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# PROCEEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

VOLUME 36

JULY 1969

NUMBER 2

## The Fiji Banana-root Nematode, *Radopholus similis*

A. L. TAYLOR<sup>1</sup>

Dr. N. A. Cobb (1891) reported finding "nearly 30 species of nematodes" in soil and diseased banana plants sent by the Secretary of the Agricultural and Industrial Association of Fiji to the Department of Agriculture of New South Wales in June 1891.<sup>2</sup> In 1893 (a, b) he published two papers describing 29 species from Fiji, giving the collecting dates for most species as July 1891.<sup>3</sup> Among the species described as new were *Tylenchus granulosus* and *T. similis*.

The description of *Tylenchus granulosus* was as follows:

"*T. granulosus*, n. sp. 2.8 10. 16. 56. 90.  
2.3 2.7 2.8 3.3 2.4

.68 mm. The cuticle is traversed by about four hundred and seventy-five transverse striae, which exist in the outer as well as the inner layers. The conoid neck terminates anteriorly in a head somewhat rounded in front and bearing six somewhat spherical lips. The stout spear is one-tenth as wide as the head, and the three bulbs as its base form a triple knot three times as wide as the shaft. Anteriorly the oesophagus is one-fourth as wide as the neck;

somewhat behind the middle of the neck it expands to form a muscular prolate bulb one-half as wide as the neck. Thence it passes through the oblique nerve-ring situated just behind the bulb, and from being there one-fifth as wide as the neck it becomes rather suddenly one-half as wide as the neck, and joins the intestine in a rather indefinite manner at 16%, as stated in the formula. The ventral excretory pore is situated at a distance behind the median bulb equal to thrice the length of that organ. The intestine is composed of cells containing coarse granules. The distance between the wings of the cuticle equals one-third of the width of the body. The tail is conoid to near the terminus, where it diminishes suddenly to a blunt point. I saw only immature females, and cannot give details concerning the sexual organs. The above formula is the average of four specimens. Male unknown.

"Hab.—Observed in numbers in brown rotten cavities three-fourths of an inch deep in the root-stock of banana plants, and also occasionally among the outer sheaths of the plants as well as in the adjacent soil, Fiji, 1891."

*T. granulosus* was not illustrated.

The description of *Tylenchus similis* consisted of only the following:

"*T. similis*, n.sp. ----- Nearly all the information I have with regard to this species is set forth in the sketches on Pl. VII.

"Hab.—Found about diseased banana plants, Fiji, July, 1891."

Figure 1 is a reproduction of the drawings. The space after "n.sp." suggests that Dr. Cobb intended to insert a formula, but this was not done.

*T. similis* was again referred to by Cobb (1915) as follows:

<sup>1</sup> Nematologist, Food and Agriculture Organization, United Nations, assigned to cooperate with the Fiji Department of Agriculture, Ministry of Natural Resources, from October, 1967 through September, 1968.

<sup>2</sup> I wish to thank Dr. C. D. Blake, Faculty of Agriculture, University of Sydney, Sydney, Australia, for finding this paper for me.

<sup>3</sup> The text of these papers is identical. Though they do not specifically state that the shipment of banana plants was the source of the specimens, I have no doubt that it was. I was unable to find any evidence that Dr. Cobb collected the specimens in Fiji. Mr. A. I. Diamond, Chief Archivist of the Central Archives of Fiji and the Western Pacific High Commission, Suva, Fiji, located correspondence dated December, 1892 which discussed arrangements for projected research by Dr. Cobb on bananas in Fiji. These letters did not mention a previous visit to Fiji, and there was no evidence that he visited Fiji before the papers were published in 1893. It is also the opinion of his daughter, Dr. Frieda Cobb Blanchard, that he never visited Fiji (private communication). I wish to thank Mr. Diamond and Dr. Blanchard for their cooperation.

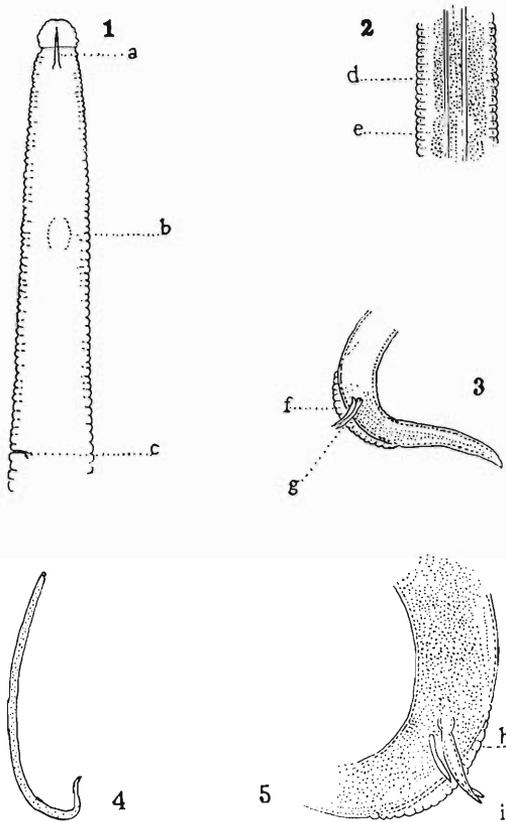


Figure 1. The original illustrations of *Tylenchus similis*. (Redrawn from Cobb, 1893a, b.) The legend was: "Fig. 1. Head and neck. Fig. 2. Portion of the body. Fig. 3. Tail of a male. Fig. 4. Male worm. Fig. 5. Anal region of a male. a, spear; b, bulb; c, excretory pore; d, striae; e, intestine; f, bursa; g, spicula; h, bursa; i, tips of the spicula."

"A serious outbreak of a disease among bananas (*Musa sapientum*) in Fiji in 1890–1891 caused the planters great uneasiness. At the request of Sir John Thurston, British High Commissioner of the Pacific, the Department of Agriculture of New South Wales, Australia, undertook an investigation, which was conducted by the writer. Most of the banana plants examined grew in the gardens adjacent to Government House at Suva, Fiji, where experimental plantings were made in connection with the disease. During the investigations roots of the banana and the soil about the roots

were examined with a view to discovering possible causes of the disease. It was during this particular part of the investigation that a new species of nematode was discovered, to which the name '*Tylenchus similis*' was applied. Only the male was seen."

This paper has descriptions and excellent drawings "prepared under the author's personal supervision by Mr. W. E. Chambers" of male and female specimens found in "diseased portions of rhizomes and true stems of the Jamaica (Gros Michel) banana." It also synonymizes *T. bififormis* Cobb, 1909, from roots of sugar cane on Kauai, one of the Hawaiian Islands. No reference was made to *T. granulatus*.

Other authors have placed *T. granulatus* in various genera as shown by the following list of synonyms: *Anguillulina granulatus* (Cobb, 1893) Goodey, 1932 (sp. inq.); *Bitylenchus granulatus* (Cobb, 1893) Filipjev, 1934; *Tylenchorhynchus granulatus* (Cobb, 1893) Filipjev, 1936, *Tetylenchus granulatus* (Cobb, 1893) Filipjev, 1936.

Two redescriptions of *T. similis* were published apparantly without study of specimens from the type host and locality. Steiner and Buhner (1933) described *T. similis* from roots of tea (*Thea sinensis*) collected in Java. Thorne (1949) based a description on specimens from roots of sugarcane (*Saccharum officinarum*) from Hawaii and roots of pepper (*Piper nigrum*) from the East Indies. He made *T. similis* the type species of a new genus *Radopholus*.

Sher (1968) after study of topotype specimens from Government House, Suva, recognized *T. granulatus* as a senior synonym or *nomen oblitum* (forgotten name) of *T. similis*.

Cobb (1891) did not mention the variety of banana, but his statement (1915) that there was a severe outbreak of disease among bananas in Fiji in 1890–91 provides a clue. Magee (1953) in a discussion of the origin of the virus disease of bananas now known as "bunchy top," cites a letter written in 1890 by the governor of Fiji in which he states that Cavendish bananas and local plantains were being attacked by a disease of unknown origin.

According to Knowles and Jepson (1912), the export trade in bananas started in Fiji in 1877, and the export variety was called "China." This variety was introduced "to the

Samoan Islands, whence in 1848 the Rev. G. Pritchard carried it to Tonga and Fiji." Simmonds (1959 and 1966, pages 102 and 103) says that the "China" variety of Knowles and Jepson was the variety now known as "Dwarf Cavendish,"<sup>4</sup> and that it and the Gros Michel variety, introduced in 1892, were the basis of the export trade in Fiji about 1900. In the absence of definite information, the importance of the Dwarf Cavendish variety suggests that was probably the one sent to New South Wales.

### Observations in Fiji

I went to Fiji in October 1967 and spent most of a year there as an employee of the Food and Agriculture Organization, United Nations, assigned to cooperate with the Fiji Department of Agriculture. I was stationed at the Koronivia Research Station on the island of Viti Levu, about 10 miles north of Suva. The Dwarf Cavendish and Gros Michel varieties of banana have been superseded in Fiji as export varieties by a Robusta type (Fijian name Veimama), but there are still numerous Dwarf Cavendish plants in the vicinity of Suva. Propagation of bananas is by suckers which grow from the corms of older plants. The suckers are almost invariably infected by the same kinds of endoparasitic nematodes as the parent plant. With ordinary planting procedures, the nematodes are transferred from planting to planting indefinitely, so it seemed probable that *Tylenchus similis* Cobb, 1893 might still be found on Dwarf Cavendish plants. Populations collected on 17 January 1968 from several Dwarf Cavendish plants growing at the Koronivia Research Station were used in preparation of the following redescription. The nematodes were found in various stages of development in the root cortex, in the outer layers of the corm, and in the adjacent soil.

<sup>4</sup> According to Simmonds (1959 and 1966, pages 52-54), the use of Latin names is inadvisable during the present state of confusion as to designation of the about 300 varieties of bananas, and the proper formal designation of this variety should be: *Musa* (AAA Group, Cavendish Sub-group) Dwarf Cavendish.

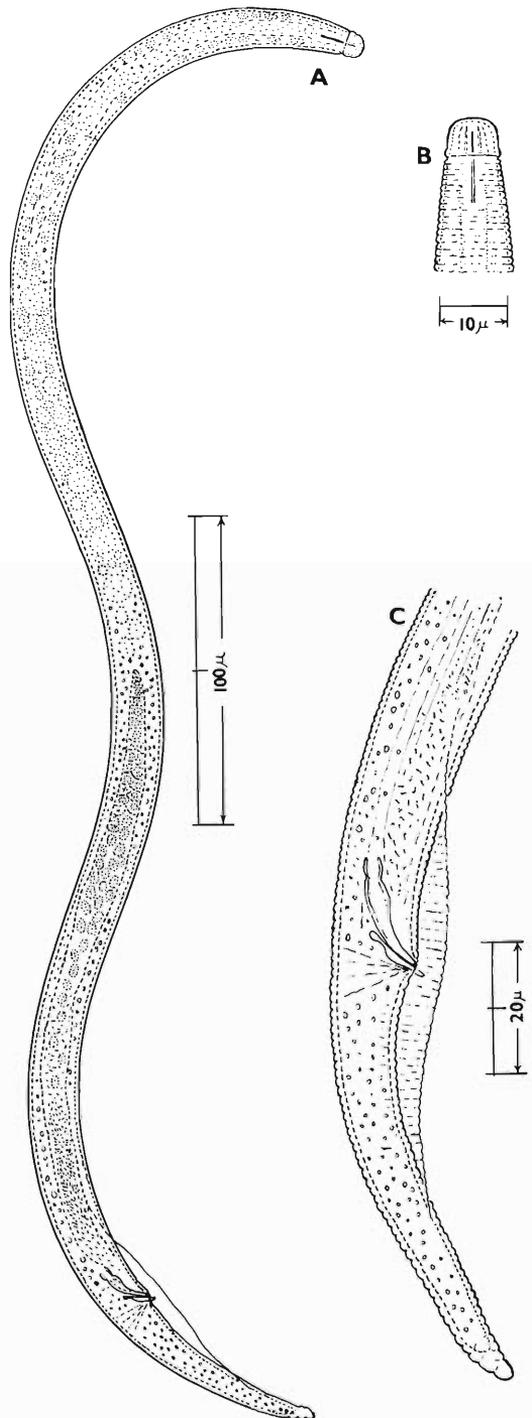


Figure 2. *Radopholus similis* male. A. Full length. B. Anterior end. C. Posterior part of body.

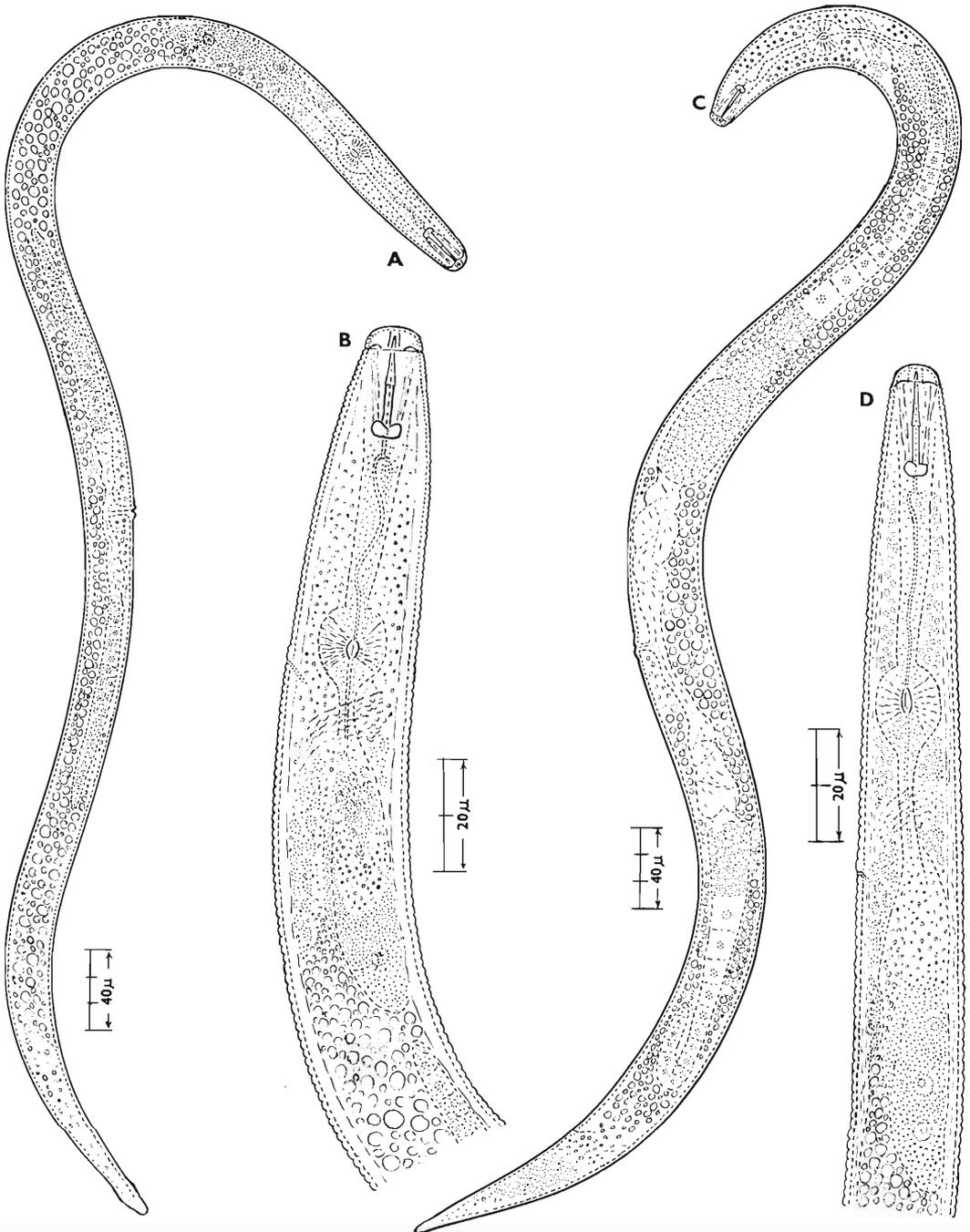


Figure 3. *Radopholus similis* female. A. Young female. B. Anterior part of body of female with eggs. C. Female with nearly mature egg in ovary. D. Anterior part of body of developing female.

### Redescription

*Radopholus similis* (Cobb, 1893) Thorne, 1949 (Figs. 2A-C, 3A-D). The males and females of this species are quite different. The males have a knob-shaped lip region, distinctly set off from the body; the females have a rounded lip region, almost continuous with the body. The male stylet is often indistinct. It is slender and without knobs, or with very small knobs. The female stylet is strong and conspicuous, with distinct knobs. The esophagus of the male is poorly developed, that of the female well developed. In many male bodies, no definite internal organs were seen except the spicules, gubernaculum, and testis. Internal organs of the female are distinct except when damaged by parasites, as is often the case in Fiji. Ovaries of many of the specimens I collected had damage apparently due to parasites. Perhaps this accounts for Cobb's (1893) inability to "give details concerning the sexual organs" of *T. granulosis*.

Average, minimum and maximum measurements of males, young females, developing females, and females with eggs are given in Table 1. All measurements were made with a calibrated camera lucida. Stylets, spicules, and gubernacula were measured to the nearest  $\mu$  at a magnification of 1220  $\times$ .

In addition to the measurements of Table 1, 15 young females were measured and the following average Cobb formula was calculated:

$$\frac{2.8 \ 10.5 \ - \ 56.6 \ 89.2}{2.2 \ 2.8 \ - \ 3.3 \ 2.4} .637 \text{ mm.}$$

**MALES** (Fig. 2A-C): The somewhat bell-shaped lip region is about 7  $\mu$  wide near the base, then tapers very gradually to the rounded anterior end. It is set off from the body by a definite constriction (Fig. 2B). The cuticle is annulated and has lateral fields about one-third of its width beginning near the anterior end and extending to the latitude of the spicules. The lateral fields are divided by four involutions into three parts, of which the middle is the widest. The outer lines are easily seen; the inner ones are faint. The bursa ends about three-fourths of the distance between the anus and the tail terminus. It has a slightly crenate edge, and faint transverse markings. The spicules and gubernaculum are of the forms shown in Figure 2C. The gubernaculum as

Table 1. Measurements of *Radopholus similis*. Average, minimum and maximum in microns.

	Male (12 speci- mens)	Young female (20 speci- mens)	Developing female (9 speci- mens)	Female with eggs (6 specimens)
Length	585.0 535-650	605.2 540-660	646.1 590-700	685.8 610-745
Width <sup>1</sup>	16.5 16-17	19.7 18-22	27.1 23-34	30.3 26-33
Vulva <sup>2</sup>		55.9 53-58	55.1 51-58	55.3 52-57
Tail length	72.8 64-86	66.9 57-77	71.6 63-80	59.8 52-74
Hyaline part of tail		11.0 8-13	13.0 11-18	13.5 11-16
Glands <sup>3</sup>		140.7 126-156	132.9 113-150	134.2 110-155
Stylet	12.0 12-12	18.0 18-18	18.0 18-18	18.0 18-18
Eggs				56.1 $\times$ 23.3 50-68 $\times$ 19-30
Spicule <sup>4</sup>	18.2 18-19			
Guber- naculum	10.3 10-11			
Phasmid <sup>5</sup>	53.7 46-58	53.0 44-61		

<sup>1</sup> At vulva of female, or at middle of male.

<sup>2</sup> Per cent of body length.

<sup>3</sup> Distance from anterior end of body to posterior end of glands.

<sup>4</sup> Straight line between tip and most distant point.

<sup>5</sup> Distance from posterior end of body.

seen in lateral view has a distinct head, a narrow neck, and a wider body, and usually protrudes slightly from the cloaca. The single testis ends about one-third of the body length anterior to the spicules.

On some specimens, a thin esophageal tube was seen attached to the stylet, and occasionally the faint outline of an esophagus with an ellipsoidal median esophageal bulb was visible. The distinctive male lip region is formed just before the last molt (Fig. 4).

**YOUNG FEMALES** (Fig. 3A): In my collections, the most abundant forms were females with slender bodies. The vulva was visible and the ovaries partly developed. Occasional specimens were found to be molting, which suggests that it is difficult to distinguish between larvae in the late fourth stage and adults. The lateral fields extend from about the latitude of the median bulb almost to the terminus. For most of their length, four incisures can be seen. The straight ovaries have small spermathecae. The esophageal glands

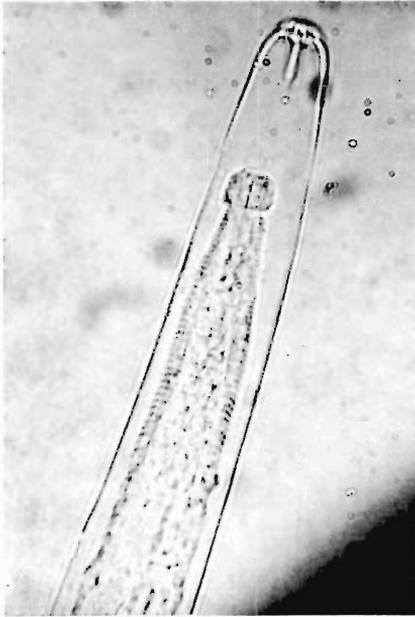


Figure 4. *Radopholus similis* male during last molt.

overlap the intestine dorsally and are in separate lobes.

**DEVELOPING FEMALES (Fig. 3C, 4):** Developing females are wider than young females. The lateral fields are visible. The ovaries are well developed, with the anterior ends often lying beside the esophageal glands, and the posterior ends extending into the tail. Both anterior and posterior ovaries may be outstretched or reflexed, and double reflexions are common. The spermathecae may or may not be visible, apparently depending on the stage of development.

**FEMALES WITH EGGS (Fig. 3B):** Females with eggs in one or both of the uteri average a little longer and wider than developing females, and the fully developed ovaries are a little longer. No spermathecae were seen. The lateral fields are indistinct or invisible.

**LARVAE:** The average length of seven larvae was  $350.0 \mu$  (315–400). Stylets were 13 or  $14 \mu$  long. The tail tapers to a bluntly rounded point and has a much shorter hyaline area than the tail of the young female. The genital primordium is near the center of the body.

## Discussion

The close agreement of my average Cobb formula for 15 females, the host plant and location all indicate that the population I studied was the same as the one studied by Cobb (1893). Sher's (1968) conclusion that *T. granulosis* is the female of *T. similis* is confirmed.

The measurements presented in Table 1 show that variations of about 15% from the average are common for most dimensions, even when the females are divided into three groups. If all females are grouped together, the variation is much greater.

The lengths of stylets, spicules, and gubernacula were remarkably constant. All of the 35 females measured to obtain the data in Table 1 had stylets  $18 \mu$  long. I found no variation from a length of  $12 \mu$  for stylets of 12 males measured for inclusion in Table 1, and only  $1 \mu$  variation in lengths of the spicules and gubernacula.

I found *R. similis* only in banana plants in Fiji, and not in the roots of other plants. In addition to Dwarf Cavendish, the Giant Cavendish, Veimama, and Lacatan varieties were often heavily infected. All of these varieties belong to the Cavendish Subgroup of the AAA Group of Simmonds (1959, 1966). The varieties Gros Michel (AAA Group), Vudi Tomoutola (AAB Group), and Blue Java (ABB Group) were lightly infected. Several other banana varieties growing at Koronivia were not found to be infected, but there was no definite evidence that they were immune. Roots of lime trees on rough lemon rootstock growing intermingled with Veimama banana roots heavily infected with *R. similis* were not infected.

This paper was written with the objective of describing what I consider to be a population most likely to be *T. similis* Cobb, 1893, so far as can be ascertained 77 years after the collection of the first specimens. Populations collected from a limited number of Dwarf Cavendish banana plants are described. However, study of numerous samples collected in different places in Fiji from plants of other banana varieties has not revealed any significant differences from the Dwarf Cavendish populations.

Study and measurement of illustrations by authors who did not have specimens from Fiji revealed many similarities and many differences from the Fiji populations in details and dimensions. Discussion of the differences would serve no useful purpose since the questions raised can only be answered by reference to the original material and by comparison with Fiji specimens.

I have deposited many specimens collected in Fiji in the USDA Nematology Collection, Nematology Investigations, Beltsville, Maryland. These will be available to anyone interested.

### Summary

*Radopholus similis* is redescribed on the basis of specimens collected from Dwarf Cavendish banana plants in Fiji. The females were divided into three groups according to stage of development, and measurements of various dimensions of the bodies were made. Variations of 15% above and below the average were common in each group; and with all groups combined, the variation was much greater. Lengths of female stylets were remarkably constant; all were 18  $\mu$  long. Similar variation was found in most measurements of the males; but all male stylets measured were 12  $\mu$  long, spicules 18–19  $\mu$  long and gubernacula 10–12  $\mu$  long.

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## IN MEMORIAM

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## Embryogenesis and Postembryogenesis in Species of *Pratylenchus* (Nematoda: Tylenchidae)<sup>1</sup>

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Although species of the genus *Pratylenchus* Filipjev, 1936, are recognized as parasites of plants of economic importance, certain aspects of their biology have not been investigated. Detailed descriptions of the early stages of embryonic development as well as the molting process and development of the reproductive system during postembryogenesis of *Pratylenchus* species are lacking. The present study is an account of certain important phases of the embryogenesis and postembryogenesis of the genus. A preliminary report of part of these studies has been presented earlier (Roman, 1968).

### Materials and Methods

*Pratylenchus* species used in these investigations were obtained from different geographical areas. They were propagated on suitable host plants in the greenhouse at 24 to 30 C or aseptically on alfalfa callus tissue in the laboratory at 27 C (Krusberg, 1961).

Studies on embryogenesis were conducted with *Pratylenchus scribneri* Steiner from alfalfa callus tissue. Freshly deposited eggs were mounted in water on glass slides. Zut rings of approximately the same thickness as the eggs were applied as supports, and the mounts were sealed with a 1:1 paraffin-lanolin mixture. The water was changed two to three times a day by opening two outlets in the sealing medium on opposite sides and passing fresh water under the coverglass. The mounts were kept at a temperature of about 24 C.

Postembryonic development was studied in *P. scribneri* and *P. brachyurus* (Godfrey) ob-

tained from infected alfalfa callus tissue fragmented in a Waring blender. Larvae were selected prior to molting and mounted singly in a small drop of warm 1.5% water agar on a glass slide. A coverglass placed on top was gently pressed down and sealed with paraffin-lanolin mixture, leaving one small opening to allow for exchange of gases. The slides were stored in a moist chamber at room temperature. Thus, single individuals could be observed throughout the entire molting process.

Development of the reproductive system was studied in *P. vulnus* Allen and Jensen, *P. coffeae* (Zimmermann), *P. penetrans* (Cobb), *P. pratensis* (de Man), *P. scribneri*, *P. zaeae* Graham, *P. brachyurus*, *P. neglectus* (Rensch), and *P. crenatus* Loof, obtained either from callus tissue or greenhouse cultures. Larvae, molting specimens and adults were stained *in toto* with 1% acetic orcein (Hirschmann, 1962).

### Results

#### Embryogenesis

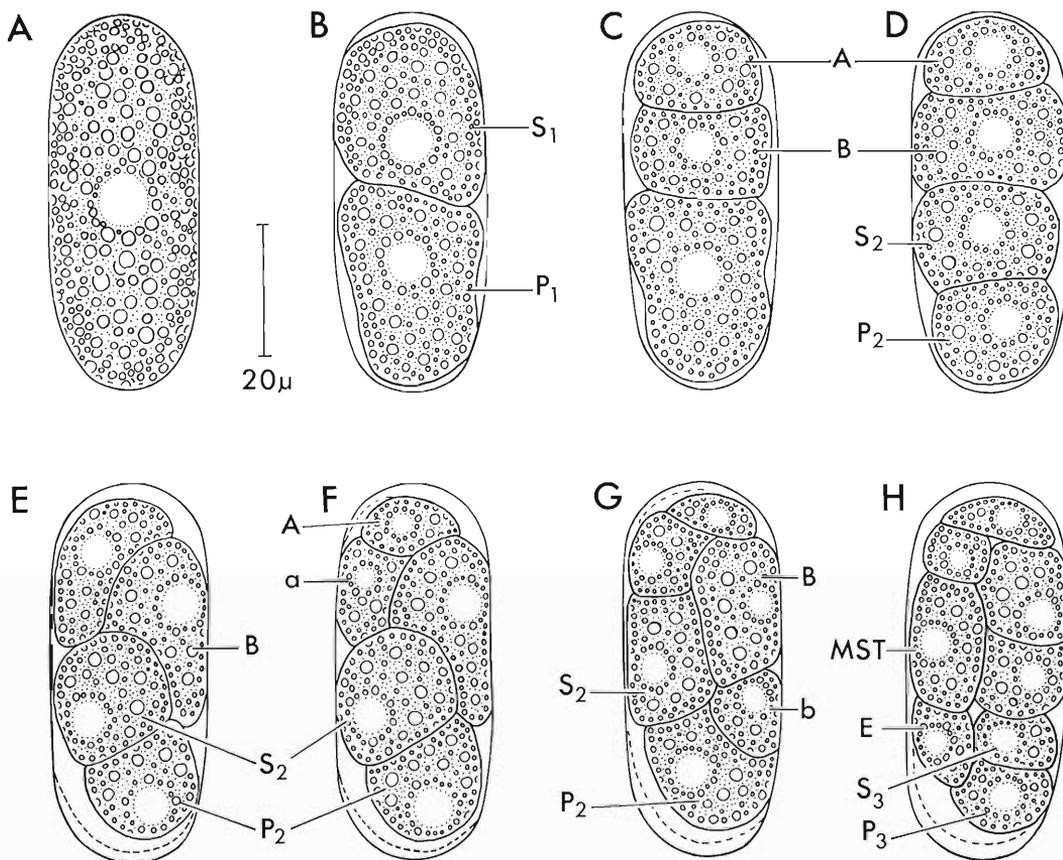
Newly deposited eggs of *Pratylenchus* are usually in the one-cell stage. In *P. scribneri*, egg size varies from 22 to 24  $\mu$  wide by 56 to 67  $\mu$  long ( $n = 14$ ). Cleavage sometimes starts in the uterus of the female so that two-celled eggs are deposited. The egg shell is thin, transparent and smooth. The lipid membrane underneath the shell is often visible, especially at the poles (Fig. 1E-H). The nucleus appears as a clear area inside the granulated cytoplasm.

The undivided egg (Fig. 1A), exhibits pronounced cytoplasmic movement and rearrangement of the various components. This movement simulates egg division, but such cytoplasmic activity decreases later, at which time the nucleus becomes indistinct and real cleavage commences. Cytoplasmic activity was also observed in multicelled eggs, especially in blastomeres shortly before division.

<sup>1</sup> Portion of a thesis submitted by the senior author in partial fulfillment of the requirements for the Ph.D. degree, North Carolina State University, Raleigh, North Carolina.

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**Figure 1. Embryonic development.** A. Undivided egg; B. First cleavage, two-celled stage; C. Second cleavage, three-celled stage; D. Second cleavage, four-celled stage in tandem arrangement; E. Second cleavage, four-celled stage in rhomboid arrangement; F. Third cleavage, five-celled stage; G. Third cleavage, six-celled stage; H. Third cleavage, eight-celled stage.

The first cleavage is completed in 6 to 11 hours after egg deposition. The division is transverse to the longitudinal axis of the egg and gives rise to two cells of about equal size. It was not possible to distinguish between the anterior and posterior blastomere at this stage. We assume that *Pratylenchus* undergoes the same pattern of cleavage as other nematodes (Boveri, 1892; Pai, 1928), and therefore, regard the blastomere that divides first as the anterior, S<sub>1</sub> blastomere (Fig. 1B).

The second cleavage is also transverse to the longitudinal axis of the egg and starts at about 7 hours after the first division has been

completed. The division of the S<sub>1</sub> blastomere takes approximately 1 hour and results in two cells of about equal size, an anterior A and a posterior B (Fig. 1C). Ten hours later, the second cleavage is completed through the division of the posterior P<sub>1</sub> cell into an anterior S<sub>2</sub> and a posterior P<sub>2</sub> cell. The resulting four cells are arranged in tandem for several hours (Fig. 1D). The cells later change position resulting in a rhomboid arrangement (Fig. 1E).

About 10 hours after the completion of the second cleavage, the third cleavage starts. The A cell divides first and gives rise to A and a (Fig. 1F). Four hours later, the B cell divides

into B and b (Fig. 1G). The cells later rotate slightly so that the  $S_2$  cell occupies the mid-ventral position, while the B and b cells migrate toward the dorsal side. The  $S_2$  cell then divides, giving rise to the E and MST cells. This is followed by the division of  $P_2$  which forms the  $S_3$  and  $P_3$  cells (Fig. 1H). Further cleavages were not studied, since divisions beyond the eight-celled stage could not be followed accurately in live material.

### Postembryogenesis

#### Molting in *P. scribneri* and *P. brachyurus*

The first of four molts takes place within the egg and the second stage larva emerges. In *P. scribneri* and *P. brachyurus*, the first stage larva is very active, but the various organ systems are not completely formed. At this stage, the larva is comprised of three main parts: an anterior clear portion; a middle granular portion; and a posterior clear portion which correspond to the esophagus, intestine and tail, respectively.

**FIRST MOLT:** At the beginning of the first molt, a small hyaline, cup-shaped cavity, containing a refractive structure which probably corresponds to the primitive stoma, appears at the terminal part of the anterior portion of the larva (Fig. 2A). Separation of the cuticle first occurs in the region adjacent to the small hyaline, cup-shaped cavity (Fig. 2B). A long line, which corresponds to the lining of the lumen of the esophagus, becomes visible in the center of the anterior region. The refractive structure within the hyaline, cup-shaped cavity now appears Y-shaped and one, two or three rings are visible below the cavity (Fig. 2C). The cuticle starts separating from the sides of the anterior end and the lip region appears greatly contracted. Meanwhile, the beginning of the point, or conical part of the developing stylet appears within a granular area directly behind the hyaline, cup-shaped cavity (Fig. 2D). As sclerotization of the stylet point proceeds, this granulation extends posteriad, and

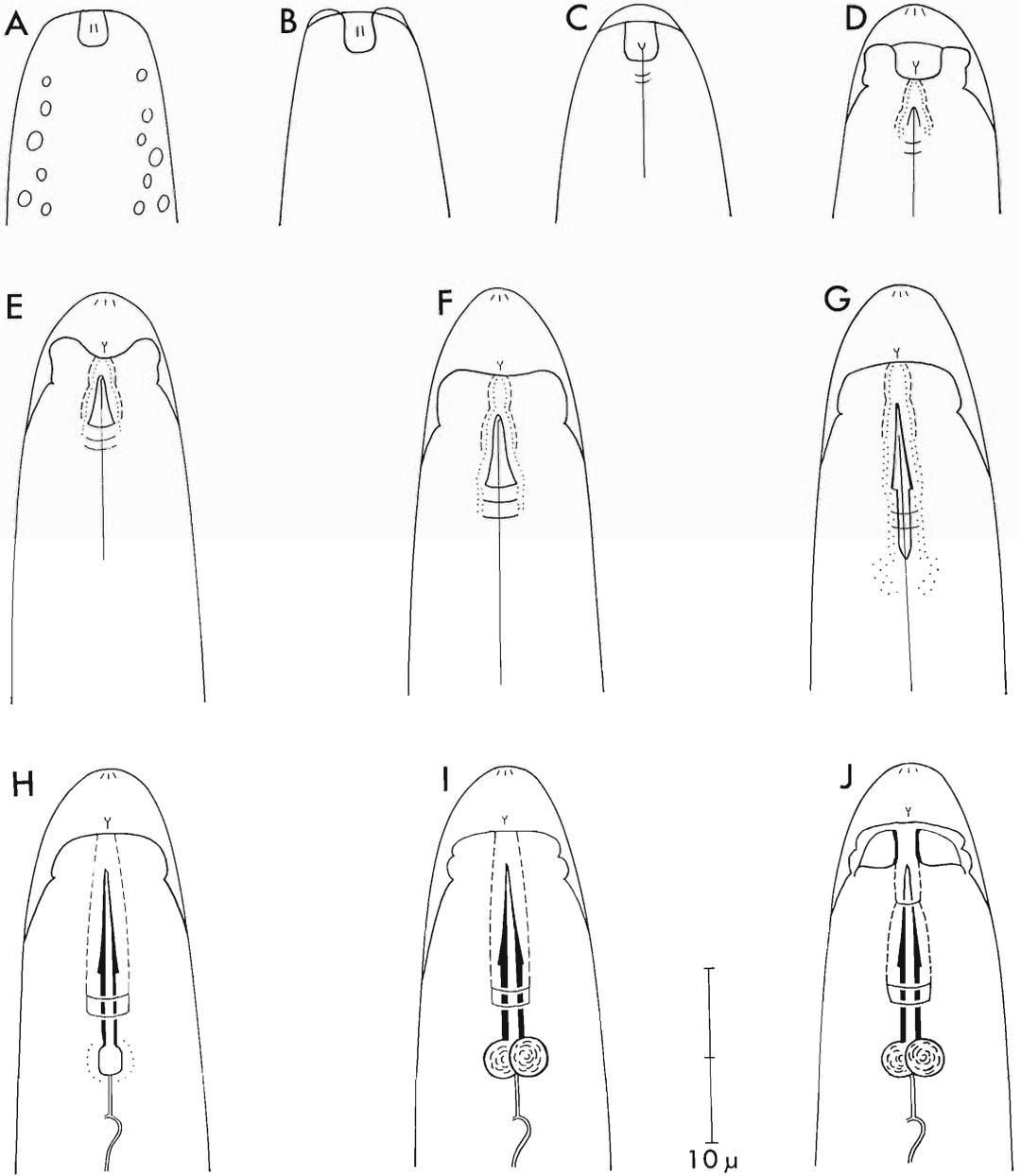
the hyaline, cup-shaped cavity gradually flattens out until it finally disappears as the lips are forming (Fig. 2E-G). At the same time, the valve plates of the esophageal metacarpus begin to appear. The small Y-shaped structure remains in front, in the center of the lip region (Fig. 2G-J). Sclerotization of the stylet shaft advances posteriad from the end of the conical part until the knobs are formed (Fig. 2G-I). During the early stages of stylet knob formation, the guiding sheath of the stylet becomes fully formed and connects with two rings encircling the shaft of the stylet (Fig. 2H). The dorsal esophageal gland orifice, excretory duct, and rectum become discernible. Sclerotization of the cephalic framework starts after the stylet is completely formed (Fig. 2J). Although the larva inside the egg moves actively during the entire process of molting, movements become more vigorous, when all the organ systems are formed. The molted cuticle finally breaks at the anterior end. The first molt lasts 3 days.

Movement of the second stage larva ceases, when it is ready to emerge from the egg. The stylet is then thrust toward one pole of the egg. This process continues for about 2 hours at a rate of approximately 47 thrusts per minute. When the stylet finally penetrates the egg shell, the larva emerges through the break. It takes about 1 minute for the larva to leave the egg shell.

**SECOND MOLT:** Prior to the second molt active motion ceases, and the larva lies straight, with occasional, slow movements (Fig. 3A). The lining of the esophageal lumen and the valve plates of the metacarpus become very faint. The whole stylet appears less refractive; however, refractivity continues to decrease gradually in shaft and knobs but not in the point. The knobs disappear first, leaving large halos through the center of which a dark line passes, probably corresponding to the lining of the stylet lumen. Refractivity of the shaft decreases antieriad until only the conical part is visible (Fig. 3B). It takes approximately 2 hours for the knobs and shaft to disappear.

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Figure 2. Postembryonic development, first molt. A. Beginning of first molt, formation of hyaline, cup-shaped cavity containing refractive structure; B. Separation of cuticle in region adjacent to hyaline, cup-shaped cavity; C. Beginning of formation of esophagus lining, rings, and Y-shaped structure; D. Lip region greatly contracted, formation of beginning of conical part of stylet; E. Sclerotization of



conical part of stylet further advanced; hyaline, cup-shaped cavity smaller; F. Sclerotization of conical part of stylet near completion; hyaline, cup-shaped cavity almost flattened; G. Conical part of stylet well developed, sclerotization of stylet shaft; disappearance of hyaline, cup-shaped cavity; H. Early stages of stylet knob formation, guiding sheath of stylet well developed, appearance of dorsal esophageal gland orifice; I. Stylet formation completed, appearance of annulation in lip region; J. Cephalic framework fully formed.

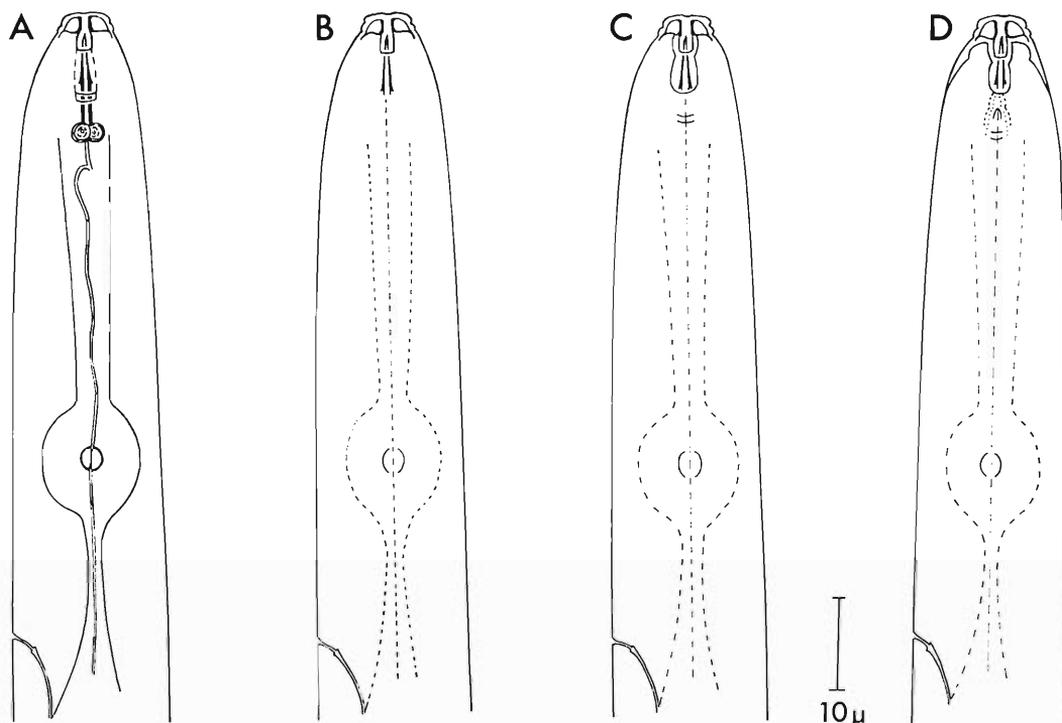


Figure 3. Postembryonic development, second molt. A. Second stage larva prior to second molt; B. Stylet knobs and shaft have become invisible, esophagus appears very faint; C. Flask-shaped hyaline cavity around conical part of stylet and rings visible; D. Head of early third stage larva beginning to retract from second cuticle; beginning of the conical part of new stylet and rings become visible.

Meanwhile, the stylet point becomes more distinct again. A flask-shaped hyaline cavity around the point and one, two or three rings below it become visible (Fig. 3C). The knob halos become less pronounced and finally disappear. At about this time, the head of the third stage larva begins to retract slowly from the second-molt cuticle. Behind the flask-shaped hyaline cavity, the beginning of the conical section of the new stylet, which is surrounded by a granular area, becomes visible in front of the rings (Fig. 3D). The second-molt cuticle carries with it the cephalic framework, as well as the conical part of the previous stylet together with a short strand of the stylet shaft lining, and the amphidial duct linings (Fig. 4A). As the sclerotization of the new stylet point advances, the opening of the lumen is seen ventrally just below the tip. The flask-shaped hyaline cavity gradually flattens

out, while the granulation extends to the area of the shaft (Fig. 4B). Sclerotization of the shaft proceeds posteriad from the end of the point as the granulation extends to the area of the knobs (Fig. 4C). After the flask-shaped hyaline cavity has disappeared, the knobs begin to form and gradually increase in size. The guiding sheath is seen attached to the rings around the shaft, and the orifice of the dorsal esophageal gland becomes visible (Fig. 4D). When the stylet is completely formed, the esophagus becomes very distinct (Fig. 5A), and sclerotization of the cephalic framework takes place (Fig. 5B). Meanwhile, the cuticle around the tail has separated. As the nematode increases its activity, the old cuticle separates completely from the new cuticle carrying with it the linings of excretory duct, rectum, and phasmidial ducts. The nematode lengthens until it fills the second-molt cuticle, pushing the

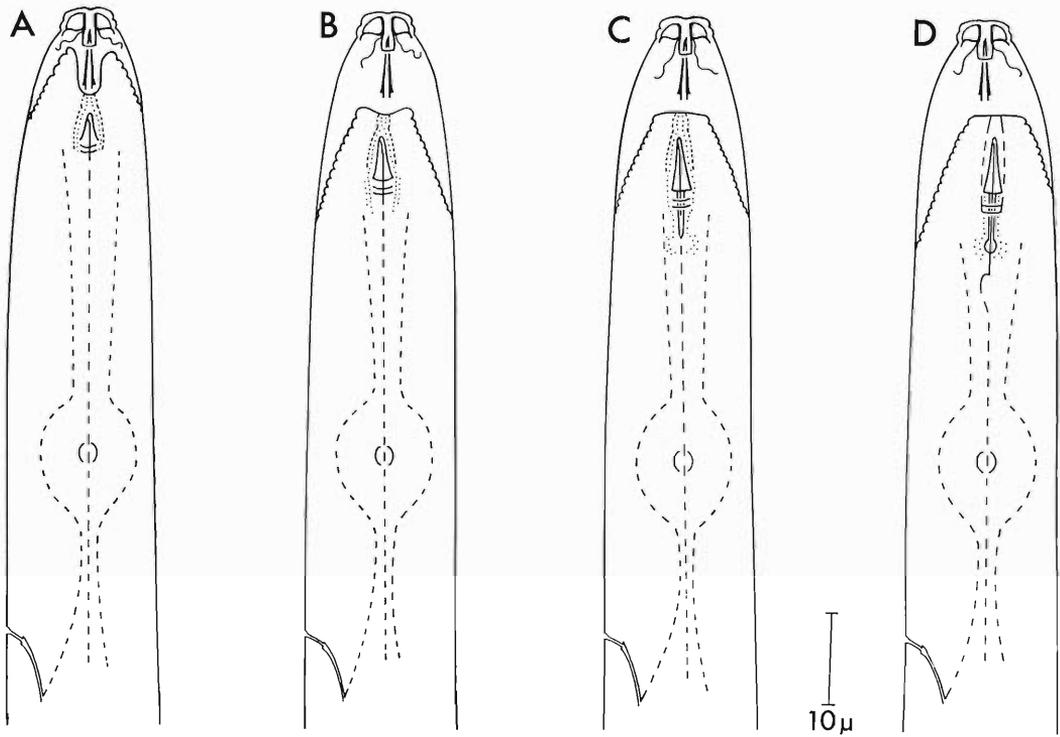


Figure 4. Postembryonic development, second molt (continued). A. Further contraction of head of third-stage larva and sclerotization of conical part of new stylet; cephalic framework, amphidial ducts and conical part of previous stylet molted with second cuticle; B. Flask-shaped hyaline cavity almost flattened; further sclerotization of conical part of new stylet; C. Flask-shaped hyaline cavity not visible; sclerotization of stylet shaft; D. Beginning of stylet knob formation, guiding sheath well developed, orifice of dorsal esophageal gland visible.

molted point of the stylet to one side. The second molt lasts 3 days.

**THIRD AND FOURTH MOLTS:** These molts proceed similarly to the second molt, except for changes in the development of the reproductive system.

#### Development of the reproductive system in various *Pratylenchus* species

The reproductive system can be initially seen in molting first stage larvae as a small, oval-shaped primordium consisting of four nuclei: two terminal epithelial nuclei and two central germinal nuclei. The epithelial nuclei later give rise to cap cell nuclei, linings of ovary or testis and female or male gonoducts, whereas the germinal nuclei form oogonia or spermatogonia. No divisions take place during the sec-

ond larval stage (Fig. 6A). Divisions of the epithelial nuclei begin during the second molt and continue thereafter during the remaining molts and larval stages.

The genital primordium of third stage male larvae of the bisexual species *P. penetrans*, *P. vulnus*, *P. coffeae* and *P. pratensis* is composed of an anterior and a posterior portion (Fig. 6D). The anterior portion consists of several epithelial nuclei; the posterior portion comprises the two germinal nuclei and one epithelial nucleus which remains terminal as the cap cell nucleus. During the third molt, the orientation of the gonad changes. The anterior portion with the epithelial nuclei turns posteriad, while the posterior portion with the germinal nuclei turns anteriorad (Fig. 6E). The two germinal nuclei divide during the third molt. At

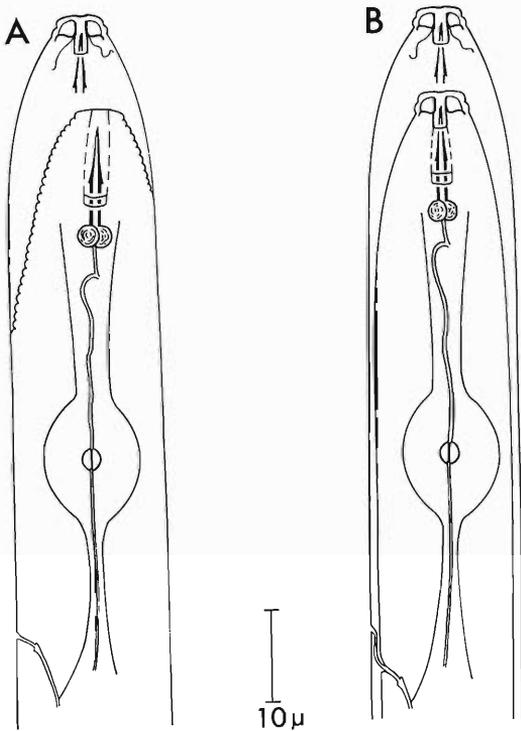


Figure 5. Postembryonic development, second molt (continued). A. Stylet completely developed, esophagus very distinct; B. Cephalic framework completed; third-stage larva fully separated from second molt cuticle.

the completion of the third molt, the gonad attains its normal position, *i.e.*, the gonoduct is located posteriad. During the fourth stage, the gonad extends in length as the number of germinal and epithelial nuclei increases (Fig. 6F).

The female gonad of *P. penetrans*, *P. vulnus*, *P. coffeae*, *P. pratensis*, *P. brachyurus*, *P. zaeae*, *P. neglectus* and *P. crenatus* follows an amphidelphic type of development. During the second molt and early in the third stage, the epithelial nuclei divide repeatedly forming two cap cell nuclei and several epithelial nuclei which concentrate in the center of the primor-

dium separating the two germinal nuclei (Fig. 6C).

Early in the fourth stage, the number of epithelial nuclei has increased considerably. The germinal nuclei are far apart and the gonad consists of two distinct branches (Fig. 6H). In species like *P. brachyurus* with a vulva value of over 80%, the number of epithelial nuclei in the anterior branch is about twice the number of that in the posterior branch. In species like *P. zaeae* with a vulva value lower than 75%, the number of epithelial nuclei may be approximately the same in both branches (Fig. 6H). In some specimens of other *Pratylenchus* species with a relatively anteriorly located vulva, the number of epithelial nuclei in both branches may be about the same, but those in the posterior branch are always closer together.

Later in the fourth stage, the germinal nucleus of the anterior gonad starts to divide, whereas that of the posterior gonad remains undivided (Fig. 6H). During the fourth molt, the germinal nucleus and most of the epithelial nuclei in the posterior gonad degenerate, and a short postvulvar uterine branch consisting of only epithelial nuclei is formed (Fig. 6I). Therefore, adult females are monodelphic, prodelphic. In some females of *P. zaeae*, however, the posterior germinal nucleus completes several divisions, and a posterior rudimentary ovary is formed that includes five to six oogonial cells (Fig. 6J).

In contrast to this amphidelphic pattern of gonad development, three different populations of the monosexual *P. scribneri* follow a monodelphic pattern of gonad development. Here, during the second molt and third stage, the two germinal nuclei remain together in the anterior part of the genital primordium, whereas the posterior part consists of epithelial nuclei only (Fig. 6B). Early in the fourth stage, the number of epithelial nuclei has increased, and two distinct gonad branches are formed with only the anterior containing germinal nuclei (Fig. 6G). During the fourth molt, most of the epithelial nuclei posteriad to the vulval area degenerate, and a short postvulvar uterine

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Abbreviations: c n, cap cell nucleus; ep n, epithelial nuclei; g n, germinal nuclei; oog, oogonia; p vu ut, postvulvar uterine branch; ut, proximal part of uterus; s ch n, specialized ventral chord nuclei; spt, spermatheca; tc, tricolumella; va in, vagina indication; vest ov, vestigial ovary.

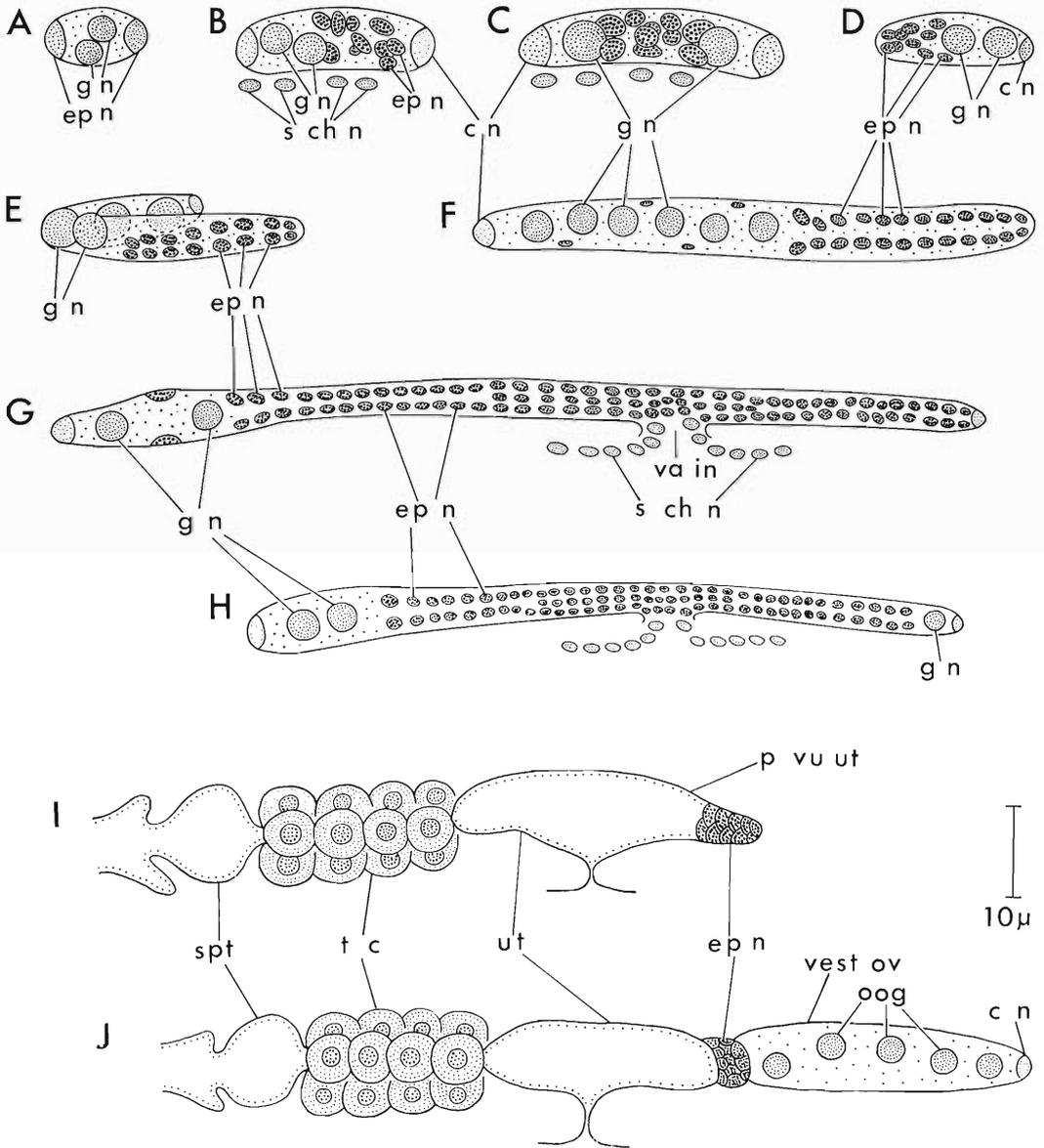


Figure 6. Development of the reproductive system. A. Genital primordium of molting first-stage and second-stage larvae; B. Genital primordium in third-stage female larva with monodelphic pattern of gonad development (*P. scribneri*); C. Genital primordium in third-stage female larva with amphidelphic pattern of gonad development; D. Genital primordium of third-stage male larva; E. Male gonad changing direction during third molt; F. Male gonad during fourth stage; G. Female gonad of monodelphic type during fourth stage; H. Female gonad of amphidelphic type during fourth stage; I. Posterior portion of adult female reproductive system with short postvulvar uterine branch containing only epithelial nuclei; J. Posterior portion of adult female reproductive system with rudimentary ovary.

branch is formed as in species with an amphidelphic type of development (Fig. 6I).

