

## PROCEEDINGS

of

# The Helminthological Society of Washington

A semiannual journal of research devoted to  
Helminthology and all branches of Parasitology

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## ***Polypocephalus* sp. (Cestoda; Lecanicephalidae): A Description of Tentaculo-Plerocercoids from Bay Scallops of the Northeastern Gulf of Mexico**

EDWIN W. CAKE, JR.

Gulf Coast Research Laboratory, Ocean Springs, Mississippi 39564

**ABSTRACT:** Tentaculo-plerocercoids of *Polypocephalus* Braun are described from digestive gland tissues of the Atlantic bay scallop, *Argopecten irradians concentricus* (Say), from the northeastern Gulf of Mexico. Forty-two of 55 scallops (76%) collected from four Florida bays adjacent to the Apalachicola River delta exhibited a mean infection intensity of  $18.5 \pm 5.5$  plerocercoids/scallop. Bay scallops appear to be intermediate hosts in the life cycle of this cestode.

Nine species of *Polypocephalus* Braun (Cestoda; Lecanicephalidae) are known worldwide (Subhadrappa, 1951). Although there are no published reports of *Polypocephalus* adults in elasmobranchs from the Gulf of Mexico, their presence has been confirmed (Tom Mattis, Gulf Coast Research Laboratory Parasitology Section, personal communication). I reported tentaculo-plerocercoids of *Polypocephalus* sp. from digestive gland tissues of the Atlantic bay scallop, *Argopecten irradians concentricus* (Say) (Mollusca; Pectinidae), at St. Teresa Beach, Florida (Cake, 1972). Six additional species of larval cestodes infect bay scallops in the eastern Gulf (Cake, 1976, 1977) including three tetraphyllideans, two trypanorhynchans, and one other lecanicephaloidean, *Tylocephalum* sp. *sensu* Burton (1963).

This report, which presents descriptive and infection data and proposes life cycle pathways, was derived, in part, from my doctoral research on larval cestodes of coastal, benthic mollusks of the eastern Gulf of Mexico (Cake, 1975).

### **Materials and Methods**

Bay scallops were collected while skin diving over marine grassflats in shallow coastal bays adjacent to the Apalachicola River delta in the northeastern Gulf of Mexico. The stomach, intestine, digestive gland, digestive diverticula, and mesenteries of each scallop were examined with the aid of a stereo-zoom, dissection microscope. After the initial examination, all visceral tissues were held in petri dishes containing filtered seawater (ca. 30‰) and permitted to putrefy. Complete recovery of the plerocercoids required partial decomposition of visceral tissues and examination of the supernatant saline. Examinations were repeated every few hours until no plerocercoids were recovered during two or more consecutive examinations.

Excysted plerocercoids were counted and either fixed, preserved, and stained using standard helminthological techniques, or maintained in glucose-enriched, artificial elasmobranch saline (vide Read et al., 1960; Hamilton and Byram, 1974) for further study. Plerocercoids were stained with Ehrlich's acid hematoxylin and mounted in Permount. Illustrations were made with the aid of a microprojector and measurements were made with an ocular micrometer. Descriptive measurements are given in micrometers and include the mean  $\pm$  the standard error, the range, and the total number of structures measured.

I have chosen to follow the taxonomy of *Polypocephalus* proposed by Southwell (1930) who considered Lecanicephaloidea separate from Tetraphyllidea, and of Pintner (1928) who separated the order into three families including Lecanicephalidae, the principal family. Freeman (1973) proposed the descriptive larval term, tentaculo-plerocercoid, that is used herein. The stingray nomenclature follows that of Bailey (1970).

*Polypocephalus* sp.

(Figs. 1, 2)

**DESCRIPTION** (based on 16 whole-mount specimens): Lecanicephaloidea; Lecanicephalidae. Small, compressed, pyriform, translucent tentaculo-plerocercoids; total length,  $328 \pm 40$  (262–408, 15); maximum width,  $184 \pm 17$  (120–260, 16) (at lateral tips); length to width ratio, 1.8:1; dorsoventral thickness, 40 (1 worm). Anterior holdfast consisting of terminal, cup-shaped cavity with 16 simple, unarmed, protrusile tentacles arising from cavity floor; cavity connecting to exterior via cylindrical tentacle canal. Holdfast  $118 \pm 8.9$  (85–143, 15) long (tentacle aperture to cavity floor) by  $132 \pm 14.5$  (85–174, 13) wide (at suckers); tentacle cavity  $81 \pm 6.6$  (62–105, 15) long by  $74 \pm 7.7$  (50–101, 15) wide; tentacle canal  $32 \pm 6.6$  (0–47, 15) long by  $40 \pm 6.6$  (16–38, 15) wide (depending on state of contraction); tentacle canal aperture constricted,  $30 \pm 4$  (19–47, 14) in diameter; tentacles  $84 \pm 7$  (58–116, 25 from 13 worms) long,  $8 \pm 1.1$  (1–12, 16 from 11 worms) in diameter. Tentacles infrequently protruding in excysted, incubated specimens.

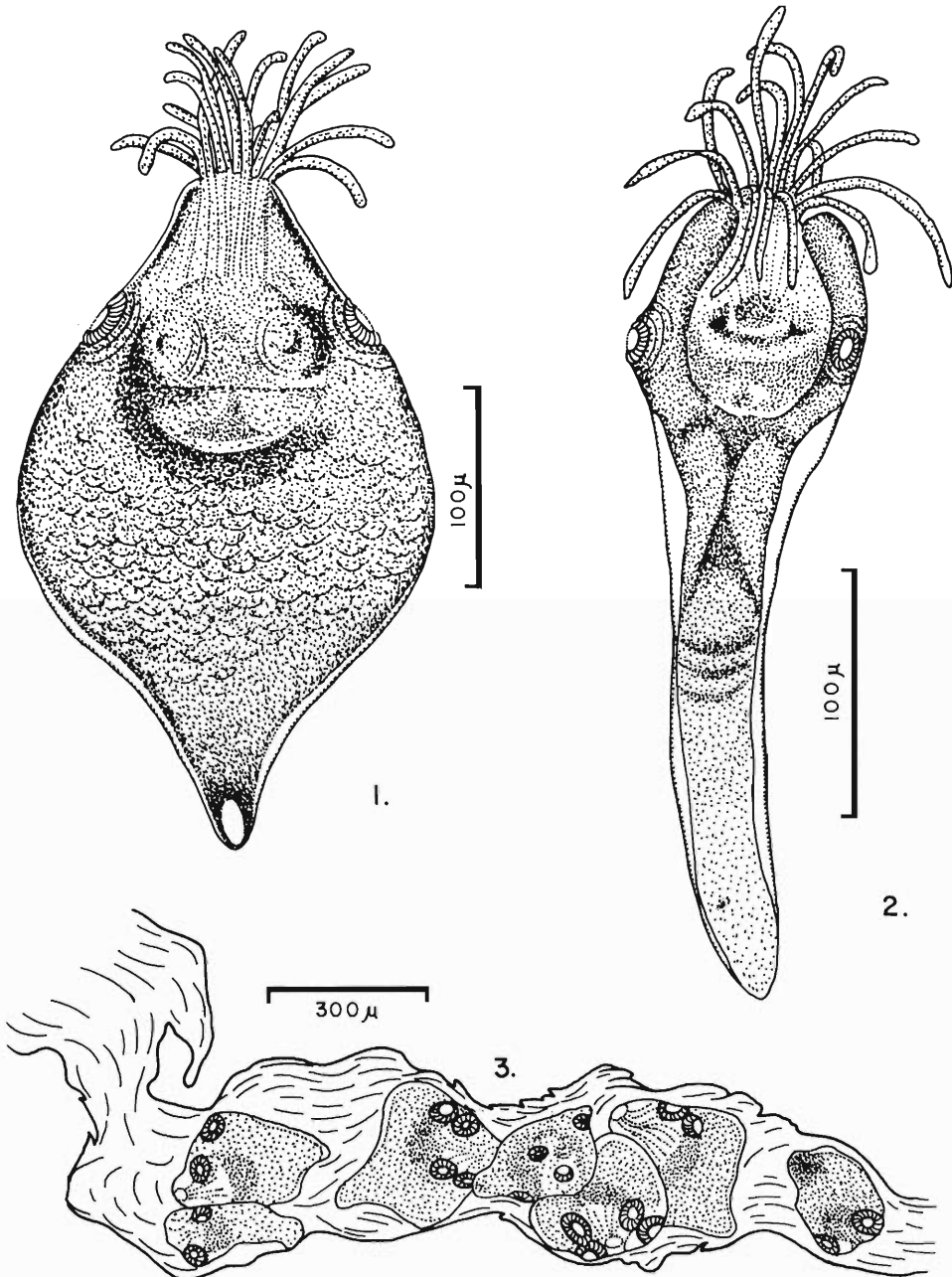
**HOST:** *Argopecten irradians concentricus* (Say) (Mollusca; Pectinidae).

**LOCATIONS:** The majority of the plerocercoids were encysted singly in thin, transparent sacs in the mesenteries and the remainder occurred in the stomach wall, digestive gland, and diverticula of infected scallops. Small groups of four to eight individually encysted plerocercoids often occurred together in mesenteries of heavily infected scallops (Fig. 3).

**LOCALITIES:** Infected scallops were collected at four localities, two on each side of the Apalachicola River delta as follows: Live Oak Point, Apalachee Bay ( $30^{\circ}3.5'N$ ,  $84^{\circ}16.7'W$ ); Turkey Point, St. George Sound ( $29^{\circ}54.8'N$ ,  $84^{\circ}29.7'W$ ); Eagle Harbor, St. Joseph Bay ( $29^{\circ}46'N$ ,  $85^{\circ}24'W$ ); and Grand Lagoon, St. Andrew Bay ( $30^{\circ}7.8'N$ ,  $85^{\circ}43.7'W$ ). Bay scallops from two localities in Apalachee Bay east of Live Oak Point (Keaton and Deckle beaches), and from two localities west of St. Andrew Bay (Little Sabin Bay and Big Lagoon near Pensacola, Florida), were uninfected.

**DISTRIBUTION:** All larvae of *Polypocephalus* sp. recovered to date were restricted to the northwestern coast of Florida from Apalachee Bay to St. Andrew Bay. Future investigations may extend that range. Bay scallops migrate into deeper water during colder months and were absent from sampling localities along the lower west coast of Florida (Cedar Key to Florida Bay). Infected scallops occurred on submerged grassflats where salinities ranged from 25 to 31‰.

**INFECTION DATA:** Table 1 summarizes the infection data from scallops examined during this investigation. Only those data from localities where *Polypocephalus* occurred are included. Forty-two of 55 scallops (76%) contained 777 plerocercoids and exhibited a mean infection intensity of  $18.5 \pm 5.5$  plerocercoids/scallop and an intensity range of 1 to 75.



Figures 1-3. Tentaculo-plerocercoids of *Polypocephalus* sp. from the digestive gland of *Argopecten irradians concentricus*. 1. Whole worm, frontal view. 2. Whole worm, lateral view. 3. Whole mount of mesentery section showing seven encapsulated plerocercoids.

LIFE HISTORY NOTES: With the exception of my preliminary report (Cake, 1972), no adults or larvae of *Polypocephalus* have been reported from the Gulf of Mexico. The nearest reported occurrence was in the vicinity of Beaufort, North Carolina, where Linton (1905) found *Polypocephalus* (syn. *Parataenia*)

**Table 1.** Summary of *Polypocephalus* sp. infection in bay scallops, *Argopecten irradians concentricus* (Say), from the northeastern Gulf of Mexico.

| Station          | Number of scallops infected/ examined | Percent infected | Total no. of plerocercoids | Mean no./ infected host ( $\pm$ S.E.) | Range infected host |
|------------------|---------------------------------------|------------------|----------------------------|---------------------------------------|---------------------|
| Live Oak Point   | 8/10                                  | 80               | 22                         | 2.8 $\pm$ 2.9                         | 1 to 12             |
| Turkey Point     | 20/25                                 | 80               | 424                        | 21.2 $\pm$ 8.0                        | 2 to 75             |
| Eagle Harbor     | 4/10                                  | 40               | 6                          | 1.5 $\pm$ 1.6                         | 1 to 3              |
| Grand Lagoon     | 10/10                                 | 100              | 325                        | 32.5 $\pm$ 12.5                       | 15 to 65            |
| Totals & (Means) | 42/55                                 | (76)             | 777                        | (18.5 $\pm$ 5.5)                      | 1 to 75*            |

\* Overall range.

*medusia* (Linton) in the bluntnose stingray, *Dasyatis sayi* (LeSueur). Linton (1889) originally described *P. medusia* from the rougthead stingray, *D. centroura* (Mitchill), at Woods Hole, Massachusetts. According to Yamaguti (1959), adults of *Polypocephalus* infect guitarfishes (Rhinobatidae) and stingrays (Dasyatidae) along the coasts of India and Sri Lanka (Ceylon) and stingrays along the Atlantic coast of the United States. Representatives of both families occur in the Gulf of Mexico.

The presence of plerocercoids in bay scallops indicates that reproducing adults of *Polypocephalus* are present in definitive hosts in the northeastern Gulf. Tom Mattis (GCRL, personal communication) obtained mature specimens of an unidentified species of *Polypocephalus* from the Atlantic stingray, *D. sabina* (LeSueur), at Turkey Point. Twenty of 25 bay scallops examined at that locality were infected with *Polypocephalus* sp. plerocercoids.

The results of this study suggest that bay scallops serve as intermediate hosts for this cestode. The manner in which scallops become infected is unknown. They may ingest demersal eggs that are released in their vicinity by definitive hosts, or they may ingest proceroids in an unknown, intermediate, crustacean host. Davis and Marshall (1963) reported that scallops (*A. irradians*) ingest benthic diatoms, detritus, bacteria, and other organic matter from the surrounding water and substratum by shell "flapping" and subsequent filter-feeding. Cestode eggs and microcrustaceans fall within the size range of those particles. Unknown symbiotic, cyclopid copepods were observed in the stomach of many infected scallops, but they were not examined for proceroids.

All plerocercoids were alive when excysted and remained active during initial incubation in artificial elasmobranch saline, but all died within 48 hr and exhibited no alteration of external morphology. No moribund, dead, or partially resorbed plerocercoids were noted among the 777 found in Gulf coast scallops. The presence of viable plerocercoids suggests that scallops are intermediate hosts in the life cycle of *Polypocephalus* sp.

The definitive hosts of recognized species of *Polypocephalus* consume a wide variety of benthic fauna including mollusks (Bigelow and Schroeder, 1953). Infected scallops and stingrays occur together in shallow coastal bays of the northeastern Gulf of Mexico (e.g., Turkey Point, St. George Sound), and those stingrays are capable of crushing and consuming infected scallops.

### Discussion

Unlike most larval cestodes that infected Gulf coast mollusks, these plerocercoids were easily identified to genus. *Polypocephalus* is the only lecanicephalid with four, simple suckers, and a ring of 16, protrusile, apical tentacles. Specific identification was impossible, however, because all specimens were immature. Complete identification of this species of *Polypocephalus* will require artificial infection of suitable definitive hosts and subsequent recovery of mature worms. The designation of a new species is deferred until Tom Mattis (GCRL) describes his adult specimens from *D. sabina*. I believe that it would be inappropriate to designate a new species solely on the basis of these plerocercoids.

### Acknowledgments

R. Winston Menzel (Florida State University, Dep. of Oceanography, Tallahassee, Florida) and James E. Byram (Peter Bent Brigham Hospital, Dep. of Pathology, Boston, Massachusetts, formerly with the FSU Dep. of Biological Sciences) provided financial, logistical, and technical assistance during the investigation from which this report was derived. Tom Mattis (Gulf Coast Research Laboratory, Parasitology Section) kindly shared his extensive knowledge of this and other cestode genera of the northern Gulf of Mexico. Adrian Lawler (GCRL Parasitol. Sect.) reviewed this manuscript. GCRL provided financial and technical support during the preparation of this manuscript.

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## BOOK REVIEW

*Principal Parasites of Domestic Animals in the United States. Biological and Diagnostic Information*, by Virginia R. Ivens, Daniel L. Mark, and Norman D. Levine. Special Publication 52, Colleges of Agriculture and Veterinary Medicine, University of Illinois, Urbana—Champaign, 1978, 270 pp., illus., \$10.

This well-illustrated, spiral-bound book presents information on the life cycles, biology and diagnosis of many common parasites of farm animals in the United States. The first part is a host-parasite list. The second section provides biological data, life cycle diagrams, and diagnostically useful photomicrographs of cysts, eggs and adult parasites of cat, cattle, chicken, dog, horse, sheep, swine, and turkey. The parasites of each host are grouped according to the principal organ system in which they live. The third section includes diagnostic techniques for examination of feces and blood and keys of infective larval stages of some gastrointestinal nematodes of cattle, sheep and horses. There is a bibliography of some 60 references and an index. Overall this attractive manual should be useful to veterinarians, laboratory technicians and students alike.—  
A. James Haley, University of Maryland, College Park.



## The Structure and Function of the Scolex Glands of Three Caryophyllid Tapeworms<sup>1</sup>

EUGENE G. HAYUNGA<sup>2</sup>

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**ABSTRACT:** The histology, histochemistry, and fine structure of the scolex glands are described for adults of *Hunterella nodulosa*, *Glaridacris catostomi*, and *Glaridacris laruei*. Frontal glands, found in *H. nodulosa*, consist of a mass of cells at the scolex apex with ducts leading to the syncytial tegument. Faserzellen, observed in *G. catostomi* and *G. laruei*, consist of a deeply staining mass of cells in the medullary parenchyma of the neck. Histochemical tests revealed the cytoplasm of the scolex glands of all three species to be rich in RNA and protein, but alkaline and acid phosphatase activity was not detected. Electron microscope observation showed these cells to have extensive endoplasmic reticulum, abundant ribosomes, Golgi, and numerous electron lucent vesicles in the cytoplasm. Vesicles originating from the frontal glands of *H. nodulosa* were observed releasing their contents to the surface; it appears that the frontal glands of this species secrete an adhesive substance which aids the parasite in attaching to its host. The function of the Faserzellen, on the other hand, could not be demonstrated for the adult worm. The role of these glands in determining site selection and causing intestinal pathology in the host is discussed.

Scolex glands in tapeworms have been shown to serve a variety of functions. In the Pseudophyllidea, for example, they are generally considered to be penetration glands (Williams, 1966; Bråten, 1968). A proteolytic function was also demonstrated for the cyclophyllideans *Aploparaxis furcigera* and *Hymenolepis parvula* by Šlais (1961) and *Taenia solium* by Faroqi (1958). However, when Ohman-James (1973) was unable to demonstrate the presence of proteolytic enzymes in the scolex glands of *Diphyllobothrium ditremum*, she suggested that the secretory product of this species might serve as an adhesive instead. In contrast, cells in the rostellum of *Hymenolepis diminuta* were clearly shown to play a neurosecretory role; it was found that the release of secretory material from these cells triggered the strobilization of the worm (Davey and Breckenridge, 1967). There is also evidence that scolex glands are involved in initiating the strobilization of *Echinococcus granulosus* (Smyth, 1971).

In the Caryophyllidea two kinds of glands have been reported in the anterior end of the worm: frontal glands found near the scolex apex, and Faserzellen found in the medullary parenchyma of the neck. According to some authorities (Pintner, 1906; Sekutowicz, 1934; Wiśniewski, 1930) the two glands are homologous, but this point remains to be proven. The present study, part of a Ph.D. Thesis (Hayunga, 1977), was undertaken to examine the histology, histochemistry, and fine structure of these glands in *Glaridacris catostomi* Cooper, 1920, *Glaridacris laruei* (Lamont, 1921) Hunter, 1927, and *Hunterella nodulosa* Mackiewicz and McCrae, 1962, in order to determine their function and their role in causing intestinal pathology in the host.

<sup>1</sup> Presented at the 507th meeting of the Helminthological Society of Washington, April 1977.

<sup>2</sup> Present (reprint) address: Biomedical Research Institute, American Foundation for Biological Research, 12111 Parklawn Drive, Rockville, Maryland 20852.

### Materials and Methods

Host fish, *Catostomus commersoni* Lacépède, were collected by seine from several streams and ponds in the vicinity of Albany, New York. Fish were killed by a sharp blow to the head, dissected rapidly, and the intestinal helminths fixed within minutes after the fish were killed.

Worms were fixed for 16 hr in 10% neutralized formalin at 4°C, washed, and stored in 70% ethanol, then subsequently dehydrated and embedded in either a mixture of paraffin and beeswax (4:1) or in JB4 glycolmethacrylate plastic (Ruddell, 1967). Paraffin sections were cut at 7  $\mu$ m on an AO Spencer 820 microtome; plastic sections were cut at 1–3  $\mu$ m on a Sorvall JB4 microtome using glass knives. JB4 plastic was obtained from Polysciences, Inc. Paraffin sections were stained as described in Clark (1973) and Humason (1972); protocols for staining plastic sections may be found in Hayunga (1977).

Localization of enzymatic activity was accomplished using naphthol substrates coupled with azo dyes, following the methods described by Burstone (1958) for alkaline phosphatase, by Barka and Anderson (1962) for acid phosphatase, and by Chayen et al. (1969) for leucyl-aminopeptidase and succinic dehydrogenase. Tissue was fixed in either 10% formalin or 100% acetone for 4 hr at 4°C and infiltrated with liquid Tissue-Tek® mounting medium for 16 hr at 4°C. Frozen sections 20  $\mu$ m thick were cut on an AO Cryo-cut cryostat at –20°C. Controls consisted of sections processed without the appropriate substrate.

Specimens were fixed for electron microscopy with 3% glutaraldehyde in 0.1 M sodium cacodylate buffer for 16 hr at 4°C, washed in buffer, then postfixed with 1% osmium tetroxide in cacodylate buffer for 90 min. Specimens were dehydrated with ethanol followed by propylene oxide, then embedded in Epon 812. Ultrathin sections were stained with a 2% solution of uranyl acetate in 50% methanol for 30 min at 60°C, and counterstained with saturated aqueous lead citrate for 2 min at room temperature. Sections were examined using the AEI EM6B transmission electron microscope at an accelerating voltage of 60 kV.

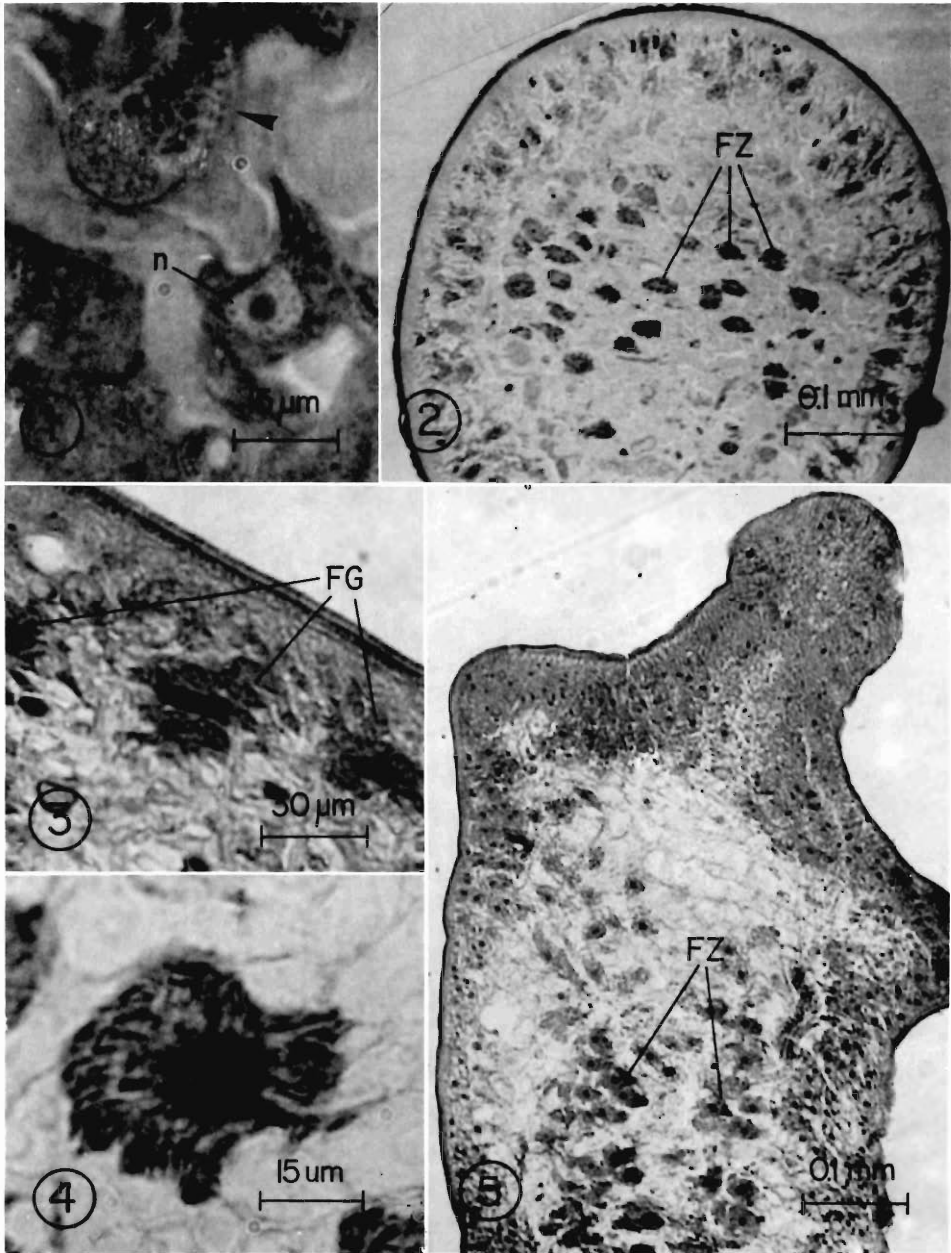
All observations reported are of adult worms; larval tapeworms could not be found in the field and attempts to raise procercoids in the laboratory were unsuccessful.

### Observations

#### *Hunterella nodulosa*

Longitudinal sections of *H. nodulosa* reveal a conspicuous mass of cells near the scolex apex. These cells have an affinity for neutral red and aldehyde fuchsin, and appear to be homologous to the frontal glands described by Will (1893), Mrázek (1901), and Wiśniewski (1930) for other caryophyllid species. Faserzellen (neck cells), described below, were not found in *H. nodulosa*.

The frontal glands of *H. nodulosa* are very large cells with numerous cytoplasmic processes that anastomose to form an extensive syncytial network. Most of their cytoplasm stains purple with hematoxylin and eosin, but there are also large aggregations of eosinophilic granules in these cells (Fig. 1). The nucleus is basophilic and contains numerous chromatin granules and a prominent nucleolus. The frontal glands occur quite close to the surface near the scolex apex (Fig. 3) and cytoplasmic processes from the cells nearest to the surface can be traced



Figures 1-5. 1. Frontal gland cell of *H. nodulosa* showing an aggregation of eosinophilic granules (arrow). Note also the large nucleus (*n*) and prominent nucleolus. Epon section, toluidine blue. 2. Cross section through the neck of *G. laruei* showing prominent Faserzellen (*FZ*) in the medullary parenchyma. Plastic section, toluidine blue. 3. Section through the cortical parenchyma of *H. nodulosa* near the scolex apex. Note the proximity of the frontal glands (*FG*) to the surface; tegumental cells are not found in this part of the worm. Plastic section, hematoxylin and eosin. 4. Faserzellen of *G. laruei*. This cell is characterized by its prominent nucleolus and by the mottled appearance of its cytoplasm. Plastic section, hematoxylin and eosin. 5. Mid-sagittal section of *G. laruei*. Note Faserzellen (*FZ*) in the medullary parenchyma. Paraffin section, trichrome stain.

**Table 1. Histochemical localization of various substances in the cytoplasm of the scolex glands.**

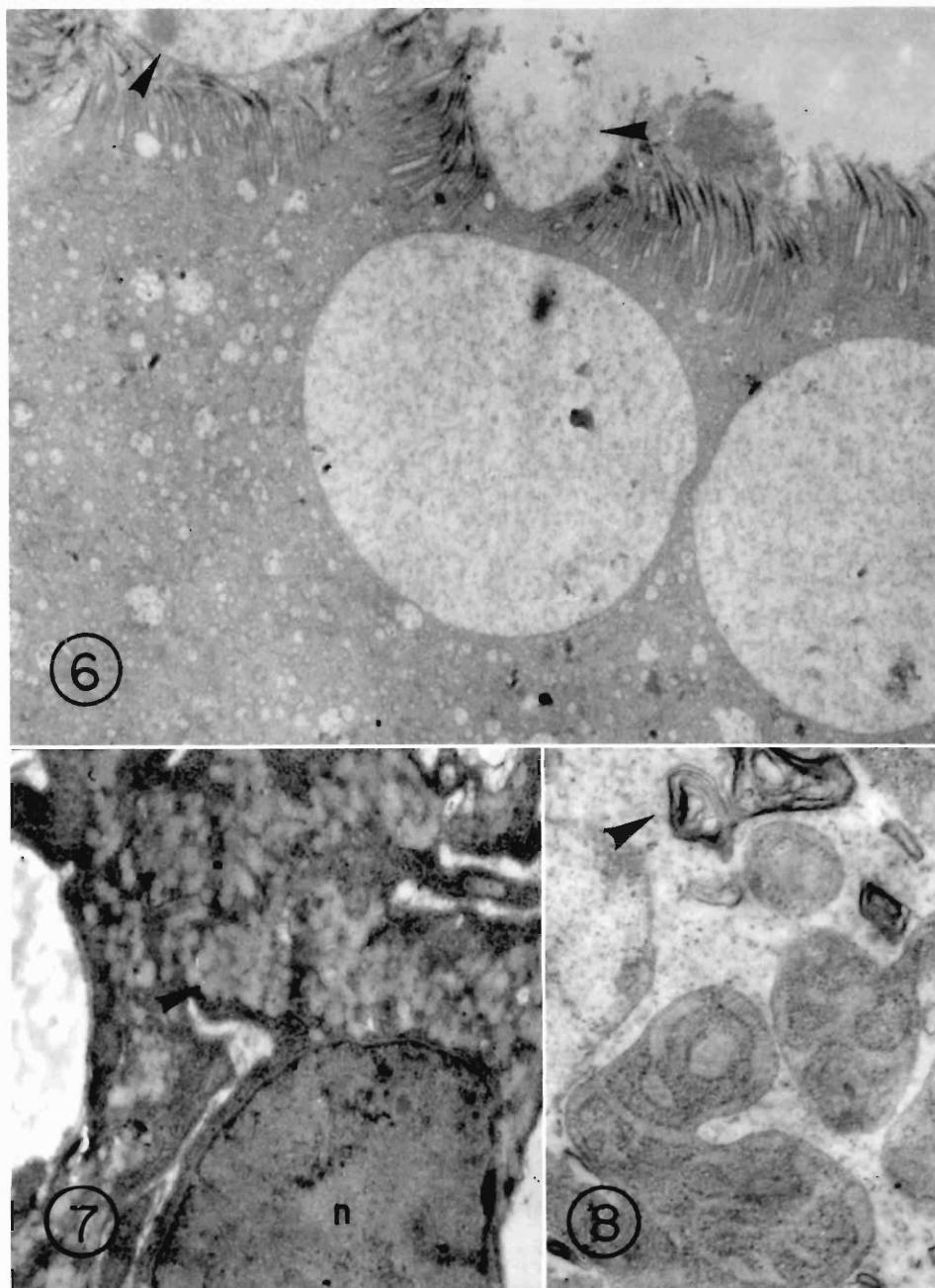
|                        | <i>G. laruei</i> | <i>G. catostomi</i> | <i>H. nodulosa</i> |
|------------------------|------------------|---------------------|--------------------|
| Alcian blue            | —                | —                   | —                  |
| PAS                    | +                | ++                  | ++                 |
| PAS control            | —                | —                   | +                  |
| Best carmine           | —                | +                   | +                  |
| Pyronin                | ++++             | ++++                | ++++               |
| Neutral red            | ++++             | +++                 | ++++               |
| Aldehyde fuchsin       | +++              | ++                  | ++                 |
| Millon's               | +                | +                   | +                  |
| Bromophenol blue       | ++               | ++                  | ++                 |
| Oil Red O              | —                | —                   | —                  |
| Alkaline phosphatase   | —                | —                   | ±                  |
| Acid phosphatase       | —                | —                   | —                  |
| Leucyl aminopeptidase  | 0                | 0                   | —                  |
| Succinic dehydrogenase | 0                | 0                   | —                  |

+ positive reaction; ++ stronger reaction; +++ very strong reaction; ++++ extremely strong reaction; — negative reaction; ± inconclusive results; 0 no data.

directly to the syncytial tegument. In terms of their staining properties the frontal glands most closely resemble the tegumental cells. (Tegumental cells are not found at the scolex apex.) Frontal glands stain positively with pyronin, Millon's reagent and bromophenol blue, indicating that they are active in protein synthesis (Table 1). The cells are PAS positive and there are small deposits of glycogen (as shown by PAS control and Best carmine) in the intercellular spaces. Attempts to localize alkaline phosphatase in these cells were inconclusive. However, acid phosphatase activity (which is present in the tegumental cells and in most of the syncytial tegument) was absent from both the scolex glands and from the tegument immediately above the glands. This suggests that the syncytium covering the body is different, chemically, from that covering the scolex apex, and that this difference is due to the nature of the secretory products synthesized by the cells immediately beneath the syncytium.

Ultrastructural observations show that the frontal glands transport their secretory products along the cytoplasmic processes in much the same manner as do the tegumental cells (see Hayunga and Mackiewicz, 1975, for comparison). Membrane bound pores passing through the tegument (as reported by Kwa, 1972; Öhman-James, 1973; Arme and Threadgold, 1976) were not observed in *H. nodulosa*; the secretion of the frontal glands of this species appears to be intra-tegumental. Electron lucent vesicles, found in the cytoplasm of the frontal glands (Fig. 7), can be followed along the cytoplasmic processes and into the syncytium, where they fuse with the surface membrane and release their contents (Fig. 6). Aggregations of these electron lucent vesicles correspond to the eosinophilic deposits seen with the light microscope; large electron lucent vesicles are not found in other parts of the tegument but only near the scolex apex. The cytoplasm of the frontal glands is rich in ribosomes and endoplasmic reticulum.

Previous investigations (Mackiewicz and McCrae, 1962; Mackiewicz et al., 1972; Hayunga and Mackiewicz, 1975) have reported that the scolex of *H. nodulosa* is separated from host tissue by a thin layer of amorphous eosinophilic material, the so-called "eosinophilic matrix." The present study indicates that



Figures 6-8. 6. Tegument of *H. nodulosa* near the scolex apex. Note the large electron lucent vesicles in the syncytium and the granular material being released at the surface (arrow).  $\times 17,000$ . 7. Frontal gland of *H. nodulosa* showing numerous electron lucent vesicles in the cytoplasm (arrow); nucleus (*n*). Aggregations of these vesicles appear to correspond to the eosinophilic deposits seen in Figure 1.  $\times 7,000$ . 8. Faserzellen of *G. catostomi* showing the fine structure of the intercellular deposits (arrow) and arrangement of ribosomes in the cytoplasm.  $\times 17,000$ .

the eosinophilic matrix is a secretory product of the frontal glands. Further observations on this matrix and the fine structure of the parasite-host interface are described elsewhere (Hayunga, 1977, 1979).

### *Glaridacris laruei*

In *G. laruei* there are no frontal glands but there is a conspicuous mass of cells, the Faserzellen,<sup>3</sup> found in the medullary parenchyma of the neck anterior to the vitellaria. The Faserzellen appear as individual cells when viewed in cross section (Fig. 2), but longitudinal sections reveal that they are arranged as a syncytial network (Fig. 5). In addition, isolated Faserzellen cells can be found in the medullary parenchyma among the first three or four vitelline follicles. The cytoplasm of these cells is characterized by numerous dark basophilic strands, and the nucleus by a large nucleolus (Fig. 4). The Faserzellen have an affinity for toluidine blue, fast green, neutral red, pyronin, and aldehyde fuchsin (Table 1). They stain positively for protein with Millon's reagent and bromophenol blue, and give a slightly positive PAS reaction, but no glycogen deposits could be demonstrated in either the cytoplasm or the intercellular spaces. Electron microscope examination shows the Faserzellen of *G. laruei* to be almost identical in appearance to the frontal glands of *H. nodulosa*; their cytoplasm is rich in ribosomes and endoplasmic reticulum and contains numerous aggregations of electron lucent vesicles.

### *Glaridacris catostomi*

Frontal glands are also lacking in *G. catostomi*. In this species, the Faserzellen are confined to the neck region anterior to the vitellaria. The syncytial cytoplasm appears mottled with hematoxylin and eosin staining; each nucleus is basophilic and has a prominent nucleolus. The Faserzellen are rich in RNA and protein, as demonstrated by methyl green-pyronin and bromophenol blue, and positive staining with neutral red and aldehyde fuchsin suggests secretory activity (Table 1). The spaces between the cells contain numerous glycogen deposits. (Such intercellular deposits of glycogen are found throughout the body of *G. catostomi*, primarily in the medullary parenchyma.)

Electron microscope examination reveals the Faserzellen of this species to be rich in ribosomes, endoplasmic reticulum, and Golgi. The ribosomes are found surrounding areas of electron lucent cytoplasm, but no aggregations of vesicles are found in these cells (Fig. 8). In the spaces between the Faserzellen there are numerous inclusions comprised of concentric dark and light bands (arrow, Fig. 8). These deposits bear a striking resemblance to the calcareous corpuscles (which are larger) of other species; the chemical composition of the deposits was not ascertained and their function remains obscure.

### Discussion

According to Wiśniewski (1930), the frontal glands of *Archigetes* were presumed to be penetration glands, and thus homologous to similar glands found in

<sup>3</sup> This term has been handed down from the early descriptive literature, much of which is in German. The word "Faserzellenstränge," which means "bundle of fibrous cells," was introduced by Will (1893) to describe these conspicuous cells in the neck. Mrázek (1901) used the shorter word "Faserzellen" ("fibrous cells"), while Wiśniewski (1930) preferred the term "Halszellen" ("neck cells").

pseudophyllid larvae. On the other hand, Hunter (1930) and Szidat (1937) suggested that the frontal glands of caryophyllids might aid in attachment. This latter view is supported by the observation of Mackiewicz (1972) that frontal glands appear more numerous in species with poorly developed attachment organs. Furthermore, caryophyllid tapeworms generally do not penetrate the gut, and there is no solid evidence that the frontal glands secrete proteolytic enzymes.

In *H. nodulosa* no evidence of proteolytic activity could be detected for the frontal glands. They were found, instead, to secrete a mucoprotein that forms the "eosinophilic matrix" found between parasite and host. This secretion appears to function primarily as an adhesive. Electron microscope examination shows that tegumental microtriches are attached to the matrix, and that the matrix closely adheres to host tissue (see Hayunga, 1979). It was originally suggested that, in the absence of any holdfast organs on the scolex of *H. nodulosa*, attachment was maintained by the hooklike microtriches (Mackiewicz and McCrae, 1962; Hayunga and Mackiewicz, 1975). It now appears that for this species attachment is accomplished by the microtriches in conjunction with the adhesive secretions of the frontal glands.

If the frontal glands of *H. nodulosa* are not penetration glands, then how does one account for the severe damage caused by this species, or for the observation that frontal glands appear most numerous in species that cause considerable damage to host tissue, while little pathology is caused by species lacking these glands? Hayunga (1977, 1979) suggested that the secretion of the frontal glands, although primarily an adhesive, also acts as a strong irritant, and that the nodule is formed as part of the inflammatory response of the fish. Thus, the frontal glands would be indirectly responsible for the pathology associated with this species. Clearly, more work is needed both in following the pathogenesis of the nodule caused by *H. nodulosa*, and in examining the scolex glands of other pathogenic species such as *Monobothrium ulmeri* and *Djombangia penetrans*.

The function of the Faserzellen remains obscure. Pintner (1906) and Wiśniewski (1930) both considered the Faserzellen to be homologous to the frontal glands, while Mrázek (1901) believed them to be the vestigial remains of a digestive system once present in the original protocestode. The positive staining of the Faserzellen with aldehyde fuchsin suggests a neurosecretory role, because scolex neurosecretory cells that triggered the strobilization of *H. diminuta* were shown to have an affinity for that dye (Davey and Breckenridge, 1967). However, if the Faserzellen are neurosecretory cells, they clearly must serve some other function because caryophyllid tapeworms do not undergo strobilization. A final possibility is suggested by the work of Sekutowicz (1934), who reported that Faserzellen found in adults of *C. laticeps* were the vestigial remains of frontal glands found in the proceroid stage. Histological studies of proceroids of *Glaridacris* and experimental studies of developing caryophyllids would be of great value in elucidating the function of these cells.

Although there is no direct correlation between the presence of scolex glands and the preferred site of attachment, there is evidence that scolex morphology can play a major role in determining site selection (Mackiewicz et al., 1972; Hayunga, 1977). Undoubtedly, both scolex glands and external scolex morphology evolved concurrently for the caryophyllids. It now appears that two successful strategies have been taken by these worms. In *H. nodulosa* we see an

extreme case where the scolex is poorly developed and attachment is maintained by the adhesive secretion of the frontal glands, while in *G. catostomi* and *G. laruei* the attachment organs are well developed and there is no need for adhesive glands. Further study is needed to determine whether intermediate strategies are also followed, and to what degree scolex glands and scolex morphology influence competition between species.

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## *Alloglossidium schmidti* sp. n. (Trematoda: Macroderoididae) from Hirudinid Leeches in Manitoba

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**ABSTRACT:** *Alloglossidium schmidti* sp. n. is described from the intestine of hirudinid leeches, *Haemopsis grandis* Verrill, 1874, from a lake in eastern Manitoba. *A. schmidti* most closely resembles *A. hirudicola* but differs in having an ovary larger than the testes, a larger cirrus pouch, a preequatorial anterior testis, and eggs lacking conspicuous abopercular knobs. This is the first report of a helminth in leeches from Canada. A key to the species of *Alloglossidium* is provided.

Examination of the intestines of 52 hirudinid leeches, *Haemopsis grandis* Verrill, 1874, from Falcon Lake, Manitoba (49°42'N, 95°18'W) during the summers of 1977 and 1978, revealed mixed infections of *Alloglossidium turnbulli* Neumann and Vande Vusse, 1976, and a small number of trematodes herein described as new. The prevalence and intensity of both species of helminths found in *H. grandis* from this locality will be published in a later paper.

Worms were removed from hand-caught, freshly killed hosts, fixed in either steaming alcohol-formol-acetic fixative (AFA) with slight coverslip pressure (6 specimens) or in steaming AFA (3 specimens), stained with Gower's carmine, cleared in methyl benzoate, and mounted in Canada balsam for study as whole mounts. Measurements of six coverslipped worms are presented as ranges followed by the arithmetic mean value in brackets; measurements are given in micrometers unless otherwise stated.

### *Alloglossidium schmidti* sp. n.

(Fig. 1)

**DESCRIPTION:** Body elongate, somewhat narrowed at both ends, 2.60 to 3.43 (3.02) mm long by 0.67 to 0.73 (0.72) mm wide at level of ovary. Tegumental spines minute, extending dorsally and ventrally from anterior end to level of posterior margin of oral sucker. Suckers equal; oral sucker ventrosubterminal, 173 to 265 (200) long by 183 to 207 (195) wide; acetabulum 188 to 217 (197) long by 188 to 212 (202) wide, preequatorial,  $\frac{1}{6}$  to  $\frac{1}{7}$  of body length from anterior end. Prepharynx short; pharynx 87 to 130 (108) long by 106 to 125 (114) wide; esophagus short. Ceca extending to or somewhat beyond posterior testis. Genital pore medial or slightly sinistral, immediately anterior to acetabulum. Cirrus pouch comma-shaped, anterolateral to acetabulum, 241 to 313 (269) long by 67 to 92 (77) wide, containing seminal vesicle. Testes ovoid, tandem to slightly diagonal, intercecal; anterior testis preequatorial, postovarian, 167 to 236 (211) long by 125 to 241 (173) wide; posterior testis slightly postequatorial, 217 to 284 (248) long by 154 to 241 (189) wide. Ovary large, ovoid, pretesticular, postacetabular, 304 to 410 (366) long by 193 to 236 (216) wide. Oviduct arising from ventral surface of ovary; seminal receptacle absent; Mehlis gland medial, at level of ovary. Uterus sinuous, voluminous, descending to near posterior end of body, then ascending ventral to testes. Metraterm slightly muscular. Eggs operculate, lacking conspicuous abopercular knobs, 27 to 33 (29) long by 14 to 17 (15) wide. Vitellaria

follicular, distributed from level of oral sucker to level of posterior testis; most follicles lateral but a few medial near dorsal and ventral surfaces. Excretory bladder tubular, extending from posterior end of body to level of anterior testis.

HOST: *Haemopsis grandis* Verrill, 1874.

LOCATION: Intestine.

LOCALITY: Falcon Lake, Manitoba.

TYPE SPECIMENS: National Museum of Canada Invertebrate Collection (Parasites): holotype no. NMCIC(P) 1978-396; paratypes no. NMCIC(P) 1978-397.

ETYMOLOGY: The species is named in honor of Dr. Gerald D. Schmidt in recognition of his contributions to helminth taxonomy.

### Discussion

The genus *Alloglossidium* currently contains eight species, four of which have been described from the intestine of North American hirudinid leeches: *A. hirudicola* Schmidt and Chaloupka, 1969 in *Haemopsis* sp. from an unknown locality (Schmidt and Chaloupka, 1969); *A. macrobdellensis* Beckerdite and Corkum, 1974 in *Macrobdella ditetra* from Louisiana (Beckerdite and Corkum, 1974); and *A. turnbulli* and *A. hamrumi* Neumann and Vande Vusse, 1976 in *H. grandis* and *H. plumbea*, respectively, from Minnesota (Neumann and Vande Vusse, 1976). In addition, Taft and Kordiyak (1973) reported *A. hirudicola* in *Haemopsis* sp. and *M. decora* from Wisconsin; and Corkum and Beckerdite (1975) reported observations on the life history of *A. macrobdellensis*. *Alloglossidium progenticum* (Sullivan and Heard, 1969) Font and Corkum, 1975 is a parasite of crayfishes and catfishes (Sullivan and Heard, 1969), *A. renale* Font and Corkum, 1975 was described from freshwater shrimps (Font and Corkum, 1975), and *A. corti* (Lamont, 1921) Van Cleave and Mueller, 1934 and *A. geminum* (Mueller, 1930) Van Cleave and Mueller, 1934 are parasites of fishes.

*Alloglossidium geminum* was described from the bullhead, *Ameiurus nebulosus*, as *Plagiorchis geminus* and transferred to *Alloglossidium* by Van Cleave and Mueller (1934). Yamaguti (1958) transferred *A. geminus* to *Glossidium* and emended the specific name to conform to the generic name; *A. corti* was retained in *Alloglossidium*. *Glossidium* and *Alloglossidium* were separated (Yamaguti, 1971) in that the former had vitellaria not extending into the forebody and an ovary not separated from the acetabulum by the cirrus pouch while the latter had vitellaria extending into the forebody and an ovary separated from the acetabulum by the cirrus pouch. Although the extent of vitellaria varies between *A. corti* (from the level of the pharynx to the posterior testis) and *A. geminum* (from the level of the acetabulum to the posterior testis), it is no more variable than in recently described species of *Alloglossidium* from hirudinid leeches and probably should not be taken as a generic determinant in this instance. Furthermore, figures of *A. geminum* (Mueller, 1930; Van Cleave and Mueller, 1934) clearly show that the ovary and acetabulum are separated by the cirrus pouch. Therefore, *G. geminum*, as proposed by Yamaguti (1958, 1971), is transferred to *Alloglossidium* and the specific name *geminum* is retained to conform to the generic name.

