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## The Microscopic Anatomy of *Lepidodermella squamata* (Dujardin, 1841)

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THE MICROSCOPIC ANATOMY OF LEPIDODERMELLA SQUAMATA (DUJARDIN, 1841)

by

Raymond W. Ulbrich

A Thesis Submitted to the Faculty of the Graduate School  
of Loyola University of Chicago in Partial Fulfillment  
of the Requirements for the Degree of  
Master of Science

December

1977

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## LIFE

The author, Raymond W. Ulbrich, is the son of Edmund Peter Ulbrich and Willie Lucille (Gilmer) Ulbrich. He was born January 27, 1943, in Detroit, Michigan.

His elementary education was obtained in the parish school of Farmington, Michigan, and secondary education at the University of Detroit High School, Detroit, Michigan, where he graduated in 1960.

In September, 1960, he entered Loyola University of Chicago, and was awarded the Bachelor of Science degree with a major in biology in June, 1965.

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## INTRODUCTION

Lepidodermella squamata (Dujardin, 1841), a common fresh-water gastrotrich, is the subject of this research thesis. Considering the amount of information available in biology, our knowledge of the gastrotrichs is, at best, sketchy.

Lepidodermella was first described by Dujardin in 1841. Until 1889 when Zelinka published his major work on the gastrotrich anatomy, little was known about these animals. Remane in 1936 published what could be considered a summation of all the available information on the gastrotrichs.

The work between 1936 and the present time has been either of a taxonomic or ecological nature or a combination of both. It has only been recently that any work of a detailed nature has been done on these animals; the Riegers and their associates (1976) have made several studies of an ultrastructural nature on gastrotrichs.

Excepting the taxonomic, ecological, and the detailed ultrastructural studies, there exists very little information which deals with the internal anatomy of these animals.

It is the purpose of this thesis to describe the microscopic anatomy of Lepidodermella squamata through a study of serial transverse cross-sections. Only then can it be determined if this species fits the "generalized" structure described for all gastrotrichs.

## LITERATURE REVIEW

The gastrotrich, Lepidodermella squamata, was first described by Dujardin in 1841, but it was not until 1889 that any detailed study of the gastrotrichs was published. In 1889 Zelinka published a very complete study of both the external and internal anatomy of many gastrotrich species. His purpose was not to study the gastrotrichs exclusively, but rather to establish a phylogenetic relationship between them and the rotifers. Each of these groups is considered to be a class in the phylum Aschelminthes.

In the original material written by Zelinka, the genus Lepidoderma appears to be equivalent to the genus that we know as Lepidodermella. In 1933, Blake proposed that the genus name, Lepidodermella, be substituted for Lepidoderma.

In 1936, Remane published a work on gastrotrichs, a work now recognized as a complete summary of all of the available information up to that year. He had surveyed the microscopic fauna of many European regions looking for new species of gastrotrichs. In addition to the taxonomic studies, he incorporated much of Zelinka's work with his own relative to the microscopic anatomy and the end result was almost a "handbook" on the gastrotrichs.

Lepidodermella squamata has been the subject of four extensive works in the United States. The first of these, published by Packard

in 1936, studied the life history of this species and was also the first to suggest using malted milk powder as a culturing medium. Packard followed the progeny of a single animal for as many as six consecutive generations detailing the life span, the number of eggs produced by a single individual, and the time required for the development of an egg.

Brunson (1949) provided a more detailed study of the biology and some of the embryology of this species. For culturing, he used Packard's malted milk medium, but he also achieved limited success using depleted protozoan cultures. These depleted cultures would sustain a population of gastrotrichs but were not suitable for pure-line studies.

In collecting field samples for culturing in the laboratory, Brunson stressed the importance of conditioning the animals for laboratory culture. He accomplished this by adding sample debris collected along with the animals to the culture fluid, waiting from one to four weeks, removing half of the volume and replacing it with new medium.

His embryological studies, although not complete, provided more information than had existed previously. He studied the number of eggs produced by each individual and the age at which the eggs were produced. His attempts to study development within an individual egg

were not completely successful. He was unable to follow cleavages past the four-cell stage and his observations of later stages were incomplete.

Goldberg, in 1949, also studied the biology of this species and arrived at many of the same conclusions as Brunson. Goldberg dealt primarily with the biology and not with the embryology of this species. He achieved success utilizing rice grains in the culture fluid. He was unable, however, to observe the opisblastic or "winter" egg that had been described by Brunson.

The most definitive embryological study of Lepidodermella squamata was the subject of a doctoral dissertation by Sacks in 1955. His work details the stages in the embryology from the time of oviposition until the egg hatches as a new individual.

Sacks found, as did Brunson, that generally Lepidodermella produces a maximum of four eggs in its life span. The average life span of the gastrotrich was found to be 10.11 days; and the eggs are produced when the animal is 1.50, 2.18, 2.96, and 3.72 days old respectively. The average hatching time was found to be 1.12 days.

Sacks compared his data with Brunson's and felt that any deviations in the average figures could be due to differences in culturing technique or temperature.

His observations of the embryology were facilitated by a water immersion objective. Using this objective, he followed the development from the time a polar body is given off and cleavage begins, until the new gastrotrich makes its way out of the shell.

When the new animal emerges from the shell, it is quite transparent and smaller than the adult. The most distinguishing feature is the presence of refractile granules in the gut. Although the exact nature of these granules is unknown, Zelinka (1889) considered them to be reserve food. Sacks feels that since they are released into the lumen of the gut and eliminated from the animal within two hours of hatching, the current view that they are excretory in nature seems more likely.

A recent study of Hummon (1976) deals with the effects of DDT on the longevity of the animals and their reproductive rate. He found a positive correlation between the amount of DDT and a lessening of the life span, a reduction in the number of eggs produced, and an increase in the hatching time.

Ultrastructural studies of gastrotrichs were made by Rieger and his associates (1974, 1976) . Utilizing a transmission electron microscope or TEM, they have arrived at some important conclusions not only on the possible evolutionary position of the gastrotrichs but also on the fine structure of a new species of gastrotrich they have discovered.

The evolutionary studies are directed at establishing the possible ancestor of the gastrotrichs and determining their position in the Metazoa. These studies were based on a survey of the monociliated cells found in the ventral epidermis of 24 different species.

In his text, Remane (1936) described the gastrotrich epidermis as syncytial although in a few of his drawings he clearly illustrates the ventral epidermis as a monociliated epithelium. The Rieger group feel that their studies, although not absolutely conclusive, seem to indicate that the epidermis is monociliated rather than syncytial. There are forms with a multiciliated epidermis but the point in question is whether the epidermis is syncytial and not the number of cilia per cell. Because he could not clearly establish cell borders with his techniques in 1936, apparently Remane was unaware of the agreement between the number of nuclei and the number of cilia in the monociliated forms.

The multiciliated cells are felt to have evolved from the monociliated cells which are considered to be more primitive. From an evolutionary standpoint, if we consider certain characteristics of these monociliated cells and unflagellated cells of other forms, e.g., those with a diplosomal basal body, a striated rootlet fiber, a ciliary pit, and a well developed collar around the base of the cilium, then a number of observations can be made. The Rieger group has shown these four features to be evident in the larval or adult

forms of more primitive Metazoa (Porifera, Cnideria), in the Aschelminthes (Gnathostomulida and Gastrotricha), and more advanced groups (Lophophorates, Hemichordata, Echinodermata).

The fine-structure study of the gastrotrich deals with a new form that they first observed in 1970 and again in 1973. Chordodasys antenatus is a macrodasyoid and the studies provide an in-depth look at this new species on the ultrastructural level and also show positive correlations with structural details already known for gastrotrichs in general.

Remane (1936) made two points regarding the anatomy of gastrotrichs which were confirmed by the TEM studies: That the gastrotrichs lack a distinct hind-gut and that in the adult forms there is an absence of a large intercellular space or body cavity. This last point, the absence of a body cavity or pseudocoel, seems to challenge the position of the Class Gastrotricha within the Phylum Aschelminthes. All of the Aschelminthes are pseudocoelomates, but this new information would seem to indicate that the gastrotrichs are acoelomates. It is true that Remane and others have seen three distinct chambers within the gastrotrich body: One centrally located which contains the gut and mature eggs, and two lateral cavities enclosing the testes (in macrodasyoid forms). Just what these chambers are remains of a speculative nature. The TEM studies could not find any evidence of an endothelial lining of the

cavities but neither could they conclusively prove that they were not coelomic in origin.

