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Title THE MORPHOLOGY AND SEXUAL MATURATION OF PHILOMETRA AMERICANA EKBAUM, 1933 (DRACUNCULOIDEA: PHILOMETRIDAE)

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The female Philometra americana Ekbaum, 1933 is a common subcutaneous parasite of the starry flounder, Platichthyes stellatus (Pallas) of Yaquina Bay, Lincoln County, Oregon. Maturation of this worm produces noticeable red cysts subcutaneously between the fin and tail rays and similarly on the operculum. The gravid female penetrates the epidermis and leaves the fish. In sea water, the female bursts to release thousands of larvae which are presumably then ingested by an intermediate host.

One thousand two hundred twenty-four starry flounders were examined and 280 fish were found to be infected. Three hundred forty-three female P. americana were collected and studied. No male was found. The percent of infection decreased as the size of the flounders increased. This suggests that the intermediate host for P. americana is primarily in the diet of the smaller fish.

Collection data indicates that there is no seasonal occurrence of the infection in this locality, but no conclusion was reached as to the time required for the parasite to become gravid

and leave the host fish.

The digestive and reproductive systems of *P. americana* change drastically as the animal matures. There is a multiplication of cells in the intestinal wall during maturation. There is an atrophy of the anus, vagina and vulva by the time the animal has become pink in color with worm-like embryos in the uterus. The growth of the embryos in the uterus causes the worm to become two to three times larger than the immature worm.

THE MORPHOLOGY AND SEXUAL MATURATION OF PHILOMETRA AMERICANA EKBAUM, 1933 (DRACUNCULOIDEA: PHILOMETRIDAE)

by

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TABLE OF CONTENTS

LIST OF TABLE AND FIGURES

THE MORPHOLOGY AND SEXUAL MATURATION OF PHILOMETRA AMERICANA EKBAUM, 1933 (DRACUNCULOIDEA: PHILOMETRIDAE)

INTRODUCTION

The most infamous of the nematodes are the animal parasites which have been examined since the time of the ancients -- no doubt due to the large size of many of them. The nematodes parasitize all animal and plant phyla. While many of the nematodes are microscopic in size, a great number of the parasitic species reach considerable length.

In Numbers of the fourth book of Moses, it is said:

> And the Lord sent fiery serpents among the people, and they bit the people; and much of the people of Israel died.

These "fiery serpents" probably were Dracunculus medinensis, a nematode which parasitizes humans if they drink water containing copepods infected with Dracunculus larvae (Kuchenmeister, 1857). The female worm eventually migrates to the subcutaneous tissues just under the skin of the body and appears somewhat like a moving, coiled varicose vein. The "fiery serpents", now known as guinea worms, are most noteworthy in that the females may reach lengths of a meter or more. Closely related to Dracunculus is the genus Philometra, which commonly inhabits the coelom and tissues of marine and fresh water fishes. York and Maplestone (1926) stated that Filaria globiceps was first reported by Rudolphi in 1819, and that Costa in 1846 proposed the name Philometra recticaudata to replace the name Filaria globiceps. York and Maplestone (1926) accepted the genus Philometra of Costa and considered the genus Ichthyonema of

Diesing (1861) as a synonym.

Today, there are twenty known species of Philometra. The description of Philometra is found in York and Maplestone (1926) and Baylis and Daubney (1930) with Philometra globiceps Rudolphi as the type species. The female is enormously larger than the male; body filiform; anterior and posterior extremities rounded; mouth with or without lips and head papilla; esophagus cylindrical; very short; viviparous; anus and vulva atrophied; the vulva in young worms is located at the junction of the middle and posterior thirds of the body; body occupied almost entirely by the uterus with embryos; there is a small ovary at each end of the body. The male has the posterior extremity rounded; cloaca terminal, bordered by two lips; spicules equal and needle-like; gubernaculum present.

Publications concerning the Philometridae are almost entirely descriptive. Very little is known of their morphological development or their life histories. Most investigators have restricted their work to species descriptions with some notes on the life histories.

Philometra americana n. sp. was described by Ekbaum (1933) from five species of Pacific coastal fishes. They were most common from Platichthyes stellatus, the starry flounder. This fish harbors the female parasite subcutaneously between the fin and tail rays and similarly in the operculum (Figure 16). The minute male was reported only twice in subcutaneous pockets in association with immature females. The male is minute, thread-like, 3.8 - 4.5 mm long and 0.04 mm wide. Morphologically, the posterior extremity of the male

was reported to be identical with Philometra globiceps Rudolphi (Ekbaum, 1933). The delicate female worm lies in pockets in the tissues and, when gravid, produces noticeable deep red-colored cysts under the skin of the fish (Figure 16).

Partial life cycle work by Ekbaum (1933) (June to August) suggested a seasonal cycle. In the fall, the gravid females would penetrate the integument and leave the fish. In sea water the worms burst the body wall and uterus in the upper one-third of the body. The active larvae are thus extruded. The larvae presumably are ingested by an intermediate host. This intermediate host is later ingested by a fish. This part of the life cycle is not understood, although various copepod species have been studied as intermediate hosts for related Philometra species (Thomas, 1929; Furuyama, 1934, and Schaperclaus, 1954).

