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Ultrastructure of the Intestine of Second and Third Juvenile Stages of the Soybean Cyst Nematode, *Heterodera glycines*

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ABSTRACT: The intestine of juvenile stages of *Heterodera glycines* consists of large epithelial cells filled with lipid droplets, glycogen rosettes, mitochondria, endoplasmic reticulum, and ribosomes. The esophago–intestinal valve consists of cells that are laterally attached with distinct junctional complexes. Apical boundaries of the cells have apposed membranes that separate during food ingestion. Cell surfaces of the intestinal epithelium that line the lumen have membrane folds, many of which resemble microvilli; however, they lack central actin filament cores. Sculpturing the surface membrane of the intestinal lumen membrane folds is an enteric coating. The membrane folds are sparse in the intestine of infective juveniles but extensive in parasitic juveniles. The intestino–rectal valve is similar to the structure of the esophago–intestinal valve in that junctional complexes attach the lateral membranes of the apposing cells that comprise the valve. Muscle fibers are associated with the intestino–rectal valve but not with the esophago–intestinal valve.

KEY WORDS: esophago–intestinal valve, *Heterodera glycines*, intestinal morphology, soybean cyst nematode, ultrastructure.

The intestinal epithelium of many nematode species forms microvilli that project into the lumen (Munn and Greenwood, 1984). Many animal-parasitic species of nematodes have regularly arranged, closely packed microvilli that constitute the surface of the intestinal epithelium (Wright, 1963; Sheffield, 1964; Bruce, 1966; Munn and Greenwood, 1984). Microvilli of *Meloidogyne hapla*, a plant-parasitic species, are similar to those of animal–parasitic species (Ibrahim, 1971). However, in other plant-parasitic species, the microvilli are blunt in the anterior intestine but greatly attenuated in the midintestinal regions (Shepherd and Clark, 1976). The anterior intestinal lumen of the infective J2 of *H. glycines* has minimal membrane projections that might be termed microvilli (Endo, 1984). Except for a brief description of microvilli (Wisse and Daems, 1968) in the J2 *Heterodera rostochiensis*, a paucity of information is available on the morphology and ultrastructure of microvilli among cyst nematodes.

This paper describes the ultrastructure of the esophago–intestinal valve and the microvilli-like membrane folds associated with the lumen formed by the intestinal epithelium of infective, advanced second-stage, and third-stage juveniles of *H. glycines*.

Materials and Methods

Samples used in these observations were processed in the same manner as those described by Endo (1984, 1987) and Endo and Wergin (1973) for previous ultrastructural studies of infective and parasitic juveniles of *H. glycines* and the host, soybean, *Glycine max*.

Results

The intestine of the soybean cyst nematode consists of a single layer of epithelial cells that surrounds a lumen in which ingested food is digested, products absorbed, and waste material expelled (Fig. 1). The intestine is joined to the esophagus by an esophago–intestinal valve (Figs. 1–3). The waste products of the intestine are released into the rectum through the intestino–rectal valve.

The cuticle-lined esophageal lumen changes from a triangular to a slit-shaped cross-sectional opening prior to forming the esophago–intestinal valve (Figs. 1, 9, 12–14). A transitional zone exists in which the lumen cuticle becomes thin and contacts the noncuticularized or unlined membranes of the esophago–intestinal valve (Figs. 1, 4, 14). Beyond this point, the esophago–intestinal valve is formed by 2 esophageal cells that are attached to each other by junctional complexes at their lateral boundaries (Figs. 1, 7, 9). The apical membranes of the valve cells are apposed to each other with minimal intermembrane connections (Fig. 4). Apposed membranes of the apical region of the cells of the esophago–intestinal valve are separated and filled with intermembrane substances when nematodes are observed 18 hr after inoculation (Fig. 7). The esophago–intestinal valve consists of 2 distinct, tightly attached cells with dense cytoplasm. In Figure 9,

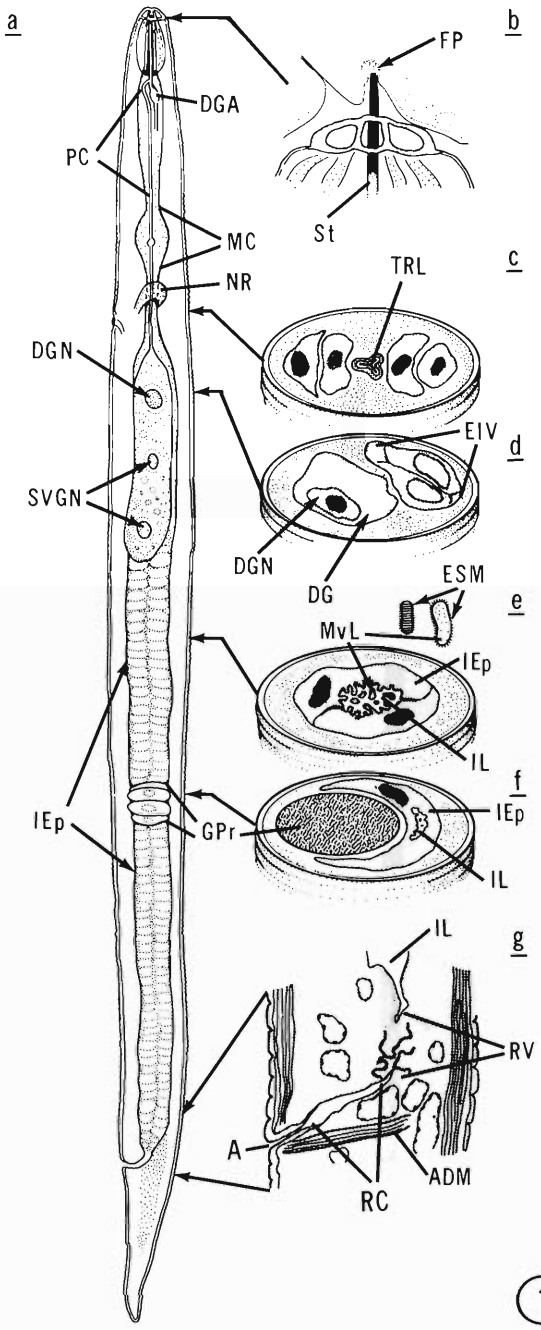


Figure 1. (a) Diagram of an infective J2 of *Heterodera glycines*. Arrows indicate sector used for other diagrams. ADM, anus depressor muscle; DG, dorsal gland; DGA, dorsal gland ampulla; DGN, dorsal gland nucleus; IL, intestinal lumen; MC, metacorpus; NR, nerve ring; PC, procorpus; SVGN, subventral gland nucleus. (b) Nematode at feeding site showing stylet (St) in contact with feeding plug (FP). (c) Diagram of the isthmus region showing a triradiate lumen (TRL) of the esophagus. (d) Diagram showing a cross section

only 1 of 2 cells is shown in longitudinal section; the valve is flanked by the anterior extremity of the intestinal epithelium.

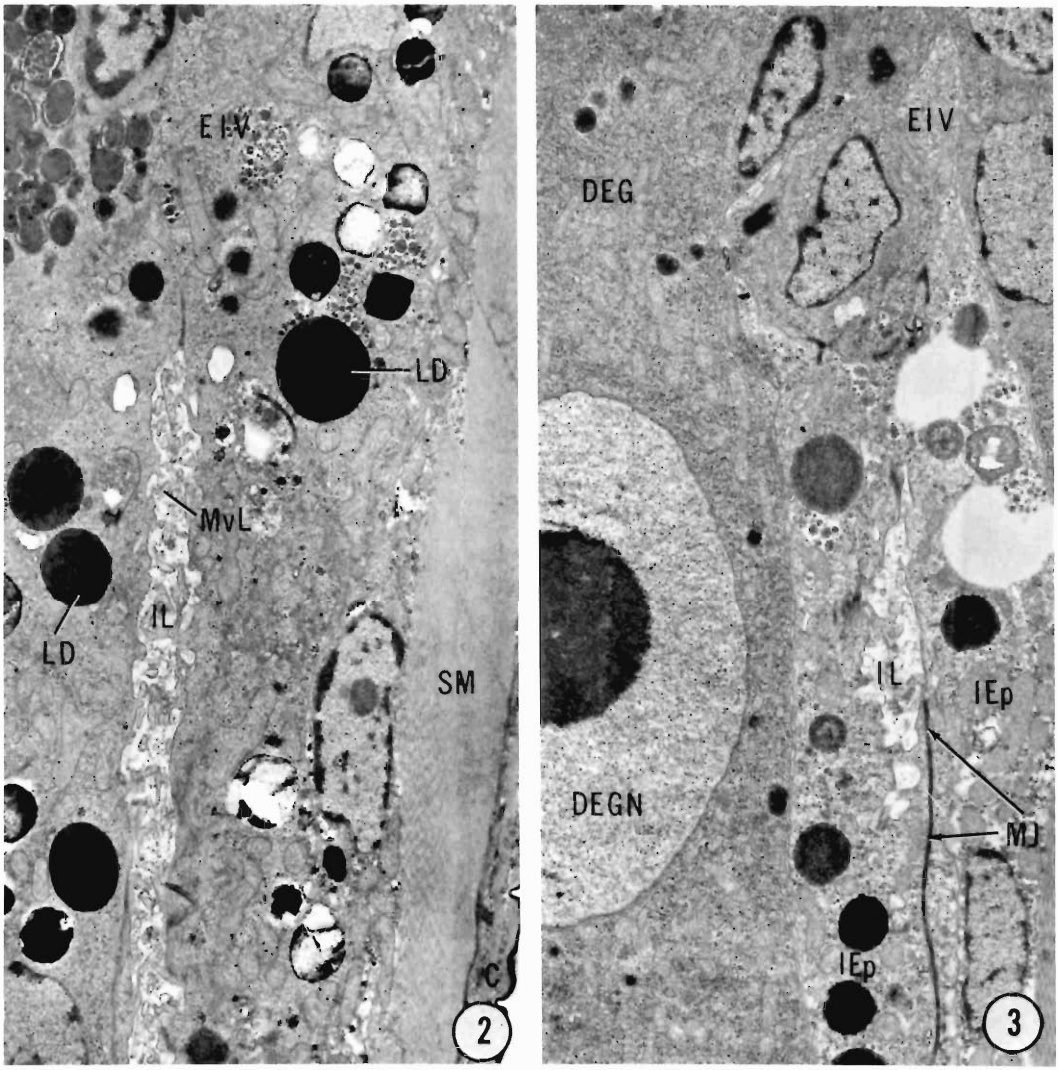
When viewed in cross section, the intestinal epithelium, posteriad to the esophago–intestinal valve, consists of 3 clearly defined cells whose lateral membranes are joined with junctional complexes just below the lumen surface (Figs. 5, 8, 19).

The intestinal epithelium of infective J2 have only a few microvilli-like surface membrane folds, whereas in parasitic juveniles, 18 hr after inoculation, the surface membrane folds occur throughout the anterior intestinal lumen (Figs. 2, 3, 10).

In contrast, at 3 days after inoculation, the intestinal lumen is partially blocked by microvilli-like surface membrane folds (Figs. 14–18). The internal contents of the folds consist of cytoplasm with organelles that are similar to and continuous with the cytoplasm of the epithelial cells (Figs. 18, 21). Furthermore, surface folds of the intestinal epithelium are more abundant among juveniles observed 3 days after inoculation than at later stages of development (Figs. 19–22). Surface sculpturing occurs on the microvilli-like surface folds and adjacent membranes that form the boundaries of the intestinal lumen. The sculpturing can be interpreted as an enteric coating material accumulating on the surface membranes (Fig. 20).

The tubelike morphology of the intestinal lumen of infective J2 changes into an irregular-shaped lumen in most developing parasitic juveniles (Figs. 16–22). Longitudinal sections of advanced second-stage and third-stage juveniles show extensive branching of the intestinal lumen. This branching is apparent in the cross sections of the intestinal epithelium that has a lumen lined with microvilli-like membrane folds (Figs. 19, 20). Intestinal epithelial cells of ad-

← (CS) of the esophago–intestinal valve (EIV) comprised of 2 esophageal cells with apposed *noncuticular* apical membranes. The EIV lies in the proximity of the dorsal gland nucleus (DGN). (e) Diagram showing CS of intestinal epithelium (IEp). Intestinal lumen lined with microvilli-like (MvL) invaginations and surface folds. Enlargement of microvilli invaginations and surface folds sculptured with enteric surface materials (ESM) shown in tangential and cross sections. (f) Intestinal epithelium (IEp) displaced by genital primordium (GPr). (g) Diagram of a longitudinal section showing the intestino–rectal valve (RV), rectal channel (RC), and anus (A).



Figures 2, 3. Infective Stage (J2) *Heterodera glycines* to show 2 levels of sectioning through the anterior intestinal lumen. 2. Longitudinal section of an infective J2 of *Heterodera glycines* showing the esophago–intestinal valve (EIV) and lumen of the anterior intestine. The lumen (IL) is partially blocked by microvilli-like (MvL) and related surface folds of the lumen membrane. The intestine lies between the epithelial cells that characteristically contain lipid droplets (LD). C, cuticle; SM, somatic muscles. ×8,800. 3. Longitudinal section of an infective J2 of *Heterodera glycines* showing the spatial relationship of the cells of the esophago–intestinal valve (EIV) with their nuclei and the dorsal esophageal gland with a single prominent nucleus (DEGN). The tangential section into the lumen of the anterior intestine reveals 1 of 3 membrane junctions (MJ) that joins epithelial cells of the intestine. IEp, intestinal epithelium; IL, intestinal lumen; DEG, dorsal esophageal gland. ×8,500.

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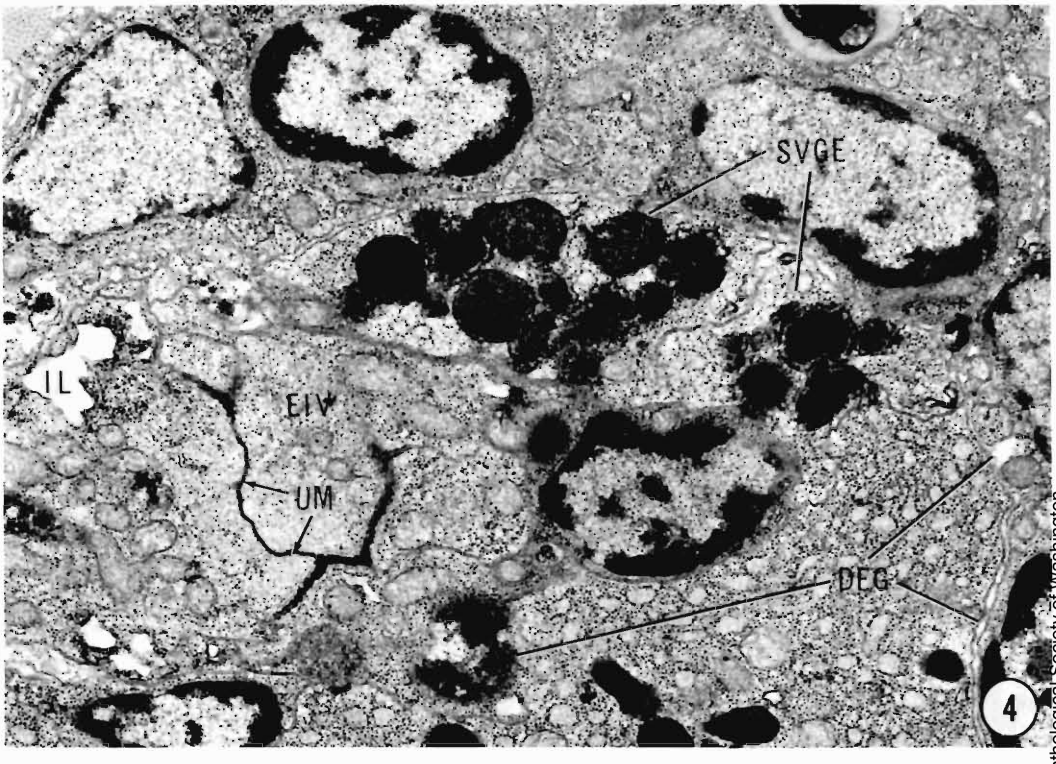
Figures 4, 5. Cross sections of the infective stage of *Heterodera glycines* to show esophago–intestinal valve and intestinal epithelium. 4. Cross section of an infective J2 of *Heterodera glycines* showing a portion of the esophago–intestinal valve (EIV). The closed mode of the valve is depicted by the unlined noncuticularized membranes (UM) bordered by electron-lucent cytoplasm and supported by membrane junctions. The EIV is bordered by a branch of the intestinal lumen (IL), the dorsal esophageal gland (DEG), and extensions of the subventral glands (SVGE). ×20,000. 5. Cross section of an infective J2 of *Heterodera glycines* showing the tripartite nature of the intestinal epithelium. Boundary of the lumen in its unexpanded state is designated by 2 of the 3 membrane junctions (MJ) that join the 3 intestinal epithelial cells. EIVL, esophago–intestinal valve lumen; Mc, mitochondria. ×54,000.