*Alloglossidium schmidti* most closely resembles *A. hirudicola* in extent of spination, extent of vitellaria and host, but differs in having an ovary larger than the testes, a larger cirrus pouch, a preequatorial anterior testis, and eggs lacking conspicuous abopercular knobs. *A. schmidti* differs from *A. turnbulli*, *A. ham-*

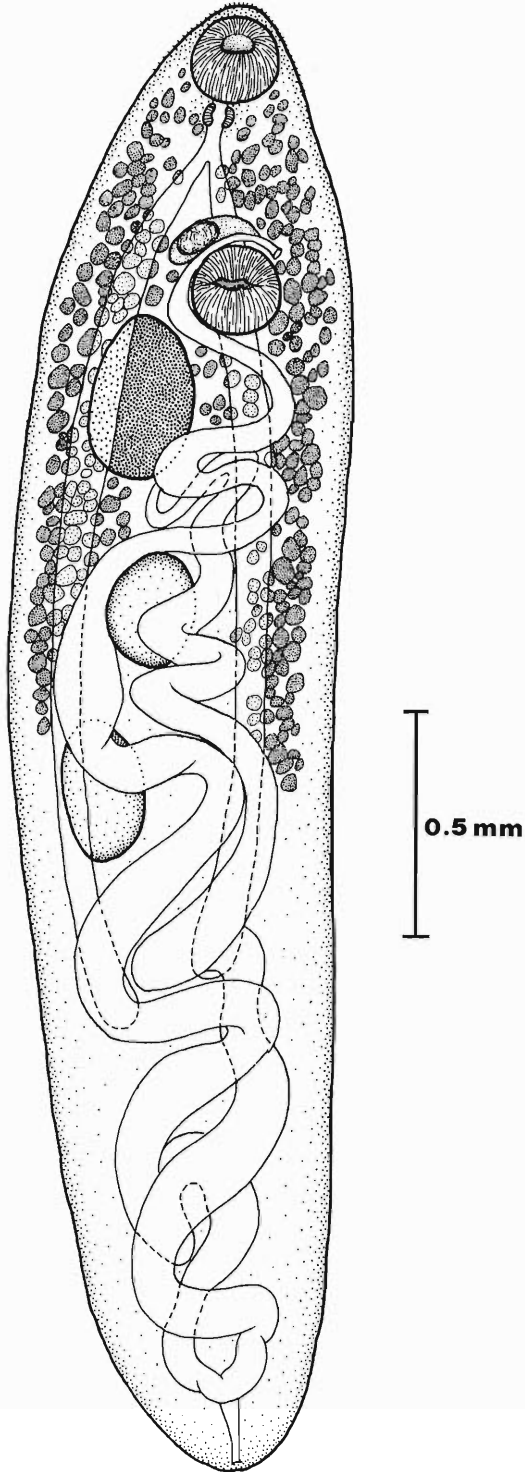


Figure 1. *Alloglossidium schmidti* sp. n. Whole mount, ventral view. Figure drawn with the aid of a microprojector.

*rumi*, and *A. macrobdellensis* in having smaller spines and less extensive spination, a short prepharynx, vitellaria from the level of the oral sucker, and a less elongate body; from *A. corti* and *A. geminum* in having an ovary larger than the testes, less extensive spination, vitellaria from the level of the oral sucker, shorter ceca, and host; and from *A. renale* and *A. progeneticum* in having a nonlobate ovary, less extensive spination, vitellaria from the level of the oral sucker, a preequatorial anterior testis, and host.

The following key will serve to differentiate the known species of *Alloglossidium*.

- |   |   |
|---|---|
| 1a. Ovary equal to or smaller than anterior testis .....  | 2 |
| 1b. Ovary larger than anterior testis .....   | 6 |
| 2a. Prepharynx short (<50 $\mu\text{m}$ ) .....   | 3 |
| 2b. Prepharynx long (>50 $\mu\text{m}$ ) .....  | 5 |
| 3a. Spines extending posterior to midbody; vitellaria reaching from level of pharynx or acetabulum to posterior testis; parasites of fishes .....           | 4 |
| 3b. Spines extending only to posterior margin of oral sucker; vitellaria reaching from level of oral sucker to posterior testis; parasites of leeches ..... |   |
| ..... <i>hirudicola</i> Schmidt and Chaloupka, 1969   |   |
| 4a. Vitellaria reaching from level of acetabulum to posterior testis .....  |   |
| ..... <i>geminum</i> (Mueller, 1930)  |   |
| 4b. Vitellaria reaching from level of pharynx to posterior testis .....   |   |
| ..... <i>corti</i> (Lamont, 1921)   |   |
| 5a. Spines smaller (3 $\mu\text{m}$ at base), reaching to level of posterior testis; cirrus pouch short, reaching just posterior to acetabulum .....        |   |
| ..... <i>hamrumi</i> Neumann and Vande Vusse, 1976  |   |
| 5b. Spines larger (7 $\mu\text{m}$ at base), reaching to posterior end of body; cirrus pouch long, extending twice the length of acetabulum .....           |   |
| ..... <i>macrobdellensis</i> Beckerdite and Corkum, 1974  |   |
| 6a. Ovary scalloped or lobate .....   | 7 |
| 6b. Ovary not so .....  | 8 |
| 7a. Spines extending to posterior end of body; ascending arm of uterus non-voluminous, with small coils; parasites of freshwater shrimps .....              |   |
| ..... <i>renale</i> Font and Corkum, 1975   |   |
| 7b. Spines extending posterior to midbody; ascending arm of uterus voluminous, sigmoid in shape; parasites of crayfishes and catfishes .....                |   |
| ..... <i>progeneticum</i> (Sullivan and Heard, 1969)  |   |
| 8a. Spines extending posterior to level of ovary; prepharynx long; vitellaria reaching from level of acetabulum to posterior testis .....                   |   |
| ..... <i>turnbulli</i> Neumann and Vande Vusse, 1976  |   |
| 8b. Spines extending only to posterior margin of oral sucker; prepharynx short; vitellaria reaching from level of oral sucker to posterior testis .....     |   |
| ..... <i>schmidti</i> sp. n.  |   |

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### Erratum

Vol. 44, No. 2, p. 239, July, 1977

**NORMAN RUDOLPH STOLL**

September 4, 1892-December 30, 1977

to

September 4, 1892-December 30, 1976

***Sphaeridiotrema echinosauense* sp. n. (Trematoda: Psilostomidae)  
from *Echinosaura horrida horrida* in Ecuador**

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**ABSTRACT:** *Sphaeridiotrema echinosauense* sp. n. is described from the intestine of the lizard, *Echinosaura horrida horrida* from Ecuador. Similarities with the other members of the genus, *S. globulus*, *S. macrocotyla*, and *S. spinoacetabulum*, are discussed. *Sphaeridiotrema echinosauense* differs from these species in having a reptilian host, smaller sized and greater number of eggs, and more extensive vitellaria.

Seven of 34 *Echinosaura horrida horrida* Boulenger, 1890 from western Ecuador examined for intestinal parasites were found to harbor a psilostome trematode not readily assigned to any species listed by Yamaguti (1971). The lizards had been fixed in 10% formalin and preserved in 70% ethanol. Worms collected from the preserved lizards were stored in 70% ethanol, stained in Semichon's acetocarmine, dehydrated in alcohol, cleared in xylene, and mounted in gum Damar. Serial sections of six worms stained in Semichon's acetocarmine or Harris' hematoxylin were cut at eight micrometers. Measurements, based on 12 worms, are expressed in micrometers. Descriptions of certain fine morphological features were made from serial sections, and drawings were made from projected photomicrographs.

***Sphaeridiotrema echinosauense* sp. n.**

(Fig. 1)

**HOST:** *Echinosaura horrida horrida* Boulenger, 1890.

**LOCALITY:** Centro Cientifico Rio Palenque, Pinchicha Province, Ecuador.

**LOCATION:** Intestine.

**HOLOTYPE:** USNM Helminth. Coll. No. 74926.

**PARATYPES:** USNM Helminth. Coll. No. 74927.

**DESCRIPTION:** Body 1,026 (930-1,180) by 652 (570-750), tegument thick; oral sucker subterminal, 156 (112-203) by 193 (130-229); acetabulum anterior, 323 (280-350) by 404 (340-430); prepharynx very short; pharynx subspherical, 126 (99-143) in diameter; esophagus short, thick walled; intestinal caecae terminate just posterior to testes; genital pore sinistral, extracecal, anterior to acetabulum; testes transversely elongate, entire, adjacent or oblique (sinistral testis usually anterior to dextral testis), midway between acetabulum and posterior end of body; sinistral testis 98 (65-138) by 218 (187-252); dextral testis 104 (73-156) by 226 (161-268); cirrus sac elongate, claviform, originating adjacent to anterior edge of acetabulum, enclosing large simple seminal vesicle and winding, muscular cirrus with very extensive pars prostatica; ovary ovate, entire, anterior to dextral testis, 101 (60-216) by 116 (94-146); vitellaria consisting of multinucleate follicles, in lateral bands extending from oral sucker to posterior end, confluent in preovarian to midacetabular region; seminal receptacle a sac adjacent to ovary; uterine convolutions extend transversely in midovarian to postacetabular region; eggs operculate, thick walled, few in number (40-60), 60 (49-70) by 32 (26-36); excretory vesicle Y-shaped, thick walled, extending to level of ovary.

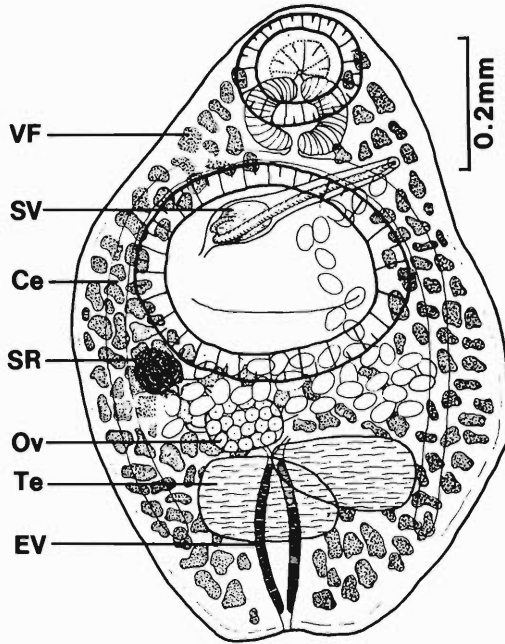


Figure 1. *Sphaeridiotrema echinosaurense* sp. n., from *Echinosaura horrida horrida*. Composite drawing from holotype and sectioned paratypes. Abbreviations: Ce, intestinal ceca; EV, excretory vesicle; Ov, ovary; SR, seminal receptacle; SV, seminal vesicle; Te, testis; VF, vitelline follicle.

### Discussion

The only previously described member of the family Psilostomidae parasitic in reptiles is *Cotylotretus rugosus* Odhner, 1902, from the intestine of the snake, *Spilotes pullatis* from Brazil and Sudan (Yamaguti, 1971). *Cotylotretus rugosus* belongs in the subfamily Cotylotretinae and is diagnostically dissimilar from Sphaeridiotrematinae.

*Sphaeridiotrema echinosaurense* appears similar to the described species of *Sphaeridiotrema*, namely *S. globulus* (Rudolphi, 1819) Odhner, 1913, *S. macrocotyla* (Macy and Bell, 1968) Yamaguti, 1971, and *S. spinoacetabulum* Burns, 1961. The existing and proposed species share an ovoid body shape, very large acetabulum, subterminal oral sucker, extracecal genital pore, anterior cirrus pouch, submedian ovary, and bilateral vitelline bands extending to or near the posterior extremity.

*Sphaeridiotrema echinosaurense* is parasitic in a reptile while other members of the genus were found in birds. Macy and Bell (1968) described the tegument of *S. macrocotyla* as having small spines on the anterior fourth of the body. Burns (1961) described acetabular spines on *S. spinoacetabulum*. No spines were observed on *S. echinosaurense*. Although Price (1934) redescribed *S. globulus* as possessing "as many as 60 eggs," this was considered a misprint by Burns (1961), since descriptions of the other species suggest that possession of a smaller number of eggs was more common (one to several). *Sphaeridiotrema echinosaurense* possesses more (40–60) and much smaller sized eggs. The eggs of *S. globulus*, as redescribed by Price (1934), are almost twice the size of the eggs of *S.*



*echinosauense* (90–105 by 60–67 compared with 49–70 by 26–36). The vitelline follicles of *S. echinosauense* are smaller than those of the other species, extend to the anterior extremity and are confluent behind the acetabulum. In the other three species the vitellaria are restricted to lateral bands adjacent the caeca in the postpharyngeal region. The pars prostatica in the cirrus pouch of *S. echinosauense* also is more extensive than in the other species.

The lack of proper fixation prevents a more detailed description of the ootype region. Elucidation of the life cycle and proper treatment of specimens of *Sphaeridiotrema echinosauense* are necessary to determine an exact relationship with other members of the family Psilostomidae.

### Acknowledgments

The authors express their sincere thanks to Greg Vigle who collected and preserved the lizards, and allowed us to recover their intestinal parasites.

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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.

## Life Cycle Studies of Three Digenetic Trematodes, Including Descriptions of Two New Species (Digenea: Cryptogonimidae)

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**ABSTRACT:** Two new species of cryptogonimid trematodes, *Caecincola latostoma* and *Cryptogonimus spinovum*, are described. These new species and *Textrema hopkinsi* employ the hydrobiid snail *Cincinnatia peracuta* as the molluscan host. *Micropterus salmoides*, *Elassoma zonatum*, and several members of the genus *Lepomis* act as second intermediate hosts and *M. salmoides* and *M. punctulatus* serve as definitive hosts. In view of life cycle and morphological similarities among these trematodes, the subfamily Caecincolinae is suppressed and its members, along with the genus *Textrema*, are assigned to the subfamily Cryptogoniminae.

Freshwater fishes belonging to the genus *Micropterus* serve as hosts in the southern United States for several recently described species of cryptogonimid trematodes. Premvati (1967) described two species, *Caecincola wakullata* and *Multigonotylus micropteri*, from the largemouth bass, *M. salmoides* (Lacépède), collected in Florida. Sullivan (1975), also working in Florida, described *Turgecaecum longifauces* from Suwanee bass, *M. notius* Bailey and Hubbs, and *Textrema hopkinsi* Dronen, Underwood, and Sunderman, 1977, was reported from *M. salmoides* taken in central Texas. Two new species of cryptogonimid trematodes, in addition to *T. hopkinsi*, were recovered from *M. salmoides* and *M. punctulatus* (Rafinesque) collected in south Louisiana. Life cycle studies were undertaken to elucidate the taxonomic and ecological relationships among these species.

Complete or nearly complete life cycles have been described for four cryptogonimid trematodes. Takahashi (1929) worked out the life cycle of *Pseudexorchis major* (Hasegawa, 1935) which Yamaguti (1938) and Ito (1956) described in greater detail. Okabe (1936, 1937) and Komiya and Tajimi (1940) determined the life cycle of *Metadena oviformis* (Kobayashi, 1915) Overstreet, 1971. Lundahl (1941) characterized the life cycle and excretory development of *Caecincola parvulus* Marshall and Gilbert, 1905, while Cable and Hunninen (1942) described the life cycle of *Siphodera vinalwardsii* (Linton, 1901).

Observations made in the present study are in accordance with previously described accounts of life cycles for members of the family Cryptogonimidae Ciurea, 1933 and indicate a close relationship among the species presently under consideration. A realignment of Yamaguti's (1971) proposed arrangement for subfamilies Caecincolinae Yamaguti and Cryptogoniminae is necessary to express taxonomically the affinities revealed by these life cycle studies.

### Materials and Methods

Infected vertebrate and invertebrate hosts were collected from False River, an oxbow lake in Pointe Coupee Parish, Louisiana. *Micropterus salmoides* and sunfishes, *Lepomis macrochirus* Rafinesque and *L. megalotis* (Rafinesque), for use

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in laboratory experimentation were obtained from areas where infections were shown previously not to occur.

Crude screening for snail infections was accomplished by placing uninfected sunfishes in containers with several hundred recently collected snails. Snails from jars in which fish became infected with cryptogonimid metacercariae were subsequently separated and the infected snails isolated. Identification of the species of cryptogonimid harbored by each snail was based on fully developed metacercariae reared out in uninfected sunfishes that had been exposed to cercariae from a single snail.

Observations made from living material, sections, and stained whole mounts formed the basis for descriptions. Vital stains: neutral red, Nile blue, light green, and toluidine blue were used on developmental stages. Adult worms were stained with Semichon's carmine. Sections of paraffin-embedded adults and infected snails were stained with hematoxylin and eosin. Miracidia were obtained from eggs under coverslip pressure. Measurements, except miracidial, were taken from a large series of specimens fixed by the rapid addition of hot 10% formalin. Unless otherwise indicated, measurements, are in micrometers with ranges in parentheses. Drawings were made with the aid of a microprojector.

*Caecincola latostoma* sp. n.

(Figs. 1–3)

TYPE HOST: *Micropterus salmoides* (Lacépède).

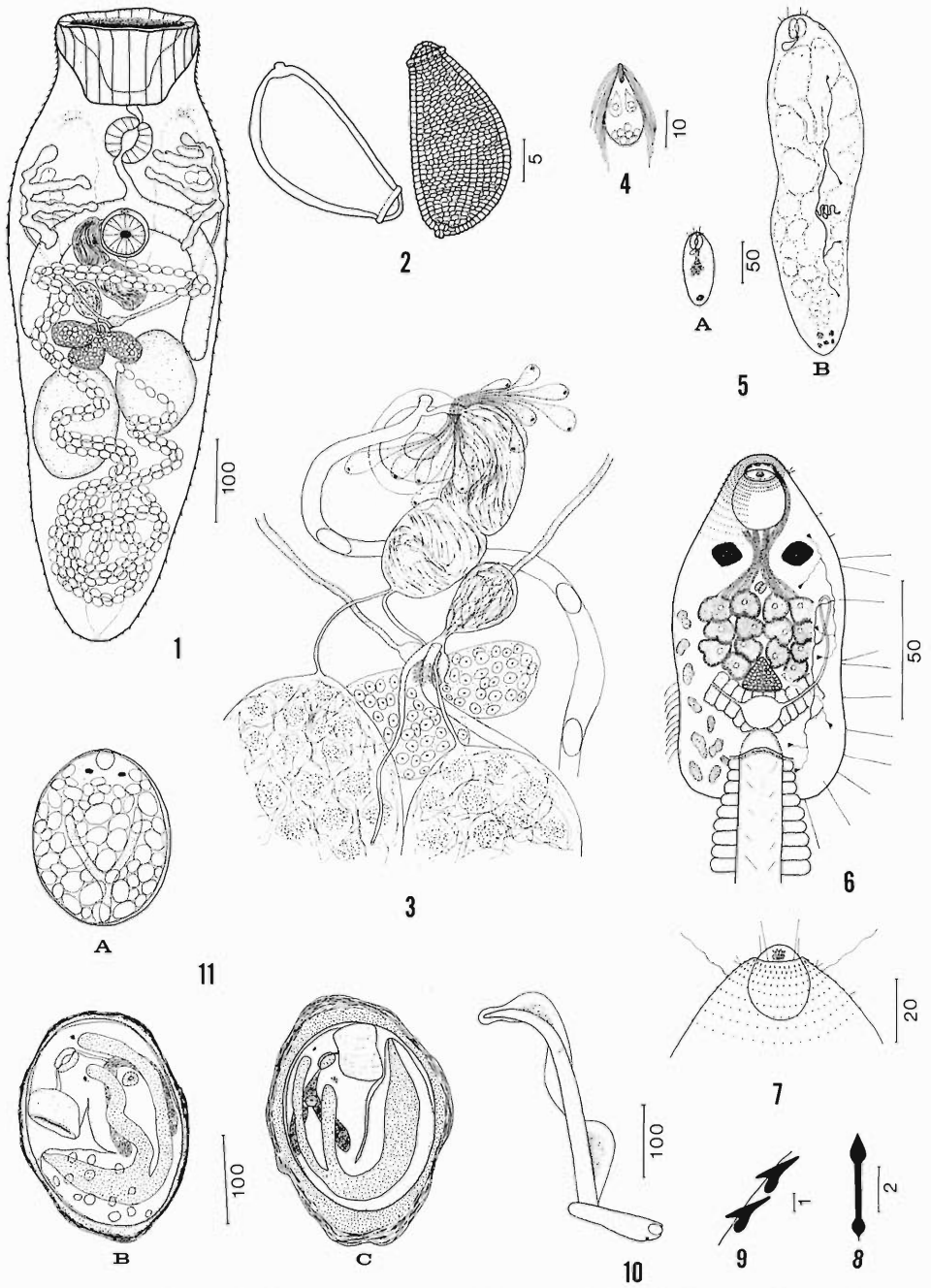
OTHER HOST: *Micropterus punctulatus* (Rafinesque).

HABITAT: Pyloric ceca and intestine.

TYPE LOCALITY: False River, Pointe Coupee Parish, Louisiana.

TYPE SPECIMEN: USNM Helm. Coll.: Holotype no.: 74810; paratype no.: 74811.

DESCRIPTION: Body elongate, 0.656 (0.516–0.856) mm long by 0.272 (0.200–0.336) mm wide. Entire body densely spined. Dermal glands numerous, concentrated in anterior ½ of body. Oculate. Oral sucker cup- to saucer-shaped, 101 (87–134) by 161 (134–184). Mouth terminal. Prepharynx short. Pharynx muscular, 46 (38–58) by 51 (42–64). Esophagus short, bifurcating near posterior margin of anterior ⅓ of body. Ceca short, extend to level of anterior testis. Acetabulum near level of cecal bifurcation, 47 (38–56) by 51 (48–51). Gonotyl absent. Ventrogenital sac present, weakly muscular. Testes oblique, smooth, oval, contiguous, in middle ⅓ of body. Anterior testis, 112 (68–140) by 88 (62–108); posterior testis, 118 (70–160) by 91 (64–120). Seminal vesicle bipartite, sacculate, courses laterally around, and extends slightly anterior to acetabulum. Short, bulbous pars prostatica immediately follows seminal vesicle and receives numerous ducts from unicellular prostatic cells. Short ejaculatory duct unites with metraterm to form common genital duct that opens through a common genital pore slightly anterior to acetabulum. Ovary trilobed, at level of anterior testis in middle ⅓ of body, 57 (40–70) by 103 (60–134). Seminal receptacle sacculate, preovarian. Oviduct ciliated. Proximal half of Laurer's canal ciliated, opens on dorsal midline at level of posterior testis. Vitellaria extending from zone of acetabulum to the level of the pharynx, elongate clumps extend dorsal and ventral to excretory arms. Vitelline ducts course posterior to acetabulum joining to form preovarian vitelline reservoir. Uterus proceeds from ootype posteriorly along left side of body filling



Figures 1–11. *Caecincola latostoma* sp. n. 1. Adult, ventral view. 2. Eggs. 3. Detail of genital complex, dorsal view. 4. Miracidium. 5. Rediae: (A) immature, (B) mature. 6. Cercaria, ventral view. 7. Detail of anterior end of immature cercaria, ventral view with oral sucker protruded. 8. Cercarial boring spine. 9. Cercarial body spines. 10. Cercaria, side view. 11. Metacercariae: (A) 1 day old, (B) 10 days old, (C) 25 days old.

posttesticular region; an ascending loop courses anteriorly along right side of body to level of ovary at which point it crosses to opposite side, recurves to midline, and joins ejaculatory duct dorsal to acetabulum. Eggs small, numerous, textured with scalelike pattern, 24 (22–26) by 12 (11–13), small knob at abopercular end. Excretory bladder large, Y-shaped, bifurcation at level of testes, dilated arms pass ventral to ceca and reach level of pharynx.

**AFFINITIES:** *Caecincola latostoma* most closely resembles *C. parvulus*, the morphology of which has been worked out in detail by Van Cleave and Mueller (1934), Mueller (1934), and Lundahl (1941). However, *C. latostoma* is larger in overall size and distinct in possessing the following features: vitellaria in the form of elongate clusters, a somewhat broader oral sucker, seminal vesicle lateral to acetabulum, and eggs lacking a spinous process. Designation of a new species is also supported by differences in the developmental stages and life histories of *C. latostoma* and *C. parvulus*.

The morphological features of *C. wakullata*, the only other member of the genus, were enumerated by Premvati (1967). Inclusion of *C. latostoma* and *C. wakullata* in the genus *Caecincola* necessitates an expansion of the generic diagnosis that should read as follows: body small, somewhat elongate, spined. Oral sucker terminal, large, funnel to saucer-shaped; prepharynx present or absent; pharynx well developed; esophagus short; ceca short, terminate near level of anterior testis. Acetabulum small, may be recessed into ventrogenital sac. Genital pore immediately anterior to acetabulum. Testes oblique. Seminal vesicle bipartite. Ovary lobed, anterior to or slightly overlapping testes. Seminal receptacle and Laurer's canal present. Vitellaria follicular, in anterior  $\frac{1}{3}$  of body. Uterus extends to near posterior end of body. Excretory vesicle Y-shaped, arms reach near level of pharynx.

### **Redia (Fig. 5)**

Rediae are elongate, opaque, and lack locomotor appendages. Rediae containing eyespotted cercariae, 437 (367–496) by 104 (76–160). Pharynx oval, 27 (24–30) by 25 (24–28). Gut short, sacculate. Birth pore near level of pharynx. Long and short sensory hairs located around anterior end of body. Young rediae possess a set of gland cells, usually 6, located in midbody with ducts that course anteriorly and open into the pharynx. Paired excretory system consists of 2 small bladders that open separately and slightly postequatorially. Short primary trunks divide into anterior and posterior secondary trunks, each receiving capillaries from 2 flame cells. One side of the excretory system is shown in Fig. 5. Rediae of various sizes and stages of development are typically present in infected snails, but only 2 mother rediae, each containing a single daughter redia, were observed.

### **Cercaria (Figs. 6–10)**

Freshwater, pleurolophocercous. Body opaque, devoid of general pigment, oculate, spinous from anterior end to level of eyespots. Body spines with blunt anchor. Body bell-shaped (contracted) to spatulate (extended), 128 (118–136) by 69 (60–80). Eyespots block shaped, 10 (9–11) by 12 (9–14). Tegument covered with several distinct types of hairlike sensory structures which are easily lost as a result of coverslip pressure. Long hairs, about 20  $\mu\text{m}$ , are distributed along body from slightly posterior to eyespots to end of body; curved hairs, 6  $\mu\text{m}$ , are

laterally positioned on body from anterior edge of genital primordium to level of tail socket; 4 pairs of short stubs, 3  $\mu\text{m}$ , are located between the anterior end of body and the anteriormost long hair. Ventral sucker a small, weak muscular depression at level of genital primordium. Protrusible oral sucker, 23 (21–24) by 20 (18–23), possesses a pair of sensory hairs, 8  $\mu\text{m}$ . Seven boring spines, approximately 5  $\mu\text{m}$  in length, are arranged in 2 rows, 3 ventral and 4 dorsal, on the dorsal lip of the mouth. Pharynx poorly developed, posterior to eyespots; remainder of digestive system indiscernible. Numerous cystogenous glands occur in posterior  $\frac{2}{3}$  of body. Seven pairs of penetration gland cells occupy middle  $\frac{1}{3}$  of body. Ducts from these cells converge between eyespots then separate into 4 bundles, 2 dorsal and 2 lateral, which course over and open around the anterior rim of the oral sucker in either a 4:3:3:4 or a 3:4:4:3 arrangement. Triangular genital primordium lies immediately anterior to excretory bladder. Single layer of thick epithelial cells surrounds spherical excretory bladder and basal portion of primary tubules. Primary arms course anteriorly to near level of pharynx then recurve and give off secondary trunks at level of genital primordium. Flame cell formula is 2 (2 + 2) + (2 + 2). Tail, 339 (324–356) by 30 (28–34), inserted on ventral surface in posterior  $\frac{1}{5}$  of body, possesses conspicuous rings in proximal  $\frac{1}{2}$  and 3 fin folds (depicted in Fig. 10).

HOST: *Cincinnatia* (= *Ammicola*) *peracuta* (Pilsbury and Walker, 1889) Thompson, 1968.

HOST CATALOGUE: Del. Mus. Nat. Hist. #120166.

LOCALITY: False River, Pointe Coupee Parish, Louisiana.

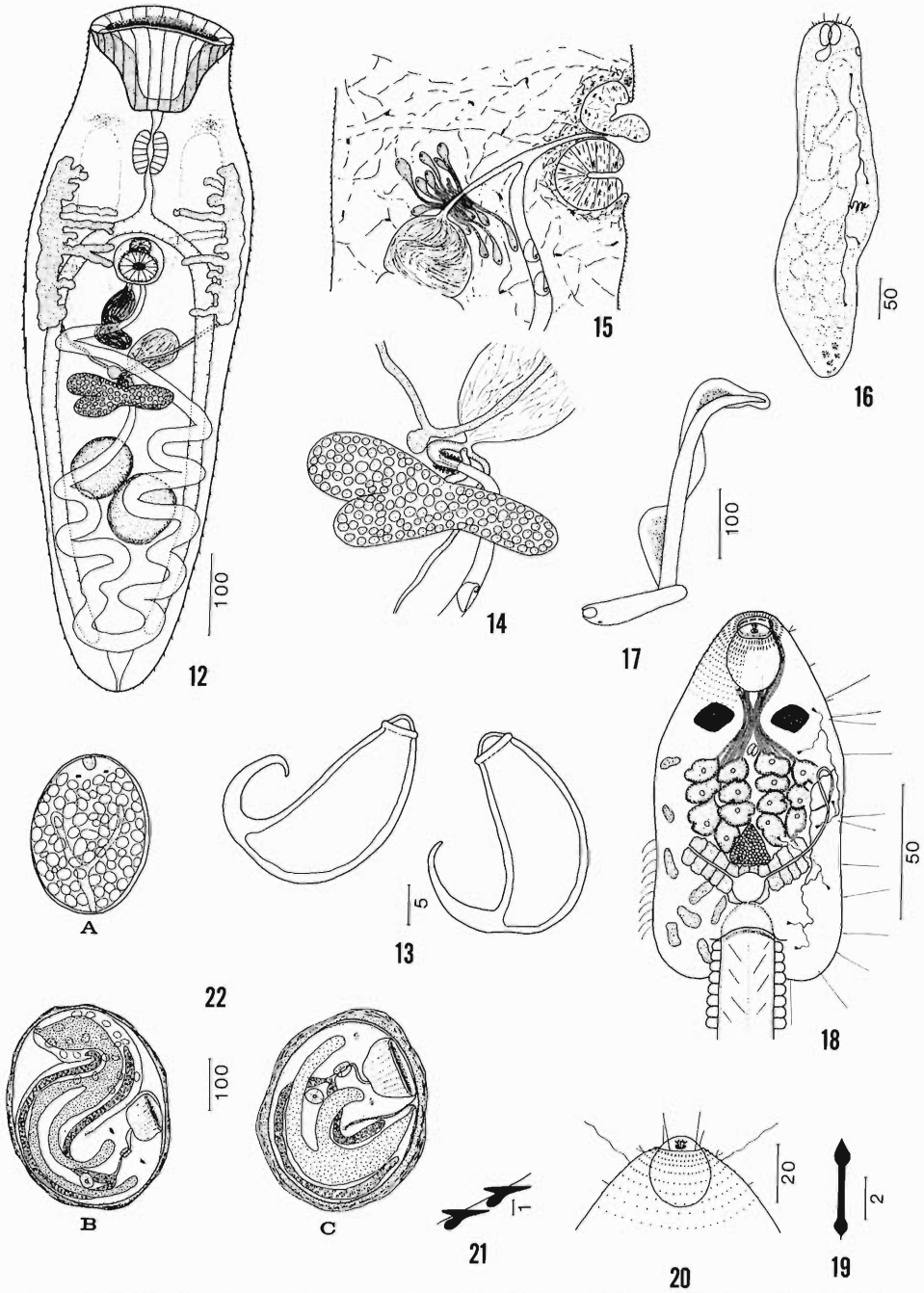
Two commonly encountered and easily distinguishable variants of this cercaria were found. One, with a 4:3:3:4 penetration duct arrangement was produced in 59% of the infected snails, and the other with a 3:4:4:3 arrangement was released from the remainder. Snails shed cercariae of one or the other, but never both forms. Metacercariae and adults obtained from the two cercariae were indistinguishable. These forms most probably represent genetic morphs, since production of the variants could not be correlated with any environmental or host conditions.

Cercariae produced by two snails exhibited another anomalous character. In both cases the relative position of the anterior long hair and posterior stub is reversed on one side of the cercarial body, reminiscent of the normal position of these structures in *Cryptogonimus spinovum* (see below). This arrangement is suggestive of hybridization, but fully developed metacercariae reared from these cercariae were morphologically identical to other *C. latostoma*.

### Metacercaria (Fig. 11)

Metacercariae are usually found encysted in body muscles just beneath the skin and in the fins of *M. salmoides*, *Elassoma zonatum* Jordan, *L. macrochirus*, *L. megalotis*, *L. gulosus* (Cuvier), and *L. symmetricus* Forbes. Young metacercariae are enclosed by a 2  $\mu\text{m}$  thick, transparent cyst of parasite origin. An irregularly shaped host cyst with a maximum thickness of approximately 37 usually develops around older metacercariae. The host cyst is comprised of an inner semifluid layer, a thick fibrous layer, and usually, a thin, partially pigmented, outer layer.

Shortly after encystment, cells of the metacercariae become so enlarged and vacuolated that in 1-day-old metacercariae only the oral sucker and excretory



Figures 12–22. *Cryptogonimus spinovum* sp. n. 12. Adult, ventral view. 13. Eggs. 14. Detail female genital complex, ventral view. 15. Detail terminal genitalia, sagittal section. 16. Redia. 17. Cercaria, side view. 18. Cercaria, ventral view. 19. Cercarial boring spine. 20. Detail of anterior end of immature cercaria, ventral view with oral sucker protruded. 21. Cercarial body spines. 22. Metacercariae: (A) 1 day old, (B) 10 days old, (C) 25 days old.

bladder remain recognizable. Most adult features are discernible in 10-day-old metacercariae, but a few vacuolated cells persist in the posterior end of the body. By 25 days, further development is evident, no vacuolated cells are present, and cysts, containing infective metacercariae at this stage, measure 194 (164–216) by 148 (124–176).

*Cryptogonimus spinovum* sp. n.

(Figs. 12–15)

TYPE HOST: *Micropterus salmoides* (Lacépède).

OTHER HOST: *Micropterus punctulatus* (Rafinesque).

HABITAT: Pyloric ceca and anterior intestine.

TYPE LOCALITY: False River, Pointe Coupee Parish, Louisiana.

TYPE SPECIMEN: USNM Helm. Coll.: Holotype no.: 74808; paratype no.: 74809.

DESCRIPTION: Body elongate, 0.765 (0.560–1.088) mm long by 0.210 (0.168–0.288) mm wide. Oculate. Entire body densely spined. Dermal glands concentrated in anterior ½ of body. Oral sucker cup-shaped, 123 (80–144) by 148 (132–210). Mouth terminal. Prepharynx short. Pharynx muscular, 55 (44–70) by 47 (34–56). Esophagus short, bifurcating near posterior margin of anterior ⅓ of body. Acetabulum preequatorial, slightly posterior to cecal bifurcation, 40 (34–44) by 44 (40–52). Gonotyl either a deep- or shallow-cupped, muscular pad immediately anterior to acetabulum. Gonotyl and acetabulum contained within ventrogenital sac. Testes tandem to slightly oblique, subspherical, contiguous. Anterior testis, 74 (60–94) by 73 (52–112); posterior testis, 79 (60–108) by 75 (56–112). Seminal vesicle bipartite, sacculate, overlaps, and extends posterior to acetabulum but does not reach level of ovary. Short, bulbous pars prostatica immediately anterior to seminal vesicle, receives ducts from numerous unicellular prostatic gland cells. Short ejaculatory duct unites with metraterm to form common genital duct which opens through a common genital pore located between the gonotyl and acetabulum. Trilobed ovary pretesticular, 52 (46–62) by 90 (60–136). Seminal receptacle sacculate, dorsal, and anterior to ovary. Oviduct ciliated. Proximal half of Laurer's canal ciliated, opens middorsally anterior to anterior testis. Vitellaria lateral to ceca, extend from level of pharynx to postacetabular, preovarian region; fingerlike projections extend dorsal and ventral to ceca. Vitelline ducts course posterior to seminal vesicle joining to form vitelline reservoir anterior to ovary. Uterus proceeds sinuously along right side of body to near posterior end where several coils are piled before coursing anteriorly; at level of ovary, the uterus crosses to opposite side and then recrosses, joining the ejaculatory duct dorsal to acetabulum. Eggs smooth, numerous, 25 (23–30) by 11 (10–12). Curved filamentous process drawn from abopercular end of egg at about right angles to longitudinal axis of shell. Excretory bladder large, Y-shaped, bifurcation at level of anterior testis, dilated arms of Y pass ventral to ceca and reach level of pharynx.

AFFINITIES: In general morphology *C. spinovum* most closely resembles its congener *C. chili* Osborn, 1903, but differs in having vitellaria mostly anterior, rather than posterior, to acetabulum; long ceca; and a spined egg without shell sculpturing. The shape, position, and occurrence of the gonotyl is rather diverse



throughout the family Cryptogonimidae and has been used extensively for taxonomic purposes. Consequently, similarities in the gonotyls of *C. spinovum* and *C. chili* are, we believe, indicative of a close affinity between these species.

To accommodate *C. spinovum*, the generic diagnosis is expanded and the following statements replace their counterparts in Yamaguti (1971): Vitellaria extend from acetabulovarian region either anteriorly or posteriorly. Ceca half long to long. Egg with or without spinous process.

#### Redia (Fig. 16)

Rediae are similar in general morphology to those of *C. latostoma*, but mature rediae containing eyespotted cercariae are slightly larger in overall size than those of *C. latostoma*, measuring 423 (320–504) by 100 (88–120); pharynx, 30 (26–34) by 29 (26–30). Notwithstanding the coincidence of rediae at various stages of development in most infected snails, no mother rediae were found.

#### Cercaria (Figs. 17–21)

The cercaria is so similar in appearance to *C. latostoma* cercariae that, except for the differences noted below, the descriptions are identical. *Cryptogonimus spinovum* cercariae are larger in average overall size as indicated by the following measurements: body, 136 (122–146) by 75 (68–84); eyespots, 11 (10–12) by 15 (13–16); oral sucker, 23 (22–26) by 21 (19–22); tail, 341 (332–350) by 27 (24–30). Other morphological differences include: a shift in the position of the anteriormost pair of long sensory hairs; they are located anterior to the eyespots and the posteriormost pair of stubs; all cercariae have a 4:3:3:4 penetration gland duct formula.

HOST: *Cincinnatia* (= *Amnicola*) *peracuta* (Pilsbury and Walker, 1889) Thompson 1968.

HOST CATALOGUE: Del. Mus. Nat. Hist. #120166.

LOCALITY: False River, Pointe Coupee Parish, Louisiana.

#### Metacercaria (Fig. 22)

Metacercariae were recovered from the muscles and often the fins of *M. salmoides*, *E. zonatum*, *L. macrochirus*, *L. megalotis*, and *L. gulosus*. Small bloody lesions could be seen for several days following cercarial penetration. The composition of parasite and host cysts, and metacercarial development is similar to *C. latostoma*, but fully developed metacercarial cysts are larger, measuring 360 (252–448) by 276 (196–348) when 25 days old.

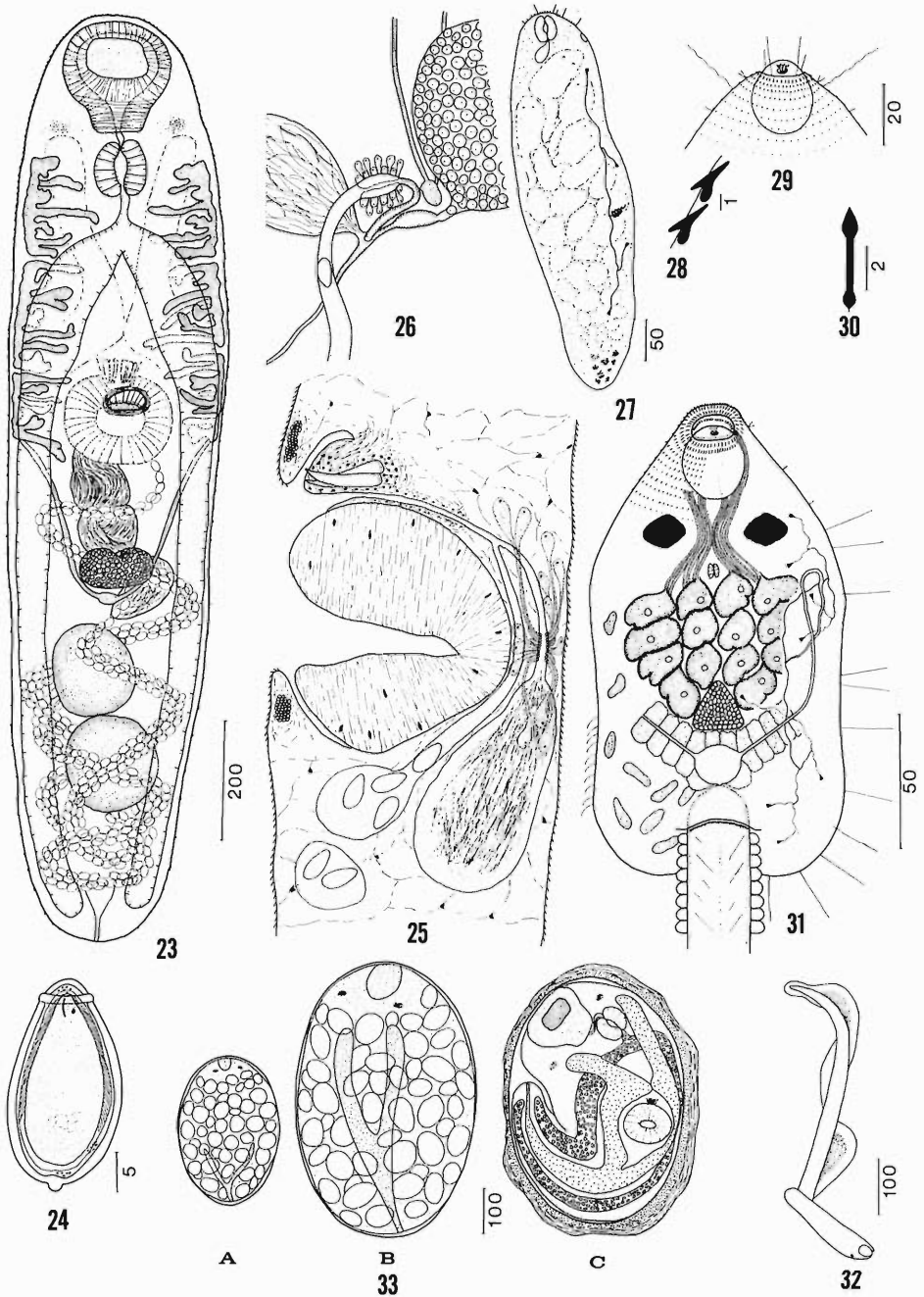
#### *Textrema hopkinsi* Dronen, Underwood, and Sunderman, 1977 (Figs. 23–33)

HOSTS: *Micropterus salmoides* (Lacépède) and *Micropterus punctulatus* (Rafinesque).

HABITAT: Middle one-third intestine.

LOCALITY: False River, Pointe Coupee Parish, Louisiana.

Identification of this worm was verified by comparison with paratype specimens. However, worms collected in Louisiana are larger in overall size as indicated by the body measurements, 1.19 (0.92–1.76) mm long by 0.342 (0.300–0.400) mm wide. These specimens also differ from the original description in the follow-



Figures 23–33. *Textrema hopkinsi*. 23. Adult, ventral view. 24. Egg containing fully developed miracidium. 25. Detail terminal genitalia, sagittal section. 26. Detail female genital complex, side view. 27. Redia. 28. Cercarial body spines. 29. Detail of anterior end of immature cercaria, ventral view with oral sucker protruded. 30. Cercarial boring spine. 31. Cercaria, ventral view. 32. Cercaria, side view. 33. Metacercariae: (A) 1 day old, (B) 10 days old, (C) 25 days old.

ing ways: 2 sets of gonotyl support rods may contain from 2 to 6 rods each, a partially ciliated Laurer's canal opens middorsally above the anterior testis, eggs possess a small abopercular knob.

### Redia (Fig. 27)

Rediae are similar in general morphology to *C. latostoma* but mature rediae containing eyespotted cercariae are larger measuring 448 (383–509) by 107 (88–145); pharynx, 31 (28–34) by 29 (28–30). In spite of the coincidence of small and large rediae in most infections, no mother rediae were seen.

### Cercaria (Figs. 28–32)

Although slightly larger in overall size, the cercaria of *T. hopkinsi* is so similar to *C. latostoma* cercariae that the descriptions differ only by substitution of the following: body, 178 (163–204) by 94 (84–104); eyespots, 12 (11–14) by 16 (13–18); oral sucker, 26 (24–27) by 23 (21–35); tail, 422 (370–470) by 30 (28–34). All cercariae possess a 4:3:3:4 penetration gland duct formula.

HOST: *Cincinnatia* (= *Amnicola*) *peracuta* (Pilsbury and Walker, 1889) Thompson, 1968.

HOST CATALOGUE: Del. Mus. Nat. Hist. #120166.

LOCALITY: False River, Pointe Coupee Parish, Louisiana.

### Metacercaria (Fig. 33)

Metacercariae are usually found deep in the body muscles and rarely in the fins of *M. salmoides*, *E. zonatum*, *L. macrochirus*, and *L. gulosus*. Bloody lesions are apparent on fish for up to 2 weeks after cercarial penetration and encystment.

Metacercarial development and cyst composition are basically similar to *C. latostoma*, but *T. hopkinsi* is larger and develops more slowly. One-day-old metacercariae resemble *C. latostoma*, but unlike the latter, the oral sucker and excretory bladder remain the only recognizable internal structures in 10-day-old *T. hopkinsi* metacercariae. Vacuolated cells are not present by 25 days, and all adult features, except the vitellaria, are easily discernible; these metacercariae are infective and cysts measure 542 (512–576) by 346 (264–400).

## Discussion

### Affinities and biology of developmental stages

MIRACIDIA: The eggs of *C. latostoma*, *C. spinovum*, and *T. hopkinsi* are fully embryonated when released by adult worms, but shell density prevents any detailed study of the unhatched miracidium. Miracidia that are obtained by crushing the egg capsule are short lived, thus allowing only cursory observations.

Miracidia of *C. latostoma*, depicted in Figure 4, are pyriform and measure approximately 23 by 8. The anterior two-thirds of the body, except for the apical papilla, is covered with cilia about 15 in length. A pigment spot is located near the anterior end and two gland cells occupy the midbody. Miracidia of both *C. spinovum* and *T. hopkinsi* have a similar morphology.

