# PROCEEDINGS

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# The Helminthological Society of Washington

## CONTENTS

# Calcareous Corpuscle Distribution in Caryophyllid Cestodes: Possible Evidence of Cryptic Segmentation

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ABSTRACT: The distribution of calcareous corpuscles was studied in whole mounts of the following four species of North American caryophyllid cestodes: *Glaridacris laruei, G. catostomi, Hunterella nodulosa,* and *Monobothrium hunteri.* In *Glaridacris,* the corpuscles were arranged in discrete clusters distributed in two dorsal and ventral rows; clusters but no rows were found in *Hunterella;* neither clusters nor rows occurred in *Monobothrium.* The resemblance of the corpuscle distribution in *Glaridacris* to segmentation is unmistakable and has led to the following theoretical considerations: that the serially arranged clusters are a form of cryptic segmentation; that they reflect a type of "physiological segmentation" that preceded somatic segmentation and the formation of a strobila. The evolutionary significance of serially arranged clusters of corpuscles in monozoic cestodes to true segmentation in polyzoic cestodes is discussed. Photographs and details of corpuscle distribution complement the text.

It is well known that the monozoic Caryophyllidea (Cestoidea) lack all traces of segmentation, even in early developmental stages (Freeman, 1973; Wiśniewski, 1930). Whenever multiple reproductive systems occur, they are not serially arranged as in strobilate tapeworms, but are found on lateral branches (Mackiewicz, 1978; Williams, 1979). It is, therefore, of great interest that the calcareous corpuscles of some caryophyllids are distributed in a discrete, serially repeated pattern that strongly suggests the presence of cryptic segmentation. The nature of this remarkable distribution and its possible significance to the origin of segmentation in cestodes form the basis of this paper.

Earlier work on calcareous corpuscles in the Caryophyllidea was reviewed by Mankes and Mackiewicz (1972), who also demonstrated that those from *Glaridacris laruei* had a laminated structure, similar to corpuscles from polyzoic tapeworms. Because this work had been done on sectioned material, the cluster arrangement of the corpuscles went undetected until whole mounts were studied.

## Materials and Methods

The species and numbers studied were: 129 *Glaridacris laruei* (Lamont, 1921) Hunter, 1927, 39 *G. catostomi* Cooper, 1920, 20 *Hunterella nodulosa* Mackiewicz and McCrae, 1962, and 11 *Monobothrium hunteri* Mackiewicz 1963, all of the family Caryophyllaeidae, and collected from the white sucker, *Catostomus commersoni* (Forster) in New York state during 1975 and 1976. Controls were plerocercoids of *Proteocephalus* sp. from largemouth bass, *Micropterus salmoides* (L.), and cysticercoids and adults of experimentally reared *Hymenolepis diminuta* Rud.

Collection, solutions, prestaining treatment, and staining procedures were slightly modified from Chowdhury and DeRycke (1974a). For example, collections were made in modified Ringers solution at a temperature of 11 to 20°C, staining with silver nitrate was from 50 to 60 min, and the exposure time to light was 60 min. Control sections were stained with alizarin red S as outlined by Mankes and Mackiewicz (1972).

## Observations

Corpuscles were easily visible on whole mounts as small dark bodies varying in size from 2.5 to 20 $\mu$m in diameter (Figs. 1–5, 9–17). Corpuscles were in the cortical parenchyma and, depending on the species, generally arranged in a distinct pattern.

### Glaridacris laruei

The most discrete pattern was found in *G. laruei* (Figs. 1–3, 5, 17). In both mature and immature specimens the corpuscles were oriented in two lateral, dorsal, and ventral rows that joined each other at each end of the worm (Figs. 1, 2, 5). In each row the corpuscles occurred in obvious clusters (Fig. 17), serially arranged and with approximately 10 to 25 corpuscles per cluster. Generally, the positions of clusters so corresponded with each other from row to row that the corresponding clusters on either side are designated as a "cluster pair." In some cases, however, the number of clusters varied slightly between rows. The serial arrangement was most pronounced in the anterior part of the worm, particularly in the previtelline region (Fig. 1), and often became obscured posterior the gonopore (Fig. 5). For this reason, all counts of cluster pairs for all species were made in the pregonopore region.

Counts of the number of cluster pairs in the pregonopore region of 31 gravid and 98 non-gravid *G. laruei* revealed that: there was a mean of 22.4 cluster pairs (SD 2.3; range 17–28); there was no correlation between the number of cluster pairs and worm length; the distance between cluster pairs increased with an increase in pregonopore length.

The numbers of corpuscles in a single row from 18 non-gravid worms (1.9–4.9 mm long; mean 3.3) and 13 gravid ones (4.2–9.7 mm long; mean 6.4) were compared with each other. For comparative purposes, the counts were made in three body regions: previtelline (tip of scolex to most anterior vitellarium), vitelline (most anterior vitellarium to gonopore), and postgonopore (gonopore to posterior tip). There was a great deal of variation in the number of corpuscles in each region in each group of worms; gravid ones had a greater mean number than non-gravid worms, however. While some pairs of worms of similar size (2.9, 4.9 mm) had differences as little as 5 and 20, respectively, in the number of corpuscles in a single row, others (2.4, 5.4 mm) had differences of 91 and 288, respectively.

A similar pattern was observed in the number of corpuscles per cluster. For example, in a specimen 1.5 mm long, 10 of 14 clusters in the vitelline region had six corpuscles while the others ranged from five to nine; on the other hand, in a worm 1.9 mm long, three of 13 clusters had six corpuscles and the others ranged from two to 13. Utilizing the Wilcoxon Matched Pairs Signed Ranks Test (Siegel, 1956), there was no significant difference ($P = 0.05$) between the number of corpuscles in the right and left clusters of cluster pairs from the vitelline portion of 13 immature and five mature *G. laruei*. These data indicated that the variation in corpuscle number per cluster or per cluster pair is due to chance and, therefore, the cluster pair, rather than individual clusters, can be regarded as a discrete unit.

### Glaridacris catostomi

Clustering patterns in *G. catostomi* were most distinct in the previtelline region and generally more diffuse in the posterior half of the worm, particularly in those
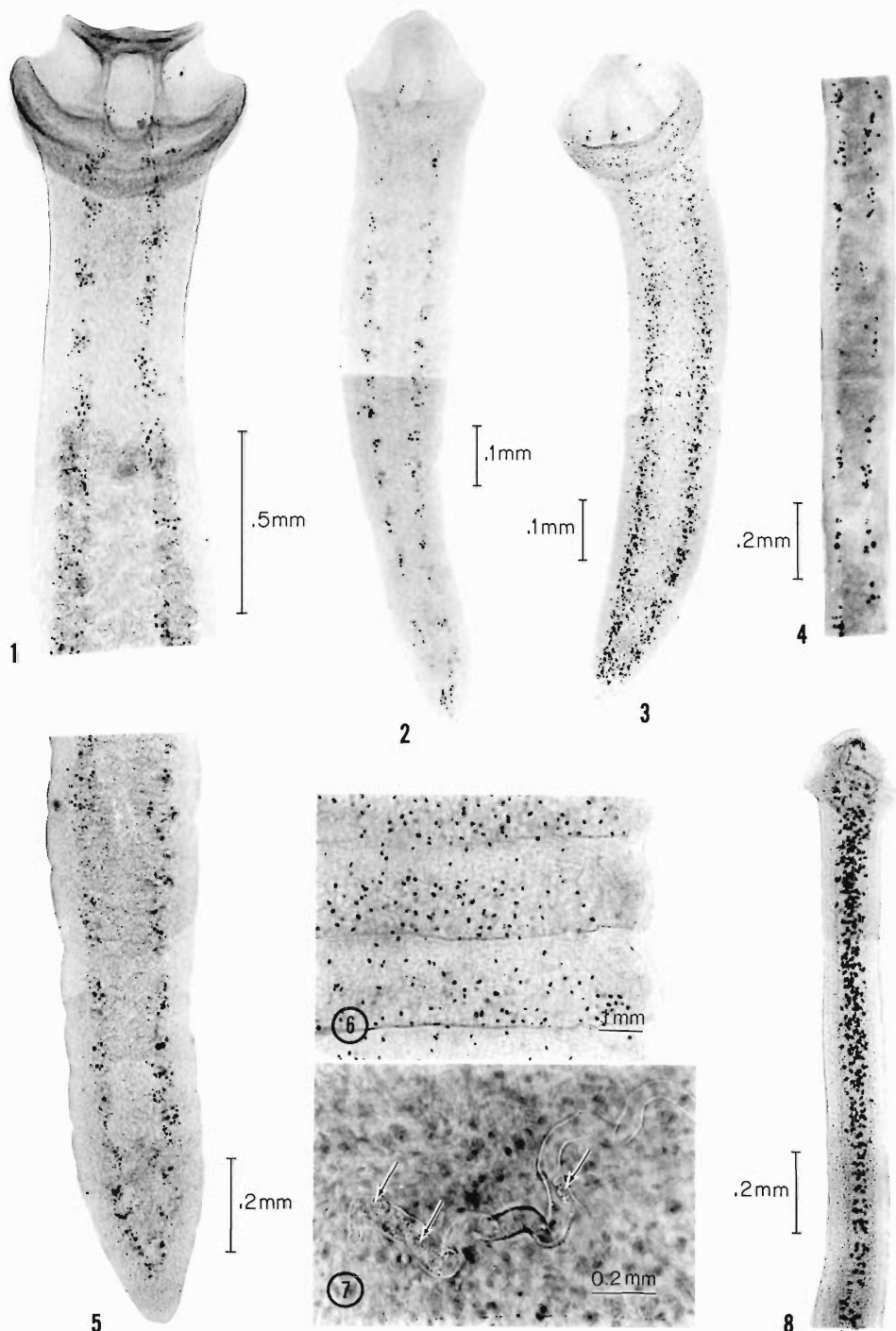
Figures 1–8. Calcareous corpuscles (black particles) in various cestodes. 1. Anterior end of gravid *Glaridacris laruei*. 2. Immature *G. laruei*. 3. Immature *G. catostomi*. 4. Lateral view of vitelline region of *G. laruei*. 5. Posterior end of gravid *G. laruei*. 6. Portion of mature proglottids, *Hymenolepis diminuta*. 7. Osmoregulatory canal with particulate matter (arrows) from live, immature *G. laruei*. 8. Anterior end of plerocercoid of *Proteocephalus* sp.
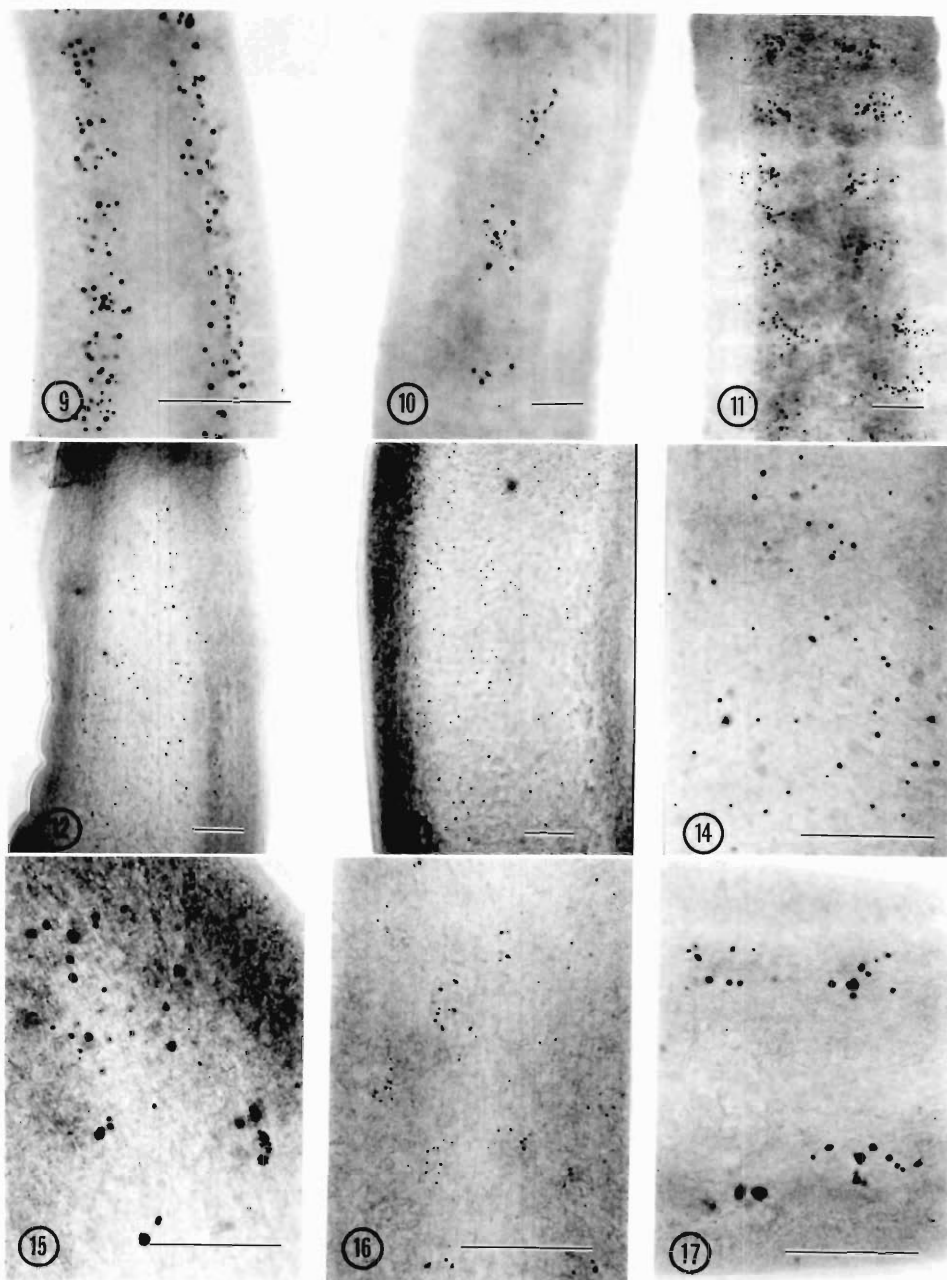
Figures 9–17. Calcareous corpuscle distribution in various caryophyllid species; all specimens are immature. All scales equal 0.1 mm. 9–11. *G. catostomi*. 9. Neck region. 10. Medial clusters. 11. Testes region. 12–14. *Monobothrium hunteri*. 12. Neck region. 13. Testes region. 14. Testes region, higher magnification. 15–16. *Hunterella nodulosa*. 15. Scolex. 16. Body. 17. *G. laruei*, two cluster pairs, scolex end on the right.

worms over 10 mm long. In individuals less than 2 mm long, the lateral rows are especially pronounced (Fig. 3); upon close examination it is possible to see that each row consists of a series of clusters (Fig. 9). Unlike *G. laruei* where the lateral clusters are distinct from each other (Figs. 1, 5, 17), in *G. catostomi* the clusters are less well defined (Fig. 11); in some cases they may be loosely connected together by a few medial corpuscles. In one case, there were well-developed medial clusters as well as lateral ones (Fig. 10). In 17 immature worms ranging in length from 1.5 to 9.6 mm (mean 4.5), there were from 17 to 33 cluster pairs with a mean of 23, surprisingly close to the mean for *G. laruei*. Corpuscles generally varied from 7.5 to 12.5 $\mu$m in diameter with some as large as 18 $\mu$m.

With increase in size and maturity of cestodes, the pattern changed through a loss of clusters. Even in the largest immature worm (9.6 mm), the clusters were staggered, rather than being opposite each other as in smaller worms. In 22 gravid worms ranging from 17 to 41 mm (mean 26) there was a loss of clusters in the larger specimens. For example, a worm 30 mm long had only 19 clusters, yet there were 78 in a specimen 4.7 mm long. Clusters remain longest in the relatively short previtelline region, $\frac{1}{10}$ to $\frac{1}{20}$ of the total body length. In 10 mature worms 22–36.6 mm long there were from 10 to 14 clusters in the short previtelline region, in contrast to approximately the same or smaller numbers in the rest of the cestode.

### Hunterella nodulosa

The development of corpuscle clusters in nine gravid and 11 immature *H. nodulosa* was much less defined than in the previous two species (Figs. 15, 16). Clusters consisting of four to 10 corpuscles 5–10 $\mu$m in diameter were most common in the broad swollen scolex. In the body, on the other hand, the corpuscles were scattered, with no apparent pattern (Fig. 16).

### Monobothrium hunteri

The condition of the corpuscles for 11 immature *M. hunteri*, ranging in size from 2.3 to 6.4 mm, differed greatly from that of any of the other caryophyllids. Not only could we not discern a pattern of any type in their distribution but also they were the smallest observed, less than 5 $\mu$m, with many 2.5 $\mu$m in diameter (Figs. 12–14). Corpuscles were numerous, not confined to lateral rows, and appeared to encircle the medullary parenchyma. They appeared to be randomly, but not homogeneously, distributed throughout the length of the worm.

### Controls

There was no systematic arrangement in the corpuscles from plerocercoids of *Proteocephalus* sp. (Fig. 8) or strobilae of *H. diminuta* (Fig. 6). Corpuscle distribution in *H. diminuta* was similar to that of *H. microstoma*, as reported by Chowdhury and DeRycke (1974b).

### Discussion

The cluster arrangement and lateral distribution of calcareous corpuscles of *G. catostomi* and *G. laruei* have not been found in any other cestode. Neither Will (1893), Mrázek (1898), Wiśniewski (1930), nor anyone else dealing with caryophyllid morphology or histology reported any unusual corpuscle distribution.

Furthermore, discrete corpuscle patterns have not been found in the Pseudo-phyllidea in studies on the morphology of procercoids or plerocercoids of *Diphyllobothrium dendriticum, D. osmeri,* and *D. vogeli* by Kuhlow (1953a,b), cellular structure of *D. dendriticum* plerocercoids by von Bonsdorff et al. (1971) or histology of *Triaenophorus* plerocercoids by Gustafsson (1973).

There appear to be only two reports of definite corpuscle patterns from cyclophyllidean cestodes. Chowdhury and DeRycke (1974b) found a "substantial accumulation" of calcareous corpuscles along the osmoregulatory canals of 8-day-old *Hymenolepis microstoma* with the result that corpuscles were generally in lateral rows, especially toward the posterior part of the worm. In the second case, Chowdhury and Singh (1978) reported that the calcareous corpuscles of *Raillietina tetragona* and *R. tunetensis* were less numerous near the egg-pouches at the anterior end of the proglottid and gradually increased to form a concentration near the posterior margin of the proglottid. The distribution in *Raillietina* is thus evidence of a gradient created by the use of calcium during egg-pouch development. It is doubtful that the functional relationships described above will explain the corpuscle distribution in caryophyllids because (a) the osmoregulatory system in these monozoic cestodes is of the net type and not confined to lateral canals, and (b) egg-pouches are not present. It is possible, however, that other functional relationships may exist between corpuscles and organ systems or physiological processes.

From recent papers on the role of calcareous corpuscles in invertebrates (Simkiss, 1976), trematodes (Erasmus and Davies, 1979), and cestodes (Chowdhury and DeRycke, 1977), it is clear that they may function in many different ways. In cestodes, calcareous corpuscles may serve: as reserve food material for the embryo; to neutralize metabolic products in the intermediate host; to protect the worm against the gastric HCl of the definitive host; to act in osmoregulation; and to act also in the fixation of metabolic waste products (Chowdhury and DeRycke, 1977). They may also serve as a source of buffer anions to counteract the acidifying effect of $CO_2$ accumulation in tissue (Befus and Podesta, 1976; Podesta and Mettrick, 1976). Except for involvement in osmoregulation and in egg-pouch formation, most of the other functions are more related to general physiological processes, i.e., metabolism, than to the function of any specific organ or system. If this is the case, then it does not seem unreasonable to assume that such ubiquitous structures have generally similar functions in polyzoic and monozoic cestodes. If this assumption is correct, and too little is known of the physiology of caryophyllids to make any other, then the unusual corpuscle arrangement is probably more related to some developmental or morphological feature *peculiar to the monozoic body plan* rather than to a generalized physiological function.

There does not appear to be any obvious correlation between corpuscle arrangement and worm development. Nowhere in caryophyllid development is there any trace of serially repeating structures, except for the ganglia on the lateral nerve cords (see below). The acaudate adult develops directly from a cercomer-bearing stage (caudate postplerocercoid of Freeman, 1973) with little change in basic morphology, including the scolex and arrangement of genitalia. In some cases, i.e., *Archigetes,* the cercomer is retained in the adult (gravid) stage. As shown in Figures 2 and 3, the corpuscle pattern is established very

early and is not modified in the acaudate adult, even though there is great change in worm size. The pattern thus appears to be intrinsic in nature, changing only after the adult stage is achieved. The loss of clusters from the posterior half of the worm is probably correlated with increased calcium utilization and does not involve a rearrangement of the basic pattern. As seen in Figure 1, the least amount of change is in the neck region that is free of organs and, as far as we know, is not a growth center as is true for polyzoic cestodes (Mackiewicz, 1972). With respect to the role of development on corpuscle distribution, Smyth and Davies (1975) reported no apparent change in the corpuscle pattern in monozoic forms of the normally polyzoic *Echinococcus multilocularis.*

Attempts to associate the corpuscle clusters with a particular anatomical feature of caryophyllids were unsuccessful. Although the morphology of *G. laruei,* with lateral vitellaria, and *G. catostomi,* with lateral and medial vitellaria, are basically different, the corpuscle distribution is essentially the same. On the other hand, species of similar basic morphology, viz., *G. catostomi* and *M. hunteri,* have different corpuscle patterns, indicating that morphology per se is not the sole determinant of corpuscle distribution. Whether each genus or species has its own characteristic corpuscle pattern remains to be seen.

Since clusters occur throughout the length of a worm, only the osmoregulatory and nervous systems seem likely candidates to influence corpuscle distribution. The report of fine granular calcareous deposits in the lining of capillaries of flame cells and the conspicuous association of corpuscles with osmoregulatory canals of *H. microstoma* by Chowdhury and DeRycke (1977) strengthens this view. Fine granules, of unknown composition (Fig. 7), were also found in the osmoregulatory canals of *G. laruei,* reinforcing the speculation that corpuscles or their constituents may be actively translocated via the osmoregulatory system. The osmoregulatory canals form so complex a netlike pattern that it is difficult to relate it to the lateral cluster pattern. Unfortunately, the distribution of flame cells for any caryophyllid is not known. If the osmoregulatory system is indeed intimately associated with the corpuscle pattern, then this association is more closely related to the physiology of osmoregulation than to a simple spatial relationship.

According to the work of Mrázek (1898) and Wiśniewski (1930) on *Archigetes,* which is morphologically similar to *G. laruei,* there are two main lateral nerve cords with from 15 to 16 ganglial nodes. Attempts to discover if the locations of ganglia in *G. laruei* were correlated with the positions of corpuscle clusters by the use of Nomarski differential interference contrast optics were unsuccessful. Although none of the functions stated above for calcareous corpuscles applies to the nervous system, the morphology of this system, with lateral cords and regularly spaced ganglia, would seem to offer the best possibility for correlating morphology and corpuscle distribution.

Perhaps the most important question regarding the unusual corpuscle arrangement is: Is the serial cluster arrangement in caryophyllid cestodes related to true somatic segmentation? Unfortunately, we are not able to answer this question unequivocally because too little is known of corpuscle function in caryophyllids. It is possible to offer the following ideas concerning this intriguing question. (1) The serially arranged clusters are a form of cryptic segmentation that reflects differing physiological states in adjacent groups of cells. Perhaps careful histochemical studies of the neck region may also reveal a serial pattern to cell types

other than those connected with corpuscle formation. (2) The serially arranged clusters reflect a type of "physiological segmentation" that preceded somatic segmentation and the formation of a strobila. Of course this possibility assumes that caryophyllids had a strobilate stage that was subsequently lost, thus giving rise to a cycle of neotenic stages, as Wiśniewski (1930) and Janicki (1930) proposed long ago. On the other hand, one can argue that "physiological segmentation" had to precede somatic segmentation and that, in this case, somatic segmentation in the caryophyllids has yet to evolve. Before either of these possibilities, or any other, can be critically evaluated, we will have to learn much more of corpuscle function and histology in these cestodes. Until this is done, the discovery of serially arranged calcareous corpuscle clusters in caryophyllid cestodes provides still further evidence of the unique nature of monozoic tapeworms and, for the first time, evidence that these cestodes may indeed have traces of segmentation after all. If this last conclusion is correct, then we may have to revise our ideas concerning the relationship of monozoic tapeworms to polyzoic ones.

## Acknowledgments

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All papers are sent to at least two reviewers usually within a day or two after I receive them. When authors provide two copies with the original, as requested, I send both out for simultaneous review. This, of course, greatly hastens the review process, which ordinarily takes two to four weeks. Most papers are returned to authors for some revision. Revised manuscripts may be accepted as received or after further review. Sometimes the additional review leads to a further revision. Material for the January issue is sent to Allen Press about October 1 and that for the July issue about April 1.

Traditionally, the *Proceedings* has had a high component of papers in parasite systematics and I expect this will continue to be the case. However, I have been receiving more manuscripts representing other areas of parasitology, including parasite ecology, and I hope this trend will continue. Every effort is being made to make the *Proceedings* the finest journal of its kind. You can help by sending me excellent manuscripts in any and all areas of parasitology.

The Editor

# Two New Species of Cestode (Trypanorhyncha, Eutetrarhynchidae) from the Yellow-spotted Stingray, *Urolophus jamaicensis*

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ABSTRACT: *Eutetrarhynchus thalassius* sp.n. and *Eutetrarhynchus caribbensis* sp.n. were collected from the spiral valves of three yellow-spotted stingrays, *Urolophus jamaicensis,* in Discovery Bay, Jamaica. Both species most closely resemble *Eutetrarhynchus geraschmidti* Dollfus, 1974. *Eutetrarhynchus thalassius* differs from *E. geraschmidti* in the arrangement, sizes, and shapes of tentacular armature. *Eutetrarhynchus caribbensis* differs from *E. geraschmidti* and *E. thalassius* on the basis of metabasal armature. The tentacular armature of *E. geraschmidti* is redescribed.

This report is based upon specimens of trypanorhynch cestodes collected at Discovery Bay, Jamaica. Several worms, recovered from the spiral valves of three yellow-spotted stingrays, *Urolophus jamaicensis,* represent two species new to science. The following descriptions are based on these specimens. The redescription of *E. geraschmidti* is based upon the type specimen, No. 13 (of the series), borrowed from the Muséum National d'Histoire Naturelle, Paris, France.

The spiral valves were removed and placed into dishes containing seawater. The worms were freed from the host tissue and each placed in its own vial of fresh water. The water was then pipetted out and replaced with alcohol-formol-acetic acid (AFA). Acetic carmine stain was used. Beechwood creosote was used for clearing, followed by permanent mounting with Canada balsam. All measurements in micrometers unless otherwise stated.

## *Eutetrarhynchus thalassius* sp.n.
### (Figs. 1–6)

DESCRIPTION (based on 12 specimens): Scolex (Fig. 1) 740–940 long, 120–180 greatest width across bothridia. Bothridia fused into 2 organs, lacking ventral notches. Pars bothridialis 190–210 long, 120–180 wide. Minute spines arm bothridial margins and outer surface of entire scolex. Two tentacles emerge from anterior end of each bothridium. Bases of tentacles somewhat swollen. Bulbs much longer than wide; pars bulbosa 380–580 long, 100–200 wide. Tentacle sheaths sinuous, with enigmatic organs at points of attachment to bulbs. Retractor muscles originate at bases of bulbs. Neck absent.

Mature strobila (Fig. 2) delicate, about 4 mm long, consisting of 3 proglottids. Proglottids acraspedote, anapolytic. Mature proglottids (Fig. 3) 700–800 long, 120–200 at greatest width. Gravid proglottid (Fig. 2) (attached to strobila) about 2.5 mm long, 200–400 at greatest width. Genital atrium absent. Reproductive systems protandrous. Genital pores postequatorial, irregularly alternating.

MALE GENITALIA: Twenty-eight to 35 testes in 2 longitudinal rows (Fig. 3) with some overlap; 5 or 6 posterior to cirrus pouch. Each testis 30–50 wide in mature segment. Cirrus pouch pyriform, transverse, thick-walled, 90–120 long, 40–100 wide. Cirrus unarmed or spines have been lost.

FEMALE GENITALIA: Ovary near posterior end of segment (Fig. 3), H-shaped
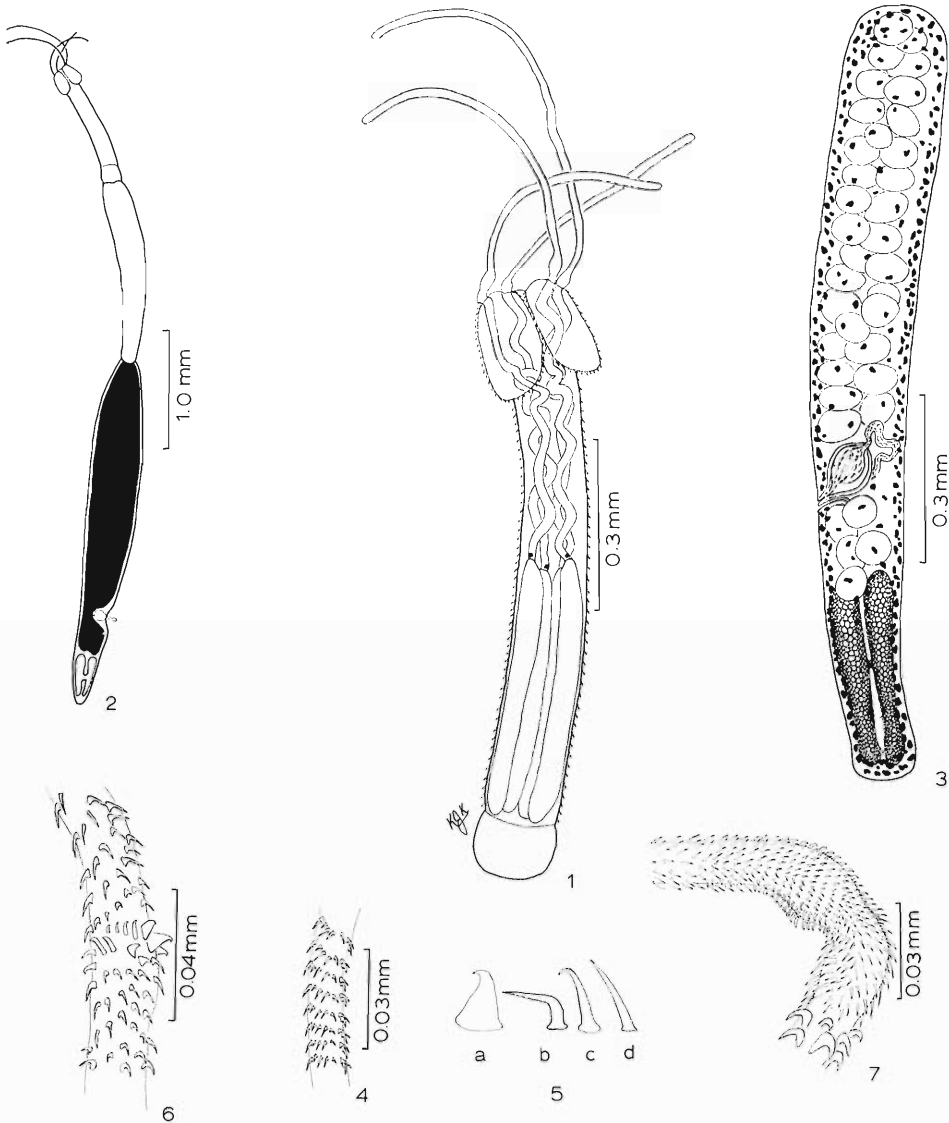
Figures 1–7. 1–6. *Eutetrarhynchus thalassius* sp.n. from a yellow-spotted stingray. 1. Scolex. 2. Entire worm. The black area illustrates the gravid uterus. 3. Mature proglottid. 4. Metabasal portion of the tentacle, lateral surface. 5. Types of hooks present: a. rosethorn-shaped, b. bent spiniform-shaped, c. falciform, d. spiniform. 6. Basal portion of the tentacle, lateral surface. 7. *Eutetrarhynchus geraschmidti* Dollfus, 1974. Basal and metabasal armature, lateral view.

with 2 equal, anteriorly directed lobes 300–400 long, 150–190 across tips of both anterior lobes. Vitellaria cortical, surrounding entire proglottid, only lateral at level of ovary; postovarian follicles present. Distal end of vagina posterior to cirrus pouch, with thick lining and muscular wall. Uterus a median, longitudinal tube, becoming a thin-walled sac filled with eggs extending posterior to cirrus pouch (Fig. 2). Eggs collapsed during preparation so could not be measured.

ARMATURE: Hook arrangement heteroacanthous, heteromorphous. Metabasal armature (Fig. 4) consisting of oblique half-circle rows of hooks, 8 hooks per row. Each row originates on inner surface of tentacle with rosethorn-shaped hook (Fig. 5a) 4–6 long, 1 falciform hook (Fig. 5c) 6–8 long, and 6 spiniform hooks (Fig. 5d) 6–11 long, decreasing in length toward tip of tentacle. Two regions of enlarged basal hooks (Fig. 6) separated by zone, about 140 long, of straight spiniform hooks, each 5–6 long. Proximal zone of enlarged hooks consisting of 2 alternating rows of bent spiniform hooks (Fig. 5b) 10–11 long. Distal zone of enlarged hooks consisting of a row of rosethorn-shaped hooks 5–10 long, making 3 turns around tentacle in a continuous spiral.

TYPE HOST: Yellow-spotted stingray, *Urolophus jamaicensis* (Cuvier, 1817).

LOCATION: Spiral valve.

TYPE LOCALITY: Discovery Bay, Jamaica.

TYPE SPECIMENS: USNM Helm. Coll.; holotype No. 75228, paratype No. 75229.

ETYMOLOGY: The specific name *thalassius* comes from the Greek and refers to the sea, from which this parasite was obtained.

**Remarks**

*Eutetrarhynchus thalassius* sp.n. most closely resembles *Eutetrarhynchus schmidti* Heinz and Dailey, 1974 and *Eutetrarhynchus geraschmidti* Dollfus, 1974, which also are the only species in this genus that have been reported from any species of *Urolophus*. On the basis of overall size and other measurements, *Eutetrarhynchus thalassius* most closely resembles *E. geraschmidti*. Drawings of the metabasal and basal armature of *E. geraschmidti* in the original description (Dollfus, 1974) are unclear, so a redrawing of the tentacular armature of *E. geraschmidti* (Fig. 7) is provided (based upon the type specimen of *E. geraschmidti* borrowed from the Muséum National d'Histoire Naturelle, Paris). The metabasal armature of *E. geraschmidti* consists of spiniform and falciform hooks only, with many more hooks than *Eutetrarhynchus thalassius*. The basal armature of *E. geraschmidti* consists of only one region of enlarged bent spiniform hooks, in two alternating rows. *Eutetrarhynchus thalassius* has two regions of enlarged basal hooks.

On the basis of these differences in the metabasal and basal armature, it is clear that *Eutetrarhynchus thalassius* represents a species new to science.

*Eutetrarhynchus caribbensis* sp.n.
(Figs. 8–13)

DESCRIPTION (based on 8 specimens): Scolex (Fig. 8) 640–750 long, 230–320 greatest width across bothridia. Bothridia fused into two organs, lacking ventral notches. Pars bothridialis 130–160 long, 230–320 wide. Minute spines arm bothridial margins and outer surface of entire scolex. Two tentacles emerge from anterior end of each bothridium. Bulbs much longer than wide; pars bulbosa 260–300 long, 80–140 wide. Tentacle sheaths sinuous, with enigmatic organs at points of attachment to bulbs. Retractor muscles insert at bases of bulbs. Neck absent. Mature strobila (Fig. 9) delicate; proglottids acraspedote, anapolytic. Mature proglottids (Fig. 10) 1,000–1,750 long, 200–240 at greatest width. Genital atrium
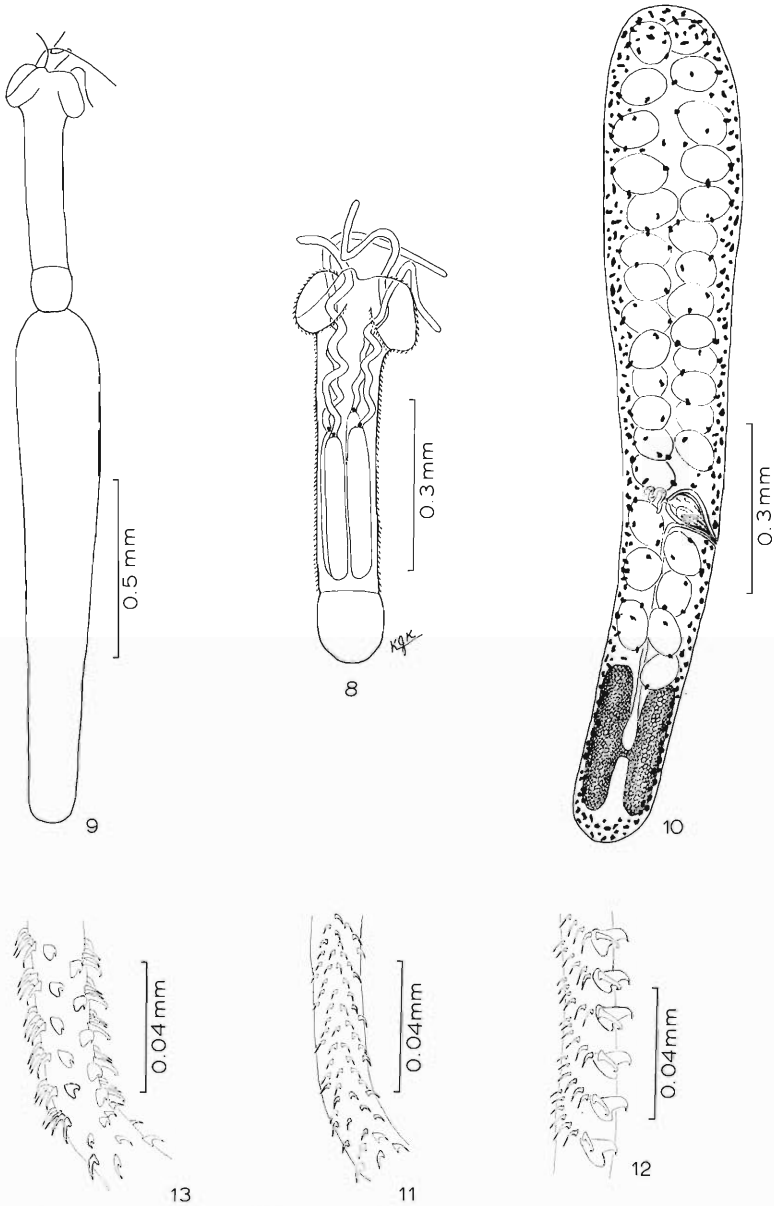
**Figures 8–13.** *Eutetrarhynchus caribbensis* sp.n. from a yellow-spotted stingray. 8. Scolex. 9. Mature worm with scolex attached to mature proglottid. 10. Mature proglottid. 11. Outer surface of tentacle showing metabasal and basal armature. 12. Lateral surface of tentacle showing oblique half-circle rows of hooks. 13. Inner surface of tentacle showing metabasal and basal armature.

absent. Reproductive systems protandrous. Genital pores postequatorial, irregularly alternating.

MALE GENITALIA: Thirty-two or 33 testes in 2 longitudinal rows (Fig. 10) with some overlap; 6 or 7 posterior to cirrus pouch. Each testis 50–60 wide in mature

segment. Cirrus pouch pyriform, transverse, thick-walled, 60–120 long, 40–50 wide. Cirrus not clearly visible.

FEMALE GENITALIA: Ovary near posterior end of segment (Fig. 10), H-shaped with 2 equal, anteriorly directed lobes 200–230 long, 110–130 across tips of both anterior lobes. Vitellaria cortical, surrounding entire proglottid, only lateral at level of ovary, postovarian follicles present. Distal end of vagina posterior to cirrus pouch, with thick lining and muscular wall. Uterus a median, longitudinal tube. Specimens not gravid, so eggs not available for measurement.

ARMATURE: Hook arrangement heteroacanthous, heteromorphous. Metabasal armature (Figs. 11, 12) consisting of oblique half-circle rows of hooks, 10 hooks per row. Each row originates on inner surface of tentacle with 1 rosethorn-shaped hook 9–15 long, followed by 1 falciform hook 11–13 long, 2 bent spiniform hooks 11–15 long, and 6 straight spiniform hooks 7–14 long; decreasing in length from inner to outer surface, and from basal region to tip of tentacle. One region of enlarged basal hooks (Figs. 11, 13) consisting of 2 alternating rows of bent spiniform hooks 10–15 long, followed by 1 row of small rosethorn-shaped hooks 6–7 long.

TYPE HOST: Yellow-spotted stingray, *Urolophus jamaicensis* (Cuvier, 1817).

LOCATION: Spiral valve.

TYPE LOCALITY: Discovery Bay, Jamaica.

TYPE SPECIMENS: USNM Helm. Coll.; holotype No. 75226, paratypes No. 75227.

ETYMOLOGY: The specific name *caribbensis* refers to the location at which this parasite was recovered.

## Remarks

On the basis of overall size and other measurements, *Eutetrarhynchus caribbensis* most closely resembles *E. geraschmidti* and *E. thalassius*. The metabasal armature of *E. caribbensis* consists of rosethorn-shaped, falciform, bent spiniform, and straight spiniform hooks. *Eutetrarhynchus geraschmidti* has no rosethorn-shaped hooks. The basal armature of *E. caribbensis* consists of one region of enlarged, bent spiniform hooks. *Eutetrarhynchus thalassius* has two regions of enlarged basal hooks, one of which is a continuous spiral of rosethorn-shaped hooks. On the basis of these differences in tentacular armature, it is clear that *Eutetrarhynchus caribbensis* represents a species new to science.

## Acknowledgments

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# Three Proteocephalid Cestodes from Colombian Siluriform Fishes, Including *Nomimoscolex alovarius* sp.n. (Monticelliidae: Zygobothriinae)[1]

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ABSTRACT: Three species of proteocephalid cestodes were collected from Colombian siluriform fishes. *Goezeella siluri* Fuhrmann, 1916 is reported from *Ageneiosus caucanus* for the first time, and Colombia is a new locality. *Goezeella siluri* has a metascolex, biloculate suckers, and cortical gonads. Proteocephalideans possessing uniloculate suckers and cortical gonads represent two groupings. Those species possessing metascolices represent the genus *Spatulifer* Woodland, 1934, containing the species *surubim, piramutab, piracatinga, rugosa,* and *rugata.* Those lacking metascolices belong in *Monticellia* LaRue, 1911, comprising *coryphicephala, lenha, megacephala,* and *spinulifera.* New combinations include *Spatulifer piramutab* for *Goezeella piramutab, S. piracatinga* for *Monticellia piracatinga, S. rugosa* for *M. rugosa,* and *S. rugata* for *M. rugata. Nomimoscolex alovarius* sp.n. from *Pimelodus clarias* most closely resembles *N. kaparari* by having fewer than 100 testes per proglottid and exhibiting two papilla-like protuberances on each sucker, but the new species differs by having 63–100 rather than 40 total lateral uterine branches, single lateral vitelline fields rather than dorsal and ventral lateral fields, anteriorly expanded ovarian lobes, and by lacking an expanded apical portion of the scolex. *Corallotaenia* sp. from *Ageneiosus caucanus* represents the first report of the genus in South America.

A small collection of cestodes infecting fishes in the Magdalena River of Colombia contained three species of proteocephalideans representing three genera, one of which has not been previously reported from South America. Two appear to be new, but the condition of our material permits description of only one.

Worms were removed from host intestines, relaxed in river water, fixed with AFA, and stored in 70% ethanol. They were stained with Van Cleave's or Mayer's hematoxylin and mounted in Histoclad for study as whole mounts. Serial cross sections cut at 8 $\mu$m and stained with hematoxylin-eosin were used to confirm certain aspects of proglottid morphology. All measurements are in $\mu$m unless otherwise noted; $N$ = number of measurements used; figures were drawn with the aid of a drawing tube.

### *Goezeella siluri* Fuhrmann, 1916
### (Figs. 1–6)

DESCRIPTION (based on single complete specimen with some proglottids cut in serial cross sections): Strobila approximately 40 mm long. Scolex (Fig. 1) a conical elevation containing 4 biloculate suckers each 358–414 long by 222–266 wide; anterior loculus 185–204 long, posterior loculus 173–229 long. Apical organ lacking. Massive folded metascolex 1.1 mm wide. Immature proglottids wider than long. Mature proglottids (Fig. 3) 605–615 long by 896–1,038 wide ($N$ = 10). Ratio of proglottid width to length 1:0.6–0.8 ($N$ = 10). Testes in dorsal cortex (Fig. 5), in anterior ¾ of proglottid, 183–310 ($N$ = 10) in number, 20–28 preporally, 73–
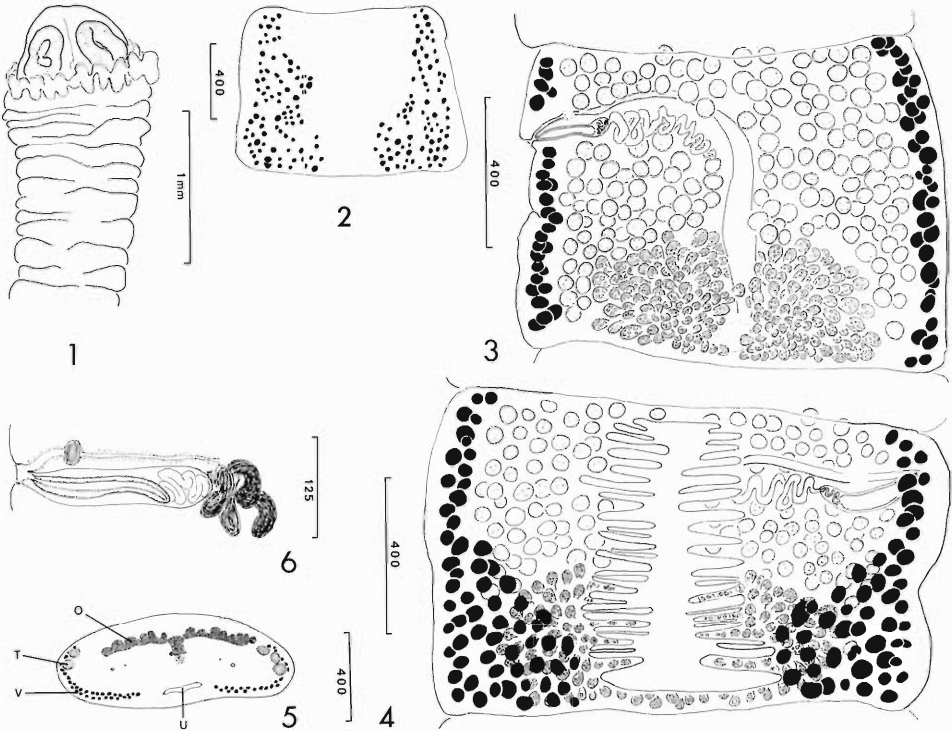
Figures 1–6. *Goezeella siluri.* 1. Scolex. 2. Ventral view of proglottid showing vitelline configuration. 3. Mature proglottid. 4. Gravid proglottid. 5. Cross section of early gravid proglottid. 6. Terminal genitalia. Abbreviations: O = ovary, T = testes, U = uterus, V = vitelline follicles.

96 postporally, 90–190 antiporally; 32–66 in diameter. Cirrus sac (Fig. 6) 116–290 long by 51–58 wide; ratio of cirrus sac length to proglottid width 1:3.5–4.3 ($N = 10$); basal portion of cirrus thin-walled, up to 7 thick. Genital atrium lacking papillae. Ovary posteromedian with lobes in dorsal cortex; lobes 928–1,044 wide at isthmus, 348–429 long. Vagina anterior to cirrus sac and never crossing it; vaginal sphincter and seminal receptacle present. Uterus not preformed in mature proglottids. Vitelline follicles (Fig. 2) extending entire length of proglottid; follicles not in single file; follicles in dorsal and ventral lateral fields; ventral fields converging toward midline posteriorly; follicles 29–57 in diameter. Gravid proglottids (Fig. 4) 802–1,044 long by 1,264–1,470 wide; ratio of width to length 1:0.5–0.8 ($N = 6$). Uterus medial, with 27–43 ($N = 6$) total lateral branches occupying 34–43% of total proglottid width; preformed ventral uterine pores not seen. Eggs 14–23 in diameter; oncospheres 9–14 in diameter, lacking hooks *in utero*. Excretory system composed of paired dorsal and ventral lateral longitudinal medullary ducts and cortical network of reticulate tubules.

HOST: *Ageneiosus caucanus* Steindachner, 1880 (Siluriformes: Ageneiosidae).
LOCALITY: Magdalena River, vicinity of San Cristóbal, Bolívar, Colombia.
SITE OF INFECTION: Small intestine near juncture of stomach and intestine.
SPECIMEN DEPOSITED: USNM Helm. Coll. No. 74544.

## Remarks

Fuhrmann (1916) reported more than 400 testes per proglottid for *Goezeella siluri*, but Rego et al. (1974) and Rego (1975) listed 200–300 testes per proglottid. The Colombian specimen has 183–310 testes, similar to specimens of *G. siluri* from Venezuela (Brooks, unpublished). We conclude that Fuhrmann's number represented an over-approximation of testes number.

LaRue (1911) proposed *Monticellia* for proteocephalidean cestodes possessing simple scolices and having all gonads situated in the proglottid cortex. Fuhrmann (1916) erected *Goezeella* for *G. siluri*, which reportedly resembled *Monticellia* species by having simple scolices and cortical gonads, but which possessed a metascolex. Rego et al. (1974) reported specimens identified as Fuhrmann's species, and illustrated uniloculate (=simple) suckers, but later Rego (1975) reexamined the material and discovered biloculate suckers. Woodland (1933a) previously reported biloculate suckers for specimens of *G. siluri* collected from the type host, *Cetopsis caecutiens*, and emended the generic diagnosis of *Goezeella* to include biloculate suckers. Later, Woodland (1933b) reported specimens identified as *Peltidocotyle rugosa* Diesing, 1850 and described cortical gonads as well as a scolex with simple suckers and a metascolex. After receiving a personal communication from Dr. Otto Fuhrmann, who stated that Diesing's type material of *P. rugosa* possessed biloculate suckers and medullary ovaries (see Fuhrmann, 1934), Woodland (1934) proposed the replacement name *Spatulifer surubim* for *P. rugosa* of Woodland, 1933. Subsequently, however, Woodland (1935), Wardle and MacLeod (1952), and Yamaguti (1959) as well as Rego et al. (1974) and Rego (1975) all considered *Monticellia* the senior synonym of both *Goezeella* and *Spatulifer*. Freze (1965) recognized three groupings among the species assigned to *Monticellia* by the above authors. Those possessing strongly developed metascolices he placed in *Goezeella*; those lacking metascolices or possessing weakly developed ones along with aspinose strobilae and relatively wide uterine branches he assigned to *Monticellia* (synonym *Spatulifer*); and for species lacking metascolices but having spinose strobilae and narrow uterine branches, Freze proposed *Spasskyellina* Freze, 1965.

Brooks (1978a) presented data suggesting that the relative width of uterine branches and the presence or absence of scolex spination were homoplastic traits among proteocephalideans; therefore, we do not consider them useful generic characters. We do consider the Monticelliinae Mola, 1929 a monophyletic group containing several lineages (Brooks, 1978b). Those groupings correspond more to the conceptions of Woodland before 1935 than to any other proposed scheme. Those species lacking a metascolex and possessing uniloculate suckers constitute *Monticellia* LaRue, 1911 (synonym *Spasskyellina* Freze, 1965), containing *M. coryphicephala* (Monticelli, 1891) LaRue, 1911 (type species), *M. lenha* Woodland, 1933, *M. megacephala* Woodland, 1934, and *M. spinulifera* Woodland, 1934. *Goezeella* Fuhrmann, 1916, as defined by Woodland (1933a) and by us, contains species with biloculate suckers and metascolices, namely *G. siluri* Fuhrmann, 1916. We further resurrect *Spatulifer* in its original meaning for monticelliines possessing metascolices and uniloculate suckers. *Spatulifer* thus contains *S. surubim* Woodland, 1934 (type species) (synonym *Peltidocotyle rugosa* Woodland, 1933), *S. piracatinga* (Woodland, 1935) comb.n. (synonym *Monticellia piracatinga* Woodland, 1935, *S. piramutab* (Woodland, 1933) comb.n. (synonym
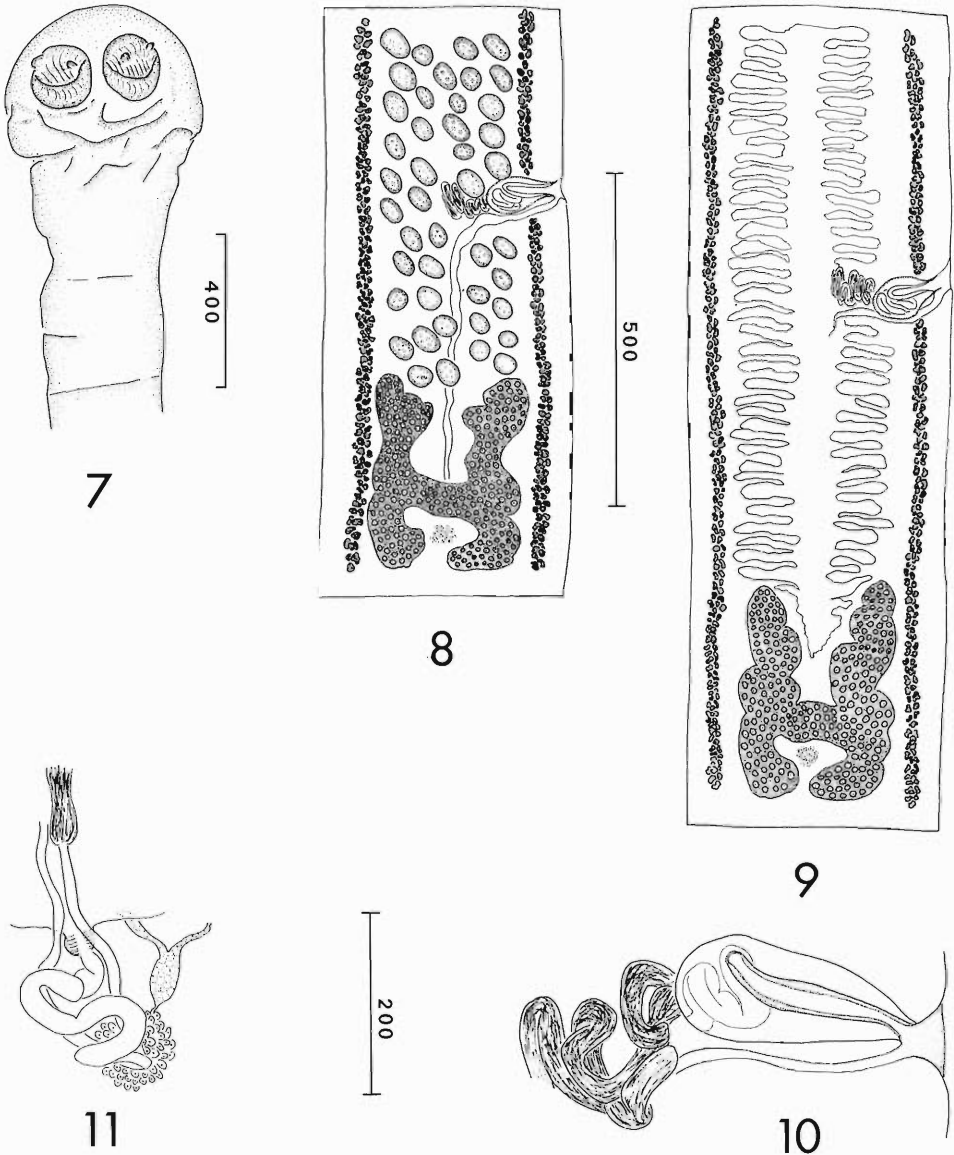
**Figures 7–11.** *Nomimoscolex alovarius.* **7.** Scolex. **8.** Mature proglottid. **9.** Gravid proglottid. **10.** Terminal genitalia. **11.** Ootype region.

