, 6 spines each, with the

fourth group at the ventral lobe being located more aboral than the rest and having 4 spines in the group. The first group measures .007 to .018 (.011) in length and .007 to .011 (.008) in width; the second group .011 to .018 (.015) in length and .007 to .011 (.009) in width; the third group .014 to .018 (.016) in length and .007 to .011 (.008) in width; and the fourth group .011 to .018 (.013) in length and .007 to .007 (.007) in width. The median groups (2nd and 3rd) are the largest with the groups at the extremes (1st and 4th) being the smallest. There are also cuticular spines which cover the general surface of the body. Immediately behind the anterior collar there are many transverse rows of small spines that terminate at the level of or just beyond the posterior testis.

The subterminal oral sucker is nearly round to oval in shape; measures 0.08 to 0.11 (0.09) in longitudinal diameter and 0.08 to 0.11 (0.09) in transverse diameter. The oral sucker is followed by a short prepharynx; measuring up to 0.05 (0.02) in length and opens into an oval shaped pharynx (larger than the oral sucker) which measures 0.09 to 0.17 (0.12) in longitudinal diameter and 0.09 to 0.11 (0.10) in transverse diameter. The esophagus leading from the pharynx is equally as long as the prepharynx and measures up to 0.05 (0.02) in length. The short esophagus opens into the bifurcated intestinal ceca which continue toward the posterior end of the worm and terminates at 0.05 to 0.09 (0.06) from the end of the body.

The acetabulum is located just below the bifurcation

of the digestive tract. It measures 0.08 to 0.16 (0.12) in longitudinal diameter and 0.11 to 0.16 (0.13) in transverse diameter. It is more nearly located in the upper half of the body. The distance from the anterior border of the oral sucker to the posterior border of the acetabulum is 0.35 to 0.54 (0.43); from the anterior border of the acetabulum to the end of the body there is a distance of 0.36 to 1.10 (0.63).

The cirrus pouch is usually located in back of the acetabulum and opens through a median genital pore located behind the bifurcation of the intestinal ceca. The cirrus pouch measures 0.06 to 0.16 (0.10) in length and 0.03 to 0.11 (0.08) in width. The position of the cirrus pouch in relation to the acetabulum ranges from completely in back of the anterior border of the acetabulum, one half of it projecting above the anterior border of the acetabulum, to completely in front of the anterior border of the acetabulum. The mean length of the cirrus pouch is nearly 2/3 of the mean longitudinal diameter of the acetabulum.

The ovary is oval in shape and is located above the anterior testis and below the posterior border of the acetabulum; occupying a position that is nearly in the midline of the body. It measures 0.03 to 0.09 (0.06) in longitudinal diameter and 0.05 to 0.12 (0.08) in transverse diameter. The short uterus fills the space between the anterior testis and the acetabulum. It contains 3 to 49 eggs (16). The eggs measure 0.07 to 0.09 (0.08) in length and 0.05 to 0.06 (0.05) in width.

The testes are in back of the ovary and short uterus. The distance from the anterior border of the anterior testis to the end of the body is 0.30 to 0.90 (0.48). The distance from the posterior border of the posterior testis to the oral sucker is 0.51 to 0.96 (0.74). The anterior testis is much wider than long and is oval to rectangular in shape. It measures 0.05 to 0.14 (0.09) in longitudinal diameter and 0.11 to 0.28 (0.17) in transverse diameter. The posterior testis is oval in shape; slightly smaller than the anterior testis in transverse diameter but larger in longitudinal diameter. It measures 0.08 to 0.17 (0.13) in longitudinal diameter and 0.09 to 0.24 (0.17) in transverse diameter.

The excretory vesicle is tubular with its anterior border just in back of the posterior testis. It gives rise to two lateral branches on either side of its anterior border. The branches continue forward (to the pharynx) as the lateral excretory canals.

The vitellaria is broken up into many scattered follicles that occupy the region immediately behind the testes. They continue along the side of the body and terminate at the level of the acetabulum. The point of termination varies from the posterior border of the acetabulum, mid acetabular, or at the anterior border of the acetabulum. The vitellaria never go beyond the acetabulum. Just above the anterior border of the anterior testis on both sides of the worm, a vitelline duct leads to the ovary.

Hosts: Second intermediate, Fundulus heteroclitus.
Experimental definitive, white mice, white rats, day-old chicks.

Locality: Salt marshes of Southeastern New Hampshire, near Johnson's Creek at Durham; near Great Bay at South Newington; and near the Hampton River at Hampton, New Hampshire.

Metacercariae

Diagnosis: Small and oval in shape; width about 2/3 of the length. They measure 0.06 to 0.11 (0.07) in length and 0.05 to 0.09 (0.06) in width. The metacercariae are positioned either at right angles or diagonally with respect to the longer axis of the gill branchiae. Their positional relationship distinguish them from the metacercariae of Ascocotyle which have their longer axis parallel to the longer axis of the gill branchiae and are 1½ to 3 times as large. Since the metacercariae of Echinochasmus are nearly round, the position of the oral end (which is distinguished by the presence of spines) or the adoral end (distinguished by the excretory vesicle), should be used as criteria in determining their arrangement on the gill branchiae.

The excretory vesicle is tubular and the lateral tubules leading away from the vesicle are either parallel or cross each other in "X" formation; depending upon how much the worm is folded on the inside of the cyst. The shape of the excretory vesicle in the metacercariae of Echinochasmus serve in distinguishing them from those of Ascocotyle since in the latter species, the excretory vesicle is "V" shaped.

C. Discussion

Four species of Echinochasmus have been described from North America, namely E. magnovatum (Stunkard and Haviland, 1924) Price, 1931; E. schwartzi Price, 1931; E. donaldsoni Beaver, 1941; and E. cohensi Rao, 1951. All species are intestinal parasites of birds and mammals. E. magnovatum was described from wild rats of New York; E. schwartzi from muskrats and dogs of Maryland and the District of Columbia; E. donaldsoni from Pied-billed Grebes of Michigan; and E. cohensi from a sea gull in Canada. Of these four species, only the life cycle of E. donaldsoni is completely known.

Beaver, 1941 reported that small gymnocephalous cercariae from Amnicola limnosa and A. lustrica are those of Echinochasmus donaldsoni. He was able to infect parasite free guppies, mollies, perch and bluegills with the cercaria. He also demonstrated that additional species such as mudminnows, bullheads, and shiners served as suitable second intermediate hosts. Though he fed infective material to canaries, a chicken, a kitten, a rat, mice, and pigeons adult worms were obtained only from the latter of these hosts. In nature the Pied-billed Grebe was found to be infected.

Fundulus heteroclitus collected near Johnson Creek, near Great Bay in South Newington, and near the Hampton River in Hampton, New Hampshire are infected with the metacercariae. Fundulus from the first two of these habitats have small numbers of metacercariae on their gills and when fed to white mice yielded a few adult worms. However, feeding experiments involving the gills of Fundulus heteroclitus from Hampton,

the latter habitat, and white mice yielded large numbers of worms (see Appendix I).

The difference in the habitats are snails and salinity. The habitats near Johnson Creek and Great Bay are brackish and the snail species are Hydrobia salsa and Hydrobia minuta respectively. The habitat near the Hampton River is fresh water and the snail species is Amnicola sp. A fairly large cercaria with the same swimming movements described by Beaver (1941) for the cercaria of E. donaldsoni have been seen to emerge from all three snail species. However, the infection in Amnicola was higher and only this type of cercariae were emerging. Fundulus collected from Hampton have tremendous numbers of echinochasmid metacercariae on their gills. Therefore, the exposure of parasite free poeciliids to the cercaria of Amnicola sp. from Hampton might yield interesting results.

While studying longevity of Echinochasmus magnovatum in different experimental hosts (day-old chicks and mice), differences in time periods of maturity and rate of growth were noted (see Appendix I). For an example, E. magnovatum reaches maturity in day-old chicks between 4 and 5 days of development. Reproductive organs, ovaries and testes are completely developed and a number of large eggs were found in the uterus. This was in marked contrast to E. magnovatum allowed to develop nearly the same time period in the mouse (6 days). Worms in this animal were hardly more than metacercariae, i.e., ovaries and testes are quite small and not fully developed, and without eggs in the uterus. From

other experiments, it has been estimated that E. magnovatum requires between 10 and 12 days in the mouse to reach a stage in development that is acquired in 5 days in day-old chicks. However, in chicks worms are voided after the six day. In mice, E. magnovatum are able to live and continue to grow up to 30 days (no experiments were conducted over this period).

Table XVI shows that means for morphological characters of 30 day old worms from the mouse are consistently larger than those for worms that had been allowed 18 days of development in the mouse (all experiments are based on 12 worms). The means for worms allowed 4 days of development in the chicks are very close to those for worms allowed to develop 18 days in the mouse. However, looking at worms from mice at 6 days, 18 days, and 30 days indicate that they grow about as long as they live. There are enough differences in adult worms from the chick, 18 day old worms from the mouse, and 30 day old worms to make them separate species. Had such differences in worm populations been encounted in nature, no doubt, an investigator would have been tempted to split them up into separate species. When a researcher considers the number of described 20 and 22 spines species that differ from each other only in relative sizes of morphological characters, and the growth rate is not known, the validity of these species must certainly be questioned (see Table XI). Only life cycles can accurately determine species or synonyms; determinations not based on life cycle work are, at best, a matter of opinion.

TABLE XI. COMPARISON OF SPECIES OF *Echinochasmus* OF NORTH AMERICA FROM NATURAL AND EXPERIMENTAL DEFINITIVE HOSTS.

Characters	*E. magnovatum* (Stunkard and Haviland, 1924) Price, 1931 a	*E. schwartzi* Price, 1931 b	*E. donaldsoni* Beaver, 1941 c	*E. cohensi* Raio, 1951 d	*E. magnovatum* Scott e
LENGTH					
Body	0.8 - 1.0	1.5 - 2.1	0.88	2.2	1.01 - 1.56
Oral sucker	0.06 - 0.07	*	0.07	0.09	0.08 - 0.11
Pharynx	0.06 - 0.10	0.10 - 0.15	0.08	0.08	0.10 - 0.10
A. Testis	*	0.15 - 0.27	0.12	0.24	0.15 - 0.25
P. Testis	*	0.18 - 0.31	0.11	0.22	0.15 - 0.25
Cirrus sac	*	*	0.16	*	0.08 - 0.14
Ovary	*	0.10 - 0.12	*	0.12	0.06 - 0.12
WIDTH					
Body	0.13 - 0.23	0.45 - 0.62	0.32	*	0.39 - 0.60
Oral sucker	0.05 - 0.07	*	0.06	0.08	0.08 - 0.12
Pharynx	0.04 - 0.07	0.09 - 0.10	0.06	0.07	0.08 - 0.12
Acetabulum	0.07 - 0.10	0.17 - 0.18	0.15	0.25	0.11 - 0.21
A. Testis	0.05 - 0.09	0.31 - 0.43	0.14	0.21	0.17 - 0.33
P. Testis	0.05 - 0.09	0.26 - 0.37	0.14	0.25	0.19 - 0.31
Cirrus sac	*	*	0.08	*	0.08 - 0.12
Ovary	0.03 - 0.04	0.12 - 0.17	0.07	0.08	0.06 - 0.15
Collar	*	0.24 - 0.27	0.24	*	0.13 - 0.22
SPINES	20	22	20	22	20 - 22

a. From wild rats (*R. norvegicus*); b. From muskrats (*Ondata zibethica*) and dogs (*Canis familiaris*) c. From pigeons (experimental) and Pied-billed grebe (*Podilymbus podiceps*) d. From a sea gull (*Larus argentatus*); and e. Experimentally obtained from feeding gills of *Fundulus heteroclitus* to white mice.

* Not given.

SECTION VIII.

DISCRIMINANT ANALYSIS OF MORPHOLOGICAL VARIATION OF ADULT TREMATODES FROM FINAL HOST ANIMALS (EXPERIMENTAL)

Heterophyid Trematodes of the Ascocotyle Complex

It is well known, among Parasitologists, that many trematodes are polyxenous, i.e., capable of utilizing a variety of animals as definitive hosts. For example, members of the family Heterophyidae are noted for their reputation of completing their development in either birds or mammals. Consequently, some of the variation in morphological characters, at least for heterophyids are, no doubt, due to host connected differences. Trematodes have been known to develop to one size in one mammal and still another size in a different mammal (Witenberg, 1929; Stunkard and Uzmann, 1955). In spite of this knowledge, difference in morphological structures (especially size of the body and reproductive organs) are given considerable rank among the deciding factors in describing species where the life cycle is unknown. An important case in point is differentiation, by Stunkard and Haviland (1924) of their species, Ascocotyle (P.) diminuta, from A. minuta Looss, 1899. These authors gave their reasons for describing this form as a different species from A. minuta (see page 29, above). Even the name of the species, diminuta, means smaller than ordinary or average; very small, or tiny. It is evident (page 29) A. (P.) diminuta was

described as a new species, mainly, on the basis of the size of the body and reproductive organs.

Observations and Experiments

Ascocotyle (P.) diminuta from the chick tend to be larger than those from the mouse. Table XII, below, is presented with measurements of morphological characters; arranged in a manner that one may scrutinize all lengths (ranges and means) or all widths (ranges and means) separately. From this table, it is obvious that worms from the chick and the mouse differ considerably in the length of the body, but only slightly in width of the body, reproductive organs, and other morphological characters. When A. (P.) diminuta from the chick is compared with A. tenuicollis (a species that is considered different because of arrangement of spines and distribution of vitellaria) from the chick, the greater differences are mainly in length and width of the body; differences in other characters are not too pronounced. When A. (P.) diminuta (experimentally obtained by completing the life cycle from the cercaria to the adult stage; 6 to 8 weeks) from the rat is compared with the other species from the chick and the mouse, greater differences, again, appear to be in the length and width of the body; less for other characters.

A null hypothesis was postulated, i.e., there is no real difference in worms from mammals and birds when multiple measurements are considered. Since Fisher (1936) had used multiple measurement in taxonomic problems and developed the discriminant function, Mr. Owen B. Durgin, statistician at

TABLE XII. MEASUREMENTS OF *Ascocotyle* COMPLEX TREMATODES FROM EXPERIMENTAL HOSTS.

Characters	*A.* (*P.*) *diminuta* from the chick (4 days of dev.)		*A.* (*P.*) *diminuta* from the mouse (5 days of dev.)	
LENGTH	R	M	R	M
Body	0.28 - 0.40	0.34	0.18 - 0.30	0.24
O. Sucker	0.04 - 0.05	0.04	0.02 - 0.05	0.03
O. Cecum	0.06 - 0.15	0.10	0.05 - 0.08	0.06
Pharynx	0.03 - 0.04	0.04	0.02 - 0.04	0.03
Acetabulum	0.03 - 0.04	0.04	0.02 - 0.04	0.03
L. Testis	0.03 - 0.06	0.04	0.03 - 0.06	0.05
R. Testis	0.04 - 0.06	0.05	0.03 - 0.05	0.05
Ovary	0.03 - 0.05	0.04	0.02 - 0.03	0.03
WIDTH				
Body	0.12 - 0.17	0.15	0.12 - 0.16	0.13
O. Sucker	0.03 - 0.05	0.04	0.02 - 0.04	0.03
O. Cecum	*		*	
Pharynx	0.03 - 0.04	0.03	0.02 - 0.03	0.02
Acetabulum	0.04 - 0.05	0.04	0.02 - 0.04	0.03
L. Testis	0.03 - 0.07	0.05	0.04 - 0.07	0.05
R. Testis	0.04 - 0.07	0.06	0.04 - 0.06	0.05
Ovary	0.03 - 0.06	0.05	0.02 - 0.03	0.03

* not measured; R/Range, M/Mean

TABLE XII. (Continued)

Characters	A. (P.) diminuta exp. from the rat (2 - 3 days dev.)		A. tenuicollis from the chick (3 days dev.)	
LENGTH	R	M	R	M
Body	0.13 - 0.23	0.17	0.34 - 0.43	0.38
O. Sucker	0.02 - 0.04	0.03	0.03 - 0.05	0.04
O. Cecum	0.04 - 0.08	0.06	0.08 - 0.14	0.11
Pharynx	0.02 - 0.03	0.03	0.03 - 0.05	0.04
Acetabulum	0.02 - 0.04	0.03	0.02 - 0.05	0.04
L. Testis	0.03 - 0.05	0.04	0.05 - 0.07	0.05
R. Testis	0.03 - 0.05	0.04	0.03 - 0.08	0.05
Ovary	0.01 - 0.04	0.02	0.02 - 0.03	0.03
WIDTH				
Body	0.07 - 0.17	0.09	0.08 - 0.17	0.11
O. Sucker	0.02 - 0.04	0.03	0.03 - 0.05	0.04
O. Cecum	*		*	
Pharynx	0.01 - 0.03	0.02	0.03 - 0.04	0.03
Acetabulum	0.02 - 0.05	0.03	0.03 - 0.06	0.05
L. Testis	0.03 - 0.08	0.04	0.03 - 0.06	0.04
R. Testis	0.03 - 0.08	0.04	0.04 - 0.07	0.05
Ovary	0.02 - 0.04	0.03	0.03 - 0.06	0.04

* not measured; R/Range, M/Mean

the University of New Hampshire suggested that discriminant analysis would test the hypothesis. Though I read Fisher's original paper, I found it easier to follow Wert, Neidt, and Ahmann (1954). Twelve specimens each of A. (P.) diminuta were selected from a chick, a mouse, and a rat. An additional, 12 specimens of A. tenuicollis were chosen from a chick. The measurements made on each worm were as follows: Body length; body width; testis length; testis width; ovary length; and ovary width (see Appendix V for actual measurements). These measurements along with the difference in means were evaluated with an IBM Computer (programmed by Mr. O. B. Durgin). The following combinations were evaluated:

1	2	3	4
A. diminuta from the chick	A. diminuta from the mouse	A. diminuta from the rat	A. tenuicollis from the chick

Therefore, when all worms were compared with respect to the above measurements, weights which would produce maximum distinction between groups were obtained:

All Comparisons

	2	3	4
1	(I)	II	III
2		IV	V
3			VI

For actual weight see Table XIII below. The weights in Table XIII, first horizontal row of figures, may be used to obtain a vector for A. (P.) diminuta in the chick vs A. (P.) diminuta

in the mouse (I):

$$V = A_1(X_{1a} - X_{1b}) + A_2(X_{2a} - X_{2b}) + A_3(X_{3a} - X_{3b}) + A_5(X_{5a} - X_{5b}) + A_6(X_{6a} - X_{6b})$$

Table XIII. Weights for Ascocotyle Complex Species

I	3.57045 14.9508	-1.92206	-8.0338	-7.2548	8.0296
II	35.6752 26.8157	10.7445	-114.244	-64.610	84.565
III	-1.06734 -1.99001	3.15165	-14.3073	6.6894	15.2192
IV	1.54779 5.4148	4.01411	-.165780	-5.0272	1.82458
V	6.3980 -1.13101	-.52012	-.194386	-4.07852	17.9054
VI	22.8721 33.878	3.50697	-4.2308	-16.1583	-37.2089

The A's are the weights and the X's are the differences between means for the measurements of worms from the chick and the mouse. When the difference in means between A. (P.) diminuta from the chick and A. (P.) diminuta from the mouse is substituted in the equation the values of A_1, A_2, A_3, A_4, A_5, A_6 obtained by simultaneous solution yield the vector, $V = .7782538$, from the discriminant function. The vector is also the within group sum of squares. The number of degrees of freedom for the discriminant function is the number of variables (= the number of measurement) which, in this instance, is six. The sum of squares for the function is

$$\frac{K_1 K_2}{N} D^2$$

where K_1 and K_2 = 12 worms each for the chick and the mouse and D^2 is the squared within sum of squares. N = 24, the total number of worms from the chick and the mouse. The mean square for the function is

$$\frac{\frac{K_1 K_2}{N} D^2}{m}$$

where m is the degrees of freedom. The mean square for the within is

$$\frac{D}{N-m-1}$$

When the needed values are substituted in the above formula, an analysis of maximum separation can be made as was done in Table XIV-A, below. Such a table shows the test of significance of the discriminantion between A. (P.) diminuta-chick vs A. (P.) diminuta-mouse by means of an F-test:

$$F = \frac{N-m-1}{m} \left(\frac{K_1 K_2}{N}\right)_D$$

$$F = \frac{24-6-1}{8} \quad \left(\frac{12x12}{24}\right) \quad .7782538$$

$$F = 13.0883$$

The F-test is significant at the .01 level. Therefore, two populations of worms exist. Since it has been shown that two populations exist, there is strong evidence that both populations could have resulted by random sampling from two homogeneous populations. Further, the computer has evaluated not only the difference between two means but also the difference between the two variances.