REDIAE: General morphology and location of *C. latostoma*, *C. spinovum*, and *T. hopkinsi* rediae agree with previous reports for other cryptogonimids, but differences are apparent in the biology of redial infections. Lundahl (1941) re-

ported a synchronous maturation and only several weeks of cercarial production by *C. parvulus* in the snail host. Contrary to this, we found that snails typically harbor rediae of various sizes and stages of development, and that cercariae of *C. latostoma*, *C. spinovum*, and *T. hopkinsi* were usually shed for several months or until death of the snail. These findings suggest that more than one redial generation is produced, but evidence of such production is scanty. Only two *C. latostoma* mother rediae were encountered during our investigations and no *C. spinovum* or *T. hopkinsi* mother rediae were found. Cable (1934), similarly unable to establish the source of redial production for the heterophyid *Cryptocotyle lingua*, speculated that young rediae may originate either from persistent, and perhaps diffuse, sporocysts or as the result of multiple infections. Neither possibility seems a likely explanation for our observations, since on the one hand, no evidence of sporocysts was found, and on the other, the high percentage of multiple infections required by our data make this an untenable explanation. The source of redial production in these species remains an enigma.

Comparison of infected and uninfected snails revealed no particular size or sex related susceptibility of the snail host to *C. latostoma*, *C. spinovum*, or *T. hopkinsi*. No gigantism was noted in infected snails, but parasitic castration was common. Castration not only affected sperm production, but the verge was usually reduced and often deformed.

**CERCARIAE:** Although *C. latostoma*, *C. spinovum*, and *T. hopkinsi* cercariae possess many features typical of other cryptogonimid cercariae, they do exhibit characteristics which set them apart. These three cercariae most closely resemble the cercaria of *C. parvulus* described by Lundahl (1941), but differ in the number and arrangement of boring spines. *Caecincola parvulus* is equipped with nine spines located on the dorsal lip of the mouth, three in a ventral row, and six in a dorsal row. In contrast *C. latostoma*, *C. spinovum*, and *T. hopkinsi* exhibit a three ventral and four dorsal arrangement.

All cryptogonimid cercariae are alike in possessing seven pairs of penetration gland cells, but ducts from these cells open around the lip of the oral sucker in two distinct patterns. One of the *C. latostoma* morphs, *C. spinovum*, and *T. hopkinsi* have a 4:3:3:4 formula, while the other *C. latostoma* morph and *C. parvulus* exhibit a 3:4:4:3 arrangement.

In addition to these morphological differences, behavioral differences were also evident. The emergence of *C. latostoma*, *C. spinovum*, and *T. hopkinsi* cercariae is elicited by light, while *C. parvulus* emerges during periods of darkness (Lundahl, 1941). Cercariae of the former three species are photopositive and display rapid, sporadic swimming which can be stimulated by shadowing the cercariae.

*Caecincola latostoma*, *C. spinovum*, and *T. hopkinsi* cercariae dissected from snails possess two pairs of sensory hairs not present on those that emerge naturally. A 20-long hair, exhibiting a flagellarlike motion, is located near the anterior end of the body. The second pair, about 11 long, occurs on the ventral side of the oral sucker and is only visible when the sucker is protruded (Figs. 7, 20, and 29). These hairs are lost either immediately before or after emergence, since neither pair was seen on emerged cercariae.

**METACERCARIAE:** Although the morphological differences between mature metacercariae of *C. latostoma*, *C. spinovum*, and *T. hopkinsi* are pronounced, the general course of development and the fish hosts employed are similar. Subtle

differences were noted in the relative rates of development, location, and pathology in experimentally infected fishes. *Caecicola latostoma* metacercariae are the most rapid in development. They are found predominantly in the fins and in muscles just beneath the skin, and leave little pathological evidence of penetration and encystment. Metacercariae of *Cryptogonimus spinovum*, intermediate in developmental rate, occur most commonly in the body muscles but also frequently in the fins, and produce small bloody lesions which persist for several days following infection. *Textrema hopkinsi* metacercariae are the slowest in development, occur deep in the body muscles and rarely in the fins. They produce bloody lesions that may be visible for 2 weeks following cercarial penetration and encystment.

### Subfamily designation

Lundahl (1941) presented a thorough historical resume on the placement and affinities of *Caecicola* and *Cryptogonimus*. It is clear from Lundahl's account that, while family designation was often disputed, most authors concurred that a close relationship exists between these genera and included them in the same subfamily. More recently, Yamaguti (1971) assigned *Caecicola* and *Cryptogonimus* to different subfamilies by designating the former to the monotypic subfamily Caecincolinae.

Dronen et al. (1977) placed *Textrema* in the family Cryptogonimidae and pointed out its resemblance to the genus *Multigonotylus*.

According to Yamaguti's (1971) diagnosis, members of the Cryptogoniminae consistently differ from Caecincolinae in having vitellaria situated in a more posterior position and by possessing a funnel-shaped, rather than saucer-shaped, oral sucker. With inclusion of *C. spinovum* in the subfamily Cryptogoniminae, only tenuous differences in the shape of the oral sucker may be used to separate members of these subfamilies. Furthermore, the life cycle similarities indicate a close relationship among *C. latostoma*, *C. spinovum*, and *T. hopkinsi*. We, therefore, propose that Caecincolinae be suppressed and its members, *Caecicola* and *Turgecaecum*, be included, along with *Textrema*, in the subfamily Cryptogoniminae. The amended subfamily diagnosis is:

### Cryptogoniminae Osborn, 1903

Cryptogonimidae. Body more or less elongate. Circumoral spines absent. Oral sucker funnel to saucer-shaped; prepharynx present or absent; pharynx present; esophagus short or long; cecal bifurcation pre- or postacetabular; ceca short or long. Acetabulum median usually contained within a ventrogenital sac. Gonotyl present or absent. Testes slightly oblique, opposite, spherical, or divided into longitudinal series of lobes. Ovary compact or lobate. Vitellaria follicular to dendritic, acetabular and more extensive, or clumped pre- or postacetabular.

### Acknowledgments

We wish to thank Dr. Fred G. Thompson of the Florida State Museum for his assistance in identification of hydrobiid snails.

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## The Morphology of Crystalline Inclusions in Primary Oocytes of *Aspidogaster conchicola* von Baer, 1827 (Trematoda: Aspidobothria)

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**ABSTRACT:** Electron microscopy of primary oocytes of *Aspidogaster conchicola* revealed numerous crystalline cytoplasmic inclusions. These inclusions first appear in association with aggregates of free ribosomes in a cytoplasmic region surrounded by whorls of rough endoplasmic reticulum. Their fine structure is described and compared with similar crystals reported from a variety of animal oocytes.

A comprehensive anatomical study of *Aspidogaster conchicola* by Stafford (1896) greatly expanded earlier studies by Huxley (1856) and Voeltzkow (1888). Stafford's account of the female reproductive system was precise, and his description of the position of oogonia and primary oocytes within the ovary was accurate. But, aside from their size and nuclear morphology, these cells were not described in detail.

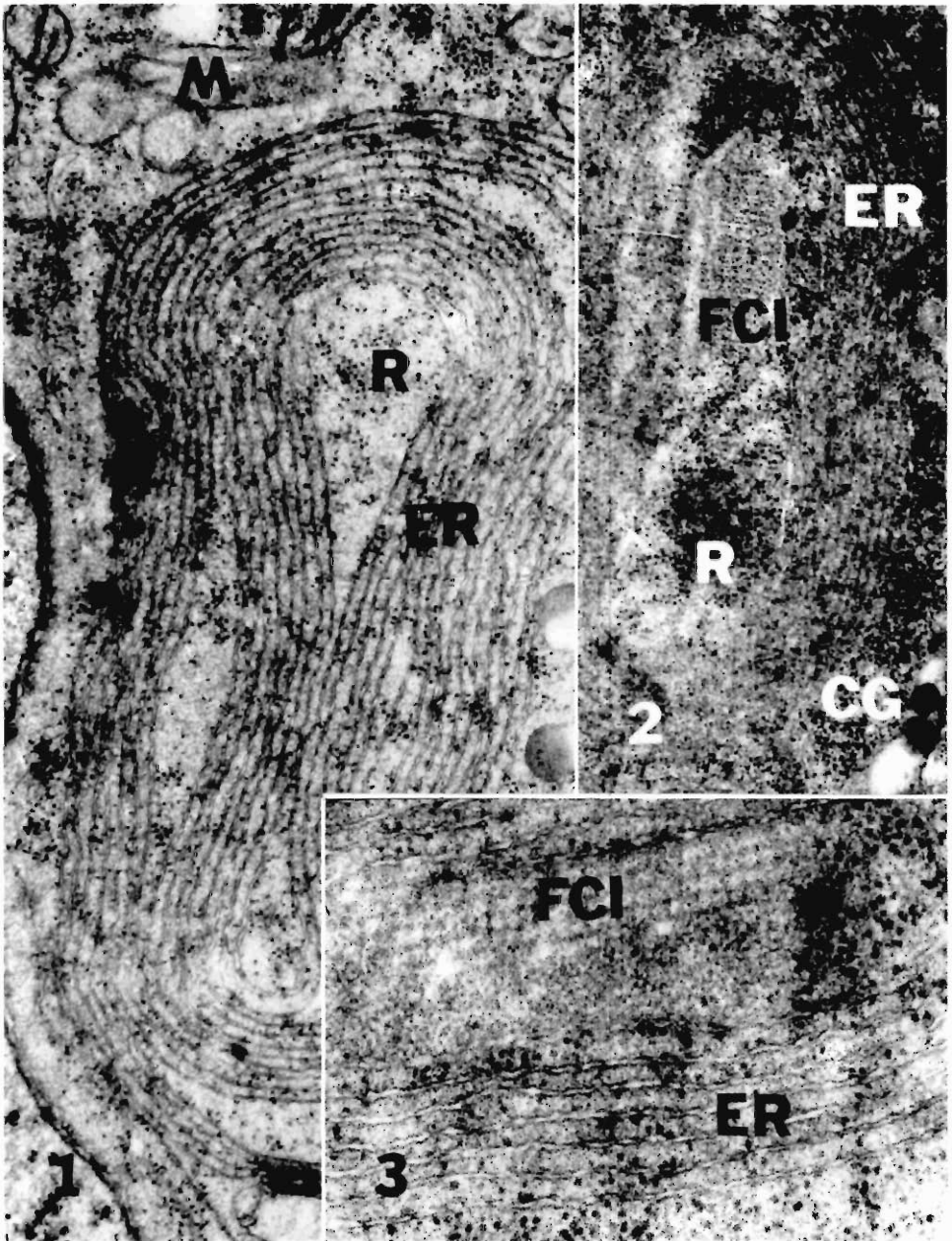
In a more recent study I found previously undescribed cytoplasmic inclusions in primary oocytes of *A. conchicola* from sections of Epon embedded material stained with toluidine blue and viewed with the light microscope. The present electron microscope study describes these inclusions. Materials and preparation methods used in this work follow Hathaway (1972).

### Observations

The cytoplasm of primary oocytes contains the usual assortment of organelles characteristic of eucaryotic cells including abundant mitochondria, rough endoplasmic reticulum, Golgi bodies, and free ribosomes. Membrane-bound vesicles, also common, have contents which appear compact and electron dense at one end and flocculent and nearly electron transparent at the other. These vesicles are usually found at the periphery of the cell (Figs. 2, 6), form in association with Golgi bodies (Fig. 7), and generally fit the description of cortical granules.

Primary oocyte cytoplasm also contains large and prominent inclusions having a crystalline appearance. These inclusions first appear in regions of cytoplasm surrounded by whorls of rough endoplasmic reticulum which surround numerous free ribosomes (Fig. 1). The first indication of an organized crystalline lattice appears in close proximity of these sequestered free ribosomes (Figs. 2, 3). As the crystal lattice becomes more compact and highly ordered, the whorled configuration of the endoplasmic reticulum is no longer observed surrounding the crystal.

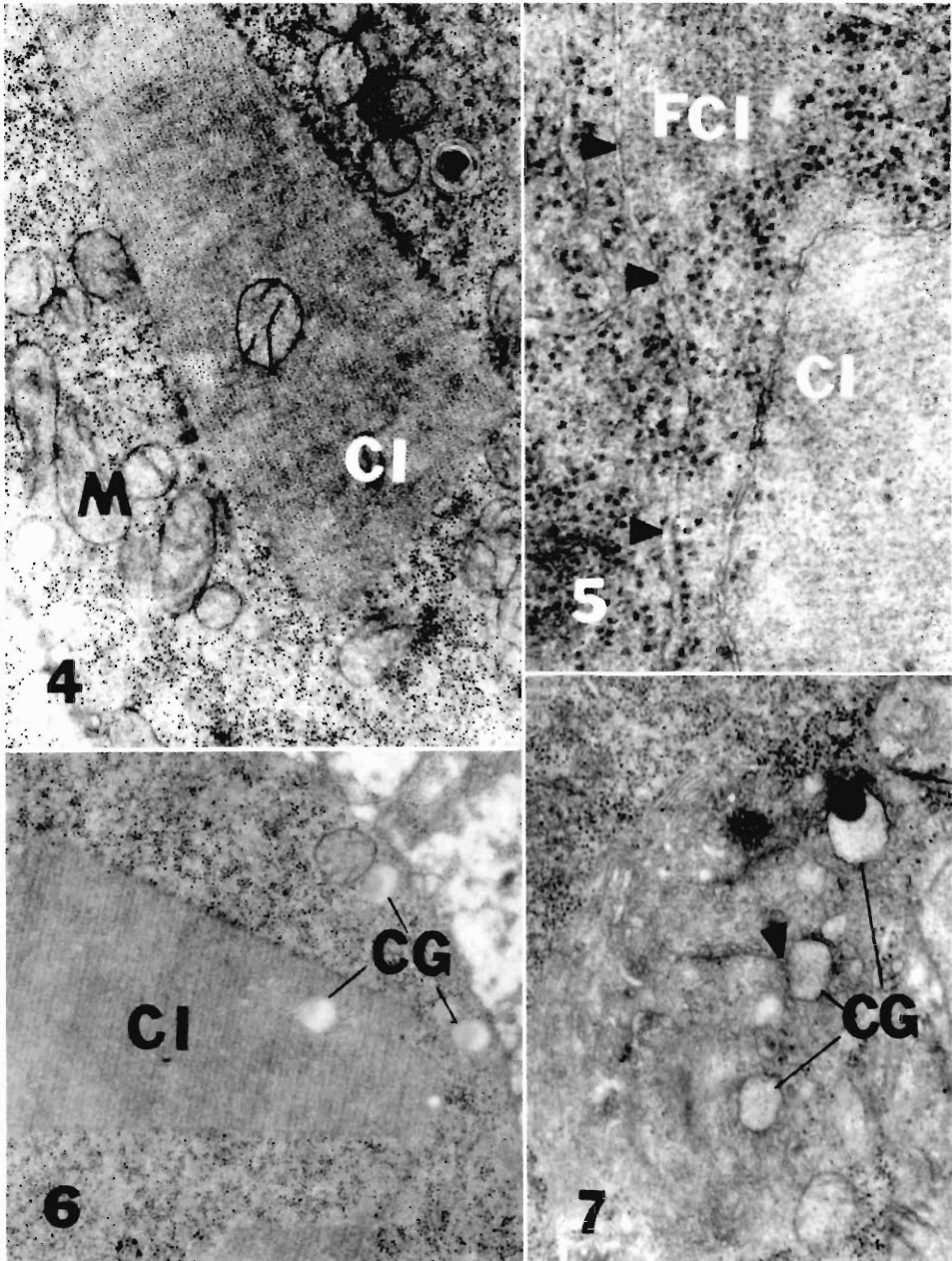
The matrix of the inclusion is laid down in parallel layers and shows a definite periodicity in which two distinct patterns have been observed, a dot pattern and a banded pattern. The dot pattern is characterized by evenly spaced, electron dense dots separated on all sides by a lighter interspace (Fig. 4). The banded pattern consists of straight, parallel dense lines traversing the crystal matrix and separated by a lighter interspace (Fig. 6). In some inclusions a secondary system of bands, also parallel but less distinct, intersects the primary band system (Fig.



Figures 1–8. Electron micrographs of primary oocytes of *Aspidogaster conchicola*. Abbreviations: CG, cortical granules; CI, crystalline inclusion; ER, rough endoplasmic reticulum; FCI, forming crystalline inclusion; M, mitochondria; R, free ribosomes.

Figures 1–3. 1. Whorls of rough endoplasmic reticulum (ER) surrounding a region of cytoplasm containing free ribosomes (R). It is in this region that crystals first appear.  $\times 35,000$ . 2. The crystal lattice of a forming inclusion (FCI) begins to become apparent. At the same time, the well-developed whorls of endoplasmic reticulum are becoming less apparent in the upper left and lower central region of the micrograph. Two cortical granules (CG) are at the periphery of the oocyte.  $\times 24,500$ . 3. A forming crystalline inclusion (FCI) demonstrating its position with respect to the rough endoplasmic reticulum (ER) and free ribosomes (R).  $\times 70,000$ .





Figures 4-7. A triangular shaped crystalline inclusion (CI) with a mitochondrion in its matrix. More commonly, mitochondria are found outside the crystal around its edges. Note the crystal matrix appears as a series of dark dots against a lighter background.  $\times 20,000$ . 5. Crystalline inclusions (CI) are surrounded by a double membrane similar to that of the endoplasmic reticulum. The arrows designate membranes of rough endoplasmic reticulum bordering a forming crystal (FCI) and extending into the cytoplasm.  $\times 70,000$ . 6. A crystalline inclusion (CI) demonstrating the banded pattern. Cortical granules (CG) are usually found near the periphery of the oocyte, but occasionally one is observed in the crystal matrix.  $\times 20,000$ . 7. Cortical granule formation is associated with Golgi bodies. The granule near the arrow appears as a swollen continuation of a Golgi membrane. Fully formed granules are membrane-bound with a compact and electron dense content at one end and a flocculent and nearly electron transparent substance at the other.  $\times 28,000$ .

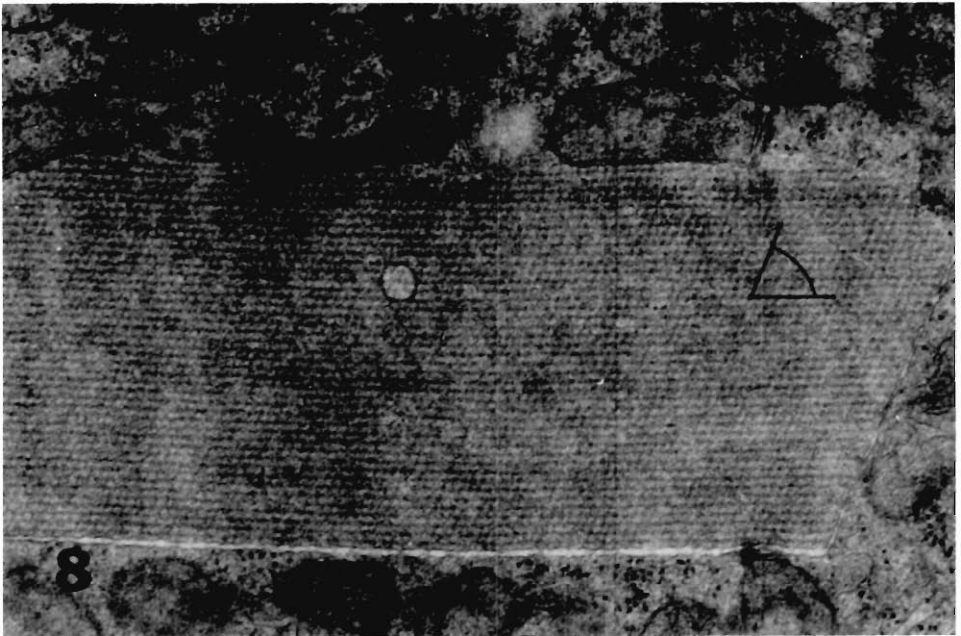


Figure 8. A crystalline inclusion illustrating the banded pattern. The acute angle of interception between primary and secondary band systems is  $67^\circ$ .  $\times 45,000$ .

8). The acute angle of interception between primary and secondary band systems is  $67^\circ$ . The center-to-center distances between dense lines (banded pattern) and dots (dot pattern) is in the range of 275–290 Å.

Occasionally mitochondria occur within a crystal (Fig. 4), though they are most abundant outside and around the edges of crystals. Rarely, cortical granules are observed within the crystal matrix (Fig. 6). However, neither mitochondria nor cortical granules, whether found within the crystal matrix or free in the cytoplasm, show internal organization similar to that of the crystal.

Crystal size varies and is difficult to ascertain in thin sections. However, many have been observed whose greatest length exceeds  $5 \mu\text{m}$ . Shape also varies, though most crystals are rectangular or triangular. Each is enclosed by a double membrane having the same appearance as membranes of the endoplasmic reticulum. The limiting membranes of some crystals, presumably in a formative state, can be seen trailing off into the cytoplasm where they are associated with ribosomes and are obviously a part of the endoplasmic reticulum (arrows, Fig. 5).

#### Discussion

There have been several reports of deeply staining cytoplasmic inclusions in trematode oocytes. Schubmann, in 1905, and Schellenberg, in 1911, noted their presence in *Fasciola hepatica* (Markell, 1943). Markell (1943) mentioned granules in oocyte cytoplasm of *Probolitrema californiense* and concluded they function as reserve food bodies. Burton (1960, 1967) described nucleolus-like bodies in oocytes of *Haematoloechus medioplexus* from light and electron microscopy. Koulisch (1965) reported on the fine structure of similar bodies in *Gorgoderina*

*attenuata*, and Grant et al. (1977) noted nucleolus-like bodies in oocyte cytoplasm of *Pharyngostomoides procyonis*. Koulisch (1965) regarded these bodies as masses of ribosome-like particles which may produce nutrient proteins for the developing embryo, but their function remains uncertain. The difference in the ultrastructure of these nucleolus-like bodies as compared to the crystalline inclusions herein described is striking. Further, the crystals, unlike nucleolus-like bodies, are membrane bound.

Crystalline inclusions similar to those presently described have been noted by several authors from a variety of animal oocytes. In a detailed study of amphibian oocytes Karasaki (1963) described the fine structure of yolk platelets from five different species. He found the platelets to have a crystalline structure which demonstrated both a dot and banded pattern. In some of the crystals having the banded pattern, he noted the presence of a primary and secondary system of bands having an acute angle of interception ranging from 20–90° depending on the species studied. He further noted that the periodic pattern displayed by the crystal yolk platelets (banded or dot) depended entirely on the angle at which the yolk platelet was sectioned and was not due to differences in crystal structure. The center-to-center spacing between dots and bands varied slightly for the amphibians studied, but it averaged 70 Å between repeating bands and 81 Å between adjacent dots.

A similar study of yolk platelets in oocytes of *Rana pipiens* (Ward, 1962) also demonstrated a banded and dot pattern inherent to the crystals. Ward measured the acute angle of interception between primary and secondary bands at 65° with an average spacing between bands ranging from 70–85 Å.

Among invertebrate animals Ward (1962) notes that Favard and Carasso measured a period of 85 Å in crystal yolk bodies of the fresh water snail *Planorbis*, and Elbers found a periodicity of 50 Å in protein inclusions of *Lymnaea stagnalis* oocytes. Taylor and Anderson (1969) reported a crystalline appearance in yolk bodies of the gastropod, *Ilyanassa obsoleta*. They mentioned a "fine-banded or striated pattern" associated with the yolk crystals, but did not provide a detailed description or measurements.

Boyer (1972) studied oocyte differentiation in the polyclad turbellarian, *Prostheceraeus floridanus*. She demonstrated the mechanism of yolk formation and showed yolk bodies to be surrounded by membranes derived from the endoplasmic reticulum. Yolk bodies were shown to have "ordered structures" resembling a crystal lattice, but only after extensive digestion (18 hr) with pronase. She considered the ordered structures to be a framework laid down during vitellogenesis upon which protein is deposited.

Based on their morphology and the fact that enzyme digestion was not necessary to demonstrate their highly ordered nature, the interesting observation emerges that crystalline inclusions in *A. conchicola* oocytes resemble crystals described from amphibia and gastropods more closely than the ordered structure associated with yolk bodies in the polyclad, *Prostheceraeus floridanus*. The basic difference between inclusions found in *A. conchicola* and those reviewed from amphibia and gastropods is in the center-to-center spacing between subunits comprising the crystals. In amphibian yolk platelets Karasaki (1963) was of the opinion that alternating dense and light regions (seen as a dot or banded pattern) were due to the regular packing of macromolecules forming the crystal lattice. If such

a view is correct, the increased distance between bands and dots in *A. conchicola* crystals may reflect the presence of different ratios and kinds of macromolecules.

The presence of numerous crystalline inclusions in the oocyte cytoplasm as well as their similarity to yolk crystals described from other animals suggests that these inclusions may represent yolk platelets in *A. conchicola*.

#### Acknowledgments

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## Trematode Parasites of the Opossum, *Didelphis virginiana*, from Florida

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**ABSTRACT:** Six different species of trematodes were recovered from the opossum *Didelphis virginiana* from Florida. Of these one is a new species, namely *Brachylaima didelphus* sp. n., characterized by having vitellaria extending from the level of pharynx to the hind end of the body. *Rhopalias macracanthus* Chandler, 1932 is redescribed to show differences in the body size and size of various organs; the absence of seminal receptacle; presence of separate male and female genital pores, and the presence of gland cells on either side of prepharynx with their ducts opening in the proboscis sacs.

The other four species recovered are: *Parascocotyle lageniformis*, the first record from Opossum; *Didelphodiplostomum variable*; *Fibricola cratera*; and *Neodiplostomum lucidum*.

During 1966-1967, nine opossum, *Didelphis virginiana* Kerr, from Leon County, Florida, were examined for helminth parasites. Six different species of trematodes recovered from the intestine are reported here. Of these, one is a new species, another is redescribed to show certain variations, and for a third, a new host is reported. The trematodes were studied alive as well as in whole mounts after fixing in hot AFA and staining in Semichon's carmine or aceto-alum carmine. For sections, specimens were fixed in hot Bouin's fluid and stained in hematoxylin and eosin. All measurements are in micrometers unless otherwise stated.

### *Brachylaima didelphus* sp. n. (Fig. 1)

HOST: *Didelphis virginiana* Kerr.

LOCALITY: Leon County, Florida, USA.

LOCATION: Intestine.

NUMBER OF WORMS: Three from one host.

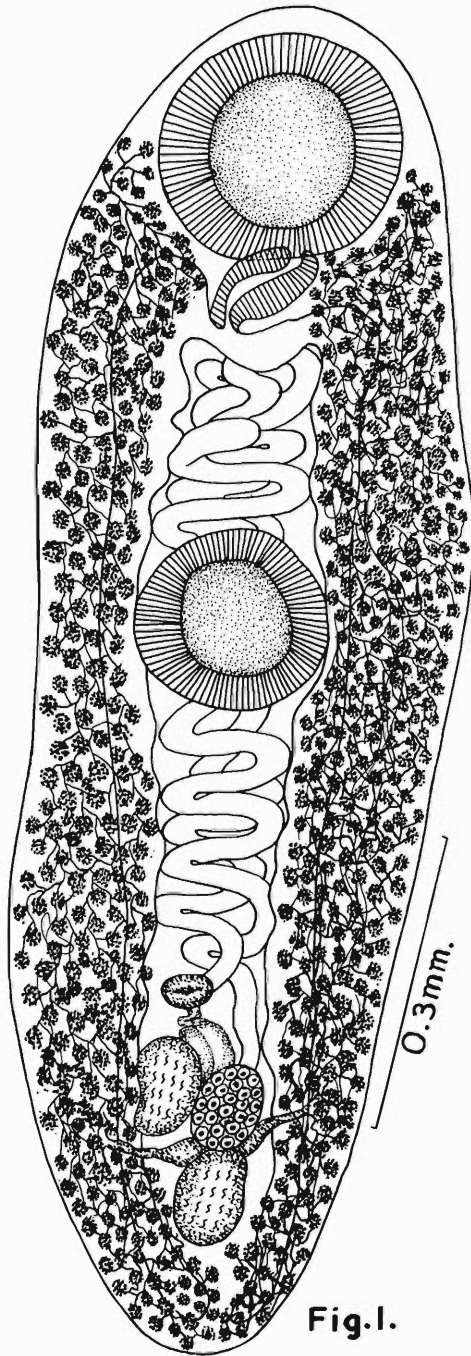
TYPE SPECIMEN: Holotype in USNM Helminth. Coll. No. 75090; paratype 75091.

DESCRIPTION: Body 1.38 mm in length and 470 in width, distance between anterior end and ventral sucker 490, and between ventral sucker and posterior end 680. Oral sucker subterminal, 230 in diameter; ventral sucker in anterior 1/2 of body, 190 in diameter. Pharynx 130 by 60, large, oval; prepharynx and esophagus absent, ceca terminating at posterior end of body.

Testes 90 to 100 by 70 to 75, slightly oval, diagonal, intercecal, near posterior end of body; both equal or anterior one slightly smaller. Cirrus sac small, seminal vesicle outside cirrus sac. Genital pore anterior to anterior testis. Ovary 85 by 65, to left of anterior testis or intertesticular. Uterus intercecal, extends forward to intestinal bifurcation. Vitellaria follicular, lateral, and extending from pharynx to posterior end of body; vitelline reservoir intertesticular. Eggs not present because of immature specimens.

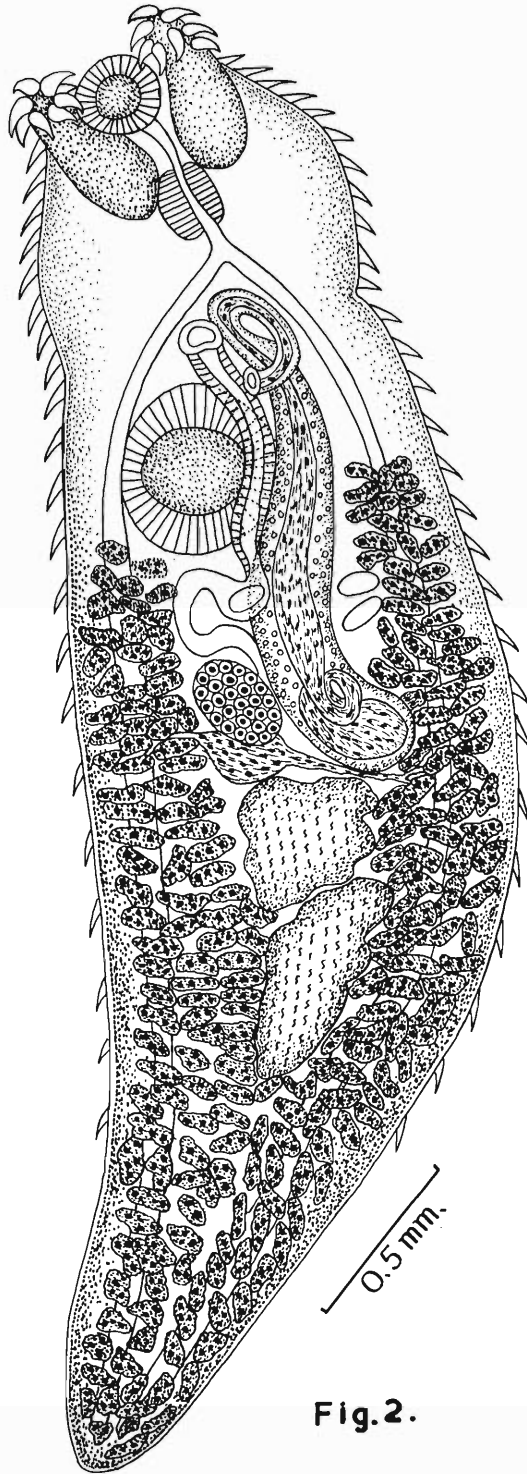
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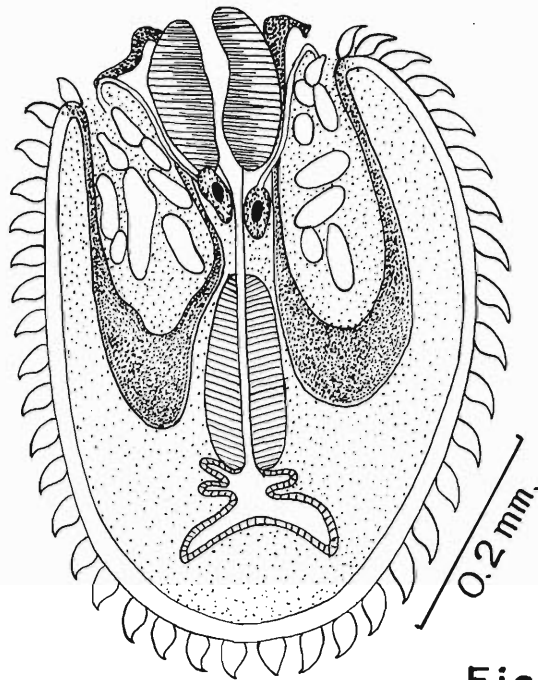
**Fig. 1.**

**Figure 1.** *Brachylaima didelphus* sp. n. Holotype, ventral view.



**Fig. 2.**

**Figure 2.** *Rhopalias macracanthus*, dorsal view.



**Fig.3.**

Figure 3. *Rhopalias macracanthus*, frontal section from the anterior region to show proboscis sacs, proboscis, glands, oral sucker, prepharynx, pharynx, and bifurcation of intestinal ceca.

#### Discussion

Three species of *Brachylaima* have been described from opossum (*Didelphis virginiana*) namely *B. opisthotrias* (Lutz, 1895) Dollfus, 1935, *B. spinulosus* (Hoffmann, 1899) Krull, 1933, and *B. virginianus* (Dickerson, 1930) Krull, 1934. *B. didelphus* sp. n. resembles *B. virginianus*, but differs most strikingly in the disposition of vitellaria in extending from pharynx to posterior end, while in the latter vitellaria begin immediately behind ventral sucker and extend to the level of anterior testis.

Since the position of vitellaria is thought to be a character of specific value, *Brachylaima didelphus* is regarded as a new species.

#### *Rhopalias macracanthus* Chandler, 1932

(Figs. 2-4)

#### Redescription

The present species is being redescribed because of: (1) variation in sizes, (2) separate male and female genital pores, (3) absence of seminal receptacle, and (4) presence of gland cells located on either side of prepharynx, with their ducts opening in the proboscis sacs—these have been described for the first time in the genus *Rhopalias*.

HOST: *Didelphis virginiana* Kerr.



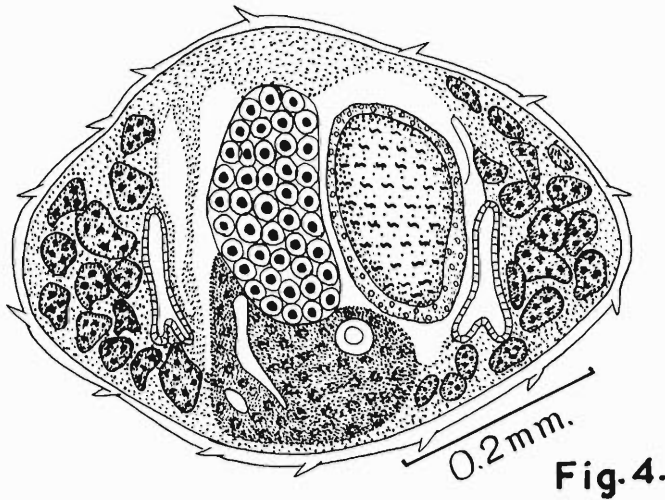


Figure 4. *Rhopalias macracanthus*, cross section from the region of ovary, cirrus sac, and shell glands.

LOCALITY: Leon County, Florida, USA.

LOCATION: Intestine.

NUMBER OF WORMS: Eight from three hosts.

SPECIMENS DEPOSITED: USNM Coll. No. 75092.

DESCRIPTION (based on four whole mounts, cross sections of two, and frontal sections of two): Body elongate, spinous, measures 3.0 to 3.72 mm in length and 1.00 to 1.18 mm in width; hind body 2 to 2½ times longer than forebody. Well-developed retractable proboscis sac on either side of oral sucker enclosing a small spiny proboscis. Wall of proboscis sac continuous with body wall; proboscis 500 to 700 in length, armed with 8 to 10 spines; largest spines posterior and measure 130 to 150 by 30 to 35; anterior smaller spines measure 30 to 40 (Fig. 2). Duct coming from a unicellular gland located on either side of prepharynx, open into each proboscis sac (Fig. 3). Oral sucker 200 by 220 to 230; ventral sucker 400 to 420 by 430 to 440. Prepharynx 160 to 170 in length; pharynx oval, 140 to 150 by 200 to 230; esophagus short, bifurcating into intestinal ceca terminating at posterior end of body.

Testes tandem, slightly lobed, in middle of hindbody. Anterior testis usually broader than long, 270 to 310 by 410 to 510; posterior testis elongate, usually trilobed, 410 to 440 by 470 to 610. Cirrus sac very large, extend up to margin of anterior testis, and open at genital pore located in space between ventral sucker and intestinal bifurcation. Seminal vesicle well developed; pars prostatica greatly elongated; prostate glands present. Ovary oval, 240 to 260 by 150 to 170, pretesticular, postacetabular, submedian, and located in middle of body. Seminal receptacle absent. Shell glands between ovary and anterior testis. Uterus entirely pretesticular, occupying area between ovary and genital pore; metraterm well developed. Vitellaria follicular, extend from level of ventral sucker, along sides of body, confluent posterior to testes. Eggs 94 to 98 by 52 (20 measured).

### Discussion

The opossum of the genus *Didelphis* seems to be a common host for the genus *Rhopalias*. Five species of the genus *Rhopalias* Stiles and Hassall, 1898 have been described from mammals; *R. coronatus* (Rudolphi, 1819) Stiles and Hassall 1898; *R. horridus* (Diesing, 1850) Stiles and Hassall 1898; *R. baculifer* Braun, 1901; *R. macracanthus* Chandler, 1932; and *R. louisiana* Hearin, 1938. As a published account was not available for the species *R. louisiana*, comparison of this species could not be made. *Rhopalias coronatus* is characterized by very large proboscis sac, extending up to the level of intestinal bifurcation or even to the acetabulum, and with the spines arranged in a row. In the other three species the proboscis sacs are small and do not extend beyond the level of the pharynx. *Rhopalias horridus* is differentiated from the other two species by the presence of numerous small spines on the proboscis. *Rhopalias baculifer* measures 10 to 12 mm as compared to *R. macracanthus* having a length of less than 5 mm. The number and size of the spines on the proboscis also differ in the two species. In *R. baculifer*, the larger spines measure 729, and the smaller ones 260. In *R. macracanthus*, having 10 spines, larger and smaller spines are 125 and 20 respectively according to Chandler and 130 and 100 according to Byrd et al. (1942), and by the present study the spines measure 130 to 150 and 30 to 40 respectively.

#### *Parascocotyle lageniformis* (Chandler, 1941) Morozov, 1952

NUMBER OF WORMS: Three from one host.

This is the first record of this species from opossum.

#### *Didelphodiplostomum variabile* (Chandler, 1932) Dubois, 1945

NUMBER OF WORMS: Ten from one host.

#### *Fibricola cratera* (Baker and Noll, 1915) Dubois, 1932

NUMBER OF WORMS: Fifteen from two hosts.

#### *Neodiplostomum lucidum* LaRue and Bosma, 1927

NUMBER OF WORMS: Ten from one host.

### Acknowledgments

This material was collected by one of us (G.P.) during a Research Associateship at the Department of Biological Sciences, Florida State University, Tallahassee, Florida, USA. We are grateful to Professor Robert B. Short for providing laboratory facilities during the period.

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## Trichomycetes and Oxyuroid Nematodes in the Millipede, *Narceus annularis*

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**ABSTRACT:** Oxyuroid nematodes belonging to the genera *Rhigonema*, *Johnstonia*, and *Aorurus* were recovered from the hindgut of millipedes, *Narceus annularis*. The trichomycete fungus *Enterobryus elegans* was found attached to the ileum of the millipede's hindgut as well as to *Rhigonema infecta* that also occurred in the ileum. A comparative electron microscope study was carried out of the holdfast of the fungus attached to millipede's ileum cuticle and to the nematode's cuticle. Since millipede ileum cuticle bears regularly spaced pits, while *Rhigonema* cuticle is variably spinous, surface contour is probably of little significance in attachment of the fungus to its substrates. The holdfast appears as a cement applied to the surface. It is speculated that the restricted distribution of *Enterobryus* may be due to its poor ability to compete with the heavy bacterial flora characteristic of the more posterior hindguts of the millipede.

In 1853 Joseph Leidy described the enteric flora and fauna of some arthropods, including those of the millipede *Julus marginatus*. At that time he elegantly illustrated the association of a fungus *Enterobryus elegans* with the hindgut of the millipede and with nematodes described as *Ascaris infecta* (now *Rhigonema infecta*) that also occurred in the millipede's hindgut. Since then the fungus has been extensively studied by mycologists (see Lichtwardt, 1973) but only scattered references to its association with nematodes have been given (d'Udekem, 1859; Thomas, 1930; Dolfus, 1952; Tuzet and Manier, 1952; Lichtwardt, 1954, 1958, 1973). *Enterobryus elegans* along with others are considered to constitute a separate group, the Trichomycetes, within the phycomycete fungi (Lichtwardt, 1973).

Recently, a population of the millipede *Narceus annularis* (Rafinesque) was located that harbored a large number of *Rhigonema infecta* (Leidy, 1849) and *Enterobryus elegans* (Leidy, 1849) as well as two other genera of oxyuroid nematodes. The infections were studied and a light and electron microscopic study of the attachment of *Enterobryus elegans* to the cuticles of the millipede and the nematode, *Rhigonema infecta* was undertaken. The association is described and illustrated in some detail in order to recall this interesting association to the general attention of helminthologists.

### Materials and Methods

Millipedes were collected on August 25, 1977 from Beausoleil Island, Georgian Bay, Ontario.<sup>1</sup> They were kept alive, either in a refrigerator, or in a sawdust terrarium fed oatmeal and cornmeal, until examined over the following 1-2 months. For dissection, the millipede's body wall was cut down both sides, the top portion removed, and the entire intestine lifted out into Tyrode's salt solution. The lengths of the whole millipede and of the various parts of the intestine were

<sup>1</sup> Following submission of this manuscript, similar nematode and fungus populations were found in *Narceus annularis* from Renfrew County, Ontario, and by M. Adamson (Univ. of Guelph) from Longpoint and Sharbot Lake Provincial Parks in Ontario.

**Table 1.** Summary of nematodes recovered from hindgut of millipedes, *Narceus annularis*.

|   | <i>Rhigonema infecta</i> |                 | <i>Johnstonia</i> sp. | <i>Aorurus</i> sp. |
|---|--------------------------|-----------------|-----------------------|--------------------|
|   | adults                   | larvae          | adults                | adults             |
| Total worms recovered   | 513                      | 690             | 210                   | 204                |
| Average no./host<br>(range)                                   | 18.3<br>(3,043)          | 26.5<br>(2–123) | 7.3<br>(0–27)         | 7.3<br>(0–51)      |
| Average % of worms/host<br>with <i>Enterobryus</i><br>(range) | 36.8%<br>(0–88)          | 7.3%<br>(0–60)  | 3%<br>(0–50)          | 5%<br>(0–8.3)      |

recorded, and the location and numbers of nematodes and fungi recorded from each. The nematodes were fixed in hot FAA (formalin-acetic acid-alcohol), or in ~2% glutaraldehyde-cacodylate (300 mOsmol. final concentration). Portions of the millipede's gut along with its fungi were fixed similarly, some after being pinned onto wax plates.

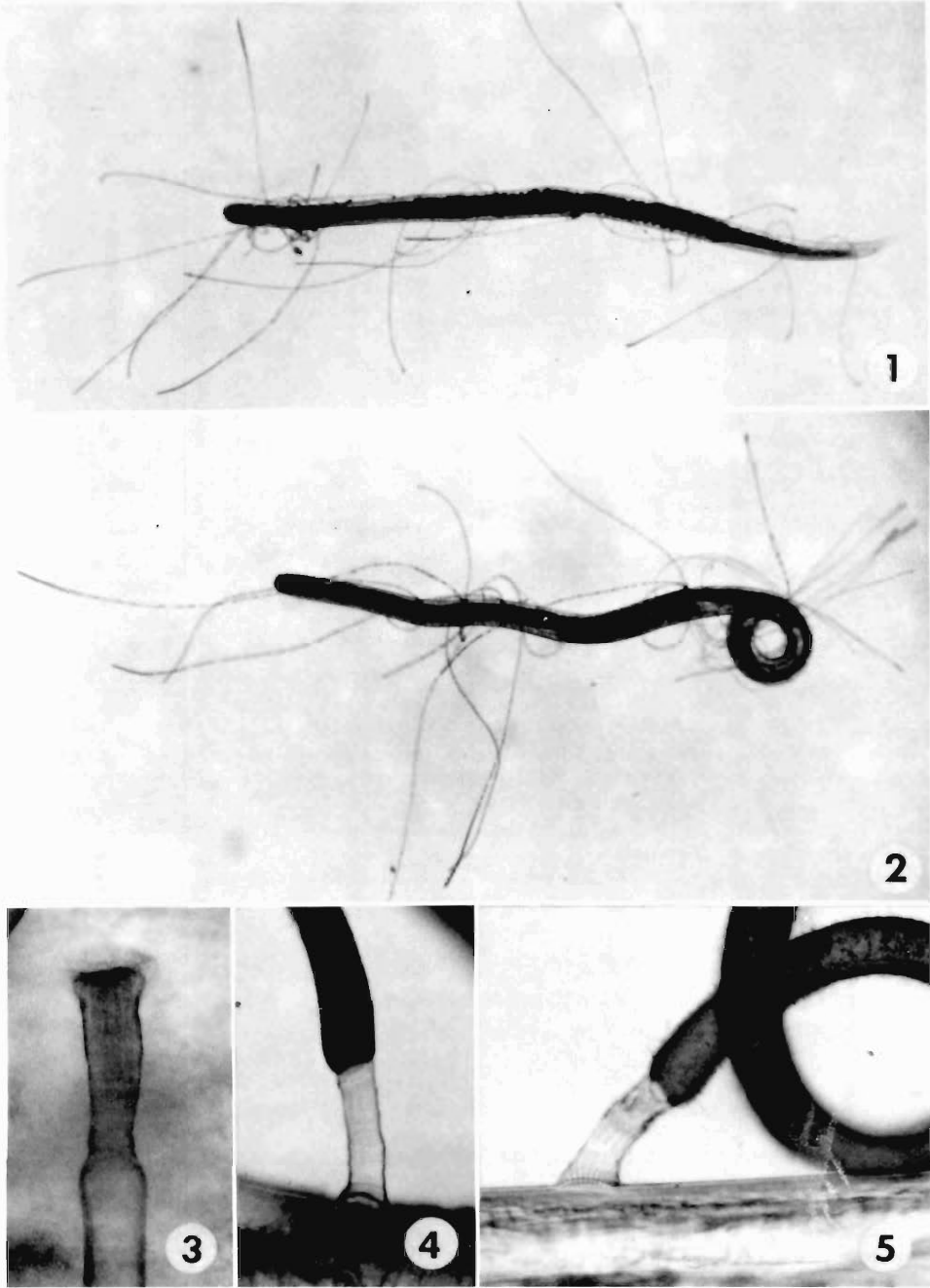
Both nematodes and host tissue were extensively examined alive (in Tyrode's solution) and photographed when necessary with electronic flash. Specimens were examined by scanning electron microscopy following fixation either in FAA, or glutaraldehyde. Some were postfixed in 1% OsO<sub>4</sub> for 2 hr. They were dehydrated through ethanol, transferred to freon 113, critical point dried from liquid CO<sub>2</sub> and both evaporative and sputter coated with gold before examination in a Coates-Welter microscope. For transmission microscopy glutaraldehyde-fixed tissues and worms were postfixed in OsO<sub>4</sub> for 1 hr, embedded in Spurr's resin, and sectioned. Sections were stained with 1% KMnO<sub>4</sub> followed by lead citrate.