*Goezeella piramutab* Woodland, 1933), *S. rugosa* (Woodland, 1935) comb.n. (synonym *Monticellia rugosa* Woodland, 1935), and *S. rugata* (Rego, 1975) comb.n. (synonym *Monticellia rugata* Rego, 1975.

### *Nomimoscolex alovarius* sp.n.
### (Figs. 7–11)

DESCRIPTION (based on 4 complete and 4 fragmented specimens): Strobila fragile, up to 30 mm long. Scolex (Fig. 7) aspinose, 340–445 wide, lacking apical

organ, with 4 uniloculate suckers; suckers 135–179 long by 142–185 wide, each with 2 papillae on anterior surface. Immature proglottids wider than long. Mature proglottids (Fig. 8) 482–882 long by 330–368 wide; ratio of proglottid width to length 1:1.3–2.6 ($N = 20$). Testes in anterior ¾ of proglottid, medullary, 40–61 ($N = 20$) in number, 7–13 preporally, 9–18 postporally, 23–33 antiporally; 41–68 in diameter. Cirrus sac (Fig. 10) 156–217 long by 41–72 wide; ratio of cirrus sac length to proglottid width 1:2.1–2.9 ($N = 20$); basal portion of unarmed cirrus up to 10 thick. Genital pores marginal, alternating irregularly in anterior 31–38% ($N = 20$) of proglottid. Genital atrium prominent, lacking papillae. Ovary posteromedian, medullary, bilobed with lobes expanded anteriorly; 90–185 wide at isthmus, lobes 200–358 long. Vagina anterior or posterior to cirrus sac, may cross it; vaginal sphincter lacking, seminal receptacle present. Uterus medial, preformed in mature proglottids. Vitelline follicles in single paired lateral cortical fields extending nearly entire length of proglottid; follicles not in single file. Gravid proglottids (Fig. 9) 882–3,259 long by 414–588 wide; ratio of width to length 1:1.5–7.9 ($N = 20$). Ratio of cirrus sac length to proglottid width 1:3.1–3.6 ($N = 20$). Genital pores in anterior 31–39% ($N = 20$) of proglottid. Ovarian lobes in gravid proglottids expanded greatly, 259–618 long by 247–377 wide at isthmus. Uterus medullary, with 63–100 ($N = 20$) total lateral branches occupying 48–56% of proglottid width; preformed ventral uterine pores lacking. Eggs 16–21 in diameter; oncospheres 15–19, hooks lacking. Excretory system composed of paired lateral longitudinal dorsal and ventral medullary ducts and cortical network of reticulate tubules.

HOST: *Pimelodus clarias* (Bloch, 1785) (Siluriformes: Pimelodidae).

LOCALITY: Quebrada Doña Juana, vicinity of La Dorada, Caldas, Colombia.

SITE OF INFECTION: Anterior ⅓ of small intestine.

HOLOTYPE: USNM Helm. Coll. No. 74499.

PARATYPES: USNM Helm. Coll. No. 74500.

ETYMOLOGY: The specific epithet is derived from the Latin *ala*, meaning wing, and *ovarium*, meaning ovary, and refers to the structure of the ovarian lobes in gravid proglottids.

## Remarks

*Nomimoscolex* Woodland, 1934 presently contains eight species parasitizing Amazon siluriform fishes in addition to the new species from the Magdalena River. *Nomimoscolex alovarius* differs from all but two species by possessing papilla-like structures associated with the suckers. *Nomimoscolex woodlandi* Freze, 1965 (=*Myzophorus sudobim* Woodland, 1934) and *N. kaparari* Woodland, 1935 possess similar structures, which Freze (1965) termed unguiculate protuberances. The protuberances of *N. woodlandi* appear as four structures situated around each sucker, whereas those of *N. kaparari* appear as two protuberances on the anterior edges of each sucker. Woodland called the structures cornified projections, and specifically stated that they could not be called hooks, so Freze's (1965) term *unguiculate protuberances* has misleading connotations. The papilla-like structures exhibited by *N. alovarius* thus appear to be similar to those which *N. kaparari* possesses, and different from those of *N. woodlandi*. *Nomimoscolex alovarius* and *N. kaparari* resemble each other further by possessing fewer than 100 testes per proglottid. The new species differs from *N.*

*kaparari* by lacking an umbelliform apical portion of the scolex, by possessing expanded ovarian lobes, and by exhibiting vitelline follicles in single, rather than dorsal and ventral, lateral lines (visible in whole mounts as well as sections). Woodland's (1935) figure of *N. kaparari* further suggests that gravid proglottids of that species possess fewer than 40 total lateral uterine branches, markedly different from the 63 to 100 exhibited by *N. alovarius*. *Nomimoscolex magna* Rego, Dos Santos, and Silva, 1974 parasitizes *Pimelodus clarias* in the Mato Grosso region of Brazil but bears little resemblance to *N. alovarius* beyond the generic characteristics. It lacks the sucker projections, has flat ovarian lobes and approximately 150 testes per proglottid, and possesses approximately 50 lateral uterine branches.

## *Corallotaenia* sp.

HOST: *Ageneiosus caucanus*.
LOCALITY: Magdalena River, vicinity of San Cristóbal, Bolívar, Colombia.
SITE OF INFECTION: Anterior ⅓ of small intestine.
SPECIMENS DEPOSITED: USNM Helm. Coll. No. 74501.

### Remarks

This is the first report of the genus from South America. Our specimens, which lack gravid proglottids, possess 18–30 testes per proglottid, thus resembling *C. parva* (Larsh, 1941) Freze, 1965 more than any other species. They differ from all known species by having a well-developed metascolex and vitelline follicles which may be larger than the testes. They possess proglottids that are longer than wide, have vitellaria not entering the medial portion of the proglottid, and they are relatively small worms, 10–15 mm long. Thus, with the exception of possessing a well-, rather than poorly-, developed metascolex, the Colombian specimens conform to Freze's (1965) generic diagnosis of *Corallotaenia* Freze, 1965 and are assigned to that genus.

### Acknowledgments

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# Cestodes in Four Species of Euryhaline Stingrays from Colombia[1]

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ABSTRACT: Seven species of cestodes are reported from coastal stingrays in the vicinity of Cartagena, Colombia. *Acanthobothrium colombianum* sp.n. from *Aetobatis narinari* resembles *A. paulum* and *A. mathiasi* but differs by having relatively shorter, more squared proglottids at the end of the strobila, a pre-equatorial genital pore, relatively less elongate ovarian lobes in terminal proglottids, and less prominent apical suckers and pads. *Acanthobothrium urotrygoni* sp.n. from *Urotrygon venezuelae* most closely resembles *A. olseni* in bothridial hook length but differs in cirrus sac size, resembles *A. southwelli* in cirrus sac size but differs in bothridial hook length, and differs from the former two and resembles *A. lineatum* by having a long and spinose cephalic peduncle; the new species differs from *A. lineatum* by possessing smaller bothridial hooks and cirrus sacs. *Acanthobothrium cartagenensis* sp.n. from *Urolophus jamaicensis* most closely resembles *A. urolophi* but differs in bothridial hook length, length of cirrus sac, number of testes and proglottids, and position of genital pore. *Rhinebothrium magniphallum* infected the new hosts *Urolophus jamaicensis*, *Urotrygon venezuelae*, and *Dasyatis americana* in the new locality of Cartagena, Colombia. *Phyllobothrium* cf. *kingae* occurred in *Dasyatis americana* and *Urolophus jamaicensis*. *Polypocephalus medusius* and *Lecanicephalum peltatum* infected the new host *Dasyatis americana*. Cartagena is a new locality for both cestode species. A host-parasite checklist for cestodes collected from 121 stingrays examined in Colombia during 1975 and 1976 is presented.

This report is the sixth in a series detailing the cestodes collected from 121 stingrays examined during 1975 and 1976 in the Republic of Colombia (Brooks and Thorson, 1976; Brooks, 1977; Brooks and Mayes, 1978; Mayes et al., 1978; Brooks et al., in press). Herein we report cestodes infecting *Aetobatis narinari* (Euphrasen), *Urotrygon venezuelae* Schultz, *Urolophus jamaicensis* (Cuvier), and *Dasyatis americana* Hildebrand and Schroeder; additionally, we present a checklist of hosts examined and cestodes collected (Table 1).

Helminths were removed from hosts and fixed with gentle heat and AFA or fixed *in situ* with 10% formalin. They were stained with Mayer's or Ehrlich's hematoxylin and mounted in Histoclad or Canada balsam for study as whole mounts. Serial cross sections cut at 8 μm and stained with hematoxylin-eosin were used to confirm some aspects of proglottid morphology. Mean values ($\bar{x}$) and sample size ($N$) are listed for certain meristic characters. Figures were drawn with the aid of a drawing tube; all measurements are in μm unless otherwise stated.

### *Acanthobothrium colombianum* sp.n.
### (Figs. 1–3)

DESCRIPTION (based on 3 specimens): Strobila craspedote, apolytic, up to 35 mm long, composed of 31–48 proglottids. Scolex 230–276 long by 230 wide, com-

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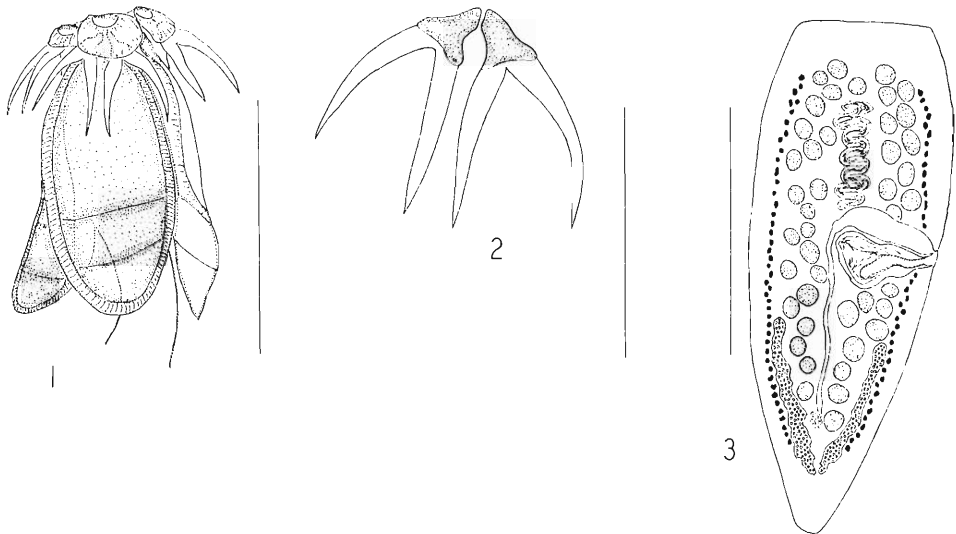
**Figures 1–3.** *Acanthobothrium colombianum*. 1. Scolex. 2. Bothridial hooks. 3. Mature proglottid. Scale for Figure 1 = 400 $\mu$m; for Figure 2 = 200 $\mu$m; for Figure 3 = 400 $\mu$m.

posed of 4 trilocular bothridia each armed with pair of bifid hooks and surmounted by apical sucker and pad. Bothridia 299–391 long by 115–161 wide; anterior loculus 200–230 long, middle loculus 39–50 long, posterior loculus 39–50 long. Ratio of locular lengths 1:0.2:0.2. Apical pad 90–100 in diameter, suckers 45–54 in diameter. Hook formula (modified from Euzet, 1956, to include mean values) for 12 hooks:

$$\frac{48\text{–}53\ (50) \qquad 120\text{–}146\ (132) \qquad 131\text{–}150\ (140)}{175\text{–}193\ (185)}$$

Cephalic peduncle 805–989 long, spinose; spines 5–7 long. Immature proglottids wider than long. Mature proglottids 667–690 long by 230–306 wide. Genital pores alternating irregularly in anterior 40–46% ($\bar{x} = 43\%$, $N = 15$) of proglottid. Cirrus sac 150–195 long by 75–143 wide, containing spined eversible cirrus. Testes in anterior ¾ of proglottid, 30–45 in diameter, 39–53 ($\bar{x} = 46.2$, $N = 15$) in number; 11–14 (13) preporally, 6–10 (8) postporally, 21–29 (25) antiporally. Ovary H-shaped with equal-length lobes not reaching anteriorly to posterior margin of cirrus sac; lobes 150–270 long by 90–135 wide at isthmus. Vitelline follicles 5–30 in diameter, extending along lateral portions of proglottid from ovarian isthmus to near anterior end.

HOST: *Aetobatis narinari* (Euphrasen).

SITE OF INFECTION: Spiral valve.

LOCALITY: Cartagena, Colombia.

HOLOTYPE: USNM Helm. Coll. No. 75160.

PARATYPES: USNM Helm. Coll. No. 75161.

ETYMOLOGY: The species is named after the Republic of Colombia, in which we collected stingray helminths for two summers.

**Table 1.** Host-parasite list of elasmobranchs examined and cestodes collected in Colombia during 1975 and 1976. Numbers in parentheses represent numbers of hosts examined.

MYLIOBATIFORMES

  Dasyatoidea

  Dasyatidae

    *Dasyatis americana* (1)

      *Phyllobothrium* cf. *kingae*

      *Polypocephalus medusius* (Linton, 1889) Woodland, 1930

      *Lecanicephalum peltatum* Linton, 1890

    *Dasyatis guttata* (10) negative

    *Himantura schmardae* (12)

      *Acanthobothroides thorsoni* Brooks, 1977

      *Acanthobothrium tasajerasi* Brooks, 1977

      *Acanthobothrium himanturi* Brooks, 1977

      *Rhinebothrium magniphallum* Brooks, 1977

      *Rhinebothrium tetralobatum* Brooks, 1977

      *Caulobothrium anacolum* Brooks, 1977

  Urolophidae

    *Urolophus jamaicensis* (5)

      *Acanthobothrium cartagenensis* sp.n.

      *Phyllobothrium* cf. *kingae*

      *Rhinebothrium magniphallum* Brooks, 1977

    *Urotrygon venezuelae* (16)

      *Acanthobothrium urotrygoni* sp.n.

      *Rhinebothrium magniphallum* Brooks, 1977

  Potamotrygonidae

    *Potamotrygon magdalenae* (51)

      *Potamotrygonocestus magdalenensis* Brooks and Thorson, 1976

      *Rhinebothrium moralarai* Brooks and Thorson, 1976

      *Acanthobothrium quinonesi* Mayes, Brooks, and Thorson, 1978

    *Potamotrygon circularis* (6)

      *Potamotrygonocestus* sp. Brooks, Mayes, and Thorson, in press

      *Rhinebothrium* sp. Brooks, Mayes, and Thorson, in press

      *Acanthobothrium amazonensis* Mayes, Brooks, and Thorson, 1978

  Myliobatioidea

  Myliobatidae

    *Aetabatis narinari* (2)

      *Acanthobothrium colombianum* sp.n.

TORPEDINIFORMES

  Narcinidae

    *Narcine brasiliensis* (17)

      *Acanthobothrium lintoni* Goldstein, Henson, and Schlicht, 1969

      *Acanthobothrium electricolum* Brooks and Mayes, 1978

RHINOBATIFORMES

  Rhinobatidae

    *Rhinobatus productus* (1) negative

## Remarks

*Acanthobothrium colombianum* most closely resembles *A. paulum* Linton, 1890 and *A. mathiasi* Euzet, 1956. *Acanthobothrium paulum* differs by having an ovary which is more follicular (less compact) than that of *A. colombianum*
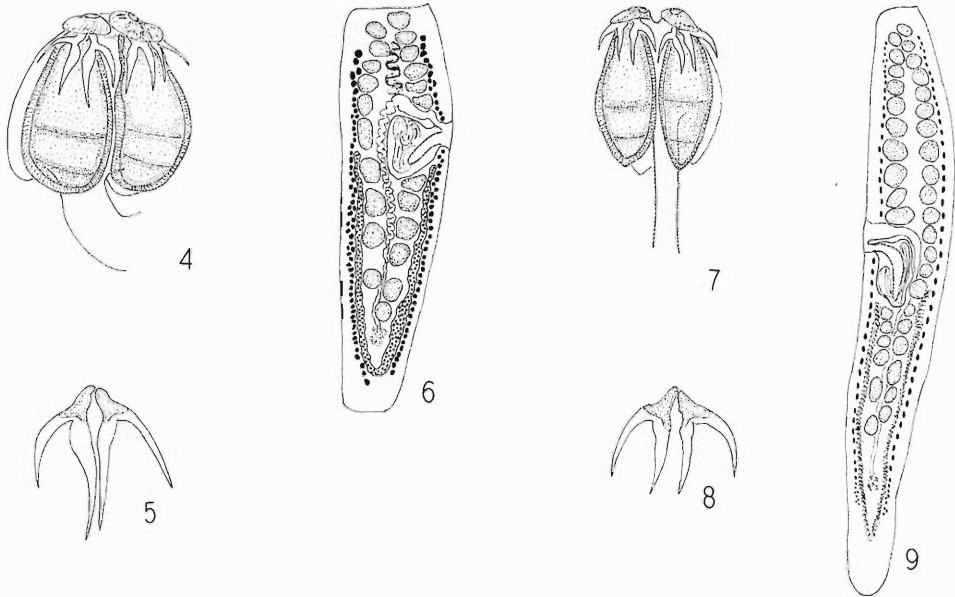
**Figures 4–9.** 4–6. *Acanthobothrium cartagenensis.* 4. Scolex. 5. Bothridial hooks. 6. Mature proglottid. 7–9. *Acanthobothrium urotrygoni.* 7. Scolex. 8. Bothridial hooks. 9. Mature proglottid. Scales for Figures 4, 6, 7, 9 = 400 μm; for Figures 5, 8 = 200 μm.

and which possesses lobes extending to near the posterior margin of the cirrus sac, rather than being distinctly posterior to the cirrus sac. The new species differs from *A. mathiasi* by having an average of 46 rather than 35 testes per proglottid (26–43 for *A. mathiasi* and 39–53 for *A. colombianum*) and by having relatively less elongate bothridia (see Euzet, 1956). *Acanthobothrium colombianum* also possesses a pre-equatorial rather than postequatorial genital pore in contrast to both *A. paulum* and *A. mathiasi*; it resembles *A. paulum* and *A. mathiasi* by having similarly sized bothridial hooks, similar numbers of proglottids per strobila, and similarly sized cirrus sacs, and by exhibiting a scolex, at the end of a relatively long and spinose cephalic peduncle which is not expanded at its junction with the scolex, whose bothridia are not attached at their posterior ends by a velum.

### *Acanthobothrium urotrygoni* sp.n.
### (Figs. 7–9)

DESCRIPTION (based on 10 specimens): Strobila acraspedote, apolytic, composed of 4–6 proglottids, up to 15 mm long. Scolex 154–161 long by 161–173 wide, composed of 4 trilocular bothridia each armed with pair of bifid hooks and surmounted by apical sucker and pad. Bothridia 184–196 long by 61–69 wide; anterior loculus 58–81 long, middle loculus 23–34 long, posterior loculus 34–46 long. Average ratio of locular lengths 1:0.4:0.6 ($N = 15$). Apical pads 58–69 in diameter, suckers 23–35 in diameter. Hook formula for 28 hooks:

$$\frac{27\text{--}33\ (30)\qquad 60\text{--}68\ (65)\qquad 60\text{--}68\ (65)}{87\text{--}101\ (95)}$$

Cephalic peduncle 207–391 long, spinose; spines 3–7 long. Immature proglottids longer than wide. Mature proglottids 465–675 long by 72–173 wide. Genital pores alternating irregularly in anterior 34–43% ($\bar{x} = 38\%$, $N = 15$) of proglottid. Cirrus sac subspherical, 60–98 long by 60–83 wide, containing spined eversible cirrus. Testes in anterior ¾ of proglottid, 29–45 in diameter, 24–33 ($\bar{x} = 28.4$, $N = 20$) in number; 7–10 (9) preporally, 4–5 (4) postporally, 11–19 (15) antiporally. Ovary V-shaped with poral lobe reaching posterior margin of cirrus sac and aporal lobe reaching lateral margin of cirrus sac; lobes 180–464 long by 45–68 wide at isthmus. Vitelline follicles 8–15 in diameter, extending in 2 lateral rows from level of ovarian isthmus to near anterior end of proglottid.

HOST: *Urotrygon venezuelae* Schultz.

SITE OF INFECTION: Spiral valve.

LOCALITY: Cartagena, Colombia.

HOLOTYPE: USNM Helm. Coll. No. 75162.

PARATYPES: USNM Helm. Coll. No. 75163; Univ. Nebraska State Museum, Manter Laboratory No. 20917.

ETYMOLOGY: This species is named for its host genus.

**Remarks**

By possessing a V-shaped ovary with arms reaching to the level of the cirrus sac, pre-equatorial genital pores, fewer than 60 testes per proglottid, and fewer than 35 proglottids per strobila, *A. urotrygoni* most closely resembles *A. lineatum* Campbell, 1969, *A. southwelli* Subhapradha, 1955, *A. brevissime* Linton, 1908, *A. tasajerasi* Brooks, 1977, *A. himanturi* Brooks, 1977, and *A. olseni* Dailey and Mudry, 1968. By having bothridial hooks only 87–101 $\mu$m long, the new species resembles *A. olseni* more than any of the others, but *A. olseni* possesses cirrus sacs which reach 168 $\mu$m in length, whereas those of *A. urotrygoni* do not exceed 90 $\mu$m. *Acanthobothrium southwelli* has cirrus sacs 80 $\mu$m long and *A. lineatum* has a long and spinose cephalic peduncle, but *A. southwelli* and *A. lineatum* differ from *A. urotrygoni* in bothridial hook and peduncle size and bothridial hook and cirrus sac size, respectively.

## *Acanthobothrium cartagenensis* sp.n.
### (Figs. 4–6)

DESCRIPTION (based on single complete specimen): Strobila apolytic, acraspedote, 25 mm long, composed of 13 proglottids. Scolex 300 long by 300 wide; composed of 4 trilocular bothridia, each armed with pair of bifid hooks and surmounted by apical sucker and pad. Bothridia 255 long by 150 wide; anterior loculus 100–130 long, middle loculus 37–40 long, posterior loculus 38–40 long. Ratio of locular lengths 1:0.3:0.3. Apical pads 67 in diameter, suckers 30 in diameter. Hook formula for 6 hooks:

$$\frac{33\text{–}35\ (34) \qquad 69\text{–}75\ (72) \qquad 90\text{–}98\ (95)}{121\text{–}131\ (127)}$$

Cephalic peduncle 180 long, spinose; spines 3–5 long. Neck expanded at juncture with scolex, 120 wide. Immature proglottids wider than long. Last 3 mature proglottids 360–660 long by 157–183 wide. Genital pores alternating irregularly in anterior 34–38% (35%) of proglottid. Cirrus sac spherical, 67–90 in diameter.

Testes in anterior ¾ of proglottid, 30–45 in diameter, 21–26 (24) in number; 4–6 preporally, 5–6 postporally, 10–14 antiporally. Ovary V-shaped, poral lobe extending anteriorly to posterior margin of cirrus sac, aporal lobe reaching anteriorly to lateral margin of cirrus sac; lobes 210–390 long by 60–112 wide at isthmus. Vitelline follicles 5–10 in diameter, extending in 2 lateral longitudinal rows from level of ovarian isthmus to near anterior end of proglottid.

HOST: *Urolophus jamaicensis* (Cuvier).

SITE OF INFECTION: Spiral valve.

LOCALITY: Cartagena, Colombia.

HOLOTYPE: USNM Helm. Coll. No. 75159.

ETYMOLOGY: This species is named after the city of Cartagena, where its host was collected.

## Remarks

*Acanthobothrium cartagenensis* has a relatively short strobila, a short and spinose cephalic peduncle which is expanded at its juncture with the scolex, and relatively short and broad bothridia which are not attached to the scolex by a velum. In these respects, the new species most closely resembles *A. urolophi* Schmidt, 1973, *A. holorhini* Alexander, 1953, *A. quinonesi* Mayes, Brooks, and Thorson, 1978, *A. amazonensis* Mayes, Brooks, and Thorson, 1978, and *A. terezae* Rego and Dias, 1977. By having a V-shaped rather than H-shaped ovary, *A. cartagenensis* more closely resembles *A. urolophi* and *A. holorhini* than the other three, all of which parasitize South American freshwater stingrays. *Acanthobothrium holorhini* differs from the new species by possessing bothridial hooks up to 218 μm long rather than 123–131 μm, by having 63–80 rather than 13 proglottids per strobila, and by having 60–77 rather than 21–26 testes per proglottid. *Acanthobothrium urolophi*, the only other member of the genus known from hosts of the genus *Urolophus*, differs from *A. cartagenensis* by having cirrus sacs up to 200 μm long rather than 90 μm, 23–26 rather than 13 proglottids per strobila, bothridial hooks 105–115 μm long rather than 123–131 μm long, and equatorial rather than pre-equatorial genital pores.

### *Rhinebothrium magniphallum* Brooks, 1977

HOSTS: *Urolophus jamaicensis, Urotrygon venezuelae, Dasyatis americana, new hosts.*

SITE OF INFECTION: Spiral valve.

LOCALITY: Cartagena, Colombia, *new locality.*

SPECIMENS DEPOSITED: University of Nebraska State Museum, Manter Laboratory No. 20915 (*Urolophus*), 20914 (*Urotrygon*), 20928 (*Dasyatis*).

## Remarks

Brooks (1977) described this species from the dasyatid ray *Himantura schmardae* (Werner) collected near Santa Marta, Colombia. This report extends the known range of *R. magniphallum* and expands the host specificity of the species to include four species representing four genera and two families (Dasyatidae and Urolophidae). We noted no differences in range or in mean number of bothridial loculi or testes in our specimens; thus, for *R. magniphallum* in natural infections, these characteristics are apparently not altered by host influence.

### *Phyllobothrium* cf. *kingae* Schmidt, 1978

HOSTS: *Urolophus jamaicensis, Dasyatis americana.*
SITE OF INFECTION: Spiral valve.
LOCALITY: Cartagena, Colombia.
SPECIMENS DEPOSITED: University of Nebraska State Museum, Manter Laboratory No. 20926, 20927.

### Remarks

The specimens to which the above refers more closely resemble *P. kingae* Schmidt, 1978 than any other species because they possess bothridia with marginal loculi and incomplete horizontal loculi (Schmidt, 1978). Our specimens differ from those of *P. kingae* in testes number and cirrus sac size, but we refrain from describing a new species because our specimens all possess bothridia which are too contracted for adequate characterization.

### *Polypocephalus medusius* (Linton, 1889) Woodland, 1930

HOST: *Dasyatis americana, new host.*
SITE OF INFECTION: Spiral valve.
LOCALITY: Cartagena, Colombia, *new locality.*
SPECIMENS DEPOSITED: University of Nebraska State Museum, Manter Laboratory No. 20916.

### Remarks

Linton (1889) described this species as *Parataenia medusia* from *Dasyatis centroura* (listed as *Trygon centroura*) collected at Woods Hole, Massachusetts. Woodland (1930) transferred the species to *Polypocephalus*, the senior synonym of *Parataenia*. Yamaguti (1959) reported *P. medusius* from *Dasyatis sayi* from Beaufort, North Carolina and Subhapradha (1955) reported this species from *Rhinobatus granulatus, R. schlegelli*, and *Rhynchobatis djeddensis* in Indian waters and presented detailed morphological accounts of his specimens.

### *Lecanicephalum peltatum* Linton, 1890

HOST: *Dasyatis americana, new host.*
SITE OF INFECTION: Spiral valve.
LOCALITY: Cartagena, Colombia, *new locality.*
SPECIMENS DEPOSITED: University of Nebraska State Museum, Manter Laboratory No. 20937.

### Remarks

*Lecanicephalum peltatum* was originally described from specimens in *Dasyatis centroura* at Woods Hole, Massachusetts (Linton, 1890). Subsequently, Southwell (1911) reported the species from Ceylon in *Trygon kuhli, Pteroplatea* (=*Gymnura*) *micrura*, and *Pristis cuspidatus*. Baer (1948) presented a detailed study of the morphology of this species based on specimens he collected from *D. centroura* in France.

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# Editor's Note

Authors submitting manuscripts of a survey or taxonomic nature for publication in the Proceedings of the Helminthological Society of Washington are urged to deposit representative specimens in a recognized depository such as the National Parasite Collection at Beltsville, Maryland and include the accession numbers in the manuscript.

# Host-Parasite Relationships of *Spathebothrium simplex* Linton, 1922 (Cestoda: Spathebothriidae) Infecting the Seasnail, *Liparis atlanticus* (Jordan and Everman, 1898) (Osteichthyes: Liparidae)

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ABSTRACT: The host-parasite relationships of *Spathebothrium simplex* Linton, 1922 (Cestoda: Spathebothriidae) infecting the seasnail (*Liparis atlanticus*) were investigated. The fish hosts were collected from one tide pool on the New Hampshire coast during September 1978 through February 1979. The prevalence and mean intensity of *S. simplex* for the entire sampling period were 86% and 3.1, respectively. The coefficient of dispersion of this cestode in parasitized fish was 1.12, indicating that infection by *S. simplex* neither favored nor precluded further infection. The mean intensity of gravid worms was highest in October 1978. Non-gravid individuals were not found in January and February 1979.

*Spathebothrium simplex* was distributed throughout the six fish length classes established, and no size group appeared particularly prone or immune. However, the numbers of gravid and non-gravid worms increased and decreased, respectively, as host length increased. *S. simplex* occurred in the caecal and anterior intestinal area. Male and female seasnails had similar prevalences and mean intensities. Amphipods of the genera *Gammarus* and *Marinogammarus* examined for procercoids of *S. simplex* were negative.

*Spathebothrium simplex* has been found in fish definitive hosts from Woods Hole (Linton, 1922), Puget Sound (Hart and Guberlet, 1936), and California (Nahhas and Krupin, 1977) in North America, and from the Sea of Japan (Yamaguti, 1934; Zhukov, 1960). The histopathological damage to the seasnail, *Liparis atlanticus,* caused by this cestode from the New England coast of North America was investigated by Munson (1974). Investigations on the host-parasite relationships of this species infecting its definitive hosts have not been found in the literature. This is the first study to investigate the host-parasite relationships of *S. simplex* infecting the seasnail.

## Materials and Methods

A total of 106 seasnails (*Liparis atlanticus*) were collected by dip net from one tide pool at Odiorne's Point, Rye, New Hampshire in the period September 1978 through February 1979. Attempts to collect fish in other months were not successful. Seasnails were brought to the laboratory alive, measured, sexed, and necropsied within 12 hr of capture. The entire alimentary canal (from the esophagus to the vent) was removed. The position, number, and degree of development of *Spathebothrium simplex* recovered were recorded. Cestodes were killed and relaxed in distilled water. Two hundred fifty-two individuals were measured in mm by a binocular microscope fitted with an ocular micrometer. Cestodes recovered were divided into two developmental stages: (1) non-gravid—eggs not present in the uterus; and (2) gravid—eggs present in the uterus.

Prevalence is the percent of infected hosts in a given sample. Mean intensity refers to the mean number of worms per infected host. The value following a mean is the standard deviation. The coefficient of dispersion (Sokal and Rohlf,

Table 1.    Prevalence, mean intensity, and maturation of *S. simplex* in 106 *L. atlanticus* examined during September 1978 through February 1979.

| Month | No. fish examined | No. infected (%) | Mean intensity ± SD | Gravid worms | | Non-gravid worms | |
|---|---|---|---|---|---|---|---|
| | | | | Mean intensity ± SD | Mean length (mm) ± SD | Mean intensity ± SD | Mean length (mm) ± SD |
| Sep (78) | 4 | 3 (75) | 2.33 ± 1.5 | 2.01 ± 1.4 | 12.8 ± 3.1 | 1.0 | 13.0 ± 2.9 |
| Oct | 26 | 21 (81) | 3.90 ± 2.2 | 3.90 ± 2.2 | 17.3 ± 4.6 | 3.21 ± 1.8 | 11.3 ± 2.6 |
| Nov | 21 | 17 (81) | 2.71 ± 1.7 | 2.43 ± 1.7 | 17.2 ± 3.6 | 1.75 ± 1.5 | 8.9 ± 2.0 |
| Dec | 26 | 23 (88) | 3.39 ± 1.9 | 2.90 ± 1.6 | 17.5 ± 3.8 | 2.38 ± 2.2 | 11.5 ± 2.6 |
| Jan (79) | 17 | 16 (94) | 2.00 ± 1.3 | 2.00 ± 1.3 | 22.3 ± 5.8 | | |
| Feb | 12 | 9 (75) | 2.56 ± 1.3 | 2.56 ± 1.3 | 20.5 ± 4.2 | | |

1969) was used to test whether the infection of a seasnail by *S. simplex* favored or precluded further infection. Specimens of *S. simplex* from the seasnails have been deposited at the Manter Parasitology Lab of the University of Nebraska State Museum, UNSM, Manter Lab. No. 20976.

## Results and Discussion

Eighty-four percent of 106 seasnails examined from the Rye tide pool were infected with *Spathebothrium simplex*. Similarly, Munson (1974) found that 93% of the seasnails collected from different tide pools along the New Hampshire coast were infected with this cestode. The prevalence of *S. simplex* was above 74% in all months sampled (Table 1). The mean intensity and infection range for the entire sampling period were $3.10 \pm 1.8$ and 0–9. Monthly mean intensities fluctuated and were not significantly different ($P > 0.05$).

The mean intensity of gravid worms was highest in October 1978 and thereafter decreased. The mean length of gravid worms was smallest in September 1978 and then increased to its maximum in January 1979. Non-gravid cestodes were not found in January and February 1979, and their mean intensity, as well as mean length, fluctuated in September through December 1978. A total of 268 (199 gravid and 69 non-gravid) worms were found in the sampling period. The coefficient of dispersion for the infected seasnails was 1.12, and indicates that infection by *S. simplex* neither favored nor precluded further infection.

The prevalence and mean intensity of *S. simplex* did not vary significantly ($P > 0.05$) from one fish length class to another (Table 2). The largest number of

Table 2.    Prevalence, mean intensity, and maturation of *S. simplex* in 106 *L. atlanticus* in six length classes (mm), examined during September 1978 through February 1979.

| Fish length class (mm) | Mean length infected fish | No. examined | No. infected (%) | Mean intensity ± SD | Mean intensity ± SD | |
|---|---|---|---|---|---|---|
| | | | | | Gravid worms | Non-gravid worms |
| 30–39 | 36.3 | 15 | 12 (80) | 3.01 ± 2.1 | 1.33 ± 0.7 | 3.02 ± 1.7 |
| 40–49 | 44.8 | 24 | 17 (71) | 3.29 ± 2.4 | 1.70 ± 0.8 | 3.01 ± 2.0 |
| 50–59 | 54.7 | 26 | 22 (85) | 2.40 ± 1.3 | 2.09 ± 0.9 | 1.40 ± 0.9 |
| 60–69 | 63.7 | 22 | 20 (91) | 3.50 ± 1.7 | 3.45 ± 1.6 | 1.0 |
| 70–79 | 73.1 | 11 | 11 (100) | 2.54 ± 2.0 | 2.54 ± 2.0 | |
| 80–89 | 84.0 | 8 | 7 (88) | 3.42 ± 2.1 | 3.28 ± 1.9 | 1.0 |

worms (a total of 70 for all examined fish of the length class) was found in seasnails that were 60–69 mm in length. The number of gravid worms increased from 12 in hosts 30–39 mm in length to a maximum of 69 in seasnails 60–69 mm in length, and then decreased. A significant negative relationship existed between the mean fish length of each fish length class and the number of non-gravid worms ($r = -0.87$). *S. simplex* infected 39 out of 47 male hosts examined and 50 out of 59 females examined. The mean intensities of infected male and female hosts were $3.36 \pm 1.9$ and $2.76 \pm 1.7$, respectively.

*Spathebothrium simplex* occurred in the anterior intestinal area of seasnails, just below the stomach. On several occasions, the scolices of worms protruded into the pyloric caeca. Similarly, Awachie (1966) found *Cyathocephalus truncatus* in the caeca of brown trout (*Salmo trutta*). Amphipods of the genera *Gammarus* and *Marinogammarus* were found in the stomachs of several of the seasnails examined. One hundred six and 118 individuals of *Gammarus* and *Marinogammarus*, respectively, collected from the same tide pool as fish during September through December 1979, were examined for procercoids of *S. simplex*; all were negative. The possibility of amphipods serving as intermediate hosts should not be ruled out, however, since only a small number of amphipods was examined, and other cestodes, *Cyathocephalus truncatus* and *Bothriomonus sturionus* (Spathebothriidea), are known to use amphipods as intermediate hosts (Awachie, 1966; Burt and Sandeman, 1969; Scott and Bullock, 1974; Amin, 1978).

## Acknowledgment

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# Macracanthorhynchus hirudinaceus from Swine: An Eighteen-Year Record of Acanthocephala from Southern Illinois

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ABSTRACT: An 18-year record of collecting *Macracanthorhynchus hirudinaceus* from southern Illinois meat packing plants has revealed a major decline in infected pigs from a small local packer (DuQuoin Packing Company). This decline reached bottom in 1969 and has remained at that level since that time. However, regional packers (Swift Fresh Meats Company and Hunter Packing Company) have had a stable population of infected pigs and a higher infection rate than that previously observed in pigs processed by the local packer. A comparison of the number of infected pigs per month for the years 1965 and 1975 indicates the worm population, while different in different locations, was stable throughout the year in each site. The decline in infection rate at DuQuoin can be attributed to changes in animal husbandry practices in that area.

We have been routinely collecting Acanthocephala from pigs in southern Illinois for 18 years. During this time, it never occurred to us that parasitologists north and south of Carbondale, Illinois were gradually developing the idea that *Macracanthorhynchus hirudinaceus* was seldom observed north of the Mason-Dixon Line. However, in 1976 Dr. Arlie C. Todd (Department of Veterinary Science, University of Wisconsin) startled us by stating that he did not see this parasite in the swine passing through their clinic and mused that *M. hirudinaceus* "may be on its way out." We thought little about this until Dr. George Cain (Department of Zoology, University of Iowa) remarked that they had difficulty collecting live *M. hirudinaceus*. Dr. S. Patton (Department of Veterinary Pathobiology, University of Tennessee) also discussed this issue with us and added that she seldom saw the parasite. Such widespread difficulty in collecting this helminth suggested to us that a literature search and an analysis of our own collection records would be worth pursuing.

## Materials and Methods

Throughout the year we make regular trips to area packing houses to obtain living specimens of *M. hirudinaceus* in order to study their physiology. Intestines containing these worms are separated from the remaining viscera shortly after evisceration and placed in 35-gallon plastic containers. Intestines are normally collected until the employees have a rest period (90 minutes from our arrival) and then the parasites are removed. Collections have been made through the courtesy of plant managers at DuQuoin Packing Company, DuQuoin, Illinois as well as Hunter Packing Company and Swift Fresh Meats Company in East St. Louis, Illinois. DuQuoin Packing Company is a local meat packer, which during the time of these observations processed 200–300 pigs daily. The animals processed were obtained from local farmers in southern Illinois. In contrast, Hunter Packing Company and Swift Fresh Meats Company are regional meat packers that process 3,000–5,000 animals daily. These animals come from Illinois, Missouri, and Arkansas. We have collected worms at these packing companies throughout the
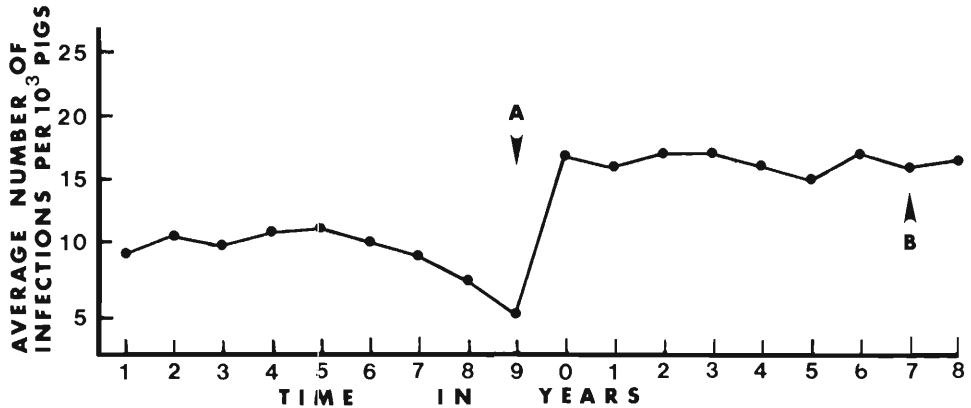
Figure 1. Average number of infections of *M. hirudinaceus* per 1,000 pigs, plotted on a yearly basis beginning in 1961 (1) and ending in 1978 (8). The 1961–1969 collections were made at DuQuoin Packing Company, DuQuoin, Illinois; 1970–1976 were made at Hunter Packing Company, East St. Louis, Illinois; 1976–1978 were made at Swift Fresh Meats Company, East St. Louis, Illinois.

year, but more frequently during the summer when the weather is more suitable for travel. An "exact" determination of the infection rate of animals observed during these trips was not possible by our procedures. We were restricted to those infections which could be determined without interfering with the assembly line procedures of a modern meat packer. While we believe we missed very few, if any, acanthocephalan infections, we could be sure of this only by a much more extensive examination including opening each intestine. Clearly an animal infected with one or two worms is not as likely to be noticed using our collection techniques as one heavily infected. Nevertheless, we believe that the 6–7 seconds available to examine each intestine was adequate to detect 95% of the infections. During this time, the duodenum was visually scanned while being manually rotated and palpated. All initial determinations were based on the presence of "knots" formed as a result of the embedded proboscis. We believe these "knots" indicate all but the earliest infections since even the smallest worms seem to cause this host response.

## Results

Figure 1 shows the average number of infections per thousand pigs for each year since 1960. The collections were made at the DuQuoin Packing Company through 1969. However, the decline in the number of infections during 1967–1969 forced us to begin collecting from a company that processed a larger number of animals. Figure 1-A shows the large abrupt increase in infected animals following our shift to Hunter Packing Company. Figure 1-B indicates when we began collecting from Swift Fresh Meats Company. This change was the result of a prolonged labor strike at Hunter Packing Company and, as is evident from the graph, not because of a decline in infections.

The gradual decline in infections at DuQuoin Packing Company in the 1967–1969 period has no obvious explanation. However, in contrast to the East St. Louis companies, DuQuoin Packing Company is a local packing company that processed animals which were and are obtained largely from southern Illinois
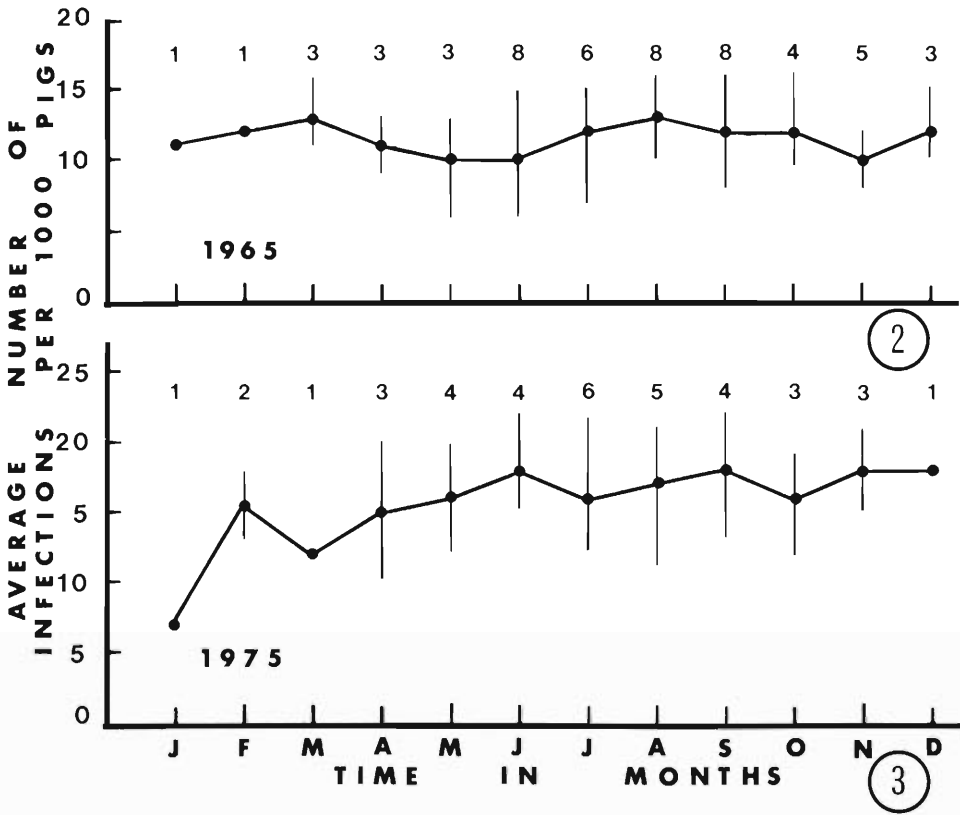
Figures 2, 3. Average number of infections of *M. hirudinaceus* per 1,000 pigs, plotted on a monthly basis for 1965 and 1975. Vertical lines indicate ranges of infections. Numbers above graph indicate number of collections made for the month.

farmers. Since 1970, we have occasionally attempted to collect parasites from DuQuoin Packing Company but without success. We believe the worms still occur locally but in much smaller numbers than formerly. This may be the same trend Drs. Todd and Cain have observed and reflect conditions for the Midwest generally. If that is true, investigators may soon be required to go south of the Mason-Dixon Line to obtain adequate quantities of live worms.

Animal husbandry of pigs in this area has gradually changed during the last two decades. Pigs are presently seldom allowed on the ground and therefore are not exposed to intermediate stages of this parasite. We are unable to explain why results of this shift in farm management practices appear in our data between 1966 and 1970 (Fig. 1). The dramatic increase in the number of infections observed in 1970 can only be attributed to the change in location of collection and to the much larger number of animals obtained from Missouri and Arkansas, which are processed by East St. Louis plants. While the packer has records containing the origin of all animals processed, we have not routinely collected these data.

Figures 2 and 3 compare infection rates at DuQuoin Packing Company in 1965 with those at Hunter Packing Company in 1975. The data show a difference in infection rate between the two sites; however, the rate does not have a seasonal

fluctuation as we expected. We thought that it was more difficult to get worms in the spring of the year but this "feeling" was not substantiated by our data.

These data do not show the intensity of infections or weight of worms per host. We have found as few as one worm and just recently as many as 232 (125 males, 107 females) in a single host. According to Kates (1944), an infection may last for ten months or longer, and since these pigs are normally marketed between 5.5 and 6 months of age, any infection prior to marketing should still be in the pig.

The stability of the population of infected pigs seems to suggest the following. Most commercial hog raisers use practices that preclude infections, whereas the small farmer who markets less than 30 animals at a time is more likely to let his animals run in the field where they may become infected. These animals form a reservoir of infections, albeit in a small number of the total hogs marketed. These "sales" hogs, so called because they are sold to a buyer at a local auction who in turn will sell them to the meat packer, are much more likely to be infected. This lot or group of hogs will be processed together so that when one infection is detected, it is usually followed by several others.

We have also noted that the chances of finding *M. hirudinaceus* are much better when the pigs contain ascaris infections. This probably reflects health care practices of a given producer which, together with his confinement practices, determine the probability of infections. Drug therapy against acanthocephala will also improve. As it does, it will become increasingly difficult to obtain living material to study.

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## Obituary Notice
THOMAS WRIGHT MOIR CAMERON
29 April 1894–1 January 1980

# The Ecology of *Acanthocephalus parksidei* Amin, 1975 (Acanthocephala: Echinorhynchidae) in its Isopod Intermediate Host[1]

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ABSTRACT: The isopod intermediate host of *Acanthocephalus parksidei* Amin, 1975 in the Pike River in southeastern Wisconsin, *Caecidotea militaris* (Hay), appears to have one major breeding season in the late spring–early summer. Cystacanth infections and developmental cycle were closely related to that of its host with initiation of recruitment in the summer following a peak adult *A. parksidei* reproduction in its fish hosts during the spring. Prevalence markedly increased beginning from July, but decreased somewhat toward the spring, possibly affected, at least in part, by parasite-induced mortality of multiply infected hosts. Cystacanth size increased with season and host size. Frequency of infection was higher in male than in female isopods; juveniles and gravid females were never infected. Sex ratio was 1 male:1.6 females. No significant association between parasitism and host depigmentation was observed. It is concluded that an *A. parksidei* generation has a seasonally well-defined life cycle of 1 year in both intermediate and definitive hosts, with a limited degree of carry-over. Cystacanths are shown to be more commonly over-dispersed in their isopod hosts; the negative binomial or truncated negative binomial distributions are the best descriptive patterns in three of five monthly samples analyzed. Regulation of isopods multiply infected with cystacanths (death rate of >0.50) during early spring is indicated.

*Acanthocephalus parksidei* Amin, 1975 was described from 11 fish species in the Pike River, southeastern Wisconsin by Amin (1975a). Its seasonal and host associations from various collecting sites were also reported (Amin, 1975c). The present report deals with the ecology of its cystacanths in the isopod intermediate host collected primarily from a Pike River site from which fish were most heavily and frequently infected with adult worms.

## Materials and Methods

Of the seven Pike River sites from which *A. parksidei* adults were described, fish from site $\alpha 6$ were most heavily and frequently infected (Amin, 1975c). This main stream site is relatively wide and bordered by a strip of southern mesic hardwoods providing an ample supply of leaf litter. Except for 2 months, regular monthly collections of isopods from leaf litter and occasionally from algae were made from May 1976 to May 1977. The hard river freeze during this record cold winter made collections impossible in January and February 1977. Isopods were then refrigerated in stream water until examined shortly thereafter. Upon dissection, they were sexed and measured to nearest mm from anterior tip of cephalothorax to posterior tip of telson. Recovered cystacanths were refrigerated overnight in distilled water and then fixed in cold AFA before being processed and permanently mounted.

Comparative collections from site 2 (a small polluted channeled branch in an open field) from which fish hosts were minimally infected (Amin, 1975c) yielded some isopods only during the summer of 1976.

