8-1964

# A Histological Study Of Cleidodiscus Robustus Muller, 1934

Prince V. McCann
*Prairie View Agricultural and Mechanical College*

# A HISTOLOGICAL STUDY OF CLEIDODISCUS ROBUSTUS MULLER, 1934

McCANN

1964

# A HISTOLOGICAL STUDY OF CLEIDODISCUS ROBUSTUS MULLER, 1934

By

Prince V. McCann

A Thesis Submitted in Partial Fulfillment of the
Requirements for the Degree of
Master of Science

In The
Graduate Division
of



Prairie View Agricultural and Mechanical College
Prairie View, Texas

Date: August 19, 1964

This Thesis for the Master of Science Degree by Prince V. McCann has been approved for the Department of Biology of the School of Arts and Sciences by:

[redacted] 8/19/1964

[redacted] 8/19/64

[redacted] 8/19/64

[redacted] 8/19/64

_____ _____

ACKNOWLEDGEMENT

The writer wishes to thank Dr. J. E. Berry, Professor of Biology, Prairie View Agricultural and Mechanical College, for his guidance through this investigation.

P.V.M.

Table of Contents

Morphological description based on histological technique is a phase of parasitology which has been, in general, neglected. Almost all the previous authors, including the most recent ones such as Bychowsky (1957), Caballero, Blavo-Hollis and Grocott (1955), Chauhan (1953), Hargis (1959), Jain (1960), Llewellyn (1941), Meserve (1937), Mizelle (1938, 1955), Price (1959), Ramalingam (1953), and Sproston (1946) have placed great emphasis on external morphology; particularly, on the cuticularized or sclerotized parts of the body such as haptoral anchors, clamp sclerites, copulatory apparatus, etc. These hard parts are, of course, of special value as systematic criteria. The internal anatomy, especially the genitalia, is also of great taxonomic value, although as pointed out by Brinkmann (1952) not much attention has been paid to them by the majority of helminthologists. Some authors, however, including Saint-Remy (1890), Cerfontaine (1895), Brinkmann (1942), Palombi (1943), and Yamaguti (1936), have endeavored to work out the details of the internal anatomy of some species.

For the purpose of orientation, the taxonomical history and general morphology of the organism are given.

The trematodes were named by Rudolphi in 1808, as an

order, which included the following genera: Monostomata, Amphistomata, Distoma and Polystomata. In 1885, van Beneden proposed the term, monogeneses, for trematodes which develope without metamorphosis and digeneses for those which develope with metamorphosis. The former category generally consisted of ectoparasites and the latter, exclusively, of endoparasites. In 1863, Carus proposed the terms, Monogenea and Digenea, respectively, to replace the two terms of van Beneden. In 1892, Monticelli divided the order Trematoda into the suborders Heterocotylea, Aspidocotylea, and Malacotylea. The suborder Heterocotylea coincides with the Monogenea (Pratt, 1900). The Aspidocotylea and the Malacotylea are divisions of the Digenea. Odhner (1912) divided the Monogenea of Carus into the Polyopisthocotylea and Monopisthocotylea, respectively, on the basis of the presence or absence of a genito-intestinal canal. According to Fuhrmann (1928), the Monogenea and Digenea of Carus are accepted as orders and the order Trematoda Rudolphi, is elevated to the status of class. The order Monogenea Carus, as given by Fuhrmann, embraces three suborders, Monopisthodiscinea, Monoposthocotylinea, and Polyopisthocotylea. Price (1937) prefers the divisions Monopisthocotylea and Polyopisthocotylea of Odhner

as suborders to those of Fuhrmann. Price's classification is followed in this investigation.