How this problem of classification will be resolved remains to be seen, but the Rieger group emphasizes the need for more light microscope studies to be done on the gastrotrichs to help fill in the gaps that remain regarding structural detail.

## LIFE HISTORY

The gastrotrichs belong to a class within the pseudocoelomate phylum Aschelminthes. They were at one time considered to be rotifers but they lack both the corona and the mastax which are rotifer characteristics.

The Class Gastrotricha is subdivided into two orders: Order Macrodasyoidea and Order Chaetonotida.

The macrodasyooids are, except for a few species, marine organisms that are hermaphroditic and reproduce by cross fertilization.

The chaetonotids are fresh-water forms although a few marine species are known. Only females occur in this order and the eggs produced develop by parthenogenesis.

Lepidodermella squamata (Dujardin, 1841), a chaetonotid, is a common fresh-water form. The following material pertaining to the gastrotrichs is a composite of all of the information available. Wherever possible, known material pertaining specifically to the genus Lepidodermella has been incorporated into this discussion. Some of the structural details will be verified by the material contained in some of the subsequent chapters.

Externals. The body of the gastrotrich is elongated and in Lepidodermella it is covered by spineless scales. The head is lobed and is

covered by cilia. In addition there are elongated ciliary tufts found on the head. These tufts are thought to have a sensory function. The head is separated from the trunk by a narrowed neck region. The trunk extends posteriorly from the neck and terminates in a fork-like structure which contains adhesive glands. On the ventral surface of the body are two parallel ciliary tracts which run the length of the body and are responsible for the gliding movement of the animal.

Integument and Muscles. The integument consists of a syncytial epidermis which secretes the overlying cuticle. Circular muscle may be found beneath the epidermis. Ventro-lateral bands of muscle extend from the mouth-pharyngeal region to the adhesive glands. A variable number of dorsal longitudinal muscles originate in the anterior portion of the animal and run the length of the body.

Digestive System. The mouth is terminal of slightly ventral and opens into a muscular pharynx which has a tri-radiate lumen similar to the nematode pharynx. In the Order Macrodasyoidea one angle is mid-dorsal and the remaining two ventro-lateral. In the Chaetonotida the opposite situation is observed, one angle is mid-ventral and the remaining two are dorso-lateral.

The pharynx leads into a midgut region which in turn leads into an intestine. The short intestine terminates in an anus which is dorsal, just anterior to the caudal forks.

Excretory System. The excretory system consists of a pair of protonephridia. They occur laterally at about the middle of the gut and each consists of an extended flame bulb from which a highly coiled tubule extends. These tubules open ventrally in the middle of the body.

Nervous System. A relatively large bilobed brain is situated on the antero-dorsal part of the pharynx. Extending posteriorly are a pair of lateral nerve cords. Ganglion cells incorporated into the brain innervate the cephalic ciliary tufts.

Reproductive System. Parthenogenic females are the only known forms in the Chaetonotida. Eggs are produced by the paired ovaries which lie lateral to the intestine. Mature eggs move to the mid-trunk region into a body cavity thought to be the pseudocoel. The mature eggs emerge from a pore on the ventral surface. The eggs are deposited on vegetation in the natural state and on the bottom of culture dishes in the laboratory.

Two types of eggs have been described: Opisblastic and tachyblastic. Visually the only difference between the eggs is that the opisblastic egg has a thick, sculptured shell.

Development proceeds almost immediately in the tachyblastic egg while the opisblastic or "winter egg" is considered to be a dormant stage capable of surviving desiccation and temperature extremes.

Development is direct and the eggs hatch as miniature adults.

## METHODS AND MATERIALS

The contents of this chapter will be discussed in three phases: The culturing of Lepidodermella, the making of the cross section slides, and the photography of the sections.

Culturing. Because of the small size and transparency of Lepidodermella, it was assumed that there would be a loss of animals in the various steps in the slide-making. To compensate for this, bulk cultures were ordered from Carolina Biological Supply Company. These were pure-line cultures and were ordered in units sufficient for a class of 100 students to ensure sufficient numbers to work with.

Although some of the cultures were maintained using malted milk powder (Packard, 1936), greater success was achieved using wheat grains as suggested by Powell (1972). In this method, four wheat grains were added to 200 ml. of distilled water and the solution was allowed to boil for approximately 10 minutes. The flask was loosely covered and allowed to stand at room temperature for at least 24 hours. After this time, the medium was poured into the culture dishes and the animals added.

Slide Preparation. The gastrotrichs were narcotized according to the method suggested by Zinn and Kneeland (1966). Using this technique, a few drops of aqueous benzamine hydrochloride (1%) were added to the culture. The number of drops varied with the volume of liquid in the culture, but 4-6 drops were sufficient for a container with a volume of

approximately 50 ml. Within a few minutes the narcotized specimens floated to the top of the container, they were collected with a micro-pipette, and transferred to a centrifuge tube. After the specimens were collected, the tube was centrifuged at the lowest speed for 5 minutes. Half of the liquid was pipetted off and replaced with Bouin's fixative. Again the tube was centrifuged at the lowest speed for 5-10 minutes. Usually by this time, the animals will have formed a button at the bottom of the tube. The supernatant was carefully drawn off and the button containing the animals was removed using a medicine dropper. They were placed on a piece of filter paper backed with a paper towel. The paper towel absorbs the excess liquid leaving the animals on the filter paper. The filter paper was then carefully folded and placed into a covered screen capsule.

The capsule was placed into an Auto-Technicon and set to perform the following steps:

1. the capsule was fixed in a 10% buffered formaldehyde solution for 6-8 hours,
2. placed in a single change of 80% alcohol for 1 hour,
3. transferred to three changes of 95% alcohol at 1 hour each,
4. moved to absolute alcohol, three changes, each lasting 1 hour,
5. two changes of toluene, 1 hour each,
6. paraffin, two changes, 1 hour each.

Following this last step (#6), the capsule was placed in a vacuum oven for 1 hour at 25 lbs. of pressure to facilitate the penetration of the paraffin into the body of the animals.

When the capsule was opened, the filter paper was carefully unwrapped and the animals transferred to a metal paraffin container partially filled with solid paraffin. The embedding was done on a Tissue-Tek II in which liquid paraffin was allowed to fill the metal block and then quickly cooled to solidify the paraffin.

The sections were cut on a rotary microtome at 5 microns thickness. The ribbon was floated in a water bath before it was placed on a slide coated with Mayer's albumin solution. After the sections were cut and mounted, the slides were placed in a drying oven for a minimum of 1 hour at 58°-60°C.

The slides were stained using hematoxylin-eosin stain. The steps are a modification of Gray's technique (1954) and are as follows:

1. The slides in a slide rack were placed in a parasol solution for 5 minutes. Parasol is preferred to xylene since it is less oily and dries quicker.
2. The rack was then dipped 10-15 times in the following solutions: Parasol, half parasol half absolute alcohol, 2 changes of absolute alcohol, 1 change of 95% alcohol, 1 change of 80% alcohol, 1 change of 70% alcohol, and 1 change of distilled water.
3. The slides were rinsed well in distilled water.
4. The rack was placed in Harris hematoxylin solution for 8 minutes.

5. Rinse well in tap water.
6. Dip the rack once quickly in acid-alcohol to remove any excess hematoxylin.
7. Rinse well in two changes of tap water.
8. Dip the rack 4-5 times in a saturated lithium carbonate solution to give a blue tint to the hematoxylin.
9. Rinse well in distilled water.
10. Dip in 70% alcohol for 5 seconds.
11. Stain in eosin solution for 2½-3 minutes.
12. Dip slides 6-8 times in 2 changes of 95% alcohol.
13. Dip slides 10-15 times in 3 changes of absolute alcohol.
14. Dip 10-15 times in 3 changes of parasol.
15. Remove the rack and coverslip the slides with Permount.

Photography. Pictures of the sections were taken under high power and an oil-immersion objective utilizing 2 photographic setups. The first set of photographs were taken with a Nikon Microflex, Model AFMM. The film used was Ilford, Pan F 135 with an ASA 50. The second set of photographs were taken using a Zeiss microscope with a built-in 35 mm. camera. The film for these photographs was Kodak Panatomic X, FX 135 with an ASA 32.