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[1] Presented at the Fourth International Congress of Parasitology, Warsaw, Poland, August 19–26, 1978 by the first author.
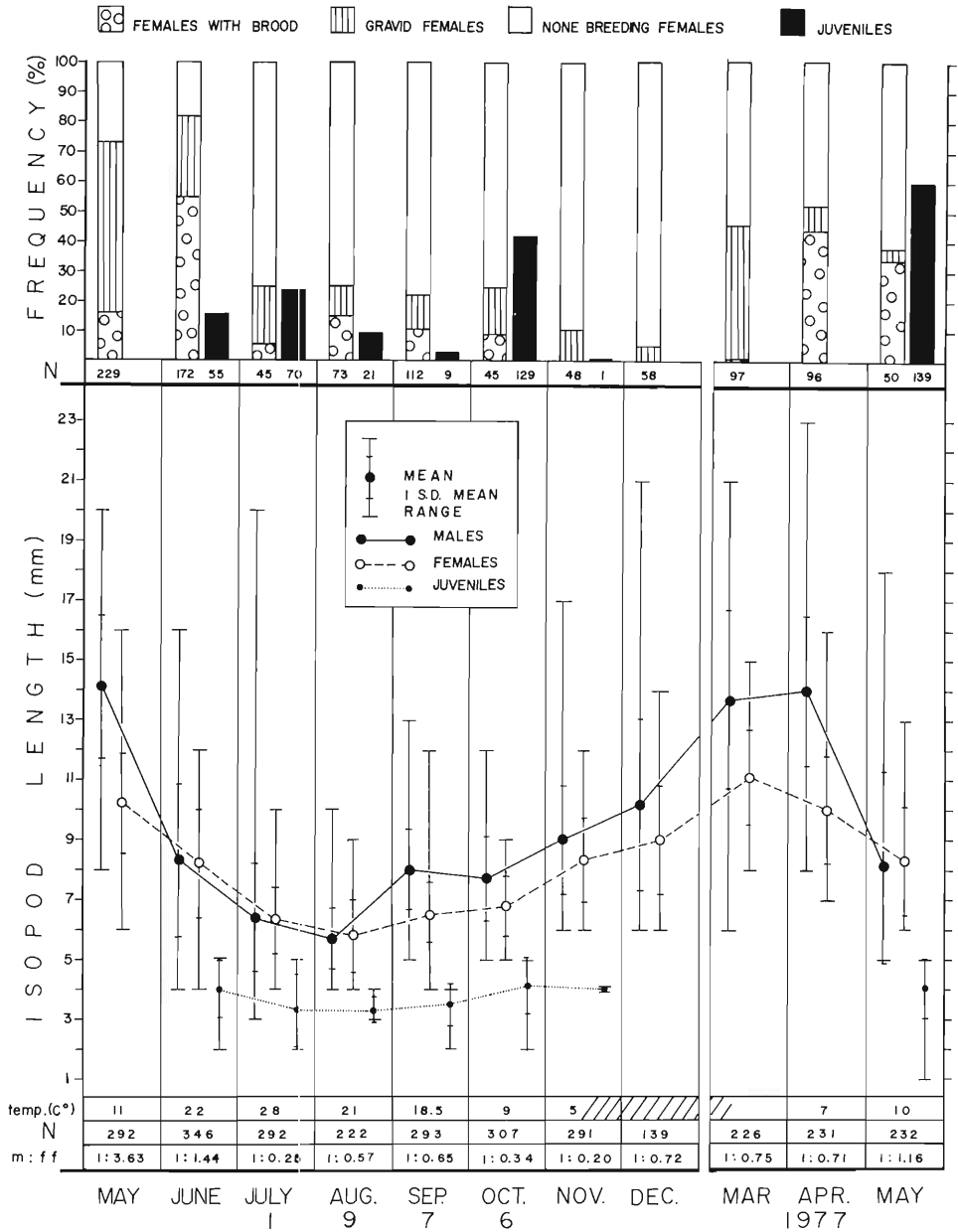
**Figure 1.** Seasonal variations in frequency, size (length), and sex ratio of *Caecidotea militaris* from the Pike River 1976–1977. Top: monthly frequency of juveniles in whole collection and of breeding and nonbreeding stages in female collection. Bottom: monthly changes in size and sex ratio. The shaded area (water temperature) indicates that the river was frozen over.

Unless otherwise specified, mean values are based on the number of infected hosts. The term cystacanth is used collectively and includes, where applicable, the few acanthellas recovered early in the season. Description of these developmental stages will be published elsewhere.

Types of dispersion of cystacanths were tested by chi-square analyses of variance:mean ratios. Observed distributions of cystacanths among their hosts were compared with five theoretical distributions. These were Poisson, negative binomial, positive binomial, normal, and lognormal distributions generated by algorithms adapted from those of Davies (1971). Tests of goodness of fit were accomplished by using chi-square analyses corrected for bias (Zar, 1974). Cystacanth populations that failed to fit any of the five theoretical distributions ($P < 0.05$) were compared to truncated negative binomial, positive binomial, and Poisson distributions. These distributions were generated in an iterative program described by Hirsch (1980). Basically, the program tests goodness of fit to theoretical distributions over a user-specified range of parameter required to characterize each distribution ($\bar{x}$, $k$ and/or $P$). Truncation begins at various user-specified infection levels and intensity. Four patterns of host mortality were tested: (1) absolute, (2) linear, (3) exponential, and (4) logarithmic.

## Results and Discussion

### I. The host

IDENTITY: Only *Asellus militaris* Hay, 1878 was recovered and found infected with *A. parksidei* cystacanths in this study. This isopod species was synonymized with *A. intermedius* Forbes, 1876 by Williams (1970) and later relegated to the genus *Conasellus* Stammer, 1932 by Henry and Magniez (1970). *Conasellus* is now regarded as a junior synonym of *Caecidotea* Packard, 1871 by Bowman (1975). Accordingly, the correct name for the Pike River asellid is to be *Caecidotea intermedius* (Forbes).

However, (1) local asellids are clearly referable to *militaris* populations as distinct from those of *intermedius* sensu Forbes according to Makin (1940) and Pennak (1953); (2) although Van Name (1936) synonymized *A. militaris* with *A. communis* Say, 1818 as correctly stated by Williams (1970), he (Van Name, 1942) later recognized *A. militaris* as a valid species; (3) Williams (1970) based his synonymy on the examination of only two male and two female *A. militaris* and parts of a few others "believed" to be Hay's material; (4) Ellis (1961) distinguished *A. militaris* from *A. intermedius* from Michigan and noted distinct biological differences, e.g., length of incubation period was 25% shorter in the first than in the second form. Based on the above, the Pike River asellid will be referred to as *Caecidotea militaris* (Hay).

In a recent study by Amin (1978), the amphipod *Pontoporeia affinis* (Lindström) from Lake Michigan locations (where the Pike River drains) was found infrequently infected with *A. parksidei* cystacanths.

SEASONAL PICTURE: A total of 2,871 isopods was examined: 1,422 males (49.5%), 1,025 females (35.7%), and 424 juveniles (14.8%). The overall length of these forms was 3–23 mm (mean of 9.50), 4–16 (8.66), and 1–5 (3.92), respectively. The 1,025 females examined were classified as gravid (carrying eggs, 27.1%), with brood (carrying young, 21.6%), or non-breeding (51.3%). The seasonal pattern of frequencies within each of the breeding classes indicates one major breeding season in late spring–early summer (peak of breeding females with eggs or young) followed by an increasing proportion of juveniles (Fig. 1, top). This does not exclude the possibility of more than one isopod generation per year. The breeding season appears to extend through the autumn and to have occurred earlier in

Table 1. Seasonal sex ratio and frequency and intensity of *Acanthocephalus parksidei* cystacanth infections in adult *Caecidotea militaris* from the Pike River, 1976–1977.

| Month* | Infections in male isopods | | | | | Infections in female isopods | | | | | Infections in males and females | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Isopods examined | No. (%) infected | Parasites recovered | Mean/ host (SD) | Maximum/ host | Isopods examined | No. (%) infected | Parasites recovered | Mean host (SD) | Maximum/ host | Isopods examined | No. (%) infected | Parasites recovered | Mean host (SD) | Maximum host | Parasite sex ratio (M:F) |
| 1976 | | | | | | | | | | | | | | | | |
| May | 63 | 0 | 0 | 0 | 0 | 229 | 3 (1.3) | 5 | 1.67 (0.47) | 2 | 292 | 3 (1.0) | 5 | 1.67 (0.47) | 2 | 1:1.5 |
| Jun | 119 | 1 (0.8) | 1 | 1.00 | 1 | 172 | 2 (1.1) | 2 | 1.00 | 1 | 291 | 3 (1.0) | 3 | 1.00 | 1 | 0:3.0 |
| Jul | 177 | 14 (7.9) | 17 | 1.21 (0.41) | 2 | 45 | 1 (2.2) | 1 | 1.00 | 1 | 222 | 15 (6.8) | 18 | 1.20 (0.44) | 2 | 1:2.0 |
| Aug | 128 | 27 (21.1) | 47 | 1.74 (1.73) | 10 | 73 | 21 (28.8) | 33 | 1.57 (0.58) | 3 | 201 | 48 (24.0) | 80 | 1.67 (1.36) | 10 | 1:1.2 |
| Oct | 133 | 1 (0.7) | 1 | 1.00 | 1 | 45 | 1 (2.2) | 1 | 1.00 | 1 | 178 | 2 (1.1) | 2 | 1.00 | 1 | 0:2.0 |
| Nov | 242 | 31 (12.8) | 39 | 1.27 (0.81) | 5 | 48 | 8 (16.7) | 11 | 1.38 (0.48) | 2 | 290 | 39 (13.4) | 50 | 1.28 (0.75) | 5 | 1:1.6 |
| Dec | 81 | 22 (27.2) | 25 | 1.14 (0.34) | 2 | 58 | 3 (5.2) | 3 | 1.00 | 1 | 139 | 25 (18.0) | 28 | 1.12 (0.32) | 2 | 1:2.1 |
| 1977 | | | | | | | | | | | | | | | | |
| Mar | 129 | 15 (11.6) | 21 | 1.40 (0.90) | 4 | 97 | 11 (11.3) | 15 | 1.36 (0.77) | 3 | 226 | 26 (11.5) | 36 | 1.38 (0.84) | 4 | 1:1.8 |
| Apr | 135 | 15 (11.1) | 18 | 1.20 (0.75) | 4 | 96 | 5 (5.2) | 6 | 1.20 (0.40) | 2 | 231 | 20 (8.7) | 24 | 1.20 (0.68) | 4 | 1:1.4 |
| Total | 1,207 | 126 (10.44) | 169 | 1.34 (1.03) | 10 | 863 | 55 (6.37) | 77 | 1.40 (0.61) | 3 | 2,070 | 181 (8.74) | 246 | 1.36 (0.94) | 10 | 1:1.6 |

* No collections were available in January and February 1977. Isopods examined during September 1976 (293) and May 1977 (232) were not infected and are not included.

Table 2. Seasonal variation in the length of *Acanthocephalus parksidei* cystanths.

| Month | Male parasite length (mm) | | | Female parasite length (mm) | | |
|---|---|---|---|---|---|---|
| | N* | Mean (SD) | Range | N* | Mean (SD) | Range |
| 1976 | | | | | | |
| May | 2 | 0.84 (0.40) | 0.44–1.24 | 3 | 2.85 (1.43) | 1.32–4.76 |
| Jun | 0 | | | 2 | 5.37 (0.03) | 5.34–5.40 |
| Jul | 4 | 1.60 (0.72) | 0.92–2.68 | 8 | 3.40 (2.46) | 1.11–7.20 |
| Aug | 36 | 1.56 (0.36) | 0.48–2.12 | 39 | 3.14 (1.31) | 1.40–6.24 |
| Oct | 0 | | | 2 | 2.47 (0.11) | 2.36–2.58 |
| Nov | 17 | 3.27 (0.26) | 2.72–3.68 | 29 | 7.61 (0.77) | 5.24–8.68 |
| Dec | 5 | 1.78 (0.39) | 1.06–2.20 | 16 | 4.11 (1.31) | 1.58–7.80 |
| 1977 | | | | | | |
| Mar | 12 | 3.57 (0.37) | 2.72–4.16 | 21 | 9.27 (1.32) | 6.52–12.56 |
| Apr | 9 | 3.55 (0.20) | 3.24–3.92 | 13 | 9.54 (1.09) | 6.64–11.26 |
| Total | 85 | 2.27 (1.03) | 0.44–4.16 | 133 | 4.72 (3.17) | 1.11–11.26 |

* Number of specimens measured.

1977 than in 1976. The high frequency of juveniles during October may not be significant, as it was not directly preceded by a peak of breeding females, but might, however, indicate the presence of additional summer–fall breeding generation(s).

The seasonal frequency in the sizes of males, females, and juveniles corresponded with their seasonal development, being smallest in the summer and increasing with growth and maturity to a peak in the spring. Sex ratio was more highly in favor of females than males only in the breeding populations of May and June (Fig. 1, bottom).

## II. The parasite

FREQUENCY AND INTENSITY OF INFECTION: None of the 424 juveniles or 278 gravid females examined were infected. The pattern of their feeding activity during the breeding season when they are most abundant (Fig. 1, top) might be involved; relative activity of female *Caecidotea communis* (Say) was shown to decrease significantly in breeding populations (Allee, 1929). Overall, frequency of infection was higher in male isopods (10.44%) than in females (6.37%), but the mean per host was similar (1.34, 1.40) (Table 1). As gravid females accounted for 27.1% of all females examined, there were proportionally fewer females available for infection. Mean and prevalence of *Acanthocephalus dirus* Van Cleave, 1931 cystacanth infections were similar in both sexes of *Caecidotea intermedius* (Forbes) up to 8 mm in length but the prevalence pattern subsequently varied in larger males and females (Seidenberg, 1973). Figures from our study are considerably lower than those found by Seidenberg (1973) for *A. dirus* cystacanths in *C. intermedius*, but more comparable to those of Muzzall and Rabalais (1975) for *A. jacksoni* Bullock, 1962 in *Lirceus lineatus* (Say) and Muzzall (1978) for *Fessisentis friedi* (Haley and Bullock, 1953) in *C. communis*. Many individuals of an acanthocephalan species, currently deposited in Amin's collection and determined by him as *A. dirus*, were collected by J. Camp (Illinois State University, Normal) from the creek chub, *Semotilus atromaculatus*, in a tributary of the

**Table 3.** The length of *Acanthocephalus parksidei* cystacanth in relation to host length.

| Isopod length (mm) | Male parasite length (mm) | | | Female parasite length (mm) | | |
|---|---|---|---|---|---|---|
| | N* | Mean (SD) | Range | N* | Mean (SD) | Range |
| 4 | 1 | 0.96 | | 0 | | |
| 5 | 5 | 1.54 (0.21) | 1.24–1.80 | 5 | 2.19 (0.59) | 1.40–3.04 |
| 6 | 21 | 1.63 (0.55) | 0.48–3.36 | 25 | 3.04 (1.76) | 1.16–8.72 |
| 7 | 10 | 1.84 (0.69) | 1.00–3.24 | 14 | 5.81 (2.28) | 2.16–8.26 |
| 8 | 13 | 2.43 (1.02) | 1.06–3.80 | 22 | 5.08 (2.79) | 1.04–10.88 |
| 9 | 7 | 2.82 (0.65) | 1.34–3.40 | 12 | 5.55 (2.21) | 1.11–8.68 |
| 10 | 6 | 3.18 (0.57) | 2.20–3.90 | 11 | 7.68 (1.42) | 3.84–9.48 |
| 11 | 6 | 3.17 (0.89) | 1.24–3.92 | 12 | 7.50 (2.28) | 2.46–11.12 |
| 12 | 6 | 2.95 (1.13) | 0.44–3.68 | 13 | 6.32 (3.25) | 1.32–10.34 |
| 13 | 4 | 3.61 (0.27) | 3.36–4.04 | 9 | 8.27 (2.14) | 4.76–12.56 |
| 14 | 3 | 3.69 (0.25) | 3.36–3.96 | 3 | 10.09 (0.51) | 9.44–10.68 |
| 15 | 3 | 2.96 (1.24) | 1.28–4.24 | 2 | 9.02 (0.10) | 8.92–9.12 |
| 16 | 0 | | | 2 | 10.58 (0.68) | 9.90–11.26 |
| 17 | 2 | 3.78 (0.38) | 3.40–4.16 | 0 | | |
| 18 | 0 | | | 1 | 10.64 | |

* Number of specimens measured.

Illinois River near Mud Creek, McLean County, where Seidenberg (1973) made his collections, suggesting that Seidenberg's species was *A. dirus* as correctly reported.

Intensity of infection did not show marked seasonal variation. Prevalence of infection was lowest in May and June 1976 (with high proportions of non-infected juveniles and gravid females), significantly picked up in July and August, but declined relatively afterwards. The lower prevalences during the supposed peak spring months might suggest a higher rate of predation of infected isopods by fish hosts in this system than in Seidenberg's (1973; with a pronounced spring peak). The termination of the cycle after April is apparently brought about by the turnover of the host generation (Fig. 1). The zero or very low figures of September and October might suggest two cystacanth generations per year. However, seasonal variations in the lengths of cystacanths (Table 2) and isopods (Fig. 1) do not support this. The larval cycle in isopods appeared compatible with that of adults in their fish hosts and preceded it by 1 to 2 months. The cystacanth cycle started significantly in July and ended in April (above); the adult cycle began in late summer and ended in May (Amin, 1975c).

COLLECTING SITE: Fish were heavily infected with adult *A. parksidei* in site α6 from which the above isopod collections were made, but virtually none were infected in site 2 (Amin, 1975c). Attempted isopod collections from the latter site produced only 238 specimens during the summer of 1976; none was infected. The absence of leaf litter in this polluted open field site and the resulting scarcity of isopods there appear to be responsible for this adult infection pattern.

SEX RATIO: Overall cystacanth sex ratio was 1 male:1.6 females. The ratio was consistently in favor of females during all months (Table 1) and the reason for its deviation from the expected genetic sex ratio of 1:1 is not clear.

SIZE: Parasite length was found to be affected by two variables: season and host length. Male and female cystacanths increased in length from 0.44 to 1.24

Table 4. Distributions of cystacanths in their isopod hosts.

| No. cystacanths per host | Aug | Nov | Dec | Mar | Apr |
|---|---|---|---|---|---|
| 0 | 174 | 252 | 114 | 200 | 211 |
| 1 | 32 | 32 | 22 | 21 | 18 |
| 2 | 13 | 5 | 3 | 1 | 1 |
| 3 | 1 | 1 | | 3 | |
| 4 | 1 | | | 1 | 1 |
| 5 | | 1 | | | |
| 10 | 1 | | | | |
| Mean | 0.338 | 0.172 | 0.201 | 0.159 | 0.104 |
| Variance | 0.831 | 0.267 | 0.206 | 0.277 | 0.154 |
| $k$ | 0.373 | 0.395 | * | * | * |

\* No good fit to negative binomial.

and 1.32 to 4.76 mm in May 1976 to 3.24 to 3.92 and 6.64 to 11.26 mm in April 1977, respectively (Table 2). The latter measurements approximate those of adult *A. parksidei* from fish hosts, 2.28 to 4.24 and 4.00 to 14.64 (Amin, 1975a). Similarly, male and female cystacanths increased in length with increased isopod size (Table 3), which is also a function of season (Fig. 1). The possible effect of host sex on parasite length was considered but found to be not significant ($t$-test at 5%).

PIGMENTATION: The total frequency of infection was 6.58% in pigmented isopods and 18.18% in nonpigmented specimens. Using a $\chi^2$ test, the difference was significant at the 5% level but not at 1%. Seidenberg (1973) reported up to 91% of *C. intermedius* infected with *A. dirus* cystacanths being nonpigmented. The corresponding percentage in this study was 3%. *Acanthocephalus parksidei* cystacanths do not appear significantly to cause *C. militaris* depigmentation. The difference in these values between the two closely related parasite and host species is clearly parasite and/or host species specific.

POPULATION DISTRIBUTION: Data for five of the monthly samples were adequate for analyses of the dispersion of cystacanths in their isopod hosts. Observed distributions (Table 4) were tested against expected normal, Poisson, binomial, negative binomial, and lognormal patterns. For two samples (December and April), the observed distributions gave good fits to Poisson (or binomial) distributions (Table 5) but not to others. Two others (August and November) fit the negative binomial, but not other distributions. The March distribution did not fit any of these five distributions, but gave good fits ($P > 0.80$) to a variety of

Table 5. Probability of fit of observed against expected distributions.

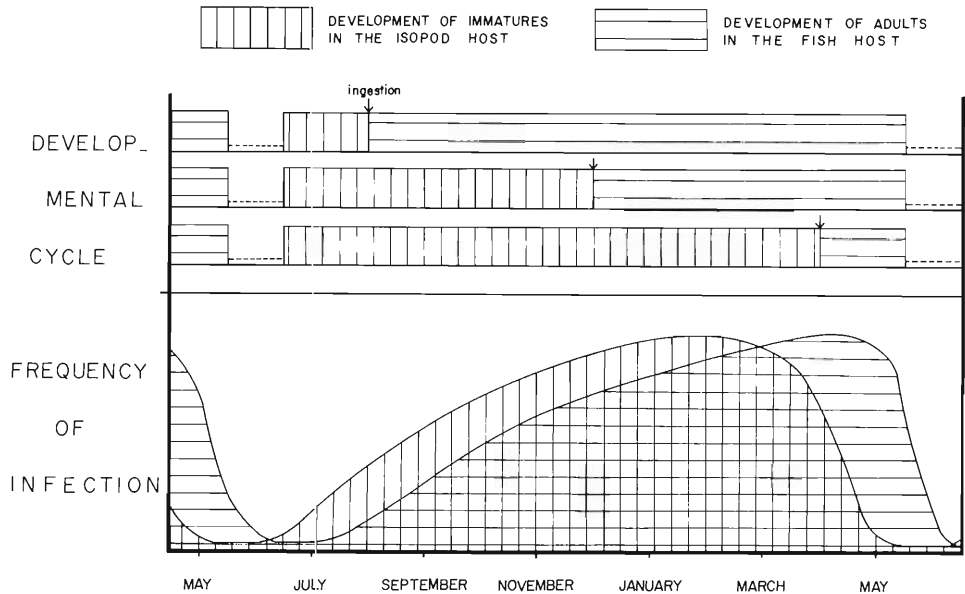| Distribution | Aug | Nov | Dec | Mar | Apr |
|---|---|---|---|---|---|
| Normal | $10^{-4}$ | $10^{-6}$ | $10^{-6}$ | $10^{-6}$ | $10^{-6}$ |
| Poisson | $10^{-3}$ | 0.02 | 0.70 | 0.02 | 0.26 |
| Binomial | $10^{-4}$ | 0.01 | 0.58 | 0.01 | 0.21 |
| Negative binomial | 0.37 | 0.52 | $10^{-6}$ | 0.03 | $10^{-6}$ |
| Lognormal | $10^{-4}$ | $10^{-6}$ | $10^{-6}$ | $10^{-6}$ | $10^{-6}$ |

Figure 2. A schematic representation (not to scale) of the developmental cycle of immature and adult *Acanthocephalus parksidei* in their respective hosts in the Pike River. The summer decline in adult frequency and the early spring peak in that of immatures are exaggerated.

truncated negative binomial distributions, using any of the four patterns of host mortality, *k* values of 0.20–0.35 and death rates of 0.50–0.99.

The fact that the observed pattern best fitting the truncated negative binomial distribution was obtained only in March when cystacanth populations reached their theoretical peak (Fig. 2) is significant. This distribution pattern suggests that there was host mortality due to multiple parasitic infections, which agrees with previous field and laboratory observations that the slightest touch of a multiply infected isopod will literally burst its abdomen and release the relatively large and variably positioned cystacanths. This observation was noted during November and March but was most frequent in the latter month and occasionally with as few as two worms per isopod. This further agrees with Seidenberg's (1973 and others quoted therein) suggestion that "the disappearance of the heavily infected isopods may result from mortality caused by growth of the numerous larvae present in the early part of the life cycle of the parasite." The March data suggest that truncation may begin with two worms per host. It is in early spring, e.g., March, that cystacanths attain their largest size (Table 2) and highest theoretical level of infection (Fig. 2). Accordingly, one would expect that it is in the same season (early spring) that such possible regulation due to mortality of heavily infected hosts will lower the levels of infections. This happens to be the case (Table 1). It should be noted, however, that removal of infected individuals from the isopod population may also be caused by increased predation of these isopods by the definitive host during the spring, to which these data do not directly pertain.

## Conclusions

Cystacanth developmental cycle was closely related to that of its isopod host, each with one generation per year. The cystacanth cycle was also closely synchronized with that of the adults in the fish hosts and preceded it by 1 to 2 months in terms of initiation (July in cystacanths and later in the summer in adults), peaking ("November–April" and May), and termination (May and June); infected isopods are not totally absent during the summer (Fig. 2). The major initial recruitment of acanthors in July closely follows the peak adult *A. parksidei* breeding population of May (Amin, 1975c). In addition, there is a corresponding seasonal increase in frequency of infection which parallels another seasonal increase in size of cystacanths as well as isopods. It is thus concluded that, although *A. parksidei* adults and cystacanths can be found throughout the year, the parasite has a well-defined seasonal cycle with a life span of about 1 year in both intermediate and definitive hosts (Fig. 2). A collection of 137 cystacanths during July 1978 indicates that carry-over does occur but not to any extent that might significantly alter the basic pattern shown in Figure 2; about 25% were relatively large well-developed cystacanths like those recovered later in the season. In the autumn, a similar proportion of sexually mature adults was recovered from fish (Amin, 1975c). These were probably ingested as older cystacanths (above). "A partial overlap of generations was observed" (Amin, 1975c).

Growth of adult worms in their fish hosts was studied by Amin (1975b) and its seasonal pattern parallels that of cystacanth seasonal increase in size. In other words, cystacanths ingested during the winter are larger and more developed than those ingested earlier during the autumn and will require less time to mature to adulthood and to reproduce in the fish host. The longer time required for the development in the fish host of the younger cystacanths ingested during the autumn would bring their sexual maturity in the fish in line with that of the first group above (Fig. 2). No evidence of a fish paratenic host due to ingestion of incompletely developed *A. parksidei* (in their normal isopod host) has been encountered.

Cystacanths are shown to be more commonly overdispersed in their isopod hosts, with the negative binomial or truncated negative binomial distributions being the best descriptive patterns in three of the 5 months analyzed. A truncated negative binomial distribution in early spring supports other observation suggesting mortality of hosts multiply infected with larger worms during this season.

## Acknowledgments

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# Notes on the Biology of Three Trematodes (Digenea: Cryptogonimidae)

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ABSTRACT: We studied several aspects of the life histories of *Caecincola latostoma* Greer and Corkum, 1979, *Cryptogonimus spinovum* Greer and Corkum, 1979, and *Textrema hopkinsi* Dronen, Underwood, and Sunderman, 1977 in south Louisiana. Under natural conditions *C. latostoma* and *T. hopkinsi* cercariae exhibit a bimodal, diurnal emergence. In the three species we noted an inverse relationship between the rates of metacercarial development and adult maturation and a strong correlation between the prevalence of each species in the molluscan host and its prevalence and intensity in the second intermediate and definitive hosts.

In a previous report (Greer and Corkum, 1979) we described the life cycles of three cryptogonimid trematodes: *Caecincola latostoma* and *Cryptogonimus spinovum,* described in that study, and *Textrema hopkinsi* Dronen, Underwood, and Sunderman, 1977. The three species use the same hosts: *Cincinnatia peracuta* (Pilsbury and Walker) is the molluscan host; *Micropterus salmoides* (Lacépède), *Elassoma zonatum* (Jordan), and several species of *Lepomis* are the second intermediate hosts; and *M. salmoides* and *M. punctulatus* (Rafinesque) are the definitive hosts.

In the present study we report patterns of cercarial emergence, metacercarial and adult development, and population densities in the vertebrate and invertebrate hosts of these three sympatric species.

## Materials and Methods

Infected hosts were collected from False River, an oxbow lake in Pointe Coupee Parish, and from Beaver Pond Branch, a small stream in Livingston Parish, Louisiana. *Micropterus salmoides, Lepomis machrochirus* (Rafinesque), and *L. megalotis* (Rafinesque) were collected for experimental use from areas where infections are absent.

Positive identification of cercariae was determined by rearing representatives to the metacercarial stage in previously uninfected sunfishes.

To determine cercarial emergence patterns under natural conditions, four *C. peracuta* infected with *C. latostoma* and four with *T. hopkinsi* were isolated and maintained at Beaver Pond Branch in vials of filtered pond water which were partially submerged along the bank of the stream. During the experimental periods, water temperature in and around the vials varied not more than 1°C. On August 25 and 26, 1975, at 2-hour intervals for 48 hours, the contents of the vials were removed and preserved in 70% ethanol. Measured samples were aspirated through Whatman No. 1 filter paper on a Buchner funnel. Dried filter papers were sprayed with 1% ninhydrin and cercariae counted after color development.

The entire body musculature of nine False River *L. macrochirus,* 2.0–2.8 cm
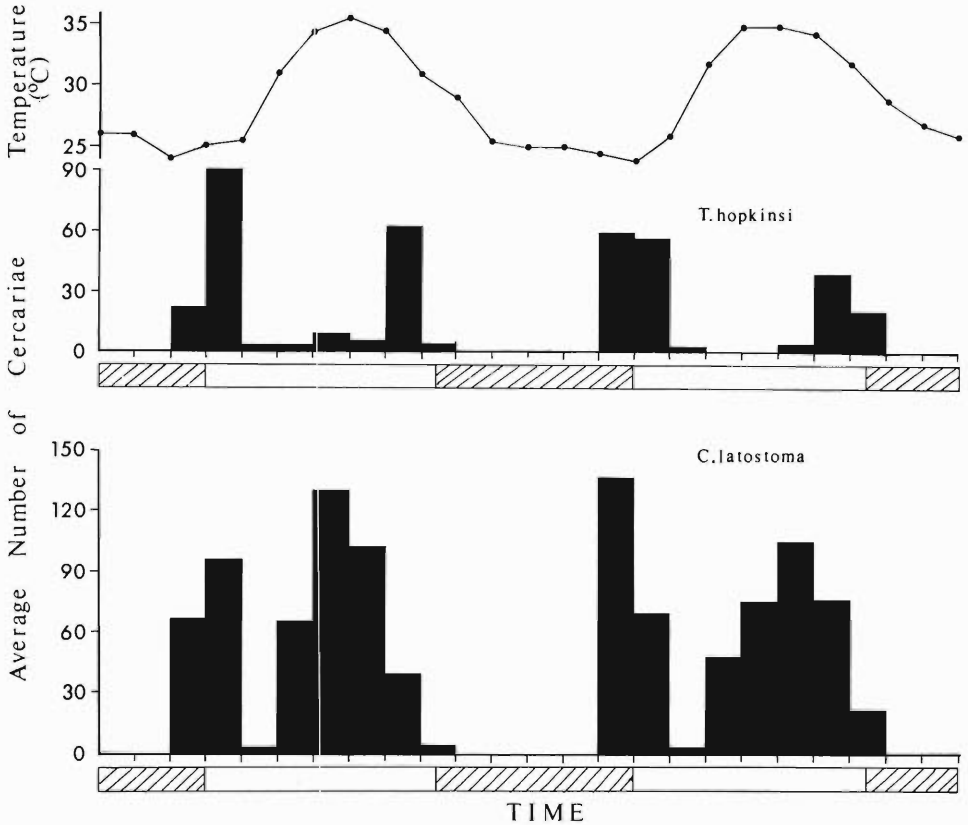
Figure 1.    Cercarial emergence patterns for *Caecincola latostoma* and *Textrema hopkinsi* from naturally infected *Cincinnatia peracuta*. Eight snails, four infected with *C. latostoma* and four with *T. hopkinsi*, were maintained under natural conditions at Beaver Pond Branch, Livingston Parish, Louisiana. Samples were taken at 2-hour intervals over a 48-hour period from August 25 to 26, 1975. □ represents day and ■ represents night, as determined by the official sunrise and sunset. Temperatures are of habitat water.

in length, was carefully dissected and examined for metacercarial infections and the results were quantified. Adult worm burdens were determined for 41 *M. salmoides* of 0.2–2.5 kg collected from False River between April 7, 1975 and December 8, 1976. The intestinal tracts were cut into three equal lengths and examined for parasites.

## Results and Discussion

### Cercarial emergence (Fig. 1)

Cercariae of *C. latostoma, C. spinovum,* and *T. hopkinsi* are morphologically similar and exhibit no behavioral differences in the laboratory (Greer and Corkum, 1979). However, we observed slight differences in the emergence patterns of *C. latostoma* and *T. hopkinsi* cercariae under natural conditions. The similar diel rhythms of both species suggest a correlation between daylight and cercarial emergence, but light alone does not explain their bimodal patterns. Likewise, the timing of the peak emergence periods indicates that neither light intensity nor

| Parasite | Minimum Development Period for Metacercariae (Days) | | | | | Minimum Period Before Egg Production by Adults (Days) | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 5 | 10 | 15 | 20 | 0 | 5 | 10 |
| C. latostoma | | | | | | | | |
| C. spinovum | | | | | | | | |
| T. hopkinsi | | | | | | | | |

Figure 2. Comparisons of *C. latostoma*, *C. spinovum*, and *T. hopkinsi* metacercarial and adult development in *L. macrochirus* and *M. salmoides*, respectively, under laboratory conditions.

temperature is solely responsible for eliciting emergence of *C. latostoma*. These factors may be of importance for *T. hopkinsi* where both peaks occur during periods of the day (morning and evening) when temperatures are nearly equivalent and light intensities are approximately at the same level. Two potential sources of stimuli may be eliciting cercarial emergence: changes in the external environment and physiological changes in the host. Neither light nor temperature directly elicits emergence of *C. latostoma* and *T. hopkinsi*, but these physical factors, in conjunction with snail-mediated stimuli, may account for the emergence patterns we observed.

Lundahl (1941) reported two periods of emergence for *C. parvulus* cercariae in the laboratory: between 4 and 7 P.M. and between midnight and dawn. Though some of the disparities between *C. parvulus* and *C. latostoma* may be due to different experimental designs, the patterns probably reflect a fundamental difference between these congeners. Cable (1972) cited several examples of distinct emergence patterns among closely related trematode genera. The differences undoubtedly have evolved as a result of selection that favors cercarial transmission to the next host. Centrarchid and cyprinid fishes are the second intermediate hosts for *C. parvulus* (Lundahl, 1941), whereas *M. salmoides*, *E. zonatum*, and several species of *Lepomis* are such hosts for *C. latostoma*. Differences in cercarial emergence of these congeners may have evolved as a result of selective pressures derived from differences in the behavior of the fish hosts.

**Metacercarial and adult development** (Fig. 2)

Feeding experiments showed different rates of development for metacercariae and adults of *C. latostoma*, *C. spinovum*, and *T. hopkinsi*. Uninfected sunfishes, *L. macrochirus* and *L. megalotis*, were individually exposed for 1 day to cercariae of one of the three species. At intervals thereafter the sunfishes were fed to *M. salmoides*, which were subsequently killed and examined. The minimum time for cercariae to develop to infective metacercariae was 14 days for *C. latostoma*, 16 days for *C. spinovum*, and 18 days for *T. hopkinsi*. In repeated experiments we found the egg production begins by the 8th day after ingestion of *C. latostoma* metacercariae, by the 5th day in *C. spinovum*, and as early as
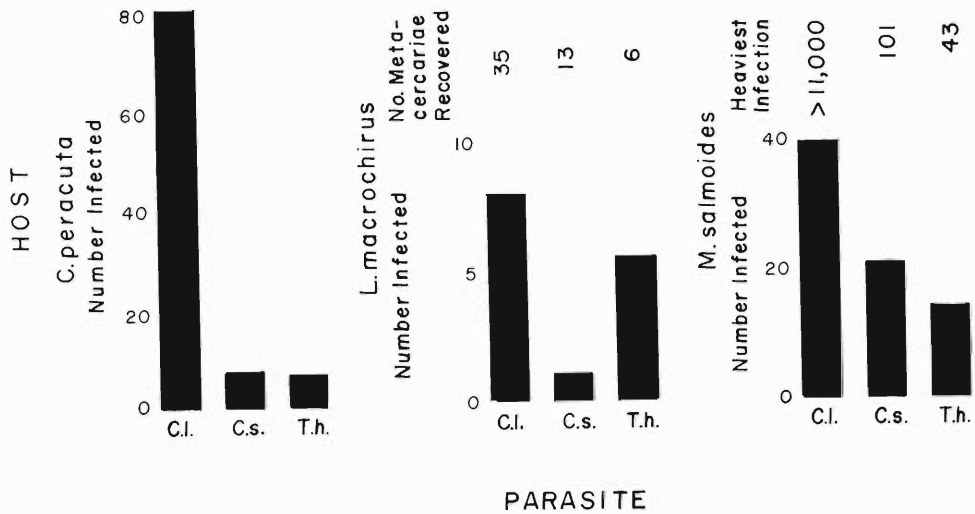
Figure 3.   Comparison of *C. latostoma* (C.l.), *C. spinovum* (C.s.), and *T. hopkinsi* (T.h.) infection levels in the snail (*C. peracuta*), second intermediate (*L. macrochirus*), and definitive (*M. salmoides*) hosts from False River, Pointe Coupee Parish, Louisiana. Results are based on examination of 6,204 *C. peracuta*, nine *L. macrochirus*, and 41 *M. salmoides*.

the 3rd day in *T. hopkinsi*. These data imply an inverse relationship between the developmental periods for the metacercariae and adults of these species.

### Parasite populations at False River (Fig. 3)

Of various snails examined in quantities, only *C. peracuta* shed the cryptogonimid cercariae under study. About 1.5% of 6,204 snails screened were infected: 83 with *C. latostoma,* seven with *C. spinovum,* six with *T. hopkinsi,* and one with both *C. latostoma* and *C. spinovum.*

We recovered metacercariae of *C. latostoma,* *C. spinovum,* and *T. hopkinsi* from the body musculature of *L. machrochirus,* *L. megalotis,* *L. gulosus,* (Cuvier) and *E. zonatum,* but not from other centrarchid or cyprinid fishes. Numbers of metacercariae of each cryptogonimid species were recovered from *L. macrochirus* in roughly the same relative proportions as were parthenitae from the snail host and adults from the definitive hosts.

*Caecincola latostoma* was found in the pyloric ceca and anterior intestine of 39 of 41 *M. salmoides.* The worms usually extended into the middle, and occasionally to the posterior third, of the intestine. The large numbers of worms found in *M. salmoides* made exact determination of worm burdens impracticable. However, based on counts made from pyloric ceca from several fish we projected an average infection of several thousand worms.

*Cryptogonimus spinovum* occupied the pyloric ceca and occasionally the anterior intestine of 21 of 41 bass. The most heavily infected fish harbored 101 worms.

Adults of *T. hopkinsi* were present in 14 bass and were restricted to the middle third of the intestine. The heaviest infection encountered was 43 worms.

Of the 39 largemouth bass harboring at least one cryptogonimid species, 23%

were infected with all three; 13% with both *C. latostoma* and *T. hopkinsi*; 31% with *C. latostoma* and *C. spinovum*; and 33%, mostly smaller fish, with only *C. latostoma*. Experimental infections indicated that location of these parasites in the host was not affected by the number of species present.

## Conclusions

Similarities in the life histories and microhabitats of *C. latostoma, C. spinovum,* and *T. hopkinsi* from False River raise questions about the possible effects of interspecific competition on these populations. Pianka (1973) considered the three main resources apportioned among competitors to be space, food, and time. An obvious, but not absolute, apportioning of the microhabitat exists between adults of *T. hopkinsi* and the other two species. However, no spatial segregation was evident between *C. latostoma* and *C. spinovum*.

Although an unequivocal determination of competition was not demonstrated, the greater abundance and broader niche of *C. latostoma* would indicate that this species represents a superior competitor (Ayala, 1972) or an ecological dominant (McNaughton and Wolf, 1970).

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# *Pseudomagnivitellinum ictalurum* gen. et sp.n. (Digenea: Macroderoididae) from the Black Bullhead of South-central Texas

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Abstract: *Pseudomagnivitellinum ictalurum* gen. et sp.n. is described from *Ictalurus melas* from south-central Texas. *Pseudomagnivitellinum* is most similar to the genus *Glossidium* but it is smaller, has an esophagus, the ovary is positioned some distance posterior to the acetabulum, and it has large vitelline follicles. This genus also bears some superficial likeness to the genus *Magnivitellinum*. The new genus is tentatively placed in the family Macroderoididae.

*Ictalurus melas* (Rafinesque), the black bullhead, is a common inhabitant of the local farm ponds and roadside ditches in south-central Texas. During 1976 and 1977, 15 black bullheads were examined for endohelminths in conjunction with a study of the seasonal dynamics of ictalurid monogenetic trematodes. Two separate samples in March and April of 1977 from the same roadside ditch, located 6 miles west of Bryan, Texas, in Brazos County, showed each of six black bullheads to be infected with an undescribed digenean genus which appeared to be in the family Macroderoididae McMullen, 1937 (22/fish). Specimens were studied alive, fixed in AFA, and stained in Semichon's carmine for additional study. Measurements are in micrometers, the mean followed by the range in parentheses.

### *Pseudomagnivitellinum* gen.n.

Body elongate, with rounded extremities, finely spined anteriorly. Oral sucker subterminal; prepharynx and esophagus short; pharynx well developed; ceca terminating near posterior extremity. Acetabulum in anterior third of body. Testes diagonal, in posterior half of body. Cirrus pouch claviform, enclosing a bipartite seminal vesicle, prostatic complex, and cirrus. Genital pore ventral, medium, immediately preacetabular. Ovary submedian, between acetabulum and anterior testis. Uterus extending to posterior extremity, largely postacetabular. Vitelline follicles large. Excretory vesicle I-shaped.

Type species: *Pseudomagnivitellinum ictalurum* sp.n.

### *Pseudomagnivitellinum ictalurum* sp.n.
### (Figs. 1–4)

Specific diagnosis (based on 20 adult specimens): With characteristics of genus. Body spinose 562 (352–865) by 148 (64–276); remnants of what appears to

→

Figures 1–4. *Pseudomagnivitellinum ictalurum* from black bullhead, Texas. 1. Camera lucida drawing of an adult *Pseudomagnivitellinum ictalurum* gen. et sp.n., ventral view showing testes (T), ovary (O), uterus (U), and vitelline follicles (V). 2. Lateral view showing seminal vesicle (S), testes (T), ovary (O), pharynx (P), and cecum (C). 3. Terminal genitalia showing bipartite seminal vesicle (S), prostate glands, protruded cirrus (PC), and uterus. 4. Camera lucida drawing of posterior end of an immature worm showing ovary (O), testes (T), ceca (C), and excretory vesicle (E).

be cercarial eyespots sometimes present. Oral sucker 61 (47–93) in diameter. Acetabulum smaller than oral sucker, 51 (38–79) in diameter, located in upper ⅓ of body. Ratio of transverse diameter of oral sucker to acetabulum, 1:0.84. Mouth subterminal, prepharynx 12 (0–17) long; pharynx 41 (24–62) by 37 (21–60); esophagus short. Ratio of transverse diameter of pharynx to oral sucker, 1:1.6. Cecal bifurcation immediately anterior to acetabulum. Testes generally smooth; anterior testis 83 (60–121) by 48 (38–91); posterior testis 72 (56–117) by 49 (34–60). Position of testes dependent on size of specimen, usually in middle ⅓ of body; body region posterior to testes greatly enlarged in larger worms. Cirrus sac slightly submedian, extending to level of posterior margin of acetabulum. Ovary round, 73 (49–120) by 53 (38–102); oötype not observed. Vitelline follicles large, in lateral fields, extending from acetabulum to level of posterior testis. Eggs small, operculate, 23 (19–25) by 12 (9–14).

HOLOTYPE: Adults; USNM Helm. Coll. No. 74868.

PARATYPES: Adults; USNM Helm. Coll. (6) No. 74869; Texas A&M University Regional Invertebrate Collection (10) No. 77-128, College of Science, Department of Biology, Texas A&M University.

HOST: *Ictalurus melas*.

SITE: Intestine.

TYPE LOCALITY: Brazos County, Texas.

## Remarks

*Pseudomagnivitellinum* gen.n. superficially resembles the genus *Magnivitellinum* Kloss, 1966 in that it has a similar body style and large vitelline follicles. The new genus differs from *Magnivitellinum* because it has a large bipartite seminal vesicle, the acetabulum is smaller than the oral sucker, and the ovary is situated some distance posterior of the acetabulum. This new genus is most like the genus *Glossidium* Looss, 1899, in its general appearance but differs in that an esophagus is present, the ovary is positioned some distance posterior of the acetabulum, the excretory vesicle is I-shaped, and it has large vitelline follicles.

*Pseudomagnivitellinum ictalurum* gen. et sp.n. resembles members of the family Allocreadiidae, however, its tegument is spined and the arrangement of the testes, ovary, uterus, genital pore, cirrus sac, and vitellaria is more typical of the family Macroderoididae. For these reasons, this new genus is tentatively assigned to the family Macroderoididae until life cycle studies have been completed.

## Acknowledgment

# Cephalic Papillae of Giant Kidney Nematode *Dioctophyma renale* (Goeze, 1782) and Comparison with *Eustrongylides* spp.

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ABSTRACT: Cephalic papillae of third- and fifth-stage *Dioctophyma renale* and fourth- and fifth-stage *Eustrongylides* spp. were found to be of three kinds in addition to the amphids. In all the stages of both genera studied, six papillae were in an internal circle, six in an external circle, and eight to 10 in two lateral fields of four or five each between the internal and external circles. Amphids were closely associated with the externolateral papillae. Another porelike papilla was found between the ventroventral papillae in all but fifth-stage *D. renale*. In third-stage *D. renale*, lateral rows of somatic papillae were spatially separated from the cephalic papillae, but in fifth-stage *D. renale* and fourth- and fifth-stage *Eustrongylides*, the somatic papillae were adjacent to the cephalic papillae.

The development and life cycle of *Dioctophyma renale* have been described recently by Mace and Anderson (1975), who have confirmed the earlier work of Karmanova (1962). Eggs hatch in an aquatic oligochaete, *Lumbriculus variegatus* (Muller), develop to the third stage, and are infective to the definitive host, usually the mink, *Mustela vison* Schreber. Third-stage larvae from the oligochaete will also survive in frogs and fish, which may be important paratenic hosts (Mace and Anderson, 1975). Larvae of another dioctophymatid, *Eustrongylides*, also are found in frogs and fish, and third-stage *D. renale* are difficult to distinguish from third-stage *Eustrongylides* spp. (Mace and Anderson, 1975; Fastzkie and Crites, 1977). Fourth-stage larvae of *D. renale* are unknown. This study provides a detailed description of cephalic papillae of third-stage and adult *D. renale* with the objective of finding morphological data for identifying larval stages. Others (Crites and Jilek, 1978) are describing the cephalic papillae of larval and adult *Eustrongylides tubifex* (Nitzsch, 1819).

## Materials and Methods

### Specimens

Four fifth-stage *Dioctophyma renale* were studied. One from a dog, *Canis familiaris* Linnaeus, was collected in Washington, D.C., in 1916; and three from a mink were collected in Ontario, Canada, in 1978 by Dr. Roy Anderson, University of Guelph, Ontario, Canada. Third-stage *D. renale* from experimentally infected leopard frogs, *Rana pipiens* Schreber, were obtained from Dr. Thomas F. Mace, University of Victoria, British Columbia, Canada.

Fourth-stage *Eustrongylides* sp., ?*Eustrongylides wenrichi* Canavan, 1929 from bullfrogs, *Rana catesbeiana* Shaw, were collected in Nevada by Dr. Bert B. Babero, University of Nevada, Las Vegas, in 1971. Fifth-stage *Eustrongylides ignotus* Jägerskiöld, 1909 from a "blue heron," *Ardea* sp., were collected in Washington, D.C., in 1929.

### Procedures

Terminology for papillae follows Chitwood (1950) except where indicated otherwise. Scanning electron micrographs were obtained by the methods of Madden and Tromba (1976).
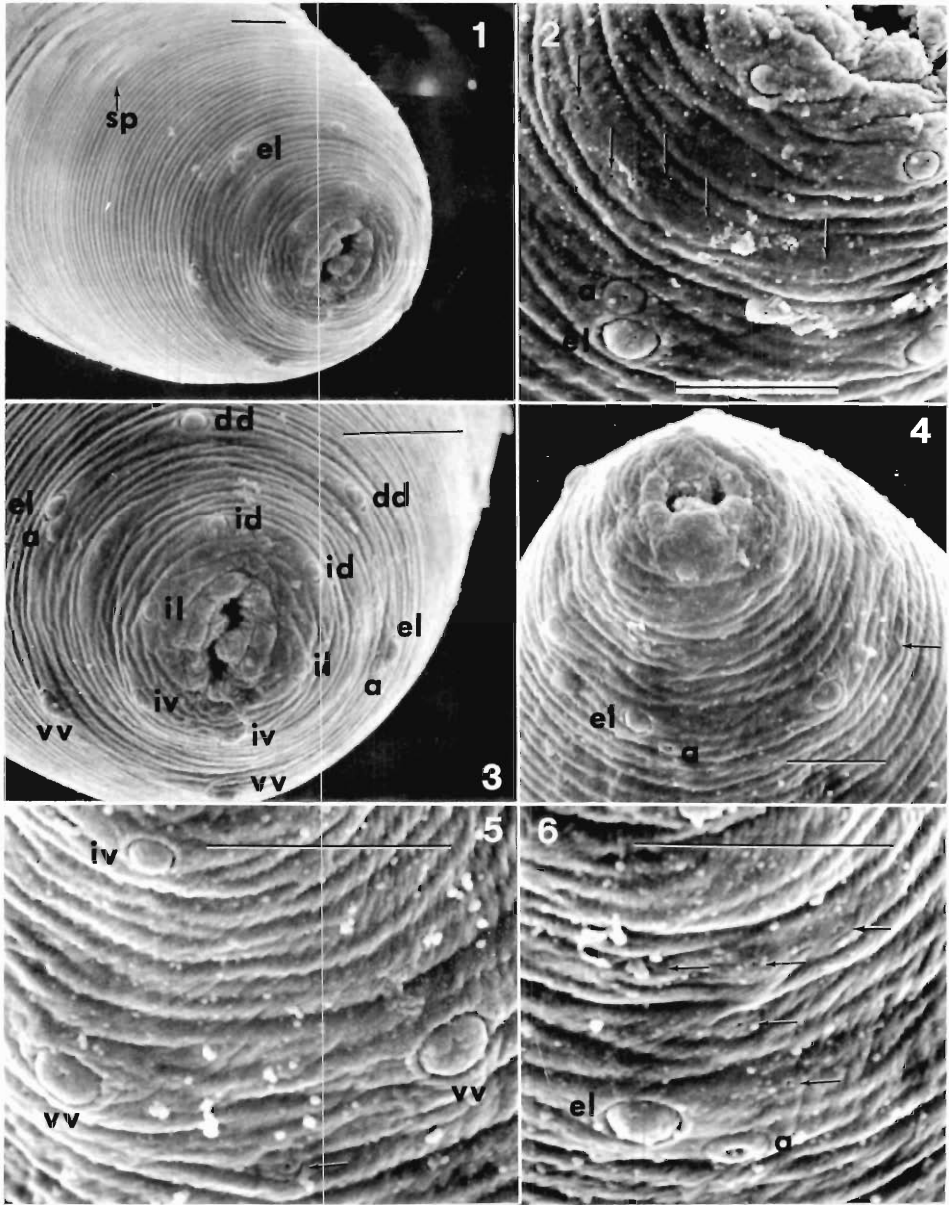
Figures 1–6. *Dioctophyma renale* third-stage larvae from leopard frogs. Scale bars 10 μm. 1. Anterior end showing separation between somatic papillae (sp) and externolateral cephalic papillae (el). 2. Left lateral cephalic region showing amphid (a) slightly anterior and ventral to externolateral papillae (el). Arrows indicate five small lateral field cephalic papillae. 3. *En face* view of internal and external circles of cephalic papillae and the amphids (a). The internal circle includes two internodorsals (id), two internolaterals (il), and two internoventrals (iv). The external circle includes two dorsodorsals (dd), two externolaterals (el), and two ventroventrals (vv). 4. Right lateral view of different specimen showing that the amphid (a) is sometimes posterior and ventral to the externolateral cephalic papilla (el). An arrow indicates a ventral porelike papilla slightly posterior to the external circle of cephalic papillae. 5. Ventral view of ventral porelike papilla (arrow), ventroventral papillae (vv), and an internoventral papilla (iv). 6. Right lateral view showing amphid (a), externolateral papilla (el), and five small lateral field papillae (arrows).

## Observations and Results

Three kinds of papillae, in addition to the amphids, were observed in the cephalic region. The papillae are arranged in an internal circle of six, an external circle of six, and in two lateral fields of four or five each between the two circles. Lateral somatic papillae extend posteriorly from the cephalic region to the caudal region.

### Third-stage *Dioctophyma renale* larvae (Figs. 1–6)

The six papillae of the internal circle are buttonlike, almost round, without projecting spines, and with a central porelike depression. They are symmetrically arranged in an oval ring around the dorsoventrally elongated mouth. Papillae of the external circle are similar in shape to those of the internal circle but are twice as large. The amphids are similar in size to the papillae of the internal circle but have a larger central pore. The amphids usually are located slightly anterior and ventral to the externolateral papillae (Figs. 1–3), but they may also be located posterior to the externolateral papillae (Figs. 4, 6). Small porelike papillae are located in two irregular and asymmetrical lateral cephalic fields of five each between the internal and external circles (Figs. 2, 6). Lateral somatic papillae (Fig. 1) are located in two lateral rows in the cervical region and extend posteriorly to the caudal region. The somatic papillae are separated from the cephalic papillae by a space of 40–60 $\mu$m in third-stage larvae. The somatic papillae are morphologically similar to the papillae of the internal circle.

A single porelike papilla is located just posterior to the external circle in what is believed to be a ventral position (Figs. 4, 5). There is no corresponding papilla on the opposite side.

### Fifth-stage (adult) *Dioctophyma renale* (Figs. 7–12)

The six papillae of the internal circle are located on a narrow, flat, shelflike rim around the round mouth. The papillae are moundlike, have an oval base delimited by a ring-shaped depression in the cuticle, and are topped by a digitiform tip (Fig. 12). The six papillae of the external circle are found on large, dome-shaped elevations that surround the shelflike rim around the mouth. Each elevation bears a single, round, buttonlike papilla delimited by a ring-shaped depression in the cuticle. The amphids are slightly smaller than the papillae of the external circle and are located either anterior or posterior and dorsal or ventral to the externolateral papillae (Figs. 7–11). Eight small papillae are located between the internal and external circles in two irregular lateral fields of four each (Figs. 7, 10). Lateral somatic papillae extend posteriorly from the externolateral papillae. The anteriormost somatic papillae are sometimes paired (Fig. 11), but the more posterior ones are single.