Ekbaum (1933) described the female as brownish-red in color, up to 75 mm long by 2.3 mm wide. The average size is 30 - 50 mm by 1.2 to 1.6 mm. In the living state the parasite is translucent, but after being killed it becomes entirely opaque. The body is cylindrical, covered with a smooth cuticule, the anterior extremity is rounded, but tapering slightly, while the posterior extremity is rounded and often has a peculiar shape due to the contraction of the longitudinal muscles. The lateral lines are broad and conspicuous. The mouth is triangular in shape, surrounded by four weakly-developed papillae. The pharynx is muscular, leading to a straight esophagus 0.9 mm long by 0.1 mm wide. The intestine is

dark brown, 0.4 mm wide, compressed to one side and running along the body to the posterior extremity where it tapers abruptly and is attached to the body wall. The intestinal wall has a reticular structure. The vagina and vulva are lacking in the adult. The entire body cavity is chiefly occupied by the large uterus, crowded with embryos which leaves a free space of 0.3 - 0.4 mm at the anterior end and about 0.1 mm from the tail end. Anteriorly and posteriorly, the uterus terminates abruptly in small ovaries, each being 1.2 mm in length, 0.1 mm in diameter and rounded at the free end.

Characteristically, the philometrid females show an atrophy of the vagina (Figure 6) and anus (Figure 8) as well as the lower intestine, as they mature and the uterus swells with embryos (York and Maplestone, 1926).

This investigation is a description of the morphology of the female Philometra americana, including morphological changes which occur as the animal matures sexually. This work is supplemented with collection data and life history experimentation carried out by the author.

MATERIALS AND METHODS

The starry flounder, Platichthyes stellatus, were collected over a period of eighteen months (Table 1, Page 22) in Yaquina Bay, Lincoln County, Oregon. Collection was carried out using a small otter trawl towed by an outboard-powered boat during low tide.

All flounders were examined for parasites and a record was taken of the percent of fish infected, size of fish infected and the number of worms per fish (Table 1, Page 22).

Because the very young nematodes are nearly invisible under the skin of the flounder, many samples of fish were taken to the laboratory for microscopic examination of possible infection sites.

The living nematodes were removed from the subcutaneous pockets on the fish by making a careful incision and gently squeezing to remove the female worm. Extreme care was required to prevent the worm from bursting.

The live nematodes were placed in fish saline. By the use of blunt dissecting needles, the worms were guided into glass tubing of a diameter large enough to enclose them so they could be fixed in a straight position. The glass tubes were then stored in Carnoy's fixative (1 part formic acid, 3 parts chloroform and 6 parts absolute alcohol) for later study. Small sections of living fish tissue were also cut out and fixed in Carnoy's fixative. The entire worm cyst (Figure 16), as well as normal tissue blocks, were taken. The purpose of this was to examine the relationship of the worm and the host fish tissue and to note any tissue reaction of the fish caused by the

parasite (Figures 10 and 11).

Since the extent of contraction varied a great deal in living specimens, all measurements were taken in the straight fixed position. The worms were grouped into three classes based on size, color in life and degree of uterine embryo development. All measurements were taken in millimeters. The worms ranged from translucent to deep red. The maturity of the female was checked by comparison of larvae apparent in cross section of the uterus at the middle one-third of the body.

Immature females - translucent, 10 - 15 by 0.8 - 1.4, no uterine embryo with worm-like development (Figure 12).

Mature females - pink to red in color, 15 - 35 by 1.3 - 1.5, uterine embryos with worm-like development (Figure 14).

Gravid females - deep red in color, 30 - 53 by 1.6 - 1.8, uterus with fully-developed microfilaria larvae (Figure 4).

Approximately 1,224 Platichthyes stellatus were examined and 339 females of Philometra americana were collected. No males were found associated with the females, or elsewhere in the fish.

Representative specimens in each of the three size classes (Figure 3) were transferred in sequential changes from Carnoy's fixative to 100 percent chloroform. Paraffin chips (56 - 58°C) were added to the worms in 100 percent chloroform and placed in an imbedding oven at 60°C. As the chloroform evaporated, more paraffin chips were added. Three changes of paraffin were made and the imbedded specimens were blocked and serially sectioned at 10 microns.

Sections were stained with Harris hematoxylin and eosin Y and mounted in piccolyte.

Normal fish tissue blocks from the same area as worm cysts and intact cysts (Figure 16) in the fish tissues were prepared in the same manner as the above, except that high melting point paraffin (60°C) was used to give more rigidity for sectioning of the enclosed fin rays.

Mature larvae were obtained by placing the gravid Philometra in sea water where most commonly the larvae are extruded by rupture of the uterus and body wall in the anterior one-third of the body. Living larvae were fixed and stained by using the Honorico Cioraia technique (Yetwin, 1944) using Hammermill filler paper as a stain. Larvae were measured and illustrated (Figure 4).

Using living, preserved and serially sectioned specimens, a morphological description of the female Philometra americana was carried out, recording changes which occur in organ size, organ atrophy and uterine embryo development. Drawings were made with the Wild drawing arm and measurements taken with an ocular micrometer. Photomicrographs were made with a 35 mm Honeywell Pentax camera adapted to a Tiyoda microscope.