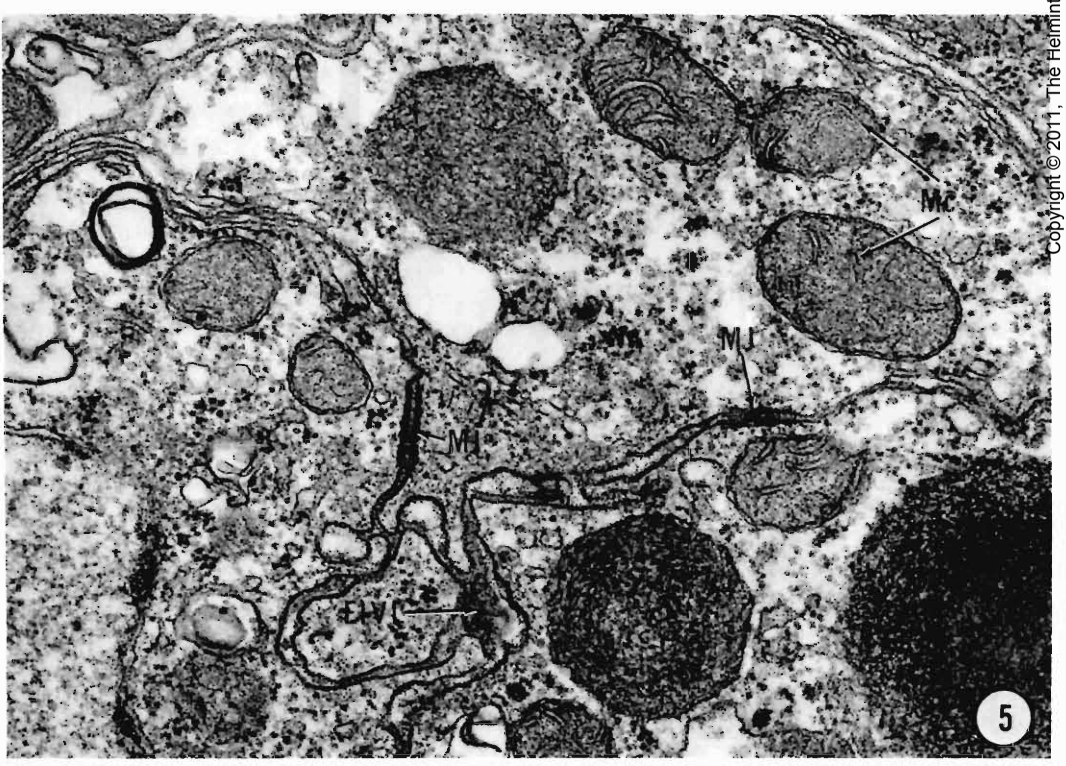
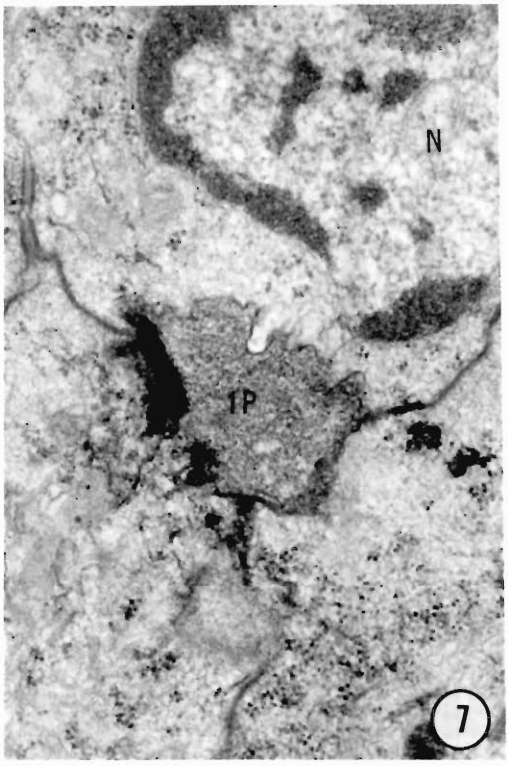
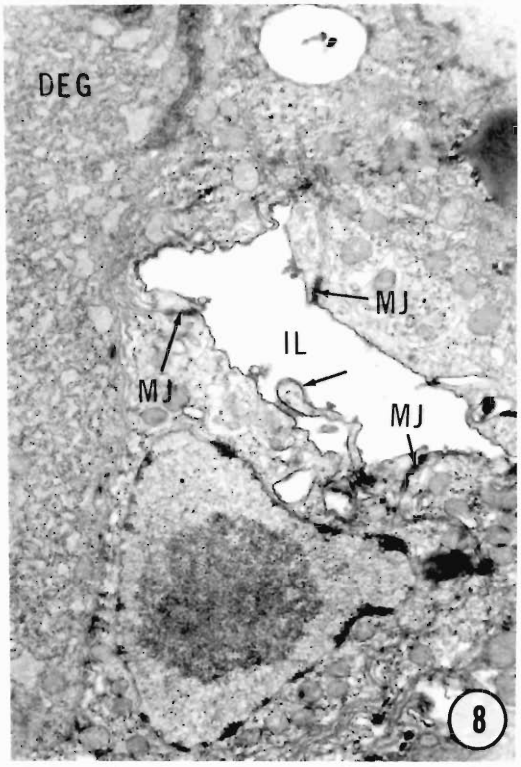


Figure 6. Longitudinal section of the rectal and anal regions of an infective J2 of *Heterodera glycines*. The lumen of the intestine (IL) is shown anterior to the intestino–rectal valve. Unlined membranes and membrane junctions constitute parts of the intestino–rectal valve (RV). The rectum (R) extends from the posterior of the intestino–rectal valve to the cuticularized anal opening. The dorsal lip of the anus (A) is attached to anal muscle fibers (AM) that extend to the dorsolateral sides of the nematode body. C, cuticle; SM, somatic muscle. ×15,100.





Figures 7, 8. J2 *Heterodera glycines,* 18 hr after inoculation to show the esophago–intestinal valve and lumen of the anterior intestine. 7. Cross section through the esophago–intestinal valve of a nematode at a feeding site. The noncuticularized cell membranes of the EIV are separated by apparent ingested products (IP) from the host. Prominent nucleus (N) is typical of the 2 nuclei that form the EIV. ×40,500. 8. A cross section of the same specimen, posteriad to the region shown in Figure 7, shows an open lumen of the intestine (IL) formed by 3 cells joined at their inner cell boundaries with membrane junctions (MJ). The lumen surface ranges from smooth to irregular with invaginations of portions of the lumen membrane, some of which are sculptured (→). DEG, dorsal esophageal gland. ×16,500.

vanced second-stage juveniles contain dense cytoplasm consisting of enlarged lipid droplets, glycogen rosettes, Golgi bodies, mitochondria, endoplasmic reticulum, and ribosomes (Figs. 15, 16, 19, 20).

Among the infective J2, the anterior intestine is long and narrow and extends from the esophago–intestinal valve to the terminus of the esophageal glands (Figs. 1–3). Three days after inoculation, the anterior intestinal region expands to about ⅓ of the nematode's diameter in the space adjacent to the esophageal glands (Fig. 15). Extending posteriad from the ends of the glands, the midintestinal region occupies a major portion of the body cavity of the nematode with the exception of space occupied by the genital pri-

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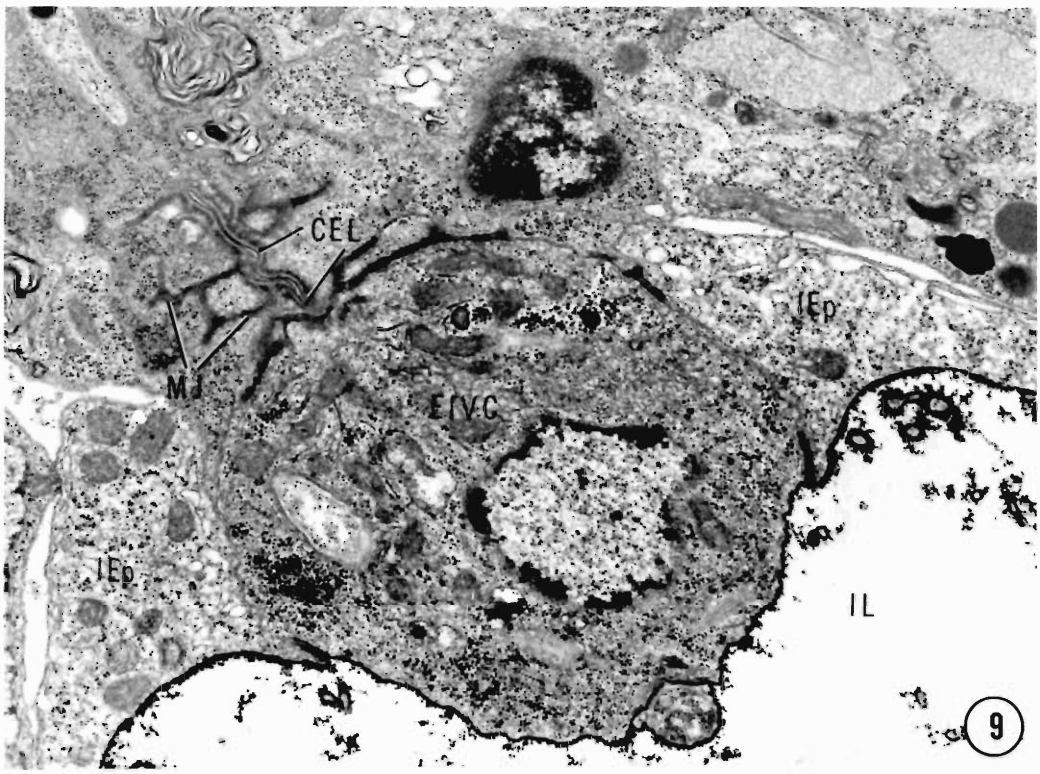
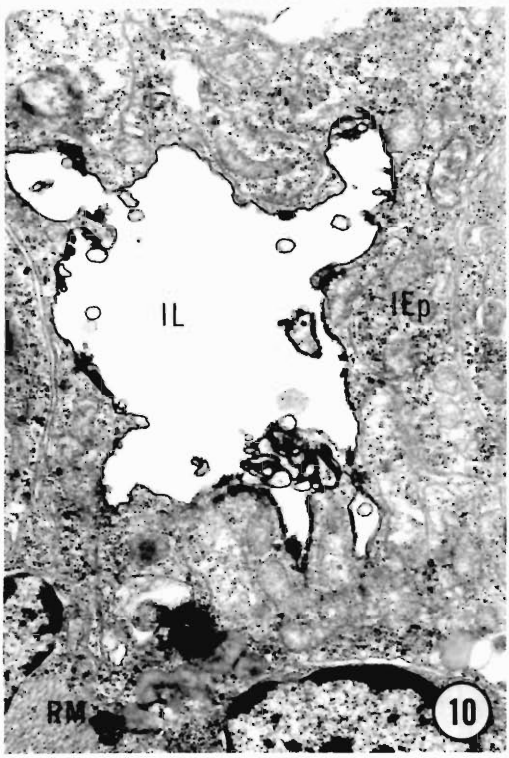
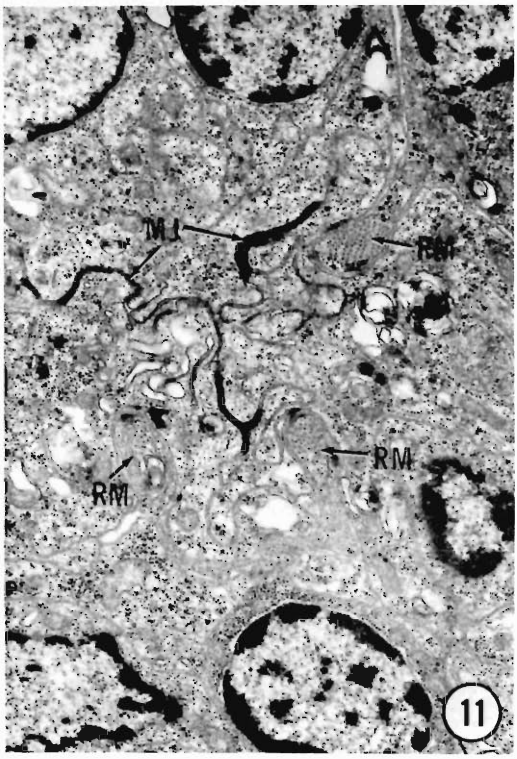
Figures 9–11. J2 *Heterodera glycines,* 2 days after inoculation to show the esophago–intestinal valve, the intestinal lumen, and the rectal valve. 9. Longitudinal section through the esophago–intestinal valve complex (EIVC) and the anteriormost sector of the intestine. The EIVC merges anteriorly with the cuticular esophageal lumen lining (CEL), which is supported by a network of membrane junctions (MJ). The EIVC is surrounded by the intestinal epithelium (IEp). IL, intestinal lumen. ×19,000. 10. Cross section of the same specimen as Figure 9 through the intestinal lumen (IL) just anterior to the rectal valve. A muscle fiber (RM) is related to other muscle elements that are part of the intestino–rectal system. IEp, intestinal epithelium. ×16,000. 11. Section through the rectal valve of the same specimen as in Figure 10. The network of membranes and membrane junctions (MJ) constitutes parts of the intestino–rectal valve. Muscle elements (RM) in 3 of the 4 corners of the micrograph are part of the intestino–rectal muscle system. ×15,500.







mordium (Fig. 1). Four days after inoculation, the intestinal epithelium in the midregion of the nematode is 2-celled (Fig. 20) and rectangular in cross section. Five days after inoculation, an intestinal epithelium, containing numerous lipid droplets and glycogen rosettes, occupies ⅗ of the diameter of a specimen.

Near the tail of the nematode, the broad expanse of the intestinal epithelium and the enlarged intestinal lumen narrows and joins the intestino–rectal valve (Figs. 1, 6). The membrane complex of the rectal valve is similar to the esophago–intestinal valve in that noncuticularized apical membranes of cells are apposed and joined at their lateral surfaces by membrane junctions (Figs. 11, 23). The intestino–rectal valve differs from the esophago–intestinal valve in that the rectal valve has muscle fibers (Figs. 10, 11, 23) in close proximity to the apposed apical membranes of the rectal valve cells. The muscle fibers appear distinct and separated from the anus depressor muscle.