The sex in *Pratylenchus* species can be recognized early in the second molt. Larvae which become females possess four specialized nuclei located in the ventral chord opposite the genital primordium (Fig. 6B, C). These specialized ventral chord nuclei, which are not present in male larvae (Fig. 6D), participate in the formation of the vagina. During the third molt, the number of specialized ventral chord nuclei increases to 16, eight of which are located in a row anteriorly with the other eight occurring posteriorly to the indication of the vagina. The four central nuclei, *i.e.*, the two of each row adjacent to the vagina indication, then migrate inward, leaving six nuclei on each side of the vaginal indication (Fig. 6G, H). In late fourth stage larvae and during the fourth molt, these specialized ventral chord nuclei also move inward as the vaginal tube is forming.

### Discussion

The study of the early stages of embryonic development of *Pratylenchus* indicates that the cleavage pattern is similar to that of other tylenchids such as observed for *Radopholus similis* (Cobb) by van Weerd (1960), *Nacobus serendipiticus* Franklin by Clark (1967), *Criconemoides xenoplax* Raski by Seshadri (1965), *Hemicriconemoides chitwoodi* Esser by Fassuliotis (1962), *Rotylenchulus parvus* (Williams) by Dasgupta and Raski (1968) and for several species of *Seinura* by Hechler and Taylor (1966a). It is different, however, than the cleavage pattern reported for *Ditylenchus dipsaci* (Kühn) by Yuksel (1960) and for *D. destructor* Thorne by Anderson and Darling (1964a). The second cleavage in eggs of *Pratylenchus* is completed in both blastomeres before the third cleavage is initiated. At the end of the second cleavage, the egg has four blastomeres arranged in tandem. In contrast to this, the posterior blastomere of eggs of *D. dipsaci* and *D. destructor* undergoes the second cleavage after the anterior blastomeres have completed the third cleavage. Thus, the eggs have six cells at the time the posterior blastomere completes its second cleavage.

The pronounced cytoplasmic movement and rearrangement of the various components observed in the cells prior to cleavage is apparently a normal condition associated with cell

division and has been reported also by van Weerd (1960) for *R. similis*, by Clark (1967) for *N. serendipiticus* and by Anderson and Darling (1964a) for *D. destructor*.

The divisions after the eight-celled stage proceed so rapidly that it is impossible to follow them in live material. This, in part, may explain, why there are no accurate records about embryogenesis in the Tylenchida.

In general, molting in *Pratylenchus* larvae is similar to the molting process in other nematodes. There appear to be certain differences with some genera, however, with regard to the sclerotization of the structure of the anterior end. The formation of the sclerotized portions of the anterior end of *Pratylenchus* follows the same pattern as described for *R. similis* (van Weerd, 1960). In both these related genera, the conical part of the stylet develops first followed by the shaft and knobs. After the stylet is completely formed, the cephalic framework develops. In *N. serendipiticus* the head skeleton and stylet tip are formed followed by the posterior portion of the stylet (Clark, 1967). Sclerotization of the stylet in *Seinura* spp. begins just behind the junction of the anterior conical and posterior cylindrical sections, advances anteriorly toward the conical section, and before this part is entirely formed, sclerotization proceeds posteriorly toward the stylet knobs (Hechler and Taylor, 1966b). The sclerotized rings in the region of the stylet shaft of *Pratylenchus* do not seem to have any function in spear formation as reported by Anderson and Darling (1964b) for *D. destructor*. In *Pratylenchus* these rings are clearly connected to the guiding sheath of the stylet.

When the second stage larvae of *Pratylenchus* is ready to emerge, the stylet is thrust toward the egg membrane until it pierces it, and the larva emerges through the ruptured shell. This agrees with Clark's (1967) observation in *N. serendipiticus*. The larva of *Heterodera rostochiensis* Wollenweber uses the stylet tip to make a line of very close perforations through the shell which results in a continuous, almost straight cut across the end of the egg through which the larva emerges (Doncaster and Shepherd, 1967). In contrast to this, the larva of *C. xenoplax* emerges by rupturing the egg membranes through active movements inside the egg without the help of the stylet (Seshadri, 1965).

The genital primordium of second stage male and female larvae of the species of *Pratylenchus* studied has two germinal nuclei. The males of *Pratylenchus* follow the general pattern of gonad development as reported by van Weerd (1960) for the males of *R. similis* and by Hirschmann (1962) for the males of *Ditylenchus triformis* Hirschmann and Sasser. The females, on the other hand, follow either an amphidelphic or a monodelphic type of gonad development. The amphidelphic type is characteristic for the majority of the species studied. However, the general tendency is that the posterior gonad degenerates during the fourth molt and early adult stage, and a short post-ovular uterine branch is formed. Although only females of *P. zaeae* from one population were found to retain a posterior vestigial ovary in the adult, all the other species with amphidelphic developmental pattern are considered to be potentially capable of retaining a posterior vestigial ovary. The monodelphic type of gonad development was observed only in *P. scribneri*. Thus this species is not considered to be potentially capable of developing a posterior vestigial ovary.

Dickerson (1962) reported that the development of the gonad in *P. crenatus* and *P. penetrans* is at first amphidelphic, and that during the adult stage a posterior vestigial ovary about three cells long is present in most specimens. The presence of a posterior vestigial ovary has also been reported in *P. vulnus* by Sher and Allen (1953), *P. subpenetrans* Taylor and Jenkins by Taylor and Jenkins (1957) and in *P. coffeae* by Loof (1960). In these cases, however, the investigators did not determine, whether this posterior gonad branch was composed of germinal and epithelial cells or only epithelial cells.

The occurrence of two patterns of gonad development in the genus *Pratylenchus* indicates that the various species of this genus are in a state of active evolution. Prodelphic species with an amphidelphic pattern of gonad development may represent the first step in evolution. Prodelphic species with a monodelphic pattern of gonad development are probably more highly evolved.

Cell multiplication in the reproductive system of all *Pratylenchus* species studied is continuous throughout molts and stages. This type of cell multiplication has also been reported in

*D. triformis* by Hirschmann (1962), *D. destructor* by Anderson and Darling (1964a) and in *Scimura* spp. by Hechler and Taylor (1966a). In contrast to this, cell divisions have been observed only during molting in *Helicotylenchus vulgaris* Yuen by Yuen (1966) and in *H. dihystra* (Cobb) by Hirschmann and Triantaphyllou (1968).

The sex in *Pratylenchus* species can be recognized early in the second molt on the basis of the presence of specialized ventral chord nuclei in female larvae. Sex can be determined in the third stage larva of *N. serendipiticus* according to Clark (1967) and of *D. destructor* as reported by Anderson and Darling (1964a). Females and males have been distinguished in the third and fourth larval stages of *D. dipsaci* by Yuksel (1960) and in the second stage as reported for *Tylenchulus semipenetrans* Cobb by van Gundy (1958), *Meloidogyne incognita* (Kofoid and White) by Triantaphyllou and Hirschmann (1960) and for *D. triformis* by Hirschmann (1962).

### Summary

The cleavage pattern of *Pratylenchus* eggs was followed accurately to the eight-celled stage. The blastomeres exhibited pronounced cytoplasmic movement before undergoing cleavage. The first two cleavages were transverse to the longitudinal axis of the egg and resulted in four cells.

The first of four molts took place within the egg and the second stage larva emerged by piercing the egg shell with its stylet. Prior to each post-hatching molt, active motion of the larva ceased, the esophagus became faint, and the stylet shaft and knobs disappeared. This was followed by separation of the cuticle from the anterior end of the nematode. The discarded cuticle carried with it the conical part of the old stylet, cephalic framework and lining of amphidial ducts. Formation of the new sclerotized parts of the anterior end of the nematode started with the conical section of the stylet, proceeded with shaft and knobs and was completed with the cephalic framework. The esophagus became visible again, and the new cuticle separated completely from the old cuticle.

Two distinct types of gonad development were observed in females during postembryogenesis. The first type, common to most of the

species studied, was the amphidelphic type in which two gonads developed up to the fourth molt and then the posterior gonad degenerated. Some females of *P. zaeae* retained the posterior gonad even in the adult stage. The second type was the monodelphic type and was observed only in *P. scribneri*. In conclusion, all species studied, except *P. scribneri*, are potentially amphidelphic, *i.e.*, capable of developing a posterior gonad which in some cases can be maintained in the adult stage.

The sex in *Pratylenchus* could be recognized early in the second molt by the presence of four specialized ventral chord nuclei opposite the genital primordium in female larvae. Such nuclei were absent in male larvae.

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## Studies on Freshwater Larval Trematodes. XXV. Two New Species of Echinostome Cercariae<sup>1</sup>

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Lühe (1909) defined Leptocercous cercariae as distomes with slender tails narrower than body, even when contracted. He subdivided this group into Echinostome cercariae, with a collar and collar spines, Xiphidiocercariae, bearing a stylet, and Gymnocephalous, which were unarmed. This latter is a very unnatural and heterogenous assemblage, because there are many cases in which the cercaria is without a head collar and collar spines but these structures invariably appear in the metacercarial and adult stages, e.g., *Echinochasmus donaldsoni* Beaver, 1941 and *E. zubedakhanam* Nasir and Díaz, 1968. Had it not been for the knowledge of subsequent cyclic forms, these cercariae, which are true echinostomes, could have been erroneously considered as gymnocephalics. Likewise, the cercariae involved in this paper, *Cercaria udoi* sp. n. and *C. paraudo* sp. n., are without collar and collar spines but these are always present in the metacercariae. Therefore, they are treated as definite echinostomes. In feeding experiments with chicks, ducklings, pigeons and cats, we have thus far failed to obtain adult parasites; however, efforts are in progress.

All observations are based on freshly emerged cercariae and measurements are in microns.

### Results

#### 1. *Cercaria udoi* sp. n. (Fig. 1)

HOST: *Marisa cornuarietis* (L.) and *Pomacea glauca* (L.).

LOCALITY: Laguna de Campoma and Laguna de Chamariapa, Cariaco, Edo. Sucre, Venezuela.

DESCRIPTION: Body aspinose, with thick granular cuticle and several sensillae along anterior border. Tail aspinose, with undetermined

rows of sensillae, and without finfold. Twelve minute spines, not collar spines, in a semicircle, anterior to oral orifice. Prepharynx present. Pharynx antero-posteriorly elongate. Esophagus extremely short, together with ceca forming an anchor. Oral orifice surrounded by a row of papillae. Ventral sucker beset with two rows of spines. Cystogenous glands with rhabditiform contents. Excretory vesicle bipartite. Main excretory tubes after forming a loop in oral region extend posteriorly, as secondary excretory tubules, till excretory vesicle where another loop is formed, and continuing again anteriorly as primary excretory tubules. Secondary excretory tubules lined internally with vibratile cilia. Main excretory tubes enclosing refractile excretory granules. Caudal excretory duct ending at tip of tail. Flame cell formula:  $2(2+2+2+2) = 16$ . Measurements: body 180–222 by 132–144; tail 165–210 by 27–42; oral sucker 42–50 in diam.; ventral sucker 33–48 in diam.; prepharynx 6–15 long; pharynx 21–30 by 15–18. Development in rediae with a pharynx, an undivided collar, a pair of posterior locomotor appendages and a saccate gut.

FURTHER DEVELOPMENT: Encysting on gills and in visceral mesenteries of freshwater fishes, *Rivulus harti* (Boulenger) and *Lebistes reticulatus* (Peters). Cysts measuring 37–150 by 31–112 and enclose a metacercaria with definite collar spines.

#### 2. *Cercaria paraudo* sp. n. (Fig. 2)

HOST: *Marisa cornuarietis* (L.) and *Pomacea glauca* (L.).

LOCALITY: Canal de Riego, Calabozo, Edo. Guárico, Venezuela.

DESCRIPTION: Body aspinose, with sensillae and thick granular cuticle. Tail aspinose, with sensillae and without a finfold. A semicircle of 12 minute spines, not collar spines, in front of oral orifice. Oral and acetabular orifices bordered by a single row of papillae. Ventral

<sup>1</sup> Supported in part by a grant from Comisión de Desarrollo e Coordinación Científicas de Universidad de Oriente, Venezuela.

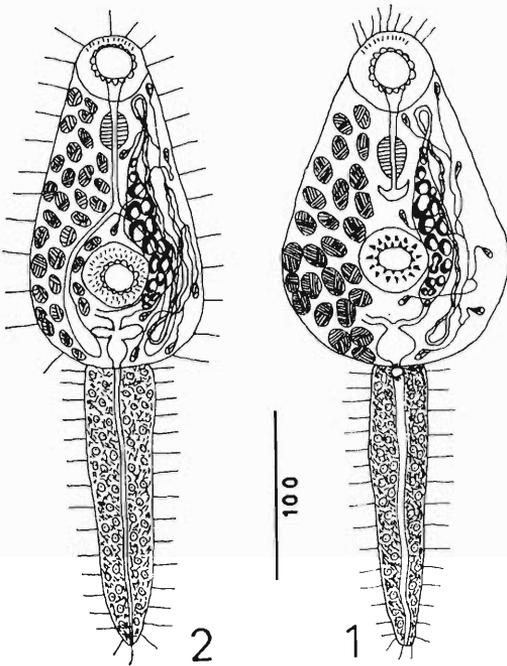


Figure 1. *Cercaria udoi* sp. n., cystogenous glands and parts of excretory tubes drawn on one side only.

Figure 2. *Cercaria paraudoii* sp. n., cystogenous glands and parts of excretory tubes drawn on one side only.

sucker located in posterior third of body and furnished with two rows of acetabular spines. Prepharynx present. Pharynx prominent. Esophagus long. Intestinal ceca extending to posterior end of body. Cystogenous glands with rhabditiform contents. Secondary excretory tubules after forming another loop, near excretory vesicle, continue again anteriorly and considerably anterior to ventral sucker divide into anterior and posterior lateral collecting excretory tubules. Main excretory tubes enclosing refractile excretory granules. Secondary excretory tubules lined internally with vibratile cilia. Caudal excretory duct extending to posterior tip of tail. Flame cell formula:  $2(2+2+2+2) = 16$ . Measurements: body 174–204 by 84–108; tail 162–192 by 36–45; oral sucker 36–45 in diam.; ventral sucker 36–45 in diam.; pharynx 18–21 by 15–18. Development in rediae with a pharynx, and un-

divided collar, a pair of posterior locomotor appendages and a saccate gut.

**FURTHER DEVELOPMENT:** Encysting on gills and visceral mesenteries of freshwater fishes, *Rivulus harti* (Boulenger) and *Lebistes reticulatus* (Peters). Metacercaria with a definite collar and collar spines.

**REMARKS:** *Cercaria udoi* and *C. paraudoii* are indistinguishable on the basis of aspinose body and tail, presence of a semicircle of 12 minute spines anterior to oral orifice, two rows of acetabular spines, rhabditiform contents of cystogenous glands, presence of cilia in secondary excretory tubes, refractile excretory granules in main excretory tubes, formation of excretory loops in oral and posterior regions, identical flame cell formulae, developing in same intermediate hosts, encysting in same site in same species of fishes and finally in the possession of collar spines in metacercarial stages. On the other hand *C. udoi* differs from *C. paraudoii* in having a short esophagus, short appendage-like intestinal ceca, anisodiametric suckers, different shape of excretory vesicle and secondary excretory tubules not dividing into anterior and posterior lateral collecting excretory tubules.

### Discussion

The cercariae of *Echinochasmus donaldsoni* Beaver, 1941 and *E. zubedakhaname* Nasir and Diaz, 1968 are, like *C. udoi* and *C. paraudoii*, devoid of collar spines which are present in their metacercariae. Other points of similarity include: the rod-like contents of cystogenous glands; pattern of principal excretory system; absence of finfold on tail; rediae with an undivided collar; and in the employment of fishes as the second intermediate hosts. However, there are several distinguishing characters which set them apart as independent entities.

*Cercaria udoi* has an anchorlike gut and this feature alone is enough for its separate designation.

In the cercariae of *Echinochasmus donaldsoni* and *C. paraudoii* the intestinal ceca extend to posterior end of body and there are spines on ventral sucker, thus these are very closely related. On the other hand, *C. paraudoii* is distinguished from *E. donaldsoni* by having papillae around oral and acetabular orifices,

isodiametric suckers, which are twice as large and the pharynx is also twice as large. The division of main excretory tubes and flame cell formula are unknown in *E. donaldsoni*.

In the cercaria of *E. zubedakhaname* the intestinal ceca reach as far as equatorial level of ventral sucker; oral sucker is larger than ventral sucker; acetabular papillae are lacking; secondary excretory tubes, after forming a loop in pharyngeal region, divide into anterior and posterior lateral collecting excretory tubules; and there are 12 flame cells in all. On the other hand, in *C. paraudoi* the ceca extend to posterior end of body; suckers are isodiametric; acetabular papillae are present; secondary excretory tubes, after forming another loop posteriorly, run again anteriorly, dividing into lateral collecting tubules; and there is a total

of 16 flame cells. At the same time, they resemble each other in the presence of oral papillae, acetabular spines, cilia in secondary excretory tubes, and finally both develop in the same intermediate host, *Pomacea glauca*.

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## Acanthocephala of Louisiana Turtles with a Redescription of *Neoechinorhynchus stunkardi* Cable and Fisher, 1961<sup>1</sup>

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Some limited investigations have been conducted on the acanthocephalan parasites in North American turtles. Leidy (1851) described *Neoechinorhynchus emydis* as the first species in turtles. In 1954, Cable and Hopp described two species, viz., *N. pseudemydis*, and *N. chrysemydis*. Fisher (1960) made a survey of the Acanthocephala from North American turtles in the Mississippi drainage system, and examined specimens deposited in the United States National Museum. The study resulted in the recovery of the three known species of Acanthocephala and the description of a new and the fourth species of *N. emyditoides*. Cable and Fisher (1961) described the female of *N. stunkardi* and Little and Hopkins (1968) described *N. constrictus*, bringing the number of species in turtles to six.

A review of literature shows that very little

significant investigation has been conducted on the acanthocephalan parasites of Louisiana turtles. Fisher's (1960) study included the examination of 12 *Pseudemys scripta elegans* (Wied) from Louisiana with the recovery of *N. pseudemydis* and *N. emyditoides*. He also obtained from Dr. F. Sogandares of Tulane University some specimens identified as *N. chrysemydis* and *P. scripta* subsp.

The present study is a survey conducted between the spring of 1965 and the summer of 1968 on turtles collected from southeastern Louisiana.

#### Materials and Methods

Turtles were decapitated and opened by cutting the connection between the carapace and plastron with an electric hand saw. The gut was then removed, opened and examined for acanthocephalan parasites.

The methods employed in treating obtained

<sup>1</sup>This investigation was supported by NSF Institutional Grant No. 8-1281.

specimens are essentially similar to those of Fisher (1960) and Bullock (1962) but with some modifications. The specimens were placed in tap water and left in the refrigerator until they were turgid and the proboscis fully everted. The parasites were then fixed in Demke's AFA, transferred to 70% alcohol, and pricked by means of fine pins to permit passage of staining fluids and prevent opacity. Pricking the worms before fixation was noted to cause their contraction and retraction of the proboscis. They were stained with Meyer's acid carmine, destained, dehydrated, and gradually cleared by successive treatment for several hours in 35, 50, 75, and then 100% methyl salicylate. Beechwood creosote was occasionally used as a clearing agent. The female specimens were easily identified by simply observing their posterior ends under the dissecting microscope and examining the live eggs under the compound microscope. All drawings were made with the aid of camera lucida.

### Results

Since the author's preliminary report (Acholou, 1966) on this investigation, 81 additional turtles have been examined bringing the total to 150 comprising 11 species and made up of 85 females and 65 males. Of this number, 37 (43.5%) of 85 females and 26 (40%) of 65 males, constituting five species were infected. Five species of Acanthocephala were recovered (see Table 2).

*N. emyditoides* is the most prevalent species. It was found in 41 turtles of three different species. There were 23 cases of mixed infection in three species of turtles viz., *P. s. elegans*, *P. floridana hoyi* (Holbrook), and *Trionyx spinifer* Le Sueur. One *P. s. elegans* harbored a multiple infection of *N. pseudemydis*, *N. emyditoides*, *N. chrysemydis* and *N. constrictus*.

Cable and Fisher (1961) described the female of *Neoechinorhynchus stunkardi* from two specimens recovered from *Graptemys pseudogeographica* (Gray) and museum material collected by Dr. H. W. Stunkard. They indicated that the male of this species had not

been recognized with certainty. From one *Graptemys kohni* Baur (Mississippi map turtle) collected from Bayou Goula, Louisiana, 34 specimens of this species (26 females and 8 males) were found. From these specimens, this species is redescribed below.

### *Neoechinorhynchus stunkardi* Cable and Fisher, 1961 (Figs. 1-3)

**DESCRIPTION:** (all measurements in millimeters) with characteristics of the genus *Neoechinorhynchus* Hamann, 1892. Live gravid specimens yellowish in color. Body long and slender, curved ventrally. Proboscis hooks in three circlets of six hooks each arranged quin-cunxially. Left lemniscus binucleate, longer than uninucleate right one.

**MALE:** Trunk up to 15.05 long and 1.152 wide. Proboscis 0.144-0.173 long and 0.158-0.180 wide. Lateral hooks of anterior circllet posterior to others 0.065-0.072 long; lateroventral and laterodorsal hooks of that circllet, 0.054-0.058. Hooks of middle circllet similar and measuring 0.038-0.050 long; lateral hooks of basal circllet 0.021-0.029 long, others 0.023-0.043. Proboscis receptacle 0.437-0.576 long, 0.158-0.187 wide. Testes contiguous, reproductive system occupying posterior 43-60% of trunk length; anterior testis 1.353-1.497 long, 0.417-0.489 wide; posterior testis 1.123-1.339 by 0.316-0.496, immediately followed by cement gland measuring 1.857-2.390 long, 0.388-0.561 wide. Cement receptacle globular, posterior to cement gland, its duct extending posteriorly to open in bursa. Fully extruded bursa 0.590-0.792 long.

**FEMALE:** Trunk up to 22.6 long and 1.182 in maximum width at about level of first ventral subcuticula nucleus, then tapering gradually until reaching caudal swelling terminating with conical papilla. Proboscis 0.163-0.201 long and 0.163-0.180 wide. Lateral hooks of anterior circllet posterior to others 0.074-0.083 long; lateroventral and laterodorsal hooks of that circllet, 0.064-0.073. Hooks of middle circllet similar and measuring 0.042-

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Figures 1-3. *Neoechinorhynchus stunkardi*. 1. Female, anterior end, lateral view. 2. Female, posterior end, lateral view. 3. Male, posterior end, lateral view.

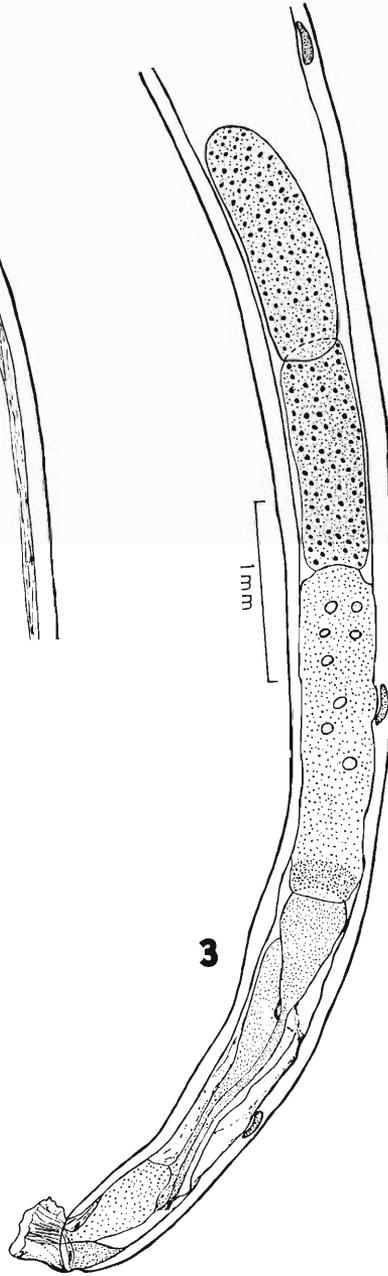
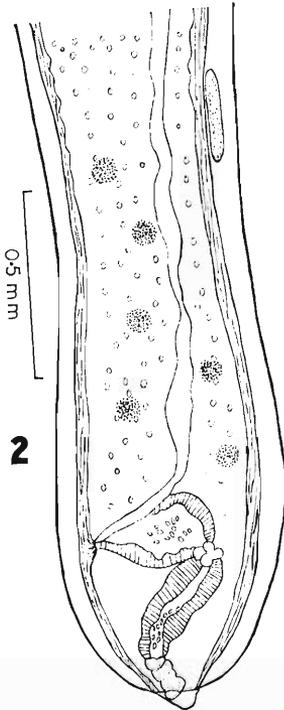
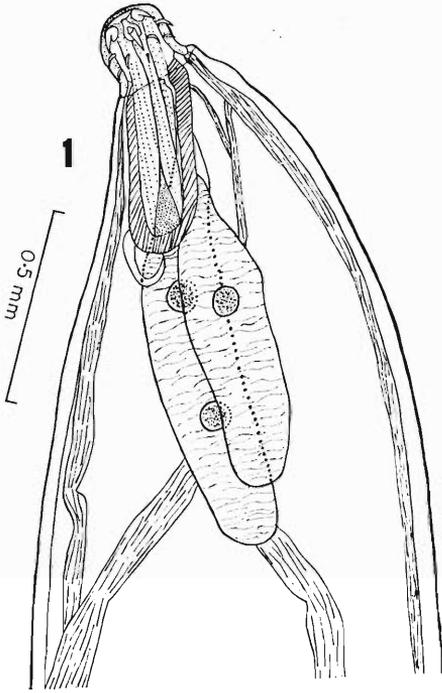


Table 1. Incidence of infection.<sup>1</sup>

Turtles species	No. examined	No. infected	Species of Acanthocephala obtained	% of infection
1. <i>Pseudemys scripta elegans</i> (Red-eared turtle)	78	44	<i>Neoechinorhynchus pseudemydis</i> <i>N. emyditoides</i> <i>N. chrysemydis</i> <i>N. constrictus</i>	56.4
2. <i>Pseudemys floridana hoyi</i> (Missouri slider)	12	8	<i>N. chrysemydis</i> <i>N. emyditoides</i>	66.6
3. <i>Chelydra serpentina serpentina</i> (common snapping turtle)	13	1	<i>Neoechinorhynchus</i> sp. (3 immature specimens)	7.6
4. <i>Trionyx spinifer</i> (spiny softshell)	18	9	<i>N. emyditoides</i> <i>N. chrysemydis</i>	50.0
5. <i>Graptemys kohni</i> (Mississippi map turtle)	1	1	<i>N. stunkardi</i>	100

<sup>1</sup> *Chrysemys picta dorsalis* (1), *Kinosternon subrubrum hippocrepis* (9), *Sternotherus odoratus* (5), *Terrapene carolina carolina* (7), *T. c. triunguis* (5), and *Trionyx muticus* (1) were negative.

0.063 long; lateral hooks of basal circlet 0.026–0.031 long, others 0.035–0.046. Proboscis receptacle 0.461–0.576 long, 0.163–0.194 wide. Terminal female apparatus sigmoid in lateral aspect. Mouth of uterine bell 0.648–0.770 in straight line from tip of posterior papilla; uterus exclusive of bell and selector apparatus 0.252–0.331 long, 0.107–0.198 wide; vagina 0.107–0.187 long, 0.058–0.079 wide; genital pore on ventral slope of caudal papilla. Fully formed eggs living or preserved in formalin, 0.024–0.026 long, 0.015–0.017 wide; inner shell with few (usually four) minute head-line excrescences, subequatorial in position. Acanthor 0.020–0.022 long, 0.006–0.008 wide.

Host: (1) The false map turtle, *Graptemys pseudogeographica* (Gray), Illinois. (2) Mississippi map turtle, *Graptemys kohni* Baur, Bayou Goula, Louisiana. Material deposited; USNM Helm. Coll. No. 71292.

### Discussion

Fisher (1960) concluded from his survey of Acanthocephala from North American turtles that *Neoechinorhynchus emydis* is the species most restricted in its distribution while *N. chrysemydis* and *N. emyditoides* are most southern in distribution. The results of this present study give credence to his statement. Of 150 turtles autopsied, none had *N. emydis* infection. It must, however, be noted that no *Graptemys geographica*, its most natural host, was collected or examined. It is the author's

opinion that the nonrecovery of *N. emydis* in this study does not reflect its complete absence from Louisiana, but may rather be due to the paucity of its appropriate host in this state.

There were several cases of multiple infection in the turtles examined. This is therefore of common occurrence among the acanthocephalan parasites of turtles. Fisher (1960) also reported encountering mixed infection in some turtles he autopsied. It is plausible to infer that host specificity is not pronounced in turtle Acanthocephala. The author is in agreement with Fisher's statement: "Parasitism of turtles by Acanthocephala seems to depend more on ecology and geographical distribution of parasites and host's food habits than on host specificity." None of the land turtles examined harbored any infection (see Table 1). This scarcity or lack of infection may probably be attributed to the food habit of the turtles rather than to their resistance to infection. A confirmation of this statement may, however, require experimental infection of some land turtles, or the discovery of natural infection by a future investigator.

Several turtles examined harbored more than 80 parasites. The heaviest infection was recorded from one *Pseudemys scripta elegans* which had a double infection of over 600 *N. pseudemydis* and *N. emyditoides*. The worms were very entangled and seemed to have caused an occlusion of the intestine of the turtle.

**Table 2. Species of Acanthocephala found.**

Parasite	Species of turtle	No. examined	No. infected	% of infection
1. <i>Neoechinorhynchus pseudemydis</i>	<i>Pseudemys scripta elegans</i>	78	14	17.8
2. <i>N. chrysemydis</i>	<i>P. s. elegans</i>	78	16	20.5
	<i>P. floridana hoyi</i>	12	8	66.6
	<i>Trionyx spinifer</i>	18	1	5.5
3. <i>N. emyditoides</i>	<i>P. s. elegans</i>	78	35	44.8
	<i>P. f. hoyi</i>	12	3	25.0
	<i>T. spinifer</i>	18	3	16.6
4. <i>N. constrictus</i>	<i>P. s. elegans</i>	78	1	1.2
5. <i>N. stunkardi</i>	<i>Graptemys kolmi</i>	1	1	100
6. <i>Neoechinorhynchus</i> sp.	<i>Chelydra serpentina serpentina</i>	13	1	7.6
7. <i>Neoechinorhynchus</i> sp.	<i>P. s. elegans</i>	78	2	2.5
8. <i>Neoechinorhynchus</i> sp.	<i>T. spinifer</i>	18	4	22.2

### *Neoechinorhynchus pseudemydis* Cable and Hopp, 1954

This species and *N. chrysemydis* were adequately refigured and redescribed by Fisher (1960) and no additional information is deemed necessary. It was recovered from the small and large intestine of 14 (17.8%) of 78 *Pseudemys scripta elegans* examined. It was collected as a single infection from four turtles. The largest number of specimens collected from one turtle was 50 and the smallest one was one. It occurred as a quadruple infection with *N. emyditoides*, *N. chrysemydis*, and *N. constrictus* in one turtle, triple in three, and double in six.

### *Neoechinorhynchus chrysemydis* Cable and Hopp, 1954

This species was collected from the small and large intestine of 25 (16.6%) of 150 turtles autopsied. Three species of turtles were infected with this parasite (see Table 2). It was collected as a single infection from eight turtles. The specimens collected from a turtle ranged from nine to 95. It occurred as a double infection with *N. emyditoides* in nine *P. s. elegans*, three *P. f. hoyi*, and one *T. spinifer*. The two latter species are new host records for *N. chrysemydis*.

### *Neoechinorhynchus emyditoides* Fisher, 1960

This species was adequately described by Fisher and requires no further description. It was the most prevalent species found and was collected mainly from the small intestine of 41 (27.3%) of 150 turtles examined. Table 2

shows that three species of turtles harbored this species. It was collected as a single infection from 18 turtles. The largest number of specimens recovered from one turtle was 70 and the smallest was one. It occurred as a double infection with *N. chrysemydis* in 13 turtles and with *N. pseudemydis* in six. *P. floridana hoyi* and *T. spinifer* are new host records for this species.

### *Neoechinorhynchus constrictus* Little and Hopkins, 1968

This worm was collected from the small intestine of only one *P. s. elegans* which in addition harbored *N. chrysemydis*, *N. emyditoides* and *N. pseudemydis*. Of over 100 specimens recovered from this turtle, 13 females and an undetermined number of males were found.

### *Neoechinorhynchus* spp.

A single specimen was collected from one *Pseudemys scripta elegans*. It was a wrinkled and opaque worm with the proboscis retracted, thus precluding specific identification. Two immature worms were collected from another turtle of the same species, three from one *Chelydra serpentina serpentina* and two from each of two *T. spinifer*. In addition, one male worm was collected from each of two other *T. spinifer*. None of these could be identified to species.

### Summary

One hundred and fifty turtles representing 11 species were examined. *Neoechinorhynchus pseudemydis* Cable and Hopp, 1954, *N. chryse-*

Table 3. Comparison of known species of turtle *Neoechinorhynchus*.

	<i>N. emydis</i>	<i>N. pseudemydis</i>	<i>N. chrysemydis</i>	<i>N. emyditoides</i>	<i>N. stunkardi</i>	<i>N. constrictus</i>
<i>Male</i>						
Trunk						
length	up to 14.5	up to 26.4	up to 12.9	up to 23.1	15.05	up to 19.2
width	up to 1.04	up to 0.89	up to 0.69	up to 0.842	up to 1.152	up to 0.800
Percentage occupied by reproductive system	42-50%	35-42%	45-51%	42-49%	43-60%	41-60%
Anterior testis						
length	0.90-1.04	1.85-2.14	0.645-0.980	1.386-1.504	1.353-1.497	1.050-1.710
width	0.29-0.30	0.40-0.44	0.190-0.263	0.380-0.472	0.417-0.489	0.270-0.410
Posterior testis						
length	0.88-0.93	2.60-2.90	0.638-0.810	1.118-1.386	1.123-1.339	1.150-1.950
width	0.31-0.36	0.43-0.45	0.190-0.278	0.311-0.483	0.316-0.496	0.300-0.370
Cement gland						
length	1.34-1.76	1.65-3.00	1.13-1.22	1.45-1.80	1.857-2.390	1.70-2.63
width	0.225-0.307	0.255-0.375	0.263-0.360	0.248-0.300	0.388-0.561	0.280-0.370
<i>Female</i>						
Trunk						
length	up to 22.2	up to 39.2	up to 15.34	up to 34.3	up to 22.6	up to 23
width	up to 1.5	up to 1.25	up to 0.73	up to 0.940	up to 1.182	up to 0.820
Uterus						
length	0.277-0.360	0.233-0.360	0.199-0.248	0.175-0.375	0.252-0.331	0.248-0.343
width	0.46-0.91	0.100-0.157	0.058-0.096	0.083-0.158	0.107-0.198	0.046-0.121
Posterior end	rounded to irregular; somewhat pointed	with 2 lateral somewhat swollen lobes	with rounded papilla	usually rounded but slightly irregular in some	swollen, with conical papilla	deeply constricted
Living eggs						
length	0.023-0.025	0.042-0.054	0.055-0.060	0.025-0.030	0.024-0.026	0.030-0.037
width	0.015-0.019	0.018-0.028	0.019-0.022	0.010-0.013	0.015-0.017	0.007-0.012
<i>Hosts</i>						
	<i>Graptemys geographica</i>	<i>Pseudemys scripta</i>	<i>Pseudemys s. elegans</i>	<i>Pseudemys s. elegans</i>	<i>Graptemys pseudo-geographica</i>	<i>Pseudemys s. elegans</i>
	<i>G. pseudo-geographica</i>	<i>Pseudemys elegans</i>	<i>P. floridana hoyi</i>	<i>P. s. troosti</i>	<i>G. kohni</i>	
	<i>Emys blandingii</i>	<i>Pseudemys s. troosti</i>	<i>Chrysemydis picta</i>	<i>P. floridana hoyi</i>		
	<i>Pseudemys scripta</i>	<i>Chrysemydis picta marginata</i>	<i>Emys blandingii</i>	<i>Emys blandingii</i>		
	subsp.	<i>Emys marginata blandingii</i>	<i>Trionyx spinifer</i>	<i>Trionyx spinifer</i>		

*emydis*, Cable and Hopp, 1954, *N. emyditoides* Fisher, 1960, *N. stunkardi* Cable and Fisher, 1961, and *N. constrictus* Little and Hopkins, 1968, were identified from the 63 infected turtles. *N. emyditoides* was most prevalent. Several cases of multiple infection were encountered. *Pseudemys floridana hoyi* (Holbrook) and *Trionyx spinifer* Le Sueur are new host records for *N. chrysemydis* and *N. emyditoides*, and *Graptemys kohni* Baur is new for *N. stunkardi*. *N. constrictus* and *N. stunkardi*

are new Louisiana records. A redescription of *N. stunkardi* which includes the male, is given.

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## Research Notes

Beginning with v. 37 (January 1970) the Proceedings of the Helminthological Society of Washington will publish short papers of the type best known as "Research Notes." Becker (1961, *J. Parasit.* 47: 396) pointed out that the research note is difficult to define. Nevertheless he gave a concept of the research note which has provided workable guidelines. A summary of these views, with some minor modifications, is presented here as the editorial policy of the Proceedings in regard to research notes.

Research notes should not exceed 1-2 printed pages; longer manuscripts should be prepared as regular articles. Research notes will not be a vehicle for preliminary reports or condensa-

tions of work to be published *in extenso* elsewhere. They should not be used to describe new taxa.

The typescript and style should conform to that of regular articles in the Proceedings except that there are no formal divisions. An abstract is not required. Citations, in the abbreviated form used in this announcement, are given in the text. The author's name and address appear at the end of the article. Examples of the format appear in this number of the Proceedings (Coil, 1969, *Proc. Helm. Soc. Wash.* 36: 204 and Little, 1969, *Proc. Helm. Soc. Wash.* 36: 286-287).—FRANCIS G. TROMBA, *Editor*.

## Abstracts

Beginning with v. 37 (January, 1970) all articles published in the Proceedings of the Helminthological Society of Washington, except research notes, shall be accompanied by an abstract. A summary will no longer be re-

quired. In print the abstract will precede the article. Authors should prepare abstracts with care as they will appear without change in Biological Abstracts.

## Some Trematodes from Louisiana Snakes with an Evaluation of the Specific Characters of *Stomotrema pusilla*

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A study of reptilian trematodes in Louisiana was begun in September, 1963 and concluded in September 1966. The following is a preliminary report on the examination of 307 snakes representing 11 genera and 14 species.

All measurements are in millimeters. Numbers in parentheses following the ranges are averages. Table 1 lists the parasite, hosts and incidence of parasitism.

### Family Plagiorchidae Luhe, 1901

#### Subfamily Styphlorodinae Dollfus, 1937

#### *Dasymetra villicaeca* Byrd, 1935

Observations were based on 96 specimens from the mouth, esophagus and/or small intestine of 33 snakes representing eight host species. *C. constrictor flaviventris*, *N. fasciata confluens*, *N. fasciata fasciata*, *N. fasciata pleuralis* and *T. sauritus* are new hosts for this parasite, other hosts are listed in Table 1.

#### *Ochetosoma laterotrema* (Byrd & Denton, 1938)

Observations are based on 22 specimens from the mouth, esophagus, stomach, and small intestine of two *Agkistrodon piscivorus leucostoma*. Several minor variations exist between the present material and that originally described. The maximum body length recorded here was 4.91 as compared to 4.3 and the minimum body width was 0.552 compared to 0.80. Pharyngeal measurements ranged from 0.132–0.179 by 0.142–0.217. The original authors listed the pharynx as being 0.18 in diameter.

Testes size was somewhat different, 0.236–0.287 long by 0.17–0.264 wide as compared to 0.18–0.36 long by 0.11–0.24 wide. Cirrus sac length ranged from 0.65 to 1.2 as compared to 0.8 given originally.

Egg size was given originally as 0.048 long by 0.02 wide, ranges of 0.04–0.053 in length by 0.019–0.026 in width were recorded in this study.

*O. laterotrema* is reported for the second time from Louisiana in *Agkistrodon piscivorus leucostoma*, the trophotype.

#### *Ochetosoma kansense* (Crow, 1913)

Observations of *Ochetosoma kansense* from Louisiana snakes were based on 128 specimens from the mouth, esophagus and/or stomach of 14 snakes representing four host species.

The present material agrees with the concept of this species as reported by Dubois and Mahon (1959).

*Diadophis punctatus stictogenys* represents a new host for this parasite.

#### *Ochetosoma aniarum* (Leidy, 1891)

Observations were made on 174 specimens from the mouth and esophagus of 33 snakes representing six host species.

The present material differed somewhat from the published data on *O. aniarum*. Ovaries as large as 0.30 were encountered, contrasted with a maximum of 0.18 in the literature. The testes, for the most part, were larger in the present material. The cirrus sac ranged from 0.19–0.95 (0.55), which represents much more variation in the size of this organ than previously reported.

Two hosts, *Agkistrodon piscivorus leucostoma* and *Lampropeltis getulus holbrooki* represent new hosts for this parasite.

#### *Stomotrema pusilla* (Guberlet, 1928)

SYNONYMS: *Stomotrema guberleti* Byrd, 1937; *Stomotrema faranciae* Parker, 1941.

Observations were based on 24 specimens from the mouth of three *Farancia abacura reinwardti* (Schlegel).

Dubois and Mahon (1959) presented evidence for considering *S. guberleti* a synonym of *S. pusilla*; the author is in agreement with these workers.

There are a few points in which the present material differs from the published data. The body length, 2.24, given by Byrd (1937) and

Table 1. Hosts, parasites, and incidence.

Host	Parasite	No. infected/ No. examined
<i>Agkistrodon piscivorus leucostoma</i>	<i>O. laterotrema</i>	2/19
	<i>O. aniarum</i>	1/19
	<i>S. magna</i>	2/19
	<i>O. kansense</i>	7/19
<i>Coluber constrictor flaviventris</i>	<i>D. villicaeca</i>	1/16
	<i>S. magna</i>	1/16
	<i>O. kansense</i>	5/16
<i>Diadophis punctatus stictogenys</i>	<i>O. kansense</i>	1/13
<i>Farancia abacura reinwardti</i>	<i>S. pusilla</i>	3/5
<i>Elaphe obsoleta linheimeri</i> (Say)	<i>O. kansense</i>	1/6
<i>Lampropeltis getulus holbrooki</i>	<i>O. aniarum</i>	1/17
<i>Natrix cyclopiion cyclopiion</i> (Dumeril and Bibron)	<i>D. villicaeca</i>	8/25
	<i>O. aniarum</i>	3/25
<i>N. erythrogaster flavigaster</i>	<i>D. villicaeca</i>	11/30
	<i>O. aniarum</i>	9/30
	<i>S. magna</i>	1/30
<i>N. fasciata confluens</i>	<i>D. villicaeca</i>	3/34
	<i>O. aniarum</i>	8/34
<i>N. fasciata fasciata</i>	<i>D. villicaeca</i>	1/15
	<i>O. aniarum</i>	6/15
	<i>L. megasorchis</i>	1/15
<i>N. rhombifera rhombifera</i>	<i>D. villicaeca</i>	7/42
	<i>O. aniarum</i>	5/42
	<i>S. magna</i>	1/42
<i>N. fasciata pleuralis</i>	<i>D. villicaeca</i>	1/5
<i>Opheodrys aestivus</i>	<i>B. salamandrac</i>	6/32
<i>Regina grahami</i>	<i>T. pseudoaculeatus</i>	2/17
<i>Storeria dekayi tropica</i>	<i>B. salamandrac</i>	1/6
<i>Thamnophis sauritus</i>	<i>D. villicaeca</i>	1/35
	<i>L. primus</i>	3/35

that given by Guberlet (1928), 1–2.3 are essentially the same as the average, 2.09, for the present material. The oral sucker, acetabulum and ovary were smaller than that given by either Guberlet or Byrd. The above mentioned differences are of no taxonomic importance and may be readily explained on the basis of individual variation and state of contraction of the worm when killed.

Parker (1941) described *S. faranciae* and stated, "This species is closely related to *Stomotrema guberleti* Byrd, 1937; in fact superficially the resemblance is so great that at first they were considered identical." He stated that *S. faranciae* differed in that the body size, acetabulum, ovary, testes, cirrus sac and eggs were smaller than described by Byrd.

With the use of the type specimens involved and the material collected in this study the author concluded that *S. faranciae* is in fact identical with *S. pusilla* and should be considered a synonym of the latter. Table 2 shows a comparison of the published data of *S. pusilla* and *S. faranciae* as well as the material collected in Louisiana.

*Stomotrema pusilla* is reported for the second time from Louisiana. The host, *Farancia abacura reinwardti*, is the trophotype for this species.

### *Styphlodora magna* (Byrd & Denton, 1938)

Observations are based on six specimens from the gall bladder of five snakes representing four host species. The specimens studied here agree with the description of *S. magna* except for a few minor variations. The maximum length given was 8.4, worms as large as 9.9 were recorded in this study, however, the average was 8.05. The only character of taxonomic importance that deviated from the published data was the ovary. The maximum size was given as 0.38, some ovaries in this study were as large as 0.42.

*S. magna* is reported from *N. erythrogaster flavigaster*, *N. rhombifera rhombifera*, *Coluber constrictor flaviventris* and *Agkistrodon piscivorus leucostoma*, all of which are new hosts for this parasite. This represents the second report of this parasite from Louisiana.

### *Lechriorchis megasorchis* (Crow, 1913)

Observations were based on two specimens from the mouth of one of 15 *Natrix fasciata fasciata* examined. The material from this study was considerably smaller in every respect than the original material, however, the material used by Crow (1913) was older than the present specimens. Both specimens were just beginning to produce eggs while those figured by Crow were fully mature.

The relative positions of the testes and vitellaria leave no doubt that this material is conspecific with *L. megasorchis*.

This represents the first report of *L. megasorchis* from Louisiana. The host, *N. fasciata fasciata*, represents a new host record.

### *Lechriorchis primus* (Stafford, 1905)

Observations were based on two mature and one immature specimens from the esophagus

Table 2. Comparative measurements of *S. faranciae*, *S. pusilla*, and present material.

	<i>S. faranciae</i>	<i>S. pusilla</i>	Present Material
Body			
length	1.22-2.19(1.56)	1.0-2.24	1.47-3.15(2.09)
width	0.64-0.83(0.76)	0.5-0.84	0.50-1.01(0.68)
Oral sucker			
length	0.28-0.37(0.35)	0.26-0.37	0.27-0.48(0.34)
width	0.30-0.37(0.36)	0.40	0.28-0.45(0.35)
Acetabulum			
length	0.27-0.44(0.48)	0.38-0.98	0.26-0.50(0.34)
width	0.31-0.44(0.38)	0.22-0.46	0.25-0.53(0.36)
Pharynx			
length	0.10-0.14(0.12)	0.13-0.17	0.08-0.14(0.11)
width	0.10-0.19(0.14)	0.117	0.10-0.19(0.14)
Ovary			
length	0.07-0.14(0.11)	0.12-0.14	0.08-0.16(0.12)
width	0.08-0.15(0.11)	0.16-0.175	0.10-0.15(0.12)
Left testis			
length	0.16-0.26(0.21)	0.16-0.25	0.16-0.30(0.21)
width	0.11-0.18(0.15)	0.12-0.21	0.11-0.23(0.17)
Right testis			
length	0.15-0.29(0.23)	0.22-0.36	0.14-0.37(0.24)
width	0.15-0.18(0.15)	0.14-0.15	0.09-0.24(0.17)
Cirrus sac			
length	0.27-0.43(0.34)	0.59	0.22-0.56(0.39)
width	0.09-0.13(0.11)	0.15	0.05-0.13(0.09)
Seminal vesicle			
length	—	—	0.09-0.28(0.18)
width	—	—	0.05-0.11(0.07)
Egg			
length	0.034-0.044	0.027-0.03	0.03-0.04(0.035)
width	0.017-0.021	0.015-0.017	0.015-0.023(0.017)

of three *Thamnophis sauritus*. There were no significant differences in the values given by Talbot (1933) and those recorded from this study. In addition to the measurements there was agreement with other features pointed out in the original description, such as the condition of the vitellaria, length of the intestinal caeca and the relative positions of the other internal organs.

#### Family Telorchiidae Stunkard, 1924

##### Subfamily Telorchiinae Looss, 1899

#### *Telorchis pseudoaculeatus* Dollfus, 1929

Observations were based on 26 specimens from the small intestine of two *Regina grahami* (Baird and Girard). The present material agrees in every respect with the published data.

This represents the first report of this trematode from Louisiana.

#### Family Brachycoeliidae Johnston, 1912

##### Subfamily Brachycoeliinae Looss, 1899

#### *Brachycoelium salamandrae* (Froelich) Dujardin, 1845

Observations were based on 26 specimens from the small and large intestine of seven snakes representing two host species. Rankin (1938) in his discussion of the genus reduced to synonymy all of the members of this genus occurring in the United States. He concluded that characteristics upon which these species were based were too variable to be considered of specific importance. The author is in agreement with Rankin.

All of the measurements of the present material fall well within the limits of this species as outlined by Rankin.

Both hosts recorded in this study represent new host records for Louisiana.

### Summary

Based on material from Louisiana snakes it is proposed that *Stomotrema faranciae* become a synonym of *S. pusilla*.

New hosts are reported for *Dasymetra villicaeca*, namely, *Coluber constrictor flaviventris* (Say), *Natrix fasciata confluens* Blanchard, *N. fasciata fasciata* (Linné.), *N. fasciata pleuralis* (Cope), and *Thamnophis sauritus* (Linné.). *Styphlodora magna* is reported for the first time from *N. erythrogaster flavigaster* Conant, *N. rhombifera rhombifera* (Hallowell), *C. constrictor flaviventris* and *Agkistrodon piscivorus leucostoma* (Troost). *Lechriorchis megasorchis* is reported for the first time from Louisiana in *N. fasciata fasciata* which represents a new host. *Diadophis punctatus stictogenys* Cope is reported as a new host for *Ochetosoma kansense*. *Agkistrodon piscivorus leucostoma* and *Lampropeltis getulus holbrooki* (Stejneger) are new hosts for *Ochetosoma aniarum*. *Brachycoelium salamandrae* is reported for the first time from *Opheodrys aestivus* (Linné.) and *Storeria dekayi tropica* Cope. *Ochetosoma laterotrema*, *Lechriorchis primus* and *Telorchis pseudoaculeatus* are also reported from Louisiana snakes.

### Acknowledgments

Gratitude is expressed to Dr. Harry J. Bennett for his guidance and comments during this study. Acknowledgments are also due Drs. Brent B. Nickol and Kenneth C. Corkum and Mr. Harry L. Henson.

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## Report of the Brayton H. Ransom Memorial Trust Fund

FUNDS ON HAND, 1 January 1968 .....	\$2,645.96
RECEIPTS: Interest received in 1968 .....	127.93
DISBURSEMENTS: Grant to Helminthological Society of Washington .....	10.00
BALANCE ON HAND, 31 December 1968 .....	2,763.89

A. O. FOSTER  
Secretary-Treasurer

## Passage of Leptomonad *Leishmania tarentolae* Through the Digestive Tract of Nematodes, *Neoaplectana glaseri*

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Leptomonad *Leishmania tarentolae* Parrot, 1949, cultivated from the blood of an African gecko, can be adapted to grow in a chemically defined medium (Trager, 1957). This medium also sustains the development of *Neoaplectana glaseri* Steiner, 1929, a nematode parasitic in beetle grubs, although the nutritional requirements of the protozoan are not identical with those of the worm (Jackson, 1962a, b; 1969). Grown together in culture, the small protozoa may be ingested by worms and with light microscopy one can see living leptomonads in the large anterior intestinal lagoon of adult female *N. glaseri*. Few, if any, living leptomonads can be seen directly in the posterior intestinal lagoon and the following experiments were devised to see whether the leptomonads could survive passage through the nematode's digestive tract.

### Materials and Methods

Preparation of media and maintenance of axenic conditions for culturing leptomonad *Leishmania tarentolae* have been detailed by Trager (1957) and for culturing *Neoaplectana glaseri* by Jackson (1962a, b; 1966). The standard temperature for the leptomonad cultures, 27 C, was used in these experiments. This is not optimal for *N. glaseri* but development of third stage larvae into adults and low levels of reproduction occur (Jackson, 1962a).

### Experimental

Twenty-five ml Erlenmeyer flasks containing 5 cc of the complete "C" medium for leptomonad *L. tarentolae* were inoculated with ca. 100 third stage *N. glaseri* contained in 0.5 cc H<sub>2</sub>O and with three drops of a week old, Medium C culture of *L. tarentolae*. If, alternatively, third stage *N. glaseri* were inoculated into an already populous culture of the leptomonads ( $20 \times 10^6$  or more organisms/ml), the development of the worms was inhibited.

Six days after simultaneous inoculation with both species, culture flasks were tipped in order to settle worms in an area and 1 cc samples containing as many of the worms as

possible were withdrawn for transfer to a pointed 12 ml centrifuge tube. When the worms had accumulated at the bottom of this tube the supernatant was removed and three drop aliquots were inoculated into 5 cc of fresh culture medium (flasks A, positive leptomonad control). The worms were washed 3 times by settling in 30 cc of H<sub>2</sub>O in pointed 50 ml centrifuge tubes, then inoculated into 2 or 3 flasks of fresh culture medium (flasks B, experimental). 0.5 cc samples of the final wash water without worms were also inoculated into fresh culture medium (flasks C, negative leptomonad control).

Flasks of the A, B, C series were examined microscopically for *N. glaseri* and, especially, for *L. tarentolae* during a minimum period of 2 weeks.

### Results

Under consideration are the results of only those experiments in which A cultures were positive for leptomonads (control for good medium) and C cultures were negative (control for thorough external washing of worms). Of 22 *Neoaplectana glaseri* lots (30 to 50 worms per lot) that had been grown in Medium C with leptomonads, then washed and inoculated into fresh Medium C, three or 13.6% gave rise to a new, large, leptomonad population in the medium; four lots or 18.1% gave rise to a small but discernible number of apparently motile leptomonads that did not reproduce significantly and the fresh medium remained clear; 15 lots or 68.1% did not give rise to discernibly living leptomonads in the fresh medium.

### Discussion

That leptomonad *Leishmania tarentolae* may be passed through the digestive tract of nematodes, *Neoaplectana glaseri*, undamaged and in sufficient numbers to inoculate new cultures is suggested by these experiments in which controls for good medium were positive and controls for thorough external washing of worms were negative. Direct microscopic observation

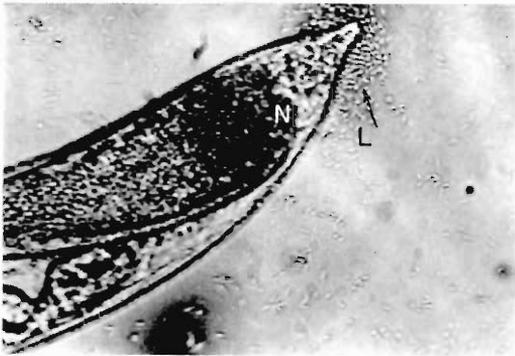


Figure 1. Anal excretion by a female nematode, *Neoaplectana glaseri*, (N) of leptomonad *Leishmania tarentolae* (L).  $\times 150$ .

of anal excretion by the large, female *N. glaseri* (Fig. 1) corroborates this suggestion since motile leptomonads were occasionally seen in the expelled materials. However, these excreted, fully active leptomonads are hardly of epidemiologic consequence.

Some washed worms excreted a few leptomonads that were motile but did not reproduce significantly. While, theoretically one leptomonad ought to suffice in starting a population, it is well known that in routine practice a culture may not grow out if the inoculum is so dilute. A small population may, by chance, include no reproductively competent individuals. However, in these experiments there is the additional possibility that the excreted leptomonads which were motile but apparently sterile had been damaged reproductively by nematode digestive processes.

Of further significance, perhaps, are those leptomonads that are digested or killed as they pass down the nematode digestive tract. Previous work with organisms and dye particles swallowed by female *N. glaseri* indicates that ingested materials are packed in the anterior lagoon of the gut tract but that major digestive degradation occurs posteriorly (Jackson, 1969). Protozoa fed to nematodes have already been used as gut lumen markers in morphological studies with the electron microscope (Jamar, 1966); the present work suggests that physiological aspects of nematode digestion might also be usefully studied with electron microscopy.

## Summary

Of 22 *Neoaplectana glaseri* lots (30–50 nematodes per lot) that had been grown in a chemically defined medium containing a small protozoan, leptomonad *Leishmania tarentolae*, then thoroughly washed and inoculated into fresh medium, 14% gave rise to a new and large leptomonad population in the fresh medium; 18% gave rise to a small but discernible number of motile leptomonads that did not reproduce significantly; 68% did not give rise to discernibly living leptomonads in the fresh culture. Controls for thorough external washing of the worms were negative and for goodness of the fresh medium were positive. It is concluded that some few leptomonads passed undamaged through the digestive tract of the nematodes. Direct observation of motile leptomonads being excreted by the large female *N. glaseri* supports the conclusion.