DATA

The fish collected ranged in length from 10 - 55 cm with a 22.9 percent rate of infection out of 1,224 fish examined. Ninety-one and eight tenths percent of the infected fish were 10 - 30 cm in length, as compared to 8.2 percent of the larger fish (31 - 55 cm), which were rarely infected. The degree of infection for all sizes of fish was 1.21 female worms per fish. The incidence of infection per fish was relatively constant, but a few fish of small size (15 cm $\pm$) harbored many worms (Figure 16), all at about the same stage of maturity. One noteworthy example was a 17 cm flounder with three mature and thirty gravid worms along the lateral fins, two gravid worms in the tail and one mature and four gravid worms in the opercular chamber.

Sampling over the eighteen month period yielded fish with all stages of maturation of the females. The percent of infection fluctuated somewhat from month to month. Maturity stages (immature, mature, gravid) of worms present in every infected fish was recorded on a monthly basis. This showed consistently low numbers of immature females and a relatively high percent of mature and gravid females in all collections. The highest rates of infection were found during March, 1964 and June, 1965 (50.0 percent and 29.2 percent, respectively). The lowest rates of infection were found during January and February, 1964 (8.8 percent and 10.0 percent, respectively). The infection rate during the other ten months recorded fluctuated from 15.1 percent to 25.9 percent (Table 1, Page 22).

The pink to red color of the worms becomes apparent by the time the females approach the mature stage (Figure 3). This color is apparently due to an iron-containing pigment substance in the fluid of the pseudocoelom (Dogiel *et al.*, 1961).

The digestive tract of *P. americana* changes in two respects as the animal matures sexually. First, there is a steady augmentation of intestinal wall cells from immaturity to maturity (Figures 5, 6 and 7). Second, there is an atrophy of the anus by the time the animal reaches maturity (Figure 8).

The mouth opens directly into a tripartite bulbous portion of the esophagus 0.6 long by 0.3 wide (Figures 2 and 15). Ekbaum (1933) reported four weakly-developed oral papillae, but did not illustrate them. Living and preserved specimens studied by the author lacked oral papillae.

The esophagus tapers from the muscular bulbous portion with cuticular lining (Figure 15) into a longer, constricted, glandular region 0.9 - 1.4 long by 0.185 wide with no cuticular lining (Figure 1). This glandular esophagus enters the brown, thin-walled intestine via the esophagointestinal valve, which is 0.095 in diameter. The intestine is extremely flaccid and its walls are commonly folded back on themselves or compressed, making measurements difficult. In immature worms the intestine is approximately 0.15 at its widest point, while mature and gravid worm intestines are about 0.9 and 1.9, respectively, at their widest points. The lumen of the intestine is nearly filled with fish red blood corpuscles.

Sections of fish tissue with the enclosed parasite

repeatedly showed the mouth of the parasite in close apposition with connective tissue capillary networks. There is no report of fish red blood corpuscles in the digestive tract of other Philometra. Lee (1966) stated that nematodes in body tissues of a host feed on body liquids, such as lymph and serum. It appears that P. americana draws nutrition from the blood supply of the flounder.

The intestine tapers at the posterior end of the worm and by the time the worm is mature, the anus is atrophied and non-functional. The intestine is anchored at the posterior end of the worm by strands of connective tissue (Figures 1 and 8). In very immature worms the anus appears joined with the intestine with a continuous constricted lumen. Wastes were never seen to be eliminated from worms placed in fish saline.

The reproductive system of P. americana is a single tubular organ with an ovary at each end of the tube (didelphic type) reflexed back over the large uterus (Figure 1). The only attachment of the reproductive tract with the body wall appears at the level of the posterior one-third of the body where the vagina and vulva are situated (Figure 1). These latter structures appear to close completely in the mature worm stage (Figure 6). Even in the very immature worm the external orifice does not appear distinct.

The ovary of the immature worm is distinguished from the uterus only by its lesser diameter (0.10 - 0.12) and by the apparent greater proliferation of eggs from the ovarian wall (Figure 7). As the nematode matures there is a decrease in size of the ovaries (Figure 1) and a constriction develops between the ovary and the

uterus. The constriction is 0.04 long by 0.055 wide with a lumen of only 0.005. In the gravid worm the ovary appears densely granular in cross section and is 0.10 - 0.12 wide by 1.2 - 1.4 long.

The eggs are 0.014 - 0.020 in diameter. The size of the eggs is greater than the constricted lumen leading from the ovary to the uterus. The size of the lumen from the ovary to the uterus, as well as the densely granular appearance of the ovary in mature and gravid worms, suggests that the ovary no longer contributes to egg production. In cross sections of the uterus at all levels throughout the period of maturation, one finds some proliferation of eggs from the uterine wall, particularly the distal ends. This suggests that the uterus is of the hologonic type and that the entire uterus is germinal and not just the ovaries.

The increase in size of the uterus as the worm matures is probably due to growth of larvae resulting in swelling from within. Mackin (1927) suggests that this occurs in Dracunculus globocephalus which parasitizes Chelydra serpentina and has a three-fold body size increase as it matures. The thickness of the uterine wall decreases from about 0.05 in an immature P. americana to 0.020 - 0.025 in mature worms and thus appears to be stretched as the larvae grow.