Discussion

During feeding by *H. glycines* and related tylenchids, plant nutrients are drawn through the stylet orifice and passed through the esophagus via the tubular procorpus esophageal lumen and the triradiate lumen of the metacorpus pump. Posteriad to the metacorpus, the lumen of the esophagus is triangular in cross section, and contents apparently move through the esophago–intestinal valve (EIV) and into the lumen of the anterior intestine. The cells of the EIV form projections of the anterior intestinal epithelium (Baldwin et al., 1977; Endo, 1984). The structure and proposed function of the esophago–intestinal valve (EIV) are similar among various nematode species. The EIV of *Aphelenchoides blastophthorus* consists of cells with many inter-

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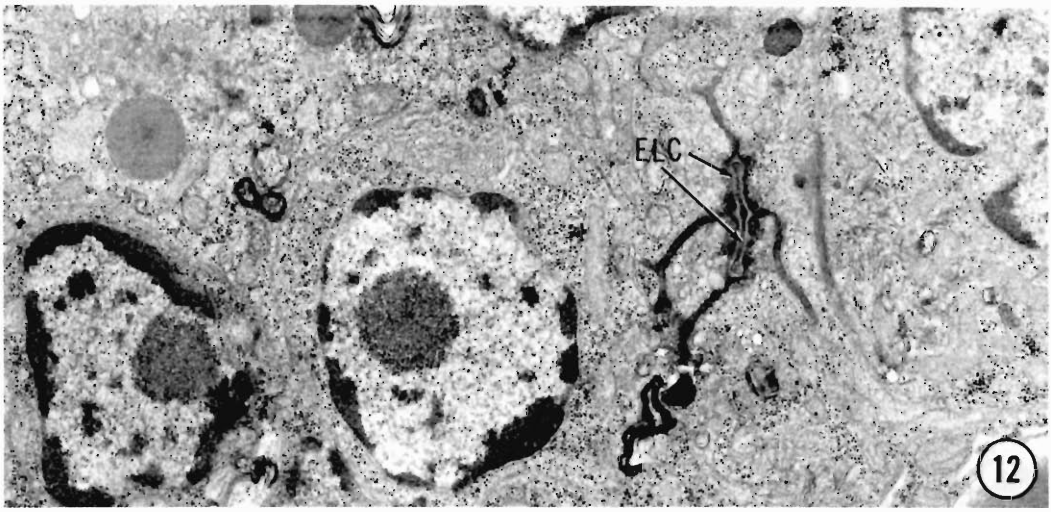
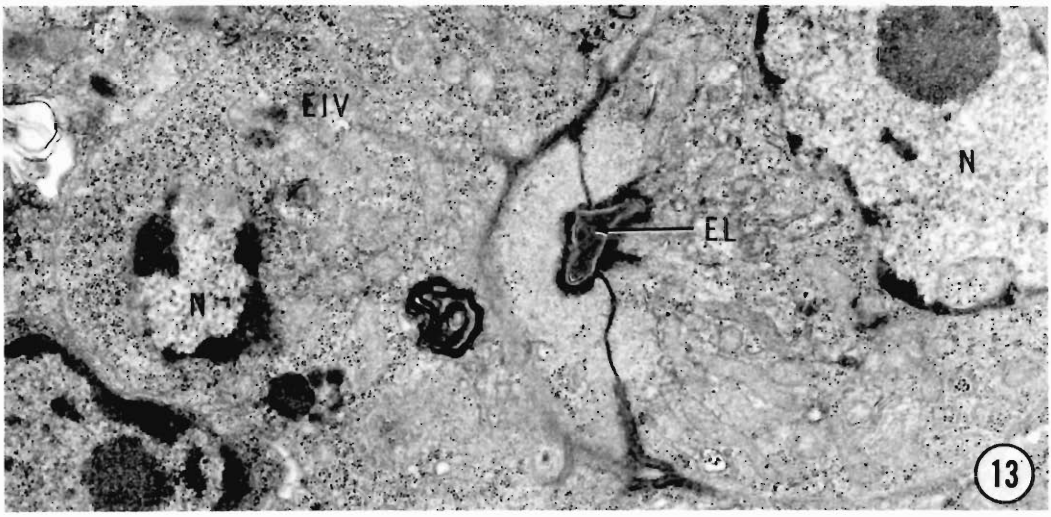
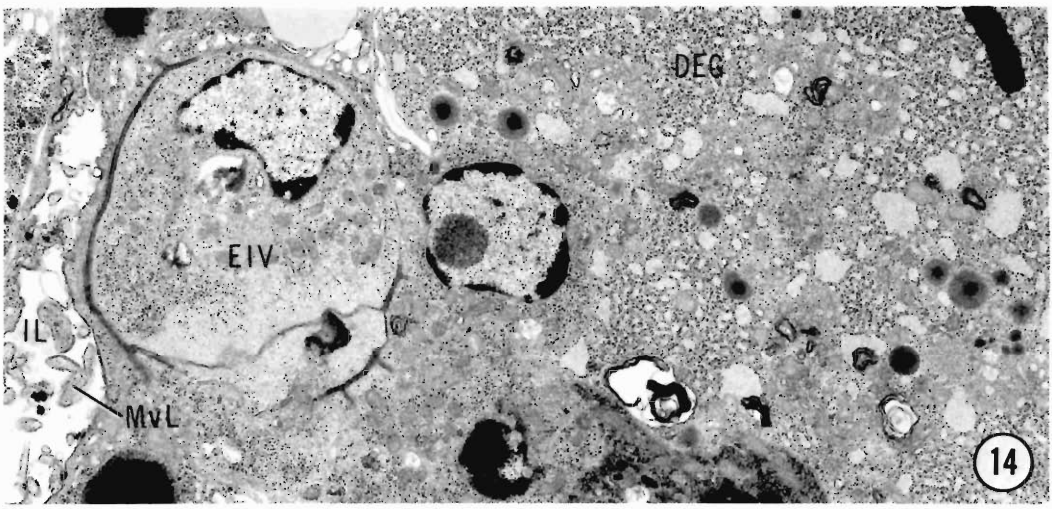
Figures 12–14. J2 *Heterodera glycines,* 3 days after inoculation, shows transition of cuticular to noncuticular lumen boundaries of the esophago–intestinal valve. 12. Section through the terminus of the esophageal lumen cuticle (ELC). The lumen is closed and has cuticular contact at 4 points with membrane junctions. ×19,200. 13. Transition between cuticular esophageal lumen (EL) and membranous portion of the esophago–intestinal valve (EIV). Same specimen as shown in Figure 12. N, nucleus of EIV cell. ×18,700. 14. Cross section of EIV of Figure 12 with various gradations of membrane junctions and noncuticularized cell membranes. Anterior extremity of the intestinal lumen (IL) is adjacent to the EIV. DEG, dorsal esophageal gland; EIV, esophago–intestinal valve; MvL, microvilli-like membrane folds. ×10,600.

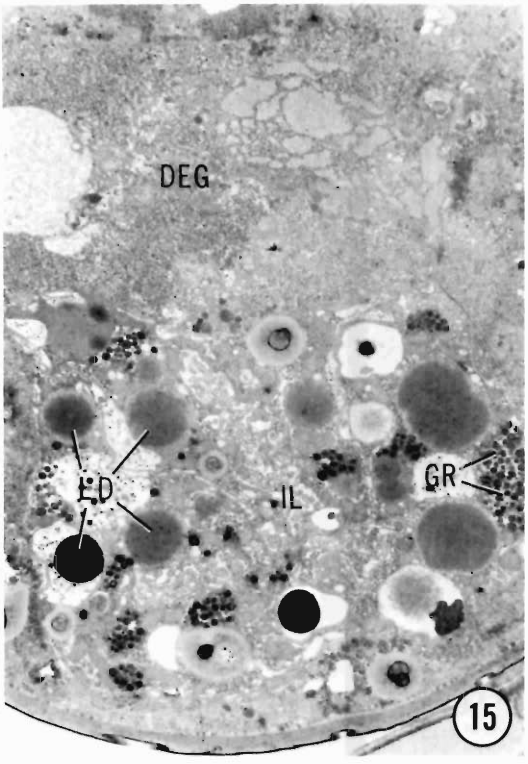
Figures 15–18. Sections of J2 *Heterodera glycines,* 3 days after inoculation, shows the anterior and midintestinal lumen and presence of microvilli-like surface membranes. 15. Cross section of the anterior intestinal region. The intestine lies adjacent to the esophageal glands and occupies about ½ the diameter of the nematode. Individual epithelial cells are not clearly defined by membrane junctions as noted among infective juveniles. The lumen of the intestine (IL) is irregular and blocked with numerous microvilli-like membrane folds. Epithelial cells contain prominent lipid droplets (LD) and numerous glycogen rosettes (GR). DEG, dorsal esophageal gland. ×6,100. 16. Cross section through midsection of the same specimen. Islands of the branched lumen are blocked with microvilli-like surface membrane folds (MvL). Epithelial cells contain densely stained lipid droplets (LD) and numerous glycogen rosettes (GR). The intestinal epithelium (IEp) occupies about ½ the cross-sectional area of the nematode and is bounded on each side by hypodermal (H) tissue. ×5,800. 17. Enlargement of microvilli-like membrane folds within the intestinal lumen of J2, 3 days after inoculation. Microvilli-like membrane folds (MvL) vary widely in thickness as they form elongated evaginations of the apical membranes of cells of the intestinal epithelium. Surfaces of microvilli are sculptured with an enteric coating (EC) when viewed in cross, longitudinal, and tangential sections. CL, central lumen. ×20,600. 18. Enlargement of a sector of the lumen of Figures 16 and 17 showing the enteric coating (EC) on the surface of the microvilli-like membrane folds (MvL). Longitudinal and cross sections of the microvilli-like membrane surfaces show the uniformity of the projections made by the enteric coating. GR, glycogen rosettes; IL, intestinal lumen. ×66,000.

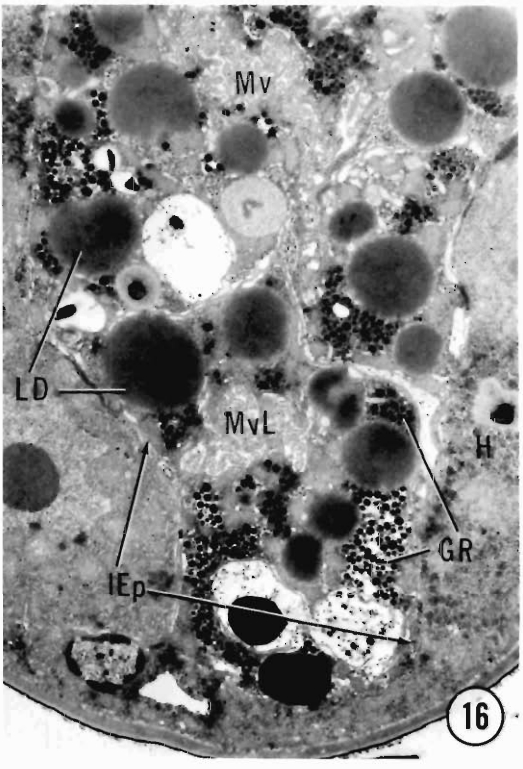
Figures 19, 20. J2 *Heterodera glycines,* 4 days after inoculation, shows the 2- and 3-celled cross section of the intestinal epithelium. 19. Cross section of central region of the intestine showing the 3-celled structure of the intestinal epithelium indicated by the membrane junctions (MJ) near the surface of the central lumen (IL). Lipid droplets (LD) of wide size range occur throughout the intestinal epithelium (IEp). Cell membranes extend from the membrane junctions to the periphery of the intestine. ×14,500. 20. Cross section of the specimen described in Figure 19 shows the 2-celled structure of the intestine in a posteriad region of the nematode; lipid droplets (LD) and glycogen rosettes (GR) are abundant in the epithelium. Microvilli are varied in morphology as the lumen expands. Sculpturing from enteric coating materials occurs on microvilli-like (MvL) and other surface areas within the lumen (IL). H, hypodermis; IEp, intestinal epithelium; LD, lipid droplets; MJ, membrane junction. ×8,900.