## Acknowledgments

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## *Chondronema passali* (Leidy, 1852) Christie and Chitwood, 1931, Redescribed with Observations on Its Early Development

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Leidy (1852) found a larval nematode parasite in the abdominal cavity of the betsy-beetle, *Popilius disjunctus* (Ill.). He described the nematode as *Nematoideum cavitatis abdominis Passali cornuti* and in the same paper referred to it as *Nematoideum passali*. At the same time, he also described from the thorax of the beetle a younger larval stage, which appears to be the infective stage of this same nematode and called it *Nematoideum thoracis cavitatis Passali cornuti*. In 1861 Deising renamed Leidy's nematodes from the betsy-beetle, calling those from the abdomen *Uracanthus brevispinosis* and those from the thorax *Agamonematodum Passali cornuti*. In his large collective work, von Linstow (1878) accepted Diesing's name of *A. Passali cornuti*. Chitwood (1932) pointed out that the generic name *Uracanthus* Diesing was a junior homonym and the specific epithet *brevispinosis* Diesing, 1861 was a synonym of *passali* Leidy, 1852. Christie and Chitwood (1931) found the adult nematodes in the beetle frass, described the various stages and probable life history, and placed the nematode in a new genus *Chondronema*. Chitwood (1932) suggested that *Chondronema* be placed in the Sphaerulariinae, which is now in the Tylenchoidea. Other workers have studied this association from an entomological point of view (Pearse et al., 1936, Gray, 1946), and they noted the presence of up to 4260 larval nematodes in one adult beetle. Contrary to the usual sphaerulariid life cycle, only larval stages, and not adults are found in the body cavity of the adult beetles. This difference, along with the apparent rarity of adult specimens, prompted the writers to pursue the current study in an attempt to determine its correct taxonomic status.

### Materials and Methods

A rotting log containing a colony of adult betsy-beetles was obtained from the Plant Industry Station grounds and placed in a glass cage in the laboratory. Almost 100% of the

Beltsville beetle population was parasitized, with hundreds of larval nematodes in various stages of development within the body cavities of the beetles. Frass was periodically obtained from the galleries and examined microscopically in attempts to find adult nematodes. Specimens were fixed and permanently mounted in glycerine.

### Results

Fifty *C. passali* adults (40 ♀♀, 10 ♂♂) were collected over a period of one year from the frass of the caged laboratory population of beetles. The females were usually replete with larvae of various forms (Fig. 2), including one larval stage which could be confused with an egg (Fig. 1). This stage, which appeared as an oval capsule with unique digitate appendages, was found for the first time in a ruptured female and later observed in other females. After more extensive investigation, it became apparent that the appendage was the cast cuticle of an earlier larval stage. This idea was further substantiated by the presence of remnants of a moulted stoma and rectum (Figs. 1, 2C, 3C), visible in the cast cuticle. The larvae are coiled within the cast cuticle in the same manner as unhatched nematodes, thus causing confusion with eggs. The extra cuticle, perhaps, serves as protection from the digestive juices of the beetle larvae and adults after ingestion.

The larva inside the cast cuticle had a stylet-like stoma with coalesced rhabdions, 2  $\mu$  in length, reminiscent of certain rhabditids. On several occasions these rhabdions were observed to be separated from each other, which does not occur in the shaft of a stylet. The esophagus of this larval stage was not discernible. In the same broken female specimen and within the body cavity of other older females, several larvae were seen re-entering the cast cuticle (Fig. 2D). This sequence was determined on the basis of the morphology of the stoma. Other female specimens contained



Figure 1. Egglike larvae of *Chondronema passali*.

larvae, as seen in Fig. 2B, which had an open panagrolaimoid stoma and a cylindrical esophagus. The youngest female recovered from the frass contained larvae which were in the first stage of development (Fig. 2A). The morphology of the female and male adults was studied and illustrated (Fig. 3A, B). The generic diagnosis is emended.

**Genus: *Chondronema* Christie and Chitwood, 1931**

**DIAGNOSIS (Emended):** Head with four distinct papillae. Amphidial openings lateral, slightly closer to oral opening than to papillae; amphidial pouches large. Stoma unarmed, not prominent. Esophagus with well developed dorsal esophageal gland overlapping intestine; dorsal gland orifice prominent; ampulla packed with esophageal secretions. Excretory pore just posterior to nerve ring. Caudal pores large, lateral, present in both sexes.

**MALE:** Testis short and flexed, with two spicules. Gubernaculum and caudal alae absent. Tail with four postanal papillae.

**FEMALE:** Body replete with eggs or larvae.

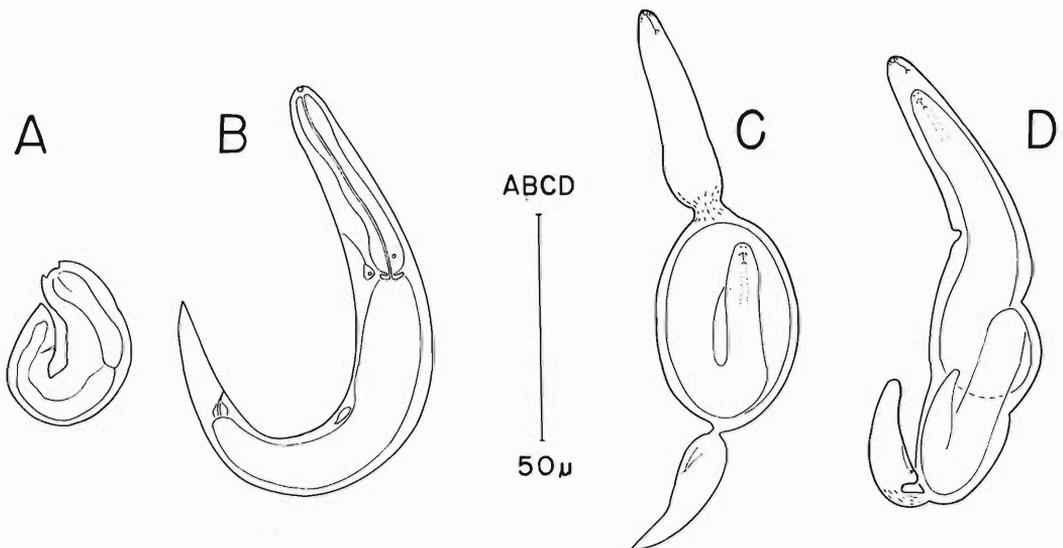
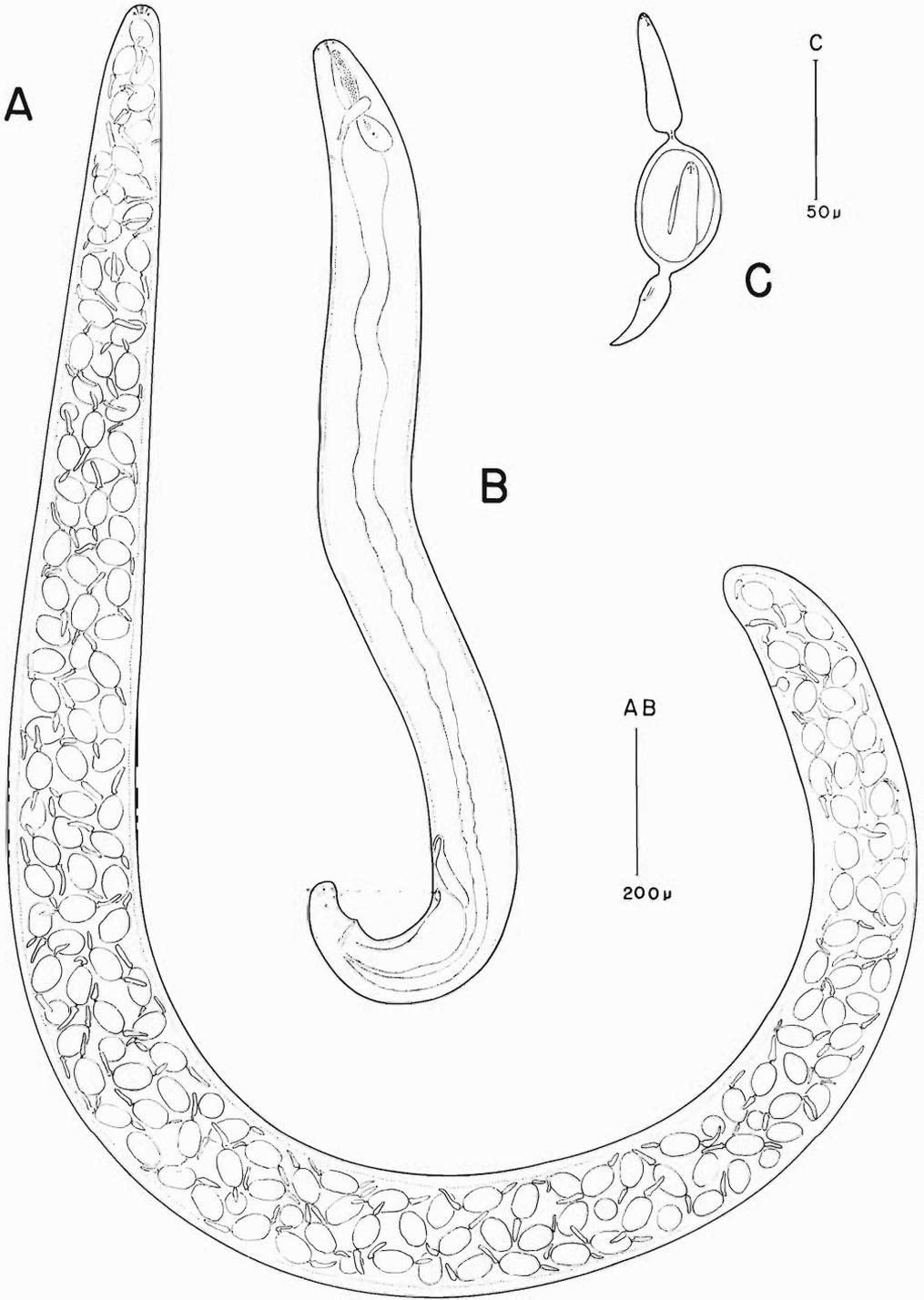


Figure 2. Larval development of *Chondronema*. A. Youngest larva found, probable first stage larva. B. Second-stage larva, found within body cavity of female nematode. C. Egglike larva, encased in earlier larval cuticle. D. Larva re-entering cuticle.



Cuticle of young specimens thick and smooth, becoming thin and with protruberances in old age. Esophagus degenerate. Vulva vestigial in function, located at 94% of body length. Anus and rectum prominent.

**EGGLIKE LARVA:** Encased in a retained larval cuticle, which appears to be the second stage larval cuticle.

**TYPE SPECIES:** *Chondronema passali* (Leidy, 1852) Christie and Chitwood, 1931.

**Syn. *Nematoideum cavitatis abdominis Passali cornuti* Leidy, 1852.**

***Nematoideum thoracis cavitatis Passali cornuti* Leidy, 1852.**

***Nematoideum passali* Leidy, 1852.**

***Uracanthus brevispinosus* Diesing, 1861.**

***Agamonematodum Passali cornuti* Diesing, 1861.**

***Agamonematodum Passali cornuti* Leidy (in Linstow, 1878).**

**MALES (5):** L = 1.59 (1.42–1.72) mm; W = 0.115 (0.104–0.126) mm; a = 13.9 (11.9–16.4); c = 19.8 (18.4–22.3); Spicule L = 38.8 (34.9–39.1)  $\mu$ ; Spicule W = 5.0 (4.2–5.9)  $\mu$ .

**FEMALES (5):** L = 3.16 (2.89–3.34) mm; W = 0.165 (0.152–0.182) mm; a = 19.2 (17.3–20.9); c = 41.6 (38.8–47.5); V% = 94.2 (92.4–94.9).

**EGGLIKE LARVA (5):** Anterior Appendage L = 46.6 (44.1–50.0)  $\mu$ ; Body L = 45.0 (44.0–47.1)  $\mu$ ; Posterior Appendage L = 35.5 (33.3–40.0)  $\mu$ .

Representative specimens are deposited in the U.S.D.A. Nematode Collection, Beltsville, Md., The University of California Survey Collection, Davis, California, and Canada National Collection, Ottawa, Canada.

### Discussion

*Chondronema passali* is a primitive nematode with no close relatives. It had been placed in the family Sphaerulariidae of the Tylenchida on the basis of having a tylenchid stylet. Our observations show that this structure is not a stylet, thus *Chondronema* is temporarily con-

sidered a genus of uncertain position. It has certain characteristics found in the strongylids, rhabditids, spirurids, and even the drilonematids. Further work on this nematode, and future discoveries of other similar worms, will be necessary before its proper status in the classification system can be determined.

### Summary

*Chondronema passali*, parasitic in the betsy-beetle, is unique in its development. Adult nematodes occur, not in the insect, but in the frass from the beetle tunnels. Morphologically different larvae were found within the body cavity of the female nematodes. The egglike larvae are of interest because they are enclosed within an earlier larval cuticle. This enclosure probably serves as protection from the digestive juices of the beetles. *C. passali* is redescribed and illustrated, and the taxonomy and morphology of this primitive nematode are given.

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← **Figure 3.** *Chondronema passali*. **A.** Female, lateral view, containing egglike larvae. **B.** Male, lateral view, showing short gonad and spicules. **C.** Egglike larva, encased in larval cuticle.

## Some Digenetic Trematodes of Marine Fishes of New Caledonia. Part IV. Hemiuridae and Summary.\*

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This is the fourth and last of a series of papers based on a collection of Digenea made at the Noumea Aquarium during three weeks in 1963 (see Durio and Manter 1968a, b, 1969). A total of 46 species of Digenea were collected from 49 species of fishes examined. A somewhat similar sampling was obtained in Fiji (Manter 1961, 1963a, b, c). The two collections will be compared (below) with the author's more extensive collections from Australia.

The trematodes found in New Caledonia must represent hardly more than a sampling of the rich trematode fauna which evidently occurs there, along with the varied fish and molluscan population of the coral reefs around the island. The last day of collecting yielded almost as many additional species as did any other day.

Measurements are in millimeters unless otherwise indicated. Sucker ratios are based on transverse diameters.

### Hemiuridae Lühe, 1901

#### *Lecithochirium magnaporum* Manter, 1940

HOSTS AND LOCALITIES: *Epinephelus* sp.; Serranidae; New Caledonia; *Lethrinus miniatus* Forskål; Lutjanidae; New Caledonia; *Lutjanus johnii* (Bloch); Lutjanidae; Heron Island, Queensland, Australia (collected by Peter Young).

LOCATION: Stomach.

NUMBER: One from *Epinephelus*; one from *Lethrinus*; three from *Lutjanus*.

DISCUSSION: This species is known from various hosts in the Galapagos Islands, American Pacific, Hawaii, and Philippines.

#### *Lecithochirium polynemi* Chauhan, 1945 (Figs. 1-2)

HOST: *Lutjanus vaiensis* (Quoy and Gaimard); Lutjanidae.

LOCATION: Stomach.

NUMBER: One.

DISCUSSION: The thin-walled sinus sac containing small cells, and the egg size resemble *L. microstomum* Chandler, 1935, but *L. polynemi* differs in that both the uterus and the ceca enter the ecsoma. *L. polynemi* is previously known from the west coast of India.

#### *Lecithocladium aegyptensis* Fischthal and Kuntz, 1963

HOSTS AND LOCALITIES: "mackerel"; Scombridae; New Caledonia. *Monodactylus argentatus* (Linn.); butter bream; Monodactylidae; Moreton Bay, Queensland, Australia.

LOCATION: Stomach.

NUMBER: Three in one host; one in Australian host.

DISCUSSION: These specimens agree with the description of this species in all important respects. Cuticular denticulations had been lost in the New Caledonian specimens but evidence of their former presence could be seen. *L. aegyptensis* was originally collected by Kuntz from *Pomadasys olivaceus* (Day) from the Giza Fish Market, Egypt. This fish was probably from the Red Sea. Weber and deBeaufort's *The Fishes of the Indo-Australian Archipelago* (vol. 7, p. 408) lists this species of fish as known from Singapore, Madagascar, South Arabia, and Malay Straits.

*Lecithocladium parviovum* Yamaguti, 1953, from *Scomber hanagunta* (Russel) in Macassar seems a very similar species. However, it is twice as large and has wider, thick-shelled eggs.

#### *Erilepturus tiegsi* Woolcock, 1935 (Figs. 3-4)

HOSTS: *Epinephelus* sp.; Serranidae. Serranidae; a mottled "grouper." Serranidae: "loche bleue."

LOCATION: Stomach.

NUMBER: One or two specimens in each of five hosts.

DISCUSSION: This species is previously known from Port Philip Bay, Victoria, Australia. It

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has not been collected from warmer waters of Australia.

The sucker ratio (given as approximately 1:2.5 by Woolcock) is 1:1.95–2 in my specimens. The base of the sinus sac is thick-walled and contains radial muscles; the sinus organ is thick-walled with circular muscles; the atrium is separated from the sinus sac.

The *sinus organ* (Fig. 4), a structure named by Manter (1969), was well described but not clearly figured by Woolcock (1935). A sinus organ is a muscular, cylindrical organ lying more or less free in the sinus sac and penetrated by a lumen, the hermaphroditic duct. It is long, coiled, and conspicuous in the genus *Elytrophallus* Manter, 1940, where it lies in a thin-walled portion of the sinus sac (Fig. 5) and is protrusible into the genital atrium. The duct of the sinus organ may contain sperm cells, eggs, or both. Manter (1969) showed that a sinus organ occurs in several species of *Dinurus* Looss, 1907 (Fig. 6). It also occurs to varying degree in *Elytrophalloides* Szidat, 1955, and *Ectenurus* Looss, 1907 (Fig. 7). Thus, the sinus organ of Manter (1969) equals the "sinus tube" of Manter (1940), "cirrus" of Looss (1907), and "terminal muscular bulb of the hermaphroditic duct" of Fischthal & Kuntz (1963). The genus *Lampritrema* Yamaguti, 1941, has a similar long, muscular tube lying in a tubular genital atrium but in this species it is a male tube only, the uterus opening separately into the base of the tubular genital atrium (see Margolis, 1962). The tube here is technically a cirrus but it seems evident that it has evolved from a sinus organ and is not homologous with the cirrus of other families of trematodes.

***Lecithaster testilobatus* sp. n.**  
(Figs. 8–11)

HOSTS AND LOCALITIES: *Scarus* (= *Callydon*) sp.; New Caledonia; Green Island, Queensland, Australia.

LOCATION: Intestine.

NUMBER: Single holotype from Australia; two paratypes from New Caledonia.

HOLOTYPE: USNM Helm. Coll. No. 63326.

DESCRIPTION (based on three specimens): Length 1.824–2.033; width 0.760–0.855; almost uniformly wide; broadly rounded at anterior end. Oral sucker 0.214–0.241 wide; acetabulum 0.355–0.435 wide. Sucker ratio

1:1.56–1.8. Forebody 0.435–0.532 or almost one-fourth body length. Pharynx 0.144–0.166 long by 0.140–0.156 wide; esophagus with thick chitinous (refractive) wall, with thick, median, dorsal, longitudinal ridge (Fig. 9) partially dividing cavity into right and left halves; inner surface of wall tuberculated. Each cecum arising from ventral side of esophagus as a short thin-walled tube enlarging to form prececal sac with well-developed microvilli; ceca extending to near posterior end of body.

Testes symmetrical, widely separated at posterior edge of acetabulum; each testis deeply divided into four rounded lobes (Fig. 10). Seminal vesicle an elongate sac partly or entirely dorsal to acetabulum, intertesticular; pars prostatica a long tube slightly sinuous, dorsal to acetabulum, lined with vesicular cells, entering base of sinus sac at level of esophagus. Genital pore ventral to pharynx; pore cavity shallow.

Ovary deeply four-lobed, lobes with rounded ends, length of lobes about twice width. Vitellaria consisting of seven claviform lobes, length of lobes three or four times width; ventral to ovary. Seminal receptacle ovoid, dorsal to ovary. Uterus filling most of body, coils chiefly longitudinal or diagonal. Sinus sac ovoid, 0.099–0.128 long by 0.093–0.112 wide; thick-walled. Uterus and sperm duct entering at posterior end of sinus sac, remaining separate for short distance before forming thin-walled hermaphroditic tube. Eggs 14–17 by 8–9  $\mu$ . Arms of excretory vesicle not seen.

DISCUSSION: This species differs from all others in the subfamily in its four-lobed testes. In other respects it is probably most similar to *L. stellatus* Looss, 1907 (= *L. sayori* Yamaguti, 1938) which also occurs in Australia.

The peculiar esophagus or esophageal bulb with its thick chitinous wall has not been described for other species of *Lecithaster* Lühe, 1901, but it may have been overlooked. In one case the dorsal ridge resembles an invagination of the dorsal wall. In all cases, the thin-walled beginning portion of the cecum is sharply demarked from the cellular portion.

***Hysterolecitha sigani* sp. n.**  
(Figs. 12–13)

HOSTS AND LOCALITIES: *Siganus* sp.; Siganiidae (type host); New Caledonia (type locality). *Siganus rivulatus* (Forskål); Green Is-

land and Moreton Bay, Queensland, Australia. *Abudejduf palmeri* (Ogilby); Pomacentridae; Green Island, Queensland, Australia. *Micracanthus strigosus* (Cuv. & Val.); Chaetodontidae; Moreton Bay, Australia.

LOCATION: Stomach.

NUMBER: Numerous.

HOLOTYPE: USNM Helm. Coll. No. 63327.

DESCRIPTION (based on 12 specimens): Length 1.389–2.793; greatest width near acetabulum, 0.321–0.627; only slightly tapered and rounded at each end. Preoral lobe usually well developed; cuticula with fine transverse striae dorsal to oral sucker. Oral sucker 0.147–0.201 wide; acetabulum 0.301–0.368 wide. Sucker ratio 1:1.5–2.0, usually 1:1.8. Forebody 0.301–0.741, usually one-third to one-fourth body length. Pharynx 0.064–0.080 long by 0.064–0.096 wide; very short esophagus forking at right angles to form a transverse muscular tube connecting on each side to a spherical prececal sac; prececal sac with pavement epithelium and fine microvilli; ceca with transverse cellular ridges, containing solid particles of food, ending near posterior end of body.

Genital pore median at level of intestinal bifurcation. Testes diagonal, smooth, in anterior half of hindbody, close together or (usually) separated by coil of uterus. Seminal vesicle tubular, its posterior end at anterior edge of acetabulum, usually with two bends but rarely almost straight; prostatic vesicle short, ovoid, surrounded by prostatic cells. Sinus sac (Fig. 13) distinct, with definite wall, ovoid, usually wider than long, 0.064–0.160 long by 0.080–0.121 wide. Genital sinus (hermaphroditic duct) straight; differentiated into three regions: (1) posterior portion with thin wall containing longitudinal muscles and surrounded by fine circular muscles; (2) thin-walled vesicular portion; and (3) terminal

thick-walled portion opening into short atrium close to genital pore. Definite bands of longitudinal muscles curving around anterior portion of sinus sac (Fig. 13).

Ovary subtriangular, in posterior one-half to one-third of body; vitelline lobes seven, claviform, extending to near posterior end of body; postvitelline space 0.147–0.335 long, usually less than 0.2. Seminal receptacle lacking. Uterus usually extending short distance posterior to vitellaria but not posterior to ceca; voluminous, filling most of hindbody even to near sides of body; entering posterior end of sinus sac. Eggs not filamented, 17–24 by 9–11  $\mu$ .

Excretory vesicle forking near acetabulum, arms uniting dorsal to pharynx by a narrow, often invisible, connection.

DISCUSSION: This species is characterized by its wide sinus sac, short prostatic vesicle, the three divisions of the genital sinus, and the extensive uterus filling most of the hindbody. It is probably most like *H. xesuri* Yamaguti, 1938. I consider *H. nahaensis* Yamaguti, 1942, a synonym of *H. xesuri* (new synonymy) since the difference in body size alone does not seem sufficient to separate the species. *H. sigani* differs in details of the sinus sac and esophagus.

*Theletrum frontilatum* sp. n.  
(Figs. 14–15)

HOSTS AND LOCALITIES: *Siganus rivulatus* (Forskål); Siganidae; type host; Moreton Bay, Queensland, Australia; type locality. *Siganus* sp.; New Caledonia.

LOCATION: Stomach.

NUMBER: Eight in Australian host; one in New Caledonian host.

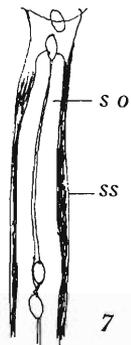
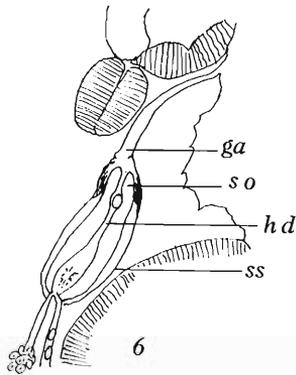
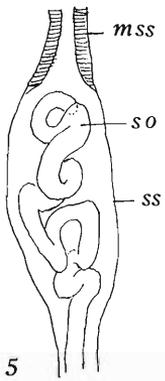
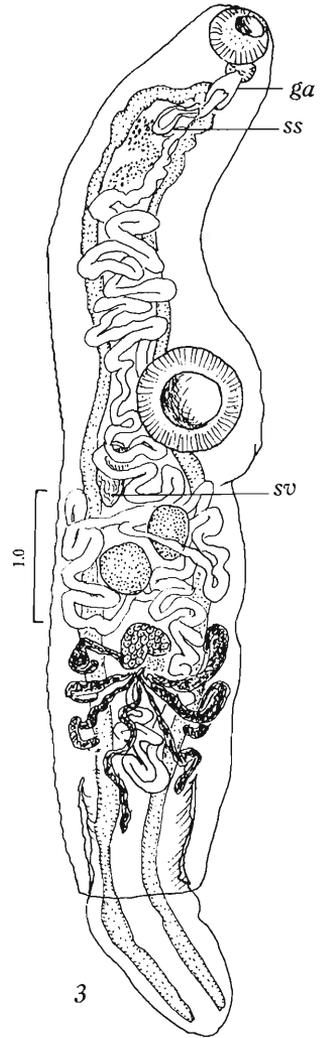
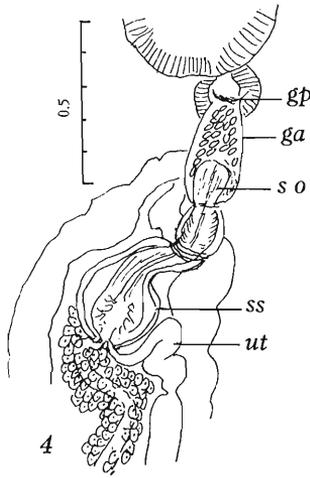
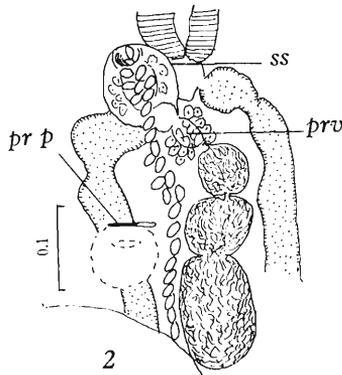
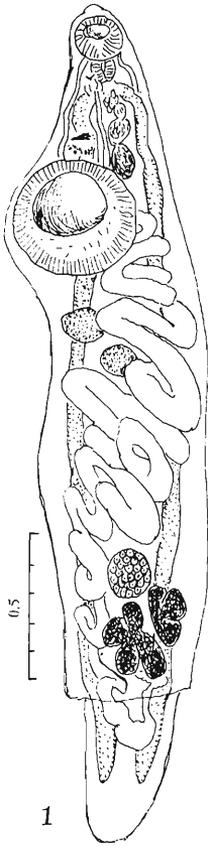
HOLOTYPE: USNM Helm. Coll. No. 63328.

DESCRIPTION (measurements on 5 specimens): Length 2.489–5.719; width near ace-

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Drawings were made with the aid of a camera lucida. Abbreviations: *es*, esophagus; *ce*, intestinal cecum; *ga*, genital atrium; *gp*, genital pore; *hd*, hermaphroditic duct; *mss*, muscular portion of sinus sac; *ph*, pharynx; *pp*, pars prostatica; *prp*, preacetabular pit; *prv*, prostatic vesicle; *ov*, ovary; *so*, sinus organ; *ss*, sinus sac; *sv*, seminal vesicle; *t*, testis; *ut*, uterus; *vt*, vitellaria.

Figures 1–7. 1. *Lecithochirium polynemi*. Ventral view. 2. Same. Region of terminal ducts. Ventral view. 3. *Erelepturus tiegsi*. Ventral view. 4. Same. Terminal ducts, showing sinus organ. 5. *Elytrophalus mexicanus* Manter, 1940. Portion of sinus sac and sinus organ. 6. Portion of anterior end of *Dimurus longisimus* Looss, 1907, showing sinus organ. Lateral view. After Manter (1969). 7. Portion of terminal ducts of *Ectenurus lepidus* Looss, 1907. Ventral view. After Manter (1969).



tabulum 0.627–1.653. Forebody wide, little tapered, broadly rounded; hindbody tapering to pointed posterior end. Preoral lobe well developed. Oral sucker 0.301–0.502 wide; acetabulum 0.536–0.874 wide; sucker ratio 1:1.62–2. Forebody 0.855–1.995, about one-third body length; anterior part of forebody with diagonal, almost transverse muscles on each side of oral sucker to level of intestinal bifurcation. Pharynx 0.107–0.147 long by 0.134–0.167 wide; esophagus about same length as pharynx; ceca not far apart, not quite reaching posterior end of body. Genital pore median, about midway between suckers.

Testes ovoid, smooth, symmetrical (or rarely slightly diagonal), just posterior to acetabulum, separated by uterine coils. Seminal vesicle free, tubular, coiled, its posterior end reaching or slightly overlapping acetabulum. Pars prostatica tubular, relatively wide but without vesicular cells, surrounded by conspicuous prostatic cells. Sinus sac (Fig. 15) subglobular, slightly longer than wide, 0.096–0.208 long by 0.088–0.192 wide, with muscular wall; genital sinus a straight tube; gland cells between sinus and sinus sac; short genital atrium present.

Ovary median, not far posterior to testes, globular, smooth. Vitellaria immediately post-ovarian; consisting of three lobes: an anterior pair side by side and a single median, larger posterior lobe; anterior lobes sometimes sublobed. Uterus much coiled posterior to ovary, extending to near posterior end of body, few coils between ovary and testes. Eggs usually 24–25 by 12–13  $\mu$ ; rarely down to about 20 by 11  $\mu$ .

Excretory vesicle forking at anterior edge of acetabulum; arms ending blindly at each side of oral sucker.

The name *frontilatum* is from *frons* = fore part, and *latum* = wide, and refers to the wide forebody.

DISCUSSION: Other species in the genus *Theletrum* Linton, 1910 are: *T. fustiforme* Linton, 1910; *T. gravidum* Manter, 1940; *T. lissosomum* Manter, 1940; and *T. magnasaccum*

Sogandares and Sogandares, 1961. *T. frontilatum* differs from all of these in body shape (very broad forebody and tapering hindbody) and in location of testes close to the acetabulum. The crura of the excretory vesicle clearly end blindly opposite the oral sucker, whereas they are described as uniting dorsal to the pharynx in both *T. gravidum* and *T. lissosomum*. A restudy of specimens of both these species confirms the union of the crura in *T. lissosomum*. In *T. gravidum*, the crura at least meet dorsal to the pharynx but actual union is not clear. Thus, this character seems to vary within the genus. All species of *Theletrum* possess a muscular knob-like structure at the extreme anterior tip of the body although it is not well indicated in descriptions. *T. frontilatum* has in addition conspicuous diagonal muscles near the anterior end.

Of the four previously named species, two are in the Caribbean region, two from the tropical American Pacific.

#### *Dichadena obesa* (Manter, 1961) n. comb.

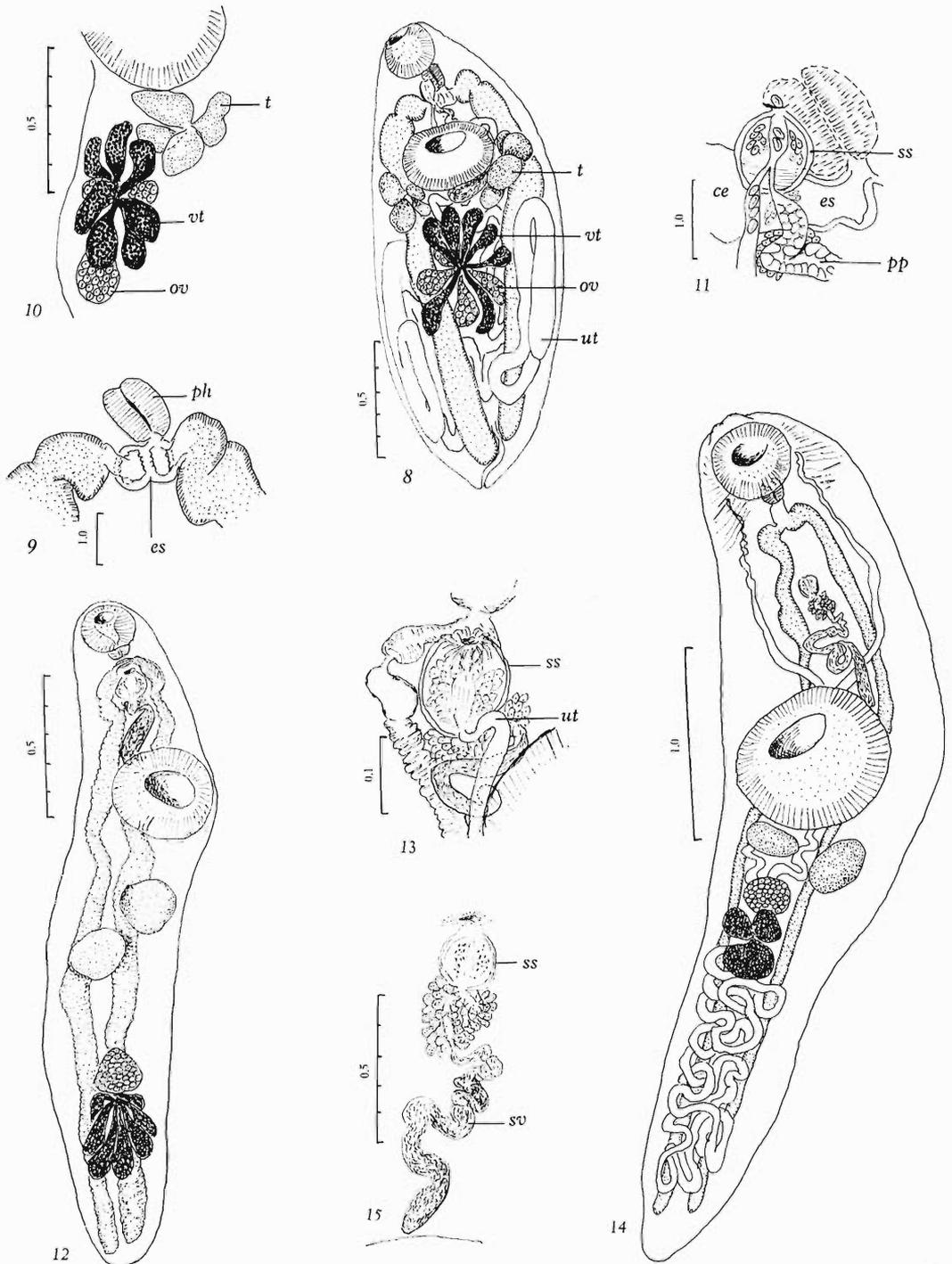
SYNONYMS: *Prolecitha obesa* Manter, 1961; new synonymy. *Prolecitha beloni* Nagaty and Abdel Aal, 1962; new synonymy.

HOSTS AND LOCALITIES: One adult specimen from *Tylosurus leirurus* (Bleeker) ?; needlefish; Belonidae; New Caledonia. One immature specimen in muscles of *Pranesus capricornis* Woodland, 1961; Atherinidae; hardyhead; Heron Isl., Australia.

LOCATION OF ADULT: Intestine.

DISCUSSION: The immature specimen was collected by Dr. J. C. Pearson at Heron Island in 1963. It reveals characters which led me to restudy paratypes of *Prolecitha obesa* from Fiji. The most outstanding of the characters was the union of the two ceca to form a cyclocoel at or near the level of the ovary. The ends of the ceca are obscured by eggs or by the ovary in most of the Fijian specimens but one shows such union clearly. A seminal receptacle is present.

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 Figures 8–15. 8. *Lecithaster testilobatus*. Holotype. Ventral view. 9. Same. Pharynx and esophagus. Ventral view. 10. Same. Region of gonads. Ventral view. 11. Same. Region of terminal ducts. Ventral view. 12. *Hysterolecitha sigani*. Holotype. Ventral view. Uterus omitted. 13. Same. Region of terminal ducts. Ventral view. 14. *Theletrum frontilatum*. Holotype. Ventral view. 15. Same. Region of terminal ducts. Ventral view.



List of Digenea collected in New Caledonia, hosts, and known geographical distribution.<sup>1</sup>

Trematode	Hosts (in New Caledonia and Australia)	Elsewhere Known
<b>Acanthocolpidae</b>		
<i>Stephanostomum japonocasum</i>	<i>Epinephelus</i> sp.	
<i>S. casum</i> (Linton, 1910)	<i>Lutjanus argentimaculatus</i> (Forskål)	Caribbean; Mexican Pacific; Galapagos Isl.; Red Sea; Philippines; Ghana
<b>Bivesiculidae</b>		
<i>Bivesiculoides posterotestis</i>	Myctophid	
<b>Bucephalidae</b>		
<i>Neidhartia coronata</i>	Serranid	
<b>Myorhynchus pritchardae</b>	Serranid	
<i>Prosorhynchus freitasi</i> Nagaty, 1937	<i>Epinephelus</i> sp.	Red Sea
	<i>Plectropomus maculatus</i> (Bloch) (A)	Australia (Heron Isl.)
<b>P. longisaccatus</b>	Serranid	
<b>P. serrani</b>	<i>Serranus louti</i> (Forskål)	Red Sea
<b>Cryptogonimidae</b>		
<i>Paracryptogonimus catalae</i>	<i>Lutjanus</i> sp.	
<b>P. longitestis</b>	<i>Lutjanus</i> sp.	
	<i>Lutjanus johnii</i> (Bloch)	Australia (Green Isl.)
	(A, new record)	
<b>P. provitellosus</b>	<i>Lutjanus vaigiensis</i> (Quoy and Gaimard)	
<i>P. saccatus</i> Manter, 1963	<i>Siganus</i> sp.	Fiji
<b>P. testitactus</b>	<i>Lutjanus</i> sp.	
<i>Siphoderina paracatalae</i>	<i>Lutjanus</i> sp.	
<b>Fellodistomatidae</b>		
<i>Tergestia clonacantha</i> Manter, 1963	<i>Hemiramphus</i> sp.	Fiji
<b>Gyliauchenidae</b>		
<i>Gyliauchen papillatus</i> (Goto and Matsudaira, 1918)	<i>Siganus</i> sp.	
	<i>Siganus lineatus</i> (Cuv. and Val.)	Australia (Green Isl.); Japan; Celebes; Philippines
<b>Halplporidae</b>		
<i>Atractotrema sigani</i>	<i>Siganus</i> sp.	Australia (Green Isl.)
<i>Hapladena tanyorchis</i> Manter and Pritchard, 1961	<i>Naso</i> sp.	Hawaii
<b>Isorchis parvus</b>	<i>Chanos chanos</i> (Forskål)	
<b>Haplosp.anchnidae</b>		
<i>Hymenocotta mulli</i> Manter, 1961	mullet	Fiji
	<i>Mugil cephalus</i> Linn. (A)	Australia (Heron Isl.)
<b>Hemiuridae</b>		
<i>Dichadena obesa</i> (Manter, 1961)	<i>Tylosurus</i> sp.	Fiji; Red Sea
	metacercaria in <i>Pranesus capri-cornensis</i> Woodland (A)	Australia (Heron Isl.)
<i>Erilepturus tiegsi</i> Woolcock, 1935	<i>Epinephelus</i> sp.	
	<i>Arripis trutta esper</i> Whitley (A)	Australia (Victoria)
<b>Hysterolecitha sigani</b>	<i>Siganus</i> sp.	
	<i>Siganus rivulatus</i> (Forskål) (A)	Australia (Green Isl.; Moreton Bay)
	<i>Micracanthus strigosus</i> (Cuv.) (A)	
	<i>Abudejduf palmeri</i> (Ogilbey) (A)	
<b>Lecithaster testilobatus</b>	<i>Scarus</i> sp. (A)	Australia (Green Isl.)
<i>Lecithochirium magnaporum</i> Manter, 1940	<i>Lethrinus miniatus</i> Forskål	

<sup>1</sup> Taxa named in the present series of papers are in boldface. Australian hosts, indicated by (A), are included. Hosts known from other regions are not included.

## List of Digenea collected in New Caledonia, hosts, and known geographical distribution (continued).

Trematode	Hosts (in New Caledonia and Australia)	Elsewhere Known
	<i>Epinephelus</i> sp.	Australia (Heron Isl.); American Pacific;
<i>L. polynemi</i> Chauhan, 1945	<i>Lutjanus johnii</i> (Bloch) (A) <i>Lutjanus vaigiensis</i> (Quoy and Gaimard) mackerel	Hawaii; Philippines Indian Ocean
<i>Lecithocladium aegyptensis</i> Fischthal and Kuntz, 1963		Red Sea
<i>Quadrifoliovarium pritchardae</i> Yamaguti, 1965	<i>Naso</i> sp.	Hawaii
<i>Theletrum frontilatum</i>	<i>Siganus</i> sp. <i>Siganus rivulatus</i> (Forskål) (A)	Australia (Moreton Bay)
Lepocreadiidae		
<i>Holorchis plectorhynchi</i>	<i>Plectorhynchus goldmani</i> (Bleeker) <i>Lethrinus miniatus</i> (Forskål)	
<i>Inusatrium robustum</i>	<i>Lepidaplois perditio</i> (Quoy and Gaimard)	
<i>I. secundum</i>	parrot fish	
<i>Neolepidapedon dollfusi</i>	<i>Epinephelus</i> sp.	
Microscophiidae		
<i>Hexangium sigani</i> Goto and Ozaki, 1931	<i>Siganus</i> sp. <i>Lutjanus vaigiensis</i> (Quoy and Gaimard) <i>Siganus</i> (six species) (A)	Japan; Celebes; Madagascar; Borneo; Philippines; Australia (Green Isl.; Heron Isl.; Moreton Bay)
Monorchhiidae		
<i>Hysterorchis vitellosus</i>	<i>Plectorhynchus</i> sp. <i>Plectorhynchus pictus</i> (Thunberg) (A)	Australia (Heron Isl.)
<i>Lasiotocus longitestis</i>	<i>Plectorhynchus</i> sp.	
Opecoelidae		
<i>Allopodocotyle serrani</i> (Yamaguti, 1952)	Serranid	Celebes; Japan
<i>Choanostoma secundum</i>	<i>Lutjanus vitta</i> (Quoy and Gaimard) <i>Plectorhynchus</i> sp.	
<i>Hamacreadium diacopae</i> Nagaty and Abdel Aal, 1962	<i>Lethrinus</i> sp. <i>Lutjanus</i> sp.	Red Sea Caribbean; Galapagos Isl.;
<i>H. mutabile</i> Linton, 1910	<i>L. amabilis</i> (DeVis) (A) <i>L. fluviiflamma</i> (Forskål) (A) <i>Lethrinus miniatus</i> Forskål	Hawaii; Red Sea; Australia (Green Isl. and Heron Isl.). The record from Fiji is prob- ably incorrect.
<i>Helicometra fasciata</i> (Rud., 1819)	<i>Epinephelus</i> sp.	Mediterranean; N. Atlantic; Japan; Caribbean; Tasmania
<i>Orthodena tropica</i>	<i>Lethrinus</i> sp. (A) <i>L. fletus</i> Whitley (A) <i>L. glyphodon</i> Günther (A) <i>L. reticulatus</i> (Cuv. and Val.) (A)	Australia (Green Isl.; Moreton Bay)
<i>Pacificreadium serrani</i> (Nagaty and Abdel Aal, 1962)	<i>Epinephelus</i> sp. <i>E. merra</i> Bloch (A) <i>Plectropomus maculatus</i> (Bloch) (A)	Australia (Heron Isl.); Red Sea
<i>Pseudoplagioporus interruptus</i>	<i>Lethrinus</i> sp. <i>L. glyphodon</i> Günther (A) <i>Choerodon albigenea</i> (DeVis) (A) <i>Epinephelus merra</i> Bloch (A) <i>Plectorhynchus</i> sp. (A)	Australia (Green Isl.; Heron Isl.)
<i>P. lethrimi</i> Yamaguti, 1938	<i>Lethrinus</i> sp. <i>Plectorhynchus</i> sp. (A)	Australia (Heron Isl.)
Zoogonidae		
<i>Diphtherostomum tropicum</i>	<i>Lethrinus</i> sp. <i>L. glyphodon</i> Günther (A)	Australia (Green Isl.)

Siddiqi and Cable (1960) showed that in *Dichadena* Linton, 1910, the ceca unite dorsal to the ovary. The vitellaria are preovarian, the ovary lobed, and a seminal receptacle is present. These characters also occur in *Prolecitha* Manter, 1961, which now does not seem to deserve generic rank and is considered a synonym of *Dichadena* Linton, 1910.

*Dichadena obesa* differs from *D. acuta* Linton, 1910, in its much shorter hindbody, much shorter pars prostatica, preacetabular seminal vesicle, and smaller eggs. The figure of *Prolecitha beloni* from *Belone strongylurus* (= *Tylosurus strongylurus* (Van Hasselt)) in the Red Sea suggests a cyclocoel. What the authors interpreted as excretory arms were probably the uniting ceca. The ovary is four-lobed in the New Caledonian and Australian specimens and a 4th lobe is present in at least one of the Fijian specimens. The other characters which supposedly separate *P. beloni* from *P. obesa* are individual variations seen in Fijian specimens. Thus, *P. beloni* should be considered a synonym of *Dichadena obesa*. The single specimen from New Caledonia shows the seminal vesicle bent once, with both parts inflated with sperm cells. The pars prostatica is actually a short tubular prostatic vesicle surrounded by conspicuous prostatic cells.

Cable and Nahhas (1963) described the cercaria of *Dichadena acuta* developing in the snail *Zebrina browniana* D'Orbigny in the Caribbean. The occurrence of immature *D. obesa* in the muscles of the hardyhead indicates that such fishes may serve either as intermediate or as paratenic hosts.

***Quadrifoliovarium pritchardae*  
Yamaguti, 1965**

HOST: *Naso* sp.; unicorn fish; Acanthuridae.

LOCATION: Intestine.

NUMBER: Two from one host.

The species name, originally spelled *pritchardi*, should be *pritchardae*. The species was described from *Naso unicornis* (Forskål) in Hawaii.

No Digenea were found in the following 13 species of fishes; *Abudefduf septemfasciatus* (Cuv. and Val.), a bonito, *Caranx* sp., *Chaetodon* sp., "communard," *Gerres* sp., *Lethrinus nebulosus* (Forskål), *Mylio berda* (Forskål), *Percanthurus teuthis* Fowler, *Polyamblydon* sp., *Scatophagus argus* (Linn.), *Siganus oranim*

(Bloch and Schneider), and *Sillago sihama* (Forskål). Since only one specimen of most of these fishes was examined, these negative findings are not significant. A total of 49 species of fishes were examined in New Caledonia.

**Geographical Distribution**

New Caledonia is a large, oceanic island midway between Fiji (to the east) and northern Australia (to the west), each of which is about 600 miles distant. Unlike most south sea islands, New Caledonia is nonvolcanic and evidently has been isolated for a very long time. Most of the native terrestrial plants and animals are endemic. The Fijian Islands, although volcanic in origin, are also geologically ancient. I have found that trematodes are abundant in all three regions. The small samplings collected, compared with the several hundred species which probably occur in each region, make comparisons tentative and perhaps premature. Present collections indicate that New Caledonian trematodes are much more similar to those of tropical Australia than to those of equidistant Fiji.

Of 46 species of New Caledonian Digenea, one species only is now also known from both the North Atlantic and Mediterranean; one each from Tasmania, southern Australia (Victoria), Borneo, Indian Ocean, Madagascar, and Ghana; two species from the Celebes; three from the American Pacific; three from the Caribbean; four from the Philippines; four from Fiji; four from Hawaii; four from Japan; seven from the Red Sea; 19 from Queensland, Australia.

An understanding of such geographical distribution must eventually consider the kinds of trematodes involved as well as a comparison of species of fishes and molluscs in the regions. Regarding the trematodes, it might be noted that families with most similarities with Australia are the Hemiuridae and the Opecoelidae. Only two genera, *Intusatrium* and *Myorhynchus*, are known only from New Caledonia. Three genera (*Hysterorchis*, *Isorchis*, *Orthodena*) are known as yet only from New Caledonia and Australia.

The presence of Cryptogonimidae in New Caledonia (six species), Fiji (four species), and Queensland, Australia (six species) would question the conclusion of Morozov (1964)

that cryptogonimids are mostly in the western hemisphere. I have, however, found no cryptogonimids in my New Zealand collection (of 66 species) nor from South Australia (of about 43 species). Thus, the family is predominantly one of warm water.

Only four species of Digenea are known both in Fiji and in Australia; two of these were found in New Caledonia.

The marked similarity of trematodes of New Caledonia to those of Australia, contrasted with slight similarity to those of equidistant Fiji is probably partly due to the fact that more collections were made in Australia than in Fiji. However, only four species of Fiji were found in Australia. Present ocean currents might explain differences in trematode faunas in these regions. The East Australian Current flows southwesterly between Fiji and New Caledonia away from tropical Australia, whereas the South Equatorial Current flows northwesterly from New Caledonia toward northern Queensland. In this connection, it might be noted that although relatively few fishes were examined at Green Island (in northern Queensland), 10 New Caledonian species occurred there, compared with nine at Heron Island (near the south end of the Great Barrier Reef), and only four in Moreton Bay (south of the Great Barrier Reef). The implication is that some of the species of trematodes originated in New Caledonia rather than in Australia.

### Summary

Nine species of hemiurid trematodes are reported. New species are: *Lecithaster testilobatus* from *Scarus* sp., *Hysterolecitha sigani* from *Siganus* sp. and other hosts, *Theletrum frontilatum* from *Siganus* sp. New host and locality records are reported for *Lecithochirium magnaporum*, *L. polynemi*, *Lecithocladium aegyptensis*, *Erilepturus tiegsi*, *Dichadena obesa*, *Quadrifoliovarium pritchardae*. *Prolecitha* Manter, 1961, is considered a synonym of *Dichadena* Linton, 1910. *Prolecitha beloni* Nagaty & Abdel Aal, 1961, syn. of *Dichadena obesa* (Manter, 1961); *Hysterolecitha nahaensis* Yamaguti, 1938, syn. of *H. xesuri* Yamaguti, 1942.

Presently known New Caledonian Digenea (46) have many more species in common with Australia (19) than with Fiji (5).

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### Research Note

## The Taxonomy of *Cercaria lamsilae* Coil, 1954

Nasir et al. (1969, Proc. Helm. Soc. Wash. 36: 102-106) published the descriptions of two gorgoderid cercariae. In the same paper they attempted to evaluate *Cercaria eriensis* Coil, 1953 and *Cercariae lamsilae* Coil, 1954 without the study of living specimens concluding: . . . "that *C. lamsilae* should be synonymized with *C. eriensis*."

They present the following arguments to support this contention, (1) the differences in the stylets are not impressive, (2) the arrangement of the papillae is a "matter of interpretation," (3) the differences in body length are "questionable." They did not comment on the different flame cell formulae.

It is not the intention of this note to discuss in detail the merits of certain criteria used in the differentiation of gorgoderid cercariae, but it is deemed essential to point out certain published facts.

Gorgoderid cercariae can be differentiated

	<i>C. eriensis</i>	<i>C. lamsilae</i>
Body length	0.978 (0.952-1.008)	0.625 (0.504-0.904)
Flame cell formula	2 [(12 + 12 + 12) + (12 + 12 + 6)]	2 [(11 + 14 + 12) + (12 + 12 + 14)]

Arrangement of sensory papillae different.

Stylet shapes and sizes different.

readily on the basis of the arrangement of the sensory papillae (Fischthal, 1951, Amer. Mid. Nat. 46: 395-443, Fischthal, 1954, Tran. Amer. Microsc. Soc. 73: 210-215, Coil, 1954, Proc. Helm. Soc. Wash. 22: 17-29, Coil, 1955, Proc. Helm. Soc. Wash. 22: 64-66 and Coil, 1960, Proc. Helm. Soc. Wash. 27: 39-41). The arrangement is consistent enough for taxonomic purposes and any paper dealing with the taxonomy of gorgoderid cercariae is incomplete without such a description. The use of flame cell formulae for separating species of trematodes has stood the scrutiny of innumerable investigators and it remains today one of the most useful characters for differentiating among the various taxa of digenetic trematodes. The only rational conclusion which can be drawn here then is that the flame cell formulae and the arrangement of the sensory papillae are adequate to separate these two species.

The differences are summarized below:

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## Anthelmintic Efficacy of Thiabendazole Fed in Low Level Dosages to Calves

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The excellent anthelmintic efficacy of thiabendazole in domestic animals has been extensively described in the world literature. (Douglas and Baker, 1968). The fact that it has a wide margin of safety, is palatable and can be administered without stressing the animals makes thiabendazole, in theory, an ideal drug to be administered as a feed additive on a daily low level basis. This method of medication would be ideal for cattle in feedlots and on pastures.

The present report deals with two separate field trials undertaken to determine the anthelmintic activity of thiabendazole when administered as a medicated feed additive in low level dosages to calves with natural infections of gastrointestinal parasites.

### Materials and Methods

**TEST 1:** Thirty calves of unknown origin were purchased from local markets. All had natural infections with the species of nematodes of importance in Georgia. The calves were assigned to three groups on the basis of restricted randomization. Group averages were balanced according to degree of parasitism (as determined by nematode egg counts made on two successive days, using a modified Stoll technique), weight, sex, and breed. Each group was placed in separate Bermudagrass pastures, but the groups were rotated every 7 days from August 1 to October 17 to allow the calves the same opportunity to share the available larvae.

All calves were group-fed a grain supplement ration, consisting of 93.7% ground corn, 4% cottonseed meal, 0.6% urea, 0.2% Vitamin A, and 1.5% trace mineral salts. The daily amount of the supplement grain fed to each group was increased from 11.3 kg at the beginning of the trial to 38.6 kg at the end of

the trial. Each calf was weighed every two weeks to calculate the dose of medication and the amount of feed supplement to be provided for the next 2 weeks.

Three premixes were used, each with soya flour as a carrier. The premix without medication was added to the grain supplement for the animals from Group I, which served as a control group. A second premix, containing 8.0 g of thiabendazole in each kg was used in Group II (low level). A third premix, which contained 32.0 g thiabendazole/kg, was added to the grain supplement offered the animals from Group III (high level). The three premixes were mixed with the grain ration at the rate of 30 g for every 226.8 kg body weight per day. Thus, the calves from Groups II and III received a daily dose of 1 and 4 mg/kg of body weight, respectively, during the 108 days covered by the experiment.

Fecal samples were obtained from the rectum of each calf every 28 days for making nematode egg counts. Feces-egg cultures were prepared to determine percentage of larval development and subsequent identification of the species collected from cultures. Feces remaining from the individual samples for egg counts were used to prepare the cultures. Known amounts of feces from each of several of the calves from one group were combined to obtain a 1,000 g sample, which was mixed with 166 g vermiculite (Porter et al., 1965). This mixture was placed in stainless steel pans and incubated at 25C for 9 days. The potential number of larvae, as determined by adding the total number of eggs contributed by the individual samples used (gm feces  $\times$  EPG), was compared to the actual number of larvae recovered from the cultures placed on Baermann funnels. Four calves from each of the groups were selected at the beginning of the test to be killed for the postmortem recovery of gastrointestinal parasites.

**TEST 2:** Forty calves, similar to those used in the first test, were allotted to four groups, rotated on pasture from June 1 to August 24, weighed and fecal samples obtained as de-

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scribed for the first test. Feces-egg cultures were not prepared for larval studies. Four calves from each group were killed at the termination of the test for parasite studies.

The grain feed supplement used was the same as used in the first test. The daily amount of feed given to each group of calves increased from 11.3 to 40.8 kg as the test progressed. Four premixes, containing soya flour, were mixed with the feed at the rate of 30 g/226.8 kg body weight. The first premix did not contain any thiabendazole, and the animals served as the unmedicated control group. The second premix contained 8.0 g of thiabendazole in each kg and enough of it was mixed with the feed to supply the animals with a daily dose of 1 mg/kg of body weight during 91 days of the test. The other two premixes had 16.0 and 32.3 g/kg and provided a dose level of 2 and 4 mg/kg of body weight to the calves from Groups III and IV, respectively.