### Results

Twenty-eight millipedes ranging from 30–90 mm long were examined. Three species of oxyuroid nematodes were consistently recovered; *Rhigonema infecta* from all hosts, *Johnstonia* sp. from all but two hosts, and *Aorurus* sp. from all but five. Details relating the intensity of infections are given in Table 1.

The millipede's digestive tract consists of a short esophagus (a few mm), the midgut that constitutes half the total length of the tract, and the hindgut. The midgut forms a peritrophic membrane; none of the nematodes occurred there. The hindgut is lined by cuticle; its anteriormost portion or ileum (about 5–8 mm long) is separated from the rest of the hindgut by a slight constriction. All *Rhigonema infecta* were recovered from the ileum. The nematodes are almost the same length as this portion and in heavy infections completely occlude its lumen. Almost no intestinal contents are found here. In contrast, the posterior hindgut contains ample digested material, often recognizable as plant remains. Both *Johnstonia* sp. and *Aorurus* sp. occurred here. Even in their heaviest infections, they did not occlude the lumen.

Fungal filaments of *Enterobryus elegans* were attached to both the cuticle lining the ileum, and the nematodes. *Rhigonema infecta* occurred in the same region as the fungi and bore the greatest numbers of filaments (Figs. 1, 2) while only rarely were the fungi found on *Johnstonia* sp. or *Aorurus* sp. (Table 1). *E. elegans* were found attached at all levels of *Rhigonema*, from head to tail. Few *E. elegans*



Figures 1-5. 1. A female *Rhigonema infecta* with numerous attached filaments of *Enterobryus elegans*.  $\times 21$ . 2. A male *Rhigonema infecta* with numerous attached filaments of *Enterobryus elegans*.  $\times 23$ . 3. The holdfast of *Enterobryus elegans* attached to *Narceus ileum* cuticle.  $\times 360$ . 4 and 5. The holdfasts of *E. elegans* attached to *Rhigonema* cuticle.  $\times 360$ .

were attached to larvae (Table 1). Larvae of *R. infecta* were arbitrarily classified into two size groups; 11 large larvae bore *E. elegans* filaments while only four small larvae did. The number of *E. elegans* filaments per larva (usually only 1 or 2) was noticeably smaller than on adult *Rhigonema*. Although a less accurate count was kept of larval stages of *Johnstonia* sp. and *Aorurus* sp., they were present in equal, or slightly greater numbers than the adults; no *E. elegans* filaments were found on these larvae.

All but one of the 28 millipedes examined had *E. elegans* filaments attached to the cuticle of the ileum. The one millipede lacking the fungus had 31 *Rhigonema* of which 11 adults and two large larvae bore numerous *E. elegans* filaments. These fungi however were producing sporangiospores. Six millipedes examined after being kept for 6 weeks in a terrarium at room temperature were found to have some *E. elegans* filaments attached to the posterior hindgut cuticle (seen by dissecting microscope examination) as well as to the ileum. It is not certain that this reflects a spreading of the distribution of the fungus as a result of the unusual diet of the millipedes since examination (using a compound microscope) of the hindgut of one millipede kept the same length of time in the refrigerator revealed many small filaments of the sort noted by Lichtwardt (1954). More detailed examination of the posterior hindgut might have revealed such "young" filaments in freshly caught millipedes.

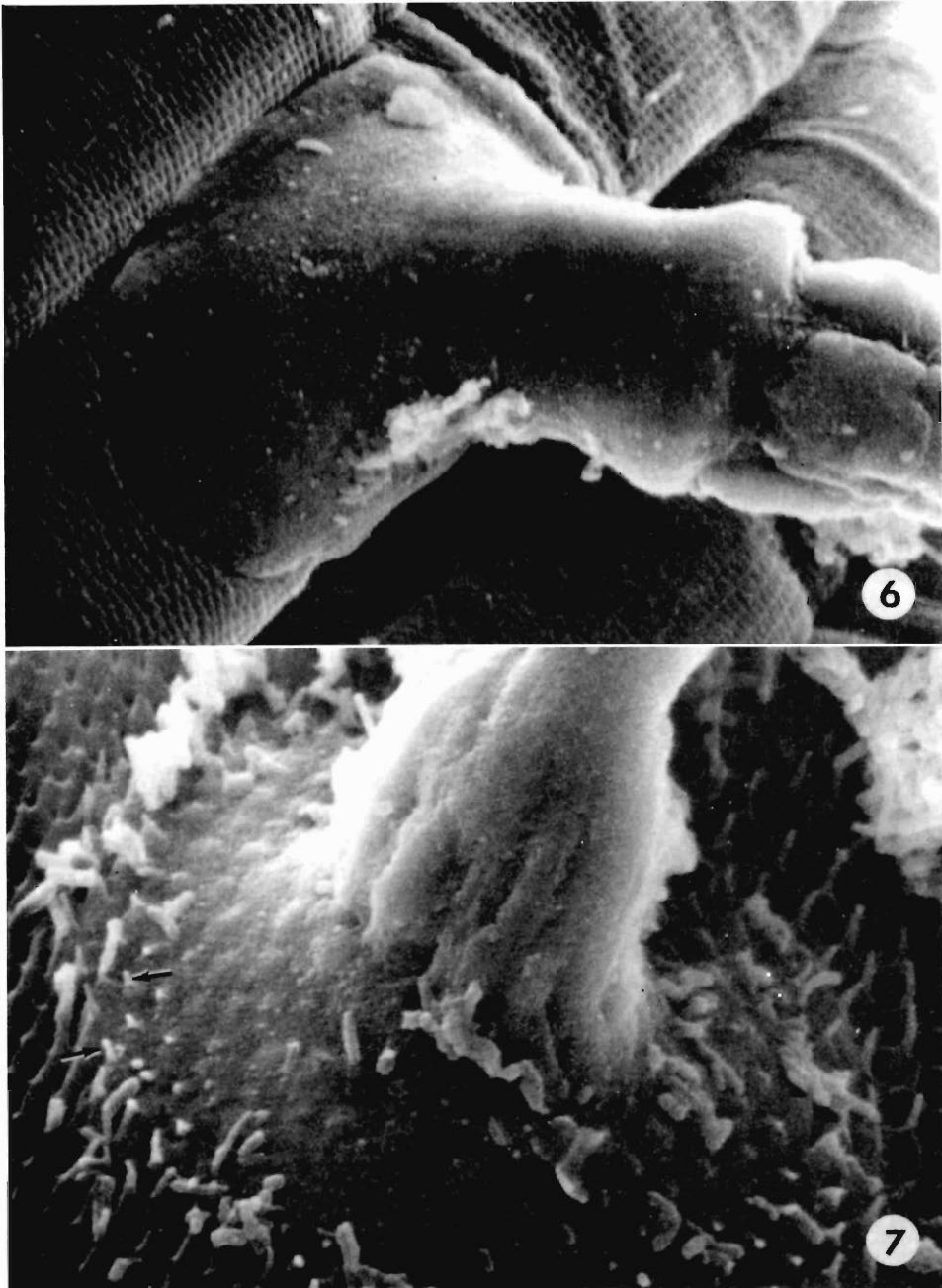
Both nematodes and fungi often are coated extensively with bacteria. Of the 18 specimens of *Johnstonia* and *Aorurus* with *E. elegans* attached to them, many filaments were extensively coated with filamentous strings of bacteria. Such heavy coatings were not noted on fungi occurring on *Rhigonema* or attached to the cuticle of the millipede's ileum. However, the posterior hindgut cuticle was often heavily matted with apparently similar bacteria.

#### Attachment of *Enterobryus elegans* filaments

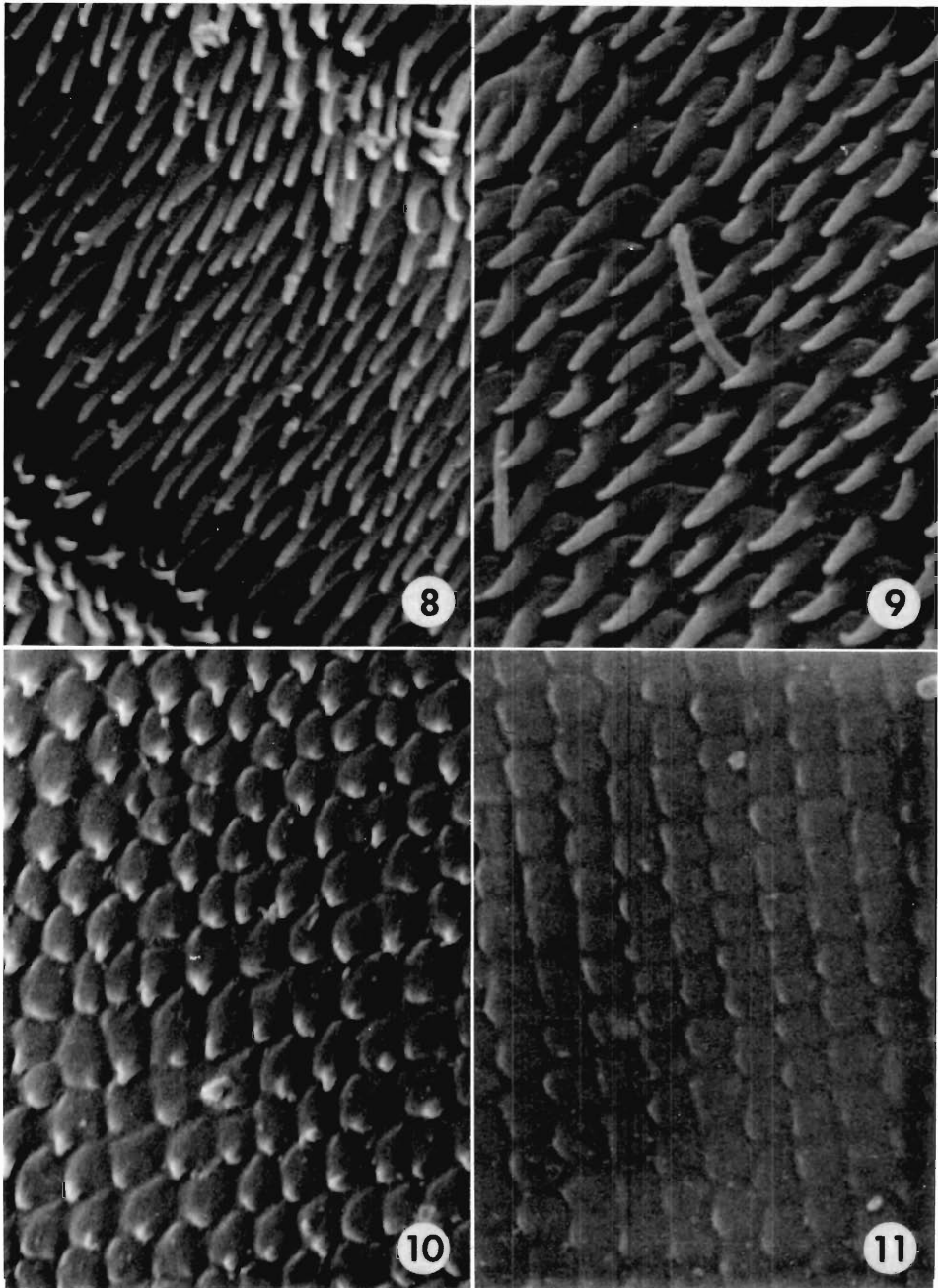
Filaments of *E. elegans* are attached to substrates by means of a holdfast unit that extends from the cellular filament to the cuticle (Figs. 3–5). There is no difference in the general shape of holdfasts attached to nematodes or to the millipede hindgut.

Scanning electron microscopy shows the distinct constriction between cell wall of the filament and the more irregular surface of the holdfast (Figs. 6, 12, 13). Many holdfasts expand onto the surface of the cuticle with a thick, clearly defined rim or margin (Fig. 6). However, when holdfasts attach to the more heavily spined cuticle of *Rhigonema* their margin may be thin and indistinct (Fig. 7). The expanded lower portion of the holdfasts attached to the millipede's ileum cuticle show numerous pits or pores apparently opening through the holdfast material (Fig. 14). Such pores are absent from holdfasts attached to *Rhigonema* cuticle. However, longer spines of the *Rhigonema* cuticle may project upwards through the margin of the holdfast (Fig. 7). Scanning electron microscopy also illustrates numerous bacteria as well as erect branching filamentous microorganisms coating the fungal filaments and sometimes the holdfast.

The contours of the surfaces to which the *E. elegans* attach vary greatly. At the head end of *Rhigonema* the cuticle bears many long retrorse spines. The length, and hence amount of overlap between spines, becomes progressively less until by the midbody, the cuticle has cobblestoned surface contours (Figs. 8–11).

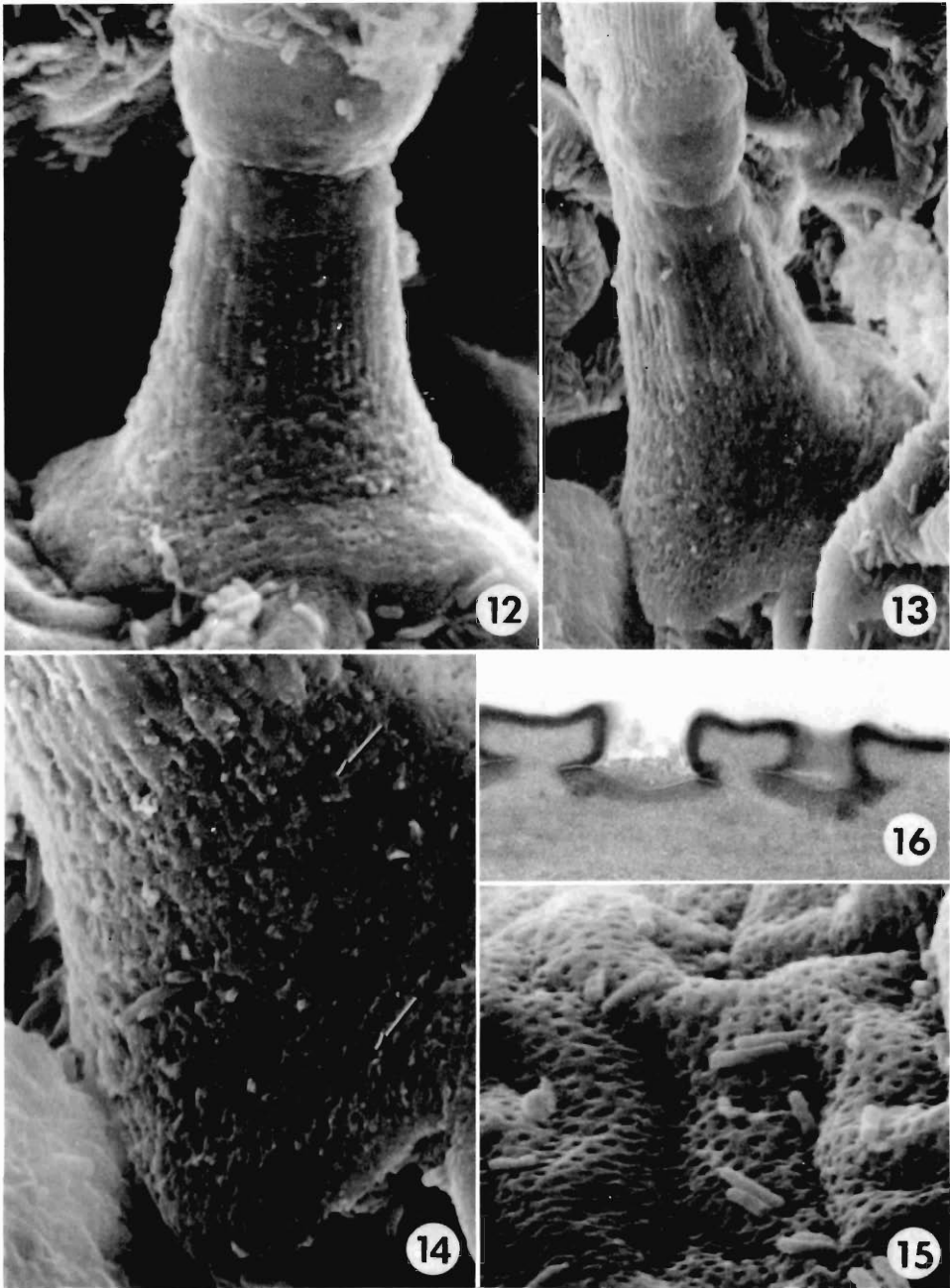


Figures 6, 7. 6. The holdfast of *E. elegans* attached to the posterior body cuticle of *Rhigonema*.  $\times 3,400$ . 7. The holdfast of *E. elegans* attached to the more anterior cuticle of *Rhigonema*. Arrows note tips of cuticular spines projecting through the holdfast.  $\times 3,600$ .



Figures 8–11. 8. Cuticle of *Rhigonema* just behind the head showing long overlapping spines.  $\times 5,900$ . 9. Cuticle of *Rhigonema* approximately one third of distance from the head. Two long bacteria also lie on the cuticle.  $\times 5,900$ . 10. Cuticle of *Rhigonema* just anterior to the vulva.  $\times 5,900$ . 11. Cuticle of *Rhigonema* posterior to the vulva showing only faintly cobblestone indentations.  $\times 5,900$ .





Figures 12–16. 12 and 13. The holdfast of *E. elegans* attached to the ileum of *Narceus*. Figure 12  $\times 3,300$ ; Figure 13  $\times 2,400$ . 14. Detail of Figure 13 showing pores (arrows) opening through the lower region of the holdfast.  $\times 4,950$ . 15. SEM view of the surface of ileum cuticle of *Narceus* showing regularly spaced pits and a few adhering bacteria.  $\times 9,900$ . 16. Transverse section of the ileum cuticle of *Narceus* showing two pits and the lower density of their epicuticle as compared to epicuticle on the surface.  $\times 60,800$ .

In contrast, the cuticle of the millipede ileum bears a very regular pattern of approximately circular shallow pits (Fig. 15). In transmission microscopy, the epicuticle lining the bottom of the pits appears less dense than epicuticle on the outer surface (Fig. 16). Bacteria coat most of the surface of the millipede hindgut cuticle and often major areas of *Rhigonema* cuticle. Filamentous microorganisms also attach to the millipede hindgut cuticle.

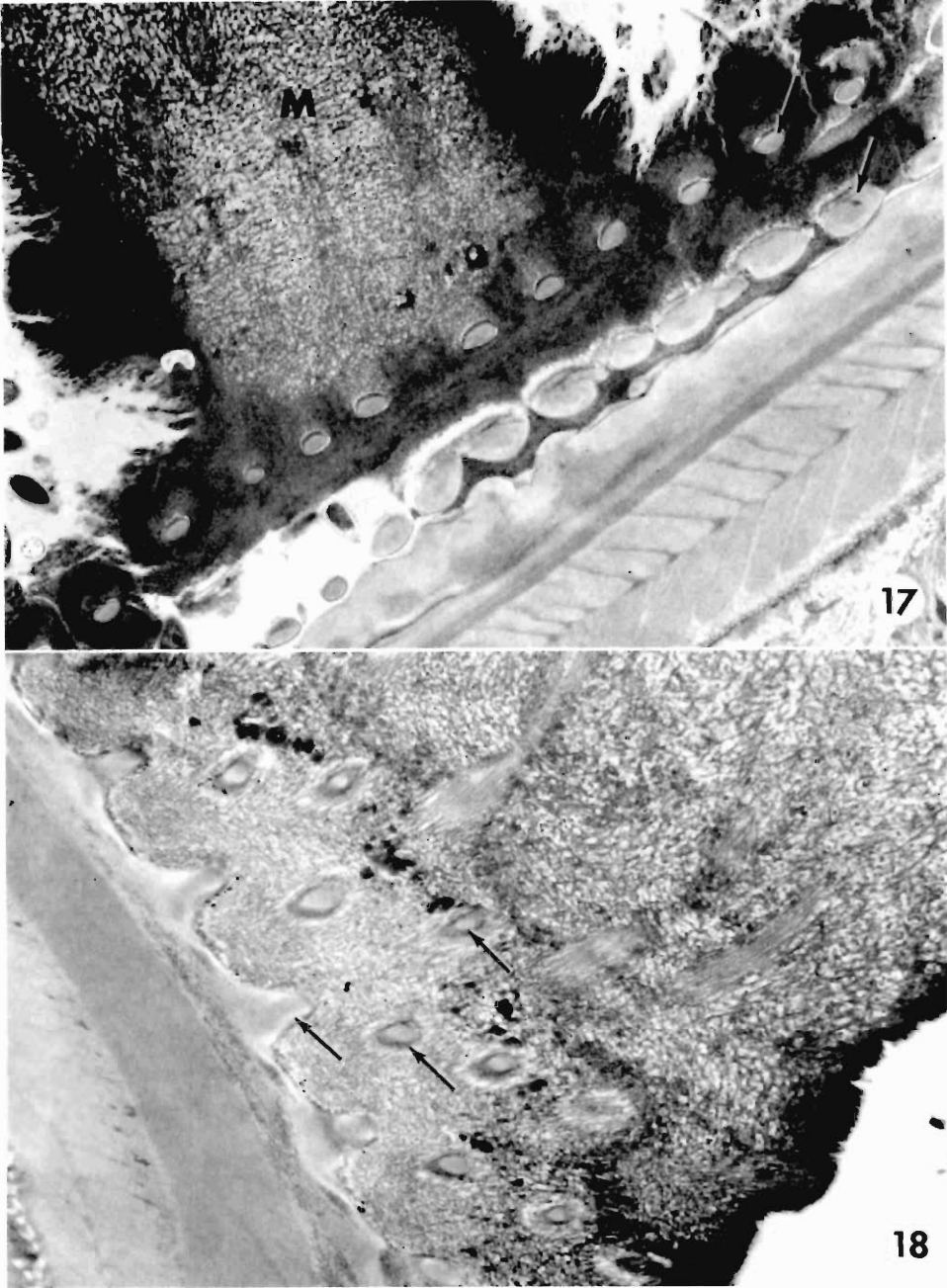
Transmission electron microscopy reveals the complex organizations of the cuticles characteristic of arthropods and nematodes. Holdfast material from *E. elegans* filaments makes intimate contact with the surfaces of these cuticles (Figs. 17–19). The holdfasts show a densely stained cortex of fine irregular filaments or granules, and a less dense medulla with low density spaces between dense irregular, perhaps reticular threads. The medullary region makes direct contact with the cuticle surface and extends to the filament of the fungus. In those holdfasts attached to the millipede hindgut, clear pores radiate from the shallow pits of the millipede cuticle into, and through, the holdfast material (Fig. 19). These correspond to the pores seen on the outer surface by scanning microscopy. Where the holdfast material contacts the rest of the fungal filament, the cell wall formed by the fungus is absent (Fig. 20).

### Discussion

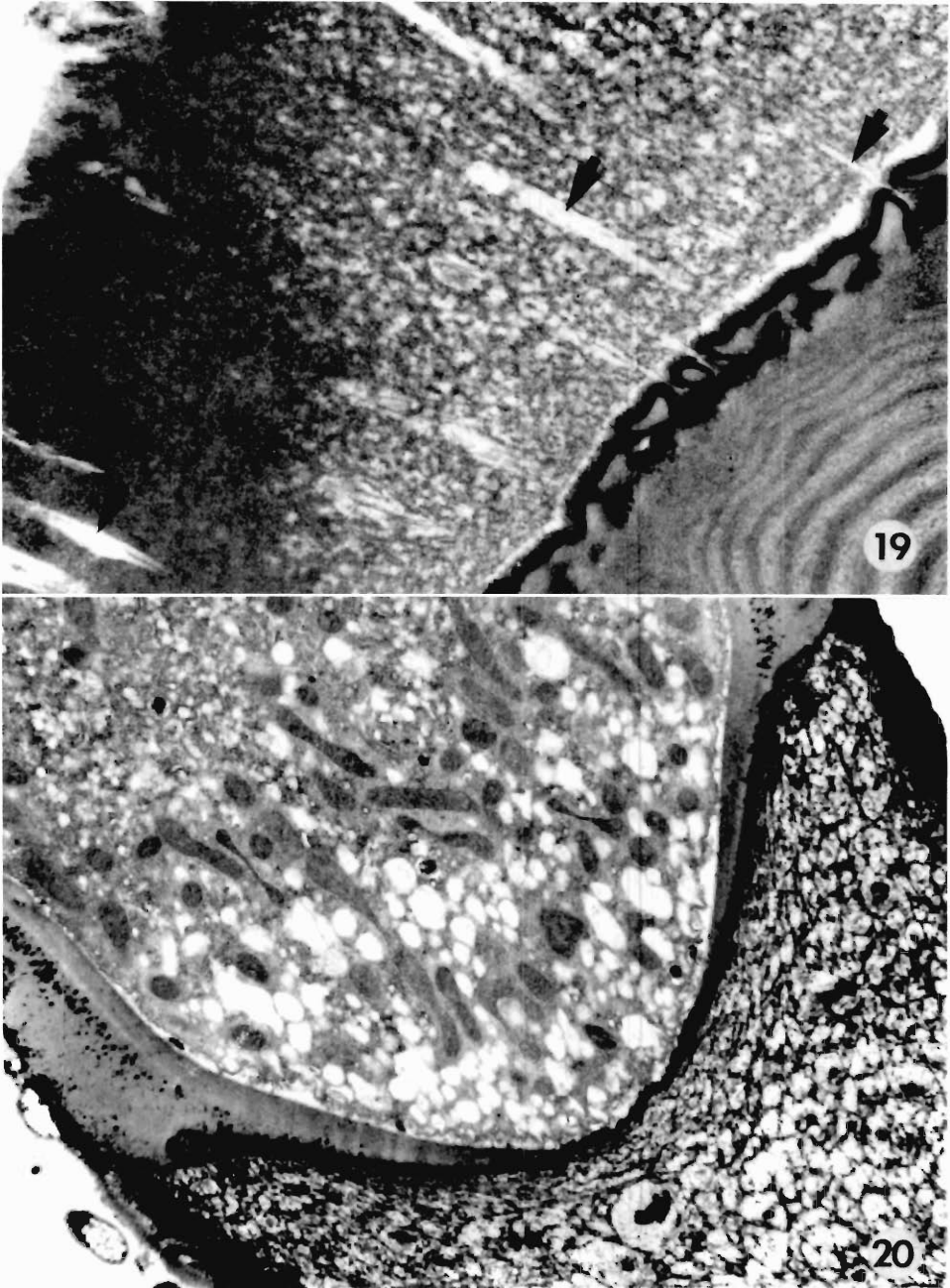
Analysis of collection data combined with the microscopic examination allows some consideration of factors that might be related to the mechanism by which *Enterobryus* holdfasts adhere to various substrates. The material forming the holdfast looks like a cement secreted by the protoplast of the fungus through the end of the filament where its wall is absent. The greater cortical density of the holdfast may be the result of interaction with physical factors of its external medium. Holdfast material appears to have been preserved in a more reticulate pattern than that shown for *Eccrinidus flexilis* by Manier and Grizel (1972).

The open pores that extend through the holdfast from the surface pits in the millipede's cuticle do not occur in holdfasts attached to the nematodes. The arthropod hindgut serves to dehydrate intestinal contents by rapid absorption of water. If the pits of *Narceus* hindgut cuticle are areas of more permeable epicuticle, fluid flow through these points might prevent consolidation of holdfast material, and hence result in formation of the pores. (Thin depressions in the epicuticle of the hindgut of cockroaches have been noted by Noirot and Noirot-Timothee, 1976).

The cementlike holdfasts adhere to surfaces with either outward projecting spines (*Rhigonema* cuticle) or indented pits (*Narceus* cuticle). Furthermore, filaments were attached at all levels of the body of *Rhigonema* where strikingly different degrees of spination occur. Thus surface contour cannot have a primary effect on adherence of the holdfast. An affinity for the major chemical constituent of cuticles has also been suggested to be important in holdfast adherence (e.g., Lichtwardt, 1958). However, while arthropod cuticle contains primarily chitin, nematode cuticle is primarily collagen. Nevertheless, both types of cuticle bear a surface layer of waxes or lipids; this may be significant. Although the *Enterobryus* filaments attach preferentially to the millipede's ileum and to *Rhigonema*, they can attach to the more posterior cuticle of the millipede and to other nematodes (*Johnstonia* and *Aorurus*) located there.



Figures 17, 18. 17. Oblique section from the edge of a holdfast of *E. elegans* attached to spinous cuticle of *Rhigonema*. Note that holdfast material surrounds the overlapping spines (arrows); C = cortex; M = medulla.  $\times 9,500$ . 18. Section through the middle of a holdfast of *E. elegans* attached to *Rhigonema* cuticle. Spines project into the holdfast (arrows).  $\times 12,600$ .



Figures 19, 20. 19. Section through part of the holdfast of *E. elegans* attached to *Narceus ileum* cuticle. Note clear pores (arrows) in both cortex and medulla and aligned with the pits in the cuticle surface.  $\times 30,000$ . 20. Section of *E. elegans* filament at the beginning of the holdfast. Note gap in cell wall in middle of the holdfast.  $\times 11,700$ .

The normally restricted distribution of *Enterobryus* in the millipede's hindgut may be related more to its ability to compete with other microorganisms, rather than its ability to adhere to various sorts of cuticle. Both the nematodes *Johnstonia* sp. and *Aorurus* sp. as well as the posterior hindgut cuticle itself may be very heavily coated with a variety of bacteria. Filaments of *Enterobryus* establishing in these areas may also become heavily coated with bacteria. Since nutrients are presumably absorbed across the wall of the filament (not through the holdfast), such a bacterial coating may hinder their growth.

Statistical analysis of the collection data did not show any correlation between host size and intensity of infection of *Rhigonema* with *Enterobryus*, or between occurrence of *Enterobryus* on males or females of *Rhigonema*. However, many more *Enterobryus* occurred on adult *Rhigonema* than on larvae. Since larvae molt during growth, their low incidence of infection may reflect time available to acquire the fungi. It is of interest that in the only millipede lacking *Enterobryus* attached to the gut, the fungi on the *Rhigonema* were sporulating. Lichtwardt (1954, 1958) has suggested that fungus attached to nematodes may be important to repopulate the gut of the millipede when its fungi are lost at molting.

No sign of pathogenesis was noted in either the cuticles or their underlying epithelia as a result of attachment of *Enterobryus*. This confirms observations by Lichtwardt (1958). Filamentous microorganisms associated with the millipede gut and the fungi were identified by Leidy (1853) as *Arthromitus cristatus* and *Cladophytum comatum*.

#### Acknowledgments

I would like to thank mycological friends Dr. D. W. Malloch and Dr. R. W. Lichtwardt for their interest and help and Miss C. Narusevicius for technical assistance. Research was supported by grant A3757 from National Research Council of Canada.

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## ***Rhabdochona catostomi* sp. n. (Nematoda: Rhabdochoniidae) from the Intestine of *Catostomus* spp. (Catostomidae)<sup>1</sup>**

ROBERT J. KAYTON,<sup>2</sup> DELANE C. KRITSKY,<sup>3</sup> AND RICHARD C. TOBIAS<sup>4</sup>

ABSTRACT: *Rhabdochona catostomi* sp. n. is described from *Catostomus catostomus* (Forster) collected from the Nose River, Alberta. It is also reported from *C. ardens* Jordan and Gilbert and *C. sp.* from Idaho and Colorado, respectively. *Rhabdochona catostomi* is characterized by having a terminal ventral barb on the left spicule, bifurcate deirids, eggs with polar filaments, eight or nine caudal papillae, and a terminal cuticular spike on the caudal tip. Moravec's (1972) subgeneric definitions of *R. (Rhabdochona)*, *R. (Filochona)*, and *R. (Globochona)* which were based primarily on egg morphology are considered of little value in determining specific relationships within the genus.

While investigating the helminth parasites of the Upper Snake River in Idaho during 1976, a new species, *Rhabdochona catostomi*, was recovered from the intestine of the Utah sucker, *Catostomus ardens* Jordan and Gilbert. This constituted the third time we recovered this worm from *Catostomus* spp. in North America. It was first found (spring 1974) in *Catostomus* sp. from the North Platte River, Colorado, and subsequently (fall 1974) in *C. catostomus* (Forster) in Nose River, Calgary, Alberta.

The nematodes were fixed alive in hot AFA or 70% ethanol and cleared in glycerin by infiltration. Several specimens were dissected to remove the spicules and eggs which were permanently mounted on slides in Grey and Wess' media (Humason, 1967) (slides deposited in USNM Helm. Coll. No. 74896). *En face* views were prepared by decapitation and subsequent mounting in glycerin. Specimens for scanning electron microscopy were dehydrated to 100% ethanol, passed through a graded series of ethanol and acetone, critical-point dried, and gold coated. Drawings were made with the aid of a camera lucida and microprojector. Measurements are in micrometers unless otherwise stated.

### ***Rhabdochona catostomi* sp. n.**

(Figs. 1-9)

TYPE HOST AND LOCALITY: *Catostomus catostomus* (Forster), Nose River, Calgary, Alberta.

OTHER RECORDS: *Catostomus ardens* Jordan and Gilbert; Snake River below American Falls Dam, Power Co., Idaho (USNM Helm. Coll. No. 74898, 74900). *Catostomus* sp.; North Platte River near Cowdery, Jackson Co., Colorado (USNM Helm. Coll. No. 74897, 74899).

LOCATION: Intestine.

TYPE SPECIMENS: 2 males, 5 females (all from Alberta); holotype (male), USNM Helm. Coll. No. 74893; allotype (female), No. 74894; paratypes, No. 74895.

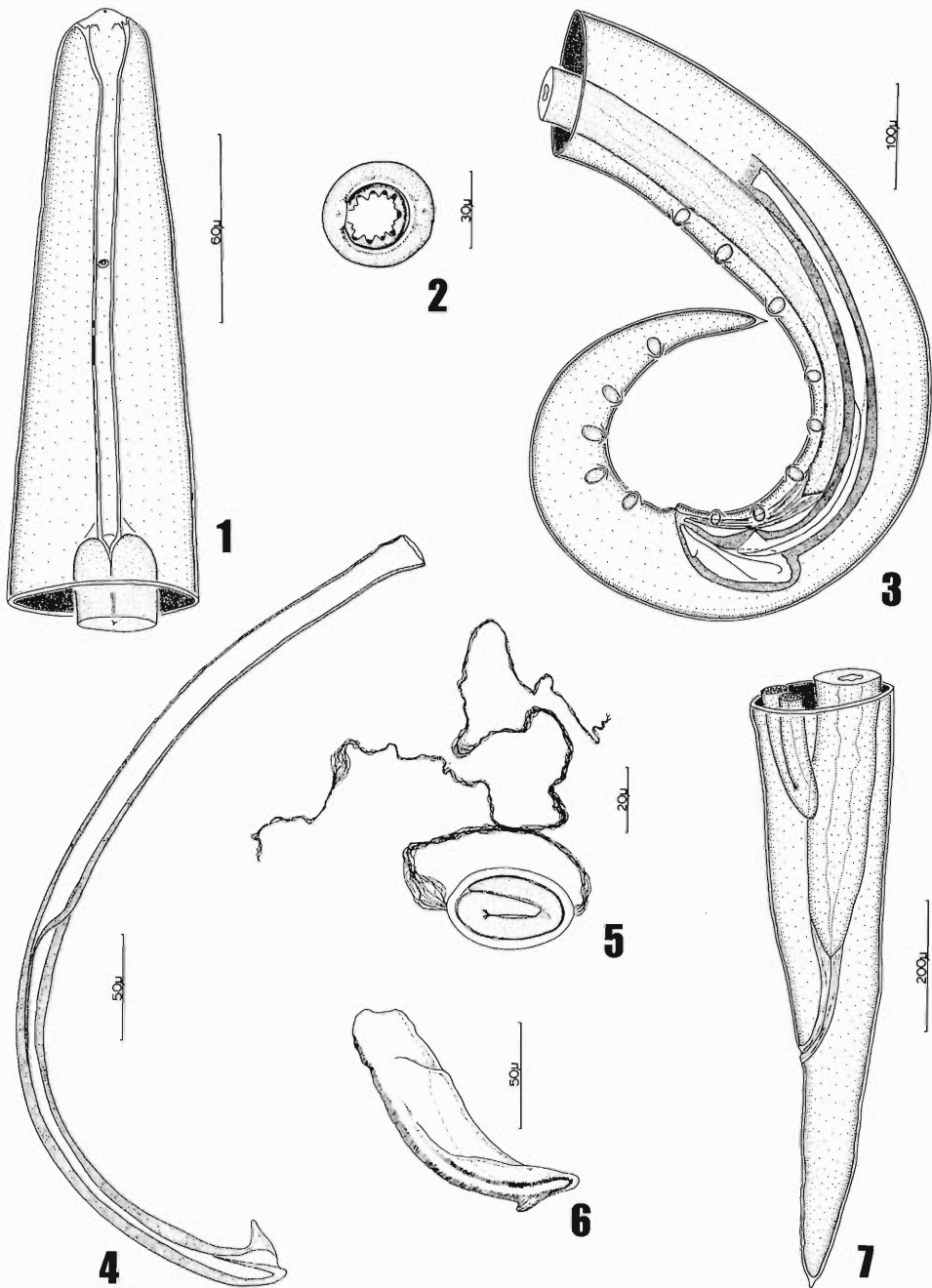
DESCRIPTION: Body filiform; males smaller than females. Cuticle with incon-

<sup>1</sup> This study was supported by a grant from the Council of Academic Deans, Idaho State University.

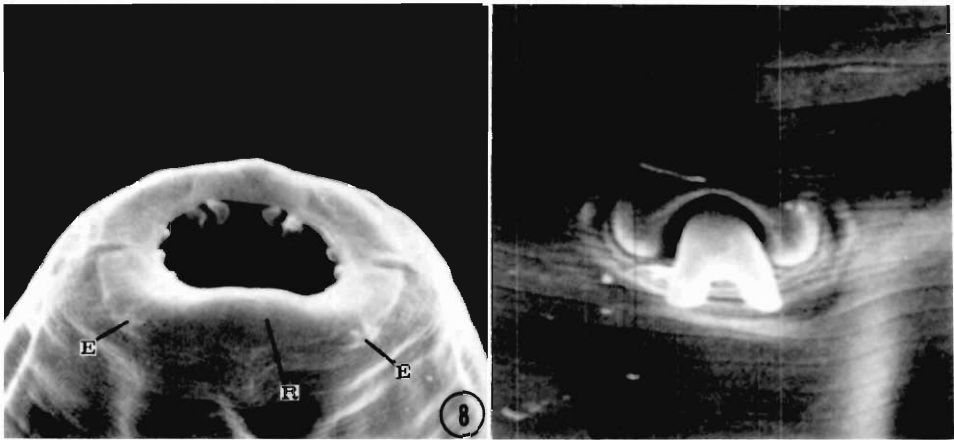
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Figures 1-7. *Rhabdochona catostomi* sp. n. 1. Anterior end of male (lateral view). 2. En face (slightly tilted). 3. Posterior end of male (lateral view). 4. Left spicule. 5. Mature egg. 6. Right spicule. 7. Posterior end of female (lateral view).



Figures 8, 9. Scanning electron micrographs of *Rhabdochona catostomi* sp. n. 8. Cephalic tip,  $\times 2,000$ . 9. Deirid,  $\times 4,500$ . (E, external papilla. R, rudimentary pseudolabium.)

spicuous transverse annulations (visible only in SEM preparations). Caudal end conical, with terminal spike. Mouth with 2 bilateral rudimentary pseudolabia; 2 large bilateral amphids, cephalic papillae arranged in outer circle of 4 (Fig. 8); inner circle apparently absent. Prostome funnel shaped, lacking basal teeth; interior wall of prostome with 10 longitudinal cuticular ridges; ridges terminating in anterior teeth, lateral ridges (4) bifurcate anteriorly forming 2 teeth each (Fig. 8). Mesostome elongate, smooth. Cuticle lining entire vestibule (=prostome + mesostome). Esophagus with anterior muscular and wider posterior glandular regions. Deirids small, bifurcate (Fig. 9), lying slightly anterior to midvestibule. Nerve ring at level of anterior  $\frac{1}{4}$  of muscular esophagus.

**MALE:** Body 8.9 to 14.3 mm long; maximum width 173 to 211 at level of junction of esophagus and intestine. Prostome 22 to 34 long; vestibule 134 to 174 long. Muscular portion of esophagus 336 to 372 long, 25 to 28 wide at its posterior end. Glandular esophagus 3.5 to 4.8 mm long, 59 to 62 wide near junction with intestine. Nerve ring 204 to 238, deirids 76 to 85, excretory pore 484 (all) from cephalic tip. Tail 336 to 392 long, curled ventrally. Caudal alae absent. Subventral preanal and postanal papillae variable in number; with 8 or 9 preanal and 5 postanal pairs; second postanal pair (from cloaca) slightly more lateral. Ventral surface of caudal end with several preanal cuticular ridges (6 in cross section of one specimen collected in Idaho) oriented slightly diagonal to the longitudinal body axis. Spicules enclosed within sheath; right spicule 151 to 154 long, with reflected distal barb. Left spicule 546 to 562 long, slender, with ventral groove; spicule tip with ventral barb.

**FEMALE:** Body 15.3 to 16.7 mm long; maximum width 248 to 292 near junction of esophagus and intestine. Prostome 28 to 31 long; vestibule 154 to 176 long. Muscular esophagus 375 to 417 long; 25 to 34 wide at posterior end. Glandular esophagus 4.97 to 6.10 mm long; 56 to 73 wide near junction with intestine. Nerve ring 227 to 284, deirids 90 to 97 (both) from cephalic tip. Tail 227 to 280 long; usually straight. Vulva ventral, postequatorial, comprising a transverse slit surrounded by elevated labia. Mature eggs ellipsoidal, embryonated; poles usually with complex elongate filaments. Eggs 31 to 36 by 20 to 24; surface smooth.



### Discussion

The closest relative of *Rhabdochona catostomi* sp. n. is apparently *R. denudata* (Dujardin, 1845). *Rhabdochona catostomi* differs from this species by having a more prominent ventral barb on the left spicule and by possessing filamented eggs (filaments lacking in *R. denudata*). *Rhabdochona catostomi* was recovered from at least two species of *Catostomus*; the specific name indicates this host-parasite relationship.

Three other *Rhabdochona* spp. have been reported from suckers of the Catostomidae: *R. ovifilamenta* Weller, 1938, from *Catostomus commersoni* (Lacépède) and *C. platyrhynchus* (Cope); *R. cascadilla* Wigdor, 1918, from *C. commersoni*; and *R. milleri* Choquette, 1951, from *Moxostoma macrolepidotum* (LeSueur). *Rhabdochona ovifilamenta* differs from *R. catostomi* by having short polar filaments on the eggs, shorter spicules of a different shape, conspicuously large deirids on the female, and more prostomal teeth. *Rhabdochona catostomi* is distinguished from *R. milleri* by possessing larger spicules, smaller deirids, an overall larger body size, and by lacking basal prostomal teeth. *Rhabdochona catostomi* differs from *R. cascadilla* by being much larger, and by having a ventral barb on the left spicule (absent in *R. cascadilla*) and filamented eggs (filaments lacking in *R. cascadilla*).

Moravec (1972) divided *Rhabdochona*, s. l., into three subgenera, *R.* (*Rhabdochona*), *R.* (*Filochona*), *R.* (*Globochona*), based primarily on the presence or absence of filaments or floates on the eggs. These subgeneric taxa were considered to be artificial by Margolis et al. (1975) primarily because species assigned to one subgenus were often most similar (except in egg morphology) to a member of another. They indicated that confusion has resulted because of the past utilization of only these criteria for separation of subgenera, and proposed that more features be incorporated. Also, Moravec and Arai (1971) found that during manipulation of the eggs, polar filaments were easily lost; and, in the present study, both filamented and nonfilamented mature eggs were observed in the same specimen. Thus, our assignment of *R. catostomi* to a subgenus pends clearer subgeneric definitions.

### Acknowledgments

The authors wish to thank Dr. G. D. Schmidt, University of Northern Colorado, for consultations concerning *Rhabdochona catostomi*. Drs. Robert Anderson and Allen Linder, Idaho State University, kindly allowed us to use their laboratory facilities and helped in the identification of hosts.

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## *Eutylenchus vitiensis* sp. n. (Nematoda: Atylenchidae) from Fiji

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ABSTRACT: *Eutylenchus vitiensis* sp. n. from three localities on Viti Levu, Fiji, is described and figured. The new species can be separated from known members of the genus *Eutylenchus* by a combination of characters which include the length of body, stylet and postuterine sac, the anterior position of the excretory pore in relation to the hemizonid, and the shape of the male caudal alae.

When Sher et al., 1966 revised the Family Atylenchidae Skarbilovich, 1959, they recognized two species of *Eutylenchus*; the type species *E. setiferus* (Cobb, 1893) Cobb, 1913 which they redescribed and *E. africanus* which was described as new. The only subsequently described species, *E. orientalis* Husain and Khan, 1968 was later synonymized with *E. africanus* by Choi and Geraert (1972), a synonymy with which I agree. Small numbers of a third species of *Eutylenchus* were collected in Fiji in 1966 by the author who was then engaged on a nematode survey of the area. More abundant material was subsequently received from Mr. M. Kirby, formerly of Koronivia Research Station, Fiji. This species is described below and named from "Viti" the Fijian word for the Fiji islands.

### Materials and Methods

Specimens were heat killed, fixed in F.A. 4:10 and processed to glycerine containing traces of picric acid by a modified Baker method. This was done unfortunately before a scanning electron microscope became available for use and only very few specimens remained for stereoscanning. These were processed by the method of Clark and Stone, 1975 and examined with an ISI-60 SEM at an accelerating voltage of 15 kV.

### *Eutylenchus vitiensis* sp. n.

(Figs. 1, 2)

MEASUREMENTS. FEMALES (n = 18): L = 710-920  $\mu\text{m}$  (816); a = 40.7-52.5 (46.2); b = 5.9-7.4 (6.4); c = 5.6-7.4 (6.7); V = 68.7-72.1 (70.8); stylet = 24-28  $\mu\text{m}$  (26).

HOLOTYPE FEMALE: L = 820  $\mu\text{m}$ ; a = 45.6; b = 6.3; c = 6.0; V = 69.3; stylet = 26  $\mu\text{m}$ .

MALES (n = 15): L = 675-880  $\mu\text{m}$  (788); a = 45.3-55.1 (50.1); b = 6.1-6.9 (6.3); c = 5.7-7.3 (6.7); stylet = 24-28  $\mu\text{m}$  (26); spicules = 17-21  $\mu\text{m}$  (19); gubernaculum = 7-9.5  $\mu\text{m}$  (8).