In the darkroom the film was removed from the cartridge, placed in a processing container and processed using the following steps:

1. Into Kodak UHOL developer for 3¼ minutes.
2. Kodak Rapid-Fix fixative for 5 minutes.

3. Into an Orbit Bath solution for 3 minutes to neutralize the fixative.
4. Washed in tap water for 5 minutes.
5. Allowed to air dry.

Developing and printing were done commercially.

## DISCUSSION

The discussion portion of this thesis will closely parallel the areas covered in the section dealing with the life history of Lepidodermella squamata (Dujardin, 1841).

Certain of the structures described for the "typical" gastrotrich have been observed; other structures which have not been demonstrated by this investigation suggest areas for further study.

Two subjects, the oviduct and the yolk gland, will be introduced into this discussion. The oviduct has been described (Zelinka, 1889; Remane, 1936; Hyman, 1951) but it has not been seen in L. squamata. The yolk gland and its relationship to the X-organ will be discussed.

Externals. The whole mount photograph of L. squamata (Fig. 1) clearly shows an elongated body divisible into head, neck, trunk, and caudal fork regions. Overlapping spineless scales (Fig. 1) cover the surface of the body but they are more evident in the cross sections (Figs. 4, 7, 10, 19, and 23).

Lobes on the head are visible (Figs. 2-5) and ciliary tufts can be seen emerging from them. The narrow neck region is evidenced by the decrease in the diameter of the animal (Figs. 6-9) which expands again as the trunk is reached (Figs. 10-33).

Because of the position of the specimen while it was sectioned, several longitudinal cuts of the caudal fork were obtained (Figs. 25,

27, 29, and 30). These sections show that the adhesive gland contained in the fork consists of a band of parallel columnar cells covered by a nucleated epidermis (Fig. 29). Some of these columnar cells appear to be nucleated and scattered among them are clear, ovoid mucous cells. The external opening of the gland is bulb-shaped and is surrounded by a ring of darkened cells (Fig. 30).

The ciliary tracts on the ventral surface of the animal were not visible in this study.

Integument and Muscles. The spineless scales secreted by the epidermis are visible (Figs. 4, 7, 10, 19, and 23). It is impossible, however, to determine whether the epidermis is syncytial as described by Remane (1936). Nuclei are evident in the epidermis (Fig. 29) but the cell membranes are impossible to discern. The circular muscle which underlies the epidermis was not seen in this study. The dorsal-longitudinal bands of muscle, usually 3 on each side in L. squamata, are clearly seen (Figs. 6, 8, 13, 20, and 23). Ventral-longitudinal muscles are visible in a few sections (Figs. 16, 26, and 32), and seem to consist of 2 bands on each side of the animal.

Digestive System. The mouth is not visible because of the position of the specimen. The mouth leads into a pharynx (Figs. 2-7) found in the anterior third of the body, a mid-gut region (Figs. 8-14) found in the middle third, and an intestine (Figs. 15-33) which begins about the

middle of the trunk and runs posteriorly to the anus. This investigation shows the intestine to be longer than reported by Remane (1936).

The triangular pharynx characteristic of the chaetonotids is very apparent (Figs. 2-7) and it shows the typical pattern of one angle in the mid-ventral position with the other two angles dorso-lateral. The pharynx gradually loses its triangular shape as the mid-gut region is approached (Figs. 6-9). The mid-gut is characterized by lateral expansions of the lumen seen in the sections (Figs. 12-14). These lateral expansions disappear as the intestine is reached; the lumen of the intestine becomes oval or rounded (Figs. 15-33) and continues to the posterior end of the animal.

The wall of the gut is made up of low columnar cells (Figs. 7, 9, 12, and 23) and there is no difference in the shape of the cells in any of the gut regions. A clear, cuticular lining of the lumen has been described (Remane, 1936) and is visible in several sections (Figs. 10, 11, and 21).

The anus is not conclusively demonstrated although an external opening appears in the last few sections (Figs. 31-33). This opening leads dorsally and connects to a narrow transverse tube which then runs medially below the intestine (Fig. 32-33). No visible connection between this tube and the intestine can be seen. If further investigation shows this structure, it seems that the anus of L. squamata

might be ventral and not dorsal as described for some of the gastrotrichs (Zelinka, 1889). The tube is definitely separated from the body cavity or pseudocoel by a narrow band of cells visible in the sections (Figs. 31-33) so it does not appear to be an artifact. It will be necessary to see if this arrangement is observed in future studies before a definitive statement can be made.

Excretory System. The protonephridia with its extended flame cell is not seen. Masses of coiled excretory tubules are visible in many of the sections (Figs. 14, 26, and 31) and appear to be extensive throughout the posterior half of the animal.

Nervous System. Anterior sections of the head of L. squamata show a bilobed brain dorsal to the pharynx (Figs. 2-5). These lobes are connected by a transverse commissure (Figs. 3-5) and they appear to be heavily nucleated with ganglion cells (Figs. 4-5). Extending from the lobes of the brain are ciliary tufts (Figs. 2, 3, and 5). The nerve cords extending posteriorly from the brain are not visible.

Reproductive System. According to Hyman (1951), the paired ovaries are posterior in the gastrotrich body and contain a fixed number of egg cells although it has been demonstrated that usually no more than 4 actually mature (Brunson, 1949 and Sacks, 1955). As the eggs mature, they move anteriorly and can be found on both sides of the gut. As they become more mature, they move to a position ventral to the gut

and move posteriorly in preparation for oviposition. They were thought to lie free in the pseudocoel and not to be contained within a structure (Zelinka, 1889 and Remane, 1936). Hyman (1951) mentions the existence of an oviduct but states that it has not been clearly demonstrated in the majority of chaetonotids.

The present study shows eggs lateral to the gut at about the beginning of the intestine (Figs. 11-13, 15). From the thickness of the sections, it is assumed that the eggs are from 5-15 microns in length. In two of the sections (Figs. 15 and 24) a nucleus and nucleolus can be seen in an egg.

As the eggs mature, they begin to shift toward the midline (Fig. 15) to a position ventral to the gut. The next series of photographs (Figs. 16-26) shows a hollow, tubular structure, the oviduct, appearing below the gut (Fig. 16). As the oviduct is followed posteriorly, an egg appears in the lumen (Fig. 19). The egg increases in size as we move posteriorly, and it is possible to differentiate the wall of the tube from the egg in a few of the sections (Figs. 20, 22-24). As sections are traced farther posteriorly a dark staining mass appears on both sides of the egg (Figs. 25-26) and eventually this mass occupies the ventral position below the gut (Fig. 28).

Brunson (1949) and Sacks (1955) have established that the mature egg at the time of oviposition is between 50-60 microns in length or

roughly one-third of the total body length. In this study, the sections of the eggs show an egg maturing lateral to the gut but its size is 10-15 microns in length.

If the eggs were to reach their full size in the lateral position, they would compress the intestine. Apparently they reach their full size in the mid-line position below the gut where only one egg is seen. One egg maturing in this position would be free to grow without interfering with any of the other organs. Its growth would only compress the body cavity.

The dark mass (Fig. 28) that appears in the posterior end of the body is in the position Remane (1936) describes for the X-organ or vestigial testis. It should be remembered that chaetonotid gastrotrichs reproduce by parthenogenesis. Therefore this vestigial testis occupies a sizable amount of body space without having any known function.

It is suggested by this study that, instead of a vestigial testis, this dark mass is a yolk gland. It is in the proper position for the egg to mature and increase in size; it is located just anterior to the opening through which the mature egg emerges at oviposition. Hyman (1951) has shown the yolk gland in other species but not in L. squamata.

This area in the mid-line would allow the rapid and extensive growth of the egg. Since the length of the egg increases by approximately 4 times, the growth could be accomplished in this area without

affecting the other organs. This growth may explain why the intestine is shifted laterally in some sections (Figs. 25-26, and 28) and why the pseudocoel is compressed and reduced in size (Figs. 31-33).

Gastrotrichs are very compact, efficient organisms and a vestigial organ of this size does not seem plausible. If the animal were going to have a non-functional organ of this size it would seem more logical to have a functioning organ. The forces of natural selection should have operated against forms carrying a vestigial structure of this size.