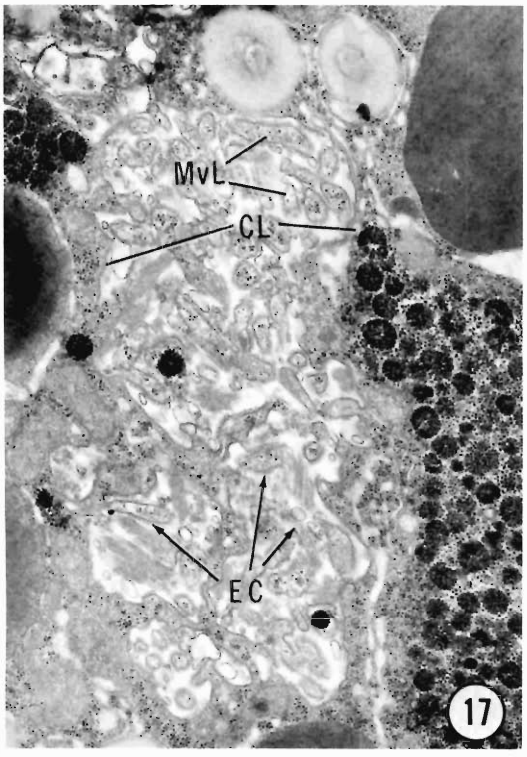


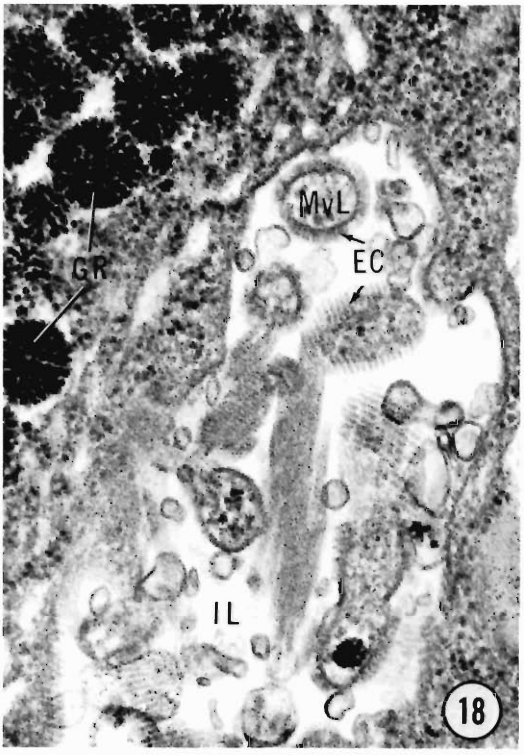


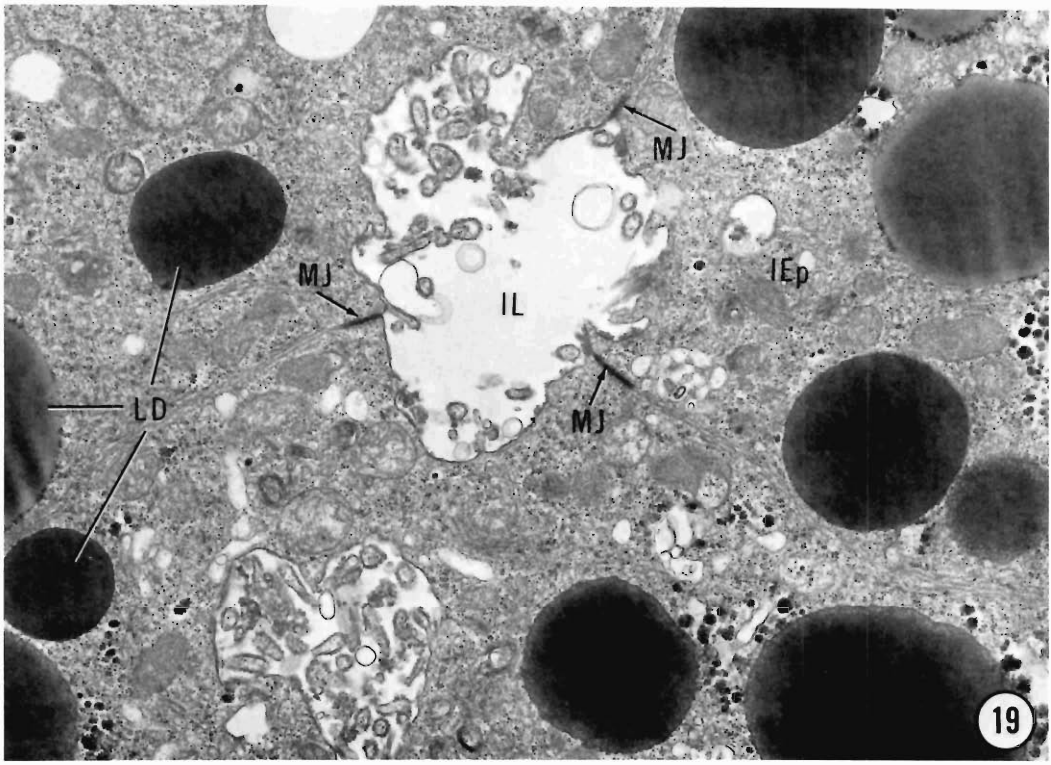


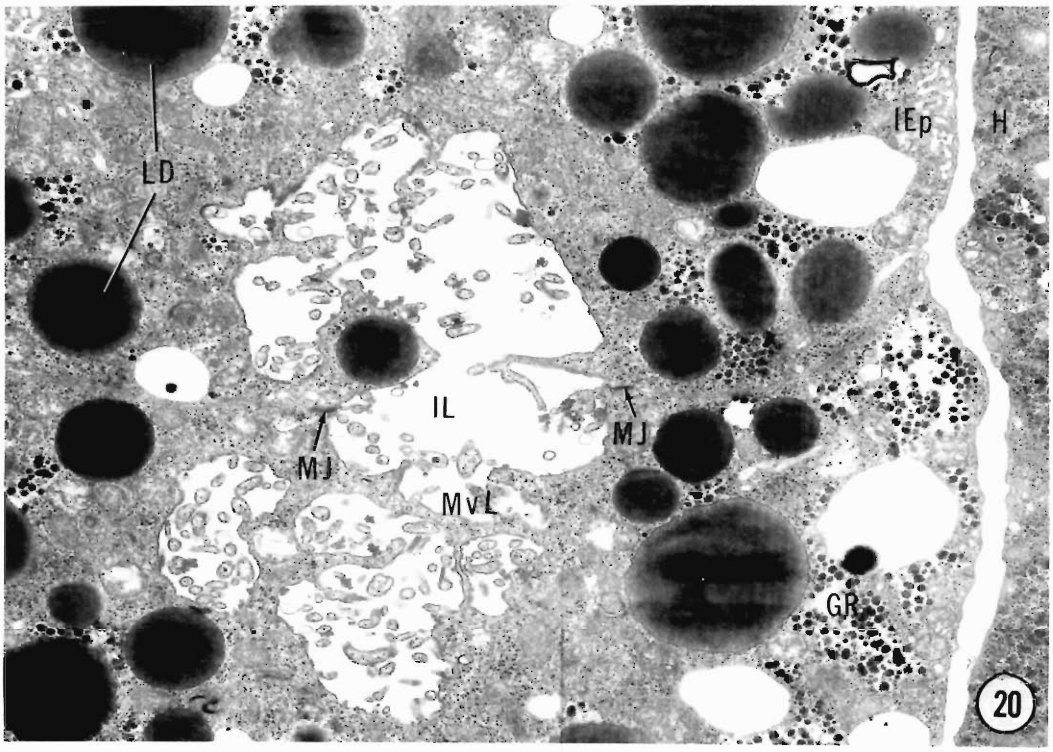












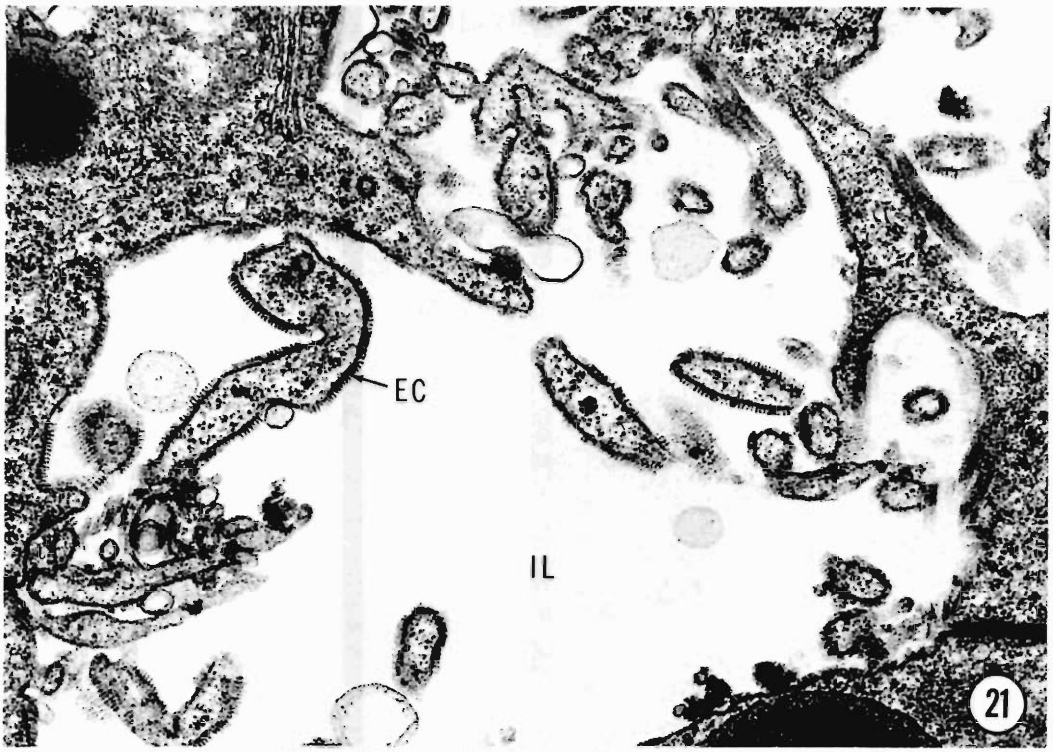


Figure 21. J2 *Heterodera glycines*, 4 days after inoculation, shows an enlargement of Figure 20 with uniform surface sculptures of an enteric coating (EC) on microvilli-like and other membrane surface folds. IL, intestinal lumen. ×32,200.

digitating branches separated by cell membranes having areas of conspicuous junctional complexes. The cells appear different from those of the esophagus and the intestines, and appear to have cuticularized lumen linings and microvilli (Shepherd et al., 1980). In contrast, the valve formed by 2 esophageal cells at the esophago–intestinal junction of *Hexatylus viviparus* are not cuticularized or thickened (Shepherd and Clark, 1976). The esophago–intestinal junction of *Ditylenchus dipsaci* contains a typical unlined or noncuticularized valve formed by 2 specialized esophago–intestinal junction cells that are closely apposed when the valve is closed (Shepherd and Clark, 1983).

The concept of cell junctions as intestinal valves in nematodes was first dicussed by Seymour and Shepherd (1974) in their study of the rectal valve in *Aphelenchoides blastophthorus* and the esophago–intestinal valve in *Thornenema wickeni*. Each valve, when closed, appeared convoluted and consisted of 2 closely apposed tripartite membranes. They reported that both valves prevented intestinal leakage; they opened briefly and rapidly by forcible dilation and closed by pressure from surrounding tissues. In a similar way, infective J2 of *H. glycines* have esophago–intestinal valves that consist of 2 esophageal cells with closely apposed noncuticularized apical membranes that are joined laterally by cell junctions.

The lining of the anterior intestinal lumen of second-stage juveniles of *H. glycines* and *H. rostochiensis* consists of irregular membrane ingrowths (Wisse and Daems, 1968; Endo, 1984). This differs from the regular array of microvilli reported for juveniles of *M. hapla* and advanced stages of other Tylenchida (Ibrahim, 1971; Shepherd and Clark, 1976; Sheperd et al., 1980). The microvilli in the anterior intestine of *A. blastophthorus* are short and bulbous with fibrillar cores, whereas the microvilli of the midintestinal region are bottle shaped (Shepherd et al., 1980). In the fungus feeder, *H. viviparus*, the anterior intestinal region has a narrow lumen that is lined with very short microvilli. The midintestinal region has a broad lumen filled with microvilli with long slender appendages and with spirally oriented flanges on the outer coat that extend to the

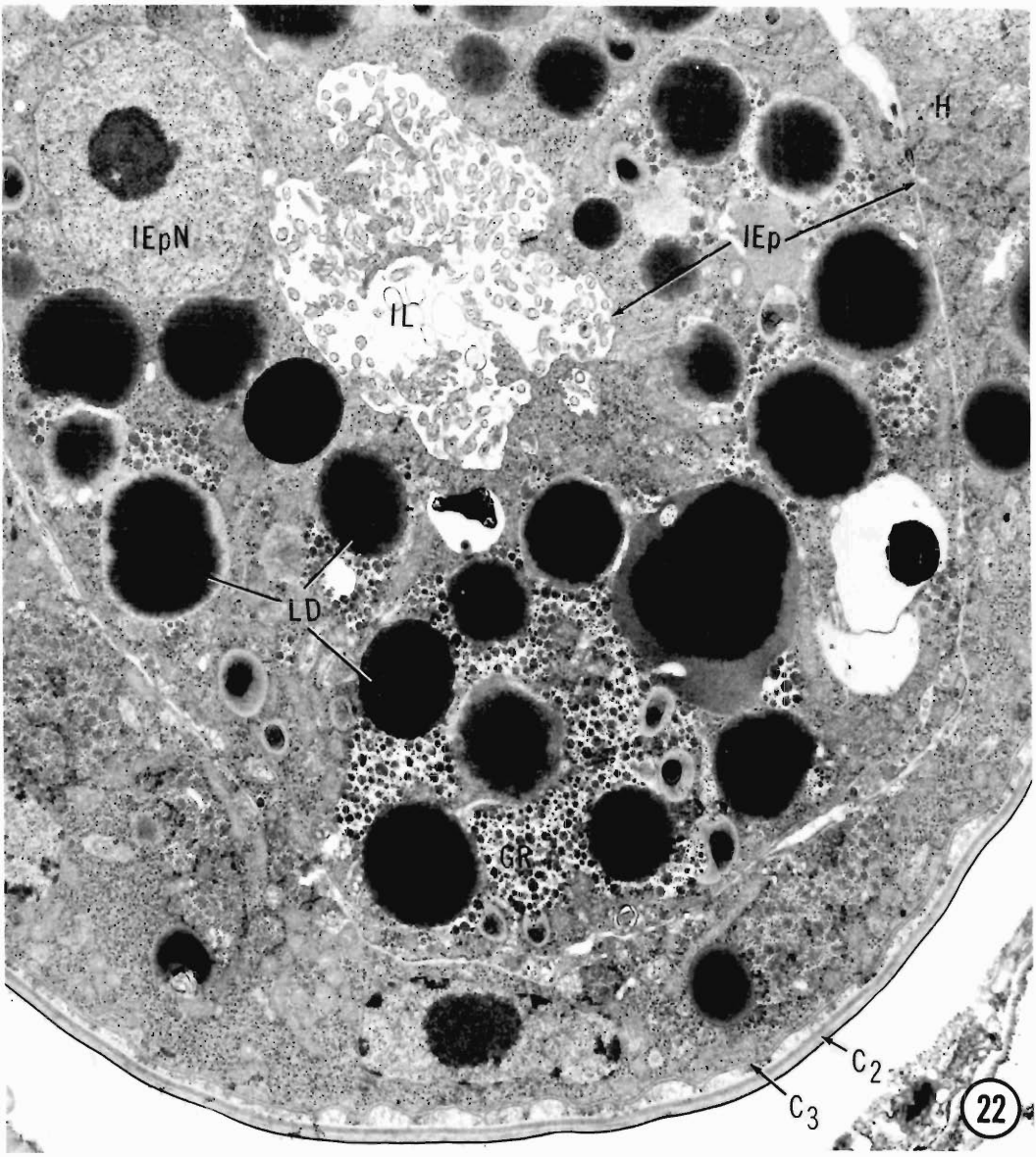


Figure 22. *Cross section of J3 Heterodera glycines,* **5 days after inoculation, showing intestine that comprises about ⅗ of the cross section of the nematode. The cuticle (C_3) has separated from the second-stage cuticle (C_2). The lumen (IL) has a moderate proliferation of microvilli in addition to broad units of cell extensions into the lumen. Lipid droplets (LD) occur throughout the epithelium, almost completely surrounded by glycogen rosettes (GR). IEp, intestinal epithelium; IEpN, intestinal epithelial nucleus; H, hypodermis. ×7,600.**

ends of the attenuated tips (Shepherd and Clark, 1976). In *D. dipsaci,* the cells of the anterior intestine are lined with slender smooth-surfaced microvilli that have a thin but distinct central fibrillar core. The midintestinal microvilli are occasionally branched and are broader with a sculptured surface formed by a tight spiral of flanges, and a broader but less conspicuous central fibrillar core (Shepherd and Clark, 1983).

Observations of *H. glycines* that are established in the host after the syncytium had been induced show a proliferation of microvilli-like surface membrane folds that at times almost fill the intestinal lumen (Figs. 15–17). In contrast to

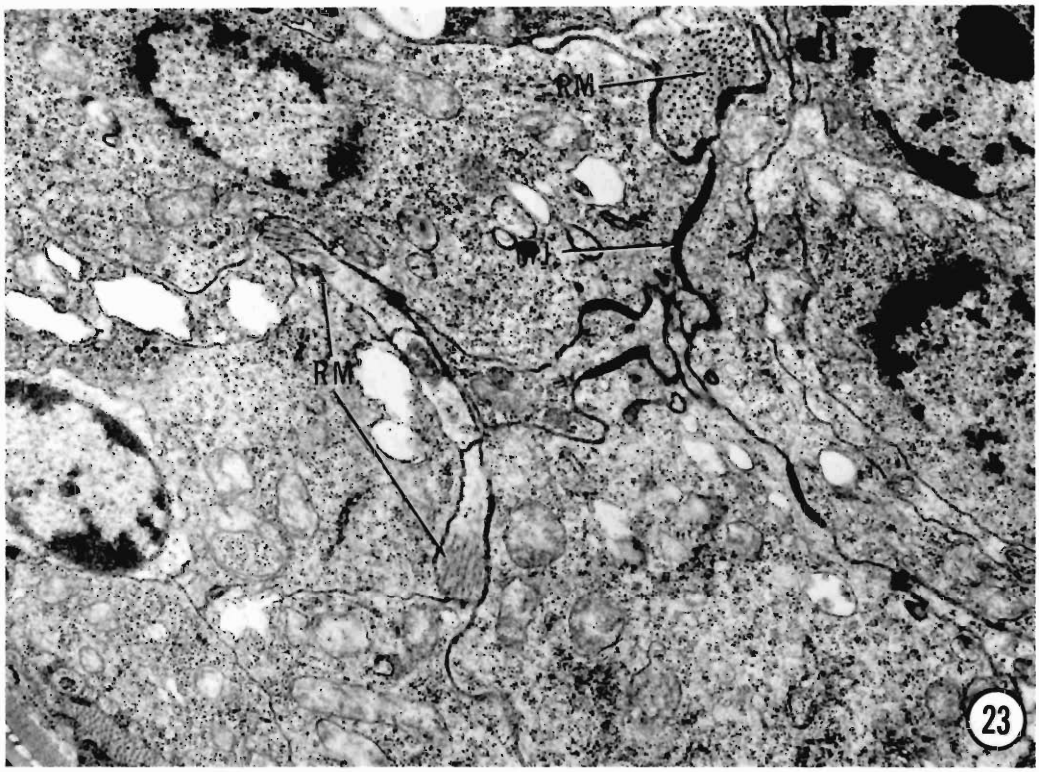


Figure 23. Cross section of J3 *Heterodera glycines*, 5 days after inoculation, showing the intestino–rectal valve with intercellular membranes and membrane junctions (MJ) and muscle elements (RM). ×18,900.

H. viviparus, the microvilli-like membrane folds of *H. glycines* do not have long slender appendages, but tend to vary in thickness throughout their lengths. They emerge from the intestinal lumen surface in irregular patterns within the main and branch channels of the lumen.

The sculptured coating of the surface membrane of intestinal microvilli has been reported for *H. viviparus* and *D. dipsaci*. It consists of a series of parallel raised flanges with spiral orientation along the length of the microvilli (Shepherd and Clark, 1976, 1983). The surface coating of microvilli of *A. blastophthorus* is a thin "fuzzy" coat, which is continuous over the epithelial surface (Shepherd et al., 1980). A few microvilli of infective juveniles of *H. rostochiensis* are reported to have surfaces bearing striated fibrillar materials (Wisse and Daems, 1968). Microvilli of *Capillaria hepatica* are covered by extracellular, fibrous mucopolysaccharide or mucoprotein (Wright, 1963).

The lumen surface of the intestinal epithelium of *H. glycines* differs widely from the animal parasite *Ascaris lumbricoides* and the tylenchids *A. blastophthorus* and *H. viviparus*, where distinct microvilli are reported. *Heterodera glycines* lacks uniformity of the intestinal epithelial surface and distinct microvilli with inner cores of fibers; juveniles of *H. glycines* do not form a terminal web or an endotube as described by Munn and Greenwood (1984).

The intestino–rectal valve membranes of *H. glycines* resemble those of the rectal valves of *A. blastophthorus* according to Seymour and Shepherd (1974). However, the presence of muscle fibers that occur in close proximity to the intestino–rectal valve indicates that these muscles have a role in the opening and closing of the intestino–rectal valve. No "intestino–rectal" sphincter muscle cell has been reported for *A. blastophthorus*; therefore it is uncertain as to how the rectal and esophago–intestinal valve function. Seymour and Shepherd (1974) proposed that valve function by local relaxation of the somatic muscles allows that part of the body containing the valves to elongate slightly due to elastic recoil of the cuticle. Simultaneously, the valve would be dilated by pressure of the gut contents displaced

by muscular activity in an adjoining part of the body. With *H. glycines,* muscle fibers and cells observed near the rectal valve could be involved in the direct function of the intestino–rectal valve as a sphincter muscle or operate as an adjunct to the system proposed by Seymour and Shepherd (1974).

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Volume 55 marks the completion of our 5-year term as editors of the *Proceedings.* It has been a pleasure and an education working with the Editorial Board, authors, and the staff of Allen Press. We want to thank all of them for maintaining the *Proceedings* as a first rate scientific journal.