The body wall is covered by a thin, transparent cuticle 0.0038 over a subcuticle (epidermis) 0.0063. Beneath the subcuticle is the weakly-developed body wall musculature which consists of four strips of longitudinal fibers divided by the usual two lateral, a dorsal and a ventral longitudinal line.

The lateral lines at mid-body are broad and appear

transparent in the living worm. They are 0.20 - 0.25 broad in the immature worms and 0.85 - 0.90 broad in mature worms. Cross section shows the cellular nature in the subcuticle adjacent only to longitudinal muscle strips, but no evidence of lateral excretory canals was seen at any stage of maturity.

The dorsal and ventral longitudinal cords are 0.023 wide in the immature P. americana (Figures 7 and 13) and approximately 0.0125 wide in mature and gravid worms.

The complete development of the fertilized eggs has not been undertaken in this study. The degree of embryo development was used rather as a criterion for judging the maturity of the female P. americana. There is a great asyncrony in embryo development such that at any one level of the uterus unfertilized eggs are often found with blastulae in immature worms and gastrulae, along with worm-like embryos, in mature worms. There is never a syncronous development of embryos as in many nematodes. This asyncrony suggests a continued production of eggs in the reproductive tract with fertilization occurring as the eggs continue to be proliferated.

When the largest of the gravid P. americana was studied, an undeveloped egg was rarely found. Some debris (which appeared to be degenerating egg cells) was seen among the active larvae.

The larvae (Figure 4) show a nearly constant size of 0.370 by 0.019. The length from the mouth to the anterior end of the rhabditin mass[1] is 0.120. The pharynx (esophagus) is 0.047 long

[1] Rhabditin is apparently primarily a carbohydrate substance found in most nematode larvae. Cobb (1914) reported that this material does not disappear with starvation and suggests that it may be utilized in gonad development.

by 0.005 wide. The esophagus is 0.073 long by 0.0036 wide. The mass of rhabditin is 0.119 long and the anal opening is 0.076 from the tip of the tail.

The larvae could be kept alive and active up to fifteen days under refrigeration in fish saline at 10°C, but died in four to five days in sea water. It was noted that the larvae were much more active in sea water than in fish saline. This finding was first reported by Mackin (1927) for the larvae of Dracunculus globocephalus.

DISCUSSION

My first approach to the study of Philometra americana was an attempt to discover the life cycle of the parasite. Other investigators have had success only with fresh water studies of this nature. Philometra nodulosa is found under the skin of the lip of the common sucker, Catastomus commersonii, in Douglas Lake, Michigan (Thomas, 1929). He found that P. nodulosa emerges from a subcutaneous pocket similarly to P. americana and bursts in fresh water to release larvae. The larvae were fed to Cyclops brevispinosus and one to four of the larvae were found to move from the mid-gut into the body cavity. Reinfection of the fish with the infected Cyclops was not successful. Furuyama (1934) also reported that larvae of Philometra fujimotoi were ingested by a Cyclops sp. and migrated into the body cavity of the copepod. The life histories of Philometra (Filaria) sanguinea Rudolphi and P. abdominalis Nybelin were reported by Schäperclaus (1954). He reported that both Philometra species had Cyclops as an intermediate host and fresh water cyprinid fishes as definitive hosts. The time and place of copulation of philometrid worms is not yet known.

In a preliminary study, active larvae of P. americana were fed to flounders by capillary tube with no infection over a period of 45 days. Since infection did not appear to occur by direct means, and in light of life cycles in related species, it was felt that a copepod intermediate host was a possibility in the life cycle of P. americana. Plankton tows were taken in the locality

of fish collection. Several copepod species were segregated and placed in separate watch glasses with active larvae. These experiments met with little success. Only one species of harpacticoid copepod ingested the larvae, but unfortunately digested them as well. None of the copepods was ever seen with larvae within the body cavity as a result of direct penetration by the larvae as suggested by Strassen (1907).

Ekbaum (1933) postulated a seasonal life cycle for P. americana, with gravid females leaving their fish hosts in August and September. Since my feeding experiments were carried out in January, it was felt that perhaps the larvae were not physiologically ready for infecting an intermediate host. This led to a monthly collection of flounders and an examination of the maturity stages of P. americana. It was found that gravid female worms with fully-mature larvae were present all twelve months of the year, as well as immature and mature female nematodes (Table 1, Page 22). Vacated cysts were also present during all months of collection. It now appears that the life cycle of P. americana is not seasonal and occurs throughout the year in this locality.

It was interesting that the incidence of infection was extremely high in fish smaller than 30 cm (Table 1, Page 22) and often extremely high in fish of the 15 cm range (See Paragraph 1, Page 8).

Orcutt (1950) reported from stomach analysis of the starry flounder studied in Monterey Bay, California, changes in feeding habits as the fish grows. Fish less than 3.9 cm feed mostly on

copepods; those 4.0 cm to 17.9 cm feed mostly on amphipods and those over 18.0 cm feed primarily on clams and small crabs. These data suggest that the probable reason for low infection incidence of the large fish in this study was a result of a change in feeding habits so that the fish no longer eat the intermediate host of P. americana. The parasite infection is not seasonal in this locality and the most probable intermediate host would appear to be a microcrustean, perhaps a copepod. Further work with the life history of P. americana should be a search for an intermediate host which is present the year around in this locality and one that is a common food source of 4 - 15 cm starry flounders.

