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Studies on the Trematode Larva Neascus

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STUDIES ON THE TREMATODE LARVA NEASCUS

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INTRODUCTION

During the spring semesters of 1955 and 1956, while the writer was under the supervision of Dr. A. E. Noble, a preliminary survey was initiated on parasites in local fishes, amphibians, and reptiles occurring in the vicinity of Stockton, California. The collection of specimens in this survey included sixteen green sunfish, Lepomis cyanella, and two bluegills, L. macrochirus. Larval trematodes recovered from the heart and liver of these specimens belonged to the family Diplostomatidae Poirier (1886) which is characterized by a conspicuous constriction separating the fore and hind-bodies. The leaf-like forebody with the lateral borders converging ventrally to form a spoon-like concavity containing the holdfast organ and acetabulum, a hind-body enclosing the reproductive organs, and a bursa copulatrix at the posterior end are diagnostic characteristics.

Haderlie (1953), whose survey of parasites in fishes from Northern California included the hosts Lepomis cyanella and L. macrechirus, listed larval diplostomids belonging to the genus Neascus Hughes (1927). He also stated that the unhealthy appearance of the hosts infected with these larvae made this parasite unpopular with fishermen. Since the delta area surrounding Stockton provided diversified

facilities for the fisherman, an identification of this parasite with its possible importance to the industry added further interest to the investigation.

METHODS AND MATERIALS

Site of collection for the green sunfish, *Lepomis cyanella*, was a small irrigation canal adjoining the Al Formaciari Ranch in Robert's Island, San Joaquin County, California. The hosts were caught by angling using sardine and earthworms for bait. The weather was usually chilly and cloudy with the water containing moderate amounts of vegetation, the temperature of the water varying little from 17° C, and the appearance of the liquid muddy. Two bluegills were contributed by a fisherman who caught them at Jenny Lind, California.

The hosts were taken to the laboratory alive and examined under the dissecting scope for ectoparasites on the fins, integument, gills, and eyes. The visceral organs were then inspected for parasites. When infected organs were found, they were placed in syracuse dishes filled with 0.7% sodium bicarbonate solution which dissolves and clears the excess mucus, and ebris surrounding the tissue. The cysts were held to the organ by a small pedicles, as observed by Linton (1898), and at times it

became necessary to tear the tissue to release the cysts. Great care had to be taken when attempting to break the cyst because disturbance of the animal forced it to exude dense and heavy material. It was found that keeping the cysts in the solium bicarbonate solution over-night weakened the cyst wall so that emergence of the parasite was facilitated, but overexposure retarded the activity of the parasite and rendered it more susceptible to injury.

The excysted metacercariae were dropped into Bouin's fixative heated to 50° C. in order to prevent contraction of the animals. Traces of lithium carbonate were added in the process to the 70% alcohol used for washing in order to hasten removal of the picric acid. Some cysts and whole mounts were stained in Grenacher's acid alum carmine and others were stained, sometimes, in Heidenhain's iron hematoxylin, and at other times in Proecher's celestine blue counterstained with acid fuchsin. Although the combination of celestine blue and acid fuchsin would seem to violate standard procedures, the results were superior to other combinations used.

Figures were drawn freehand and also with the aid of a camera lucida. Three composite figures were made.

HISTORICAL BACKGROUND

Hughes (1928) states that Leidy (1888), Linton (1911), Cooper (1915), Ward and Whipple (1919) and Pearse (1924) were early American workers who noted metacercarian cysts in the visceral organs of fresh water fishes. From their brief descriptions, he surmised that they were describing the larval genus Neascus Hughes (1927).

Dr. G. R. LaRue (1926), with his associated and students, conducted a series of studies on the Strigeidae (Holostomidae), contributing to our knowledge of the anatomy, relationships and life-histories of this family. One of these studies was made by Hughes (1927) who described Neascus amblopltis as representing a new larval group from the rock-bass Ambloplitis rupesris. He diagnosed this new group as:

> Strigeid metacercaria with both fore and hind-bodies well developed and distinctly set apart by a constriction; no lateral sucking-cups, fore-body leaf-like, holdfash organ well-developed; reserve bladder highly developed, the smaller branches of which are usually anastomoses; calcreous granules mostly free in the circumbient fluid; encysted.

Hughes (1927) also redescribes and transfers Diplostomum cuticola von Nordmann (1832) to Neascus cuticola (von Nordmann).

Hughes (1928) in his examination of the hosts Eupomotis

gibbosus, Enotis megalotis perlatus, Ambloplitis repestris and Helioperca incisor, found heavy infection of a new species Neascus van-cleavei (Agersborg) in the visceral organs. According to Hughes, Agersborg (1926) in his descripttions of Diplostomum van-cleavei made many misinterpretations in his descriptions such as designating anterior for posterior, holdfast organ for acetabuum, acetabulum for genital papilla, etc., but the new species resembled that of Agersborg closely. In the same study, Holostomum brevicaudum von Nordmann (1832) and Holostomum musculicola Waldenburg (1860) were reclassified as Neascus brevicaudatus (von Nordmann) Hughes (1927) and Neascus musculicola respectively (Waldenburg) Hughes (1927).

In an unpublished paper, Van Haitsma determined that Neascus bulboglossa described as new by Hughes (1928) developed in to the adult Crassiphiala bulboglessa Van Haitsma (1925) from Ceryl alcyon. Cooper (1915 described a worm similar to and perhaps identical with this new species as Cercaria sp. from black pigmented cysts in a minnow. Hughes collaborated with Piszczek in 1928 to describe Neascus ptychocheilus in Netropsis delicious stramineus. Hughes and Piszczed (1928) noted that the description of Cercaria ptychosheilus Faust (1917, 1917a) was identical with that of the new species. McCoy (1928) working on the life history of several cercariae found that Cercaria Hemata Miller

(1923) in Eupemotis gibbosus developed a metacercaria belonging to the larval genus Neascus. In the same year, Hunter (1928) described Neascus wardi, but Van Cleave and Mueller (1934) declared it to be synonymous with Neascus amploplitis. Ciurea (1929) noting numerous cysts embedded in the integument of a carp, described Neascus perlatus. In an experimental study of this larva, the adult Neodiplostomum perlatum was obtained.

Hunter (1933) described the metacrercaria Neascus rhinichthysi from Rhinichthys astromasus and R. cataractae which was mentioned but not described by Hunter and Hunter (1931). Van Cleave and Mueller (1932, 1934) described Neascus oneidensis from Perca Flavescens, Esox Lusius, and Neascus grandis from Umbra limi. Other Neascus types similar to N. van-cleavei were classified according to their respective hosts. Chandler (1951) in his studies on metacarcariae from Perca flavescens described Neascus pyriformis, N. Longicollis, and N. ellipticus.

Haderlie (1953) who studied parasites of fresh-water fishes from Northern California noted Neascus vancleavei as being the most common encysted larvae, but other seemingly new species were not given specific names. They were, however, classified according to their respective hosts in the manner of Van Cleave and Mueller (1934).

Haderlie (1953) stated:

> The adult flukes of this family (strigeidae) are very much unlike the metacercariae in morphology. Therefore, when larval stages are encountered it is not possible to assign them to their respective genera. In order to classify the larval stages into some sort of workable arrangement, investigators have followed the custom of assigning names to what seem to be closely related forms. These larval names are regarded as tentative generic groups and then as the life cycle of the individual types are determined, the metacercariae are assigned to their respective genera. Since the life histories of but few of these have been worked out, most of the metacercariae retain their temporary larval names.

LIFE-HISTORY OF NEASCUS

Previously mentioned experiments were made by Van Haitsma who developed adult _Crassiphiala bulboglossa_ from _Neascus bulboglossa_ Hughes (1928). Ciurea (1929) obtained the adult _Neodiplostomum Perlatum_ from feeding experiments of _Neascus perlatus_ cysts to birds. A more recent study was made by Hoffman (1954) who infected fish hosts with cercaria of _Neascus bulboglossa_ and noted its subsequent development to the metacercaria. Other experimental studies have been published in North America by Van Haitsma (1930, 1931, 1931a), Price (1931) and Bosma (1937). In Europe, Szidat (1924, 1924a, 1929, etc.) worked on the life cycle of this group. Only two like cycles have been completely demonstrated; one being _Posthodiplostonum minimum_

(MacCallum), and the other Crassiphiala ambloplitis. Hunter and Hunter (1930).