Class----Trematoda
Order----Monogenea
Suborder---Monopisthocotylea
Superfamily---Gyrodactyloidea
Family--------Dactylogyridae
Subfamily----Tetraonchinae
Genus-------Cleidodiscus
Species----C. robustus

The classification according to Bychowsky is as following:

Class----Monogenoidea
Subclass---Polyonchoinea
Order------Dactylogyridea
Suborder----Dactylogyrinea
Family-----Dactylogyridae
Subfamily---Ancyrocephalinae
Genus-------Cleidodiscus
Species-----C. robustus

Other systems of classification were contributed by Fuhrmann (1932), Poche (1925), Johnston and Tiegs (1922), Odhner (1912), Monticelli (1903), and Zeder (1800).

The monogenetic trematodes are hermaphroditic flatworms that are parasitic on or in aquatic or amphibious vertebrates, and occasionally, are found on aquatic invertebrates such as crustaceans, mollusks, etc. These parasites most commonly occur in the gill chamber but may be on the skin, in the buccopharyngeal cavity, or in other organs communicating with the exterior, such as nostrils, eyes, ears, cloaca, rectal gland, urinary bladder, etc. Carus, (1863). Members of this group inhabit only one host and have at the posterior end well-developed attaching organs which are used to anchor the animals to their hosts. The anterior end of the body bears attaching organs which are employed during feeding. Many of these parasites are host specific and even have certain locations that they will occupy on certain gill arches. For example, some species prefer the second and third pairs of arches; some occupy the ends of a particular part; whereas, others are found in the middle of specific gill arches. However, certain species occur on all four pairs of gills.

The trematodes of this group are somewhat flattened dorsoventrally and have a trunk narrowly elliptical in outline. A pair of lateral lobe-like projections are present in the head region. Two pairs of eye spots are present, and are located dorsally near the anterior end.

A group of glandular cells occurs on each side of the body in the region of the pharynx. The haptor is hexagonal in shape and contains four anchors, two bars and seven pairs of hooks. These are relatively large parasites, having an average length of 1.4 mm. and an average width of 0.2 mm. However, the measurement of size is hampered by the fact that the body is capable of contracting and stretching excessively.

Their color is determined by the color of the internal organs, but the body itself is either colorless or grayish white. With the organs showing through, the color may vary considerably. The intestine is blackish in color while the vitelline material and gonads are milky-white. The uterus is yellow or tan.

## Materials and Methods

Specimens of *Cleidodiscus robustus* were collected from sunfish (*Helioperca macrochira*) found in lakes and ponds in the Prairie View vicinity. The gill arches were removed from the fishes, placed in a Syracuse watch glass containing saline solution and examined with a dissecting microscope. The parasites were dislodged by scraping the gills with a dissecting needle and transferred to a Syracuse watch glass where they were narcotized with a 0.1 per cent chloral hydrate solution. For sectioning purposes, each organism was pipetted on a square piece of rice cigarette paper which had been treated previously with India ink. This treatment was necessary because of the small size and refractive index of the parasite. The chloral hydrate solution was allowed to evaporate and the specimens were fixed with Carnoy's fluid. The cigarette paper was then cut in the shape of the letter, "T", with the parasite on the unpaired limb. This arrangement allowed for proper orientation. The specimens were allowed to remain in Carnoy's fluid for an hour, then dehydrated, infiltrated with paraffin, and finally embedded. Sections were cut at five microns and stained in Heidenhain's "Azan" stain.

In the preparation of the whole mounts, the organisms were placed on cover glasses, fixed with Carnoy's fluid and stained in Heidenhain's "Azan" stain.

## Results

The body of *Cleidodiscus robustus* is flattened dorsoventrally and is elongated in shape (Pl. I). The average length of the specimen is 1.411 mm. and the average greatest body width is 0.216 mm. The external covering is represented by a thin cuticle which is typical of the parasitic flatworms (Bychowsky, 1957). It is double-layered and contains scattered scale-like structures. The narrow anterior end is elliptical and has two pairs of eyespots located dorsally with one pair behind the other (Pl. II). The anterior pair is smaller than the posterior one. The average diameter of the former is 0.013 mm. and that of the latter, 0.023 mm. Each eyespot is composed of an aggregation of melanistic granules which are easily separated from each other by cover-glass pressure. A pair of lateral lobe-like projections known as cephalic lobes are present in the head region (Pl. I). On each side of the cephalic region, three to five ducts, comprising the head organs, anastomose to form a single duct which continues posteriad for a short distance ending in glandular cells in the region of the pharynx (Pl. II). They are believed to have an adhesive function.