A study was begun with fish infected with immature and mature worms to determine the time required for nematode maturation. Due to a failure in the water supply at the end of two and one-half months, all fish died. The author can only conclude that longer than two and one-half months is required for the worm to become gravid and leave the host.

Ekbaum (1933) reported finding the male P. americana only twice, and each time associated with a very immature female in a subcutaneous cyst. In this study, no males were found in association with even the most immature female worms. One female with very early embryo development contained eggs which had very recently been fertilized. This was indicated by the presence of male and female pronuclei (Figure 12), yet careful microscopic examination yielded no male worm. Repeated attempts were made to recover a male worm, with no success. The author feels that either

the male nematode does not migrate into the subcutaneous tissues with the female worm or that it migrates with the female at a stage when both male and female are microscopic and unnoticed.

The male P. americana description by Ekbaum (1933) is not complete and even the most careful examination in this study has not verified her findings. Two attempts to correspond with Dr. Ekbaum to clarify this finding were unanswered.

Among the Philometridae it is not uncommon for the male worm to be unknown. Only the female of Philometra (Ichthyonema) cylindracea (Ward and Magnath, 1916) found in the body cavity of Perca flavescens has been reported. Ward and Magnath (1916) suggested that the minute size is probably the reason for the lack of reports of males. Dogiel et al., (1961) reported that in the Philometridae the male worm dies after copulation and the female worm alone migrates to the tissue or skin, there maturing and giving birth to live larvae. This would appear to be the most probable occurrence in the case of P. americana.

The significance of the parasitic infection of P. americana in the starry flounder would appear to be detrimental considering the occurrence of fish red blood corpuscles in the intestine of the parasite, as well as the reaction of the fish to the parasite (Figures 10 and 11). When the worm leaves the fish, an open sore is exposed which could easily be invaded by pathogens of various sorts. Either of the above results of infection by P. americana would tend to increase mortality in the starry flounder.

At this time, there is no evidence that philometrosis[2] of the starry flounder is of economic significance. It would seem that with the increasing fishery demands in this country, the flounder may become a more important food fish. If *P. americana* is pathogenic to the fish, then this parasite would be of significance since approximately 20 percent of young flounders are infected. For this reason, a complete understanding of the parasite is desired. Both its life history and its physiological effects on the starry flounder need yet to be investigated.

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[2] Philometrosis is a term coined by Wierzbicki in 1916 referring to infection caused by a philometrid worm.

SUMMARY

A description of the morphological changes which occur in the maturation of the female *Philometra americana* from the starry flounder of Yaquina Bay has been discussed. A description of the larval stage is also included.

One thousand two hundred twenty-four starry flounders were examined and 280 fish found to be infected. Three hundred forty-three female *P. americana* were collected from subcutaneous cysts between the fin rays and in the opercular chamber. No male worm was found.

Collection data indicated that the percent of infection decreases as the fish increase in size.

The starry flounder contains all stages of *P. americana* in this locality during all months of the year; the life cycle is not seasonal.

The digestive and reproductive tracts change drastically as the female worm matures. Intestinal wall cells multiply and there is an atrophy of the anus, vulva and vagina by the time there are worm-like embryos in the uterus. The worm enlarges two to three times as it matures, due primarily to growth of the embryos in the uterus.

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APPENDIX

Table 1. Collection data of *Philometra americana* from January, 1964 through June, 1965.

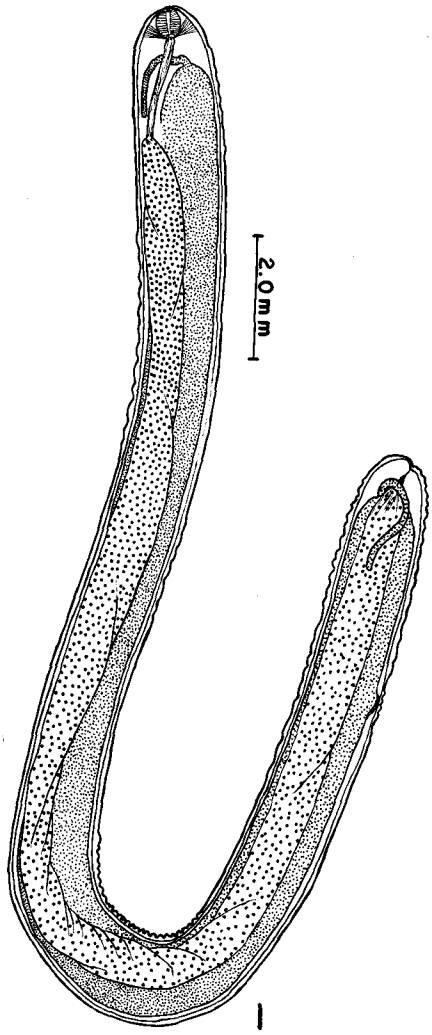
Collection Months	Fish Examined	% of Fish Infected	Fish (10-30 cm) Infected	Fish (31-55 cm) Infected	Worms Collected	Immature Worms Collected	Mature Worms Collected	Gravid Worms Collected
January	34	8.8	3	0	4	1	2	1
February	40	10.0	3	1	5	0	2	3
March	50	50.0	21	4	27	3	10	14
April	87	21.8	17	2	21	2	10	9
July	130	24.6	27	5	32	7	15	10
August	88	19.3	16	1	17	1	6	10
September	13	15.4	2	0	6	0	2	4
October	61	23.0	14	0	22	0	2	20
November	130	23.8	29	2	34	4	14	16
December	143	25.9	35	2	40	6	16	18
January	56	17.9	9	1	13	1	7	5
March	126	15.1	18	1	23	2	12	9
May	105	19.0	20	0	24	1	13	10
June	161	29.2	43	4	71	14	22	35
Totals	1224	22.9	257	23	339	42	133	164

Figure 1. Detailed drawing of entire, mature *Philometra americana*.