### Fourth-stage *Eustrongylides* sp. (Figs. 13, 14)

The six papillae of the internal circle each have a large, flat, round base delimited by a cuticular depression, and a long, tapering, spinelike central process (Figs. 13, 14). The six papillae of the external circle have an umbonate shape with a large, round base and a short, blunt central process with an apical, porelike depression (Fig. 14). The amphids are slightly ventral to and at about the same level as the externolateral papillae (Figs. 13, 14). The amphids are smaller than
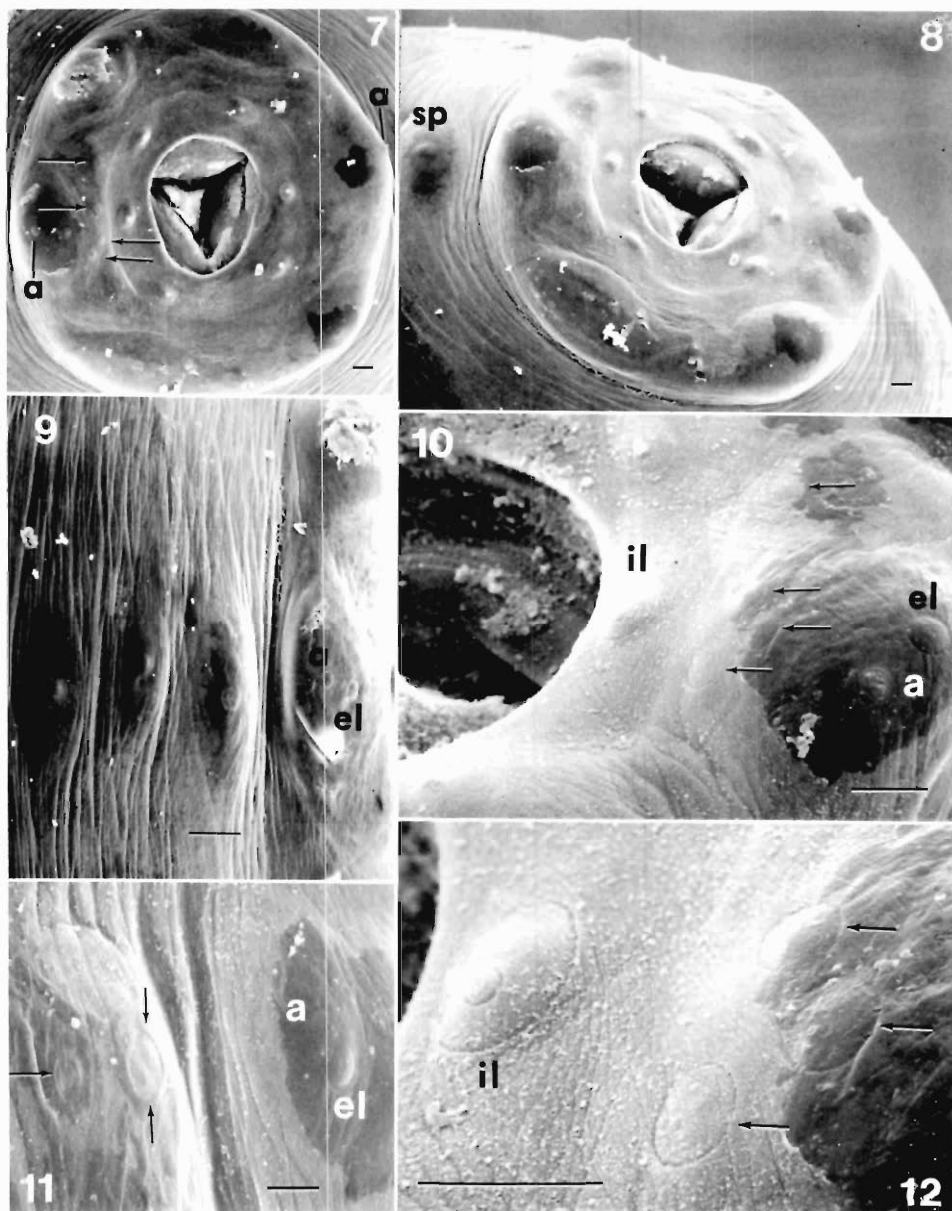
Figures 7–12. *Dioctophyma renale* adults from kidney of mink. Scale bars 20 μm. 7. *En face* view of internal circle of six papillae on shelflike rim around mouth, amphids (a) posterior to the papillae of the external circle, and four right lateral field cephalic papillae (arrows) between the internal and external circles. 8. Anteroventral view of specimen shown in Figure 7 showing anteriormost somatic *papilla (sp)* on left side and the dome-shaped elevations that bear the papillae of the external circle. 9. Right lateral cephalic region of specimen in Figures 7 and 8 showing amphid (a), externolateral papilla (el), and three somatic papillae. 10. Left cephalic region showing internolateral papilla (il), four lateral field cephalic papillae (arrows), amphid (a), and externolateral papilla (el). 11. Right side of specimen in Figure 10 showing amphid (a) slightly dorsal and posterior to externolateral papilla (el), and double (double arrows) and single (single arrow) somatic papillae. 12. Higher magnification of specimen in Figures 10 and 11 showing internolateral papilla (il) with digitiform tip and three of the four lateral field cephalic papillae (arrows).

either the papillae of the external circle or the lateral somatic papillae (Figs. 13, 14). There are four small, round or oval papillae in each of two lateral cephalic fields between the internal and external circles (Figs. 13, 14), and a single ventral porelike papilla is located between the ventroventral papillae (Fig. 14).

### Fifth-stage *Eustrongylides ignotus* (Figs. 15–18)

Each of the six papillae of the internal circle has a raised base and an apical digitiform process (Fig. 17) which is frequently missing in fixed specimens. The six papillae of the external circle have broad, low, convex, circular bases delimited by a depression in the cuticle. Small, buttonlike elevations with a central porelike depression are located in the centers of the circular bases. The amphid on the right side (Fig. 18) is located on the base of the right externolateral papilla and is porelike. The left amphid, however, is apparently anterior to the base of the left externolateral papilla and indistinguishable from the small lateral cephalic papillae between the internal and external circles (Fig. 16). A single porelike papilla is located ventrally, slightly anterior to the outer circle of papillae.

### Discussion

Previous interpretations of cephalic papillae of Dioctophymatoidea differ from that presented above. Chitwood (1950) considered as cephalic papillae only the six papillae of the internal circle, the six large papillae of the external circle, and four of the eight small lateral field cephalic papillae. Thus, in Chitwood's system, the external circle had six large and four small papillae, an arrangement that agrees with that of some other aphasmidian nematodes, the Enoploidea (Chitwood, 1950). Chitwood considered the lateralmost two of the four small papillae between the internal and external circles to be somatic papillae extending anteriorly from the lateral areas.

Another interpretation of the papillae of the anterior region of nematodes is that of de Coninck (1942, 1965). In de Coninck's system are an inner circle of six labial papillae, an outer circle of six labial papillae, and two bilateral groups of two cephalic bristles or papillae. Hyman (1951) adopted de Coninck's system and noted that the number of bristles (=papillae) in the cephalic circle is often augmented by doubling or by the forward migration of body (=somatic) papillae. In the present study, the eight to 10 small lateral papillae between the internal and external circles were found to be of uniform morphology. Furthermore, in third-stage larvae of *D. renale,* a considerable distance separates the cephalic papillae from the lateral rows of somatic papillae in the cervical region. We have, therefore, labeled as cephalic papillae all small papillae located in lateral fields between the two circles of large labial papillae. Our observations appear to support de Coninck's system of hexaradiate labial and bilateral cephalic papillae. We interpret the cephalic papillae of *Dioctophyma* and *Eustrongylides* to represent an inner circle of six labial papillae, an outer circle of six labial papillae, and two bilateral groups of four or five cephalic papillae.

In a light micropscopy study of third-stage larval *D. renale,* Mace and Anderson (1975) placed the amphid midway between the internal and external circles of labial papillae and showed the externolateral papillae as double. We believe that what they labelled as amphids were some of the eight to 10 small cephalic papillae between the circles and that the ventral partners of their double exter-
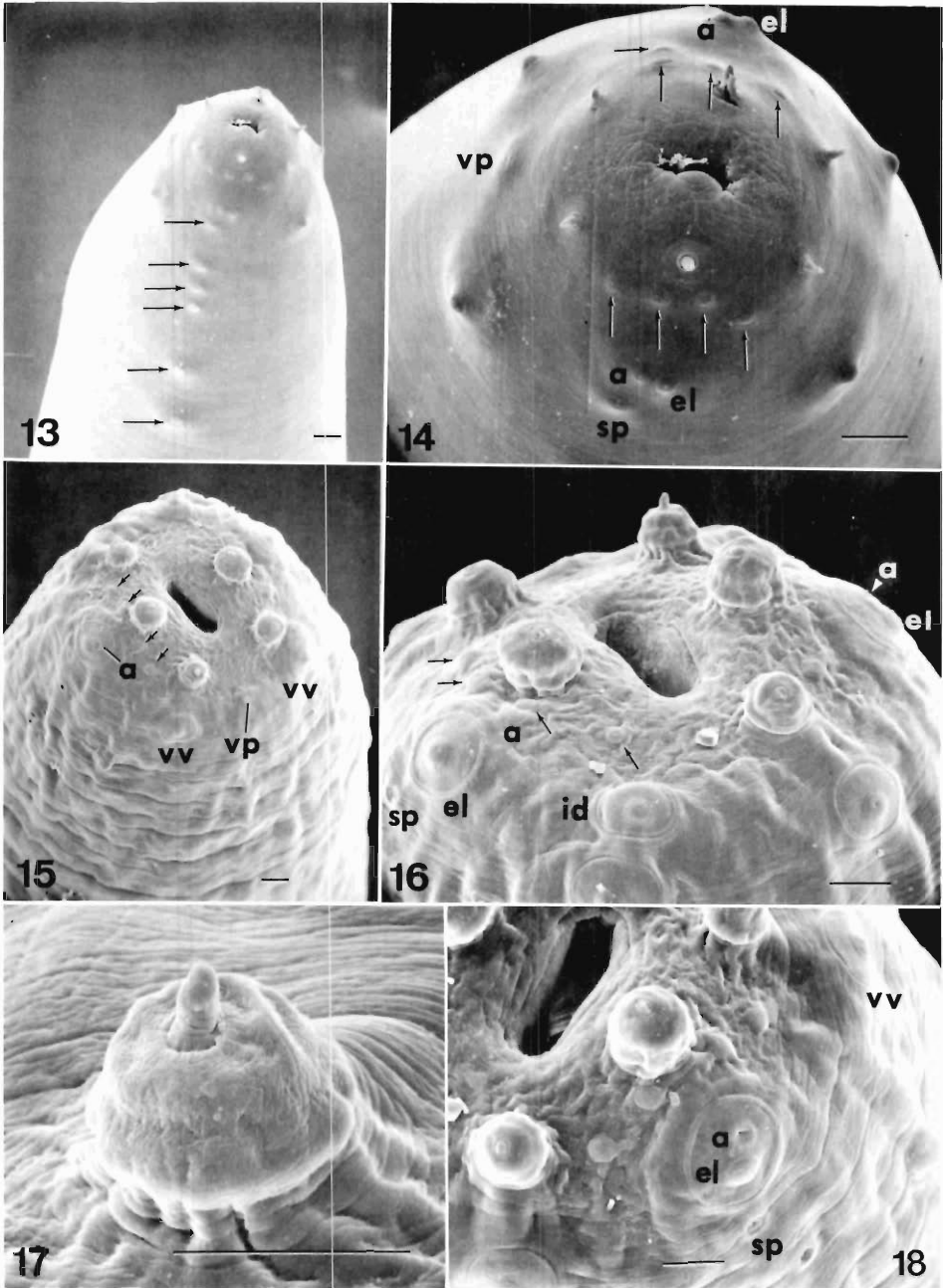
Figures 13–18. 13, 14. *Eustrongylides* sp. fourth-stage larva from bullfrogs. Scale bars 20 μm. 13. Anterolateral view of anterior end showing cephalic papillae and somatic papillae (arrows). 14. Higher magnification of specimen in Figure 13 showing six spinelike papillae of the internal circle, six umbonate papillae of the external circle, two amphids (a), the anteriormost somatic papilla (sp), an unpaired ventral papilla (vp), and four small lateral field cephalic papillae on each side between the internal and external circles (arrows) (el = externolateral papillae). 15–18. *Eustrongylides ignotus* adult from a "blue heron." Scale bars 20 μm. 15. Ventrolateral view of anterior end showing a dorsoventrally elongated mouth surrounded by an internal circle of six raised papillae, a ventral porelike papilla (vp) between the

nolaterals are actually the amphids. This interpretation is supported by the different morphology of these porelike papillae in third-stage larvae. In adult *D. renale*, only their smaller size helps in identifying the amphids. In *Eustrongylides* spp., the identification of the amphids is especially difficult, and we relied somewhat on a position equivalent to that found in *D. renale* because all other labial and cephalic papillae have equivalent positions. Chitwood (1950), however, indicated that the amphids of dioctophymatids are posterior to the externolaterals. However, Gibson and McKiel (1972) figured amphids anterior to papillae of the outer circle in *Eustrongylides* sp. from a muskrat. Panesar and Beaver (1979) were unable to recognize amphids in fourth-stage larvae of *Eustrongylides wenrichi*, although they described the externolateral papillae as double. Fastzkie and Crites (1977) also were unable to recognize amphids in adult *Eustrongylides tubifex* (Nitzsch, 1819). Jones (1978) described the cephalic papillae of *Eustrongylides acrochordi* Jones, 1978 without distinguishing amphids. Additional studies are needed to determine conclusively which of the labial and cephalic papillae are the amphids.

The single, small, porelike papilla found between the ventroventral papillae of third-stage larval *D. renale* and fourth-stage larval and adult *Eustrongylides* spp. could not be found in the limited material of adult *D. renale*. However, Karmanova (1968) and Roman (1965) illustrated this papilla in a drawing by M. Rather of an adult *D. renale*. Jones (1978) figured a papilla between the ventroventral papillae and another between the dorsodorsal papillae in *E. acrochordi*. Despite a careful search, a dorsal papilla was not found in the present study. The position of the ventral papilla was confirmed only in fourth-stage *Eustrongylides* sp. by relating the papilla position to that of the large ventral nerve cord and the glandular subventral sections of the esophagus. The function of this porelike papilla is unknown.

Some evidence shows that the morphology of cephalic papillae may be determined by their position on the face of the nematode. In Figures 15–17, the left internodorsal papilla is displaced toward the external circle and resembles the papillae of the external circle more than it resembles other internal papillae. The right amphid (Fig. 18), on the base of the right externolateral papilla, is different morphologically from the left amphid (Fig. 16), which is displaced anteriorly. The left amphid is morphologically similar to the four adjacent small lateral field cephalic papillae.

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ventroventral papillae (vv) of the external circle, an amphid (a) slightly anterior and ventral to the externolateral papilla, somatic papillae posterior to the externolateral papilla, and four right lateral field cephalic papillae (arrows) between the two circles. 16. Higher magnification of the specimen in Figure 15 viewed anterodorsally, showing asymmetrical arrangement of amphids, with the left amphid (black a) apparently grouped with the four left lateral field cephalic papillae (arrows) rather than on the base of the externolateral papilla (el) like the right amphid (white a). The left internodorsal papilla (id) is also asymmetrically arranged, being closer to the external circle than the others (sp = somatic papilla). 17. Higher magnification of the right internoventral papilla showing apical digitiform process that is frequently missing in fixed specimens. 18. Higher magnification of right lateral region showing amphid (a) on the base of the externolateral papilla (el), four small lateral field cephalic papillae between the papillae of the internal and external circles, and somatic papillae (sp) posterior to the externolateral papilla (vv = ventrolateral papilla).

Determination of the usefulness of labial cephalic papillae for separating larvae of *D. renale* and *Eustrongylides* spp. must await the publication of studies of third-stage *Eustrongylides*, but the usefulness of the small lateral field cephalic papillae must be questioned because of the difficulty in observing them with the light micropscope. However, Crites and Jilek (1978) described the larger cephalic papillae of the internal circle of third-stage *Eustrongylides tubifex* as having a "cone-shaped, almost spine-like central projection." Thus, the papillae of the internal circle appear to be useful in separating third-stage larvae of *D. renale* and *E. tubifex*.

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# Larval Development of *Spirocamallanus cricotus* (Nematoda: Camallanidae)[1]

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ABSTRACT: The larval development of *Spirocamallanus cricotus* in the harpacticoid copepod *Tigriopus californicus* is described. The larvae pass through two molts at 23–26°C, one at 6 days post-infection (p.i.) and the other between 10 and 11 days p.i., reaching the infective third stage. *Mesochra* sp., a common harpacticoid in the northern Gulf of Mexico, could not be infected with first-stage larvae of *S. cricotus*. Experimental infections of *Penaeus setiferus* (white shrimp) were obtained, suggesting that this crustacean may help to transmit *S. cricotus* infections in the Gulf of Mexico.

*Spirocamallanus cricotus* Fusco and Overstreet, 1978 is one of the most frequently occurring nematode parasites in the northern Gulf of Mexico, infecting at least 13 different species of marine and estuarine fishes (Fusco and Overstreet, 1978). This nematode attaches to the host's intestine by means of a sclerotized buccal capsule and feeds on host blood (Fusco, 1978). Of the 62 nominal species of *Spirocamallanus* parasitizing freshwater and marine fish, the larval development of only four freshwater species has been reported. This is the first study of the larval development of a marine member of the Procamallaninae.

## Materials and Methods

Adult *Spirocamallanus cricotus* were obtained from the intestine of *Micropogonias undulatus* (Atlantic croaker) collected in Davis Bayou, Mississippi. Gravid female worms were individually placed in stender dishes containing seawater diluted with distilled water to 20 parts per thousand (ppt). Larvae were obtained by teasing apart the uterus of adult worms.

Two species of harpacticoid copepods and one species of penaeid shrimp were selected as potential hosts. *Mesochra* sp. (an undescribed copepod) was collected from a saline pool on Horn Island, Mississippi, cultured in one-liter stender dishes in seawater (salinity 20 ppt), and fed an algae-protozoan mixture obtained at the site of collection. The copepod *Tigriopus californicus* was obtained from Sea-Life Supply (Sand City, California) and cultured according to supplied instructions. *Penaeus setiferus,* white shrimp, averaging 5 cm in total length were collected by seining along the mouths of several tributaries emptying into Davis Bayou and maintained on a diet of TetraMin Staple Fish Food® in aerated 38–76 liter aquaria containing seawater (8 ppt). No natural nematode infections were found in an examination of 100 *Mesochra* and 100 white shrimp. Between 20 and 100 copepods that were starved for 24 hr were added to stender dishes containing the larval contents of one gravid female worm. Copepods were examined at various intervals from 18 hr to 31 days post-infection (p.i.).

Larvae collected from infected copepods were fixed in glacial acetic acid or hot

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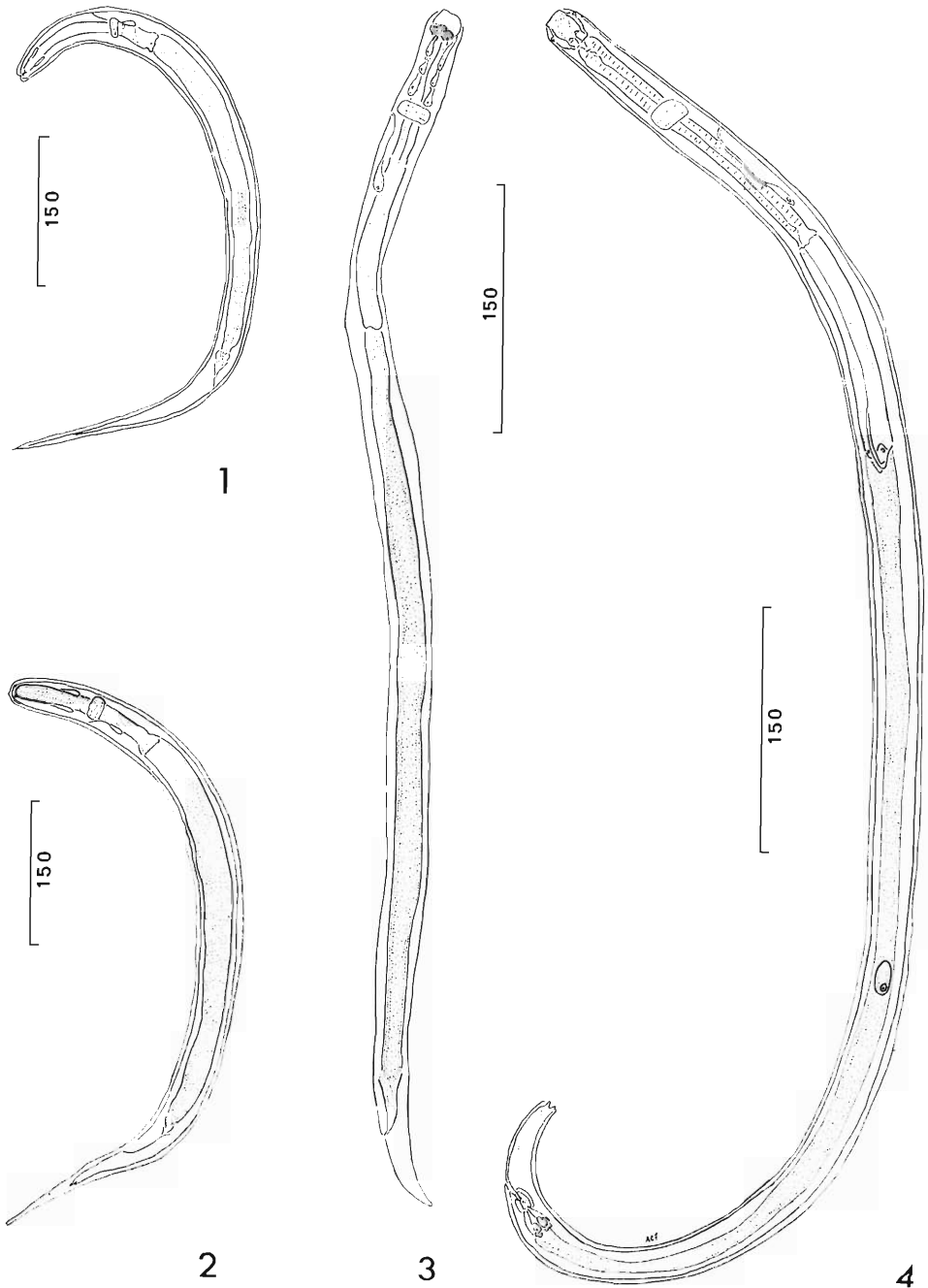
Figures 1–4. *Spirocamallanus cricotus*, larval stages (from *Tigriopus californicus*, Figs. 3, 4). Scales are in micrometers. 1. First-stage larva from the uterus of a gravid female. 2. First-stage larva, 48 hr p.i. 3. Second-stage larva, 9 days p.i. 4. Third-stage larva, 31 days p.i.

(70°C) glycerine-alcohol (95 parts 70% ethanol and 5 parts glycerine), cleared in glycerine, and mounted in glycerine jelly. In several instances, larvae in infected copepods were fixed *in situ* with hot ethanol and subsequently dissected from the hemocoel. Measurements are in micrometers unless otherwise stated; figures were drawn with the aid of a drawing tube.

## Results

### Experimental infection of copepods

Larvae did not infect *Mesochra* sp. The harpacticoid was not observed eating or attempting to eat larvae. Several white shrimp that were fed larvae became infected (three out of eight in one instance and one out of 20 in another; intensities ranged from one to two larvae per shrimp). First-stage larvae were collected 24 hr and 5½ days p.i., and second-stage larvae were collected on day 12. Shrimp that ingested copepods infected with third-stage larvae did not acquire the infection.

*Tigriopus californicus* readily ate wiggling larvae. Copepods infected with from four to eight larvae died within three days. Those containing three larvae were noticeably less active than those containing either one or two larvae. Prevalence of larvae in experimentally infected copepods was between 82 and 100%. The average intensity of infection was two larvae per copepod (range one to three larvae per copepod; one experiment had an intensity of three to eight with an average of five larvae per copepod). Figures 1–4 show larval stages, Figures 5–10 show buccal capsule development, and Figures 11–13 show development of the posterior region of larval *S. cricotus* recovered from *T. californicus*.

First-stage larvae ($L_1$) occupy the hemocoel of the copepod within 18 hr p.i. The first molt was observed at 6 days p.i. The second molt was not observed; however, three third-stage larvae ($L_3$) obtained at day 11 showed sheath fragments near the rectal region and an empty sheath was obtained from the hemocoel of a copepod at 12 days p.i. These observations suggest the second molt takes place between 10 and 11 days. Developmental changes in third-stage larvae were observed between days 11 and 16, after which no morphological change was detected. Fully developed larvae were curved or folded within the hemocoel.

### Description of developmental stages

FIRST-STAGE LARVA FROM THE UTERUS (from several females; measurements based on 18 specimens): Body ensheathed, slender, colorless, nearly transparent, 444–493 long by 22–24 wide, 18–22 times longer than wide, tapering to a point posteriorly. Cuticle with fine transverse striations 1–2 apart. Cephalic end with dorsal penetration tooth 2–3 long. Buccal vestibule short, weakly sclerotized, 4–5 long. Arcade cells (of Chitwood and Chitwood, 1933) 4 in number. Esophagus 78–105 long, 17–22% of body length; muscular portion 65–82 long by 9–11 wide, comprising 76–92% of entire esophagus; glandular portion poorly defined, 7–26 long by 9–11 wide. Excretory pore 31–60 from cephalic end; renette cell globular with single nucleus, at level of esophago-intestinal junction. Nerve ring 54–74 from anterior end, 6–7 in height, 1–16 posterior to excretory pore. Intestinal contents granular. Rectum an indistinct, narrow, short tube. Tail elongate, slender, 87–121 long, 19–25% of body length.

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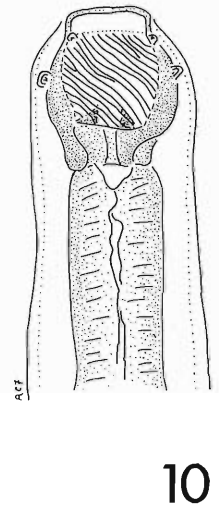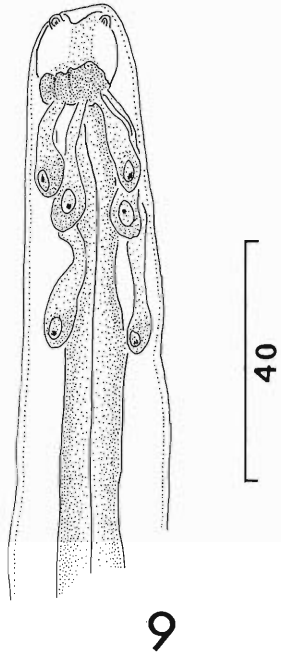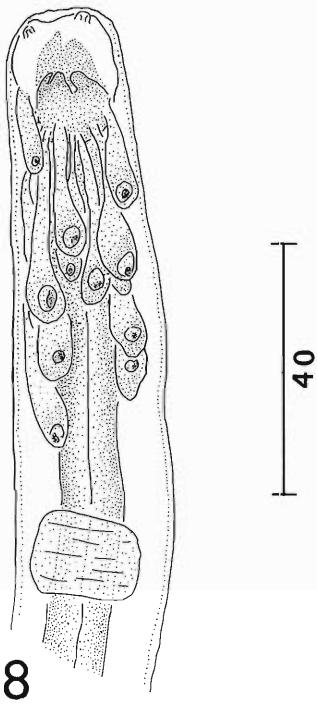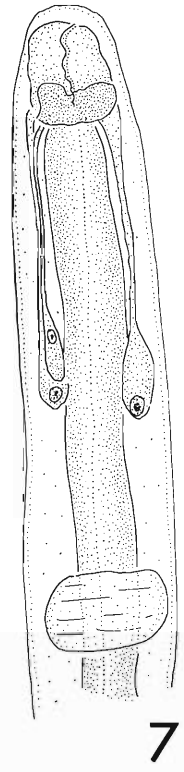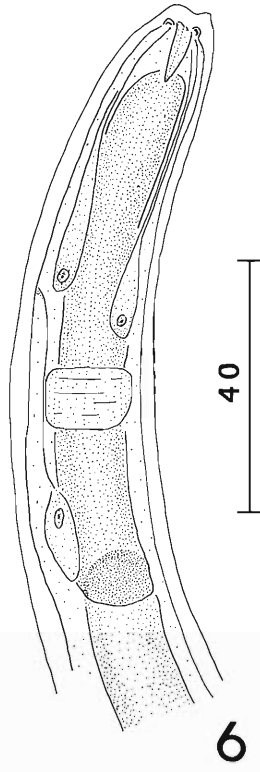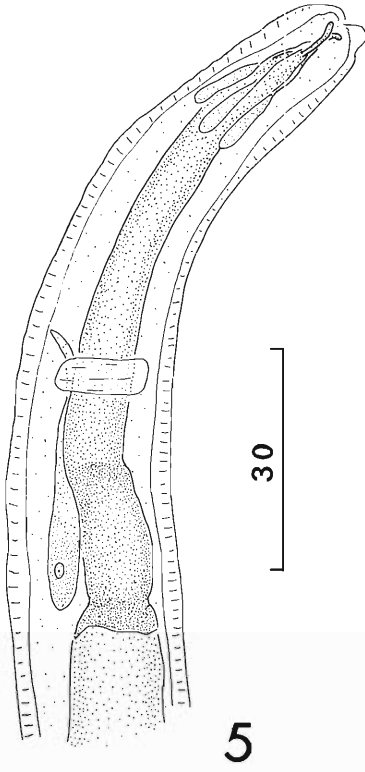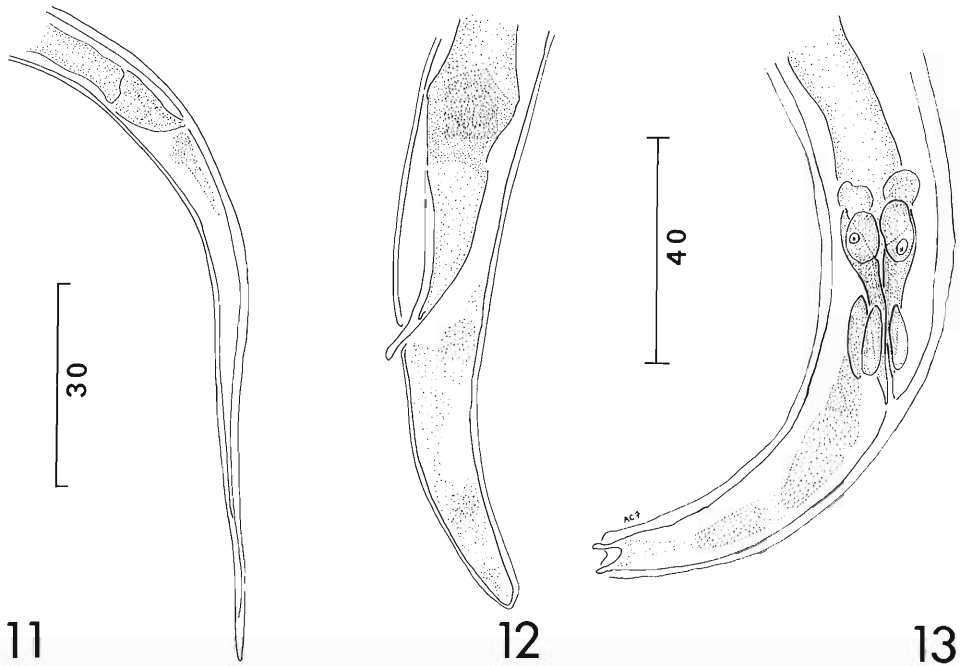
Figures 11–13.  *Spirocamallanus cricotus*, development of the posterior region of larval stages. Scales are in micrometers.  11. First-stage larva from the uterus of a gravid female.  12. Second-stage larva from *Tigriopus californicus*, 9 days p.i.  13. Third stage larva from *T. californicus*, 31 days p.i.

FIRST-STAGE LARVA IN *TIGRIOPUS CALIFORNICUS* (measurements based on 22 specimens): Body ensheathed, slender, colorless, tapering toward extremities, 398–533 long ensheathed, 378–487 long excluding sheath, 20–30 wide, 16–21 times longer than wide. Transverse cuticular striations 1–2 apart in early $L_1$, absent in late $L_1$. Cephalic papillae 4 in number, visible in late $L_1$. Dorsal penetration tooth 2–3 long (early $L_1$), indistinct between 36 and 48 hr p.i.; buccal cavity compressed, 6–8 long by 1–2 wide before molt (late $L_1$). Arcade cells 4 in number, 10–52 long, increasing in length with age. Esophagus 83–134 long, 22–34% of body length; muscular portion 63–109 long by 7–13 wide, comprising 65–85% of entire esophagus; glandular portion poorly defined, 20–46 long by 7–15 wide. Excretory pore 38–64 from cephalic end; renette cell occasionally indistinct. Nerve ring 48–81 from anterior end, 6–7 (early $L_1$) to 9–12 (late $L_1$) in height, 10–36 posterior to excretory pore. Intestine colorless, with granular contents. Rectum weakly sclerotized at 24 hr p.i., 18–49 long; rectal glands not visible. Tail elongate, slender, 49–72 long; 19–20% (early $L_1$) to 10–17% (late $L_1$) of body length.

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Figures 5–10.  *Spirocamallanus cricotus*, development of buccal capsule in larval stages (from *Tigriopus californicus*, Figs. 6–10). Scales are in micrometers.  5. First-stage larva from the uterus of a gravid female.  6. Late first-stage larva, 6 days p.i.  7. Early second-stage larva, 6 days p.i.  8. Second-stage larva, 9 days p.i.  9. Late second-stage larva, 9 days p.i.  10. Late third-stage larva, 31 days p.i.

SECOND-STAGE LARVA IN *TIGRIOPUS CALIFORNICUS* (measurements based on 7 specimens): Body ensheathed, slender, reddish, tapering toward extremities, 627–911 long by 22–33 wide, 24–35 times longer than wide. Cephalic papillae 4 in number. Cuticular striations absent. Buccal cavity clear, not striated, faint longitudinal division dividing buccal capsule into 2 parts (early $L_2$); late $L_2$ buccal capsule 1 piece, 2 to 3 bulbous projections at base of capsule; buccal capsule 9–12 long by 12–19 wide. Arcade cells elongate 20–53 long, emptying into base of buccal capsule, increasing in number from 4 (early $L_2$) to 22, 6 in number before molt. Esophagus 168–216 long, 22–33% of body length; muscular portion 62–115 long by 9–15 wide, comprising 34–56% of entire esophagus; glandular portion well defined by 9 days p.i., 79–120 long by 10–15 wide. Nerve ring 55–100 from cephalic end, 8–11 in height. Excretory pore 65–121 from anterior end, at level of or up to 25 posterior to nerve ring. Intestine with large reddish globular contents. Rectum 25–53 long, rectal glands not visible. Tail blunt to 3-spined, 48–61 long; 7% of body length.

THIRD-STAGE LARVA IN *TIGRIOPUS CALIFORNICUS* (measurements based on 33 specimens): Body elongate, slender, reddish, tapering toward extremities, 548–1,000 long by 24–37 wide, 16–36 times longer than wide. Cephalic papillae 8, in 2 circles of 4. Cuticular striations absent. Buccal capsule golden, moderately sclerotized, 22–30 long by 18–25 wide; traces of second buccal ring apparent as 2 or 3 toothlike projections; buccal capsule striated with 19–23 (early $L_3$) to 16–19 (mid $L_3$) narrow, continuous, spiraling bands. Arcade cells absent. Esophagus 215–258 (early $L_3$) to 240–348 (mid $L_3$) long, 24–27% of body length; muscular portion with distinct striations, 124–188 long by 10–17 wide, comprising 47–67% of entire esophagus; glandular esophagus well defined, 100–200 long by 11–20 wide; esophago-intestinal valves distinct by 12 days p.i. Nerve ring 69–105 from cephalic end, 10–15 in height. Excretory pore 80–169 from anterior end, 5–64 posterior to nerve ring. Intestine with large reddish globules. Gential primordium oval, 15–18 long by 8–12 wide at ⅓ of body length from posterior end. Rectum distinct, 25–50 long; rectal glands number 8, in 2 rows of 2 anteriorly and 1 ring of 4 posteriorly. Tail 40–62 long, 4–9% of body length, with 2 or 3 terminal spines.

## Discussion

This study demonstrated larval development of *Spirocamallanus cricotus* in *Tigriopus californicus* and *Penaeus setiferus*, but not in *Mesochra* sp. The small size of *Mesochra* (500) in relation to the size of the larva (444–493) was probably the major factor inhibiting its infection with *S. cricotus*. Moravec (1975), investigating the life cycle of *Procamallanus laeviconchis*, stated that the ability of a copepod to become infected depends upon its size in relation to that of the first-stage larva and on the copepod's ability to ingest intact larvae. *Mesochra* sp. was apparently unable to ingest intact larvae, but larger members of other copepod orders, such as calanoids, could possibly transmit the infection in the Gulf of Mexico. Calanoids were not investigated as possible intermediate hosts because of the difficulty in culturing them.

*Tigriopus californicus* was chosen for developmental experiments because of the ease of obtaining and culturing this copepod and because of its size (1 mm). The assumption is made that, since development occurs in *T. californicus*, de-

velopment may also occur in copepods of suitable size and specificity in the Gulf of Mexico and, if so, that a similar pattern of development may be seen.

The results of this study show that the development of *S. cricotus* in *T. californicus* occurs under the stated experimental conditions in the following manner. A larva may be released either via the vulva or by the rupture of a gravid female worm. The larva settles to the bottom of the water column, attaches to sediment or debris by its adhesive tail and shows wiggling motions which might serve to attract a suitable crustacean host. After ingestion, the larva enters the host's hemocoel within 18 hr, presumably with the aid of a dorsal penetration tooth. Before the first molt, a first-stage larva loses its cuticular striations, the dorsal tooth degenerates, a short buccal capsule forms, the nerve ring increases in size, and the tail decreases in size and develops as many as three terminal spines. The first molt occurs on the 6th day p.i.

During the second larval stage, the buccal capsule starts to form as a weakly sclerotized structure lacking striations. The arcade cells elongate and increase in size and number. As the body lengthens, the excretory pore is displaced posteriad, the muscular and glandular portions of the exophagus become distinct, and the tail continues to shorten in relation to total body length. The second molt occurs between the 10th and 11th day.

The third-stage larva has a well-developed, adultlike buccal capsule which is finely striated. These striations decrease in number as the larva increases in age. Arcade cells are not observed during this stage. Other characteristics of third-stage larvae include well-defined valves between the glandular esophagus and the intestinal tract, and a tail having two to three terminal spines. No morphological changes in the third-stage larva occur after 16 days.

The term arcade cell was first used by Chitwood and Chitwood (1933) for a group of nine club-shaped cells just posterior to the buccal cavity of *Cephalobellus papilliger* Cobb, 1920. The arcade cells in *S. cricotus* differ from the original description by varying in number from four to 22 during the developmental process, instead of remaining constant at nine cells. In all other respects the arcade cells of *S. cricotus* agree with the description by Chitwood and Chitwood (1933). While the function of arcade cells has never been determined, Chitwood and Chitwood (1933) believed these cells were involved in the formation of the buccal cavity. My observations support this view, in that the arcade cells of *S. cricotus* have ducts leading into the buccal capsule and are prominent during the second stage when buccal capsule development is at its peak.

This study strongly suggests that *S. cricotus* will develop in shrimp. Previously, all camallanids were thought to use only copepods as intermediate hosts. In 1973, Overstreet reported two third-stage larvae of *S. pereirae* (=*S. cricotus*) in the intestine of *Penaeus setiferus* in the Gulf of Mexico. Later, Feigenbaum (1975) reported obtaining a third-stage larva of *S. pereirae* in *P. vannamei* from the Pacific coast of Mexico. Overstreet (1973) fed 18 shrimp the larval contents of one mature female and recovered one third-stage larva on the 21st day. I obtained second-stage larvae from the hepatopancreas of experimentally infected white shrimp after 12 days. The second-stage larva I collected and the third-stage larva Overstreet (1973) reported correspond morphologically to the respective larvae I obtained from *T. californicus*. In addition, infection did not result from the

exposure of 20 shrimp to copepods infected with third-stage larvae, suggesting that *P. setiferus* does not act as a transfer host. Hence, I conclude that penaeid shrimp may serve as intermediate hosts of *S. cricotus*; however, nothing is known about the significance of this role in the transmission of infections in the Gulf of Mexico.

The development and life histories of four species of *Spirocamallanus* have been investigated: *S. fulvidraconis* (Li, 1935) Olsen, 1952 by Li (1935); *S. cearensis* (Pereira, Dias, and Azevedo, 1936) Olsen, 1952 by Pereira et al. (1936); *S. xenopodis* (Baylis, 1929) Olsen, 1952 by Thurston (1970); and *S. intestinecolas* Bashirullah, 1973 by Bashirullah and Ahmed (1976). All of these species probably reach the third stage within the hemocoel of a copepod after molting twice. However, Li (1935) and Pereira et al. (1936) both reported only one molt. Their descriptions of the first and second stage led Moravec (1975) and Ivashkin et al. (1971) to conclude that the first molt was probably overlooked.

*Spirocamallanus cricotus*, *S. fulvidraconis*, *S. cearensis*, and *S. intestinecolas* have the following similarities in their developmental pattern (morphological data on *S. xenopodis*, a parasite of frogs, were not supplied by Thurston [1970]). The first stage is characterized by a dorsal penetration tooth and elongated tail. Before the first molt, the dorsal penetration tooth degenerates and the tail shortens. In the second larval stage the major characteristics are a primitive, weakly sclerotized buccal capsule, a distinct two-part esophagus, and a spined tail. By the second larval stage a reddish intestinal color results from the ingestion of host pigment granules. During the third stage the buccal capsule is well formed and the tail has two or three spines.

In spite of these similarities, several differences exist between *S. cricotus* and other species of *Spirocamallanus*. *Spirocamallanus fulvidraconis* and *S. intestinecolas* do not form spiral bands in their buccal capsule during the third stage as does *S. cricotus*. The third-stage larva of *S. fulvidraconis* coils into a spiral when development is completed instead of folding back on itself. The number of spiral striations in the buccal capsule of *S. cearensis* increases from 10 in the third stage to 18 in the adult, whereas the number decreases from 19 to 23 in the third stage to 10 to 15 in the adult of *S. cricotus*.

Experimental infections of the definitive host were not attempted due to the difficulty of culturing Atlantic croaker.

## Acknowledgment

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## *Heliconema serpens* sp.n. (Nematoda: Physalopteridae) and *Camallanides malayensis* sp.n. (Nematoda: Camallanidae) from *Cerberus rhynchops* (Schneider) (Reptilia: Colubridae) in Malaysia[1]

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ABSTRACT: Two species of nematodes are described from the dog-faced water snake in West Malaysia. The presence of *Heliconema serpens* sp.n. confirms an earlier report that the genus can parasitize snakes. All other reports of *Heliconema* have been from anguilliform fish. *Heliconema serpens*, the sixth species in the genus, is characterized by possessing eight to nine tessellated longitudinal ridges in the last quarter of the body, four precloacal and four postcloacal papillae, a spicule ratio of 1:1.5–2.0, and indistinct vulvar lips. *Camallanides malayensis* sp.n. is the seventh species in the genus and is characterized by possessing long similar spicules 492–539 and 415–492 long; five precloacal, one cloacal, and five postcloacal papillae; and larvae 492–692 long. Keys to the species of both genera are given.

During a helminthological investigation of reptiles and amphibians of West Malaysia, five *Cerberus rhynchops* (Schneider), dog-faced water snakes, were examined from 1964 to 1965. Two of the hosts contained a single infection of a new camallanid with intensities of three females, and 14 females and three males, respectively. One snake was infected with four female camallanids and 30 females and 32 males of a new species of physalopterid. Two snakes each contained one unidentified oxyurid. Previously, three nematode species have been reported from *C. rhynchops*: an immature species of *Kalicephalus* (Myers and Kuntz, 1969) and two species of *Camallanides* (Jones, 1978).

Worms were removed from the host's intestinal tract and fixed in hot (80°C) 90 parts 70% ethanol plus 10 parts glycerine. For study, worms were cleared in phenol-alcohol (80 parts phenol, 20 parts absolute ethyl alcohol) and studied as wet mounts. Measurements are in micrometers unless otherwise stated; ranges are followed by statistical means in parentheses. Figures were drawn with the aid of a camera lucida.

### *Heliconema serpens* sp.n.
### (Figs. 1–7)

DESCRIPTION: Two lateral pseudolabia present, each with 2 submedian papillae and 1 lateral amphid; internal border of each pseudolabium with 1 internal lateral tooth and a single submedian tooth at each dorsal-ventral extremity. Mouth dorsoventrally elongated. Cephalic collarette formed by anteriad folding of cuticle. Cuticle with transverse striations 4–5 apart. Esophagus with short muscular anterior portion and long posterior glandular portion. Nerve ring slightly posterior

to midpoint of muscular esophagus. Excretory pore posterior to nerve ring. Deirids (=cervical papillae) lateral at approximate level of nerve ring. Tail blunt.

MALE (based on 10 mature specimens): Body 24.9–33.8 mm (29.4 mm) long by 206–257 (229) wide at junction of muscular and glandular portions of esophagus, increasing posteriorly to 354–470 (408) at level of greatest width, 65–88 (72) times longer than wide. Cephalic collarette 130–162 (141) in width, 18–27 (23) in height. Esophagus 3.0–4.3 mm (3.7 mm) long, 11.4–14.1% (12.7%) of body length; muscular portion 395–426 (422) long by 58–72 (67) wide, 9.9–13.9% (11.4%) of entire esophagus; glandular portion 2.6–3.8 mm (3.3 mm) long by 139–186 (158) wide. Nerve ring 260–301 (275) from cephalic end, 40–54 (45) in height. Deirids 224–305 (288) from anterior end. Excretory pore located 328–440 (387) from anterior end, 68–162 (112) posterior to nerve ring. Testis sinuous, not reflexed, 6.3–12.2 mm (9.4 mm) from cephalic end. Spicules dissimilar, unequal in length; left spicule tapering to a point distally, 404–525 (464) long; right spicule thicker with a blunt distal end and widened proximal end, 247–314 (274) long; spicule ratio 1:1.5–2.0 (1:1.7). Gubernaculum absent. Caudal alae not united anteriorly on ventral surface, 833–1,154 (994) long, supported by 8 elongated, symmetrical pairs of pedunculated papillae; precloacal pairs 4 of equal length in 2 groups of 2 pairs each; postcloacal papillae 4 in number, in 1 group of 3 pairs anteriad and 1 smaller pair posteriad, 1st and 3rd pairs similar in shape. Phasmids not observed. Longitudinal tessellated ridges in 8, occasionally 9, rows extending from approximately midway along spicule length to 2.0–3.7 mm (2.9 mm) anteriorly. Posterior end strongly coiled ventrally, tail flexed ventrad, 284–398 (332) long.

FEMALE (based on 10 mature specimens): Body 28.5–47.5 mm (37.3 mm) long by 202–314 (234) wide at junction of muscular and glandular portions of esophagus, increasing posteriorly to 400–647 (533) at level of greatest width, 55–78 (71) times longer than wide. Cephalic collarette 153–180 (170) in width, 27–40 (30) in height. Esophagus 3.6–5.0 mm (4.4 mm) long, 9.0–14.6% (11.7%) of body length; muscular portion 399–567 (477) long by 72–85 (76) wide, 9.5–12.8% (11.0%) of entire esophagus; glandular portion 3.2–4.5 mm (3.9 mm) long by 144–251 (173) wide. Nerve ring 287–314 (297) from cephalic end, 40–58 (48) in height. Deirids 265–301 (281) from anterior end. Excretory pore located 359–443 (402) from anterior end, 81–156 (112) posterior to nerve ring. Vulva situated 14.0–23.8 mm (19.4 mm), 47.6–55.9% (51.9%) of body length, from anterior end. Vagina 3.2–4.2 mm (3.7 mm) long by 63–93 (77) wide, 7–11% (10%) of body length; uterus didelphic, amphidelphic with eggs 49–54 long by 27–31 wide (52 × 30) containing larvae, 1 ovary associated with each uterine sac. Anterior reproductive structures: ovary 4.98–7.18 mm (5.73 mm) long, oviduct 773–1,610 (1,010) long forming an oval seminal receptacle 591–909 long by 81–168 wide (718 × 113). Posterior reproductive structure: ovary 4.82–6.64 mm (5.90 mm) long, oviduct 773–1,091 (943) long, forming oval seminal receptacle 523–841 long by 90–159 wide (666 × 121). Anterior ovary 0.84–1.40 (0.98) times the length of posterior ovary. Tail blunt 79–148 (118) long. Rectum 314–425 (341) long surrounded by 3 rectal glands anteriorly.

HOST: *Cerberus rhynchops* (Schneider) (Reptilia: Colubridae), dog-faced water snake.

SITE OF INFECTION: Small intestine.

LOCALITY: Pasir Malacca, West Malaysia.
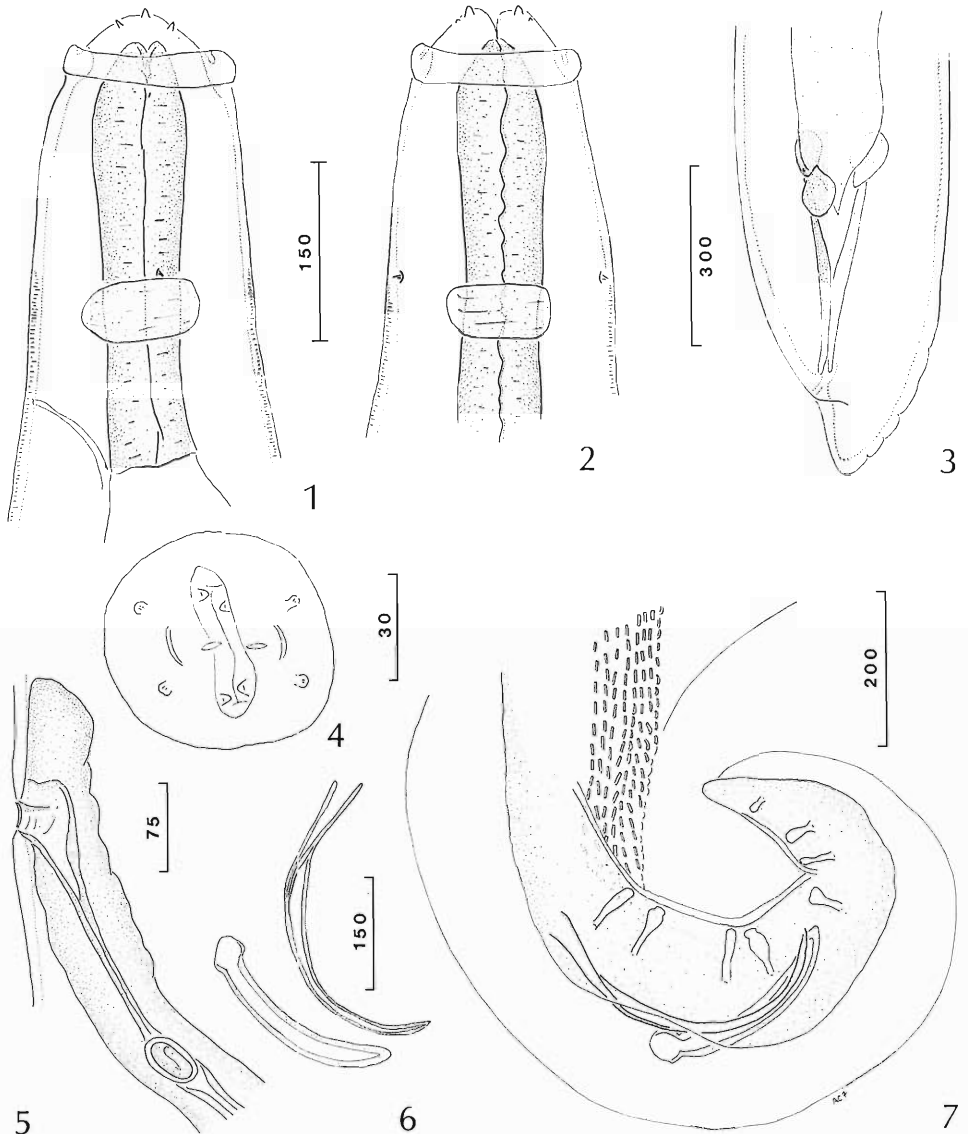
Figures 1–7. *Heliconema serpens* sp.n. Measurements are in micrometers. 1. Anterior portion of female, lateral view. 2. Anterior portion of same female, dorsoventral view. 3. Female tail, lateral view. 4. *En face* view of female. 5. Female reproductive structures: vulva, vagina, and egg. 6. Right (smaller) and left spicules. 7. Male tail, lateral view showing eight tessellated longitudinal ridges.

HOLOTYPE: USNM Helm. Coll. No. 75093.

ALLOTYPE: USNM Helm. Coll. No. 75094.

PARATYPES: USNM Helm. Coll. No. 75095 (5 males, 5 females) and British Museum (Natural History) Reg. No. 1978.1339-45 (4 males, 4 females).

ETYMOLOGY: The Latin *serpens* means snake, referring to the type host of this parasite.

**Remarks**

Ogden (1969), in his review of the genus *Heliconema,* listed five species: *H. heliconema* Travassos, 1919 (type species); *H. ahiri* Karve, 1941; *H. baylisi* Ogden, 1969; *H. brevispiculum* Baylis, 1923; and *H. longissima* (Ortlepp, 1923). Bilquees and Khanum (1970) described a sixth species, *H. hamiltonii,* from 14 female specimens; however, their description and drawings do not agree with the emended generic description given by Ogden (1969), hence we consider *H. hamiltonii* a *species inquirenda.*

The genus *Heliconema* commonly infects anguilliform fishes and has been reported from Africa, India, the Indian Ocean, Australia, China, Japan, and the Gulf of Mexico. Ortlepp's (1923) description of *H. longissima* from "snakes" in Australia was the only report of *Heliconema* in other than anguilliform fish. The fact that all other reports of *H. longissima* had been from anguilliforms led Ogden (1969) to agree with Chabaud and Campana-Rouget's (1956) suggestion that the host record of "snakes" given by Ortlepp (1923) was doubtful. Thus, the discovery of *H. serpens* confirms the occurrence of *Heliconema* in snakes.