The nature of the X-organ or yolk gland is a matter of conjecture at this time and further investigations using differential staining may aid in resolving the exact nature of this mass.

## SUMMARY

1. Lepidodermella squamata (Dujardin, 1841) a chaetonotid gastrotrich, is the subject of this research thesis.
2. L. squamata was successfully cultured using wheat grains in a technique suggested by Powell (1972).
3. Dorsal-longitudinal muscles, usually three bands per side, were demonstrated. Ventral-longitudinal muscles which occurred in double bands were also seen.
4. A structure, the oviduct, was shown which had not previously been seen in L. squamata.
5. A suggestion that the vestigial X-organ might function as a yolk gland was made.

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**APPENDIX A**

LEGEND FOR APPENDIX

B	Brain
CF	Caudal Fork
CT	Ciliary Tuft
CC	Columnar Cells
C	Cuticle
DLM	Dorsal Longitudinal Muscles
E	Egg
EP	Epidermis
ES	Epidermal Scales
ET	Excretory Tubules
G	Ganglion Cells
H	Head
I	Intestine
MG	Mid-gut
MC	Mucous Cell
N	Neck
NU	Nucleus
OP	Opening
O	Oviduct
P	Pharynx
PC	Pseudocoel
TC	Transverse Commisure
T	Trunk
VLM	Ventral Longitudinal Muscle
YG	Yolk Gland

PLATE I

- Figure 1. Whole mount of Lepidodermella squamata  
(Dujardin, 1841) x 1000
- Figure 2. Cross section through head x 1000
- Figure 3. Cross section through head x 1000
- Figure 4. Cross section through head x 1200

PLATE I

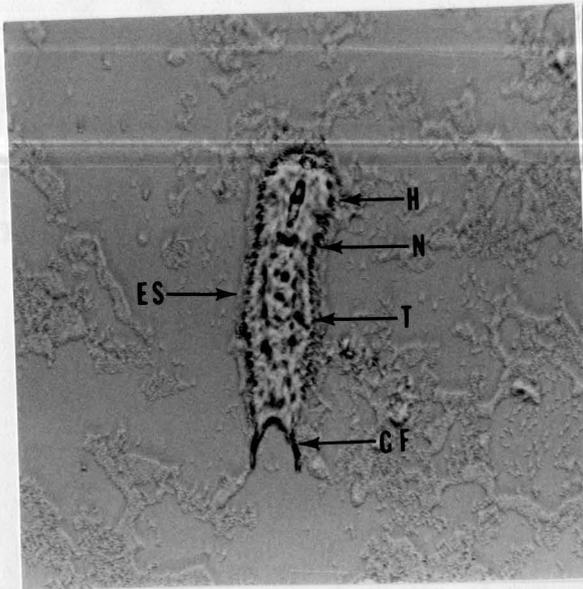


Figure 1

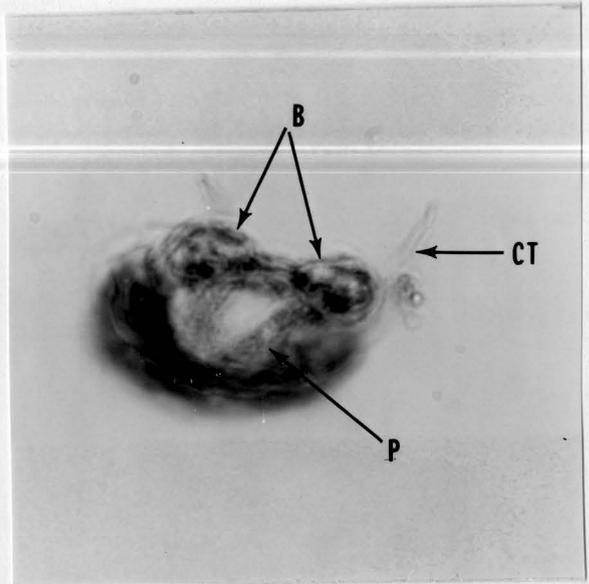


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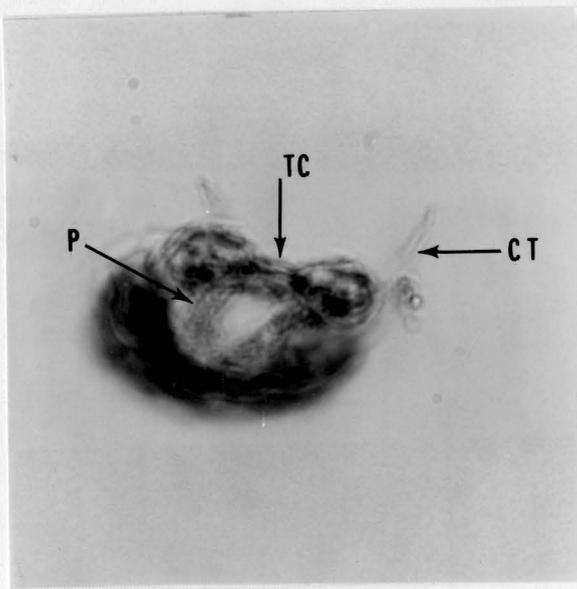


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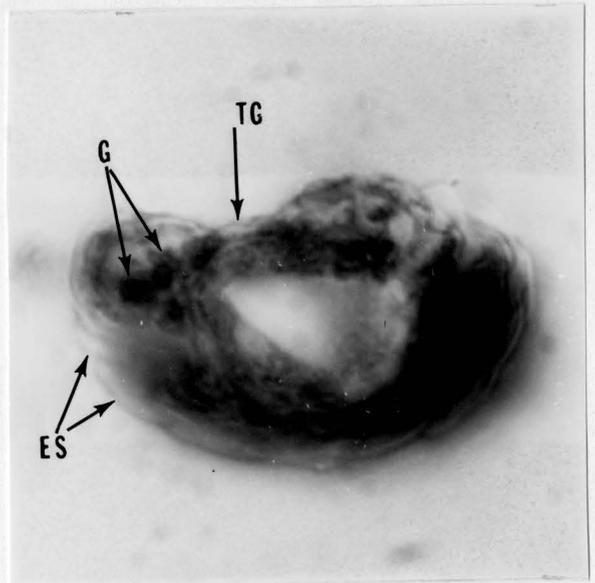


Figure 4

PLATE II

- Figure 5. Cross section through head x 1200
- Figure 6. Cross section through pharynx x 1000
- Figure 7. Cross section through pharynx x 1000
- Figure 8. Cross section through junction of pharynx and  
mid-gut x 1000

PLATE II

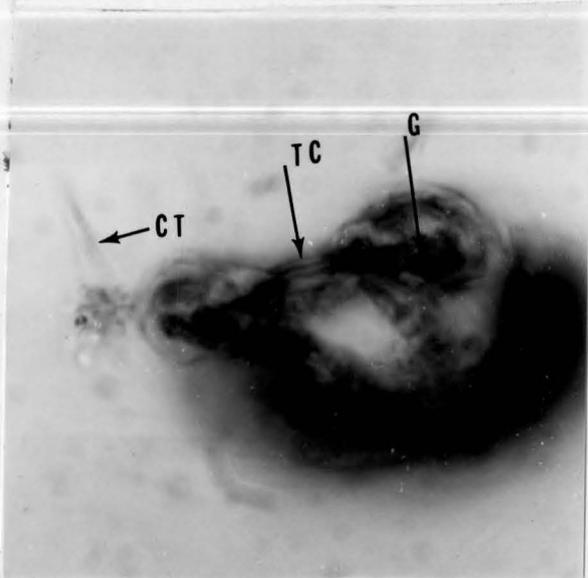


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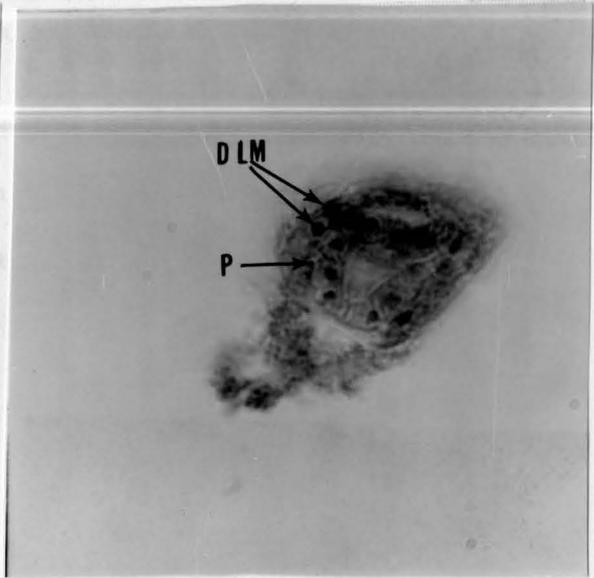