The posterior end is broad and hexagonal (Pl. I). The

average width is 0.111 mm. and the average length, 0.106 mm. (Mizelle, 1938). Two bars are always present, each of which connects the bases of a pair of anchors (Pl. III, Figs. 1, 2). The ventral bar is similar in shape to the dorsal but slightly longer and more complicated in construction (Pl. III, Fig 2). On the ventral and dorsal centers of the ventral bar, there are two sharp peaks giving it the appearance of a candle holder. These areas on the dorsal bar are smooth (Pl. III, Fig. 1). The ends of both bars are curved toward their centers. The bars do not come in contact with each other but connect the bases of a pair of anchors (Pl. III, Figs. 1, 2). The average length of the ventral bar is 0.028 mm. and that of the dorsal bar is 0.027 mm. (Mizelle, 1938). There are always four anchors present which are similar in shape with their bases bifurcated into superficial and knob-like deep roots (Pl. III, Figs. 1, 2). The ventral anchors are slightly longer than the dorsal ones and the bases of the two pairs are approximately the same width. The average length of the ventral anchor from the base to the curvature is 0.026 mm. and the average width is 0.012 mm. The dorsal anchor has an average length of 0.024 mm. and an average width of 0.012 mm. Seven pairs of hooks are present on the

haptor with five located ventrally and two dorsally (Pl. I). A hook is a tapering rod ending distally in a sickle-shaped process and an opposable piece (Pl. III, Fig. 3). The sizes of the hooks vary and, like the anchors, are divided into two parts: an ovate base which is very short and a shaft which is curved at the end away from the base (Pl. IV, Fig. 3). The average length of the various pairs of hooks is as follows:

| Hook pair | Average length in mm. |
|---|---|
| 1 | 0.015 |
| 2 | 0.017 |
| 3 | 0.019 |
| 4 | 0.019 |
| 5 | 0.016 |
| 6 | 0.019 |
| 7 | 0.019 |

The subcuticle material includes the circular, longitudinal and diagonal muscle layers which are very thin.

In the reproductive system, the gonads lie near the middle of the body (Pl. I). The testis is single, ovate in shape, very large and is located posterior to the ovary (Pl. I). The average length of the testis is 0.310 mm.

and the average width is 0.165 mm. The vas deferens, composed of epithelial and circular muscle layers, passes forward on the left side of the body and expands to form a conspicuous seminal vesicle just posterior to the cirrus base (Pl. V). Only one prostate gland has been observed. It is bulbshaped, located posterior to the cirrus base, and empties into it by a single duct arising from the anterior end (Pl. V). The average length being 0.072 mm. and the width 0.036 mm. The copulatory complex is well developed and situated in a relatively large vestibule (Pl. VI). The cirrus is a simple, curved hard tube, which is 0.054 mm. long and 0.0072 mm. wide. The accessory piece is a blade-like structure with a knob near the middle which serves as a site for muscle attachment. On contraction of these structures, the cirrus and accessory piece separate distally and the cirrus is projected ventrally, for a short distance, through the pore of the vestibule. The retraction mechanism of the cirrus is not understood. The ovary is ovate in shape, smaller than the testis, and situated anterior to it (Pl. I). The single ovary is composed of compact follicle cells and averages 0.09 mm. in diameter (Pl. IV). A short oviduct arises from the anterior surface of the ovary and opens into the ootype (Pl. IV). The uterus originates from the ootype

and passes cephalad on the ventral surface and opens to the outside through the uterine pore in the vicinity of the copulatory complex (Pl. I). The vitelline ducts also arise from the ootype and give rise to the vitellaria which consist of innumerable minute follicles arranged in two lateral longitudinal bands (Pl. IV). They are powerfully developed and occupy almost the entire body starting from the head end and extending to the haptor. Surrounding the ootype is Mehlis gland which is believed to secrete a lubricant that aids the passage of eggs along this tube and possible activates spermatozoa (Pl. IV). A vagina is present on the left side near the junction of the anterior and middle thirds of the body length (Pl. IV). The vaginal canal is tubular and composed of thin muscular and epithelial layers. It is short and extends a short distance into the seminal receptacle (Pl. IV).