The above procedures were followed for the remaining comparisons:

II *A.* (*P.*) *diminuta*-chick vs *A.* (*P.*) *diminuta*-rat

III *A.* (*P.*) *diminuta*-chick vs *A. tenuicollis*-chick

IV *A.* (*P.*) *diminuta*-mouse vs *A.* (*P.*) *diminuta*-rat

V *A.* (*P.*) *diminuta*-mouse vs *A.* (*P.*) *diminuta*-chick

VI *A.* (*P.*) *diminuta*-rat vs *A.* (*P.*) *diminuta*-chick

For these comparisons, the following tables were prepared:

TABLE XIV. ANALYSIS OF MAXIMUM SEPARATION OF ASCOCOTYLE COMPLEX SPECIES

(A) *A.* (*P.*) *diminuta*-chick vs *A.* (*P.*) *diminuta* mouse

SOURCE OF VARIATION	DF	SS	MS	F
Function	6	3.5574	.5929	13.0883
Within	17	.77	.0453	-
Total	23	4.3274	-	-

(B) *A.* (*P.*) *diminuta*-chick vs *A.* (*P.*) *diminuta*-rat

SOURCE OF VARIATION	DF	SS	MS	F
Function	6	793.5000	132.2500	195.5204
Within	17	11.50	.6764	-
Total	23	805.0000	-	-

(C) A. (P.) diminuta-chick vs A. tenuicollis-chick

SOURCE OF VARIATION	DF	SS	MS	F
Function	6	.8214	.1369	6.3087
Within	17	.37	.0217	-
Total	23	1.1914	-	-

(D) A. (P.) diminuta-mouse vs A. (P.) diminuta-rat

SOURCE OF VARIATION	DF	SS	MS	F
Function	6	.2904	.0484	3.7519
Within	17	.22	.0129	-
Total	23	.5104	-	-

(E) A. (P.) diminuta-mouse vs A. (P.) diminuta-chick

SOURCE OF VARIATION	DF	SS	MS	F
Function	6	4.4646	.7743	14.9767
Within	17	.88	.0517	-
Total	23	5.3446	-	-

(F) A. (P.) diminuta-rat vs A. (P.) diminuta-chick

SOURCE OF VARIATION	DF	S	MS	F
Function	6	90.7926	15.1321	66.1368
Within	17	3.89	.2288	-
Total	23	94.6826	-	-

From this information, so far, it is possible to answer other questions. For example, whether the vector or within sum of squares from the discriminant function in Table XIV-A is due to influence of the chick on growth pattern of worms or influence of the mouse. This information may be obtained by deriving a critical vector from the function. The critical vector may be found by solving the discriminant function twice, once by inserting the actual measurements (from Appendix V) for X_{1a} to X_{6a} for the chick and, again, by inserting actual measurements (from Appendix V) X_{1b} to X_{6b} for the mouse. The critical vector is considered to lie midway between the two vectors. When the values were inserted into the discriminant function, the vectors were:

for A. (P.) diminuta-chick, 1.3102760

for A. (P.) diminuta-mouse, .5320222

The critical vector is then, 0.9211491, midway between vectors for the chick and mouse. Therefore, the chick had a greater influence on the growth pattern of A. (P.) diminuta than the mouse. The same procedures were followed for the other comparisons (II to VI) and is shown in Table XV.

TABLE XV. INFLUENCE OF HOST ON GROWTH PATTERN OF Ascocotyle COMPLEX SPECIES.

Comparison	Animals	VECTORS Parasite 1	Parasite 2	Both	Critical
I	Chick	A. diminuta 1.3102760			
	Mouse		A. diminuta .532020222		
				.7782538	
					0.9211491
II	Chick	A. diminuta 10.664343			
	Rat		A. diminuta 1.730152		
				11.501736	
					6.1972647
III	Chick	A. diminuta 1.1070886			
	Chick		A. tenuicollis .6814473		
				.3740457	
					.8942679
IV	Mouse	A. diminuta .5259483			
	Rat		A. diminuta .3062005		
				.2214056	
					.41407 44

TABLE XV. (Continued)

Comparison	Animals	VECTORS Parasite 1	Parasite 2	Both	Critical
V	Mouse	A. diminuta .6831668			
	Chick		A. tenuicollis 1.6187647		
				.8853176	
					1.1509657
VI	Chick	A. tenuicollis 5.7479388			
	Rat		A. diminuta 1.8565233		
				3.8913014	
					3.8022310

Discussion of Results

Statistical inference has shown that the size of the body and reproductive organs are variable and that different populations can be establish by allowing worms to develop in different experimental host (birds and mammals). Tables XIV-A to XIV-F showed the analysis of maximum separation for all comparisons and tests of significance of discrimination. All were significant at the .01 percent level, except IV where A. (P.) diminuta from the mouse and rat were compared. The F-test indicated (at .01 percent level) two populations do not exist. This comparison was of interest for the following reasons: (1) The worms from the rat were from my experimental life cycle; and (2) worms of the same species (A. (P.) diminuta) from mammals could not be separated into two populations. In all other comparisons (I to III) where worms of the same species (A. (P.) diminuta) were allowed to develop in both birds and mammals, two populations existed. In comparisons (V to VI) the species in the chick was a different species, Ascocotyle tenuicollis, and when compared with A. (P.) diminuta from mammals, they were separated into two populations. In comparison (III) where A. (P.) diminuta in the chick is compared with A. tenuicollis in the chick, two populations were shown to exist. It is clear that the null hypothesis, i.e., no difference in populations of worms from birds and mammals when multiple measurements are considered is rejected at .01 percent level of significance.

The vectors in Table XV yielded possible answers as to which host had greater influence on growth pattern of worms. The vector under the column headed, Both is for two parasites (Parasite 1 and Parasite 2), each from a different host. The vector under the column headed, Parasite 1 or Parasite 2 is for individual parasites from different host. The vector under the column headed, Critical is the combined vectors for Parasite 1 and Parasite 2 divided by 2. Therefore, the Critical vector is midway between those for individual parasites. If the vector for either Parasite 1 or Parasite 2 is above the Critical vector, there is evidence that the particular host in which the parasite was allowed to develop had a greater influence on the growth pattern of the parasite. Hence; the differences in the size of the parasite is due to host connected differences. In all comparisons, regardless of species involved, chicks had a greater influence on the growth rate of worms than mammals.

A criticism of the time periods in which the worms were allowed to develop in the host would not affect the statistical inference that chicks had a greater influence on the growth rate of worms than mammals. For example, in comparison 1 the greater influence is due to the chick though worms developed in chicks for four days as compared to five days in the mouse.

Echinochasmus magnovatum

Although species of *Echinochasmus* have been described from birds and mammals, only one species (*Echinochasmus liliputanous* Looss, 1896) is known to be capable of completing its development in either bird or mammal. It appears that members of the genus are not polyxenous species and probably host specific for either birds or mammals. However, I have been able to infect day-old chicks with metacercariae of *E. magnovatum* and adults have been recovered after five days of development.

Observations and Experiments

Worms from mammals had slower growth rates, maturity rates, and remained in the intestine longer than in birds. Worms from chicks grew nearly three times as fast as those in mammals, but remained in the intestine only 1/6 as long. Worms in chicks were voided in feces after five days of development whereas worms in mammals were able to remain in the intestine for 30 days.

A null hypothesis is postulated, i.e., there are no real differences in worms from birds or mammals when multiple measurements are considered. Twelve worms each from the chick (5 days of development); mouse (6 days of development); mouse (18 days of development); and mouse (30 days of development) were selected for measurements (see Appendix VI). The following measurements were used: Body length; body width; anterior testis length; anterior testis width; posterior testis length; posterior width; ovary length; and ovary width (see Table XVI).

TABLE XVI. MEASUREMENTS OF *Echinochasmus magnovatum* FROM EXPERIMENTAL HOSTS.

Characters	*E. magnovatum* from the chick (4 days dev.)		*E. magnovatum* from the mouse (6 days dev.)	
LENGTH	R	M	R	M
Body	0.09 - 0.79	0.68	0.23 - 0.37	0.29
O. Sucker	0.04 - 0.07	0.06	0.04 - 0.06	0.05
Acetabulum	0.07 - 0.11	0.09	0.05 - 0.10	0.06
Prepharynx	0.06	0.02	0.03	0.01
Pharynx	0.04 - 0.08	0.07	0.03 - 0.07	0.05
Ovary	0.03 - 0.07	0.05	0.01 - 0.02	0.02
A. Testis	0.04 - 0.12	0.07	0.01 - 0.02	0.02
P. Testis	0.06 - 0.12	0.08	0.01 - 0.02	0.02
C. Pouch	0.06 - 0.08	0.07	0.01 - 0.06	0.02
WIDTH				
Body	0.13 - 0.29	0.22	0.07 - 0.16	0.10
O. Sucker	0.04 - 0.07	0.05	0.03 - 0.05	0.04
Acetabulum	0.07 - 0.11	0.09	0.05 - 0.10	0.06
Prepharynx	*		*	
Pharynx	0.07	0.05	0.02 - 0.05	0.04
Ovary	0.04 - 0.08	0.06	0.01 - 0.02	0.01
A. Testis	0.08 - 0.12	0.11	0.01 - 0.04	0.03
P. Testis	0.10 - 0.15	0.12	0.02 - 0.03	0.02
C. Pouch	0.04 - 0.07	0.06	0.01 - 0.02	0.01

* not measured; R/Range, M/Mean

TABLE XVI. (Continued)

Characters	E. magnovatum from the mouse (18 days dev.)		E. magnovatum from the mouse (30 days dev.)	
LENGTH	R	M	R	M
Body	0.71 - 1.55	1.00	1.01 - 1.56	1.33
O. Sucker	0.08 - 0.11	0.09	0.08 - 0.12	0.11
Acetabulum	0.08 - 0.17	0.11	0.10 - 0.18	0.15
Prepharynx	0.02 - 0.05	0.03	0.07	0.02
Pharynx	0.08 - 0.15	0.11	0.10 - 0.18	0.14
Ovary	0.03 - 0.09	0.06	0.06 - 0.12	0.09
A. Testis	0.05 - 0.14	0.09	0.11 - 0.28	0.18
P. Testis	0.06 - 0.17	0.12	0.15 - 0.25	0.20
C. Pouch	0.06 - 0.12	0.09	0.08 - 0.14	0.12
WIDTH				
Body	0.22 - 0.41	0.31	0.39 - 0.60	0.46
O. Sucker	0.06 - 0.09	0.08	0.08 - 0.12	0.10
Acetabulum	0.11 - 0.16	0.12	0.11 - 0.21	0.15
Prepharynx	*		*	
Pharynx	0.08 - 0.11	0.10	0.08 - 0.12	0.10
Ovary	0.05 - 0.11	0.08	0.06 - 0.15	0.09
A. Testis	0.11 - 0.25	0.17	0.17 - 0.33	0.24
P. Testis	0.09 - 0.24	0.16	0.19 - 0.31	0.24
C. Pouch	0.03 - 0.11	0.08	0.08 - 0.12	0.10

* not measured; R/Range, M/Mean

Table XVI, above shows lengths (ranges and means) and widths (ranges and means) for measurements of morphological characters. The measurements along with the differences in means were evaluated with an IBM Computer. The following comparisons were evaluated:

1	2	3	4
E. magnovatum from the chick 5 days dev.	E. magnovatum from the mouse 6 days dev.	E. magnovatum from the mouse 18 days dev.	E. magnovatum from the mouse 30 days dev.

All Comparisons

	2	3	4
1	I	II	III
2		IV	V
3			VI

Weights from the computer for these comparisons are shown in the Table XVII below:

Table XVII. Weights for Echinochasmus magnovatum

I	1.51646 .97883	-.332821 -4.8535	-29.1694 22.6489	1.99857	20.4201
II	12.2228 -7.1448	10.2420 -20.8173	-1.69715 17.2369	28.7083	6.0780
III	2.20614 -1.22316	7.2962 -12.8583	1.93296 -5.4409	.63402	-5.8603
IV	4.7615 63.362	-5.5855 16.3691	28.7348 52.610	11.5270	-28.2804
V	1.40783 -1.01920	1.53972 -4.6718	-1.22569 -4.04477	4.9209	-3.20112
VI	3.94249 2.33806	2.71088 -8.9254	-.355770 6.5846	11.2992	3.04507

The weight served as the discriminant function. The procedures outlined on page 151 to 154 were followed and vectors were obtained for the following comparisons:

I *E. magnovatum*-chick vs *E. magnovatum*-mouse

II *E. magnovatum*-chick vs *E. magnovatum*-mouse

III *E. magnovatum*-chick vs *E. magnovatum*-mouse

IV *E. magnovatum*-mouse vs *E. magnovatum*-mouse

V *E. magnovatum*-mouse vs *E. magnovatum*-mouse

VI *E. magnovatum*-mouse vs *E. magnovatum*-mouse

TABLE XVIII. ANALYSIS OF MAXIMUM SEPARATION OF ECHINOCHASMUS MAGNOVATUM FROM EXPERIMENTAL HOSTS

(A) *E. magnovatum*-chick vs *E. magnovatum*-mouse

SOURCE OF VARIATION	DF	SS	MS	F
Function	8	14.0454	1.7556	17.2117
Within	15	1.53	.1020	-
Total	23	15.5754	-	-

(B) *E. magnovatum*-chick vs *E. magnovatum*-mouse

SOURCE OF VARIATION	DF	SS	MS	F
Function	8	63.3750	7.9218	36.5734
Within	15	3.25	.2166	-
Total	23	66.6250	-	-

(C) E. magnovatum-chick vs E. magnovatum-mouse

SOURCE OF VARIATION	DF	SS	MS	F
Function	8	1332.06	166.507	167.6301
Within	15	14.90	.9933	-
Total	23	1346.96	-	-

(D) E. magnovatum-mouse vs E. magnovatum-mouse

SOURCE OF VARIATION	DF	SS	MS	F
Function	8	1721.7816	215.2227	190.5844
Within	15	16.94	1.1293	-
Total	23	1737.7216	-	-

(E) E. magnovatum-mouse vs E. magnovatum-mouse

SOURCE OF VARIATION	DF	SS	MS	F
Function	8	11.7600	1.4700	15.7556
Within	15	1.40	.0933	-
Total	23	13.1600	-	-

(F) E. magnovatum-mouse vs E. magnovatum-mouse

SOURCE OF VARIATION	DF	SS	MS	F
Function	8	29.0520	3.6315	24.7714
Within	15	2.20	.1466	-
Total	23	31.2520	-	-

In Table XVIII-F, above, the F-test is significant at the .01 percent level of significance. This is enough evidence to conclude that two populations exist. However other questions may be answered. For example, is the growth of Echinochasmus magnovatum influenced more by the type of host or the length of time they are allowed to develop in a given host? The answer to this question may be found by the the following procedures: (1) deriving a vector from the function for each host in comparisons; and (2) finding the critical vectors. The procedures for these steps are outlined on page 156. The vectors for all comparisons and critical vectors are shown in Table XIX.

Discussion of Results

The analysis of maximum separation tables for all comparisons and F-tests of significance of discrimination indicated two populations existed. The null hypothesis is rejected at the .01 percent level of significance. The rate at which Echinochasmus developed to maturity depends upon which host worms are allowed to develop. In comparison I, the greater influence on growth pattern is shown to be in

favor of the chick host rather than the mouse host though worms developed two days longer in the latter host.

In comparisons II and III, the greater influence on growth pattern of Echinochasmus is in favor of mice rather than chicks. Therefore, the influence of either the chick or mouse host on growth pattern of Echinochasmus must be equated with both type of host and length of time the worm is allowed to develop in any one host (comparison I to III). For example, in comparison IV to VI, where only mammals are involved it is clear that influence on growth pattern is due to number of days of development rather than host.

Discussion of Biometrics and Trematode Taxonomy

Mettrick (1963) studied the morphological variation observed between populations of the dicrocoeliid, Zonorchis petiolatum Railliet, 1900, from the crow family (Corvidae); the thrush family (Turdidae); and the starling family (Sturnidae). He noticed considerable variation in every morphological character examined. Further, he was able to show statistically that there were significant differences in the egg size of the trematodes from hosts in different families of birds. He commented:

> If when sufficient material is available to carry out further work comparing other morphological characters, similar differences are found, the question of speciation must be reexamined.

Mettrick used a coefficient of difference, i.e., difference of means divided by the sum of standard deviations, to show the joint nonoverlap percent between populations (based on

TABLE XIX. INFLUENCE OF HOST ON GROWTH PATTERN OF Echinochasmus magnovatum.

Comparison	Animals	VECTORS Parasite 1	Parasite 2	Both	Critical
I	Chick	E. magnovatum 1.3677509			
	Mouse		E. magnovatum .4371124		
				1.5357185	
					.9024316
II	Chick	E. magnovatum 12.1608835			
	Mouse		E. magnovatum 18.3252305		
				3.2523826	
					15.2430570
III	Chick	E. magnovatum 1.6646860			
	Mouse		E. magnovatum 3.6636104		
				14.9048434	
					2.6641482
IV	Chick	E. magnovatum 3.242050			
	Mouse		E. magnovatum 17.044617		
				16.947249	
					10.1433335

TABLE XIX. (Continued)

Comparison Animals		VECTORS Parasite 1	Parasite 2	Both	Critical
V	Mouse	E. magnovatum .4824630			
	Mouse		E. magnovatum 1.8883676		
				1.4058966	
					1.1854153
VI	Mouse	E. magnovatum 7.5423345			
	Mouse		E. magnovatum 9.4638554		
				2.2022890	
					8.5030949

differences in egg size). He stated:

> Because of the large percentage nonoverlap between populations when compared by a coefficient of difference, it is suggested that the standard of species determination, as far as helminthological work is concerned, may lie near Amadon's (1949) concept of subspecific distinction. This expressed in terms of coefficient of difference, would indicate specific difference if the CD value was 1.96 or above. If large populations are being compared a higher CD value (2.18) should be used.

According to Mayr, Linsley, and Usinger (1953) the coefficient of difference is a method used for rough approximation of subspeciation. They concluded that two populations could not be distinguished unequivocally by a single character, but could be separated by multiple character analysis, i.e., using in the analysis simultaneously two or more characters. They suggested several methods of multiple character analysis, but thought Fisher's method of discriminant functions was the most useful. This method has been used by Stone (1947) for studies on fish; Carson and Stalker (1947) for Drosophila and Storer (1950) for birds.

I wish to emphatically point out that my work was not to suggest that trematode species could or could not be distinguished by using multiple character analysis (discriminant functions developed by Fisher, 1936). However, the method strongly indicates that populations exist within trematode species as a result of host connected differences and length of time of development in any one host. The existence of these populations within known species indicated that variation occurs within the species. Studies on populations of worms could lead to better descriptions of

species and hence a more satisfactory concept of speciation.

It is shown in Appendix VIII and IX that it would be unwise to use a coefficient of difference, which assumes only one variable, to separate multiple variable trematodes into species or subspecies.

Observations on variability in other helminth groups have been made by other authors. For example, Bullock (1962) noted that the genus Acanthocephalus exhibited variability in series of worms from different hosts and different geographical locations. He indicated that a plurality of species might be involved, but found considerable overlap in all measurements even when differences in distribution of measurements occurred with host or with location. At the end of his study, he remarked:

> It would appear that even though some species of Acanthocephala are most stable morphologically, others are prone to vary considerably. Wherever possible, descriptions of species of Acanthocephala should include measurements of a long series of worms. It would also be of value to indicate in such a description the number of worms used.

My study on populations of trematodes from different final hosts might serve to remind helminth taxonomists of comments of Mayr, Linsley, and Usinger (1953):

> Most taxonomic characters are variable, and a study of this variability is part of the taxonomic procedure. It is obvious that taxonomic characters should not be drawn from single representatives of populations, but rather from adequate samples.

SECTION IX.

GENERAL SUMMARY

This investigation reports the morphology and life history of Ascocotyle (Phagicola) diminuta (Stunkard and Haviland, 1924) Stunkard and Uzmann, 1955, a trematode belonging to the Ascocotyle Complex. Its complete life history, from the cercaria to the adult has been worked out in the laboratory at the University of New Hampshire. The first intermediate host was found to be Hydrobia salsa Pilsbry, a brackish water snail collected from depressions in the salt marshes near Johnson Creek in Durham, New Hampshire. The incidence of infection of these snails with the heterophyid cercaria was 1 to 2 percent.

The gills of various species and varieties of parasite free poeciliids (marble mollies, red swordtails, green tuxedo swordtails, red platies, red wagtail platies, and red tuxedo platy) have been experimentally infected with the heterophyid cercaria from Hydrobia salsa. The gills from the experimental tropical fish were fed to laboratory-reared day-old chicks, white rats, and white mice after the metacercariae had been allowed to mature for three weeks. Worms have been recovered from experimental hosts after 2 to 4 days of development. Adults were described from day-old chicks after 4 days of development. Cercaria and redia stages were described from Hydrobia salsa. The metacercaria was described from wild Fundulus heteroclitus and experimental tropical fish.

Studies have also been made on the sexual cycle of Ascocotyle tenuicollis Price, 1935, a species restricted to the conus arteriosus of Fundulus heteroclitus collected from depressions in salt marshes near Johnson Creek in Durham, New Hampshire. This species was also found in the conus arteriosus of Fundulus heteroclitus collected from depressions of salt marshes near Great Bay in South Newington; and near the Hampton River in Hampton, New Hampshire. However, only metacercariae from the hearts of Fundulus collected in Durham were fed to day-old chicks. Morphological studies were made on adult worms recovered from day-old chicks after 3 days of development. Variation in the oral coronet of spines and size of the body were noted. The species was redescribed. This is the first report of Fundulus heteroclitus serving as second intermediate host for this species. Therefore, this is a new host and locality record.