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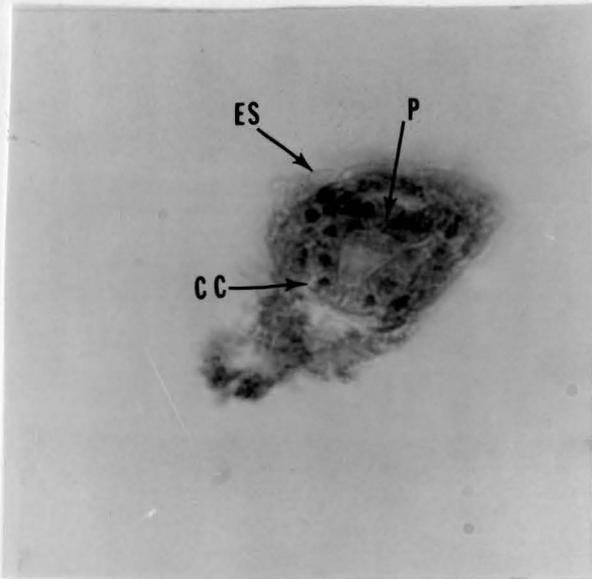


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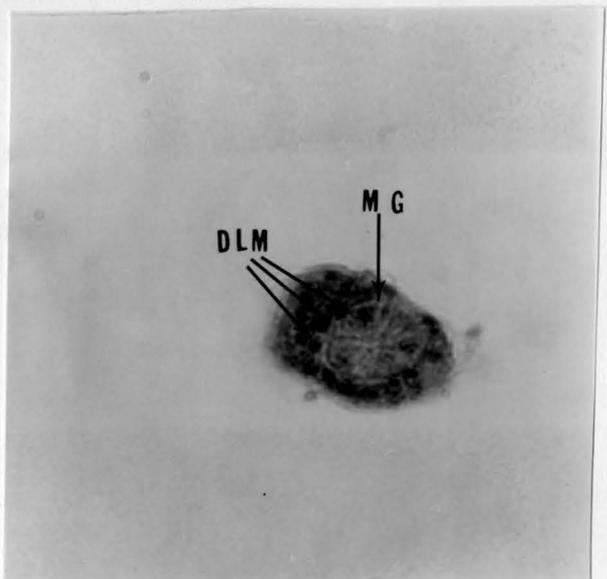


Figure 8

PLATE III

- Figure 9. Cross section through junction of pharynx and mid-gut x 1000
- Figure 10. Cross section through mid-gut x 1000
- Figure 11. Cross section through mid-gut x 1000
- Figure 12. Cross section through mid-gut x 1000

PLATE III

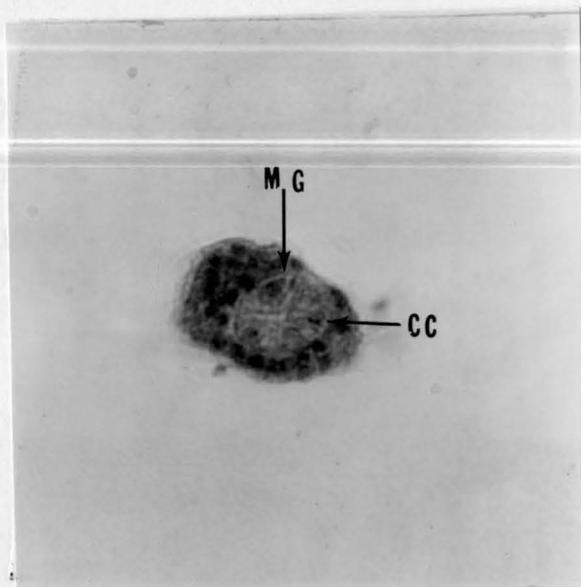


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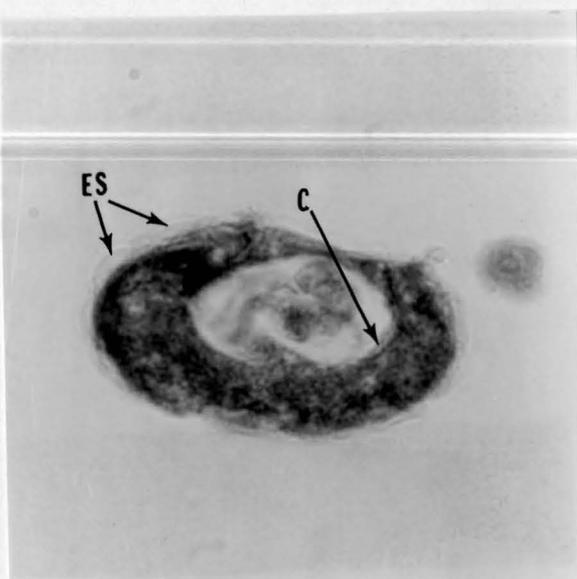


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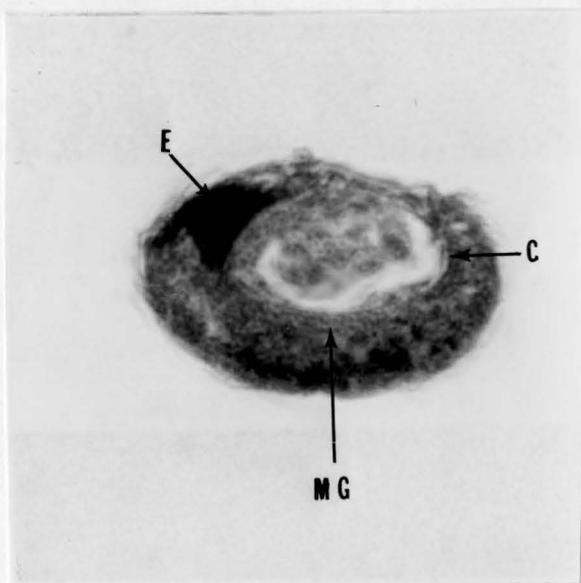


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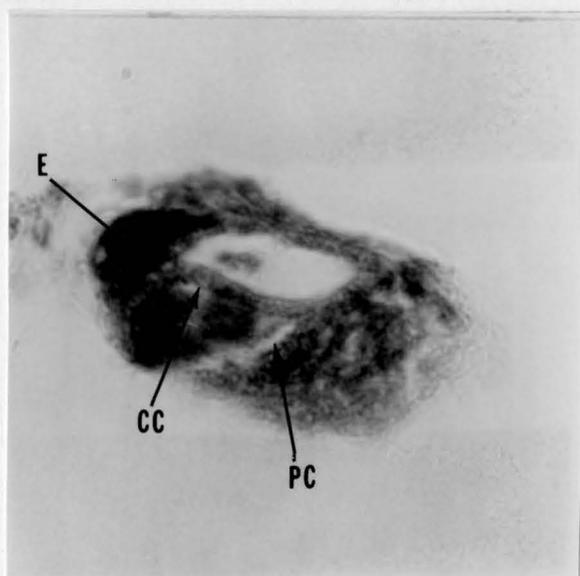


Figure 12

PLATE IV

- Figure 13. Cross section through mid-gut x 1000  
Figure 14. Cross section through mid-gut x 1000  
Figure 15. Cross section through intestine x 1000  
Figure 16. Cross section through intestine x 1000