The digestive system is well developed. The mouth is located in the mid-ventral region near the level of the anterior eyespots (Pl. II). A short buccal canal passes posterodorsad to enter the well-developed pharynx which is rounded, composed of circular and radial muscles and has a diameter of 0.0774 mm. (Pl. I) The short esophagus bifurcates to form two lateral intestinal crura which unite posteriorly in the region of confluency of the vit-

ellaria bands (Pl. I).

According to Mizelle (1938), the excretory system consists of a maze of ramifying tubules presumable terminating in flame cells. Basically a single pair of lateral collecting ducts occurs in the posterior part of the anterior body half. Anteriorly each tube bifurcates to form two ducts. One of these passes laterally at an angle of about forty-five degrees and empties on the dorsolateral body surface near the level of the copulatory complex. The other passes anteriorly and terminates laterally to the anterior eyespot.

According to Bychowsky (1957), the nervous system is relatively strongly developed. The cephalic brain is located dorsally in front of the pharynx and consists of two large ganglia joined by dorsal and ventral commissures thus forming a nerve ring. Three to four pairs of anterior nerves emerge from the "brain" and, usually, three pairs of nerve trunks emerge behind it. The ventral pair is the most powerfully developed and gives off a network of nerves which innervate the entire periphery of the animal and internal organs.

Discussion

The external covering or cuticle of Cleidodiscus robustus was found to possess elongated, splinter-like structures which were very pronounced in unstained specimens. Mizelle (1934) mentioned the presence of scale-like structures in the cuticle of the same species. These characteristic scales are absent in most species of this genus. According to Bychowsky (1957), a more or less well-developed basal membrane is located under the cuticle. This investigation revealed no such structure.

The description of the anterior end was found to coincide with that of Mizelle (1934) and Yamaguti (1961) There are four eyespots located on the dorsal surface which are composed or rod-like granules. According to Bychowsky (1957), they are believed to function as organs of feeling. However, it is probable that they act as photoreceptors. The head organs are a series of ducts located anterior to each cephalic lobe and empty the secretions, produced by the cephalic glands in the pharyngeal region, to the exterior. These glands are lobulated and extend beyond the pharynx. According to Noble (1961), the products produced by these glands aid in the adhesiveness of the mouth to the region of feeding.

The posterior end, which is called the haptor, con-

tains the attaching organs and may be embedded in the tissues of the host. In some cases the organisms were so fixed that it was impossible to isolate the specimens from their host without injuring these organs. The dorsal and ventral bars are strongly developed, dissimilar and have curved extremities with knob-like structures on their dorsal surfaces. These structures were not mentioned by Mizelle (1938), Yamaguti (1936, 1961) and Bychowsky (1957) in their descriptions. They serve as a connection between the dorsal and ventral pairs of anchors, respectively. The anchors are typical for the species. The arrangement of the seven pairs of hooks is an outstanding characteristic of these Monogenea with five pairs being on the ventral surface and two on the dorsal one.

The reproductive system is well developed. The male reproductive system is composed of a single testis which gives rise to the vas deferens. The latter expands to form a conspicuous seminal vesicle, which empties into the cirrus base. A duct from the prostate gland also opens into the base of the cirrus. The copulatory complex consists of the cirrus and the accessory piece, both of which is well developed and located below the esophagus. The ovary of the female reproductive system is located anterior to the testis and gives rise to the oviduct which enlarges

to form the ootype. The vitelline ducts, the seminal receptacle and the uterus communicate with the ootype. The vagina communicates with the seminal receptacle via the vaginal canal. According to Mizelle (1936), a row of spines encircles the base of the vaginal canal at its junction with the seminal receptacle. Yamaguti (1937) did not report this and the investigator observed no such structures. They appeared to be thin muscular layers at this junction. According to Mizelle (1936) and Yamaguti (1937) there is only one prostate gland present but the observer noted a structure which appeared to be a second one.