The Cercaria of Posthodiplostomum minimum was described by Miller (1923) as Cercaria multicelluata, the metacercaria Neascus van-cleavei by Hughes (1928), and the adult Postnodiplostomum minimum by MacCallum (1931). Hunter (1936) achieved penetration in the common sunfish with Cercaria multicellulata after exposure from 24-36 hours. The parasites appeared in the liver within 8-11 days and, by the end of 14-17 days, the larval had developed many of the characteristics of the metacercaria. Then Ferguson (1936), through experimental feeding of chicks recorded adult worms after 35 hours and the eggs appeared in the feces a few hours later. The eggs hatched in about 3 weeks. Hunter (1937) again performed the penetration experiment and noted the entry of the Cercaria into the gills and under the scales, although he was unable to demonstrate the entire migration. His graphic illustration of the life cycle is reproduced in Plate I. Ferguson (1938, 1940, 1943) added finer datails to the life cycle, but in 1940 he warrants an additional note since the successfully developed P. minimum in sterile cultures. Miller (1953) noted that the cercaria migrating via the circulatory

system to the site of infection was of a different species than that of _C. multicellulata_.

The developmental sequence of _Crassiphilala amblopli-tis_ Hunter and Hunter (1930) is essentially the same. Hunter and Hunter (1930, 1931) developed the metacercaria _Neascus amblonlitis_, first described by Hughes (1927), into the adult. Meanwhile Krull (1934) developed the meta-cercaria from infections with _Cercaria bessiae_ Cort and Brooks (1928). Hunter and Hunter (1934, 1935) reported on the experimentally completed life-cyle as follows: undeveloped eggs are passed in droppings, and the miracidium develops after 21 days. Then penetration of the host takes place where after approximately 41 days mother sporocysts are produced followed by the development of daughter sporocysts. _Cercaria bessiae_ emerge, then penetrate, migrate, and develop into the metacercaria within the second intermediate after 22 days. Final adult development in the definitive host take twenty-seven days. Price (1931), Van Haitsma (1931), and Hunter (1931) suggested that the miracicium belonged to the family Schistosoma-tidae LaRue (1926).

HOST-PARASITE RELATIONSHIPS

Butler (1919) first recorded large infections of

cysts in the eyes of fishes which seem to be the natural site of infection. Agesborg (1926) studied the eggects of parasitism on the tissues and reported that three trematodes seemed to be sufficient to upset metabolism with several marked symptoms: the minnow swam with spasmodic, asymmetrical strokes, and sometimes whirled in a circle; it lost its balance and the right or left side became uppermost; it became filled with gas; it came to the surface and gasped for air which it seemed to swallow (this may be characterized as a strangulation period); it may become relieved, if not, it succumbs life. The liver in infected individuals, dead or alive is far more delicate than in uninfected ones, post-mortem decomposition is much more rapid in parasitized minnows than in non-infected ones.

Hubbs (1927) notes the following results in heavily infected Platygopis gacilis: a retardation of early growth, retension of larval characters, pale coloe due to poor development of melanophores, short and feeble fin, flabby abdomen, large eye, united nostrils, small mouth, absence of barbels and an increase in the number of scales. Hunter (1928) reported a definite correlation between the number of cysts in a host and the size of the fish examined, Wesenburg-lund (1934) noted the emaciation of

the host, Hunter (1937) and Krull (1936) that heavy infestations of cysts cause loss of weight of the host and death of fishes, while Bangham (1938) noticed that fish heavily infected with cysts were often thin and lacked fat about their visceral organs.

Klak (1940) examined dead fish harboring more than 12,000 cysts reported that the pressure created by the cysts caused these to flow out from the body cavity in a yellow fluid when the fish was opened. His findings also indicate that the pressure of cysts inhibited formationof eggs and produced sterility. Bangham (1938) noticed that fish heavily infected with cysts were often thin and lacked fat about their visceral organs. Other host-parasite relationships received attention by Todd (1929), Hunter and Hunter (1940, 1942), and Hunter and Hunter (1921) whose studies revealed that larvae found encysted in the organs consist of two layers; and outer, cellular, connective tissue wall of host origin, and an inner tough non-cellular hyalin layer, apparently of parasitic origin.

DISTRIBUTION OF THE METACERCARIA

The larval genus <u>Neascus</u> is cosmopolitan in its distribution. Its hosts are found from East to West Coasts.

Pearse (1942) listed the following factors affecting the distribution of these parasites within a large body of water; (1) fishes associated with vegetation show the highest infection, (2) bottom and open water fish with intermediate incidence; shallow water fishes show the least. Incidence is, of course, correlated with the availability of the intermediate and definitive hosts. Temperature, specific physiological differences, seasonal changes, sizes of stream, and the calmness of the water all affect distribution. Van Cleave and Mueller (1932, 1934) in a comprehensive ecological study of the fauna in Oneida Lake stressed the importance of such studies to improve recreational facilities, to reduce the death rate of fishes from heavy infection, and to trace the possible migratory habits of fishes harboring a particular parasite.

TAXONOMY

The taxonomic picture of this group has been the object of extensive work and revision, but Dubois (1938, 1953) monographed all of the known species into a complex and thorough classification on which this thesis is based.

Class Trematoda Rudolphi (1819)

  Order Strigeatoidea La Rue (1926)

    Sub-order Strigeata La Rue (1926)

Supersuperfamily Strigeida Poche (1925)

The supersuperfamily Strigeida is divided into two superfamilies. If the cirrus pouch is absent and the disposition of vitellarian follicles axial, it belongs to the Strigeides; if not, then to the Cyathocotylides.

The superfamily Strigeidae is divided into three subsuperfamilies Strigeines, Diplosomatines, and Bolbocephalodines. The animal in this study belongs to the Diplostomatines for the following reasons: the forebody is foliate, cochearial, or spathula, holdfast organ with definite form, rounded or elliptical, tongue-shaped or heart-shaped, with or without cavity. This group reunites all the known forms otherwise under the names of Hemistomes and Diplostomes.

The subsuperfamily is divided into two families Diplostomatidae and Proterodiplostomatidae. The family Diplostomatidae is characterized as parasites of birds and mammals, absence of a paraprostate gland (prostate gland independent of the genital male duct, by returning to that which is always situated dorsally, constituted by a tubular or bag-shaped, more or less tubular, surround- ing those which flow into it their secretion, and is elongated by an efferent canal), and vitellarian follicles

dividing in the two segments of the body or confined in the hind-body. The hold-fast organ, small in the middle, opens generally by a small slit.

The family is further divided into two sub-families Diplostomainae or Alariinae. If the animal is found parasitized in the bird, has vitelline follicles in both segments of the body or confined to the hind-body, has a hold-fast organ a little to the middle which generally opens itself by a slit in the middle and whose diameter does not exceed the half-length of the forebody in the second half in which it is located, then it belongs to the subfamily Diplostomatinae.

The subfamily is divided into two subsubfamilies by the distribution of the vitellaria. If the follicles are distributed in both segments of the body, then referral is made to diplostomatini; if not, then to Crassiphalini. The genus Posthodiplostomum is characterized as follows: absence of pseudo-suckers, the bursa copulatrix invaginable, the body distinctly divided into two halves, the ovary situated anterior to the testes; and parasites of the bird family Ardeae.

MORPHOLOGY OF NEASCUS SP. FROM THE BLUEGILL

Host-Lepomis macrochirus

Two bluegills were found to have heavily infected hearts and livers. The non-cellular and non-pigmented cysts, 1.10 (0.97-1.22) x 0.75 (0.162-0.813) mm., appear conspicuously on the surface of the infected organ as grayish-white, hyaline capsules, ovoid, spherical, or elliptical in shape (Plate II). The walls are translucent making the appearance of the parasite evident, but the two walls, an outer wall of host origin and an inner wall of parasitic origin, does not become evident until the cyst is opened. The cavity of the cyst is sufficiently spacious to permit free motion of the animal within it. The body is flexed in a horse-shoe shape. The axis of the body is often, but not always, slightly flexed dorsally in the region of the constriction, and the animals are capable of vigorous contractual movements if strongly stimulated by injury or chemicals. The worm within the cyst revealed larger organs such as the oral sucker, holdfast organ, reserve bladder system, and acetabulum (Figure 1). The forebody, separated from the hindbody by a distinct constriction, is thin, leaflike with the lateral edges of the forebody converging to form a spoonlike, cup-shaped cavity for attachment. The forebody, separated from the hind-body by a distinct constriction, is thin, leaflike with the lateral edges of the forebody converging to form

a spoon-like, cup-shaped cavity for attachment. The hind-body is conical, bulbous, or spherical. Hughes (1928) states that freshly killed specimens put in fresh water increased in size and attributes this to the gelatinous content of the crust and excretory spaces of the parasite which may be expected to have a high osmotic pressure. Gelatinous material can be seen within the cyst as part of the excretion.