Figure 2. Detailed drawing of mouth and bulbous portion of esophagus of *Philometra americana*.

Figure 3. Scale drawing of gravid, mature and immature *Philometra americana* to show relative size.

Figure 4. Detailed drawing of mature *Philometra americana* larva.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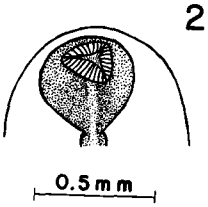
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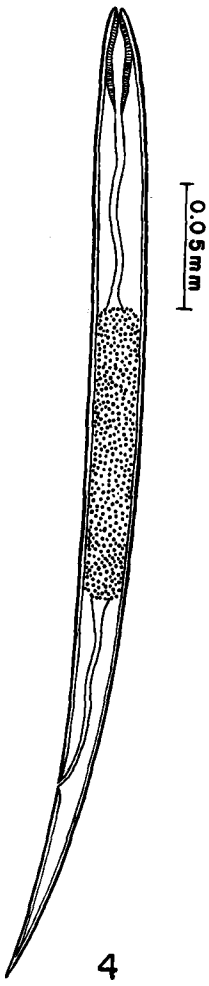
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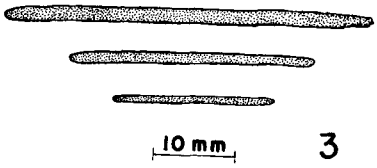

10 mm
3

Key to figures on Page 26

E - Esophagus
AV - Atrophied vulva
DL - Dorsal line
LL - Lateral line
A - Anus

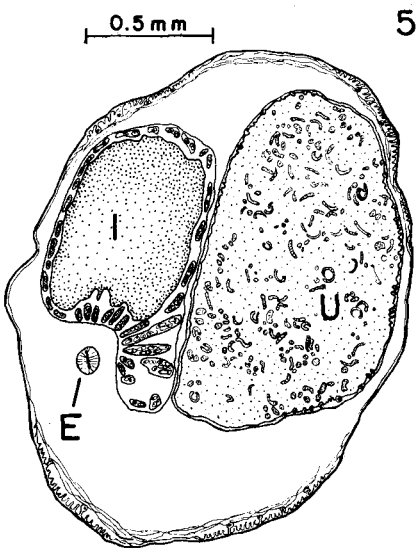
Figure 5. Detailed drawing of a cross section of mature *Philometra americana* at level of junction of esophagus and intestine showing syncitial nuclear arrangement of intestine.

Figure 6. Detailed drawing of a cross section of mature *Philometra americana* at junction of atrophied vagina and vulva.

Figure 7. Detailed drawing of a cross section of an immature *Philometra americana* at level of posterior ovary.

Figure 8. Detailed drawing of a cross section of a mature *Philometra americana* at level of atrophied anus.

Figure 9. Detailed drawing of a cross section of an immature *Philometra americana* at level of junction of esophagus and intestine.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5
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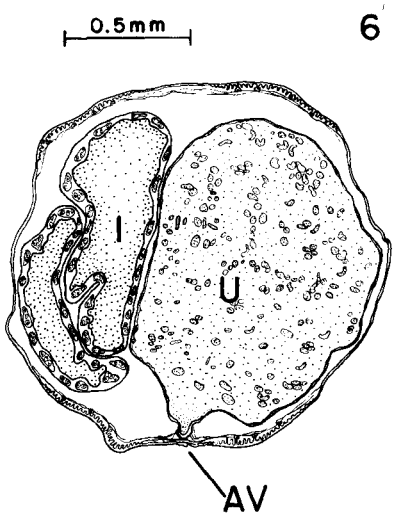
E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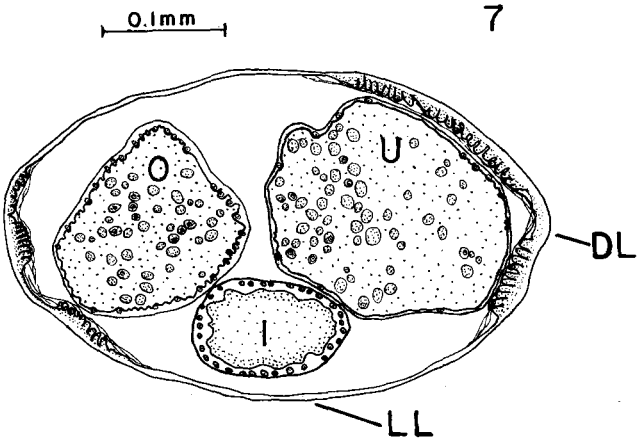
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DL
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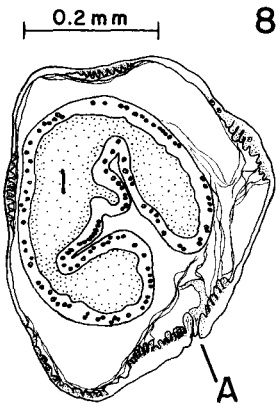
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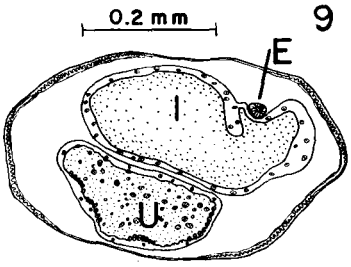
8
I
A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9
E
I
U