ALLOTYPE MALE: L = 798; a = 51.5; b = 6.1; c = 6.0; stylet = 27  $\mu\text{m}$ ; spicules = 17.5  $\mu\text{m}$ ; gubernaculum = 7.5  $\mu\text{m}$ .

DESCRIPTION. FEMALES: Body slightly curved, elongate, cylindrical, tapering regularly towards head from oesophageal region and towards terminus just posterior to vulva. Cuticle with 12 longitudinal ridges of cuticular blocks alternating with 12 clear longitudinal bands over most of the body. Anteriorly the rows of blocks commence at different levels. Immediately behind head the first 1 or 2 body annules are broken only midlaterally on either side of the body by a single

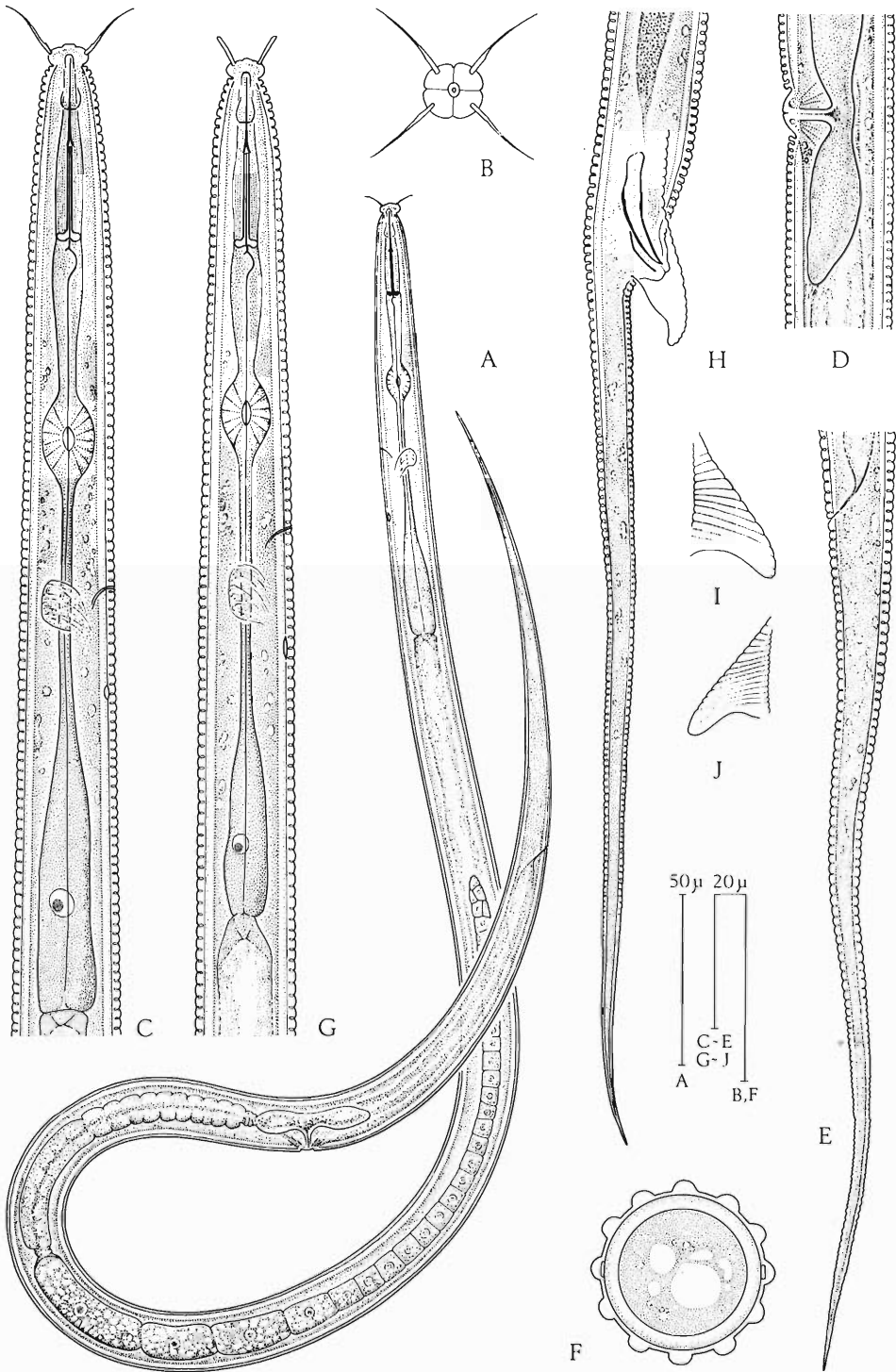


Figure 1. *Eutylenchus vitiensis* sp. n. A-F. Female. G-J. Male. A. Entire. B. Face view. C, G. Anterior ends. D. Vulval region. E, H. Posterior ends. F. Cross section midbody. I, J. Caudal alae.

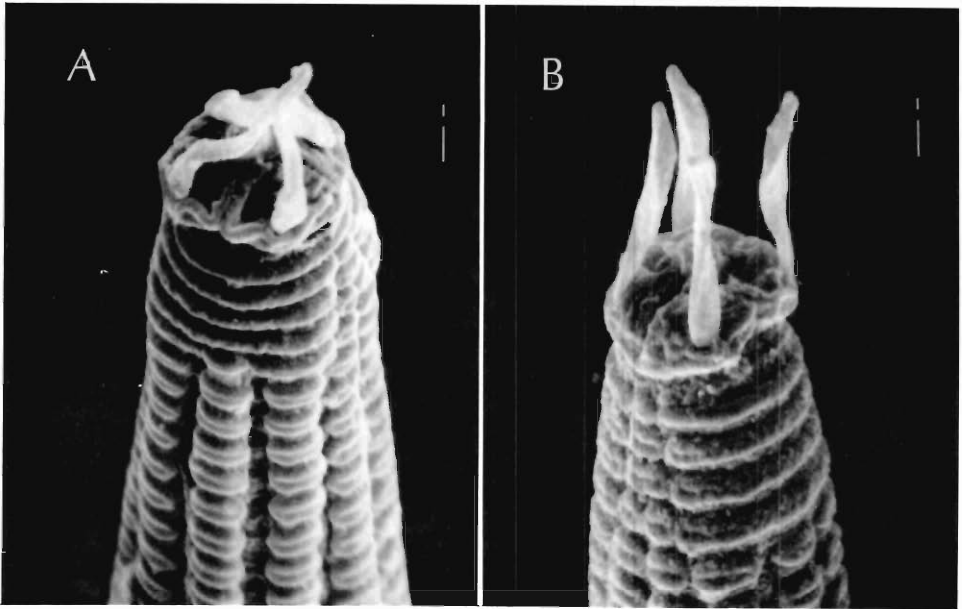


Figure 2. *Eutylenchus vitiensis* sp. n. A. Female, anterior end. (The setae have become attached to each other during processing.) B. Male, anterior end. In A and B the lower scale line is 1  $\mu\text{m}$  long.

cuticular block which immediately divides on subsequent annules to form 2 rows. At about the 5th body annule, 3 dorsal and 3 ventral rows of blocks commence at or nearly at the same level, giving rise to a total of 10 rows which continue for some way behind head. This arrangement, the same in both sexes, is best seen in stereomicrographs (Fig. 2A, B). The final 2 rows of blocks arise midlaterally in the anterior  $\frac{1}{3}$  of the oesophageal region. The number of rows is reduced posterior to anus, the midlateral rows disappearing first. Annules 1.4–1.7  $\mu\text{m}$  in width at midbody.

Cephalic region set off by deep constriction, not annulated, and bearing 4 setae. Lip region projecting slightly from head contour. In face view (Fig. 1B) head clearly composed of four lobes separated by dorsal, ventral, and lateral incisures, each lobe bearing a seta. Setae 8–13  $\mu\text{m}$  long with a wider basal portion and finer distal portion which tapers to a point, the junction of the 2 parts marked by a fine inwardly directed process. Amphid apertures not seen in face view (2 females). Conus of stylet fine, 10.5–13.5  $\mu\text{m}$  long, shorter than shaft. Stylet knobs flattened or slightly concave anteriorly. Dorsal oesophageal gland orifice about 2  $\mu\text{m}$  from stylet base. Median bulb well developed, isthmus slender with nerve ring near middle, widening to elongate basal bulb. Oesophagus 115–142  $\mu\text{m}$  long. Excretory pore 68–81  $\mu\text{m}$  from anterior end, at level of nerve ring or just anterior. Hemizonid two or three body annules long, situated 6–16 clear annules (8–23  $\mu\text{m}$ ) posterior to excretory pore.

Gonad single, outstretched; spermatheca not seen. Postuterine sac 1.1–1.8 times body width at vulva, sometimes with a rudimentary cell or cells at its posterior end. Vulva–anus distance 105–128  $\mu\text{m}$  varying from 33  $\mu\text{m}$  less than

**Table 1. The main characters differentiating the species of *Eutylenchus* Cobb, 1913.**

| Character  | Females               |                       |                     | Males                 |                                  |                                   |
|--|-----------------------|-----------------------|---------------------|-----------------------|----------------------------------|-----------------------------------|
|  | <i>E. setiferus</i> * | <i>E. africanus</i> * | <i>E. vitiensis</i> | <i>E. setiferus</i> * | <i>E. africanus</i> *            | <i>E. vitiensis</i>               |
| Body length (in $\mu\text{m}$ )                  | 560–700               | 750–1,030             | 710–920             | 500–650               | 710–1,010                        | 675–880                           |
| Stylet length (in $\mu\text{m}$ )                | 18–21                 | 20–24                 | 24–28               | 18–20                 | 20–23                            | 24–28                             |
| Cephalic lobes                                   | 6                     | 6                     | 4                   | ?                     | ?                                | 4                                 |
| Position of excretory pore relative to hemizonid | anterior              | posterior             | anterior            | anterior              | posterior                        | anterior                          |
| Postuterine sac                                  | 1.0 or less           | more than 1.0         | 1.1–1.8             | —                     | —                                | —                                 |
| Body width at vulva                              | —                     | —                     | —                   | 15–18                 | 21–27                            | 17–21                             |
| Spicule length (in $\mu\text{m}$ )               | —                     | —                     | —                   | 15–18                 | 21–27                            | 17–21                             |
| Shape of caudal alae                             | —                     | —                     | —                   | rounded               | triangular, apex broadly rounded | triangular, apex narrowly rounded |

\* Data from Sher et al., 1966.

tail length to 12  $\mu\text{m}$  more, generally a few micrometers less. Tail 99–138  $\mu\text{m}$  long, 10–14 times body width at anus, tapering regularly to a pointed terminus.

**MALES:** Body straight or slightly curved, tapering regularly towards head from oesophageal region, constricted after cloaca. Number and anterior origin of cuticular ridges similar to that of female. Posteriorly, number of ridges reduced at bursa. Annules 1.2–1.6  $\mu\text{m}$  in width at midbody.

Cephalic region set off by deep constriction, not annulated, and bearing 4 setae. Lip region projecting slightly from head contour. Setae 5–7.5  $\mu\text{m}$  long, rod shaped with rounded distal ends, inwardly directed processes absent.

Conus of stylet fine, 11.5–13  $\mu\text{m}$  long, shorter than shaft. Stylet knobs flattened or slightly concave anteriorly. Dorsal oesophageal gland orifice about 2  $\mu\text{m}$  from stylet base. Oesophagus resembling that of female, 110–135  $\mu\text{m}$  long. Excretory pore 64–83  $\mu\text{m}$  from anterior end, at level of nerve ring or just anterior. Hemizonid about 2 body annules long, situated 5–16 clear annules (8–21  $\mu\text{m}$ ) posterior to excretory pore.

Testis single, outstretched. Ventral body wall protruding in cloacal region, anterior lip of cloaca projecting slightly further than posterior lip. Caudal alae prominent, triangular or "fin-shaped" with a finely rounded apex and crenate outer edge. Alar base usually extending less than one cloacal body width in front of cloacal aperture. Spicules slightly curved; gubernaculum simple, slightly curved. Body narrowing posterior to cloaca. Tail 98–137  $\mu\text{m}$  long, 8–11 times body width at cloaca, tapering regularly to a pointed terminus. Males present in about the same numbers as females.

**JUVENILES:** Similar to adults except for gonad development.

**HABITATS AND LOCALITIES:** *E. vitiensis* sp. n. was identified from the following three localities on Viti Levu, the largest and main island in the Fiji group. Soil around the roots of a fern (unidentified), growing in bush, Savura Creek, Naitasiri District (Type locality).

Soil around the roots of a tree fern, *Cyathea* sp., growing in bush beside the Queen's Road, 1 mile west of the bridge at Wainadoi village, Veivatuloa District.

Soil, from bush at Nanuku Creek, Nadrau Plateau, Navosa District.

**TYPES:** The holotype female, 10 female paratypes, allotype male, and nine male paratypes have been deposited at the Commonwealth Institute of Helminthology, St. Albans, England. One female and one male paratype have been sent to each of the following institutions: Nematology Department, Rothamsted Experimental Station, Harpenden, England; Laboratoire des Vers, Muséum national d'Histoire naturelle, Paris, France; Laboratoria voor Morfologie en Systematiek, Rijksuniversiteit, Gent, Belgium; Laboratorium voor Nematologie, Landbouwhogeschool, Wageningen, Netherlands; USDA Nematode Collection, Beltsville, Maryland, USA; DSIR, Auckland, New Zealand.

**DIAGNOSIS:** The main characters that can be used to differentiate the new species from *E. setiferus* and *E. africanus* are set out in Table 1.

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## Effects of *Ostertagia ostertagi* on Pepsinogen Granules of Chief Cells from Calf Abomasa Correlated with Selected Plasma and Abomasal Proteins

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**ABSTRACT:** Ostertagiasis and its effects on the pepsinogen granules of chief cells and the consequences were assayed without the complications of plasma proteins leaking into the gastric contents particularly at 22-30 DAI days after infection (DAI). Albumin and globulin values of the gastric contents were significantly less from infected calves than values from uninfected calves. The pepsin content of abomasa from infected calves peaked at 14 DAI; then it dropped precipitously 22-30 DAI. Chief cells were denuded of pepsinogen granules in severe infections as shown histologically, histochemically, and ultrastructurally. Concurrently, plasma pepsinogen concentrations were significantly high from 22 DAI onward. Horseradish peroxidase (HPR), injected intravenously in tests to determine whether plasma proteins leaked into the gastric contents, gave similar values in gastric contents from uninfected and infected calves. The results indicate that pepsinogen was retained in the circulation giving abnormally high plasma pepsinogen values. It also implies that chief cell pepsinogen was released directly into the circulation rather than taken up from the gastric contents through a damaged vasculature.

Blood and the gastric contents from cattle infected with *Ostertagia ostertagi* were studied by Anderson et al. (1966) and Jennings et al. (1966). However, the interpretation of most studies of ostertagiasis was complicated by the possibility of plasma proteins leaking between the circulation and the gastric contents. The variables including interplay between plasma protein leaked, pepsinogen secreted by chief cells, and progressive changes in the pH of the gastric contents during infection, with its subsequent effects on pepsin digestion made it difficult to correlate the effects of *O. ostertagi* on the pepsinogen granules of the chief cells with the biochemical consequences. Current ideas about the pathogenesis of parasitic gastritis in cattle were updated by Armour (1974); and concepts about the value of plasma pepsinogen as an aid for diagnosing parasitic diseases were reviewed recently by Ford (1976). However, these reviews show how little is known about the mechanism by which plasma pepsinogen is elevated during Type I ostertagiasis (direct development).

The present report results from studies of tissue, gastric contents, and serum from calves infected with *O. ostertagi* (Stringfellow, 1974, 1977). Ostertagiasis and its effects on the pepsinogen granules of chief cells and selected plasma and abomasal proteins are correlated at 22-30 days after infection (DAI). These studies shed further light on the mechanism by which plasma pepsinogen is elevated in Type I ostertagiasis.

### Materials and Methods

#### Infections and treatment of samples

Each of 27 3-month-old calves was inoculated orally with 250,000 larvae of *O. ostertagi*. Infected and control calves were fasted 12 hr before necropsy. All calves were injected via the jugular vein with 170 purpurogallin units/kg of body

**Table 1.** Analysis of gastric contents from calves infected with 250,000 larvae of *Ostertagia ostertagi* and from uninfected control calves.

| Days after infection       | No. of calves | Gastric contents |                     |                     |   |                        |
|----------------------------|---------------|------------------|---------------------|---------------------|---|------------------------|
|                            |               | pH               | Albumin (g/100 ml)  | Globulin            | Ave. Range<br>Pepsin (milliunits tyrosine)* | HPR (Units)†           |
| 0<br>(uninfected controls) | 6             | 2.3<br>(2.1–2.5) | 0.36<br>(0.15–0.51) | 0.14<br>(0.02–0.26) | 23,263<br>(20,832–27,776)                   | 630<br>(210–1,260)     |
| 3                          | 3             | 2.1<br>(1.9–2.4) | 0.38<br>(0.01–0.49) | 0.05<br>(0.03–0.08) | 29,798<br>(27,719–31,877)                   | 420<br>(0–840)         |
| 5                          | 3             | 2.5<br>(2.1–3.3) | 0.04<br>(0.02–0.09) | 0.03<br>(0.02–0.05) | 22,474<br>(20,367–24,580)                   | 1,260<br>(0–3,780)     |
| 7                          | 3             | 2.7<br>(2.1–3.9) | 0.01<br>(0–0.03)    | 0.04<br>(0–0.09)    | 38,383<br>(20,833–55,829)                   | 1,470<br>(1,050–1,890) |
| 14                         | 6             | 2.4<br>(1.9–3.3) | 0.09<br>(0.01–0.25) | 0.06<br>(0.02–0.08) | 55,555<br>(27,777–98,221)                   | 1,050<br>(210–1,890)   |
| 22                         | 6             | 6.5<br>(4.6–7.2) | 0.15<br>(0.02–0.30) | 0.11<br>(0.01–0.16) | 31,250<br>(27,777–38,194)                   | 630<br>(210–2,520)     |
| 26                         | 3             | 7.3<br>(7.2–7.5) | 0.13<br>(0.10–0.17) | 0.08<br>(0.07–0.10) | 0   | 1,470<br>(630–2,100)   |
| 30                         | 3             | 6.7<br>(6.8–7.0) | 0.18<br>(0.14–0.22) | 0.07<br>(0.06–0.09) | 347<br>(0–1,041)                            | 840<br>(420–1,890)     |

\* Micromoles of tyrosine/minute/liter released at 37°C × 1,000 (Reid et al., 1967).

† Purpurogallin units.

weight of Type II Sigma<sup>1</sup> horseradish peroxidase (HPR) as a tracer for vascular leakage at least 30 min before necropsy. Horseradish peroxidase (mol wt 40,000) has a molecular weight less than that of albumin and globulin but similar to that of plasma pepsinogen, the molecule it was selected to simulate. It is also easily measured both chemically and histochemically. The calves were killed with a captive bolt gun at 3, 5, 7, 14, 22, 26, and 30 DAI. The numbers of calves per group on the various DAI and of uninfected controls are given in Tables 1 and 2. Whole blood was collected in large blood tubes from the slit jugular vein and allowed to clot overnight in a refrigerator. Serum samples were dispensed into vials and stored on dry ice. In this paper, blood pepsinogen is designated plasma pepsinogen although values were estimated from serum. The abomasum were slit at the pylorus in such a way that there was negligible to no bleeding and their contents were drained into a bucket. The pH of the gastric contents was measured electrometrically immediately. The contents then were filtered through gauze, the volume was measured and centrifuged, and the clear supernatant was recovered and stored in vials on dry ice. Abomasal cannulae were not used because we wanted to get tissue and contents simultaneously without the complicating factor of cannular leakage.

<sup>1</sup> Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.



**Table 2.** Analysis of serum from calves infected with 250,000 larvae of *Ostertagia ostertagi* and from uninfected control calves.

| Days after infection       | No. of calves | Serum                 |                     |                                      |                                  |
|----------------------------|---------------|-----------------------|---------------------|--------------------------------------|----------------------------------|
|                            |               | Albumin<br>(g/100 ml) | Globulin            | Ave.<br>Range<br>Albumin<br>Globulin | Pepsin<br>(milliunits tyrosine)* |
| 0<br>(uninfected controls) | 6             | 3.92<br>(1.73-7.94)   | 4.02<br>(2.91-7.25) | 0.98                                 | 992<br>(0-6,944)                 |
| 3                          | 3             | 5.53<br>(4.13-8.01)   | 5.06<br>(3.61-7.19) | 1.09                                 | 4,514<br>(0-10,417)              |
| 5                          | 3             | 4.01<br>(3.70-4.30)   | 4.20<br>(4.00-4.31) | 0.95                                 | 5,208<br>(3,472-6,944)           |
| 7                          | 3             | 3.12<br>(2.61-4.02)   | 3.02<br>(2.11-3.80) | 1.03                                 | 3,472<br>(3,472-3,472)           |
| 14                         | 6             | 3.13<br>(1.65-4.69)   | 3.58<br>(2.35-4.63) | 0.87                                 | 9,375<br>(6,944-17,361)          |
| 22                         | 6             | 2.98<br>(1.56-4.84)   | 4.28<br>(2.41-7.62) | 0.70                                 | 32,986<br>(24,305-41,666)        |
| 26                         | 3             | 2.58<br>(2.18-2.91)   | 3.77<br>(3.10-4.12) | 0.68                                 | 68,402<br>(45,138-107,637)       |
| 30                         | 3             | 2.42<br>(2.33-2.49)   | 4.89<br>(4.39-5.29) | 0.49                                 | 26,736<br>(20,833-38,194)        |

\* Micromoles of tyrosine/minute/liter released at 37°C × 1,000 (Reid et al., 1967).

### Light and electron microscopy

Fundic mucosa excised from the abomasa was sectioned (8  $\mu\text{m}$ ) and stained (H&E) for pathologic study. The following histochemical methods were used: Lillie's nucleic acid stain, DMAB nitrite method, Häusler's variant of Kurata's method for carbonic anhydrase, succinic dehydrogenase, Wachstein-Meisel adenosine triphosphatase technique, and Van Duijn's improved benzidine peroxidase reaction for HPR (Pearse, 1972). Specimens were prepared for electron microscopy by fixing in 2% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.2) for 24 hr at 4°C and postfixing in 1% OsO<sub>4</sub> in 0.05 M cacodylate buffer with 0.15 M sucrose (pH 7.2) for 3 hr at 4°C. The fixed specimens were dehydrated in ethanol and embedded in SPURR low viscosity embedding medium, thin sectioned, stained with lead citrate and uranyl acetate, and examined with the electron microscope.

### Analysis of gastric contents and serum

Additional measurements were made on both gastric contents and sera. Albumin and globulins in serum samples and gastric contents were determined with Harleco kits on a Clinocard Analyser Model 368. Plasma pepsinogen and gastric pepsin levels were measured according to the procedure given by Edwards et al. (1960) from sera. Sigma Fraction V albumin was incubated at pH 2 for 24 hr at 37°C. The tyrosine-like products were estimated with Folin-Ciocalteu reagent,

and the samples were read in a Spectronic 20 spectrophotometer at 540 nm. Standard tyrosine (0.2 mmole) and a water blank served as a reference and results were expressed as milliunits of tyrosine ( $\mu\text{moles tyrosine/liter/minute} \times 1,000$ ) (Reid et al., 1967). Horseradish peroxidase from serum and gastric contents was estimated according to the procedure provided by Sigma Chemical Company with the product. The activity of HPR was expressed as purpurogallin units. One purpurogallin unit caused the formation of 1 mg of purpurogallin in 20 sec at 20°C. Results were read at 420 nm in the spectrophotometer. Samples were run in triplicate. Results in Tables 1 and 2 are expressed as averages; the range represents the dispersion of the data. Differences between arithmetic means were tested with Student's *t*-test at the 0.05 level of significance (Spiegel, 1961). The data were also tested with the analysis of variance (Snedecor, 1940).

## Results and Discussion

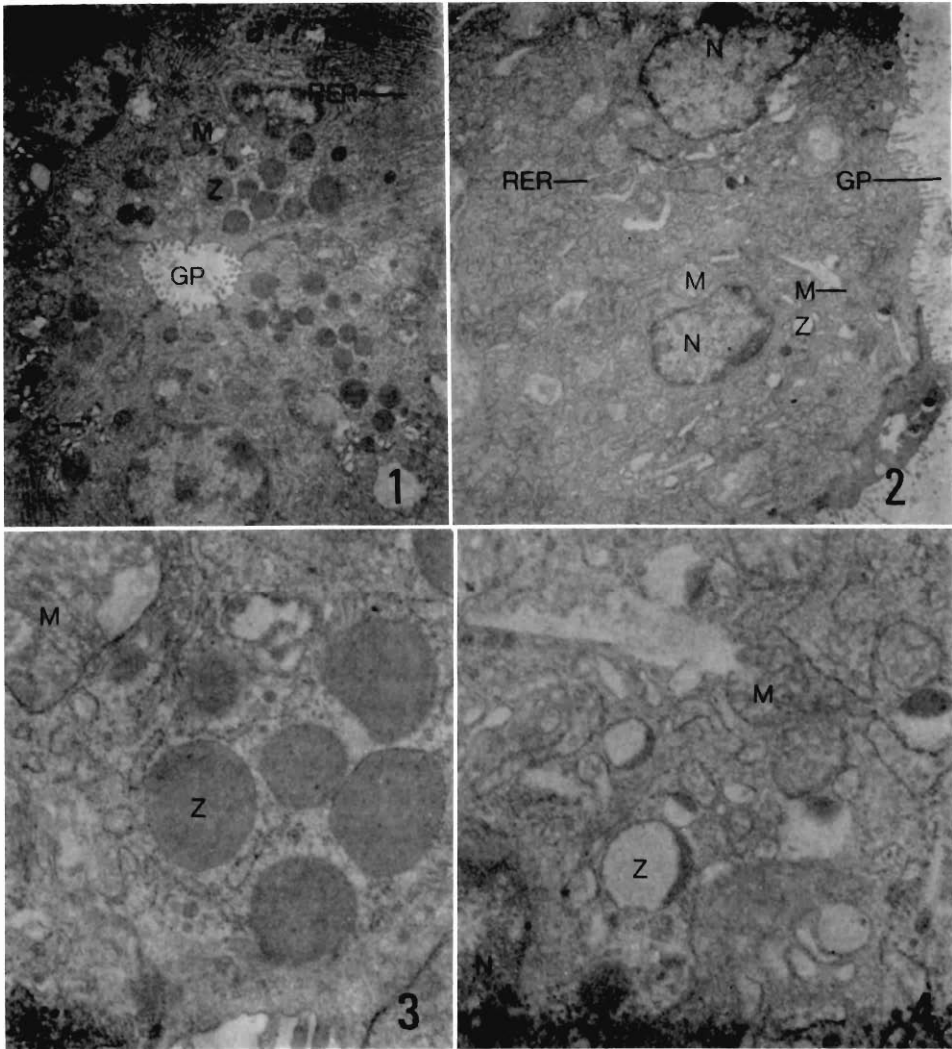
### Infections

The results of analyses of both gastric contents and sera are summarized in Tables 1 and 2. This series of infections presented a fairly typical syndrome of ostertagiasis in calves: a rise in the pH of the gastric contents (Table 1) and a decrease in plasma albumin (Table 2) with progressing infection. These studies show that albumin/globulin ratios decreased from 5 to 30 DAI. However, the hypoalbuminemia was not reflected by increased levels of albumin in the gastric contents. These values were less in the infected calves than in the controls. Perhaps hypoalbuminemia was caused by factors other than leakage since plasma albumin leaked negligibly into the gastric contents of calves infected 22–30 DAI. Further investigations to clarify the basis of hypoalbuminemia are warranted.

### Light and electron microscopy

The gross pathology and histopathology in the present infections were similar to those reported by Ritchie et al. (1966). Histochemical findings 0–26 DAI were similar to those reported previously by Stringfellow (1974, 1977) but with these additional observations. Pepsinogen granules were completely denuded from chief cells from 26 DAI onwards. A large parietal cell mass was present throughout the fundus even at 30 DAI although fewer parietal cells were detected in the immediate vicinity of infected gastric glands. Methods for carbonic anhydrase detected less visible precipitate around the infected gland and detected almost no stainable activity throughout the tissue at 26 DAI or later. Otherwise, stainable carbonic anhydrase activity at 0–26 DAI was similar to that previously reported by Stringfellow (1977).

Murray (1970) and Ross et al. (1971) described the fine structure of chief cells and other fundic cells from uninfected calves (Fig. 1). Murray and Jennings (1970) and Murray (1969) basically described the changes in the fine structure of fundic cells from calves infected with *O. ostertagi* (Fig. 2). Ultrastructural study of pepsinogen granules from heavily damaged chief cells showed that there was variation both between cells and between cells in different areas. Generally badly damaged chief cells were denuded of pepsinogen granules. Pepsinogen granules in chief cells from uninfected calves are shown in Figure 3. There were occasional crescent shaped indentations of the granules (Fig. 4), and in severely damaged cells, the granule membrane was lost. The nucleus of the chief cell was indented



Figures 1-4. Pepsinogen granules of chief cells from calf abomasum uninfected (1, 3) and infected (2, 4) with *Ostertagia ostertagi* (26 DAI). 1. Gastric gland with pepsinogen granules (Z) in chief cells from infected calves.  $\times 5,000$ . 2. Abnormal pepsinogen granules in chief cells from infected calves.  $\times 5,000$ . 3. Structure of pepsinogen granules in chief cells from uninfected calves.  $\times 20,000$ . 4. Structure of abnormal pepsinogen granules in chief cells from infected calves.  $\times 20,000$ . Other abbreviations: rough endoplasmic reticulum (RER), gastric pit (GP), Golgi apparatus (G), nucleus (N), mitochondria (M).

and shrunken in badly damaged cells. In general, these results at the ultrastructural level were predictable from results observed by light microscopy (Stringfellow, 1974).

#### Gastric contents and serum

The results of these infections indicate that plasma proteins leaked negligibly into the gastric contents, particularly at 26-30 DAI. Plasma proteins that leaked naturally into the gastric contents served as normal controls (Table 1). Leakage

of plasma proteins was relative rather than absolute; that is, it was measured as HPR or plasma protein values in the gastric contents from infected calves relative to controls. Albumin and globulin values were significantly less in the gastric contents of infected calves at 22–30 DAI than in those of controls. Plasma proteins were probably negligibly digested by pepsin between 22–30 DAI because pepsinogen is negligibly converted to pepsin above pH 5 and gastric pepsin values dropped to very low values at 26–30 DAI. On the other hand, plasma proteins probably were digested by pepsin at 0–14 DAI because pepsin was abundantly present and the pH of the gastric contents was optimal to activate pepsinogen to pepsin. Albumin and globulins then may have been digested somewhat by pepsin. However, that made the interpretation of the data more conservative because control values were equal to or less than they might have been. The pepsin concentration of gastric contents from infected calves peaked at 14 DAI then it dropped precipitously at 22–30 DAI. Plasma pepsinogen would have been detected if it had leaked into the gastric contents particularly at 26–30 DAI. Instead it was hardly detectable. One could not conclude if plasma proteins leaked significantly into the gastric contents from the circulation at 0–14 DAI. Digestion of plasma protein and pepsin secreted by chief cells complicated the interpretation as was also true in a previous study (Jennings et al., 1966). In general, HPR values in the gastric contents from infected calves were similar to those from uninfected calves. The results of these infections suggest strongly, but not unequivocally, that plasma proteins leaked negligibly into the gastric contents.

The pH of the gastric contents is a good measure of the damage to the functional stomach. The pH of the gastric contents of infected calves increased significantly from control values of 2.1–2.5 to as high as 7.2–7.5 at 26 DAI. In general, the correlation between plasma pepsinogen values and the pH of the gastric contents is positive (Anderson et al., 1966). The present studies showed also that if the pH of the gastric contents rose significantly (22–30 DAI) so too did the plasma pepsinogen values (Tables 1, 2). In general, serum values were similar to those reported by Anderson et al. (1966) and Jennings et al. (1966) except that plasma pepsinogen values were unusually high at 14–22 DAI onward in infected calves. Concurrently, the pepsin values of the gastric contents peaked then dropped to significantly low values. Chief cells (Figs. 1–4) were denuded of pepsinogen granules. These results imply that pepsinogen was retained in the circulation and thus caused unusually high plasma pepsinogen values at 22–30 DAI. Plasma pepsinogen, which elevated appreciably without leaking significantly into the gastric contents, implies that pepsinogen secreted by chief cells was released directly into the circulation rather than taken up from the gastric contents through a damaged vasculature as was suggested by Armour (1974) and discussed by Ford (1976).

These studies elucidate a probable mechanism by which plasma pepsinogen is elevated in Type I ostertagiasis. They also show that in Type I ostertagiasis: plasma pepsinogen is not always leaked significantly from the circulation into the gastric contents and abnormally high plasma pepsinogen values may occur if they are not leaked.

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## Evidence of Spring and Post-Parturient Fecal Nematode Ova Count Rises in Arkansas Sheep

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**ABSTRACT:** Thirty-three Hampshire × Suffolk crossbred sheep were sampled for feces and EPG (nematode eggs per gram of feces) counts determined from 11/12/77 to 5/8/78. All animals were treated for parasitic, gastrointestinal (GI) nematodes in November. The EPG levels displayed thereafter represented nematode populations acquired from contaminated pasture and/or the maturation of hypobiotic forms. Both spring and post-parturient fecal nematode ova level rises were noted. *Haemonchus* was the genus which precipitated both rises. *Trichostrongylus* and *Ostertagia* genera ova were also noted in significant levels.

Although springtime and post-parturient EPG rises may occur concurrently in sheep, they are apparently of separate etiology and additive in effect (Gibbs, 1964). These phenomena are well founded evolutionarily in that they provide for enhanced parasite fecundity and dissemination during the time of optimum moisture, ambient temperature, and susceptible host concentration (the lambs). *Haemonchus* and *Ostertagia* genera appear to be the ones primarily responsible for this enhanced parasite fecundity (Gibbs, 1968, 1977).

The data reported herein were obtained to seek evidence of springtime and post-parturient EPG rise in Arkansas sheep, the nematode genera involved, and the parasitic helminth population fluctuations following routine fall anthelmintic treatment; as revealed by EPG determinations.

### Materials and Methods

The experiment consisted of two parts done concurrently utilizing two groups of Hampshire × Suffolk sheep. Group I consisted of 14 mature ewes (1-5 years of age) that were rectally sampled for feces and EPG (nematode eggs per gram of feces) counts determined periodically from 11/12/77 to 5/8/78. Ten of the ewes lambed in March and the other four were open throughout the trial.

Group II consisted of six mature rams (3-7 years of age) and 13 mature ewes (1-5 years of age). Determinations of EPG levels were done on 1/30, 2/13, 2/23, and 2/27/78, only. All Group II ewes lambed in January.

Animals of both groups were managed alike. Anthelmintic treatment<sup>1</sup> was given on 11/21 and 11/29. All animals grazed the same small grain and grass pasture throughout the study. Only when a ewe was lambing or lactating was it penned in an open barn with straw bedding and fed hay and grain as supplement.

For each EPG determination, from 0.1 to 1.0 gm of feces (varied inversely with expected ova number) was weighed and recorded for the sample. The feces were then homogenized in 10 ml of water and the homogenate strained through a tea strainer (aperture of 1.5 mm). The filtrate was equally divided into two 15-ml centrifuge tubes. The tubes were then filled with sugar solution (prepared according to Sloss, 1976) covered with coverslips and centrifuged for 3 min at 227 ×

<sup>1</sup> Tramisol; American Cyanamid.

**Table 1.** Mean nematode EPG counts ( $\log_{10}$ ).

| Date     | Group I Ewes         |                          |
|----------|----------------------|--------------------------|
|          | Pregnant-lambing     | Open                     |
| 11/21/77 | 1.573 <sup>d</sup>   | 1.710 <sup>a,b,c,d</sup> |
| 11/29/77 | 0.060 <sup>f</sup>   | 0.000 <sup>e</sup>       |
| 12/12/77 | 0.095 <sup>f</sup>   | 0.119 <sup>e</sup>       |
| 12/19/77 | 0.000 <sup>f</sup>   | 0.250 <sup>e</sup>       |
| 1/5/78   | 0.835 <sup>e</sup>   | 0.443 <sup>d,e</sup>     |
| 1/16/78  | 1.122 <sup>d,e</sup> | 0.766 <sup>e,d,e</sup>   |
| 1/30/78  | 1.500 <sup>d</sup>   | 0.998 <sup>b,c,d,e</sup> |
| 2/13/78  | 2.154 <sup>c</sup>   | 1.864 <sup>a,b,c</sup>   |
| 2/27/78  | 2.708 <sup>b</sup>   | 2.469 <sup>a</sup>       |
| 3/13/78  | 2.856 <sup>a,b</sup> | 2.390 <sup>a</sup>       |
| 3/27/78  | 3.221 <sup>a,b</sup> | 2.433 <sup>a</sup>       |
| 4/10/78  | 3.264 <sup>a</sup>   | 2.544 <sup>a</sup>       |
| 4/24/78  | 3.152 <sup>a,b</sup> | 2.176 <sup>a,b</sup>     |
| 5/8/78   | 2.758 <sup>a,b</sup> | 2.192 <sup>a,b</sup>     |

<sup>a,b,c,d,e</sup> Means in the same column with unlike superscripts are significantly different ( $P < 0.05$ ).

g. The slips were placed on a microscope slide and the total number of ova counted. The number of ova divided by the sample weight was recorded as the EPG value.

In order to assess the distribution of the nematode ova amongst the specific genera where ova characteristics were not sufficient, egg culturing was employed and the third-stage larvae identified according to Dikmans and Andrews (1933).

In order to compare EPG means for the various sheep groupings at various times, all EPG counts were transformed to  $\log_{10}$ , thereby reducing the tremendous variability inherent to the EPG count. The resultant data were then subjected to standard analysis of variance, the Student's *t*-test for paired comparisons and the Duncan's Multiple Range test for multiple mean comparisons (Snedecor and Cochran, 1967; Steele and Torrie, 1960).

### Results and Discussion

The mean  $\log_{10}$  nematode EPG counts for the Group I ewes are presented in Table 1. The trend for the ewes which lambed in March showed a significant elevation about the time of lambing. On the other hand, the EPG level of the open ewes in spring did not exceed that seen in November before routine anthelmintic treatment. The additive effect of parturition therefore was evident in the Group I ewes. The lowering of ova counts in May is sooner than that recorded for sheep in northern areas (Gibbs, 1977), probably due to differences in the onset of seasons. Perhaps also, hypobiosis is not as pronounced in worm burdens in the southern part of the United States as it is in the north, where change of season is much more dramatic. In other work done on the epizootiology of ovine helminthiasis in the southern United States (Ciordia and Neville, 1969) no spring rise was noted, but rather a fall rise due to increased moisture at the time of decreased pasture forage. Undoubtedly, a similar rise exists in Arkansas.

Figure 1 depicts individual nematode genera EPG trends for the Group I ewes that lambed in March. The mean lambing date was March 16. *Trichostrongylus*

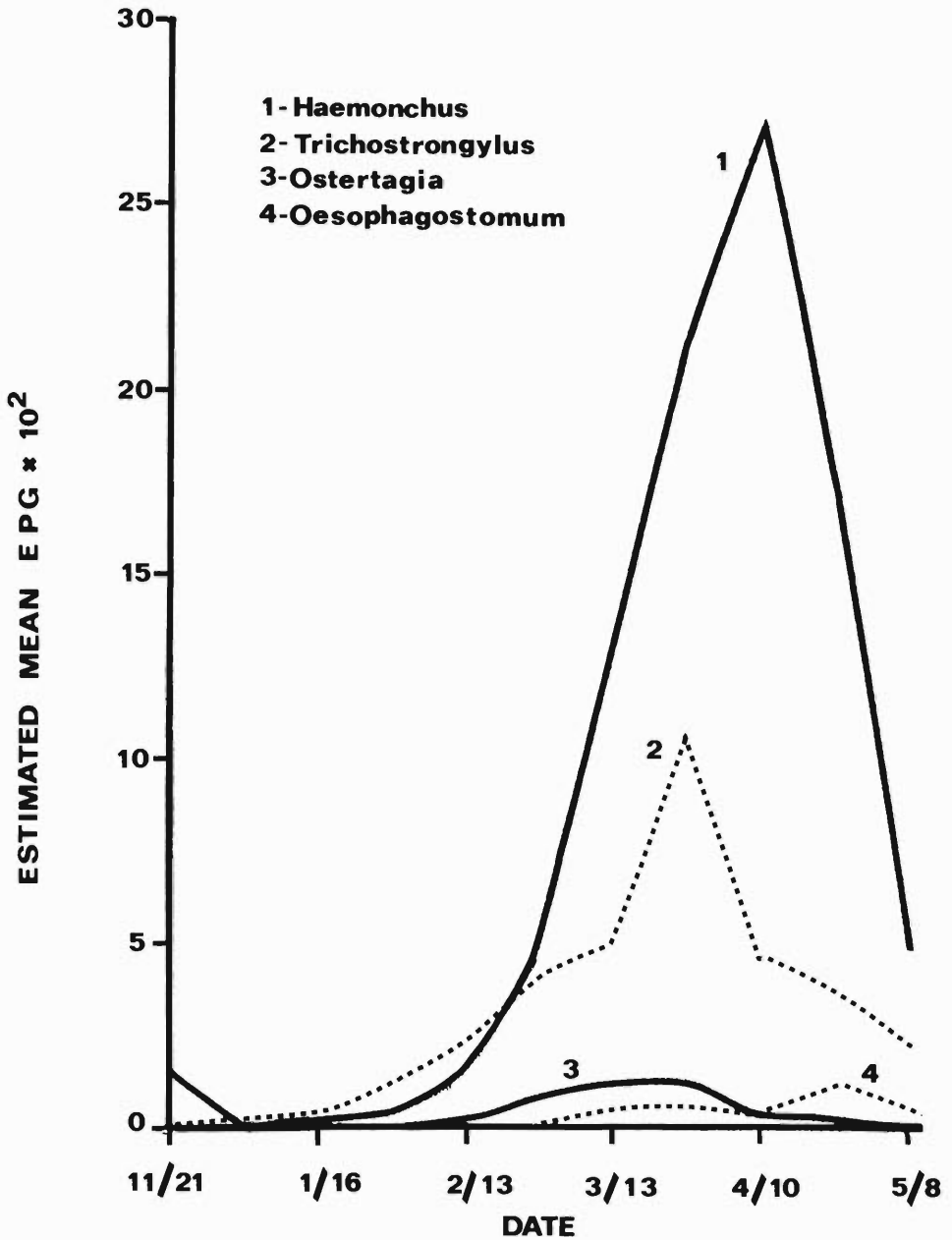


Figure 1. Estimated mean, per-genera EPG counts for Group 1 ewes (pregnant-lambing).

ova levels were the earliest to rise posttreatment. After 2/13 however, the point at which the rise became significant, *Haemonchus* was the major egg-layer. Both genera peaked in fecundity at the same time, indicating commonality of host-parasite-season interactions. *Ostertagia* and *Oesophagostomum* genera ova were present in light proportions.



**Table 2. Mean nematode EPG levels ( $\log_{10}$ ) and percentage contributions by the various nematode genera for Groups I and II.**

| Group                           | Mean EPG level ( $\log_{10}$ ) | % of ova as:      |                         |                   |                        |                 |
|---------------------------------|--------------------------------|-------------------|-------------------------|-------------------|------------------------|-----------------|
|                                 |                                | <i>Haemonchus</i> | <i>Trichostrongylus</i> | <i>Ostertagia</i> | <i>Oesophagostomum</i> | <i>Cooperia</i> |
| Group I lambed ewes (3/27/78)   | 3.221 <sup>a</sup>             | 67.5              | 26.5                    | 4.3               | 0.9                    | 0.9             |
| Group II lambed ewes (1/30/78)  | 2.537 <sup>b</sup>             | 67.3              | 13.5                    | 19.2              | 0.0                    | 0.0             |
| Group I open ewes (3/27/78)     | 2.433 <sup>b,c</sup>           | 65.5              | 26.0                    | 5.0               | 3.5                    | 0.0             |
| Group II rams (1/30/78)         | 1.705 <sup>c,d</sup>           | 27.7              | 72.0                    | 0.0               | 0.3                    | 0.0             |
| Group I pregnant ewes (1/30/78) | 1.500 <sup>c,d</sup>           | 25.2              | 73.0                    | 1.8               | 0.0                    | 0.0             |
| Group I open ewes (1/30/78)     | 0.998 <sup>d</sup>             | 0.0               | 100.0                   | 0.0               | 0.0                    | 0.0             |

<sup>a,b,c,d</sup> Means in the same column with unlike superscripts are significantly different ( $P < .05$ ).

Table 2 is a comparison between groups of certain EPG values ( $\log_{10}$ ) which were obtained during the study. The animals displaying the highest EPG counts were those ewes which lambed in the spring. The March 27 EPG counts were obtained, on the average, 11.2 days post-parturition. The group with the next highest counts was the Group II ewes which lambed in January. The January 30 EPG counts were taken, on the average, 14.8 days post-parturition. The significant difference between the two EPG means was due to the compounding effect of lactation and spring conditions in the Group I lambing ewes only. EPG levels due to lactation (January 30 Group II lambed ewes) and spring conditions (March 27 Group I open ewes) were not significantly different. This suggests that season and lactation are of equal importance in initiating fecal egg count rises. The lowest EPG levels were seen in nonlactating ewes and rams in the winter (January 30 levels). From these results, it is apparent that reproduction and season were the stimuli for enhanced nematode fecundity; a finding which has been reported previously (Crofton, 1958; Gibbs, 1968). It was also interesting to note, that the greater the percentage of ova as *Haemonchus*, the higher the total EPG level. *Haemonchus* accounted for the elevated ova levels in the spring, and also, during lactation.

#### Acknowledgments

The author thanks Dr. A. H. Brown for the statistical analysis of the data and Mr. D. Nash for his technical assistance.

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## BOOK REVIEW

*Pathology of Tropical and Extraordinary Diseases. An Atlas*. Volume I and Volume II, edited by Chapman H. Binford and Daniel H. Connor. Published by the Armed Forces Institute of Pathology, Washington, D.C., Vol. I, 1976, 339 pp., illus., \$15; Vol. II, 1976, 355 pp., illus., \$20.

This very handsome two volume set succeeds the classic, "Pathology of Tropical Diseases—an Atlas," by Colonel James E. Ash and Dr. Sophie Spitz, which was published in 1945. In the new work, 44 authors have contributed 112 authoritative chapters dealing with 218 diseases, approximately one-fourth of which had not been described in man by 1945. Volume I consists of 7 sections which successively describe diseases caused by viruses, chlamydial agents, rickettsiae, spirochetes, bacteria, mycobacteria and protozoa. Volume II comprises sections dealing with diseases caused by filarial nematodes, other nematodes, trematodes, cestodes, fungi and actinomycetes, arthropods and unusual, undefined and other agents. There is also a section on nutritional, metabolic and neoplastic diseases. The text, throughout, is profusely illustrated with nearly 1900 good quality maps, photographs and photomicrographs, of which 276 are in color.

These attractively priced volumes are well produced and well edited and will be valuable references for physicians, parasitologists, and others interested in this area for many years to come.—A. James Haley, University of Maryland, College Park.