Upon removal from the cyst, contractions continued, but actual locomotion was not observed. The animals reacted negatively to intense light, but resumed their natural positions in ordinary light.

Due to the fact, however, that the worms were almost constantly in motion, it was difficult to make satisfactory measurements. The writer endeavored to measure the fore and hindbodies of the parasite in its most contracted and in its most extended positions as well as in a condition of rest. Measurements were taken from ten specimens, without pressure, and are presented in Table II. Relative distance of the acetabulum and holdfast organ are included for possible diagnostic purposes.

The free metacercaria (Figure 2) shows a fore and hind-body divided by a constriction. The forebody is leaf-

like with the widest breadth in the anterior third of the body. It is approximately twice the length of the hind-body and its lateral borders, beginning at the anterior third of its length, fold ventrally and meet just posterior to the holdfast organ, forming a spoon-like cavity which is a distinct characteristic of the group. When contracted, the body becomes thick and broad with notches along the lateral borders. Elongated, it becomes ribbon-like with a small apical cone in the region of the oral sucker. Lateral sucking cups and spines on the cuticle are absent. The forebody measures 1.47 (1.23-1.70 x 0.541 (0.442-0.646) mm. extended and 0.843 (0.656-0.984) x 0.714 (0.544-0.749) mm. contracted. (Table II)

The hind-body varies in shape from spherical when contracted to conical when extended. There is a shallow median groove which marks an internal septum between the large excretory spaces, thus producing a bilobate appearance. When stimulated by cover-glass or probe the body compresses laterally and discharges fluids through the excretory pore. The hind-body is contracted less under preservation. Hughes (1928) states that this may be due to the fact that the excretory content is gelatinous and probably does not diffuse readily through the body-walls; whereas, in the forebody, the excretory fluid is thin and watery, and partly to the fact that the musculature of the

forebody, being stronger than that of the hind-body, contracts more in fixation, thereby forcing more excretory fluid into the hindbody. Its measurements are 0.704 (0.442-0.884) x 0.374 (0.357-0.510) mm. extended and 0.476 (0.357-0.527) x 0.476 (0.427-0.565) mm. contracted. (Table II)

The oral sucker, sub-terminal, is situated at the anterior tip of the forebody. It faces ventrally and its diameter varies from 0.051-0.085 mm. It is approximately twice as large as the pharynx and the opening, when at rest, is a longitudinal slit. (Figure 3) The pharynx, circular in outline, immediately follows the oral sucker with the diameter 0.034 mm. of organ enclosing a longitudinal or ovoid aperture. (Figure 3)

A short esophagus precedes the intestinal cecum which bifurcates and proceeds laterally to the anterior tip of the acetabulum which they encircle briefly, then diverge slightly between the acetabulum and holdfast organ. They then proceed outward and around the latter organ where they again come together for a short distance at the level of the constriction and then encircle the reproductive fundaments following the septa and ending blindly in the region lateral to the bursa copulatrix. At the bifurcation of the esophagus the caeca are slender but becoming broader

as they proceed posteriorly. This system is obscured in live specimens due to the opaqueness of the body. (Figure 3)

The acetabulum, 0.092 (0.068-0.102) mm. in diameter, is situated 1.0 (0.765-1.088) mm. from the anterior tip of the oral sucker. In live specimens, opening and closing of the entrance can be seen. It is circular in shape and is elevated with its size approximately twice as large as the oral sucker. (Figue 3, ACE)

The holdfast organ is in the posterior third of the forebody and is contained in the cavity formed by the lateral folds of that body. Its distance from the oral sucker is 1.292 (0.935-1.360) mm. In preserved specimens it is longer than broad with the external opening a longitudinal median cleft. In living specimens, opening and closing of the entrance was observed. Its diameter varied from 0.187 (0.170-0.222) mm. It is circular in outline with the hold-fast gland lying in a dorsad position just posterior of the organ. (Plate VI, HF, HFG)

The nervous system is composed of a commissure at the level of the pharynx with paired branches extending posteriorily as far as the holdfast organ where they could not be traced further. Longitudinal muscle fibers are sub-cuticular and can be distinguished in sections. (Figure 3 N, Plate III, MF). The reproductive fundaments consist of

three or more irregular large and dark-stained bodies contained by the septum in the hind-body. The posterior testes in a few mounted specimens is transversly elongated with the apex of the inversely V-shaped fundament extending dorsally into the septum between the dorso-lateral vessels. The septum lies ventrally and extending from the anterior tip to the posterior end of the hind-body. (Figue 3, RF,S)

According to Faust (1922) and Hughes (1927) the excretory system of the Neascus larva is of two parts, the primary excretory apparatus and the reserve bladder. In living specimens, the arrangement of the flame cells was obscured by the thickness and opaqueness of the forebody and the elaborate reserve bladder system. Study of the reserve bladder system was made only on living specimens since preserved materials did not reveal any orderly pattern. To avoid confusion the terminology is that employed by Hughes (1927).

In live specimens small calcareous granules of various sizes can be seen flowing through the channels of the reserve bladder system from anterior to the posterior length of the parasite, Szidat (1924a), emersing the parasite in HCL, evolved gas and demonstrated the presence of phosphate in the granules by a test with ammonium molybdate. Under pressure of cover glass, these granules are exuded from the excretory pore with a heavy fluid not

readily miscible with water and as yet of unknown character. The arrangement of the system is as follows (Figure 4): The median dorsal vessel (MDV) extends from the region immediately back of the oral sucker to the posterior margin of the holdfast organ where it then extends as a small channel and emptying through the excretory pore. At the region of the oral sucker, two to three pairs of anterior transverse commissural (ATCV) vessels connect with the median dorsal vessel. Immediately lateral to the median dorsal vessel is a pair of intra-lateral vessels (ILV) connected by the posterior transverse commissural vessels (PTCV). The primary lateral vessel (PLV), externally adjacent to the intra-lateral vessel, begins anteriorly with a connection to the first pair of anterior commissural vessels and extends posteriorly where it encircles the holdfast organ and is joined by the lateral vessels to form the median ventral vessel (MVV). External to the primary lateral vessel, the smaller external lateral-vessel (ELV) runs along the lateral borders of the forebody, and joining at the lips of the cup forming the marginal vessel (MV). The primary lateral vessels are connected to their respective intra and extra-lateral vessels by smaller anastomoses (ANAS). In the hind-body, the large space divided by the thing septum constitute the dorso-lateral vessels (DV).

MORPHOLOGY OF *NEASCUS* SPP. FROM THE GREEN SUNFISH

Host-*Lepomis cyanella*

The morphological characteristics of this parasite is essentially the same as described for the host *L. machrochirus* so the following data will be limited to measurements and additional notes.

Cyst: The outer cyst wall of this parasite was thicker, and less transparent, while the inner cyst wall of the animal was thinner. Both cyst walls were well-defined and separate. Larvae found in the undifferentiated state (Plate IV) showed the cyst walls to be thicker, spherical, with enclosed dense fluid. As the worm matures, the cyst walls became thinner, more elastic and took the shape of the larval stage. In live and mounted specimens, the cysts measured from 1.237 (0.966-1.40) x 0.667 (0.087-0.812) mm.

Forebody: Spinous elements on the cuticle were not observed. Measurements were 0.964 (0.700-1.260) x 0.462 mm. and 0.613 (0.555-0.742) x 0.486 (0.455-0.560) mm. Hind-body: Spheroidal containing the reproductive fundaments and bursa copulatrix. Measurements were 0.570 (0.42-0.728) x 0.511 (0.483-0.558) and 0.342 (0.306-0.378) x 0.479 (0.419-0.554).mm.

Digestive system: Begins at the oral sucker which measures 0.070 mm. in diameter and 0.019 mm. thick. The canal then passes through the pharynx 0.030 (0.038-0.032) mm. in diameter and 0.004 mm. thick. A short esophagus 0.080 mm. long then bifurcates, proceeds posteriorly and laterally of the acetabulum, where it converges momentarily between the acetabulum and holdfast organ; it then encircles the holdfast organ, bends mesiad at the level of the constriction and ends blindly at the bursa copulatrix. Acetabulum: 0.686 mm. from the oral sucker and 0.066 (0.059-0.070) mm. in diameter is circular in shape and muscular fibers can be seen radiating from the slit to the outer edge of the organ.