Key to figures on Page 28

E - Epidermis
P - Pronuclei
DL- Dorsal line

Figure 10. Cross section of normal integument of the starry flounder, Platichthyes stellatus. h.p. H and E staining.

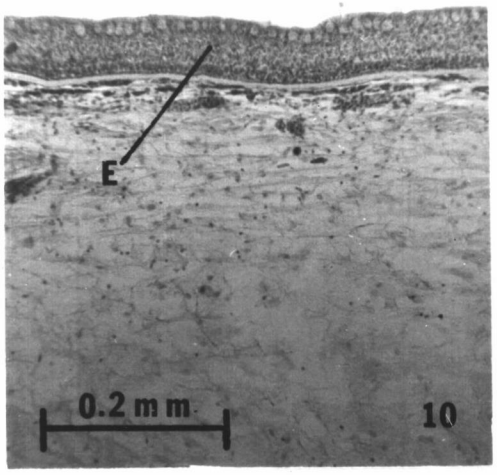
Figure 11. Cross section of integument of the starry flounder, Platichthyes stellatus taken from surface of gravid Philometra americana cyst showing inflammatory reaction. h.p. H and E staining.

Figure 12. Cross section of uterus of immature Philometra americana showing early embryo stages. h.p. H and E staining.

Figure 13. Cross section of the body wall of Philometra americana showing the dorsal line and adjacent longitudinal, muscular strips. h.p. H and E staining.

Figure 14. Cross section of uterus of mature Philometra americana showing worm-like embryos with stomodael invagination. h.p. H and E staining.