## Helminth Parasitism in the Badger, *Taxidea taxus* (Schreber, 1778), from the Western Great Plains<sup>1</sup>

DANNY B. PENCE<sup>2</sup> AND ROBERT C. DOWLER<sup>3</sup>

**ABSTRACT:** One cestode and eight nematode species were collected from 30 badgers, *Taxidea taxus*, from Kansas and West Texas. These included *Mesocestoides corti* Hoeppli, 1925 (23% of hosts infected), *Filaroides milksi* Whitlock, 1956 (7%), *Filaria taxideae* Keppner, 1970 (33%), *Metathelazia capsulata* Gerichter, 1948 (47%), *Physaloptera torquata* Leidy, 1886 (100%), *Molineus* sp. (37%), *Ancylostoma taxideae* Kalkan and Hansen, 1966 (80%), *Ascaris columnaris* Leidy, 1856 (30%), and *Capillaria aerophila* (Creplin, 1839) Travassos, 1915 (10%). Badgers were infected with from 1 to 7 ( $\bar{x}$  = 3.5) helminth species. Simpson's index was low (0.17) indicating a dispersed helminth fauna in this host. Intensities of infection were low for most helminth species except *P. torquata* which ranged from 3 to 644 ( $\bar{x}$  = 112) worms per host. Low similarity indexes indicated the helminth faunas from badgers in different localities of North America are basically different. The female-male ratio of *A. taxideae* was above unity (3.2:1) and positively correlated with the worm burden in badgers. The mean number of helminth species in 17 male hosts was 3.6 species while that of 13 female badgers was 3.1 species. There was no significant difference in frequency of occurrence of helminth species in different host sexes. New host records are established for *M. capsulata*, *F. milksi*, and *C. aerophila*. *Metathelazia capsulata* is redescribed from the North American badger. The host-parasite relationships and pathology of the various helminth species are discussed.

Badgers, *Taxidea taxus* (Schreber, 1778), are important carnivorous mammals in western North America. Although the literature consists of numerous sporadic reports of helminths from these hosts, there are only three comprehensive studies on their helminth fauna. Erickson (1946) reported six helminth species from Minnesota badgers. Leiby et al. (1971) documented nine helminth species in this host from South Dakota, and Wittrock and Ulmer (1974) found 13 helminth species from Iowa badgers. Leiby et al. (1971) provided a checklist of helminths reported from this host in North America. The present study examines the nature and extent of the helminth fauna from 30 badgers collected in the Western Great Plains of Kansas and the High Plains of Texas.

### Materials and Methods

Badgers were obtained as carcasses from fur trappers in western Kansas and the Texas Panhandle in the winter, 1976-1977. Carcasses were frozen for later necropsy. Total recovery of all helminths was attempted. Tissue from lungs, liver, kidney, lymph nodes, and subcutaneous tissues was preserved in 10% formalin for later histological studies. Sections were cut at 6  $\mu$ m and stained with hematoxylin and eosin. Nematodes were fixed in glacial acetic acid, preserved in 70% ethyl alcohol with 5% glycerine, and examined in glycerine wet mounts. Cestodes were fixed in AFA, preserved in 70% ethyl alcohol, stained in Celestin blue B,

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Table 1. Helminths of the badger from the Western Great Plains.

| Helminth   | No. infected/no. examined | %   | Intensity |      | Locality        |
|--|---------------------------|-----|-----------|------|-----------------|
|  |                           |     | Range     | Mean |                 |
| Cestoda  |                           |     |           |      |                 |
| <i>Mesocestoides corti</i><br>Hoepli, 1925 (I)                     | 7/30                      | 23  | 1-300+    | —    | Kansas<br>Texas |
| Nematoda   |                           |     |           |      |                 |
| <i>Filaroides milksi</i><br>Whitlock, 1956 (L)                     | 2/30                      | 7   | —         | —    | Kansas          |
| <i>Filaria taxideae</i><br>Keppner, 1970 (T)                       | 10/30                     | 33  | 1-6       | —    | Kansas          |
| <i>Metathelazia capsulata</i><br>Gerichter, 1948 (L)               | 14/30                     | 47  | 2-25      | 4    | Kansas          |
| <i>Physaloptera torquata</i><br>Leidy, 1886 (S)                    | 30/30                     | 100 | 3-644     | 112  | Kansas<br>Texas |
| <i>Molineus</i> sp. (I)  | 11/30                     | 37  | 1-26      | 13   | Kansas<br>Texas |
| <i>Ancylostoma taxideae</i><br>Kalkan and Hansen, 1966 (I)         | 24/30                     | 80  | 1-80      | 12   | Kansas<br>Texas |
| <i>Ascaris columnaris</i><br>Leidy, 1856 (I)                       | 10/30                     | 30  | 1-25      | 8    | Kansas          |
| <i>Capillaria aerophila</i><br>(Creplin, 1839) Tranassos, 1915 (L) | 3/30                      | 10  | 1         | 1    | Kansas          |

(I) Intestine, (S) Stomach, (L) Lung, (T) Subcutaneous.

and mounted in Canada balsam. Calculation of Simpson's index was according to Holmes and Podesta (1968). An index of similarity comparing the helminth faunas of the Western Great Plains with that reported in previous studies from the badgers in Minnesota, Iowa, and South Dakota was calculated according to Holmes and Podesta (1968). A coefficient of correlation ( $r$ ) was calculated for intensity of infection and the parasite female-male ratio (FMR) of *Ancylostoma taxideae* from badgers with significance of the  $r$ -value determined with a  $t$ -test (Sokal and Rohlf, 1969). Significance of mean numbers of helminth species in 17 male and 13 female badgers was analyzed by chi-square analysis (Sokal and Rohlf, 1969). Figures were prepared with the aid of a Leitz drawing tube. All measurements are in  $\mu\text{m}$  unless otherwise indicated. In the following description the means follow in parentheses the range of all measured values. Skins and skulls of badgers collected in this study are deposited in the Collection of Mammals, Museum of the High Plains, Fort Hays State University.

## Results

### Ecological implications

One cestode and eight nematode species were recovered from 30 badgers in western Kansas and the Texas Panhandle (Table 1). All animals were infected with from one to seven helminth species ( $\bar{x} = 3.5$  species). Intensities of infection

**Table 2. Helminths of badgers from different regions of North America.**

| Helminth                         | Locality                         |  |   |  |
|----------------------------------|----------------------------------|--|---|--|
|                                  | Minnesota<br>(Erickson,<br>1946) | Iowa<br>(Whitlock<br>and Ulmer,<br>1974) | South Dakota<br>(Leiby et al.,<br>1971) | Western Great<br>Plains<br>(Present study) |
| <b>Trematoda</b>                 |                                  |  |   |  |
| <i>Alaria taxideae</i>           | 13*                              | 79                                       | 29                                      | —  |
| <i>Eupharyphium</i> sp.          | 8                                | —  | —                                       | —  |
| <i>Eupharyphium melis</i>        | —                                | 4  | 12                                      | —  |
| <i>Fibricola cratera</i>         | —                                | 4  | —                                       | —  |
| <b>Cestoda</b>                   |                                  |  |   |  |
| <i>Atriotaenia procyonis</i>     | —                                | 42                                       | 16                                      | —  |
| <i>Mesocestoides lineatus</i>    | —                                | 8  | —                                       | —  |
| <i>Mesocestoides corti</i>       | —                                | —  | —                                       | 23   |
| <i>Monordotaenia taxidiensis</i> | —                                | 8  | 25                                      | —  |
| <b>Nematoda</b>                  |                                  |  |   |  |
| <i>Capillaria aerophila</i>      | —                                | —  | —                                       | 10   |
| <i>Capillaria plica</i>          | —                                | 13                                       | —                                       | —  |
| <i>Trichinella spiralis</i>      | —                                | 4  | —                                       | —  |
| <i>Dracunculus insignis</i>      | —                                | 8  | —                                       | —  |
| <i>Filaria taxideae</i>          | —                                | —  | 6                                       | 33   |
| <i>Filaroides milksi</i>         | —                                | —  | —                                       | 7  |
| <i>Ancylostoma taxideae</i>      | —                                | 54                                       | 29                                      | 80   |
| <i>Molineus mustelae</i>         | —                                | 83                                       | —                                       | —  |
| <i>Molineus</i> sp.              | —                                | —  | —                                       | 37   |
| <i>Molineus patens</i>           | 13                               | —  | 65                                      | —  |
| <i>Metathelazia capsulata</i>    | —                                | —  | —                                       | 47   |
| <i>Physaloptera torquata</i>     | 13                               | 67                                       | 100                                     | 100  |
| <i>Physaloptera maxillaris</i>   | 13                               | —  | —                                       | —  |
| <i>Ascaris columnaris</i>        | 13                               | 29                                       | 35                                      | 30   |

\* % infected.

of most helminth species were low, except *Physaloptera torquata* in which all animals were infected and intensities ranged from three to 644 ( $\bar{x} = 112$ ) worms.

Simpson's index was low (0.17) indicating a lack of dominance of particular helminth species in this host. Indexes of similarity comparing the helminth fauna of the Western Great Plains badgers with those reported in previous studies from Minnesota (Erickson, 1946), South Dakota (Leiby et al., 1971), and Iowa (Whitlock and Ulmer, 1974) were low for all compared areas (Table 2). These data were arranged in a trellis diagram for comparative purposes (Fig. 11). Simpson's indexes calculated for the helminth faunas for Minnesota, South Dakota, and Iowa were 0.19, 0.18, and 0.15, respectively, also indicating an equitable distribution (lack of dominance) of helminth species in these areas.

The mean number of helminth species from 17 male badgers was 3.6 species while 13 female hosts harbored a mean number of 3.1 helminth species. There was no statistical difference in frequency of occurrence of helminth species between male and female badgers. Since all animals collected were adults, no comparisons of helminth parasitism in different age-classes were possible.

The female-male ratio of *Ancylostoma taxideae* was 3.2:1 in combined sexes of badger hosts. The *r*-value was 0.889 between worm burden and the FMR. The

Table 3. FMR of *Ancylostoma taxideae* from the badger.

| FMR   | N  | ♂  | ♀   | r      | ts    | P      |
|-------|----|----|-----|--------|-------|--------|
| 3.2:1 | 24 | 66 | 208 | 0.8893 | 9.124 | <0.001 |

calculated *t*-value was highly significant ( $P < 0.001$ ) indicating a positive correlation (Table 3). As the number of total worms increase within the host the relative number of males to females increase, thus decreasing the FMR. Because of the small sample size or, in the case of *Physaloptera torquata*, the large number of sexually immature stages the FMR was not calculated for other nematode species.

### Taxonomic remarks

Three new host records are established for nematodes from the badger. These are *Metathelazia capsulata*, *Filaroides milksi*, and *Capillaria aerophila*. Additionally, a new species of *Molineus* was recovered. Its description is to be presented elsewhere (Platt and Pence, in preparation). Also, the following redescription of *M. capsulata* is presented in lieu of certain morphological features which differ from its original description in the Old World badger, *Meles meles*. Specimens of representative species of helminths recovered from badgers in this study are deposited in the Medical Zoology Collection, The Museum of Texas Tech University, TTUM-MZ No. 12648–12669, 12811–12920.

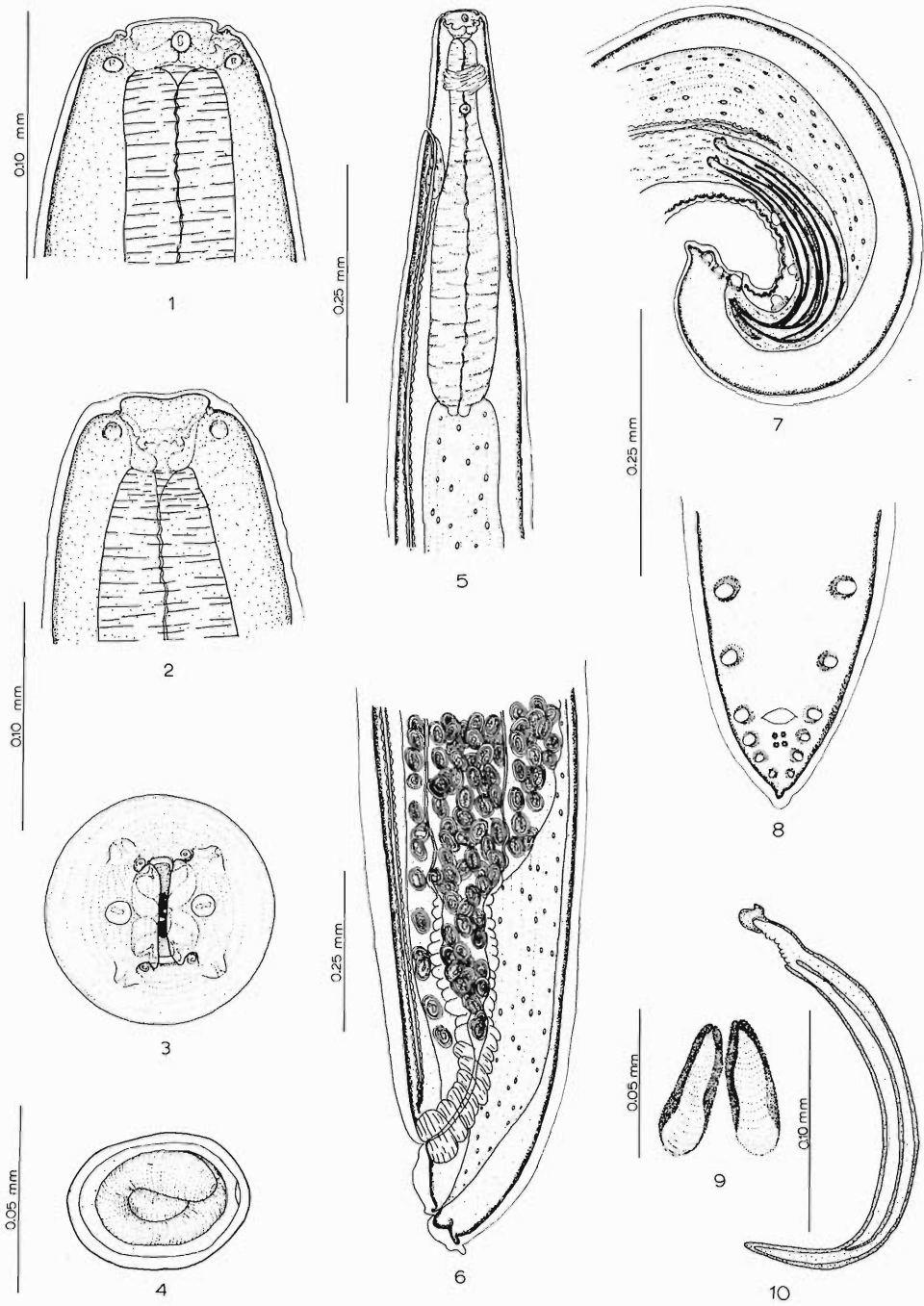
#### *Metathelazia capsulata* Gerichter, 1948

(Figs. 1–10)

Pneumospiruridae Wu and Hu, 1938; *Metathelazia* Skinker, 1931; *M. capsulata* Gerichter, 1948.

HOST RECORDS: 14 or 25 badgers, *Taxidea taxus*, infected from western Kansas (Trego, Graham, Wallace, and Phillips counties) collected November 1976 through January 1977 by R. C. Dowler. None of the five badgers infected from King and Knox counties, Texas collected December 1976 through February 1977 by D. P. Pence.

DESCRIPTION (based on 18 ♂♂ and 22 ♀♀): With characters of the genus. Body short, white, cuticle transparent without striations or subcuticular tuberculations. Pseudolabia weak; buccal capsule sclerotized, aperture elongate dorsoventrally, capsule wall thickened to base of esophagus forming 6 lip-shaped folds with the 4 dorsoventral folds smaller and more angulate and the 2 lateral folds shallow and massive (Figs. 1–3). Lateral margins of each fold of internal buccal capsule with a small toothlike structure arising from its outer margin (Fig. 3). Four large single papillae in outer circle, a pair each located laterally at dorsal and ventral margins of buccal capsule (Fig. 3). Two pairs smaller sublateral circumoral papillae located in the inward fold of superficial thickening of dorsal and ventral margins of buccal capsule (Fig. 3). Amphids lateral. Esophagus distinctly divided into anterior muscular and posterior glandular portions (Fig. 5). Intestine straight, smooth, without caecae. A pair of lateral deirids just posterior to nerve ring (Fig. 5). Excretory pore ventral, below level of nerve ring, joining a small oval gland diverging into a pair of small ventral tubules which extend posteriorly to near level of the anus (Figs. 5, 6). Female tail blunt, conical, truncate (Fig. 6).



Figures 1-10. *Metathelazia capsulata* from *Taxidea taxus*. 1. Lateral view of head. 2. Ventral view of head. 3. En face view of head. 4. Egg. 5. Anterior extremity of female. 6. Posterior extremity of female. 7. Lateral view of male posterior extremity. 8. Ventral view of male posterior extremity. 9. Gubernaculum. 10. Spicule.

|           |              |      |                      |                      |
|-----------|--------------|------|----------------------|----------------------|
|           |              |      | —                    | Western Great Plains |
|           |              |      | 38                   | Iowa                 |
|           |              | —    | 50                   | South Dakota         |
| —         | —            | 56   | 42                   | 35                   |
| —         | —            | 56   | 42                   | 35                   |
| Minnesota | South Dakota | Iowa | Western Great Plains |                      |

Figure 11. Trellis diagram of indexes of similarity of helminth faunas from badgers in different geographic regions of North America.

Vulva opening near anus, with a heavy sphincter muscle in terminal portion of vagina, ovjector muscular, closed by a weak sphincter at level of junction of vestibule and uterus (Fig. 6). Male tail spirally coiled, caudal alae absent, end conical terminating in a small sharp tip (Fig. 7). Six pairs large lateral pedunculate papillae; 2 pairs preanal, 3 pairs postanal, 1 pair adanal (Fig. 8). Two pairs small ventral papillae just posterior to anus, a pair of phasmids near tip of tail (Fig. 8). Spicules equal, similar, falciform, sharp-tipped, lateral wing on central shaft (Fig. 10). Gubernaculum divided into two identical scoop-shaped parts,  $\frac{1}{3}$  to  $\frac{1}{4}$  length of spicules (Fig. 9). Eggs thick shelled, with well-developed larvae in terminal portion of uterus (Fig. 4).

MALE: 8.63–12.05 (10.07) mm long, 131–346 (273) wide (maximum). Buccal capsule 12–54 (30) deep, 12–44 (28) wide. Muscular esophagus 205–345 (269) long, glandular esophagus 51–179 (117) long. Nerve ring and excretory pore 49–140 (89) and 72–130 (100) from anterior extremity, respectively. Spicules 183–315 (260) long, 12–15 (18) wide (maximum). Gubernaculum 25–53 (41) long, each section 8–18 (12) wide. Cloaca opening 36–76 (54) from tip of tail.

FEMALE: 11.56–21.83 (17.13) mm long, 294–603 (442) wide (maximum). Buccal capsule 10–50 (27) deep, 12–47 (34) wide. Muscular esophagus 222–345 (293) long, glandular esophagus 96–164 (137) long. Nerve ring and excretory pore 82–132 (110) and 62–204 (152) from anterior end, respectively. Vulva and anus 125–249 (187) and 35–94 (71) from posterior extremity, respectively. Eggs 29–37 (33) wide, 42–50 (44) long.

DISPOSITION OF SPECIMENS: 2 ♂♂ and 2 ♀♀ USNM Helm. Coll. No. 74892. Remaining specimens in Medical Zoology Collection, The Museum of Texas Tech University.

### Remarks

*Metathelazia capsulata* was described from the Old World badger, *Meles meles*, the fox, *Vulpes nilotica*, and the marbled polecat, *Vormela peregusna*, in Israel (Gerichter, 1948). It was also recovered from *Meles meles* in Russia (Sulimov, 1968). The present specimens conform in most respects to the original description by Gerichter (1948). However, the specimens examined herein (1) demonstrate a wider range of metric variability, (2) lack diverticulae or caecae on



the anterior intestine, (3) have a shorter muscular portion of the uterine tube, and (4) possess two pairs of small papillae just ventral to the anus in the male. This species differs from the other North American representative of the genus, *Metathelazia bassarisci* Pence and Stone, 1978, by (1) presence of four bifid teeth at the base of the buccal capsule in the latter (6 simple teeth in *M. capsulata*), (2) different shape of the buccal capsule with less indented lateral margins in *M. bassarisci*, (3) absence of subcuticular tuberculations arising from the hypodermis in the former (numerous in *M. bassarisci*), and (4) only six pairs pedunculate caudal papillae in *M. capsulata* (8 pairs in the latter species). This is the first report of this species from the New World.

**PATHOLOGY:** A number of badgers from both Kansas and Texas were heavily infected with *Physaloptera torquata*. Although some stomachs contained in excess of 500 worms, there was little evidence of gross or histopathological response in these hosts. Most animals were in prime condition with an abundance of subcutaneous and omental fat.

The lungs of two badgers infected with *Filaroides milksi* were mildly edematous and presented histologically with similar lesions to those reported from the hog-nosed skunk (Pence, 1978). There were only focal areas containing nematodes surrounded by a mildly granulomatous response with lymphocytes, epithelioid cells, plasma cells, and eosinophils. The lung parenchyma was mildly congested and alveolar septae were mildly edematous adjacent to the lesions. The intensity of infection and severity of the reaction was much less pronounced than that reported for skunks (Pence, 1978; Levine et al., 1965).

*Metathelazia capsulata* infections were light. Bronchioles containing this nematode were mildly congested with an exudate containing a few lymphocytes and plasma cells. There was bronchiectasis and hypertrophy of some smooth muscle fibers surrounding the infected bronchiole. The pathologic response in this host was similar to that reported for *Metathelazia bassarisci* in the ringtail (Pence and Stone, 1977).

The pathology of *Filaria taxideae* appeared identical to that previously described by Keppner (1971). Infections with the remaining helminth species were light to moderate in intensity and evoked no discernible pathological response.

### Discussion

The results of this study indicate badgers have a variable helminth fauna which is not a characteristic fauna throughout their range. Simpson's indexes for helminth faunas of badgers from the Western Great Plains, Minnesota, South Dakota, and Iowa are low indicating a lack of dominance of particular species. *Physaloptera torquata* and *Ascaris columnaris* are the only two species common to all four areas. Only two additional species, *Alaria taxideae* and *Ancylostoma taxideae*, are common to three of the four geographic areas. Thus, indexes of similarity for badger helminth faunas from different areas were low confirming a variable, noncharacteristic fauna throughout their range. Similar results were noted in other top carnivores such as the coyote (Holmes and Podesta, 1968; Pence and Meinzer, 1978) and bobcat (Stone and Pence, 1978) which occupy variable habitats over wide geographic areas.

The helminth species composition of badgers from the Western Great Plains reflects the semiarid habitat of this region and is composed primarily of species

with direct life histories (*Ancylostoma taxideae* and probably *F. milksi*) or terrestrial arthropod intermediate hosts (remaining helminth species). There is a conspicuous absence of species, especially trematodes, requiring more humid or aquatic environments common to those found on the Eastern Great Plains reported in previous studies (Minnesota, Iowa, and South Dakota).

There was no significant difference between sex of the badger hosts and extent of helminth parasitism. This is also the situation in other carnivores (Pence and Meinzer, 1978; Stone and Pence, 1977). Unfortunately, the small host sample size did not permit multispecies analyses in terms of frequency of occurrence of helminth species, mean levels of infection, and helminth parasitism versus age of the host.

As noted in hookworm infections from other carnivores such as in dogs (Roche and Patrzek, 1966) and coyotes (Young and Pence, 1979) the FMR of *A. taxideae* in the badger is above unity and positively correlated with the worm burden. As the intensity of infection increases, the FMR decreases. Possible causes for this phenomenon are reviewed by Young and Pence (1979).

The taxonomy of the primitive pneumospirurid lungworms was recently reviewed by Pence and Stone (1977) who described a new species, *Pneumospirura bassarisci*, from the ringtail, *Bassariscus astutus*, and redescribed two species from the bobcat, *Felis rufus*, in North America. These authors considered that "the genus *Pneumospirura*, represented by *P. hainanensis*, *P. capsulata*, and *P. bassarisci*, is localized in the bronchioles of carnivorous mammals . . . [and] . . . are short robust nematodes closely resembling the genus *Vogeloides* except for the presence of a well developed sclerotized buccal capsule with teeth and reduced lips." Wertheim and Chahaud (1977) revised the family Pneumospiruridae based on SEM studies of cephalic structures of several species and considered the genus *Pneumospirura* a subjective synonym of *Metathelazia*. Thus, the species described as *P. bassarisci* from the ringtail by Pence and Stone (1977) should now be considered as *Metathelazia bassarisci* (Pence and Stone, 1977). Additionally, recent evidence based on a new species from an Australian marsupial which is intermediate between the remaining pneumospirurid genera, *Vogeloides* and *Metathelazia*, suggests that only a single genus, *Metathelazia*, should be recognized in the Pneumospiruridae (Spratt, 1979, pers. commun.). The redescription of *M. capsulata* is presented herein to clarify certain morphological features of this species and to compare specimens from New and Old World hosts. Apparently, the species is circumboreal in distribution extending as far south as Israel and Texas.

The symptoms of heavy infections of *Physaloptera* sp. in badgers and their response to anthelmintic treatment was examined by Ehlers (1931). In the present study there was no evidence of a pathologic response to heavy *P. torquata* infections in adult badgers. Also, there was no pathological response to other internal helminth species, except *F. milksi* in the lungs. Even in this infection, however, there were few worms present and the concordant response was minimal, but very similar to that reported in skunks (Levine et al., 1965; Pence, 1978). The pathology of *Filaria taxideae* in the skin and subcutaneous tissues of badgers is documented by Keppner (1971).

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## Taxonomy, Biology, and Occurrence of Some Marine Leeches in Newfoundland Waters

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**ABSTRACT:** Taxonomy, biology, and occurrence of four species of marine leeches, from the NW Atlantic, in the vicinity of Newfoundland were studied. Species include *Johanssonia arctica* (Johansson), *Platybdella olriki* Malm, *P. anarrhichae* (Diesing), and *Calliobdella nodulifera* (Malm).

This paper is the second in our studies on marine leeches from the northwestern North Atlantic, in the vicinity of Newfoundland. Previously we (Khan and Meyer, 1976) discussed the taxonomy and biology of four species of the *Malmiana-Oceanobdella* complex.

Taxonomy of marine leeches is poorly known, and information concerning North American representatives is particularly meager. The principal limitation on availability of material is because it is related to the degree of difficulty of obtaining the host, which is usually fish. A few species infest Reptilia and Arthropoda. The arthropod hosts are usually decapod Crustacea, but in a few instances Pycnogonida are utilized. Some of the host-parasite data have been summarized by Knight-Jones (1962).

The best faunistic records are provided by the excellent knowledge of their occurrence on the coasts of Scandinavia and Greenland (Malm, 1863, 1865; Johansson, 1896, 1899), the Arctic Ocean and northern regions of the Atlantic and Pacific Oceans (Selensky, 1914b, 1915, 1923; Vasileyev, 1939). Occurrence records have been extracted and brought together by Epshtein (1967). Soós (1965) and Epshtein (1961, 1962, 1967, 1968) are particularly useful in coordinating early and recent nomenclature.

### Materials and Methods

Animals were studied alive and fixed, in serial sections, dissections, and whole mounts. Before fixation leeches were anesthetized with 5 to 15% ethanol in seawater. Following anesthetization, specimens were transferred to a slide and straightened, and fixative (80% ethanol) added slowly. This resulted in straight, normally relaxed, and unflattened specimens. In mature and unengorged worms properly anesthetized prior to fixation there is uniformity of characters, with only slight variability.

Descriptive measurements include the means, followed by the range in parentheses. Length measurements are overall, i.e., both suckers are included. Measurements are in mm, unless otherwise indicated, and are based upon 10 or more readings. No attempt has been made to compile every host and all occurrence records for each locality as this would be redundant; however, all original host species and locality records are included. Vernacular and scientific names of fish are from Leim and Scott (1966).

A stock population of *Johanssonia arctica* was established on spider crabs, *Chionoecetes opilio* (Fabricius), in May 1973 and maintained throughout the

study in holding tanks (100 × 80 × 65 cm), with a water flow of approximately 3 liters/min. Longhorn sculpins (*Myoxocephalus octodecemspinosus*) were also placed in the same tank. Fasted leeches fed on the sculpins and subsequently attached to the crabs. Water temperature varied between -1 and 3°C. *Chionoecetes opilio* and *Hyas coarctatus* Leach were collected in crab pots (160 to 360 m deep) off coastal Newfoundland and maintained alive in tanks at the Marine Sciences Research Laboratory, Logy Bay.

Wolffish, Atlantic and spotted (*Anarhichas lupus* and *A. minor*, respectively), Atlantic cod (*Gadus morhua*), eelpouts: Laval's, Vahl's, and Arctic (*Lycodes lavalaei*, *L. vahlii*, *L. reticulatus*, respectively) were collected by trawls (80 to 210 m) on the Grand Banks, maintained alive in tanks aboard the *A. T. Cameron*, and examined for leeches at the laboratory. Other species of fish, which included winter flounders (*Pseudopleuronectes americanus*), and Atlantic sea raven (*Hemitripterus americanus*), collected by divers, were negative for leeches. Fish examined for leeches were obtained in April through June.

### *Johanssonia arctica* (Johansson 1899)

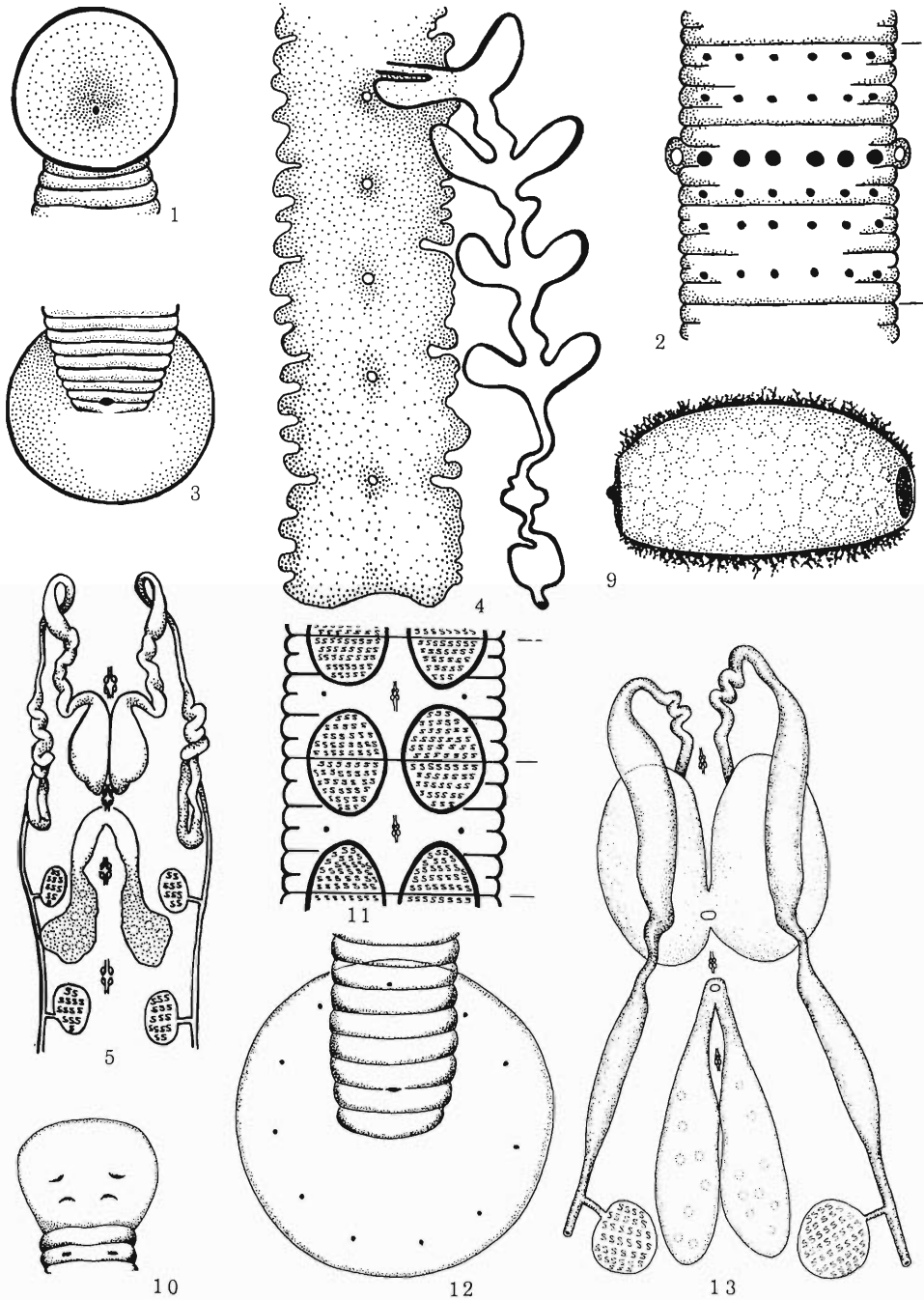
A brief history of our knowledge of the species involved is necessary. Selensky (1914b:209) proposed the generic name *Johanssonia* for leeches infesting Atlantic wolffish (*Anarhichas lupus*), from the Gulf of Kola, near the Biological Station at Murmansk, which he described as *Johanssonia kolaensis*. In the same paper Selensky assigned to *Johanssonia* a species infesting a Pycnogonida (*Nymphon strömi*), from the same general locality, which he had earlier described (1914a) as *Ichthyobdella pantopodium*. In using *Ichthyobdella* Selensky followed Johansson (1899:687), who used *Ichthyobdella* as a provisional category for species of Piscicolidae of uncertain generic assignment.

Based upon specimens from the Karischen Sea and Greenland, Johansson (1899:671) described *Oxytonostoma arctica*. In a critical review of the species of *Oxytonostoma* and *Johanssonia*, in the Zoological Institute of the Academy of Sciences, USSR, which included many *O. arctica* identified by Johansson and Vasilejev, Epshtein (1961, 1968 [in the second paper, based on dissections, the author authenticated his provisional decisions in the earlier paper]) made two taxonomic changes. He synonymized *Johanssonia pantopodium* with *Oxytonostoma arctica* and, because *O. arctica* has structural characters that are not compatible with those of *Oxytonostoma*, transferred the species to *Johanssonia*.

### Anatomy

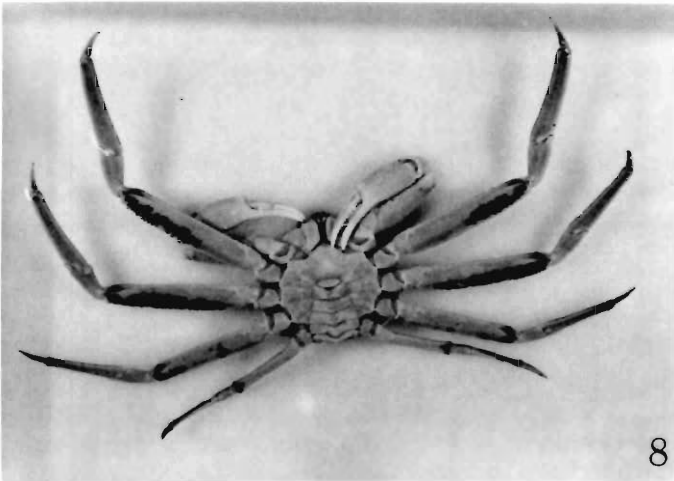
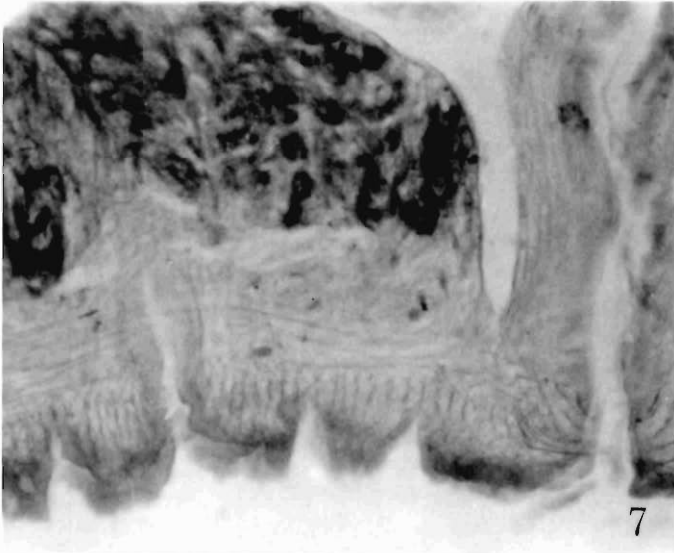
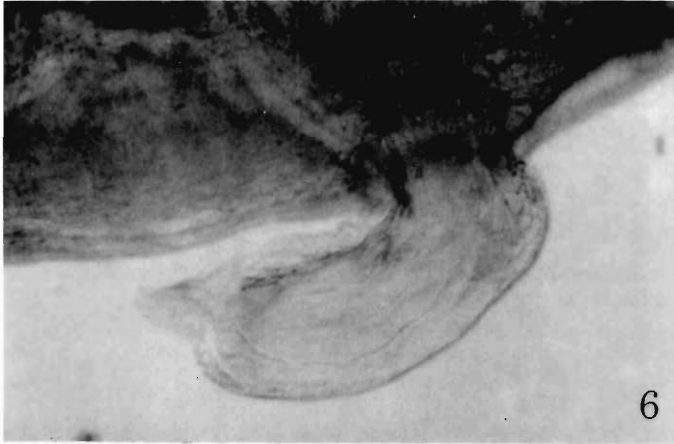
**EXTERNAL FEATURES:** Body rounded, becoming narrower toward the ends; clitellum indistinct; trachelosome only slightly narrower than urosome. Body shape varies only slightly. Anterior sucker discoidal, eccentrically attached, anterior exposed portion nearly 3 times as great as posterior ventral surface, and sharply separated from trachelosome by a prominent peduncle (Fig. 1). Posterior sucker cupuliform, weakly developed, and not sharply separated from urosome (Fig. 3). Lacking eyes, metameric ocelli and ocelli on anal sucker, and color markings on body surface.

**MEASUREMENTS:** length 21.5 (18.2–29.3); width of trachelosome and urosome, 0.9 (0.7–1.1) and 1.2 (0.9–2.1) respectively; transverse diameter of oral and anal suckers 1.2 (0.7–1.4) and 1.3 (1.1–1.4), respectively.



Figures 1–5. *Johanssonia arctica*. 1. Anterior sucker and adjacent trachelosomal rings, ventral view. 2. Complete segment, showing annulation, respiratory vesicles, and papillae, dorsal view. 3. Posterior sucker and adjacent urosomal rings, dorsal view. 4. Posterior region of digestive system, with intestine displaced to the right to show postcaeca and fenestrae, dorsal view. 5. Reproductive system, dorsal view.

Figures 9–13. 9. *Johanssonia arctica*, cocoon, lateral view. 10–13. *Platybdella olriki*. 10. Anterior sucker and adjacent trachelosomal rings, dorsal view. 11. Complete segments, showing annulation, nerve ganglia, segmental ocelli, and testes. 12. Posterior sucker and adjacent urosomal rings, dorsal view. 13. Reproductive system, dorsal view.



Figures 6–8. *Johanssonia arctica*. 6. Cirrus extended, directed posteriorly, lateral view. 7. Mid-sagittal section through gonopores, showing one large and two small annuli separating pores and vector tissue in vicinity of female pore. 8. Cocoons on *Chionoecetes opilio*, ventral view.

Respiratory vesicles 11 pairs, beginning in segment XIII, not always apparent externally but visible in serial sections. In complete segments, primary annuli are each divided twice, resulting in a 12-annulate segment (Fig. 2) expressed by formula ( $c1 - c4 + c5 - c8 + c9 - c12$ ). If greater recognition is given smaller annuli and depth of furrows, the number of annuli per segment increases. According to Epshtein (1968:1012 and Fig. 2)  $c9$  and  $c11$  were each divided into discrete annuli, resulting in the 14-annulate segment. Thus, mapping of annulation is fraught with difficulty because of subtle variations among specimens and the vagaries of personal interpretation.

Papillae in 12 rows on body surface, the dorsal 6 are slightly larger, apparent in living and most preserved specimens. Largest and most regular papillae on neural annulus ( $c6$ ) of complete segments. Papillae usually in straight lines, a pair of paramedians, supramarginals, and marginals, but they may be somewhat scattered and some may be missing. Position and relative size of the most constant papillae shown in Figure 2.

**REPRODUCTIVE SYSTEM:** The reproductive system, especially that of the male, is rather complicated in structure (Fig. 5). Testisacs 6 pairs, intrasegmentally at XIII through XVIII, anterior margins reaching to nerve ganglia, and alternating with gastric chambers.

Vas deferens, a capillary tube in testicular region, expands into a convoluted and loosely folded epididymis in segments XIII and XII, then reduced in diameter and continues cephalad in a relatively straight course, lateral to atrium, before increasing again in diameter to form ejaculatory duct which bends ventrad to join atrium. Ejaculatory ducts and adjacent atrial cornua, which form a conspicuous part of vas deferens, are remarkable in that they extend far forward of atrium, to 4th free ganglion (of segment X) or beyond; but their exact position and form differ somewhat among specimens.

Beginning with epididymis all parts of sperm duct contain sperm. Terminally there is a large cirrus pouch, or bursa, containing an extensible cirrus, occupying a space between 5th and 6th free ganglia (of XI and XII), which extends horizontally backwards, before bending ventrad and ending with male gonopore. Cirrus is a cylindrical, muscular troughlike structure, fused ventrally and open dorsally. Cirrus when extended in preserved specimens, directed posteriorly (Fig. 6).

Ovisacs elongated thin-walled sacs, between ganglia of XII and XIV. Each consists of 2 parts, a posterior globoid or ellipsoidal, thin-walled sac filled with developing ova, and an anterior tubular portion containing mostly mature ova. Passing beneath esophagus the ducts become thickened with connective tissue and muscle fibers, representing vector tissue (Fig. 7), and converge below nerve cord to unite just before the female gonopore, so that there is practically no common oviduct. Gonopores separated by 3 annuli, 1 large and 2 small.

**DIGESTIVE SYSTEM:** Mouth or proboscis pore on small elevation, situated slightly postequatorially in sucker cavity; proboscis at rest very long, extending to 4th free ganglion (of segment X), where it joins the esophagus. Esophageal caeca small, in anterior  $\frac{1}{2}$  of XI. Stomach, beginning at XII/XIII, with 7 lateral caeca, which tend to alternate with the testisacs. Size and shape of these caeca somewhat variable, depending on recency of feeding, but even if relatively empty, they tend to assume the shape and degree of expansion when animal is gorged.



At XIX/XX, off the last stomach caecum, alimentary tract branches into a postcaecum and an intestine (Fig. 4). The ventrally situated postcaecum has 5 small fenestrae, aligned with the ganglia; the dorsal intestine has 4 paired, anterolaterally directed caeca, decreasing in size from fore to aft. Posterior to the last caecum, intestine continues as a convoluted tube, ending in a spacious rectum which tapers to a minute anus.

**OCCURRENCE:** Greenland, and Karischen Sea, Cape Middendorff, Johansson (1899:672); Kola Bay, Murmansk, Selensky (1914a:273); Kara Sea, Wesenberg-Lund (1926:98); Alaska Peninsula, Stepovak Bay, Moore and Meyer (1951:55); Barents Sea, Eastern Siberian Sea, Laptev Sea, Epshtein (1961:1121); Newfoundland (this paper).

**HOST-PARASITE RELATIONSHIP:** Selensky (1914a:273) reported *A. arctica* [=his *Ichthyobdella pantopodium*] from a Pycnogonida (*Nymphon strömi*); Epshtein (1961:1121) [=his *Oxytonostoma arctica*] from Pycnogonida (*Colossendeis* sp.); and (this paper) decapod Crustacea, *Chionoecetes opilio* and *Hyas coarctatus*, and Atlantic cod, *Gadus morhua*.

*Johanssonia arctica* lays its cocoons on spider crabs, primarily *Chionoecetes opilio*, and occasionally on *Hyas coarctatus* attaching them on the ventral surface of the meropodite (merus) of second and third pereopods; a few on the fourth leg (Fig. 8). Cocoons turgid, of tawny or horn-brown color, elliptical; measurements of 15 produced in the laboratory on the host are 1.5 (1.4–1.8) × 1.0 (0.9–1.0). Upper surface strongly convex; under surface flat. At each end of convex surface there is an orifice sealed with an opercular plug, slightly darker in color, with a terminal papilla; one opening about 255 μm in diameter through which the young probably escape, and the other only about half as large. Free surface of cocoon covered by a network of filaments, which are tangled at the base and branched terminally (Fig. 9).

This species, as does *Myzobdella lugubris* according to Daniels and Sawyer (1975), is the second to infest fish and subsequently leave the fish host to deposit its cocoons on decapod Crustacea.

### Remarks

This species is characterized by 12 rows of papillae, 11 pairs of respiratory vesicles, the absence of eyes, segmental ocelli and ocelli on anal sucker, six pairs of testes, and long proboscis, reaching to fourth free ganglion (of segment X).

#### *Platybdella olriki* Malm 1865

#### *Platybdella fabricii*: Levinsen 1883:254

In 1865 Malm described *Platybdella olriki* as follows (in translation):

Body not very expanded, somewhat less high than broad, spoon-shaped, tapering toward front, dorsally at the extreme rear with 4 longitudinal rows of small warts. The anal sucker about half as wide as the posterior part of the body at its broadest point. The mouth is approximately the width of the anterior part of the body. Eyes 4: the front ones wide apart and crescent-shaped with the concave surface forward. The distance between the anterior ones 4 times as great as the distance between one of the front ones and the

one behind it. The body is clay-grey. Irregular dark spots dorsally. The anal disc immediately inside the edge with a circle of blackish dots.

Length between suckers 9; width at broadest point 3; height at same point 2; width immediately behind oral disc  $\frac{3}{4}$ ; immediately anterior to anal disc  $1\frac{1}{6}$  mm. All measurements taken from specimens preserved in alcohol.

Some specimens of this little leech, which is perhaps to be regarded as a type for its own genus, were taken off Greenland on *Hyas araneus*, by Mr. Olrik . . . . [Description unaccompanied by figures.]

Like most descriptions of leeches of that day there is little to distinguish it from related species. Except that Johansson (1899:685) added the third pair of eyes, which were overlooked by Malm, nothing further has been added to the description. Yet in view of the recognition given to the species by such qualified workers as Johansson (1899:684), Selensky (1923:406), Vasilejev (1939:42), Ringuet (1945:114), Epshtein (1962:648), and Sóos (1965:448), combined with the tendency of conserving names of long standing, *Platybdella olriki* is herein retained.

### Anatomy

**EXTERNAL FEATURES:** Body rounded to subcylindrical, normally not flattened; trachelosome not, or only slightly, narrower than urosome. Urosome is usually fusiform, but this varies somewhat with the state of development of the gonads and recency of feeding, whereas the trachelosome undergoes no such change. Young and unengorged animals are quite slender; gravid and fully gorged ones are robust. Unlike that of many leeches, the clitellum is neither conspicuously enlarged nor distinctively colored. Occasionally the region is slightly enlarged but in most cases it is faintly narrower and recessed. No lateral pulsatile vesicles or other special respiratory appendages. Anterior sucker small, cupuliform, eccentrically attached, only little larger than trachelosome (Figs. 10, 12). Posterior sucker large, discoidal, only slightly eccentrically attached, about twice diameter of oral sucker.