The mouth is ventrally located in the cephalic part of the body and opens into a buccal canal which is connected to the pharynx. The latter is muscular and joins the esophagus which bifurcates immediately to form two lateral intestinal crura which are very thin-walled and appear as canals in the parenchyma. According to Bychowsky (1957), salivary glands open into the lower end of the pharynx and are responsible for chemical digestion. The food substances are absorbed by the walls of the intestine along its length.

In this investigation, no observations were available concerning the excretory and nervous systems; however, some investigators have been successful in describ-

ing these systems in certain other groups. It is also important to note that the tissues composing the wall of organs are extremly thin. In fact, they are so thin that it is impossible to differentiate the cells composing the tissue. According to the literature, this is the first time sections have been made in an attempt to describe the morphology of C. robustus.

Summary

Parasites were collected from Helioperca macrochira found in ponds and streams in the Prairie View vicinity. Sections and wholemounts were prepared and observed after staining.

A description of the integumentary, digestive, muscular and reproductive systems is given. Features that have not been mentioned before in the literature are: the presence of knob-like structures found at the extremities of the bars, the presence of a second prostate gland and the absence of spines around the base of the vaginal canal at its junction with the seminal receptacle.

The attaching organs and the copulatory structures are well developed and serve as definite classification markings for the species.

The parasites concerned in this investigation have only been found in fresh waters and on the gills of a limited number of species of fish. Since there is so much information that is not known about these trematodes, there is a need for much more research in this area.

# LEGEND

Plate I Wholemount - *Cleidodiscus robustus*
Plate II Head Organs
Plate III Attaching organs
Fig. 1 Dorsal anchors and bar
Fig. 2 Ventral anchors and bar
Fig. 3 Hooks
Plate IV Female reproductive organs
Plate V Male reproductive organs
Plate VI Copulatory complex
Plate VII Sections
Fig. 1 Pharynx
Fig. 2 Prostate gland and seminal vesicle
Fig. 3 Copulatory complex
Fig. 4 Testis
Fig. 5 Ovary

M mouth
ES eyespot
UP uterine pore
P prostate gland
UT uterus
OV ovary
IC intestinal crura
H haptor
HO head organs
CL cephalic lobe
CG cephalic gland
PH pharynx
CC copulatory complex
SV seminal vesicle
V vagina
SR seminal receptacle
VD vas deferns
T testis
VIT vitelline