This investigation also reports the finding of the second intermediate host (Fundulus heteroclitus) of Echinochasmus magnovatum (Stunkard and Haviland, 1924) Price, 1931, one of the three species of this genus described from this country. The sexual cycle was studied. Its morphology and development in experimental definitive host (day-old chicks and white mice) from 5 to 30 days was studied. Variation in the number of spines and size of the body were noted and the species was redescribed from the mouse after 18 days of development. Other observations were made on uterine egg counts and the hatching of the miracidium in various solutions.

Discriminant analysis of morphological variation of trematodes from final host animals (experimental) was considered. Heterophyid trematodes of the Ascocotyle Complex showed considerable variation in regards to the size of the body and reproductive organs. Statistical inference from multiple character analysis indicated populations in experiments where worms were allowed to develop in different experimental hosts (day-old chicks, white mice, and a white rat). It was found that regardless of species involved, chick hosts had a greater influence on growth pattern of worms than mammalian hosts.

Echinochasmus magnovatum from final host animals was also subjected to discriminant analysis. The size of the body and reproductive organs varied considerably. Multiple character analysis indicated populations existed when worms were allowed to develop in different experimental hosts (a day-old chick and white mice). It was found that the influence of either chicks or mice on the growth pattern of E. magnovatum was equated with both type of host and number of days worms were allowed to develop in any one host.

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APPENDIX I.

FEEDING EXPERIMENTS INVOLVING WILD *Fundulus heteroclitus* (FROM DEPRESSIONS OF SALT MARSHES IN SOUTHEASTERN N. H.) AND LABORATORY-REARED ANIMALS.

A. *Fundulus* from Johnson Creek

Experimental Animal	*A. (P.) diminuta*	*E. magnovatum*	Remarks
			All mice in this table were fed gills from two fish on 2 consecutive days and fed rat food on the third day after the first infective feeding.
Mouse No. 1	Numerous	Few	Mouse No. 1 and No. 2 were killed and examined for worms on the tenth day following the first infective feeding. All specimens of *A. (P.) diminuta* were mature (uterus filled with eggs). Most of the specimens of *Echinochasmus magnovatum* were immature though some had one or two eggs in the uterus.
Mouse No. 2	"	"	
Mouse No. 3	"	"	Mouse No. 3 and No. 4 were killed on the third day after the first infective feeding. Specimens of *A. (P.) diminuta* were both mature and immature while those of *Echinochasmus magnovatum* were all immature.
Mouse No. 4	"	"	

A. Fundulus from Johnson Creek (Continued)

Experimental Animal	A. (P.) diminuta	E. magnovatum	Remarks
Mouse No. 5	Numerous	Few	Mouse No. 5 and No. 6 were killed thirteen days after the first infective feeding. Some moribund specimens of A. (P.) diminuta were observed. All specimens of Echinochasmus magnovatum had reached maturity and had two to six eggs in the uterus.
Mouse No. 6	"	"	
Mouse No. 7	"	"	Mouse No. 7 and No. 8 were killed on the sixth day after the first infective feeding. All specimens of A. (P.) diminuta had reached maturity while none of the Echinochasmus magnovatum had reached maturity.
Mouse No. 8	"	"	
Chick No. 8	220	8	Chick No. 8 was fed a daily diet of gills (from 2 fish each day) and killed on the fourth day. All specimens of A. (P.) diminuta were mature while those of E. magnovatum were immature.

A. Fundulus from Johnson Creek (Continued)

Experimental Animal	A. (P.) diminuta	E. magnovatum	Remarks*
Chick No. 9	70	5	Chick No. 9 and No. 10 were fed the same diet as Chick No. 8, but the diet was increased to include 5 fish on the fifth and sixth day. The chicks were all killed on the sixth day. Specimens of all worms recovered were mature.
Chick No. 10	86	39	
Chick No. 11	280	14	Chick No. 11 was fed the same as Chick No. 8 and killed on the fourth day. Specimens of A. (P.) diminuta were all mature. Specimens of E. magnovatum were all immature.
Chick No. 12	Negative	Negative	Chicks No. 12, 13, and 14 were fed a daily diet of gills (from 3 fish each day) for 6 days. Chick No. 12 and No. 13 were killed on the sixth day.
Chick No. 13	"	"	
Chick No. 14	4	1	Chick No. 14 died on the seventh day. Four moribund specimens of E. magnovatum were recovered. All specimens were mature.

* All chicks were one day-old. They were given their first infective feedings while their feathers were still moist. All other feedings were infective. They were never given chicken food.

B. Fundulus from Crommet Creek (Continued)

Experimental Animal	A. (P.) diminuta	E. magnovatum	Remarks*
Mouse No. 9	Numerous	Few	Mouse No. 9 and No. 10 were killed three days after the first infective feeding. Both mature and immature specimen of A. (P.) diminuta were recovered. All specimens of Echinochasmus magnovatum were immature.
Mouse No. 10	"	"	
Mouse No. 11	"	"	Mouse No. 11 and No. 12 were killed ten days after the first infective feeding. All specimens of A. (P.) diminuta were mature while those of Echinochasmus were all immature.
Mouse No. 12	"	"	

* All animals were fed the gills from six fish in one infective feeding.

C. *Fundulus* from South Newington (Continued)

Experimental Animal	*A.* (*P.*) *diminuta*	*E. magnovatum*	Remarks*
Chick No. 15	Negative	Negative	Chick No. 15 was examined for worms on the second day after the infective feeding. Chicks No. 16, 17, 18, and 19 were examined on the 3rd, 4th, 5th and 6th day.
Chick No. 16	"	"	
Chick No. 17	"	"	
Chick No. 18	"	"	
Chick No. 19	"	"	
Mouse No. 19	Numerous	Few	Mouse No. 19 and No. 20 were examined for worms on the 4th day. Specimens of *A.* (*P.*) *diminuta* were both mature and immature. All specimens of *E. magnovatum* were immature.
Mouse No. 20	"	"	
Mouse No. 21	"	"	Mouse No. 21 and No. 22 were killed on the 10th day. All specimens of *A.* (*P.*) *diminuta* were mature. Specimens of *E. magnovatum* were immature.
Mouse No. 22	"	"	

C. Fundulus from South Newington (Continued)

Experimental Animal	A. (P.) diminuta	E. magnovatum	Remarks*
Mouse No. 23	Numerous	Few	Mouse No. 23 was examined for worms on the 13th day. All specimens of A. (P.) diminuta were mature. Some morbound specimens of this species was observed. Specimens of E. magnovatum were both mature and immature. Mature specimens of this species had 1 to 3 eggs in the uterus.

* All chicks were 10 days old. They were given one infective feeding of gills from 18 fish (divided into equal portions). On the following day, they were placed on a regular diet of chicken food.

All mice were given one infective feeding from the gills of 25 fish (divided into equal portions).

D. *Fundulus* from Hampton (Continued)

Experimental Animal	*A.* (*P.*) *diminuta*	*E. magnovatum*	Remarks*
Mouse No. 13	Negative	1,192	Mouse No. 13 was given one infective feeding of gills from 3 fish. The animal was killed 18 days after the infective feeding. The infection was very extensive since 1,097 worms were taken from the small intestine, 20 from the large intestine and 75 from the cecum. These worms were all mature though some had reached maturity at a smaller size.
Mouse No. 14	Few	228	Mouse No. 14 was fed the gills from one fish in one infective feeding and killed on the 6th day. All *Echinochasmus* were immature and all *Ascocotyle* were mature.
Mouse No. 15	"	361	Mouse No. 15 was fed gills from one fish and killed on the 14th day. All worms were mature.

D. *Fundulus* from Hampton (Continued)

Experimental Animal	*A.* (*P.*) *diminuta*	*E. magnovatum*	Remarks
Mouse No. 16	Negative	15	Mouse No. 16 was fed gills from one fish and died on the 17th day. The cause of death was unknown. All worms recovered were mature.
Mouse No. 17	"	26	Mouse No. 17 was also fed one infective feeding. The animal was killed on the 22nd day. All worms were mature.
Mouse No. 18	"	925	Mouse No. 18 was given one infective feeding which included the gills of seven fish. The animal was killed on the 20th day and all worms were mature. However, many had reached maturity at a different size.
Rat No. 1	"	2	Rat No. 1 was given one infective feeding of gills from seven fish and killed on the 14th day. The worms recovered were mature.
Chick No. 1	"	Negative	Chick No. 1 and No. 2 were 7 days old. The gills from seven fish were divided between the 2 chicks. Both died 3 days after the infective feeding. The were autopsied and found negative.
Chick No. 2	"	"	

APPENDIX II.

MEASUREMENTS OF METACERCARIAE ON THE GILLS OF WILD *Fundulus heteroclitus*.

A. *Fundulus* from Johnson Creek

Number of Metacercariae	Length	Width
1.	0.20	0.11
2.	0.14	0.09
3.	0.13	0.08

0.13 - 0.20 Range of Size 0.08 - 0.11

0.16 Mean 0.09

±.037 Standard Deviation ±.016

23% Coefficient of Variation 17.7%

* Gill Arch No. 1

4.	0.14	0.08
5.	0.19	0.14
6.	0.22	0.13
7.	0.16	0.09
8.	0.16	0.13
9.	0.20	0.13
10.	0.14	0.08
11.	0.17	0.09
12.	0.19	0.14
13.	0.19	0.14
14.	0.16	0.09
15.	0.17	0.11

0.14 - 0.22 Range of Size 0.08 - 0.14

0.17 Mean 0.11

±.024 Standard Deviation ±.024

14.1% Coefficient of Variation 21.8%

* Gill Arch No. 2

A. Fundulus from Johnson Creek (Continued)

Number of Metacercariae	Length	Width
16.	0.22	0.13
17.	0.16	0.13
18.	0.14	0.11
19.	0.13	0.08

0.13 - 0.22 Range of Size 0.08 - 0.13

0.16 Mean 0.11

±.04 Standard Deviation ±.024

25% Coefficient of Variation 21.8%

* Gill Arch No. 3

20.	0.08	0.05
21.	0.16	0.09
22.	0.17	0.09
23.	0.16	0.11
24.	0.17	0.14
25.	0.14	0.09

0.08 - 0.17 Range of Size 0.05 - 0.14

0.15 Mean 0.09

±.034 Standard Deviation ±.03

22.6% Coefficient of Variation 33.3%

* Gill Arch No. 4

A. Fundulus from Johnson Creek (Continued)

Number of Metacercariae	Length	Width
26.	0.16	0.08
27.	0.09	0.06
28.	0.09	0.06
29.	0.19	0.13
30.	0.17	0.13
31.	0.20	0.17
32.	0.16	0.13
33.	0.20	0.13
34.	0.16	0.09
35.	0.15	0.16
36.	0.14	0.09
37.	0.16	0.13
38.	0.16	0.09
39.	0.17	0.11
	0.09 - 0.20 Range of Size	0.06 - 0.17
	0.16 Mean	0.11
	±.01 Standard Deviation	±.034
	6.2% Coefficient of Variation	30.9%

* Gill Arch No. 5

40.	0.13	0.09
41.	0.20	0.13
42.	0.16	0.08
43.	0.16	0.08
44.	0.17	0.08
45.	0.16	0.09
46.	0.22	0.13
47.	0.17	0.14
48.	0.17	0.09
49.	0.22	0.13
50.	0.19	0.14
51.	0.20	0.13
52.	0.16	0.09
	0.13 - 0.22 Range of Size	0.08 - 0.14

A. Fundulus from Johnson Creek (Continued)

Number of Metacercariae	Length	Width
	0.18 Mean	0.11
	±.026 Standard Deviation	±.024
	14.4% Coefficient of Variation	21.8%

* Gill Arch No. 6

53.	0.14	0.08
54.	0.11	0.08
55.	0.11	0.09
56.	0.14	0.09
57.	0.16	0.11
58.	0.14	0.09
59.	0.20	0.14
60.	0.14	0.09
61.	0.17	0.11
62.	0.17	0.14
63.	0.13	0.09
64.	0.14	0.08
65.	0.14	0.13
66.	0.22	0.17
67.	0.16	0.09
68.	0.14	0.09
69.	0.14	0.11
70.	0.22	0.13
	0.11 - 0.22 Range of Size	0.08 - 0.17
	0.15 Mean	0.11
	±.031 Standard Deviation	±.026
	20.6% Coefficient of Variation	23.6%

* Gill Arch No. 7

A. Fundulus from Johnson Creek (Continued)

Number of Metacercariae	Length	Width
71.	0.27	0.17
72.	0.19	0.13
73.	0.20	0.11
74.	0.16	0.11
75.	0.20	0.13
76.	0.13	0.11
77.	0.11	0.06
78.	0.17	0.14
79.	0.14	0.08
80.	0.14	0.08
81.	0.14	0.08
82.	0.09	0.06
83.	0.17	0.09
84.	0.16	0.09
85.	0.19	0.11
86.	0.11	0.08
87.	0.14	0.08
88.	0.17	0.09
89.	0.16	0.11
90.	0.14	0.09
91.	0.14	0.09
92.	0.17	0.09
93.	0.22	0.13
94.	0.16	0.13
95.	0.16	0.08
96.	0.16	0.11
97.	0.14	0.08
98.	0.17	0.11
99.	0.13	0.08
100.	0.16	0.08
101.	0.19	0.11
102.	0.17	0.09
103.	0.11	0.06
104.	0.17	0.11
	0.09 - 0.27 Range of Size	0.06 - 0.17
	0.16 Mean	0.10
	±.035 Standard Deviation	±.024
	21.8% Coefficient of Variation	24%

* Gill Arch No. 8

B. Fundulus from Crommet Creek (Continued)

Number of Metacercariae	Length	Width
1.	0.17	0.14
2.	0.19	0.13
3.	0.14	0.13
4.	0.14	0.11
5.	0.14	0.09
6.	0.16	0.09
7.	0.14	0.11
8.	0.14	0.09
9.	0.13	0.08
10.	0.16	0.09
11.	0.19	0.09
12.	0.16	0.08
13.	0.17	0.11
14.	0.16	0.09
15.	0.14	0.08
	0.13 - 0.19 Range of Size	0.08 - 0.14
	0.15 Mean	0.10
	±.02 Standard Deviation	±.02
	13.3% Coefficient of Variation	20%

* Gill Arch No. 1

16.	0.14	0.08
17.	0.16	0.09
18.	0.17	0.11
19.	0.16	0.09
20.	0.17	0.09
21.	0.22	0.14
22.	0.16	0.09
23.	0.16	0.09
24.	0.13	0.08
25.	0.16	0.11
26.	0.14	0.09
27.	0.09	0.08
28.	0.19	0.14
29.	0.16	0.09
30.	0.14	0.08
31.	0.17	0.11

B. Fundulus from Crommet Creek (Continued)

Number of Metacercariae	Length	Width
32.	0.13	0.08
33.	0.19	0.11
34.	0.17	0.13
35.	0.14	0.09
36.	0.19	0.13
37.	0.14	0.08
38.	0.14	0.08
39.	0.13	0.08
40.	0.13	0.09
41.	0.19	0.09
42.	0.14	0.09
43.	0.14	0.11
	0.09 - 0.22 Range of Size	0.08 - 0.14
	0.15 Mean	0.10
	±.026 Standard Deviation	±.02
	10.6% Coefficient of Variation	20%

* Gill Arch No. 2

44.	0.16	0.09
45.	0.16	0.09
46.	0.14	0.09
47.	0.14	0.08
48.	0.16	0.08
49.	0.22	0.16
50.	0.14	0.09
51.	0.16	0.11
52.	0.14	0.08
53.	0.22	0.11
54.	0.22	0.13
55.	0.14	0.08
56.	0.21	0.14
57.	0.16	0.08
58.	0.14	0.09
59.	0.19	0.14
60.	0.16	0.11
61.	0.16	0.09
62.	0.16	0.13

B. Fundulus from Crommet Creek (Continued)

Number of Metacercariae	Length	Width
63.	0.16	0.11
64.	0.16	0.11
65.	0.21	0.13
66.	0.13	0.09
67.	0.22	0.14
68.	0.16	0.11
69.	0.16	0.09
	0.13 - 0.22 Range of Size	0.08 - 0.16
	0.17 Mean	0.10
	±.03 Standard Deviation	±.024
	17.6% Coefficient of Variation	24%

* Gill Arch No. 3

70.	0.14	0.08
71.	0.14	0.08
72.	0.14	0.08
73.	0.13	0.09
74.	0.13	0.09
75.	0.19	0.09
76.	0.14	0.08
77.	0.14	0.08
78.	0.13	0.09
79.	0.16	0.09
80.	0.20	0.14
81.	0.20	0.13
82.	0.14	0.09
83.	0.22	0.16
84.	0.13	0.09
85.	0.11	0.08
86.	0.19	0.13
87.	0.20	0.13
	0.11 - 0.22 Range of Size	0.08 - 0.16

B. Fundulus from Crommet Creek (Continued)

Number of Metacercariae	Length	Width
0.16	Mean	0.10
±.032	Standard Deviation	±.024
20%	Coefficient of Variation	24%

* Gill Arch No. 4

88.	0.14	0.09
89.	0.14	0.09
90.	0.09	0.06
91.	0.16	0.09
92.	0.13	0.09
93.	0.19	0.11
94.	0.13	0.08
95.	0.21	0.14
96.	0.16	0.09
97.	0.19	0.13
98.	0.13	0.08
99.	0.13	0.08
100.	0.16	0.11
101.	0.16	0.08
102.	0.14	0.09
103.	0.17	0.11
104.	0.16	0.09
105.	0.16	0.11
106.	0.16	0.09
107.	0.13	0.09
108.	0.14	0.09
109.	0.14	0.08
110.	0.16	0.09
0.09 - 0.21	Range of Size	0.06 - 0.14
0.15	Mean	0.09
±.024	Standard Deviation	±.02
16%	Coefficient of Variation	22.2%

* Gill Arch No. 5

B. Fundulus from Crommet Creek (Continued)

Number of Metacercariae	Length	Width
111.	0.16	0.08
112.	0.14	0.09
113.	0.16	0.08
114.	0.22	0.11
115.	0.14	0.08
116.	0.14	0.09
117.	0.22	0.11
118.	0.11	0.08
119.	0.17	0.09
120.	0.19	0.13
121.	0.17	0.11
122.	0.17	0.14
123.	0.14	0.08
124.	0.17	0.11
125.	0.11	0.09
126.	0.17	0.11
127.	0.16	0.11
128.	0.13	0.09
129.	0.11	0.08
130.	0.14	0.08
131.	0.16	0.08
132.	0.14	0.09
133.	0.14	0.09
134.	0.14	0.08
135.	0.14	0.08
136.	0.08	0.16
137.	0.14	0.09
138.	0.16	0.09
139.	0.16	0.08
140.	0.17	0.11
141.	0.14	0.11
142.	0.17	0.14
143.	0.16	0.08

0.08 - 0.22	Range of Size	0.08 - 0.16
0.15	Mean	0.10
±.028	Standard Deviation	±.02
18.6%	Coefficient of Variation	20%

* Gill Arch No. 6

B. Fundulus from Crommet Creek (Continued)

Number of Metacercariae	Length	Width
144.	0.14	0.11
145.	0.14	0.09
146.	0.16	0.09
147.	0.16	0.09
148.	0.17	0.11
149.	0.16	0.08
150.	0.20	0.14
151.	0.16	0.11
152.	0.14	0.08
153.	0.14	0.09
154.	0.16	0.08
155.	0.11	0.08
156.	0.13	0.08
157.	0.17	0.11
158.	0.13	0.08
159.	0.09	0.16
160.	0.14	0.14
161.	0.22	0.13
162.	0.14	0.09
163.	0.20	0.13
164.	0.11	0.08
	0.09 - 0.22 Range of Size	0.08 - 0.16
	0.15 Mean	0.10
	±.032 Standard Deviation	±.024
	20.3% Coefficient of Variation	24%

* Gill Arch No. 7

165.	0.14	0.11
166.	0.22	0.13
167.	0.19	0.13
168.	0.19	0.13
169.	0.17	0.13
170.	0.14	0.06
171.	0.16	0.09
172.	0.16	0.11

B. Fundulus from Crommet Creek (Continued)

Number of Metacercariae	Length	Width
173.	0.19	0.13
174.	0.14	0.09
175.	0.14	0.09
176.	0.17	0.09
177.	0.24	0.14
178.	0.22	0.14
179.	0.22	0.16
180.	0.14	0.08
	0.14 - 0.24 Range of Size	0.06 - 0.16
	0.18 Mean	0.11
	±.034 Standard Deviation	±.026
	18.8% Coefficient of Variation	23.6%