**MEASUREMENTS:** Overall length 12.6 (9.8–15.4); width of trachelosome and urosome 0.7 (0.5–0.9) and 1.1 (0.7–1.4), respectively; transverse diameter of oral and anal suckers 0.8 (0.7–1.4) and 1.7 (1.4–2.1), respectively.

Complete segments basically 3-annulate, with each annulus further but less distinctly divided, so that the segments are secondarily 6-annulate (Fig. 11). Body not pigmented and no radiating rays on anal sucker.

Four longitudinal rows of papillalike structures on the last 3 or 4 rings dorsally, as described by Malm (1865) and confirmed by Johansson (1899:684). These small projections are usually noticeable in living but not in preserved specimens, because they are not true papillae, as in *Johanssonia arctica*, but consist of cuticle separated from the epidermis.

Three pairs of eyes, anterior 2 pairs somewhat crescent-shaped. First pair, situated about equatorially on sucker, have crescents facing anteriorly; crescents of 2nd pair, slightly closer together, face posteriorly. Punctate 3rd pair, on 2nd ring of trachelosome, are farther removed from 2nd pair than is the 2nd pair from the 1st pair. One or both eyes of a pair may be altered in shape, divided, or missing.

Up to 12 segments, XIII through XXIV, with ocelli, situated midlaterally on *a2*. Some segments with 2 pairs, both dorsally and ventrally. Up to 11 ocelli, in a circle inside margin of anal sucker.

In some specimens of a lot, subjected to the same fixation and preservation, even after clearing, the eyes, segmental ocelli, and ocelli on anal sucker are not discernible. These occasional deficiencies should be kept in mind when working with a few preserved specimens.

**REPRODUCTIVE SYSTEM:** Testisacs 5 pairs, at XIV/XV through XVIII/XIX, alternating with segmental ocelli and stomach caeca. Fine thin-walled vasa efferentia connect testisacs with vas deferens, situated lateral to gonads. Vas deferens continues forward to XIV/XIII, where it expands slightly, forms an epididymis in XII before expanding into a prominent ejaculatory bulb or seminal vesicle, situated dorsad to atrial cornu (Fig. 13). Ejaculatory bulbs continue anteriorly to X/IX where they bend ventromedially, become convoluted ejaculatory ducts and enter the apex of the atrial cornua. The cornua, located in XI and contiguous halves of X and XII, are large, bulbous, muscular, and unite to form a small median atrium in posterior portion of XI.

The clavate, tubular ovisacs normally occupy segments XIV and XIII, the caudal end bulbous, tapering to narrow ducts, which unite beneath the nerve cord in a short common oviduct ending at the female gonopore.

**DIGESTIVE SYSTEM:** Mouth or proboscis pore situated equatorially in ventral face of oral sucker. The proboscis has the typical Rhynchobdellae structure, being a slender hollow muscular tube. Esophagus a narrow thin-walled tube extending from base of proboscis to stomach or crop in XIII and lying dorsad of atrium between cornua. Paired esophageal pouches in segment X. Stomach, largest part of the digestive system, consists of 6 chambers, aligned with the segmental ocelli, occupies segments XIV through XV. The thin walls of the chambers adapt themselves to the amount of food so that they may vary somewhat in size and form, being distended and directed anteriorly and slightly lobulated at the margins when distended with food. At XIX/XX the stomach divides into the large postcaecum and intestine. Postcaecum, extending into XXV or XXVI, consists of 6 chambers: the first 4 are large and their margins are usually bilobate; the last 2 are short and are not divided. Whether postcaecum is completely fused throughout or has fenestrae, cannot be determined from available material; but it is not completely separated. There are no intestinal caeca and only very slight intersegmental constrictions except the one at XXIV/XXV, marking the beginning of the slightly expanded rectum, which ends at the anus.

**OCCURRENCE:** Greenland, Malm (1865:414); Spitzbergen, Johansson (1899:685); Bering and Okhotsk Seas, Kamtchatka Biological Station, Vasilev (199:42); Iturup Isl. and Shikotan Isl. (Kuril group), Epshtein (1962:649); Newfoundland (this paper).

**HOST-PARASITE RELATIONSHIP:** Malm (1865:414) reported *P. olriki* from *Hyas araneus*; Wesenberg-Lund (1926:100), from *Sclerocrangon boreas* and *Hippoglossus hippoglossus* [=her *Crangon borealis* and *Hippoglossus maximus*, respectively]; and (this paper) from body surface of *Lycodes reticulatus*, from Grand Banks. An Atlantic seasnail (*Liparis atlanticus*), Atlantic sea raven (*Hemistriperus americanus*), and a winter flounder (*Pseudopleuronectes americanus*), in an aquarium with running water, each had a leech. But until these host records

are confirmed on fish in the wild, they are of little or no value for host predilection purposes.

#### Remarks

*Platybdella olriki* closely resembles *P. fabricii* (following Epshtein, 1967) in form, eyes, segmental ocelli, and ocelli on anal sucker. But it has no longitudinal color striping on the body and diverging rays on the anal sucker mentioned by Malm (1863:249) and described in detail by Selensky (1923:411) in *P. fabricii* [=his *Crangonobdella murmanica* Selensky 1914].

The reproductive system, with its prominent atrial cornua, also separates it from *P. fabricii*. One or the other character may not be of much individual importance but the combination of anatomical characters together is important.

#### *Platybdella anarrichae* (Diesing 1859)

For synonymy see Sóos 1965:447.

For a description of the species, which is abundantly recorded in the literature, readers are referred to Leigh-Sharpe (1916).

OCCURRENCE: Scandinavia, Malm (1863:223), Johansson (1896:39, 1899:684); Scotland, Leigh-Sharpe (1916:275); Greenland, Vanhoffen (1897:224), Wesenberg-Lund (1926:100); Faroes Islands and Iceland, Bruun (1928:3, 1938:2, respectively); Netherlands, Dresscher and Engel (1960:38); Barents Sea, Polyanskii (1955:89); Newfoundland (this paper).

HOST-PARASITE RELATIONSHIP: Preferred hosts are wolffish, especially *Anarrhichas lupus*, but Herter (1935:52) reported it also from pale eelpout (*Lycodes pallidus*). Our material came from wolffish: Atlantic (*A. lupus*) and spotted (*A. minor*); attached to gills and inner operculum.

#### Remarks

This species is characterized by the unpigmented, transparent body flecked with whitish spots, the absence of respiratory vesicles, eyes and segmental ocelli and ocelli on anal sucker, five pairs of testes, and its relatively high degree of host specificity.

#### *Calliobdella nodulifera* (Malm 1963)

For synonymy see Johansson 1896:17.

For a description of the species, which is abundantly recorded in the literature, readers are referred to Epshtein (1973).

OCCURRENCE: Scandinavia, Malm (1863:236), Johansson (1896:19, 1899:676); Scotland, Leigh-Sharpe (1917:118); Faroes and Iceland, Bruun (1928:3, 1938:2, respectively); Newfoundland, Threlfall (1969:807).

HOST-PARASITE RELATIONSHIP: Malm (1863:236) reported *C. nodulifera* from *Gadus morhua*, *Odontogadus merlangus* [=his *Gadus merlangus*] (p. 241), *Trigla gurnardus* (p. 244); Olsson (1876:4) *Molva molva*, *Pollachius virens*, *Sebastes marinus*, *Squalus acanthias* [=his *Molva molva*, *Gadus virens*, *Sebastes norvegicus*, *Acanthias vulgaris*, respectively], *Raja batis*, *R. fullonica*, *Chimaera monstrosa*; Johansson (1896:19) *Melanogrammus aeglefinus*, *Merluccius merluccius* [= *Gadus aeglefinus*, *Merluccius vulgaris*, respectively], *Hippoglossus vulgaris*, *Anarrhichas lupus*; Leigh-Sharpe (1917:118) *Pollachius virens* [=his *Gadus*

*carbonarius*]; Bruun (1928:3) *Anarhichas minor*; Threlfall (1969:807) *Squalus acanthias*. Our material came from eelpouts: Laval's (*Lycodes lavalaei*) and Vahl's (*L. vahlii*).

### Remarks

This species is characterized by the yellowish-brown pigment spots covering the body, 11 pairs of respiratory vesicles (discernible in living animals, and usually in preserved specimens), the absence of eyes, segmental ocelli and ocelli on anal sucker, six pairs of testes, and its broad host tolerance.

### Acknowledgments

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## ***Eimeria tenella* in Chickens: Resistance to a Mixture of Sulfadimethoxine and Ormetoprim (Rofenaid)**

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**ABSTRACT:** Rofenaid (a combination of sulfadimethoxine and ormetoprim) protected chickens against cecal coccidiosis infections initiated by a strain of the parasite that had no previous exposure to drugs. No cross resistance was found to 13 strains resistant to other anticoccidials. A strain of *Eimeria tenella* that was serially propagated in chickens fed mash containing Rofenaid became resistant to the chemical. This strain was cross resistant to robenidine, but not to eight other anticoccidials tested against it.

Sulfonamides were the first of the modern anticoccidials. Since Levine's (1939) demonstration of the anticoccidial activity of sulfanilamide, sulfonamides have been used alone or in combination with other chemicals for coccidiosis control. Lux (1954) first reported the enhancement of anticoccidial activity of sulfonamides by folic-, folenic-acid antagonists. His results with sulfaquinoxaline and pyrimethamine led to the formulation of other combinations of sulfonamides and potentiating agents. Among these is Rofenaid<sup>1</sup> (a combination of sulfadimethoxine and ormetoprim); the anticoccidial activity of Rofenaid was described by Mitrovic et al. (1969).

Sulfonamides also were the first class of compounds to which coccidia developed resistance. Waletzky et al. (1954) described a field strain of *Eimeria tenella* that was resistant to sulfaquinoxaline. Subsequently, there have been numerous reports of sulfonamide resistance including reports of field strains resistant to sulfadimethoxine (Oikawa et al., 1975).

In this report, we describe the activity of Rofenaid against 14 strains of *E. tenella* and the development of a strain resistant to this sulfadimethoxine-ormetoprim combination.

### **Materials and Methods**

The same general procedures were used throughout. Three-week-old White Leghorn cockerels were grouped by weight as suggested by Gardiner and Wehr (1950). Each group was started on the appropriate mash 24 hr before birds of the infected groups were orally inoculated with 100,000 sporulated oocysts of *E. tenella*.

The birds were weighed and killed on the 8th day after inoculation. Ceca were removed, lesions evaluated, and ceca and cecal contents cultured. Subsequently, oocysts were counted, and the number recovered per surviving bird was calculated for each group. Mortality, weight gains, lesion scores, and oocyst production data were used to calculate the Anticoccidial Index (McManus et al., 1968)

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<sup>1</sup> Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be available.

for each group. The indices form the basis for comparison; less than 160 is interpreted as poor control, 160–179 moderate, and above 180 good.

### **Efficacy trials**

In each of two trials groups of birds were fed mash containing Rofenaid premix (supplied through the courtesy of Hoffman-La Roche, Nutley, New Jersey) at a level that provided 125 ppm sulfadimethoxine and 75 ppm ormetoprim. These birds were inoculated with oocysts from a strain of *E. tenella* that had no previous exposure to drugs (sensitive strain) or with oocysts from laboratory strains resistant to amprolium, arsenosobenzene, buquinolate, clopidol, decoquinate, glycarbylamide, nequinatate, nitrofurazone, nicarbazin, Novastat (a combination of aklomide and sulfanitran), robenidine, Unistat (a combination of nitromide and sulfanitran), or zoalene. The sensitive strain also was used to inoculate a control group of unmedicated birds. Additionally, uninoculated, unmedicated groups and uninoculated, Rofenaid-medicated groups served as controls.

### **Development of a resistant strain**

For the first 29 passages, the experimental strain was serially propagated in groups of birds fed mash containing 62 ppm sulfadimethoxine and 37 ppm ormetoprim. This suboptimal level was necessary to continuously assure sufficient oocyst recovery for subsequent propagation and assay. The drug level was doubled to the usual recommended level for the final 11 passages. A second strain (control) from the same parental stock concomitantly was serially propagated in unmedicated chickens. The response of both strains to the higher level of Rofenaid was assayed at each passage after the first; oocysts recovered from the preceding passage were used. A group of unmedicated birds inoculated with the experimental strain and a Rofenaid-medicated group inoculated with the sensitive strain served as controls as did an uninoculated, unmedicated group.

### **Tests for cross resistance**

Oocysts recovered from the 40th passage were used in tests for cross resistance to other anticoccidials. In addition to the usual uninoculated, unmedicated controls, groups of birds fed mash containing the recommended level of amprolium, buquinolate, clopidol, glycarbylamide, lasalocid, monensin, nicarbazin, Novastat, robenidine, or Rofenaid were inoculated with Rofenaid-resistant oocysts. Corresponding groups were inoculated with control strain oocysts. All trials were replicated; and for buquinolate, lasalocid, monensin, and robenidine, there was a third trial.

## **Results**

### **Efficacy trials**

The data, expressed as Anticoccidial Indices, for the two trials are given in Table 1. Rofenaid afforded good protection against infections initiated with the strains resistant to other anticoccidials as well as with the sensitive strain. Anticoccidial Indices for the medicated groups ranged from 177 to 207, with an aggregate average of 198. Moreover, as judged by the uninoculated, medicated groups, Rofenaid neither enhanced nor adversely affected weight gains in uninfected birds.



**Table 1. Efficacy (expressed as Anticoccidial Indices) of Rofenaid\* against sensitive and resistant strains of *Eimeria tenella*.**

| Inoculum resistant to   | Anticoccidial Index† |         |      |
|-------------------------|----------------------|---------|------|
|                         | Trial 1              | Trial 2 | Avg. |
| No resistance           | 205                  | 196     | 200  |
| Amprolium               | 188                  | 192     | 190  |
| Arsenosobenzene         | 199                  | 202     | 200  |
| Buquinolate             | 197                  | 198     | 197  |
| Clopidol                | 203                  | 177     | 190  |
| Decoquate               | 202                  | 198     | 200  |
| Glycarbylamide          | 205                  | 207     | 206  |
| Nequinat                | 200                  | 206     | 203  |
| Nicarbazin              | 201                  | 198     | 199  |
| Nitrofurazone           | 201                  | 203     | 201  |
| Novastat                | 200                  | 206     | 203  |
| Robenidine              | 195                  | 189     | 192  |
| Unistat                 | 197                  | 200     | 198  |
| Zoalene                 | 195                  | 199     | 197  |
| Avg.                    | 199                  | 198     | —    |
| Inoculated, unmedicated | 76                   | 83      | 79   |
| Uninoculated, medicated | 190                  | 200     | 195  |

\* 125 ppm sulfadimethoxine and 75 ppm ormetoprim.

† Less than 160 = poor control; 160–179 = moderate control; above 180 = good control.

### Development of a resistant strain

The pertinent data for the last 11 passages (30 through 40) are given in Table 2. These were the only passages in which groups fed the higher drug level and inoculated with experimental strain oocysts consistently had Anticoccidial Indices of less than 160. However, from passage 11 on, the indices for this group

**Table 2. Response (expressed as Anticoccidial Indices) to Rofenaid\* by the Rofenaid-resistant and -sensitive strains of *Eimeria tenella*.**

| Passage no. | Anticoccidial Index† |                  |                  |                  |
|-------------|----------------------|------------------|------------------|------------------|
|             | Unmedicated          |                  | Medicated        |                  |
|             | Sensitive strain     | Resistant strain | Sensitive strain | Resistant strain |
| 30          | 98                   | 56               | 189              | 130              |
| 31          | 7                    | 93               | 189              | 106              |
| 32          | 7                    | 73               | 194              | 158              |
| 33          | 48                   | 92               | 197              | 90               |
| 34          | 121                  | 110              | 201              | 71               |
| 35          | 112                  | 135              | 195              | 145              |
| 36          | 111                  | 107              | 176              | 119              |
| 37          | 139                  | 66               | 195              | 121              |
| 38          | 66                   | 92               | 191              | 111              |
| 39          | 93                   | 76               | 193              | 88               |
| 40          | 85                   | 67               | 196              | 117              |

\* 125 ppm sulfadimethoxine and 75 ppm ormetoprim.

† Less than 160 = poor control; 160–179 = moderate control; above 180 = good control.

**Table 3. Response (expressed as Anticoccidial Indices)<sup>1</sup> of Rofenaid-resistant (R) and -sensitive (S) strains to field use levels of various anticoccidials.**

| Medication     | Anticoccidial Index |     |          |     |          |     |          |     |          |     |            |     |
|----------------|---------------------|-----|----------|-----|----------|-----|----------|-----|----------|-----|------------|-----|
|                | Trial 1 Strain      |     | 2 Strain |     | 3 Strain |     | 4 Strain |     | 5 Strain |     | Avg Strain |     |
|                | S                   | R   | S        | R   | S        | R   | S        | R   | S        | R   | S          | R   |
| None           | 104                 | 39  | 99       | 61  | 85       | 84  | 105      | 53  | 108      | 107 | 100        | 69  |
| Rofenaid       | 199                 | 86  | 193      | 104 | 182      | 132 | 200      | 122 | 195      | 129 | 194        | 115 |
| Amprolium      | 207                 | 195 | —        | —   | 186      | 190 | —        | —   | —        | —   | 196        | 192 |
| Buquinolate    | 175                 | 163 | —        | —   | 183      | 193 | —        | —   | 188      | 182 | 182        | 179 |
| Clopidol       | 195                 | 192 | —        | —   | 187      | 197 | —        | —   | —        | —   | 191        | 194 |
| Glycarbylamide | 175                 | 180 | —        | —   | 194      | 190 | —        | —   | —        | —   | 184        | 185 |
| Lasalocid      | —                   | —   | 181      | 173 | —        | —   | 200      | 195 | 203      | 184 | 195        | 184 |
| Monensin       | —                   | —   | 186      | 190 | —        | —   | 176      | 173 | 179      | 172 | 180        | 178 |
| Nicarbazin     | —                   | —   | 182      | 170 | —        | —   | 190      | 192 | —        | —   | 186        | 181 |
| Novostat       | —                   | —   | 193      | 191 | —        | —   | 196      | 186 | —        | —   | 194        | 188 |
| Robenidine     | —                   | —   | 192      | 133 | —        | —   | 196      | 108 | 206      | 82  | 198        | 108 |

<sup>1</sup> Less than 160 = poor control; 160–179 = moderate control; above 180 = good control.

generally were lower than were those of the corresponding group inoculated with control oocysts.

Although the lower level of Rofenaid (62 ppm sulfadimethoxine and 37 ppm ormetoprim) provided poor control of infections from the start, it was not until the experimental strain had 24 serial exposures to this level that the Anticoccidial Indices for groups medicated at this level fell and thereafter remained below 120.

### Tests for cross resistance

The pertinent results, expressed as Anticoccidial Indices, are given in Table 3. There was an apparent cross resistance to robenidine but not to any of the 8 other compounds tested. The average Anticoccidial Indices for groups medicated with anticoccidials other than robenidine or Rofenaid were comparable for infections initiated with either the Rofenaid-resistant or the -sensitive strain.

### Discussion

Tsunoda (1963) reported more favorable therapeutic effects with sulfadimethoxine than with other sulfonamides tested against field strains of *E. tenella* that had previous sulfonamide exposure. Mitrovic and Baurenfeind (1967) also reported therapeutic efficacy against single and mixed infections in chickens and turkeys. Subsequently, Mitrovic et al. (1969) described the synergistic anticoccidial activity of the sulfadimethoxine-ormetoprim combination in chickens. Our results with 13 laboratory strains essentially agree with those of Mitrovic et al. (1969) for *E. tenella*. Moreover, we found no cross resistance to Rofenaid by any of the resistant strains tested, even by those resistant to anticoccidials that contain a sulfonamide component (Novostat and Unistat).

Oikawa et al. (1975) found field strains of *E. acervulina*, *E. necatrix*, and *E. tenella* that were resistant to sulfadimethoxine; to our knowledge, there are no reports of coccidial resistance to the sulfadimethoxine-ormetoprim combination. Although our strain that was serially exposed to half the recommended level of

Rofenaid (62 ppm sulfadimethoxine and 37 ppm ormetoprim) was poorly controlled by this level from the start, the efficacy against infections initiated with oocysts recovered from this group in birds fed the recommended level of Rofenaid was comparable with that of infections initiated with control oocysts for the first 10 passages.

The cross resistance of the Rofenaid-resistant strain to robenidine was not reciprocal as Rofenaid adequately controlled infections initiated with oocysts from an experimental robenidine-resistant strain. There was no apparent cross resistance between the Rofenaid-resistant strain and Novostat nor between a Novostat-resistant or a Unistat-resistant strain and Rofenaid although all three anticoccidials have a sulfonamide component.

Our results suggest that continuous exposure to Rofenaid will lead to the emergence of coccidial populations that are resistant to the compound. The resistance may not develop as rapidly as it does to some other classes of anticoccidials such as the quinoline compounds. However, the initial efficacy of Rofenaid probably would not be compromised by the previous use of other anticoccidials on the same premises as there was no indication of cross resistance to Rofenaid by strains resistant to any of the anticoccidials we tested. Conversely, the activity of any of the anticoccidials we tested, except robenidine, should not be adversely affected by the previous use of Rofenaid as there was no indication of cross resistance, except to robenidine, by the Rofenaid-resistant strain.

#### Acknowledgment

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### Research Note

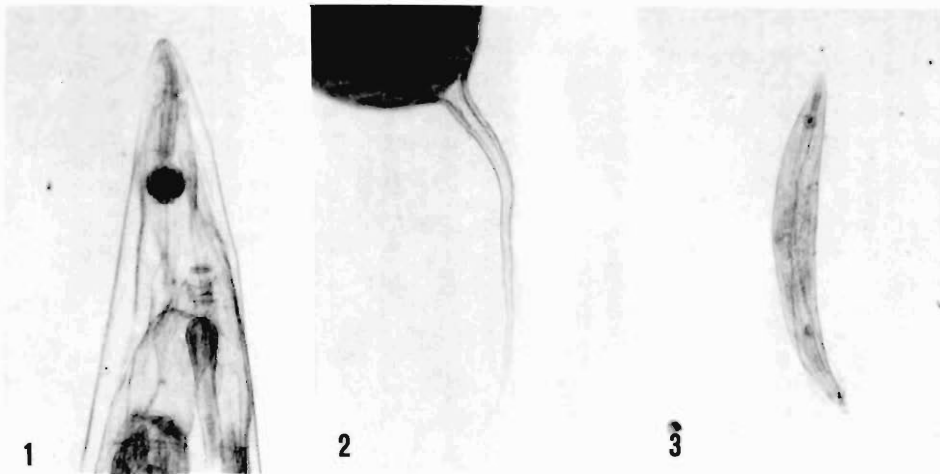
## Helminth Parasites of Sceloporine (Iguanidae) Lizards from Central Oregon

Reports on the parasite species found in the transmontane west sceloporine lizards have been limited to southern California (Telford, 1970, *Am. Midl. Nat.* 83:516-554); the Great Basin and Upper Colorado Plateau of Utah (Pearce and Tanner, 1973, *Great Basin Nat.* 33:1-18); and Idaho (Waitz, 1961, *J. Parasitol.* 47:51). The literature concerning the parasitism of *Sceloporus* was reviewed by Pearce and Tanner (1973, loc. cit.). During a population biology study of *Sceloporus occidentalis* and *S. graciosus* from Central Oregon, *Spauligodon giganticus* (Nematoda: Pharyngodonidae) was found in both species of lizards and *Oochoristica scelopori* (Cestoda: Linstowiidae) was found in *S. occidentalis*.

Host lizards were collected from three sites in Deschutes County, Oregon near the city of Bend. The study was conducted from April to October 1975. Lizards were shot with 22 caliber dust shot. Morphological measurements were recorded, and the lizards were fixed in AFA for 24 hr and transferred to 70% alcohol. The helminths were removed from the digestive tracts and preserved in alcohol until their subsequent identification. The nematodes were cleared in a solution of lactophenol while the cestodes were stained in Harris's Hemotoxylin (diluted 1:9). An ocular micrometer was used to make the diagnostic measurements. Helminths were classified according to Hyman (1951, *The Invertebrates: Acanthocephala, Aschelminthes, and Entoprocta. Vol. III*) and Yamaguti (1959, *Systema helminthum. Vols. II and III*) for the higher taxonomic categories, and Voge and Fox (1950, *Trans. Am. Microsc. Soc.* 69:236-242) and Read and Amrein (1953, *J. Parasitol.* 39:365-370) for the specific identifications.

While the present study does not include descriptions of any new parasite species it does report new distribution records for parasites of *Sceloporus*. The results are based on data from 60 *S. occidentalis* and 40 *S. graciosus*. Cestodes were found only in the small intestine of 20 *S. occidentalis*. The cestodes were identified as *Oochoristica scelopori*. All structural components resemble the description of Voge and Fox (1950, loc. cit.). Nematodes were recovered from the ceca of 40 *S. occidentalis* and two *S. graciosus*. We identified the single nematode species to be *Spauligodon giganticus* (Figs. 1, 2, 3) based upon the description of Read and Amrein (1953, loc. cit.). There was considerable variation in the diagnostic measurements for this species (Table 1). We have used the generic designation *Spauligodon*, rather than *Pharyngodon*, in accordance with the revision by Skrjabin et al. (1960, in K. I. Skrjabin, ed. *Essentials of nematology. Vol. VIII. Translated from Russian, 1974*). The two infested *S. graciosus* were sympatric with their congener while allopatric *S. graciosus* were not infested. In addition only one male nematode was recovered. Fourteen of the *S. occidentalis* were infested with both parasite species.

*Oochoristica scelopori* was reported as a parasite of both *S. occidentalis* and *S. graciosus*, but the incidence of *O. scelopori* in *S. graciosus* seems to be geographically discontinuous. The parasite was reported by Waitz (1961, loc. cit.), Telford (1970, loc. cit.), Pearce and Tanner (1973, loc. cit.). We did not find



Figures 1–3. 1. Anterior portion of a female *Spauligodon giganteus* (20×). 2. Posterior portion of a female *Spauligodon giganteus* (40.3×). 3. Male *Spauligodon giganteus* (10×).

*O. scelopori* in *S. graciosus* from central Oregon. Likewise, Burkholder and Tanner (1974, Brigham Young Univ. Sci. Bull. Biol. Ser. 19:1–44) examined 690 specimens of *S. graciosus* from the Great Basin of Utah without finding *O. scelopori*. These findings might suggest the absence of a suitable intermediate host. We cannot explain the apparent absence of male specimens of *S. giganteus* (only one was found); however, we recognize the possibility that some of the small males could have been overlooked during the initial retrieval from the hosts. Pearce and Tanner (1973, loc. cit.) suggested that *O. scelopori* and *S. giganteus* were mutually exclusive of one another and that they appear to exert interference mechanisms against other parasites. We presented evidence to the contrary with 23% of the hosts in this study doubly infested. One of us (White, unpublished

Table 1. Comparative diagnostic measurements for female specimens of *Spauligodon giganteus*. Units are in mm and means are in parentheses.

| Diagnostic characteristic              | Read and Amrein (1953) | Central Oregon*     |
|--|------------------------|---------------------|
| Maximum length                         | 6.40–11.52             | 3.18–9.84 (6.28)    |
| Maximum width                          | 0.480–1.104            | 0.350–1.094 (0.709) |
| Width at vulva                         | 0.384–0.792            | 0.300–1.051 (0.477) |
| Esophagus length                       | 0.792–0.912            | 0.527–0.700 (0.600) |
| Esophageal bulb length                 | 0.099–0.138            | 0.100–0.149 (0.121) |
| Esophageal bulb width                  | 0.112–0.122            | 0.119–0.179 (0.141) |
| Nerve ring from anterior extremity     | 0.268–0.313            | 0.109–0.199 (0.130) |
| Excretory pore from anterior extremity | 0.816–0.980            | 0.280–1.100 (0.802) |
| Vulva from anterior extremity          | 0.960–1.200            | 0.363–1.238 (0.911) |
| Anus from posterior extremity          | 1.440–1.920            | 0.403–1.625 (1.155) |
| Cuticular spines                       | 10–11                  | 5–12 (8.620)        |
| Ova length                             | 0.112–0.125            | 0.104–0.140 (0.128) |
| Ova width                              | 0.033                  | 0.027–0.041 (0.035) |

\* Oregon data are based upon 50 specimens and 186 ova.

data) also found a spiruroid nematode in the cecum of an *S. occidentalis* from southern Oregon which contained *S. giganticus*. There is great doubt that an interference mechanism between these parasites exists.

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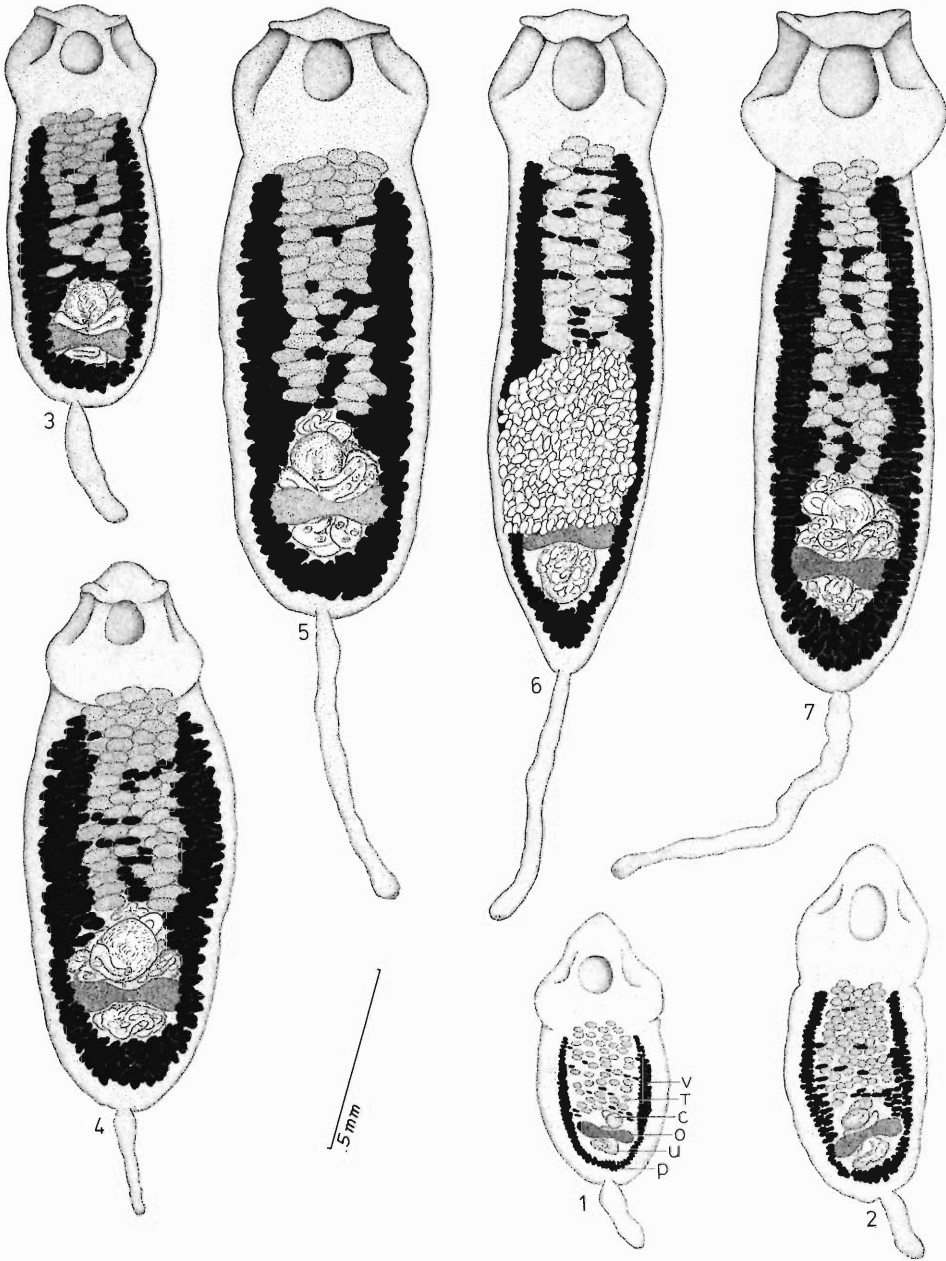
Proc. Helminthol. Soc. Wash.  
46(2), 1979, pp. 272-274

#### *Research Note*

### ***Archigetes iowensis* (Cestoda: Caryophyllidae) from *Limnodrilus hoffmeisteri* (Annelida: Tubificidae) in Wisconsin**

While studying the oligochaete annelids of western Wisconsin for parasitism by caryophyllidean cestodes, 19% of 1984 *Limnodrilus hoffmeisteri* Claparède, collected from the Red Cedar River (sections 8, Tainter tp., and 18, Grant tp., Dunn Co.) were found to be parasitized by a species of *Archigetes*, similar to *A. iowensis* Calentine, 1962, except that median preovarian vitellaria are present (Figs. 1-7). Although the species diagnosis of *A. iowensis* (Calentine, 1962, J. Parasitol. 48:513-524) describes preovarian vitellaria as being "located in two lateral bands," implying that median preovarian vitellaria is absent, figure 2 of Calentine (1962, loc. cit.) shows median vitellaria. In addition, specimens of *A. iowensis*, collected from *Cyprinus carpio* L. from the type locality in 1960 and 1978, also possess median vitellaria (personal communication: Mr. D. R. Sutherland, Iowa State University). Since comparison of the *Archigetes* from Iowa and Wisconsin (Table 1) reveals no significant differences, *Archigetes* from Wisconsin are considered to be *A. iowensis*. The presence of *A. iowensis* in Wisconsin extends its known geographical distribution.

Incidence of parasitism for spring and summer 1978 varied from 41% in May to 3% in July. Parasitized oligochaetes possessed one to seven proceroids within seminal vesicles (between segments 9 and 18). Two specimens were submitted to the USNM Helm. Coll. (No. 74792, 74793). No species of *Archigetes* parasitized any of the *L. hoffmeisteri*, *L. cervix* Brinkhurst, *L. udekemianus* Ratzel, *L. profundicola* (Verrill), *Dero digitata* (Muller), *Ilyodrilus templetoni* (Southern), or *Aulodrilus limnobius* collected from the following 17 Wisconsin locations;



Figures 1-7. *Archigetes iowensis*. 1-3. Mature cestodes. 4-7. Gravid cestodes. Abbreviations: C—cirrus sac; O—ovary; P—postovarian vitellaria; T—testis; U—uterus; V—preovarian vitellaria.

habitats where *Cyprinus carpio* L., the fish host of *A. iowensis*, also occur: Barron Co. (Chetek R., Red Cedar R.), Chippewa Co. (Duncan Creek), Crawford Co. (Kickapoo R.), Dunn Co. (Red Cedar R.), Eau Claire Co. (Chippewa R., Eau Claire R., Lake Altoona), Pepin Co. (Lake Pepin), and Pierce Co. (Lake Pepin).

**Table 1.** Comparison of gravid *Archigetes iowensis* from Iowa, Calentine (1962, loc. cit.) and Wisconsin. Measurements, from unflattened specimens, are expressed in  $\mu\text{m}$ . Mean is given (minimum and maximum in parentheses).

| Location                     | Iowa River, Iowa  | Red Cedar River, Wisconsin                       |
|------------------------------|---|--|
| Body length (w/o cercomer)   | 1,600 (1,100–2,400)                                       | 1,680 (1,100–1,860)                              |
| Width (at gonopore)          | 520 (370–720)   | 560 (470–590)                                    |
| Cercomer length              | 500 (300–700)   | 520 (280–950)                                    |
| Testes number                | 66 (57–76)  | 68 (54–86)                                       |
| Cirrus sac diameter          | 150 (140–170)   | 152 (137–176)                                    |
| External seminal vesicle     | 110 (90–120) by<br>80 (70–100)                            | 120 (106–136) by<br>85 (68–103)                  |
| Anterior extent of uterus    | Extending just anterior to cirrus pouch                   | May encircle cirrus sac, anteriorly              |
| Median preovarian vitellaria | Present or absent   | Present: seven to 37 ( $\bar{X}$ = 21) follicles |
| Fixation                     | A.F.A. "room temperature"                                 | 10% formalin at 21 to 25°C                       |
| Scolex                       | Bothrioloculodiscate                                      | Bothrioloculodiscate                             |
| Eggs                         | 32 × 43   | 43 × 76 (N = 15)<br>Dissected from oligochaetes  |
| Specimens studied            | 20 ?  | 42   |
| Host(s)                      | <i>Limnodrilus hoffmeisteri</i><br><i>Cyprinus carpio</i> | <i>L. hoffmeisteri</i>                           |

Nor were any of the more than 450 *C. carpio* collected 1967 to 1978 from rivers or their reservoirs in Wisconsin (Chetek, Mississippi, Red Cedar, St. Croix), Iowa (Boone, Des Moines, Little Sioux, Lake Macbride, Mississippi, Raccoon, Skunk, Wapsipinicon), or Nebraska (Lake McConaughy) parasitized by *A. iowensis*. Studies are continuing to determine if *A. iowensis* parasitizes oligochaetes or *C. carpio* elsewhere in Wisconsin and Minnesota.

Appreciation is expressed to Dr. Robert J. Muncy, Leader, Iowa Cooperative Fishery Unit, Iowa State University, for assistance in obtaining *C. carpio* in Iowa, Nebraska, and Wisconsin and to Mr. Monte L. Madsen, Nebraska Department of Game, Forestation and Parks, for assistance in Nebraska.

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*Research Note*

**American Opossums (*Didelphis marsupialis*) Exposed to Different Strains of *Schistosoma mansoni***

Different parameters including various aspects of definitive host-parasite relationships (susceptibility determined by schistosome returns from hosts exposed to a given number of cercariae, distribution of schistosomes in the host, tissue egg deposits as an indication of fecundity, host compatibility or pathologic potential, and tissue eggs/worm pair) have been employed in an effort to gain a better understanding of the basic biology of the mammalian schistosomes. These features have also been used in describing or characterizing the behavior of schistosome strains, particularly *Schistosoma mansoni* (Anderson and Cheever, 1972, Bull. W.H.O. 46:233-242; Powers and Cheever, 1972, Bull. W.H.O. 46:295-300; Saoud, 1965, J. Helminthol. 39:101-112; 1966, Trans. R. Soc. Trop. Med. Hyg. 60:585-600; Warren, 1967, Trans. R. Soc. Trop. Med. Hyg. 61:795-802). Further studies of strain differences were undertaken by Lee et al. (1971, Bull. W.H.O. 45:147-158) and Saeed and Hussein (1974, J. Helminthol. 48:205-212), respectively, who evaluated parasite differences with reference to chemotherapy and pathogenicity. The present authors concur with Powers and Cheever (1972, loc. cit.) that a full appreciation of schistosome behavior and characterization can only be attained by studying the parasite in different host species. A number of mammals, including the American opossum, has been exposed to *S. mansoni* (Bruce et al., 1961, J. Parasitol. 47:752-756; Lichtenberg et al., 1962, Am. J. Trop. Med. Hyg. 11:347-356) to evaluate susceptibility and certain aspects of pathology, and the opossum has been found naturally infected with *Heterobilharzia americana* (Kaplan, 1964, J. Parasitol. 50:797).

The present report is based upon a study of American opossums exposed to four geographic strains (Egypt, Kenya, Puerto Rico, and South Africa) of *S. mansoni*. Baby opossums were removed from mothers captured in the vicinity of the Southwest Foundation (southwest San Antonio). After weaning, babies were fed on a mush prepared from dog biscuits (Purina) mixed with milk, then at a later date, on dry dog biscuits. Test subjects were maintained in rabbit cages with wire mesh bottoms and all were of approximately the same weight (sexes approximately 50:50) when exposed to infection at 18-24 weeks of age. All hosts used for a given strain were exposed from one lot of cercariae. Host exposures and examinations were made by conventional parasitologic technics (Kuntz et al., 1975, Int. J. Parasitol. 5:21-26). There was a wide range in schistosome numbers in different organs with only a few parasites from the lungs, Table 1(a) and, based on the limited number of hosts employed, a wide range in total worm recoveries, i.e., 18.6 and 39.9%, respectively, for the Egypt and Kenya strains. Egg distribution was greater than that of adult worms, the heaviest tissue egg burden by far recorded for the Kenya strain, Table 1(b). As expected, this strain also showed the largest number of tissue eggs/worm pair. The South Africa strain

Table 1. Host-parasite relationships in opossums exposed to 1,000 *Schistosoma mansoni* cercariae and examined 26–28 weeks postexposure.

| Geographic strains | No. of hosts | a. Parasites recovered                               |                  |         |          |        |         |         |                |      |  | Total recovery |             |
|--------------------|--------------|--|------------------|---------|----------|--------|---------|---------|----------------|------|--|----------------|-------------|
|                    |              | Distribution of parasites in visceral organs (range) |                  |         |          |        |         |         |                |      |  | Range          | Average (%) |
|                    |              | Lungs  | Liver/Hep. Port. | Stomach | Pancreas | Spleen | S. Int. | L. Int. | Total recovery |      |  |                |             |
| Egypt              | 5            | 0  | 32–121           | 0       | 2–4      | 0      | 17–76   | 38–84   | 118–245        | 18.6 |  |                |             |
| Kenya              | 6            | 0  | 77–386           | 2–4     | 4–22     | 0–16   | 51–246  | 5–144   | 242–476        | 39.9 |  |                |             |
| Puerto Rico        | 6            | 0–2  | 43–216           | 0       | 0–14     | 0–8    | 64–170  | 20–79   | 146–420        | 31.2 |  |                |             |
| South Africa       | 5            | 0  | 5–212            | 0       | 0–15     | 0–4    | 20–108  | 21–107  | 147–263        | 20.3 |  |                |             |

| Geographic strains | No. of hosts | b. Total number of parasite eggs in organs (10 <sup>3</sup> )* |        |         |          |        |         |         |                      |     |       | Total body egg count |         | Eggs/worm pr.† (Average) |
|--------------------|--------------|--|--------|---------|----------|--------|---------|---------|----------------------|-----|-------|----------------------|---------|--------------------------|
|                    |              | Distribution of eggs in visceral organs (range)                |        |         |          |        |         |         |                      |     |       | Range                | Average |                          |
|                    |              | Lungs  | Liver  | Stomach | Pancreas | Spleen | S. Int. | L. Int. | Total body egg count |     |       |                      |         |                          |
| Egypt              | 5            | 0–1‡   | 27–95  | 0       | 1–12     | 0      | 17–318  | 63–274  | 123–631              | 494 | 5,259 |                      |         |                          |
| Kenya              | 6            | 6–2  | 19–249 | <1–32   | 14–49    | 0      | 655–281 | 56–184  | 496–910              | 723 | 6,336 |                      |         |                          |
| Puerto Rico        | 6            | 0–3  | 24–75  | 0–1     | 2–47     | 0      | 120–717 | 32–301  | 185–1,105            | 602 | 5,311 |                      |         |                          |
| South Africa       | 5            | 0–7  | 42–106 | 0–<1    | 1–14     | 0–<1   | 55–190  | 19–76   | 118–393              | 267 | 4,570 |                      |         |                          |

\* Determined by KOH digest technic (Cheever, 1970. Bull. W.H.O. 43:601–603).

† Eggs/worm pair = total number of tissue eggs determined by KOH digest/worm pair (actual count).

‡ Rounded out to nearest thousand.

had the lowest total body egg count as well as the least number of eggs/worm pair. No significant differences were detected in the histopathology produced by different strains, although grossly there was more extensive intestinal involvement in hosts exposed to the Kenya *S. mansoni*.

The opossum is classed as a good host for the four strains of *S. mansoni* employed in this study. Information on host-parasite relationships and the potential for pathology in opossums infected with the Puerto Rico strain are similar to that given by Bruce et al. (1961, loc. cit.) and Lichtenberg (1962, loc. cit.). In the present investigation, eggs were passed with the feces of opossums infected with the four strains of *S. mansoni*. It is biologically impractical to compare definitive host-parasite relationships of *S. mansoni* in opossums with those demonstrated by authors employing rodents (Anderson and Cheever, 1972 and Saoud, 1965, 1966, loc. cit.) and nonhuman primates (Powers and Cheever, 1972, Saeed and Hussein, 1974, loc. cit.), but present data tend to support the contention that there are detectable biologic differences in geographic strains of *S. mansoni*. Differences in susceptibility, i.e., parasite returns, total body egg counts, as well as eggs/worm pair seem to suggest a greater potential for pathogenicity for the Kenya parasite based upon observations in the American opossum.

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### *Research Note*

## **Reinfection of Domestic Chicks with *Zygodontia lunata* (Trematoda)**

Reinfection studies on enteric digenes have been reported. Cable (1937, J. Parasitol. 23:559) noted that an established infection with *Parorchis acanthus* failed to protect Herring Gulls against a subsequent infection. Willey (1941, Zoologica 26:65-88) reported that infection with *Zygodontia lunata* in rats and ducks prevented reinfection. Anderson and Cable (1950, J. Parasitol. 36:395-410) reported that repeated infection of a heron with *Linstowiella szidati* rendered it immune to infection with that species. Ulmer (1951, Trans. Am. Microsc. Soc.

**Table 1.** Infection of chicks with *Zygodotyle lunata*.

| Exp.          | No. of chicks exposed | No. of metacercariae fed |          | Age (in days) of chick at necropsy | No. of worms recovered and (%) |              |
|---------------|-----------------------|--------------------------|----------|------------------------------------|--------------------------------|--------------|
|               |                       | 1st inf.                 | 2nd inf. |                                    | 1st inf. (%)                   | 2nd inf. (%) |
| Experimentals | 13                    | 5                        | 5*       | 14                                 | 41 (63)                        | 19 (30)      |
| Controls      | 3                     | 10                       | ND       | 7                                  | 10 (33)                        | — —          |
| Controls      | 3                     | 10                       | ND       | 14                                 | 16 (53)                        | — —          |
| Controls      | 4*                    | 10*                      | ND       | 14                                 | 9 (23)                         | — —          |

\* Indicates that chicks were 7 days old at time of exposure to cysts; otherwise chicks were day-old.  
 ND = Not done.

70:189–238) indicated that *Postharmostomum helicis* infection in rats reduced the number of worms developing from the second exposure. Fried and Weaver (1969, Proc. Helminthol. Soc. Wash. 36:153–155) reported that a primary infection of *Echinostoma revolutum* did not protect the domestic chick against a secondary infection. The purpose of this note is to report our observations on reinfection of the domestic chick with the cecal trematode, *Zygodotyle lunata* Diesing, 1836.

Domestic chicks were experimentally infected with *Z. lunata* as described previously (Fried, Robbins and Nelson, 1978, J. Parasitol. 64:395–397). For reinfection studies, each of 13, day-old chicks were fed five cysts initially, five again 7 days later and necropsied at 14 days of age. A single feeding of 10 cysts was given to each of 10 control chicks: six at 1 day of age with three necropsied 7 days later and three on the 14th day; and four at 7 days of age with necropsy 7 days thereafter. Food was withheld from the 7-day-old group for 24–48 hr before infection and from all chicks for 24 hr before necropsy.