**Table 1. Effect of Thiabendazole in Calves as a Feed Additive in Low Level Daily Dosages (Test No. 1).**

	I Control	II 1 mg/kg	III 4 mg/kg
No. of calves	10	10	10
No. of days on test	108	108	108
Avg weight on test, kg	173.7	173.7	174.8
Avg weight gain/calf, kg	50.8	52.2	64.0
A.D.G., kg	0.47	0.49	0.59
Feed/head/day; kg	2.57	2.56	2.55
Avg EPC			
8/1/66	96	54	122
8/25/66	335	174	108
9/21/66	493	856	1768
10/17/66	716	808	1662
Percentage larval recovery*			
8/1/66 (start)	52.3	47.6	51.3
8/25/66	53.2	0.4	1.7
9/21/66	43.5	25.7	5.4
10/17/66 (end)	41.5	27.7	23.6
Avg no. worms recovered**	15,978	9,030	11,676
<i>Haemonchus placei</i>	896	208	542
<i>Ostertagia ostertagi</i>	6,486	3,829	4,411
<i>Trichostrongylus axei</i>	3,324	2,932	1,345
<i>Cooperia punctata</i>	4,625	1,375	4,167
<i>C. oncophora</i>	318	58	767
<i>T. colubriformis</i>	1	63	200
<i>Bunostomum phlebotomum</i>	5	33	2
<i>Nematodirus</i> spp.	50	0	0
<i>Oesophagostomum radiatum</i>	1	7	1
All larvae	273	525	241

\* Percentage larval recovery = actual recovery/potential recovery × 100.

\*\* Four calves from each group necropsied.

## Results

**TEST 1:** The data in Table I show that the calves from Group III made higher average daily weight gains (A.D.G.) than those from the other two groups. The average number of eggs per g of feces (EPG) collected from the calves from the two medicated groups was lower than from the controls at the first sampling, but higher at the end of the test. The percentage of larvae recovered from cultures made from feces from the treated calves was lower than that from the control calves. The relative percentages of the various species of larvae obtained from the cultures did not vary appreciably between the three groups. Larvae of *Haemonchus* spp. predominated in all cultures. Larvae of *Cooperia oncophora* and *Ostertagia ostertagi* were next in numerical importance.

The calves treated with thiabendazole had a lower number of nematodes at necropsy than those from the untreated group although the differences are not significant.

**TEST 2:** The addition of thiabendazole to a grain feed supplement at the daily dose level of 1, 2, or 4 mg/kg of body weight failed to reduce the number of nematodes recovered at necropsy from the medicated animals. The numbers of *H. placei* recovered were reduced as the dosage was increased (Table 2). However, the apparent reduction was not statistically significant, probably because of the large range in the number recovered from the calves within the same group, and because the sampling used was perhaps too small. The 4 mg/kg dose appeared to reduce the average number of *T. axei* recovered, but this was also not significant. One specimen of *Oesophagostomum radiatum* was recovered from one calf of Group I and one of *Capillaria bovis* from one calf of Group IV.

The average number of eggs passed by the treated calves was lower than that from the untreated controls. The weight gains of the calves were not affected by the medication.

## Discussion

In general, our results showed that treatment of calves with daily doses of thiabendazole administered as an additive in a grain feed ration, failed to reduce the number of gastrointestinal nematodes at necropsy. *H. placei* apparently responded to the treatments, as

**Table 2.** Effect of thiabendazole in calves as a feed additive in low level daily doses (Test No. 2).

	I Control	II 1 mg/kg	III 2 mg/kg	IV 4 mg/kg
No. of calves	10	10	10	10
No. of days on test	91	91	91	91
Avg weight on test, kg	167.0	169.1	169.4	161.7
Avg weight gain, kg	63.8	66.2	57.9	63.3
A.D.G., kg	0.70	0.73	0.64	0.69
Feed/head/day, kg	2.56	2.57	2.55	2.56
Avg EPG				
6/1/67	155	155	157	153
6/30/67	95	114	86	80
7/27/67	389	52	113	34
8/24/67	345	139	121	27
Avg no. worms recovered*	33,490	74,005	125,332	52,732
<i>H. placei</i>	5,369	2,950	981	250
<i>O. ostertagi</i>	4,094	13,716	25,723	11,789
<i>T. axei</i>	12,269	10,900	14,975	4,679
<i>C. punctata</i>	154	400	1,765	45
<i>C. oncophora</i>	15	0	4,450	0
<i>T. colubriformis</i>	30	135	50	78
<i>B. phlebotomum</i>	15	4	9	35
All larvae	11,544	45,900	77,379	35,856

\* Four calves from each group necropsied.

their number was generally reduced as the dose of thiabendazole increased, but the reduction was not significant. The gains in weight made by the treated calves in both tests were erratic, as compared with the control animals. The percentage of larvae recovered was lower from the cultures made from treated animals, although this study was conducted in Test 1 only. However, this reduction perhaps may be related to the ovicidal activity of thiabendazole reported by Southcott (1963) and Barnett et al. (1964). The number of eggs passed by the medicated calves, was reduced in Test 2 only. Therefore, it appears that low level dosages of thiabendazole are of no help in controlling parasitism in calves, and show no economic advantages. This type of treatment may be of future epizootiological implication as some strains of nematodes common in cattle may be naturally selected for their resistance to medication, as already reported by Drudge et al. (1964) and by Smeal et al. (1968).

### Summary

Two experiments were conducted to evaluate the anthelmintic efficacy of thiabendazole as a feed additive in daily low level dosages of 1, 2, and 4 mg/kg of body weight in calves. These dosages did not reduce the number of nematodes recovered at necropsy to any statis-

tical significance. None of the treatments consistently resulted in changes in the weight and in the number of nematode eggs passed by the calves. There was a reduction in the percentage of infective larvae recovered from cultures from the treated animals as compared with those from the control group.

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## *Peltamigratus thornei* sp. n. (Nematoda: Hoplolaimidae) from Soil in Central America

NATALIE A. KNOBLOCH<sup>1</sup>

Sher (1963) revised the *Hoplolaiminae* and erected a new genus, *Peltamigratus*. He proposed *P. christiei* (Golden and Taylor), 1956, as the type species and described four new species. Since 1963, two additional species have been included, *P. pachyurus* Loof (1964) and *P. sheri* Andrassy (1968).

*Peltamigratus thornei* sp. n. was recovered from sandy soil collected August 17, 1968, from soil around the roots of two coconut palms growing at sea level on the Salt Creek Estates, Ltd. in British Honduras. The collection site was located approximately one mile from the Caribbean Sea. Several hundred young females were recovered; no males were found. Presence of only young females indicates that the time was outside the period in the life cycle when reproduction occurs.

The description of the species is based on preserved specimens. Measurements were made on specimens which had been heat relaxed, fixed in F.A. 4:10, preserved and mounted according to the glycerol-ethanol method of Seinhorst (1959).

### *Peltamigratus thornei* sp. n. (Figs. 1-7)

HOLOTYPE FEMALE: Length = 0.79 mm; a = 26; b = 6.0; c = 48; V = <sup>15</sup>55<sup>15</sup>; spear = 32.5  $\mu$ .

PARATYPE FEMALES (20): Length = 0.78-1.1 mm; a = 26-35; b = 5.8-7.4; c = 32-50; V = <sup>13-21</sup>51-56<sup>13-21</sup>; spear = 32-33  $\mu$ ; anterior phasmid 78-88% and posterior phasmid 83-92% from anterior end of body.

FEMALE: Body usually C shaped, longer specimens spiral shaped. Body cuticle in thick

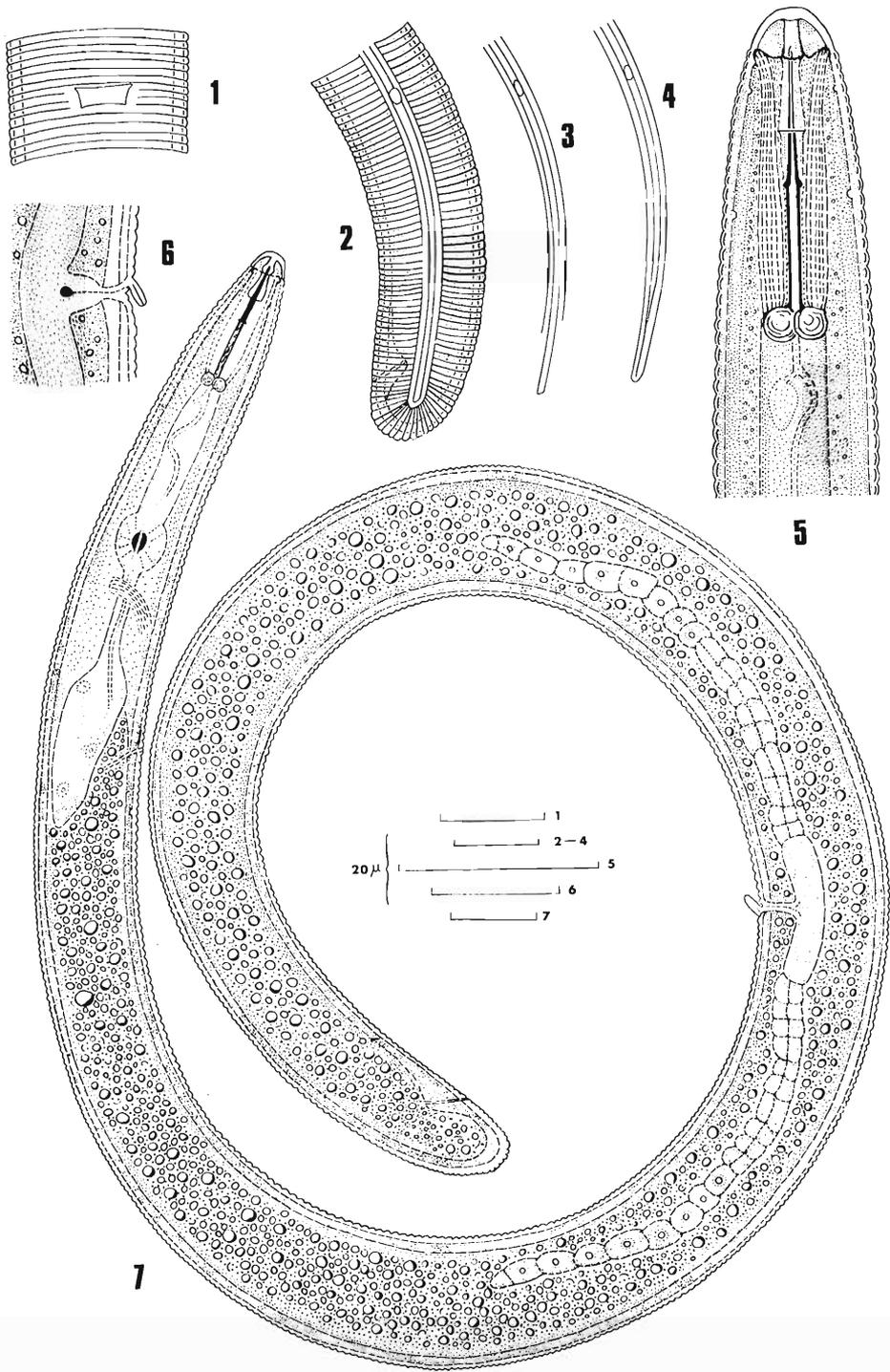
layers. Lateral field with four incisures, the outer usually disappearing somewhat anterior to anal region but with considerable variation occurring (Figs. 2, 3, 4). Right phasmid at 82% and left 88% from anterior end of body. Lip region not set off, smooth with no annules observed. Anterior cephalids about 1 lip region behind cephalic framework. Posterior cephalids about opposite middle of spear (Fig. 5). Outlet of dorsal oesophageal gland about 6  $\mu$  behind spear base. Excretory pore usually opposite middle of basal oesophageal lobe but its position is somewhat variable. Hemizonid an obscure refractive line about two annules long, adjacent or slightly anterior to excretory pore. Hemizonion about eight annules posterior to excretory pore. Epiptygma double, conspicuous; lying flattened or slightly elevated over vulva. Vulva transverse. A refractive structure, variable in form and size at base of vagina (Fig. 6). Ovaries outstretched with oocytes arranged in single file in young females in which ovaries are immature and egg production has not commenced. No spermatheca seen. Rectum attached to ventral side of intestine which forms a post-anal blind sac. Tail bluntly rounded with 12-14 annules (Fig. 7); in some specimens a slight constriction is apparent between distal annules of tail.

HOLOTYPE, Female: Collected 17 August 1968 by the author from a coconut plantation in British Honduras. Slide T-142t deposited with U.S.D.A. Nematology Collection, Beltsville, Maryland.

PARATYPES, Females: Same data as holotype. Slides T-694p and T-695p deposited with USDA Nematology Collection, Beltsville, Maryland. Additional slides in the following collections: Specimens filed under *Peltamigratus* 1, Entomology Department, Michigan State University, East Lansing, Michigan.

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Figures 1-7. *Peltamigratus thornei* sp. n. 1. Female, ventral view of anterior epiptygma. 2-4. Female, posterior end with variations in lateral field. 5. Female, head region. 6. Female, vulvar region with dorsal view of epiptygma and refractive structure at base of vagina. 7. Female.



Specimens filed under *Peltamigratus* 1, Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin. Specimens filed under *Peltamigratus* 1, Department of Plant Pathology, South Dakota State University, Brookings, South Dakota.

TYPE HABITAT: Sandy soil around roots of coconut palm, *Cocos nucifera* L.

TYPE LOCALITY: Salt Creek Estates Ltd., near Belize, British Honduras.

DIAGNOSIS: *Peltamigratus thornei* sp. n. can be distinguished from the closely related species *P. luci* Sher, 1963, by the rounded spear knobs, longer spear (32–33  $\mu$ ) against 26–29  $\mu$  in *P. luci*), an obscure hemizonid anterior to excretory pore, no spermatheca, rounded tail terminus. *P. luci* has a well developed hemizonid posterior to excretory pore, oval spermatheca with sperm and a bluntly conical tail with distal annules narrower than other annules of tail. It differs from *P. pachyurus* Loof, 1964, in a projecting epiptygma and an annulated tail terminus. *P. pachyurus* has a conspicuous thickened cuticle with smooth surface on the terminus. *P. thornei* can be distinguished from the type species, *P. christiei* (Golden and Taylor), by four incisures in the lateral field, no spermatheca, no deep constriction of the annules on the female tail. It differs from *P. holdemani* Sher, 1963, by the larger conspicuous epiptygma, single hemizonid anterior to excretory pore, no spermatheca, and 12 to 14 annules on tail against 10 annules in *P. holdemani*. From *P. macbethi* Sher 1963, it differs in hemizonid anterior to excretory pore, no spermatheca, shape of epiptygma which in *P. thornei* does not project straight out from the body but lies flattened back over the vulva, four incisures in lateral field, longer spear (32–33  $\mu$  against 27–29  $\mu$  in *P. macbethi*). Distinguished from *P. nigeriensis* Sher, 1963, by the longer spear (32–33  $\mu$  against 26–30  $\mu$  in *P. nigeriensis*), hemizonid anterior to excretory pore, no spermatheca, epiptygma does not project straight out from body, number of tail annules (12–14 against 7 in *P. nigeriensis*). Distinguished from *P. sheri* Andrassy, 1968, by four incisures in lateral field, a projecting epiptygma flattened over the vulva, head which is not set off, no spermatheca.

### Key to Species of *Peltamigratus*

1. Females with spermatheca, males known 3  
Females without spermatheca, males  
unknown ..... 2
2. Terminus with annules ..... *thornei* sp. n.  
Terminus without  
annules ..... *pachyurus* Loof, 1964
3. Epiptygma double, conspicuous ..... 4  
Epiptygma single,  
inconspicuous ..... *holdemani* Sher, 1963
4. Two or less incisures in lateral field ..... 5  
Four incisures in lateral field ..... 7
5. Hemizonid anterior to excretory pore .... 6  
Hemizonid posterior to excretory  
pore ..... *macbethi* Sher, 1963
6. Head well set off, annules of tail  
not separated by deep  
constrictions ..... *sheri* Andrassy, 1968  
Head not set off, or if so, very slightly,  
annules of tail separated by deep con-  
strictions (1 or more annule width  
deep) ..... *christiei* Sher, 1963
7. Female tail with more than 10 annules  
(12), distal annules narrower than  
other tail annules ..... *luci* Sher, 1963  
Female tail with less than 10 annules  
(7), distal annules wider than other  
tail annules ..... *nigeriensis* Sher, 1963

### Acknowledgment

The writer thanks Professor Gerald Thorne, Department of Plant Pathology, University of Wisconsin for continued guidance and assistance.

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## Observations on the Effects of Fish Serum on Cercarial and Metacercarial Stages of *Posthodiplostomum minimum* (Trematoda: Diplostomidae)<sup>1</sup>

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The effects of vertebrate sera on cercariae have been reported occasionally in the literature. Culbertson and Talbot (1935) observed cercaricidal activity of serum from uninfected mice, rats, snakes, frogs, and fish. True cercaricidal activity was preceded by the formation of a granular or globular precipitate which surrounded first the tail and later the body. A period of reduced, uncoordinated larval movement occurred immediately prior to death, this state being determined by an absence of motility and flame cell activity. Experimental studies indicated the responsible factor to be storage and heat labile (56 C for 30 min). Papirmeister and Bang (1948) recorded another phenomenon when cercariae were placed in either uninfected or infected *Schistosoma mansoni* mouse and rat serum. Finely granular or globular surface deposits accumulated around the larvae in what they termed the precipitin reaction. When these workers exposed cercariae to heat inactivated serum, a pericercarial envelope always formed. Liu and Bang (1950) reported agglutination of cercariae into large clumps in infected mouse and hamster serum. Studies by Stirewalt and Evans (1955) indicated that agglutination might be a stage in a weak or slowly developing *cercarienhüllenreaktion* (CHR) of Vogel and Minning (1949). This reaction was produced when *S. mansoni* cercariae were placed in serum of infected mice and hamsters and in heat inactivated serum of infected rats (Stirewalt and Evans, 1955). Stirewalt (1963) demonstrated that newly recovered schistosomules did not give the CHR as did cercariae, this indicating a change in the outer surfaces of the cercarial integument. Further, cercaricidal serum was found to be ineffective against the schistosomule, suggesting a lack of correlation between in vitro cercaricidal activity and the susceptibility of individual hosts.

The studies of Culbertson and Talbot (1935), as noted earlier, dealt with cercaricidal effects of serum from uninfected *Ictalurus nebulosus* (LeSueur) and did not include the metacercarial stage. In the present study, serum from centrarchid fishes both infected and uninfected with metacercariae of the trematode *Posthodiplostomum minimum* (MacCallum, 1921) Dubois 1936 were analyzed for their cercaricidal and metacercaricidal activities.

### Materials and Methods

Fourteen fishes were used in the investigation including five *Lepomis macrochirus* Rafinesque, one *L. megalotus* (Rafinesque), four *L. microlophus* (Gunther), and four *Chaenobryttus gulosus* (Cuvier), collected by either seine or hook from Club Lake, an area in east Texas which has a high incidence of *P. minimum* and from Lake Granite Shoals, central Texas, where the parasite is essentially absent. Blood was obtained by cardiac puncture, allowed to clot at 5 C, and serum extracted using a Lourdes refrigerated centrifuge. All fishes were given thorough post-mortem examinations to determine the extent of metacercarial infection with *P. minimum* as well as to eliminate those possessing other parasites. Cercariae used in the study were collected after their spontaneous emergence from snails, *Physa halei* Lea; whereas, metacercariae were obtained from heart and liver tissue of fishes and excysted and washed in physiological saline before being exposed to serum. *Schistosoma mansoni* were obtained from snails, *Australorbus glabratus* Say. Eight larvae were added to each unpooled serum sample in a depression slide with several samples being taken from each of the 14 fishes.

### Results

Cercariae exposed to fresh unheated serum from infected and uninfected fish reacted similarly in being initially hyperactive and vigorous

<sup>1</sup> Supported in part by grants from the Penrose Fund of the American Philosophical Society and the Sport Fishing Institute.

in movement. Within three minutes globular secretions were copiously exuded from the oral end of the cercariae, and this continued until death. Detachment of tails normally occurred within 10 minutes, although some were retained for 30 min (Fig. 1). After 30 min a soft mucoid sheath enveloped the larvae causing debris to adhere to the surfaces. Most cercariae were immobilized and appeared dead after several hours. Those placed in serum heated at 56 C for 30 min on the other hand produced no oral exudate, failed to detach their tails during the first hr, and remained motile after 12 hr. A similar reaction was observed using *Schistosoma mansoni* cercariae indicating the nonspecific nature of the reaction (Fig. 2).

Excysted metacercariae exposed to infected and uninfected serum likewise secreted copious quantities of water-insoluble exudate in amounts approaching the size of the metacercariae itself, and continued to secrete it until succumbing. Within 10 min a thin mucoid sheath began forming around the excysted metacercariae and in 15–20 min the metacercarial membranes of many larvae appeared to weaken and balloon, often in several different places on each parasite (Fig. 3). Excysted metacercariae began to lyse at the weakened surfaces 30–60 min after exposure to serum, all dying within 2 hr. Excysted larvae placed in heat inactivated serum produced small amounts of oral exudate within 30 min, but no lysis of membranes occurred and all were viable after 2 hours. Intact metacercariae in cysts were unaffected by serum. When they were exposed to serum for 2 hr, mechanically excysted, and the excysted larvae subjected to direct exposure to serum, lysis occurred within the hour. Reactions obtained were identical to metacercariae not incubated in their cysts prior to exposure. Larvae incubated in cysts, excysted, and placed in heat inactivated serum were unaffected. Controls placed in saline showed normal motility after several hours with no obvious deleterious effects.

## Discussion

The present study is the first to report a metacercaricidal factor present in centrarchid fish serum taken from hosts uninfected or infected with metacercariae of *Posthodiplostomum minimum*. That this is possibly a non-specific reaction is evidenced by the fact that cercaricidal effects on both *P. minimum* and *S. mansoni* were likewise displayed. Metacercariae were obviously shielded from the factor(s) while in intact cysts. Even when incubated in serum for several hours prior to excystment and exposure, no differences in survival rate were apparent. Both cercaricidal and metacercaricidal properties of the serum were destroyed by heating at 56 C for 30 min and by storage for 48 hr.

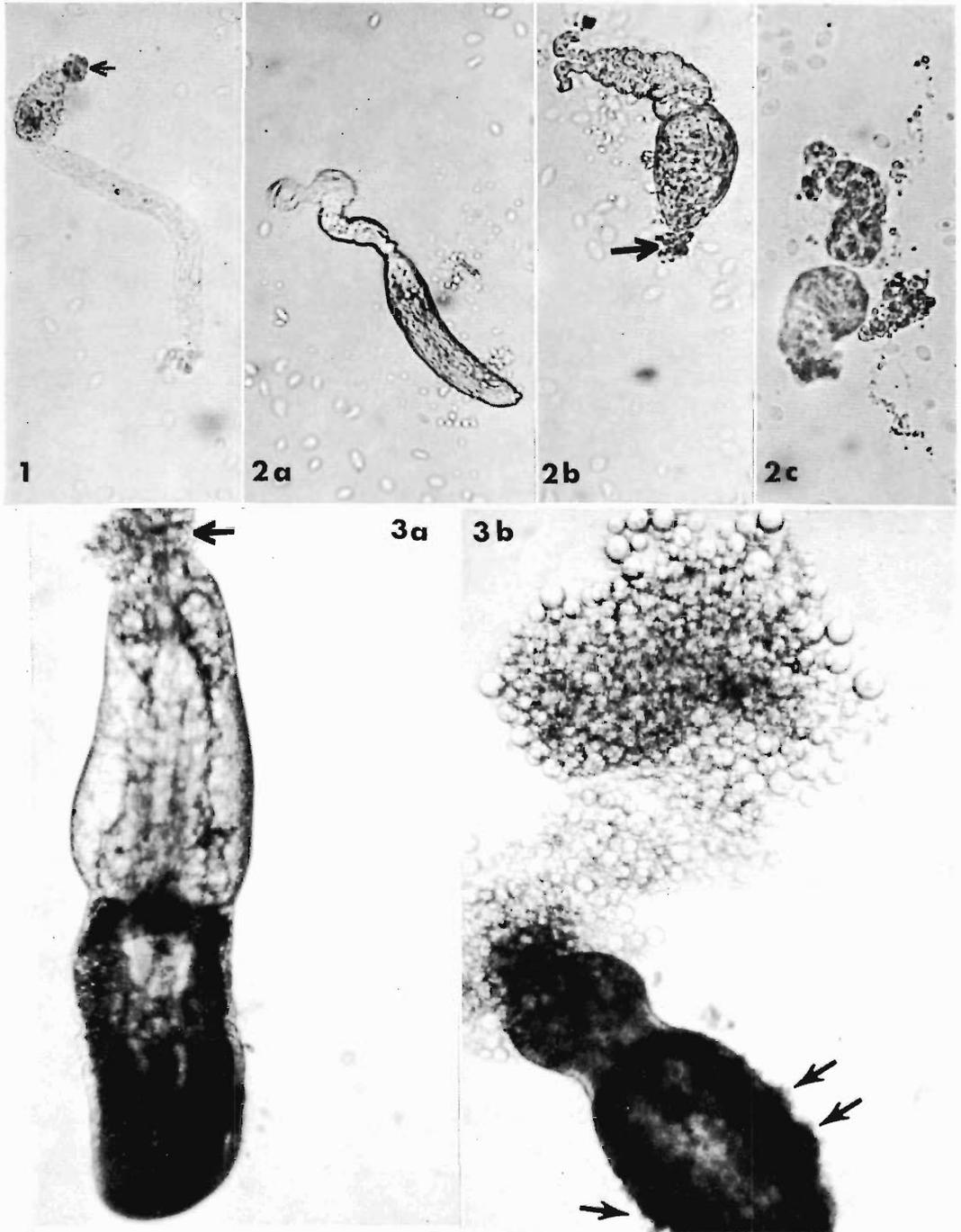
The comparative responses of *P. minimum* and *S. mansoni* larval stages when exposed to fish serum are of interest. Stirewalt (1963) reported cercariae of *S. mansoni* reacted in host serum while schistosomules were unaffected. This is supported by recent micrographs by Lichtenberg (1967) that illustrate distinct differences between the cercarial and schistosomule integuments of *S. mansoni*, a fact which suggests a possible physiological alteration of the worm as a prerequisite for survival in the host. *Posthodiplostomum minimum* apparently does not undergo such alteration upon excysting since both larval stages are serum-sensitive. In view of the nonspecificity of the reaction with respect to types of cercariae employed and the loss of both cercaricidal and metacercaricidal factors by heat inactivation, it may be suggested that identical serum factor(s) are involved. The movement of cercariae through the host circulatory system without damage from cercaricidal agents prior to encystment as metacercariae is unexplained.

## Summary

Serum from fishes both infected and uninfected with metacercariae of *Posthodiplostomum minimum* were found to have cercari-

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Figures 1–3. 1. *P. minimum* cercaria at 10 min exposure to *Lepomis macrochirus* serum. Note oral exudate (arrow) and adherence of debris.  $\times 30$ . 2. Sequence illustrating the effects of *L. macrochirus* serum on *S. mansoni* cercaria: (a) at 5 min, (b) at 10 min, (c) at 25 min.  $\times 30$ . 3. Metacercaricidal effect of *L. macrochirus* serum on *P. minimum*. Note oral exudate (arrow) at 5 min. (a) Compared to copious amount at 60 min. (b) Arrows at 60 min denote areas of membrane lysis.  $\times 16$ .



cidal and metacercaricidal activities. Metacercariae exposed to serum in intact cysts were unaffected but displayed metacercaricidal behavior when exposed without cysts. When heated for 30 min at 56 C, serum produced no response from either larval stage.

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## *Diplectanum lacustris* sp. nov. (Dactylogyroidea: Diplectanidae), a Monogenetic Trematode from the Gills of the Nile Perch

JUNE P. THURSTON<sup>1</sup> AND I. PAPERNA<sup>2</sup>

During surveys of fish parasites in Ghana and Uganda, specimens of a monogenetic trematode were obtained from the gills of two species of *Lates*, the Nile Perch. The trematode was identified as a new species of *Diplectanum* (Dactylogyroidea: Diplectanidae). *Diplectanum* is predominantly a parasite of marine teleosts, and the present species is therefore unusual in occurring on a fresh water fish. Interestingly, however, the genus *Lates* is classified by Greenwood (1966) in the Family Centropomidae, which is composed mainly of marine fish. *Lates calcarifer*, which is the host of *Diplectanum latesi* Tripathi, 1955 in India, is an estuarine species.

#### Materials and Methods

Five specimens of *Lates albertianus* were obtained in Uganda from Lake Albert and nine

from the River Nile between Lakes Victoria and Kyoga, while three specimens of *Lates niloticus* were obtained from the newly formed Volta Lake in Ghana.

Methods used in collecting the monogeneids, and in their fixation, mounting and measurement were similar to those used in earlier studies (Paperna and Thurston, 1969). In addition, some specimens were stained with Semichon's carmine and cleared in clove oil, but examination of these preparations revealed little more anatomical detail than the examination of specimens mounted in glycerin jelly.

#### *Diplectanum lacustris* sp. nov.

##### Description

This parasite exhibits a wide range of shapes and sizes, from typical "slender" forms in which the opisthaptor is well delineated from the body, to "gravid" forms which are proportionately wider and usually longer than the "slender" forms and in which the opisthaptor is almost completely embedded in the pos-

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terior end of the body (Figs. 1, 2). The opisthaptor armature (Fig. 3) remains of the same shape and size in the two forms.

The postero-dorsal pair of anchors have only a vestigial inner root, while in the antero-ventral pair of anchors the outer root is extremely long. Hooklets are vestigial. The dorsal and ventral squamodiscs consist of 10–11 concentric rows of rodlets, the two distal rows of which are composed of only rudimentary rodlets.

Reproductive organs appear well developed in both “slender” and “gravid” forms, but only one uterine egg (Fig. 4) was found in 12 “gravid” specimens that were examined, and none was found in 80 “slender” specimens. The “slender” forms were fully mature because in several specimens ova were observed lying in the oviduct, having just been shed from the ovary. “Gravid” specimens contain dense masses of vitelline follicles. The copulatory organs lack any sclerotization or additional structures (Fig. 5). All measurements are in microns.

#### Measurements

“SLENDER” SPECIMENS (based on 6 specimens): Total length, 650–1000; breadth, 150–250; opisthaptor 50–150 in depth, 100–200 in breadth; squamodiscs 30–40 in depth, 50–70 in width; postero-dorsal pair of anchors 60–70, inner root vestigial; antero-ventral pair of anchors 70–80, inner root 10–20, outer root 40–60; lateral bars 35–40, median bar 50–60; hooklets vestigial.

“GRAVID” SPECIMENS (based on 8 specimens): Total length 1,000–2,000; breadth 300–500; opisthaptor 50–150 × 150–250; anchors 60–80; uterine egg 46 × 23 (without the filament). Measurements of anchors, bars and squamodiscs as in “slender” specimens.

HOSTS AND LOCALITIES: *Lates albertianus*, Lake Albert and the Victoria Nile, Uganda. *Lates niloticus*, Volta Lake, Ghana. The holotype is in the collection of the second author, and paratypes are deposited in the British Museum of Natural History, London.

#### Differential diagnosis

The long outer root in one of the pairs of anchors and the absence of sclerotized cirrus are distinct characters which separate *Diplectanum lacustris* from all other known species of *Diplectanum*.

*Diplectanum latesi*, which was described by Tripathi (1955) from *Lates calcarifer* in India, differs from *Diplectanum lacustris* in the number of concentric rows in the squamodiscs in addition to differences in the morphological pattern of the cirrus and the shape of the anchors. The size of *D. latesi*, 550–940 by 110–250, corresponds to that of the “slender” form of *D. lacustris*.

#### Comparison of the parasite fauna of fish from different localities

Superficial examination of *Lates niloticus* from the Volta Lake showed that almost all were infested with the “gravid” form of *D. lacustris*, while “slender” forms were found as well on two fish which were subjected to more detailed examination. Crustacean parasites, *Ergasilus kandti*, were few in number.

Both “gravid” and “slender” forms were likewise found on *Lates albertianus* from Lake Albert, as is shown in Table 1. The parasite was found on three out of the five fish examined. The mean number of parasites in these three infected fish was 16, and the maximum number in this limited survey was 39. On the other hand, these Nile Perch were heavily infested with the crustacean gill parasite *Ergasilus kandti*.

Nine specimens of *Lates albertianus* from the Victoria Nile have been examined, and all were infested with *Diplectanum lacustris*; only the “slender” form was found. The mean number of parasites per fish was 104, and the maximum number recorded was 405. There seems to be no correlation between the size of the Nile Perch and the number of *D. lacustris*. *Ergasilus kandti* was not found on *Lates albertianus* from the Victoria Nile. Another crustacean parasite, *Dolops ranarum*, was frequently found on Nile Perch from both the lake and the river, but has not been included in the table.

At present, no reason can be given for the absence of *Ergasilus kandti* from Nile Perch in the Victoria Nile, nor for the heavy infestations of *D. lacustris* in these fish. It is possible that in Lake Albert the heavy infestations with *Ergasilus kandti* may make the gills less suitable for monogeneans to become established, and therefore may be the reason for the low rate of infestation with *D. lacustris*. Fryer (1965) recorded very heavy infestations of *E.*

Table 1. Numbers of *Diplectanum lacustris* and crustacean parasites from the gills of *Lates albertianus* from Lake Albert and the Victoria Nile. An asterisk indicates that gills from only one side of the fish were examined. *Dolops ranarum* is not included in this table.

Date	Locality	Fish Standard length cm	Fish weight kg	No. <i>D. lacustris</i>		No. Crustacea	
				"slender"	"gravid"		
<i>Lake Albert</i>							
Sept. 1967	Butiaba	25	—	0	8	55	<i>Ergasilus kandti</i> Note 1
Sept. 1967	Butiaba	30	—	0	0	80	<i>Ergasilus kandti</i> Note 1
Sept. 1967	Butiaba	30	—	25	14	43	<i>Ergasilus kandti</i> Note 1
Sept. 1967	Butiaba	70	—	0*	1*	963*	<i>Ergasilus kandti</i> Note 1
Sept. 1968	Butiaba	18	—	0	0	0	Note 2
<i>Victoria Nile</i>							
Jan. 1966	Kalagala Falls	—	2.5	17	0	0	Note 3
Jan. 1966	Kalagala Falls	—	3.2	88	0	0	Note 3
Feb. 1966	Mbulamuti	—	22.0	16	0	0	Note 4
Sept. 1967	Mbulamuti	—	2.7	105	0	0	Note 4
Sept. 1967	Mbulamuti	100	25.0	94*	0*	0*	Note 4
Sept. 1967	Mbulamuti	130	41.0	12*	0*	0*	Note 4
Oct. 1968	Mbulamuti	55	—	84	0	0	Note 4
Oct. 1968	Mbulamuti	75	—	10	0	1	larval lernaedid Note 4
Nov. 1968	Mbulamuti	67	4.5	405	0	0	Note 4

- Note 1. Fish bought from local fishermen; exact locality unknown.  
 Note 2. Fish caught in shallow water.  
 Note 3. Fish caught in rapidly flowing water.  
 Note 4. Fish caught in water flowing at a medium rate.

*kandti* on *Lates albertianus* from Lake Albert but noted that the gills appeared to suffer little damage from the parasite. Greenwood (1966), however, listed heavy parasitization of the gills as a contributory factor in the periodic mass mortality of *Lates albertianus* in Lake Albert, which is probably chiefly associated with de-oxygenation of the water. The crustacean parasites, being the more numerous, are likely to be more important than the monogeneans in contributing to these deaths.

It is interesting to note that the parasite faunas of the two samples of *Lates albertianus* are now different, although the fish in the Victoria Nile originated from Lake Albert. Nile Perch had been restricted to Lake Albert and the Albert Nile, but on a number of occasions between 1954 and 1960 specimens were taken from the Butiaba region of Lake Albert and were released at various places along the Victoria Nile above the Murchison Falls. More than 500 fish were transported during this

time (Anderson, 1961). They are now well established and are being fished commercially and by anglers. No precautions were taken against transferring parasites from Lake Albert to the Victoria Nile.

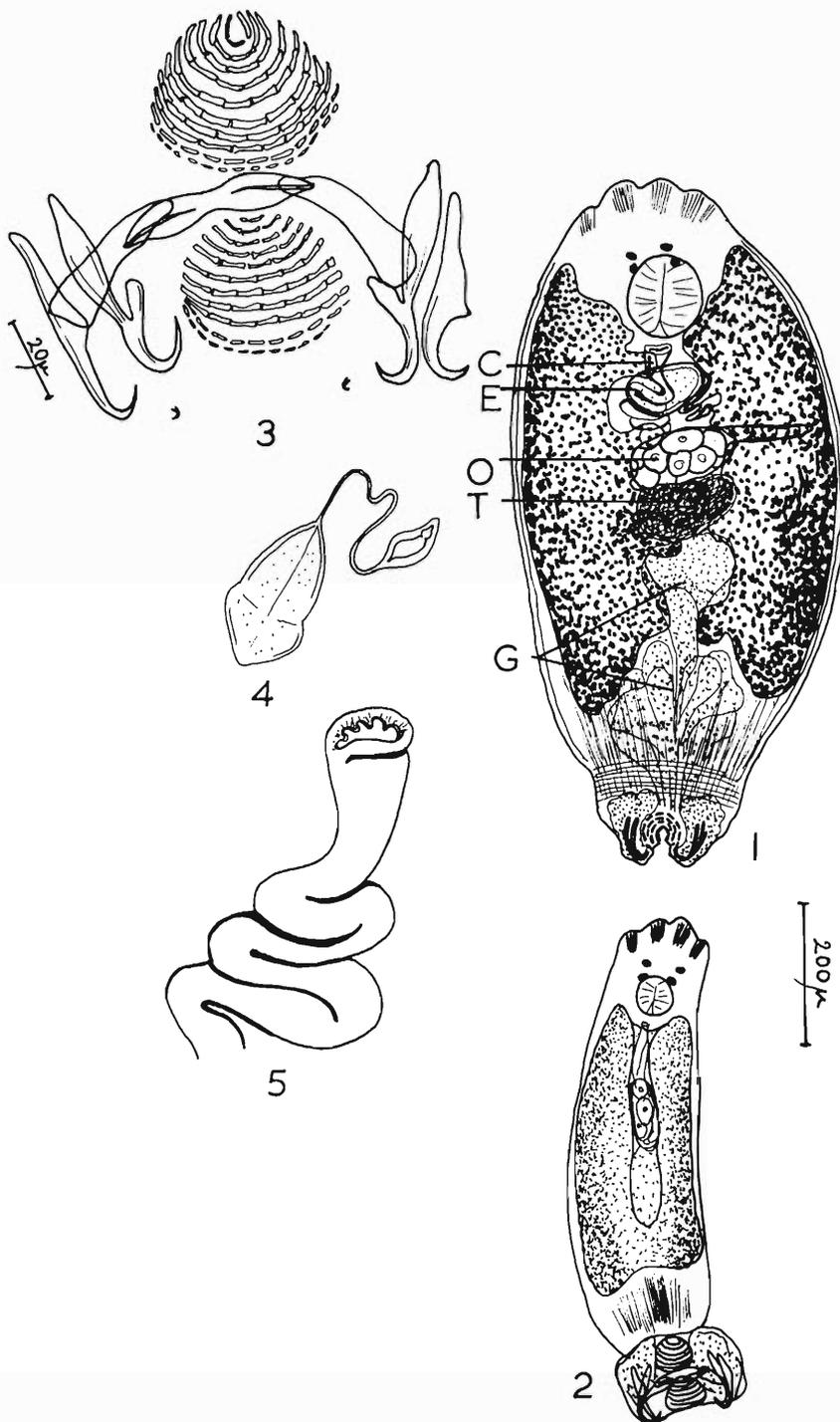
### Summary

1. A new species of monogenetic trematode, *Diplectanum lacustris*, is described from the gills of two Nile Perch species, *Lates albertianus* in Uganda and *Lates niloticus* in Ghana. It differs from other species of *Diplectanum* in possessing a long outer root to one of the pairs of anchors, and also in lacking a sclerotized cirrus.

2. A broad "gravid" form of *D. lacustris* is found on fish from the Volta Lake and from Lake Albert, in addition to typical "slender" specimens; but only the "slender" form has been found on Nile Perch from the Victoria Nile.

Figures 1-5. *Diplectanum lacustris* from the gills of the Nile Perch. 1. "Gravid" form. 2. "Slender" form. 3. Anchors, bars, and squamodiscs. 4. Intrauterine egg. 5. Copulatory organ.

Abbreviations: C, copulatory organ; E, egg; G, cement glands; O, ovary; T, testes.



3. Specimens of *Lates albertianus* from Lake Albert are less heavily infested with *D. lacustris* than specimens from the Victoria Nile. This may be because the fish from Lake Albert are heavily infested with the crustacean gill parasite *Ergasilus kandti*, whereas this parasite is absent from the Victoria Nile fish.

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## *Litobothrium alopias* and *L. coniformis*, Two New Cestodes Representing a New Order from Elasmobranch Fishes<sup>1</sup>

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A massive infection of two unusual cestodes was found in the spiral valve of two bigeye thresher sharks, *Alopias superciliosus* (Lowe, 1840) (O. Pleurotremata, Fam. Alopiidae). The first shark was caught on 22 October 1966, in about 183 meter (100 fathoms) of water, one and one half nautical miles off Newport Beach, California. The second host was found on 24 August 1968, shot through the head, on Bolsa Chica State Beach, Huntington Beach, California.

Under the existing systems of cestode classification (Hyman, 1951; Wardle and McLeod,

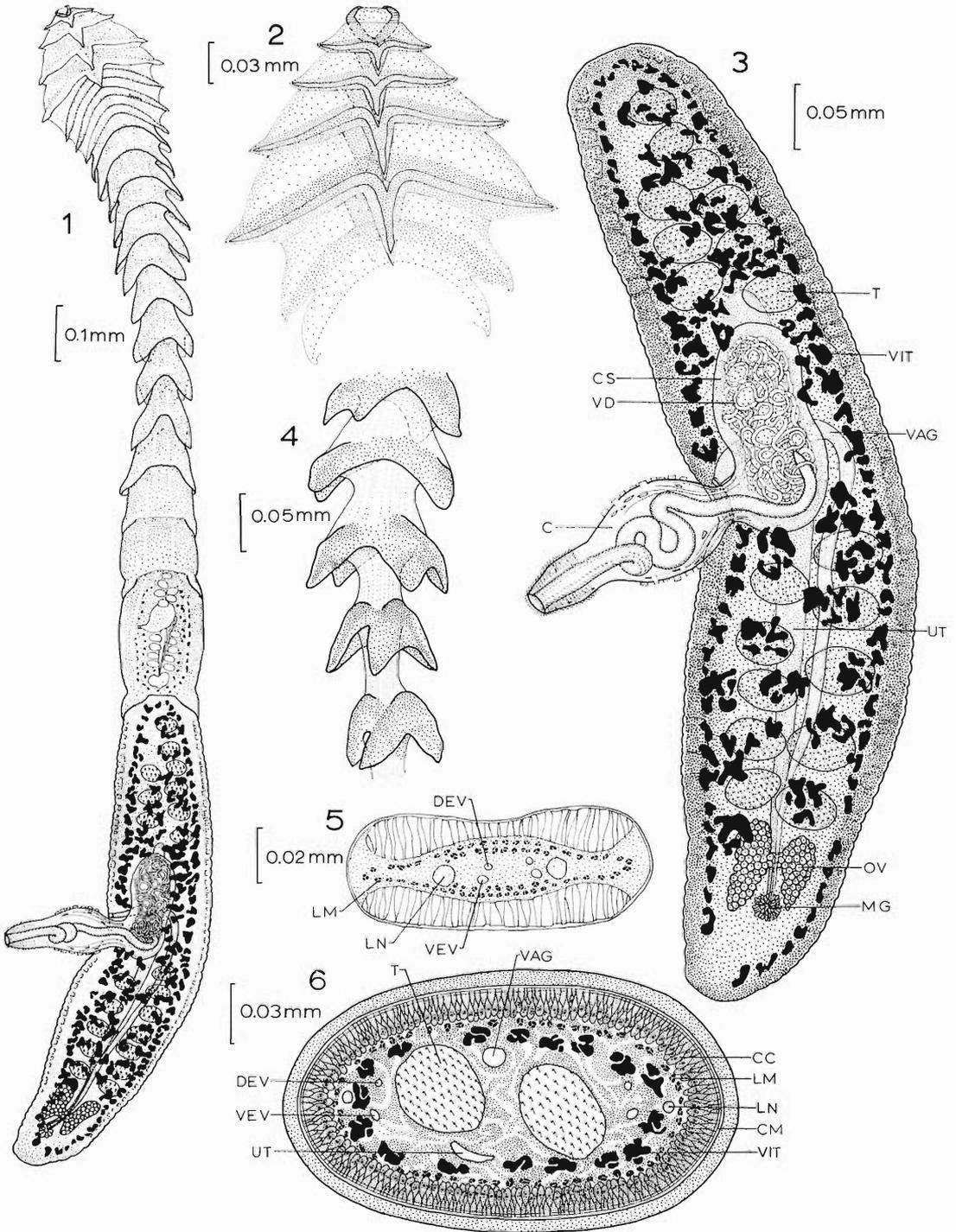
1952; Yamaguti, 1959; Joyeux and Baer, 1961), holdfast morphology is used as the distinguishing characteristic at the ordinal level. In light of the unique holdfast features which restrict placement of these two distinct cestodes in any existing orders, coupled with the fact that the parasite is well established, being found in large numbers in two separate hosts examined almost two years apart, the new order Litobothridea is proposed.

#### Methods

Worms were removed from the spiral valve and fixed in Lavdowsky's fluid (AFA) and Bouin's fluid. Whole mounts were stained with

<sup>1</sup> This study was supported by the Long Beach California State College Foundation under Grant #NSF IG 212.73.

Figures 1-6. *Litobothrium alopias* gen. n., sp. n. 1. Strobilate worm. 2. Anterior end of specimen showing apical sucker and modified segments. 3. Mature proglottid. 4. Segments number 16-19 showing isthmus between four muscular, lacinated projections. 5. Transverse section through muscular region of Figure 4. 6. Transverse section through preovarian region of mature proglottid. Abbreviations: C, cirrus; CC, cuticular cells; CM, circular muscle; CS, cirrus sac; DEV, dorsal excretory vessel; LM, longitudinal muscle; LN, lateral nerve; MG, Mehlis gland; OV, ovary; T, testis; UT, uterus; VAG, vagina; VD, vas deferens; VEV, ventral excretory vessel; VIT, vitellarium.



celestine blue B and Semichon's carmine. Specimens were sectioned singly and *in situ* at 6, 8, and 10  $\mu$  and stained with haematoxylin-eosin and Mallory's trichrome. All material was dehydrated in ethanol, cleared in xylene and mounted in Piccolyte. Drawings were made with the aid of a drawing tube. All measurements are in microns unless otherwise stated. Average measurements are given, with ranges in parentheses.

*Litobothrium alopias* gen. n., sp. n.  
(Figs. 1-6, 11-13)

The following description is based on 30 specimens. Specific diagnosis: small, laciniated, craspedote, anapolytic worms, measuring 2.27 mm (1.65-3.70) in length. Strobila consists of 29 (20-34) segments (Fig. 1). In gravid worms, mature proglottid approximately 7 times longer than broad, 780 (330-1570) long by 190 (125-290) wide. Apical sucker cup or clamp shaped, strongly muscled, measuring 38.0 (30.0-59.0) in diameter by 26.5 (18-37) in depth (Fig. 2). Anterior four segments modified into accessory holdfast structures which are cruciform in cross section. Strobila swelling in width immediately posterior to apical sucker, reaching maximum body width at fourth segment 220 (120-340) decreasing to 190 (125-290) in mature and gravid segments (Fig. 2). Segments 16-19 on all specimens show an unusual formation with an isthmus between four muscular, laciniated projections which fold about the next segment (Figs. 4, 5). Testes spherical to subspherical, 20 (15-27) in number, 39 (28-50) in diameter, in two distinct rows, approximately equal numbers occurring pre- and postporally. Vas deferens forming large mass of coils in mature proglottid. Cirrus sac large, extending more than half proglottid width, 164 (108-210) long by 87 (70-125) wide. Cirrus armed with minute spines distally and small peg-like projections proximally. Genital aperture lateral, irregularly alternating, approximately midsegment. Ovary posterior, bilobed, X-shaped in transverse section. Vitellaria large amorphous follicles encircling proglottid (Fig. 6).

HOST: Bigeye thresher shark *Alopias superciliosus*.

LOCATION: Spiral valve.

LOCALITY: Newport Beach, California.

TYPE SPECIMENS: Holotype and paratypes USNM Helm. Coll. Nos. 71324, 71325.

REMARKS: In transverse section the X-shaped cerebral ganglion is found 90-100 posterior to the apical sucker. It is similar to that shown by Rees (1959) for *Ditrachybothrium macrocephalum*. The apical sucker is elliptical in shape (Fig. 12) and in *en face* view an internal, horizontal slit is seen extending the diameter of the sucker. The sucker functions in a clamp-like manner during attachment to the spiral valve mucosa (Fig. 13).

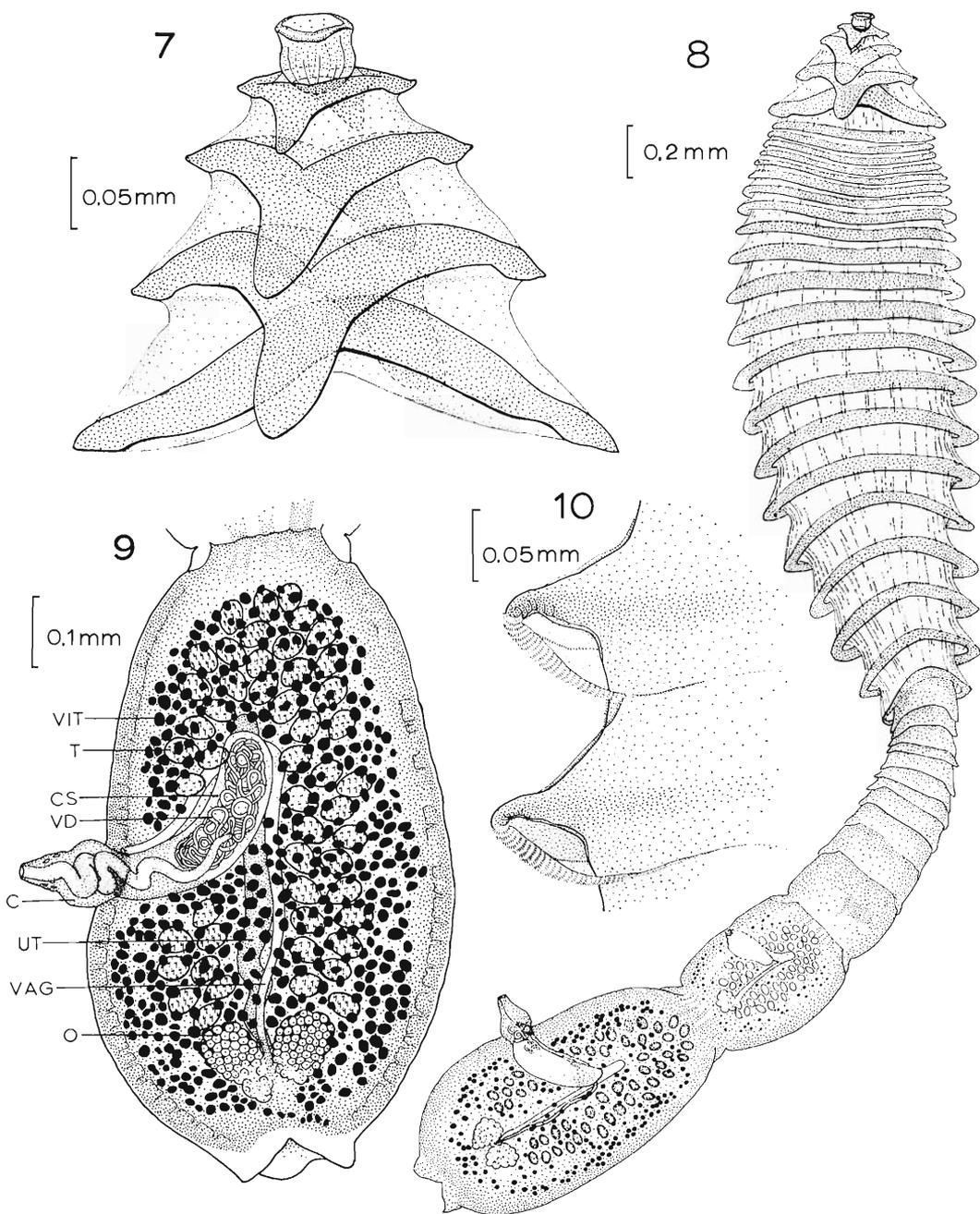
*Litobothrium coniformis* sp. n.  
(Figs. 7-10)

The following description is based on 25 specimens recovered from the spiral valve of two bigeye thresher sharks from Southern California. Specific diagnosis: Small, craspedote, apolytic worms measuring 4.14 mm (2.0-8.0) in length. Strobila consists of 41 (29-51) segments. Only terminal proglottid mature, approximately 4 times longer than broad, 1,103 (600-2,160) long by 363 (200-600) wide. Apical sucker bowl shaped, not clamp like, weakly muscled, measuring 62.0 (46-90) in diameter by 46.0 (22.0-70.0) deep (Fig. 7). Anterior three segments with dorso-ventral projections. Strobila swelling in width immediately posterior to apical sucker, reaching maximum width at 18-19 segment 515 (320-790) narrowing to 363 (200-600) in mature segment (Fig. 8). Segments numbering approximately 4-24 fit into each other and possess rows of minute spines under their lateral projections (Fig. 10). Testes 50 (47-52) in number, spherical or subspherical, 36 (22-50) in diameter; 10 (1-11) preporal, 32 (31-34) antiporal, 8 (7-9) postporal. Vas deferens highly coiled in mature proglottid. Cirrus sac moderate, extending just to center of praglottid, 209 (120-320) long by 205 (110-320). Cirrus unarmed. Genital aperture lateral, irregularly alternating, approximately midsegment. Ovary bilobed, irregularly shaped. Vitellaria follicular, occurring as small discrete spheres encircling proglottid (Fig. 9).

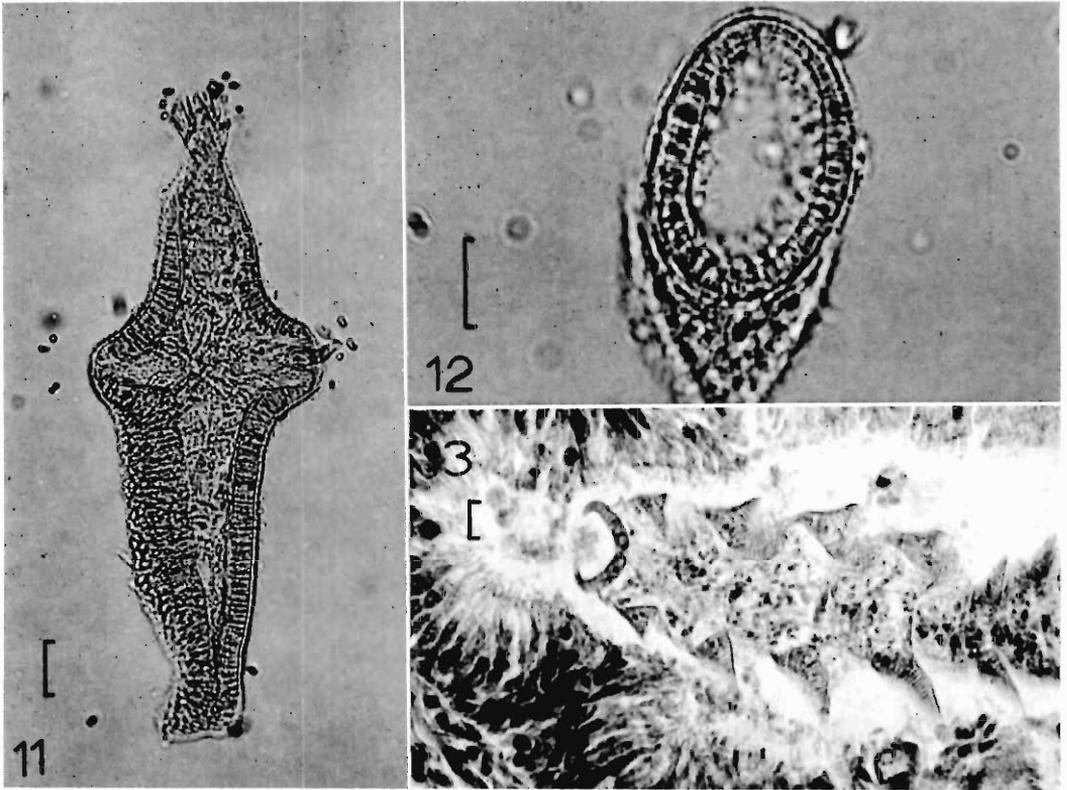
HOST: Bigeye thresher shark *Alopias superciliosus*.

LOCATION: Spiral valve.

LOCALITY: Newport Beach, California.



Figures 7-10. *Litobothrium coniformis* sp. n. 7. Anterior end of specimen showing apical sucker and modified segments. 8. Strobilate worm. 9. Mature terminal proglottid. 10. Minute spines found under lateral projections of segments 4-24.



Figures 11–13. *Litobothrium alopas* gen. n., sp. n. 11. Transverse section approximately 90–100  $\mu$  posterior to apical sucker showing cruciform shape of modified segments. 12. Transverse section of apical sucker. 13. Section of specimen *in situ* showing attachment in spiral valve. Bars indicate 10  $\mu$ .

TYPE SPECIMENS: Holotype and paratypes USNM Helm. Coll. Nos. 71364, 71365.