*Heliconema serpens* is easily distinguished from other species in the genus by possessing eight to nine instead of 12 or 20 tessellated longitudinal ridges in the last quarter of the body, four postcloacal papillae instead of five or more, and a spicule ratio of 1:1.5–2.0. In possessing indistinct vulvar lips, *H. serpens* most closely resembles the females of *H. baylisi, H. longissima,* and *H. ahiri;* however, *H. longissima* and *H. ahiri* are shorter in length (14–35.8 mm) and *H. baylisi* has smaller eggs (32–42 × 21–29) in addition to a shorter length (18.4–26.5 mm).

### Key to the Species of the Genus *Heliconema,* Travassos, 1919

1. Males with 20 tessellated longitudinal ridges in last quarter of body, females with prominent vulvar ridges _____ 2
   Males with less than 20 tessellated longitudinal ridges, females with indistinct vulvar ridges _____ 3
2. Spicule ratio (right:left) averaging 1:20_____ *H. heliconema*
   Spicule ratio averaging 1:2.4 _____ *H. brevispiculum*
3. Males with 8–9 tessellated longitudinal ridges, spicule ratio averaging 1:1.7_____ *H. serpens*
   Males with 12 tessellated longitudinal ridges _____ 4
4. Spicule ratio averaging 1:1.5 _____ *H. baylisi*
   Spicule ratio averaging 1:2.7 _____ 5
5. Caudal papillae 9 pairs_____ *H. longissima*
   Caudal papillae 11 pairs_____ *H. ahiri*

### *Camallanides malayensis* sp.n.
#### (Figs. 8–15)

DESCRIPTION: Body reddish with region of greatest width anterior to midpoint. Lips lacking. Four cephalic papillae, 1 associated with each quadrant of buccal capsule; amphids lateral. Mouth dorsoventrally elongated. Cuticle with transverse striations 4–9 apart, coarser in esophageal region. Buccal capsule reddish-brown, with from 10 to 17 chitinous longitudinal bands separated by a mediolateral groove
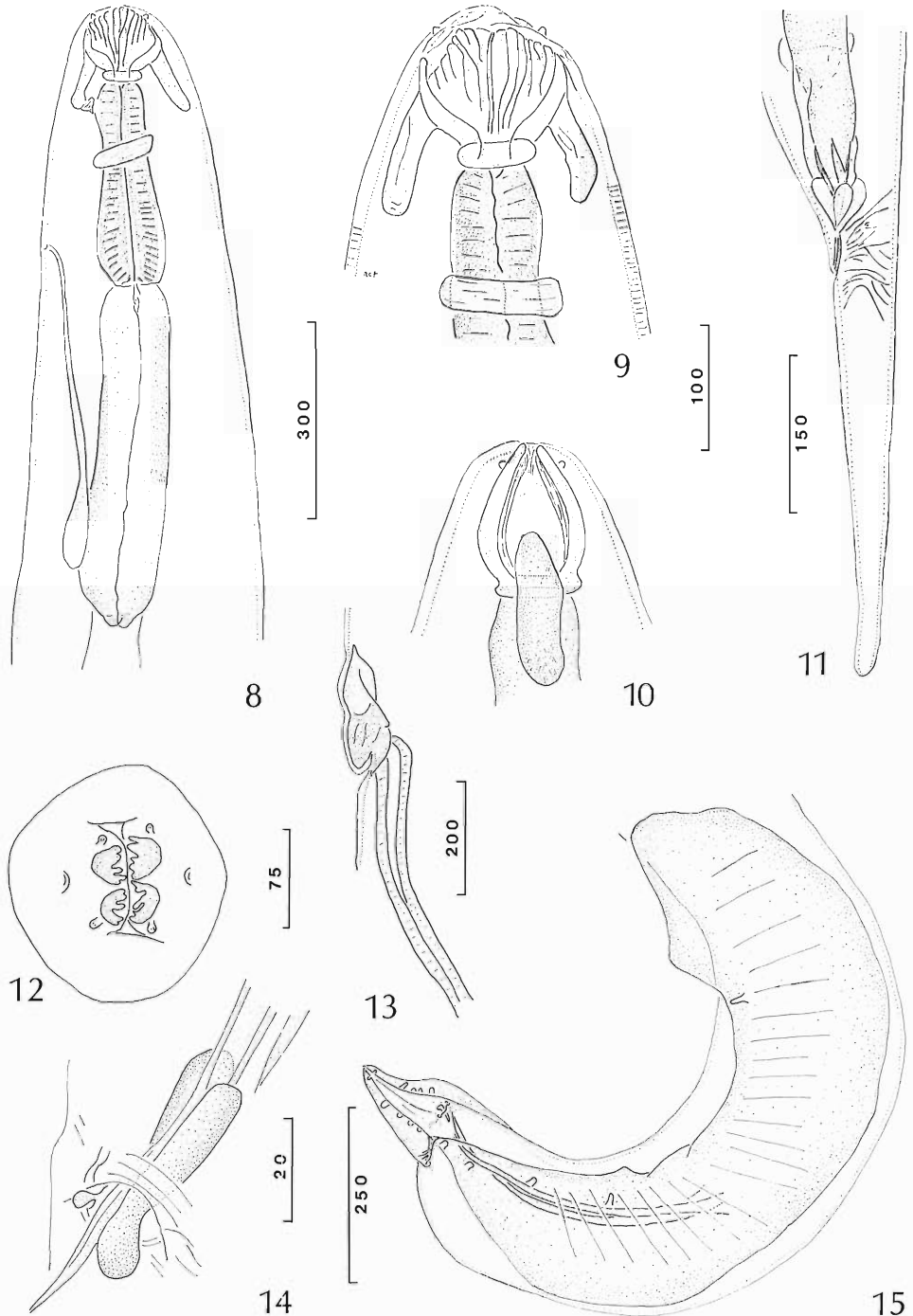
**Figures 8–15.** *Camallanides malayensis* sp.n. Measurements are in micrometers. **8.** Anterior portion of female, lateral view. **9.** Cephalic region of female, lateral view. **10.** Cephalic region of female, dorsoventral view. **11.** Female tail, lateral view. **12.** *En face* of female. **13.** Vulva showing posterior bend and vulvar flap along with a portion of the vagina vera. **14.** Gubernaculum of male, also illustrating one of a pair of cloacal papillae and spicule tips. **15.** Male tail, lateral view except for ventrally folded region posterior to cloaca.

(Fig. 12). One dorsal and 1 ventral rodlike formation (monodent) associated with buccal capsule. Deirids absent. Esophagus with anterior muscular portion slightly club-shaped and posterior glandular portion slightly longer than muscular portion ending in paired, lobed valves. Nerve ring at level of anterior ½ to ⅓ of muscular esophagus. Excretory pore located posterior to nerve ring opposite posterior ½ of muscular esophagus. Tail shape varying with sex.

MALE (based on 3 mature specimens): Body 10.0–11.7 mm (11.0 mm) long by 166–242 (206) wide at junction of muscular and glandular portions of esophagus, increasing posteriorly to 260–319 (285) at level of greatest width, 31–45 (39) times longer than wide. Buccal capsule 85–90 (88) long by 76–85 (82) wide, striated with from 12 to 15 (14) longitudinal bands; monodent 72–81 (78) long, 0.84–0.90 (0.88) times buccal capsule length. Esophagus 651–705 (672) long, 5.6–7.1% (6.2%) of body length; muscular portion 238–247 (242) long by 81–85 (82) wide, comprising 35–37% (36%) of entire esophagus; glandular portion 413–458 (430) long by 99 wide. Nerve ring 153–180 (163) from cephalic end, 22–31 (25) in height. Excretory pore located 283–292 (288) from anterior end, 130–135 (133) posterior to nerve ring. Testis moderately sinuous, not reflexed, 4.0–5.0 mm (4.4 mm) from cephalic end. Spicules gradually tapering posteriorly to a point, similar in shape; left spicule 415–492 (446) long; right spicule 492–539 (515) long; spicule ratio 1:1.1–1.2. Gubernaculum small, approximately 45 long. Caudal alae extending 893–1,123 (995) from posterior end, supported by 11 symmetrical pairs of papillae; 5 precloacal pairs, 2nd to 5th pairs supporting alae along length adjacent to spicules, separated from 1st anteriormost pair; 5 postcloacal pairs, 4th pair longer than others; 1 cloacal pair, located along cloacal opening. Phasmids not observed. Ventral prominent muscular bands in anal region extending anteriorly from region anteriad to 3rd pair preanal papillae to beginning of alae. Tail pointed, flexed ventrad, 62–108 (87) long.

FEMALE (based on 10 mature specimens): Body 15.4–25.8 mm (19.5 mm) long by 211–292 (250) wide at junction of muscular and glandular portions of esophagus, increasing posteriorly to between 301–540 (393) at level of greatest width, 41–58 (50) times longer than wide. Buccal capsule 99–121 (112) long by 94–117 (111) wide, striated with from 10 to 17 (12.8) longitudinal bands; monodents 85–108 (96) long, 0.80–0.95 (0.86) times buccal capsule length. Esophagus 709–881 (809) long, 3.3–5.2% (4.2%) of body length; muscular portion 287–332 (331) long by 85–103 (94) wide, 36–42% (39%) of entire esophagus; glandular portion 408–567 (498) long by 99–227 (108) wide. Nerve ring 193–242 (220) from cephalic end, 27–31 (28) in height. Excretory pore located between 337–427 (380) from anterior end, 113–189 (125) posterior to nerve ring. Vulva with posterior bend and vulvar flap, situated 7.3–11.7 mm (8.8 mm) from anterior end, 43–47% (45%) of body length; vagina vera 198–269 (237) long, extending posteriorly from vulva, 51–67 (60) at widest point; vagina uterina 938–2,252 (1,629) long by 31–49 (40) wide, 4.3–10.0 (6.9) times longer than vagina vera; uterus monodelphic, saclike, ending in a blind sac posteriorly; oviduct 916–1,285 (1,100) long forming a seminal receptacle 143–262 (190) long by 71–119 (89) wide, 277–761 (492) from uterus; ovary 1,904–2,761 (2,441) long, directed posteriorly, 7.6–14.4% (12.4%) of body length, 1.7–2.6 (2.2) times longer than oviduct. Larvae 492–692 (595) long by 22–27 (23) wide. Tail blunt, 335–450 (395) long. Rectum 134–206 (175) long surrounded by 3 rectal glands comprising a circular arrangement along base of rectum.

HOST: *Cerberus rhynchops* (Schneider) (Reptilia: Colubridae), dog-faced water snake.

SITE OF INFECTION: Small intestine and stomach.

LOCALITIES: Kampong Pasir Alai Malacca (type locality), Pasir Malacca, and Port Swettenhan, all in West Malaysia.

HOLOTYPE: USNM Helm. Coll. No. 75096.

ALLOTYPE: USNM Helm. Coll. No. 75097.

PARATYPES: USNM Helm. Coll. No. 75098 (7 females, 2 males—one with broken caudal region).

ETYMOLOGY: The specific name *malayensis* refers to the type locality of the worm.

### Remarks

Six species of *Camallanides* have been previously described (Ivashkin et al., 1971; Jones, 1978): *C. prashadi* Baylis and Daubney, 1922 (type species); *C. cerberi* Jones, 1978; *C. dhamini* Deshmukh, 1968; *C. hemidenta* Majumdar, 1965; *C. piscatori* Khera, 1954; and *C. ptyas* Khera, 1954. A seventh species is listed by Ivashkin et al. (1971), *C. bungari* (MacCallum, 1918) Yamaguti, 1961, but the description and illustrations are based on females and are not consistent with the generic description. This, in addition to the species not being reported since MacCallum (1918), leads us to consider *C. bungari* a *species inquirenda*. Members of the genus *Camallanides* usually infect snakes of the families Elaphidae and Colubridae, although *C. prashidi* has been reported from a ranid frog and *C. hemidenta* was described from a channid fish. Three morphologically distinct species of *Camallanides* have now been reported from *Cerberus rhynchops*: *C. prashadi* from Thailand (Jones, 1978), *C. cerberi* from Australia (Jones, 1978), and now *C. malayensis* from Malaysia. The genus has also been reported from India, Pakistan, and Java.

The males of *C. malayensis* can be distinguished easily from other members of the genus by possessing large and similar spicules rather than comparatively short, dissimilar spicules. In addition, the papillae number and arrangement of *C. malayensis* differs from all other species except *C. ptyas,* which has a similar number but different arrangement. The male *C. ptyas* further differs from *C. malayensis* by generally having much smaller measurements and possessing deirids.

The females of *C. malayensis* can be distinguished from the other species by the large size of the larvae. Only *C. dhamini* and *C. cerberi* have larvae close to the length of *C. malayensis* (490 and 600 compared to 492–692). However, female *C. dhamini* are smaller and possess a more anteriorly located vulva than *C. malayensis*. The description of female *C. cerberi* gives no characters that could easily be used to distinguish it from *C. malayensis*.

### Key to the Species of *Camallanides* Baylis and Daubney, 1922

1. Gubernaculum absent ................................................................................................. 2
   Gubernaculum present.............................................................................................. 3
2. Caudal papillae 6 in number ........................................................... *C. hemidenta*
   Caudal papillae 14–18 in number ..................................................... *C. cerberi*

3. Caudal papillae 14–15 in number _____ 4
   Caudal papillae 11 or less in number _____ 6
4. Gubernaculum averaging 170 in length _____ *C. dhamini*
   Gubernaculum 25–29 in length_____ 5
5. Spicule ratio averaging 1:1.4, 5 postcloacal papillae _____ *C. piscatori*
   Spicule ratio averaging 1:1.8, 7 postcloacal papillae _____ *C. prashadi*
6. Spicule ratio 1:1.1–1.2, gubernaculum 45 long _____ *C. malayensis*
   Spicule ratio 1:2.0–2.1, gubernaculum 28 long _____ *C. ptyas*

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# Influence of Different Crops on the Dimensions of *Meloidogyne arenaria* Isolated from Fig

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ABSTRACT: A more striking effect of crops was observed on the dimensions of adult egg-laying females than on the pre-parasitic larvae and matured eggs of *Meloidogyne arenaria*. Generally, adult egg-laying females particularly from tomato, potato, and tobacco were larger with shorter neck length than those in barley, corn, lettuce, and carrot. The variations in the mean body length and width and neck length were highly significant in most of the crops. The size of matured eggs was also significantly affected by some crops. Those *M. arenaria* reared in carrots produced the smallest eggs (88.4 × 37.5 $\mu$m), while the largest eggs (104.3 × 39.9 $\mu$m) were produced by the nematodes in figs. Most of the crops had no apparent effect on the body width and stylet length of the larvae that hatched within 24 hours. It was only in the body length, tail length, and esophagus length to the end of the valve in the median bulb that most of the crops had significant influence. It was also shown that the age of pre-parasitic larvae had some effect on the body length but not on the body width, stylet, esophagus, and tail lengths.

Descriptions of root-knot nematode species are mainly based on morphological features and dimensions, as well as the proportions of parts of the nematode body at different stages of development. Systematic nematologists have attempted to emphasize dimensions and proportions of parts of the nematode in separating different species. This is particularly true in using larvae in species diagnosis. For instance, in his proposed key to larvae of *Meloidogyne* spp., Whitehead (1968) mainly used the dimensions of the larvae to separate and define the species of the nematodes. For example, larvae of *M. indica* could be distinguished from larvae of *M. artiellia* in that the former has a mean body length of 415 $\mu$m and 17-$\mu$m mean tail length whereas the latter has a mean body length of around 336 $\mu$m and 22-$\mu$m mean tail length. Following this key, however, Mulvey et al. (1975) claimed that larvae of *M. microtyla* which they described as a new species would easily key to *M. incognita*. This may be expected as the degree of variability of those dimensions within and among the species has not yet been established; neither are the factors of environment that influence such variations known and defined.

Goodey (1952) showed that host plants could exert considerable influence on the dimensions of *Ditylenchus destructor* Thorne. In root-knot nematodes, however, the extent of variation in their dimensions due to the influence of host crops and other environmental conditions has not yet been thoroughly investigated. In a previous paper (Davide, 1979), I obtained evidence that the dimensions of this fig isolate of *M. arenaria* not only vary at different stages of development but also vary with the two different host crops tested. It was observed that the adult egg-laying females were relatively bigger or broader in tomato plants than in cucumber plants and, in addition, they also have greater neck length and esophagus length to the end of median bulb.

This study, therefore, was primarily conducted to obtain further information on the effects of various crops on the dimensions of adult egg-laying females,

matured eggs, and pre-parasitic larvae of this fig isolate of *M. arenaria*. The effect of age of larvae was also included in the study.

## Materials and Methods

### Measurements of larvae

Larvae that hatched in water within 24 hr from egg masses taken from adult females in galls of different crops were randomly picked up and mounted on ringed slides with a few drops of water. They were killed gently by heat. The larvae were immediately examined and measured under a calibrated microscope. Measurements of the body length and width, and stylet, esophagus, and tail lengths of 20 random samples of larvae were made from each crop. Since it was difficult to measure the esophagus length to its posterior end, the measurement was made only up to the end of the valve in the median bulb.

To determine the effect of age of larvae on their dimensions, egg masses from adult females in galls of tobacco plants were picked up and placed in hatching dishes with water. After 24 hr, all the larvae that had hatched were pipetted out and placed on a small dish. From these larvae 20 individuals were randomly picked up and mounted on ringed slides with a few drops of water, killed gently by heat, and measured immediately to represent the 24-hr-old treatment. The remaining larvae were then stored in the same dish in the laboratory for subsequent sampling at 48, 72, and 144 hr after hatching. Measurements also were made on the body length and width, and stylet, esophagus, and tail lengths using 20 random samples of larvae in each period of examination.

### Measurements of adult females

Adult females that had already laid eggs in egg sacs were used in this study, as it has been shown in another study (Davide, 1979) that at this stage they have already attained the maximum size. They were dissected from gall samples (1 g) taken from each crop 2 months after inoculation. The galls were first fixed in FAA for at least 48 hr, washed in tap water, and stained in boiling acid–fuchsin lactophenol for 2 minutes. They were then kept in vials with clear lactophenol for at least 5 days before the adult egg-laying females were dissected and mounted on ringed slides with a few drops of clear lactophenol. To prevent breakage or distortion of the nematode body, no coverslips were placed over them. From each crop, 20 random samples of adult egg-laying females were examined and measured under a calibrated microscope. Measurements were made only on the body length and width and neck length, as the other parts of the body were not clearly seen on the stained specimens.

### Measurements of matured eggs

From the stained galls of different crops, egg masses were removed from adult females. They were placed on ringed slides with a few drops of clear lactophenol. From each crop, 20 random samples of matured eggs were taken and measured for their lengths and widths.

In this study no measurement was made on the males because they were not found in most of the crops.

**Table 1.** Effects of different crops on the dimensions of about 24-hr-old larvae of *M. arenaria* isolated from fig.

| Crops | Larval measurement* ($\mu$m) | | | | |
| | Body length | Body width | Stylet length | Esophagus length† | Tail length |
|---|---|---|---|---|---|
| Barley | 418.95 b | 14.28 a | 14.53 ab | 65.16 bc | 45.5 a |
| Bean (Dwarf) | 410.9 a | 14.16 a | 14.35 a | 61.68 ab | 48.7 b |
| Cauliflower | 438.6 c | 14.61 a | 14.61 ab | 68.78 d | 52.5 c |
| Corn | 407.4 a | 14.96 ab | 14.44 a | 61.43 a | 45.1 a |
| Cucumber | 409.8 a | 14.97 ab | 14.88 ab | 66.01 c | 51.92 c |
| Fig (Weeping) | 408.8 a | 14.70 ab | 14.70 ab | 62.38 ab | 50.4 bc |
| Lettuce | 406.0 a | 15.23 b | 14.53 ab | 62.29 ab | 49.48 b |
| Potato | 410.2 a | 14.70 ab | 14.53 ab | 63.29 b | 51.19 bcd |
| Radish | 438.5 c | 14.61 a | 14.44 a | 67.03 c | 50.58 bc |
| Spinach | 428.4 bc | 14.53 a | 14.70 ab | 64.05 bc | 48.83 b |
| Soybean | 423.8 b | 15.45 b | 15.25 b | 66.24 c | 50.23 bc |
| Tobacco | 410.0 a | 15.17 b | 15.00 b | 62.56 ab | 51.89 c |
| Tomato | 445.2 c | 15.49 b | 15.14 b | 66.50 c | 54.43 d |
| Turnip | 434.3 bc | 14.82 ab | 14.70 ab | 67.11 cd | 51.89 c |
| Watermelon | 420.0 ab | 15.23 b | 15.05 b | 65.27 c | 49.52 b |
| Wheat | 428.6 b | 14.88 a | 14.56 ab | 62.74 ab | 48.91 b |

\* Data are means of 20 random samples of the nematode larvae. Means followed by the same letter within each column are not significantly different at $P = 0.05$.

† Measured to the end of the median valve.

## Results

### Measurements of 24-hr-old larvae from different crops

As shown in the results summarized in Table 1, it is evident that the 16 different crops tested had some significant effects on the dimensions of the larvae that hatched within 24 hr. Statistical analysis of the data using Student's *t*-test indicated that a great number of the crops gave a highly significant difference in the mean body length, esophagus length to the end of the median valve, and tail length. However, only very few of the crops had any significant influence on the body width and stylet length.

### Effect of age of larvae

It is evident from the results presented in Table 2 that the age of larvae had significant influence only on the body length. It did not affect the body width, or stylet, tail, and esophagus lengths. The mean length of 48-hr-old larvae was about 8 $\mu$m greater than that of 24-hr-old larvae, while those 72 and 144 hr old were longer by 17 and 30 $\mu$m, respectively. This indicates that there was a continuous lengthwise growth of the larvae in water suspension when kept for several days after hatching.

### Measurements of adult egg-laying females and matured eggs

As shown in the data of Table 3, different crops had pronounced effects on the dimensions of adult egg-laying females. Statistical comparison of individual means indicated that a great majority of the crops had significant influence on the body length and width and neck length. However, for matured eggs there were more crops showing significant influence on the length than on the width.

Table 2.  Effects of age on the dimensions of pre-parasitic larvae of *M. arenaria* isolated from fig.

| Larval age (hr) | Larval measurement* ($\mu$m) | | | | |
|---|---|---|---|---|---|
| | Body length | Body width | Stylet length | Esophagus length† | Tail length |
| 24 | 410.0 a‡ | 15.17 | 15.0 | 62.6 | 51.9 |
| 48 | 417.9 ab | 14.96 | 14.9 | 61.3 | 51.9 |
| 72 | 426.6 b | 14.61 | 15.0 | 64.2 | 52.8 |
| 144 | 440.2 c | 14.61 | 14.7 | 62.8 | 52.7 |

* Data are means of 20 random samples of the nematode larvae.
† Measured to the end of the median valve.
‡ Means followed by the same letter are not significantly different at $P = 0.05$.

## Discussion

The influence of the different crops on the dimensions of the nematodes was more striking on the adult egg-laying females than on the pre-parasitic larvae. This may be due to the fact that these adult females have been in direct contact with the host plants since the time they entered, have been nourished, and have developed to maturity inside the gall tissues, whereas the pre-parasitic larvae have not yet made any direct contact with the plants. It seems, however, that nutrition or the availability of nutrients in the host plants is the main factor that determines the growth and increase in size of the nematode body. It was generally observed that those nematodes in less susceptible crops like barley, corn, and radish were relatively smaller than those in more susceptible crops such as potato, tomato, cucumber, tobacco, and others.

Table 3.  Effects of different crops on the dimensions of adult females and eggs of *M. arenaria* isolated from fig.*

| Crop | Adult egg-laying female ($\mu$m) | | | Matured eggs ($\mu$m) | |
|---|---|---|---|---|---|
| | Body length | Body width | Neck length | Length | Width |
| Barley | 468.3 a | 283.1 b | 211.8 cd | 91.0 ab | 36.3 a |
| Bean (Bread) | 710.5 de | 434.0 deg | 193.6 bc | 97.8 c | 37.4 ab |
| Bean (Dwarf) | 608.3 bc | 411.6 de | 149.5 ab | 91.6 b | 39.1 bc |
| Carrot | 602.7 bc | 194.8 a | 231.0 bcd | 88.4 ab | 37.5 ab |
| Cauliflower | 602.7 bc | 365.7 cd | 154.0 ab | 92.9 b | 40.0 c |
| Corn | 574.0 b | 360.7 cd | 162.7 a | 90.6 ab | 39.9 c |
| Cucumber (Explorer) | 691.6 cde | 386.1 d | 223.3 cd | 97.4 c | 39.9 c |
| Cucumber (Long Green) | 741.6 e | 348.9 cd | 241.2 d | 99.7 c | 38.6 bc |
| Fig (Weeping) | 663.0 cd | 335.0 c | 212.0 cd | 104.3 d | 39.9 c |
| Lettuce | 647.2 cd | 384.7 d | 239.1 cd | 90.4 ab | 39.1 bc |
| Potato | 725.5 de | 438.2 c | 191.1 bc | 88.4 ab | |
| Radish | 579.6 b | 324.8 bc | 163.1 ab | 98.0 c | 38.1 b |
| Spinach | 709.4 de | 429.1 c | 201.9 c | 97.3 c | 38.5 bc |
| Tobacco | 692.6 de | 526.7 f | 143.5 a | 90.0 ab | 39.3 c |
| Tomato | 711.5 de | 479.5 g | 194.6 b | 94.7 bc | 39.5 c |
| Turnip | 684.6 d | 441.5 eg | 170.5 b | 94.9 bc | 38.4 bc |
| Watermelon | 700.2 de | 386.7 d | 243.5 d | 90.0 ab | 38.4 bc |
| Wheat | 637.0 c | 360.3 cd | 186.9 b | 92.2 b | 38.8 bc |

* Data are means of 20 random samples of adult egg-laying females and matured eggs of the nematode. Means followed by the same letter within each column are not significantly different at $P = 0.05$.

From the taxonomic point of view, this wide variation in body length and width of adult females as influenced by the host crops would certainly affect the usefulness of these parts in species diagnosis. I fully support the view of Esser et al. (1976) that body length of adult females as well as alpha measurements be rejected as criteria in species diagnosis.

Therefore, in considering the dimensions and body ratios as criteria in specific descriptions of root-knot nematodes, it may be important to specify the host plants and other environmental conditions to which the nematodes were subjected prior to their diagnosis. This is, in effect, emphasizing Goodey's (1952) statement that systematic nematologists should adopt what is essentially an ecological attitude in studying nematode behavior and the reaction of their host, as well as their appearance.

## Acknowledgment

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# Two New Nematode Genera, *Safianema* (Anguinidae) and *Discotylenchus* (Tylenchidae), with Descriptions of Three New Species

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ABSTRACT: *Safianema* gen.n. is proposed under Anguininae, family Anguinidae. It differs from *Ditylenchus* in having esophageal glands forming a long diverticulum over the intestine. It is compared with *Pseudhalenchus* whose diagnosis is amended. *Safianema lutonense* gen.n., sp.n. is described from peaty soil under oak in Luton, England. *Safianema anchilisposomum* (Tarjan, 1958) comb.n., *S. damnatum* (Massey, 1966) comb.n., and *S. hylobii* (Massey, 1966) comb.n. are proposed for species previously in *Pseudhalenchus*. *Discotylenchus* gen.n. is close to *Filenchus* but has a strongly tapering lip region with a distinct disc at the apex. Two new species of this genus are described—*D. discretus* (type species) from apple and cabbage soils at Damascus, Syria, and *D. attenuatus* from bush soil at Ibadan, Nigeria.

From peaty soil underneath an oak tree at Luton Hoo, Luton, Bedfordshire, England, *Safianema lutonense* gen.n., sp.n. was collected by my daughter Safia Fatima Siddiqi; the new genus is named after her and the name is neuter in gender. *Discotylenchus* gen.n. is proposed under Tylenchidae for two new species, *D. discretus* (type species) and *D. attenuatus,* described below.

The families Anguinidae and Tylenchidae were defined and differentiated from each other by Siddiqi (1971). Thus the genera *Ditylenchus* Filipjev, 1936 and *Tylenchus* Bastian, 1865 which were associated together under Tylenchidae for a long time were separated into two families, the former genus belonging to Anguinidae and the latter to Tylenchidae. Siddiqi (1970) and Golden (1971) proposed new subfamilies, Pseudhalenchinae and Ditylenchinae, respectively. In the present article, *Safianema* gen.n. is assigned to the Anguininae (syn. Ditylenchinae) of the Anguinidae (see definitions and compositions of these in Siddiqi, 1971) because of its close affinities with *Ditylenchus* and *Anguina* in the characters of the lip region, spear, gonads, spicules, and the absence of the prominent cardia. The genus *Pseudhalenchus* and its subfamily Pseudhalenchinae are removed from Anguinidae to Tylenchidae and the diagnosis of *Pseudhalenchus* Tarjan, 1958 is amended.

The specimens were killed by applying gradual heat, fixed in F.A. 4:10, and mounted in dehydrated glycerine containing traces of picric acid, after processing through warm lactophenol.

## *Safianema* gen.n.

DIAGNOSIS: Anguininae; Anguinidae. Body small-sized (about 1 mm or less long), slender. Lateral fields with 6 incisures. Deirids present near level of excretory pore. Phasmids not observed. Lip region low, flattened, smooth. Spear small, with conus less than ½ its length and small rounded basal knobs. Median esophageal bulb oval, valvate. Cardia absent. Anterior region of intestine at esophageal glands usually very narrow and without distinct lumen. Esophageal glands extending over intestine mostly laterally; dorsal gland enormously developed. Anterior branch of female reproductive organs well developed, out-
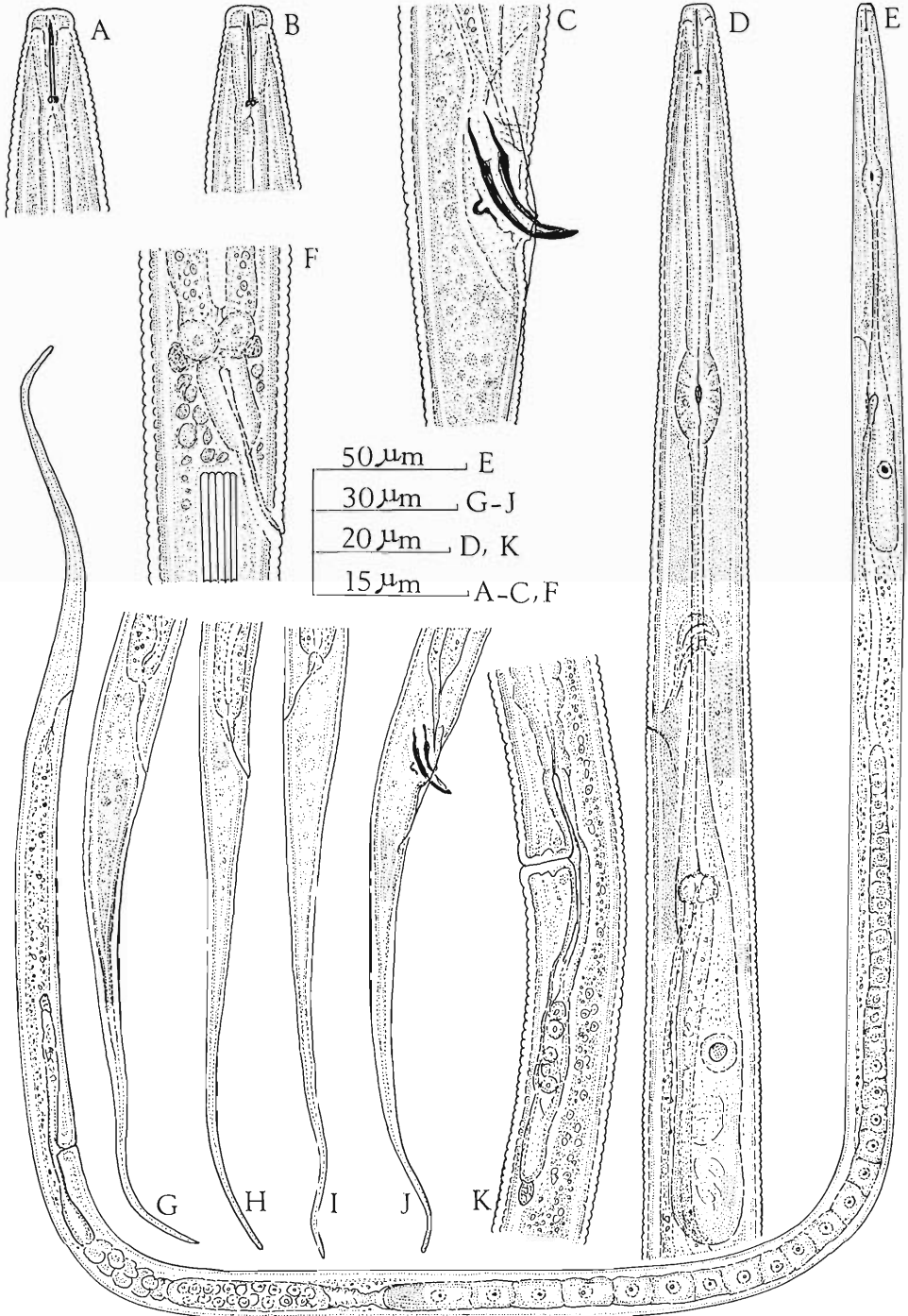
Figure 1. *Safianema lutonense* gen.n., sp.n. A. Female head end. B. Male head end. C. Spicular region. D. Female esophageal region. E. Female. F. Female rectal region. G–I. Female tails. J. Male tail. K. Vulval region. (D, E, K, holotype.)

stretched. Quadricolumella well developed. Spermatheca elongated, axial. Ovary with a single row of oocytes. Posterior branch reduced to a uterine sac. Tail in both sexes elongate-conoid to filiform. Spicules ventrally arcuate, anteriorly expanded. Gubernaculum small, trough-shaped, fixed. Sperm cells large, rounded. Caudal alae adanal or may extend to cover up to ⅔ of tail.

TYPE SPECIES: *Safianema lutonense* gen.n., sp.n.

OTHER SPECIES: *Safianema anchilisposomum* (Tarjan, 1958) comb.n., syn. *Pseudhalenchus anchilisposomus* Tarjan, 1958; *S. damnatum* (Massey, 1966) comb.n., syn. *P. damnatus* Massey, 1966; *S. hylobii* (Massey, 1966) comb.n., syn. *P. hylobii* Massey, 1966.

### Relationship and discussion

In the structure of the body, spear, female gonad, lip region, and spicules, *Safianema* gen.n. comes close to *Ditylenchus* Filipjev, 1936 but differs in having the esophageal glands extending as an elongated lobe over the intestine and narrowed intestinal region at the esophageal glands.

In having overlapping esophageal glands and slender body, *Safianema* is similar to *Pseudhalenchus* Tarjan, 1958, which, as shown below, is more nearly related to members of the Tylenchidae than to those of the Anguinidae.

### *Safianema lutonense* gen.n., sp.n.
### (Fig. 1A–K)

MEASUREMENTS: Eleven ♀♀ (paratypes): L = 0.72–0.95 (0.83) mm; a = 42–60 (46.5); b = 5.5–7.5 (6.4); b′ = 3.7–5.3 (4.3); c = 7.2–8.5 (7.7); c′ = 8–11 (9.2); V = 70–74 (71.8); spear = 8.0–9.4 (8.6) $\mu$m. Eleven ♂♂ (paratypes): L = 0.61–0.85 (0.71) mm; a = 45–58 (52); b = 5.5–6.8 (6.3); b′ = 3.8–4.5 (4.2); c = 6.5–7.8 (7.1); c′ = 8.2–11.0 (9.0); T = 45–59 (53); spear = 8–9 (8.6) $\mu$m; spicules = 15–17 (16) $\mu$m; gubernaculum = 4–5 (4.5) $\mu$m. Holotype ♀: L = 0.95 mm; a = 59; b = 7.5; b′ = 5.3; c = 8; c′ = 10; V = $^{47\text{-}}71.5^{\text{-}5.2}$; spear = 8 $\mu$m.

DESCRIPTION: FEMALE: Body almost straight to arcuate, usually with a slight bend at vulva, when relaxed by gentle heat. Transverse striae fine, 0.8–1.0 $\mu$m apart at midbody. Lateral fields about ¼ as wide as body, with 6 distinct incisures, reducing in number towards extremities. Lip region low, caplike, smooth, continuous; cephalic sclerotization light. Spear delicate, with conus about ⅓ of its length; basal knobs minute, rounded, 1.5 $\mu$m across. Orifice of dorsal esophageal gland 1.5 $\mu$m behind spear base. Median esophageal bulb oval, about 15 $\mu$m or corresponding body diameter long and half as much wide, with a distinct valvular apparatus in center. Distance from anterior end to center of median esophageal bulb 54–60 (57) $\mu$m. Isthmus narrower than slender part of the corpus, 45–50 (48) $\mu$m long, crossed by nerve ring near its middle or slightly anteriorly. Dorsal esophageal gland lobelike, extending over intestine 45–59 (52) $\mu$m, mostly laterally; its nucleus near middle. Subventral esophageal glands small, near level of esophago-intestinal junction. Latter controlled by 2 modified anteriormost cells of intestine apparently acting as a valve. Lumen of intestine narrower and indistinct in region of dorsal esophageal gland (Fig. 1D). Excretory pore 84–106 (94) $\mu$m from anterior end; hemizonid 4–5 annules long, just anterior to excretory pore; deirids distinct, in region of excretory pore.

Vulva a transverse slit, neither raised nor depressed. Uterus with a proximal muscular and a distal glandular part represented by a prominent quadricolumella. Spermatheca elongate-oval, about 3 body-widths long, packed with sperms which are about 4.5 $\mu$m in diameter. Uterine egg 76 × 16 $\mu$m long. Post-vulval uterine sac 35–53 (46) $\mu$m or 2.6–3.3 (2.9) body-widths long. Ovary single, anteriorly outstretched, with oocytes in a row, may extend to the esophageal gland region.

Rectum about 1½ anal body-widths long, opening with a distinct anus. Tail elongate, filiform, 85–113 (105) $\mu$m or 8–11 anal body-widths long, transversely striated, ending in an acute to minutely rounded tip.

MALE: Body straight to slightly arcuate. Lip region, spear and esophagus as in female. Anterior end to center of median esophageal bulb 48–56 (54) $\mu$m. Dorsal esophageal gland extending over intestine for 49–59 (52.4) $\mu$m. Excretory pore 80–85 (82) $\mu$m from anterior end, just behind hemizonid which is 4–5 annules long. Testis single, anteriorly outstretched. Spicules cephalated, arcuate; gubernaculum small, fixed, trough-shaped in lateral view but inverted U- or S-shaped when spicules are protruding. Caudal alae smooth, sometimes with wavy margins, covering ⅙ to ⅕ of tail. Tail elongate, filiform, ending in a minutely rounded tip.

TYPE MATERIAL: Holotype ♀ and 3 ♀♀, 3 ♂♂ paratypes at C.I.H., St. Albans, England; 2 ♀♀ and 2♂♂ paratypes at each of these centers: Nematology Department, Rothamsted Experimental Station, Harpenden, England; USDA Nematode Collection, Beltsville, Maryland, USA; Department of Nematology, Landbouwhogeschool, Wageningen, The Netherlands; Division of Nematology, Indian Agricultural Research Institute, New Delhi, India.

TYPE HABITAT AND LOCALITY: Peaty soil underneath an oak tree (*Quercus* sp.) in picnic area, Luton Hoo, Luton, Bedfordshire, England.

## Relationship

*Safianema lutonense* gen.n., sp.n. differs from *S. hylobii* (Massey, 1966) comb.n. in having a smaller body size (♀ L = 1.04–1.06 mm in *S. hylobii*), longer overlap of the dorsal esophageal gland over the intestine, and longer post-vulval uterine sac (less than a body-width long in *S. hylobii*).

The nominal species of *Pseudhalenchus* show considerable morphological diversity. It was shown by Geraert and Kheiri (1970) that the type species, *Pseudhalenchus minutus* Tarjan, 1958, has a female gonad similar to that of *Tylenchus* and several other members of the Tylenchidae. My study of the paratypes of this species and some other populations from Yugoslavia and England confirms this. The spermatheca in these is rounded and sperm cells are small, with very little cytoplasm. The spicules are slender and not prominently thickened. It is, therefore, proposed to revise the diagnosis of the genus *Pseudhalenchus* and to transfer the subfamily Pseudhalenchinae Siddiqi, 1970 from Anguinidae to Tylenchidae.

### *Pseudhalenchus* Tarjan, 1958

DIAGNOSIS (amended): Pseudhalenchinae; Tylenchidae. Body small-sized (less than 1 mm long), with distinct cuticular annules. Lateral fields with 4 distinct incisures. Deirids present near excretory pore; phasmids not seen. Lip region low, flattened, with moderate to indistinct annulation; framework hexaradiate, lightly sclerotized. Spear about 10 $\mu$m long, with rounded basal knobs. Median

esophageal bulb oval, valvate. Sub-ventral esophageal glands forming a pseudob-ulb at base of esophagus but the dorsal gland lobelike and extends over intestine laterally. Anterior end of intestine in region of dorsal esophageal gland very slender and with indistinct lumen, attached to base of esophagus through 2 mod-ified anteriormost intestinal cells which possibly act as an esophago-intestinal valve. Vulva at about 70–80% of body from anterior end. Vagina at right angles to body axis. Post-vulval uterine sac present. Spermatheca rounded, axial; sperm cells small-sized, rounded, with very little cytoplasm. Ovary single, outstretched. Tail in both sexes elongate-conoid, straight to arcuate. Spicules slender, arcuate, cephalated but not prominently expanded anteriorly. Gubernaculum small, trough-shaped, fixed. Caudal alae short, may extend to about ⅓ of tail length.

TYPE SPECIES: *Pseudhalenchus minutus* Tarjan, 1958.

OTHER SPECIES: *P. acutus* Khan and Nanjappa, 1971; *P. indicus* Sethi and Swarup, 1967.

### Relationship and discussion

*Pseudhalenchus* Tarjan, 1958, sensu stricto, is unique among the Tylenchidae in having the dorsal esophageal gland forming a lobe extending over the intestine and the anteriormost part of the intestine in the region of the dorsal gland being slender and with indistinct lumen. Thus it belongs to a separate subfamily of its own, Pseudhalenchinae Siddiqi, 1970, which is now removed from the Anguinidae and assigned to the Tylenchidae on account of the structure of the female gonad, sperm, and spicules as discussed above.

### *Discotylenchus* gen.n.

DIAGNOSIS:   Tylenchinae; Tylenchidae. Body small-sized (under 1 mm long), with thin, finely annulated cuticle. Lateral fields with 4 incisures; deirids present; phasmids in postmedian region of body, in female at or close to vulva level. Lip region strongly tapering, anteriorly truncate, smooth, with a distinct disc at the apex. Spear slender, less than 10 $\mu$m long, conus less than half its length, knobs tiny, rounded. Median esophageal bulb oval, valvate; basal bulb saccate, offset from intestine, with a rounded to discoidal cardia at base. Vulva postmedian (at about 60–70% of body), transverse; vagina at right angles to body axis. Anterior branch of reproductive organs well developed; spermatheca rounded, offset, 2- or 3-lobed; ovary short, not reaching esophagus, with less than 20 oocytes. Pos-terior branch represented by a short post-vulval uterine sac. Tail in both sexes elongate, filiform. Spicules arcuate, cephalated. Gubernaculum small, trough-shaped, fixed. Caudal alae adanal.

TYPE SPECIES: *Discotylenchus discretus* gen.n., sp.n.

OTHER SPECIES: *D. attenuatus* sp.n.

### Relationship and discussion

*Discotylenchus* gen.n. is recognized by its smooth, tapering lip region bearing a terminal flat disc. This disc appears to have been formed by the four submedian lobes which are pronounced in this genus. In other morphological characters the genus is close to *Filenchus* (Andrássy, 1954) Meyl, 1961. The postmedian phas-mids of *Discotylenchus discretus* are similar in position to those of the genus *Filenchus* and other Tylenchinae as reported by Siddiqi (1978).
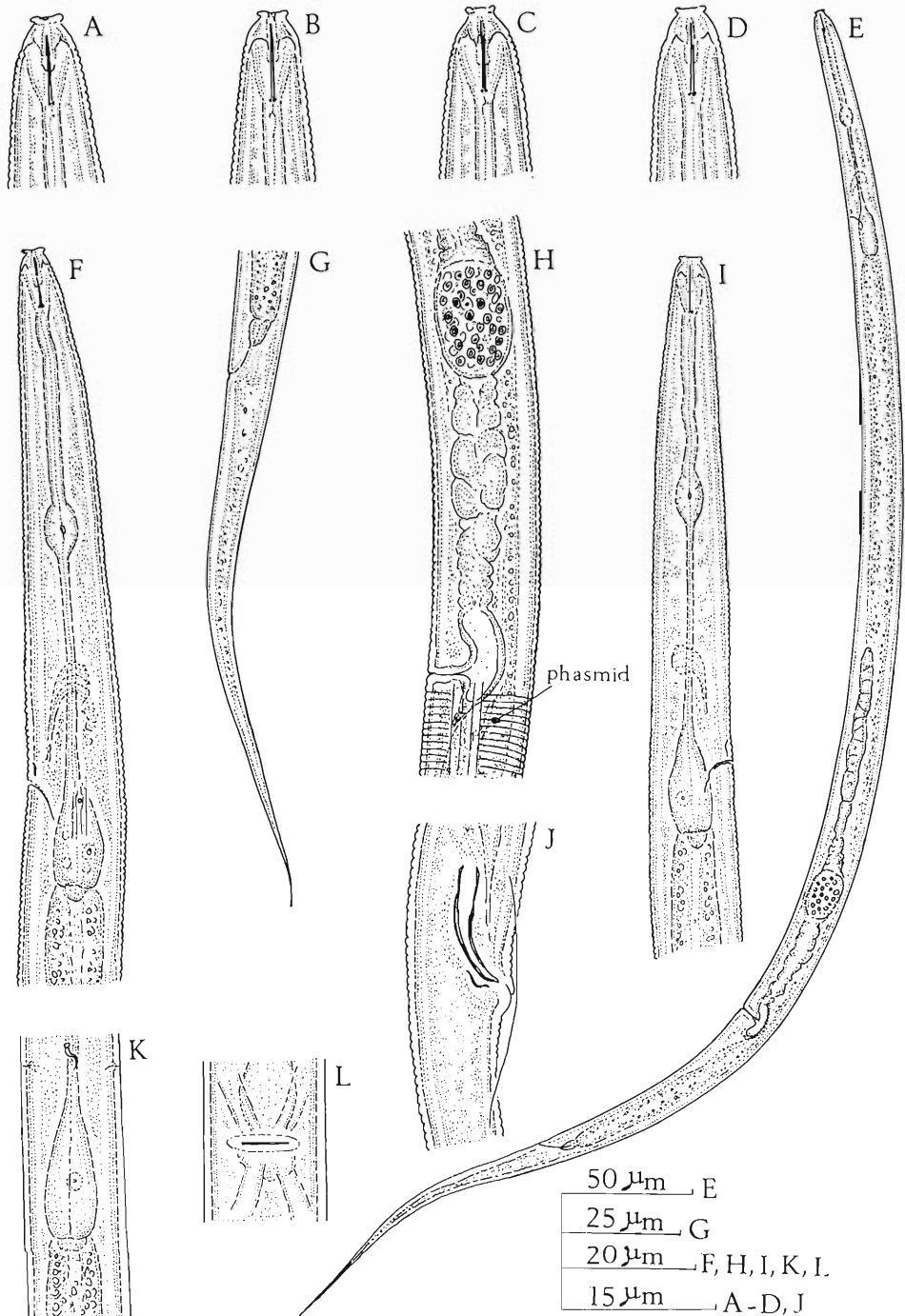
Figure 2. *Discotylenchus discretus* gen.n., sp.n. A–C. Female head ends. D. Male head end. E. Female. F. Female esophageal region. G. Female tail. H. Vulval region. I. Male esophageal region. J. Spicular region. K. Basal region of esophagus in ventral view showing excretory pore and deirids. L. Vulva in ventral view. (A, E–H, holotype.)

### *Discotylenchus discretus* gen.n., sp.n.
### (Fig. 2A–L)

MEASUREMENTS: Seven ♀♀ (paratypes): L = 0.44–0.61 (0.56) mm; a = 29–37 (33); b = 5.0–6.8 (5.7); c = 5.0–6.2 (5.6); c' = 9–11 (10.5); V = 63–67 (65.5); spear = 7.0–7.5 (7.2) μm; MB = 40–49 (45). Two ♂♂ (paratypes): L = 0.40, 0.46 mm; a = 31, 40; b = 4.3, 5.4; c = 5.4, 5.5; c' = 8.3, 10.3; T = 31, 40; spear = 7.0, 7.5 μm; MB = 41, 48; spicules = 11, 13 μm; gubernaculum = 3.5, 4.0 μm. Holotype ♀: L = 0.61 mm; a = 36; b = 6.6; c = 5.3; c' = 10.3; V = 65.5; spear = 7 μm; MB = 43.4.

DESCRIPTION: FEMALE: Body straight to C-shaped upon fixation, marked by fine transverse striae about 1 μm apart near middle. Lateral fields 0.3 of body width, with 4 incisures, not areolated. Lip region smooth, markedly tapering to an offset, distinct labial disc 3 μm in diameter; maximum width of body at lip region base 5 μm (Fig. 2A–C). Cephalic framework hexaradiate, slightly sclerotized. Amphid aperture minute, appears as longitudinal slit in lateral view, near labial disc. Spear extremely delicate, with conus less than ½ its length and minute, rounded basal knobs. Orifice of dorsal esophageal gland 2 μm behind spear base. Median esophageal bulb oval, muscular, valvate, about ½ as wide as corresponding body width; distance from anterior end to its center 39–47 (43) μm. Isthmus elongate, slenderer than precorpus, crossed by nerve ring slightly behind its middle. Basal esophageal bulb saccate, a little more than body width long and about ½ as wide as body at neck base, offset from intestine, with a flat base carrying a large rounded cardia. Excretory pore midway between nerve ring and basal bulb or near distal end of basal bulb, 1–2 annules behind hemizonid which is 3 annules long. Deirids distinct, a little behind level of excretory pore. Intestine with small, round granules. Rectum distinct, 1.5 anal body-widths long. Phasmids outside lateral field, latero-subdorsal in position, near vulva (7 μm behind to 5 μm anterior to vulva) (Fig. 2H).

Vulva a transverse slit, occupying ²/₅ of body width. Vagina at right angles to body axis, extending ²/₅ across body. Post-vulval uterine sac vestigial, ⅓–½ body width long. Gonad single, prodelphic, outstretched. Spermatheca large, round to oval, filled with minute rounded sperm. Oviduct short. Ovary also short, with 6–15 oocytes arranged serially. Vulva–anus distance 70–103 (93) μm. Anus distinct. Tail gradually tapering, filiform, 80–115 (100) μm long.

MALE: Body, lip region, spear, and esophagus similar to that of female. Phasmids at 64–65% of body length from anterior end. Testis anteriorly outstretched; spermatocytes in a row. Spicules arcuate, prominently cephalated, with pointed distal end. Gubernaculum trough-shaped, fixed. Cloacal lips smooth, slightly protuberant. Caudal alae smooth, adanal. Tail filiform.

TYPE MATERIAL: Holotype ♀, 3 ♀♀ and 2 ♂♂ paratypes at C.I.H., St. Albans, England; 1 ♀ paratype at each of these centers: Department of Nematology, Rothamsted Experimental Station, Harpenden, England; USDA Nematode Collection, Beltsville, Maryland, USA; Nematology Department, Landbouwhogeschool, Wageningen, The Netherlands; Division of Nematology, I.A.R.I., New Delhi, India.