Holdfast organ: This is slightly oval with a longitudinal slit. In preserved material the spinous inner surface of the holdfast organ is shown protruding through the external opening. The holdfast gland is situated dorsal to the organ where a fine network of canals of undetermined nature can be readily observed. Its distance from the oral sucker is 0.812 mm., and 0.182 (0.168-0.196) x 0.147 (0.140-0.154) x 0.190 (0.170-0.210) mm. Holdfast gland measured 0.049 (0.0418-0.057) mm.

Reproductive fundaments: Consists of 2-3 large deeply stained irregular shaped bodies which measure 0.196 (0.183-0.111) x 0.059 (0.052-0.063) x 0.053 (0.051-0.056)mm. The bursa copulatrix encloses multinucleated and thick

muscular tissue 0.019 mm. which is much convoluted. It measured 0.095 (0.093-0.098) mm. in diameter.

DISCUSSION

Twelve species of Neascus are known. The metacercariae reported in this study are synonymous with Neascus van-cleavi, a larval trematode which develops into the adult trematode Posthodiplostomum minimum. Minor differences such as measurements, canals of the reserve bladder system and morphological features are evident but such differences may be related to the age of the host, stage of development of the parasite, influence of physiological or environmental factors, and methods of preparation.

Haderlie (1953) reported only three of thirty-seven green sunfish infected, whereas twelve of sixteen hosts in this study were parasitized. This suggests that the infection rate in this area is higher, but matters such as the size of the host, type and season of collection as well as availability of the intermediate and definitive hosts must be investigated before this suggestion can be confirmed. Organs of the green sunfish were sparsely infected but numerous developmental cysts in the periphery of the liver indicated that the fish were recently infected. These hosts were collected during the winter months, a period which

coincides with the absence of definitive hosts. In the laboratory, the behavior of the hosts appeared normal, but further studies involving a larger quantity of specimens should be conducted during the summer months.

SUMMARY

A larval trematode embedded in the livers of the green sunfishes *Lepomis cyanella* and *L. macrochirus* is described as the metacercaria of *Posthodiplostomum minimum*. The history of previously described species is presented. Two experimental life cycles are elucidated; host-parasite relationships were noted; a survey of literature on the distribution of the metacercaria cited. A detailed taxonomic picture was presented. In addition to morphological data, notes on behavior are presented.

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APPENDIX

TABLE I

REPORTED METACERCARIAE OF THE TREMATODE FAMILY DIPLOSTOMATIDAE

Name with synonym(s)	Fish Host(s)
Diplostomum cuticola von Nordmann 1832 Neascus cuticola	
Holostomum brevicaudatum von Nordmann 1832 N. brevicaudatus	
Holostomum musculicola Waldenburg 1860 N. musculicola	
Cercaria sp. Cooper 1915[1] N. bulboglossa Crassiphiala bulboglossa	Perca flavescens Notropsis cornutus Boleosoma nigrum Umbra limi Leucosomus corporalis
Cercaria ptychocheilus Faust 1917 N. ptychocheilus	Notropsis delicious stamineus
Diplostomum van-cleavei Agersborg Agersborg 1926[2] N. van-cleavei N. oneidensis Posthodiplostomum minimum	Lepomis macrochirus L. cyanella Mollinesia latippina Notropsis anogenus Gambusia affinis Lavinia exilicauda Micropterus dolomieu Micropterus salmoides Ptychocheilus grandis Hesperoleucus symonetricus venustus Syphatoles obesus bicolor Rhinichthys osculus R. osculus klamthensis Mylopharidon conocephalus Hyborhynchus notatus Eupomotis gibbosus

TABLE I (Continued)

Name with synonym (s)	Fish Host(s)
	Enotis megalotis perlatus *Helioperca incisor* *Esox lucius*
Neascus amblopitis Hughes 1927[2] *N. wardi* *Crassiphiala amblolitis*	*Amblopitis rupestris* *Micropterus dolomieu* *Aplitis salmoides* *Eupomotis gibbosus* *Lepomis auritus*
N. perlatum Ciurea 1911 *Neodiplostomum perlatum*	Carp
N. rhinichthys Hunter 1933	*Rhinichthys astromasus* *R. cataractae* *Semotilus atromaculatus atromacula* *Notropsis cornutus*
N. grandis Van Cleave and Mueller 1934	*Umbra limi*
Unidentified sp. Bangham 1946	*Margariscus margarita neogaes* *Hybognathus hankinsosni* *Pfrille neogaes*
N. ellipticus Chandler 1951	*Perca flavescens*
N. longicollis Chandler 1951	*Perca flavescens*
N. pyriformes Chandler 1951	*Perca flavescens* *Fundulus diaphanus* *Stizostedion vitreus*

TABLE I (Continued)

Name with synonym(s)	Fish Host (s)
Unidentified species Haderlie 1953	*Catostomus occidentalis* *C. humboldtianus* *C. rimiculus* *Salmo gairdnerii* *Gasterosteus aculeatus* *Ptychocheilus grandis* *Hesperoleucus s. venustus*
Unidentified species DeRoth 1953	*Lepomis gibbosus* *L. auritus*

[1]Part of life cycle demonstrated.

[2]Life cycle demonstrated experimentally.

TABLE II

MEASUREMENTS OF TEN METACERCARIAE FROM THE HOST *LEPOMIS MACROCHIRUS* FREE OF COVER GLASS PRESSURE

	1	2	3	4	5
FOREBODY:					
EXTENDED LENGTH	1.230 mm.	1.476 mm.	1.705 mm.	1.525 mm.	1.459 mm.
EXTENDED WIDTH	0.656	0.984	0.819	1.034	0.918
CONTRACTED LENGTH	0.623	0.442	0.612	0.545	0.578
CONTRACTED WIDTH	0.544	0.714	0.544	0.765	0.798
HIND-BODY:					
EXTENDED LENGTH	0.442	0.656	0.820	0.820	0.782
EXTENDED WIDTH	0.367	0.510	0.510	0.527	0.510
CONTRACTED LENGTH	0.510	0.480	0.374	0.340	0.357
CONTRACTED WIDTH	0.544	0.578	0.544	0.544	0.560
DIAMETER OF ORAL SUCKER	0.186	0.186	0.186	0.221	0.186
DIAMETER OF PHARYNX	0.051	0.051	0.068	0.068	0.085
DIAMETER OF ACETABULUM TO TIP OF ORAL SUCKER	0.765	0.816	1.088	1.055	1.088
DIAMETER OF ACETABULUM	0.034	0.034	0.034	0.034	0.034
DIAMETER OF HOLDFAST	0.068	0.085	0.085	0.102	0.102
DISTANCE OF HOLDFAST TO TIP OF ORAL SUCKER	0.936	1.275	1.326	1.360	1.360

TABLE II (Continued)

6	7	8	9	10	Range	Ave.
1.492 mm.	1.394 mm.	1.427 mm.	1.575 mm.	1.476 mm.	1.230-1.705 mm.	1.470 mm.
0.863	0.836	0.706	0.803	0.820	0.656-1.034	0.843
0.442	0.476	0.544	0.578	0.510	0.442-0.623	0.541
0.706	0.782	0.748	0.798	0.714	0.544-0.798	0.714
0.853	0.697	0.836	0.833	0.706	0.442-0.853	0.765
0.459	0.510	0.493	0.510	0.425	0.367-0.527	0.476
0.357	0.374	0.391	0.374	0.323	0.323-0.510	0.374
0.544	0.527	0.595	0.578	0.527	0.527-0.595	0.561
0.204	0.186	0.170	0.186	0.204	0.170-0.222	0.187
0.051	0.051	0.051	0.068	0.051	0.051-0.085	0.060
1.105	0.970	1.072	1.038	0.987	0.765-1.105	1.000
0.034	0.034	0.034	0.034	0.034	0.034	0.034
0.102	0.085	0.102	0.102	0.085	0.068-0.102	0.092
1.275	1.411	1.310	1.360	1.293	0.935-1.411	1.292

EXPLANATION OF PLATES AND FIGURES

All drawings of the trematode larva Neascus except Plates I, II, and Figures 1, 2, and 4 were made with the aid of camera lucida. Plates II, III, and Figures 1, 2, 3, and 4 represent parasites from the bluegill *Lepomis macrochirus*; while Plates IV, V, VI, and Figures 10 to 21 represent parasites from the green sunfish *L. cyanella*. All animals were fixed in Bouin's and whole mounts were stained in Grenacher's acid alum carmine or borax carmine. Longitudinal and cross-sections of 10, 12, and 15 micra were stained either in Heidenhain's iron hematoxylin or Procher's celestine blue counter-stained with acid fuchsin.