* Gill Arch No. 8

C. Fundulus from South Newington (Continued)

Number of Metacercariae	Length	Width
1.	0.14	0.09
2.	0.14	0.08
3.	0.16	0.09
4.	0.20	0.11
5.	0.14	0.11
6.	0.13	0.11
7.	0.20	0.11
8.	0.19	0.11
9.	0.16	0.13
10.	0.25	0.16
11.	0.19	0.11
12.	0.20	0.11
13.	0.19	0.11
14.	0.13	0.13
15.	0.22	0.13
16.	0.14	0.13
17.	0.16	0.14
18.	0.20	0.11
19.	0.20	0.13
20.	0.20	0.13
21.	0.20	0.13
22.	0.19	0.13
23.	0.19	0.13
24.	0.14	0.08
25.	0.22	0.13
26.	0.17	0.09
27.	0.22	0.13
28.	0.20	0.11
29.	0.19	0.13
30.	0.19	0.13
31.	0.14	0.08
32.	0.16	0.13
33.	0.27	0.13
34.	0.16	0.14
35.	0.17	0.13
36.	0.24	0.14
37.	0.16	0.08
38.	0.24	0.11
39.	0.22	0.13
40.	0.13	0.08
41.	0.16	0.09
42.	0.20	0.11
43.	0.25	0.11
44.	0.19	0.13
45.	0.19	0.13

C. Fundulus from South Newington (Continued)

Number of Metacercariae	Length	Width
46.	0.20	0.14
47.	0.24	0.13
48.	0.20	0.13
49.	0.14	0.09
50.	0.22	0.14
	0.13 - 0.27 Range of Size	0.08 - 0.16
	0.19 Mean	0.12
	±.036 Standard Deviation	±.02
	19% Coefficient of Variation	16.6%

* Gill Arch No. 1

51.	0.20	0.14
52.	0.17	0.13
53.	0.22	0.14
54.	0.24	0.13
55.	0.19	0.14
56.	0.17	0.09
57.	0.22	0.13
58.	0.14	0.08
59.	0.13	0.13
60.	0.22	0.11
61.	0.13	0.11
62.	0.20	0.13
63.	0.20	0.13
64.	0.20	0.16
65.	0.20	0.11
66.	0.24	0.14
67.	0.19	0.13
68.	0.17	0.11
69.	0.16	0.11
70.	0.16	0.11
71.	0.24	0.14
72.	0.24	0.17
73.	0.22	0.14
74.	0.19	0.14
75.	0.19	0.16

C. Fundulus from South Newington (Continued)

Number of Metacercariae	Length	Width
76.	0.22	0.13
77.	0.17	0.11
78.	0.24	0.14
79.	0.19	0.11
80.	0.19	0.13
81.	0.20	0.13
82.	0.22	0.13
83.	0.19	0.11
84.	0.22	0.14
85.	0.19	0.11
86.	0.22	0.13
87.	0.19	0.13
88.	0.16	0.09
	0.13 - 0.24 Range of Size	0.08 - 0.17
	0.19 Mean	0.13
	±.03 Standard Deviation	±.02
	15.7% Coefficient of Variation	15.3%

* Gill Arch No. 2

89.	0.19	0.11
90.	0.24	0.13
91.	0.25	0.14
92.	0.16	0.11
93.	0.24	0.14
94.	0.20	0.13
95.	0.19	0.14
96.	0.19	0.11
97.	0.25	0.13
98.	0.20	0.11
99.	0.22	0.08
100.	0.20	0.14
101.	0.16	0.11
102.	0.20	0.14
103.	0.19	0.14
104.	0.22	0.14
105.	0.22	0.13
106.	0.22	0.13

C. Fundulus from South Newington (Continued)

Number of Metacercariae	Length	Width
107.	0.16	0.09
108.	0.13	0.09
109.	0.17	0.11
110.	0.14	0.08
111.	0.20	0.11
112.	0.09	0.08
113.	0.24	0.14
114.	0.20	0.13
115.	0.24	0.13
116.	0.24	0.14
117.	0.19	0.11
118.	0.19	0.14
119.	0.24	0.16
120.	0.20	0.13
121.	0.25	0.14
122.	0.24	0.14
123.	0.19	0.14
124.	0.24	0.13
125.	0.20	0.13
	0.09 - 0.25 Range of Size	0.08 - 0.16
	0.20 Mean	0.12
	±.036 Standard Deviation	±.02
	18% Coefficient of Variation	16.6%

* Gill Arch No. 3

126.	0.19	0.09
127.	0.17	0.11
128.	0.19	0.14
129.	0.24	0.14
130.	0.24	0.13
131.	0.25	0.16
132.	0.24	0.16
133.	0.24	0.13
134.	0.24	0.14
135.	0.13	0.06.
136.	0.22	0.13
137.	0.20	0.14

C. Fundulus from South Newington (Continued)

Number of Metacercariae	Length	Width
138.	0.17	0.09
139.	0.24	0.14
140.	0.22	0.14
141.	0.20	0.13
142.	0.20	0.13
143.	0.22	0.16
144.	0.24	0.16
145.	0.17	0.13
146.	0.19	0.13
147.	0.20	0.13
148.	0.22	0.14
149.	0.20	0.14
150.	0.22	0.13
151.	0.16	0.11
152.	0.16	0.13
153.	0.16	0.09
154.	0.17	0.14
155.	0.16	0.19
156.	0.11	0.09
157.	0.09	0.06
158.	0.20	0.14
159.	0.20	0.14
160.	0.20	0.13
161.	0.17	0.11
162.	0.20	0.13
163.	0.24	0.13
164.	0.25	0.14
165.	0.20	0.13
166.	0.24	0.14
167.	0.20	0.14
168.	0.22	0.13
169.	0.22	0.13
170.	0.19	0.14
171.	0.24	0.14
172.	0.19	0.14
173.	0.20	0.14
174.	0.16	0.09
175.	0.19	0.13
176.	0.19	0.14
177.	0.22	0.11
178.	0.22	0.13
179.	0.24	0.14
180.	0.22	0.13
181.	0.24	0.14

C. Fundulus from South Newington (Continued)

Number of Metacercariae	Length	Width
	0.09 - 0.25 Range of Size	0.06 - 0.19
	0.20 Mean	0.13
	±.034 Standard Deviation	±.022
	17% Coefficient of Variation	17%
* Gill Arch No. 4		
182.	0.20	0.14
183.	0.24	0.16
184.	0.20	0.14
185.	0.16	0.11
186.	0.17	0.09
187.	0.16	0.11
188.	0.22	0.14
189.	0.19	0.11
190.	0.19	0.14
191.	0.20	0.09
192.	0.16	0.09
193.	0.20	0.14
194.	0.19	0.11
195.	0.19	0.14
196.	0.20	0.13
197.	0.24	0.11
198.	0.14	0.11
199.	0.16	0.09
200.	0.16	0.09
201.	0.16	0.11
202.	0.22	0.16
203.	0.16	0.09
204.	0.20	0.16
205.	0.22	0.14
206.	0.25	0.13
207.	0.20	0.13
208.	0.13	0.09
209.	0.16	0.13
210.	0.22	0.13
211.	0.22	0.13
212.	0.14	0.09

C. Fundulus from South Newington (Continued)

Number of Metacercariae	Length	Width
213.	0.22	0.11
214.	0.24	0.14
215.	0.19	0.11
216.	0.22	0.13
217.	0.20	0.13
218.	0.17	0.11
219.	0.22	0.14
220.	0.20	0.13
221.	0.22	0.13
222.	0.19	0.11
223.	0.20	0.11
224.	0.24	0.16
225.	0.20	0.11
226.	0.17	0.11
227.	0.19	0.13
228.	0.17	0.13
229.	0.22	0.14
230.	0.20	0.14
231.	0.17	0.13
232.	0.22	0.14
233.	0.13	0.11
234.	0.20	0.14
235.	0.19	0.13
236.	0.19	0.09
237.	0.20	0.14
238.	0.17	0.13
239.	0.20	0.13
240.	0.22	0.16
241.	0.17	0.14
242.	0.20	0.13
243.	0.17	0.13
244.	0.16	0.08
245.	0.20	0.14
246.	0.19	0.11
247.	0.19	0.11
248.	0.22	0.11
249.	0.19	0.11
250.	0.22	0.13
251.	0.17	0.09
	0.13 - 0.25 Range of Size	0.08 - 0.16
	0.19 Mean	0.12

C. Fundulus from South Newington (Continued)

Number of Metacercariae	Length	Width
	±.026 Standard Deviation	±.02
	13.7% Coefficient of Variation	16.6%
* Gill Arch No. 5		
252.	0.22	0.13
253.	0.24	0.13
254.	0.24	0.13
255.	0.19	0.13
256.	0.14	0.09
257.	0.24	0.13
258.	0.17	0.13
259.	0.22	0.13
260.	0.24	0.16
261.	0.19	0.11
262.	0.14	0.09
263.	0.16	0.08
264.	0.19	0.14
265.	0.22	0.13
266.	0.22	0.13
267.	0.19	0.11
268.	0.19	0.13
269.	0.19	0.13
270.	0.19	0.14
271.	0.22	0.14
272.	0.16	0.11
273.	0.24	0.11
274.	0.17	0.13
275.	0.20	0.14
276.	0.14	0.08
277.	0.17	0.11
278.	0.20	0.14
279.	0.17	0.09
280.	0.19	0.11
281.	0.19	0.13
282.	0.20	0.11
	0.14 - 0.24 Range of Size	0.08 - 0.16
	0.19 Mean	0.12

C. Fundulus from South Newington (Continued)

Number of Metacercariae	Length	Width
	±.03 Standard Deviation	±.02
	15.8% Coefficient of Variation	16.6%

* Gill Arch No. 6

283.	0.19	0.14
284.	0.22	0.11
285.	0.20	0.13
286.	0.20	0.13
287.	0.19	0.14
288.	0.20	0.14
289.	0.09	0.06
290.	0.16	0.11
291.	0.16	0.11
292.	0.24	0.14
293.	0.14	0.09
294.	0.20	0.13
295.	0.20	0.14
296.	0.22	0.11
297.	0.19	0.13
298.	0.22	0.13
299.	0.19	0.13
300.	0.22	0.14
301.	0.20	0.14
302.	0.17	0.13
303.	0.24	0.14
304.	0.19	0.13
305.	0.17	0.11
	0.09 - 0.24 Range of Size	0.06 - 0.14
	0.19 Mean	0.12
	±.033 Standard Deviation	±.02
	17.3% Coefficient of Variation	17.3%

* Gill Arch No. 7

C. Fundulus from South Newington (Continued)

Number of Metacercariae	Length	Width
306.	0.24	0.09
307.	0.22	0.16
308.	0.22	0.14
309.	0.20	0.13
310.	0.22	0.14
311.	0.19	0.13
312.	0.22	0.14
313.	0.24	0.22
314.	0.24	0.13
315.	0.16	0.13
316.	0.19	0.11
317.	0.17	0.11
318.	0.22	0.14
319.	0.20	0.13
320.	0.16	0.11
321.	0.20	0.14
322.	0.20	0.13
323.	0.19	0.13
324.	0.22	0.11
325.	0.17	0.14
326.	0.20	0.13
327.	0.17	0.13
328.	0.24	0.14
329.	0.24	0.14
330.	0.20	0.13
331.	0.22	0.13
332.	0.16	0.11
333.	0.22	0.14
334.	0.24	0.13
335.	0.16	0.11
0.16 - 0.24	Range of Size	0.09 - 0.22
0.20	Mean	0.13
±.028	Standard Deviation	±.022
14%	Coefficient of Variation	17%

* Gill Arch No. 8

D. Fundulus from Hampton** (Continued)

Number of Metacercariae	Length	Width
1.	0.09	0.06
2.	0.08	0.06
3.	0.08	0.06
4.	0.09	0.06
5.	0.06	0.06
6.	0.06	0.06
7.	0.06	0.06

0.06 - 0.09 Range of Size 0.06 - ____

0.07 Mean 0.06

±.014 Standard Deviation ____

20% Coefficient of Variation ____

* Gill Arch No. 1

8.	0.08	0.06
9.	0.06	0.06
10.	0.06	0.06
11.	0.09	0.06
12.	0.06	0.06
13.	0.08	0.06

0.06 - 0.09 Range of Size 0.06 - ____

0.07 Mean 0.06

±.014 Standard Deviation ____

20% Coefficient of Variation ____

* Gill Arch No. 2

D. Fundulus from Hampton (Continued)

Number of Metacercariae	Length	Width
14.	0.09	0.06
15.	0.09	0.06
16.	0.09	0.06
17.	0.09	0.06
18.	0.09	0.06
19.	0.09	0.06
20.	0.09	0.06
21.	0.09	0.06

0.09 - ____ Range of Size 0.06 - ____

0.09 Mean 0.06

____ Standard Deviation ____

____ Coefficient of Variation ____

* Gill Arch No. 3

22.	0.08	0.06
23.	0.08	0.06
24.	0.09	0.06
25.	0.06	0.05
26.	0.06	0.05

0.06 - 0.09 Range of Size 0.05 - 0.06

0.07 Mean 0.06

±.014 Standard Deviation ±.0022

20% Coefficient of Variation 3.7%

* Gill Arch No. 4

D. Fundulus from Hampton (Continued)

Number of Metacercariae	Length	Width
27.	0.08	0.06
28.	0.06	0.05
29.	0.08	0.05
30.	0.08	0.06
31.	0.08	0.06
32.	0.08	0.06
	0.06 - 0.08 Range of Size	0.05 - 0.06
	0.08 Mean	0.06
	±.0028 Standard Deviation	±.002
	3.5% Coefficient of Variation	3.3%

* Gill Arch No. 5

33.	0.06	0.05
34.	0.06	0.05
35.	0.11	0.08
36.	0.09	0.08
37.	0.09	0.06
38.	0.11	0.09
39.	0.09	0.06
40.	0.08	0.06
41.	0.08	0.05
	0.06 - 0.11 Range of Size	0.05 - 0.09
	0.08 Mean	0.06
	±.02 Standard Deviation	±.014
	25% Coefficient of Variation	23%

* Gill Arch No. 6

D. Fundulus from Hampton (Continued)

Number of Metacercariae	Length	Width
42.	0.08	0.05
43.	0.06	0.05
44.	0.09	0.08
45.	0.08	0.06
46.	0.06	0.06
	0.06 - 0.09 Range of Size	0.05 - 0.08
	0.07 Mean	0.06
	±.014 Standard Deviation	±.01
	20% Coefficient of Variation	16%

* Gill Arch No. 7

47.	0.08	0.05
48.	0.08	0.05
49.	0.08	0.05
50.	0.06	0.05
51.	0.08	0.05
52.	0.06	0.05
	0.06 - 0.08 Range of Size	0.05 - ____
	0.07 Mean	0.05
	±.01 Standard Deviation	____
	14% Coefficient of Variation	____

* Gill Arch No. 8

** All metacercariae are those of Echinochasmus magnovatum.

APPENDIX III.

MEASUREMENTS OF METACERCARIAE ON THE GILLS OF EXPERIMENTAL TROPICAL FISH.

A. Red tuxedo platy (one day of development)

Number of Metacercariae	Length	Width
1.	0.06	0.06
2.	0.08	0.06
3.	0.08	0.06
4.	0.08	0.06
5.	0.06	0.05
6.	0.06	0.05
7.	0.06	0.05
8.	0.08	0.06
9.	0.08	0.06
10.	0.06	0.05
11.	0.06	0.08
12.	0.08	0.06
13.	0.08	0.05
14.	0.08	0.06
15.	0.08	0.06
16.	0.06	0.06
17.	0.08	0.06
18.	0.08	0.06

0.06 - 0.08	Range of Size	0.05 - 0.08
0.07	Mean	0.06
±.01	Standard Deviation	±.0022
14.3%	Coefficient of Variation	3.7%

* Oral spines are present

B. Red swordtail (three days of development)

Number of Metacercariae	Length	Width
1.	0.08	0.05
2.	0.09	0.05
3.	0.09	0.06
4.	0.09	0.06
5.	0.08	0.06
6.	0.08	0.05
7.	0.08	0.06
8.	0.08	0.05
9.	0.11	0.05
10.	0.11	0.06
11.	0.08	0.06
12.	0.08	0.05
13.	0.05	0.05
14.	0.05	0.03
15.	0.08	0.06
	0.05 - 0.11 Range of Size	0.03 - 0.06
	0.08 Mean	0.05
	±.017 Standard Deviation	±.01
	21.2% Coefficient of Variation	20%

* Eye spots are normal

C. Red platy (16 days of development)

1.	0.17	0.11
2.	0.16	0.08
3.	0.17	0.09
4.	0.16	0.11
5.	0.17	0.09
6.	0.16	0.11
7.	0.16	0.09
8.	0.16	0.11
9.	0.16	0.08
10.	0.14	0.09
11.	0.16	0.09
12.	0.16	0.09

C. Red platy (16 days of development)

Number of Metacercariae	Length	Width
13.	0.16	0.09
14.	0.17	0.11
15.	0.17	0.09

0.14 - 0.17 Range of Size 0.08 - 0.11

0.16 Mean 0.09

±.01 Standard Deviation ±.01

6.2% Coefficient of Variation 11%

* Oral spines are present

D. Red swordtail (18 days of development)

1.	0.16	0.09
2.	0.14	0.09
3.	0.14	0.09
4.	0.16	0.09
5.	0.14	0.09
6.	0.14	0.08
7.	0.17	0.09
8.	0.17	0.09
9.	0.16	0.08
10.	0.14	0.08
11.	0.14	0.09
12.	0.16	0.09
13.	0.14	0.08
14.	0.16	0.09
15.	0.16	0.09

0.14 - 0.17 Range of Size 0.08 - 0.09

0.15 Mean 0.09

±.01 Standard Deviation ±.0017

6.6% Coefficient of Variation 1.8%

* Eye spots are scattered/ Oral spines are present

E. Green tuxedo swordtail (14 days of development)

Number of Metacercariae	Length	Width
1.	0.16	0.11
2.	0.16	0.09
3.	0.16	0.11
4.	0.14	0.09
5.	0.11	0.11
6.	0.16	0.09
7.	0.13	0.08
8.	0.16	0.09
9.	0.16	0.09
10.	0.14	0.08
11.	0.14	0.08
12.	0.14	0.06
13.	0.16	0.09
14.	0.16	0.09
15.	0.16	0.09
16.	0.16	0.11
17.	0.16	0.11
18.	0.14	0.09
19.	0.16	0.09
20.	0.16	0.09
	0.11 - 0.16 Range of Size	0.06 - 0.11
	0.15 Mean	0.09
	±.014 Standard Deviation	±.014
	9.3% Coefficient of Variation	13.3%

* Eye spots are scattered/ Oral spines are present

F. Green tuxedo swordtail (18 days of development)

1.	0.16	0.11
2.	0.16	0.08
3.	0.16	0.09
4.	0.14	0.09
5.	0.17	0.08
6.	0.16	0.09
7.	0.14	0.09
8.	0.16	0.08

F. Green tuxedo swordtail (18 days of development)

Number of Metacercariae	Length	Width
9.	0.09	0.06
10.	0.16	0.09

0.09 - 0.17 Range of Size 0.06 - 0.11

0.15 Mean 0.09

±.022 Standard Deviation ±.014

15% Coefficient of Variation 13.3%

* Eye spots are scattered/ Oral spines are present

G. Green tuxedo swordtail (19 days of development)

1.	0.13	0.09
2.	0.16	0.11
3.	0.13	0.09
4.	0.16	0.08
5.	0.13	0.08
6.	0.16	0.09
7.	0.14	0.09
8.	0.16	0.09
9.	0.16	0.09
10.	0.14	0.09
11.	0.14	0.08
12.	0.16	0.08
13.	0.14	0.09
14.	0.16	0.09
15.	0.14	0.09

0.13 - 0.16 Range of Size 0.08 - 0.11

0.15 Mean 0.09

±.014 Standard Deviation ±.0024

9.3% Coefficient of Variation 2.66%

* Eye spots are scattered/ Oral spines are present

H. Green tuxedo swordtail (21 days of development)

Number of Metacercariae	Length	Width
1.	0.16	0.09
2.	0.16	0.09
3.	0.13	0.08
4.	0.17	0.09
5.	0.17	0.09
6.	0.17	0.09
7.	0.17	0.11
8.	0.17	0.09
9.	0.16	0.11
10.	0.19	0.11
11.	0.17	0.11
12.	0.16	0.11
13.	0.14	0.09
14.	0.16	0.09
15.	0.16	0.11
	0.13 - 0.19 Range of Size	0.08 - 0.11
	0.16 Mean	0.10
	±.014 Standard Deviation	±.01
	8.7% Coefficient of Variation	10%

* Eye spots are scattered/ Oral spines are present

I. Marble molly (21 days of development)

1.	0.11	0.09
2.	0.16	0.09
3.	0.16	0.09
4.	0.17	0.09
5.	0.16	0.08
6.	0.16	0.09
7.	0.17	0.09
8.	0.17	0.09
9.	0.11	0.09
10.	0.16	0.08
11.	0.16	0.09
12.	0.16	0.09

I. Marble molly (21 days of development)

Number of Metacercariae	Length	Width
13.	0.17	0.09
14.	0.16	0.09
15.	0.16	0.08

0.11 - 0.17 Range of Size 0.08 - 0.09

0.16 Mean 0.09

±.02 Standard Deviation ±.0014

12.5% Coefficient of Variation 1.66%

* Eye spots are scattered/ Oral spines are present

J. Red swordtail (30 days of development)

1.	0.16	0.08
2.	0.14	0.09
3.	0.16	0.11
4.	0.08	0.05
5.	0.13	0.08
6.	0.16	0.09
7.	0.16	0.09
8.	0.14	0.11
9.	0.14	0.11
10.	0.16	0.08
11.	0.14	0.11
12.	0.16	0.09
13.	0.17	0.11
14.	0.16	0.09
15.	0.14	0.11

0.08 - 0.17 Range of Size 0.05 - 0.11

0.15 Mean 0.09

±.022 Standard Deviation ±.017

1.4% Coefficient of Variation 18.8%

* Eye spots are scattered

K. Red swordtail (33 days of development)

Number of Metacercariae	Length	Width
1.	0.14	0.09
2.	0.14	0.11
3.	0.16	0.09
4.	0.14	0.08
5.	0.16	0.08
6.	0.13	0.09
7.	0.16	0.09
8.	0.16	0.09
9.	0.14	0.11
10.	0.14	0.09
11.	0.14	0.08
12.	0.16	0.09
13.	0.16	0.09
14.	0.14	0.08
15.	0.14	0.08

0.13 - 0.16 Range of Size 0.08 - 0.11

0.15 Mean 0.09

±.01 Standard Deviation ±.003

6.6% Coefficient of Variation 3.3%

* Eye spots are scattered/ Oral spines are present

L. Marble molly (30 days of development)

Number of Metacercariae	Length	Width
1.	0.14	0.08
2.	0.13	0.08
3.	0.14	0.09
4.	0.16	0.08
5.	0.19	0.11
6.	0.14	0.09
7.	0.16	0.09
8.	0.11	0.06
9.	0.16	0.08
10.	0.14	0.08
11.	0.16	0.09
12.	0.16	0.09
13.	0.14	0.08
14.	0.16	0.09
15.	0.14	0.08
	0.11 - 0.19 Range of Size	0.06 - 0.11
	0.15 Mean	0.08
	±.017 Standard Deviation	±.01
	11.3% Coefficient of Variation	12.5%

* Eye spots are scattered/ Oral spines are present

APPENDIX IV.