TYPE HABITAT AND LOCALITY: Soil around roots of apple trees, Damascus, Syrian Arab Republic. Also collected from soil around cabbage roots in the same locality.
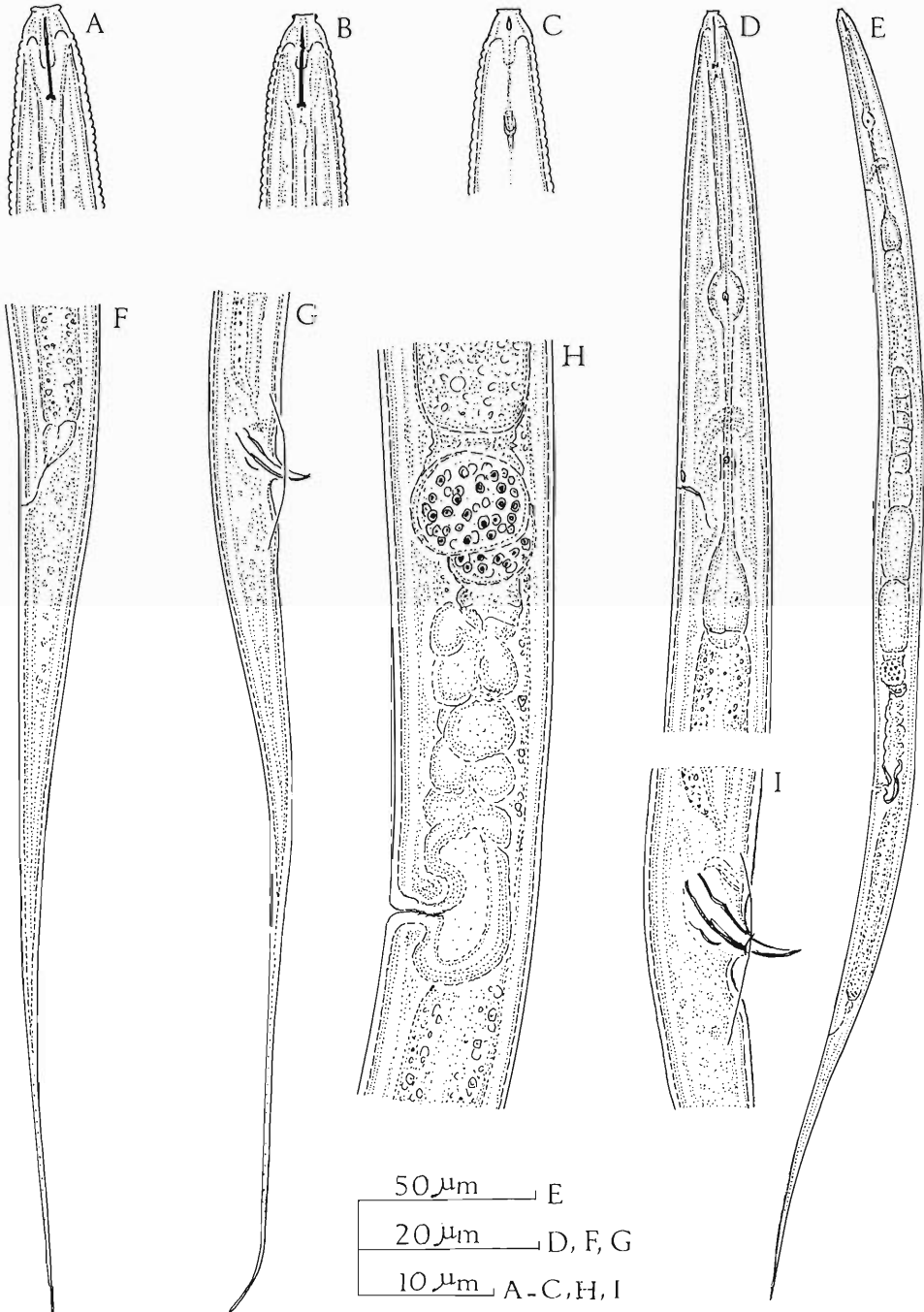
**Figure 3.** *Discotylenchus attenuatus* sp.n. A. Female head end. B, C. Male head ends (C showing amphids). D. Female esophageal region. E. Female. F. Female tail. G. Male tail. H. Vulval regon. I. Spicular region. (A, D–F, H, holotype.)

### *Discotylenchus attenuatus* sp.n.
### (Fig. 3A–I)

MEASUREMENTS: Fifteen ♀♀ (paratypes): L = 0.33–0.40 (0.36) mm; a = 25–33 (29); b = 4.1–5.4 (4.8); c = 4.1–5.0 (4.3); c′ = 9–12 (10.5); V = 60–64 (61.4); spear = 6.0–6.5 (6.2) $\mu$m; MB = 43–46 (44.7). Ten ♂♂ (paratypes): L = 0.34–0.37 (0.35) mm; a = 31–37 (34); b = 4.6–5.2 (4.9); c = 3.7–4.8 (4.2); c′ = 9.5–11.7 (10.3); T = 30–38 (34); spear = 6.0–6.5 (6.1) $\mu$m; MB = 41–46 (44); spicules = 11–12 (11.6) $\mu$m; gubernaculum = 3.0–4.5 (3.8) $\mu$m. Holotype ♀: L = 0.37 mm; a = 30; b = 5.4; c = 4.2; c′ = 12; V = $^{32}60^{-1.3}$; spear = 6 $\mu$m; MB = 45.

DESCRIPTION: FEMALE: Body straight to slightly arcuate, widest at about 3 body-widths anterior to vulva, regularly tapering and considerably attenuated towards tail end, anteriorly tapering regularly in esophageal region until lip region base is about ⅓ of maximum body width. Cuticular annules fine, 0.8–1.0 $\mu$m wide, sometimes indistinct. Lateral fields with 4 incisures, indistinct on most specimens. Lip region smooth, tapering, elevated, with labial disc 2.1 $\mu$m in diameter; framework lightly sclerotized; maximum width of lip region at base 3.7–4.0 $\mu$m and its height about 2 $\mu$m. Spear only 6.0–6.5 $\mu$m long, with a tiny conus and minutely rounded basal knobs. Orifice of dorsal esophageal gland 1.25 $\mu$m behind spear base. Median esophageal bulb oval, valvate; distance from anterior end of body to its center 32–34 $\mu$m and from latter to base of esophagus 38–40 $\mu$m. Isthmus elongate-slender, crossed by nerve ring near its middle. Basal esophageal bulb saccate or pyriform, with flat base and a rounded cardia. Excretory pore 50–55 (52.5) $\mu$m from anterior end, 1–1.5 body-widths behind nerve ring; 0–3 annules behind hemizonid which is about 3 annules long; hemizonion about 1.5 body-widths behind hemizonid. Deirids slightly above level of excretory pore. Phasmids close to vulva.

Vulva a transverse slit, its lips very slightly raised. Vagina extending ²/₅ into body. Post-vulval uterine sac about ²/₅ body-width long. Spermatheca offset, rounded, with sperm. Ovary short, with less than 15 oocytes, outstretched. Vulva-anus distance 50–62 (58) $\mu$m. Rectum 1.5 times anal body-width long; anus distinct. Tail attenuate, filiform, with extremely thin end, 68–96 (80) $\mu$m long.

MALE: Body, lip region, spear and esophagus as in female. Phasmids 59–62% of body length from anterior end. Testis single, outstretched. Spicules arcuate, cephalated, pointed distally. Gubernaculum trough-shaped, fixed. Caudal alae smooth, adanal. Tail filiform, 72–90 (81) $\mu$m long.

TYPE MATERIAL: Holotype ♀, 10 ♀♀, 5 ♂♂ paratypes at C.I.H., St. Albans, England; 5 ♀♀, 1 ♂ paratypes at each of these centers: Department of Nematology, Rothamsted Experimental Station, Harpenden, England; USDA Nematode Collection, Beltsville, Maryland, USA, Nematology Department, Landbouwhogeschool, Wageningen, The Netherlands; Division of Nematology, I.A.R.I., New Delhi, India.

TYPE HABITAT AND LOCALITY: Bush soil in Block F of the International Institute of Tropical Agriculture, Oyo Road, Ibadan, Nigeria.

### Relationship

*Discotylenchus attenuatus* sp.n. differs from *D. discretus* sp.n. in having a smaller body size, a less conspicuous labial disc, and a shorter spear.

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# Gastrointestinal Helminths in Relation to Sex and Age of *Desmognathus fuscus* (Green, 1818) from Illinois

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ABSTRACT: Gastrointestinal helminths recovered from 442 *Desmognathus fuscus* of one population in southern Illinois between 1974 and 1978 included *Thelandros magnavulvaris* (26.9%), *Cosmocercoides dukae* (1.4%), *Brachycoelium obesum* (11.1%), and unidentified juvenile acanthocephalans (1.1%). Both sexes were equally infected with each species of helminth. *Thelandros magnavulvaris* and *B. obesum* exhibited a significant increase in both prevalence and mean number per salamander with increasing age in both sexes of the host. Infections with *C. dukae* and acanthocephalans were infrequent, the former occurring in animals 1 year of age or older.

With the exception of comprehensive surveys on helminth parasites of salamanders in the contiguous United States conducted by Harwood (1932), Mann (1932), Rankin (1937a, 1945) and Fischthal (1955a, b), little is known of salamander parasites. Probably the most comprehensive study was that conducted by Rankin (1937a), who examined over a 1,000 specimens comprising 19 species. Rankin concluded that studies of salamander parasites should prove to be of more interest from a scientific point of view and of more fundamental value than those on parasites of frogs and toads. Despite the rationale for this statement (see Rankin, 1937a), a review of the literature reveals that, while reports on parasites of amphibians are numerous, most are confined to those of anurans. The majority of reports pertaining to salamander parasites are restricted either to descriptions of new species or to lists of helminths in a few salamanders made in connection with broad taxonomic surveys of amphibians and reptiles.

Although the biology and habitats of several species of salamanders in southern Illinois have been investigated, helminth parasitism, with the exception of the report by Dyer and Brandon (1973), has only been sporadically examined. The present study was initiated in order to better understand the relationship of prevalence and intensity of helminth parasitism to age and sex of the dusky salamander, *Desmognathus fuscus* (Green, 1818), a species currently listed as endangered in this state because of its tiny range in Illinois, being limited to Pulaski County (Brandon and Huheey, 1979).

## Materials and Methods

Four hundred seventy-three *Desmognathus fuscus* (USNM 210200–210672) were collected in Pulaski County, southern Illinois between September 7, 1974, and April 14, 1978, as part of a study of their reproductive ecology. All but 31 were examined for gastrointestinal helminths. The study area in Chestnut Hills Nature Preserve is characterized by spring-fed streams in a mature forest of beech and oak in deep ravines fronting the Ohio River.

The animals were killed in Chloretone, fixed in 10% formalin, and preserved in 70% ethyl alcohol. Trematodes were stained with Harris' hematoxylin and acanthocephalans in Schneider's alcoholic borax carmine, cleared in beechwood creosote, and mounted in Canada balsam. Nematodes were identified from glycerin wet mounts.

**Table 1.** Incidence of single, mixed, and combined infections in relation to sex of 442 *Desmognathus fuscus* from Pulaski County, Illinois.

| Parasite | Incidence—(number) percent | | |
|---|---|---|---|
| | Sexes combined (442) | Male (226) | Female (216) |
| **Single infection** | | | |
| *Thelandros magnavulvaris* | (90) 20.4 | (48) 21.2 | (42) 19.4 |
| *Cosmocercoides dukae* | (3) 0.7 | (2) 0.9 | (1) 0.5 |
| *Brachycoelium obesum* | (25) 5.7 | (15) 6.6 | (10) 4.6 |
| Acanthocephalans | (2) 0.5 | (1) 0.4 | (1) 0.5 |
| **Mixed infection** | | | |
| *T. m. + C. d.* | (3) 0.7 | (2) 0.9 | (1) 0.5 |
| *T. m. + B. s.* | (23) 5.2 | (14) 6.2 | (9) 4.2 |
| *T. m. + A.* | (2) 0.5 | (2) 0.9 | (0) |
| *T. m. + B. s. + A.* | (1) 0.2 | (1) 0.4 | (0) |
| **Combined (single and mixed) infection** | | | |
| *Thelandros magnavulvaris* | (119) 26.9 | (67) 29.6 | (52) 24.1 |
| *Cosmocercoides dukae* | (6) 1.4 | (4) 1.8 | (2) 0.9 |
| *Brachycoelium obesum* | (49) 11.1 | (30) 13.3 | (19) 8.8 |
| Acanthocephalans | (5) 1.1 | (4) 1.8 | (1) 0.5 |

Salamanders were assigned to age cohorts on the basis of (1) polymodal size-frequency analysis (Harding, 1949), (2) testis and vas deferens morphology (Huheey and Brandon, 1973; Humphrey, 1922), and (3) oocyte and oviduct diameters (Tilley, 1973 a, b). Cohort 0 animals were less than 1 year old and higher cohort numbers give age at last "birthday" (=date of hatching). Males, because of the annual change in testis morphology, could be assigned to cohorts reliably, but females above cohort 2 (age at first reproduction) had to be lumped.

## Results

The incidence of gastrointestinal helminths in 226 male and 216 female *D. fuscus* is summarized in Table 1. Two species of nematodes, one of trematodes, and a few unidentified juvenile acanthocephalans were found in 149 (33.7%) of the 442 animals examined. There was no significant difference in frequency of occurrence of helminth species between the sexes.

One hundred twenty hosts were positive for only one parasitic species (27.3%), 28 for two (6.4%) and one for three (0.2%) (Table 1).

**Table 2.** Incidence of parasitism in male and female *Desmognathus fuscus* in relation to age.

| Parasite | Incidence—(number) percent | | | | | | |
|---|---|---|---|---|---|---|---|
| | Male—cohort (N) | | | | Female—cohort (N) | | |
| | 0 (81) | 1 (50) | 2–3 (50) | 4–8 (45) | 0 (87) | 1 (68) | 2–n (61) |
| *Thalandros magnavulvaris* | (8) 9.9 | (13) 26.0 | (22) 44.0 | (24) 53.3 | (6) 6.9 | (18) 26.5 | (27) 44.3 |
| *Cosmocercoides dukae* | (0) | (2) 4.0 | (4) 8.0 | (1) 4.2 | (0) | (0) | (2) 3.3 |
| *Brachycoelium obesum* | (1) 1.2 | (5) 10.0 | (10) 20.0 | (13) 28.9 | (2) 2.3 | (7) 10.3 | (10) 16.4 |
| Acanthocephalans | (0) | (2) 4.0 | (0) | (2) 4.4 | (0) | (1) 1.5 | (0) |

Table 3.  Mean level of parasites in relation to sex and age of 442 *Desmognathus fuscus* from Pulaski County, Illinois.

| | Mean number of parasites by cohort (N) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Male | | | | Female | | |
| Parasite | 0 (81) | 1 (50) | 2–3 (50) | 4–8 (45) | 0 (87) | 1 (87) | 2–n (61) |
| *Thelandros magnavulvaris* | 0.16 | 0.40 | 0.82 | 1.29 | 0.10 | 0.43 | 0.74 |
| *Cosmocercodes dukae* | 0 | 0.06 | 0.06 | 0.04 | 0 | 0 | 0.03 |
| *Brachycoelium obesum* | 0.02 | 0.42 | 0.32 | 0.76 | 0.02 | 0.24 | 0.28 |
| Acanthocephalans | 0 | 0.04 | 0 | 0.07 | 0 | 0.01 | 0 |

Table 2 lists the prevalence of parasitism in male and female salamanders in relation to age. Male salamanders ranged from cohort 1 to cohort 8 but, due to low numbers of older animals, cohorts 2 and 3 and cohorts 4 through 8 were combined to approximate more equal composition in the various age groups. Female salamanders were from cohorts 1 and older; cohorts 2 and older were combined.

An increase in prevalence with increasing age in both sexes was observed in the date for *Thelandros magnavulvaris* (Rankin, 1937) Schad, 1960 from 9.9% in males of cohort 0 to 53.3% in cohorts 4–8 and from 26.5% in females of cohort 0 to 44.3% in cohorts 2–n. The data for *Brachycoelium obesum* Nicoll, 1914, likewise reveals an increase in prevalence with increasing age in both sexes from 1.2% in males of cohort 0 to 28.9% in cohorts 4–8 and from 10.3% in females of cohort 0 to 16.4% in cohorts 2–n. The occurrence of *Cosmocercoides dukae* (Hall, 1928) Wilkie, 1930 shows some variability in males. Acanthocephalans were found in males of cohorts 2 and 4–8 and in females of cohort 1.

The intensities (mean levels) of parasites in relation to sex and age appear in Table 3. *Thelandros magnavulvaris* shows an increase in number of parasites per salamander in each cohort for both males and females. *Brachycoelium obesum* shows a decline in numbers in males of cohorts 2–3, followed by a significant sharp increase in cohorts 4–8. Age differences in *C. dukae* and acanthocephalans could not be detected because of a very low overall prevalence.

### Discussion

Because salamanders were collected at only four seasonal intervals between 1974 and 1978 and not systematically by month over a period of several consecutive years, there are insufficient data to form any convincing conclusions on the seasonal dynamics of *D. fuscus* parasites.

With the exception of *T. magnavulvaris*, the overall incidence of gastrointestinal parasites was very low, with the majority of salamanders infected with but a single species. Both *T. magnavulvaris* and *B. obesum* show a significant increase in both prevalence and numbers with increasing age in both sexes of the host.

*Thelandros magnavulvaris* was described by Rankin (1937b) on the basis of female specimens only. Fischthal (1955a) reported this species in 18 of 178 *D. fuscus* from New York. Schad (1963) described the male and redescribed the female from the green salamander, *Aneides aeneus* (Cope and Packard, 1881),

from Cooper's Rock, West Virginia. A review of the literature reveals that male specimens are rare. In a study of the gastrointestinal parasites of 225 cave salamanders, *Eurycea lucifuga* Rafinesque, 1822, from the southeastern United States by Dyer and Peck (1975), only 0.8% were positive for *T. magnavulvaris*, none of which were males. In the present study, only five male specimens were encountered, two of which were from a single host. Representative samples have been deposited in the USNM Helm. Coll., Nos. 75217, 75218.

Judged from this study, *C. dukae* is an infrequent nematode of *D. fuscus* in southern Illinois, primarily infecting older animals. In a study of the helminths of three sympatric species of cave-dwelling salamanders in southern Illinois, Dyer and Brandon (1973) reported this parasite in 13 (4.0%) of 322 zigzag salamanders, *Plethodon dorsalis* Cope, 1889 and 3 (0.9%) of 17 cave salamanders, *Eurycea lucifuga*. None was recovered from the 41 slimy salamanders, *Plethodon glutinous* (Green, 1818), examined. This species has also been reported in *D. fuscus* from N. Carolina (Rankin 1937a), Ohio (Odlaug, 1954; McGraw, 1968), New York (Fischthal, 1955a), and Pennsylvania (Fischthal, 1955b). Representative samples have been deposited in the USNM Helm. Coll., No. 75219.

Species of *Brachycoelium* are the most common flukes encountered in salamanders, and in the present study *B. obesum* was the only fluke collected. *Brachycoelium obesum* has also been reported in *D. fuscus* from Georgia (Byrd, 1937; Parker, 1941). The finding of this parasite in *D. fuscus* in Illinois constitutes a new locality record. Representative samples have been deposited in the USNM Helm. Coll., No. 75220. Considerable difference of opinion exists in regard to the number of species in this genus. For a review of the literature pertaining to this controversy and a summary of the geographical distribution of *Brachycoelium* reported from various salamander hosts in the contiguous United States, the reader is referred to the report by Dyer and Brandon (1973).

Of the 442 salamanders examined in this study, acanthocephalans were detected in only five. Because of the immature nature of the specimens, specific identification could not be made. To our knowledge, *Acanthocephalus acutulus* Van Cleave, 1931, is the only acanthocephalan reported from *D. fuscus*. It has also been reported from the red-spotted newt, *Notophthalmus viridescens* (Rafinesque, 1820), the marbled salamander, *Ambystoma opacum* (Gravenhorst, 1807), and *Plethodon glutinosus*.

## Acknowledgments

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# Helminths of the Common Crow, *Corvus brachyrhynchos* Brehm, from West Texas

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ABSTRACT: Three nematode, two cestode, one trematode, and two acanthocephalan species were collected from 65 of 68 (96% infected) common crows, *Corvus brachyrhynchos,* from western Texas. These included *Acuaria anthuris* (33.8% infected), *Microtetrameres helix* (69.0%), *Splendidofilaria* sp. (2.9%), *Hymenolepis corvi* (55.9%), *Anomotaenia constricta* (26.5%), *Zonorchis petiolatus* (2.9%), *Mediorhynchus grandis* (5.9%), and *Centrorhynchus* sp. (1.5%). Hosts were infected with one to four ($\bar{x} = 2.1$) helminth species per individual. Simpson's index for west Texas crows was low (0.25), indicating a lack of dominance by particular helminth species. Indices of similarity of the helminth faunas of crows from different geographic areas in North America were low, indicating a dispersed fauna, except between the west Texas and Wisconsin-Iowa helminth faunas, which appeared very similar. The only significant association between common helminth species in terms of frequency of occurrence and intensity of infection was between *M. helix* and *H. corvi.* Possibly these species share a common intermediate arthropod host. A new host record is established for *Zonorchis petiolatus.*

The common crow, *Corvus brachyrhynchos,* is the most common, widely distributed, and omnivorous species of the Corvidae in North America. The Texas population is a resident breeding population, but during autumn and winter it is supplemented by migrants from other areas. Although the helminth parasites of the crow have been investigated in Oklahoma (Ward, 1935), southern Wisconsin and Iowa (Morgan and Waller, 1941), Virginia (Daly, 1959), North Carolina (Hendricks et al., 1969), Newfoundland (Andrews and Threlfall, 1975), and Ohio (Jones, 1968; Fendinger, 1952), there is no available information on the helminth fauna of this species from west Texas. Consequently, the present study was initiated in order to (1) examine the composition and prevalence of helminth parasites of the common crow from this area, (2) compare helminth faunas of this host from different geographic regions in North America, and (3) examine species interactions of common helminths in this host.

## Materials and Methods

Sixty-eight crows were collected by shooting near Brownfield, Terry Co., Texas in October and November 1975. Entire carcasses were frozen for later necropsy. Sex and age data were not obtained. Viscera were examined for helminths grossly and with a dissecting microscope. Nematodes were briefly fixed in glacial acetic acid, preserved in a mixture of 70% ethyl alcohol with 5% glycerine, and examined in glycerine wet mounts after evaporation of the alcohol. Trematodes, cestodes, and acanthocephalans were briefly fixed in AFA, stained in Celestin blue B or Semicohn's acetic carmin, and mounted in Canada balsam. Simpson's index and a similarity index comparing the helminth faunas of crows from different geographic regions in North America were computed according to Holmes and Podesta (1968). The latter values were arranged in a trellis diagram. Significant interactions of the four common helminth species (*Acuaria anthuris, Micro-*

Table 1. Helminths of the common crow from west Texas.

| | Site of infec- tion* | Prevalence | | Intensity | |
|---|---|---|---|---|---|
| | | No. infected/ No. examined | % | Range | Mean |
| Nematoda | | | | | |
| *Acuaria anthuris* | | | | | |
| (Rudolphi, 1819) Williams, 1929 | G | 23/68 | 33.8 | 1–30 | 3.9 |
| *Microtetrameres helix* Cram, 1927 | P | 48/68 | 70.5 | 1–74 | 13.3 |
| *Splendidofiliaria* sp. | B | 2/68 | 2.9 | 2–3 | 2.5 |
| Cestoda | | | | | |
| *Anomotaenia constricta* | | | | | |
| (Molin, 1858) Mettrick, 1958 | I | 18/68 | 26.5 | 1–15 | 3.8 |
| *Hymenolepis corvi* | | | | | |
| (Mayhew, 1925) Yam, 1956 | I | 38/68 | 55.9 | 1–31 | 4.7 |
| Trematoda | | | | | |
| *Zonorchis petiolatus* | | | | | |
| (Railliet, 1900) Denton and Byrd, 1951 | L | 2/68 | 2.9 | 1 | 1 |
| Acanthocephala | | | | | |
| *Mediorhynchus grandis* | | | | | |
| (Van Cleave, 1916) Pachyman and Berry, 1948 | I | 4/68 | 5.9 | 1–2 | 1.5 |
| *Centrorhynchus* sp. | I | 1/68 | 1.5 | 1 | 1 |

* G, gizzard; P, proventriculus; B, body cavity; I, intestine; L, liver.

*tetrameres helix, Anomotaenia constricta,* and *Hymenolepis corvi*) were determined in terms of frequency of occurrence by total chi-square analysis using 2 × 2 contingency tables where species pairs were considered in terms of both species present, one present to the exclusion of the other, and both species absent (Sokal and Rohlf, 1969). Cole's coefficients of association were computed to determine

Table 2. Comparison by *t*-tests of mean levels of infection of common helminths in the common crow from west Texas.

| | | *A. constricta* | *H. corvi* | *A. anthuris* | *M. helix* |
|---|---|---|---|---|---|
| *A. constricta* | + | | 2.6 (11)* | 2.5 (4) | 8.5 (15) |
| | | | 1.185† | 0.519 | 1.530 |
| | – | | 5.5 (27) | 4.2 (19) | 15.5 (32) |
| *H. corvi* | + | 4.5 (8) | | 2.7 (14) | 10.4 (23) |
| | | 0.956 | | 1.232 | 1.343 |
| | – | 2.9 (10) | | 5.8 (9) | 16.1 (24) |
| *A. anthuris* | + | 6.0 (4) | 5.8 (14) | | 16.5 (15) |
| | | 1.455 | 0.782 | | 1.014 |
| | – | 3.1 (14) | 4.0 (24) | | 11.8 (32) |
| *M. helix* | + | 4.4 (14) | 5.0 (23) | 4.3 (15) | |
| | | 1.497 | 0.431 | 0.460 | |
| | – | 1.5 (4) | 4.1 (15) | 3.1 (8) | |

* Mean infection level followed by sample size in parentheses, value for *t* between values for compared means.
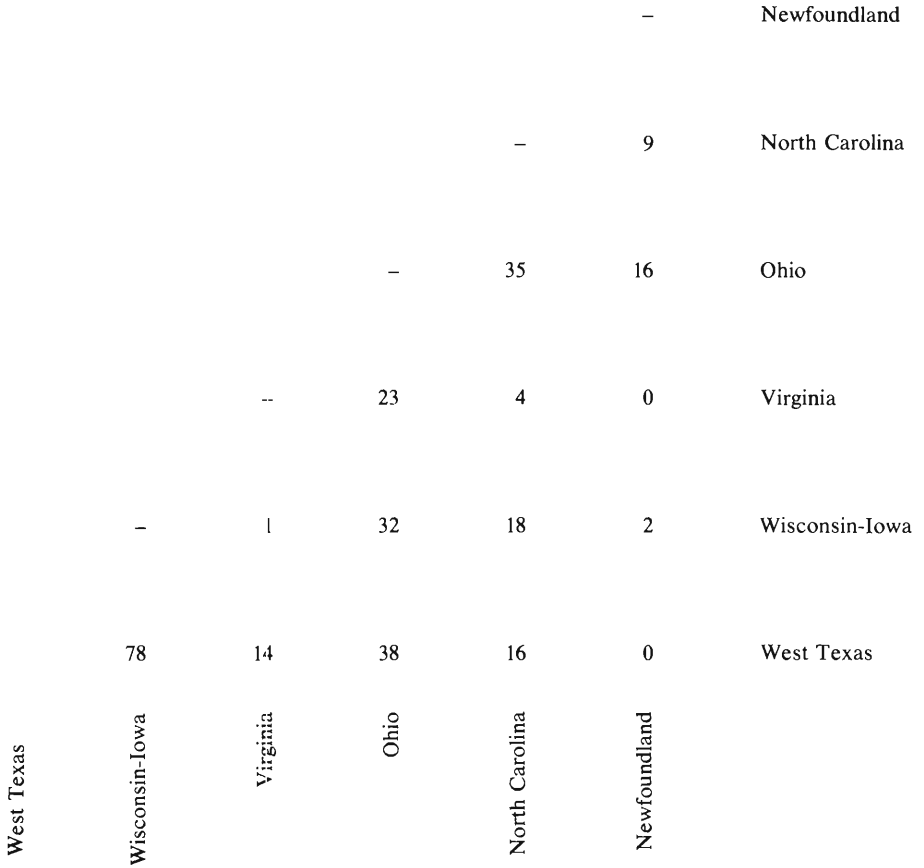† Significant at $P \leqslant 0.050$ ($N_1 + N_2 - 2$ df).

| West Texas | Wisconsin-Iowa | Virginia | Ohio | North Carolina | Newfoundland | Region |
|---|---|---|---|---|---|---|
| | | | | | – | Newfoundland |
| | | | | – | 9 | North Carolina |
| | | | – | 35 | 16 | Ohio |
| | | -- | 23 | 4 | 0 | Virginia |
| | – | 1 | 32 | 18 | 2 | Wisconsin-Iowa |
| | 78 | 14 | 38 | 16 | 0 | West Texas |

Figure 1. Trellis diagram of indices of similarity of helminth faunas of common crows from different regions in North America.

a positive or negative interaction (Cole, 1957). For intensity of infection, each of the above common helminths was compared to every other common helminth species using $t$-tests on mean levels of infection (Snedecor and Cochran, 1967). Statistical inferences are reported at the $P \leq 0.05$ level. Representative specimens of helminths collected in this study are deposited in the Medical Zoology Collection, The Museum of Texas Tech University, TTUSMZ Nos. 14,636–14,972 and 14,993–15,003.

## Results

Three nematode, two cestode, one trematode, and two acanthocephalan species were recovered from 65 of 68 common crows in west Texas (Table 1). Ninety-six percent of these hosts harbored from one to four ($\bar{x} = 2.1$) helminth species. Intensities of infection ranged from one to 104 ($\bar{x} = 15$) individual helminths per host.

Simpson's index for west Texas crows was low (0.25), indicating a lack of dominance of particular helminth species in this host. Likewise, Simpson's indices calculated for crows from other areas including North Carolina (Hendricks

| M. helix | A. anthuris | H. corvi | A. constricta | |
|---|---|---|---|---|
| | | | – | A. constricta |
| | | | 0.147 (−0.048) | H. corvi |
| | – | 0.177 (0.063) | 2.120 (−0.391) | A. anthuris |
| – | 0.875 (−0.138) | *6.587 (−0.600) | 0.806 (0.245) | M. helix |

Figure 2. Total $\chi^2$ values and Cole's coefficients ($C_{ab}$) of expected versus observed values of frequency of occurrence for pairs of common helminth species in the common crow from west Texas. * $P \leq 0.05$ (1 df).

et al., 1969), Ohio (Jones, 1968), Virginia (Daly, 1959), Newfoundland (Andrews and Threlfall, 1975), and southern Wisconsin and Iowa (Morgan and Waller, 1941) had correspondingly low values of 0.31, 0.11, 0.30, 0.15, and 0.29, respectively, indicating a lack of dominance.

Comparison of the helminth faunas of crows from the above different geographic areas in North America using an index of similarity indicated a basically different helminth fauna between most regions (Fig. 1). The highest value (78) was between the west Texas fauna and that of Wisconsin and Iowa, indicating a basically similar helminth fauna in these two regions. However, even in this case only three species were shared, although these were the overwhelmingly dominant (in terms of prevalence) species.

Multispecies interactions between common helminth species, as determined using chi-square analysis of frequency of occurrence, implicated only one association between helminth pairs (Fig. 2). There was a significant association between *Hymenolepis corvi* and *Microtetrameres helix*. Cole's coefficients indicated a negative association with the number of instances where both species were absent more frequently than expected, leading to a greatly increased chi-square value. There were no significant associations between the remaining pairs of helminth species. There were no significantly positive or negative associations in terms of intensity of infection between pairs of common helminth species in

the crow (Table 2). Likewise, it was not possible to analyze the prevalence or intensity of infection of helminth species in terms of host age or sex.

A new host record for *Zonorchis petiolatus* from the common crow is established. The remaining helminth species recovered in this study have been previously reported from this host.

## Discussion

The results of the present study suggest *Corvus brachyrhynchos* is commonly infected (96% of hosts infected) with one or more helminth species in west Texas. Moreover, all but one of these helminth species have been reported previously from this host in North America. Andrews and Threlfall (1973) have provided a comprehensive list of parasites reported from the common crow in North America.

The low Simpson's indices for helminth faunas of this host from the several areas in North America where sufficient data are available (North Carolina, Ohio, Virginia, Wisconsin and Iowa, Newfoundland, and west Texas) indicate a lack of dominance of particular helminth species. Likewise, the low values for indices of similarity for crow helminth faunas between most of the above areas indicate a fairly diverse fauna. From the above six areas where surveys have been completed, only 14 of the 41 (35%) total helminth species are common to more than one area. Of these species, *Acuaria anthuris, Capillaria contorta,* and *Mediorhynchus grandis* occur in four of the six regions. Two nematodes (*Diplotriaena tricuspis* and *Microtetrameres helix*), two cestodes (*Anomotaenia constricta* and *Hymenolepsis corvi*), and one trematode (*Echinostoma revolutum*) each occur in three of the six areas. The low prevalence of shared species between areas, coupled with the low levels of infection of many of these species, accounts for the low values in the similarity indices between most areas. A notable exception is the high similarity index of the west Texas with the Wisconsin and Iowa crow helminth faunas. Although only three of 13 helminth species (*Acuaria anthuris, Microtetrameres helix,* and *Hymenolepis corvi*) are shared between the two regions, these were overwhelmingly the most prevalent species from both areas. The remaining helminths encountered in both areas were very infrequently encountered (low percent prevalence). Although the crow is a migratory species, this does not seem to have a pronounced effect on their helminth faunas, as reflected by the low similarity indices between most geographic regions. In this respect the helminth fauna of this species more closely resembles that of other vertebrates which are of wide geographic distribution occupying variable habitat types and are omnivorous in food habits, such as the coyote (Holmes and Podesta, 1968; Pence and Meinzer, 1979; Custer and Pence, in press) and bobcat (Stone and Pence, 1978) which have a diverse helminth fauna with a lack of dominance by particular helminth species.

Of the four common helminths found in the present study, only one species pair, *M. helix* and *H. corvi,* demonstrated a significant association in terms of frequency of occurrence. There were no significant associations of species pairs in terms of intensity of infection. Although the above species occur in different locations within the host, proventriculus and small intestine, both utilize arthropod intermediate hosts (Schell, 1953; Fendinger, 1952), which could account for the above association in terms of prevalence.

## Acknowledgments

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# Development of *Sarcocystis hemionilatrantis* Hudkins and Kistner, 1977 in the Small Intestine of Coyotes

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ABSTRACT: The prepatent and patent periods and the endogenous development of *Sarcocystis hemionilatrantis* were studied in coyotes (*Canis latrans*) which had ingested infected mule deer (*Odocoileus hemionus*) musculature. Oocysts measured 21.3 × 17.5 μm, and were shed sporulated and (or) unsporulated in the feces of coyotes which had previously ingested *Sarcocystis*-infected meat. Sporocysts, which were also shed in the feces, measured 16.2 × 9.9 μm and had four sporozoites and a granular residuum. The prepatent period was 9 to 13 days; the patent period was 31 to 35 days. At 3 and 5 days after infection, small spheroid stages which evidently formed zygotes were found in the lamina propria of the small intestine. Zygotes and oocysts in various stages of sporulation occurred in the lamina propria of the villi in the distal one-third of the duodenum, and all of the jejunum and ileum. No parasites were seen in the cecum, large intestine, or other organs. No meronts or gamonts were found. Coyotes were susceptible to reinfection with the same meat used to establish the original infection. Little or no histological response occurred in a 12-day primary infection; however, a pronounced cellular response occurred in a 14-day primary infection as well as in all days of reinfection.

*Sarcocystis* is an obligate two-host parasite in which carnivores or omnivores serve as final hosts and herbivores or omnivores serve as intermediate hosts. In the western hemisphere, *Sarcocystis* has been reported in various free-ranging herbivores, including moose (*Alces alces andersoni*) (Kelly et al., 1950), mule deer (*Odocoileus hemionus*) (Hudkins et al., 1976; Hudkins and Kistner, 1977; Mills, 1936; Pond and Speer, 1979; Scott, 1943), white-tailed deer (*O. virginianus*) (Karstad and Trainer, 1969; Pond and Speer, 1979) and elk (*Cervus canadensis*) (Mills, 1936; Pond and Speer, 1979; Sayama, 1952; Scott, 1943). There are only a few reports of a *Sarcocystis* infection transmitted between a free-ranging carnivore and a free-ranging herbivore (Dubey et al., 1978; Fayer and Johnson, 1975; Hudkins and Kistner, 1977). There are no previous descriptions of the endogenous stages of *Sarcocystis* in free-ranging carnivores. The endogenous development of *S. hemionilatrantis* in the small intestine of coyotes infected with mule deer musculature is reported herein.

## Materials and Methods

Eight 3- to 4-week-old coyote pups were taken from dens on the National Bison Range (NBR) about 45 miles north of Missoula, Montana. Each pup was placed in an isolation cage, fed canned evaporated goat milk *ad lib* for 7 days, and then weaned and maintained on commercial dry dog food. For 21 days, fecal samples were obtained from each coyote, floated by Sheather's sugar solution, and examined for parasites. None of the coyotes passed oocysts or sporocysts during this period.

In order to study the prepatent and patent periods and the endogenous development of *S. hemionilatrantis*, coyotes were fed *Sarcocystis*-infected ground

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musculature of a mule deer (*Odocoileus hemionus*) obtained from the NBR. The entire skeletal musculature of the deer was ground in a commercial meat grinder, divided into 500-g portions, wrapped in plastic, and stored at 4°C. The deer had macroscopic cysts of *Sarcocystis* in its esophagus as well as microscopic cysts in skeletal muscle, as determined by the digestion technique of Box and Mc-Guinness (1978) and by histological examination. The concentration of *Sarcocystis* zoites was $1.4 \times 10^5$ zoites/g muscle tissue, determined by counting with a hemacytometer the number of parasites in the fluid obtained from digested tissue. Each of the eight coyote pups was fed 500 g ground mule deer meat each day for 2 days. The extent of development of *S. hemionilatrantis* and the cellular response of previously infected animals was determined by feeding each of three coyotes another 500 g of ground meat each day at 16 and 17 days after the original feeding. Daily fecal samples from each coyote were floated by Sheather's sugar solution and examined for oocysts and sporocysts for 66 days after the initial feeding or until the coyotes were killed.

At various intervals after the initial or second feeding of *Sarcocystis*-infected meat, five of the eight coyotes were euthanized by an overdose of Sernylan (phencyclidine hydrochloride)[3] and portions of tissue were obtained at 6-inch intervals along the lengths of the small and large intestines, and cecum, and from the heart, lung, liver, spleen, diaphragm, adrenals, kidney, and mesentetric lymph nodes, and prepared for histological examination. Two coyotes were killed at 12 and 14 days after the initial feeding of infected meat, whereas three other coyotes were killed at 3, 5, and 7 days after the second feeding. Tissues were removed from each animal and fixed in 10% (v/v) formalin or 3% (v/v) glutaraldehyde in cacodylate buffer. Tissues fixed in formalin were embedded in paraffin, sectioned at 3–5 μm and stained with hematoxylin and eosin. Tissues fixed in glutaraldehyde were embedded in Spurr's or Epon 812 medium, sectioned at 1 μm, stained with toluidine blue, and examined by bright-field microscopy. Thin sections were cut from these same blocks, stained with uranyl acetate and lead citrate and examined by a Zeiss EM9S-2 electron microscope.

## Results

No oocysts or sporocysts were found in the feces of the coyote pups before the feeding of infected meat. Nine to 13 days after the initial feeding of infected mule deer meat each of the eight coyotes began to shed *S. hemionilatrantis* sporocysts (Fig. 16) or oocysts (Figs. 14, 15) in their feces. This shedding continued for 22 days (31 to 35 days after infection) or until the coyotes were killed. The three coyotes which were not killed during the study shed oocysts and sporocysts intermittently until day 43 and were then negative up to 66 days, after which their feces were no longer examined for parasites.

*Sarcocystis hemionilatrantis* sporocysts contained four sporozoites and a granular residuum and were usually passed singly (Fig. 16), but occasionally were seen in pairs surrounded by an oocyst wall (Fig. 14). At 2 to 4 days of the patent period (10 to 13 days after the original feeding), partially sporulated oocysts with

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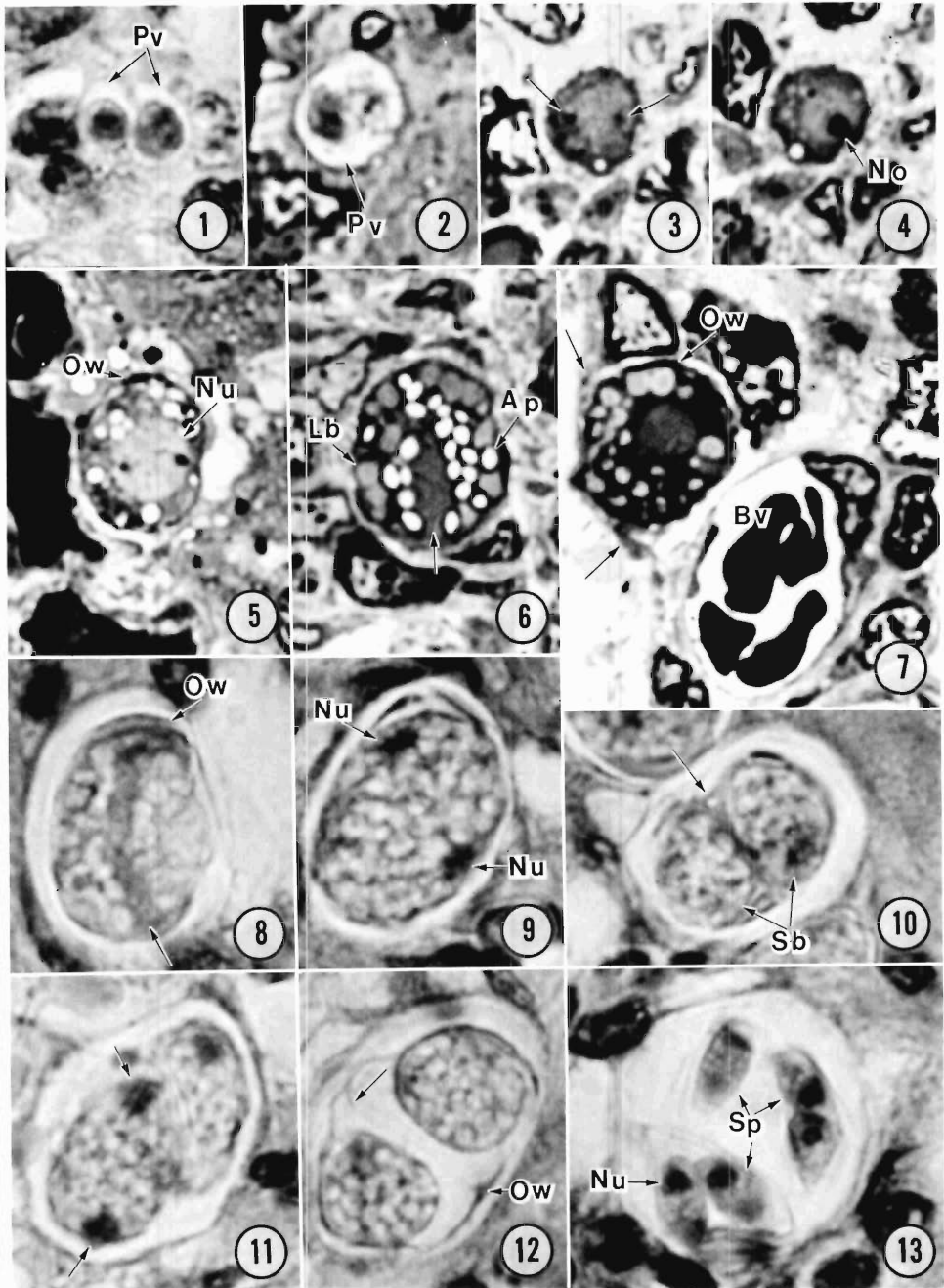
Figures 1–13. Photomicrographs of the endogenous stages of *Sarcocystis hemionilatrantis* and the cellular response to reinfection in the small intestine of coyotes. Figures 1, 8–13, paraffin-embedded sections, H & E stain; Figures 2–7, 17, 18, 1-μm epoxy resin–embedded sections, toluidine blue stain; Figures 14–16, 19, fresh preparations. Day after ingestion of *Sarcocystis*-infected mule deer meat and magnification listed in parentheses. Abbreviations for figures: Ap, amylopectin; Bl, basal lamina; Bv, blood vessel; Lb, lipid body; Lc, lymphocyte; Mc, mast cell; Mp, macrophage; No, nucleolus of parasite; Nu, nucleus of parasite; Ow, oocyst wall; Pc, plasma cell; Pv, parasitophorous vacuole; Sb, sporoblast;

sporoblasts (Fig. 14) or single sporoblasts were observed in the feces of four of the eight coyotes. These oocysts and sporoblasts completed sporulation within 12 hr at room temperature (22°C). Sporulated oocysts measured 21.3 × 17.5 μm (range 19–22 × 16–19 μm) ($N$ = 20); sporocysts were 16.2 × 9.9 μm (range 15–17 × 9–12 μm) ($N$ = 50) and contained four sporozoites and a granular residuum (Fig. 16).

At 12 to 15 days after the initial feeding, one coyote shed unsporulated oocysts of an *Isospora* species. These oocysts sporulated within 16 hr at room temperature (22°C), measured 21.1 × 16.3 μm ($N$ = 50) (range 19–23 × 14–18 μm) and contained two sporocysts, each with four sporozoites and a granular residuum.

Histological sections of the small intestine obtained from the two coyotes killed at 12 and 14 days after the initial feeding of *Sarcocystis* had only sporulated oocysts and sporocysts (Fig. 19). Parasites occurred in the distal one-third of the duodenum in which they were most abundant, and all of the jejunum and ileum. They were located in the distal one-third of the villi immediately beneath the intestinal epithelium and adjacent to blood vessels (Fig. 7). No meronts or gamonts were found. No parasites were seen in the cecum or large intestine or other organs.

At 3 and 5 days after the second feeding of infected meat, small spheroid stages and oocysts with sporonts, sporoblasts, or sporocysts occurred in the small intestine, whereas only oocysts were present at 7 days. Parasites were located in the same areas as those described for the initial feeding except that they occurred throughout the length of the villi. Occasionally, parasites were seen in Peyer's patches but not in mesenteric lymph nodes. No parasites occurred in the large intestine or cecum or other organs.

Spheroid stages, which evidently arose from bradyzoites present in the infected deer meat, were the earliest stages observed. Such spheroid stages were present at day 3, measured 3.2 μm (range 2–4 μm) in diameter, and appeared as pairs usually in separate parasitophorous vacuoles (Fig. 1) or within the same parasitophorous vacuole (Fig. 2). Somewhat later stages which were present at days 3 and 5 were 6.3 μm (range 4–8 μm) in diameter, had a homogeneous cytoplasm and a large nucleus 4.3 μm (range 3–5 μm) in diameter with one or two nucleoli (Figs. 3–5). Specimens in Figures 3–5 which were examined by transmission electron microscopy were found to be already in the process of forming an oocyst wall. Thus, these stages were interpreted to be zygotes rather than macrogamonts. Small colorless and larger blue-staining granules occurred in the cytoplasm

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Sp, sporozoite. **1.** Two spheroid parasites; note that each organism is surrounded by a parasitophorous vacuole (3; ×2,400). **2.** Two spheroid parasites in same parasitophorous vacuole; note pinpoint nucleoli in each parasite (3; ×2,400). **3.** Zygote with 2 nucleoli within a large nucleus; note lack of parasitophorous vacuole (3; ×2,200). **4.** Zygote with large nucleus and prominent nucleolus (5; ×2,200). **5.** Zygote in early stage of oocyst wall formation; note nucleus with 2 nucleoli (5; ×2,200). **6.** Oocyst in early stage of sporulation; note nuclear band (arrow), and lipid and amylopectin bodies (5; ×2,200). **7.** Early oocyst in close proximity to a blood vessel (5; ×2,200). **8.** Oocyst with nuclear band (arrow) (7; ×2,200). **9.** Oocyst sporont with 2 nuclei (7; ×2,200). **10.** Cleavage (arrow) of sporont to form 2 sporoblasts (7; ×2,200). **11.** Oocyst with 2 sporoblasts, one of which has a nucleus (arrows) at each pole (7; ×2,200). **12.** Oocyst with 2 sporoblasts; note septum (arrow) which arises from the oocyst wall and separates the sporoblasts (7; ×2,200). **13.** Sporulated oocyst (7; ×2,200).
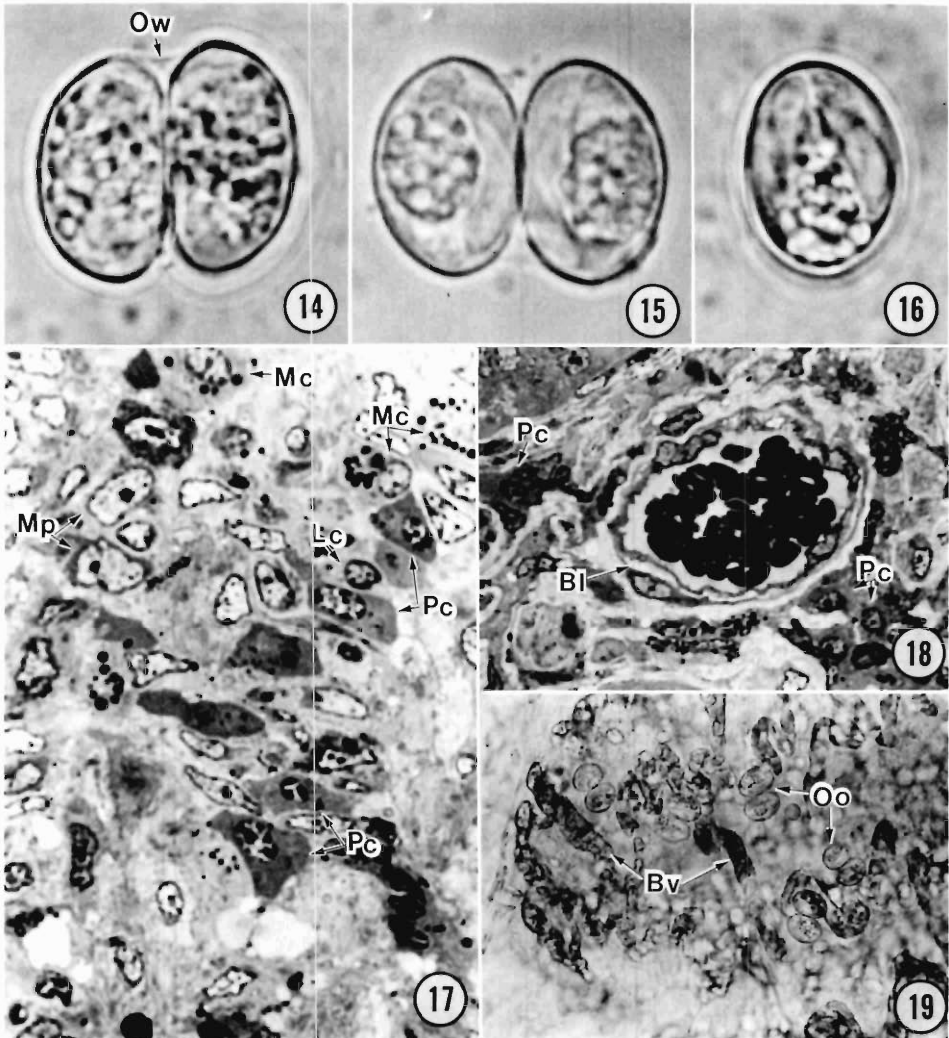
Figures 14–19. Endogenous stages of *Sarcocystis hemionilantris* and cellular response to reinfection. Explanation as for Figures 1–13. 14. Oocyst with 2 sporoblasts, each with numerous granules (×2,400). 15. Sporulated oocyst with 2 sporocysts (×2,200). 16. Sporocyst (×2,200). 17. Cellular response to reinfection with *S. hemionilatrantis* 21 days after initial feeding of infected meat, 5 days after second feeding (×1,200). 18. Cross section of blood vessel showing pale and thickened basal lamina in reinfection with *S. hemionilatrantis* 21 days after initial feeding of infected meat, 5 days after second feeding (×1,200). 19. Fresh mucosa scraping obtained from a 14-day primary infection; note oocysts and distended blood vessels (×400).

of zygotes as well as oocysts beginning sporulation (Figs. 5–7). Such bodies in electron micrographs were found to be amylopection and lipid bodies, respectively. Oocysts had a thin dense layer at their margins which apparently represented the oocyst wall (Figs. 7, 8).

Parasites were surrounded by a very thin layer of host cell cytoplasm which appeared discontinuous and was visible only in 1-$\mu$m sections stained with to-

luidine blue (Fig. 7). In electron micrographs, parasites appeared extracellular, being partially surrounded by macrophage or fibroblast pseudopodia.

At 3, 5, and 7 days after the second feeding of mule deer meat, various stages of oocyst sporulation were present. The first indication of sporulation was the appearance of a nuclear band which traversed from near one pole of the sporont to the other (Figs. 6, 8). Slightly later stages had a nucleus at each pole of the sporont (Fig. 9), after which cleavage (Fig. 10) evidently occurred to form two sporoblasts (Figs. 10–12). Each sporoblast had a nucleus located at each pole (Fig. 11). Sporulated oocysts contained two sporocysts, each with four sporozoites and a granular residuum (Figs. 13, 19). Oocysts had a thin oocyst wall and in some specimens a thin septum (Fig. 12) arose from the oocyst wall and traversed the oocyst separating the sporoblasts or sporocysts.

The lamina propria of the small intestine of the coyote killed at 12 days after the initial feeding of mule deer meat exhibited little or no tissue response to the parasite. A marked cellular response occurred in the small intestine of the coyote sacrificed at 14 days after the initial feeding of infected meat. A similar but more pronounced cellular response occurred at 3, 5, and 7 days in the three coyotes which received the second feeding. In these animals, the entire small intestinal mucosa appeared hyperemic upon gross examination. Histologically, the lamina propria was extensively infiltrated by mononuclear cells including lymphocytes, macrophages, mast cells, and plasma cells (Fig. 17). Few polymorphonuclear leucocytes were present. Arterioles, venules, capillaries, and lymphatic vessels were distended (Figs. 18, 19). Blood vessels had pale and thicker basement membranes (Fig. 18), and edema occurred between the intestinal epithelium and lamina propria.

### Discussion

Hudkins and Kistner (1977) found that coyotes passed sporocysts of S. hemionilatrantis intermittently in their feces between 12 and 36 days after ingestion of infected mule deer meat. They did not report that coyotes passed oocysts of S. hemionilatrantis. In the present study, coyotes which ingested mule deer meat infected with S. hemionilatrantis shed oocysts and (or) sporocysts continuously between 9 to 13 days and 35 days, and then intermittently up to 43 days. Fayer and Johnson (1975) found that coyotes which ingested bovine heart infected with S. cruzi (syn. S. fusiformis) shed sporulated sporocysts intermittently between 10 or 11 days and 19 or 20 days after feeding. Pairs of sporocysts, which presumably represented oocysts, were passed on day 11 only. Beginning at 9 to 13 days after ingestion of mule deer meat (present study), coyotes shed sporocysts and oocysts of S. hemionilatrantis for 5 days, after which only sporocysts were passed. Thus, the prepatent period of S. hemionilatrantis was somewhat similar to that of S. cruzi infection in coyotes, whereas the patent period was distinctly different.