ACE - Acetabulum

ATCV - Anterior commissural vessel

AV - Anastomose vessel

BC - Bursa copulatrix

DE - Developing metacercaria

DV - Dorso - lateral vessel

ELV - Extra Lateral vessel

EP - Excretory pore

ES - Esophagus

HF - Holdfast organ

HFG - Holdfast gland

ILV - Intra-lateral vessel

INC - Inner cyst

INT - Intestinal caecum

MDV - Median dorsal vessel

MF - Muscular fiber

MVV - Median ventral vessel

MV - Marginal vessel

N - Nerve fiber

OC - Outer cyst

OS - Oral sucker

P - Pharynx

PLV - Primary lateral vessel

PTC - Posterior transverse commissure

RF - Reproductive fundament

S - Septum

SPC - Supra-pharyngeal commissure

VV - Ventral vessel

PLATE I

LIFE-CYCLE OF POSTHODIPLOSTOMUM MINIMUM

AFTER G. W. HUNTER (1937)

1. Undeveloped eggs hatched in about three weeks.
2. Ciliated miracidium must penetrate snail.
3. Snail, primary host.
4. Daughter sporocysts produces cercariae.
5. Furocercous cercaria emerges from snail and penetrates beneath the scales of the fish.
6. Tailless cercaria from the flesh a few hours after penetration.
7. Fully developed metacercaria.
8. Metacercaria encysted in liver.
9. Common sunfish, rock bass, small-mouthed bass and fifteen other species serve as the intermediate host. Infected fish must be eaten by heron. About thirty-six to forty hours required to reach maturity after engesting infected fish.

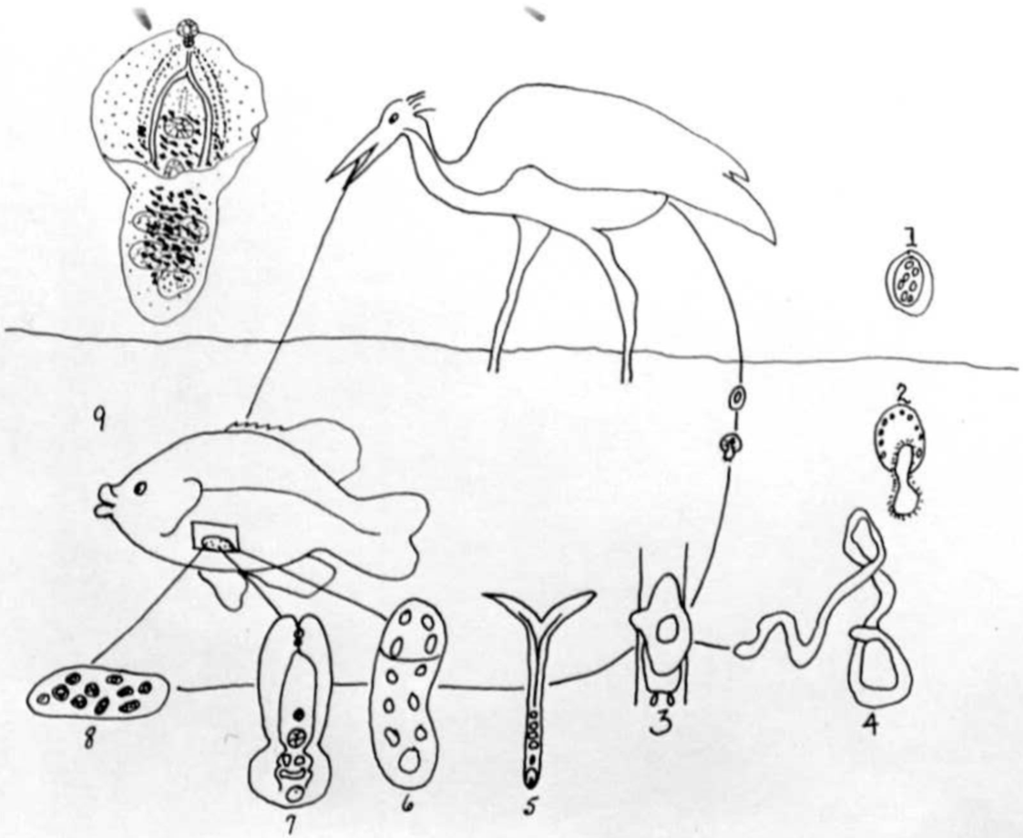


PLATE I

PLATE II

DIAGRAMMATIC DRAWING OF DISSECTED FISH SHOWING THE HEART AND LIVER INFESTED WITH CYSTS.

HOST-*LEPOMIS MACROCHIRUS*

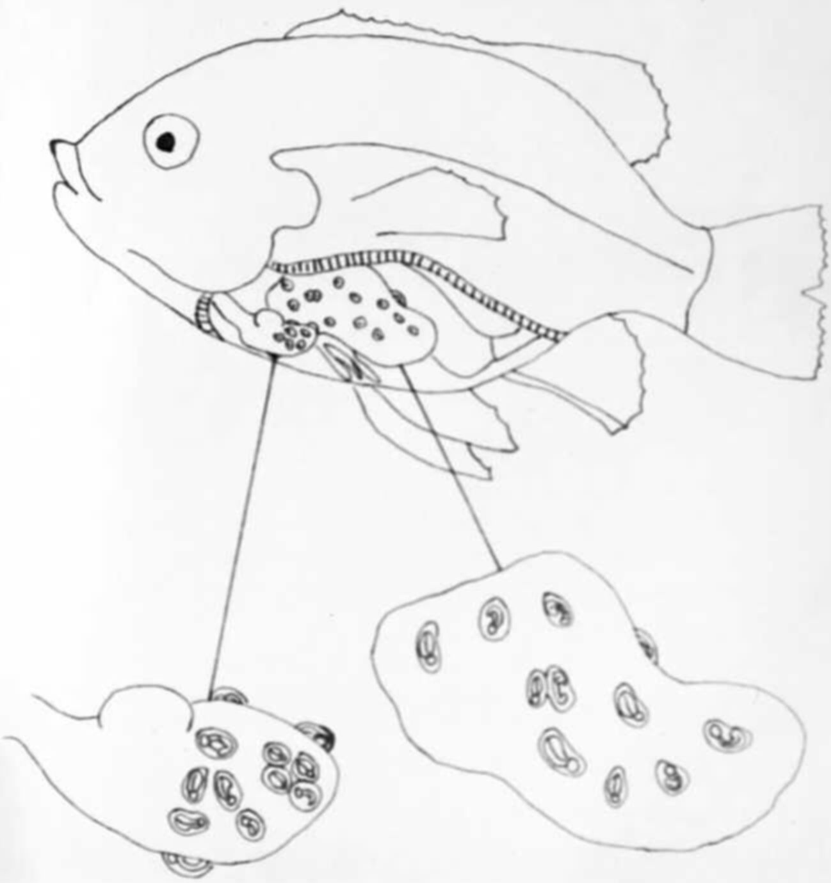


PLATE II

FIGURE 1

ENCYSTED METACERCARIA VIEWED VENTRALLY.