REMARKS: *L. confiformis* differs from *L. alopas* in shape and size of apical sucker, number of modified anterior segments, width of strobila, length of strobila, number of segments, number of testes, size of cirrus pouch, ornamentation of cirrus and shape of vitellaria.

#### Litobothridea ord. n.

ORDER DIAGNOSIS: Eucestoda. Scolex a single, well-developed apical sucker. Anterior proglottids modified; cruciform in transverse section. Neck lacking. Strobila dorso-ventrally flattened with numerous proglottids, reproductive organs single, medullary. Proglottids lacinated and craspedote; apolytic or anapolytic. Testes numerous, medullary, preovarian. Geni-

tal pores lateral. Ovary two or four lobed, posterior. Vitellaria follicular, encircling medullary parenchyma. Eggs not reaching onchosphere stage while in uterus. Adults in spiral valve of elasmobranchs.

#### Litobothridae fam. n.

FAMILY DIAGNOSIS: Litobothridea. Small worms, lacinated and craspedote. Scolex a single apical sucker followed by anterior modified segments. Strobila dorso-ventrally flattened; segmentation distinct. Inner longitudinal muscles well-developed, forming boundary between cortex and medulla. Excretory stems in medulla. Testes numerous, medullary. Cirrus sac present. Genital pores lateral, alternating irregularly. Ovary two or four lobed, posterior. Vitellaria follicular, encircling entire proglottid.

Eggs rounded to oval shaped. Parasitic in elasmobranchs.

TYPE GENUS: *Litobothrium* gen. n.

*Litobothrium* gen. n.<sup>1</sup>

GENERIC DIAGNOSIS: Litobothridae. Scolex with single apical sucker followed by modified anterior segments; neck absent. Strobila swelling in width immediately behind apical sucker. Excretory stems medial to muscle sheath. Testes numerous, nearly symmetrical, medullary. Cirrus pouch reaching mid-segment. Vas deferens, coiled. Ovary median, posterior. Vitellaria encircling proglottid, extending posterior to ovary. Parasites of elasmobranchs.

TYPE SPECIES: *Litobothrium alopias*.

**Discussion**

Many previous classifications of the Cestoda have been published. A review of these attempts is found in Southwell (1925). Southwell (1925) suggests dividing the Cestoda into five orders "based primarily on the characters of the head." He lists the orders Pseudophyllidea, Cyclophyllidea, Tetracyllidea, Trypanorhyncha, and Heterophyllidea, the latter order being erected to contain those forms found in elasmobranchs that do not fit into Tetracyllidea or Trypanorhyncha. The Proteocephala and Lecanicephala are considered families of Cyclophyllidea.

Southwell (1930) revised his classification into two orders (Cestodaria and Eucestoda) and six superfamilies (Dibothriocephaloidea, Tetrarhynchoidea, Phyllobothroidea, Lecanicephaloidea, Proteocephaloidea, and Taenioida). No mention is made of Heterophyllidea and those genera previously placed in that order are now listed as "Genera of uncertain Systematic Position."

Since Southwell (1930), some other cestode classifications have been proposed by Hyman (1951), Wardle and McLeod (1952), Riser (1955), Euzet (1959), Yamaguti (1959), and Joyeux and Baer (1961).

Hyman's (1951) classification is modified from Southwell (1930). Elasmobranch cestodes are placed into four orders: Tetracyllidea, Trypanorhyncha or Tetrarhynchoidea, Diphyllidea, and Lecanicephaloidea. These orders are retained by Yamaguti (1959) with

only a spelling modification of Lecanicephaloidea. Wardle and McLeod (1952) retain the orders Tetracyllidea, Trypanorhyncha, and Lecanicephala but differ from the preceding authors in the addition of Disculicepitidea and the deletion of Diphyllidea. Riser (1955) divided the Cestoda into two superorders, Trixenidea and Dixenidea, based on the number of hosts in the life cycle. Riser includes all the elasmobranch cestodes in the orders Tetracyllidea and Trypanorhyncha (=Tetrarhynchoidea). Euzet (1959) and Joyeux and Baer (1961) also use these two groups to include all elasmobranch cestodes with the exception of the echinobothriids, which they retain in order Diphyllidea. Of these classifications, the author agrees with the latter two workers that the Lecanicephala should be included in Tetracyllidea and Diphyllidea should be retained based on scolex types.

The scolex of Litobothridea ord. n. consists of a single apical sucker. This feature, coupled with the auxillary holdfast modification of the anterior segments, is unique and restricts placement of these two distinct cestodes in any existing order.

Features of Litobothridea ord. n. resemble a combination of cestodes found in several existing orders. The single apical sucker is reminiscent of that described by Yamaguti (1939) for *Nippotaenia chaenogobii*, the representative species for the order Nippoetaeniidea. However, this is the only common feature between these two orders and even these are morphologically distinct. The extensive swelling in width behind the holdfast organ approximates that shown for *Discobothrium cobraeformis* (= *Hornellobothrium cobraeformis*) and *Eniochobothrium gracile* Shipley and Hornell (1906).

The mature proglottid resembles that of Tetracyllidea in the preovarian position of the testes, the position of the vagina dorsal to the uterus, and the opening of the vagina anterior to the cirrus, while the continuous, sleeve-like distribution of the yolk glands and the muscular cirro-vaginal atrium are trypanorhynchan in nature.

The bigeye thresher shark has been reported from Southern California only six times prior to this report (Dr. Sheldon Applegate, Los Angeles County Museum, personal communication). The finding of massive infections of

<sup>1</sup> Litos (Gr.) = simple, plain; bothrios (Gr.) = pit.

these cestodes in both bigeye thresher sharks examined over a 2-year period indicates that this parasite is well established. It is the opinion of the author that this fact, in addition to the unique morphological features possessed by these cestodes, justifies the erection of a new order. It is highly probable that additional cestodes with this unique type of holdfast will be found on subsequent examinations of other elasmobranchs. At that time the various morphological characters can be evaluated to determine their importance at the familiar and generic levels.

#### Acknowledgments

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## Refractile Body Changes in Sporozoites of Poultry Coccidia in Cell Culture

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Little is known regarding the number, size, location, or function of refractile bodies (= eosinophilic globules) in sporozoites of poultry coccidia. The illustrations by Tyzzer et al. (1932), of Giemsa-stained smears prepared from the intestine of chickens about 1 hr after feeding *Eimeria tenella* (Railliet and Lucet, 1891) Fantham, 1909 oocysts, show: (1) sporozoites with 2 refractile bodies, one an-

terior and one posterior to the nucleus; (2) a sporozoite with one refractile body posterior to the nucleus; and (3) a sporozoite with what appears to be two small anterior refractile bodies and a single posterior refractile body. Intracellular *E. tenella* sporozoites illustrated by Tyzzer (1929) have a single refractile body, located posterior to the nucleus. Clarkson (1958, 1959) reported a posterior refrac-

**Table 1. Relative frequencies of four morphological types of *E. meleagridis* sporozoites at various time intervals in vitro.**

Number and location of refractile bodies	Exp. no.	Number of extracellular sporozoites		Number of intracellular sporozoites							
		Freshly excysted	1 hr	Hours after inoculation							
				½	1	2	3	6	8	24	
2 r.b.'s, 1 anterior and 1 posterior to nucleus		360	318								
	1			114	50	46	30	33	26	44	
	2			122	54	64	43	39	30	43	
	subtotal	360	318	236	104	110	73	72	56	87	
	% of total	72	64	47	21	22	15	14	11	17	
2 or 3 small r.b.'s anterior to nucleus, 1 r.b. posterior		101	120								
	1			114	148	145	94	45	28	13	
	2			102	140	134	104	54	30	20	
	subtotal	101	120	216	288	279	198	99	58	33	
	% of total	20	24	43	58	56	40	20	12	7	
2 r.b.'s posterior to nucleus		11	22								
	1			11	30	32	33	26	29	9	
	2			6	28	22	24	15	12	2	
	subtotal	11	22	17	58	54	57	41	41	11	
	% of total	2	4	3	12	11	11	8	8	2	
1 r.b. posterior to nucleus		28	40								
	1			11	22	27	93	146	167	184	
	2			20	28	30	79	142	178	185	
	subtotal	28	40	31	50	57	172	288	345	369	
	% of total	6	8	6	10	11	34	58	69	74	
Total		500	500	500	500	500	500	500	500	500	

tile body in sporozoites of *E. adenoides*, Moore and Brown, 1951, and *E. meleagridis*, Tyzzer, 1929, respectively. He also found granules in the pointed end of Giemsa-stained specimens of both species, but did not identify them as refractile bodies. In their work on cultivation of poultry coccidia in bovine kidney cell cultures, Doran and Vetterling (1967b) showed sporozoites with one and two refractile bodies.

The present report concerns morphological changes in the refractile bodies in sporozoites of *E. adenoides*, *E. meleagridis*, and *E. tenella* after inoculation into bovine embryonic kidney cell cultures.

### Materials and Methods

Bovine embryonic kidney cells in the 22nd-26th serial passages were grown as monolayers on coverslips in Leighton tubes. The techniques employed for cultivation of these cells were those of Doran and Vetterling (1967b).

Oocysts were collected, sporulated, and cleaned of debris by the method of Vetterling (personal communication). They were steril-

ized and excysted as described by Doran and Vetterling (1967a). Sporozoites of *E. adenoides* and *E. tenella* were freshly excysted; those of *E. meleagridis* had been frozen and maintained in liquid nitrogen vapor for 4 months. Immediately after excystation or thawing, sporozoites were placed in Medium 199 with Hanks' balanced salt solution containing 5% chicken serum. The number of organisms was then estimated with the aid of a counting chamber and final concentrations of 450,000 *E. adenoides*, 500,000 *E. meleagridis* and 680,000 *E. tenella* sporozoites per 1.5 ml were obtained by diluting the suspensions with additional medium.

After adjusting the medium to pH 7.0-7.2, 1.5 ml was pipetted into each of 7 Leighton tubes for each experiment. After 3-6 hr, the medium was replaced with Eagle's basal medium containing Hanks balanced salt solution (HBME) and 10% fetal calf serum. Cultures were kept in an incubator which alternated between 40.6 and 43 C for 12-hr intervals. Medium 199, chicken serum, and fetal calf serum were obtained from Baltimore Biological

Table 2. Relative frequencies of four morphological types of *E. adenoides* sporozoites at various time intervals in vitro.

Number and location of refractile bodies	Exp. no.	Number of extracellular sporozoites		Number of intracellular sporozoites						
		Freshly excysted	1 hr	Hours after inoculation						
				½	1	2	3	6	8	24
2 r.b.'s, 1 anterior and 1 posterior to nucleus	1	289	300	118	109	—	80	82	67	54
	2			123	107	91	98	88	71	55
	subtotal	289	300	241	216	91	178	170	138	109
	% of total	58	60	48	43	36	35	34	28	22
2 or 3 small r.b.'s anterior to nucleus, 1 r.b. posterior to nucleus	1	175	123	58	82	—	18	3	17	12
	2			55	87	39	10	5	15	6
	subtotal	175	123	113	169	39	28	8	32	18
	% of total	35	25	23	34	16	6	2	6	4
2 r.b.'s posterior to nucleus	1	10	13	6	10	—	17	10	17	2
	2			7	9	8	12	5	12	3
	subtotal	10	13	13	19	8	29	15	29	5
	% of total	2	3	3	4	3	6	3	6	1
1 r.b. posterior to nucleus	1	26	64	68	51	—	135	155	149	184
	2			65	47	112	130	152	152	187
	subtotal	26	64	133	98	112	265	307	301	371
	% of total	5	13	27	20	45	53	61	60	74
	Total		500	500	500	500	250	500	500	500

Laboratories, Cocksylville, Md. The HBME was purchased from Grand Island Laboratories, Grand Island, Nebraska.

To obtain quantitative information regarding changes in the number and location of refractile bodies, *E. adenoides* and *E. meleagridis* sporozoites were each used in two experiments. In each of these four experiments, a single coverslip was removed at 0.5, 1, 2, 3, 6, 8, and 24 hr except for one experiment with *E. adenoides* in which the 2-hr examination was omitted. These coverslips were fixed with neutral buffered formalin and stained with hematoxylin and eosin. In each of two other experiments, *E. adenoides* and *E. meleagridis* sporozoites that remained extracellular 1 hr after inoculation were decanted from the Leighton tubes. These suspended sporozoites were centrifuged into a pellet from which smears were prepared on coverslips. The smears were fixed with Spray-Cyte (Clay-Adams, Inc., New York) and stained with hematoxylin and eosin. Smears of freshly excysted sporozoites (prepared 20 min after

placing sporocysts in excystation fluid) were similarly fixed and stained.

To obtain quantitative data, regarding refractile body changes, *E. meleagridis* and *E. adenoides* sporozoites were allocated to four general morphological groups which described the number and location of their refractile bodies. These groups included those sporozoites with: (1) two refractile bodies, one located anterior and one posterior to the nucleus; (2) two or three small refractile bodies located anterior to the nucleus and one posterior to the nucleus; (3) no anterior refractile body, two refractile bodies located posterior to the nucleus; and (4) no anterior refractile body, one located posterior to the nucleus. The location and number of refractile bodies in these sporozoites were determined by examination of stained slides. For both species, 500 freshly excysted sporozoites, 500 extracellular sporozoites, and 250 intracellular sporozoites were observed at each time interval in each experiment.

To substantiate and clarify quantitative data from the previous experiments, living *E. ten-*

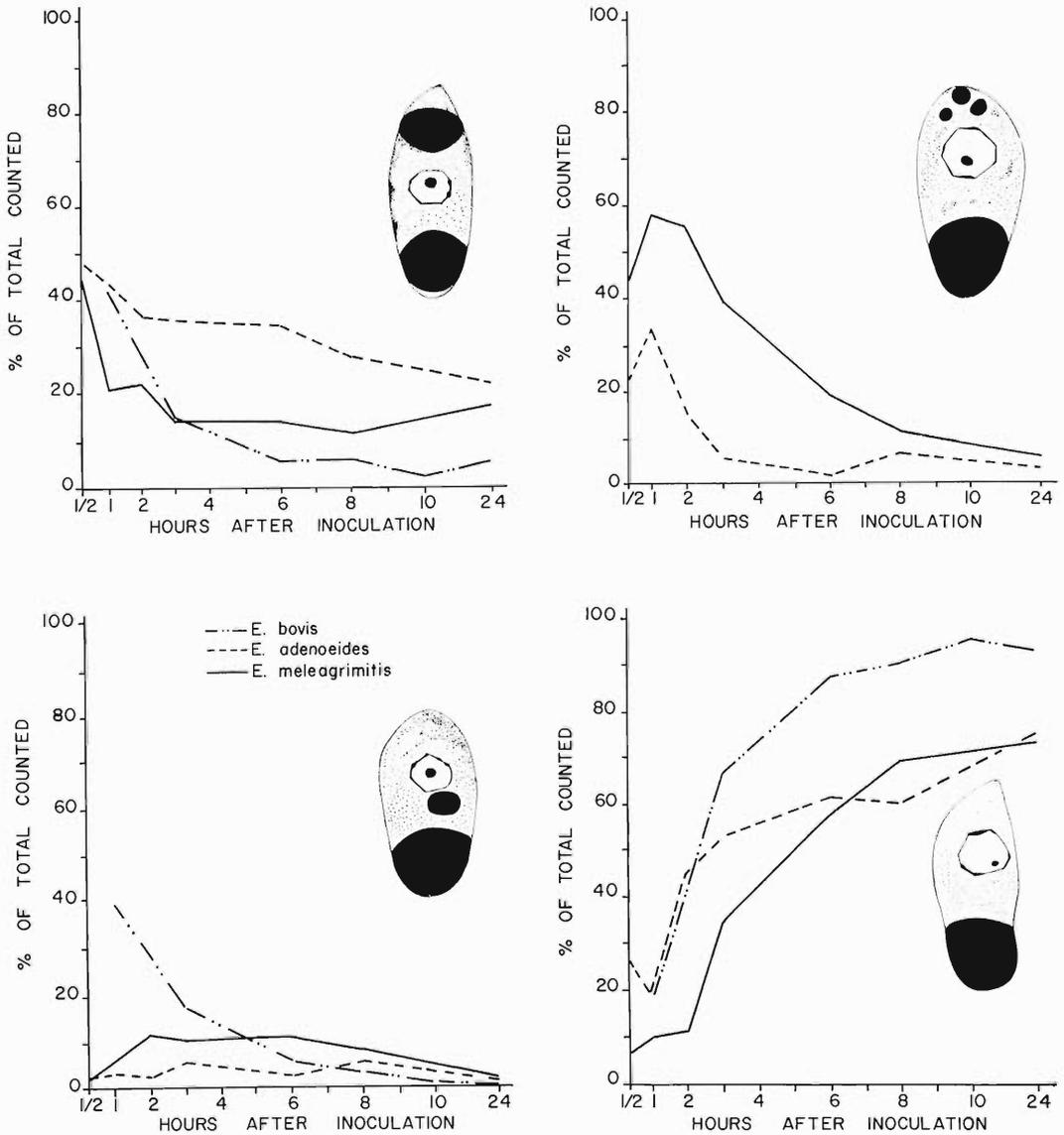


Figure 1. Relative frequency of morphological types of intracellular *E. bovis* (data from Fayer and Hammond (in press)), *E. adenoeides*, and *E. meleagrimitis* sporozoites in cultured cells.

ella, *E. adenoeides* and *E. meleagrimitis* sporozoites were studied for intervals up to 24 hr in double coverslip preparations or in Rose perfusion chambers using phase-contrast microscopy. Morphological changes in the intracellular organisms were photographed utilizing time-lapse cinemicrographic techniques.

## Results

### Stained specimens

Quantitative data regarding the number and location of refractile bodies in extracellular and intracellular *E. meleagrimitis* and *E. adenoeides* sporozoites appear in Tables 1 and 2, respec-

tively and in Fig. 1. A comparison of freshly excysted sporozoites with those removed from cell cultures after 1 hr shows an increase in the relative number of sporozoites of both species with two refractile bodies posterior to the nucleus and with a single posterior refractile body. No counts were made after this time, since many of the extracellular sporozoites appeared dead or abnormal at 1 hr.

Intracellular sporozoites of both species underwent the following relative changes during the 24 hr period: (1) those containing two refractile bodies, one anterior and one posterior to the nucleus, decreased in number; (2) those with two or three small anterior refractile bodies reached a peak 1 hr after inoculation and then declined in number; (3) sporozoites containing two refractile bodies posterior to the nucleus were most abundant from 1-8 hr after inoculation, but were not as numerous as any of the other types at or near their peak (specimens in which the anterior refractile body was found alongside the nucleus were included in counts of this morphological type); (4) sporozoites with only a posterior refractile body progressively increased in number up to 24 hr.

Qualitative observations of stained specimens with regard to the location, size, and shape of the anterior refractile body in extracellular sporozoites of *E. meleagridis* and *E. adenoeides* as well as changes in the location, size and shape of the bodies in the intracellular sporozoites were the same as described for *E. bovis* sporozoites by Fayer and Hammond

(1969). However, the occurrence of two or three small refractile bodies anterior to the nucleus has not been described. The location of these bodies varied greatly within the anterior tips of the sporozoites. In some, the bodies were close to one another and appeared to be touching; in others, they were dispersed randomly throughout the tip and sometimes were found beside the nucleus. The bodies varied in size from very small dots to round, ovoid, or irregularly shaped bodies  $\frac{1}{2}$  or  $\frac{1}{3}$  the diameter of the larger single anterior refractile body.

The posterior refractile body varied in size and shape in extracellular sporozoites within each of the two species. In intracellular sporozoites, there was no appreciable variation in location or size as compared with the freshly excysted sporozoites. Although one or two small fingerlike projections were observed at the anterior edge of the body in several intracellular organisms at various intervals up to 24 hr, the general ovoid shape remained the same.

#### Living specimens

In *E. meleagridis*, *E. adenoeides*, and *E. tenella* intracellular sporozoites with two refractile bodies, the anterior body moved to a position alongside or behind the nucleus and, within 15 min or less, merged with the posterior refractile body (Figs. 2-26). After this occurred, the sporozoite appeared to contain a single refractile body. Prior to, during, and following the merger of refractile bodies, ac-

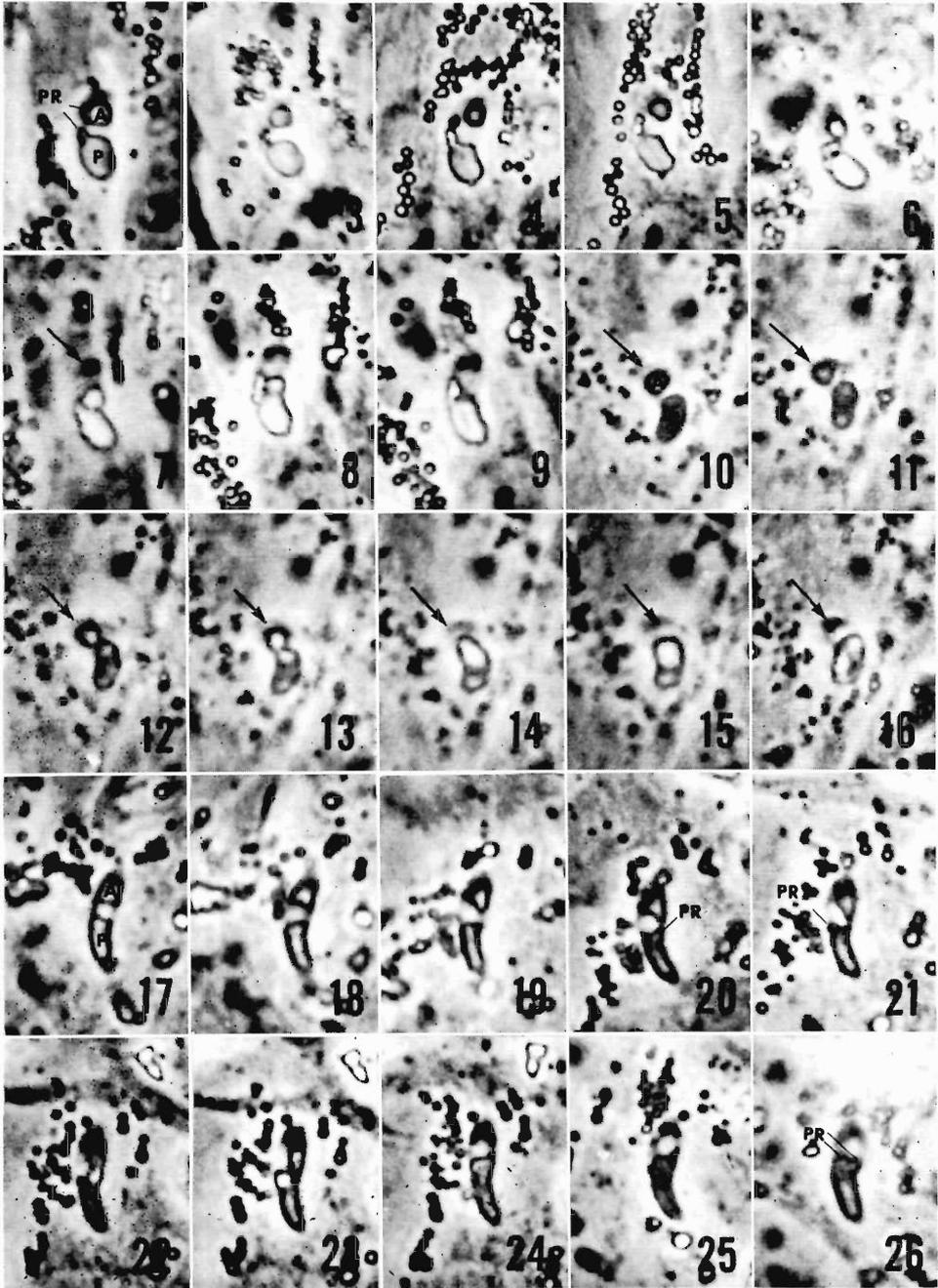
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 Figures 2-26. Photomicrographs from 16 mm motion picture film of live sporozoites in bovine kidney cell cultures 24 hr or less after inoculation (phase contrast). Abbreviations: A, anterior refractile body; P, posterior refractile body; PR, projection of refractile body material. Arrows indicate location of anterior tip of sporozoites. All sporozoites.  $\times 2,000$ .

Figures 2-9. *E. meleagridis* sporozoite. 2-6. Small projections of material detaching from anterior and posterior refractile bodies. 7, 8. The anterior refractile body merges with the posterior refractile body. 9. Only the posterior refractile body is present.

Figures 10-16. *E. adenoeides* sporozoite. 10. The anterior and posterior refractile bodies are present, but the sporozoite nucleus is not visible. 12, 13. The anterior refractile body merges with the posterior refractile body. 14-16. The posterior refractile body undergoes changes in shape.

Figures 17-26. *E. tenella* sporozoite. 17-21. The anterior refractile body changes shape as it moves posteriad on the right side of the sporozoite nucleus (clear area). In figures 22 and 23, respectively, the light colored ovoid and round body between the two refractile bodies is an extracellular particle above the sporozoite. The anterior refractile body in these photomicrographs is beside the nucleus. 24. The two refractile bodies have fused. 25, 26. Projections occur at the anterior margin of the posterior refractile body.



tivity was observed at the anterior margin of the posterior body (Figs. 2-9, 20-26). One or two small, fingerlike projections that appeared randomly along the margin, moved laterally as well as up and down and, occasionally, became detached from the refractile body. Similar projections were observed at the lateral margin of an anterior refractile body in an *E. meleagridis* sporozoite (Fig. 2) and several *E. adenoides* sporozoites. These projections became detached and were observed in the sporozoite cytoplasm.

### Discussion

The present findings indicate that by 24 hr after inoculation most intracellular *E. meleagridis*, *E. adenoides* and *E. tenella* sporozoites contain only a posterior refractile body. Such sporozoites are found after the anterior refractile body has disappeared either by moving posteriad and merging with the posterior refractile body or by remaining at the anterior tip and becoming smaller. Data obtained from *E. meleagridis* and *E. adenoides* sporozoites suggest that the anterior refractile body may undergo a reduction in size at the anterior tip by releasing small amounts of refractile body material into the cytoplasm or by forming several smaller bodies which then decrease in size and disappear.

The disappearance of the anterior refractile body following its posteriad migration was described for *E. bovis* sporozoites in cell cultures (Fayer and Hammond, 1969). A comparison of this species with *E. meleagridis* and *E. adenoides* indicates that the relative number of organisms undergoing changes and the rate of change varied with each species (Fig. 1).

The function of the refractile bodies and the significance of the changes are unknown. However, the fact that the bodies merge, supported by evidence that they are proteinaceous (Patillo and Becker, 1955; Wagner and Foerster, 1964; Hammond et al., 1968) and appear identical in fine structure (Colley, 1967; Sheffield et al., 1968), strongly suggests that they have a similar function. The disappearance of the two or three small anterior refractile bodies, the reduction in size of the single anterior refractile body, and the release of small amounts of material from both the anterior and posterior refractile bodies indi-

cate that there is a great deal of activity associated with the bodies during the first 24 hr after inoculation of cell cultures. The presence of four morphological types of sporozoites in samples of freshly excysted and extracellular organisms suggests that this activity occurs to a limited extent under extracellular conditions.

### Summary

Intracellular *E. tenella*, *E. meleagridis* and *E. adenoides* sporozoites contained a posterior refractile body (= eosinophilic globule) which was always present and most also contained an anterior refractile body which often disappeared within 24 hr after inoculation into bovine kidney cell cultures. In sporozoites of each species, the anterior refractile body moved from a site anterior to the nucleus to a position alongside or behind the nucleus and merged with the posterior refractile body. In some sporozoites of the latter two species, two or three small anterior refractile bodies, possibly originating from a single anterior refractile body, decreased in size and disappeared. Small projections of refractile body material became detached from the anterior refractile body and from the anterior margin of the posterior refractile body and were seen in the sporozoite cytoplasm. Extracellular *E. adenoides* and *E. meleagridis* sporozoites also underwent limited changes in the location and number of refractile bodies.

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## Studies on Freshwater Larval Trematodes. XXIII. Additional Five New Species of Venezuelan Cercariae<sup>1</sup>

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In previous papers of this series 34 species of cercariae have been described which fall into Gymnocephalic, Echinostome, Xiphidiocercariae, Macrocercous, Vivax, Pharyngeate Longifurcate Distomate and Apharyngeate Brevifurcate Distomate Ocellate groups of larval trematodes. This paper deals with five additional species. One of these, *Cercaria arismendii*, is the first representative of the Ubiquita group of Xiphidiocercariae in Latin America.

All observations are based on freshly emerged cercariae except measurements (in mm) which were taken from specimens killed in hot 10% formalin.

### A. Gymnocephalic Cercariae Reflexae group

#### 1. *Cercaria asaguensis* sp. n. (Fig. 1-2)

HOST: *Marisa cornuarietis* (L.).

LOCALITY: Puente de Asagua, Caripito, Edo. Monagas.

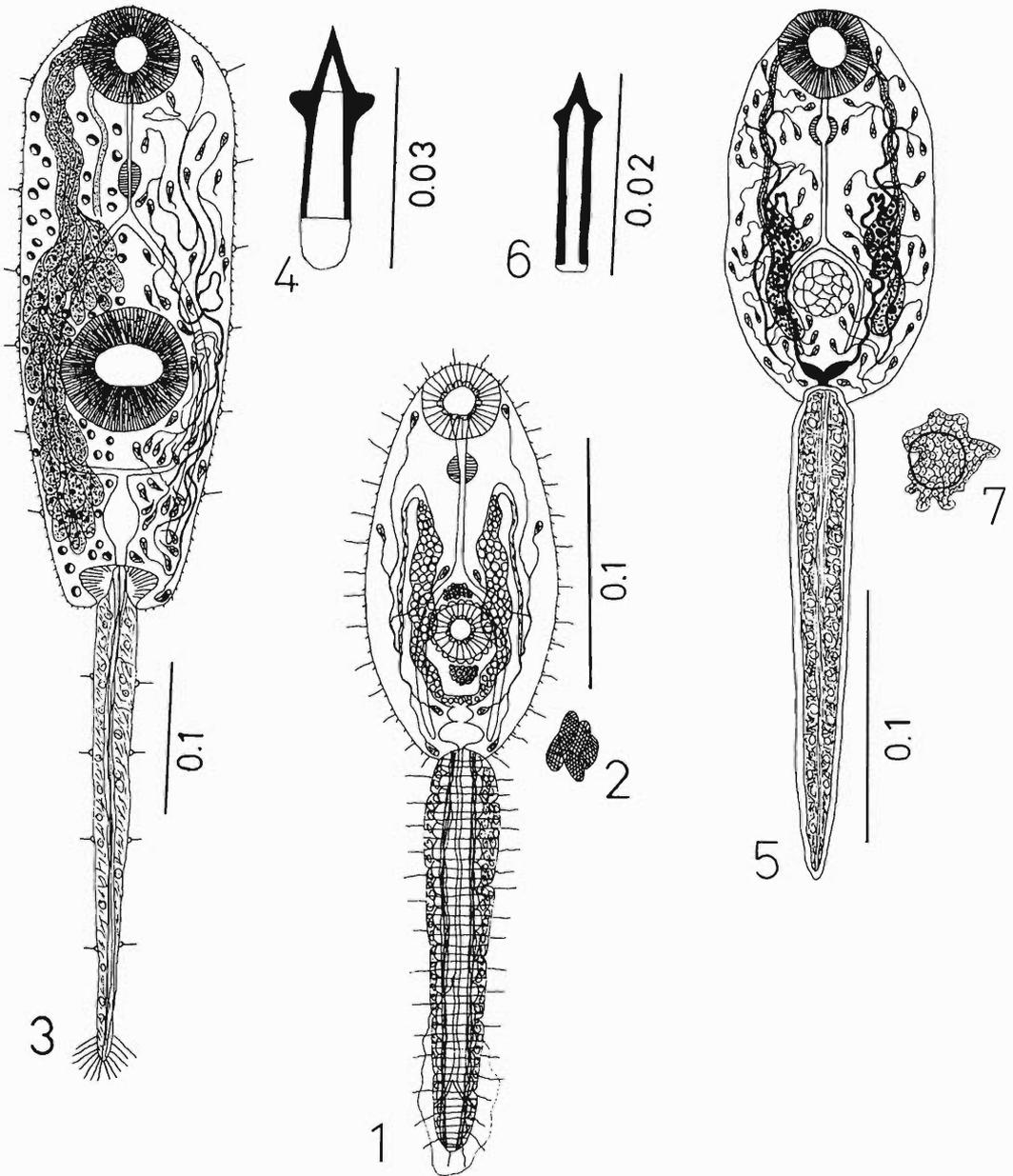
DESCRIPTION: Body spinose, with flagellets.

Tail aspinose, furnished with a dorsoventral finfold only along its posterior third. Oral and acetabular orifices bordered with a single row of papillae; a single row of papillae also surrounding periphery of acetabulum. Prepharynx present. Pharynx conspicuous. Esophagus extending to acetabulum and ceca extending to posterior division of excretory vesicle. Cystogenous glands with rod-like contents. Excretory vesicle bichambered; main excretory tubes throughout enclosing refractile excretory granules; secondary excretory tubules ciliated and dividing, at equatorial level of acetabulum, into anterior and posterior lateral collecting excretory tubules. Flame cell formula: 2 [(1+1+1) + (1+1)] = 10. Measurements: body 0.153-0.168 by 0.072-0.078; tail 0.150-0.186 by 0.027-0.036; oral sucker 0.024-0.038 in diam.; acetabulum 0.021-0.027 in diam.; pharynx 0.012-0.015 in diam. Development in rediae, with a pharynx, a complete collar, a saccate gut and a pair of posterior locomotor appendages.

### Remarks

The other gymnocephalic cercariae of the Reflexae group with rod-like contents of cyst-

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Figures 1-7. 1. *Cercaria asaguensis* sp. n. Note the extent of finfold. 2. A group of cystogenous glands of *C. asaguensis* with rod-like contents. 3. *C. pseudopifanoi* sp. n., penetration glands, refractile globular bodies, and flame cells shown on one side only. 4. Stylet of *C. pseudopifanoi* showing basal bulb. 5-7. *C. arismendii* sp. n. 5. Showing penetration glands with finely and coarsely granular contents. 6. Stylet. 7. Genital rudiments in association with rudimentary ventral sucker.

togenous glands, as found in *C. asaguensis*, are: *Cercaria helvetica* XVII Dubois, 1929 = larva of *Sphaerioditrema globulus* (Rudolphi, 1819) after Szidat (1937), *C. obscura* Wessenberg-Lund, 1934, *C. penthesilia* Faust, 1921 and *C. sucrensis* Nasir and Acuña, 1965. In *C. helvetica* XVII and *C. obscura* the tail is furnished with a finfold throughout its entire extent and the digestive system is limited only to pharynx and a part of esophagus whereas the finfold of *C. asaguensis* is confined to the distal third of its tail and the ceca extend almost to the posterior end of its body, thus alluding to the independent entity of this species.

Insofar as the pattern of finfold is concerned, *Cercaria penthesilia* and *C. sucrensis* are indistinguishable from *C. asaguensis*, but the first two species are set distinctly apart in having a very short esophagus. Moreover, the ventral sucker of *C. penthesilia* is larger than its oral sucker in contrast with the ventral sucker of *C. asaguensis* which is smaller than the oral sucker. There are 24 flame cells in all in *C. sucrensis* while *C. asaguensis* possesses a total of 10.

*Cercaria ornatocauda* Brooks, 1943, differs from *C. asaguensis* in having a larger ventral sucker than the oral one, a complete dorso-ventral finfold and its cystogenous glands have granular contents. *Cercaria sudanensis* No. 4 Archibald and Marshall, 1932 and *C. vertebraeformis* Faust, 1921, have a tail completely surrounded by a finfold, a short esophagus and the intestinal ceca extend only slightly posterior to ventral sucker; in both of these cercariae the nature of the contents of cystogenous glands and the flame cell formula are unknown.

## B. Xiphidiocercaria

### 2. *Cercaria pseudopifanoi* sp n.

(Fig. 3-4)

HOST: *Marisa cornuarietis* (L.).

LOCALITY: La Victoria, Caripito, Edo. Monagas.

DESCRIPTION: Body spinose, with six rows of setate papillae and abundant supply of refractile globular bodies. Tail aspinose, excepting a group of enlarged spines at its tip; subterminally attached, with four rows of setate papillae; caudal pockets with needle-like spines. Stylet with a basal bulb (Fig. 4). Ventral sucker protrusible and both suckers armed with

hook-like spines. Prepharynx relatively long. Pharynx anteroposteriorly elongated. Esophagus half as long as pharynx, not extending to ventral sucker. Intestinal ceca terminating considerably anterior to posterior end of body. Penetration glands numerous, pre-, para-, and postacetabular, giving rise to four ducts on each side of body; another duct, with finely granular contents, on each side of body, escaping from certain glands whose position could not be determined. Excretory vesicle more or less T-shaped; main excretory tubes dividing in pre-equatorial region of ventral sucker. More than 40 flame cells on each side of body. Measurements: body 0.310-0.439 by 0.131-0.150; tail 0.282-0.439 by 0.018-0.028; oral sucker 0.057-0.075 in diam.; ventral sucker 0.060-0.084 in diam.; stylet excluding basal bulb 0.026-0.034 by 0.005-0.007 at shoulder; basal bulb 0.006-0.008 in diam.; prepharynx 0.027-0.045 long; pharynx 0.021-0.030 by 0.012-0.015. Development in sausage-shaped sporocysts.

### Remarks

Insofar as the flame cell system and the number and the arrangement of penetration glands is concerned, *Cercaria pseudopifanoi* is unique among freshwater larval trematodes. It is very similar to *C. pifanoi* Nasir and Diaz, 1967, in the shape of stylet, but from the behavior and morphological standpoint these are two distinct species: *C. pseudopifanoi* is found throughout water but *C. pifanoi* is characteristically limited to upper layers.

### 3. *Cercaria arismendii* sp n.

(Fig. 5-7)

HOST: *Pomacea glauca* (L.).

LOCALITY: Los Pocitos, en route to San Juan de Macarapana, Edo. Sucre.

DESCRIPTION: Ubiquitous group of xiphidiocercariae. Body and tail aspinose, without papillae or flagellates. Stylet without a basal bulb (Fig. 6). Ventral sucker rudimentary, always associated with irregularly shaped genital primordia. Prepharynx present. Pharynx globular. Esophagus extending to acetabular rudiments. Intestinal ceca extending about halfway in post-acetabular region. Penetration glands in three pairs, pre-, para-, and post-acetabular; first two pairs with more coarsely

granular contents than that of third pair; two penetration ducts on each side of body. Excretory vesicle V-shaped; main excretory tubes arising terminally, and dividing between pharynx and esophageal bifurcation. Flame cell formula:  $2 [(3+3+3+3) + (3+3+3+3)] = 54$ . Measurements: body 0.141–0.165 by 0.075–0.087; tail 0.188–0.235 by 0.018–0.028; pharynx 0.009–0.012 in diam.; oral sucker 0.033–0.039 by 0.039–0.045; rudiments of ventral sucker 0.021–0.030 by 0.024–0.027; stylet 0.021–0.024 by 0.002–0.004 at shaft by 0.005 at shoulder.

### Remarks

Cercaria of *Levinseniella amincolae* Etges, 1953, *Cercaria indicae* LII Sewell, 1922 and *C. indicae* LXI Sewell, 1922, are the other freshwater cercariae of Ubiquita group but only *L. amincolae*, like that of *C. arismendii*, is marked with a rudimentary ventral sucker whereas this structure is absent in Sewell's species. At the same time, *L. amincolae* can be easily separated, from *C. arismendii*, in the possession of a considerably smaller stylet coupled with a different shape, in the flame cell formula,  $2 [(1+1) + (1+1)] = 8$ , in the absence of a digestive tract and bearing 4 pairs of penetration glands without differentiation in their contents.

### C. Furcocercariae

#### Pharyngeate longifurcate distomate

#### 4. *Cercaria cornuarietis* sp. n.

(Fig. 8)

HOST: *Marisa cornuarietis* (L.).

LOCALITY: Laguna de Chamariapa, Caricao, Edo. Sucre; canal de riego, Calabozo, Edo. Guarico.

DESCRIPTION: Body and tailstem uniformly spinose, with undetermined rows of flagellets. Furcae with scattered setiferous papillae and longitudinal rows of spines. Anterior organ, anteriorly, bordered with two staggered rows of spines. Oral cap consisting of 9–11 rows of spines. Ventral sucker armed with two rows of hook-like spines. Tailstem with 10–12 pairs of irregularly shaped caudal bodies. Pigmented or unpigmented eyespots absent. Prepharynx present. Pharynx conspicuous. Esophagus extending almost halfway between pharynx and ventral sucker. Intestinal

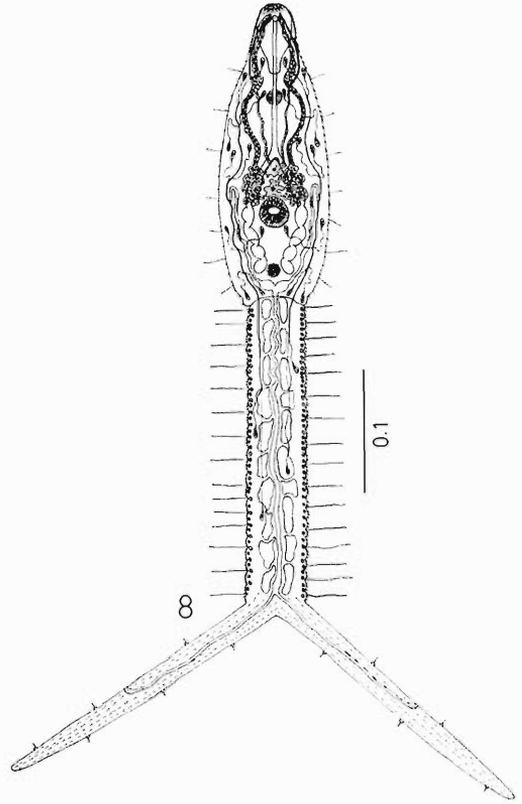
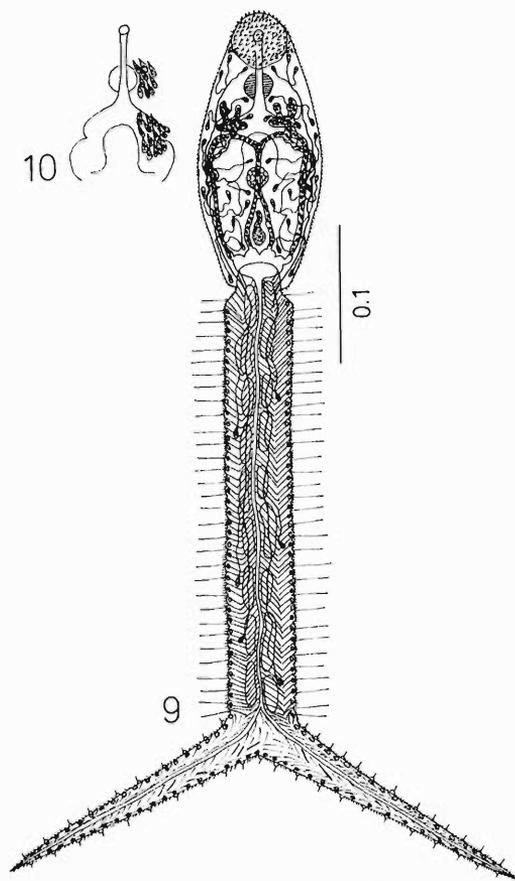


Figure 8. *Cercaria cornuarietis* sp. n.

ceca constricted into five or six segments, very prominent in some specimens, and extending just posterior to level of genital rudiments. Penetration glands in three pairs, almost always anterior to ventral sucker; two mesial glands with finely granular contents while external pairs coarsely granular. Genital rudiments represented by a cellular mass in front of excretory vesicle. Excretory vesicle bicornuate. Each of main excretory tubes lined, internally, with two ciliated patches; division of main excretory tubes at anterior level of ventral sucker; caudal excretory duct opening laterally about halfway along corresponding furca. Flame cell formula:  $2 [(2+2+2) + (2+2+(2))] = 24$ . No transverse excretory commissure. Measurements: body 0.234–0.276 by 0.066–0.094; tailstem 0.234–0.249 by 0.045–0.054; furcae 0.228–0.243; anterior organ 0.057–0.066 by 0.024–0.033; ventral sucker 0.024–0.033 in diam.;



Figures 9-10. 9. *Cercaria monagasica* sp. n. 10. Two groups of penetration glands of *C. monagasica* shown on one side only.

pharynx 0.012-0.015 in diam.; esophagus 0.039-0.057 in length. Development in thread-like sporocysts.

#### Remarks

Pharyngeate longifurcate distomate cercariae with three pairs of penetration glands pre-, para-, and post-acetabular in position are: *Cercaria bruauxi* Vercamen-Grandjean, 1960, *C. gilleti* Vercamen-Grandjean, 1960, *C. linearis* Wesenberg-Lund, 1934, *C. magalhiesia* Porter, 1938, *C. neujeani* Fain, 1953, *C. parolinearis* Goodman, 1951 and *C. rodhaimi* Fain, 1953, but in none of these species penetration glands are differentiated into finely and coarsely granular contents as found in *C. cornuarietis*. Fur-

thermore, all of these forms exhibit a lesser number of flame cells in relation to that of *C. cornuarietis*.

#### Pharyngeate longifurcate of Vivax group 5. *Cercaria monagasica* sp. n. (Fig. 9-10)

HOST: *Pomacea glauca* (L.).

LOCALITY: La Chorrera, Caripito, Edo. Monagas.

DESCRIPTION: Body, tailstem and furcae uniformly spinose. Tailstem with several transverse rows of flagellated furcae, dorsally inserted to posterior end of body. Furcae without finfolds, laterally compressed, with setiferous papillae. Caudal bodies in nine pairs. Anterior organ without particular forward-pointing spines; oral cap with 9-13 rows of hook-like spines. Ventral sucker rudimentary. Prepharynx very short. Pharynx well developed. Esophagus short. Intestinal caeca considerably dilated, tortuous, extending anterior to excretory vesicle. Penetration glands in two groups on each side of body; anterior group along each side of pharynx consisting of nine cells, and posterior group, at level of esophageal bifurcation, comprising 14 cells. Excretory vesicle transversely elongated, giving rise to four anteriorly and one posteriorly directed main excretory tubes; of four anterior tubes, two internal ones uniting posterior to rudimentary ventral sucker and dividing again, just posterior to esophageal bifurcation, to become continuous with two externals; on each side, at level of esophageal bifurcation, main excretory tubes giving rise to a system of blindly ending ramifications. All of four anterior tubes, including ramifications, filled with refractile excretory granules. From inner margins of main excretory tubes, on each side, in region of ramifications, arising a secondary excretory tubule which at posterior level of acetabular rudiments divides into anterior lateral and posterior lateral collecting excretory tubules. Secondary excretory tubules lined with ciliated patches internally. Caudal excretory tube ending at tips of corresponding furca. Flame cell formula:  $2 [(3+3+3) + (3+3+(3))] = 36$ . A pyriform cellular mass occupying a space encompassed by two main internal excretory tubes. Measurements: body 0.188-0.210 by 0.084-0.094; tailstem 0.310-0.338 by 0.047-0.050; furca 0.188-0.216; anterior organ 0.040-

0.075 in diam.; acetabular rudiments 0.012–0.080 in diam.; pharynx 0.018–0.024 in diam.; esophagus 0.009–0.015 long. Development in long thread-like sporocysts.

### Remarks

*Cercaria monagasica* is very similar to the only other species of the Vivax group, i.e., *Cercaria neoorientalis* Nasir and Guevara, 1969, in the following points: spinose body and furcae, rudimentary ventral sucker, shape and extent of intestinal ceca, blind-ending ramifications of excretory system, identical flame cell formula and absence of finfold on furcae; however, *C. neoorientalis* differs from *C. monagasica* in having aspinose tailstem, oral cap of nine rows of spines, absence of penetration glands, numerous caudal bodies, esophagus 3 times as large and no cilia in secondary excretory tubules.

### Discussion

Lühe (1909) defined Leptocercous cercariae as the distomes with undivided tails which were smaller than the bodies in width, at their insertion, even when their tails were contracted and included the Gymnocephalic cercariae, which were unarmed, the Echinostomes, with a head collar of spines, and the Xiphidiocercariae, with a stylet in oral sucker. Sewell (1922) considered gymnocephalics as an unnatural assemblage and, therefore, discarded it in favor of new subgroups, Parapleurolophocerca, Isopori, Agilis and Reflexae. Porter (1938) added another new subgroup, Paragilis, but retained the "Gymnocephalic cercariae" for the forms like *Fasciola hepatica* and *F. gigantica* which could not be accommodated in any other subgroups. Fain (1953) included the Megalura group which had been treated as an appendix to the Echinostomes by Cort (1915) and Sewell (1922). Vercamen-Grandjean (1960) agreed with these subgroups and regarded Pleurolophocerca, syn. Parapleurolophocerca, as intermediate between Echinostomatidae and Gymnocephala.

Wesenberg-Lund (1934) doubted the usefulness of this classification because the structures exhibited by the Indian and North American forms were not shared by the European species and advocated the creation of four other subgroups; also, a whole series of characters was given for the European gymnocephalics.

Cable (1938) modified the Agilis and Reflexae groups, by the addition of further species, and expressed his opinion about the inadequacy of Sewell's splitting, especially as to the Agilis and Reflexae because "it is questionable if the presence or absence of collar spines alone is sufficient basis for separating the echinostomelike species from the true echinostome cercariae which they resemble very closely in many fundamental respects." Furthermore, as pointed out by Cable (1938) as early as 1858 Filippi suggested that the adult of *Cercaria agilis*, the type species of Agilis, would prove to have collar spines, and van Beneden indicated in 1861 that in the case of *Himasthla militaris* (Rudolphi, 1802) collar spines first appeared in the metacercarial stage, being absent in cercaria. This is further supported by Stunkard (1934; 1938) who demonstrated that the larva of *H. quissetensis* (Miller and Northup, 1926) is a true echinostome cercaria. Our own observations (Nasir and Díaz, 1968), on the life cycle of *Echinochasmus zube-dakhaname* Nasir and Díaz, 1968, confirm the view of Cable; the cercaria of the species in question is absolutely without collar spines whereas these are invariably present through metacercaria to adult. The same holds true for *Stephenoprora par-denticulate* Nasir and Rodriguez, 1969. On the contrary, the cercaria, metacercaria and adult of *S. denticulata* (Rudolphi, 1802) all have a constant number of collar spines (Nasir and Scorza, 1968). Thus, not only in the same family but also in the same genus, we have instances of mistaken identity which would lead to a false taxonomic allocation. In the view of our present state of knowledge, and as it might prove right in future when more life cycles are elucidated, we believe that the groups Reflexae and Paragilis should be relegated to synonymy with Agilis which in turn should be incorporated in true echinostomes.

Sewell (1922) employed as the sole criterion the presence of a finfold in the Reflexae group to delimit it from the Agilis. Again, this character is not reliable when we take into account the life cycles of *Echinostoma nudicaudatum* Nasir, 1960, *E. donosoii* Nasir, 1964 and *E. pinnicaudatum* Nasir, 1961; in the former two species, the cercaria is without any finfold on its tail but in that of the latter there is a prom-

inent finfold on tail throughout its extent; at the same time all of these species belong to the same genus, *Echinostoma*.

Feldman (1941) connected experimentally what he thought to be *Cercaria reflexae* Cort, 1914, the type species of Reflexae group of Sewell, with a new adult parasite, *Psilostomum reflexae* (Cort). Beaver (1943) remarked that there was no justification for declaring the cercaria identical with *C. reflexae* Cort which it resembles. He therefore substituted a new name, *Protechinostoma mucronisertulatum*, for *P. reflexae*. As a matter of fact, *C. reflexae* Cort, *C. reflexae* of Feldman and the cercaria of *P. mucronisertulatum* Beaver are three independent entities. The contents of cystogenous glands in *C. reflexae* of Cort are rodlike, there is a complete finfold on entire length of its tail, and rediae possess an undivided collar. In *C. reflexae* of Feldman the "tail is provided with a dorsoventral finfold which extends from the tip to the level of excretory bifurcation" and the redial collar is divided into four lobes. The finfold of the cercaria of *P. mucronisertulatum* runs along the entire length of its tail, although it is low in certain regions, the contents of cystogenous glands are granular, there is a crown of small collar spines and the redial collar is complete. Thus, the true identity of *C. reflexae* Cort still remains obscure, and it would not be surprising, as already mentioned by Sewell, that it was probably a larval stage of a species in the genus *Himasthla*. *Cercaria asaguensis* differs from these species mainly in the finfold extent, flame cell system and in the presence of papillae around orifices of suckers.

Szidat (1937) established *Cercaria helvetica* XVII Dubois, 1929, as the larva of *Sphaeriodotremata globulus* (Rudolphi, 1818). Probert, found a cercaria, in England, which was considered, without experimental evidence, as the cercaria of *S. globulus*, but this cercaria lacks a finfold whereas in *C. helvetica* XVII = *S. globulus* there is a definite finfold throughout tail length. In words of Probert "the finfold of *C. helvetica* has been mistaken for much folded cuticle of the tail by Dubois." There is no reason to doubt the authenticity of Dubois' observation because he clearly mentioned its presence therein. Apparently, Probert was dealing with a different species.

The cercaria of *Sphaeriodotremata spinace-*

*tabulum* Burns, 1961, is another ambiguity as far as the finfold is concerned. According to Burns (1961) "margins clear suggesting short lateral fins." Even if we assume the presence of a finfold, the contents of its cystogenous glands are granular, its ventral sucker is larger than the oral sucker and there are 14 flame cells on each side of body in contrast with *C. asaguensis* in which the contents of cystogenous glands are rodlike, oral sucker is larger than the ventral sucker and there are five flame cells on each side of the body.

The main characters of the Ubiquita group of monostome cercariae Sewell (1922) are the presence of a stylet, absence of esophagus or ceca, a group of 3-6 penetration glands on each side of body and a bicornuate or rounded excretory vesicle with the main tubes dividing at about middle of body. It included two Indian forms in which the ventral sucker was absent. Although several species have been described from brackish and marine snails, only two other, *Levinseniella amnicolae* Etges, 1953, from *Amnicola pilsbryi* Walker, USA, and *Cercaria arismendii*, from *Pomacea glauca* (L.), in Venezuela, have been found in freshwater snails and possess a rudimentary ventral sucker. Lebour (1912) traced the development of *C. ubiquita*, a marine form, into a Spelotrema-like metacercaria, of the family Heterophyidae, in a green crab, and remarked "no ventral sucker is to be seen, it apparently develops afterwards as does also the alimentary canal." Stunkard (1923) pointed out that the presence or absence of acetabulum is not a character of systematic importance. Stunkard (1930) also demonstrated in the life cycle of *Cryptocotyle lingua* that a "monostome cercaria later develops an acetabulum within a genital atrium and becomes a distome belonging to the family Heterophyidae." It is, therefore, evident that the subgroup Ubiquita should be removed from monostome cercariae and be reallocated to its rightful systematic position among true distome cercariae of the xiphidiocercarial type.

Sewell (1922) subdivided his Group 3, of pharyngeate longifurcate monostome cercariae, into the Vivax and Tetis subgroups. The subgroup Vivax takes its name from the type species, *Cercaria vivax* Sonsino, 1892, and its main characters are an extremely small and rudimentary ventral sucker, furcal rami with com-

plete finfold, 12 pairs of flame cells in body and three pairs in tail. Faust (1924) added another subgroup, Leptoderma, for *C. leptoderma* Faust, 1922. Miller (1926) transferred this species to Sewell's Vivax subgroup. Szidat (1933) created another subgroup, Tauiana, for *C. tauiana* Faust, 1930, and revalidated Leptoderma subgroup; furthermore, a new subgroup, Vivipara, was introduced for the cercaria of *Linstowiella viviparae* (v. Linstow, 1877) Szidat, 1933. Dubois (1951) on the basis of flame cell formula, presence or absence of glands, ventral sucker and furcal finfolds, redefined and split these subgroups into several subdivisions, with the inclusion of a new subgroup, Novena. Goodman (1951) considered Leptoderma and Viviparae as the definitive entities of Cyathocotylid cercariae, and gave a key for their separation; it was also pointed out that a rudimentary ventral sucker may be present or absent in members of any subgroups and expressed his opinion about their retention till more knowledge is gained about their life histories. Chandler (1953) in a key of furcocercous cercariae, gave a modification of Dubois' classification. Fain (1953) described a cercaria, *C. baeri*, from the Belgian Congo, which could not be assigned in any subgroups due mainly to a peculiar excretory system consisting only of two anteriorly directed excretory canals and lacking flame cells in tail.

Cable (1935) reported the first representative of the Vivax group, *Cercaria kentuckiensis*, from USA, with the redefinition and simplification of Group 3 of Sewell into the following subgroups: Vivax of Sewell, 1922, Tetis of Sewell, 1922, and Tauinia of Szidat, 1933; several additional species were also included. Anderson (1944) proposed a further simplification and recognized only two subgroups of Vivax type of cercariae 1) the Vivax subgroup which includes forms with flame cells in tailstem and 2) the Tauiana subgroup whose members lack such flame cells.