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Comparisons of Selected Stains for Distinguishing Between Live and Dead Eggs of the Plant-parasitic Nematode *Heterodera glycines*[1]

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ABSTRACT: Seven stains were tested on *Heterodera glycines* (soybean cyst nematode) to determine whether live and dead eggs of a plant-parasitic nematode could be distinguished from each other. When observed with bright-field microscopy, the stains chrysoidin, eosin-Y, new blue R, and nile blue A were useful in differentiating dead from live eggs, particularly when the stains were combined with dimethyl sulfoxide. Acridine orange, eosin-Y, fluorescein, and fluorescein diacetate, when observed with fluorescence optics, also differentially stained live and dead eggs.

KEY WORDS: *Heterodera glycines,* soybean cyst nematode, nematode egg stains, nematode egg viability, fungus–nematode interactions, microscopy.

Evaluation of the pathogenicity and virulence of biological control agents of plant-parasitic nematodes often requires that live and dead nematodes be distinguished from each other. When nematode eggs, rather than motile vermiform nematodes, are studied, it can be particularly difficult to determine which individuals are alive and which are not. A number of stains have been used to help establish whether a nematode is dead or alive; however, as summarized by Hooper (1986), varying results have been reported for some of these stains. To test for fungal pathogenicity on eggs of plant-parasitic nematodes, it is therefore necessary to determine which stains are the most reliable for differentiating dead and live eggs of these nematodes. To this end, we applied different stains to eggs of the soybean cyst nematode, *Heterodera glycines.*

Seven stains were tested on live eggs and on eggs that had been heated-killed or that had died of natural causes. Six of these stains had been used on plant-parasitic or free-living nematodes in earlier studies. One stain—aqueous fluorescein—has not, to our knowledge, been previously reported as a stain for plant-parasitic nematodes. Even though some of these stains have been reported to be ineffective or inconsistent under certain circumstances, we evaluated them to determine whether they could be useful for studies of this nature. Because difficulty with infiltration might be one cause for variable results that have been reported in the literature, 6 of the stains were applied both with and without dimethyl sulfoxide (DMSO) to determine whether this chemical would aid penetration of stains into eggs. The objectives of this study were to find a stain that would (1) penetrate live or dead plant-parasitic nematode eggs and give a clear distinction between them, (2) be effective within an hour or 2 after application, and (3) stain fungal hyphae.

Materials and Methods

NEMATODE CULTURE AND PREPARATION OF EGGS FOR STAINING: Eggs of *Heterodera glycines* Ichinohe, races 3 and 5, were obtained from previously established monoxenic root explant cultures (Lauritis et al., 1982). Nematodes were maintained in vitro on excised root tips of *Glycine max* (L.) Merr. cv. Kent grown on Gamborg's B-5 medium (Gamborg et al., 1976; Huettel and Rebois, 1985). Egg-containing cysts from these cultures were placed into 250-µl tubes and disrupted in distilled water with a hand-held homogenizer. The resulting egg suspensions were then either removed from the tubes and stained without further treatment, or left in the tubes, immersed in boiling water for at least 5 min to heat-kill the eggs, and then removed and stained.

STAINS: The stains prepared were 200 ppm acridine orange (Homeyer, 1953), 0.005% chrysoidin (Doliwa, 1956, as cited in Hooper, 1986), 0.67% eosin-Y (Chaudhuri et al., 1966), 0.01% fluorescein diacetate in phosphate buffer (pH 7.3) (Bird, 1979), 0.05% new blue R (Shepherd, 1962), 0.1% nile blue A (Ogiga and Estey, 1974), and 0.01% fluorescein. All were in aqueous solution unless otherwise stated. Acridine orange, chrysoidin, eosin-Y, and nile blue A were also prepared in 10% aqueous DMSO. Fluorescein diacetate was made in 10% DMSO in 0.067 M phosphate buffer, and new blue R was prepared as 0.045% in 10% aqueous DMSO. DMSO was not added to fluorescein.

STAIN TECHNIQUE: For each stain test, approximately 20 µl of egg suspension were placed on a slide and mixed with a drop of stain. Unstained eggs were mixed with a drop of distilled water. Staining was al-

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lowed to proceed for at least 2 hr, and the eggs were then examined with either a Nikon Microphot FX for bright-field microscopy or a Nikon Diaphot-TMD for fluorescence microscopy. Bright-field stains without DMSO were each tested on a minimum of 4 slides of egg suspensions. Each bright-field stain with DMSO was tested on at least 6 slides. Acridine orange was an exception—it was tested 4 times with DMSO. Fluorescent stains without DMSO were each tested a minimum of 3 times; with DMSO, each fluorescent stain was tested on at least 2 slides of egg suspensions.

Fluorescence was observed using epifluorescence optics and 5 different filter systems. The filter systems are referred to in this paper as B, G, V, UV, and BV. The composition of these filter systems was as follows: B—blue light excitation (dichroic mirror 510, excitation filter 420–490, barrier filter 520), G—green excitation (dichroic mirror 580, excitation filter 546/10, barrier filter 580), V—violet excitation (dichroic mirror 430, excitation filter 380–425, barrier filter 460), UV—ultraviolet excitation (dichroic mirror 400, excitation filter 330–380, barrier filter 420), and BV—blue violet excitation (dichroic mirror 455, excitation filter 400–440, barrier filter 480). The B, G, V, UV, and BV filter combinations were all used for each of the fluorescent stains in this study. Fluorescence was observed with objective lenses of 20× and 40× magnification. Most of the dead eggs examined with the fluorescence microscope were heat-killed, and the colors reported in Table 1 are based mainly on those eggs. Observations of fungal-infected eggs are included in the text of this paper.

PREPARATION OF FUNGAL-INFECTED EGGS: Dr. L. R. Krusberg (University of Maryland) graciously provided field soil containing cysts of the soybean cyst nematode. Fungi were isolated from cysts and eggs and maintained in pure culture. For bright-field microscopy, yellow and brown egg-containing cysts from cultures over 2 mo old were placed on water agar in petri dishes and inoculated with 2 unidentified fungi. In the first experiment, the inoculated cysts were placed into a 25°C incubator for 3.5 wk. The cysts inoculated with the second fungus were incubated for 6 wk. For fluorescence microscopy, egg-containing cysts were inoculated with a third fungus and placed into the incubator for 2 wk. Following incubation in all of these experiments, the inoculated cysts were disrupted and the eggs were stained.

Results

BRIGHT-FIELD MICROSCOPY: When observed with bright-field microscopy, shells of both hatched and unhatched eggs tended to stain a little darker than the background stain color. In many of the live eggs, the shell stained but the larva inside the egg was unstained. Figure 1 is an example of this type of staining pattern in a live egg. The major color differences described here between live and dead eggs were observed in the egg contents. In eosin-Y, shells were pink, live eggs (Fig. 1) clear to pink, and dead eggs (Fig. 2) pink to dark pink. In acridine orange, shells appeared yellow, live eggs clear to yellow, and dead eggs dark yellow, orange, yellow and orange mixed, or orange-yellow. With chrysoidin, shells stained yellow, live eggs clear to yellow, and dead eggs (Fig. 3) dark yellow (often with orange droplets), or orange. Nile blue A stained shells blue, live eggs (Fig. 4) clear to blue, and dead eggs (Fig. 5) dark blue, turquoise, or mixed dark blue and orange. Shells in new blue R were blue or purple, live eggs were clear, blue, or purple, and dead eggs (Figs. 6, 7) were dark blue, dark purple, dark bluish-purple, or blue and orange.

Live eggs that had picked up some stain and dead eggs that were not darkly stained were sometimes difficult to distinguish from each other. When DMSO was used, the colors observed appeared similar to those seen when the stains were not mixed with DMSO. However, the distinction between live and dead eggs became clearer, because the dead eggs generally appeared much darker than the live stained eggs. This was most noticeable when DMSO was added to chrysoidin, eosin-Y, new blue R, and nile blue A. To determine whether 1 or more of these 4 stains was superior to the others for fungal biological control studies, DMSO was added to each stain and the stains were applied to eggs inoculated with 2 different fungi. Some of these eggs had presumably died of natural causes, including fungal-induced death. A total of 416 eggs was counted for each stain—208 eggs for each inoculation experiment. When the results of the 2 inoculation experiments were combined for each stain, the percentages of live eggs were as follows: chrysoidin + DMSO—91.1%, eosin-Y + DMSO—92.3%, new blue R + DMSO—92.5%, nile blue A + DMSO—92.3%.

The fungal hyphae that picked up the stains could be seen the most clearly with new blue R (Fig. 7) and nile blue A. When chrysoidin and eosin-Y were used, the stained hyphae tended to be similar in color to the background color on the slides.

FLUORESCENCE MICROSCOPY: The colors observed with fluorescence microscopy are indicated in Table 1, and examples of eggs treated with stains are shown in Figures 8–19. The fluorescence colors often appeared brighter when observed with the 20× objective lens than when observed with the 40× lens.

Live and dead eggs that had not been treated with a stain were difficult to distinguish from each other. In some filter systems (particularly the G), the fluorescence was either faint or faded

fairly quickly. The dead eggs were often brighter than the live eggs, especially when the B and BV filter systems were used. However, live eggs in groups also tended to be brighter than single live eggs, and therefore sometimes hard to distinguish from dead eggs.

Acridine orange, eosin-Y, fluorescein, and fluorescein diacetate all differentially stained live and dead nematode eggs. When DMSO was present, fluorescence colors or patterns did not always appear the same as those observed without DMSO. For example, dead eggs treated with acridine orange and DMSO and observed with the V filter system were greenish-yellow, orange, red, or red-orange. However, the differences that resulted from the addition of DMSO did not appear to enhance the usefulness of the stains.

Acridine orange combined with the BV filter system (Figs. 9, 10) appeared to give clearer distinctions between live and dead eggs than when used with the other filter systems. However, the other filter systems did give some distinctions with acridine orange. Overall, this was not the most effective stain used in this study. Eosin-Y distinguished between live and dead eggs with all of the filter systems, as the live eggs did not fluoresce. However, when the B and UV filter systems were used, not all of the dead eggs fluoresced, either. Consequently, the G, V (Fig. 8), and BV filter systems appeared to be the most reliable with the eosin-Y stain.

When fluorescein was used as the stain, the live and dead eggs were most easily distinguished from each other with the V and UV filter systems (Figs. 11, 14, 15). Fluorescein diacetate gave good distinctions between live and dead eggs. The results were clearest with the B and BV filter systems (Figs. 12, 13, 16–19), and with the V system. The G system did not give as distinct a difference between the eggs. With the UV system, live and dead eggs were generally distinguishable from each other. However, during one of the fluorescence experiments, the fluorescence colors of the live and dead eggs were difficult to tell apart.

Hatched eggs fluoresced in acridine orange. In eosin-Y, fluorescein, and fluorescein diacetate, these shells commonly varied from fluorescent (Fig. 11) to nonfluorescent (Fig. 13), even within a single stain-filter system treatment. In a number of treatments, eggs were observed that did not exhibit a general diffuse fluorescence, or did not fluoresce at all, but that did stand out as dark objects against the bright backgrounds. Examples of such eggs are in Figures 11 and 17 (live eggs) and Figure 12 (hatched egg). Some of these eggs had a faint fluorescence around the perimeter, whereas others had portions of the internal contents that fluoresced (Fig. 18). All of these eggs, whether or not they had any fluorescence, could be more easily discerned than the nonfluorescing eggs represented by the hatched egg in Figure 13.

The fluorescence microscopy results with fungal hyphae were as follows: When stained with acridine orange, the fungal hyphae fluoresced orange with all but the G filter system. When the G system was used, the hyphae fluoresced red. Although the hyphae often fluoresced the same color as the eggs they infected, the hyphae inside the eggs were frequently visible because of the fluorescence pattern they formed. However, internal hyphae (i.e., those inside of eggs) were obscured by the fluorescence of some eggs. Occasionally, hyphae were seen that did not appear to be fluorescing.

With eosin-Y, the hyphae were nonfluorescent to dark against the background with all but the BV filter system. When the dark hyphae filled a dead egg, it was sometimes difficult to see the

→

Figures 1–19. Light micrographs of *Heterodera glycines* eggs treated with various stains. Abbreviations: dead egg (D), hatched egg (H), live egg (L). Figures 1–7: bright-field optics. Figures 8–19: fluorescence optics. 1. Live egg treated with eosin-Y. The internal contents of the egg are clear. 2. Dead egg stained with eosin-Y. 3. Dead egg stained with chrysoidin. 4. Live egg stained with nile blue A. 5. Dead egg stained with nile blue A. 6. Dead egg stained with new blue R. 7. Dead egg and fungal hyphae (arrows) stained with new blue R. 8. Dead egg stained with eosin-Y. V filter system. 9, 10. Eggs stained with acridine orange. BV filter system. 9. Live egg. 10. Dead eggs. 11. Hatched egg and live egg stained with fluorescein. V filter system. Compare with Figures 14 and 15. 12, 13. Hatched egg and dead egg stained with fluorescein diacetate. Compare with Figures 16–19. 12. B filter system. 13. UV filter system. The hatched egg is not fluorescing. The figure demonstrates the difficulty that can arise when total egg populations are being counted, and nonfluorescing eggs are part of the population. 14, 15. Dead egg stained with fluorescein. 14. V filter system. 15. UV filter system. 16–19. Eggs stained with fluorescein diacetate. Figures 17–19 are of the same egg. 16. Dead egg. BV filter system. 17. Live egg. BV filter system. 18. Live egg. B filter system. 19. Live egg. UV filter system.

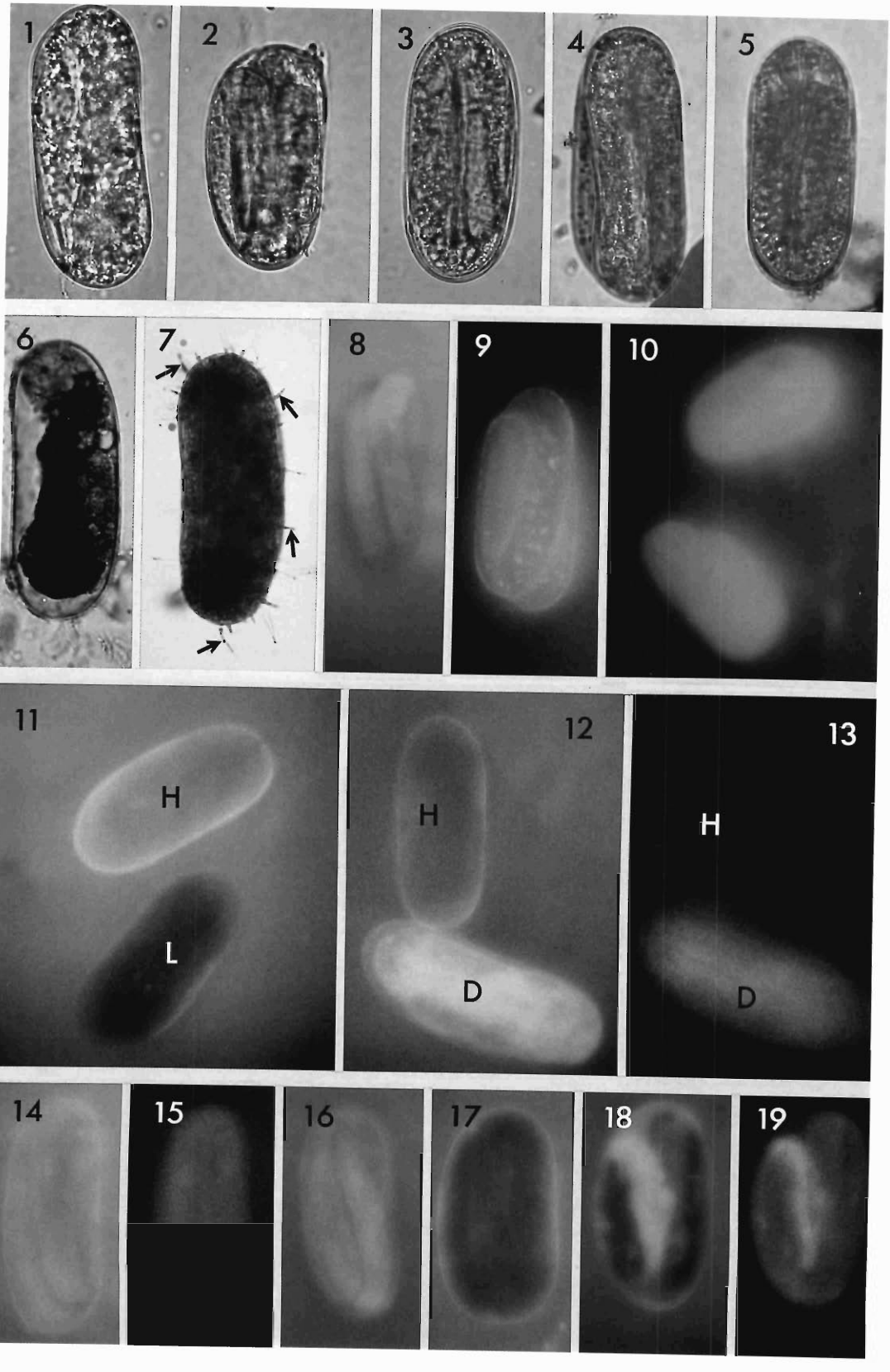

Table 1. Colors observed with the fluorescence microscope in stained and unstained eggs of *Heterodera glycines*.