HOST-*L*. *MACROCHIRUS*. X 70

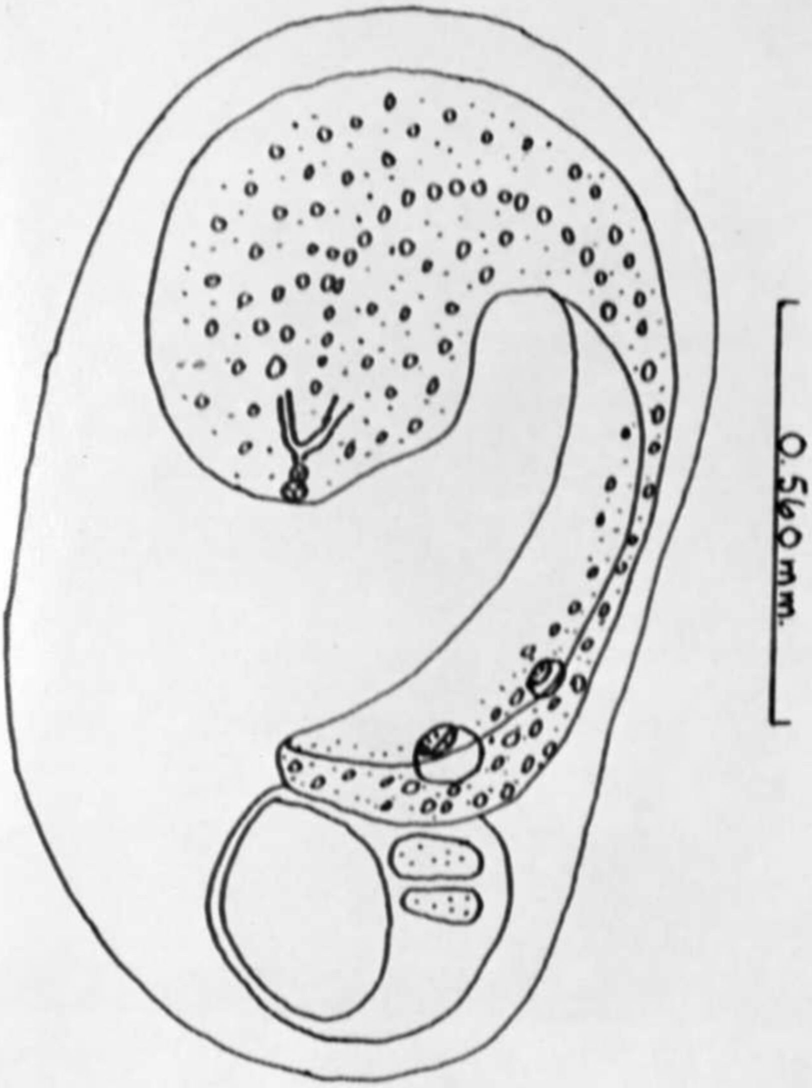


FIG. 1

FIGURE 2

EXCYSTED METACERCARIA SHOWING THE SPOON-LIKE CAVITY OF THE FOREBODY AND THE CONSTRICTION SEPARATING THE FORE AND HIND-BODY AS VIEWED VENTRALLY