There are several species of Cyathocotylid cercariae, like *Cercaria multiplicata* Premvati, 1955, *C. vivacis* Iles, 1959, *C. papillosoma* Khan, 1962, cercaria of *Cyathocotyle bushiensis* Khan, 1962, and *C. hirsuticauda* Probert, 1966, whose characters overlap to such an extent that these forms do not fit conveniently into any of the subgroups. Since the subgroups, as they

stand now, of Cyathocotylid cercariae fail to embrace all the larval forms of this type, this classification should be discarded in favor of only one group, Vivax Sewell, 1922.

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## Epizootiology of Ovine Helminthiasis in the Georgia Piedmont

H. CIORDIA AND WALTER E. NEVILLE, JR.<sup>1</sup>

The study of the course of nematode infections in sheep is not only of academic concern to parasitologists interested in understanding the complex host-parasite relationship involved, but also to veterinarians, animal scientists, and sheep raisers who design systems for the control and treatment of helminthiasis. The information is of special importance to the sheep industry in the Piedmont Region of Georgia because the climatic conditions and some of the management systems employed are conducive to parasitosis.

Sheep and lamb population in Georgia increased from 9,000 in 1950 to 50,000 in 1957, according to a USDA report (1968). During that time, breeding animals were imported from other sections of the country. The increase was accompanied by excessive economic losses by farmers. According to Becklund (1961), this loss was due to lack of experience in sheep management and lack of concrete epizootiological information as to the cause of clinical helminthiasis. According to the 1964 U. S. Census of Agriculture there were only 7,306 sheep in Georgia that year. In 1959, Georgia had 617 farms with ewes 1 year old or older, but this had decreased to 330 farms by 1964. Extension personnel have said that the great decrease in numbers of sheep was due, in no small part, to losses from intestinal parasites. Parasitism is still an important limiting factor in sheep production in the area.

This paper reports results of research under conditions of flock management, on the course of nematode infections in young ewes based on fecal egg counts made at regular intervals. Since the experimental animals were replacement ewes, they were not available for post-mortem recovery of worms, as suggested by Rossiter (1964). However, these animals were useful for this study because of their known age and parentage, and because they were to re-

main on the premises for as long as three years. Single egg counts taken at any one time do not provide all the information desired for a study of parasitosis. However, counts made at regular intervals together with "an examination of the sheep and of the farm, and a history of the flock and its management," will provide important details of a parasite population (Potet and Conway, 1966).

### Materials and Methods

This study was carried out from September 1963 through November 1965. The experimental ewes were of mixed Hampshire-Rambouillet breeding with approximately 50% of each in their genetic composition. Each year all lambs born in the preceding November-December were weaned in February or early March when they weighed about 20.4 kg. They were then pastured on winter temporary grazing on ryegrass until mid-May. Replacement ewes from three lamb crops were used in this study. Thus, a new group was introduced into the flock each year. The lambs constituting the first group (Group J) were placed on test in October 1963, and were kept under observation on the station farm during the period covered by the experiment. The lambs constituting the second group (Group K) were placed on test in June 1964, and were used for two years, and the third group of lambs (Group L) were placed on test in June 1965. Each year, replacement ewes were selected from the new crop of lambs on the basis of earliness of lambing, growth rate, and body conformation. For this experiment, the newly selected ewe lambs were placed in the same pasture with the older ewes in the latter part of May and all remained together until the older ewes were separated for breeding in June and July. During June and July, the ewe lamb replacements were pastured on similar common Bermudagrass as older ewes. After the breeding season, the sheep again were run as one flock. Groups J, K, and L consisted of 19, 20, and 15 lambs, respectively, when first put on test.

At approximately monthly intervals, each animal was weighed, and fecal samples were

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obtained directly from the rectum of each ewe for helminth egg counts and identification. Egg counts were made using a modification of the Stoll technique, and are reported as the number of eggs per gm of feces (EPG). Eggs were identified according to Kates and Shorb (1943). Because of the difficulty of identifying genera, the eggs were grouped according to their morphology, vis. *Cooperia-Trichostrongylus-Ostertagia* complex, and the *Haemonchus-Oesophagostomum* complex.

It was decided at the outset of the experiment that the ewes as a group would be drenched with an anthelmintic when their fecal egg counts rose to a level indicative of clinical parasitosis to avoid undue mortality. Maretin (Chemagro Corp.) or Ruelene (Dow Chemical Co.) were the drugs used for this purpose and they were successful in reducing the number of eggs in the feces.

### Results and Conclusions

Observations indicated a definite and predictable seasonal fluctuation in the average number of nematode eggs in the feces during the 3-year period. The seasonal pattern appeared to be similar for all species as reported by Gordon (1958). The average number of eggs of the several nematode species passed by all ewes was relatively low each year from November through August (Fig. 1). A peak for the average number of eggs was observed during the September-October period each year. Normally, as known from previous fecal examinations at this station, the number of eggs passed by untreated ewes increases in September, remains high during October and the first part of November, and declines during December. However, in this experiment, the number of eggs was relatively low in November because, before this time each year, all animals were drenched with anthelmintics. In general, egg counts declined in September and October as age of ewes increased, indicating an age effect on degree of resistance after initial infection. In these data, it was impossible to separate the effects of age from the effects of previous infection (Gibson, 1965).

Before the decision to initiate this experiment, Group J ewes were drenched with Ruelene in August 1963, in accordance with the normal parasite control procedure at this sta-

tion. This drenching was probably the reason that the average EPG obtained from Group J in September 1963 (3,982) was lower than peak infections of Groups K and L in the 2 succeeding years. Group J ewes averaged a maximum of 4,939 and 3,864 EPG in September 1964 and October 1965, respectively. For Group K, the peak number of eggs was 13,362 in September 1964, and 11,493 EPG in October 1965. The lambs from Group L had an average peak of 12,122 EPG in September and 19,201 in October 1965.

At necropsy, 37,583 nematodes were recovered from a ewe killed *in extremis* on October 15, 1963, 2 days after drenching with Ruelene. Of the worms recovered, 77% were from the stomach. Of these, 20,283 were *Haemonchus contortus*, 1,800 *Ostertagia circumcincta*, 1,400 *O. ostertagi*, 1,400 *Trichostrongylus axei*, and 3,900 larvae of *Ostertagia* spp. Of the worms from the small intestine, 95% were *Cooperia curticei* and *C. punctata*; the rest were *T. colubriformis*. The stomach wall was extremely edematous and the lesions were widespread. The same species were recovered from another ewe that died on the same day, although only 7,115 worms were recovered. However, the pathological changes observed along the entire digestive tract indicated a previous heavy infection (66,200 EPG had been counted before anthelmintic treatment).

In all cases, the eggs of the *Cooperia-Trichostrongylus-Ostertagia* species complex predominated throughout the experiment. Although the relative number of these eggs fluctuated, the peak occurred during the September-October period (Fig. 2). The number of eggs declined abruptly in November, perhaps because of the anthelmintic treatment of all the sheep during August or September; occasionally a secondary peak number of eggs was observed sometime after treatment.

The number of eggs of the *Haemonchus-Oesophagostomum* complex also reached a maximum during the September-October period (Fig. 3), dropped after treatment and remained constantly low until the following July-August. The average number of eggs of *Bunostomum* sp. fluctuated from month to month and this parasite never was abundant enough to produce clinical signs (Fig. 4). The highest average

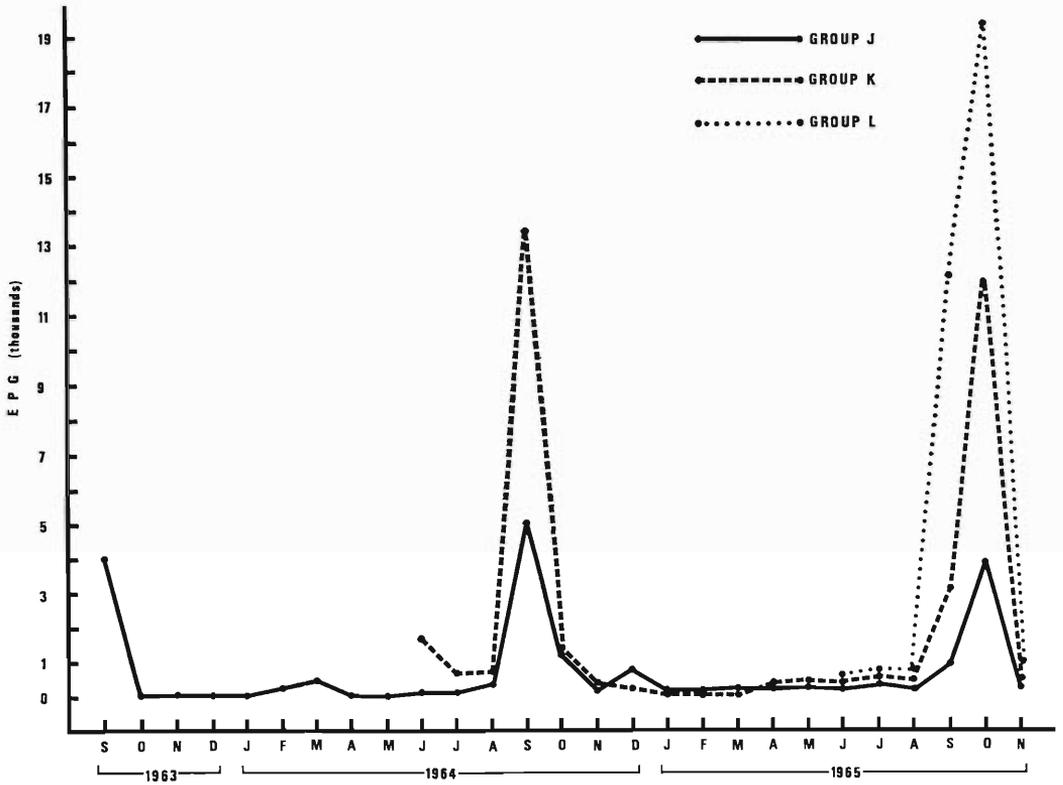


Figure 1. Average number of nematode eggs from feces obtained from sheep at monthly intervals.

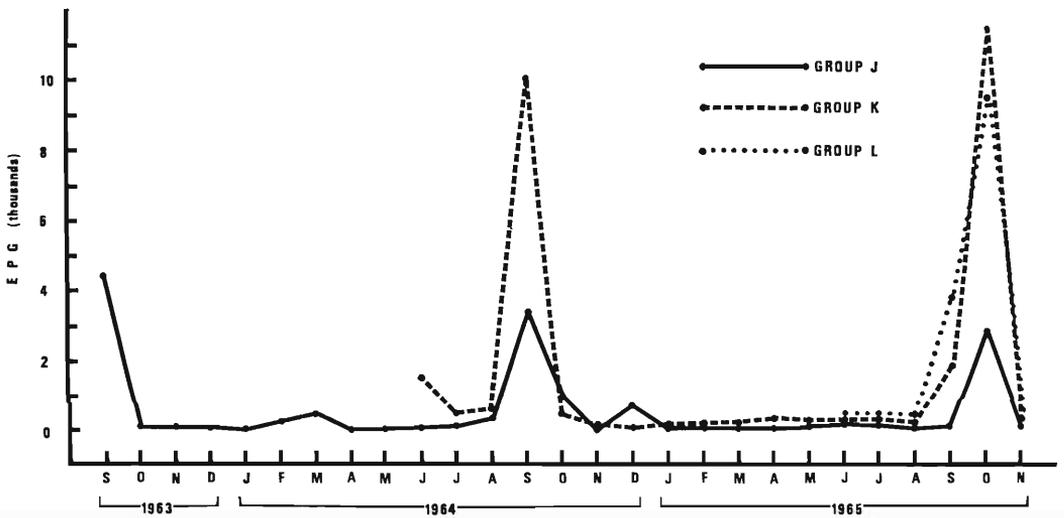


Figure 2. Average numbers of eggs of *Cooperia-Trichostrongylus-Ostertagia* complex obtained from sheep at monthly intervals.

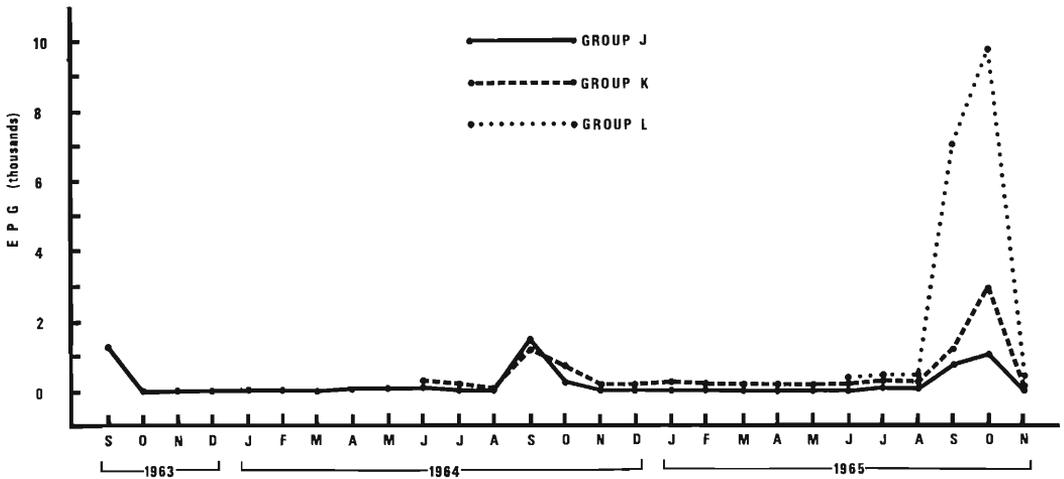


Figure 3. Average numbers of eggs of *Haemonchus-Oesophagostomum* complex obtained at monthly intervals from sheep.

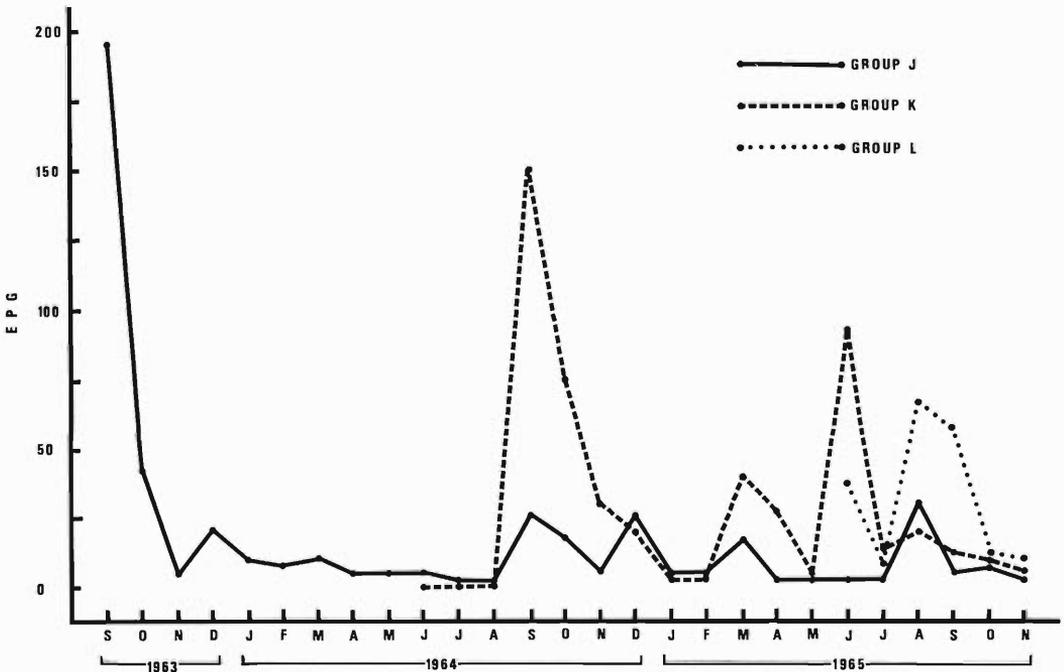


Figure 4. Average numbers of eggs of *Bunostomum* sp. obtained from sheep.

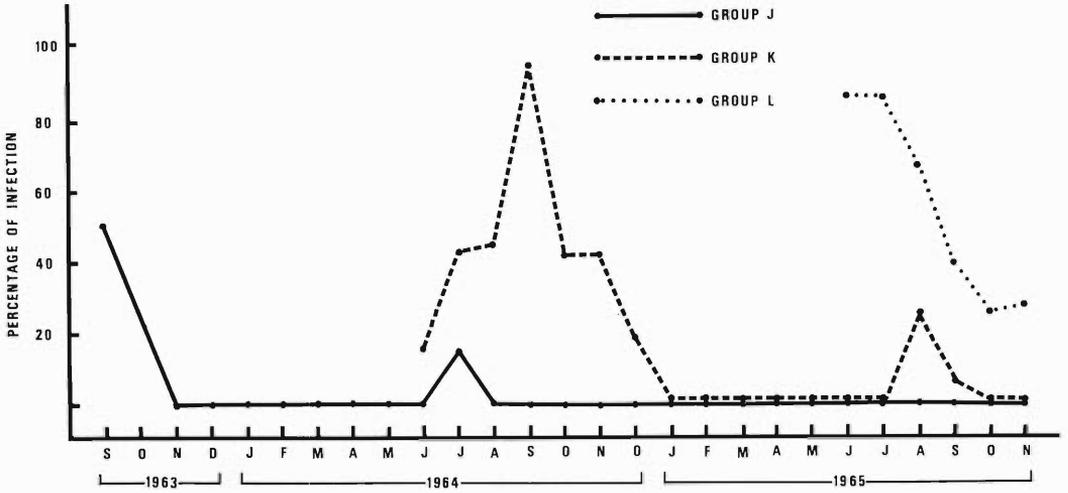


Figure 5. Percentage of ewes and lambs with *Moniezia expansa* eggs in the monthly fecal samples.

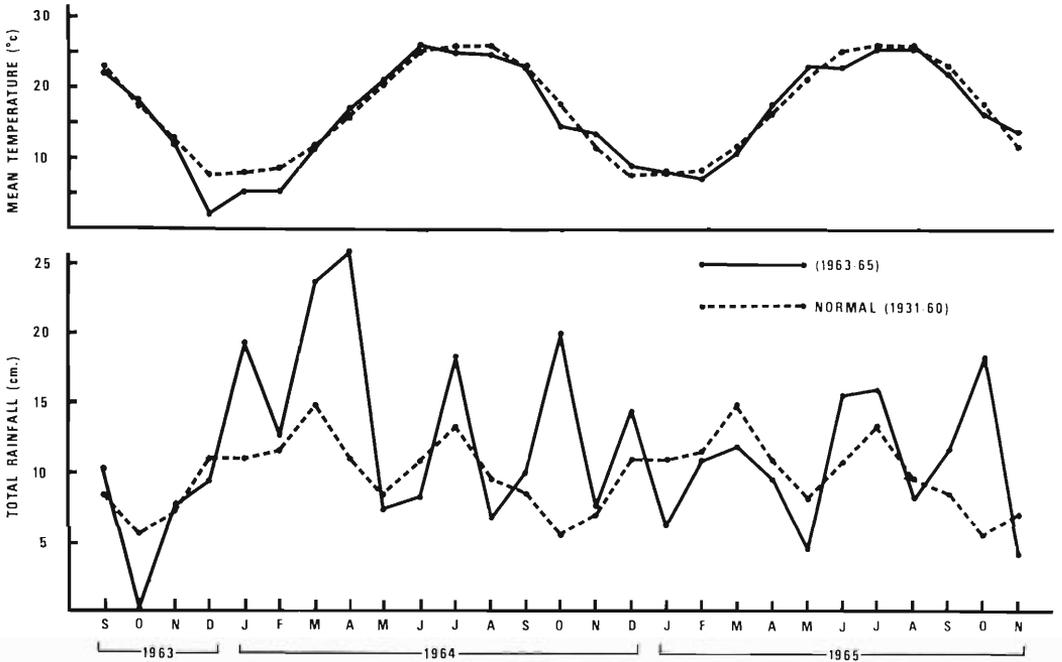


Figure 6. Monthly temperatures and rainfall at Experiment, Georgia.

Table 1. Average egg counts of ewes in September 1964 and 1965.

Ewe group	Sire	September 1964			September 1965		
		No. ewes	Average EPG	Range	No. ewes	Average EPG	Range
J	44	3	245	(208-304)	3	504	(300-812)
	57	5	279	(50-842)	4	290	(28-918)
	59	5	3,111	(60-13,528)	5	1,274	(12-3,274)
	67	2	313	(50-576)	2	376	(54-698)
K	50	4	33,301	(19,318-52,942)	3	1,475	(626-3,044)
	53	3	11,318	(112-31,952)	3	1,413	(64-4,046)
	57	2	661	(428-894)	2	407	(90-724)
	59	5	5,816	(240-17,922)	4	6,766	(240-16,214)
	72	5	1,983	(256-7,296)	5	3,316	(10-4,046)
L	57				3	6,222	(904-9,918)
	72				4	12,800	(9,428-16,268)
	85				6	11,044	(1,100-30,064)

was observed in Group J during September 1963.

*Nematodirus* sp. eggs were never abundant. Apparently lambs developed a resistance to this genus, as previously suggested by Kates and Turner (1953). Eggs of *Trichuris* spp. were present each month, but the average number was never above 46 EPG, which occurred in October 1965. Eggs of the tapeworm, *Moniezia expansa* were present from June through December of each year (Fig. 5). In September 1964, tapeworm eggs were recovered from 95% of the Group K animals. Eighty-seven per cent of the lambs from Group L passed tapeworm eggs in June and July 1965. Fifty per cent of the animals in Group J had tapeworm eggs in the feces when the experiment was started, possibly indicating that the peak had passed, as higher percentages of infection were seen in succeeding years.

The mean monthly temperatures recorded during the experiments paralleled closely the normal average temperature recorded at the Georgia Station during a 30-year period (1931-1960). The highest mean temperatures were recorded in May or June through September, whereas the period from December through February was the coldest (Fig. 6). The normal average monthly rainfall during the 30-year period was relatively uniform throughout the year, ranging from 5.6 to 14.8 cm. In general, March and July were the wettest months, and October and November were the driest. In view of the above information, it may be reasoned that the relatively wet period usually

experienced during July, when the temperature is normally moderate, may influence the availability of larvae on the pastures. This was confirmed by the increase in the number of eggs passed by the ewes and lambs beginning in August and September.

The most dangerous period, as indicated by the peak in nematode egg production and clinical outbreaks, coincides with the period when grazing is at a critical level, both in quality and quantity of forage. During that time, the summer pasture is relatively scarce and what is available is of inferior quality. Also, it is too early for grazing winter temporary pasture. These conditions are significant in the epizootiological picture. The effect of better nutrition on the incidence of nematode parasites at this time of year should be investigated in more detail, as it is thought to be more significant than other epizootiological factors, such as temperature or moisture.

None of the replacement ewes were given anthelmintics before the fecal egg count in September each year with the exception of Group J in 1963. The first drenching of these ewes in 1964 was with Ruelene on September 9. In 1965, ewes were given an anthelmintic (Maretin) for the first time on 1 October when the monthly fecal collections were made. Therefore, an appropriate examination of the data for heritable differences in egg output would include only data collected in September 1964 and 1965. The average egg counts and ranges are shown by sires and years in Table 1. With one exception, these data show that ewes by

Table 2. Analyses of variance of total egg counts in three groups of ewes.

Source	Group J			Group K			Group L		
	d/f	Mean square (000)	F	d/f	Mean square (000)	F	d/f	Mean square (000)	F
Total	28			35			12		
Sires	3	7,627	1.10	4	369,807	5.29*	2	38,902	.56
Years	1	1,964	.28	1	546,796	7.82*			
S × Y	3	2,191	.32	4	337,645	4.83*			
Error	21	6,933		26	69,930		10	69,538	

\*  $p < .01$ 

sire No. 57 had the lowest average egg counts, while those of sire No. 59 generally had the highest egg counts.

The data were analyzed by least squares (Snedecor, 1956). Total egg counts of ewes by sires, years, and their interaction were not significant for Group J (Table 2). However, for Group K, under the assumption of "fixed" main effects, differences because of sires, years, and their interaction were all highly significant. For Group L ewes, differences in egg counts because of sire effects were not significant.

In addition to sire effects, the data were examined further for the presence of heritable differences in resistance or susceptibility to parasitosis as indicated by fecal egg counts. The degree to which the same ewe had similar egg counts from one year to the next (corrected for either year or age effects, or both) is called repeatability, and in this case is measured by intraclass correlation. Egg counts classified in this way measure the percentage of variance from the combined effects of heritability (since the same ewe, same genetic composition, responds twice to a parasitic situation) and permanent environment. The proportionate contribution of these two effects to the intraclass correlation in these data are inseparable. Repeatability of the September 1964–1965 total egg counts was 0.15 for Group J ewes and 0.11 (corrected for either year or age effects, or both) for Group K ewes. When only ewes from sires No. 57 and 59 were included in Group K repeatability of total egg count was 0.99. This indicates that most of the sire × year interaction effect in Group K ewes was due to those ewes sired by rams No. 50, 53 and 72.

The authors are cognizant that the numbers of sires and of ewes per sire used in our investigations, unlike classical heritability studies

reported, are too small to draw any finite conclusion. The results are presented here, however, to provide additional information to this field of sparse research. Nevertheless, our results may be interpreted as an indication that resistance or susceptibility to internal parasitic infections are positively heritable.

Weight changes averaged by ewe groups over a 49-day period before September 17, 1965, are shown as negative daily gains in Table 3. The 9- to 10-month-old group L ewes lost 0.16 kg per day. Group K, a year older, lost 0.10 kg per day, while Group J lost 0.05 kg per day. Either age or resistance effects or both are indicated.

Also in Table 3 are correlations between the average daily loss of each ewe and her respective total egg count for September 1965. This correlation was  $-0.86$  (highly significant) for Group L, indicating a close association between total egg count and weight loss in these young ewes. There was little or no association of these effects in older ewes as indicated by correlations of  $-0.02$  and  $+0.19$  for Groups K and J, respectively.

Probably the best indication of heritable resistance to *Haemonchus contortus* in sheep was reported by Warwick *et al.* (1949). The last two years of a nine-year selection experiment produced highly significant survival percentages of 64 and 85% for the least and most resistant groups, respectively. Also heritable resistance to trichostrongylosis in sheep and its highly correlated characteristic, hematocrit values, had been reported by Whitlock (1955, 1958, 1963). Whitlock (1958) believed that the genetic resistance observed in his studies produced its anthelmintic action within the gastric mucosa and its immediate vicinity.

These data indicate that a regular seasonal treatment as suggested by Gordon (1953),

**Table 3.** Average daily weight loss for the 49-day period before 17 September 1965 and its correlation with total egg count of September 1965 for three age groups of ewes.

Group	Average weight 30 July 1965 kg	Average daily loss to 17 Sept. 1965 kg	Correlation A.D.G. with egg count Sept. 1965
J	50	-0.05	+0.19
K	45	-0.10	-0.02
L	38	-0.16	-0.86*

\*  $p < .01$

should become part of the management practices employed to raise sheep successfully in the Piedmont Region of Georgia and to prevent their death from nematode parasites. Treatment of ewes should begin either August or September, preferably in August, and continue at 3-week intervals through October.

### Summary

Field studies showed there was a definite and predictable seasonal pattern in the number of nematode eggs passed by three groups of replacement ewes in the Piedmont Region in Georgia during three consecutive years. The pattern appeared to be similar for all the species of nematodes represented, although somewhat different for *Bunostomum* sp.

A peak number of eggs passed in the feces was obtained during September and October when clinical outbreaks were common. During this period, the temperatures are normally moderate and follow a relatively wet period normally experienced in June and July. The peak egg production coincided with the period when the pasturage available was of very low nutritional quality and relatively scarce.

A certain degree of resistance was observed, which could be due to previous infection or to the age of the ewes.

There was an indication of heritable resistance or susceptibility to parasitic infections.

It is suggested that all sheep in a flock should be treated three times, at 3-week intervals starting the latter part of August.

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## Two New Species of *Cleidodiscus* (Monogenea) from the Southeastern U. S.<sup>1</sup>

WILMER A. ROGERS AND MAC V. RAWSON<sup>2</sup>

The species described herein were collected as part of a continuing survey of fish parasites being conducted by the Southeastern Cooperative Fish Parasite and Disease Project of the Auburn University Agricultural Experiment Station, Auburn, Alabama. Hosts were collected by seine or from aquaria in the lab, placed in a 1:4,000 formalin solution (Putz and Hoffman, 1963) and after 1 hr, formalin was added to make a 5% solution. Parasites were later recovered from the solution and gills in the laboratory. Specimens were treated and measured as described by Mizelle and Klucka (1953). Measurements are in microns. Averages of measurements are followed by the range in parentheses. Drawings were made with the aid of a camera lucida.

### *Cleidodiscus allisoni* sp. n. (Figure 1)

HOST AND LOCALITY: Dollar sunfish, *Lepomis marginatus* (Holbrook); from small unnamed stream 6 miles E of Slocomb, Geneva County Alabama, Choctawhatchee River System.

SPECIMENS STUDIED: Nine.

TYPE SPECIMENS: Holotype and 2 paratypes, USNM Helm. Coll. Nos. 71360 and 71361; paratypes in authors' collections.

DESCRIPTION: Length 293 (250–380), width 61 (50–70). Cephalic area lacking well defined lobes, usually truncate to convex. Head organs poorly differentiated. Eyespots four, composing granules of anterior pair often dissociated, posterior pair larger. Pharynx circular, transverse diameter 17 (14–19). Haptor subpentagonal, length 51 (45–65), width 63 (50–70). Anchor wings usually inconspicuous. Dorsal anchor length 20 (18–22), width of base 3–4. Ventral anchor length 24 (23–26); width of base 5 (4–6). Dorsal bar length 24 (21–26), ventral bar length 30 (27–35). Hooks

14, in normal arrangement (Mizelle and Crane, 1964). Hook length 13 (11–15). Cirrus a curved tapering tube with an expanded base. Accessory piece bifid, with blunt and sharp rami; sharp tip in close proximity with cirrus termination. Vagina a short sclerotized tube opening sinistromarginally. Vitellaria dense, diffuse over most of body from pharynx into peduncle.

REMARKS: The accessory piece of *C. allisoni* may be used to distinguish this species from all other *Cleidodiscus* spp. This species is named in honor of Dr. Ray Allison of Auburn University.

### *Cleidodiscus bulbus* sp. n. (Figure 2)

HOST AND LOCALITY: Peacock bass, *Cichla ocellaris* (Bloch and Schneider), experimental ponds, Dade County, Florida (originally from "Peruvian Amazon," South America).

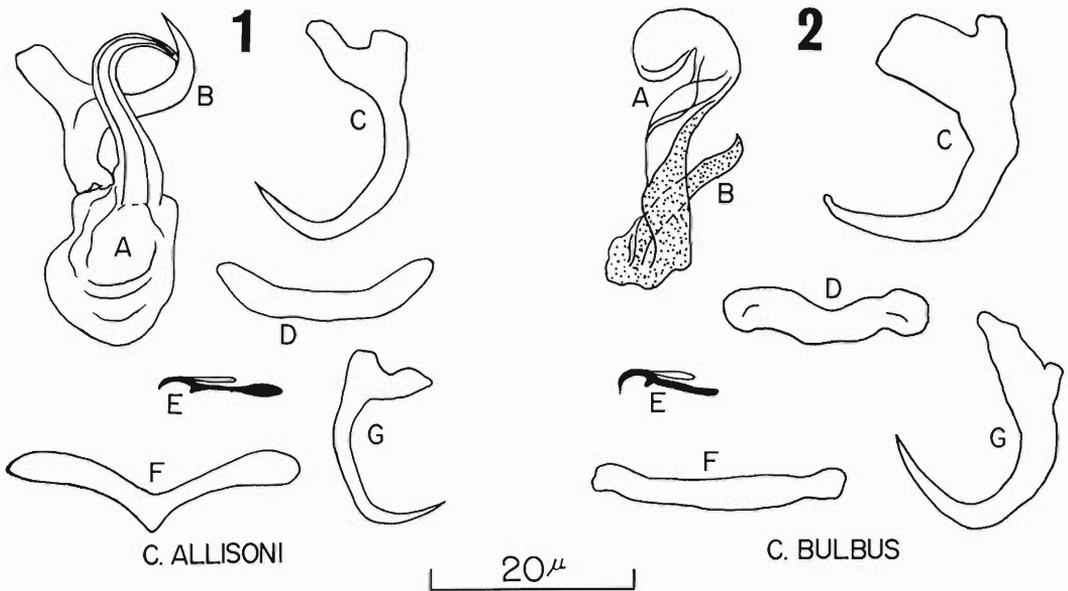
SPECIMENS STUDIED: Six.

TYPE SPECIMENS: Holotype and one paratype, USNM Helm. Coll. Nos. 71362 and 71363; paratype in authors' collections.

DESCRIPTION: Length 396 (300–510), width 173 (120–220). Cephalic area lacking well defined lobes, head organs moderately well developed. Eyespots four, members of posterior pair slightly larger and farther apart. Pharynx circular, transverse diameter 48 (40–50). Intestine bifurcate, confluent posteriorly. Haptor subpentagonal, much narrower than body width. Haptor length 49 (35–70), width 69 (50–690). Dorsal anchor with short deep root, length 23–24, width 5 (constant). Ventral anchor with distinctive angles in shaft and point with small terminal bead. Dorsal anchor length 26 (25–27), width 7 (6–8). Bars normal, dorsal bar length 26 (25–27), ventral bar length 21 to 22. Hook lacking inflated base, length 12 (11–13). Cirrus sclerotized proximally, becoming inflated and diaphanous distally, length 27 (24–30). Accessory piece articulated to cirrus base, length 21 (17–25). Testis ovate, postovarian. Ovary ovate, much smaller than

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Figures 1-2. 1. *Cleidodiscus allisoni* sp. n. 2. *Cleidodiscus bulbus* sp. n. A. Cirrus. B. Accessory piece. C. Ventral anchor. D. Ventral bar. E. Hook. F. Dorsal bar. G. Dorsal anchor.

testis. Vagina, seminal receptacle, seminal vesicle, and prostates not observed. Vitellaria dense, covering most of body from pharynx to peduncle.

REMARKS: This species may be distinguished by the characteristic copulatory complex and dorsal anchors. The name is from Latin (*bulbus*—a bulb) and refers to the inflated terminal portion of the cirrus.

**Summary**

Two new species of *Cleidodiscus* are described from the Southeastern United States; *C. allisoni* from the dollar sunfish, *Lepomis marginatus* (Holbrook) from Alabama, and *C. bulbus* from the peacock bass, *Cichla ocellaris* (Bloch and Schneider), which was introduced into Southern Florida from the "Peruvian Amazon."

**Acknowledgments**

Thanks are extended to Mr. Vernon Ogilvie for supplying specimens of peacock bass, and to Mr. William Smith-Vaniz for assistance in collecting the dollar sunfish.

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## Lecithodendriid Trematodes from the Bat *Peroteryx kappleri* in Colombia, including Discussions of Allometric Growth and Significance of Ecological Isolation<sup>1</sup>

DENNIS R. MARTIN<sup>2</sup>

During the summer of 1968 emballonurid bats, *Peroteryx kappleri* Peters, 1867, were collected from mines near Cali, Colombia, South America. Ten of these bats were examined at the Universidad del Valle. The intestine of the bats was divided into three equal parts (anterior, middle, and posterior thirds) and examined with the aid of a binocular dissecting microscope. All trematodes were fixed in an alcohol-formalin-acetic acid solution, some being flattened under a coverslip during fixation. These worms were stained with Van Cleave's (1953) hematoxylin and mounted in Permount. Drawings were made with the aid of a microprojector and a compound microscope. All measurements are in millimeters. A report and discussion of the material collected follows.

### *Castroia amplicava* Travassos, 1928 (Figure 1)

HOST: *Peroteryx kappleri* Peters, 1867 (Chiroptera: Emballonuridae).

LOCATION: Anterior one-third intestine. Some specimens in middle one-third of intestine.

INCIDENCE OF INFECTION: In 8 of 10 hosts.

LOCALITY: Abandoned coal mines south of Cali, Departamento del Valle, Colombia, South America.

SPECIMEN DEPOSITED: USNM Helm. Coll. 71407.

DIAGNOSIS (based on 17 complete and 42 incomplete adult specimens): Lecithodendriidae, *Castroia*. Body reniform, 0.36–1.23 long by 0.58–1.70 wide. Forebody, 0.194–0.416 long; hindbody, 0.273–0.685 long. Integument aspiny. Oral sucker terminal, cupuliform, 0.038–0.065 long by 0.044–0.075 wide. Prepharynx short, appearing absent. Pharynx, 0.030–0.046 long by 0.026–0.057 wide. Esophagus variable

in length, usually longer than pharynx, ceca forking laterally or slightly posterolaterally to end blindly in anterior one-third body. Acetabulum rounded, 0.098–0.206 long by 0.075–0.194 wide, lying within large acetabulo-genital sac, in anterior half of body, occasionally equatorial. Sucker ratio 1:1.46 to 3.34 (mean 1:2.45).

Genital pore mesial to submesial, preacetabular, followed by shallow genital atrium opening into large acetabulo-genital sac, which opens ventrally to outside (depending upon contraction of body) anterior to (Fig. 7a, b), at level of (Fig. 7c), or posterior to acetabulum. Testes two, ovoid, lateral or posterolateral to and separated by acetabulum; right testis, 0.176–0.320 long by 0.103 to 0.246 wide; left testis, 0.200–0.378 long by 0.137–0.262 wide. Seminal vesicle sinistral, sometimes dextral, long, coiled, lies free in body, mesial to left testis, connecting with prostatic vesicle which perforates genital atrium. Prostatic vesicle with ducts radiating to numerous prostatic gland cells in area posterior to ceca and lateral to posterolateral to acetabulum. Ovary with 3–5 lobes, dextral, sometimes sinistral, mesial to right testis, posterior to ceca, 0.234–0.394 long by 0.097–0.229 wide. Oviduct short, emerging from posterior ovarian lobe, connecting with postovarian ootype surrounded by Mehli's gland. Seminal receptacle elongate to flask-shaped, originating at ootype, base directed mesially. Laurer's canal originating approximate to seminal receptacle, opening submesially on dorsum at level of ootype.

Vitellaria follicular, extending transversely in anterior fourth of body, anterior to and overlapping ceca; vitelline ducts unite posteromesial to ovary, lead to area approximate to ootype. Uterus originating at ootype, proceeding posteromesially, coiling transversely in posterior half of body, then ascending to meet metra-terium, surrounded by gland cells, connecting with genital atrium. Uterine eggs many, light

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brown, operculated, 0.014–0.024 long by 0.006–0.010 wide (mean 0.018 by 0.009). Excretory pore mesial at posterior end of body, leads to excretory vesicle which bifurcates into two long ducts directed anterolaterally, ending blindly posterior to testes.

***Castroia silvai* Travassos, 1928**  
(Figures 2, 3)

HOST: *Peropteryx kappleri* Peters, 1867 (Chiroptera: Emballonuridae).

LOCATION: Middle one-third intestine.

INCIDENCE OF INFECTION: In 6 of 10 hosts.

LOCALITY: Abandoned coal mines south of Cali, Departamento del Valle, Colombia, South America.

SPECIMEN DEPOSITED: USNM Helm. Coll. 71406.

DIAGNOSIS (based on 13 adult specimens): Lecithodendriidae, *Castroia*. Body reniform, 0.416–1.100 long by 0.616–1.720 wide. Forebody, 0.114–0.260 long; hindbody, 0.228–0.692 long. Integument aspinose. Oral sucker terminal, cupuliform, 0.039–0.065 long by 0.052–0.075 wide. Prepharynx short, appearing absent. Pharynx, 0.030–0.046 long by 0.029–0.052 wide. Esophagus variable in length, usually longer than pharynx, ceca forking laterally or posterolaterally to end blindly in anterior one-third of body. Acetabulum rounded, 0.038–0.058 long by 0.044–0.062 wide, lying in large acetabulo-genital sac, in anterior half of body, occasionally equatorial. Sucker ratio 1:0.69–0.93 (mean 1:0.82).

Genital pore mesial to submesial, preacetabular, followed by shallow genital atrium opening into large acetabulo-genital sac, which opens ventrally to outside (depending upon contraction of body) anterior to, at level of (Fig. 7f), or posterior (Fig. 7 d, e), to acetabulum. Testes two, ovoid, lateral to posterolateral to and separated by acetabulum, right testis, 0.132–0.387 long by 0.097–0.300 wide; left testis, 0.197–0.400 long by 0.092–0.246 wide. Seminal vesicle sinistral, sometimes dextral, long, coiled, lies free in body, mesial to left testis, connecting to prostatic vesicle which perforates genital atrium. Prostatic vesicle with ducts radiating to numerous prostatic gland cells lying in area posterior to ceca and lateral or posterolateral to acetabulum (Fig. 3). Ovary with 3–5 lobes, dextral, sometimes sinistral, anteromesial to right testis, posterior to ceca, 0.185–0.370 long

by 0.108–0.246 wide. Oviduct short, emerging from posterior ovarian lobe, connecting with postovarian ootype surrounded by Mehlis' gland. Seminal receptacle elongate, originating from ootype, base directed mesially. Laurer's canal originating approximate to seminal receptacle duct, opening submesially on dorsum at level of ootype.

Vitellaria follicular, extending transversely in anterior fourth of body, anterior to and overlapping ceca; vitelline ducts unite posteromesial to ovary, lead to area approximate to ootype. Uterus originating at ootype, proceeding posteromesially, coiling transversely in posterior half of body, then ascending to meet metraterm surrounded by gland cells, which connects with genital atrium. Uterine eggs many, light brown, operculated, 0.015–0.021 long by 0.006–0.010 wide (mean 0.018 by 0.008). Excretory pore mesial at posterior end of body, excretory vesicle bifurcates anterolaterally, extent of ducts not determined.

DISCUSSION: Eight of 10 bats examined contained trematodes in the anterior one-third and middle one-third intestine. Of the three trematode species found, *Castroia amplicava* exhibited the greatest incidence and intensity of infection in the anterior one-third intestine. When *Castroia silvai* was found it always appeared concomitant with *C. amplicava* (Table 1).

The measurements of my *Castroia* specimens (Table 2) agree in most respects with those cited in the original descriptions by Travassos (1928). Caballero (1962) described two specimens of *C. silvai* found in the intestine of *Micronycteris hirsuta* (Peters, 1869) Andersen, 1906 from Costa Rica. He reported that his *C. silvai* specimens differed only in mensural characters from those described by Travassos (1928). Caballero and Monteros' (1962) measurements and illustrations indicate that their specimens were immature, perhaps accounting for the mensural differences from Travassos' (1928) and my material. His description, however, of a balled-up dextral seminal vesicle does not agree with Travassos' (1928) description or the one given here.

The previous descriptions of the type species, *C. amplicava*, and *C. silvai* were incomplete, thus justifying redescription of the following structures: (1) the prostatic vesicle, its radiating ducts and numerous prostatic

Table 1. Location, incidence, and intensity of infection of *Peropteryx kappleri* by three lecithodendriid trematodes.

Location	<i>C. amplicava</i>		<i>C. silvai</i>		<i>L. gastroides</i>	
	Incidence	Intensity*	Incidence	Intensity*	Incidence	Intensity*
Anterior 1/3 intestine	8/10	1-34(9.25)	0	0	3/10	1(1)
Middle 1/3 intestine	3/10	1-4(2.33)	6/10	1-18(5.67)	0	0
Posterior 1/3 intestine	0	0	0	0	0	0

\* Range and mean.

Table 2. Measurements of *Castroia amplicava* and *C. silvai*.

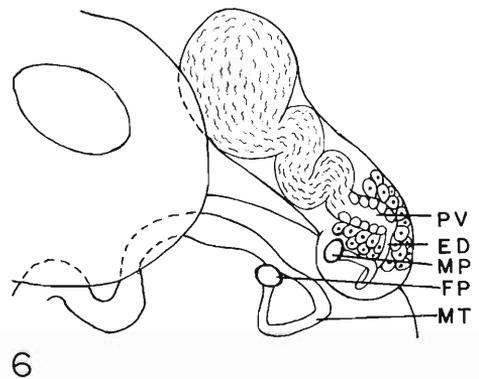
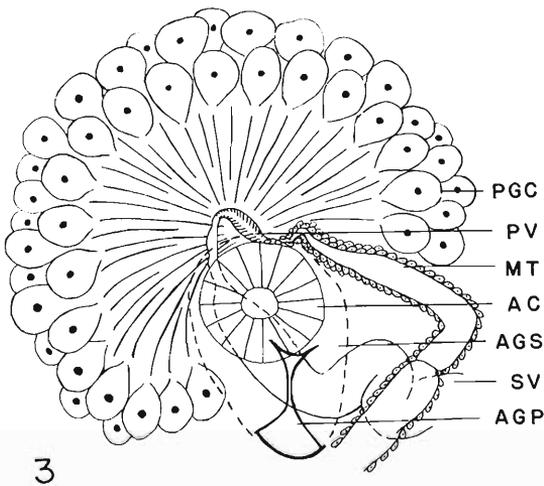
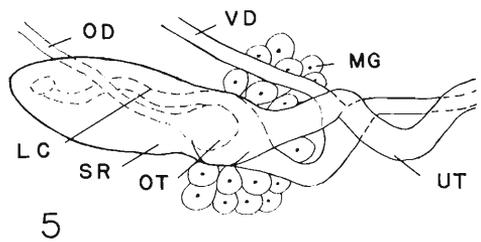
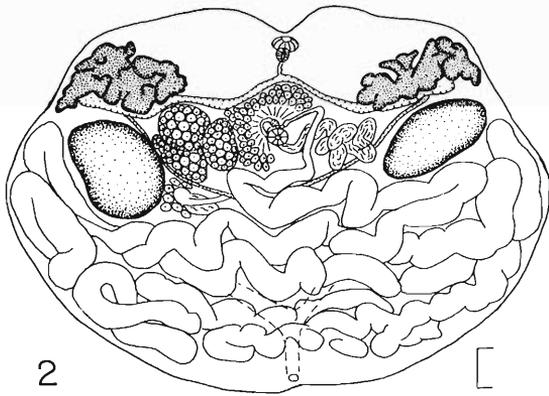
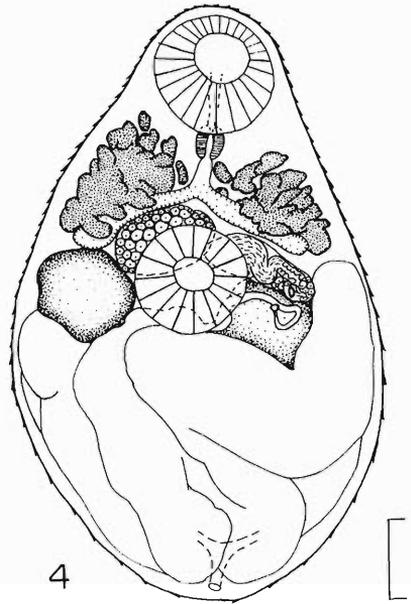
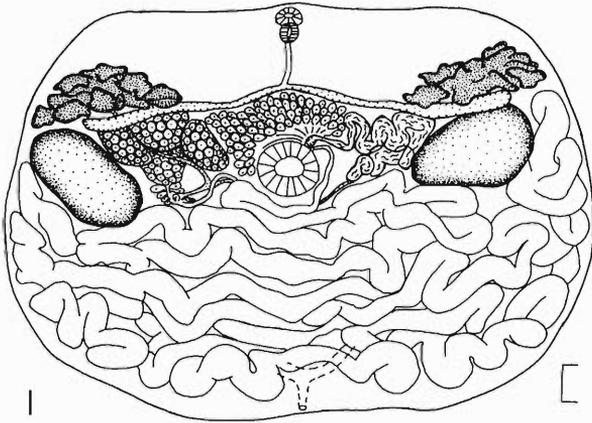
	<i>Castroia amplicava</i> Travassos, 1928		<i>Castroia silvai</i> Travassos, 1928		
	Travassos 1928	This paper	Travassos 1928	Caballero 1962	This paper
Length	0.84-0.92	0.36-1.23	0.84-1.00	0.439-0.586	0.416-1.100
Width	0.95-1.50	0.58-1.70	1.20-1.50	0.933-1.171	0.616-1.720
Oral sucker length		0.038-0.065		0.041-0.041	0.039-0.065
width	0.07-0.08	0.044-0.075	0.085-0.100	0.053-0.070	0.052-0.075
Pharynx length		0.030-0.046		0.020-0.029	0.030-0.046
width	0.42	0.026-0.057	0.028-0.035	0.029-0.049	0.029-0.052
Acetabulum length		0.098-0.206		0.049-0.061	0.038-0.058
width	0.15-0.17	0.075-0.194	0.078-0.085	0.053-0.078	0.044-0.062
Ovary length	0.24-0.27	0.234-0.394	0.21-0.27	0.135-0.164	0.185-0.370
width	0.14	0.097-0.229	0.11-0.17	0.123-0.189	0.108-0.246
Testis length	0.21-0.28	0.176-0.378	0.21-0.35	0.164-0.271	0.132-0.400
width	0.12-0.21	0.103-0.262	0.10-0.15	0.119-0.152	0.092-0.300
Egg length	0.019-0.020	0.014-0.024	0.019-0.020	0.020-0.020	0.015-0.021
width	0.010-0.011	0.006-0.010	0.101-0.011	0.010-0.012	0.006-0.010
Sucker ratio	1 : 2.14-1 : 2.42*	1 : 1.46-3.34 (1 : 2.45)	1 : 0.92-1 : 1*	1 : 1.4-1 : 1.1 1 : 1-1 : 1	1 : 0.69-0.93 (1 : 0.82)
Host	<i>Peropteryx macrotis</i>	<i>Peropteryx kappleri</i>	<i>Peropteryx macrotis</i>	<i>Micronycteris hirsuta</i>	<i>Peropteryx kappleri</i>
Geographical distribution	Brazil	Colombia	Brazil	Costa Rica	Colombia
Habitat	intestine	upper 2/3 intestine	intestine	intestine	middle 1/3 intestine

\* Estimated maximum and minimum sucker ratios determined from reported data.

→

Figures 1-6. *Castroia amplicava*, ventral view, acetabulo-genital sac pore not shown. 2. *Castroia silvai*, ventral view, acetabulo-genital sac pore not shown. 3. *C. silvai*, terminal genitalia. 4. *Limatulum gastroides*, ventral view. 5. *L. gastroides*, ovarian complex. 6. *L. gastroides*, terminal genitalia.

The projected scales have values of 0.1 mm. Abbreviations: AC, acetabulum; AGP, acetabulo-genital sac pore; AGS, acetabulo-genital sac; ED, ejaculatory duct; FP, female pore; LC, Laurer's canal; MG, Mehlis' gland cells; MP, male pore; MT, metraterm; OD, oviduct; OT, ootype; PGC, prostatic gland cells; PV, prostatic vesicle; SR, seminal receptacle; SV, seminal vesicle; UT, uterus; VD, vitelline duct.



gland cells; (2) the seminal receptacle and its relation to the uterus, ootype, Mehlis' gland and the vitelline ducts; (3) the excretory pore, vesicle, and ducts; and (4) the terminal genitalia and the acetabulo-genital sac comprising the acetabulo-genital complex.

This is the first time that an acetabulo-genital sac has been reported in *Castroia* or in the Lecithodendriidae. The sac is similar to the ventrogenital sac, or "genital atrium" referred to by Yamaguti (1958), in the Heterophyidae, but differs in the absence of a gonotyl. The sucker-like pouch found in the Vesperugidendriinae Yamaguti, 1958 (Lecithodendriidae) is bound by two muscular ridges, anteriorly and posteriorly, and is formed from a shallow depression unlike the acetabulo-genital sac of *Castroia*. There is also a superficial resemblance in size and structure of *Castroia* to the heterophyid *Euryhelmis* Poche, 1926. I prefer, however, to retain *Castroia* in the Lecithodendriidae until more is known of its life cycle.

Travassos (1928) indicated that *C. amplivava* differed from *C. silvai*, principally by sucker ratio and the presence of a sulcus at the posterior end of the body. Also, the vitelline follicles were reported to be fewer and larger, and the testes more regular in shape. My Colombian specimens do or do not show a sulcus, depending on the preparation. The appearance of the vitellaria and testes are variable enough that there is no apparent distinction between the two species. The only significantly distinguishing character between *C. amplivava* and *C. silvai* is the sucker ratio.

An analysis of allometric growth, a method proven to be significant by Beaver (1937), Rohde (1966), and others, has shown some differences between the two species that were not apparent on examination of single specimens. The average diameter (length + greatest width / 2) of the ovary, testes, forebody length, hindbody length, and sucker ratio (acetabulum width / oral sucker width) were compared to increase in body size. Regression analysis was done (with the IBM 7044 Computer) utilizing BMD (1965) programs. The sucker ratio (Fig. 7) showed little growth relative to increase in body size, indicating relative stability as a mensural character. *C. amplivava* also differs from *C. silvai* in greater variation in acetabulum size range (1.21 vs. 0.24). The forebody (Fig.

8a), the distance from mesial anterior border of acetabulum to anterior end of body, shows relative growth to be the same, with *C. amplivava* being slightly larger than *C. silvai*. The hindbody (Fig. 8b) exhibits greater rate of growth than the forebody. Rate of growth of the ovary (Fig. 8c) and the testes (Fig. 8d) of both species was less than that found for the hindbody. Ovarian measurements for the two species show smaller differences (slope = 0.1236, *C. amplivava*; 0.1776, *C. silvai*) than the testicular measurements (slope = 0.1091, *C. amplivava*; 0.2191, *C. silvai*).

My data suggest that *C. silvai* grows at a faster rate than *C. amplivava* (Fig. 8d) but establishes smaller populations in a separate part of the host intestine. Assuming normal egg release, superficial examination is suggestive of higher fecundity in *C. silvai* as judged by the larger hindbody which is packed with eggs. These features are suggestive that *C. silvai* is characteristic of a population living in a peripheral habitat.

Sogandares-Bernal (1958, 1959), was one of the first to clearly point out that certain related species of trematodes from marine fishes were separated in the host intestine, suggesting ecological isolation. He also suggested that in cases where closely related species intermingled, mechanical and / or behavioral isolating mechanisms would be important in preventing cross-fertilization. In some cases where small differences in terminal genitalia or weak recognition characters exist between closely related trematodes, possible hybrids have been reported (e.g. *Fasciola hepatica* × *F. gigantica* in livestock where the host ranges meet and the trematodes are found together (Price, 1953). Sogandares-Bernal (1958) gave reasons for believing that *Pseudocreadium myohelicatum* (Bravo and Manter, 1957) is a possible hybrid between *P. scaphosomum* Manter, 1940, and *P. spinosum* Manter, 1940. The case of *Fasciola* reported by Price (1953) may represent an intermediate form within the same species (i.e., *F. hepatica* and *F. gigantica* may be geographical variants of the same species).

The differences of opinion on whether cross-fertilization or self-fertilization is the rule has created great confusion in applying the biological species concept to the hermaphroditic trematodes. In fact, as late as 1957 and 1960, Stunkard stated that the "genetic species" of

the new systematics is impractical, if not impossible for the student of parasitic flatworms since these are hermaphroditic and self-fertilizing and do not ordinarily occur in interbreeding populations. Some evidence has shown, however, that cross-fertilization may be the more common mode of reproduction in trematodes. Sogandares-Bernal (1965) excysted and maintained the trematode *Microphallus opacus* (Ward, 1894) Ward, 1901 *in vitro* as individuals and as pairs. His results showed that a higher percentage of the paired groups produced eggs. Unfortunately, the viability of the eggs was not tested. Bacha (1966) reviewed the evidence for intraspecific crossmating and showed that miracidia of the trematode *Zygocotyle lunata* (Diesing, 1836) Stunkard, 1916 produced either by parthenogenesis or self-fertilization were inviable. Nollen (1968) also reviewed the evidence for intraspecific crossmating in trematodes and used autoradiographic techniques to study fertilization in the trematode *Philophthalmus megalurus* (Cort, 1914) Fisher and West, 1958. His results showed 28 of 37 trematodes self-inseminated when maintained individually. All of 33 trematodes were found to have cross-inseminated with 47 of 61 unlabeled trematodes when placed in groups of 2 to 4 with only one self-insemination. In cases where old trematodes were mixed with young ones there was no appreciable difference in the degree of cross-insemination even though there was a three-fold difference in size between the two age groups. Thus the possibility that cross-breeding in trematodes is the rule and not the exception should be considered and the biological species concept as applied to Digenea can no longer be so easily ignored.

An interpretation of the value of the sucker ratios as a distinguishing character between the two species of *Castroia* is difficult to assess at present. From a speculative point of view, the narrow acetabular size range of specimens of *C. silvai* is suggestive of a pleiotropic character under a tightly integrated genetic system physiologically responsible for its location in a narrow range of the host intestine. The fact that only a few specimens of *C. amplicava*, readily distinguished by their greater sucker ratio, were found concomitant with *C. silvai* in the middle one-third of the host intestine, would suggest that these specimens were at the periphery of their habitat and had possibly

migrated there or been mechanically displaced in dissecting the host, after maturation in the anterior one-third of the intestine. Factors affecting metacercarial excystment in the definitive host intestine may well determine the original site at which a trematode becomes established. The development of metacercariae in two different intermediate host species may determine metacercarial cyst composition and thickness. These factors would in turn determine the site of excystment in the definitive host intestine, possibly leading to eventual genetic segregation of populations within a single host intestine. Thus, if the sucker ratio is a pleiotropic character it would be determined at the site and moment of excystment. The overlap in ecological ranges in the case of *C. amplicava* and *C. silvai*, assuming that intraspecific cross-fertilization is the rule, would suggest that intermediate sucker ratios would be found. This, however, is not the case (Fig. 7). Thus, one must either accept the distinctness of the two species or regard the sole distinguishing character as pleiotropic to the adaptational response of the species to its initial site of excystment in the host intestine.