MEASUREMENTS OF EXCYSTED METACERCARIAE (*Ascocotyle* (*Phagicola*) *diminuta*).

A. *Ascocotyle* (*Phagicola*) *diminuta* from the green tuxedo swordtail (3 weeks of development)

Characters	Number of worms											
	1	2	3	4	5	6	7	8	9	10	11	12
LENGTH												
Body	0.17	0.10	0.11	0.10	0.13	0.12	0.13	0.13	0.13	0.12	0.12	0.11
O. Sucker	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.03
O. Cecum	0.04	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.04
Pharynx	0.02	0.02	0.01	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.02	0.02
Acetabulum	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
L. Testis	-	-	-	-	-	-	-	-	-	-	-	-
R. Testis	-	-	-	-	-	-	-	-	-	-	-	-
Ovary	-	-	-	-	-	-	-	-	-	-	-	-
WIDTH												
Body	0.06	0.07	0.07	0.07	0.06	0.07	0.08	0.06	0.07	0.07	0.07	0.05
O. Sucker	0.02	0.02	0.02	0.03	0.03	0.03	0.02	0.02	0.02	0.02	0.03	0.02
O. Cecum	*	*	*	*	*	*	*	*	*	*	*	*
Pharynx	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01
Acetabulum	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
L. Testis	-	-	-	-	-	-	-	-	-	-	-	-
R. Testis	-	-	-	-	-	-	-	-	-	-	-	-
Ovary	-	-	-	-	-	-	-	-	-	-	-	-

* not measured
\- not developed

B. *Ascocotyle* (*Phagicola*) *diminuta* from wild *Fundulus*
(? time of development)

Characters	Number of worms											
	1	2	3	4	5	6	7	8	9	10	11	12
LENGTH												
Body	0.19	0.22	0.21	0.28	0.26	0.18	0.20	0.22	0.27	0.21	0.21	0.27
O. Sucker	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.04
O. Cecum	0.06	0.07	0.06	0.08	0.06	0.06	0.06	0.07	0.07	0.06	0.06	0.08
Pharynx	0.02	0.03	0.02	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.02	0.03
Acetabulum	0.02	0.02	0.02	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.03
L. Testis	-	-	-	-	-	-	-	-	-	-	-	-
R. Testis	-	-	-	-	-	-	-	-	-	-	-	-
Ovary	-	-	-	-	-	-	-	-	-	-	-	-
WIDTH												
Body	0.09	0.10	0.09	0.09	0.10	0.09	0.07	0.07	0.08	0.08	0.10	0.07
O. Sucker	0.03	0.03	0.03	0.04	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.03
O. Cecum	*	*	*	*	*	*	*	*	*	*	*	*
Pharynx	0.01	0.02	0.02	0.03	0.03	0.02	0.03	0.03	0.02	0.02	0.02	0.02
Acetabulum	0.03	0.04	0.03	0.04	0.02	0.03	0.04	0.04	0.04	0.03	0.03	0.04
L. Testis	-	-	-	-	-	-	-	-	-	-	-	-
R. Testis	-	-	-	-	-	-	-	-	-	-	-	-
Ovary	-	-	-	-	-	-	-	-	-	-	-	-

* not measured
- not developed

APPENDIX V.

ANALYSIS OF VARIANCE OF EXPERIMENTALLY ENCYSTED METACERCARIAE (*Ascocotyle (Phagicola) diminuta*) FROM EXPERIMENTAL SECOND INTERMEDIATE HOSTS.

A. Body length (14 to 20 days of development)

Metacercariae from the Green tuxedo swordtail (14 days dev.)	Metacercariae from the Red platy (16 days dev.)	Metacercariae from the Brick red swordtail (18 days dev.)	Metacercariae from the Green T. swordtail (19 days dev.)	Metacercariae from the Green T. swordtail (20 days dev.)
X_1	X_2	X_3	X_4	X_5
0.16	0.17	0.16	0.13	0.13
0.16	0.16	0.14	0.16	0.13
0.16	0.17	0.14	0.13	0.13
0.14	0.16	0.16	0.16	0.14
0.11	0.17	0.14	0.13	0.13
0.16	0.16	0.14	0.16	0.16
0.13	0.16	0.17	0.14	0.16
0.16	0.16	0.17	0.16	0.13
0.16	0.16	0.16	0.16	0.14
0.14	0.14	0.14	0.14	0.14
0.14	0.16	0.14	0.14	0.16
0.14	0.16	0.16	0.16	0.16
0.16	0.16	0.14	0.14	0.14
0.16	0.17	0.16	0.16	0.16
0.16	0.17	0.16	0.14	0.13
2.24	2.43	2.28	2.21	2.14
M: 0.15	0.16	0.15	0.15	0.14

A. Body length (14 to 20 days of development)

SOURCE OF VARIATION	DF	SS	MS	F
Total S.S.	74	.0141		
Between S.S.	4	.0030	.0007	7.0000
Within S.S.	70	.0111	.0001	

Standard Error $S\bar{X} = \sqrt{\frac{.000100}{15}} = \sqrt{.000006} = .0244$

****

P:		(2)	(3)	(4)	(5)	
SSR:		.0917	.0956	.0983	.1005	
Expts.	X_5	X_1	X_3	X_4		X_2
Means:	0.14	0.15	0.15	0.15		0.16

Note: Any two means not underscored by the same line are significantly different.
Any two means underscored by the same line are not significantly different.

B. Body width (14 to 20 days of development)

Metacercariae from the Green tuxedo swordtail (14 days dev.)	Metacercariae from the Red platy (16 days dev.)	Metacercariae from the Brick red swordtail (18 days dev.)	Metacercariae from the Green T. swordtail (19 days dev.)	Metacercariae from the Green T. swordtail (20 days dev.)
X_1	X_2	X_3	X_4	X_5
0.11	0.11	0.09	0.09	0.08
0.09	0.08	0.09	0.11	0.08
0.11	0.09	0.09	0.09	0.08
0.09	0.11	0.09	0.08	0.08
0.11	0.09	0.09	0.08	0.08
0.09	0.11	0.08	0.09	0.08
0.08	0.09	0.09	0.09	0.09
0.09	0.11	0.09	0.09	0.09
0.09	0.08	0.08	0.09	0.08
0.08	0.09	0.08	0.09	0.09
0.08	0.09	0.09	0.08	0.09
0.06	0.09	0.09	0.08	0.08
0.09	0.09	0.08	0.09	0.09
0.09	0.11	0.09	0.09	0.09
0.09	0.09	0.09	0.09	0.08
1.35	1.43	1.31	1.33	1.26
M: 0.09	0.09	0.09	0.09	0.09

B. Body width (14 to 20 days of development)

SOURCE OF VARIATION	DF	SS	MS	F
Total S.S.	74	.0084		
Between S.S.	4	.0010	.0002	2.0000
Within S.S.	70	.0074	.0001	

Standard Error $S\bar{X} = \sqrt{\frac{.000100}{15}} = \sqrt{.000006} = .0244$

****

P:		(2)	(3)	(4)	(5)
SSR:		.0917	.0956	.0983	.1005
Expts.	X_1	X_2	X_3	X_4	X_5
Means:	0.09	0.09	0.09	0.09	0.09

Note: Any two means not underscored by the same line are significantly different.
Any two means underscored by the same line are not significantly different.

C. Body length (21 to 35 days of development)

Metacercariae from the Marble molly (21 days dev.)	Metacercariae from the Green tuxedo swordtail (21 days dev.)	Metacercariae from the Red swordtail (30 days dev.)	Metacercariae from the Red swordtail (23 days dev.)	Metacercariae from the Marble molly (35 days dev.)
X_1	X_2	X_3	X_4	X_5
0.11	0.16	0.16	0.14	0.14
0.16	0.16	0.14	0.14	0.13
0.16	0.13	0.16	0.16	0.14
0.17	0.17	0.08	0.14	0.16
0.16	0.17	0.13	0.16	0.19
0.16	0.17	0.16	0.13	0.14
0.17	0.17	0.16	0.16	0.16
0.17	0.17	0.14	0.16	0.11
0.11	0.16	0.14	0.14	0.16
0.16	0.19	0.16	0.14	0.14
0.16	0.17	0.14	0.14	0.16
0.16	0.16	0.16	0.16	0.16
0.17	0.14	0.17	0.16	0.14
0.16	0.16	0.16	0.14	0.16
0.16	0.16	0.14	0.14	0.14
2.34	2.44	2.20	2.21	2.23
M: 0.16	0.16	0.15	0.15	0.15

C. Body length (21 to 35 days of development)

SOURCE OF VARIATION	DF	SS	MS	F
Total S.S.	74	.0240		
Between S.S.	4	.0028	.0007	2.3333
Within S.S.	70	.0212	.0003	

Standard Error $S\bar{X} = \sqrt{\frac{.0003}{12}} = \sqrt{.00002} = .013$

****

P:		(2)		(3)		(4)		(5)	
SSR:		.4966		.5187		.5330		.5421	
Expts.	X_3		X_4		X_5		X_1		X_2
Means:	0.15		0.15		0.15		0.16		0.16

Note: Any two means not underscored by the same line are significantly different.
Any two means underscored by the same line are not significantly different.

D. Body width (21 to 35 days of development)

SOURCE OF VARIATION	DF	SS	MS	F
Total S.S.	74	.0104		
Between S.S.	4	.0013	.0003	3.0000
Within S.S.	70	.0091	.0001	

Standard Error $S\bar{X} = \sqrt{\frac{0.000100}{15}} = \sqrt{.000006} = .0244$

****

P:		(2)		(3)		(4)		(5)	
SSR:		.0917		.0956		.0983		.1005	
Expts.	X_1		X_3		X_4		X_5		X_2
Means:	0.09		0.09		0.09		0.09		0.10

Note: Any two means not underscored by the same line are significantly different.
Any two means underscored by the same line are not significantly different.

D. Body width (21 to 35 days of development)

Metacercariae from the Marble molly (21 days dev.)	Metacercariae from the Green tuxedo swordtail (21 days dev.)	Metacercariae from the Red swordtail (30 days dev.)	Metacercariae from the Red swordtail (23 days dev.)	Metacercariae from the Marble molly (35 days dev.)
X_1	X_2	X_3	X_4	X_5
0.09	0.09	0.08	0.09	0.08
0.09	0.09	0.09	0.11	0.08
0.09	0.08	0.11	0.09	0.09
0.09	0.09	0.05	0.08	0.08
0.08	0.09	0.08	0.08	0.11
0.09	0.09	0.09	0.09	0.09
0.09	0.11	0.09	0.09	0.09
0.09	0.09	0.11	0.09	0.06
0.09	0.11	0.11	0.11	0.08
0.08	0.11	0.08	0.09	0.08
0.09	0.11	0.11	0.08	0.09
0.09	0.11	0.09	0.09	0.09
0.09	0.09	0.11	0.09	0.08
0.09	0.09	0.09	0.08	0.09
0.08	0.11	0.11	0.08	0.08
1.32	1.46	1.40	1.34	1.27
M: 0.09	0.10	0.09	0.09	0.09

APPENDIX VI.

MEASUREMENTS OF *Ascocotyle* COMPLEX SPECIES FROM FINAL HOST ANIMALS (EXPERIMENTAL).

A. *Ascocotyle* (*Phagicola*) *diminuta* from the chick (4 days of development)

Characters	Number of worms											
	1	2	3	4	5	6	7	8	9	10	11	12
LENGTH												
Body	0.28	0.34	0.36	0.31	0.31	0.34	0.40	0.34	0.32	0.32	0.34	0.33
O. Sucker	0.05	0.05	0.04	0.05	0.04	0.04	0.04	0.05	0.04	0.04	0.04	0.05
O. Cecum	0.06	0.09	0.12	0.09	0.15	0.10	0.12	0.07	0.07	0.07	0.11	0.12
Pharynx	0.04	0.03	0.04	0.04	0.04	0.03	0.04	0.04	0.03	0.04	0.04	0.03
Acetabulum	0.04	0.03	0.03	0.04	0.04	0.04	0.04	0.04	0.04	0.03	0.03	0.04
L. Testis	0.03	0.06	0.05	0.04	0.04	0.04	0.05	0.04	0.04	0.03	0.03	0.04
R. Testis	0.05	0.06	0.05	0.05	0.04	0.05	0.05	0.05	0.04	0.04	0.05	0.05
Ovary	0.03	0.05	0.04	0.03	0.05	0.03	0.03	0.04	0.04	0.03	0.03	0.05
WIDTH												
Body	0.17	0.17	0.15	0.16	0.14	0.14	0.13	0.16	0.12	0.15	0.16	0.16
O. Sucker	0.04	0.03	0.04	0.05	0.03	0.04	0.03	0.04	0.04	0.04	0.04	0.04
O. Cecum	*	*	*	*	*	*	*	*	*	*	*	*
Pharynx	0.03	0.03	0.03	0.04	0.03	0.04	0.04	0.04	0.03	0.03	0.03	0.03
Acetabulum	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.05	0.04	0.04	0.04	0.04
L. Testis	0.04	0.07	0.05	0.05	0.06	0.05	0.05	0.06	0.03	0.05	0.05	0.05
R. Testis	0.05	0.07	0.06	0.06	0.04	0.05	0.07	0.07	0.04	0.04	0.06	0.05
Ovary	0.04	0.06	0.04	0.06	0.05	0.03	0.04	0.05	0.03	0.04	0.06	0.05

* not measured

B. Ascocotyle (Phagicola) diminuta from the chick (5 days of development)

Characters	Number of worms											
	1	2	3	4	5	6	7	8	9	10	11	12
LENGTH												
Body	0.26	0.28	0.23	0.18	0.30	0.29	0.22	0.21	0.31	0.20	0.21	0.24
O. Sucker	0.03	0.03	0.02	0.02	0.05	0.04	0.03	0.02	0.05	0.02	0.02	0.03
O. Cecum	0.05	0.05	0.06	0.06	0.05	0.05	0.05	0.06	0.08	0.05	0.05	0.06
Pharynx	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.03	0.04	0.02	0.03	0.04
Acetabulum	0.03	0.03	0.03	0.04	0.04	0.03	0.02	0.03	0.02	0.03	0.03	0.02
L. Testis	0.03	0.06	0.05	0.04	0.03	0.04	0.04	0.05	0.06	0.05	0.05	0.06
R. Testis	0.03	0.05	0.04	0.04	0.05	0.05	0.04	0.04	0.05	0.05	0.05	0.05
Ovary	0.03	0.02	0.03	0.03	0.03	0.03	0.02	0.02	0.03	0.02	0.02	0.03
WIDTH												
Body	0.16	0.13	0.13	0.13	0.16	0.12	0.13	0.14	0.15	0.12	0.12	0.12
O. Sucker	0.03	0.03	0.02	0.02	0.04	0.04	0.03	0.02	0.04	0.03	0.02	0.02
O. Cecum	*	*	*	*	*	*	*	*	*	*	*	*
Pharynx	0.02	0.02	0.02	0.03	0.03	0.02	0.02	0.02	0.03	0.02	0.02	0.03
Acetabulum	0.04	0.04	0.03	0.03	0.04	0.04	0.02	0.02	0.02	0.04	0.03	0.03
L. Testis	0.04	0.04	0.04	0.05	0.05	0.05	0.04	0.05	0.07	0.05	0.05	0.05
R. Testis	0.05	0.04	0.06	0.05	0.05	0.05	0.04	0.05	0.06	0.05	0.05	0.04
Ovary	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.03	0.02	0.03	0.03

* not measured

C. Experimental Ascocotyle (Phagicola) diminuta from the rat (2 to 3 days of development)

Characters	Number of worms											
	1	2	3	4	5	6	7	8	9	10	11	12
LENGTH												
Body	0.18	0.18	0.19	0.22	0.21	0.16	0.17	0.16	0.14	0.16	0.13	0.23
O. Sucker	0.02	0.03	0.04	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.04
O. Cecum	0.05	0.07	0.05	0.06	0.07	0.05	0.07	0.05	0.04	0.05	0.04	0.08
Pharynx	0.03	0.03	0.03	0.02	0.03	0.02	0.03	0.03	0.02	0.03	0.02	0.03
Acetabulum	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.02	0.04
L. Testis	0.05	0.03	0.04	0.05	0.04	0.04	0.04	0.03	0.03	0.03	0.03	0.05
R. Testis	0.04	0.03	0.04	0.04	0.03	0.04	0.03	0.03	0.03	0.04	0.03	0.05
Ovary	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.02	0.04
WIDTH												
Body	0.08	0.09	0.09	0.09	0.10	0.08	0.08	0.07	0.09	0.07	0.07	0.17
O. Sucker	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.03	0.03	0.02	0.04
O. Cecum	*	*	*	*	*	*	*	*	*	*	*	*
Pharynx	0.01	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03
Acetabulum	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.03	0.05
L. Testis	0.03	0.04	0.04	0.04	0.04	0.03	0.04	0.03	0.03	0.04	0.03	0.08
R. Testis	0.03	0.03	0.04	0.05	0.04	0.04	0.04	0.03	0.05	0.04	0.03	0.08
Ovary	0.03	0.03	0.02	0.04	0.04	0.03	0.04	0.03	0.03	0.03	0.02	0.03

* not measured

D. *Ascocotyle tenuicollis* from the chick
(3 days of development)

Characters	Number of worms											
	1	2	3	4	5	6	7	8	9	10	11	12
LENGTH												
Body	0.38	0.40	0.34	0.39	0.38	0.43	0.41	0.36	0.38	0.36	0.36	0.38
O. Sucker	0.05	0.05	0.03	0.05	0.05	0.05	0.03	0.03	0.04	0.05	0.03	0.03
O. Cecum	0.10	0.14	0.11	0.11	0.10	0.12	0.14	0.12	0.10	0.12	0.10	0.08
Pharynx	0.04	0.04	0.03	0.03	0.05	0.04	0.03	0.03	0.04	0.03	0.04	0.04
Acetabulum	0.04	0.04	0.04	0.04	0.05	0.04	0.04	0.03	0.02	0.03	0.03	0.04
L. Testis	0.07	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.06	0.06
R. Testis	0.07	0.04	0.06	0.05	0.08	0.04	0.04	0.05	0.04	0.03	0.05	0.07
Ovary	0.03	0.02	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.03	0.02
WIDTH												
Body	0.08	0.15	0.12	0.17	0.12	0.11	0.08	0.09	0.10	0.10	0.10	0.10
O. Sucker	0.04	0.04	0.05	0.03	0.04	0.04	0.03	0.03	0.04	0.05	0.05	0.04
O. Cecum	*	*	*	*	*	*	*	*	*	*	*	*
Pharynx	0.03	0.03	0.03	0.03	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Acetabulum	0.05	0.05	0.04	0.06	0.05	0.05	0.04	0.04	0.03	0.05	0.04	0.04
L. Testis	0.05	0.06	0.05	0.03	0.05	0.03	0.04	0.04	0.05	0.05	0.03	0.05
R. Testis	0.04	0.07	0.05	0.05	0.05	0.05	0.05	0.05	0.04	0.04	0.05	0.04
Ovary	0.03	0.06	0.03	0.05	0.03	0.05	0.03	0.04	0.03	0.03	0.04	0.03

* not measured

APPENDIX VII.

MEASUREMENTS OF *Echinochasmus magnovatum* FROM FINAL HOST ANIMALS (EXPERIMENTAL).