HOST-*L. MACROCHIRUS*. X 70

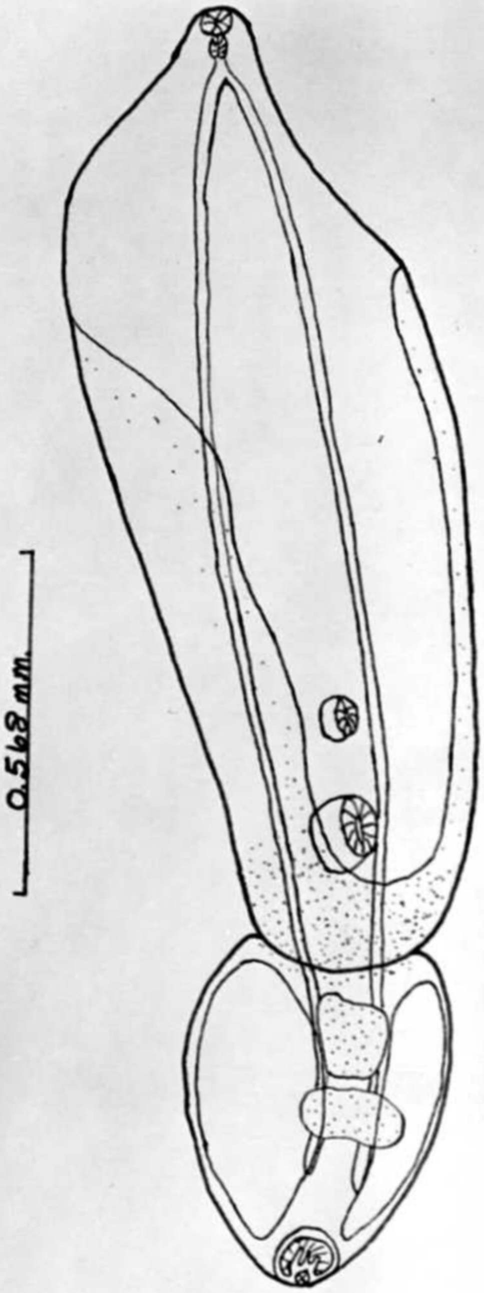


FIG. 2

FIGURE 3

COMPOSITE VENTRAL VIEW OF THE METACERCARIA

HOST-*L*. *MACROCHIRUS*. X 75

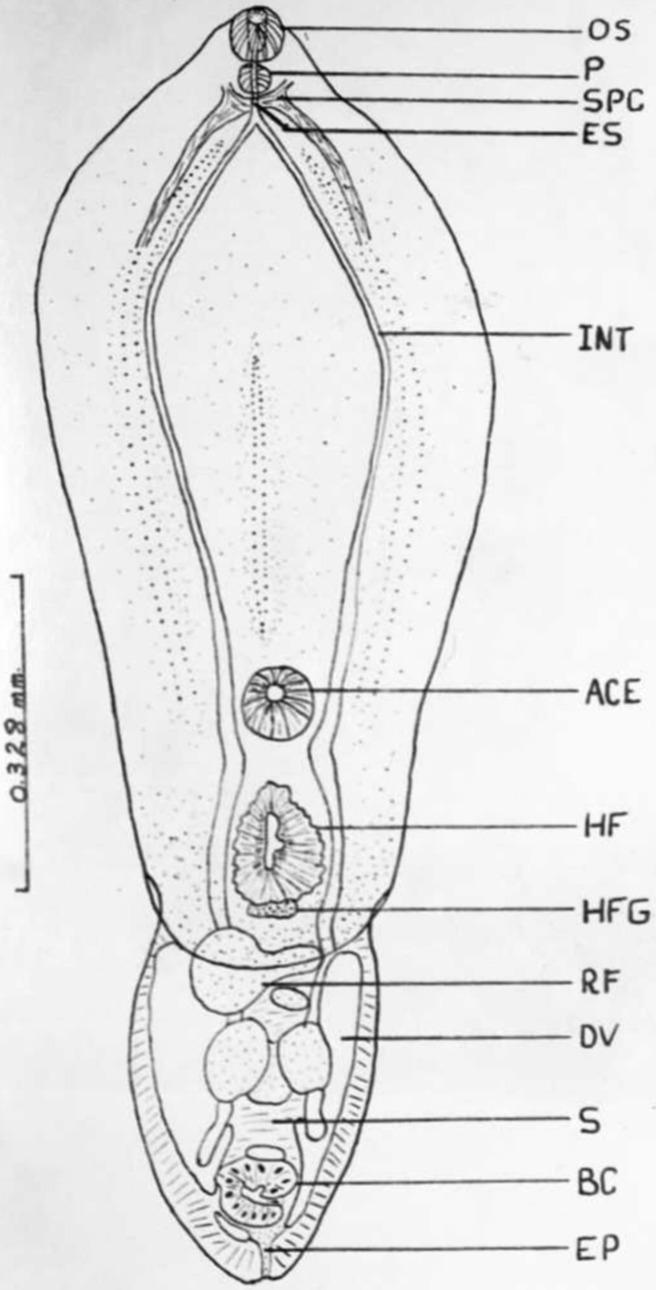


FIG. 3

FIGURE 4

DIAGRAMMATIC DORSAL VIEW OF THE RESERVE BLADDER SYSTEM

HOST-*L. MACROCHIRUS*. X 95

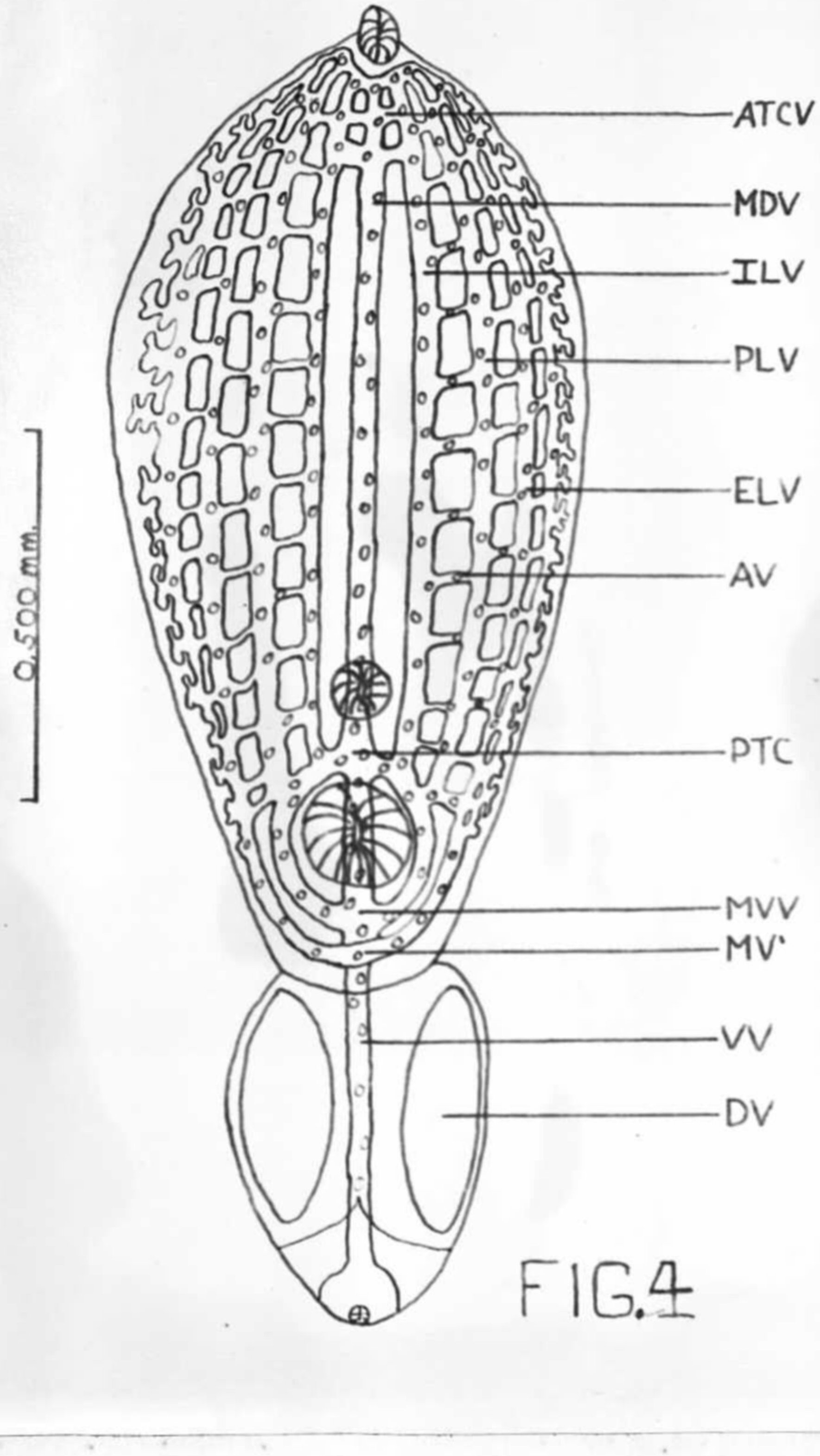


FIG.4

PLATE III

LONGITUDINAL AND CROSS-SECTIONS OF METACERCARIA

HOST-L. MACROCHIRUS. X 85

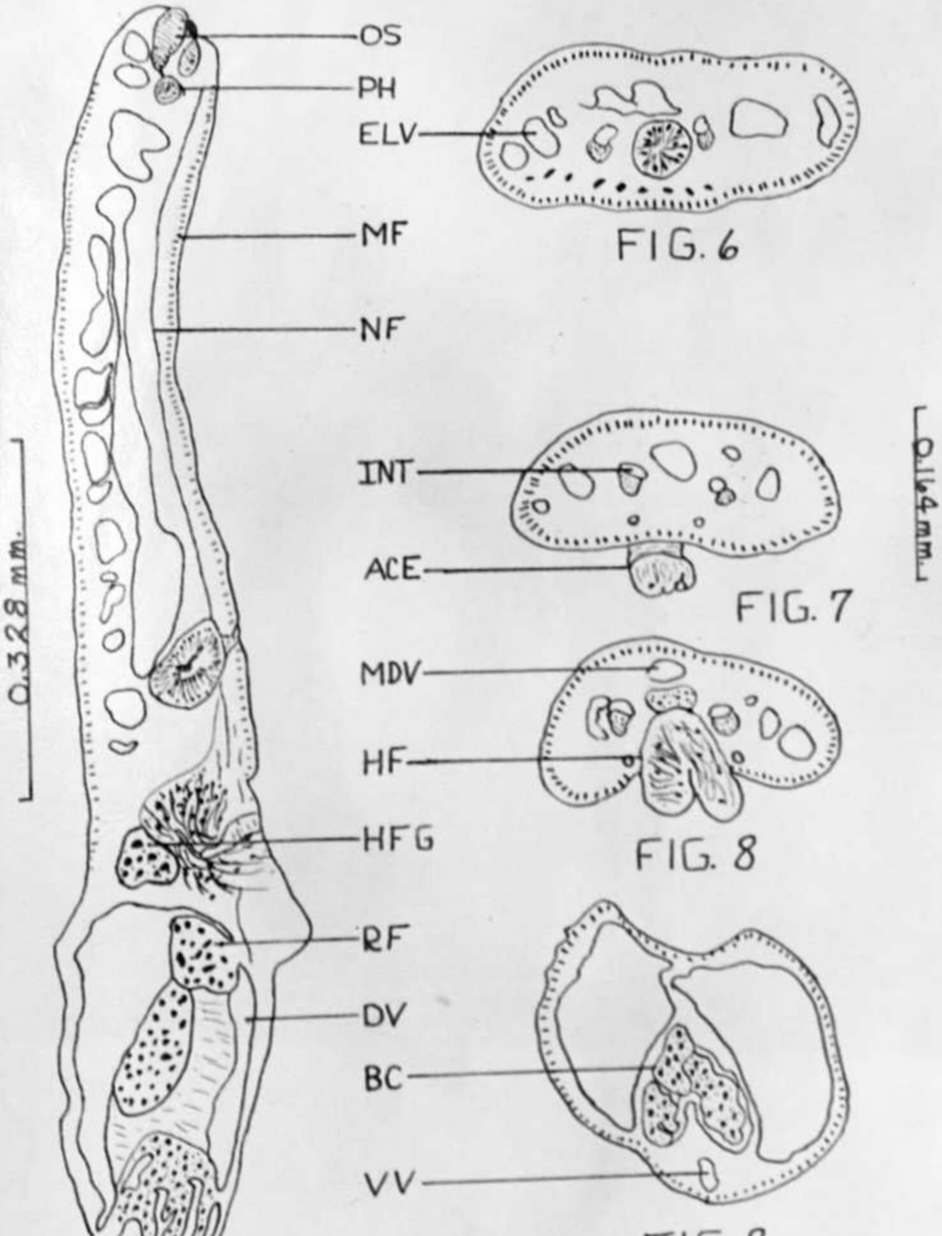
FIGURE 5. LONGITUDINAL COMPOSITE OF METACERCARIS VIEWED FROM THE RIGHT SIDE.

FIGURE 6. TRANSVERSE SECTION THROUGH THE PHARYNX.

FIGURE 7. TRANSVERSE SECTION SHOWING THE EVERTED ACETABULUM.

FIGURE 8. TRANSVERSE SECTION THROUGH THE HOLDFAST ORGAN.

FIGURE 9. TRANSVERSE SECTION THROUGH THE BURSA COPULATRIX.