PLATE IV

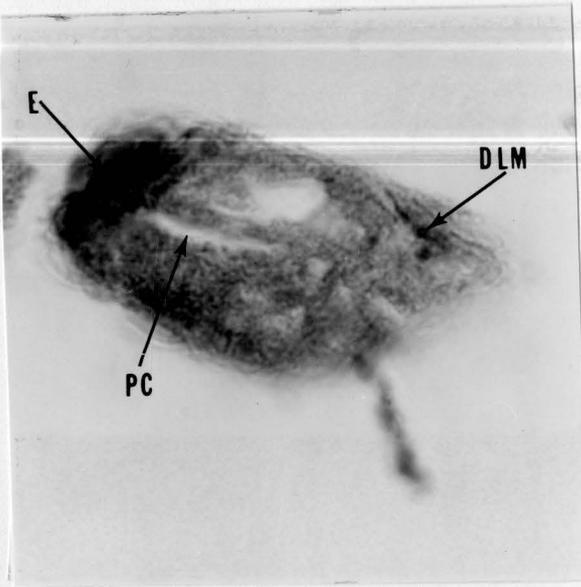


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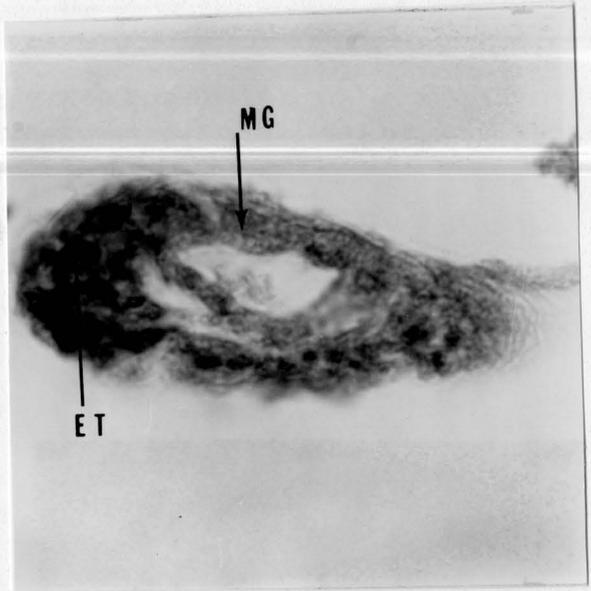


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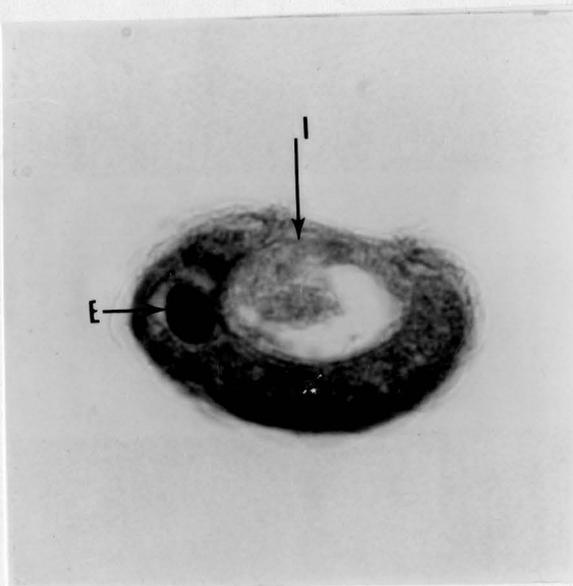


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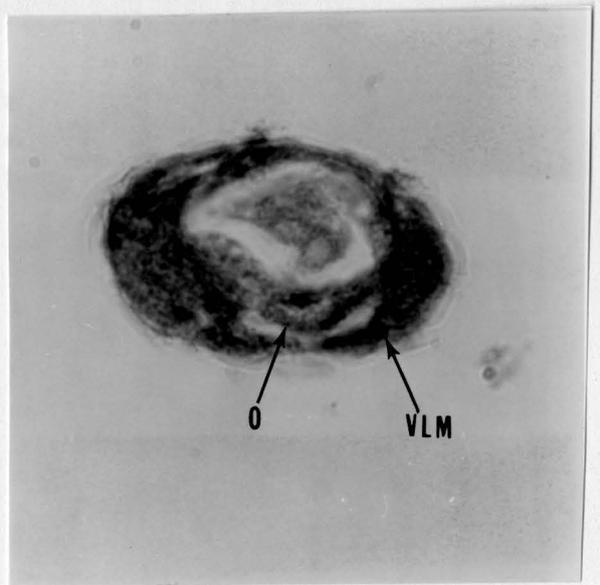


Figure 16

PLATE V

Figure 17. Cross section through intestine x 1000

Figure 18. Cross section through intestine x 1200

Figure 19. Cross section through intestine x 1200

Figure 20. Cross section through intestine x 1200

PLATE V

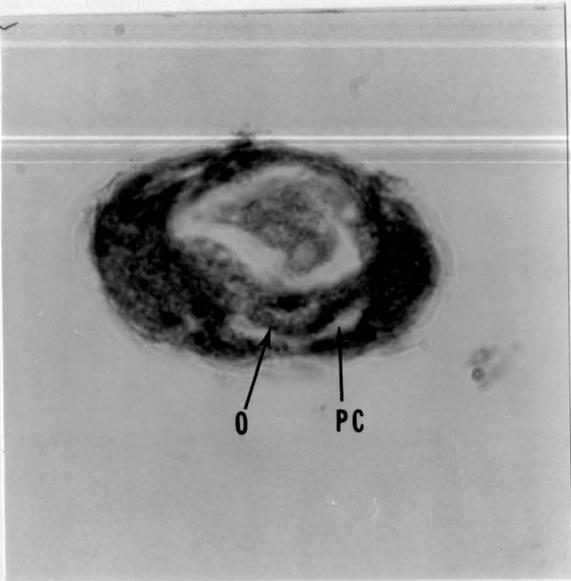


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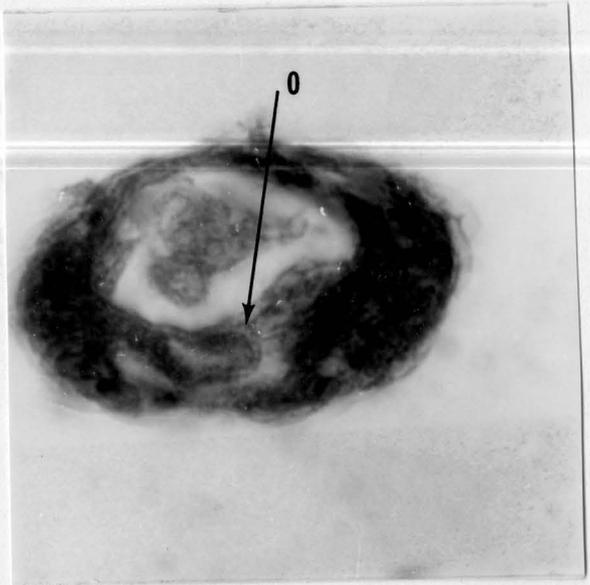


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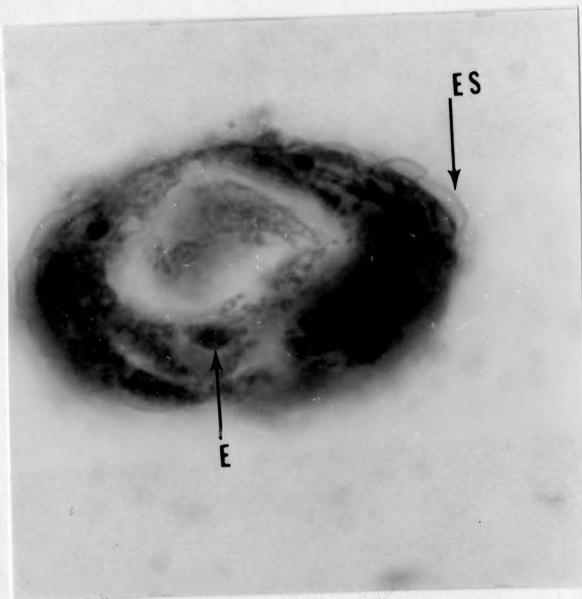


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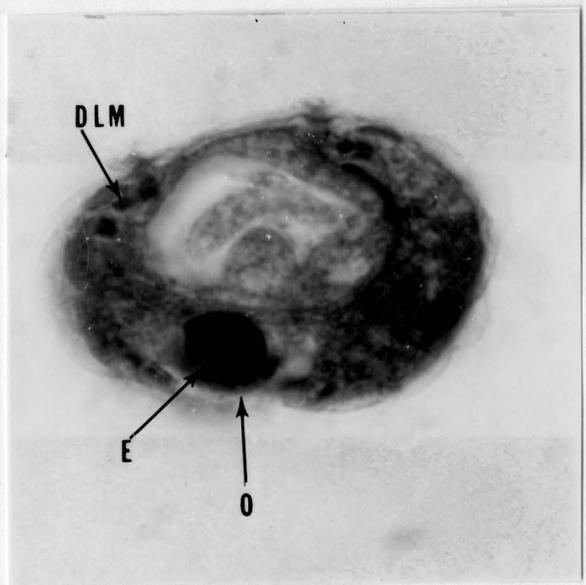