A. *Echinochasmus magnovatum* from the chick (4 days of development)

Characters	Number of worms											
	1	2	3	4	5	6	7	8	9	10	11	12
LENGTH												
Body	0.57	0.58	0.79	0.63	0.65	0.78	0.74	0.76	0.78	0.67	0.68	0.49
O. Sucker	0.06	0.06	0.04	0.06	0.04	0.06	0.06	0.06	0.07	0.06	0.04	0.07
Acetabulum	0.10	0.11	0.07	0.08	0.10	0.10	0.11	0.10	0.08	0.07	0.11	0.08
Prepharynx	0	0	0.03	0.03	0.04	0.06	0	0	0	0	0.04	0
Pharynx	0.07	0.07	0.04	0.06	0.08	0.07	0.08	0.08	0.08	0.06	0.04	0.07
Esophagus	0	0	0	0.04	0.02	0.10	0.07	0	0	0.03	0.04	0
Ovary	0.04	0.03	0.07	0.06	0.04	0.06	0.06	0.06	0.06	0.04	0.06	0.06
A. Testis	0.08	0.07	0.07	0.06	0.07	0.12	0.07	0.06	0.07	0.04	0.08	0.08
P. Testis	0.10	0.08	0.10	0.07	0.08	0.12	0.08	0.07	0.08	0.07	0.08	0.06
C. Pouch	0.08	0.07	0.06	0.06	0.07	0.06	0.08	0.08	0.07	0.06	0.08	0.07
WIDTH												
Body	0.23	0.26	0.23	0.22	0.29	0.19	0.28	0.22	0.18	0.22	0.13	0.13
O. Sucker	0.06	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.06	0.07	0.04	0.06
Acetabulum	0.10	0.10	0.11	0.08	0.11	0.08	0.10	0.08	0.08	0.08	0.11	0.07
Pharynx	0.06	0	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.07	0.04	0.06
Ovary	0.06	0.08	0.07	0.07	0.06	0.06	0.07	0.06	0.07	0.06	0.06	0.04
A. Testis	0.15	0.12	0.12	0.12	0.08	0.10	0.11	0.11	0.10	0.08	0.11	0.10
P. Testis	0.13	0.11	0.10	0.12	0.15	0.10	0.11	0.11	0.12	0.11	0.11	0.11
C. Pouch	0.06	0.04	0.07	0.06	0.07	0.06	0.06	0.04	0.06	0.06	0.04	0.06
Collar	0.13	0.12	0.15	0.15	0.13	0.17	0.15	0.15	0.13	0.13	0.13	0.10

B. Echinochasmus magnovatum from the mouse
(6 days of development)

Characters	Number of worms											
	1	2	3	4	5	6	7	8	9	10	11	12
LENGTH												
Body	0.26	0.33	0.33	0.26	0.25	0.23	0.32	0.31	0.28	0.37	0.33	0.24
O. Sucker	0.04	0.04	0.05	0.05	0.04	0.05	0.05	0.06	0.05	0.05	0.05	0.04
Acetabulum	0.06	0.06	0.05	0.06	0.06	0.05	0.07	0.07	0.06	0.10	0.05	0.06
Prepharynx	0	0	0.03	0	0	0	0	0.01	0	0.02	0.01	0
Pharynx	0.05	0.06	0.04	0.05	0.03	0.04	0.06	0.07	0.03	0.07	0.05	0.05
Esophagus	0.01	0	0.01	0.01	0.01	0.01	0.01	0.01	0	0.01	0.01	0
Ovary	0.02	0.01	0.02	0.02	0.01	0.02	0.02	0.01	0.02	0.02	0.01	0.01
A. Testis	0.01	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.01	0.01
P. Testis	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.02	0.02	0.01
C. Pouch	0.02	0.06	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.01
WIDTH												
Body	0.10	0.07	0.10	0.12	0.11	0.09	0.13	0.13	0.12	0.16	0.09	0.10
O. Sucker	0.04	0.04	0.04	0.04	0.04	0.05	0.03	0.05	0.05	0.05	0.04	0.04
Acetabulum	0.06	0.05	0.05	0.07	0.06	0.06	0.05	0.07	0.07	0.10	0.06	0.06
Pharynx	0.03	0.04	0.04	0.04	0.02	0.03	0.04	0.05	0.04	0.05	0.03	0.04
Ovary	0.01	0.01	0.01	0.02	0.01	0.02	0.01	0.01	0.02	0.02	0.01	0.02
A. Testis	0.03	0.04	0.02	0.03	0.02	0.02	0.01	0.01	0.03	0.04	0.03	0.02
P. Testis	0.03	0.03	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.02	0.02
C. Pouch	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.02	0.02
Collar	0.06	0.04	0.05	0.08	0.05	0.08	0.09	0.08	0.07	0.09	0.05	0.08

C. Echinochasmus magnovatum from the mouse
(18 days of development)

Characters	Number of worms											
	1	2	3	4	5	6	7	8	9	10	11	12
LENGTH												
Body	0.90	0.87	0.85	0.85	0.87	1.55	0.71	1.09	1.00	1.30	1.10	1.01
O. Sucker	0.09	0.09	0.09	0.11	0.09	0.08	0.08	0.11	0.08	0.09	0.08	0.09
Acetabulum	0.14	0.09	0.11	0.17	0.12	0.09	0.13	0.17	0.13	0.09	0.08	0.09
Prepharynx	0.03	0.03	0.05	0.02	0.02	0.03	0.03	0.02	0.03	0.05	0.03	0.03
Pharynx	0.14	0.11	0.13	0.15	0.09	0.11	0.08	0.14	0.11	0.11	0.11	0.11
Esophagus	0.03	0.02	0.03	0.03	0.03	0.02	0.03	0.02	0.05	0	0.03	0.05
Ovary	0.05	0.08	0.08	0.08	0.05	0.09	0.03	0.06	0.05	0.06	0.06	0.05
A. Testis	0.07	0.09	0.09	0.11	0.06	0.14	0.05	0.09	0.13	0.14	0.13	0.09
P. Testis	0.09	0.11	0.11	0.14	0.08	0.16	0.06	0.17	0.16	0.16	0.17	0.13
C. Pouch	0.06	0.08	0.11	0.09	0.08	0.11	0.06	0.11	0.11	0.08	0.12	0.11
WIDTH												
Body	0.33	0.35	0.39	0.41	0.25	0.22	0.22	0.33	0.34	0.22	0.36	0.30
O. Sucker	0.08	0.09	0.09	0.09	0.08	0.08	0.06	0.09	0.09	0.09	0.08	0.08
Acetabulum	0.13	0.14	0.14	0.14	0.11	0.11	0.11	0.11	0.16	0.11	0.14	0.11
Pharynx	0.09	0.09	0.11	0.11	0.11	0.11	0.08	0.09	0.11	0.11	0.11	0.11
Ovary	0.09	0.11	0.09	0.09	0.06	0.08	0.05	0.08	0.09	0.08	0.09	0.09
A. Testis	0.19	0.19	0.22	0.22	0.11	0.16	0.11	0.17	0.25	0.17	0.17	0.19
P. Testis	0.14	0.19	0.19	0.24	0.09	0.17	0.09	0.16	0.19	0.16	0.14	0.17
C. Pouch	0.05	0.09	0.11	0.09	0.09	0.06	0.03	0.09	0.11	0.08	0.11	0.08
Collar	0.14	0.17	0.16	0.16	0.13	0.16	0.13	0.17	0.19	0.16	0.16	0.17

D. *Echinochasmus magnovatum* from the mouse
(30 days of development)

Characters	Number of worms											
	1	2	3	4	5	6	7	8	9	10	11	12
LENGTH												
Body	1.18	1.26	1.56	1.50	1.46	1.31	1.46	1.31	1.25	1.31	1.35	1.01
O. Sucker	0.11	0.08	0.11	0.12	0.10	0.11	0.10	0.12	0.11	0.12	0.12	0.10
Acetabulum	0.18	0.17	0.15	0.18	0.11	0.17	0.10	0.17	0.15	0.13	0.11	0.12
Prepharynx	0	0	0	0.04	0.07	0	0.06	0	0.04	0	0	0.04
Pharynx	0.18	0.14	0.17	0.17	0.12	0.15	0.10	0.17	0.13	0.13	0.13	0.13
Esophagus	0.02	0.02	0	0.04	0.06	0.03	0.04	0	0	0.03	0.03	0
Ovary	0.06	0.12	0.11	0.08	0.08	0.07	0.08	0.07	0.10	0.11	0.10	0.06
A. Testis	0.14	0.12	0.11	0.22	0.17	0.15	0.17	0.26	0.17	0.11	0.28	0.13
P. Testis	0.18	0.17	0.18	0.22	0.21	0.25	0.21	0.17	0.15	0.22	0.21	0.17
C. Pouch	0.11	0.14	0.12	0.10	0.11	0.12	0.12	0.13	0.12	0.10	0.13	0.08
WIDTH												
Body	0.43	0.54	0.43	0.40	0.42	0.42	0.39	0.42	0.42	0.60	0.50	0.49
O. Sucker	0.10	0.11	0.10	0.11	0.08	0.08	0.08	0.10	0.11	0.12	0.12	0.12
Acetabulum	0.21	0.15	0.15	0.17	0.11	0.15	0.11	0.17	0.17	0.17	0.17	0.11
Pharynx	0.10	0.12	0.10	0.11	0.10	0.08	0.10	0.10	0.11	0.11	0.12	0.08
Ovary	0.15	0.10	0.08	0.08	0.10	0.07	0.06	0.10	0.06	0.11	0.11	0.10
A. Testis	0.22	0.33	0.17	0.21	0.22	0.23	0.22	0.26	0.23	0.21	0.28	0.31
P. Testis	0.19	0.29	0.29	0.26	0.22	0.22	0.19	0.23	0.22	0.26	0.31	0.25
C. Pouch	0.11	0.12	0.11	0.08	0.10	0.08	0.08	0.08	0.11	0.11	0.11	0.08
Collar	0.15	0.18	0.17	0.18	0.15	0.15	0.15	0.18	0.15	0.22	0.18	0.13

APPENDIX VIII.

COEFFICIENT OF DIFFERENCE BETWEEN POPULATIONS OF *Ascocotyle* COMPLEX SPECIES FROM FINAL HOST ANIMALS (EXPERIMENTAL).

Comparison	Animals	Worms		Coefficient of difference	
		Parasite 1	Parasite 2	Length	Width
I	Chick Mouse	*A. diminuta*	*A. diminuta*	2.27	0.67
II	Chick Mouse	*A. diminuta*	*A. diminuta*	2.66	1.25
III	Chick Chick	*A. diminuta*	*A. tenuicollis*	0.73	0.95
IV	Mouse Rat	*A. diminuta*	*A. diminuta*	1.36	0.83
V	Mouse Chick	*A. diminuta*	*A. tenuicollis*	3.59	0.48
VI	Chick Rat	*A. tenuicollis*	*A. diminuta*	3.64	0.33

APPENDIX IX.

COEFFICIENT OF DIFFERENCE BETWEEN POPULATIONS OF *Echinochasmus magnovatum* FROM FINAL HOST ANIMALS (EXPERIMENTAL).

Comparison	Animals	Worms		Coefficient of difference	
		Parasite 1	Parasite 2	Length	Width
I	Chick Mouse	*E. magnovatum*	*E. magnovatum*	2.93	1.32
II	Chick Mouse	*E. magnovatum*	*E. magnovatum*	1.00	1.39
III	Chick Mouse	*E. magnovatum*	*E. magnovatum*	2.73	3.38
IV	Mouse Mouse	*E. magnovatum*	*E. magnovatum*	2.58	4.35
V	Mouse Mouse	*E. magnovatum*	*E. magnovatum*	5.33	7.55
VI	Mouse Mouse	*E. magnovatum*	*E. magnovatum*	.87	3.41

APPENDIX X.

PERCENTAGE OF NONOVERLAP OF PARTIALLY OVERLAPPING CURVES ASSOCIATED WITH STATED VALUES OF THE COEFFICIENT OF DIFFERENCE (C.D.).

(After Mayr, Linsley, and Usinger, 1953)

Values	C.D.	Joint nonoverlap, per cent
Below the level of conventional subspecific distinctness	0.675	75
	0.84	80
	0.915	82
	0.995	84
	1.04	85
	1.08	86
	1.13	87
	1.175	88
	1.23	89
Conventional level of subspecific difference	1.28	90
Above the level of conventional subspecific difference	1.34	91
	1.405	92
	1.48	93
	1.555	94
	1.645	95
	1.75	96

APPENDIX XI

Plates

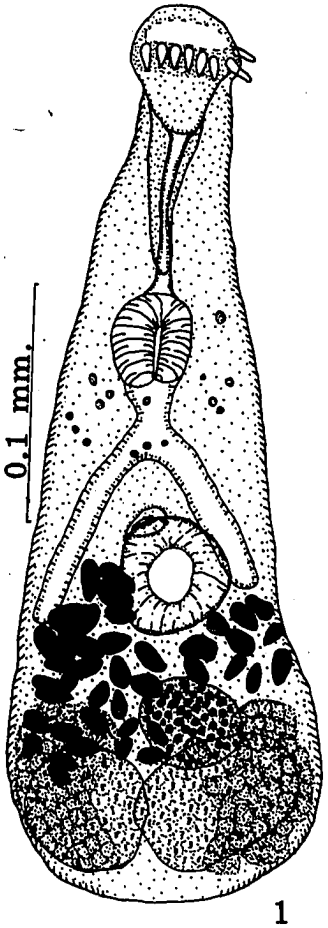
Note: All drawings were made with the aid of camera lucida unless stated otherwise.

PLATE 1

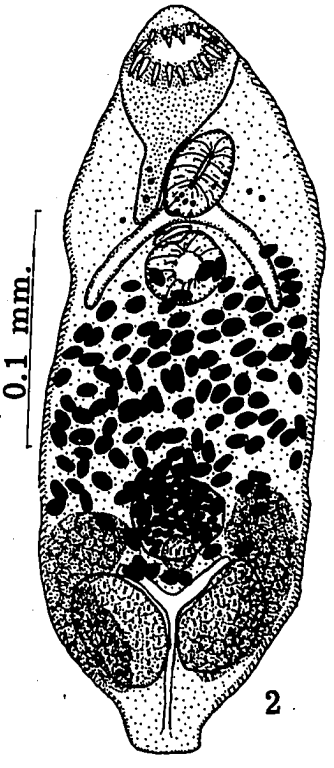
Figure 1. *Ascocotyle* (*Phagicola*) *diminuta* (four days of development). Experimentally obtained by feeding the gills of wild *Fundulus heteroclitus* to a day-old chick.

Figure 2. *A.* (*P.*) *diminuta* (five days of development). Experimentally obtained by feeding the gills of wild *Fundulus heteroclitus* to a white mouse.

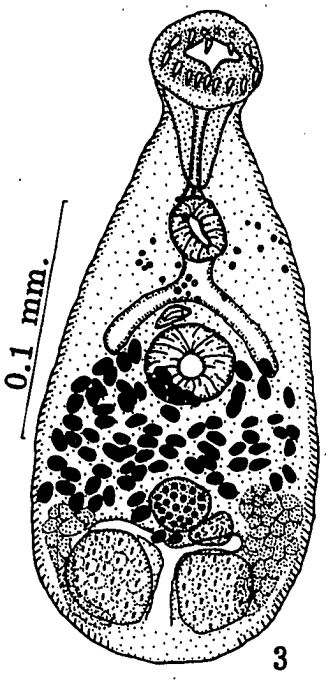
Figure 3. *A.* (*P.*) *diminuta* (three days of development). Experimentally obtained by feeding the gills of experimentally infected green tuxedo swordtails to a day-old chick.



1



2



3

PLATE 2

Figure 1. *Ascocotyle* (*Phagicola*) *diminuta* (four days of development). Experimentally obtained by feeding the gills of wild *Fundulus heteroclitus* to a day-old chick.

Figure 2. *A.* (*P.*) *diminuta* (four days of development). Experimentally obtained by feeding the gills of experimentally infected green tuxedo swordtails to a day-old chick.

Figure 3. *A.* (*P.*) *diminuta* (four days of development). Experimentally obtained by feeding the gills of wild *F. heteroclitus* to a white mouse.

Figure 4. *A.* (*P.*) *diminuta* (three days of development). Experimentally obtained by feeding the gills of experimentally infected brick red swordtails and green tuxedo swordtails to a white rat.

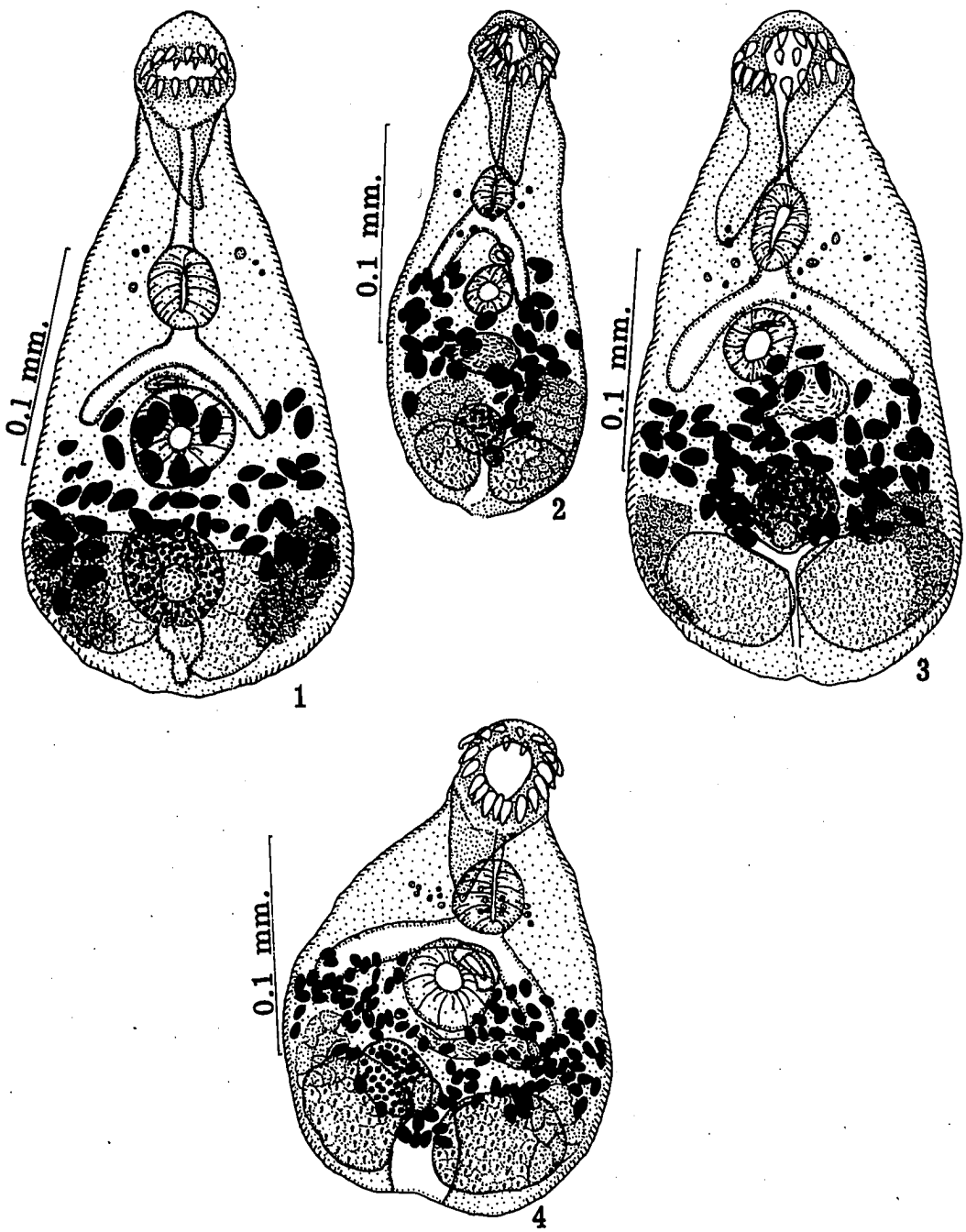


PLATE 3

Figure 1. *Ascocotyle* (*Phagicola*) *diminuta* (four days of development). Experimentally obtained by feeding the gills of experimentally infected green tuxedo swordtails to a day-old chick.

Figure 2. *A.* (*P.*) *diminuta* (five days of development). Experimentally obtained by feeding the gills of naturally infected wild *Fundulus heteroclitus* to a white mouse.

Figure 3. *A.* (*P.*) *diminuta* (three days of development). Experimentally obtained by feeding the gills of experimentally infected brick red swordtails and green tuxedo swordtails to a white rat.

Figure 4. *A.* (*P.*) *diminuta*; experimentally excysted metacercaria (three weeks of development). From experimentally infected green tuxedo swordtails.

Figure 5. *A.* (*P.*) *diminuta*; experimentally excysted metacercaria (number of days of development not known). From naturally infected wild *F. heteroclitus*.

Figure 6. *A.* (*P.*) *diminuta* (four days of development). Experimentally obtained by feeding the gills of naturally infected wild *F. heteroclitus* to a day-old chick.