The sporocyst of S. hemionilatrantis was similar in size (16.2 × 9.9 μm) to that of S. cruzi (15.7 × 9.9 μm) (Fayer, 1974) but was slightly larger than that reported previously for S. hemionilatrantis (14.4 × 9.3 μm) (Hudkins and Kistner, 1977).

Certain aspects of the endogenous development of S. hemionilatrantis in coyotes were different from S. cruzi infections in dogs (Fayer, 1974). Parasites of S.

*cruzi* (Fayer, 1974) and *S. hemionilatrantis* were found in the duodenum, jejunum, and ileum. The former was most abundant in the distal jejunum and proximal ileum, whereas the latter was most abundant in the distal one-third of the duodenum. Macrogamonts of *S. cruzi* were present at days 2 through 13. In the present study, stages of *S. hemionilatrantis* which appeared similar to macrogamonts of *S. cruzi* (Fayer, 1974) were actually found to be zygotes or developing oocysts when examined by electron microscopy. Such stages occurred at 3 and 5 days after infection but not at 7 days. At 7, 12, and 14 days after infection, oocysts of *S. hemionilatrantis* were in the process of sporulating or had completed sporulation.

Heydorn and Rommel (1972) and Fayer (1974) found that dogs could be reinfected with *S. cruzi* in which there was little or no indication of microscopic lesions or cellular reaction to the parasite. They suggested that dogs evidently have low resistance to reinfection with *S. cruzi*. In the present study, we found that coyotes could be reinfected with *S. hemionilatrantis* with meat obtained from the same mule deer used to establish the original infection. However, unlike *S. cruzi* reinfections in dogs, 14-day primary infection and especially reinfection with *S. hemionilatrantis* caused a marked mononuclear cellular infiltration, hyperemia, and vascular wall changes in the lamina propria of the small intestine of coyotes. We have yet to determine whether *S. hemionilatrantis* will infect dogs and, if it does, whether it will produce a similar histopathological response.

Microgamonts of *Sarcocystis* have been observed only in a *Sarcocystis* species from wild grackles (Fayer, 1971; Vetterling et al., 1973), in *S. suihominis* (Mehlhorn and Heydorn, 1979) in cell cultures, and in dogs infected with meat from *Sarcocystis*-infected sheep (Munday et al., 1975). Microgamonts were not observed in *S. cruzi* infections in dogs (Fayer, 1974) or in *S. hemionilatrantis* infections in coyotes in the present study. Microgamonts of *Sarcocystis* from grackles (Fayer, 1971) and *S. suihominis* (Mehlhorn and Heydorn, 1979) developed to maturity in cultured cells within 30–42 hr and 18–22 hr after inoculation, respectively. If macrogamonts of *S. hemionilatrantis* undergo a similar rapid development, they may have already reached maturity and then disappeared before 3 days, which was the earliest time that tissues were obtained from coyotes for histological examination.

Macrogamonts were also not observed in coyotes infected with *S. hemionilatrantis*. Certain stages of *S. hemionilatrantis*, which appeared similar to macrogamonts of *S. cruzi* (Fayer, 1974) when examined by light microscopy, were actually found upon ultrastructural examination to be zygotes already in the process of forming an oocyst wall. Thus, gamogony and fertilization of *S. hemionilatrantis* must evidently occur within the first 3 days of development of this parasite in coyotes.

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# Eimeria dispersa and E. meleagrimitis: Excystation in Chickens and Turkeys

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ABSTRACT: Chickens and turkeys were killed 0.5, 2, and 9 hr after they were fed 6 or 10 million sporulated oocysts of Eimeria dispersa or E. meleagrimitis, respectively. The number of oocysts and free sporocysts in the crop, proventriculus, intestine, and droppings of each bird was determined. Data on the total number of sporocysts (free and within intact oocysts) recovered indicate that (1) both species excysted more rapidly in turkeys than in chickens and (2) the percentage of sporozoites of both species that excysted in the two hosts was similar.

Andrews (1930), Kartchner and Becker (1930), Lotze et al. (1961), Marquardt (1966), and Haberkorn (1970) have shown that excystation of mammalian *Eimeria* is a nonspecific phenomenon. *Eimeria* from poultry also excyst in the "wrong" host. Haberkorn (1970) observed that *E. tenella* sporozoites excyst in the mouse, penetrate the intestinal epithelium, develop into trophozoites, and proceed into nuclear division. There are several reports of successful cross-transmission of *Eimeria* spp. to avian hosts (McLoughlin, 1969; Doran, 1978b).

Excystation of avian *Eimeria* has been studied *in vivo,* but only in the natural host (Goodrich, 1944; Itagaki, 1954; Doran and Farr, 1962; Farr and Doran, 1962). These studies, however, provide data on ratios of oocysts, liberated sporocysts, and excysted sporozoites in various areas of the digestive tract. There are no data available on rates of excystation and quantities of sporozoites excysting in either the natural or experimental hosts. The present report provides such data for two turkey coccidia, *E. dispersa* and *E. meleagrimitis,* in chickens and turkeys.

## Materials and Methods

Both species of coccidia were obtained from Dr. S. A. Edgar, Auburn University. Oocysts were collected, sporulated, and freed of fecal debris as described by Vetterling (1969). They were stored in Ringer's solution at 3–6°C, and were 8–11 weeks old when used.

Three- to 4-week-old chickens (White Leghorn cockerels) and turkeys (Beltsville Small White) were placed individually in small ($\approx$150- × 200-mm) cages that were fitted with a removable 14-mm wire mesh floor suspended $\approx$26 mm above a layer of heavy aluminum foil. Feed and water were withheld during the experiments and for 12 hr before inoculation.

Sporulated oocysts were administered per os. In three experiments, *three* chickens and three turkeys each were given 6 million *E. dispersa* oocysts; in three other experiments, three chickens and three turkeys each were given 10 million *E. meleagrimitis* oocysts. One bird of each species were killed with ether at 0.5, 2, and 9 hr after inoculation. The crop, proventriculus, gizzard, and intestine of each bird were removed. The intestine was slit open, placed in an electric blender with 250 ml of water, and mixed for 1 min. The homogenate was
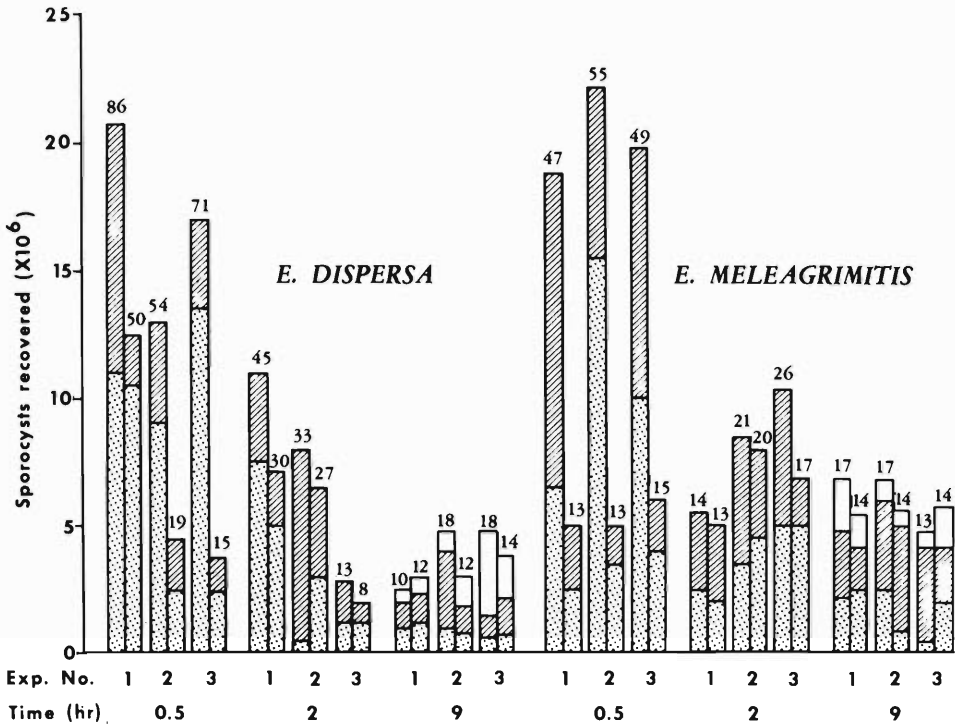
**Figure 1.** Sporocysts recovered from chickens and turkeys given either 6 million *Eimeria dispersa* oocysts (24 million sporocysts) or 10 million *E. meleagrimitis* oocysts (40 million sporocysts). In each pair of bars, left = chicken and right = turkey. Numbers above each bar represent percentage of sporocysts, free and within intact oocysts, recovered. Dotted shading = crop, proventriculus, and gizzard; lined shading = intestine; no shading = droppings.

then transferred to a measuring bottle and diluted to 350 ml. The crop, proventriculus, and gizzard were opened and the contents were washed into a beaker with 150 ml of water. This suspension was transferred to a measuring bottle and water was added to 200 ml. The wire floor was washed with 200 ml of water. Aluminum foil under the wire floor that contained fecal material was removed with a scalpel and placed in the container with the wire-floor wash. This material was then placed in an electric blender, mixed for 1 min, and transferred to a measuring bottle. Water was added to 250 ml.

The number of intact oocysts and free sporocysts in the intestinal homogenate and in each of the washings was counted with a counting chamber. The total number of sporocysts (free and within intact oocysts) recovered was used as a criterion for excystation.

## Results

The numbers and percentages of each dose that was recovered as sporocysts (free and within intact oocysts) are shown in Figure 1. Both species excysted more rapidly in turkeys. At 0.5 hr, the average recoveries in three experiments (AR's) with *E. dispersa* and *E. meleagrimitis* were 42 and 37% lower, respectively, in turkeys than in chickens.

*Eimeria meleagrimitis* excysted more rapidly than *E. dispersa* in both chickens and turkeys. At 0.5 hr, the AR's of *E. meleagrimitis* from chickens and turkeys were 20 and 14% lower, respectively, than those of *E. dispersa*. In turkeys, excystation of *E. melagrimitis* was completed at 0.5 hr; there was only a 1% change in the AR between 0.5 and 9 hr in each of the three experiments. With *E. dispersa* in turkeys, the percent changes in the AR's between these two times were 38, 7, and 1%, for the three experiments.

Overall, excystment was similar in the two hosts. At 9 hr, there was only a 3 or 4% difference in the AR of each species in chickens and turkeys.

Only intact oocysts were found in the droppings at 9 hr. The numbers of oocysts of each species at 9 hr were very similar in the two hosts. With *E. dispersa*, the 9-hr average oocyst counts from chickens and turkeys were $3.6 \times 10^5$ and $3.0 \times 10^5$, respectively; with *E. meleagrimitis*, the 9-hr average oocyst counts from the two hosts were $3.0 \times 10^5$ and $2.8 \times 10^5$, respectively.

## Discussion

Gill (1954) reported that *E. meleagrimitis* produced patent infections in chickens. Numerous attempts (unpublished) have been made at this institute over the past 25 years to produce patent infections in chickens with this species and others from turkeys. The only success with chickens not given immunosuppressants has been with *E. dispersa*. The oocyst yield, however, was far less than that obtained with the natural host. Two turkeys given 50,000 sporulated oocysts each shed a total of 121 million oocysts (Doran, 1978a), whereas two chickens given the same dosage shed a total of only 70,000 oocysts (Doran, 1978b). One of the reasons for the enormous difference in quantity of oocysts shed by the two hosts was thought to be differences in the quantity of sporozoites excysting (Doran, 1978b). Present data indicate that *E. meleagrimitis* excysts in the chicken and that the percentage of sporozoites excysting is not a contributing factor to either the inability to infect chickens with *E. meleagrimitis* or the differences in oocyst yield with *E. dispersa*. Both species excysted more rapidly in turkeys, but the percentage of sporozoites excysting in the two hosts became similar by 9 hr after inoculation. Differences in the number of sporozoites penetrating the villi, the number of sporozoites and developmental stages surviving intracellularly, or both, are probably responsible for our failure to infect chickens with *E. meleagrimitis* and the different yields obtained with *E. dispersa* in chickens and turkeys.

One reason for the more rapid excystation in turkeys might be that more of the dosage had reached the intestine sooner after inoculation. At 0.5 hr, in all experiments with *E. meleagrimitis* and two of the three experiments with *E. dispersa*, considerably less of the dose was in the crop, proventriculus, and gizzard of the turkey than in these organs of the chicken. However, before a more rapid excystation rate can be attributed to a faster passage time in turkeys, it must be determined whether species from chickens also excyst more rapidly in turkeys. Another reason for the more rapid excystation might be that conditions necessary for rapid excystation (such as bile and pancreatic enzymes) were more satisfactory in turkeys. At 0.5 hr, in all experiments with both species, there was less of the dose in the intestine of turkeys than in the intestine of chickens.

Determining excystation rates and quantities by counting the oocysts and sporocysts remaining in the digestive tract is the indirect approach to the study of

excystation. Counting sporozoites themselves would have been much better, but this could not be done for two reasons: (1) some undoubtedly had entered cells by the time birds were killed and (2) it is virtually impossible to distinguish sporozoites in an intestinal homogenate.

It is unlikely that any oocysts or sporocysts were ruptured during mixing with the electric blender. Preliminary work showed that oocysts in suspension remained intact for 10 min when mixed at the speed used to prepare the intestinal homogenate.

## Acknowledgments

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# Fertilization in the Coccidia: Fusion of *Sarcocystis bovicanis* Gametes

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Coccidian parasites of animals and man include the genera *Eimeria, Isospora, Toxoplasma, Sarcocystis,* and others. The life cycle of each of these parasites includes both asexual and sexual stages of multiplication. The sexual stages of nearly all coccidian species develop intracellularly in the intestine of the final host. Differentiation of the parasite into microgametes (male) and macrogametes (female) is followed by fertilization and development of the zygote into an oocyst stage. Thousands of species of coccidia have been described from numerous hosts, but the act of fertilization has never before been recorded, perhaps because of the concealed location of the gametes within host cells. However, eimerian microgametes have been observed on the surface of macrogametes by light (Marquardt, 1966) and scanning electron microscopy (Madden and Vetterling, 1977), and within a macrogamete by transmission electron microscopy (Hammond and Scholtyseck, 1970). The present study reports for the first time the process of coccidian fertilization by fusion of the plasma membranes of a micro- and a macrogamete and the transfer of nuclear material. This observation indicates that fertilization in the coccidia resembles that reported for other invertebrates (Epel and Vacquier, 1978) and for mammals (Bedford and Cooper, 1978), where membrane fusion occurs, rather than penetration of the female gamete by the male gamete as previously assumed for the coccidia.

Intramuscular cysts of *Sarcocystis bovicanis* develop in bovine hosts after ingestion of sporocysts from canine feces. Following ingestion of *S. bovicanis* cysts containing mature asexual zoites, infection is established in the small intestine of the dog (Fayer, 1974). Zoites enter goblet cells and differentiate, without further multiplication, into sexual stages which mature within 6–12 hr after ingestion (unpublished observations). During a study of specimens collected at these time periods, fertilization was observed.

## Materials and Methods

Myocardium containing mature cysts of *Sarcocystis bovicanis* was taken from a bovine host experimentally infected with sporocysts from dogs and ground in a commercial meat grinder. The ground myocardium was fed, within an hour after death of the bovine host, to coccidia-free beagle puppies that had never before eaten meat. The dogs were killed at 6 and 12 hr after ingesting the ground myocardium. Segments of small intestine were fixed and processed for study by both light and electron microscopy.

## Results

Fertilization of macrogametes was observed in specimens of canine small intestine fixed 12 hr after dogs were fed infected bovine heart (Fig. 1). The gametes
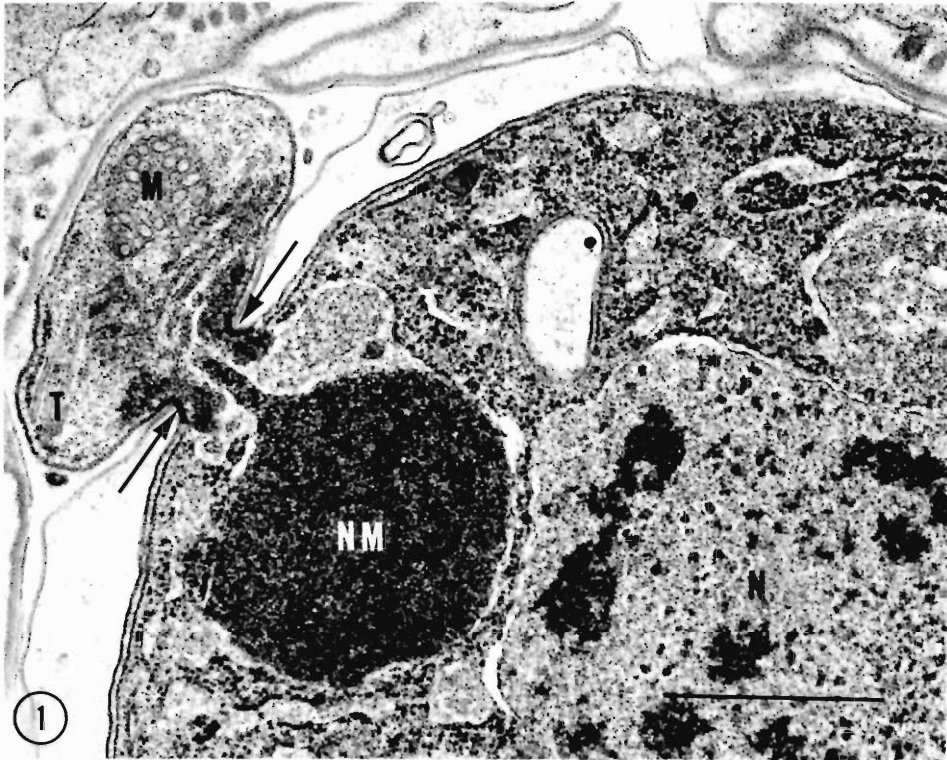
Figure 1.   Electron micrograph of *Sarcocystis bovicanis* gametes in dog small intestine. Portions of intestine were fixed in 5% glutaraldehyde buffered at pH 7.3 with 0.05 M phosphate and then were postfixed in 2% $OsO_4$. Tissues were dehydrated, embedded in Epon, sectioned, and stained with lead citrate and uranyl acetate. Section is through a microgamete (upper left) and a portion of a macrogamete. The microgamete lies inside the host layers and is fused with the macrogamete through a discontinuity in the raised parasite membrane layer. At the point of fusion, the gamete membranes are thickened (arrows) and an aggregate of dense material lines the cytoplasmic side of the fusion area. A dense mass of microgamete nucleoplasm (NM) has passed through the area of fusion into the macrogamete cytoplasm. Microtubules (T) and a mitochondrion (M) remain within the microgamete. The macrogamete nucleus (N) is visible at the lower right. Scale bar, 1 $\mu$m.

were extracellular within the lamina propria. The macrogamete was enveloped by several layers. Outermost was a nonmembranous thick layer, beneath which was a closely adherent membranous layer; both are thought to be of host cell origin. Another membranous layer, presumably derived from the parasite, was present beneath the two outer layers and separated them from the pellicle of the macrogamete. The fertilizing microgamete was found between the two outermost layers and the membranous layer of parasite origin. The latter appeared to be discontinuous at the point of fusion of the two gametes.

The body of the microgamete, which contained a typical mitochondrion and several microtubules, was surrounded by a single-membrane plasmalemma and was oriented tangential to the surface of the macrogamete. The two gametes were connected to one another by a small necklike region through which there was cytoplasmic continuity. In the necklike region, at the point of their fusion, the

gamete membranes formed an electron-dense, thickened ring. Through this ring, nucleoplasm from the microgamete passed into the macrogamete cytoplasm. Microgamete nucleoplasm was also present in the cytoplasm of macrogametes in which oocyst wall formation had begun, and no evidence of residual microgamete structure was observed. Fusion of the microgamete nucleoplasm with the macrogamete nucleus was not seen.

In several specimens, microgametes were seen in association with macrogametes but fusion was not apparent, although it could have occurred outside the plane of the sections. In each case, the microgamete was located beneath the two outermost layers as described above. Flagella were not present on any microgametes observed in association with macrogametes.

## Discussion

Observations of fertilization of *Sarcocystis bovicanis* differ from those reported for other coccidia by earlier workers. The first ultrastructural observation of fertilization of coccidia was made by Hammond and Scholtyseck (1970), who found an intact microgamete within a macrogamete of *Eimeria bovis*. The microgamete appeared to lie within a vacuole delimited by a membrane. Two flagella were observed, one of which was enclosed by a membrane that also covered the microgamete nucleus. Penetration of a macrogamete by a microgamete was reported in a scanning electron microscopy study of *Eimeria tenella* (Madden and Vetterling, 1977). Microgametes have also been seen by light microscopy on the surface of macrogametes of *Eimeria nieschulzi,* but neither penetration nor fertilization was reported (Marquardt, 1966). These studies imply that the intact coccidian microgamete attaches to the macrogamete surface, penetrates its cell membrane, and enters its cytoplasm during fertilization.

In contrast, fertilization in *S. bovicanis,* as reported herein, proceeds by fusion of the microgamete plasmalemma with that of the macrogamete and subsequent entry of microgamete nucleoplasm into the macrogamete cytoplasm. A similar process has been observed for three other genera of related protozoans. For *Parahaemoproteus velans,* the presence of the nucleus and axoneme of the microgamete free within the cytoplasm of the macrogamete suggested that fusion of the gamete membranes occurred during fertilization, resulting in entry of the "contents" of the microgamete into the macrogamete (Desser, 1972). For *Haemoproteus columbae,* fusion of the gamete membranes resulted in one continuous membrane surrounding their combined contents (Gallucci, 1974). It was also noted that microgamete axonemes did not persist within the macrogamete cytoplasm for more than two hours after fertilization. For *Plasmodium yoelii nigeriensis,* fusion of the gamete plasmalemmas allowed the naked axoneme and nucleus of the microgamete to pass into the cytoplasm of the macrogamete (Sinden et al., 1976).

Interpretation of the differences observed in fertilization among the coccidia is difficult because so few observations have been made. The lack of microgamete flagella at the time of fusion in our study may be explained as follows. Two layers of host origin surround the macrogamete and must be penetrated by the microgamete before fusion with the macrogamete. Madden and Vetterling (1977) may have observed a flagellated microgamete penetrating these layers rather than penetrating the macrogamete membrane. We suggest that the microgamete fla-

gella are lost at the time of fusion or slightly thereafter. The observation of a flagellated microgamete in a membrane delimited vacuole within a macrogamete (Hammond and Scholtyseck, 1970) differs from the other cited reports on fertilization, but resembles the entry of mammalian sperm into somatic cells where phagocytosis occurs and the sperm is membrane enclosed (Bedford and Cooper, 1978).

Only two electron microscope studies of sexual stages of *Sarcocystis* utilizing *in vitro* techniques have been reported (Vetterling et al., 1973; Mehlhorn and Heydorn, 1979). Although neither reported evidence of fertilization, Mehlhorn and Heydorn (1979) published a micrograph (fig. 22) of an oocyst with its cytoplasm connected to a cytoplasmic mass through a ringlike opening at its periphery, similar to Figure 1 (present study). However, they interpreted their micrograph as the beginning of sporogony.

Because of the difficulty of making observations *in vivo*, clarification of the fertilization process will probably necessitate further utilization of *in vitro* cultivation of the parasite.

## Acknowledgments

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# Demonstration of Thromboplastin-like Activity in Extracts from *Eimeria tenella*-Infected and Noninfected Chicken Ceca

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ABSTRACT: A component, present in an extract prepared from *Eimeria tenella*-infected chicken ceca (ICE), displayed a thromboplastin-like activity. An extract prepared from ceca of noninoculated chickens (NCE) displayed similar activity. An intravenous injection of ICE into the wing of noninoculated control birds was lethal in 20–60 sec at a level one-half to one-fifth of that necessary to kill birds with the NCE, even though both the ICE and NCE were of equal protein concentrations. The apparent cause of death was intravascular coagulation, which could be averted if heparin was administered concomitant with either extract. The activity of the ICE and NCE was quantitated using a modified prothrombin time test. In all trials the ICE resulted in lower prothrombin times than the NCE of an equal protein concentration. The thromboplastin-like activity was present on day 5 post-inoculation (PI) and continued at least through day 9 PI. The ICE activity was more heat stable at 60°C than was the NCE or brain powder thromboplastin.

Tyzzer (1929) first suggested the presence of a substance(s) in *Eimeria tenella*-infected tissue which might interfere with blood coagulation. Since then, many attempts have been made to demonstrate a toxin. Sharma and Foster (1964) examined bacteriologically sterile oocyst extracts and suggested that a lethal toxin was part of the oocyst cytoplasm. Daugherty and Herrick (1952) described a fraction from ceca of coccidia-infected chickens which decreased the capacity of chicken brain to utilize glucose. Burns (1959) prepared cecal extracts from infected chickens and demonstrated a toxic reaction in rabbits but not in chickens. In an earlier study, Wolfe et al. (1946) described various tissue extracts that, when injected into chickens, resulted in death. When tissue homogenates from either *E. tenella*-infected ceca or noninfected ceca were injected intravenously (i.v.) into healthy control birds, death by intravascular coagulation ensued. Those investigators also noted that injections of the cecal contents from *E. tenella*-infected birds were always lethal; however, injections of the cecal contents from healthy birds were nonlethal. Using mortality as the sole positive indicator of activity, Bradford et al. (1947) further examined the "thromboplastin-like" activity of the cecal core material from *E. tenella* infections. Although these authors hypothesized that the initiator of the intravascular coagulation was a tissue thromboplastin, they did not examine cecal tissue in their assay system.

The following experiments were undertaken to expand the work of Wolfe et al. (1946) and Bradford et al. (1947) because neither group quantitated the described activity or used any parameter other than mortality for assaying the lethal component found in the extracts. The experimental objectives established were to: (1) quantitate the reported "thromboplastin-like" activity found in cecal extracts and (2) determine at what day during the infection the activity is present.

## Materials and Methods

Single comb White Leghorn cockerels, 3 wk old, were used as sources of cecal tissue and blood plasma. Infected cecal tissue was obtained from chickens with

severe *Eimeria tenella* infections (+4 lesion score, Johnson and Reid, 1970). The strain of *E. tenella* used was the Beltsville (NAPL) strain maintained at the Animal Parasitology Institute.

### Preparation of extracts

The methods of preparation in each of two experiments were identical except as noted. Chickens were killed by cervical dislocation. The ceca were removed, slit lengthwise, lesion-scored, and washed thoroughly with the appropriate buffer. Buffer was added, and the tissue homogenized in a tissue homogenizer for 10 min. The homogenates were then sonicated for 2 min and centrifuged for 30 min at $17,000 \times g$. The supernatants were collected and protein concentrations determined by either a modified Lowry (Layne, 1957) or Biuret (Wooten, 1964) method. The protein concentrations of the two extracts [infected cecal tissue extract (ICE) or noninoculated cecal tissue extract (NCE)] were equalized by the addition of the appropriate buffer.

### Experimental modifications

EXPERIMENT I: Equal numbers of infected and noninfected ceca were used as extract sources. Twenty milliliters of buffer (0.1 M phosphate) at the cecal pH of 6.8 (Ruff and Reid, 1975) was added to the tissue for homogenation. Injections of either extract (ICE or NCE) were administered in the right wing vein of non-inoculated healthy chickens; all "misses" were discarded. Decreasing volumes of NCE were injected into different noninoculated chickens until the amount of NCE was no longer lethal. Decreasing volumes of ICE were injected into other birds until no death occurred. Those birds receiving nonlethal doses were monitored at least 1 hr postinjection. Dying times commenced at the start of injection, and stopped upon death of the bird. Immediately upon death, the chickens were necropsied.

Inhibition of intravascular coagulation was assayed by injecting i.v. 0.5 ml of 6,000 units sodium heparin/ml of buffer. The dosage used was calculated to give heparin as an anticoagulant at 100 units/ml of blood.

EXPERIMENT II: Both infected and noninfected ceca were collected daily from days 4 to 9 postinoculation (PI). The collected, washed ceca were blotted dry and weighed. Equal weights of ceca were used in extract preparations. Because the extracts were to serve as thromboplastin sources, the buffer used was 0.05 M imidazole in 0.025 M $CaCl_2$, pH 7.0. The ceca were homogenized with 3 ml of buffer/g of ceca. The extract was diluted 1:9 with buffer, the total protein determined, and equalized with the addition of the buffer. To determine the activity of the extract, serial $2\times$ dilutions were made from the 1:9 stock using the buffer as diluent. The dilutions were then stored on ice.

Heat lability was determined in the following manner. Tubes containing ICE, NCE, or chick brain powder thromboplastin (Doerr et al., 1975) were heated in a 60°C water bath for 5, 10, 20, 30 or 40 min. The tubes were cooled, centrifuged, and the supernatant was collected. The pellets were resuspended to original volumes with buffer and stored on ice with the supernatants.

PLASMA COLLECTION: On the day of the trial, 2.7 ml of blood from 10 non-inoculated healthy chickens was collected via cardiac puncture, immediately placed into 0.3 ml of 0.18 M sodium citrate (Doerr et al., 1975), and mixed

**Table 1.** Effect of injected substances on time of death in noninoculated chickens.

| Substance* | Amount injected†<br>(ml) | Time of death after<br>injection (sec) |
|---|---|---|
| 0.1 M phosphate buffer | 0.5 | — |
| NCE | 0.5 | — |
| ICE | 0.5 | 20 |
| ICE | 0.25 | 40 |
| ICE | 0.10 | 60 |
| Sodium heparin (6,000 units) ml | 0.5 | — |
| Sodium heparin + ICE (1:1) | 0.5 | — |

* NCE = extract from noninfected chicken cecal tissue, ICE = extract from *Eimeria tenella*-infected cecal tissue.
† Protein content (30 mg/ml) of tissue extracts from 10 pairs of infected or noninfected ceca was equal.

thoroughly. The citrated blood was then centrifuged, and the plasma was collected, pooled, and placed on ice until needed.

THROMBOPLASTIN ACTIVITY ASSAY: The assay procedure is essentially that described by Doerr et al. (1975) for determining prothrombin time, except that diluted ICE and NCE were used as the thromboplastin source instead of chick brain powder and the plasma was not prewarmed. As a control measure, prothrombin times using brain powder thromboplastin alone (Doerr et al., 1975) and buffer alone (essentially a recalcification time) were determined for the plasma pool at the start of each trial. Subsequently, these substances were used throughout the trial to monitor plasma condition. Because the extremely diluted ICE and NCE contained essentially only buffer, the trial was ended when the prothrombin time of the ICE- or NCE-treated plasma reached that of the buffer. Four prothrombin times for each protein concentration (=dilution) of ICE or NCE were conducted during each trial. Because both extracts were of equal protein concentrations, the prothrombin times at each concentration were compared with the Student's $t$-test and the log of the mean prothrombin time was plotted against the log of the protein concentration. The resulting graphs were analyzed by conventional regression analysis.

**Table 2.** Relationship of cecal wet weight and number of ceca used to prepare extracts.

| Days post-<br>inoculation | Type of<br>cecal tissue | Number<br>of ceca | Total wt<br>(g) | Avg wet<br>wt/cecum<br>(g) | mg/ml of<br>stock extracts<br>before dilution<br>($\bar{x} \pm$ SE) |
|---|---|---|---|---|---|
| 4 | Noninfected | 6 | 3.9 | 0.65 | 8.3 ± 0.3 |
| 4 | Infected | 5 | 3.9 | 0.78 | 8.3 ± 0.4 |
| 5 | Noninfected | 8 | 7.0 | 0.88 | 10.2 ± 0.5 |
| 5 | Infected | 5 | 7.1 | 1.41 | 10.1 ± 0.2 |
| 6 | Noninfected | 7 | 5.2 | 0.74 | 9.5 ± 0.2 |
| 6 | Infected | 4 | 5.2 | 1.30 | 9.6 ± 0.2 |
| 7 | Noninfected | 7 | 6.9 | 0.99 | 8.4 ± 0.1 |
| 7 | Infected | 4 | 6.3 | 1.56 | 8.4 ± 0.1 |
| 9 | Noninfected | 12 | 13.4 | 1.12 | 7.1 ± 0.1 |
| 9 | Infected | 7 | 14.3 | 2.04 | 7.3 ± 0.1 |
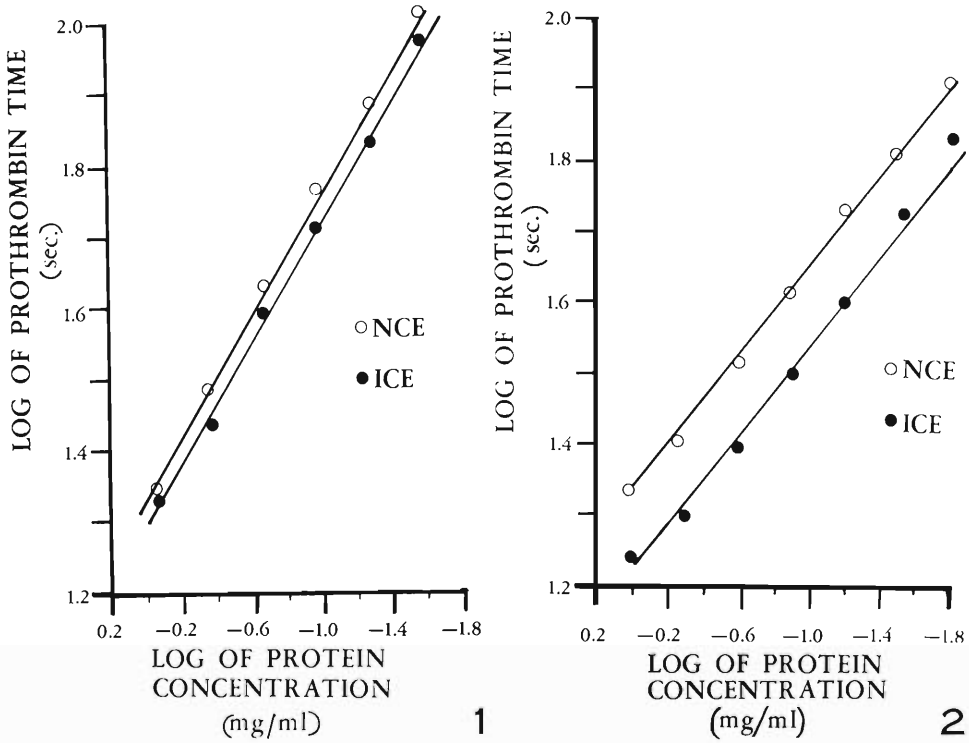
Figure 1.   Plot of the prothrombin times using extracts from noninfected ceca (NCE) (O) and from *E. tenella*-infected ceca (ICE) (●) as thromboplastin sources. Plots are representative of effects seen on days 4 postinoculation.

Figure 2.   Plot of the prothrombin time with extracts from noninfected ceca (NCE) (O) and from *E. tenella*-infected ceca (ICE) (●) as thromboplastin sources. Plots are representative of effects seen on days 5–9 PI.

## Results

### Experiment I

In all trials, the ICE, when injected i.v. into noninoculated chickens, was lethal at one-half to one-fifth of the injection volume of the nonlethal amount of NCE (Table 1). The volume of injected NCE which was nonlethal varied between trials depending on the total protein content of the extract. When a lethal volume of NCE was injected, the chicken died with the same symptoms as those chickens succumbing to injections of ICE.

Death resulting from the injection of either NCE or ICE was rapid, usually within 40 sec. The injected chickens convulsed severely, and intravascular clots were observed in cardiopulmonary vessels. Occasionally, these clots could be seen in the dorsal aorta, carotid arteries, and jugular vein. Vessels in the brain appeared enlarged, and the pancreases appeared congested. If chickens dying from injection of either extract were decapitated, minute amounts of blood were lost.

The time required for death after injection of the ICE increased as the volume of ICE injected decreased (Table 1). If 3,000 units of heparin were administered

Table 3. Effect of 60°C for variable time periods on activity of the thromboplastin and cecal extracts.

| Substance* | Time at 60°C (min) | Supernatant prothrombin time (sec) | Pellet prothrombin time (sec) |
|---|---|---|---|
| Thromboplastin | 0 | 20 | — |
| NCE | 0 | 30 | — |
| ICE | 0 | 22 | — |
| Thromboplastin | 5 | 162 | 26 |
| NCE | 5 | 89 | 36 |
| ICE | 5 | 39 | 30 |
| Thromboplastin | 10 | 174 | 19 |
| NCE | 10 | 124 | 48 |
| ICE | 10 | 44 | 33 |

* NCE = extract from noninfected cecal tissue; ICE = extract from *Eimeria tenella*-infected cecal tissue.

concomitant with either ICE or NCE, death could be averted (Table 1) and no adverse symptoms would be observed.

### Experiment II

When the modified prothrombin time test was used to measure the clot initiating activity of the two extracts, no effect was evident on day 4 PI (Fig. 1). The clot initiating activity was, however, present from days 5–9 PI, and results typical for these days are shown in Figure 2. In this time period, the linear relation between prothrombin time and protein concentration was well correlated ($P \geqslant 0.95$). However, the slopes of the NCE and ICE regressions were not significantly different even though the intercepts were ($P \geqslant 0.95$).

In most trials, approximately twice as many noninfected ceca were used to obtain the approximate wet weight of infected ceca (Table 2). When wet weights were equal, the amount of soluble protein was not significantly different within a trial. On day 4 PI the individual infected and noninfected ceca were of about equal wet weight; on days 5–9 PI, the weights of the infected ceca were greater than those of the noninfected (Table 2). Heating the cecal extracts at 60°C for 5 or 10 min only slightly decreased the activity of the ICE; however, such heating for 5 min significantly decreased the activity of NCE (Table 3).

### Discussion

The extract from infected ceca (ICE) was more active in initiating intravascular coagulation and death than a similar extract prepared from noninfected cecal tissue (NCE), even though both the ICE and NCE were of equal protein concentrations (Table 1). Enlargement of the vessels in the brain and congestion of the pancreas were also observed. Although Wolfe et al. (1946) injected similar tissue extracts into chickens and observed intravascular coagulation, they did not mention an increased activity in the extract from the infected tissue. They did not equalize the protein concentrations of their extracts; however, they did use equal numbers of ceca to prepare their extracts.

When equal numbers of ceca were used and the protein concentrations equalized, the ICE still had greater activity than the NCE (Tables 1, 2). If the extracts were not corrected for the protein differences, the ICE activity could be under-

estimated. Similarly, the use of mortality as the sole measured parameter provides no true quantitative measure of the activity in the ICE, because Wolfe et al. (1946) and the present investigators observed that heparin could prevent death (Table 1), and the previously described symptomology, intravascular coagulation, was presumed as the cause of death. This presumption implies that ICE, and to some extent, NCE, had a "thromboplastin-like activity." Bradford et al. (1947) suggested such a possibility; however, the activity they described was found only in i.v.-injected cecal contents and core material of *E. tenella*-infected chickens, and again, mortality was the only parameter measured. Thus, the conventional prothrombin time test (Doerr et al., 1975) modified to quantitate the activity observed from cecal tissue extracts was deemed an acceptable quantitative method.

There was no significant difference between the thromboplastic activity of ICE and NCE prepared on day 4 PI (Fig. 1). However, ICE prepared from infected ceca on the 5th to 9th days PI gave consistently and significantly shorter prothrombin times than did the NCE prepared the same day PI (Fig. 2 typical). The slopes of the regression lines were not significantly different; however, there were significant differences in the intercepts of the NCE and ICE regressions (Fig. 2). These differences imply that the activity found in the two types of extracts decreases at the same rate with decreasing protein concentration, but that the prothrombin times at each protein concentration were significantly different from one another.

Infection with avian coccidia prolongs the prothrombin time of plasma from infected chickens (Ruff et al., 1978; Witlock and Wyatt, 1978). The increase in prothrombin time occurs on day 5 PI with *E. tenella* and is transitory. The 5th day PI roughly coincides with the rupture of the second-generation schizonts and onset of extensive tissue damage. Similarly, the activity of ICE begins on day 5 PI.

Bradford et al. (1947) noted that the extracts from cecal contents were lethal from days 5 through 14 PI. They suggested two possible effects of the thromboplastin-like activity in the cecal contents: (1) the activity may place the blood of infected birds in a hypercoagulative state (decreased prothrombin time), or (2) the blood may be in a hypocoagulative state (increased prothrombin time). The hypocoagulative state was presumed to be due to the depletion of clotting factors resulting from the continued leakage of the thromboplastin-like activity into the blood stream. Bradford et al. (1947) suggested the hypocoagulative hypothesis after noting that sublethal injection of contents from infected ceca resulted in blood that was incoagulable.

Thus, an influx of the activity found in the ICE into the vasculature with subsequent dissemination into the systemic circulation could result in a "negative clotting phase" and cause a prolonged prothrombin time. Compensation for the decrease of clotting components by the host would then place the bird back into the steady state; this shift toward the steady state may account for the transitory nature of the prolonged prothrombin time observed in vivo. Conceivably, slight overcompensation by the host may well result in hypercoagulation (decreased prothrombin time) when compared with control animals, as Bradford et al. (1947) suggested.

The relative heat stability of ICE when compared with that of NCE and throm-

boplastin suggested that the active component of the ICE is different from that of the NCE or thromboplastin. NCE was not stable when heated to 60°C (Table 3) as evidenced by the great increase of prothrombin time; likewise the activity of the thromboplastin was greatly decreased. However, ICE could withstand such a temperature for 10 min without appreciable loss of activity. Wolfe et al. (1946) noted that the lethality of the cecal homogenates was variable after they were heated for 5 min at 60°C.

At present, more specific information on the chemical nature of the activity found in ICE is unavailable. Whether a similar activity can be isolated from birds infected with other coccidial species is also unknown.

## Acknowledgments

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# Factors Affecting the Virulence of *Naegleria fowleri* for Mice

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ABSTRACT: Incubation temperature, growth phase, and strain of parasite were examined for their effect upon the virulence of *Naegleria fowleri* for mice. Agitated cultures of amebae incubated at 30 and 37°C were more virulent than amebae cultured at 23 and 44°C. Mortality was greater for mice inoculated with amebae harvested at late logarithmic and early stationary growth phases than it was with amebae harvested at early logarithmic and late stationary growth phases. Virulence varied greatly among the 10 strains of *N. fowleri* tested. Also, it appeared that virulence of amebae decreased with increased time in axenic culture. These results suggest that factors affecting the virulence of *N. fowleri* for mice may also influence the outcome of natural exposure and infection in man.

*Naegleria fowleri* is a free-living ameboflagellate which produces a fatal meningoencephalitis in man. It is also able to produce a similar progressive and fatal disease in mice (see Griffin, 1978 for a recent review).

There are numerous publications concerning the virulence of *N. fowleri* for laboratory animals. Most of these accounts describe the invasiveness of amebae with respect to route and size of inoculum. In contrast, little information is available on the effect of culture conditions upon the virulence of *N. fowleri* amebae.

Wong et al. (1975) have suggested that pathogenicity of *N. fowleri* may be influenced by the strain virulence, growth phase, and cultural conditions of amebae. Recently (Wong et al., 1977), they demonstrated that prolonged maintenance of *N. fowleri* amebae in axenic medium reduced the organism's virulence for mice. Griffin (1972) stated that the ability of *N. fowleri* to grow at high temperatures seemed directly related to virulence.

In this study, we demonstrate that incubation temperature, growth phase, and ameba strain affect the virulence of *N. fowleri* for mice.

## Materials and Methods

### Ameba strains

Ten human isolates of *N. fowleri* were used in this study. Strains LEE, WM, CJ, and TY were isolated at the Medical College of Virginia by E. C. Nelson (Department of Microbiology, Virginia Commonwealth University); NF66 and NF69 were isolated in Australia and kindly supplied by J. L. Griffin (Armed Forces Institute of Pathology, Washington, D.C.); Lovell and GJ isolated in Florida, and O 359 and KUL isolated in Belgium, were kindly supplied by S. L. Chang (U.S. Environmental Protection Agency, Cincinnati, Ohio).

### Cultivation

The LEE strain of *N. fowleri* was used in all experiments except where noted. Amebae were grown axenically in cotton-stoppered 2.8-liter siliconized Fernbach flasks using 1 liter of Nelson medium (Weik and John, 1977) inoculated with $10^4$ amebae/ml. Cultures were adjusted to pH 5.5 and, unless otherwise specified, incubated at 37°C in a gyrotory shaker (New Brunswick) at 100 RPM.
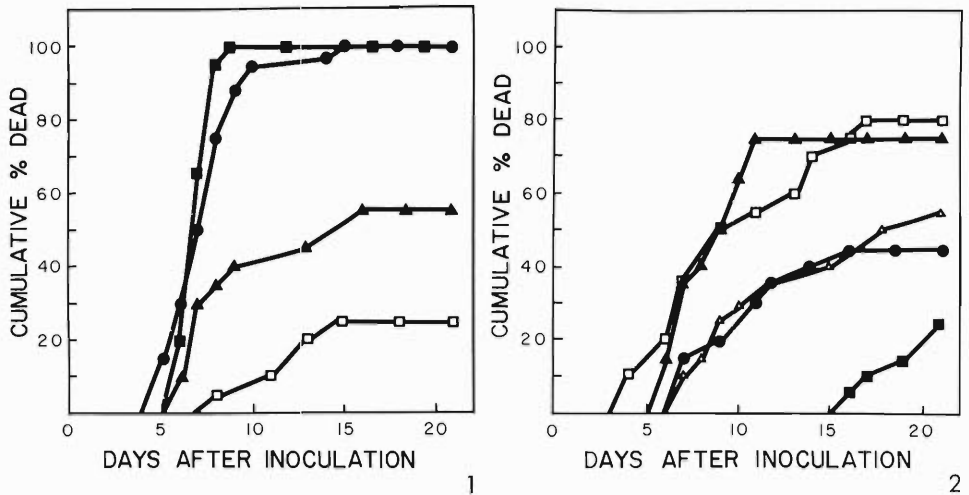
Figures 1, 2. 1. Mortality of 13–18-g male DUB/ICR mice inoculated with *N. fowleri* (LEE) from 72-hr cultures incubated at various temperatures. Forty mice/group were injected i.v. with $5 \times 10^6$ amebae/mouse. (▲) 23°C; (●) 30°C; (■) 37°C; (□) 44°C. 2. Mortality of 13–18-g male DUB/ICR mice inoculated with *N. fowleri* (*LEE*) harvested at 5 points during growth. Cultures were incubated at 37°C and 20 mice/group were injected i.v. with $5 \times 10^6$ amebae/mouse. (●) 24 hr; (□) 48 hr; (▲) 72 hr; (△) 96 hr; (■) 120 hr.

## Cell collection

Amebae were harvested by centrifugation at $2,000 \times g$ for 10 min at 20°C. The cells were washed twice with Page ameba saline (Page, 1967) and suspended in 0.15 M NaCl for counting and mouse inoculation. Amebae were judged viable using trypan blue exclusion.

For counts, 0.2 ml of cell suspension was added to 9.8 ml of electrolyte solution consisting of 0.5% (v/v) formalin and 0.4% (w/v) NaCl in distilled water and vortex-shaken to disperse cell aggregates. Cells were then counted in a Coulter counter (model $Z_{BI}$, Coulter Electronics) using settings described elsewhere (Weik and John, 1977).

## Mouse inoculation

Male DUB/ICR mice (Flow Research Animals, Inc., Dublin, Va.) weighing 13–18 g were used in all experiments. Mice were allowed to adjust to their new environment for at least 3 days prior to experimentation. The mice were given free access to water and feed (Purina Lab Chow, Ralston Purina Corp.). There were 20 mice per treatment group unless otherwise specified.

Mice were inoculated intravenously (i.v.) into a lateral tail vein using a 25-gauge needle and tuberculin syringe. Inocula contained $8.3 \times 10^5$, $2.5 \times 10^6$, or $5 \times 10^6$ live *N. fowleri* amebae in a 0.2 ml cell suspension. Mice were held for 21 days after inoculation and the cumulative percent dead was recorded on a daily basis. Infection was verified by culturing in Nelson medium amebae from brain tissue of dead or dying mice.

Table 1.   Mortality of mice inoculated i.v. with various strains of *N. fowleri.**

| Strain of ameba | Location of initial isolation | Date of isolation | Reference | Cumulative % dead† |
|---|---|---|---|---|
| $2.5 \times 10^6$ amebae/mouse | | | | |
| CJ | Virginia | 1967 | Duma et al., 1971 | 40 |
| LEE | Virginia | 1968 | Duma et al., 1971 | 85 |
| O 359 | Belgium | 1970 | Jadin et al., 1971 | 85 |
| NF69 | Australia | 1969 | Carter, 1970 | 95 |
| TY | Virginia | 1969 | Duma et al., 1971 | 100 |
| KUL (838) | Belgium | 1973 | Van Den Driessche et al., 1973 | 100 |
| NF66 | Australia | 1966 | Carter, 1970 | 100 |
| WM | Virginia | 1969 | Duma et al., 1971 | 100 |
| GJ | Florida | 1973 | Wellings et al., 1977 | 100 |
| Lovell (RN) | Florida | 1974 | De Jonckheere, 1977 | 100 |
| $8.3 \times 10^5$ amebae/mouse | | | | |
| TY | Virginia | 1969 | Duma et al., 1971 | 30 |
| KUL (838) | Belgium | 1973 | Van Den Driessche et al., 1973 | 40 |
| NF66 | Australia | 1966 | Carter, 1970 | 55 |
| WM | Virginia | 1969 | Duma et al., 1971 | 55 |
| GJ | Florida | 1973 | Wellings et al., 1977 | 80 |
| Lovell (RN) | Florida | 1974 | De Jonckheere, 1977 | 95 |

\* Twenty 13–18-g male DUB/ICR mice per group.
† At 21 days after inoculation.

## Experimental design

Initially, a 37°C incubation temperature was chosen because the amebae were isolates from fatal human infections. To determine the effect of cultivation temperature upon virulence, the following incubation temperatures were used: 23, 30, 37, and 44°C. Agitated cultures were incubated 72 hr and mice were inoculated i.v. with $5 \times 10^6$ amebae/mouse.

To determine whether there were differences in virulence at various stages of the growth cycle, amebae were harvested at five different points during agitated growth: 24, 48, 72, 96, and 120-hr culture age. Amebae, incubated at 37°C, were inoculated i.v. into mice using a dose of $5 \times 10^6$ cells/mouse.

Finally, we wanted to determine whether there were differences in virulence among the *N. fowleri* strains maintained axenically in our laboratory. Mice were injected i.v. with one of 10 different strains of *N. fowelri* isolated from clinical cases in Virginia, Florida, Australia, and Belgium. The initial dose was $2.5 \times 10^6$ amebae/mouse. However, since six strains produced 100% mortality with this dose, a threefold reduction in inoculum ($8.3 \times 10^5$ amebae/mouse) was given to a second group of mice.

## Results

Amebae cultured at 23°C produced 55% cumulative mortality, those cultured at 44°C produced 25% mortality, and at 30 and 37°C both produced 100% mortality (Fig. 1). Amebae cultured at 30 and 37°C maintained a high degree of virulence, whereas amebae cultured at the higher and lower temperatures did not. Cumu-

lative mortality was 100% by day 9 for mice inoculated with amebae from 37°C cultures, but did not reach 100% until day 15 for mice inoculated with amebae from 30°C cultures. The last deaths for mice inoculated with amebae cultured at 44°C and 23°C occurred on days 15 and 16, respectively.

Based upon these results, the 37°C incubation temperature was selected as the standard temperature for *N. fowleri* cultivation in virulence studies. Also, 21 days after inoculation appeared to be sufficient time in which to record deaths from infection.

Two patterns of clinical signs were observed for mice inoculated i.v. with *N. fowleri*. Approximately 2 days before death, mice appeared either lethargic, exhibiting malaise and anorexia, or they demonstrated noticeable hyperactivity with unilateral movement. A short time before death, cerebral edema and partial or complete posterior paralysis were observed, usually resulting in severe spinal flexure and finally coma. Some deaths occurred rapidly with little or no noticeable sign of infection. Amebae were recovered by brain culture from all dead or dying mice.

Figure 2 shows the cumulative percent dead for mice inoculated with amebae harvested at five different points during agitated growth. Amebae harvested at 24 hr produced 45% mortality, 48-hr cells produced 80% mortality, 72-hr cells 75% mortality, 96-hr cells 55% mortality, and 120-hr cells 25% mortality. Again, factors favoring virulence caused early deaths and those reducing virulence produced later deaths. The 48–72-culture period was the optimal culture age for ameba virulence. Mortality was 50% by day 9 for amebae from 48- and 72-hr cultures, compared with 25% mortality or less for amebae of earlier or later culture age.

We have shown in earlier work (Weik and John, 1977) that, with the agitated culture system described above, 24- and 48-hr cells are in logarithmic phase growth and 60-hr culture age represents transition from logarithmic to stationary phase growth. It appears that cells in the late logarithmic and early stationary phases of growth are most virulent for mice. Hence, 72-hr amebae were used in the remaining experiments because maximum mortality (75%) occurred by day 10 compared to day 17 for 48-hr amebae which produced 80% mortality.

Mortality ranged from 40 to 100% for mice inoculated with $2.5 \times 10^6$ amebae/mouse of the 10 strains of *N. fowleri* tested (Table 1). Mortality and survival time ranged from 100% in 6 days for the Lovell strain to 40% in 20 days for the CJ strain. For the six strains producing 100% mortality, a threefold reduction in inoculum resulted in mortality ranging from 30 to 95% (Table 1). From these data, it appears that, for i.v.-inoculated mice, the most virulent strain was Lovell, isolated from a fatal human infection in Florida in 1974. The least virulent strain was CJ, isolated from a human fatality in Richmond, Virginia, in 1967. Again, the more virulent strains caused death sooner after inoculation. Strains Lovell and GJ, for example, both produced 50% mortality by day 6 as compared to the other strains which caused 5% mortality or less for the same day.