***Limatulum gastroides* Macy, 1935**  
(Figures 4, 5, 6)

Host: *Peropteryx kappleri* Peters, 1867 (Chiroptera: Emballonuridae).

LOCATION: Anterior one-third intestine.

INCIDENCE OF INFECTION: In 3 of 10 hosts.

LOCALITY: Abandoned coal mines south of Cali, Departamento del Valle, Colombia.

SPECIMENS DEPOSITED: USNM Helm. Coll. 71405.

DIAGNOSIS (based on three adult specimens): Lecithodendriidae; *Limatulum*. Body oval to fusiform, 0.784–0.827 long by 0.466–0.493 wide. Forebody, 0.290–0.355 long; hindbody, 0.308–0.370 long. Integumental spination dense anteriorly, decreasing posteriorly to end of body. Oral sucker subterminal, cupuliform, 0.158–0.166 long by 0.158–0.176 wide. Prepharynx, 0.25–0.062 long. Pharynx, 0.040–0.042 long by 0.032–0.042 wide. Esophagus short, ceca short, broad, forking posterolaterally, to end blindly in anterior one-third body. Acetabulum rounded, immediately preequatorial, 0.132–0.150 long by 0.141–0.167 wide. Sucker ratio 1:0.89–0.95 (mean 1:0.91).

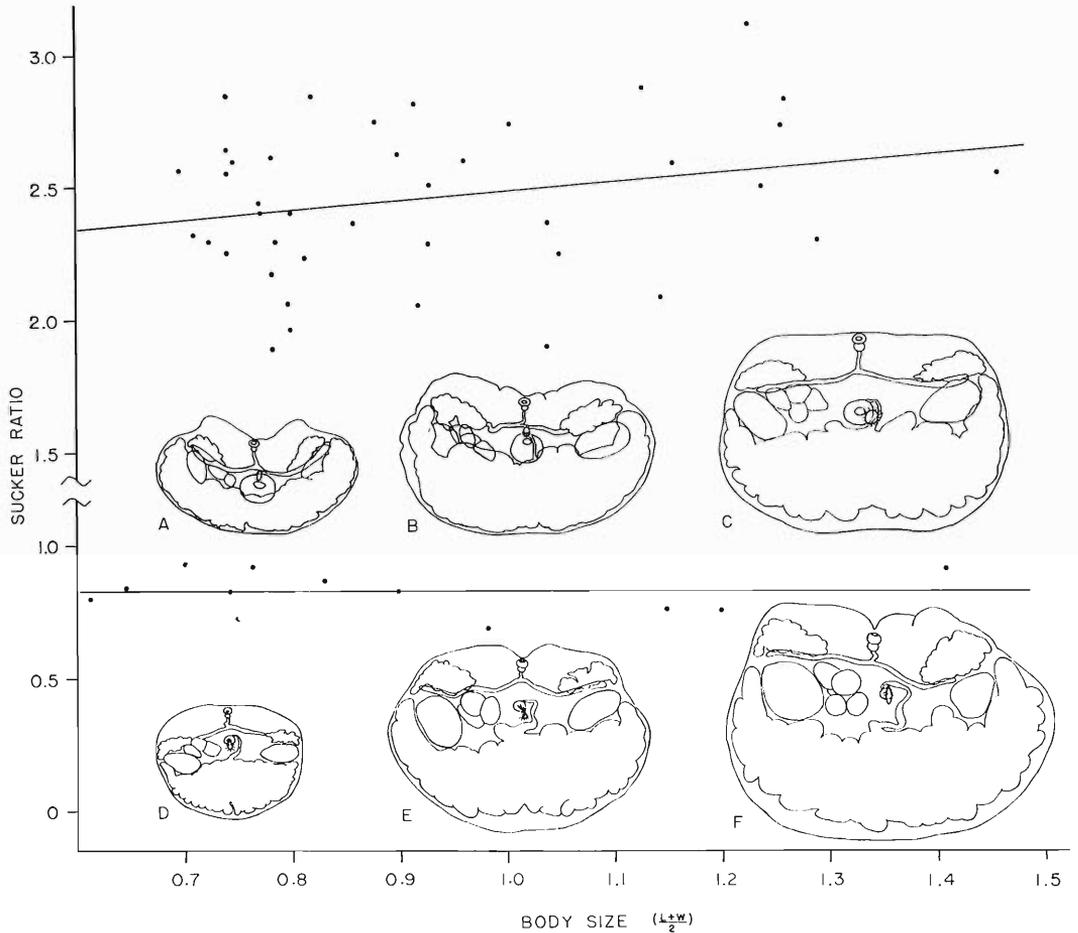


Figure 7. Sucker ratio (ordinate) vs. body size (abscissa) of *Castroia amplicava* (mean = 1:2.45) and *C. silvai* (mean = 1:0.82). A-C, *C. amplicava*; D-F, *C. silvai*. Acetabulo-genital sac pore shown.

Genital pores open separately. Male pore sinistral, lateral to acetabulum at level of anterior border of testis, opening ventrally (Fig. 6). Female pore immediately posteromesial to male pore. Testes two, side by side, ovoid, posterolateral to and separated by acetabulum; left testis, 0.132–0.176 long by 0.074–0.158 wide; right testis, 0.132–0.166 long by 0.114–0.132 wide. Cirrus sac well developed, large, elongate, 0.148–0.189 long by 0.046–0.076 wide; sinistral, located anterolateral to acetabulum but directed posterolaterally; internal seminal vesicle coiled in posterior two-thirds sac, proceeding to short prostatic vesicle, surrounded

by prostatic gland cells connecting with ejaculatory duct opening to genital pore (Fig. 6). Ovary ovoid, dextral, anterolateral to acetabulum, posterior to ceca, 0.114–0.142 long by 0.088–0.097 wide. Ootype postovarian, surrounded by Mehlis' gland, seminal receptacle flask-shaped, originating at ootype, directed laterally. Laurer's canal originating approximate to seminal receptacle duct opening submesially on dorsum at level of ootype (Fig. 5).

Vitellaria follicular, extending posterolaterally in anterior one-third of body, anterior to and overlapping ceca; vitelline ducts uniting posterior to ovary, proceeding to area of seminal

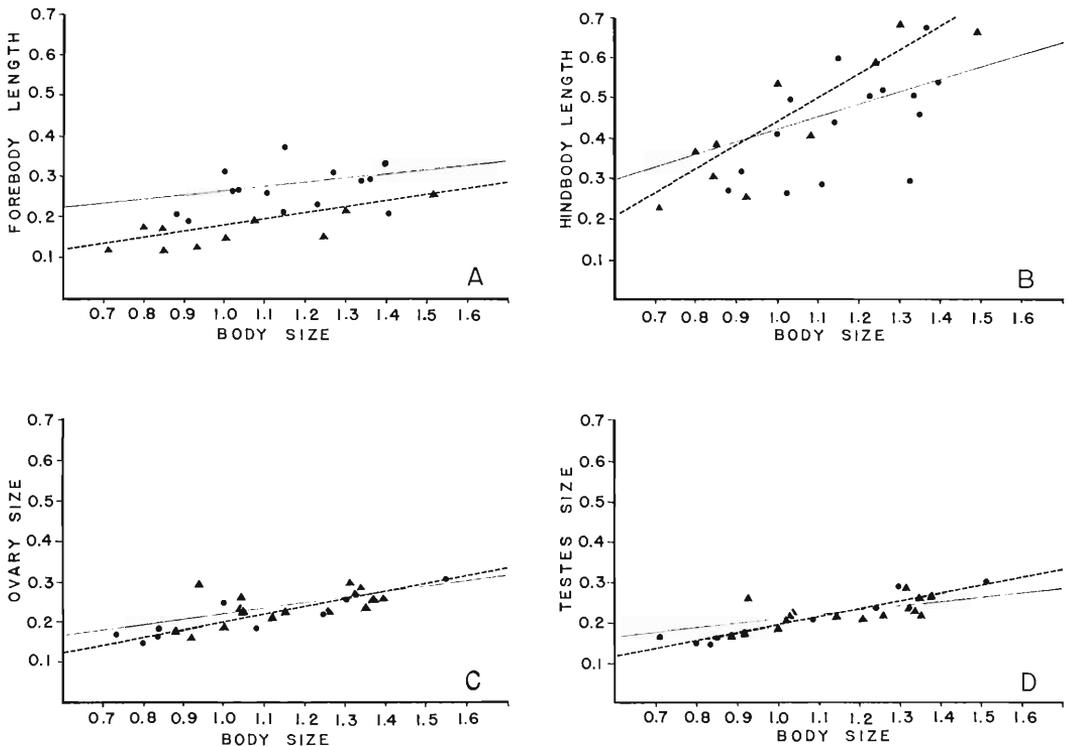


Figure 8. A. Forebody length (ordinate); B. Hindbody length; C. Ovary size; D. Testes size, vs. body size (abscissa). *C. amplicava*, ●; *C. silvai*, ▲.

receptacle. Uterus originating at ootype, proceeding posteriorly, coiling in posterior half of body, ascending to meet metraterm opening at female pore. Uterine eggs many, light brown, oval, operculated, 0.018 long by 0.010–0.012 wide (mean 0.018 by 0.011). Excretory pore ventromesial at posterior end of body, connecting with short Y-shaped excretory vesicle which bifurcates in posterior one-seventh of body.

DISCUSSION: The third species found in *P. kappleri* was identified as *Limatulum gastroides* Macy, 1935 based on the revision of the genus by Dubois (1964). One specimen each was found in three bats also infected with *Castroia amplicava* and *C. silvai*. The forms reported here correspond to the Costa Rican forms reported by Caballero and Brenes (1957) as *Ochoterentrema costarricensis* (Table 3). Measurements of the Colombian forms correspond in all respects to *L. gastroides* except for the measurements and position of the cirrus sac

and genital pore. The cirrus sac described and illustrated for the holotypes of *L. gastroides* and *L. oklahomense* (Macy, 1931, 1935; Dubois, 1964) is shorter, curved medially, and does not extend appreciably beyond the anterior border of the acetabulum. In contrast the Latin American forms have a relatively longer and narrower cirrus sac which does not curve medially and extends beyond the anterior border of the acetabulum; the genital pore is located in the ventral anterolateral area near the left testis and posterior to the distal end of the left cecum. Judging from the illustrations these differences may be accounted for by the differences in preparation (i.e. the holotypes were apparently flattened to a greater degree than were the Colombian specimens). This difference may be further affected by the more advanced state of reproduction of the Colombian forms, i.e., larger uterus and reproductive organs. Based upon these observations the

Table 3. Measurements for *Limatum gastroides* Macy, 1935 and its synonyms (adapted from Dubois, 1964).

	<i>L. gastroides</i>	<i>Ochoterentrema costarricensis</i>	<i>Limatum costarricensis</i>	<i>L. istmicus</i>	This paper
	Macy 1935	Caballero and Brenes 1957	Caballero 1964	Caballero 1964	
Length	0.54-0.70	0.66-0.77	0.439	0.732	0.784-0.827
Width	0.40-0.50	0.52-0.54	0.348	0.403	0.466-0.493
Oral sucker					
length	0.08-0.095	0.134	0.098	0.074	0.158-0.166
width	0.090-0.100		0.107	0.098	0.158-0.176
Pharynx					
length	0.03-0.04	0.037-0.054	0.041	0.020	0.040-0.042
width	0.04-0.05	0.037-0.042	0.041	0.041	0.032-0.042
Acetabulum					
length	0.077-0.09	0.096-0.125	0.102	0.106	0.132-0.150
width	0.074-0.085	0.112-0.116	0.094	0.106	0.141-0.167
Ovary					
length	0.056-0.086	0.079-0.114	0.053	0.09	0.114-0.142
width	0.036-0.054	0.112-0.125	0.082	0.098	0.088-0.097
Testis					
length	0.087-0.110	0.135-0.208	0.102	0.074, 0.082	0.132-0.176
width	0.052-0.104	0.137-0.179	0.107	0.094, 0.061	0.074-0.158
Cirrus sac					
length	0.110-0.132	0.125-0.137	0.156	0.143	0.148-0.219
width			0.061	0.061	0.046-0.076
Egg					
length	0.019-0.021	0.021	0.020	0.016	0.020
width	0.011-0.013	0.012	0.012	0.010	0.012
Host	<i>Myotis lucifugus</i> <i>M. californicus</i>	<i>Eptesicus propinquus</i>	<i>Myotis nigricans</i>	<i>Peronycteris kappleri</i>	<i>Peropteryx kappleri</i>
Geographical distribution	Wisconsin, Minnesota, Oregon	Costa Rica	Panama	Costa Rica	Colombia
Habitat	stomach, intestine	intestine	intestine	intestine	upper 1/3 intestine

disposition of the cirrus sac and the genital pore are not considered in this case to be significant in distinguishing my species of *Limatum*.

Caballero (1964) transferred *Ochoterentrema costarricensis* Caballero and Brenes, 1957, to *Limatum* Travassos, 1921. He was apparently unaware of an almost simultaneously published revision of *Limatum* by Dubois (1964) who has examined the types of *O. costarricensis* and declared it a synonym of *L. gastroides*. I concur with Dubois' (1964) views. In the same paper Caballero (1964) described a new species, *L. istmicus*, from a single specimen. The measurements (Table 3) and the illustrations do not indicate any significant difference from *L. gastroides*. The description of the position, proportions and orientation of the cirrus sac and the position of the genital pore

agree with the description of the Latin American forms of *L. gastroides* discussed previously. Caballero indicated that *L. istmicus* differed from *Ochoterentrema costarricensis* (= *Limatum gastroides*) in that the oral sucker was smaller than the acetabulum (1:1.4 by 1:1.1). However, in his description (1964b), of *L. costarricensis* (= *L. gastroides*) from Panama he indicated a sucker ratio of 1:0.9 by 1:1 which corresponds to that of my Colombian specimens and does not differ significantly from the holotype. Thus, the slight differences in sucker ratio are not considered significant. In consideration of the total data I regard *Limatum istmicus* Caballero, 1964, as a synonym of *Limatum gastroides* Macy, 1935.

This report extends the geographical range of *L. gastroides* south into Colombia, South America and extends its host occurrence to a

third family of bats, Emballonuridae, the other two being Vespertilionidae and Phyllostomidae.

### Acknowledgments

This study was done under the direction of Professor Franklin Sogandares-Bernal. Acknowledgments are also extended to Dr. Paul C. Beaver, director of ICMRT at Tulane University, and Dr. Antonio D'Alesandro, assistant director of ICMRT at Universidad del Valle, Cali, Colombia and their staff who were most helpful in making this study possible. Dr. Satyu Yamaguti and his assistant Shunya Kamigai have kindly examined my specimens and criticized the manuscript. Mr. Maurice Thomas assisted me with the field sampling.

### Summary

Eight of 10 bats, *Peropteryx kappleri* Peters, 1867, from Cali, Colombia were found infected with *Castroia amplicava* Travassos, 1928 (anterior two-thirds intestine) and *Castroia silvai* Travassos, 1928 (middle one-third intestine). One *Limatulum gastroides* Macy, 1935 was found in the anterior one-third intestine of each of three bats. The possible significance of ecological isolation and allometric growth of these helminths is discussed. Redescription of the two *Castroia* species reports for the first time the presence of a acetabulo-genital sac and the terminal-genital complex. *Limatulum istmicus* Caballero, 1964 is synonymized with *L. gastroides*. The presence of separate genital pores in this species is reported for the first time. This report extends the host occurrence and geographical range for these three trematode species.

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## The Monogenean Parasites of African Fishes. VII. Dissolution of the Family Protogyrodactylidae Johnston and Tiegs, 1922<sup>1</sup>

C. E. PRICE<sup>2</sup> AND T. PIKE<sup>3</sup>

Initial research on the Monogenea of Australian freshwater fishes was carried out by Johnston and Tiegs (1922). Although their pioneering effort is praiseworthy and constituted an important early investigation on the monogenetic trematodes (in 1922, only two species of these parasites were known from the entire Western Hemisphere), some of their observations and opinions are subject to question. One problematical portion of their study concerned the establishment of the family Protogyrodactylidae to contain their genera *Protogyrodactylus* and *Trivitellina*.

Initial examination of specimens of *Protogyrodactylus* at our disposal (plus four additional ones recently donated by Dr. P. C. Young of the Fisheries Helminthological Unit, Commonwealth Bureau of Helminthology, St. Albans, Herts, England) indicated that these forms clearly belonged to the subfamily Ancyrocephalinae Bychowsky, 1937 of the family Dactylogyridae Bychowsky, 1933.

What criteria, then, did Johnston and Tiegs employ in establishing their family Protogyrodactylidae? Their family diagnosis made reference to three strong differentiating factors, which, if actually present, would adequately serve as the bases of a valid new family. These factors were: (1) the presence of 12 haptorial hooks (the vast majority of members of Dac-

tylogyridae possess 14 hooks), (2) the existence of longitudinal and multiple transverse vitelline canals (lacking in most dactylogyrids), and (3) the presence of a genito-intestinal canal (these structures are very rarely present in members of the suborder Monopisthocotylea).

### Materials and Methods

Branchial materials were donated by members of the Natal Parks, Game and Fish Preservation Board, Pietermaritzburg, Republic of South Africa. Thanks are extended to contributing personnel.

Host materials were frozen and then preserved in 3½% formalin prior to shipment to the United States. Upon arrival, the gills and recovered parasites were treated as prescribed by Price (1966). Measurements were made as outlined by Price and McMahon (1967). Anatomical terminology employed follows the recommendations of Hargis (1958) and of Price and Arai (1967).

Observations were made with a phase microscope. Appropriate measurements and illustrations were made with the aid of a calibrated filar micrometer ocular and a camera lucida, respectively. Average measurements are given first, followed by minimum and maximum values enclosed in parentheses. All measurements are expressed in microns.

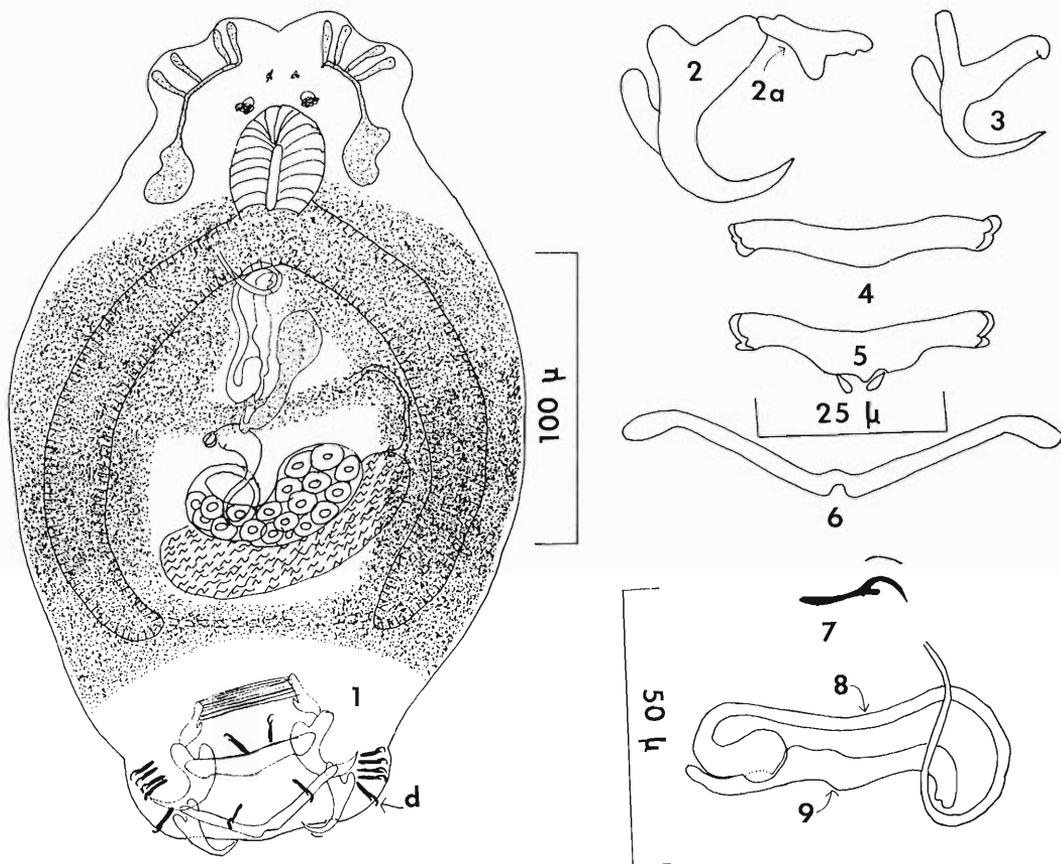
### *Protogyrodactylus johnsonettiiegsi* sp. n.

HOST AND LOCALITY: *Terapon jarbua* (Forsk.); Lake Nhlange, Kosi Bay Lake System, Natal, Republic of South Africa.

<sup>1</sup> This study jointly supported by: (1) The Natal Parks, Game and Fish Preservation Board, (2) Millersville State College and (3) a research grant from The American Philosophical Society (#4956-Penrose fund).

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Figures 1-9. Camera lucida illustrations of *Protogyrodactylus johnstonettiegi* n. sp. 1. Whole mount, ventral view (dorsally located hook designated by "d"). 2. Ventral anchor. 2a. Accessory sclerotized structure in association with ventral anchor. 3. Dorsal anchor. 4, 5. Different views of ventral bar. 6. Dorsal bar. 7. Hook. 8. Cirrus. 9. Accessory piece. (100  $\mu$  scale for Fig. 1; 50  $\mu$  scale for Figs. 8, 9; and 25  $\mu$  scale for Figs. 2-7.)

NUMBER OF SPECIMENS STUDIED: Five.

TYPES: Holotype deposited in USNM Helm. Coll., No. 71343, Washington, D.C. First paratype deposited in the Natal Museum, Republic of South Africa. Remaining paratypes are in authors' collections.

DESCRIPTION: A very robust dactylogyrid provided with a thin cuticle; length 323 (295-340), greatest body width 190 (172-212). Anterior and lateral cephalic lobes moderately well developed. Head organs (on either side) composed of four elongate glandular structures connected by a common duct; this duct in turn joins to a larger pharyngeal gland. Two

pairs of eyespots, members of posterior pair larger. Each eyespot provided with a biconvex lens-like structure. Peduncle short and stout; haptor poorly set off from body proper in all specimens (Fig. 1).

Haptor provided with two pairs of dissimilar anchors (Figs. 2, 2a, 3). Ventral anchors each composed of (1) a solid base provided with a blunt deep and a superficial root, (2) a solid shaft, and (3) a solid point. Anchor wings present. Articulated to superficial root of each ventral anchor is a sclerotized structure of irregular outline (Fig. 2a). A broad band of muscular or connective tissue extends between these addi-

tional structures (Figs. 1, 2a). Length of ventral anchor 28 (26–31); width of base 16 (14–19). Dorsal anchors smaller than ventrals; each composed of a solid base, a solid shaft and a solid point. The roots are more elongate than in ventrals. Anchor wings present. Length of dorsal anchor 22 (20–25); width of base 16 (14–18). No accessory structure in association with roots.

Bases of each pair of anchors connected by a transverse bar, the bars not joined to each other (Figs. 1, 4, 5, 6). Ventral bar much shorter and more heavily sclerotized than dorsal; length of ventral bar 33 (30–36). Dorsal bar elongate, with moderately expanded ends. A cleft is seen in the midpoint; this furnishes an erroneous impression of the bar being divided; bar bent in a slight "V" shape, the open portion of the "V" directed anteriorly. Length of dorsal bar 57 (54–63).

Haptor hooks 14 in number (seven pairs). Each hook composed of a solid elliptical base, a solid shaft and a sickle-shaped termination provided with an opposable piece (Fig. 7). A domus in association with each hook. Hooks similar in shape and size; range of hook lengths 14–16. Hook arrangement atypical for subfamily Ancyrocephalinae (further comment in Discussion section).

Copulatory complex consists of a tubular cirrus and a basally articulated accessory piece (Figs. 8, 9). Estimated total length of cirrus 101 (91–113); length of accessory piece 50 (46–54). Prostatic reservoir large, single, filled with a yellowish fluid in which small granules are suspended; reservoir opens into cirrus base via a small duct. Both gonads transversally elongate; testis postovarian and partially overlapping ovary (dorsal view). Unable to trace entire course of vas deferens, but it does not appear to loop around either intestinal limb. Oviduct appears to be in form of a single turn, widening into a uterus which has its opening to right of midline just posterior to copulatory complex. Neither vagina nor seminal receptacle observed.

Vitellaria well developed, composed of granules which form two broad lateral bands (Fig. 1). Vitellarial granules become confluent at three points: (1) near region of pharynx, (2) just posterior to copulatory complex, and (3) at posterior terminations of lateral bands. The "frond-like" appearance of the vitellaria as re-

ported by Johnston and Tiegs was not seen. Although vitellarial bands become confluent posteriorly, the simple intestinal limbs do not appear to become confluent, but terminate unjoined. No structure resembling a genito-intestinal canal observed. Neither transverse nor longitudinal vitelline ducts observed.

DISCUSSION: The parasite specimens used in this study are seemingly closely related morphologically to those Johnston and Tiegs recovered from *Terapon carbo*, *T. hilli*, and *T. fuliginosus* from Australia. Many of the features of our specimens, e.g., robust body outline, eyespots, copulatory complex, anchors, bars, etc., parallel those of the specimens described by Johnston and Tiegs. Parasites utilized in both studies were recovered from *Terapon* hosts.

Our specimens of *Protogyrodactylus* are provided with 14 hooks whereas Johnston and Tiegs gave 12 as the number present on their specimens. Essentially all members of the Dactylogyridae (the family to which we believe *Protogyrodactylus* and *Trivitellina* rightfully belong) possess 14 hooks. The authors agree with Bychowsky (1957) that probably all dactylogyrids possess 14 of these structures. The presence of only 12 hooks would seemingly constitute a rather drastic departure from normal. The spatial arrangement of the hooks on our specimens, however, is quite atypical for the Dactylogyridae. Mizelle and Crane (1964) described a generalized hook arrangement for the family; in essentially all included species, five pairs are located on the ventral aspect of the haptor, the remaining two pairs on the dorsal surface. In the present species, six pairs are located ventrally with one pair dorsal (Fig. 1d) on the haptor.

We observed no structure which could reasonably be considered a genito-intestinal canal. As pointed out by both Bychowsky (1957) and Yamaguti (1963), the presence of such a canal in a monogenean belonging to the suborder Monopisthocotylea is an extremely rare occurrence. There are a few exceptions (some of which are questionable), but these rare specimens are not very closely related to the forms under discussion.

The multiple vitelline ducts referred to by Johnston and Tiegs were not observed in our specimens and it is unlikely that they were present in the forms described by the Australians. What is possibly a single transverse

vitelline canal can be seen where the vitellarial granules become confluent in the posterior region of the trunk; this is shown in dotted outline in Fig. 1. Excessive contraction can produce wrinkles in the cuticle which can be erroneously interpreted as ducts or canals. Although the vitellarial granules become confluent at three points, it should be noted that this is a relatively common trait among monopisthocotyleans and such confluences do not necessarily denote the presence of vitelline (or other) canals. The "frond-like" appearance of the vitellaria described by Johnston and Tiegs was not seen in our specimens.

Our observations thus indicate that the unusual traits attributed to these forms by the Australian workers are artifacts. Without these special features, it appears that these parasites are eligible for inclusion in the Ancyrocephalinae.

Our opinion is strengthened considerably by observations made by P. C. Young, referred to above. Dr. Young has made an extensive recent study of monogenetic trematodes of Australian fishes (Young, 1967a; 1967b; 1967c). While engaged in Australian collections, he visited the Sydney Museum, where he examined holotypes of *Protogyrodactylus quadratus* and *Trivitellina subrotunda*. Dr. Young kindly informed the senior author (personal communication) of results of his observations. It is noted that these observations are in essential agreement with ours.

Of *P. quadratus*, he noted that: (1) although only 12 haptor hooks were observed, their positions on the haptor indicated that 14 should have been present, (2) the vitellaria were not arranged in the special manner reported by Johnston and Tiegs, (3) the "posterior transverse vitelline duct" appears to be merely two lateral bands of vitellaria joined posteriorly and (4) no longitudinal yolk duct was visible.

Of *T. subrotunda* he concluded that: (1) only 13 hooks were observed but positioning indicated that the actual number was 14, (2) no posterior transverse vitelline duct was observed, and (3) the previously reported longitudinal yolk duct was apparently lacking.

Bychowsky (1957) is credited with being the first worker to question the validity of Protogyrodactylidae. His opinions closely parallel the observations by Dr. Young and the

present authors. In view of this agreement of opinions, we propose that the family Protogyrodactylidae should be liquidated and that *Protogyrodactylus* should be considered a valid genus of the Ancyrocephalinae.

Concerning *Trivitellina*, it appears likely that this genus is a synonym of *Protogyrodactylus*. It appears from a study of the whole mount illustrations by Johnston and Tiegs that the form they referred to as *Trivitellina* was simply a severely contracted specimen of *Protogyrodactylus*. One of the principal traits of the former genus was the very unusual arrangement of the testis being located anterior to the ovary. In a parasite which is greatly distorted, the testis (which partially overlaps the ovary in our specimens) could conceivably move to a position in front of the ovary.

DERIVATION OF SCIENTIFIC NAME: The generic name of *Protogyrodactylus* is retained, according to the rules of priority. It is pointed out by Bychowsky (1957), however, that this designation is inaccurate in the meaning which it imparts. The name proposed by Johnston and Tiegs denotes a primitive condition ("protos"—first) inherent in the included parasites. This must be considered an error because characters such as reduction in number of hooks and presence of a genito-intestinal canal would be traits of advancement, not of primitiveness.

The species name, *johnstonetiegsi*, is formed by combining the names of Johnston and Tiegs; this name is chosen to honor these pioneers in parasitology.

#### **Diagnosis of *Protogyrodactylus* Johnston and Tiegs, 1922, emended**

Dactylogyridae, Ancyrocephalinae. Robust forms of moderate size. Two pairs of eyespots. Two pairs of anchors, members of ventral pair provided with an accessory sclerotized structure. Bases of each pair of anchors connected by a transverse bar, the bars not articulated to each other. Fourteen hooks (seven pairs), arranged six pairs ventrally on haptor, one pair dorsally. Copulatory complex composed of a cirrus and a basally articulated accessory piece. Prostatic reservoir single. Vas deferens not looped around intestinal limb. Vagina not sclerotized. Vitellaria moderately well developed. Intestinal crura simple, the crura terminating posteriorly without undergoing confluency.

### A Note on the Host, *Terapon jarbua* (Forsk.)

This host is quite widespread in distribution. One reason for this is the ability of the fish to withstand a great range of osmotic pressures. This enables *T. jarbua* to survive in both freshwater and marine habitats.

Its range extends from the south coast of the Republic of South Africa, northwards to and as follows: Madagascar, Mauritius, Seychelles, including the Red Sea, across the Indian Ocean Akyab, Muscat, Chilka Lake, Mardas, Ceylon, Praslin, The Philippines, Formosa, China, Japan, Riu Kiu Islands, Australia, Lord Howe Island, New Caledonia, New Britannia, Neu-Pommern, Samoa, Fiji Islands, Tonga Islands, Solomon Islands, and Admiralty Islands.

#### Summary

The monogenetic trematode family Protogyrodactylidae Johnston and Tiegs, 1922 is re-evaluated. It is concluded that this family (established to include the genera *Protodactylus* and *Trivitellina*, both of Johnston and Tiegs, 1922) is not warranted. It is recommended that: (1) *Protogyrodactylus* and *Trivitellina* are synonymous and constitute the single genus *Protogyrodactylus* and (2) the family Protogyrodactylidae be liquidated and *Protogyrodactylus* be included as a valid genus of the subfamily Ancyrocephalinae, family Dactylogyridae. A new species, *P. johnstonettiegi*, is described.

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## Pathogenesis of *Trichostrongylus colubriformis* (Nematoda) Infections in Guinea Pigs

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The nematode, *Trichostrongylus colubriformis*, an intestinal parasite of ruminants, can be readily established in the guinea pig (Herlich et al., 1956). Oral doses of 20,000 or more infective larvae can cause clinical disease and death as early as 2 days (Herlich, 1969). The small intestine of severely affected guinea pigs is distended and easily torn at necropsy. Symons (1957) reported that infection of rats with *Nippostrongylus brasiliensis* (= *N. muris*) caused a 2-fold increase in the water content of the lumen and tissues of the small intestine and 2.5-fold increase in the width of the muscularis externa of the jejunum.

This report presents results of some experiments to determine whether the guinea pig is similarly affected by *T. colubriformis*.

### Experimental Procedure

**EXPERIMENT 1.** *Determination of effect of infection on wet and dry weights of the small intestine and contents.* Thirty-five male guinea pigs weighing 400–550 gm were used. Twenty-five were each inoculated orally with 5,000 freshly isolated infective larvae of the RLS isolate (Herlich, 1966) of *T. colubriformis* suspended in 0.3 ml of water. Of ten noninoculated controls five were killed at this time. Groups of five infected guinea pigs were killed 3, 6, 9, 12, and 15 days after inoculation (DAI), and the remaining five noninfected guinea pigs were also killed on day 15. Feed and water were withheld 6 hr before necropsy.

The intact small intestine of each animal was quickly removed at necropsy, lightly blotted, and weighed in a petri dish. The intestine was slit longitudinally as rapidly as possible and lightly scraped to remove its contents. Intestine and contents were weighed in separate dishes, dried to constant weight in a hot air oven (10 hr at 100 C), and weighed again.

All guinea pigs were weighed at the start of the experiment. As guinea pigs of each group

were killed, they and those in the noninfected control group were weighed again.

**EXPERIMENT 2.** *Determination of effect of infection on circumference of the small intestine and thickness of the muscularis externa.* Thirty male guinea pigs weighing 350–520 gm were allotted randomly to four groups: three groups of nine animals each and one of three. Nine guinea pigs were each inoculated with 5,000 larvae, nine with 20,000, three with 40,000 and nine were left uninfected. Guinea pigs of the various groups were killed at three different times after infection (Table 2).

The small intestine was removed from each guinea pig, and a 2.5-cm-long piece at a point 15 cm from the pylorus was excised and fixed in Helly's fluid. Cross sections of the gut were cut; all three pieces of intestine from a group were imbedded in the same block of paraffin. Sections were stained with hematoxylin and eosin.

The gut in cross section was frequently not perfectly round, as had been noted by Symons (1957) in his work with rats. However, instead of determining the effect of infection on gut size by measuring tangential diameters perpendicular to each other as that researcher did, tracings were made of the circumference of the gut from an image in an overhead microprojector. The tracing was then calibrated with a planimeter. Width of the muscularis externa was determined by averaging four measurements made 90° apart on each gut as outlined by Symons (1957).

**EXPERIMENT 3.** *Replication of experiments 1 and 2.* Forty male guinea pigs weighing from 470–610 gm were used. Fifteen were each inoculated with 10,000 larvae (Group A), 15 with 20,000 (Group B), and ten were maintained as noninoculated controls (Group C). Five of each group were killed on days 5 and 13; the other ten in Groups A and B were killed on day 10.

**Table 1. Water and dry matter weight of tissues and contents of small intestines and body weight changes of guinea pigs inoculated with 5,000 *T. colubriformis* larvae.**

Days after inoculation	0 Control	3	6	9	12	15	15 Control
Water in tissues	9.3±1.2	9.4±1.6	9.2±1.7	8.4±1.2	9.1±1.0	10.8±1.9	8.6±1.4
Water in contents	2.5±0.8	3.0±0.9	1.9±0.7	2.4±0.9	1.2±0.7	2.2±0.8	2.1±0.7
Dry weight tissues	2.6±0.7	2.0±0.6	2.2±0.6	1.5±0.5	2.3±0.6	2.0±0.6	1.4±0.5
Dry weight contents	0.2±0.1	0.3±0.1	0.2±0.1	0.1±0.1	0.7±0.2	0.2±0.1	0.2±0.1
Body weight changes	—	+1 ±0.2	+3 ±0.1	-22±2.9	-60±5.4	-61±6.2	+22±3.1

The small intestine of each was removed and placed in a petri dish. A piece of intestine 2.5 cm long was excised 15 cm from the pylorus and fixed in Helly's fluid. The remaining portions of the intestine were processed by the technique described above in experiment 1 to determine the amount of water in the contents and tissues. The fixed tissues were sectioned, stained, and measured in the manner described for experiment 2.

All values obtained were analyzed statistically using "Student's" t-distribution test.

### Results and Discussion

All infected guinea pigs lost weight; losses occurred as early as 9 DAI with 5,000 larvae and averaged 61 g by 15 DAI, while controls gained an average of 22 g (Table 1). Guinea pigs inoculated with greater numbers of infective larvae developed diarrhea, became emaciated, and had more severe weight loss. The guinea pigs given 20,000 larvae lost as much

**Table 2. Circumference of the small intestine and width of the muscularis externa in guinea pigs infected with *Trichostrongylus colubriformis*.**

No. larvae inoculated	0	5,000	20,000	40,000
Days after inoculation				
	Circumference of gut (mm) Mean ± S.E. of 3 guinea pigs			
5	7.8±0.7	7.3±0.2	7.4±0.7	7.8±1.0
10	8.2±2.8	7.8±1.6	9.9±2.2	—
14	7.7±0.1	7.7±1.0	10.0±1.2*	—
	Width of muscularis externa (microns)			
5	50±17.7	50±12.3	32±7.8	79±23.7
10	37±26.6	44±23.1	53±11.9	—
14	36±9.4	73±7.6	89±21.0*	—

\* Guinea pigs of this group died 13 DAI, tissue underwent postmortem change.

**Table 3. Water and dry matter weight of tissues and contents of small intestines, body weight changes, circumference of small intestines and width of muscularis externa of guinea pigs inoculated with two levels of *T. colubriformis* larvae.**

No. larvae inoculated	Noninoculated control	10,000	20,000
Days after inoculation			
	Water in tissues (g) Mean ± S.E. of 5 guinea pigs		
5	8.7±0.9	9.2±1.0	8.4±1.1
10	—	10.2±1.6	9.0±2.0
13	10.2±1.4	8.9±1.2	9.3±1.6
	Dry matter of Tissues (g)		
5	2.1±0.8	1.9±0.9	1.9±0.8
10	—	1.9±0.8	2.2±1.0
13	2.1±0.7	1.8±0.9	1.9±1.0
	Water in contents (g)		
5	1.7±0.7	1.7±0.8	3.4±2.1
10	—	1.6±0.8	2.5±1.1
13	4.2±2.1	5.8±2.0	6.3±2.7
	Dry matter contents (g)		
5	0.2±0.2	0.2±0.1	0.2±0.1
10	—	0.2±0.1	0.1±0.1
13	0.3±0.2	0.1±0.1	0.2±0.1
	Body weight changes (g)		
5	+8±1.2	+2±0.9	-2±1.0
10	—	-27±9.5	-12±5.2
13	+9±2.2	-79±11.5	-122±16.8
	Circumference of gut (mm) Mean ± S.E.		
5	7.9±1.7	8.7±2.0	9.1±1.6
10	—	7.9±1.4	8.4±1.2
13	8.2±2.2	7.9±0.7	8.7±1.4
	Width of muscularis externa (microns) Mean ± S.E.		
5	51±10.9	39±3.4	57±9.6
10	—	78±9.4	92±13.4
13	49±8.2	96±11.3	122±14.8

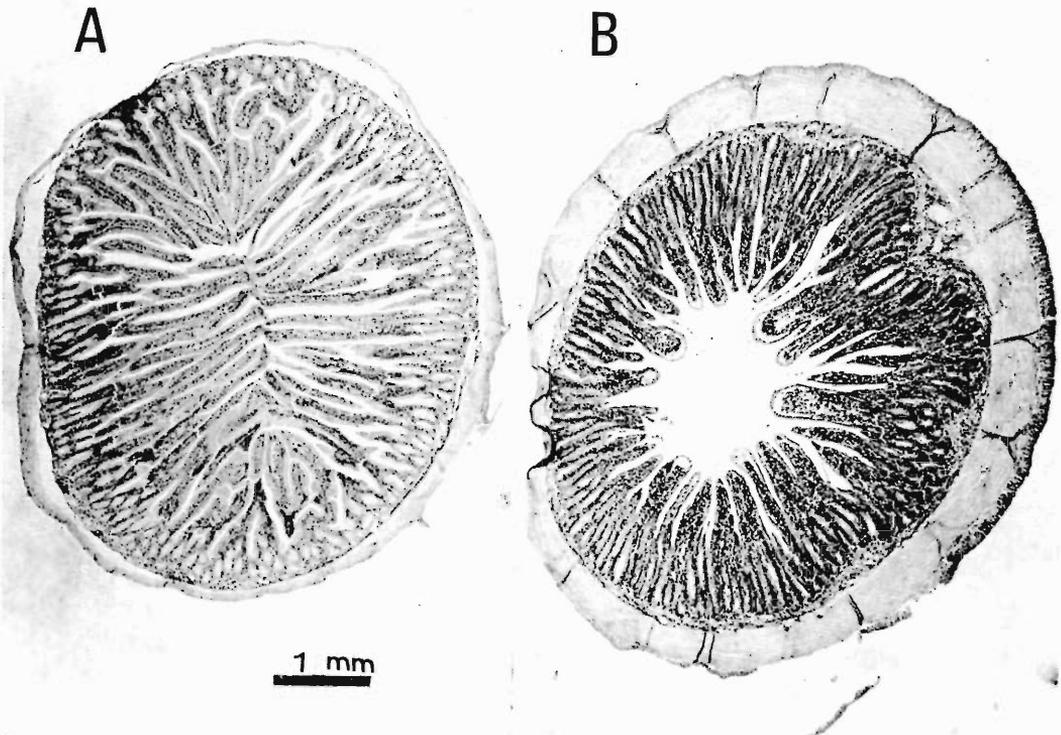


Figure 1. Cross section of guinea pig duodenum. A. Normal. B. 13 days after inoculation with 20,000 *T. colubriformis* larvae.

as 122 g (Table 3). All guinea pigs inoculated with 20,000 larvae in experiment 2 died during the night of the 13th DAI, and the tissues underwent postmortem change.

At necropsy, the small intestines of guinea pigs given 5,000 larvae were mildly inflamed and sometimes slightly distended. Guinea pigs inoculated with 10,000 or more larvae had congested and greatly distended intestines at 10 DAI and later.

The amounts of water in the intestinal tissues and contents and their dry weights in the guinea pigs of experiments 1 and 3 are shown in Tables 1 and 3, respectively. There was no evidence of consistent change in either of these parameters, and mean differences between infected and noninfected controls are not significant ( $P > 0.05$ ).

The circumference of the gut ranged from

6.7–11.2 mm; the variation within groups was greater than between them. Differences in mean circumferences between infected and control groups are not significant (Tables 2 and 3).

The muscularis externa of the duodenum in principals was significantly ( $P < 0.01$ ) wider than in controls of experiments 2 and 3 (Figs. 1A and B, Tables 2 and 3). Its width in the controls ranged from 23–84  $\mu$  and in the principals to as much as 145. The increase in width occurred in both the circular and longitudinal layers, both of which were as much as 2.5 times wider. The nuclei of the circular layer were thicker by about 30% in principals, and they seemed to be less densely stained than nuclei in tissues of controls. The increased width of the muscular layers was directly related to the number of larvae administered

and to the interval between inoculation and necropsy. The width was greatest in the guinea pigs given the most larvae and necropsied at 13 DAI, at which time the worms are young adults.

Some of the results of these experiments agree with those reported by Symons (1957) for his studies with *N. brasiliensis* in the rat; namely, there was an increase in width of the muscularis externa. However, no differences were found in the water content of the intestinal tissues and contents between principals and controls; whereas, Symons reported as much as a 2.5-fold increase in infected rats. Perhaps these differences in results are due to differences in host responses, i.e. either guinea pigs vs. rats or to the differences inherent in the parasites or both. *Trichostrongylus colubrifomis* utilizes a simple direct oral route of infection in contrast to *N. brasiliensis* which effects a skin penetration and circulatory system migration to arrive at its final destination. Conceivably, the alterations in water content in rats infected with the latter parasite reflect physiological disturbances created during the migratory phase of the life history. The question naturally arises, and is as yet unanswered, as to whether *T. colubrifomis* infections in the definitive ruminant hosts would similarly affect the muscular layers of the intestinal tract.

### Summary

Inoculation of guinea pigs with 5,000–40,000 infective larvae of *Trichostrongylus colubrifomis* did not result in significant alterations in the water content of the intestinal tissues and contents or in the circumference of the gut wall. The infections did cause an increase in the width of the muscularis externa of the duodenum. The increase in width was greatest (as much as 2.5 times) in the guinea pigs given the most infective larvae and killed 13 days after inoculation while the worms were still young adults.

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## New Locality Records for *Taenia rileyi* Loewen, 1929 and *Taenia macrocystis* Diesing, 1850, and a Comparison of Some Hook Measurements

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Although *Taenia rileyi* is a relatively large and apparently common cestode of North American felids, there has been much confusion in its identification. Riser (1956) pointed out that Skinner's *Taenia lyncis* (1935) was a composite species, based on *T. rileyi* and *T. omissa* Lühe, 1910. According to Riser, to further

complicate the matter, the description of *T. rileyi* was also based on two or possibly three species and only the strobila was new.

Recently, the intestines from three bobcats *Lynx rufus* (Schreber, 1777) from Brazos County, Texas, were examined. The cestodes recovered were fixed, stained in alum cochineal

**Table 1. Comparison of hook measurements for *Taenia pisiformis*, *Taenia rileyi*, and *Taenia macrocystis*. (All measurements are in mm.)**

Helminth	Total length	Handle (b)	Blade (c)
<i>T. pisiformis</i>			
Large (After Riser, 1956)	0.25-0.27	0.16-0.17	0.09-0.10
Small	0.14-0.15	0.09-0.10	0.08-0.09
Large (Present study)	0.24-0.26	0.16	0.09-0.13
Small	0.14-0.15	0.085-0.10	0.08-0.10
<i>T. rileyi</i>			
Large (After Riser, 1956)	0.22-0.24	0.17-0.18	0.10-0.11
Small	0.16-0.17	0.11	0.07
Large (Present study)	0.22-0.23	0.16	0.11-0.11
Small	0.16-0.18	0.09-0.12	0.07-0.09
<i>T. macrocystis</i>			
Large (After Riser, 1956)	0.32-0.34	0.17-0.18	0.16
Small	0.19	0.09-0.10	0.12-0.13
Large (Present study)	0.33-0.34	0.17-0.18	0.16
Small	0.19	0.09-0.11	0.12-0.13

and mounted in Gum Damar. Worms were identified as *T. rileyi*, *T. macrocystis* and *T. pisiformis* Bloch, 1780. However, in view of the confused taxonomic state of the taeniids, a few words concerning their identification may be appropriate. Recent work (Esch and Self, 1965) on identification based on hook measurements of members of the genus *Taenia*, including *T. pisiformis*, would be difficult to duplicate as precise techniques were omitted; therefore, the method employed here is that of Stevenson and Engberg (1904). In Table 1 we compare the hook measurements of our three species with those of Riser who also used the methods of Stevenson and Engberg.

*Taenia macrocystis* is a small, delicate worm, and our three specimens averaged 160 mm each in total length. One of the specimens possessed 13 gravid proglottids which made up about half of the total length of the strobila. The distal gravid segments of this worm are about 3 times as long as broad, and possess a very prominent genital pore in all of the gravid proglottids.

All three hosts contained *T. rileyi* and *T. pisiformis*, but only 1 host harbored *T. macrocystis*. One host contained 22 specimens of *T. rileyi* which were in all stages of development and ranged from 7 to 65 cm in length. This

would indicate that there is a lack of resistance to establishment of secondary infections with *T. rileyi*. The immature, mature and generally the gravid proglottids of *T. rileyi* are 2 to 3 times as broad as they are long.

Recently, Miller and Harkema (1968) found *T. rileyi* and *T. macrocystis* but not *T. pisiformis* in *L. rufus* from North Carolina and South Carolina. Our specimens of *T. rileyi* and *T. macrocystis* were compared with material kindly provided by these workers, and it seems certain that the two cestodes are identical with those of Miller and Harkema.

As far as we can determine, only one other helminth, *Spirometra mansonoides* (Mueller, 1935; Read, 1948) has been reported from Texas bobcats.

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## Population Fluctuations and Observations of the Life Cycle of *Xiphinema americanum* Associated with Cottonwood (*Populus deltoides*) in South Dakota<sup>1</sup>

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The American dagger nematode, *Xiphinema americanum* Cobb, is one of the most commonly encountered nematodes in South Dakota soils (Thorne and Malek, 1968). Occurring in greatest abundance around tree roots, it is thought to be a factor in stunting and premature decline of shelterbelt trees (Malek, 1968). Because of difficulties in maintaining *X. americanum* populations in laboratory or greenhouse, demonstrations of its pathogenic capabilities have been infrequent and often inconclusive. However, pathogenic relationships with certain tree species have been reported by White (1955), Ruehle and Sasser (1962), Griffin and Epstein (1964), and Krebill et al. (1967).

Although the influence of environmental conditions on *X. americanum* has been studied under controlled conditions (van Gundy et al., 1962; Lownsbey and Maggenti, 1963; Griffin and Barker, 1966), the biology of this nematode remains poorly understood. Ecological studies of *X. americanum* on alfalfa in Iowa (Norton, 1963) and on spruce in Wisconsin (Griffin and Darling, 1964) have revealed possible host- or climate-influenced differences in population fluctuations.

As part of a broad study of the relationship of *X. americanum* to unthriftiness of South Dakota shelterbelt trees, the present investigation was undertaken to determine its population fluctuations around the roots of a commonly planted tree species under the climatic conditions of the upper Great Plains. In addition, further knowledge of the field biology of this nematode was needed to develop practical techniques for its study in the laboratory and greenhouse.

### Materials and Methods

A weed-free planting of 8-year-old cottonwood (*Populus deltoides* Marsh.) known to sustain dagger nematodes and located on the Plant Pathology Research Plots, Brookings, was chosen as the study site. The sample area consisted of 30-foot trees, 14 feet apart and in rows of eight on a level Vienna loam soil. In November, 1964, one tree from the inner six in each of six alternate rows was randomly selected as a sample tree. Preliminary sampling at that time revealed nematode populations consisting of 90–98% *X. americanum* with only trace numbers of *Psilenchus hilarulus*, *Boleodorus thylactus*, *Eudorylaimus* spp., *Nygolaimus brachyurus* and *Tylencholaimellus* sp.

Sampling was resumed in mid-April, 1965, when the sample horizon had thawed and, except for the period of December through March when the horizon was frozen, was continued through mid-November, 1966. At bi-weekly intervals through August and at 4-week intervals thereafter until mid-November, approximately one liter of soil was removed from the rhizosphere in the 5–25 cm profile just inside the dripline of each sample tree. Beginning at a randomly chosen point under each tree, consecutive samples were taken from undisturbed soil until the tree had been encircled at the end of the season. This procedure was repeated in 1966, except that samples were taken from an adjacent tree in the row. Soil temperatures during the sampling period were measured at the 15 cm depth by a recording thermograph, and soil moisture percentages in the sample horizon were determined at each sampling date.

Samples were collected in the late morning and processed within 4 hours. After each sample was thoroughly mixed and the large roots were discarded, a 400 cc portion was removed for processing. Free-living stages of *X. americanum* were extracted by a modification of the

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method of Christie and Perry (1951). Residues were collected on a 270-mesh sieve and allowed to remain on a Baermann funnel overnight. During 1966, eggs were extracted from 100 cc of a sample soil composite by the centrifugal flotation method of Caveness and Jensen (1955), collected on a 325-mesh sieve, and backwashed into a petri dish.

Extracted nematodes were observed and counted in a Syracuse watch glass with a subdivided bottom. Because of seasonal temperature variations in the extraction room and their effect on extraction efficiency, numbers of live nematodes remaining in the residues were determined by the aliquot method. The two figures were then combined. Total numbers, as well as numbers of adults and gravid individuals, were recorded. The presence of dagger nematode eggs at sample dates was noted but no counts were made.

### Results and Discussion

Seasonal population fluctuations of *X. americanum* around roots of cottonwood at Brookings are presented in Figure 1. Nematode numbers were lowest in February and highest in June and July. Following a decline during the remainder of the summer, another population peak occurred in early autumn. These results agreed with those of Griffin and Darling (1964) but were dissimilar to those of Norton (1963), who reported population peaks in early spring and late summer on alfalfa in Iowa.

Population fluctuations from July through November in 1965 and 1966 were similar but the spring trends were markedly different. Soil beneath sample trees in November, 1964, showed a mean value of 2,850 nematodes per 400 cc of soil, while numbers in April, 1965, were 53% lower. Average monthly air temperatures for the period of December through March of 1964-65 were 6, 5, 5, and 7 C, respectively, below the normal of -7, -10, -8, and -1 C. Thus, the severity of the winter evidently resulted in a high mortality of dagger nematodes. During the following winter (1965-66), subnormal average temperatures occurred in January and February alone, and the April population level in 1966 was only 6% lower than that (3,400) in November, 1965. Another disparity in spring trends was the absence in 1965 of the substantial population increase in May and early June, 1966.

Despite the differences in population levels of *X. americanum* during the spring of 1965 and that of 1966, primary population peaks were the same for both years (ca. 4,600).

Adult population fluctuations, though less obvious, were similar to those of the total population. Molting of preadults was observed from April to late July and again in September or October. Dead transparent females were common in residues in June and again in August and September. Griffin and Darling (1965) observed that adults often outnumbered larvae. In the present study, larvae always were predominant, even though extraction loss of any stage during soil settling was less than 15%. This difference may have been due to a longer life cycle and a high mortality of larvae under South Dakota conditions.

Gravid females first appeared in early May in 1965. In the following spring, soil temperatures rose more slowly and reproduction began 3 weeks later. Oocyte development was noted 7-10 days before the appearance of gravid females and at a soil temperature of 10-15 C. Norton (1963) found reproduction to be most intense during the late stages of the cycle, which continued into late August in Iowa. In the present study gravid females were most abundant in May and early June. These early individuals, which were thought to be overwintered adults, were transparent except for eggs or had only sparsely granulated intestines. Reproduction increased slightly in late June and terminated in late July. These late egg-bearing females had densely granulated intestines, which obscured eggs, and may have passed the winter as third or fourth stage larvae. Adult numbers increased again in autumn but no evidence of gonad activity was observed. Dagger nematode populations were periodically observed in 1965 in an adjacent field of alfalfa (*Medicago sativa* L.) and in 1967 in a planting of American elm (*Ulmus americana* L.) in sandy loam soil and in the cottonwood plot. In all cases, the reproductive cycle was similar to that shown in Figure 1. This evidence that reproduction in the field is limited to late spring and early summer in South Dakota is in contrast to the findings of Griffin and Darling (1964), who observed a second period of reproduction in late autumn in Wisconsin.

Eggs of *X. americanum* were found at all

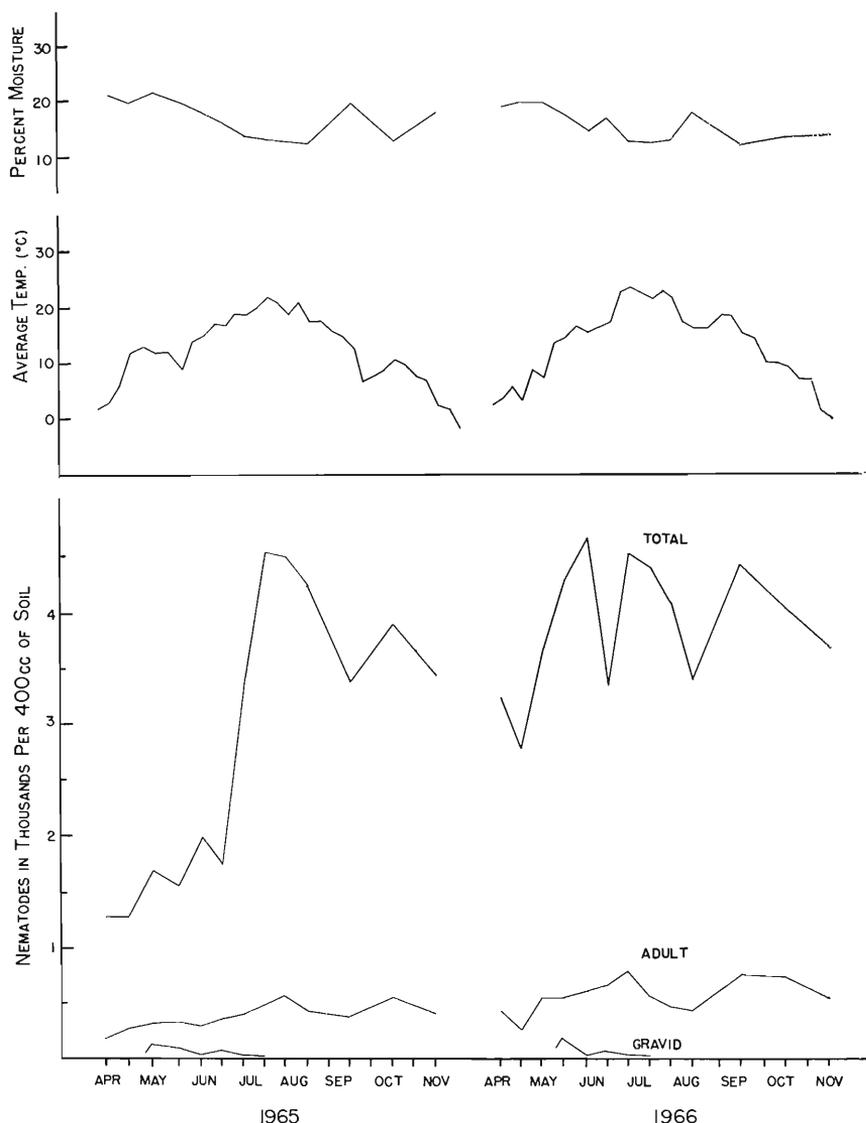


Figure 1. Population fluctuations of *Xiphinema americanum*, average soil temperature at 15 cm, and soil moisture around cottonwood roots. Plant Pathology Research Plots, Brookings.

sampling dates during 1966. However, hatching apparently occurred in spring and early summer and again in early autumn, since free-living first stage larvae were present only when total populations were increasing. These larvae were comparable in size and appearance to those artificially hatched by rupturing fully devel-

oped eggs with an eye knife. Eggs and first stage larvae were seen prior to the reproductive period in 1966, indicating that eggs may remain in the soil up to a year before hatching.