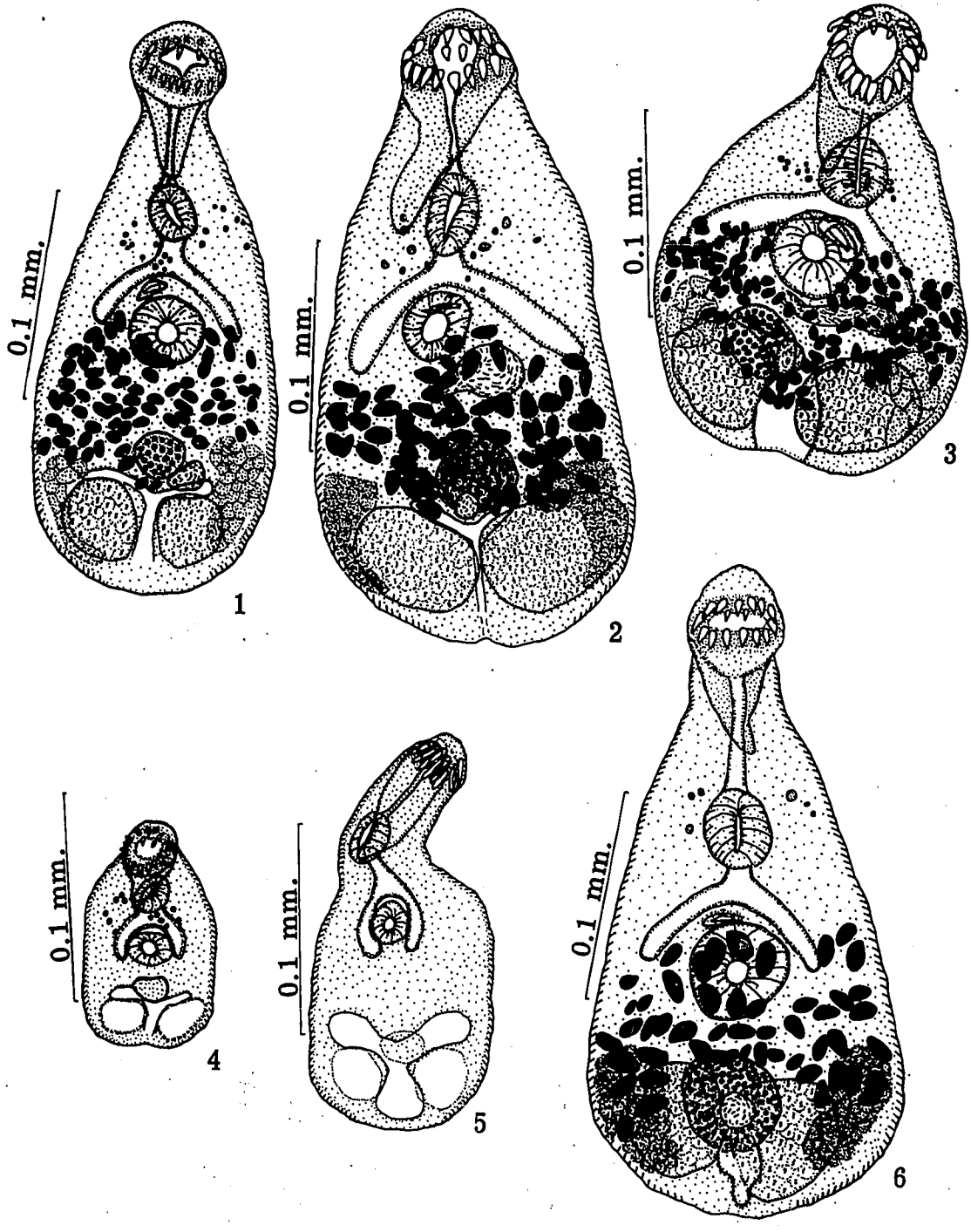


PLATE 4

Figures 1-4 Ascocotyle (Phagicola) diminuta (two days of development). Experimentally obtained by feeding the gills of experimentally infected brick red swordtails and green tuxedo swordtails to a white rat.

Figures 5-7 A. (P.) diminuta metacercariae (three weeks of development). Experimentally excysted after three weeks of development on the gills of green tuxedo swordtails.

Figure 8 A. (P.) diminuta metacercaria (number of days of development not known). From the gills of wild Fundulus heteroclitus.

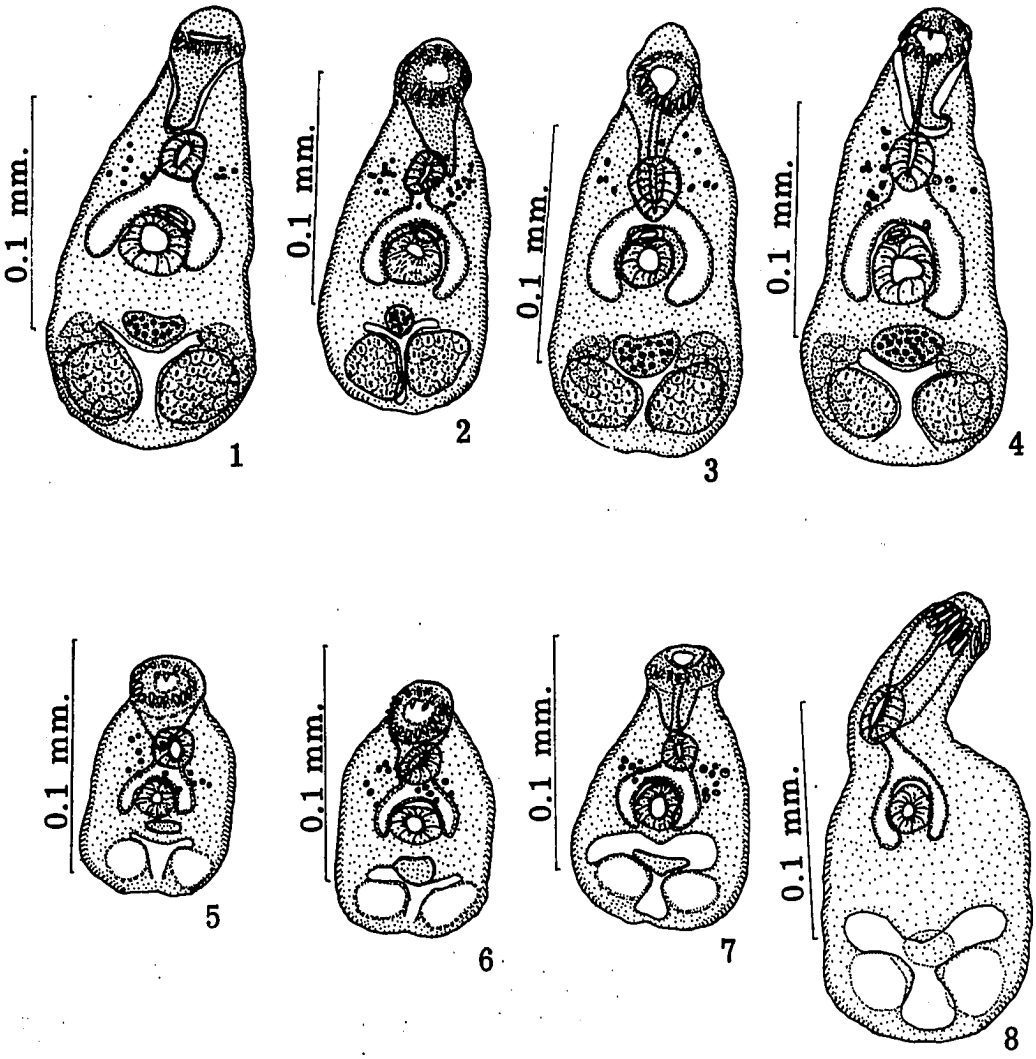
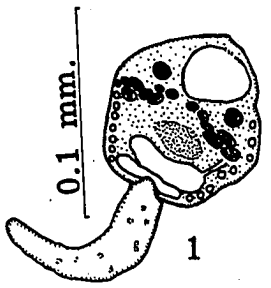


PLATE 5

Figures 1-3 Cercaria of *Ascocotyle* (*Phagicola*) *diminuta* drawn at different positions and states of contraction. From *Hydrobia salsa* Pilsbry.

Figure 4 Redia of *A.* (*P.*) *diminuta*. From *Hydrobia salsa* Pilsbry.

Figure 5 Cercaria of *A.* (*P.*) *diminuta* showing flame cell pattern.



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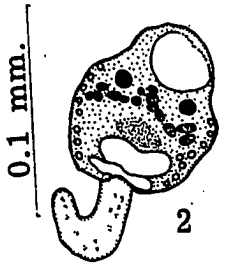

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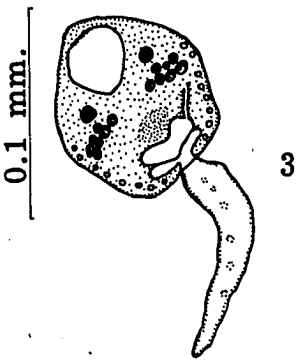

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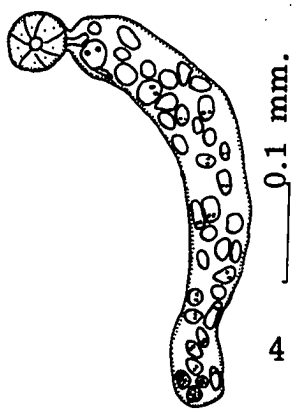

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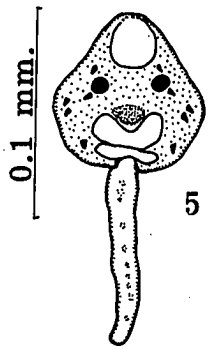

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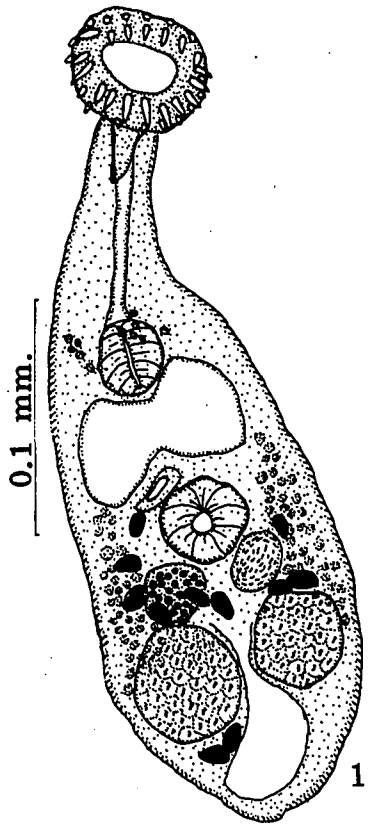
PLATE 6

Figure 1. Ascocotyle tenuicollis (three days of development. Experimentally obtained by feeding the hearts of wild Fundulus heteroclitus to a day-old chick.

Figure 2. Cotype specimen No. 38161 of Ascocotyle leighi Burton, 1956 (three days of development). Experimentally obtained by feeding hearts of wild Mollienisia latipinna LeSueur (from southern Florida) to a day-old chick.

Figure 3. Same species as in figure 1., but drawn to show different views of the oral coronet of spines.

Figures 4-6 Ascocotyle tenuicollis. Drawn to show different views of the prolonged triangular dorsal lip; oral coronet of spines; and oral appendages.



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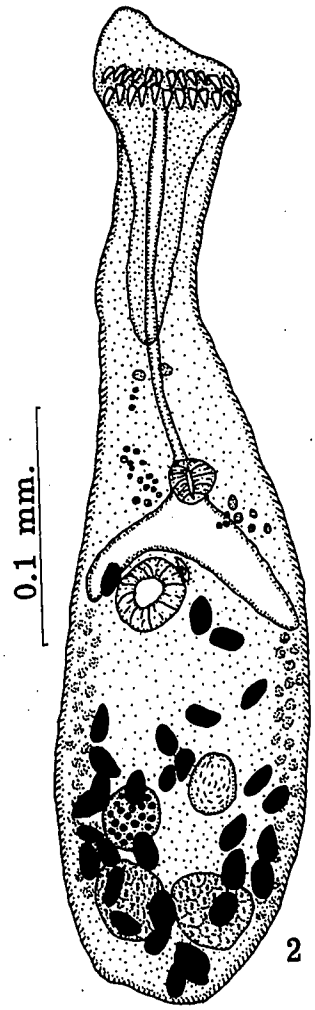

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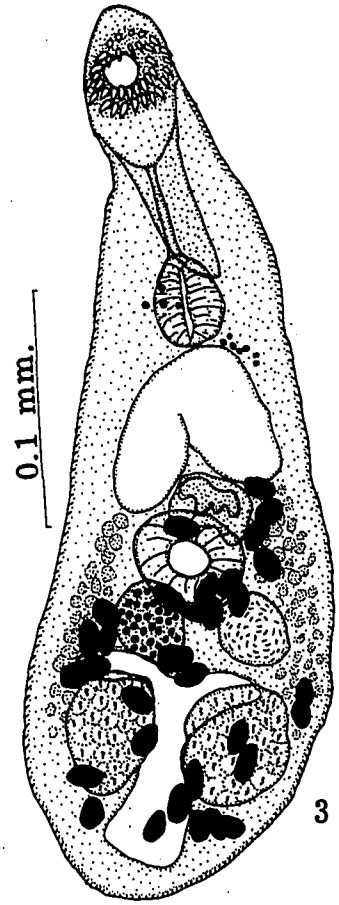

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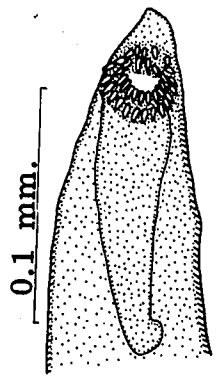

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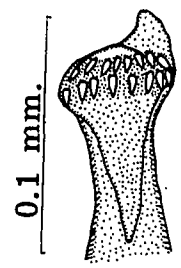

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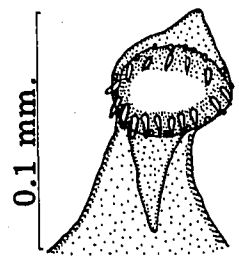

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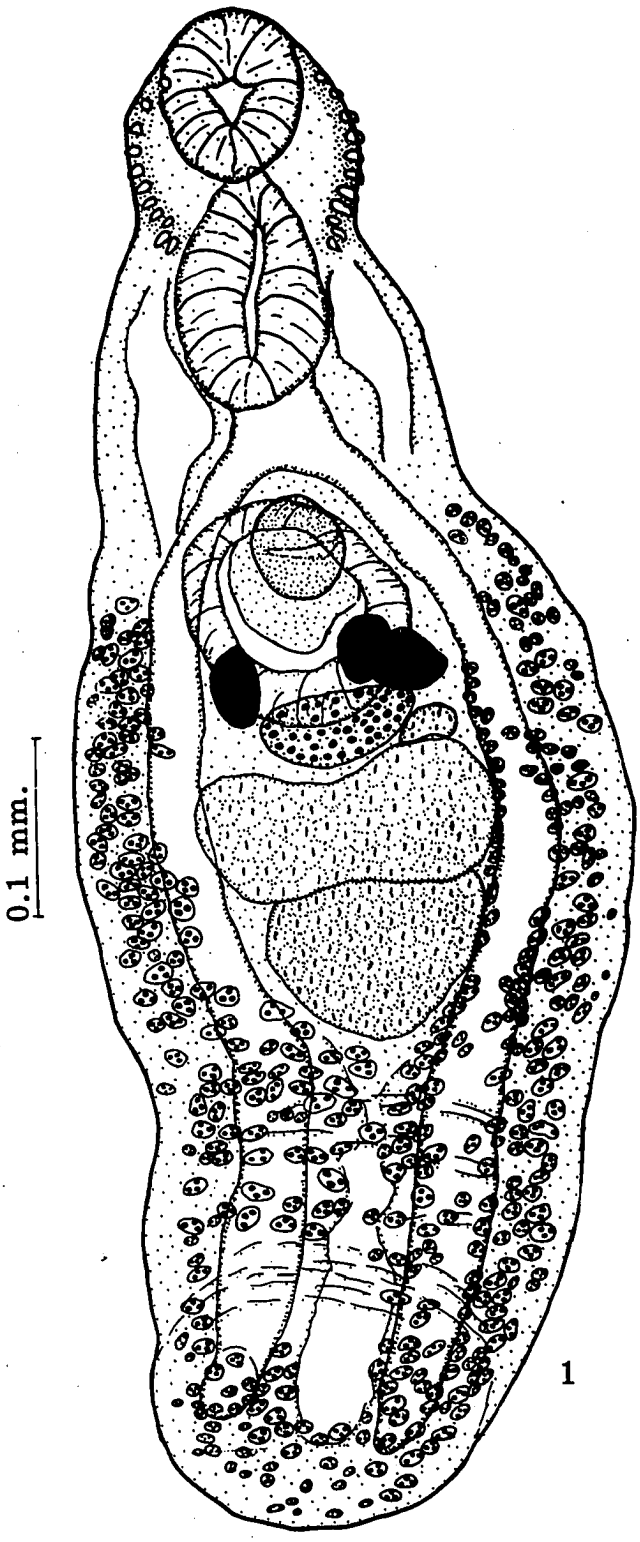
PLATE 7

Figure 1. *Echinochasmus magnovatum* (18 days of development). Experimentally obtained by feeding the gills of wild *Fundulus heteroclitus* to a white mouse. Drawing made with the aid of a microprojector.



1

PLATE 8

Figure 1. Echinochasmus magnovatum (30 days of development). Experimentally obtained by feeding the gills of wild Fundulus heteroclitus to a white mouse.

Figure 2. E. magnovatum (five days of development). Experimentally obtained by feeding the gills of wild F. heteroclitus to a day-old chicks.

Figure 3. E. magnovatum (four days of development). Experimentally obtained by feeding the gills of wild F. heteroclitus to a day-old chick.

Figure 4. E. magnovatum (six days of development). Experimentally obtained by feeding the gills of wild F. heteroclitus to a white mouse.

Figure 5. Dorsal view of E. magnovatum (18 days of development). Experimentally obtained by feeding the gills of F. heteroclitus to a white mouse.

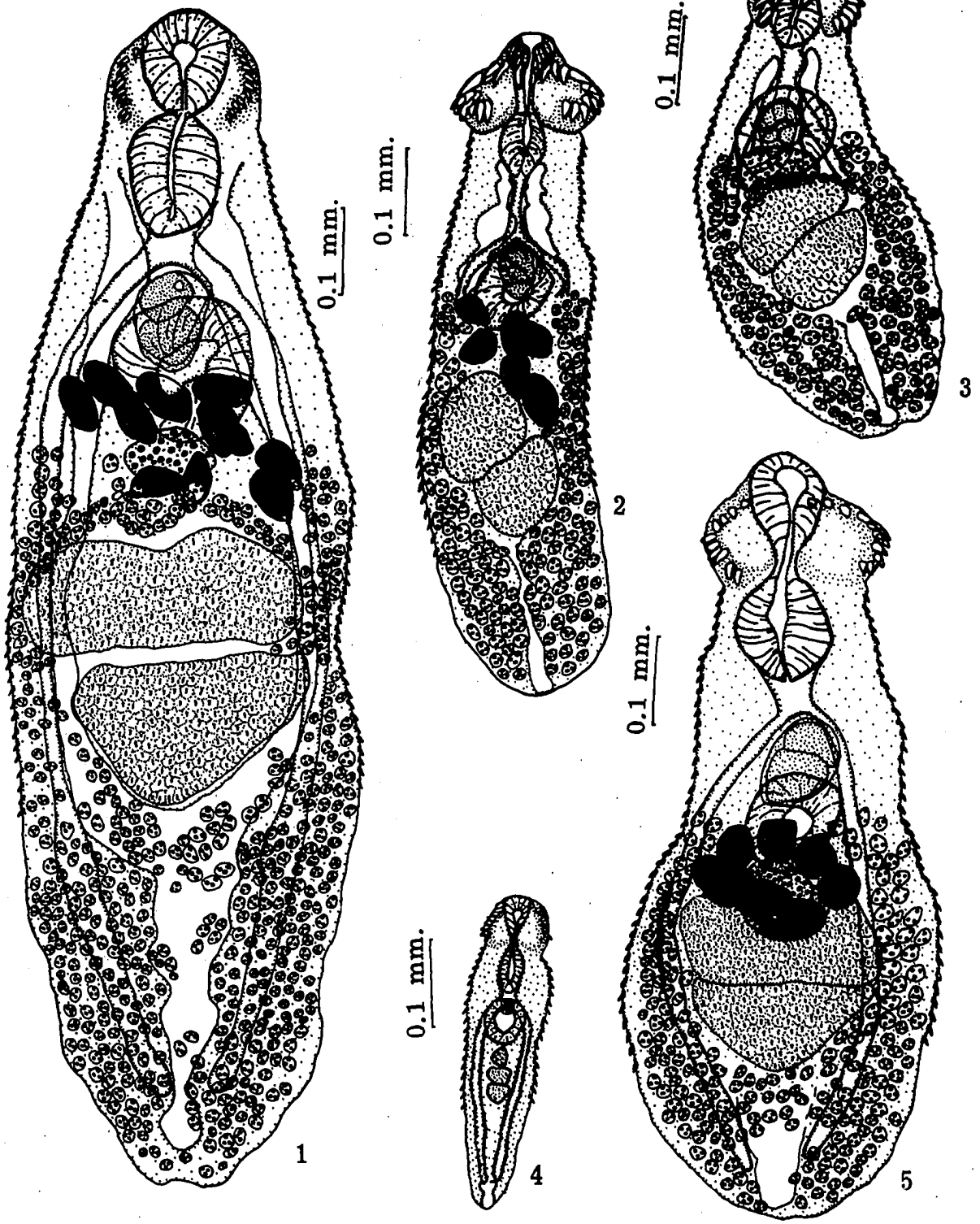


PLATE 9

Figure 1. *Echinochasmus magnovatum* (four days of development). Experimentally obtained by feeding the gills of wild *Fundulus heteroclitus* to a day-old chick.