## Discussion

Earlier virulence studies have used amebae from unagitated cultures (Adams et al., 1976; John et al., 1977; De Jonckheere, 1977). We compared the virulence of *N. fowleri* for mice using 96-hr amebae harvested from both agitated and unagitated cultures (data not shown) and observed 80% mortality among mice

inoculated with amebae from agitated cultures and 45% mortality for mice inoculated with amebae from unagitated cultures. There were 20 mice in each group. The apparent greater virulence for amebae from agitated cultures probably reflects a uniform group of cells, i.e., cells are in the same phase of growth. In unagitated cultures, the cells are in various stages of growth, hence, a mixed population of cells, because $O_2$ and nutrients probably are not available to all cells in similar amounts.

The growth phase had a marked effect upon virulence of the amebae. An incubation period of 48–72 hr yielded amebae which were most virulent for mice. At 37°C, this incubation period represents late logarithmic and early stationary growth phases. Amebae harvested at 24 hr (early logarithmic phase) and 96 or 120 hr (late stationary phase) were less virulent for mice. It is possible that the amebae in late logarithmic and early stationary growth phases present a complete antigenic structure (or perhaps digestive and cytolytic enzymes) necessary for invasiveness and virulence. Červa (1967) observed that *Acanthamoeba* from the logarithmic growth phase were more virulent for guinea pigs than were the same number of amebae from stationary growth phase.

*Naegleria fowleri* amebae proliferate over a wide temperature range. The strains we have used were isolates from human infections in which temperature ranged to 41°C. In experimental infections, mice provide a similar host temperature of 37°C or greater. Procedures for the isolation of *N. fowleri* from the environment routinely use an incubation temperature of 45°C (De Jonckheere, 1977). Amebae cultured at 37°C grew well and maintained invasiveness and virulence when introduced into animals whose body temperature was similar. It may be that amebae cultured at other temperatures take longer to establish in the host because of temperature adaptation; during the period of adaptation the amebae may be more vulnerable to host depredation. It is also possible that factors such as digestive or cytolytic enzymes which may be responsible for invasiveness and virulence are most active at 30–37°C. Alternatively, the observed difference in virulence of amebae cultured at various temperatures may reflect, again, the phase of growth. For example, cells incubated at 23°C and harvested at 72 hr may still be in early logarithmic phase growth because of a slower growth rate, whereas cells cultured at 44°C for 72 hr may be in late stationary phase growth because of a faster growth rate. We have already shown that amebae from early logarithmic and late stationary growth phases are less virulent for mice than amebae from the late logarithmic and early stationary growth phases.

We have shown that virulence varied greatly among the 10 strains of *N. fowleri* tested. Virulence also appears to be related to the length of time a strain has been maintained in axenic culture. This can be demonstrated with the *N. fowleri* strains isolated in Richmond, Virginia; all four strains have been maintained axenically in Nelson medium since primary isolation from patients. Strain CJ isolated in 1967 produced 40% mortality, LEE strain isolated in 1968 caused 85% mortality, and strains WM and TY isolated in 1969 both produced 100% mortality. We cannot make similar comparisons with the other strains because a variety of media were used for primary isolation and subsequent maintenance. However, Wong et al. (1977) also have demonstrated that strain virulence decreases with continued axenic maintenance.

In summary, agitated incubation at 37°C for 48–72 hr produced amebae of

maximum virulence. The considerable variation in virulence which occurred among the 10 strains of *N. fowleri* tested probably reflects more the length of time the strains have been maintained *in vitro* than it does the virulence of the amebae at the time of original isolation. Nonetheless, the factors which affect the virulence of *N. fowleri* for mice may influence the outcome of natural exposure and infection in man.

## Acknowledgments

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*Research Note*

# *Heterotylenchus* sp. (Nematoda: Sphaerulariidae), a Nematode Parasite of House Fly, *Musca domestica* L. (Diptera: Muscidae) in Northeastern Brazil

Members of the nematode genus *Heterotylenchus* have been previously reported to parasitize flies of the genus *Musca* (Stoffolano, 1970, Bull. Entomol. Soc. Amer. 16:194–203). Nickle (1967, J. Parasitol. 53:398–401), described a new species, *H. autumnalis* Nickle, from the face fly, *M. autumnalis* De Geer, in the U.S.; Vilagiova (1968, Biologia, Bratislava 23:397–400) reported the same species parasitizing *M. larvipara* Portchinsky and *M. tempestiva* Fallén in Czechoslovakia; while Hughes and Nicholas (1969, J. Econ. Entomol. 62:520–521) reported what is believed to be a new species from the bush fly, *M. vetustissima* Walker, from Australia. Because of its demonstrated capacity to exploit this genus, it seemed remarkable that natural *Heterotylenchus* infections had not been previously encountered in the house fly, *Musca domestica* L.

During a 2-year field survey in N.E. Brazil for the eyeworm parasite (*Thelazia*) of cattle, the authors discovered *Heterotylenchus* from the house fly. The survey was restricted to the state of Pernambuco and included 13 different cattle ranches. Flies were attracted from the faces of cattle using a board smeared with citrated bovine blood, swept from the board using a net, and placed into cages held at 29°C and 40–60% R.H. The flies were maintained on powdered milk, granulated sucrose, and water until dissection. Specimens were anesthetized in ether and dissected in physiological saline. Any nematodes recovered were transferred, along with the fly, to individual vials of warm F.A.A. to preserve them in a relaxed state.

In order to obtain free-living male and female nematodes for future species determination, flies were anesthetized and dissected on top of fresh cow manure. Larval nematodes from infected flies were added to the manure samples and left for 24 to 72 hr before removal and recovery from the manure using a Berlese technique. Following separation they were placed in vials of warm F.A.A.

After identification of the infected flies by Adrian Pont, all specimens were sent to Dr. Nickle for nematode identification. Nickle (personal correspondence) noted that the nematodes recovered from house flies represent a new, undescribed species of *Heterotylenchus* that he will describe elsewhere. All infected flies were identified by Adrian Pont to be the house fly, *Musca domestica*.

Of the 3,258 flies dissected, 34 specimens were infected with nematodes, of which 28 (0.86%) were positive for *Heterotylenchus*. Parthenogenetic and mature gamogenetic females were found in the hemocoel, while immature gamogenetic males and females filled the ovaries. Based on these findings, it is evident that the parasite can complete development in the house fly in Brazil. Collections were limited to the months of March, April, November, February, and June because the rainy season prohibited travel on unpaved roads. Six of the collection sites were east of Serra Talhada and failed to show any *Heterotylenchus* while the seven collections west of Serra Telhada contained all of the flies infected with the parasite. There is no apparent geographical barrier which could *account for*

its isolation in the west, yet no nematodes of this genus were recovered from flies east of this city.

In Massachusetts, examination of 1,633 field-collected house flies (Geden, personal correspondence) failed to reveal any *Heterotylenchus,* while experimental infections of house flies in Connecticut by the nematode, *H. autumnalis,* failed because of a host response (Stoffolano and Streams, 1971, Parasitology 63:195–211). In New England, niches of the house fly and face fly are rather discrete: house flies breed in piled manure and seldom visit the feces of cattle in the field, while face flies breed only in freshly dropped manure. The absence of *Heterotylenchus* in house flies examined from New England is due to a host response. Since other insect–nematode studies have demonstrated a genetic component of the host susceptibility to nematodes, it is obvious that a similar situation should exist within the *Musca* and the nematode genus *Heterotylenchus.* An additional factor influencing the success of the parasite in establishing new hosts may be the limitations which the various types of breeding media impose on the free-living stage of the nematode.

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*Research Note*

# Pairing Between *Echinostoma revolutum* (Trematoda) Adults and other Hermaphroditic Digeans *in vitro*

Pairing between *Leucochloridiomorpha constantiae* metacercariae and adults in vitro has been studied (Fried and Roberts, 1972, J. Parasitol. 58:88–91). Pairing between immature and mature *Echinostoma revolutum* adults in vitro has also been reported (Fried et al., 1980, J. Parasitol. 66: in submittal). Studies on interspecific pairing of hermaphroditic digeans in vitro have not been reported. Pairing between adults of *Nippostrongylus brasiliensis* (Nematoda) and other species of

Table 1. Pairing between *E. revolutum* adults and other digeans in vitro.

| Time (hr) | Mature *E. revolutum* adult vs. | | | Immature *E. revolutum* adult vs. | | |
|---|---|---|---|---|---|---|
| | Mature *Z. lunata* adult | Mature *L. constantiae* adult | Metacercaria of *L. constantiae* | Immature *Z. lunata* adult | Mature *L. constantiae* adult | Metacercaria of *L. constantiae* |
| 0.5 | 10/19 | 1/13 | 2/5 | 8/24 | 0/9 | 2/8 |
| 1 | 14/19 | 4/12 | 2/5 | 15/24 | 4/9 | 2/8 |
| 2 | 16/19 | 7/12 | 1/5 | 16/24 | 2/9 | 4/8 |
| 3 | 7/9 | 2/3 | 3/5 | 15/24 | 3/9 | 5/8 |
| 4 | 9/9 | 2/3 | 2/5 | 14/24 | 2/8 | N.O. |
| 24 | 12/17 | 10/12 | 3/5 | N.O. | N.O. | N.O. |
| Total | 68/92 | 26/55 | 13/30 | 68/120 | 11/44 | 13/32 |
| | (73.9) | (47.3) | (43.3) | (56.7) | (25.0) | (40.6) |

Numerator = number paired; denominator = total cultures examined; ( ) = percent paired; N.O. = no observations.
 Intraspecific pairing observations on *L. constantiae* metacercariae and adults showed pairing of 138/227 = 61% and 123/199 = 62%, respectively (Fried and Roberts, 1972, loc. cit.). Intraspecific pairing observations on immature and mature *E. revolutum* adults showed pairing of 363/584 = 62% and 435/858 = 51%, respectively (Fried et al., 1980, loc. cit.). Intraspecific pairing observations on mature *Z. lunata* adults showed pairing of 28/47 (60%) (present study).

nematodes in vitro has been studied (Roberts and Thorson, 1977, J. Parasitol. 63:764–766). Their study indicated that significant interspecific pairing of nematodes does occur in vitro, and that pairing may be associated with phenomena other than reproduction. The present study reports our observations on pairing between *E. revolutum* adults and other species of hermaphroditic digeans in vitro.

The in vitro attraction design used in this study was that of Fried and Roberts (1972, loc. cit.) and Roberts and Thorson (1977, loc. cit.) in which an agar substratum and a Locke's overlay were contained in a petri dish culture at 39°C on a slide warmer. Two worms, each of a different species, were placed 2 cm apart and the criterion for pairing was based on worms in contact or within 5 mm of each other from 0.5 to 24 hr later. Worms of each species were obtained as described previously: *E. revolutum* adults (Fried and Weaver, 1969, Proc. Helminthol. Soc. Wash. 36:153–155); *Zygocotyle lunata* adults (Fried, 1970, J. Parasitol. 56:44–47); *Leucochloridiomorpha constantiae* metacercariae and adults (Fried and Harris, 1971, J. Parasitol. 57:866–868). Immature adults of *Z. lunata* and *E. revolutum* were 7 days old, whereas mature flukes of both species were 14–17 days old. Mature adults of *L. constantiae* were 7–14 days old.

Previous observations on intraspecific pairing in *E. revolutum* and *L. constantiae* along with present observations on intraspecific pairing in *Z. lunata* are presented in a paragraph below Table 1. Results of interspecific pairing are presented in Table 1.

Observations on pairing from intraspecific studies range from 51 to 62% (avg. 59%). Results from the present study (Table 1) show that *E. revolutum* is capable of considerable interspecific pairing with other species of hermaphroditic digeans. The response between mature *E. revolutum* and *Z. lunata* adults was very marked, with more than 50% paired at 30 min, and total pairing of 73.9%.

Thin layer chromatographic (TLC) studies have indicated that free sterols are involved in intraspecific pairing of *L. constantiae* metacercariae and *E. revolutum* adults (Fried and Gioscia, 1976, J. Parasitol. 62:326–327; Fried et al., 1980, J. Parasitol. 66: loc. cit.). Further studies are needed to elucidate the role of free sterols as chemoattractants for interspecific pairing of digeans in vitro.

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*Research Note*

## Procercoid Development of *Isoglaridacris wisconsinensis* (Cestoda: Caryophyllaeidae)

Ten species comprise the genus *Isoglaridacris* (Williams, 1977, Proc. Helminthol. Soc. Wash. 44:91–95). This communication presents data about the experimental procercoid development of *I. wisconsinensis* Williams. The development of the nine other species is unknown.

Gravid *I. wisconsinensis*, obtained from *Hypentelium nigricans* (LeSueur) from the Red Cedar River, Barron Co., Wisconsin, were dissected to obtain eggs. Operculate eggs, after 21 days' embryonation, were placed in sterilized mud (silt-clay mixture), and laboratory-reared oligochaete annelids were exposed to this medium. After exposure for 48 hr, oligochaetes were transferred to sterilized mud and river water. All experiments were conducted at 19–23°C. Cestodes were preserved, without pressure, in cold 10% formalin. Measurements are expressed in micrometers.

Six oligochaete species were exposed to *I. wisconsinensis* eggs. The results were (number exposed: percent parasitized): (Tubificidae) *Limnodrilus hoffmeisteri* Claparède (150:0); *L. udekemianus* Ratzel (11:0); *Ilyodrilus templetoni* (Southern) (150:17); *Aulodrilus limnobius* Bretscher (160:4); (Naididae) *Dero digitata* (Muller) (86:0); and *Sylaria lacustris* (L.) (27:0). Incidence was determined at 20 days postexposure. In the three tubificid genera parasitized, procercoid



**Figure 1.** *Isoglaridacris wisconsinensis* from *L. hoffmeisteri* at 50 days. Scale = 33 micrometers.

development required 45 to 55 days. Fully developed procercoids (Fig. 1) averaged 1,170 (940–1,320) in body length with a cercomer length of 148 (72–183). Procercoids were located throughout the coelom.

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*Research Note*

# Occurrence of *Quadrigyrus torquatus* Van Cleave, 1920 (*Acanthocephala*) in North-central Colombia, South America

During June and July of 1975 and 1976, fish of the Magdalena River basin in Colombia, South America, were examined for acanthocephalans. A total of 16 species of fish were collected from the departments of Caldas and Bolivar. Worms were fixed in AFA and stained in Mayer's carmalum.

*Quadrigyrus torquatus* Van Cleave, 1920 was recovered from the intestines of two piscine species. No specimens were found in the mesenteries. One of three *Ageneiosus caucanus* taken from Rio Magdalena, vic. San Cristobal, Bolivar, contained three worms and four of seven *Hoplias malabaricus* taken from Quebrada Dona Juana, vic. La Dorada, Caldas, contained from one to six worms. *Ageneiosus caucanus* has not previously been reported as host for *Q. torquatus*. All specimens except one female from each host species were mature with females gravid. The two immature females were 7.70 mm and 6.98 mm in trunk length and each contained a single ovoid unfragmented ovary 132 $\mu$m long by 88 $\mu$m wide. Specimens correspond closely with previous descriptions (Van Cleave, 1920, Proc. U.S. Nat. Mus. 58:455–466; Diaz-Ungria and Gracia Rodrigo, 1957, Noved. Cient. Novedades Cientificas Serie Zoologia 223:1–19). Specimens were deposited in the USNM Helm. Coll., No. 75493.

*Quadrigyrus torquatus* displays an intriguing pattern of apparent discontiguous distribution. It is now known from two drainage basins in Colombia, the Rio Magdalena (present report) and the Orinoco (Schmidt and Hugghins, 1973, J. Parasitol. 59:829–835), two river basins isolated from each other by the northern extension of the Cordillera Oriental portion of the Andes Mountains. The type locality, Lake Valencia, Venezuela (Van Cleave, loc. cit.), is adjacent to the Orinoco basin. The parasite has also been collected from areas remote to these Colombian river basins, the Chagres River in the Panama Canal Zone (Thatcher and Nickol, 1972, Proc. Helminthol. Soc. Wash. 39:245–248), Surinam (Ortlepp, 1924, J. Helminthol. 2:15–40), and Brazil (Machado, 1941, Rev. Bras. Biol. 1:57–61).

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*Research Note*

## Endohelminth Parasites from Largemouth Bass, *Micropterus salmoides*, in Belton and Livingston Reservoirs, Central Texas

Although largemouth bass, *Micropterus salmoides* (Lacépède), are an important sportfish in Texas, few studies have been conducted on their endohelminth parasites in Texas lakes and reservoirs. Sparks (1951, Trans. Am. Microsc. Soc. 70:351–358) examined bass from 13 Texas counties, but did not designate the bodies of water where collections were made. In surveys of helminth parasites of Centrarchidae by Allison and McGraw (1967, Tex. J. Sci. 19:326–328) and McGraw and Allison (1967, Southwest. Nat. 12:332–334), fish were obtained from Lake Mexia, Springfield Lake, Belton Reservoir, and several rivers. It was not stated, however, which of the parasites reported were found in lakes and which had been recovered from rivers. Meade and Bedinger (1967, Southwest. Nat. 12:334–335) reported that four of five bass examined were infected with *Posthodiplostomum minimum* but did not indicate localities. In a 5-year study of helminths from freshwater fish in two eastern Texas counties, Meade and Bedinger (1972, Southwest. Nat. 16:281–295) reported five species of endohelminths from largemouth bass; however, the sites of collection were not given. Lawrence and Murphy (1967, Tex. J. Sci. 19:164–174) examined 17 *M. salmoides* from Benbrook Lake and recovered five species of endohelminth parasites while Underwood (1975, M.S. Thesis, Stephen F. Austin University, Nacogdoches, Texas) reported seven species in largemouth bass from Sam Rayburn Reservoir. Dronen et al. (1977, J. Parasitol. 63:282–284) described a new genus and species of digenetic trematode from Texas bass and reported that 80% of the bass examined from three Texas counties were infected with this parasite.

Because of the paucity of information on the parasites of largemouth bass in Texas lakes and reservoirs and the potential importance of these parasites to the

Table 1. Endohelminth parasites recovered from largemouth bass in Belton and Livingston reservoirs.

| Parasite | Belton Reservoir ($N = 129$) | | Livingston Reservoir ($N = 74$) | |
|---|---|---|---|---|
| | Percentage of infection | Average intensity | Percentage of infection | Average intensity |
| Digenea | | | | |
| *Caecincola parvulus* | 50 (42–59)* | 813.2 (1–11,533)† | 10 (5–21)* | 354.3 (5–1,735)† |
| *Clinostomum marginatum* | 3 (0–6) | 1.0 (1–1) | 38 (30–54) | 3.0 (1–10) |
| *Diplostomum* sp. | 2 (0–11) | 1.0 (1–1) | 12 (7–24) | 3.6 (1–12) |
| *Phyllodistomum lohrenzi* | 6 (3–14) | 3.5 (1–7) | — | — |
| *P. pearsei* | 13 (10–26) | 15.4 (1–79) | 7 (3–17) | 2.8 (1–6) |
| *Pisciamphistoma* sp. | 2 (0–4) | 1.0 (1–1) | — | — |
| *Posthodiplostomum minimum* | 29 (21–37) | 16.9 (1–99) | 78 (70–88) | 79.8 (1–877) |
| Cestoda | | | | |
| *Proteocephalus ambloplitis* adults | 2 (0–4) | 9.0 (2–16) | — | — |
| *P. ambloplitis* plerocercoids | 15 (9–21) | 2.6 (1–7) | 37 (29–53) | 5.0 (1–24) |
| Nematoda | | | | |
| *Camallanus oxycephalus* | 65 (57–73) | 5.0 (1–63) | 32 (25–48) | 2.4 (1–13) |
| *Contracaecum* sp. | 50 (41–58) | 2.8 (1–11) | 72 (61–83) | 9.7 (1–39) |
| *Philometra nodulosa* | — | — | 8 (4–18) | 8.0 (5–12) |
| *Spinitectus carolini* | 43 (34–51) | 3.3 (1–23) | 45 (38–61) | 6.9 (1–33) |
| Acanthocephala | | | | |
| *Neoechinorhynchus cylindratus* | 85 (79–92) | 30.3 (1–110) | 42 (35–58) | 8.4 (1–69) |

* Values in parentheses represent 95% confidence intervals for percentages.
† Values in parentheses represent the range of intensity observed.

sportfishing industry, a survey was conducted on the endohelminth parasites of largemouth bass in Belton and Livingston reservoirs.

Seventy-four largemouth bass were obtained from sport fishermen at Livingston Reservoir, while 129 bass were collected at Belton Reservoir from sport fishermen and cove rotenone surveys conducted by the Texas Parks and Wildlife Department. All fish were collected between 13 February and 15 September 1976. Bass were placed in individual plastic bags and transported on ice to Texas A&M University. The mouth and visceral organs were examined for endohelminth parasites while gill surfaces, fins, skin, body musculature, and eyes were examined for metacercariae.

The parasites recovered are listed in Table 1. Of the 16 species previously reported to infect largemouth bass in Texas, only *Crepidostomum cooperi, Textrema hopkinsi,* and *Bothriocephalus* sp. were not recovered from the bass in this survey. Although a species of *Pisciamphistoma* was found, the few specimens recovered were damaged and could not be identified as to species.

*Crepidostomum cooperi, Pisciamphistoma reynoldsi,* and *P. stunkardi* are common parasites of sunfish (*Lepomis* spp.) in Texas, but have been reported from Texas bass (*Micropterus* spp.) only infrequently (Allison and McGraw, 1967, Tex. J. Sci. 19:326–328; McGraw and Allison, 1967, Southwest. Nat. 12:332–334; Sparks, 1951, Trans. Am. Microsc. Soc. 70:351–358). The low infection level of bass by these flukes in areas where these parasites are common in other fish suggests that bass may not typically serve as the primary host. This may be due to the fact that the invertebrate secondary hosts for these flukes are

probably not regular food items in the diet of the size of bass which are usually collected in surveys (Clady, 1976, Am. Midl. Nat. 91:453–459). We speculate that *Textrema hopkinsi* was absent from this survey because the probable molluscan host (*Amnicola* spp.) is not commonly found in reservoirs. *Bothriocephalus* sp. has only been reported from Sam Rayburn Reservoir in Texas and is probably not an important parasite of bass in this state.

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*Research Note*

# Helminths of Mariculture-reared Green Turtles (*Chelonia mydas mydas*) from Grand Cayman, British West Indies

Ten mariculture-reared green turtles (4 to 4.5 years of age) were examined at necropsy during December 1978 at the Cayman Turtle Farm, Grand Cayman, British West Indies. These were selected at random from a holding tank containing several hundred turtles and included eight males weighing from 45 to 147 lb ($\bar{x} = 83$ lb) and two females (both 105 lb). Each of the following organs was placed into an individual container in which it was either macerated or cut open. Spleens, gonads, kidneys, and livers were macerated; urinary and gall bladders, the airways of the lungs and tracheae, the major vessels attached to the heart, and the heart chambers were cut open. The gastrointestinal tract was first subdivided into esophagus/stomach, small intestine, and large intestine and then each section was slit lengthwise. The surfaces of the organs cut open were examined visually and then washed through a series of 50- and 100-mesh screens. The remaining washed material from each organ was then examined for helminths under a dissecting microscope. All helminths recovered were relaxed in cold tap water, fixed in AFA and stained with Harris' hematoxylin. Kidney tissues and the proximal portions of the aortic trunk were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 7 $\mu$m and stained with hematoxylin and eosin.

Four of the 10 turtles harbored digenetic flukes. A single specimen of *Pleurogonius* sp. (Pronocephalidae) was collected from the small intestine of one turtle. This genus contains 13 species that have been described or reported from *C. mydas* and our specimen most closely resembles *P. mehrai* Ruiz 1946. Previous reports of *P. mehrai* include those from green turtles in Trinidad (Gupta, 1962, Can. J. Zool. 39:293–298) and India (Mehra, 1939, Proc. Natl. Acad. Sci. India
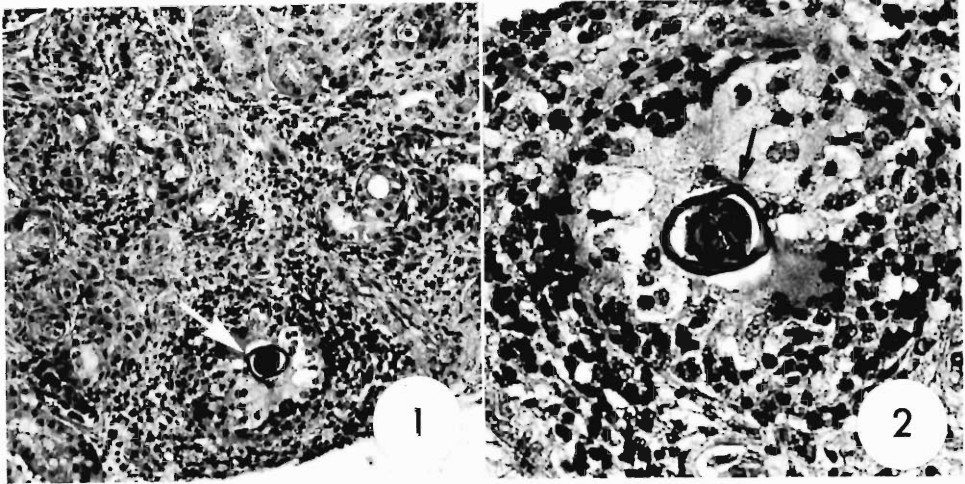
Figures 1, 2.   Pathology associated with apparent *Learedius learedi* eggs in green turtles. 1. Kidney tissue showing inflammatory reaction to the presence of *L. learedi* egg (at tip of arrow) (×140). 2. Section of *L. learedi* egg (at tip of arrow) in kidney tissue (×370).

9:99–130). The other fluke recovered was *Learedius learedi* Price 1934 (Spirorchiidae) reported previously from a green turtle in the U.S. National Zoo. It has also been found in *C. mydas* in Panama (Cabellero et al., 1955, An. Inst. Biol. Nac. Univ. Mex. 26:149–191) and in Florida (Nigrelli, 1941, J. Parasitol. 27(suppl.):15–16). Twenty-five (three to 15 per host) specimens of *L. learedi* were present in the heart and (or) associated major vessels of four of 10 turtles necropsied. Since the heart and associated major vessels were treated as one entity in the initial examinations, hearts and the major vessels from 10 additional turtles were examined after separating the vessels from the heart. All 10 of these were infected with *L. learedi*, ranging from one to 49 ($\bar{x}$ = 14) flukes per host. A total of 141 flukes was collected, of which 12 were found in the vessels and 129 in the chambers of the heart. Upon histopathological evaluation, in one animal there was found a focus of increased cellularity in the endothelial-intimal layer of the aorta comprised mostly of mononuclear cells. Whether or not this lesion was associated with fluke attachment is unknown.

Due to the location of the adult *L. learedi* in the heart and associated major vessels, its eggs probably would be found in a variety of tissues. Since the eggs must first leave the circulatory system and then eventually leave the host, organs such as the lung, liver, and kidney would be the most accessible for completion of the cycle. The eggs of *L. learedi* are morphologically similar but smaller than those of a related fluke, *Hapalotrema constrictum*, reported by Smith and Coates (1939, Zoologica [N.Y.] 24:379–383) in association with fibroepithelial tumors on green turtles in Florida waters. Their diagnosis of *Hapalotrema* was based only on eggs; adults were not found. *Hapalotrema* adults have been reported from *C. mydas* in India, but have also been reported from the Atlantic loggerhead (*Caretta caretta*) in Florida waters, and it is probable that other marine turtles are hosts for these flukes as well.

Kidneys examined showed a range in lesions from a mild multifocal interstitial

infiltrate of mononuclear cells to a much more severe chronic inflammatory reaction consisting of tubular and glomerular necrosis with diffuse interstitial infiltrate of mononuclear cells and fibrosis (Fig. 1). Tissues from several green turtles had what appeared to be trematode eggs surrounded by discrete chronic granulomatous reactions consisting of epithelial cells, multinucleated giant cells, and mononuclear cells. These eggs were seen as oblong homogenous yellow capsules surrounding a central area of undifferentiated cells measuring 43 $\mu$m by 34 $\mu$m (Fig. 2). The eggs of other spirorchiids which occur in freshwater turtles are known to cause similar tissue damage (Goodchild and Dennis, 1967, J. Parasitol. 53:38–45; Halliman et al., 1971, J. Parasitol. 57:71–77).

Since these turtles had been in the same holding tank for the past 18 months, it was probable that they had acquired the flukes during this period, suggesting infected snails and (or) cercariae were entering the tank with the incoming seawater. The tank was examined for the presence of potential intermediate hosts. Three species of snails were found on the bottom of the tank and were identified as *Nodolittorina muricatus*, *Strombus gallus*, and *Diodora listeri*. Although several living representatives of these snails were placed in seawater in containers overnight, no cercariae emerged.

Representative specimens of the flukes have been deposited in the U.S. National Parasite Collection, Beltsville, Maryland (USNM Nos. 75099 and 75100) and in the Harold W. Manter Laboratory of Parasitology, University of Nebraska State Museum, Lincoln, Nebraska (No. 20909).

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*Research Note*

# Helminths of the Ring-Necked Pheasant, *Phasianus colchicus* (Gmelin) (Phasianidae), from the Texas Panhandle

The ring-necked pheasant, *Phasianus colchicus* (Gmelin), was first established in the Texas Panhandle about 1940 (Jones and Felts, 1950, Texas Game and Fish 8:4–7). The first hunting season was established in 1958 (Guthery et al., 1980, U.S. For. Serv. Gen. Tech. Rep., in press), and this species has since become a primary resident gamebird of the region. Because of the complete dearth of information on the helminth fauna from pheasants in the Southwest, the present study was initiated.

Table 1.  Helminths of pheasants from the Texas Panhandle.

| | Prevalence | | Intensity | |
| --- | --- | --- | --- | --- |
| | No. infected/<br>No. examined | % | Range | Mean |
| *Heterakis gallinarum* | 3/78 | 3.8 | 1–2 | 1.5 |
| *Oxyspirura petrowi* | 1/30 | 3.3 | 2 | 2.0 |
| *Choanotaenea infundibulum* | 3/78 | 3.8 | 1 | 1.0 |
| *Echinoparyphium recurvatum* | 1/78 | 1.3 | 1 | 1.0 |

Viscera of 78 specimens of *P. colchicus* were collected from hunter-killed cocks in Dallam, Castro, Hale, and Floyd counties, Texas, during December 1978. Heads of 30 specimens were also examined. Specimens were frozen, later necropsied, and examined for helminths. Nematodes were briefly fixed in glacial acetic acid, stored in a mixture of 70% ethyl alcohol with 5% glycerin, and examined in glycerin wet mounts. Cestodes and trematodes were stained in Celestine blue B and Semicohn's acetic carmine, respectively, and mounted in Canada balsam. Simpson's index of diversity (Holmes and Podesta, 1968, Can. J. Zool. 46:1193–1204) was computed to indicate the concentration of dominance of helminth faunas in this and previous studies. An index of similarity (Holmes and Podesta, 1968, loc. cit.) was used to compare helminth faunas of pheasants from different geographic regions. Representative specimens of helminth species recovered in this study are deposited in the Medical Zoology Collection, The Museum of Texas Tech University, TTUS-MZ Nos. 12931–12937, and in the USNM Helm. Coll., Nos. 75403–75405.

One trematode, one cestode, and two nematode species were recovered (Table 1). Seven of 78 (9%) birds examined were infected with helminths. No individual host harbored more than one helminth species and worm burdens were very light with not more than two parasite specimens in a single host. There were no new host records established for helminths recovered from pheasants in the Texas Panhandle.

The eyeworm, *Oxyspirura petrowi* Skrjabin, 1929 from West Texas pheasants, quail, and prairie chickens has been previously documented (Pence and Sell, 1979, Proc. Helminthol. Soc. Wash. 46:146–149). Although McClure (1941, J. Wildl. Manage. 13:394–397) found a prevalence of at least 40% of Nebraska pheasants infected with this species, it appears to be more commonly a parasite of quail and prairie chickens in West Texas.

*Heterakis gallinarum* (Schrank, 1788) appears to be the most common helminth, in terms of both prevalence and intensity of infection, in the pheasant. It has been reported in this host from Illinois (Leigh, 1940, Ill. Nat. Hist. Surv. Bull. 21:185–194), Minnesota (Olsen, 1938, J. Parasitol. 24[suppl.]:24–25), New York (Cheatum, 1952, Disease and Parasite Investigations, Pitmann, Robertson Proj. 1-R, Suppl. E, N.Y. Conser. Dept. 75 pp.), South Dakota (Gilbertson and Huggins, 1964, J. Wildl. Manage. 28:543–546), Nebraska (Greiner, 1972, J. Wildl. Dis. 8:203–206), and Oregon (Morgan, 1939, A Survey of Internal Parasites of Poultry and Gamebirds in Oregon, M.S. Thesis, Oregon State University, Corvallis, 89 pp.).

The cestode *Choanotaenea infundibulum* (Bloch, 1779) is reported from pheas-

| Texas | Nebraska | South Dakota | New York | Minnesota | |
|---|---|---|---|---|---|
| | | | | - | Minnesota |
| | | | - | 81 | New York |
| | | - | 85 | 93 | South Dakota |
| | - | 56 | 42 | 56 | Nebraska |
| - | 89 | 45 | 31 | 45 | Texas |

Figure 1. Trellis diagram of indexes of similarity of helminth faunas of pheasants from different regions in North America.

ants in Minnesota (Olsen, 1938, loc. cit.), South Dakota (Gilbertson and Huggins, 1964, loc. cit.), and Nebraska (Greiner, 1972, loc. cit.). This is the most frequently reported cestode from this host in North America.

*Echinoparyphium recurvatum* (Linstow, 1873) was recovered from pheasants in Minnesota (Olsen, 1938, loc. cit.) and Nebraska (Greiner, 1972, loc. cit.). This is normally a cecal fluke of chickens, ducks, and geese (Becklund, 1964, Am. J. Vet. Res. 25:1138–1416) and reported levels of infection in pheasants are very low.

Simpson's index was 0.28 indicating a low concentration of dominance of helminth species in pheasants from western Texas. In contrast, this index for pheasants from Minnesota, South Dakota, Nebraska, and New York was 0.67, 0.74, 0.48, and 0.95, respectively. The low concentration of dominance of particular helminth species in the Texas pheasants is probably a reflection of (1) the low frequency of occurrence and infection levels and (2) lower frequency of occurrence of *H. gallinarum* in this area in contrast to other regions.

Data from previous studies on the helminth faunas of pheasants in Minnesota, New York, Nebraska, and South Dakota and from the present study were used in a similarity index for comparing helminth faunas from different regions in North America. These indexes were arranged in a trellis diagram (Fig. 1). The highest values between compared areas were Minnesota with New York and South Dakota, South Dakota with New York, and Texas with Nebraska. High values for similarity indexes between the northern tier of states indicate similar helminth faunas, while corresponding low values between these states with Texas and Nebraska indicate variation in helminth composition in the more southern and western portions of this host's range. The high index of similarity between Texas and Nebraska pheasant helminth faunas represents a similar helminth composition, undoubtedly resulting in part from similarities in climate and topography of the two areas. A striking difference, however, in the helminth faunas of Texas and Nebraska as well as all other areas previously examined, is the much lower prevalence of worm burdens in Texas pheasants.

Previous studies in South Dakota (Gilbertson and Huggins, 1964, loc. cit.) and New York (Cheatum, 1952, loc. cit.) indicate adult pheasants are more frequently parasitized than juveniles. Although the incidence of *H. gallinarum* appears independent of season in these studies, *C. infundibulum* was conspicuously absent in South Dakota pheasants collected during winter. Although the influence of sex, age, and season could not be determined, the ring-necked pheasant from the Texas Panhandle appears to have a very low prevalence rate and worm burdens of helminths based on data from fall-harvested cocks. This helminth fauna has a low concentration of dominance and is most similar in composition to that of Nebraska pheasants. Consequently, helminth parasitism is regarded as having little if any effect on the morbidity or mortality of this gamebird in western Texas.

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*Research Note*

## New Records of Leeches (Annelida: Hirudinea) from the Shortnose Sturgeon (*Acipenser brevirostrum*) in the Connecticut River

During a study on the biology of the shortnose sturgeon (*Acipenser brevirostrum*) in the Connecticut River carried out by the junior author, leeches removed from captured fish were retained for study. A review of the literature revealed a record of *Placobdella montifera* on the shovelnose sturgeon (*Scaphirhynchus platorynchus*) (Pearse, 1924, Trans. Wis. Acad. Sci. Arts Lett. 21:161–194) and *Piscicola geometra* occurring on the Asian sturgeon (*A. güldenstadti*) (Dubinin, 1952, *in* Astakhova, 1974, J. Ichthyol. [USSR] 14:788–792). No leech records for *A. brevirostrum* have been published.

Sturgeon were sampled with gill nets for 28 months (April 1976 to July 1978) throughout the Connecticut River from Enfield (Hartford Co.), Connecticut, to Montague (Franklin Co.), Massachusetts. Twenty-three of 356 sturgeon examined

Table 1. Numbers of host fish and attachment sites of collected leeches.

| Species | Number of leech specimens collected | Number of fish with leeches | Location on fish |
|---|---|---|---|
| Glossiphoniidae | | | |
| *Actinobdella inequiannulata* | 1 | 1 | venter |
| *Batracobdella phalera* | 1 | 1 | venter |
| Piscicolidae | | | |
| *Myzobdella lugubris* (=*Illinobdella moorei*) | 1 | 1 | isthmus |
| *Calliobdella vivida* (=*Cystobranchus vividus*) | 31 | 15 | venter and maxillary furrow |
| *Piscicola milneri* | 3 | 1 | pectoral fin |
| *Piscicola punctata* | 5 | 4 | barbels, isthmus, pelvic fin, and maxillary furrow |

had leeches. All preserved specimens of leeches have been deposited into the invertebrate collections of the Museum of Zoology, University of Massachusetts at Amherst.

Table 1 presents distribution data for all specimens of leeches found on sturgeon in this study. Among the species collected, only two, *Calliobdella vivida* and *Piscicola punctata*, may be considered to be usual parasites of the shortnose sturgeon. The other species collected, particularly those represented by only a single specimen, may have been accidental occurrences.

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*Research Note*

# Prevalence of *Toxoplasma* Antibodies in Humans and Various Animals in the Amazon

Worldwide, man and numerous species of animals have been found to be naturally infected with *Toxoplasma gondii*. Domestic and wild felids are considered to be the key to the epidemiology of toxoplasmosis because they are the only animals known to excrete oocysts in their feces (Hutchison et al., 1970, Brit. Med. J. 1:142–144; Frenkel et al., 1970, Science 167:893–896). Animals other

than felids, particularly mammals and birds, serve only as intermediate hosts and become infected with asexual forms by ingesting infected meat or oocysts of *T. gondii*. In response to infection, felids and other hosts form antibodies against *T. gondii* which may be detected by various immunological tests. Such tests give a good indication of the prevalence of *T. gondii* in human or animal populations.

There are only a few reports of the prevalence of *T. gondii* in animals and humans in the Amazon River Basin (Baruzzi, 1970, Rev. Inst. Med. Trop. São Paulo 12:93–104; Lamb and Feldman, 1968, Am. J. Epidemiol. 87:323–328; Walls and Kagan, 1967, Am. J. Epidemiol. 86:305–313). These studies were conducted in the eastern and east-central regions of the Amazon. The present study was undertaken to determine the prevalence of *Toxoplasma* infections in humans and various animals living in the central and northern regions of the Amazon. These regions included (1) people and various wild and domestic animals living near Manaus, Amazonas, Brazil and (2) the Sanomā Indians living in a relatively isolated area in the territory of Roraima, Brazil.

Domestic and wild animals were captured near Manaus and their sera examined for the presence of *T. gondii* antibodies. These animals, most of which were adults, included the following: domestic cats (*Felis catus*), cattle (*Bos taurus*), chickens (*Gallus domesticus*), domestic dogs (*Canis familiaris*), ducks (*Cairina moschata*), opossums (*Didelphis marsupialis* and *Marmosa cinerea*), ocelots (*Felis pardalis*), squirrel monkeys (*Saimiri sciureus*), and spiny rats (*Proechimys goyanensis riparum*). Immediately after capture, blood samples were obtained from these animals by heart or venous puncture. Blood samples were also obtained from 51 adult humans from Manaus, and 51 adults of the Sanomā Indian tribe living in a remote area in Roraima, Brazil.

By using reagents obtained from the Eiken Chemical Co. (Katsushita, Tokyo, Japan), the indirect hemagglutination (IHA) test was used to detect antibodies against *T. gondii* (Jacobs and Lunde, 1957, J. Parasitol. 43:308–314; Fujita et al., 1969, Am. J. Trop. Med. Hyg. 18:892–901). Sera obtained from humans or animals were inactivated at 56°C for 30 min, adsorbed with sheep erythrocytes, and then tested for the presence of *T. gondii* antibodies. Sera were considered positive when agglutination occurred at dilutions of 1:128 or greater.

Although the Sabin-Feldman dye test is generally considered to be a more reliable test for *Toxoplasma* antibodies than the IHA test, in remote regions of the world such as the Amazon, the IHA is easier to perform and has been accepted for the diagnosis of *Toxoplasma* in man on the basis of comparisons with the dye test (National Communicable Disease Center, Vet. Public Health Notes, July 1967, HEW, USPHS, Atlanta, Ga.).

In the present study, percentages of positive titers to *Toxoplasma* varied considerably among domestic and wild animals (Table 1). The lowest percentages occurred in cattle (12%), chickens (24%), and ducks (27%); intermediate percentages occurred in dogs (63%), ocelots (50%), opossums (55%), spiny rats (55%), and squirrel monkeys (49%); the highest percentage occurred in domestic cats (81%). The prevalence of *Toxoplasma* antibodies found in cats (81%) in the present study is one of the highest ever recorded. Surveys of *Toxoplasma* antibodies in cats throughout the world have reported prevalences that range from about 5 to 85% (for reviews see Dubey, 1968, Vet. Bull. 38:495–499; Work, 1971, Acta Pathol. Microbiol. Scand., Sect. B, Suppl. 221). Previous serologic surveys

Table 1. Prevalence of positive reactions of sera obtained from humans and various animals in the Amazon River Basin to *Toxoplasma gondii* as determined by the indirect hemagglutination test (IHA).

|  | Number examined | Number with positive IHA (%) |
|---|---|---|
| Human |  |  |
| Manaus | 51 | 30 (59) |
| Roraima | 51 | 28 (54) |
| Cattle | 25 | 3 (12) |
| Chicken | 17 | 3 (24) |
| Domestic cat | 32 | 26 (81) |
| Domestic dog | 19 | 12 (63) |
| Duck | 15 | 4 (27) |
| Ocelot | 4 | 2 (50) |
| Opossum | 33 | 18 (55) |
| Spiny rat | 18 | 8 (44) |
| Squirrel monkey | 49 | 24 (49) |

in Brazil have found that 1.8% (Nery-Guimaraes et al., 1971, Mem. Inst. Oswaldo Cruz 96:97–111) to 60% (Sogorb et al., 1972, Rev. Inst. Med. Trop. São Paulo 14:314–320) of various species of monkeys had antibodies to *Toxoplasma*. Such a wide range in positive sera may be due to differences in habitat and behavior among the various species of monkeys surveyed. However, in the present study, a relatively high percentage (49%) of squirrel monkeys was positive for *Toxoplasma*. Such a finding indicates that even though these monkeys are arboreal and eat primarily fruit, they must still have frequent contact with *Toxoplasma*-infected cat feces.

The Sanomã Indian tribe is not nomadic and lives in a relatively isolated area of Roraima, Brazil, in which there are a few ocelots but no domestic felids. They eat primarily cassava and fruits, and rarely meat. Thus, it was surprising to find that 54% of the Sanomã Indians examined in the present study had antibodies to *Toxoplasma* (Table 1). Thus, even though ocelots are rare in this area of Brazil they must somehow provide sufficient fecal contamination of the area with *Toxoplasma* oocysts in order to account for the relatively moderate infection rate in the Sanomã Indians. People surveyed in the city of Manaus (Table 1), who have contact with domestic cats, had a similar incidence (59%) of *Toxoplasma*-positive sera. In comparison, Wallace et al. (1974, Am. J. Trop. Med. Hyg. 23:8–14) found in New Guinea a low prevalence (2%) of sera positive for *Toxoplasma* in one Indian population which had no contact with wild or domestic cats and a much higher prevalence (14–34%) in another Indian population that had contact with felids. They also reported a prevalence of 50% in a Colombian Indian tribe that hunts and eats wild felids.

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# Theodor Curt Von Brand
## (1899–1978)

An illustrious career which spanned two continents came to an end on July 18, 1978, with the death of Theodor Curt Von Brand in Bethesda, Maryland. He was born in Ortenberg Castle in the Black Forest, the home of his maternal grandfather, on September 22, 1899, the son of Baron Phillip Paul and Diane (von Hirch) Von Brand. His early education was provided in part by private tutors and in part by public schools. He served in the German Army in 1917–1918 on the Russian front. Following the Armistice, Von Brand entered the University of Munich where he took his Ph.D. degree in 1922. On December 20, 1923, he married Margarethe Brandeis, who survives him together with a son and three grandchildren. He continued his education at the University of Erlangen, where he received the M.D. degree in 1928.

Dr. Von Brand was a faculty member of the University of Erlangen from 1923 to 1930, following which he joined the staff of the Institute für Schiffs-und-Tropenkrankheiten in Hamburg, as an assistant to Prof. Edward Reichenow. Because of his strong anti-Nazi feelings, he left Germany in 1933 for a fellowship at the University of Copenhagen. In 1936, he came to the United States under the sponsorship of Dr. W. W. Cort of the School of Hygiene and Public Health, Johns Hopkins University. Later, he was professor of Biology at Barat College, Lake Forest, Illinois. From 1940 to 1947, he was a member of the faculty of Catholic University, Washington, D.C. After serving as a visiting scientist for two summers, in 1947 Dr. Von Brand became a staff member of the Laboratory of Tropical Diseases, National Institute of Allergy and Infectious Diseases (then the Microbiological Institute), National Institutes of Health, where he headed the Section on Physiology and Biochemistry. He retired in 1969. Dr. Von Brand was President of the Helminthological Society of Washington in 1945.

Dr. Von Brand exerted a profound influence on the development of the science of parasite physiology both in Germany and the United States. In his Presidential address before the American Society of Parasitologists in 1969, he pointed out that 22 percent of the papers at that meeting dealt with the physiology of parasites, whereas prior to World War II less than one percent were concerned with that topic.

Dr. Von Brand never lacked for ideas. In fact, his research efforts, in spite of great accomplishments, never caught up with all of the objectives which he visualized. He was an inspiring leader to his assistants and the foreign scientists who came to his laboratory at various times. His research covered a wide field. In Erlangen, he was interested in the metabolism of *Fasciola hepatica* and *Monezia expansa* and studied the chemical composition of the snail, *Helix pomatia*. The summers of 1923 and 1924 were spent at the Biological Station in Helgoland where he studied the chemistry of free-living marine worms. These studies were continued at the Zoological Station at Naples in 1925 and 1926.

His interest in the physiology of the parasitic protozoa began at Hamburg and continued with his work on the trypanosomes until his retirement. His early interest in tapeworm physiology was renewed so that he made outstanding con-

tributions to the role of the calcareous corpuscles in these parasites. In addition to publishing some 200 papers, he was the author of six books. After his retirement, he produced his "Parasitenphysiologie" and shortly before his death he completed revision of his "Biochemistry of Parasites." He was an extremely facile writer and his manuscripts, once completed, seldom needed revision.

In 1969, Dr. Von Brand received the Superior Service Award of HEW and in 1978 he was the recipient of the Gold Medal of the Robert Koch Foundation.

It is difficult to appraise precisely the influence and multiple accomplishments of an unusually gifted scholar such at Theodor Von Brand. One must say, however, that he was the genesis, both in Europe and America, of the field of the physiology and biochemistry of protozoan and metazoan parasites, not to mention his early contributions to the metabolism of some free-living marine animals.

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# PRESENTATION

## 1979 HelmSoc Anniversary Award
## Dr. Everett Elmer Wehr

Mr. President, Guests, and Members of the Helminthological Society of Washington:

It is my pleasure to be here tonight to honor a true gentleman and tireless scientist who has made many significant contributions to the field of parasitology, Dr. Everett Elmer Wehr.

Dr. Wehr was born in Cozad, Nebraska, on November 5, 1895, and while at the tender age of 5, moved in 1901 with his parents to Star, Idaho where he spent his childhood. While pursuing a B.S. degree at the University of Idaho, he met his future wife, Ceola. After graduation in 1918, he spent two years travelling along the east coast working as a Special Field Agent for the Bureau of Entomology, Division of Insects Affecting Man and Animals, of the U.S.D.A. In 1920, he went to the Berkeley campus of the University of California where he earned an M.S. degree in 1921. He then married and moved to Lincoln, Nebraska, and accepted a teaching position as an Instructor in Entomology at the University of Nebraska. Another move in 1924 brought him to the University of Illinois and the laboratory of Dr. H. B. Ward where he worked as an Assistant in Zoology until 1928. It is here that he began his studies in avian parasitology and became a charter member of the American Society of Parasitologists. Accepting a position as a biologist at the U.S. Range Livestock Experiment Station in Miles City, Montana, Dr. Wehr worked for three years on not only horse helminths and arthropods but also western game-bird parasites. By sending notes to be read at meetings, Dr. Wehr began his association with the Helminthological Society of Washington as a corresponding member. After transfer to Washington in 1931, he worked, first at the Agriculture Building downtown and later at Beltsville until his retirement in 1965. He received his Ph.D. from the George Washington University in 1935. The subject of his dissertation was ''A Monograph of the Filarioidea and Dracunculoidea (Nematoda) of Birds,'' and was directed by Dr. Paul Bartsch.

During his active years as a scientist, Dr. Wehr authored or co-authored over 130 papers. He quickly became known as an outstanding classical avian helminthologist with expertise in the areas of systematics, morphology, life histories, and control of nematodes and cestodes from domestic and wild birds. He also proved to be an excellent epidemiologist with a sense for following up epidemics among birds and being able to gain a broad perspective on the problem. The dedication and drive of Dr. Wehr as a parasitologist can be attested to by his many outstanding contributions to our understanding of the biology and pathology of *Ascaridia, Syngamus, Capillaria,* and other nematodes of game and domestic birds. He was also continually testing new anthelminthics to control these helminths. His interests and work in parasitology also included many studies on the control of protozoans, particularly coccidia in poultry. Furthermore, he demon-

**Everett Elmer Wehr**

strated the life history and pathogenicity of *Gastrophilus intestinalis*, the horse bot fly.

To those working in his laboratory, he proved to be an effective teacher. He was patient, considerate, unassuming, yet thorough with those trying to learn from his expertise and experience. He worked well with young people by listening to them and aiding them in developing their ideas. He taught by example as well by doing his share and more of the work.

Dr. Wehr is a genuine naturalist with many interests beyond parasitology. His knowledge about living things, especially insects and botanical subjects, is known to many of you. He developed into an avid gardener, growing vegetables and flowers, especially chrysanthemums. He is one of the founders of the Takoma Park Horticultural Society and participated in many flower shows and sales where his plants usually won a prize.

Dr. Wehr has served the Helminthological Society of Washington in a variety of capacities. Among the more prominent are: the Corresponding Secretary-Treasurer in 1934; the first and fifth Vice President of the Society in 1935 and 1939; and President in 1940.

In characterizing Dr. Wehr as a person, several of his colleagues with whom I corresponded consider him with the highest regard, a quiet, kind, dedicated worker and a first-class gentleman.

I think it is clear that Dr. Wehr is an outstanding parasitologist and deserving of the 1979 Anniversary Award of the Helminthological Society of Washington. On behalf of the Society's membership and the members of the Awards Com-

mittee (Dr. Louis Diamond and Dr. Kendall Powers), I am pleased to present this token of our esteem. Unfortunately, due to the frail nature of his health, Dr. Wehr is unable to be here tonight to accept his award. Accepting on his behalf, is his friend and colleague, Dr. John Andrews.—SHERMAN S. HENDRIX, Chairman, Awards Committee

### Remarks made in accepting (on behalf of Mrs. Wehr) The Helminthological Society of Washington's Parasitologist of the Year Award for 1979 presented to Dr. Everett Elmer Wehr at its 525th Meeting. October 12, 1979

On behalf of Mrs. Wehr, it is a great honor and privilege for me to accept the Helminthological Society of Washington's Anniversary Parasitologist of the Year Award, for the 1979 recipient, Dr. Everett E. Wehr.

Having known Dr. Wehr from the day he first arrived at the Zoological Division, I would like to add a few remarks to what has been said. He was always ready to help his co-workers, whether with transportation or providing a listening ear and sounding board for projects. He was always a gentleman in his dealings and was admired as a scientist and respected for his points of view.

His interest in horticulture was centered about his expertise in raising chrysanthemums.

When he retired he was missed for his vast knowledge of parasitology, his wise counsel, and his friendly personality.

For Mrs. Wehr, I thank the Society for this signal honor you have bestowed on her husand.—JOHN S. ANDREWS, Ret. USDA Visiting Scientist API

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Goldberg, A.
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Haley, A. J.
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Hood, Martha Walker
Isenstein, R. S.
Jachowski, L. A., Jr.
Jacobs, L.
Johnson, Anthony J.
Jones, A. W.
Kates, K. C.
Kinnamon, Kenneth E.
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Loizeaux, Peter S.
Lunde, M. N.
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# CONTENTS

*(Continued from Front Cover)*