Stain	Filter system*				
	B	G	V	UV	BV
1. Acridine orange					
Hatched eggs	Orange	Pale red, red	Orange, pale red-orange	Pale red-orange, pale yellow-orange	Orange, red-orange
Live eggs	Orange	Pale red	Orange, red, red-orange	Pale orange, pale orange with green flecks	Orange (some with green or yellow flecks)
Dead eggs†	Orange (some with green or yellow flecks), green, yellow, red-orange	Red	Orange, red with green or yellow flecks, red-orange	Orange, red-orange, yellow-orange	Orange, orange and yellow, red-orange
2. Eosin-Y					
Hatched eggs	Pale red-orange, none	Pale yellow, none	Pale red-orange, none (some visible as dark eggs against bright background)‡	Pale red-orange, none (some dark against background)	None (some dark against background)
Live eggs	None (some dark against background)	None (most dark against background)	None (most dark against background)	None (most dark against background)	None (most dark against background)
Dead eggs	Orange, red-orange, none	Yellow, yellow-orange	Orange with yellow flecks, orange and yellow, red-orange	Orange with yellow flecks, orange and yellow, red-orange, none	Orange (some with yellow flecks), yellow, red-orange
3. Fluorescein					
Hatched eggs	Pale yellow, none (some dark against background)	Pale red-orange, none	Pale yellow, none (some dark against background)	Blue, none	Pale yellow, none (most dark against background)
Live eggs	Greenish-yellow, none (some dark against background)	Pale red-orange, none	None (most dark against background)	Blue to almost none	None (most dark against background)
Dead eggs	Yellow-green, none (dark against background)	Red-orange	Greenish-yellow	Blue, bluish-purple	Greenish-yellow, lime green, some almost none
4. Fluorescein diacetate					
Hatched eggs	Pale greenish-yellow, none (some dark against background)	Pale red, none	Pale yellow, pale orange-yellow	Pale yellow-blue, pale bluish-purple, none	Pale yellow, pale orange-yellow, pale greenish-yellow
Live eggs	Greenish-yellow areas, none (dark against background)	Pale red, none	Yellow areas, orange-yellow areas, none (dark against background)	Pale bluish-purple	*Pale yellow areas*, pale greenish-yellow areas, none (dark against background)
Dead eggs	Green, orange-yellow, greenish-yellow	Red	Yellow-green	Blue, blue-green	Greenish-yellow
5. Not treated with stain					
Hatched eggs	Yellow	Pale red, none	Turquoise, yellow-green	Pale blue, pale blue-green	Yellow-green

Table 1. Continued.

Stain	Filter system*				
	B	G	V	UV	BV
Live eggs	Yellow	Red	Turquoise, yellow-green	Pale blue, pale blue-green	Yellow-green
Dead eggs	Yellow	Red	Turquoise, yellow-green	Blue, blue-green	Yellow-green, lime-green

* The filters and dichroic mirrors in each filter system are described in the text.

† Dead eggs were heat killed or had died of natural causes.

‡ In some eggs, no bright fluorescence was observed, but the eggs did stand out as dark objects against a bright background. Where the word "none" appears with no further description, the eggs were indistinguishable or barely distinguishable from the background.

fluorescence of the egg. These eggs sometimes resembled live, uninfected, dark eggs. With the BV filter system some hyphae fluoresced orange, but others were difficult to see.

Hyphae in fluorescein generally appeared non-fluorescent to dark. In most cases, it was difficult to determine with fluorescence microscopy whether hyphae were present. The UV system was an exception—some of the hyphae fluoresced faint blue.

In fluorescein diacetate, fluorescence was not observed in the fungal hyphae.

When infected eggs that had not been treated with any stain were observed, no fluoresence was seen in hyphae, except with the BV filter system. Some hyphae fluoresced faint yellow-green in the BV system.

Discussion

Of the stains used with bright-field optics, chrysoidin, eosin-Y, new blue R, and nile blue A gave the clearest distinction between live eggs of *Heterodera glycines* and eggs that had been heat-killed or that had died of natural causes. The colors observed with chrysoidin were somewhat different from those previously reported by Ogiga and Estey (1974). These authors observed that dead nematodes and eggs stained yellow, whereas live specimens stained yellow with orange granules. In the current study, dead eggs were dark yellow (often with orange droplets) or orange, and live eggs were clear to yellow.

Acridine orange differentiated dead from live eggs with bright-field microscopy, but the distinction was not as easy to make as it was with the other stains. The major use of this stain for nematodes has been with fluorescence microscopy (e.g., Homeyer, 1953; Kurt, 1977; Perry and Feil, 1986).

With all of the stains some eggs could not be readily classified as either live or dead. This, along with the inability to stain certain types of dead eggs, has been mentioned before as a problem when nematodes were treated with some of these stains. For example, Moriarty (1964) observed that new blue R was good for nematodes killed by heat or chemicals, but not for nematodes less than 2 yr old that had died of natural causes in the soil. Sayre (1964) found that dead larvae that had been frozen and then warmed did not always stain well with new blue R. Ogiga and Estey (1974) found that new blue R results were not always consistent, while chrysoidin was a little more reliable, and nile blue A was more reliable than either of these stains. These variable results indicate that the best stain to use may depend on several factors, including the source of the eggs and the means of egg death. It is interesting to note that in our experiments with bright-field microscopy, DMSO added to chrysoidin, eosin-Y, new blue R, and nile blue A appeared to increase the contrast between live and dead eggs. When these 4 stains were mixed with DMSO and applied to eggs from inoculated cysts, similar percentages of live eggs were counted for each stain, indicating that these stains were equally reliable for distinguishing dead from live eggs. New blue R and nile blue A might be advantageous in studies where it is necessary to clearly discern fungal hyphae, because these 2 stains caused the hyphae to stand out distinctly.

Forge and MacGuidwin (1986), examining plant-parasitic nematodes with the fluoresence microscope, found that live and dead nematodes could be distinguished from each other by patterns of autofluorescence. The major difference was that autofluorescence of live nematodes usually appeared as particles in the intestine, while

in dead nematodes, autofluorescence was diffuse throughout the body. Their observations were of nematodes that were older than the egg stage. In our study, live and dead eggs that had not been treated with a stain were mostly indistinguishable when examined with the fluorescence microscope. In the cases where the dead eggs tended to be brighter than the live eggs, the 2 egg types were still not easy to distinguish with a rapid scan, particularly when the eggs were in groups and both live and dead appeared fairly bright. However, the fluorescent stains acridine orange, fluorescein, and fluorescein diacetate were useful for distinguishing live from dead nematode eggs. Eosin-Y, which has not generally been used as a fluorescent stain for eggs of plant-parasitic nematodes, also differentiated between live and dead eggs with fluorescence optics. The colors or patterns of staining observed with fluorescence microscopy were not always similar to those reported by previous authors (Homeyer, 1953; Perry and Feil, 1986). This might be because different types of specimens were examined, or because different filter systems were used.

No single fluorescent stain was identified as consistently better than all of the others; a number of stain–filter system combinations distinguished live and dead eggs from each other. Excitation wavelengths that have been used by some authors studying nematode stains for fluorescence microscopy (Bird, 1979; Perry and Feil, 1986) and those provided by the manufacturer include blue and broad-band blue for acridine orange, green for eosin-Y, violet, blue, green, and ultraviolet for fluorescein compounds, and blue for fluorescein diacetate. A number of the filter systems that proved useful in this study were similar to these. One problem that needed to be taken into account was that stain–filter system combinations that distinguished between live and dead eggs were not always effective in differentiating dead eggs from hatched eggs. This was because some of the hatched eggs fluoresced, and the fluorescence colors were similar to those of dead eggs. Additional problems arose when fluorescence microscopy was used. Eggs that did not fluoresce could be overlooked when counting the total egg population. Hyphae in certain stain–filter system combinations did not appear to fluoresce, whereas in other combinations, the hyphae fluoresced the same colors as the infected eggs. Consequently, the fluorescent glow of an egg could obscure internal fungal hyphae. Uptake of stains may vary with different fungi, but similar difficulties might have to be overcome with any fungus in such a study. If bright-field and fluorescence microscopy were combined to overcome these problems, increased time was necessary to count live and dead eggs. These limitations should be considered when selecting a fluorescent stain for eggs of plant-parasitic nematodes. Several filter system–stain combinations that have been shown to be useful may have to be tested to determine which one is the most reliable for the conditions of a particular experiment.

Acknowledgments

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Survey or Taxonomic Papers

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 and include the accession numbers in the manuscript. The following are acceptable, and others are described in the "Guide to Parasite Collections of the World," prepared by the ASP and available from Allen Press ($3.00).

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Department of Invertebrate Zoology
National Museum of Natural History, W-325
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Beltsville, Maryland 20705
(Phone: 301-344-3891)

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National Museum of Natural History
Smithsonian Institution
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(Phone: 202-357-2472)

Helminths and Protozoans

(voucher specimens or paratypes)

University of Nebraska State Museum
Prof. Mary Hanson Pritchard, Curator
16th and W Streets
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(Phone: 402-472-3334)

Proc. Helminthol. Soc. Wash.
55(2), 1988, pp. 140–145

Microanatomical Features of *Yatesia hydrochoerus* (Nematoda: Filarioidea) from Colombian Capybaras

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ABSTRACT: A detailed description of microanatomical features that could aid in recognizing *Yatesia hydrochoerus* (Yates and Jorgenson, 1983) Bain, Baker, and Chabaud, 1982, in biopsy specimens is presented. Unusual structural features of the cuticle that may be especially useful include external lateral alae as well as lateral internal ridges in both sexes. Alae extend from the level of the nerve ring to the level of the cloaca in males and from the nerve ring to the tip of the tail in female worms. Microfilariae also are recognized easily in tissue sections by their lateral alae that extend from the anterior extremity to the beginning of the tail. Other features that differentiate this species from closely related filariae include the size and shape of lateral chords and the number of muscle cells per quadrant in transverse sections.

KEY WORDS: *Yatesia hydrochoerus,* Nematoda, Filarioidea, capybara, tissue sections, histopathology, morphology, histology, Colombia, *Hydrochoerus hydrochaeris,* microanatomy.

In recent years the Colombian Department of Agriculture has formally encouraged domestic farming of capybaras (*Hydrochoerus hydrochaeris*), especially in the hotter savannah regions of the country where cattle and pigs often fail to thrive (Fuerbringer, 1974; Rodriguez et al., 1975). Capybaras, however, harbor many parasitic organisms that may prove to be infective to other domestic animals or people (Ojasti, 1973; Rodriguez et al., 1975; Eberhard et al., 1976, 1984; Morales et al., 1978; Yates and Jorgenson, 1983; Yates and Lowrie, 1984). *Yatesia hydrochoerus,* for example, is an extremely common parasite of capybaras in Colombia. In a recent study, 97% of 62 mature capybaras sampled in 3 states of Colombia harbored this filaria (Yates and Jorgenson, 1983), and in a smaller study in Venezuela, which included immature capybaras, 80% (12 of 15) were infected (Campo-Aasen et al., 1985). This species is apparently transmitted by ixodid ticks, especially *Amblyomma cajennense* as reported by Yates and Lowrie (1984). In light of the fact that various stages of *A. cajennense* and certain other ixodids in the enzootic regions will feed on a variety of hosts and readily take blood from humans, the potential for transmission of this filaria to people may increase with domestication of capybaras. It is in this context that a description of the microanatomical features of this species in histologic sections is warranted.

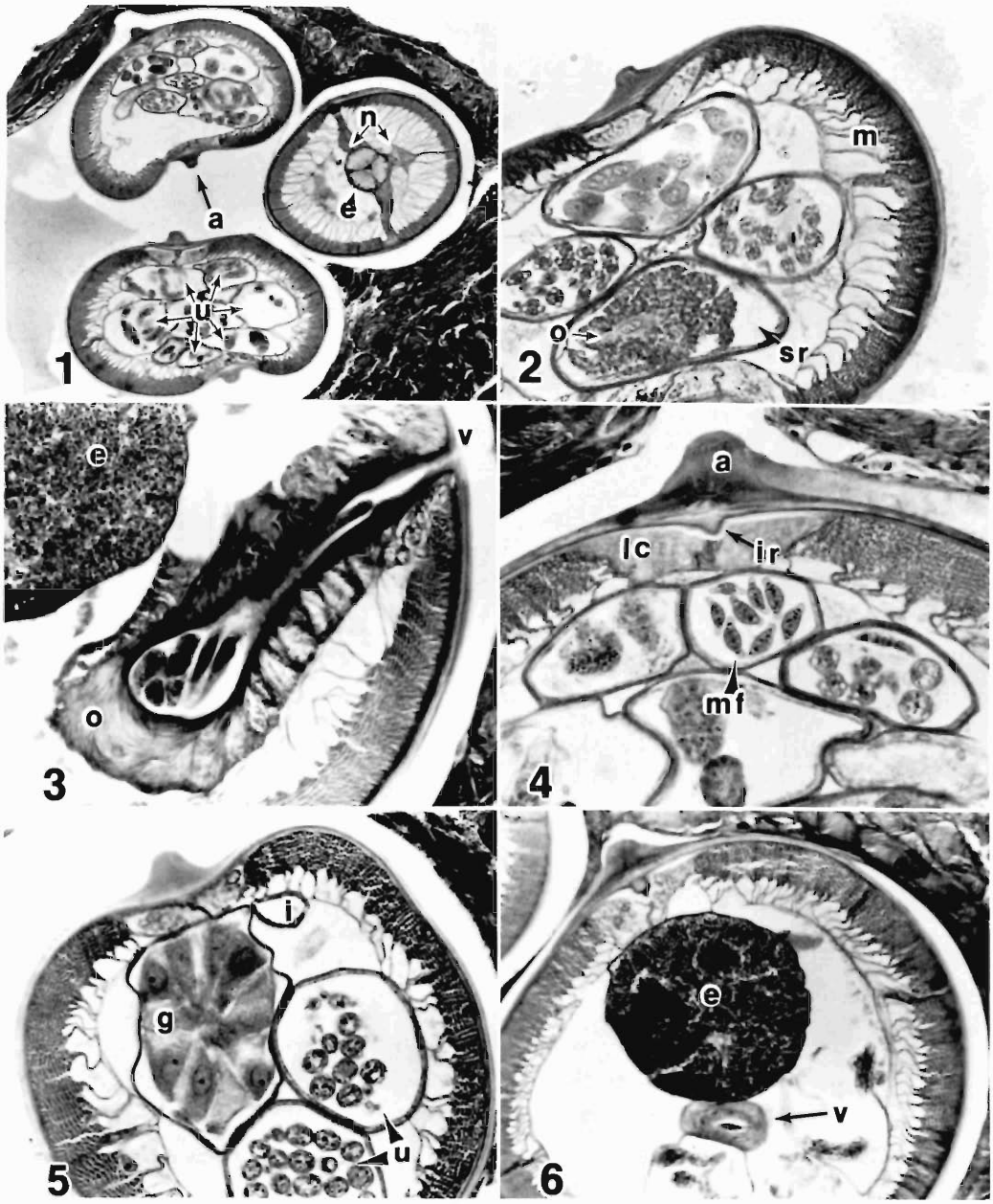
Materials and Methods

Muscle fascia containing adult male and female *Y. hydrochoerus* was collected at necropsy from capybaras obtained from hunters in Casanare, Colombia, South America. These tissues were fixed in Bouin's fluid and subsequently embedded in paraffin, serially sectioned, and stained with hematoxylin and eosin or Heidenhain's trichrome using standard histologic techniques. Photomicrographs were made with a Leitz Orthomat camera.