PLATE I

Cleidodiscus robustus

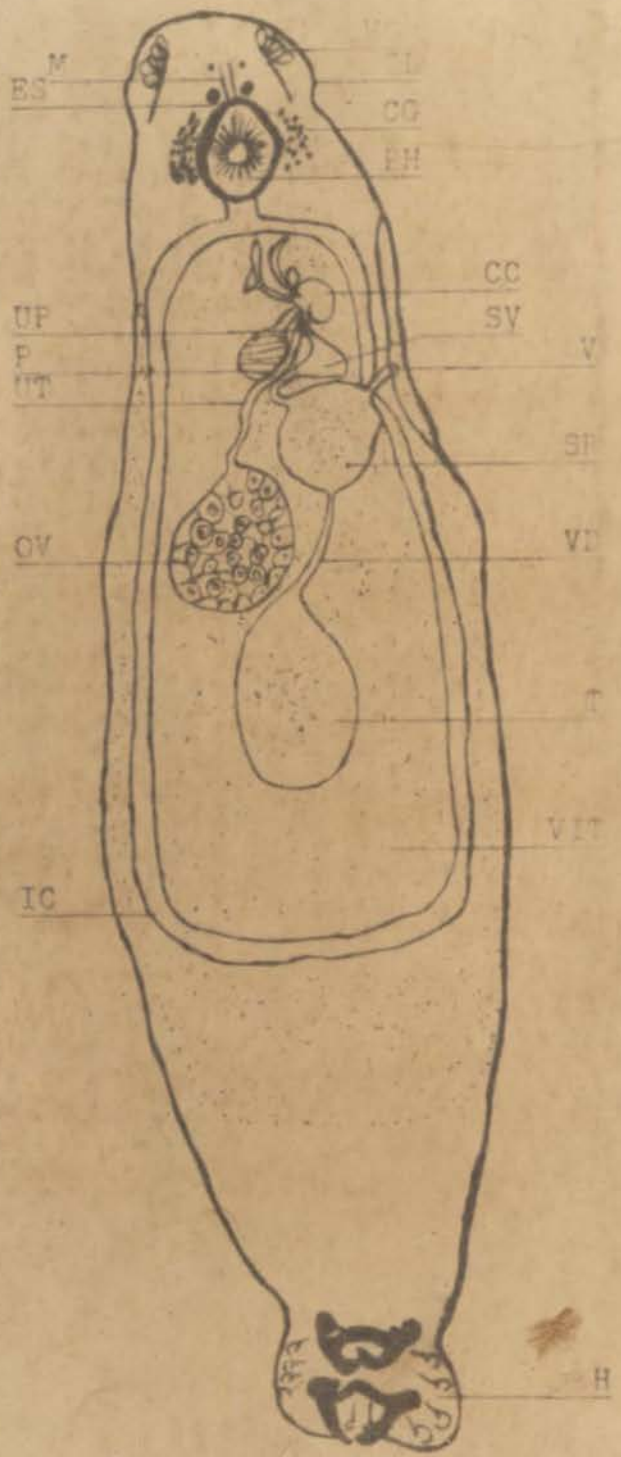


PLATE II

HEAD ORGANS

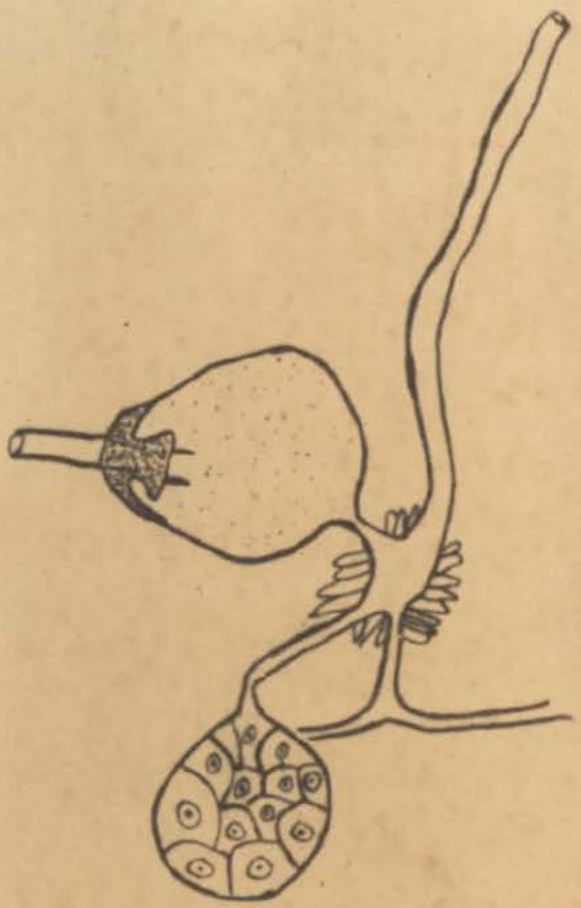
PLATE III

Fig. 1 - Dorsal anchors and bar

Fig. 2 - Ventral anchors and bar

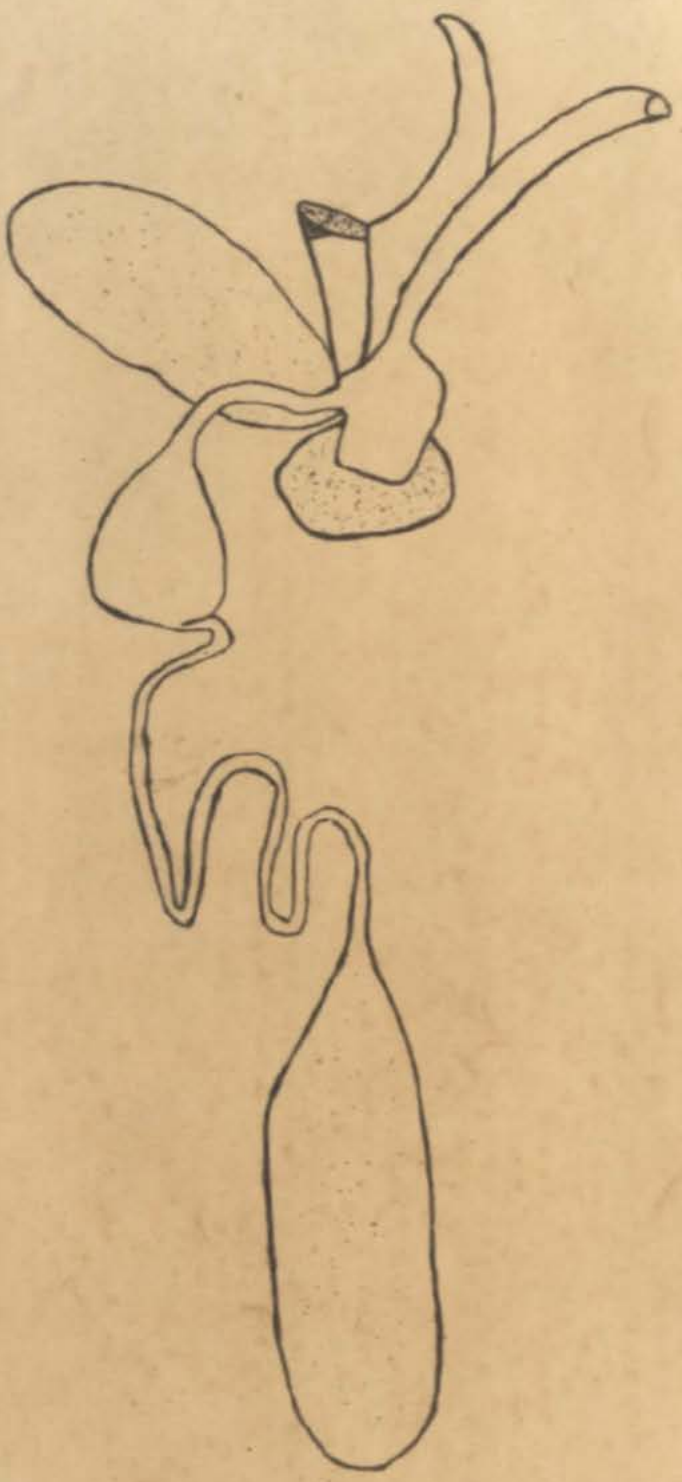
Fig. 3 - Hooks

PLATE IV



FEMALE REPRODUCTIVE ORGANS

PLATE V



MALE REPRODUCTIVE ORGANS

PLATE VI

COPULATORY COMPLEX

# PLATE VII

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

# BIBLIOGRAPHY

Brinkmann, A. 1942. On some new and little known Dactylocotyle species, with a discussion on the relations between the genus Dactylocotyle and the family Diclidophoridae. Goteborgs K. Vitensk-o. Vitterb.-Samb.Hanal., 6.,sB,1 (13).

Brinkmann, A. 1952. Soem Chilean monogenetic trematodes. Reports of the Lund University Chile expedition. Lunds University.

Bychowsky, B. E. 1957. Monogenetic Trematodes - Their Systematics and Phylogeny, American Institute of Biological Sciences.