Figure 2. *E. magnovatum* (five days of development). Experimentally obtained by feeding the gills of wild *F. heteroclitus* to a day-old chick.

Figure 3. *E. magnovatum* (six days of development). Experimentally obtained by feeding the gills of wild *F. heteroclitus* to a white mouse.

Figure 4. *E. magnovatum*, ventral view (18 days of development). Experimentally obtained by feeding the gills of wild *F. heteroclitus* to a white mouse.

Figure 5. *E. magnovatum*, dorsal view (18 days of development). Experimentally obtained by feeding the gills of wild *F. heteroclitus* to a white mouse.

Figure 6. *E. magnovatum* (18 days of development). Experimentally obtained by feeding the gills of wild *F. heteroclitus* to a white rat.

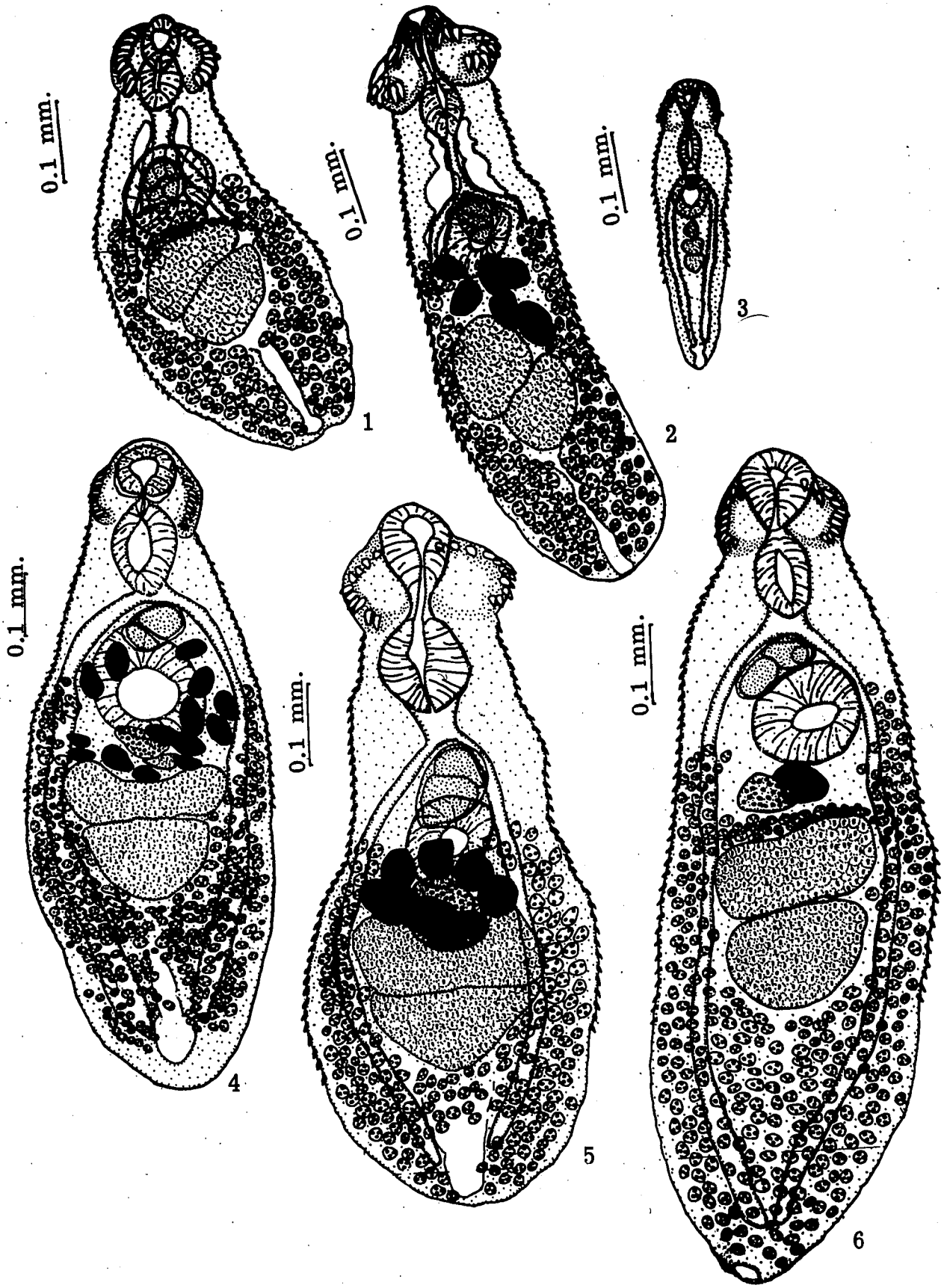


PLATE 10

Figure 1. Type specimen No. 36724 of Echinochasmus donaldsoni Beaver, 1941 (seven days of development). Experimentally obtained by feeding the gills of sticklebacks and perch to a pigeon. Drawing made with the aid of a microprojector.

Figure 2. Echinochasmus magnovatum (five days of development). Experimentally obtained by feeding the gills of wild Fundulus heteroclitus to a day-old chick.

Figure 3. E. magnovatum (four days of development). Experimentally obtained by feeding the gills of wild F. heteroclitus to a day-old chick.

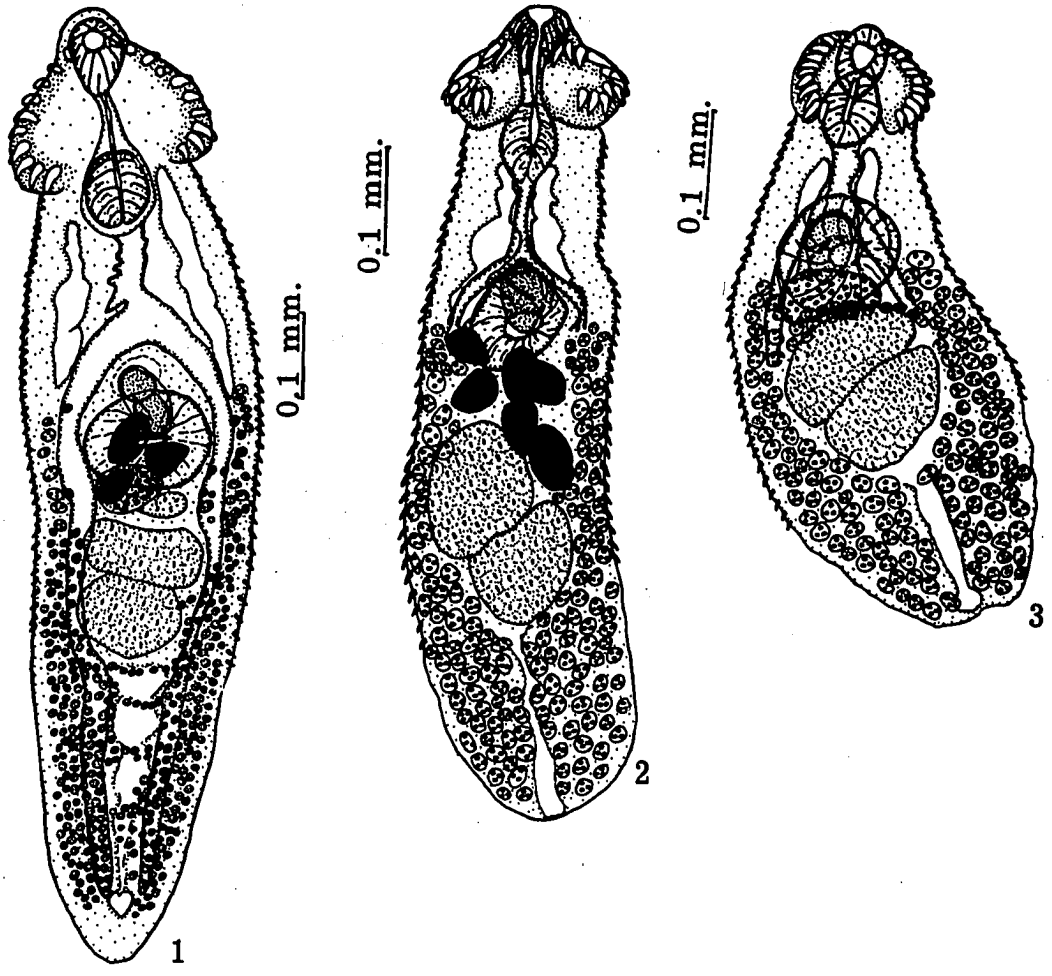


PLATE 11

Figure 1. Type specimen No. 36724 of *Echinochasmus donaldsoni* Beaver, 1941 (seven days of development). Experimentally obtained by feeding the gills of perch and sticklebacks to a pigeon. Drawing made with the aid of a microprojector.

Figure 2. *Echinochasmus magnovatum* (30 days of development). Experimentally obtained by feeding the gills of wild *Fundulus heteroclitus* to a white mouse.

Figure 3. Type specimen No. 29754 of *Echinochasmus schwartzi* Price, 1931 (days of development not known). From muskrats (*Ondatra zibethica*) of Maryland and District of Columbia. Drawing made with the aid of a microprojector.

PLATE 11.

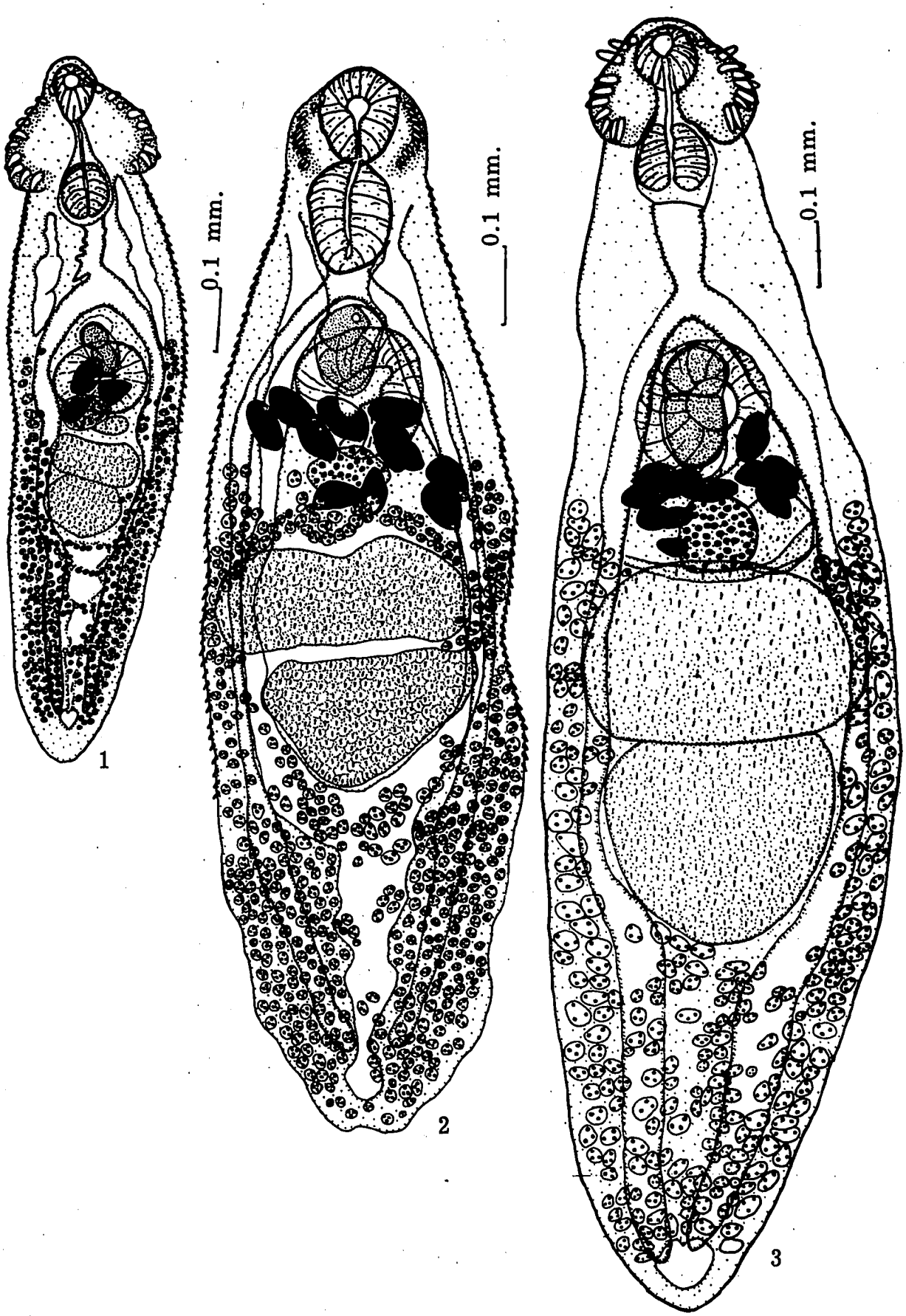


PLATE 12

Figure 1. Field location at Johnson Creek is marked by a black circle.

PLATE 12

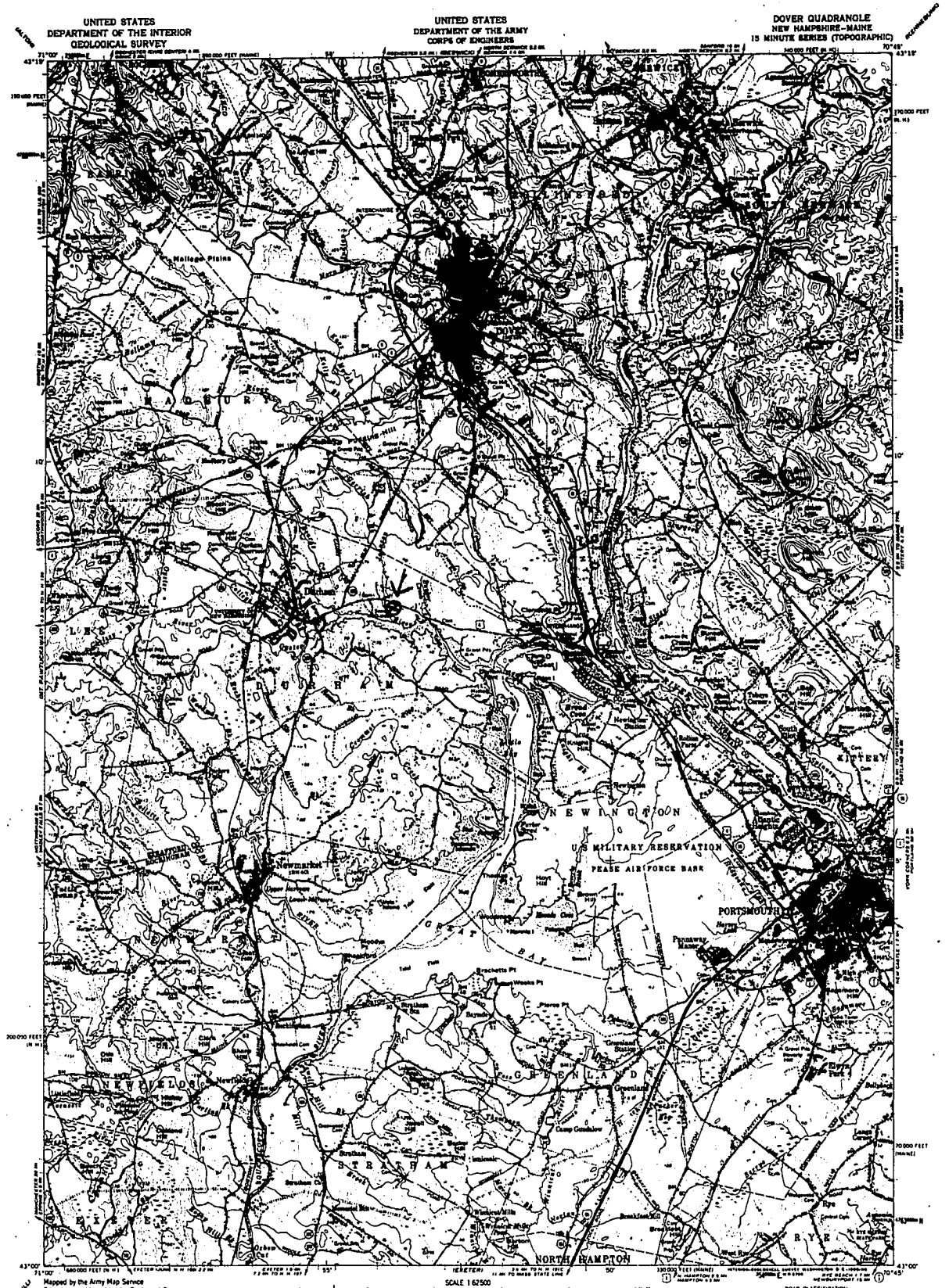


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PLATE 13

Figure 1. Field location at South Newington is marked by a black circle.

PLATE 13

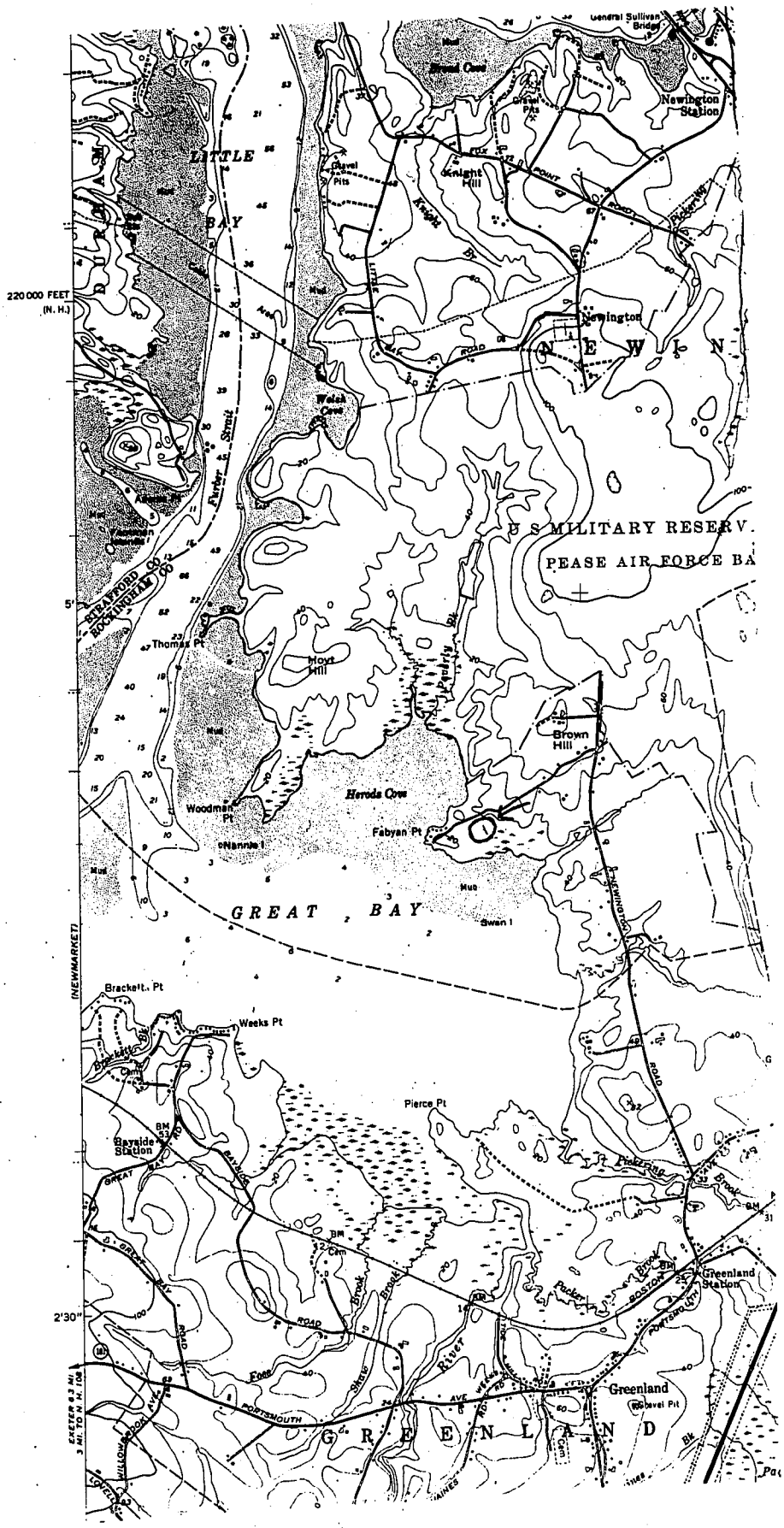

PLATE 14

Figure 1. Field location at Hampton is marked by a black circle.

PLATE 14