Results

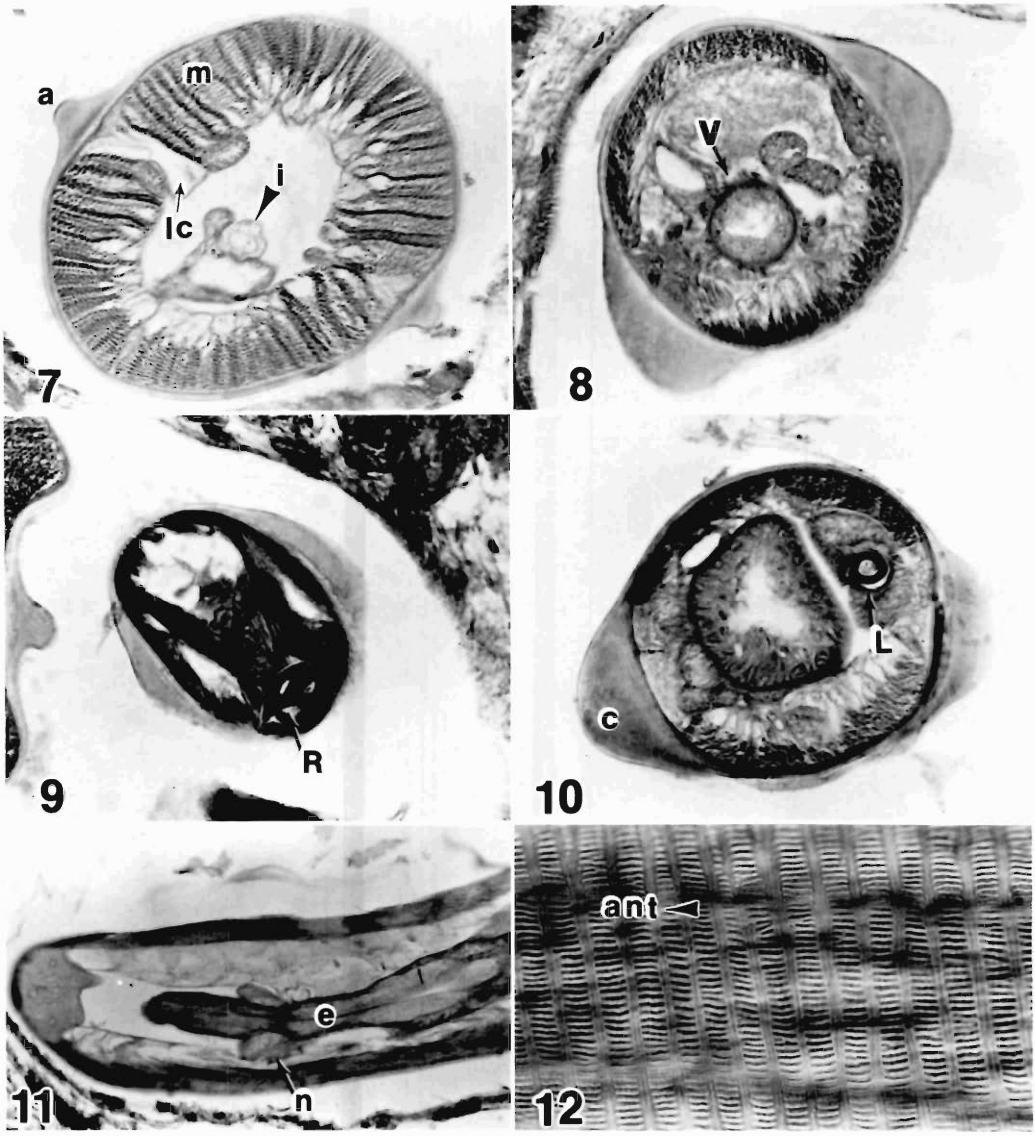
In transverse sections adult worms were of moderate size for filariae; males ranged from 220 to 290 μm in maximum diameter and females were 300–400 μm in diameter. Cuticular thickness was fairly uniform throughout the length of the bodies of worms of either sex, ranging from 3 to 4 μm in males and from 4 to 5 μm in females, except at the lateral chords where alae were formed. At least 3 cuticular layers were clearly observed at the light microscope level. The thin inner layer stained densely with Heidenhain's trichrome and was thickened at the lateral chords forming wedge-shaped internal ridges. The thick middle cuticular layer appeared homogeneous or fibrous, and stained less densely than the other layers. The thin cortical layer bore transverse striations and was thickened along the lateral chords to form low bluntly rounded alae (Figs. 1, 7). Internal ridges and alae began at the level of the nerve ring and extended to the posterior extremity in both sexes. In males the alae expanded and thickened in the caudal extremity (Figs. 8, 10), then terminated abruptly just posterior to the cloaca. An extensive area rugosa (Fig. 12) was observed in tangential sections of males, just anterior to the cloaca.

The hypodermis was thin, approximately 3 μm, in both sexes except where it thickened to form



Figures 1–6. Transverse sections of female *Yatesia hydrochoerus*. **1.** At the level of the nerve ring (n), muscular esophagus (e), and at midbody, showing 8 sectioned loops of the highly coiled uteri (u), ×100. **2.** Through the seminal receptacle (sr), showing an ovum (o), and typical musculature (m), ×200. **3.** Through the vulva (v) and ovejector (o), ×500. **4.** At midbody showing detail of ala (a), internal cuticular ridge (ir), microfilariae (mf), and lateral cord (lc), ×500. **5.** Through the growth zone of the ovary (g), showing uteri (u), and intestine (i), ×200. **6.** Through the glandular esophagus (e) at the level of the vagina (v), ×200.

lateral, dorsal, and ventral chords (Figs. 1, 5, 7). In females, the lateral chords were nearly the height of the contractile portion of the adjacent musculature and occupied approximately 20% of the circumference at midbody (Figs. 1, 2, 4). They tended to be taller and narrower in male worms (Figs. 5, 7). The lateral chords contained 2 distinct columns of vesicular cells with large



Figures 7–12. Histological sections of male *Yatesia hydrochoerus*. 7. Transverse section at midbody showing alae (a), lateral chords (lc), musculature (m), and intestine (i), ×200. 8. Transverse section at the level of the vas deferens (v), ×200. 9. Section at the level of the right spicule (R), ×200. 10. Section at the level of the left spicule (L) also showing broad caudal alae (c), ×200. 11. Longitudinal section of the muscular esophagus (e) and nerve ring (n), ×150. 12. Tangential section of the ventral cuticle at the area rugosa, arrow points toward anterior (ant) end of worm, ×500.

oval nuclei that appeared singly or in clusters of 2 or 3. No pattern of distribution was apparent for lateral chord nuclei. Dorsal and ventral chords were more readily discerned in the anterior portion of the body. However, with Heidenhain's stain, they appeared bright red and were easily seen in the posterior portion of the body as well.

Musculature was coelomyarian and polymyarian in both sexes with approximately 10–15 cells in each quadrant, depending on the body level. The contractile portion of muscle cells tended to be of uniform height but the protoplasmic portion of dorsally and ventrally placed cells in female worms tended to be taller than those more laterally placed (Figs. 1, 2). In female worms, approximately 30–50% of the muscle cell

consisted of contractile fibers that appeared dense and striated (Fig. 4). The remainder of these cells were made up of a lightly staining protoplasm; occasionally, nuclei were observed in the protoplasmic portion. They were ovoid, approximately 5 μm in diameter, and had densely stained nucleoli. In male worms, muscle cells tended to be proportionally taller than those in females; the contractile portion comprised 60–95% of the cell (Fig. 7).

The esophagus was divided into a narrow muscular portion, a short transitional area, and a broad glandular portion. The muscular and transitional portions had a triangular lumen lined with cuticle. Muscle fibers radiated from the lumen toward the periphery of the esophagus. More densely stained connective tissue fibers extended from the outer circumference of the esophagus to the corners of the lumen, dividing it into 3 triangular fields (Fig. 1). The glandular portion of the esophagus was granular and stained densely. The lumen was oval or round and was supported by numerous fibers that extended to the periphery of the esophagus.

The intestine was a simple tube with variable cellular morphology depending on the level of the section. Anteriorly, intestinal cells tended to be vesicular, light-staining, and somewhat cuboidal in shape (Fig. 7). Cell membranes were indistinct between adjacent cells and nuclei were small and few in number. Posteriorly, gut cell membranes became less distinct, but the lumen and basement membrane of the intestine, with a low band of foamy cytoplasm, could be recognized (Figs. 1, 5).

The female reproductive system included a thick, bulbous, elongate ovejector (at the level of the glandular esophagus) with dense fibrous muscular walls and a straight, cuticle-lined lumen (Fig. 3). In transverse section the vagina had a thick wall made up of muscle cells and fibers that encircled a densely staining basement membrane and 5–7 cuboidal epithelial cells. The vaginal lumen was plicate and often contained transversely sectioned microfilariae. Looped and coiled uterine tubes distended with developing microfilariae filled the body cavity from the level of the nerve ring almost to the level of the anus. Because of the extensive looping of the reproductive tract, a transverse section at midbody often contained 2–9 sections of various levels of the uterus (Figs. 1, 2, 4, 5). The distal portion of each uterine tube served as a seminal receptacle (Fig. 2). The epithelium in that region was not as flattened as more anterior regions and the lumen was filled with sperm and an occasional ovum. In the posterior portion of the body, just anterior to the level of the anus, highly coiled ovaries were observed. In transverse sections 2 distinct regions were recognized; the germinal zone was small in diameter with 6 or 7 epithelial cells on a basement membrane. The lumen was filled with developing ova clustered around a rodlike central rachis. The growth zone was larger in diameter with a similar flattened epithelium and basement membrane. The lumen was filled with larger triangular ova, each with a distinct nucleus and large nucleolus (Fig. 5).

Fully developed microfilariae within the reproductive tract appeared flattened dorsoventrally due to bluntly rounded alae that protruded approximately 3 μm laterally from each side of the cellular portion of the body (Fig. 4) and extended from the cephalic extremity to the beginning of the tail. The posterior extremity of the microfilaria was tubular and appeared circular in cross sections.

In the male worms the testis began at the level of the glandular esophagus. It was ensheathed by a thin connective tissue coat and consisted of a germinal zone, which was often looped, and a growth zone. In transverse sections through the anterior portion of the testis 1 or 2 sections of the germinal zone could be seen depending upon the degree of looping present. The germinal zone was small in diameter (approximately 25 μm) and consisted of a protoplasmic central rachis surrounded by small cells of the spermatogonia. The growth zone was larger in diameter (approximately 45 μm) and extended from the level of the glandular esophagus to midbody. In transverse sections, the growth zone appeared to be packed with large hexagonal and irregularly shaped sperm cells. A nucleus could be observed in almost every cell. No epithelial lining was observed at this level. Posteriorly the reproductive tract was straight and a tubular seminal vesicle with a sparse epithelium could be recognized. Irregularly shaped sperm cells filled the lumen at this level. Posterior to the seminal vesicle the vas deferens was recognized by its thick conspicuous basement membrane (Fig. 8). Cell boundaries at this level remained indistinct. Distal to the vas deferens, just anterior to the cloaca, the ejaculatory duct was recognized by its small diameter and thick muscular wall. It was lined with a low columnar epithelium and had a very thick basement membrane.

Discussion

The presence of adult *Y. hydrochoerus* in connective tissues of the skeletal musculature of capybaras did not appear to be accompanied by cellular infiltration or other recognizable immunopathologic changes in the vicinity of the worms. This observation, in conjunction with the high prevalence of *Yatesia* in capybaras suggests a well-adapted host–parasite relationship and does not necessarily indicate low pathogenesis in aberrant hosts such as other domestic animals or people.

Unusual microanatomical features of the cuticle of this filaria may be especially useful in recognizing it in tissue sections. Adult worms of either sex have external lateral alae as well as lateral internal ridges. The alae extend from the level of the nerve ring to the level of the cloaca in males and from the nerve ring to the tip of the tail in female worms. Microfilariae also are recognized easily in tissue sections by their lateral alae that extend from the anterior extremity to the beginning of the tail. The microfilarial tail, in cross section, appears tubular and devoid of nuclei.

At this writing, the genus *Yatesia* is monospecific; hence the usefulness of the microanatomical features described herein for specific identification cannot be determined. However, because a close taxonomic proximity for the genus *Yatesia* and the genus *Acanthocheilonema* seems likely based on morphological and biological criteria (Bain et al., 1982; Yates and Lowrie, 1984), a comparative evaluation of the microanatomy of selected species of *Acanthocheilonema* may be useful.

In general, the histologic features of *Y. hydrochoerus* resemble those described for *Acanthocheilonema viteae* and *Acanthocheilonema reconditum* (Sulahain, unpubl. dissertation, 1971; Dr. Jack Esslinger, Tulane University, New Orleans, Louisiana, pers. comm.), except for the conspicuous lateral alae of *Y. hydrochoerus*. On careful examination, however, *Y. hydrochoerus* differs from these related species in several other ways. The 3 species all have internal lateral cuticular ridges, but *A. viteae* has small inconspicuous ridges; those of *A. reconditum* are prominent, densely staining, and pedunculate, whereas *Y. hydrochoerus* has conspicuous, triangular-shaped internal ridges that often stain densely at the apex. In *A. viteae* the lateral chords are narrow and pedunculate, whereas in *Y. hydrochoerus* and *A. reconditum* they are much broader. *Yatesia hydrochoerus* also has a larger number (10–15) of muscle cells per quadrant in transverse sections than either *A. viteae* or *A. reconditum*, which have only 9–11 cells per quadrant. In *Y. hydrochoerus* and *A. reconditum* females, the reproductive tract is extensively looped and coiled. Thus, in transverse sections at midbody one may observe 2–9 segments of uterus within the body cavity. In female *A. viteae* the reproductive tract is comparatively straight so that at midbody transverse sections typically present only 1 segment of each uterine branch. Features of the digestive tract and male reproductive system were similar for all 3 species. Only the microfilariae of *Y. hydrochoerus* appear alate in sections through gravid uteri. Indeed, the presence of large lateral cuticular alae in *Y. hydrochoerus* microfilariae appears to be an unusual and taxonomically distinctive characteristic.

Acknowledgments

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Obituary Notices

ALBERT L. TAYLOR

26th Recording Secretary: 1951
Appointed to Editorial Board: 1952–1963
38th President: 1955
Elected Life Member: 1975
Deceased: 11 March 1988

JOHN SCOTT ANDREWS

Elected Member: 20 September 1930
Washington Academy of Sciences Representative: 1955, 1956
21st Vice President: 1955
41st President: 1956
Anniversary Award Recipient: 1969
Elected Life Member: 1980
Deceased: 7 March 1988

EDNA M. BUHRER

7th Corresponding Secretary-Treasurer: 1934–1971
Constitution Committee Member: May 1943
Trustee of Ransom Memorial Trust Fund: 1956
1st Anniversary Award Recipient: 1960
55th President: 1972
Deceased: 29 February 1988

A. BURNS WEATHERSBY

Elected Member: 21 January 1959
Deceased: 25 January 1988

Proc. Helminthol. Soc. Wash.
55(2), 1988, pp. 146–154

Ochoterenella figueroai sp. n. and *O. lamothei* sp. n. (Nematoda: Filarioidea) from the Toad *Bufo marinus*

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ABSTRACT: *Ochoterenella figueroai* sp. n. and *O. lamothei* sp. n. are described from giant toads, *Bufo marinus,* collected in Guatemala and Mexico, respectively. The female of *O. figueroai* is distinguished from most of the other known species of the genus by the greater length of the midbody cuticular bosses (avg. 15–18 μm) and their disposition. The size and appearance of the microfilaria further separate this species from the rest. The male of *O. figueroai* is readily distinguished from those of *O. guyanensis, O. royi,* and *O. oumari* on spicular structure, but it closely resembles *O. digiticauda.* These 2 can be separated by the structure and appearance of the midventral preanal plaque. *Ochoterenella lamothei,* for which no male is known, is separated from other species on the length (avg. 11–13 μm) and arrangement of the midbody cuticular bosses, the conical tail lacking prominent cuticular bosses, and the location of the vulva near the posterior region of the esophagus. The microfilaria, distinguishable from nearly all other species by its dimensions and appearance, very closely resembles that of *O. figueroai.* These 2 can be separated by the relatively narrower nuclear column in *O. lamothei* and by the size and appearance of the Innenkörper, with that of *O. lamothei* occupying greater than ½ the width of the body and being homogeneously hyaline, and that of *O. figueroai* occupying less than ½ the body width and being differentiated into central and peripheral regions.

KEY WORDS: Nematoda, Filarioidea, *Ochoterenella figueroai* sp. n., *Ochoterenella lamothei* sp. n., *Bufo marinus,* toad, amphibian, blood parasite, microfilariae, Mexico, Guatemala.

Specimens of filarial worms from the body cavity of giant toads, *Bufo marinus,* collected in Guatemala and Mexico, were referred to the investigator for identification. These specimens, belonging to the genus *Ochoterenella* Caballero, 1944 (sensu Esslinger, 1986), were determined to represent previously unknown species and are herein described as *Ochoterenella figueroai* sp. n. and *O. lamothei* sp. n., respectively.