Caballero, Y.C.E., Bravo-Hollis, M., and Grocott, R.C. 1955. Helmintos de la Republica de Panama. XIV. Trematodos monogeneos de peces marinoz del Oceano Pacifico del Notre, con descripcion de nuevos formas. An. Inst. Biol. 26, 117-147.

Cerfontaine, P. 1895. Le genre Dactylocotyle. Bull Acad. Belg., Cl. Sci., 29 510-527.

Chauhan, B. S. 1953. A brief review of our knowledge of monogenetic trematodes of the Indian region. Thapar Commem. Vol. 31-40.

Fuhrmann, O. 1928. Zweite Klasse des Cladus Platyhelminthese: Trematoda - In Kukenthal's Handbuch d. Zool. Berlin & Leipzig, 2, Teil 2. Lief. Bogen 1-8.

Hargis, W. J. 1959. Systematic note on the monogenetic trematodes. Proc. Helminth. Soc. Washington, 26, 14-31.

Jain, S. L. 1960. Monogenea of Indian freshwater fishes XII. New dactylogyrid trematodes from the gill filaments of Cyprinidae and Gobiidae hosts. Libr. Hom. al Dr. Caballero, 161-171.

Johnston, T.H. and Tiegs, O.W. 1922. New gyrodactylid trematodes from Australian fishes, together with a reclassification of the superfamily Gyrodactyloidea. Proc. Linn. N.S.W. 47, 83.

Llewellyn, J. 1941. A revision of the monogenean family Diclidophoridae Fuhrmann, 1928. Parasitology, 33, 431-460.

Meserve, F.G. 1937. Twenty new species of ectoparasitic trematodes (Abstract). Proc. South Dakota Acad. Sc. v. 17, 63.

Mizelle, J.D. 1938. Comparative studies on Trematodes (Gyrodactyloidea) from the Gills of North American Fresh-water Fishes, Urbana, Ill.

Monticelli, F.S. 1892. Di alcuni organi di tatto nei tristomidi. Contributo allo studio dei trematodi monogenetici - Partel 1. Boll. Soc. Nat..Napoli, 5, 99-134.

Noble, E.R., and Noble, G.A. 1961. Parasitology - The Biology of Animal Parasites. Lea & Febiger, Philadelphi, Penn. pp 171-178.

Odhner, T. 1912. Noch einmal die Homologien der weiblichen Genitalwege der monogenetishen Trematoden. Zool. Anz. 558-559.

Palombi, A. 1943. Notizie elmintologiche. III. Una specie del genere Merizocotyle Cerf. probabilmente nuova. Posizione sistematica del genere.

Poche, F. 1925. Das System der Platodaria. Arch. Naturgesch., Abt. A,91, 1-459.

Pratt, H.S. 1900. North American Invertebrates - No. 13, Trematodes. pt. 1. Heterocotylea. Amer. Nat. 34, 645-662.

Price, E. W. 1937. North American monogenetic trematodes. I. The superfamily Gyrodactyloidea. J. Wash. Acad. Sc. 27, 114-130.

Price, E.W. 1959. A proposed reclassification of the gastrocotyloid monogenea. J. Parasit. 45, Sect. 2, 59-63

Ramalingam, K. 1953. A new genus of trematode from the gills of Sphyraena acutipinnis Day. J. Zool. Soc. India, 5, 59-63.

Saint-Remy, G. 1890. Sur une espece nouvelle de polystomien du genre Onchocotyle Diew. Rev. Biol. Nord.

Sproston, N.G. 1946. A synopsis of the monogenetic trematodes. Tr. Zool. Soc London, 25, 185, 160.

Yamaguti, S. 1936. Idem. Pt. 14. Amphibian trematodes, Japan, J. Zool. 6 (4), 551-576.

Yamaguti, S. 1937. Idem. Pt. 19. Fourteen new ectoparasitic trematodes of fishes, 28 pp.

Yamaguti, S. 1963. Systema Helminthum. Monogenea and Aspidocotylea, Vol. IV.