Figure 20

PLATE VI

- Figure 21. Cross section through intestine x 1200
- Figure 22. Cross section through intestine x 1200
- Figure 23. Cross section through intestine x 1000
- Figure 24. Cross section through intestine and  
beginning of caudal fork x 1000

PLATE VI

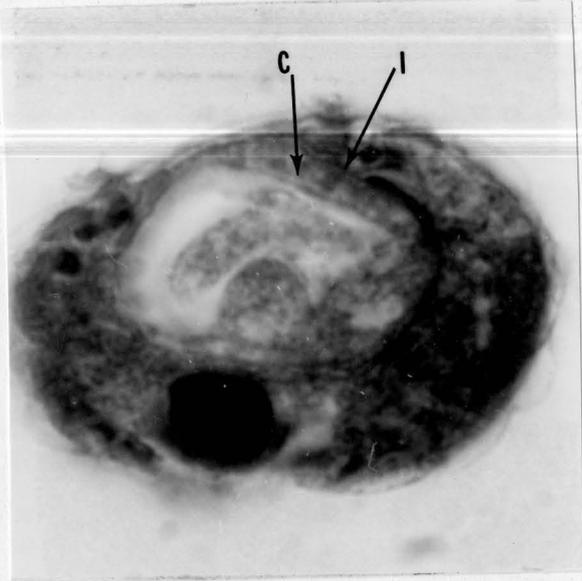


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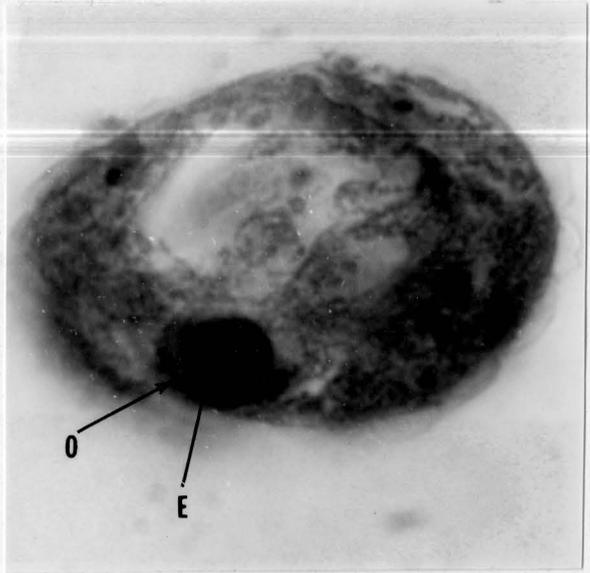


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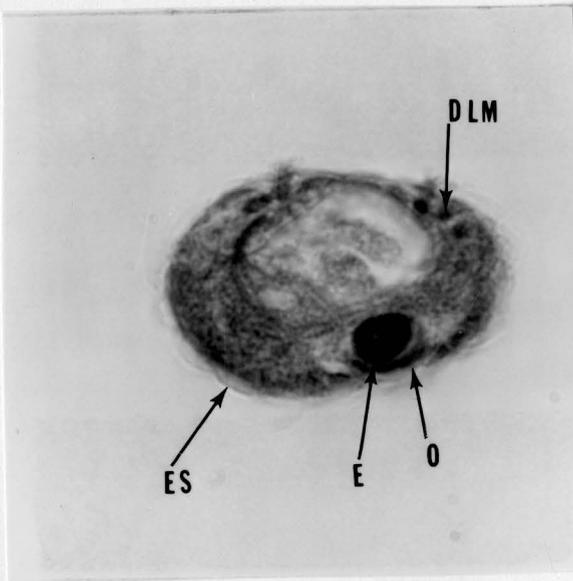


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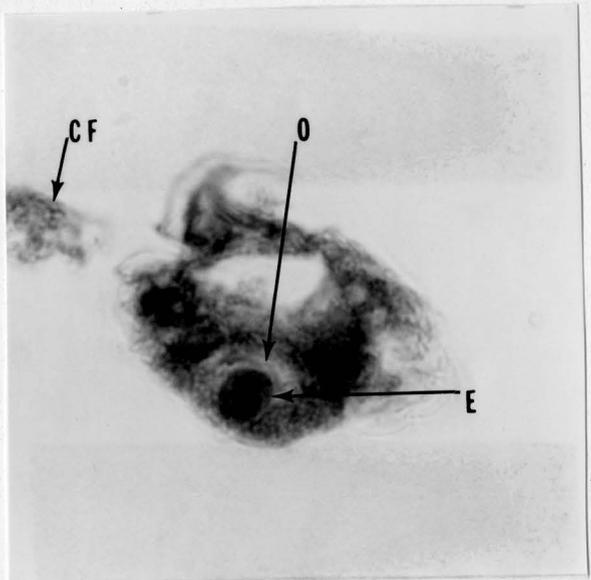


Figure 24

PLATE VII

- Figure 25. Cross section through intestine with longitudinal section through caudal fork x 440
- Figure 26. Cross section through intestine x 1000
- Figure 27. Longitudinal section of caudal fork x 1000
- Figure 28. Cross section through intestine x 1000

PLATE VII

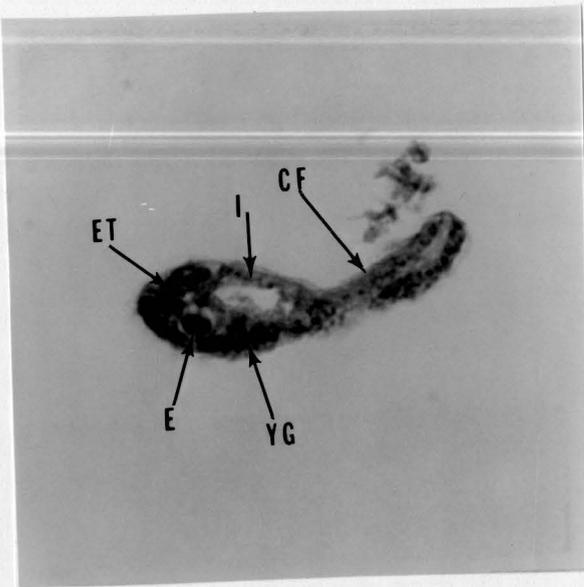


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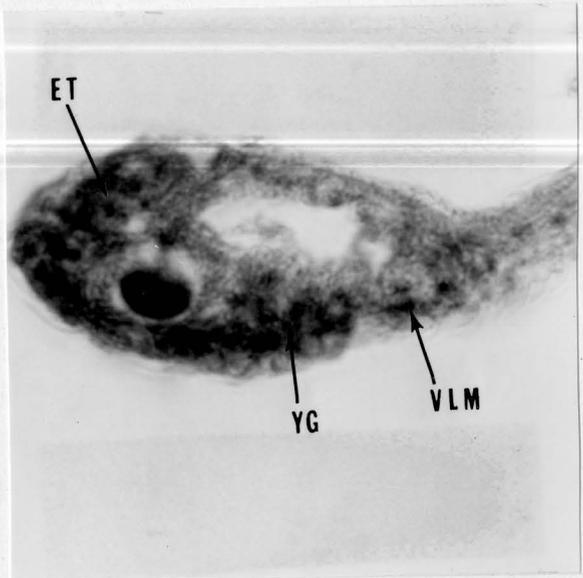


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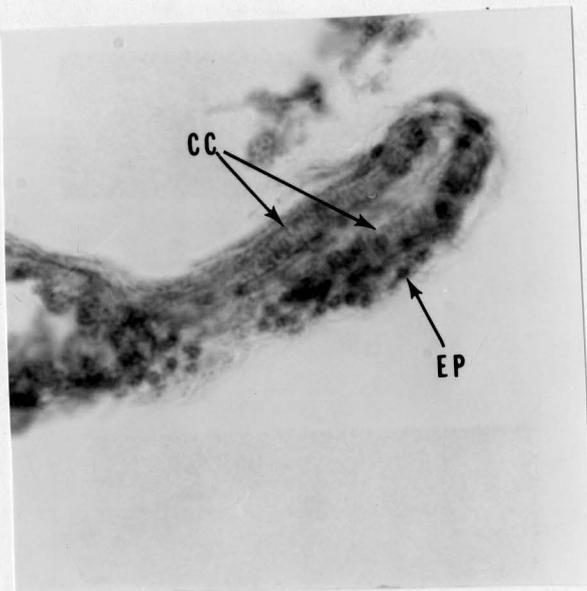