OS
PH
ELV
MF
NF
0.328 mm.
INT
ACE
0.164 mm.
MDV
HF
HFG
RF
DV
BC
VV
EP
FIG. 5
FIG. 6
FIG. 7
FIG. 8
FIG. 9

PLATE III

PLATE IV

DEVELOPMENTAL STAGES OF THE METACERCARIA

HOST-*LEPOMIS CYANELLA*. X 1000

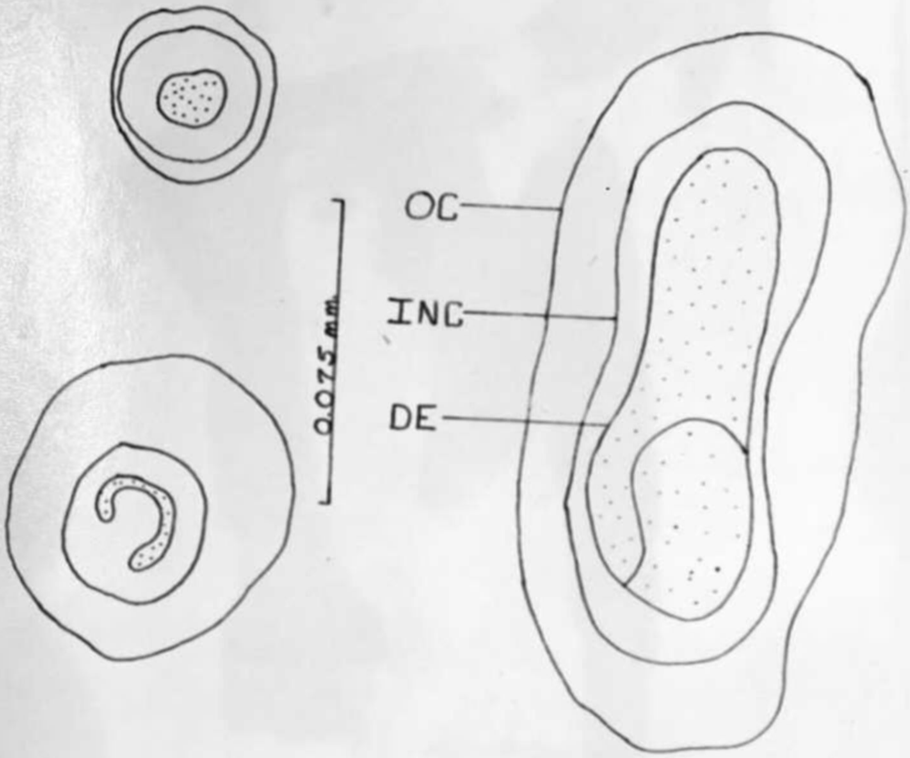


PLATE IV

FIGURE 10

DORSAL VIEW OF METACERCARIA. HOST-*L*. *CYANELLA*. X 100

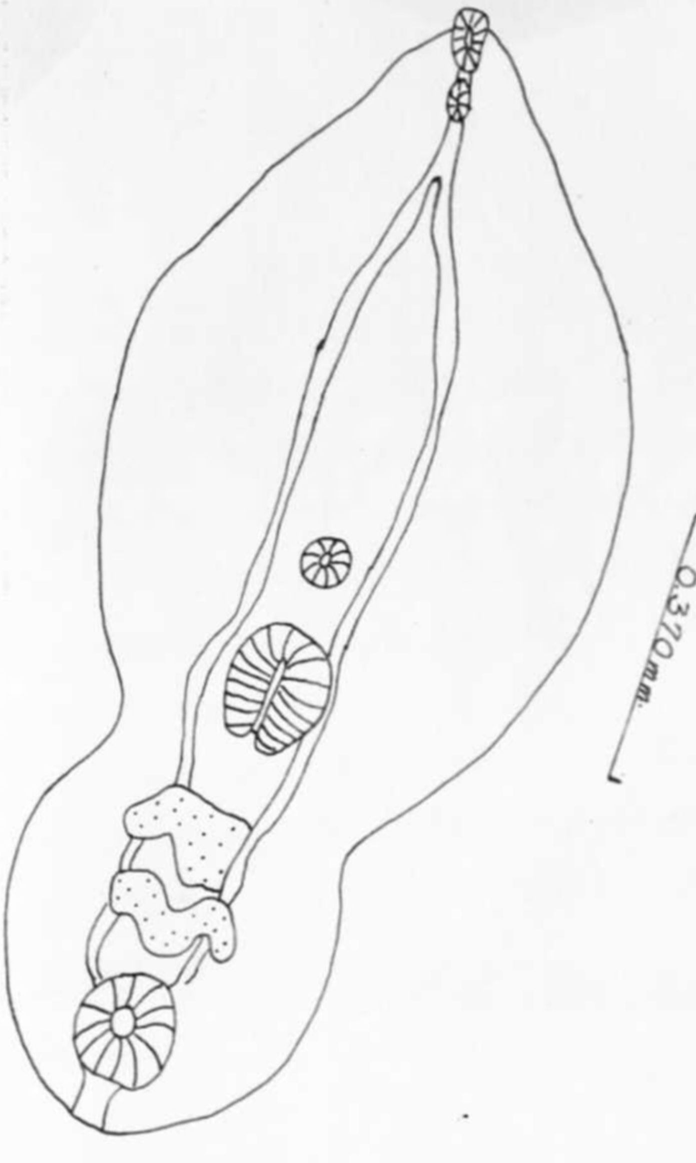


FIG. 10

PLATE V

LONGITUDINAL AND CROSS-SECTIONS OF METACERCARIA.

HOST-LEPOMIS CYANELLA X 85

FIGURE 11. COMPOSITE LATERAL VIEW FROM THE RIGHT SIDE.

FIGURE 12. TRANSVERSE SECTION THROUGH THE ORAL SUCKER.

FIGURE 13. TRANSVERSE SECTION THROUGH THE PHARYNX.

FIGURE 14. TRANSVERSE SECTION BETWEEN THE PHARYNX AND ACETABULUM.

FIGURE 15. TRANSVERSE SECTION THROUGH THE ACETABULUM.

FIGURE 16. TRANSVERSE SECTION THROUGH THE HOLDFAST ORGAN.

FIGURE 17. TRANSVERSE SECTION THROUGH THE REPRODUCTIVE FUNDAMENTS.

FIGURE 18. TRANSVERSE SECTION THROUGH THE BURSA COPULATRIX.

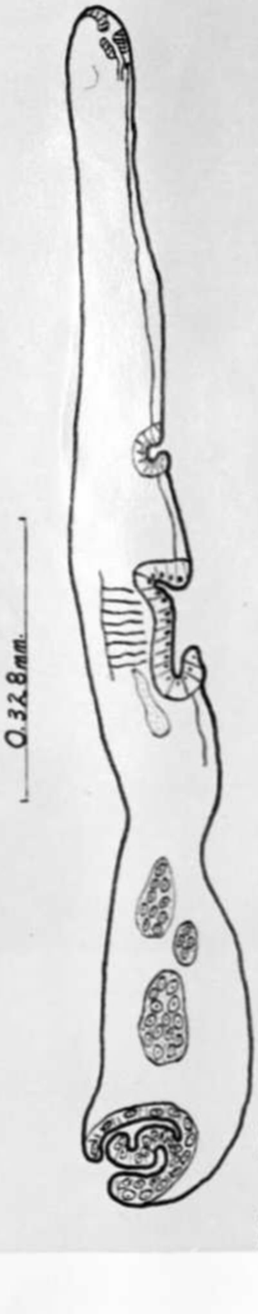


FIG. 11


FIG. 12


FIG. 13


FIG. 14


FIG. 15


FIG. 16

0.164 mm


FIG. 17


FIG. 18

PLATE V

PLATE VI

LONGITUDINAL SECTIONS OF SUCKERS SHOWING DETAILED MUSCLE FIBERS. HOST-L. CYANELLA. X 500

FIGURE 19. THROUGH THE ORAL SUCKER AND ACETABULUM.

FIGURE 20. THROUGH THE ACETABULUM.

FIGURE 21. THROUGH THE HOLDFAST ORGAN.

FIG. 19

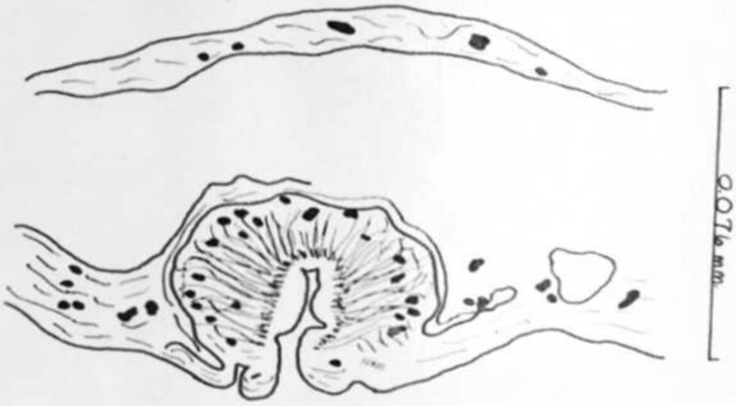


FIG. 20

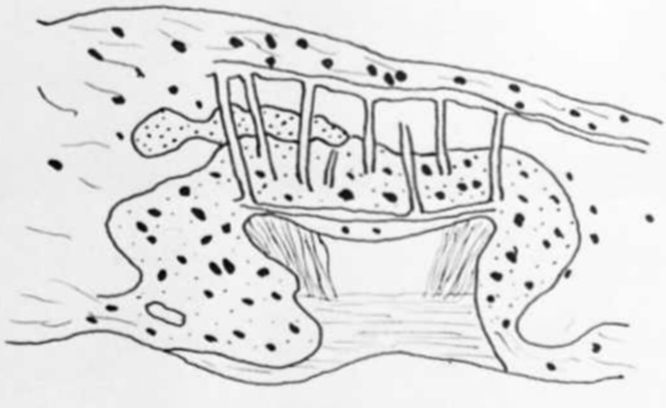


FIG. 21

PLATE VI