In late June, 1966, there was a temporary decline in total numbers of nematodes and a disappearance of first stage larvae in extracts,

which was associated with an increase in dead individuals in residues. This mortality may have resulted from failure of newly hatched larvae to find feeding sites. Many small root branches die during the summer months, and eggs deposited in a region of healthy rootlets were therefore likely to hatch some distance from a source of food. Furthermore, adults were more active in extracts than larval forms; first stage larvae seldom exhibited motion, suggesting that newly hatched larvae may be incapable of migrating any distance to live roots. First stage larvae reappeared in mid-July, 1966, and the resultant secondary population peak closely corresponded with the primary peak in 1965. Since both occurred near the end of the reproductive cycle and nearly two months after its inception, July peaks were attributable to hatch of eggs deposited in the respective seasons. It would seem that 6 to 8 weeks of incubation were necessary before hatching could occur, and eggs deposited late in the reproductive cycle may not hatch until the following autumn or spring when moisture and/or temperatures were favorable. First stage larvae were scarce in the spring of 1965; the absence of a substantial late spring population increase similar to that of 1966 suggests that a near total egg-mortality occurred during the preceding winter.

Samples taken from the frozen cottonwood rhizosphere in mid-March, 1967, revealed approximately the same ratio of adults to immature forms (ca. 1:8) as did samples taken 4 weeks later and in November, 1966. Furthermore, the ratios between second, third and fourth stage juveniles did not appear to change noticeably and eggs were present in the soil on all three occasions. In general, intestines of larvae were densely granulated, while those of adults were sparsely granulated to transparent. However, tessellated or transparent females were seen in May with eggs in the uteri. Markedly subnormal temperatures occurred only in February; averages for the remaining months were near or slightly above normal. Apparently, no one stage of *X. americanum* was most capable of overwintering under normal South Dakota conditions.

Growth and development of larval stages were confined to relatively short periods of the year. Molting of second, third, and fourth stages began approximately 2 weeks before

the appearance of gravid females, when soil temperatures rose to 5–10 C. Molting ceased in early July, but resumed briefly in early autumn. First stage larvae, which were absent after population peaks were attained, probably molted soon after hatching if feeding sites were accessible. Dead second, third and fourth stage larvae were present in residues throughout the sampling season, indicating continuous mortality, but their numbers were generally lower during these periods of activity. The cyclic nature of nematode activity may have been directly attributable to temperature and moisture effects, but tree root growth patterns, which closely correspond with periods of nematode activity, may have been a factor as well.

It could not be determined whether the egg to egg cycle could be completed within a single season. However, considering the restricted reproductive period and the length of time eggs may lie dormant, it is thought that *X. americanum* may require as least one year to complete its life cycle in the upper Great Plains.

Differences between the results of this study and those of Norton (1963) and Griffin and Darling (1964) are apparently related to host and climatic influences on the biology of *X. americanum* and emphasize the need for more extensive research on the comparative ecology of nematodes in their native habitats.

### Summary

Population fluctuations of *Xiphinema americanum* around cottonwood roots in South Dakota are described and the life cycle of the nematode under field conditions is discussed. Nematode numbers were lowest in April and highest in June and July. A population decline in August and September was followed by an early autumn peak. In consecutive years, there was a 61% difference in initial spring population levels, but maximum numbers at the primary peaks were the same. Adult population peaks occurred at the same time as those of the total population, but reproduction was limited to May through July. Egg-hatch, growth, and development apparently were confined to spring and early summer and a brief period in mid-autumn. Except for first stage larvae, which were not present during the winter months, all stages were capable of overwintering in South Dakota, but eggs ap-

peared to be most affected by subnormal winter temperatures. The restricted reproductive period and long dormancy of many eggs suggest that the life cycle of *X. americanum* may require at least one year for completion in the upper Great Plains. Differences between the results of this and similar studies were ascribed to host and climatic influences on the biology of the nematode.

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## Parasites of the Pygmy Whitefish, *Prosopium coulteri* (Eigenmann and Eigenmann) and Mountain Whitefish *Prosopium williamsoni* (Girard) from Western Montana

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The parasite fauna of the pygmy whitefish *Prosopium coulteri* (Eigenmann and Eigenmann), to our knowledge, has never been reported. In addition, the mountain whitefish *Prosopium williamsoni* (Girard) whose parasite fauna has been studied in other areas (Skinker, 1931; Wardle, 1932; Smedley, 1933; Bangham, 1951; Bangham and Adams, 1954;

and Fritts, 1959) has not been studied extensively in western Montana. The pygmy whitefish *P. coulteri*, which appears to have a disjunct distribution, has been recorded from Lake Superior (Eschmeyer and Bailey, 1954), Columbia River drainage in Washington, Montana and British Columbia (Weisel and Dillon, 1954) and from the Fraser, Skeena, Yukon, and Mac-

kenzie River systems of the Pacific and arctic slopes (Carl, *et al.*, 1959). It also occurs in both Pacific and Bering Sea drainages of southwest Alaska, having been reported from the Nushagak, Chignik, Naknek, and Kvichak River systems (Heard and Hartman, 1966). Montana Fish and Game records (personal communication) record it from the following Montana lakes: Bull, Bitterroot, Flathead, McDonald, and Whitefish. The mountain whitefish *P. williamsoni* ranges from the Lahontan Basin of Nevada to the Liard, Peace, Athabaska and Saskatchewan Rivers of Canada (Carl *et al.*, 1959), and is widely distributed in western Montana. These two species of fish are also sympatric in western Montana.

The purpose of this paper is to give the results of our study on the parasite fauna of these two fish species collected in western Montana and to compare the parasite fauna of these two sympatric species of coregonoid fishes.

### Materials and Methods

This study began in December 1967 and was terminated in June 1968. Fish were captured in December on the spawning grounds by the use of seines. Others were taken by gill nets and some mountain whitefish were caught with hook and line. Fish samples were obtained from Ross Creek, a tributary of Bull Lake, and Flathead, and Bitterroot Lakes.

All of the fish, except the specimens obtained from the Montana Fish and game Department, were examined fresh, the latter were preserved in 10% formalin. The eyes, gills, viscera, flesh, fins, and integument of fresh fish were placed in 0.6% saline and examined with the aid of a dissecting microscope. Standard methods were used in preservation, staining, and mounting of parasites. All parasite specimens were deposited in the University of Montana parasitology collection.

### Results and Discussion

Forty-eight pygmy whitefish, *P. coulteri* and 59 mountain whitefish, *P. williamsoni* were examined. Results for the pygmy whitefish are given first and in more detail. Numbers in parentheses indicate number of fish infected by a parasite species. Larval forms are designated by an asterisk.

### Parasites of the Pygmy Whitefish, *Prosopium coulteri* from Western Montana

**PROTOZOA**—*Henneguya zschokkei* (Gurley, 1894)—small white, cream or yellowish cysts about pea size. Encysted in muscle, under skin, along the vertebrae and spines of the backbone (27). First reported from the United States in the whitefish *P. williamsoni* by Mitchell (1968).

**TREMATODA**—*Crepidostomum farionis* O. F. Muller, 1884; (Nicoll, 1909). Lying free in bile of the gall bladder (1).

**CESTODA**—*Proteocephalus exiguus* (LaRue, 1911). In the intestine (2). \**Bothriocephalus* sp. (Rudolphi, 1808). Pleurocercoids encysted in gut wall and mesentery (28).

**NEMATODA**—*Cystidicola stigmatura* (Leidy, 1886); (Skinker, 1931). In swim bladder (2). \**Eustrogylides* sp. (Jägerskiöld, 1909). In the body cavity (1).

**ACANTHOCEPHALA**—*Neoechinorhynchus rutili* (Müeller, 1780). Attached to intestinal wall (1). *Pomphorhynchus bulbocolli* (Linkins, 1919); (Van Cleave, 1919). Attached to intestinal wall (1).

**ANNELIDA**—*Piscicola geometra* (Linnaeus, 1758). Attached to exterior body surface (8). Nine species of parasites were recovered from the pygmy whitefish *P. coulteri* and the percent infection was 77. Since parasites have not been reported from the pygmy whitefish prior to this report these are all new host records.

Twelve species of parasites were recovered from the mountain whitefish *P. williamsoni*. The pleurocercoid of *Bothriocephalus* sp. is the only new host record for this fish species. A tabulation of parasites recovered from the mountain whitefish is given below.

### Parasites of the Mountain Whitefish *P. williamsoni*, from Western Montana

1. *Henneguya zschokkei* (Gurley, 1894)—in muscle (40).
2. *Crepidostomum farionis* (O. F. Muller, 1884); (Nicoll, 1909). In gall bladder (14).
3. *Tetraonchus variabilis* (Mizelle and Webb, 1953). On gills (11).
- \*4. *Bothriocephalus* sp. (Rudolphi, 1808). Encysted on gut wall (3).
5. *Proteocephalus laruei* (Faust, 1919). In intestine (2).

6. *Bulbodacnitis occidentalis* (Smedley, 1933). In intestine (20).
7. *Cystidicola stigmatura* (Leidy, 1886); (Skinker, 1931). In swim-bladder (2).
- \*8. *Eustrongylides* sp. (Jägerskiöld, 1909). In body cavity (1).
9. *Hepaticola bakeri* (Müeller and Van Cleave, 1932). In intestine (2).
10. *Philonema agubernaculum* (Simon and Simon, 1936). In body cavity (4).
11. *Pomphorhynchus bulbocolli* (Linkins, 1919). In intestine (2).
12. *Salmincola* sp. (Wilson, 1915). On gills (8).

In western Montana, five of the nine parasites from the pygmy whitefish were also found in common with the sympatric mountain whitefish. In comparing the 29 parasite species listed for the mountain whitefish by Hoffman (1967) and including those recorded in this study, only one from the pygmy whitefish, the leech *Pisicola geometra* was not found in common with the mountain whitefish. This parasite information certainly indicates that these fish, where they occur together, share a great portion of the habitat and in fact may overlap niches a great deal.

### Summary

Pygmy whitefish *Prosopium coulteri* and mountain whitefish *Prosopium williamsoni* from portions of the Clark Fork and Kootenai drainages of western Montana were surveyed for parasites. No extensive parasite work had been done on the mountain whitefish in Montana and the pygmy whitefish, to our knowledge, has never been examined. Nine parasites were recovered from the pygmy whitefish, all new host records. Of the twelve parasite species recovered from the mountain whitefish, only one, *Bothriocephalus* sp. is a new host record. In comparing twenty-nine parasite species listed for the mountain whitefish by Hoffman (1967) and including those recorded in this study, only one from the pygmy whitefish, a leech, was not found in common with the mountain whitefish. The parasite information strongly indicates that these two species of fish, where they occur together, share a great portion of the habitat and in fact may overlap niches a great deal.

### Acknowledgments

We would like to thank Mr. Delano Hanzel, Montana Fish and Game Department for loaning specimens of pygmy whitefish and Dr. J. D. Mizelle, Sacramento State College, for confirming the identification of the monogenetic trematode, *Tetraonchus variabilis* (Mizelle and Webb, 1953).

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*Neoechinorhynchus magnapapillatus* sp. n. (Acanthocephala)  
from *Pseudemys scripta scripta* (Chelonia)<sup>1</sup>

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The following six species of *Neoechinorhynchus* have been described from the intestine of various North American turtles: *Neoechinorhynchus chrysemydis* Cable and Hopp; *N. pseudemydis* Cable and Hopp; *N. stunkardi* Cable and Fisher; *N. emyditoides* Fisher; *N. emydis* (Leidy); and *N. constrictus* Little and Hopkins. All except *N. stunkardi* have been reported from *Pseudemys scripta* subsp. I have recently examined many yellow-bellied turtles, *P. s. scripta* (Schoepff) from Alabama and North Carolina and found many female specimens of *Neoechinorhynchus* which appear to be new to science. The specific name refers to the large caudal papilla on the female.

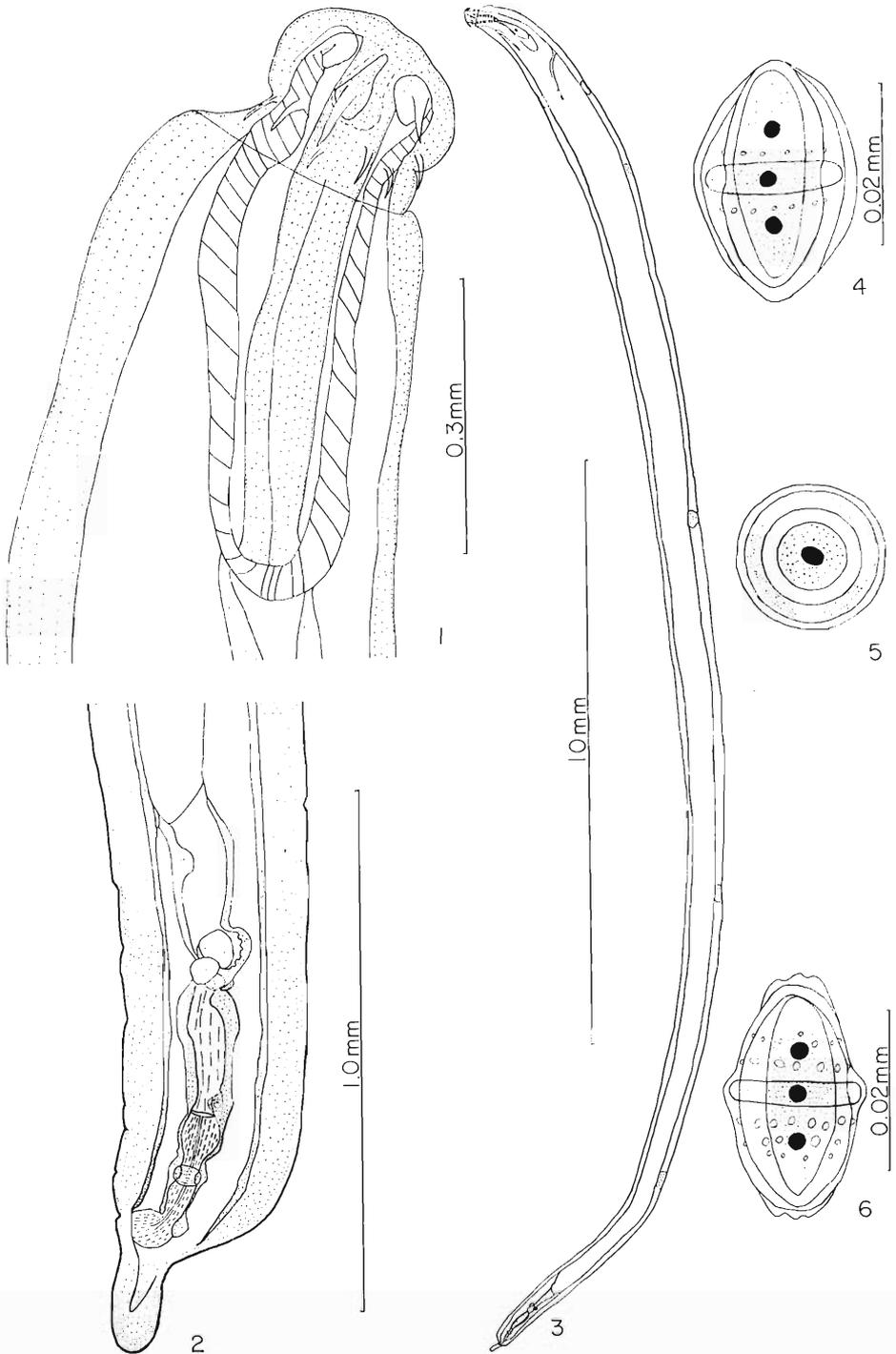
Most of the specimens of *Pseudemys scripta scripta* were collected alive from farm ponds in Alabama and North Carolina. The turtles were killed and examined for parasites. All acanthocephala were removed and placed in a 0.7% NaHCO<sub>3</sub> solution (Van Cleave and Mueller, 1934), agitated to remove debris, then placed in cold tap water until the proboscis was fully extruded. The worms were then fixed in AFA, washed in 70% ethanol and stored in a 9:1 70% ethanol-glycerine mixture. On the subsequent staining procedure, worms were hydrated in 10 minute changes of 50, 35% ethanol and deionized water, stained in a stock solution of Van Cleave's Combination Hematoxylin (1 ml stock Erlich's, 1 ml stock Delafield's, 100 ml distilled water and 6 gm potassium alum) for 15-30 minutes, washed in deionized water and dehydrated to 95% ethanol. During the washing the cuticle was pricked in several places. Worms were further dehydrated, cleared in methyl benzoate and mounted in neutral piccolyte. Worms were rotated on a slide in order to measure dorsal and ventral hooks. The system for measuring hooks as published by Fisher (1960) was followed.

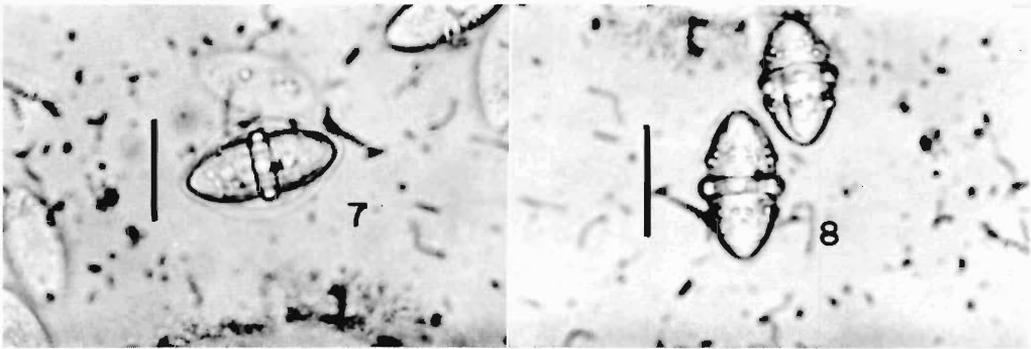
<sup>1</sup>This study is taken from a Masters Thesis submitted to the Department of Zoology-Entomology, Auburn University, Auburn, Alabama.

*Neoechinorhynchus magnapapillatus*  
sp. n.  
(Figs. 1-8)

(Based on a study of 20 female specimens. All measurements in millimeters. Mean in parentheses.)

DESCRIPTION: With characteristics of the genus. Trunk curved ventrally 15.4-36.6 (27.9) long by 0.546-1.456 (0.894) wide at level of ventral nucleus, then tapering to posterior end, terminating in a large caudal papilla 0.106-0.205 (0.150) by 0.060-0.098 (0.084). The largest females collected from Chowan Co., N. C. turtles were about twice as long as the largest form from both Lee Co., Ala. and Wake Co., N. C. hosts. External praesoma 0.165-0.221 (0.196). Proboscis hooks in three circlets of six hooks each, arranged quincunxially. Lateral hooks of anterior circlet 0.071-0.097 (0.089), posterior to others of that circlet 0.063-0.080 (0.075). Hooks of middle circlet similar 0.029-0.048 (0.040). Lateral hooks of basal circlet 0.021-0.034 (0.027), other hooks of that circlet 0.036-0.044 (0.040). Left lemniscus binucleate, slightly longer than right one. Mouth of uterine bell 0.510-1.515 (1.119) from posterior end, uterus exclusive of bell and selector apparatus 0.113-0.415 (0.251) by 0.095-0.198 (0.130), vagina 0.160-0.342 (0.255) by 0.059-0.079 (0.070) wide at vaginal sphincter. Genital pore ventral, at base of caudal papilla. Ovarian balls 0.042-0.072 (0.055) long, oblong in small specimens, round in larger specimens. Egg: Living material from Chowan County, N. C. Fully formed eggs removed from pseudocoel of worms placed in tap water 0.028-0.034 (0.031) by 0.016-0.022 (0.019). Acanthor 0.025-0.029 (0.028) by 0.009-0.011 (0.009); surrounded by three membranes and a "band" 0.002-0.004 (0.003) wide encircling and appearing to develop from inner membrane at the equator. An irregular row of tubular





Figures 7, 8. Photomicrographs, Chowan Co., N. C. 7. Same as Figure 4. 8. Same as Figure 6. Surface view. Scale 0.020 mm.

structures project from the inner membrane above and below the band. Acanthor with three "vacuoles" in midline. Eggs from feces 0.029–0.036 (0.033) by 0.014–0.021 (0.017), acanthor 0.024–0.031 (0.028) by 0.008–0.010 (0.009), band 0.003–0.005 (0.004). Tubular structures of inner membrane more numerous. Outer membrane wrinkled at poles of egg.

TYPE HOST: *Pseudemys scripta scripta* (Schoepff).

LOCALITIES: Lee County, Alabama; Wake County, North Carolina; and Edenton, Chowan County, North Carolina.

TYPE SPECIMENS: USNM Helm. Coll. 70484 (holotypes), 70485 (paratypes).

The large caudal papilla of the female; three "vacuoles" in the acanthor; circumoval "band," shape, number and arrangement of the tubular structures on the egg are the most distinctive characteristics of this new species and will separate it from all known *Neoechinorhynchus* from North American turtles. The posterior end of the female of the described species is similar in shape to females of 2 other described species of *Neoechinorhynchus* from turtles, but can be separated on the following characters. *N. chrysemydis* has a smaller caudal papilla and its tail is more rounded than *N. magnapa-*

*pillatus* sp. n., *N. stunkardi* has a small cone shaped papilla and a sigmoid posterior end. The egg of *N. magnapapillatus* sp. n. resembles that of *N. emydis* in respect to the "band," *N. emydis* however, has a C-shaped band.

Other species of acanthocephala which were found with *N. magnapapillatus* sp. n. are *N. emyditoides*, *N. chrysemydis*, and *N. pseudemydis*. The occurrence of *N. magnapapillatus* sp. n. with one, two, or three of these species has prevented the description of the male. The life cycles of these four species should be studied to possibly separate the males with more certainty.

I wish to thank Dr. C. F. Dixon under whose direction this study was conducted, Dr. R. Harkema for his review of the manuscript, Mr. N. A. Powell of the Edenton National Fish Hatchery for aid in collection of some of the turtles, Dr. F. Sogandares-Bernal for the formula of Van Cleave's Combination Hematoxylin and Dr. W. M. Brooks for use of his photographic equipment.

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Figures 1–6. *Neoechinorhynchus magnapapillatus* sp. n. Figures 1, 2, 4, 6 made with aid of camera lucida. Figure 3 made with aid of microprojector. 1. Lateral view of anterior end, Wake Co., N. C. 2. Lateral view of posterior end, Lee Co., Alabama. 3. Ventrolateral view of entire worm, Chowan Co., N. C. 4. Living egg from pseudocoel of female in tap water, Wake Co., N. C. 5. Hand drawing of optical equatorial cross section of egg in body of female, Lee Co., Ala. 6. Living egg recovered from feces of host, Chowan Co., N. C.

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## *Phlyctainophora squali* sp. nov. (Nematoda, Philometridae) from the Spiny Dogfish, *Squalis acanthias*<sup>1</sup>

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In 1921, G. Steiner described *Phlyctainophora lamnae* from a single female worm found in the subcutaneous tissue above the hyomandibular arch in the mackerel shark *Lamna nasus* (= *cornubica*) (Bonnaterre). Larva thought to be *P. lamnae* have been described by Johnston and Mawson (1943) and de Ruyck and Chabaud (1960), but no additional adult specimens have been reported since 1921.

During parasitological studies off Los Angeles, California, a spiny dogfish (*Squalis acanthias* L.) was caught at a depth of 200 meters. Grouped around its dorsal fins, caudal peduncle, and mandibular arches were raised areas about 5 mm high, which when excised were found to contain worms similar to that described by Steiner; a total of 23, all female, were taken. Subsequent external examination of 440 *S. acanthias* failed to reveal any additional specimens.

Adults of these unusual nematodes are easily distinguished by huge vesicular enlargements

on the ventral surface of the body. In older worms the vesicles totally obscure the worm's basic shape and are unlike any structure found in other nematodes.

Steiner did not give a location of capture for his host, *Lamna nasus*, but this species is normally confined to the North Atlantic Ocean (Bigelow and Schroeder, 1948). As a result of having only a single specimen, Steiner was able to give a limited description of these interesting forms. This report not only records a new species but also offers additional information on the genus *Phlyctainophora* with suggestions for its taxonomic position.

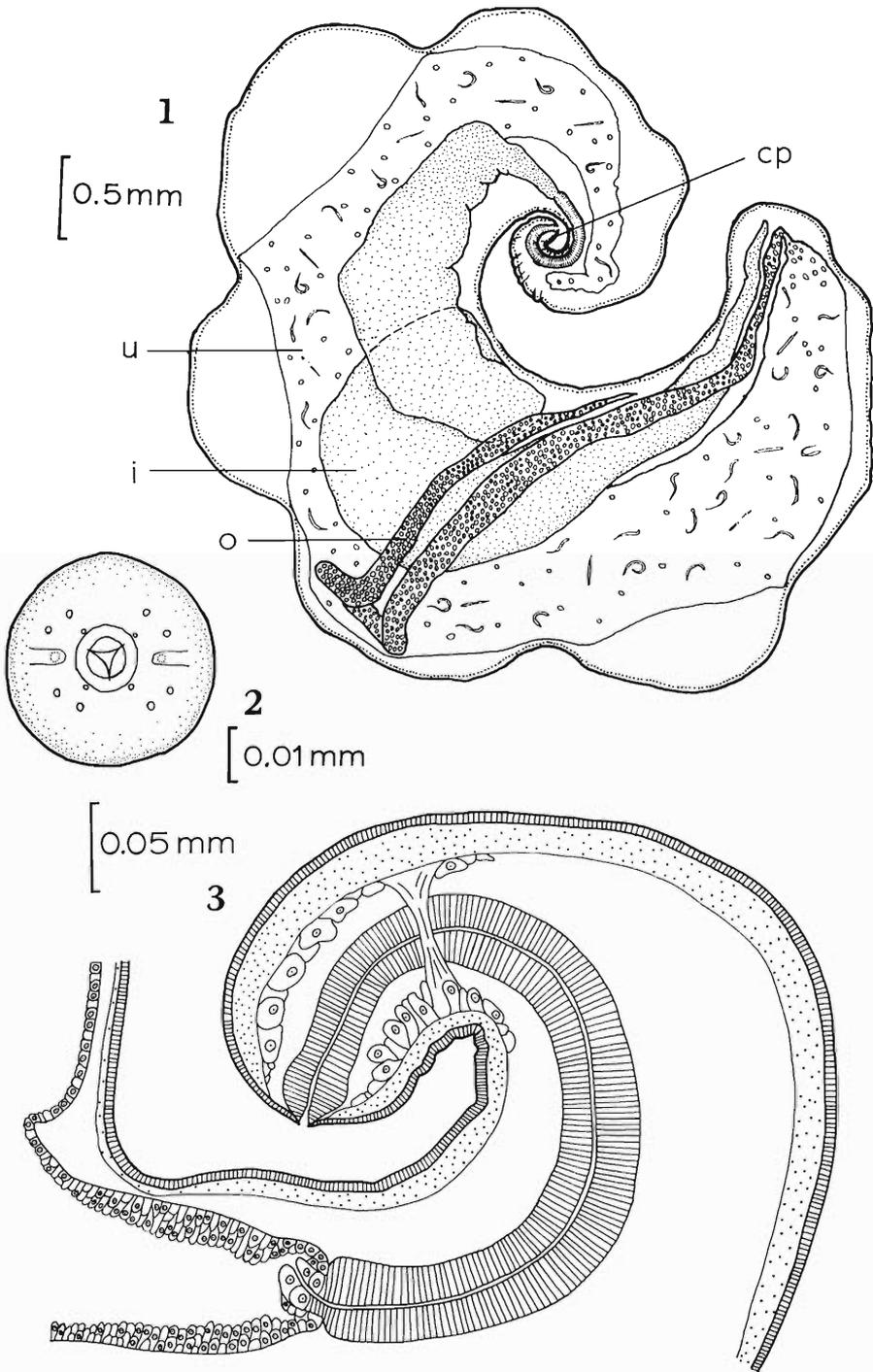
### Materials and Methods

All worms were fixed in AFA and later placed in 70% ethyl alcohol. Several were embedded, sectioned, and later stained with Mayer's hematoxylin and eosin Y. The remainder were cleared in glycerin and mounted in glycerin jelly. All drawings were made with the aid of a drawing tube. Measurements are given in microns unless otherwise stated.

<sup>1</sup>This study was supported in part by the Long Beach California State College Foundation under grant NSF IG 212.73.

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Figures 1-3. *Phlyctainophora squali*. 1. Mature female. 2. En face view. 3. Sagittal section of anterior region. Abbreviations: cp, cephalic protrusion; i, intestine; o, ovary; u, uterus.



### Description

#### *Phlyctainophora squali* sp. nov. (Figs. 1-7)

DIAGNOSIS (based on 23 specimens: 2 immature and 21 mature females). Body of mature females tightly coiled dorsally into crescent shapes. Body length 2.3-14.0 mm by 1-3 mm in diameter. Ventral surface with large outpocketings, variable in size and number, without pattern or symmetry. Body rigid, musculature reduced, very stout, narrowing at mouth; posterior end rounded. Cuticle with small parallel annules. Mouth simple, without well defined lips. Eight cephalic papillae in outer circle, four in inner circle. Amphids present. Nerve ring at or near anterior fourth of esophagus. Esophagus 500-600 long by 50 wide, narrowing slightly before joining intestine, surrounded by cells containing large nuclei. Oesophageo-intestinal valve projecting into intestine. Intestine expands immediately behind esophagus filling midbody and tapering toward posterior extremity where it terminates. Anus atrophied. Ovary single, originating posterior to midbody, anteriorly directed, looping posteriorly, emptying into uterus at posterior extremity (Fig. 1). Uterus large, filling all available body space and terminating blindly in esophageal region; capable of undulating motion.

Eggs and larva present in all stages of development. Larva 200-250 long by 14 wide. Anterior with toothlike projection; two large preanal phasmids present; anus 165 from anterior end, tail sharply pointed.

HOST: *Squalis acanthias* L.

LOCATION: Subcutaneous tissue.

LOCALITY: Eastern Pacific Ocean off Los Angeles, California. Depth 200 meters.

SPECIMENS: Holotype and two paratypes in USNM Helm. Coll. No. 63044.

### Remarks

*Phlyctainophora squali* differs from *P. lamnae* in the following characteristics: Adults and

intrauterine larvae of *P. squali* are smaller than those of *P. lamnae* measuring 2-14 mm versus 17 mm and 200-250 versus 330-350 respectively. They also differ in host and geographical distribution.

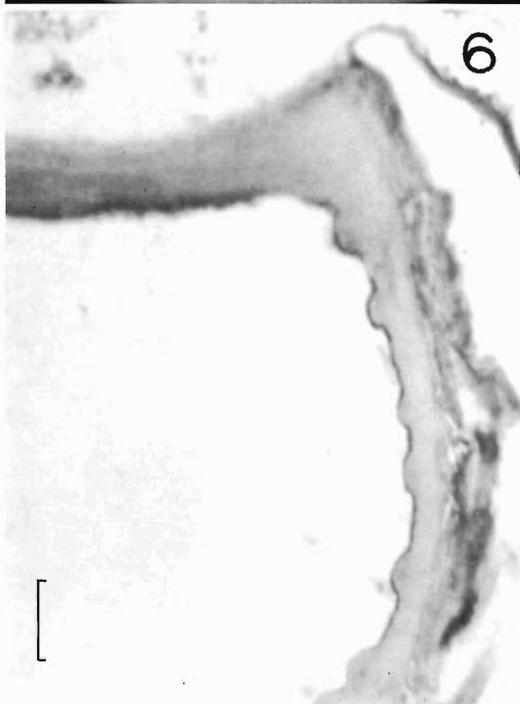
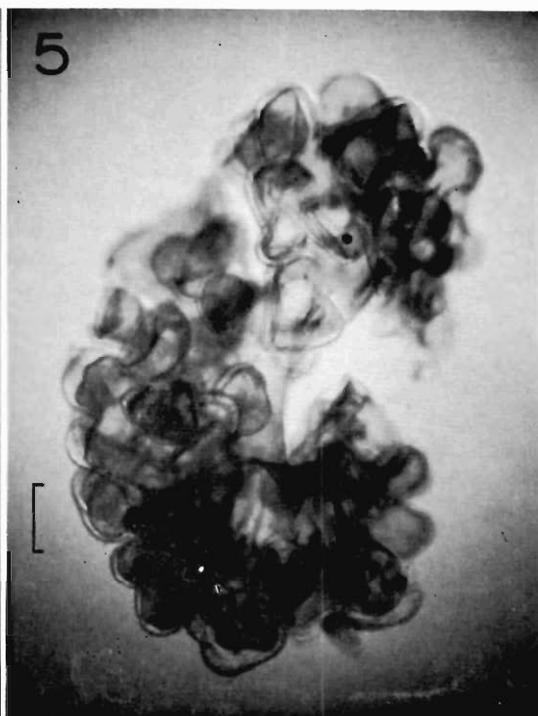
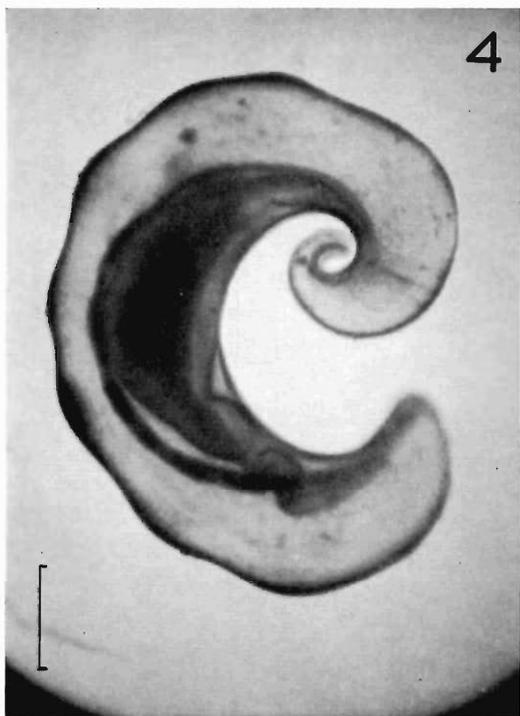
### Discussion

The systematic position of *Phlyctainophora* is somewhat unsettled. Yorke and Maplestone (1926) and Yamaguti (1961) considered it a genus *incertae sedis*, while Johnston and Mawson (1943) suggested its placement in Philometridae. De Ruyck and Chabaud (1960) disagreed with the latter placement and considered the genus more closely related to *Muspicea* Sambon, 1925, and *Robertdollfus* Chabaud and Campano, 1960, on the basis of larval characteristics. Our adult specimens demonstrate that *Phlyctainophora* is more closely related to Philometridae in that it possesses a digestive system (not found in Robertdollfusidae) and lacks the bilobed tail structure found in *Muspicea*. The atrophication of vulva and anus, presence of a simple mouth, viviparity, and location of worms in connective tissue of fishes, combine to indicate that *Phlyctainophora* belongs in the Philometridae. The absence of an anterior ovary is here recognized as a modification of the more primitive didelphic condition. In this respect *Phlyctainophora* appears to be closely related to *Ichthyofilaria* Yamaguti, 1935, which has its anterior ovary reduced. The diagnosis of Philometridae Baylis and Daubney, 1926, is amended accordingly to read "Ovaries relatively short, situated at opposite ends of body or anterior ovary rudimentary or absent."

Rasheed (1963) has discussed characteristics of systematic importance in the family Philometridae and lists them as follows: 1. Size and shape of body. 2. The cuticle and its modifications. 3. Cephalic papillae. 4. The esophagus. 5. The tail. Regarding body shape and size she concluded that the "general shape of the body does not vary enough to give it any taxonomic importance." While this is true for

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Figures 4-7. *Phlyctainophora squali*. 4. Young female showing cuticular vesicles beginning to form (scale: 0.5 mm). 5. Older female with many pronounced cuticular vesicles (scale: 0.5 mm). 6. Cross section of cuticular annules (scale: 0.02 mm). 7. Optical section of dorsal surface of young female showing cuticular annules (scale: 0.02 mm).



the majority of Philometridae, adult *Phlyctainophora* are easily recognizable by their coiled shape and vesicular enlargements (Figs. 1, 4 and 5). The vesicles involve not only the cuticle, but also the hypodermis and muscular layers of the body wall. A wide range in size of adult *Phlyctainophora* indicates they follow the general philometrid growth pattern cited by Rasheed as continuing long after fertilization.

The cuticle and its modifications have been categorized by Rasheed into the following: smooth, bosses, rods and cones. In *P. squali* the corticle layer of the cuticle possesses annules running perpendicular to the longitudinal axis (Fig. 6, 7).

The small cephalic papillae (Fig. 2) of *P. squali* appear most similar to those found on *Philometra* (*Philometra*) *lateolabracis* Yamaguti, 1935.

The esophagus (Fig. 3) is typically philometrid with a slightly enlarged anterior and cylindrical posterior portion of uniform diameter.

Several observations made by Steiner (1921) on *P. lamnae* may now be reviewed in the light of the large number of specimens recovered in this study. Steiner indicated the mouth of *P. lamnae* to be located in a slight depression and the presence of a terminal anus. In *P. squali* the mouth is located on a small cephalic protrusion (Fig. 1) and the anus is completely atrophied. Steiner noted that cuticular vesicles appeared paired with a single vesicle located at the anterior end. *Phlyctainophora squali* shows no pairing or pattern of vesicles. Generally, more vesicles occur in older, larger worms, although one large specimen was almost devoid of them.

De Ruyck and Chabaud (1960) suggest the mode of transmission for *Phlyctainophora* to be cannibalism, the larva being passively transmitted from one shark to another. While this may be possible, the present writers observed openings to the outside in vesicles containing worms. This indicates that larvae may escape and *Phlyctainophora* would more likely require a copepod or similar small crustacean as a first intermediate host, as shown by previous work on the life cycles of Philometridae (Platzer and Adams, 1967).

## Summary

*Phlyctainophora squali* sp. nov. is described from 23 female specimens recovered from the subcutaneous tissue of one of 440 *Squalis acanthias* caught off Los Angeles, California. This finding represents a new host and distribution for this genus. *Phlyctainophora* is placed in the family Philometridae on the basis of atrophication of vulva and anus, simple mouth, viviparity and location in tissue of fishes. The family diagnosis for Philometridae is amended to include *Phlyctainophora* which lacks an anterior ovary.

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**World Federation of Parasitologists**  
**SECOND INTERNATIONAL CONGRESS OF PARASITOLOGY**  
**Mayflower Hotel, Washington, D. C. 20036 U.S.A.**  
**September 6-12, 1970**

*Theme of the Congress and keynote of the plenary session, 7 September 1970 is:* Increasing international cooperation in research and communication to help solve worldwide problems of parasitic diseases affecting man, animals, and plants.

*Program:* The Program Committee is developing eight Technical Review Sessions and 30 to 60 Colloquia (workshops).

*Technical Review Session Subjects:* Genetics and Evolution; Immunity and Host Responses; Pathology of Parasitic Infections; Physiology and Biochemistry; Pharmacology of Antiparasitic Agents; Concept of Planning and Evaluation in Control of Parasitic Infections; Rise of Nematology; and Taxonomy.

*Colloquia:* Volunteer (free) communications will be grouped in appropriate Colloquia. Résumés of 500 words or less of volunteer papers must be submitted to the Office of the Secretary General not later than 1 March 1970 in order to be scheduled and included in the prepublished Congress Proceedings. It is planned that the Colloquia will be informal discussions, and the résumés of volunteer papers will be the basis for opening these discussions. Following are some examples of Colloquia topics:

Biological Control	Host Reactions—Cellular and Humoral	Parasites of Game Animals
Cultivation of Protozoa	Immunization	Parasites of Wild Birds
Cultivation of Helminths	Immunodiagnosis	Parasitic Crustacea
Carbohydrate Metabolism	Immunopathology	Parasites from Waste Water and Sewage
Lipid Metabolism	Immune Response of Arthropods to Parasites	Pathophysiology of Helminth Diseases
Protein and Nucleic Acid Metabolism	Life Cycles of Helminths	Pathophysiology of Protozoan Diseases
Ecology	Life Cycles of Protozoa	Phytnematology and Food Sources
Economics of Parasitic Diseases	Literature Retrieval	Taxonomy of Arthropods
Entomophilic Parasites	Nematode Ecology	Taxonomy of Helminths
Epidemiology and Epizootiology	Nematodes as Vectors of Viruses	Taxonomy of Protozoa
Fine structure of Flatworms	Nutrition and Parasitism	
Fine structure of Nematodes	Parasites of Fishes	
Fine structure of Protozoa		
Helminth Genetics		

(Many others depending on résumés submitted)

The Program Committee will strive to develop and schedule Colloquia which will serve the best interests of those attending the Congress.

A general reception is planned for the evening of 6 September, and a second evening reception and banquet later on during the Congress. The final Plenary Session will be held on the morning of 12 September.

*Official travel agency:* The American Express Company.

*Registration fee:* During 1969, 30 U. S. dollars; 1 January to 31 August 1970, 40 U. S. dollars; thereafter 50 U. S. dollars.

Please use the attached form to (a) Preregister, (b) indicate the title or subject of your proposed paper, and (c) make further inquiry. Please type or print.

Enclosed is my money order (bankdraft, check—U. S. banks) for the sum of \_\_\_\_\_ U. S. dollars, payable to 2ND INTERNATIONAL CONGRESS OF PARASITOLOGY for preregistration. Send receipt to \_\_\_\_\_

I intend to submit a paper on the following subject for the Congress Program: \_\_\_\_\_

I would like more information, especially about hotels, charges, etc., \_\_\_\_\_

(Signed) \_\_\_\_\_

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Return this form and address all correspondence to: Dr. G. F. Otto, Secretary General, 2nd ICP, Department of Zoology, University of Maryland, College Park, Maryland 20742, USA.

*Research Note***A Specimen of *Taenia pisiformis* Bloch, 1780  
with One Circle of Hooks**

Anomalies in cestodes are rather common and have been reported by many workers. Extensive references on the subject have been compiled by Clapham (1939, *J. Helm.* 17: 163–176) and Wardle and McLeod (1952, *The zoology of tapeworms*. University of Minnesota Press). Recently (Merdivenci, 1964, *J. Parasit.* 50: 476–477 and Velasquez and Chanco, 1969, *J. Parasit.* 55: 199) beef tapeworms with double genital pores have been found, and Lubinsky and Galaugher (1966, *Can. J. Zool.* 44: 767–768) reported a scolex of *Echinococcus multilocularis* Leuckhart, 1883 with six suckers. Clapham categorized the various types of abnormalities, but said little about hook reduction other than it occurred, or about the loss of a complete row of hooks.

Taeniid genera, with some exceptions, i.e., *Taeniarhynchus* Weiland, 1858 and *Monorodotaenia* Little, 1967 are armed with two concentric circles of hooks. These circles normally consist of one row of large hooks and another relatively small. This note reports an atypical *Taenia pisiformis* which possessed a single row of 21 small hooks.

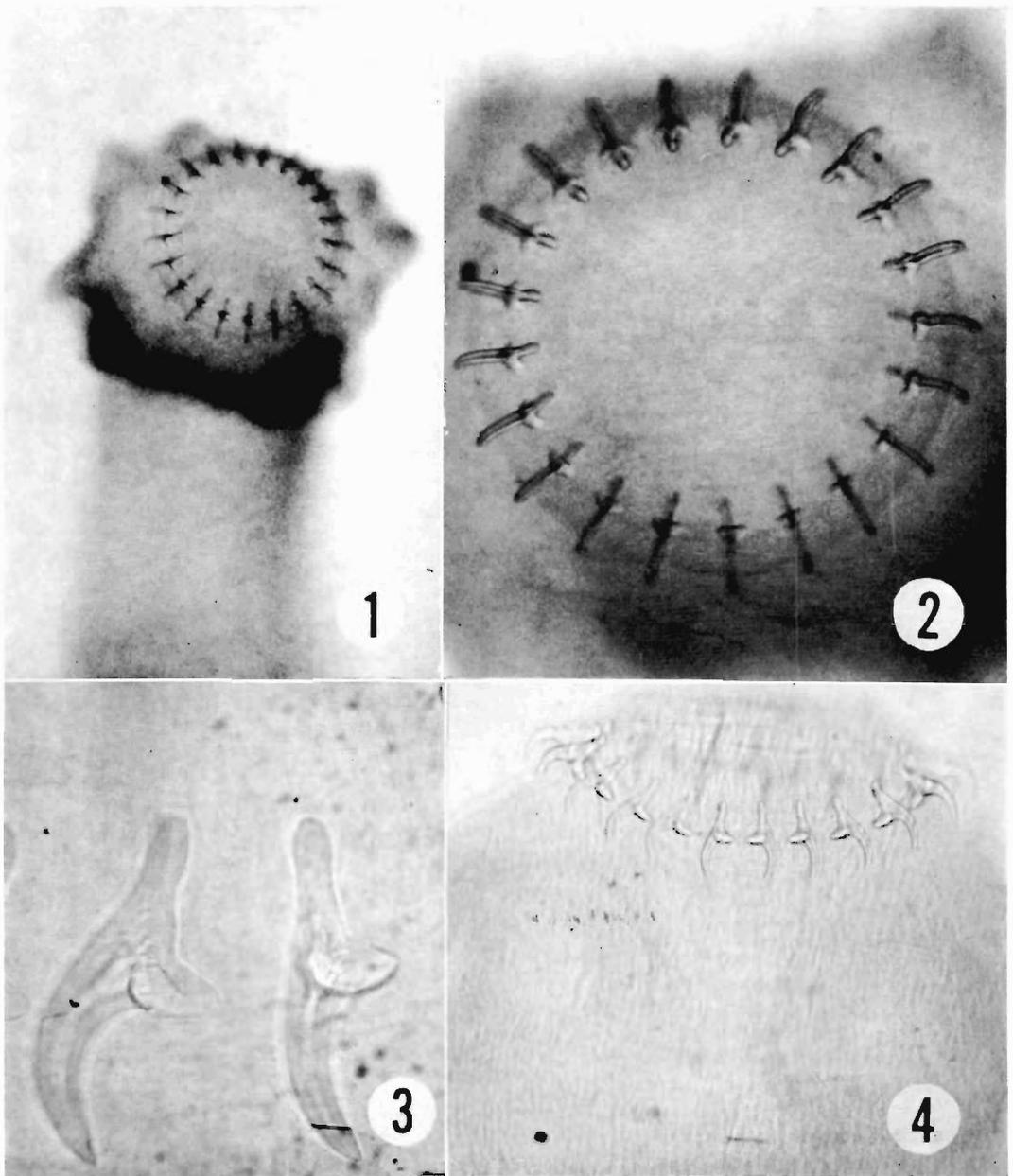
The specimen was one of a batch of about 100 *T. pisiformis* from 10 coyotes, *Canis latrans* Say trapped in Zapata County, Texas. The unusual worm was noticed with the aid of a dissecting microscope while separating various fragments of strobilae.

Figures 1 and 2 are photographs taken before the worm, which was about 35 cm in total length, was processed into permanent whole mount slides. Figures 3 and 4 show the single row of small hooks in the permanent preparation. It is unlikely that the row of missing hooks was somehow dislodged as there were no scars indicating any previous presence of hooks. It is also most unlikely that only the large hooks would have been lost or removed without disturbing other parts of the scolex.

Merdivenci (op. cit.) pointed out that, "such atypical worms have been mistakenly named as new species by some authors." Hall's *Taenia balaniceps* (1910) is probably a case in point as he recovered only one complete specimen, and according to Riser (1956, *Am. Midl. Nat.* 56: 133–137), it was "markedly distorted." It seems almost certain that if *T. balaniceps* was a valid species it would have been reported in the subsequent examinations of hundreds of canids and felids from the southwestern United States in the last 6 decades.

I thank Dr. John P. Smith, Department of Veterinary Parasitology, Texas A & M University, for the material used in this study.

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Department of Biology,  
Texas A & M University,  
College Station, Texas 77843



Figures 1-4. Photomicrographs of *Taenia pisiformis* with one row of hooks. 1. Before mounting. 2. *En face* view before mounting. 3. Permanent whole mount.  $\times 450$ . 4. Permanent whole mount.  $\times 100$ .

## MINUTES

### Four Hundred Thirty-seventh Through Four Hundred Forty-fourth Meetings

*437th Meeting:* Adult Education Center, University of Maryland, College Park, Maryland, 18 October 1968. Amendment to Article 9 of By Laws pertaining to the responsibility of the editor of the "Proceedings" and collateral changes in Articles 6 and 7 of the Constitution proposed. Dr. Benjamin G. Chitwood elected to life membership. Slate of officers for 1969 presented: A. C. Pipkin (President), A. J. Haley (Vice-President), E. J. L. Soulsby (Recording Secretary), and E. M. Buhner (Corresponding Secretary-Treasurer). Papers presented: "Field Observations of Parasitology in Pakistan," by A. J. Haley; "Immunity to Arthropod-borne Nematodes," by G. F. Otto; "The Ultrastructure of Malarial Parasites," by D. L. Price; short illustrated talk of a recent visit to the USSR by W. R. Nickle.

*438th Meeting:* Beltsville Parasitological Laboratory, Beltsville, Maryland, 22 November 1968. Approval of the transfer of the Society's business management to Allen Press. Vote of thanks and standing ovation for Miss Buhner. Increase of dues from \$6.00 to \$8.00, effective 1 January 1970 approved. Amendment to Article 9 of By Laws and collateral changes to Articles 6 and 7 of Constitution approved. Slate of officers presented at 437th meeting approved by acclamation. Papers presented: "Rapid Card-Agglutination Test for Bovine Anaplasmosis," by T. E. Amerault; "Microbial Flora Associated with the Swine Nematodes *Stephanurus dentatus* and *Ascaris lumbricoides*," by W. R. Anderson; "Preliminary Studies on Cholesterol Metabolism of *Stephanurus dentatus*," by P. Allen and F. Tromba; "Bursal Bosses as a Taxonomic Character of Nematodes," by F. Stringfellow; "In vitro Cultivation of *Eimeria bovis*," by R. Fayer.

*439th Meeting:* Naval Medical Research Institute, Bethesda, Maryland, 18 December 1968, sponsored by Laboratory of Parasitic Diseases, N.I.H. Formalities of installation of new officers completed. Papers presented: "Observations on Cestode Calcareous Corpus-

cles," by T. von Brand; "Some Observations on Sporogonic Cycles in *Plasmodium schwetzi*, *P. vivax* and *P. ovale*," by W. E. Collins; "Succinic Dehydrogenase and Cytochrome Oxidase in the Liver of Mice Infected with *Trypanosoma cruzi*," by T. I. Mercado; "Aging of Third Stage Larvae of *Ancylostoma caninum*," by F. E. Clark; "Repeated Sequences of *Entamoeba histolytica* DNA," by A. Gelderman and L. Diamond.

*440th Meeting:* Patuxent Wildlife Research Center, Laurel, Maryland, 24 January 1969. Death of Dr. Charles G. Durbin announced. Audit Committee's report given. Completion of transference of Society's business affairs to Allen Press announced. Papers presented: "Identification of Four Species of *Acanthamoeba* by Variations in Their Cultural and Immunological Habits," by T. Sawyer; "*Limax*-like Amoeba Isolated from Whooping and Sandhill Cranes," by R. Kocan; "A New Breeding Site for *Simulium rugglesi* on the Seney National Wildlife Refuge, Michigan," by I. B. Tarshis and J. Stuhrt; "Aspects of the Life History and Bionomics of *Corynosoma constrictum* (Acanthocephala; Polymorphidae)," by J. Keithly; "Some Immunological Aspects of *Trichomonas* in Columbiform Birds," by R. Kocan; "A Note on the Epizootiology of Malaria in Captive Penguins," by C. M. Herman, C. Gray and J. O. Kniseley.

*441st Meeting:* Plant Industry Station, Beltsville, Maryland, 21 February 1969. Papers presented: "Occurrence of Tonofilaments (intracellular) in the Hypodermis of the Marine Nematode, *Deontostoma californicum*," by D. Hope; "A Fungus Disease of Plant Parasitic Nematodes," by R. Sayre; "Enzyme Histochemical Studies of Soybeans Infected by the Root-knot Nematode, *M. incognita acerita*," by J. Veech; "Structure-activity Relations in a Group of Benzoic Acids and Their Derivatives," by J. Feldmesser; "*Chondronema passali*—an Innovator in 'Egg' Packaging," by P. Pilitt and W. R. Nickle; "Some Impressions of Nematol-

ogy in the USSR Today," by W. R. Nickle.

**442nd Meeting:** Naval Medical Research Institute, Bethesda, Maryland, 19 March 1969, sponsored by the Department of Zoology, Howard University. New prices for back volumes of the "Proceedings" approved. Announcement that October, 1969 meeting will be an "Anniversary Meeting." Papers presented: "Pantothenic Acid Changes in Mice Infected with *Trypanosoma duttoni*," by C. Lee; "Pyridoxine and *Trichinella spiralis* Infections in Rats," by D. K. Sen; "Host Hyperthyroidism and *Trypanosoma lewisi* Infections," by A. S. Smith.

**443rd Meeting:** Naval Medical Research Institute, Bethesda, Maryland, 23 April 1969. Proposed amendments to Constitution and By Laws read. Papers presented: "Morphological Characteristics of the Sporogonous Cycle of *Plasmodium cynomologi*," by L. A. Terzian; "Behavior of Hemoflagellates in an Insect Oriented Culture System," by D. E. Wood and A. C. Pipkin; "Philippine Capillariasis," by K. D. Murrell; "Cytochemical Analysis of the Mucinogenous Glands of *Schistosoma mansoni* Cercariae," by M. A. Stirewalt and M. Walters.

**444th Meeting:** University of Pennsylvania's New Bolton Center, Kennett Square, Pennsylvania, 24 May 1969. Amendments to Article 4 of the Constitution and Articles 7, 8, 10, 11, 12, 13 and 14 of the By Laws approved. Papers presented: "Efficacy of Parabendazol and Thiabendazole against *Haemonchus contortus*," by V. J. Theodorides; "A Comparative Study on Dimension of the Filariform Larvae of

*Strongyloides ratti* and *S. venezuelensis*," by S. R. Sylk; "Localization of Antibody Binding Sites in *Fasciola hepatica*," by T. J. Hayes; "Lymphoid Responses to *Ascaris* in the Guinea Pig," by E. J. L. Soulsby. Cocktails were served in the Allam House, courtesy of the School of Veterinary Medicine, following which members and their guests enjoyed a dinner served in Alumni House.

The following were elected to membership at the meetings indicated: **437th:** O. Amosu, M. D. Dailey, A. Druckenmiller, R. Fayer, D. J. Forrester, S. M. Gonzalez, E. Hansen, L. Jacobs, A. Jones, A. K. Lawler, R. B. Malek, O. G. Marti, Jr., E. S. McClellan, R. A. Overstreet, P. E. Pellgrino, R. L. Richardson, M. M. Robinson, S. H. Rogers, G. A. Schmunis, J. G. Stoffolano, F. Stringfellow, V. J. Theodorides, C. P. Turco, J. M. Vetterling, J. A. Winchester. **438th:** G. A. Buhler, J. E. Fisher, D. A. Munson, B. B. Nickol, H. S. Osborene, E. Rifkin, G. Samuel. **439th:** T. Blewett, A. D. Johnson, A. W. Jones, R. Leventhal, F. C. Rabalais, M. V. Rawson, Jr., F. R. Rellosa, A. J. Seidenberg, W. A. Webster, R. R. Williams. **440th:** C. D. Becker, K. K. Chakrabarti, R. Herman, A. Husain, N. A. Lapp, D. R. Martin, J. L. Murad, P. A. Pilitt, J. T. Vasquez, T. Yokoo. **442nd:** B. L. Duncan. **443rd:** J. H. Esslinger, R. Mahajan, H. A. James, P. Mehlhop, R. Salerno. **444th:** F. Adetokundo-Christian, R. A. Dunn, R. P. Hathaway, D. C. Kutsky.

E. J. L. SOULSBY  
Recording Secretary

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