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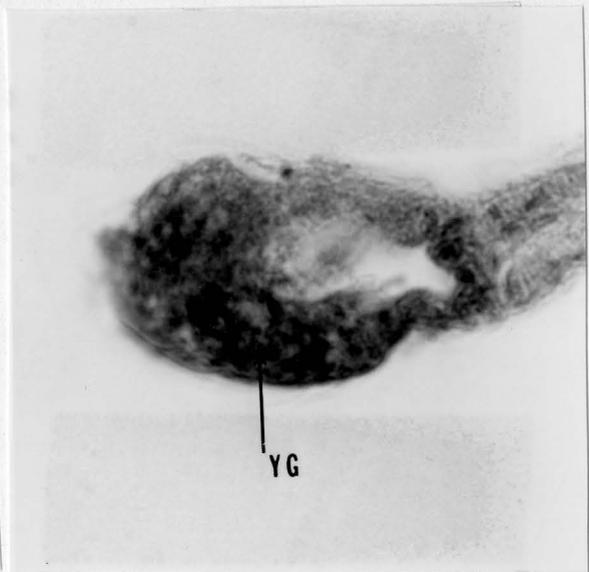


Figure 28

PLATE VIII

- Figure 29. Longitudinal section of caudal fork x 1000 <sup>l</sup>
- Figure 30. Longitudinal section of tip of caudal fork x 1000
- Figure 31. Cross section through intestine x 1000
- Figure 32. Cross section through intestine x 1000

PLATE VIII

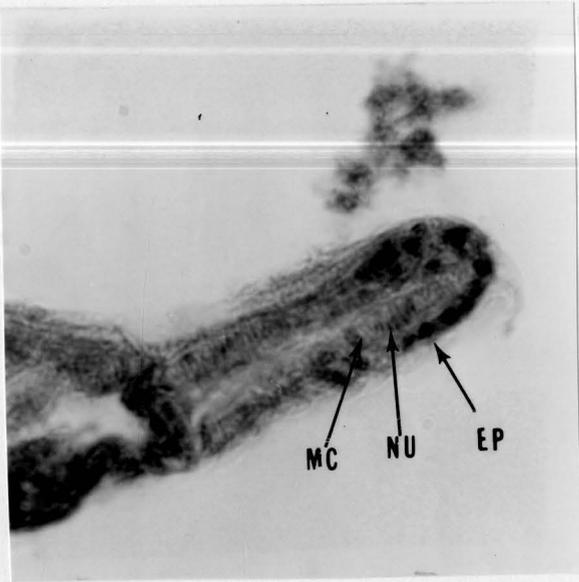


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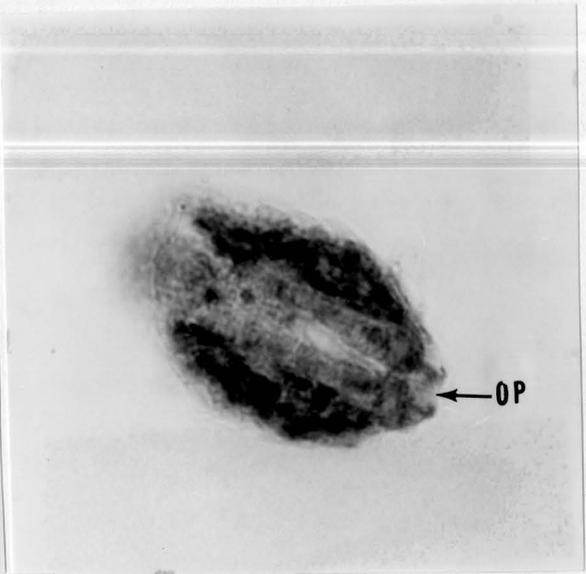


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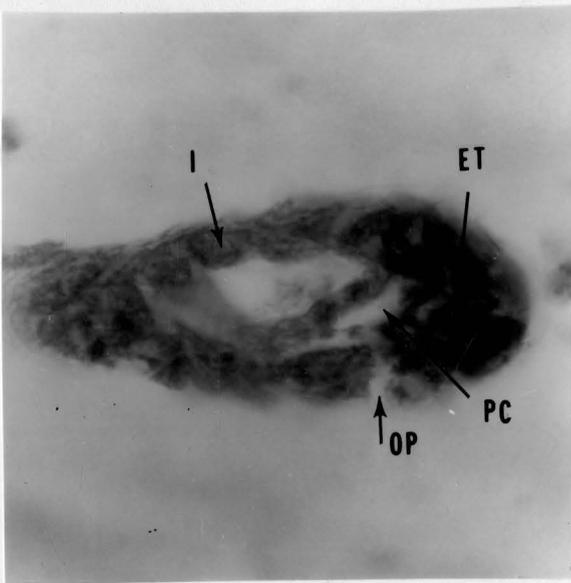


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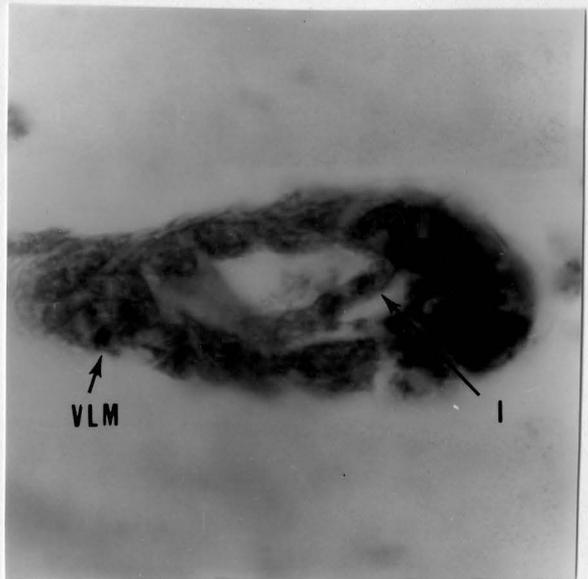


Figure 32

PLATE IX

Figure 33. Cross section through intestine x 1000

PLATE IX

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[Title]  
[Institution]

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manuscript has been  
approved by the [Committee]  
on [Date]

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manuscript has been  
approved by the [Committee]  
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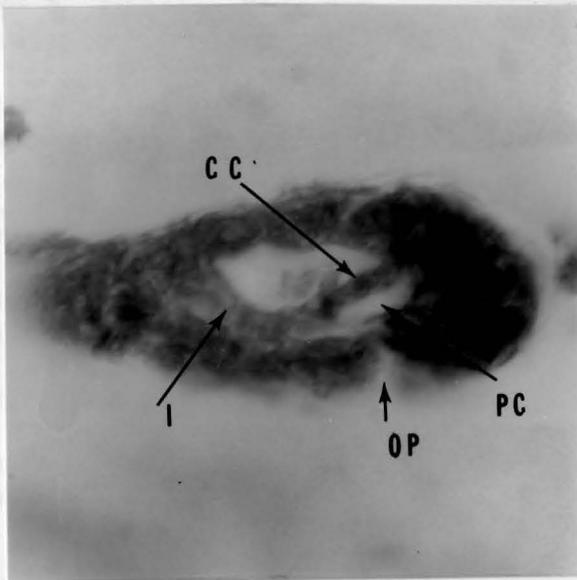


Figure 33

[Name]  
[Title]

*[Signature]*  
Director's Signature

APPROVAL SHEET

The thesis submitted by Raymond W. Ulbrich has been read and approved by the following committee:

Dr. Clyde E. Robbins, Director  
Assistant Professor, Biology, Loyola

Dr. Boris E.N. Spiroff  
Assistant Professor, Biology, Loyola

Rev. Walter P. Peters, S.J.  
Professor, Biology, Loyola

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the Committee with reference to content and form.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

Dec. 8, 1977  
Date

Clyde E. Robbins  
Director's Signature