Figure 15. Cross section through bulbous muscular esophagus of Philometra americana showing cuticular lining. h.p. H and E staining.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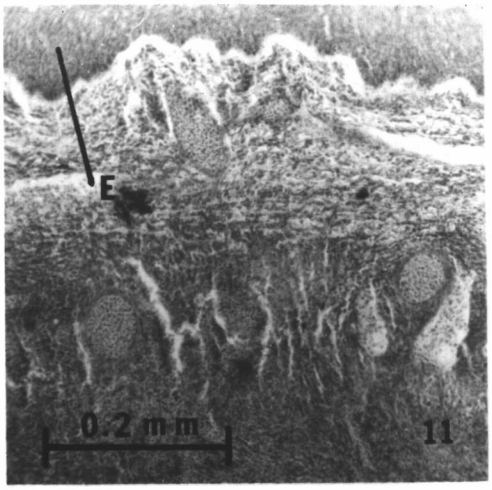
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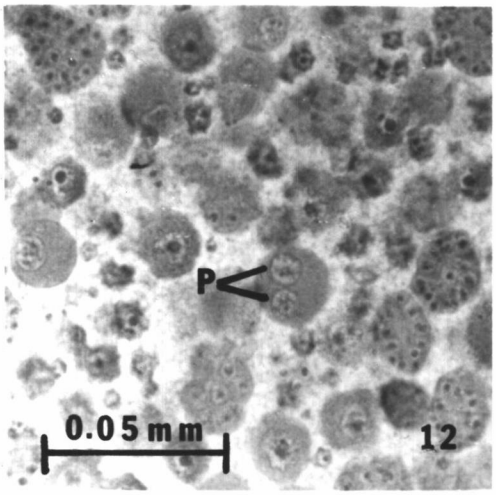
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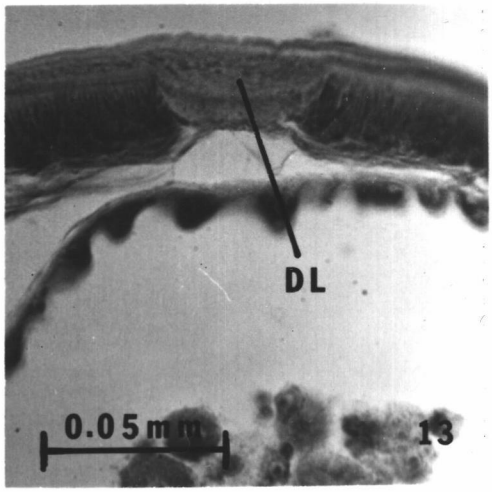
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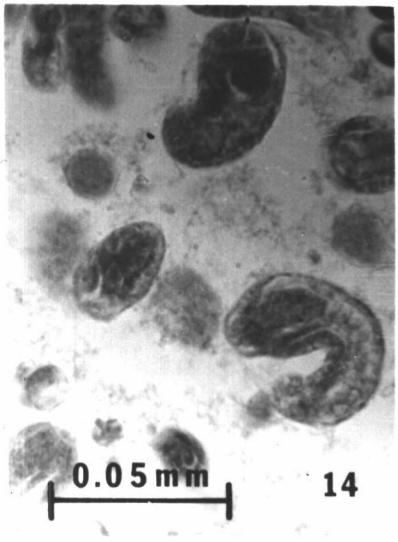
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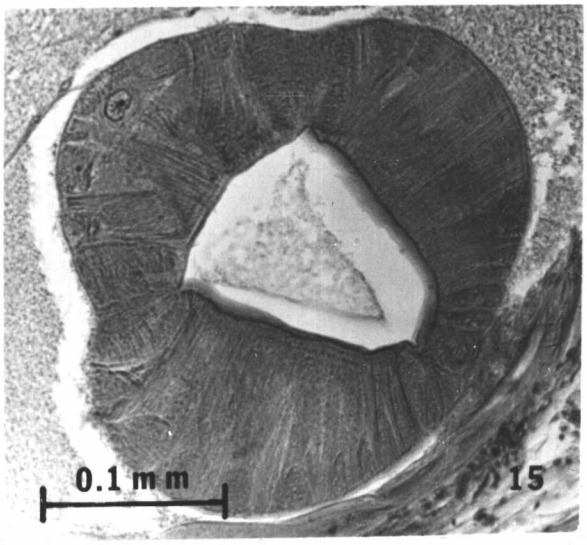
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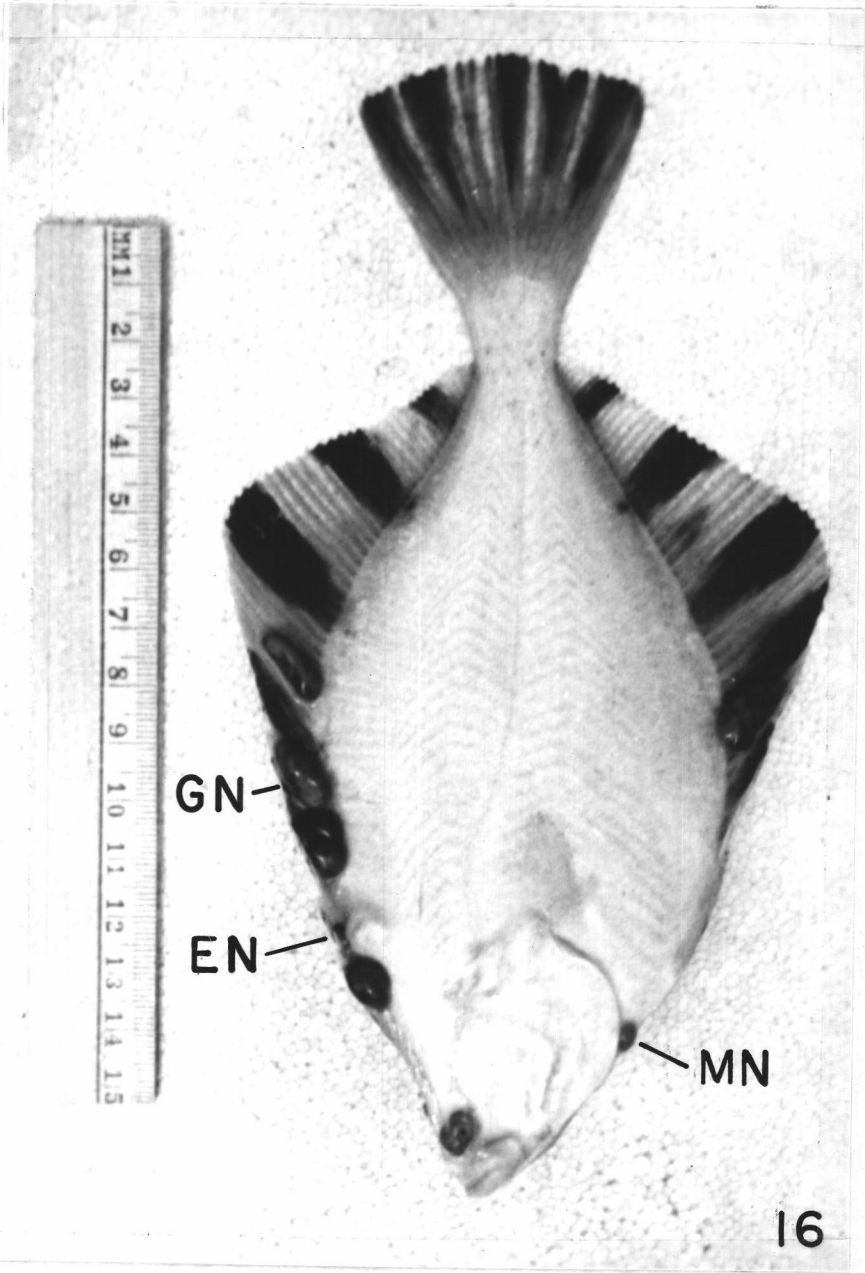
15

Key to figure on Page 30.

GN - Gravid nematode
EN - Emerging nematode
MN - Mature nematode

Figure 16. Photograph of the ventral side of a starry flounder, *Platichthyes stellatus*, showing bulbous cysts produced by *Philometra americana*.


GN
EN
MN
16