Results are presented in Table 1. The number and size differences of worms recovered at necropsy indicated that reinfection occurred. Four of the experimental chicks yielded more worms (6, 7, 7, 8) than the number of cysts in the initial feeding. Also, age resistance to infection with *Z. lunata* was indicated by the recovery of 53.6% of the worms fed as metacercariae to day-old chicks and only 27% from chicks infected at 7 days of age.

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### Research Note

## *Oligacanthorhynchus lamasi* (Freitas and Costa, 1964) comb. n. from Domestic Cats of Brazil

Freitas and Costa (1964, Arq. Esc. Vet. Univ. Fed. Minas Gerais 16:231–234) described a new species, *Echinopardalis lamasi*, from two acanthocephalans (1 ♂, 1 ♀) of a cat, *Felis domestica* (= *F. catus*), captured in Belo Horizonte, Minas Gerais. These specimens have been lost (Freitas, 1975, personal communication). Until two of 127 domestic cats, *F. catus*, from Porto Alegre, Rio Grande do Sul, captured between October 1973 and July 1976, were found to harbor respectively two and nine acanthocephalans conspecific with those described by Freitas and Costa, no additional specimens of this species were available for study. These new specimens assist in clarifying the generic affinities of *E. lamasi* and permit additions to the species description.

In a revision of the Archiacanthocephala, Schmidt (1972, J. Parasitol. 58:290–297) synonymized *Echinopardalis* and *Oligacanthorhynchus* but a list of species made no mention of *E. lamasi*. Later, without comment (Schmidt, 1977, J. Parasitol. 63:508–510), he assigned this species to *Oncicola* presumably because the anterior testis reaches into the front half of the trunk, a feature (Schmidt, 1972, loc. cit.) by which *Oncicola* differs from *Oligacanthorhynchus*. Although resembling *Oncicola* in this regard, we consider the species in question to belong to *Oligacanthorhynchus* as redefined by Schmidt (1972, loc. cit.) because of the slender body and the narrowness of the trunk anteriorly (Fig. 5).

As was to be expected, study of additional specimens revealed slightly more variation than described for the two specimens by Freitas and Costa (1964, loc. cit.). When proboscis armature is described in 12 approximately longitudinal rows of three hooks each, hook lengths, in micrometers, from anterior to posterior were 158–163, 120, and 72–86 for those in the six rows reaching farthest anteriorly and 149–154, 91–106, and 53–62 in alternate rows (Figs. 1–3). Except for the first hook in the anteriormost rows which is longer, these hooks are shorter than those described by Freitas and Costa (1964, loc. cit.). Egg dimensions, not given in the original description, averaged  $58 \times 38 \mu\text{m}$ .

*Oligacanthorhynchus lamasi* differs from all other members of the genus in features typically used to distinguish among acanthocephalans. Features of proboscis armature, however, are similar in several genera of the Oligacanthorhynchidae, and, in regard to these, *O. lamasi* most resembles *Oncicola martini* Schmidt, 1977. Schmidt (1977, loc. cit.) distinguished *O. martini* from *O. lamasi* by lengths of the first hook in the anteriormost rows and the last hooks in each row. Differences in the first hooks are apparently not so great as previously thought while those of the last hooks may be greater. The two species were further distinguished by barbs on all hooks except the last in each row of *O. martini*. According to Schmidt (1977, loc. cit.), barbs occur only on the first hook of anteriormost rows in *O. lamasi*. Freitas and Costa did not specifically mention



Figures 1-5. Camera lucida drawings of *Oligacanthorhynchus lamasi*. 1. Anteriormost hook of a longitudinal row. 2. Middle hook of a longitudinal row. 3. Basal hook of a longitudinal row. 4. Proboscis and neck. 5. Entire male. Scale for Figure 1 applies equally to Figures 2 and 3.

barbs for hooks of *O. lamasi*, but illustrated the pattern described by Schmidt. All hooks except the last in each row on Porto Alegre specimens possessed barbs (Fig. 4).

*Oligacanthorhynchus lamasi* has not been reported since its original description, although Federman, Holanda, and Evangelista (1973, Rev. Pat. Trop. 2:207–215) did report unidentified acanthocephalans in two cats from Belo Horizonte. Our study confirms the occurrence of *O. lamasi* in Brazilian domestic cats and suggests that the prevalence may not be as low as previously assumed.

Specimen deposition: Instituto Oswaldo Cruz, Rio de Janeiro (Helm. Coll. No. 31.798a, ♂; 31.798b, ♀).

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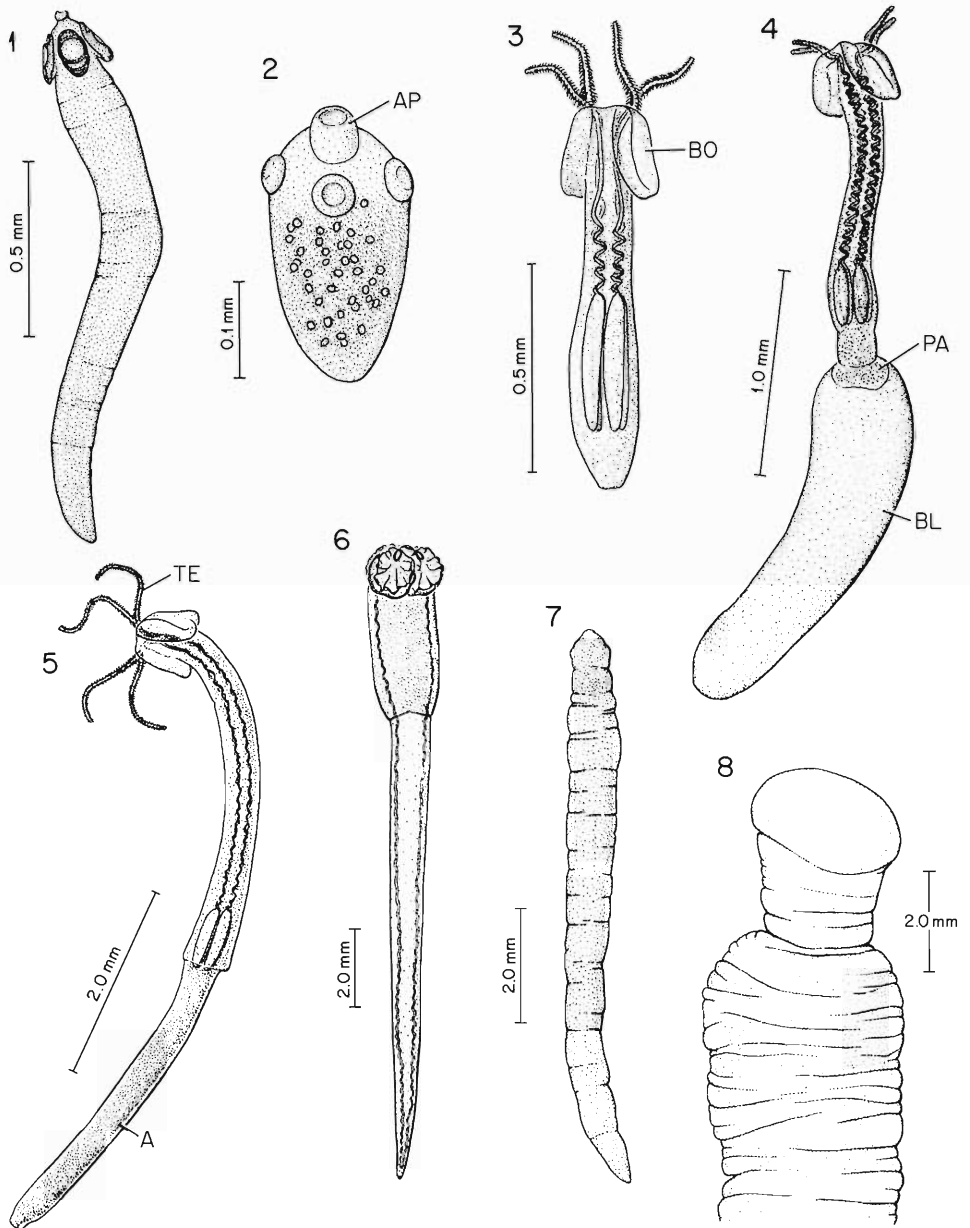
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### Research Note

## The Parasites of the California Lizardfish, *Synodus lucioceps*

From May 1976 to August 1978 21 species of adult and larval parasites were collected from 440 California lizardfish, *Synodus lucioceps*, taken off the coast of Los Angeles. The present study was undertaken due to the paucity of information on the parasitic fauna of this fish, although Arai (1962, An. Inst. Biol. Univ. Nac. México 33:113–130) reported the trematode, *Helicometrina nimia* Linton, 1910 and Jensen and Heckmann (1977, J. Parasitol. 63:471–472) described the cestode, *Anantrum histocephalum*. Parasitological findings are summarized in Table 1.

Four species of Protozoa were recovered. The microsporidan is assigned to the genus *Encephalitozoon* Levaditi, Nicolau and Schoer, 1923, based on an uninucleate spore, corrugated exospore and four and one-half coils of the polar filament. The presence of *Encephalitozoon* sp. was rare and always confined to the skeletal muscle. The three species of myxosporidians, *Ceratomyxa anoplopoma* Moser, 1976, *C. crassa* Jameson, 1929 and *C. hopkinsi* Jameson, 1929 were common inhabitants of the gall bladder, multiple infections being the rule.



Figures 1–8. Larval and adult cestodes of *Synodus lucioceps*. 1. *Scolex pleuronectis*, quadriloculate form. 2. *Scolex pleuronectis*, uniloculate form. 3. Plerocercoid of *Grillotia smarigora*. 4. Plerocercoid of *Lacistorhynchus tenuis*. 5. Plerocercoid of *Callitetrarhynchus gracilis*. 6. Plerocercoid of *Phyllobothrium* sp. 7. Preadult of *Anantrum histocephalum*, found free in the intestinal lumen. 8. Clavate scolex of *A. histocephalum*, dissected from the intestinal wall. Abbreviations: A, appendix; Ap, apical sucker; BL, blastocyst; BO, bothridia; PA, patelliform attachment to the blastocyst; TE, tentacle.



Table 1. Parasites recovered from the California lizardfish, *Synodus lucioceps*.

| Parasite   | Site of infection† |
|--|--------------------|
| Protozoa, microsporida   |                    |
| <i>Encephalitozoon</i> sp.*                                    | Sk                 |
| Protozoa, myxosporida  |                    |
| <i>Ceratomyxa anoplopoma</i> *                                 | G                  |
| <i>Ceratomyxa crassa</i> *                                     | G                  |
| <i>Ceratomyxa hopkinsi</i> *                                   | G                  |
| Trematoda  |                    |
| <i>Sterrhurus exodicus</i> *                                   | S                  |
| Cestoda  |                    |
| <i>Anantrum histocephalum</i>                                  | I                  |
| <i>Callitetrarhynchus gracilis</i> (encapsulated plerocercoid) | M                  |
| <i>Grillotia smarigora</i> (encapsulated plerocercoid)         | M, Sp, St          |
| <i>Lacistorhynchus tenuis</i> (encapsulated plerocercoid)      | L, M, Sk           |
| <i>Phyllobothrium</i> sp. (plerocercoid)                       | S                  |
| <i>Scolex pleuronectis</i> , quadriloculate form               | I                  |
| <i>Scolex pleuronectis</i> , uniloculate form                  | I                  |
| Acanthocephala   |                    |
| <i>Corynosoma strumosum</i> (cystacanth)                       | M                  |
| Nematoda   |                    |
| <i>Anisakis simplex</i> (larva)                                | L, M, Sp, St, T    |
| <i>Contracaecum</i> sp. (larva)                                | M                  |
| <i>Phocanema decipiens</i> (larva)                             | L, M, Sk, St       |
| <i>Thynnascaris aduncum</i> *                                  | I, S               |
| Crustacea  |                    |
| <i>Argulus borealis</i> *                                      | B                  |
| <i>Caligus pectinatus</i> *                                    | B                  |
| <i>Lepeophtheirus</i> sp.*                                     | B                  |
| <i>Lironeca vulgaris</i> *                                     | O                  |

\* New definitive host records.

† Abbreviations: B, body surface; G, gall bladder; I, intestine; L, liver; M, mesentery; O, opercular cavity; S, stomach; Sk, skeletal muscle; Sp, spleen; St, stomach wall (submucosa); T, testis.

The presence of the adult digenean, *Sterrhurus exodicus* (MacFarlane, 1936), in the stomach was common and ranged from one to eight worms per fish.

The adult cestode, *Anantrum histocephalum* Jensen and Heckmann, 1977 was encountered in 58 of 440 fish. The gravid worm possessed a mushroom-shaped or clavate scolex (Fig. 8) embedded in the intestinal wall, while the scolex of the preadult (Fig. 7) was unattached. Two larval tetraphyllideans are identified as *Scolex pleuronectis*, quadriloculate form (Fig. 1) and *Scolex pleuronectis*, uniloculate form (Fig. 2). The scolex of the first is adorned with four, quadriloculate bothridia and a protruding apical sucker, whereas the second is smaller, possesses four, uniloculate bothridia and an apical sucker. Both tetraphyllideans were prevalent and infestations were usually heavy. One tetraphyllidean plerocercoid of the genus *Phyllobothrium* Beneden, 1849 (Fig. 6) was also collected but may be merely an incidental or transitory parasite from squid consumption. The most abundant larval trypanorhynch was *Grillotia smarigora* (Wagener, 1854) (Fig.

3), and was found in 85.9% of 242 fish. Plerocercoids of *Lacistorhynchus tenuis* (van Beneden, 1858) (Fig. 4) and *Callitetrarhynchus gracilis* (Rudolphi, 1819) (Fig. 5) were only occasional helminths.

Of the four species of nematodes, larvae of *Anisakis simplex* (Rudolphi, 1809) and *Phocanema decipiens* (Krabbe, 1878) were commonly encountered; the submucosa of the stomach wall was the site most heavily burdened. Larval nematodes of *Contracaecum* Railliet and Henry, 1912 and adults of *Thynnascaris aduncum* (Rudolphi, 1802) were only rarely collected.

Acanthocephalan cystacanths of *Corynosoma strumosum* Rudolphi, 1802 were also of minor significance in terms of numbers and prevalence.

*Lironeca vulgaris* Stimpson, 1857 and *Argulus borealis* Wilson, 1912 were the most frequently recovered crustaceans, while *Caligus pectinatus* Shiino, 1965 and a species of *Lepeophtheirus* Nordmann, 1832 had lesser parasitic importance.

Representative specimens: *Encephalitozoon* sp. USNM Helm. Coll. No. 74823; *Ceratomyxa* spp. No. 74824; *A. histocephalum* No. 74825; *Scolex pleuronectis*, uniloculate form No. 74826; *T. aduncum* No. 74827; *A. simplex* No. 74828; *P. decipiens* No. 74829; *Contracaecum* sp. No. 74830; *L. vulgaris* No. 74831; *C. pectinatus* No. 74832; *Lepeophtheirus* sp. No. 74833; *S. exodicus* No. 74834; *G. smarisgora* No. 74835; *L. tenuis* No. 74836; *C. gracilis* No. 74837; *Scolex pleuronectis*, quadriloculate form No. 74838; *Phyllobothrium* sp. No. 74839; *C. strumosum* No. 74840; *A. borealis* No. 74841.

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*Research Note*

***Dirofilaria uniformis* and *Dirofilaria scapiceps*  
(Nematoda: Filarioidea) from Rabbits in  
Georgia and South Carolina<sup>1</sup>**

The viscera of eastern cottontail rabbits, *Sylvilagus floridanus* (Allen) were routinely examined for gastrointestinal parasites at the Animal Parasite Research Laboratory, Tifton, Georgia, from 1968 to 1973. We were particularly interested in evidence for the rabbits acting as a transport host for parasites of domestic animals. Many hunters in the area brought viscera of recently killed rabbits to the laboratory for examination. On one occasion, Dr. Ray Worley, a frequent supplier of rabbit viscera, noticed a "wad" of worms in the tarsal bursa of a rear leg of a rabbit killed in Tift County and included the leg with the viscera. These worms (6 female and 5 male) were identified as *Dirofilaria scapiceps* (Leidy, 1886) which is a common parasite of wide distribution. Dr. Worley thereafter included both the front and rear legs of the rabbits along with the viscera. Once he included the entire rear half of a carcass that had been impregnated with many shot. Examination of the carcass revealed a large nematode under the mucosal layer of the right side, between the ribs and rear leg, which was identified as *Dirofilaria uniformis* Price, 1957. Five female *D. scapiceps* were also found in the tarsal bursa of the right rear leg. *Dirofilaria uniformis* was previously reported only from the eastern cottontail rabbit in Maryland by Price (1957, Proc. Helminthol. Soc. Wash. 24:15-19). Specimens of *Dirofilaria* from three eastern cottontail rabbits taken in South Carolina were identified as: four female *D. uniformis* in a rabbit from Aiken County; one female *D. scapiceps* in a rabbit from Lancaster County and 20 female and eight male *D. scapiceps* in a rabbit from Anderson County. Examinations of cottontail rabbits from Georgia revealed four additional animals positive for *D. uniformis*. The greatest numbers of adults were from rabbits from Berrien County, Georgia, one of which had 33 female and 13 male *D. uniformis* and seven female and 10 male *D. scapiceps*. A second rabbit from the same county was infected with only *D. scapiceps*, 22 females and 13 males. One of four marsh rabbits, *Sylvilagus palustris* (Bachman), examined was positive for *D. uniformis*. These marsh rabbits were from the coastal area in McIntosh County, Georgia. Although *D. scapiceps* has previously been reported from *S. palustris* (Stringer et al., 1969, J. Parasitol. 55:328), this is the first record of *D. uniformis* in the marsh rabbit. The exact number of cottontail rabbits examined was impossible to determine because many pieces of carcasses were sometimes mixed together before they were brought to the laboratory. Our findings extend the range for *D. uniformis* to include South Carolina and Georgia. Because specimens can be easily missed when examining a rabbit since the nem-

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<sup>1</sup> In cooperation with the University of Georgia, College of Agriculture Experiment Stations, Coastal Plain Station, Tifton, Georgia 31794.

atodes resemble fibrous tissue when the skin is pulled from the carcass, the range of *D. uniformis* may include a much wider range than that reflected in the literature and by our findings.

Specimens of *Dirofilaria uniformis* and *D. scapiceps* recovered from rabbits in Georgia have been deposited in the United States National Museum Helminthological Collection, numbers 73652 and 73651, respectively.

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### *Research Note*

## **New Records for Amphibian and Reptile Trematodes**

From 1975 to 1978, 200 amphibians and reptiles from localities in Nebraska, Arkansas, Oklahoma, Louisiana, Mississippi, Florida, North Carolina, and West Virginia were examined for trematode parasites. This report provides additional information concerning the geographical distribution of North American amphibian and reptile trematodes. Some results have already been published (Brooks and Fusco, 1978, J. Miss. Acad. Sci. 23:95-99). Worms, removed from their hosts, were examined alive when possible, and fixed with AFA after flattening with slight coverslip pressure. Specimens were stored in 70% ethanol, then stained with Mayer's hematoxylin and mounted in Histoclad or Canada balsam for study as whole mounts. Voucher specimens for all species have been deposited in the Harold W. Manter Laboratory, Division of Parasitology, University of Nebraska State Museum. Numbers in parentheses represent numbers of hosts infected per hosts examined at each locality. The notation NH refers to new host, NL to new locality, L to lungs, SI to small intestine, M to mouth, UB to urinary bladder, R to rectum, H to heart, MB to mesenteric blood vessels, and SM to submucosa of esophagus. (\*) refers to specimens donated by colleagues.

*Glythelmins quieta* (Stafford, 1900) Stafford, 1905 SI

*Rana catesbeiana* Shaw—Pea Vine Creek, Oklahoma (1/1) NL\*; Ocean Springs, Mississippi (2/4) NL

- Rana clamitans* Latreille—Blind Choctaw Bayou, vicinity of Baton Rouge, Louisiana (4/15)
- Rana utricularia* Harlan—Ocean Springs, Mississippi (1/9); Blind Choctaw Bayou, Louisiana (14/32)
- Rana pipiens* Schreber—10 miles South of Berkeley Springs, West Virginia (1/2) NL
- Brachycoelium salamandrae* (Froehlich, 1789) Lühe, 1909 SI
- Rana utricularia*—Ocean Springs, Mississippi (1/9) NL
- Rana pipiens*—10 miles South of Berkeley Springs, West Virginia (1/2) NL
- Plethodon glutinosus* (Green)—Ocean Springs, Mississippi (1/1) NL; 10 miles Northeast of Oxford, Mississippi (3/3) NL
- Haematoloechus complexus* (Seely, 1906) Krull, 1933 L
- Rana utricularia*—Ocean Springs, Mississippi (2/9) NL
- Haematoloechus breviplextus* Stafford, 1902 L
- Rana catesbeiana*—Ocean Springs, Mississippi (1/4) NL
- Haematoloechus longiplextus* Stafford, 1902 L
- Rana catesbeiana*—Lake Texoma, Oklahoma (1/1)
- Megalodiscus temperatus* (Stafford, 1905) Harwood, 1932 R
- Rana clamitans*—Blind Choctaw Bayou, Louisiana (5/15)
- Rana utricularia*—Blind Choctaw Bayou, Louisiana (15/32); Ocean Springs, Mississippi (1/9) NL
- Gorgoderina minima* Cort, 1912 UB
- Rana catesbeiana*—Ocean Springs, Mississippi (2/4) NL
- Rana clamitans*—Blind Choctaw Bayou, Louisiana (2/15)
- Rana utricularia*—Blind Choctaw Bayou, Louisiana (1/32)
- Gorgoderina attenuata* (Stafford, 1902) Stafford, 1905 UB
- Rana catesbeiana*—Ocean Springs, Mississippi (2/4) NL
- Gorgoderina bilobata* Rankin, 1937 UB
- Rana utricularia*—Ocean Springs, Mississippi (2/9) NL
- Polystoma nearcticum* (Paul, 1935) Price, 1939 UB
- Hyla cinerea* (Schneider)—Ocean Springs, Mississippi (1/4) NL
- Hyla cf. versicolor*—Pea Vine Creek, Oklahoma (1/1) NH, NL\*
- Ochetosoma magna* (Byrd and Denton, 1938) Caballero and Vogelsang, 1947 L
- Coluber constrictor* L.—Wake Co., North Carolina (1/1) NH, NL\*
- Elaphe obsoleta obsoleta* (Say)—Vanderburgh Co., Indiana (1/1) NH, NL\*
- Ochetosoma aniarum* (Leidy, 1891) Skrjabin and Antipin, 1957 M
- Coluber constrictor*—Fremont, Nebraska (1/1) NH, NL
- Ochetosoma kansensis* (Crow, 1913) Skrjabin and Antipin, 1957 M
- Agkistrodon piscivorus* (Lacépède)—Lincoln Parish, Louisiana (1/3) NL
- Ochetosoma wardi* (Byrd, 1936) Skrjabin and Antipin, 1957 M
- Nerodia c. cyclopion* (Dumeril and Bibron)—Jennings, Louisiana (1/3) NH, NL
- Nerodia erythrogaster flavigaster* (Forster)—Jennings, Louisiana (1/2) NH
- Nerodia fasciata confluens* Blanchard—Jennings, Louisiana (1/3) NH
- Auritelorchis auridistomi* (Byrd, 1937) Stunkard, 1979 SI
- Farancia abacura* (Holbrook)—Payne's Prairie, Alachua County, Florida (1/1) NL\*

- Allassostoma magna* Stunkard, 1916 R  
*Chrysemys concinna* (LeConte)—Stuttgart, Arkansas (1/1) NL  
*Heronimus mollis* (Leidy, 1856) Stunkard, 1964 L  
*Chrysemys scripta* (Schoepf)—Posey County, Indiana (1/1) NL\*  
*Cotylaspis* sp. SI  
*Chrysemys scripta*—Lincoln Parish, Louisiana (1/2) NL  
*Telorchis corti* Stunkard, 1915 SI  
*Chrysemys scripta*—Shreveport, Louisiana (1/1)  
*Telorchis robustus* Goldberg, 1911 SI  
*Sternotherus carinatus* (Gray)—Richland Parish, Louisiana (3/5) NH, NL  
*Macravestibulum obtusicaudum* Mackin, 1930 SI  
*Sternotherus carinatus*—Richland Parish, Louisiana (1/5) NH, NL  
*Pleurogonius malaclemys* Hunter, 1961 SI  
*Malaclemys terrapin centrata* (Latr.)—Savannah Beach, Georgia (1/1) NL\*  
*Polystomoidella* sp. UB  
*Chrysemys scripta*—Lincoln Parish, Louisiana (2/2)  
*Polystomoides* sp. UB  
*Sternotherus carinatus*—Richland Parish, Louisiana (2/5)  
*Spirorchis artericola* (Ward, 1921) Stunkard, 1925 H  
*Chrysemys scripta*—Richland Parish, Louisiana (1/1) NL  
*Spirorchis elegans* Stunkard, 1923 SM  
*Chrysemys scripta*—10 miles NE of Oxford, Mississippi (2/2) NL  
*Spirorchis haematobium* Stunkard, 1922 H  
*Chelydra serpentina* (L.)—Ocean Springs, Mississippi (1/1) NL  
*Spirorchis parvus* (Stunkard, 1923) Yamaguti, 1958 H  
*Chrysemys scripta*—Stuttgart, Arkansas (1/1) NL  
*Unicaecum dissimilis* Byrd, 1939 MB  
*Graptemys geographica* (LeSueur)—10 miles N Yazoo City, Mississippi (1/1)  
 NH, NL  
*Unicaecum ruszowskii* Stunkard, 1925 MB  
*Chrysemys scripta*—10 miles NE Oxford, Mississippi (2/2) NL

Previous reports of digeneans infecting leopard frogs in the southeastern United States listed either *Rana sphenocephala* (Brandt, 1936, Ecol. Monogr. 6:491–532; Bennett, 1938, Proc. La. Acad. Sci. 4:178–181), *R. pipiens* (Parker, 1941, J. Tenn. Acad. Sci. 16:27–45), or *R. pipiens sphenocephala* (Sacks, 1952, Trans. Am. Microsc. Soc. 71:267–269) as host. Pace (1974, Misc. Publ. Mus. Zool. Univ. Mich. 148:1–140) ascribed all such frogs to *Rana utricularia*. Including the above and the present report, *Rana utricularia* is known to host the following digeneans: *Brachycoelium salamandrae*, *Cephalogonimus americanus* Stafford, 1902, *Gorgoderina minima*, *Gorgoderina attenuata*, *G. bilobata*, *Glypthelmins quieta*, *Haematoloechus complexus*, *Langeronia parva* Sacks, 1952, *Loxogenoides bicolor* (Krull, 1933) Kaw, 1945, and *Megalodiscus temperatus*.

Dronen and Guidry (1977, Proc. Helminthol. Soc. Wash. 44:223–225) presented data suggesting that relative size of gonads and absolute sizes of various body parts do not provide adequate means for distinguishing species of *Ochetosoma*. My identifications are based on examination of the posterior extent of the cirrus sac, amount of glandulation inside the cirrus sac, vitelline configuration, sucker

ratio and genital pore location. I have deposited 64 specimens of *Ochetosoma* spp. in hopes they will aid a much-needed revision of the group.

This paper presents only the third report of the spirorchiid genus *Unicaecum* Stunkard, 1925. Stunkard (1925, *Ann. Parasitol. Hum. Comp.* 5:117–126) erected the genus for *U. ruszowskii* Stunkard, 1925 from *Chrysemys scripta* in North Carolina and Byrd (1939, *J. Tenn. Acad. Sci.* 14:116–161) described *U. dissimilis* from *Chrysemys troostii* in Reelfoot Lake, Tennessee. Spirorchiids have occasionally been reported from sites other than the circulatory system. I found one specimen of *U. ruszowskii* in the small intestine of a *Chrysemys scripta*, but all other specimens occurred in the mesenteric blood vessels. *Spirorchis elegans* collected from near Oxford, Mississippi all occurred in the esophageal submucosa, a finding consistent with results published by Schroeder and Ulmer (1959, *Proc. Iowa Acad. Sci.* 66:443–454).

I wish to thank the following colleagues who aided this study by providing additional specimens: Dr. Richard L. Buckner, Dr. Richard W. Heard, III, Mr. Richard Franz. This study was supported in part by NIH Training Grant No. 5-T32-AI-07030-04.

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### *Research Note*

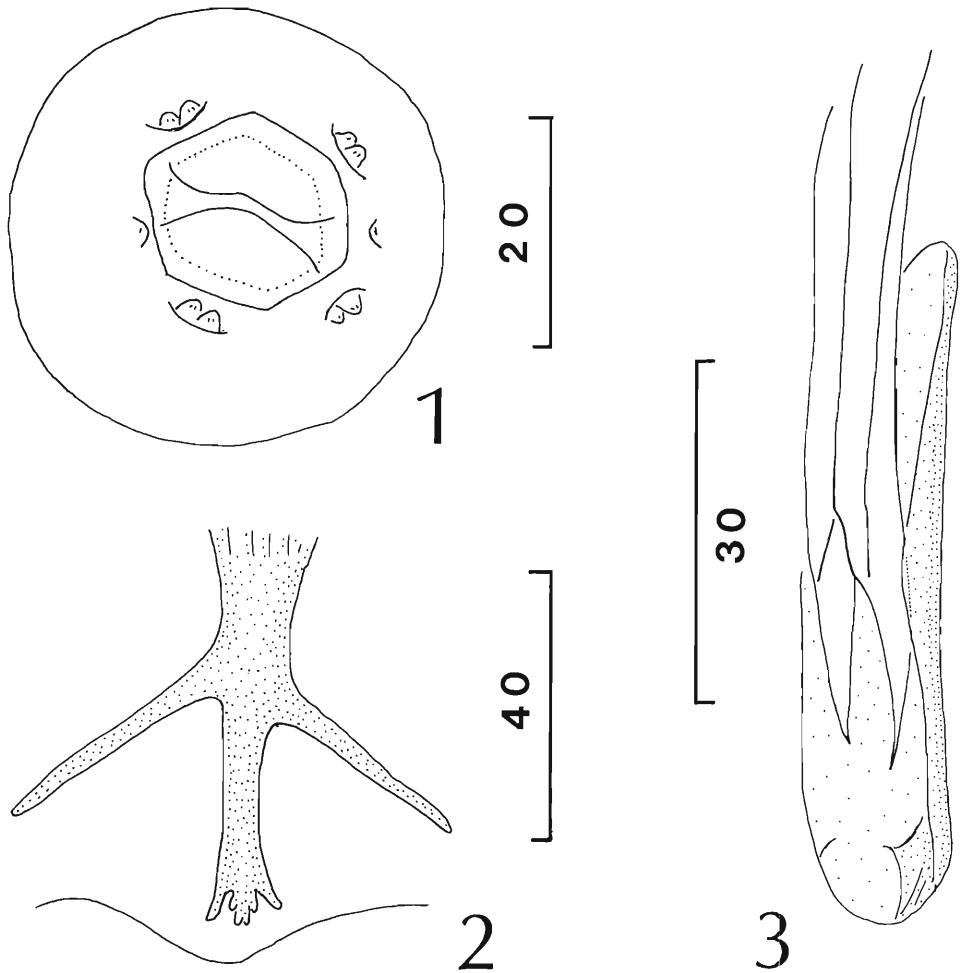
## **The Nematode Fauna of *Rana macrodon* Dumeril and Bibron with Supplementary Data on *Batrachonema synaptospicula* Yuen, 1965 (Nematoda: Amidostomatidae)<sup>1</sup>**

The intestinal tracts of 87 *Rana macrodon* collected along the Burong River, Tg. Karang, Malaysia were examined for nematodes from 1976–1977. Two nematode species were recovered: *Batrachonema synaptospicula* Yuen, 1965 (USNM Helm. Coll. No. 75173) and *Paracosmocera* sp. (USNM Helm. Coll. No. 75174). *Batrachonema synaptospicula* had a prevalence of 11.5% with intensities ranging from 7 to 35; while, *Paracosmocera* sp. had a prevalence of 41.3% with intensities ranging from 1 to 8. Concurrent infections were found in only two cases. Previously, only *Amplicaceum* sp. had been reported from this host (Myers and Kuntz, 1969, *J. Fish. Res. Bd. Can.* 26:793–797).

Our specimens of *Paracosmocerca* are similar to *P. mucronata* Kung and Wu, 1945, the only described species in the genus. However, we recovered only one

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<sup>1</sup> This study was supported in part by a grant AI 10051 (UC ICMR) to the Department of International Health, School of Medicine, University of California, San Francisco from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U.S. Public Health Service.



Figures 1–3. *Batrachonema synaptospicula* Yuen, 1965. Measurements are in micrometers. 1. *En face* of female. 2. Details of externodorsal and dorsal rays. 3. Gubernaculum and bifid spicule tips.

damaged male which was not sufficient to positively identify our specimens as *P. mucronata*.

Yuen (1965, *Can. J. Zool.* 43:411–415) described *Batrachonema synaptospicula* from *Rana* sp. in Batu Berendam, Malacca (Malaysia). *Batrachonema* was originally placed in the Trichostrongylidae; however, Durette-Desset and Chabaud (1977, *Ann. Parasitol.* 52:539–558) believe that this genus should be placed in the Amidostomatidae. We have followed the latter classification. Yuen reported the absence of a gubernaculum, the dorsal ray with a bifurcated distal end, and an egg size, which differ from our specimens. Type specimens of *B. synaptospicula* were obtained from the British Museum (Natural History), instead of the Department of Zoology, University of Singapore where they had originally been deposited, and were found to be morphologically similar to our specimens. Because of the discrepancy between the original description and the actual speci-



mens, we are adding supplementary data to the description of *B. synaptospicula* by describing our specimens in detail.

Measurements are in microns, numbers in parentheses refer to data given by Yuen. Figures were drawn with the aid of a drawing tube and serve to illustrate previously unreported details. Specimens were cleared with phenol-alcohol.

***Batrachonema synaptospicula* Yuen, 1965**  
(Figs. 1-3)

**GENERAL:** Body strongly coiled, with region of greatest width near midregion. Two liplike structures present. Cephalic papillae 8, in 4 paired groups forming a ring 45° from dorsoventral axis; amphids 2 at lateral axis. Mouth hexagonally shaped. Buccal capsule small, moderately sclerotized containing a single tooth. Cephalic vesicle inflated, striated, bullet shaped, originating at anterior portion of buccal capsule. Cuticle with fine striations approximately one apart. Esophagus muscular, claviform, with region of greatest width near base. Nerve ring near midregion of esophagus. Excretory pore located slightly posterior to or anterior to base of esophagus, posterior to nerve ring.

**MALE** (based on 14 mature specimens): Body 2.9-3.7 mm (1.8-3.4 mm) long by 36-39 wide at base of cephalic vesicle, increasing to 51-70 at junction of esophagus and intestine and between 84-96 (80-90) at level of greatest width, 33-43 times longer than wide. Buccal capsule 12-14 (16) long by 12-17 (13) wide. Cephalic vesicle 75-108 long by 39-46 wide, with between 27 and 32 transverse striations. Esophagus 334-377 (290-360) long by 39-46 (40-43) at widest point, 9-13% of body length. Nerve ring 176-212 (200-220) to cephalic end, 10-14 in height. Excretory pore located 321-381 from anterior end. Testis straight, beginning 358-485 from cephalic end. Spicules equal, fused along distal  $\frac{2}{3}$ , bifid at tip, 230-265 (260-270) long, 7-9% of body length. Gubernaculum scooplike, with distal end curved slightly dorsal, 60-70 long, 24-28% length of spicule. Bursa with wide lateral lobes and small dorsal lobe with distinct bulge at terminal margin. Anteroventral, posteroventral, anterolateral, and mediolateral rays equal, contiguous at their bases, reaching margin of bursa except for anterolateral ray. Posterolateral rays separate, about  $\frac{2}{3}$  as long as other rays. Externodorsal rays arise from common trunk with dorsal ray, slightly posterior to center of dorsal ray, not reaching margin of bursa. Dorsal ray divided at tip into four small fused distal rays and two longer separate proximal rays.

**FEMALE** (based on 14 mature specimens): Body 3.9-5.2 mm (3.4-4.8 mm) long by 39-43 wide at base of cephalic vesicle, increasing to 60-79 at junction of esophagus and intestine and between 99-111 (110-120) at level of greatest width, 38-49 times longer than wide. Buccal capsule 12-17 (16) long by 17-19 (15) wide. Cephalic vesicle 87-104 (110) long by 43-51 (50) wide with between 30-35 transverse striations. Esophagus 352-415 (370) long by 41-53 (43-46) at widest point, 8-9% of body length. Nerve ring 188-241 from cephalic end, 14-19 in height. Excretory pore located 334-426 from anterior end. Vulva with conspicuous lips situated postequatorial 800-1,100 (930-1,080) from posterior end, 3.0-4.1 mm from anterior end, 19-25% of body length from posterior end. Uteri amphidelphic, didelphic containing a single row of eggs. Ovaries both directed anteriorly, posterior ovary longer than anterior ovary. Eggs 58-65 (66-80) long by 31-43 (43-

53) wide. Rectum 36–58 long with 2–3 rectal glands. Tail 121–147 (130) long, tapering to a fine distal point.

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## JUSTUS FREDERICK MUELLER

Honorary Member



It is my very pleasant duty, on behalf of the Society, to present the Honorary Membership upon one of the most colorful parasitologists. In the past we have had our Charles Wardell Stiles. Our present recipient of Honorary Membership justifies all the accolades for his accomplishments as a research scientist, teacher, author, and editor. He has effectively used his capacity as an artist to embellish all of his professional activities and to top it all he is a perfectionist. He demands perfection of himself and his colleagues in precise but colorful expressive language with scarcely concealed disdain for less than perfection.

Justus Frederick Mueller was born in Baltimore, Maryland on November 20, 1902. He attended Johns Hopkins University (AB 1923), and the University of Illinois (M.S. 1926, Ph.D. 1928). He spent one year (1923-1924) as Scientific Assistant in the U.S. Bureau of Fisheries before beginning graduate study at the University of Illinois. His full-time appointments were Instructor, Assistant Professor, to Associate Professor in the College of Forestry 1928-1942; Associate Professor of Parasitology to Professor of Microbiology, College of Medicine 1942-1972, and acting Chairman of Microbiology 1954-1957 at Syracuse; he is now (1972-date) professionally very active as Emeritus Professor in the Upstate Medical Center, S.U.N.Y. During this long and successful career he has had many concurrent appointments such as Naturalist in the Roosevelt Wild Life Forest

Experiment Station (1928–1935); Lecturer or Visiting Professor of Parasitology at Medical Schools, Syracuse (1930–1942), Marquette (1956–1960), Pittsburgh (1965), and Yale (1968); and Consultant to a number of Diagnostic Laboratories and pharmaceutical companies.

His early contributions to our knowledge of fish parasites has been followed by a long sustained and meticulous study of the biology of pseudophyllidean cestodes including in vitro cultivation and host changes resulting from infection.

Justus has pioneered the use of art in the teaching of parasitology including the designing of Medical Exhibits in Parasitology for Winthrop Chemical Company and the Mueller-Ward Models of Wards Natural Science Establishment. He has designed the ASP Seal (on the cover of the Journal of Parasitology), the Henry Baldwin Medal and the 50th Anniversary Medal for ASP and the Bailey K. Ashford Medal for ASTM & H.

Justus Mueller's editorial contributions may well remain the most constantly visible record of his genius. In 1962 he became both an editorial consultant to the Journal of the Student American Medical Association, the *New Physician* and the Journal of Parasitology. He is a charter member of ASP and joined Helm Soc in 1963. In 1962 he took over the responsibility of the very good journal (J.P.) which has suffered some lapses since the Cort, Stoll, Stunkard, and Jacobs standards. Within a few years he converted it into a superior journal. His scientific acumen, attention both to the total unit and the component details, and his pressure on authors for perfection overlaid with his artistic instincts and capacity have developed the outstanding parasitological journal. It is second to none in biology. It is a model to be emulated by many other editors. His successor in 1979 will face a challenge to maintain the standards that Justus Frederick Mueller has set. The Helminthological Society of Washington honors itself and it is an honor and pleasure for me to present, on behalf of the society, this token of our appreciation.

GILBERT F. OTTO

#### OBITUARY NOTICE

**Donald J. Ameel**  
Apr. 24, 1907–Jan. 30, 1979  
Member since 1949

**Robert Rubin**  
May 27, 1922–Jun. 20, 1979  
Member 1954–1959

## MINUTES

### Five Hundred Seventeenth Through Five Hundred Twenty-Fourth Meetings

*517th Meeting:* Animal Parasitology Institute, USDA, Beltsville, Maryland, 19 October 1978. The 1978 Anniversary Award was presented to Dr. K. C. Kates by Merle L. Colglazier. Honorary membership in the Society was presented to Dr. Justus F. Mueller by Dr. Gilbert F. Otto. Special guests at the meeting were persons concerned with editorial policy of journals related to parasitology, including Drs. Paul C. Beaver, Thomas C. Cheng, John O. Corliss, A. James Haley, Charles P. Hibler, George J. Jackson, B. M. Honigberg and Mrs. Honigberg, David R. Lincicome, and Justus F. Mueller. The following slate of officers was presented: Ronald Fayer (President), James Burke (Vice President), Ralph Lichtenfels (Corresponding Secretary-Treasurer), Nancy Pacheco (Recording Secretary). The death of Dr. Reinhard Harkema was announced. Mr. Arly Allen of Allen Press, Inc., gave a presentation "Scientific Journals, Past, Present and Future."

*518th Meeting:* National Zoological Park, Washington, D.C., 15 November 1978. The slate of officers presented at the 517th meeting was elected. The death of Dr. Theodosia M. Welch was announced. An open forum on "Parasitology in Exotic Animals, and Problems Encountered" was held with presentations on "Chronic Intestinal Parasitism in the Bactrian Camels at the Conservation and Research Center (Front Royal, Virginia)," Richard Cambre; "Morbidity and Mortality Due to Parasitism at the National Zoological Park," Jack Hoopes; "Rectularia in the Golden Marmoset," Elizabeth Smith; "Overview of Animal Medical Care at the National Zoological Park," Mitchell Bush.

*519th Meeting:* Naval Medical Research Institute, Bethesda, Maryland, 8 December 1978. President Harley Sheffield announced that Dr. Ronald Fayer had received the Henry Baldwin Ward medal from the American Society of Parasitologists. Officers elected at the 518th meeting were installed. Papers presented from the Navy Schistosomiasis Vaccine Research Division: "Protective Immunity: Chronic Infection Model," David A. Dean; "Protective Immunity: Irradiated Cercarial Model," Wilton E. Vannier; "Schistosome Surface Antigens," David Taylor and Eugene Hayunga; "Antibodies, Antigens, and Immune Complexes by ELISA," Michael Stek.

*520th Meeting:* National Institutes of Allergy and Infectious Diseases, Bethesda, Maryland, 16 January 1979. Treasurer Ralph Lichtenfels gave a brief financial report for 1978. The death of Dr. Harry M. Martin was announced. Papers presented: "Endocrine Control of Mosquito Biting Behavior," Raymond Beach; "The Clearance of Parasitized Erythrocytes in Malaria," Thomas Quinn; "Induction of *T. cruzi*-mammalian Cell Hybrids," Mark Crane; "Studies on the Virulence of *E. histolytica*," Theodore Mattern.

*521st Meeting:* Walter Reed Army Institute of Research, Washington, D.C., 2 March 1979. President Ronald Fayer took a poll to indicate interest in purchasing a cumulative index of the *Proceedings* for \$5.00-6.00. The printing of

this index is being considered. Papers presented: "Overview of Leishmaniasis and the Army's Research Efforts in this Area," Larry Hendricks; "Kala Azar in Kenya: Epidemiology and Clinical Data," Wayne Hockmeyer; "Studies on the Effects of Skin Application of Hexachlorophene, Lipid Solvents and/or Lipids on *Schistosoma mansoni* Cercarial Penetration of Intact Mouse Tail Skins," Lyford Greene; "Mass *in vitro* cultivation of Leishmania," George Childs.

*522nd Meeting:* Food and Drug Administration, Washington, D.C. (cosponsored by Plant Protection Institute, USDA), 14 March 1979. Papers presented: "Population Genetic Studies of Parasitic Helminths," Robert Vrijenhoek; "Biochemical Profiles of Larval Nematodes in Edible Fish . . . or, What Has Cod Wrought," George Cain; "The Application of the ELISA Technique to Detect Antibodies in Dogs Infected with *Dirofilaria immitis*," Gary Weil; "Soybean Cyst Nematode: Its Economic Importance in Agriculture and Its Pathology in Resistant and Susceptible Soybean Varieties," Burton Endo; "Travelog Nematodes, Mosquitoes, Rome, Cairo, Alexandria, Luxor, Delhi, Agra, Rawalpindi," William Nickle.

*523rd Meeting:* The Johns Hopkins University, Baltimore, Maryland, 20 April 1979. Papers presented: "The mallophaga, *Trinoton anserinum*, as the Cyclo-developmental Vector for a Heartworm Parasite of Waterfowl," William Seegar; "Morphogenesis of Larval *Onchocerca volvulus* in Guatemalan Black Flies," Robert D'Antonio; "Oogenesis in *Schistosoma mansoni* with Special Emphasis on the Male Contribution to Female Development," Ellen Clough; "Phenol Oxidase and Egg Shell Formation in *Schistosoma mansoni*," John Seed; "Mating Behavior and Inhibition of Copulation of *Schistosoma mansoni* In Vitro," D'Jamshid Shirazian; "Phagocytosis in *Limulus*," Gilda Cardenosa.

*524th Meeting:* University of Pennsylvania's New Bolton Center, Kennett Square, Pennsylvania, 12 May 1979. The death of Dr. Donald J. Ameel was announced. Dr. J. F. A. Sprent was elected to Honorary membership and M. M. Farr, J. T. Lucker, and Dr. G. W. Luttermoser were elected to Life membership in the Society. Papers presented: "Equine Verminous Arteritis: Healing of Lesions Following Albendazole Larvacidal Therapy," Jay Georgi; "Effects of Loss of Adult Worms and Depressed Host Immunity on the Resumption of Development of Arrested *Ancylostoma caninum* Larvae," Michael Page; "Immune Regulation By Products of a Nematode," Omar Barriga; "Origin and Coevolution of the Schistosomes Worldwide," George Davis; "Immune Responses of Athymic Mice to *Schistosoma mansoni* Infection," Philip Khoury.

The following 25 new members were elected at the meetings indicated: *518th:* M. Maqbool Ahmad, Amy R. Campbell, George E. Childs, Jr., Reid Jilek, Robert P. Lavan, Susan E. Wade, Dale R. Witlock. *519th:* Jose F. Amato, K. Darwin Murrell. *520th:* Paul A. Coulis, Anthony J. Johnson, James R. Staub, Michael Stek, Jr., Joseph P. Sypek. *521st:* Charles R. Cabiatic, Brian D. Hansen, Joseph F. Urban, Jr. *523rd:* Herman B. Garrett II, Thomas D. Bair, Kinga J. Kovacs, Suzana B. Amato. *524th:* Mary Beverly-Burton, K. J. Orton Williams, John T. Sullivan, William S. Seegar.

NANCY D. PACHECO  
Recording Secretary

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