Materials and Methods

The specimens used in the description of *O. figueroai* sp. n. were collected in 1980 from the body cavity of *Bufo marinus* in Guatemala, Guatemala, by Dr. H. Figueroa-M. Blood films from living toads were made and subsequently stained with hematoxylin and eosin. Adult worms were fixed in 10% formalin. Three additional female specimens, not used for the present description, were found in Caballero's collection from Mexico (IBUNM No. 106-4); this collection, all specimens originally identified as *O. digiticauda,* is discussed elsewhere (Esslinger, 1986).

Specimens herein described as *O. lamothei* sp. n. were obtained from a preserved (10% formalin) *B. marinus* collected by M. en C. Rafael Lamothe-A. and M. en C. Raul Pineda-L. in 1985 in Huixtla, Chiapas, Mexico. Eight female worms from this host provided the basis for the description. No corresponding male worms of *O. lamothei* were recovered, although both males and females of *O. digiticauda* were obtained from this same host.

All adult worms were examined in pure glycerin following slow evaporation from 70% ethanol containing 5% glycerin. Microfilariae were removed from the vagina of gravid specimens of both species and examined unstained and stained with azure II in glycerine. Those from blood (*O. figueroai* fixed in 2% aqueous formalin; *O. lamothei* in 10% formalin from preserved toad) were examined as above. Illustrations were made with the aid of a Wild drawing apparatus, and measurements were made with an ocular micrometer. In the following descriptions all measurements (ranges with means in parentheses) are in micrometers unless otherwise indicated. Dimensions given for individual bosses, distances between bosses, and distances between bands of bosses are based on at least 50 measurements for each feature for each worm. Locations of structures in the microfilariae are given as the distance from the anterior extremity.

Ochoterenella figueroai sp. n. (Figs. 1–25)

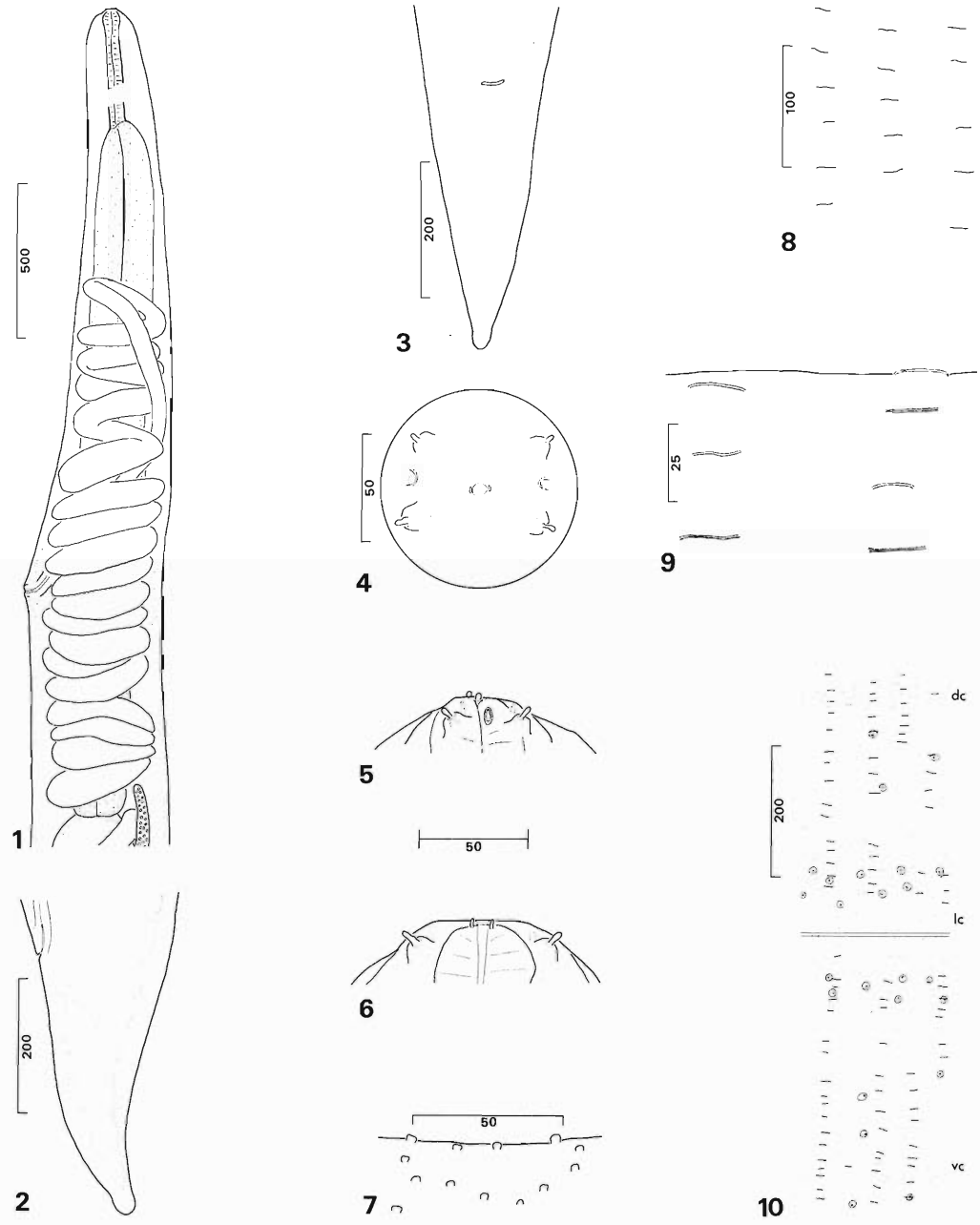
Description

GENERAL: Onchocercidae (Leiper, 1911) Chabaud and Anderson, 1959; Waltonellinae Bain and Prod'hon, 1974; *Ochoterenella* Caballero, 1944 (sensu Esslinger, 1986). Male, female, and microfilariae with characters of the genus.

FEMALE (based on 10 gravid specimens; dimensions of holotype specimen in brackets; Figs. 1–11): Body more robust throughout anterior ½, widest just posterior to esophago–intestinal junction. Both extremities (Figs. 1–3) attenuated. Cephalic extremity (Figs. 4–6) rounded, cephalic plate moderately salient; parastomal structures distinct, truncated, approximately 3 wide by 3

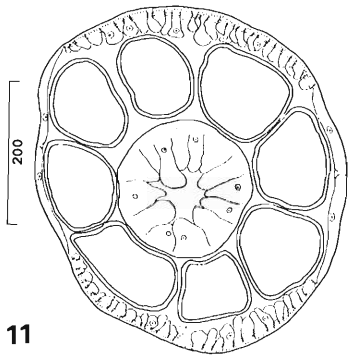
high; mouth 9–10 in diameter. Outer papillae salient, distal portion extending approximately 4 beyond surface; inner papillae sessile. Cuticle in midbody region (Figs. 8, 9) with annular bands of low, slender, longitudinally oriented bosses; distribution (Fig. 10) relatively even over dorsal and ventral quadrants of body, but irregular and sparse near lateral cords and lacking near lateral line. Body length 58–71 (65) [67] mm, maximum width 564–702 (607) [663]. Width of body at nerve ring 170–243 (209) [208], at junction of muscular and glandular portions of esophagus 208–287 (243) [262], at esophago–intestinal junction 475–609 (549) [564], at vulva 431–624 (511) [436]. Cephalic plate 34–43 (39) [41] by 52–66 (60) [64]; ratio length to width 1.4–1.8 (1.5) [1.6]. Esophagus total length 2,811–3,980 (3,159) [2,999]; muscular portion 327–534 (402) [336] long by 36–58 (45) [46] wide; glandular portion 2,406–2,792 (2,652) [2,663] long by 139–185 (170) [161] wide; ratio of length glandular to muscular 4.50–9.87 (7.18) [7.93]; ratio of width glandular to muscular 3.1–4.2 (3.8) [3.5]. Nerve ring 208–347 (280) [208] from anterior extremity. Vulva (Fig. 1) salient, extending up to 20 beyond adjacent body profile in some specimens, located near posterior ⅓ of esophagus 1,683–2,574 (2,141) [1,702] from anterior extremity. Vagina vera directed anteriad, often reaching base of muscular esophagus; vagina uterina tightly coiled around glandular portion of esophagus in some specimens. Tail (Figs. 2, 3) 293–504 (389) [360] long, gradually attenuated, conical, with slightly constricted tip in some specimens; cuticular bosses (Fig. 7) present on dorsal surface of tail, numerous, 4–5 long by 1 high near level of anus, smaller (ca. 2 long) and sparse or absent near tip; anal region not salient, dorsoventral thickness of body at level of anus 106–252 (177) [175], ratio of tail length to thickness at anus 1.76–2.67 (2.25) [2.06]. Midbody cuticular bosses as illustrated (Figs. 8–10). Length individual bosses 10–24 with averages for each worm ranging from 15.1 to 18.0 (overall mean for 10 worms 16.2) [15.9]; distance between bosses within band with range of averages 37–44 (overall mean 40) [42]; distance between bands (center to center) with range of averages 58–67 (overall mean 61) [58]. In transverse section at level of midbody (Fig. 11), cuticle thin, lateral cords broad, low, with demarcated portion at lateral line, each cord occupying slightly less than ¼ circumference of body; dorsal and ventral cords narrow but conspicuous; musculature confined to dorsal and ventral quadrants; intestine ¼–⅓ body diameter; uterine loops occupying most of body cavity.

MALE (based on 10 specimens; dimensions of allotype specimens in brackets; Figs. 15–25): Body length 22–27 (24) [26] mm, anterior portion moderately attenuated, posterior more distinctly so and helically coiled (usually 3 or 4 turns); diameter nearly uniform throughout midportion of body with maximum width 276–376 (322) [376]. Cephalic extremity (Fig. 22) resembling that of female but smaller; cephalic plate 26–32 (29) [30] by 34–48 (41) [48], ratio length to width 1.3–1.6 (1.4) [1.6]. Width of body at nerve ring 113–166 (146) [148], at junction of muscular and glandular portions of esophagus 156–180 (164) [168], at esophago–intestinal junction 223–366 (298) [347]. Esophagus total length 1,796–2,604 (2,199) [2,069]; muscular portion 252–475 (357) [267] by 30–40 (35) [34]; glandular portion 1,544–2,129 (1,842) [1,802] by 108–143 (124) [120]; ratio length glandular to muscular 4.53–6.75 (5.32) [6.75]; ratio width glandular to muscular 3.0–4.2 (3.6) [3.5]. Nerve ring 180–361 (256) [228] from anterior extremity. Tail (Fig. 15) digitiform, 132–170 (154) [146] long, dorsoventral thickness of body at level of anus 84–125 (101) [103], ratio of tail length to thickness at anus 1.34–1.69 (1.54) [1.42]. Caudal papillae (Figs. 15, 17) large, mammiform; 1 pair preanal (1 extra unilateral papilla observed on 2 specimens), 3 pairs postanal, the posteriormost distinctly separated from the second postanal papillae and located 66–93 (76) [76] from tip of tail. Distinct median ventral preanal cuticularized plaque with configuration as illustrated (Figs. 15–18), located just anterior to caudal papillae, comprised of posterior irregular hyaline bar and separate anterior transverse granular region. Spicules as illustrated (Figs. 20, 21); left 178–243 (204) [226] long with weakly cuticularized and often distorted tubular proximal portion and filamentous distal portion; right 120–147 (133) [134] long, 10–13 (12) [12] wide just distal to cuplike base; spicular ratio 1.37–1.77 (1.54) [1.69]. Area rugosa well developed, consisting of transverse bands of raised (ca. 1) longitudinally oriented cuticular bosses as illustrated (Fig. 25); measured at midportion of area rugosa, length individual bosses 3–7 with averages for each worm ranging from 4.0 to 4.9 (overall mean for 10 worms 4.4) [4.1], distance between bosses within a band 3–15 with averages for each worm ranging from 6.3 to 9.6 (overall mean 7.6) [7.5], distance between bands (center to center) 14–50 with averages for



Figures 1–10. ***Ochoterenella figueroai*** **sp. n., gravid female. 1. Anterior end, lateral view. 2. Posterior end, lateral view. 3. Posterior end, ventral view. 4. Anterior extremity, en face. 5. Anterior extremity, lateral view. 6. Anterior extremity, dorsal view. 7. Cuticular bosses on dorsal surface of tail near level of anus. 8. Midbody bosses. 9. Detail of midbody bosses; distance between bosses within band foreshortened by curvature of body. 10. Cuticular spread showing circumferential distribution of midbody bosses. dc, dorsal cord; lc, lateral cord; vc, ventral cord.**

each worm ranging from 24 to 34 (overall mean 30) [33]. Cuticular bosses on body (Figs. 23, 24; measured at distance of 3 times length of esophagus from anterior extremity) structurally resembling those of female; length individual bosses 7–14 with averages for each worm ranging from 8.6 to 10.6 (overall mean 9.7) [10.4]; distance between bosses within a band 11–48 with av-



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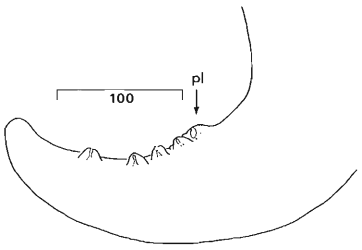

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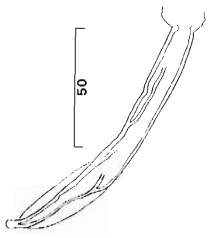

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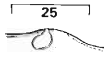

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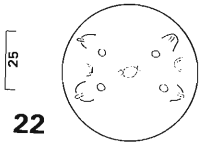

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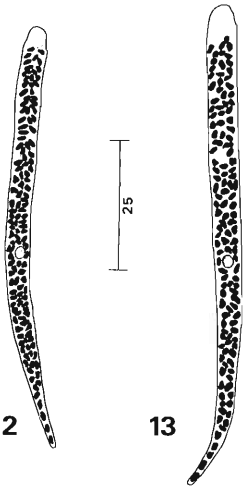

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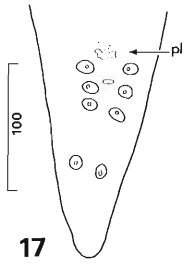

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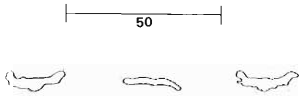

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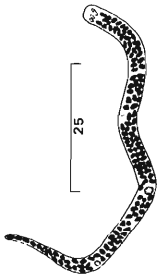

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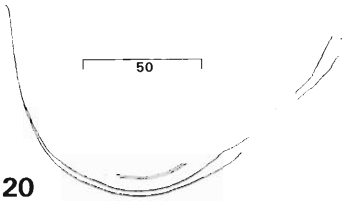

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Figures 11–25. ***Ochoterenella figueroai*** **sp. n. 11. Transverse section in midbody region of female; contents of uteri not shown. 12. Microfilaria ex vagina of fixed specimen. 13. Microfilaria from blood, fixed in 2% aqueous formalin. 14. Microfilaria in blood film from living** ***Bufo marinus.*** **15. Posterior of male, lateral view; pl, median preanal plaque. 16. Detail of median preanal plaque, lateral view; anterior is right. 17. Posterior of male, ventral view; pl, median ventral plaque. 18. Detail of median ventral plaques in surficial view, from 3 specimens of** ***O. figueroai*** **sp. n. 19. Detail of median ventral plaques in surficial view, from 3 specimens of** ***O. digiticauda.*** **20. Left spicule. 21. Right spicule. 22. Cephalic extremity of male, en face. 23. Bands of cuticular body bosses of male. 24. Detail of body bosses of male. 25. Area rugosa, detail of bosses in surficial view.**

erages ranging from 20 to 28 (overall mean 24) [29]; distance between bands (center to center) 17–63 with averages ranging from 30 to 40 (overall mean 36) [38].

MICROFILARIAE A, FROM VAGINA OF FIXED WORM (40 specimens stained with azure II in glycerin; Fig. 12): Body widest near anterior extremity, gradually attenuating to posterior; tip of tail narrow, rounded. Sheath present. Cephalic extremity rounded to slightly truncated, cuticle at apex appearing thickened. Cephalic hook small, inconspicuous. Somatic nuclei ovoid to spheroid; column 3 or 4 nuclei wide, reaching nearly to margin of body. Cuticle thin, with fine transverse striae not clearly seen on all specimens. Caudal nuclei ovoid to elongate, nearly reaching tip of tail, posteriormost 6 or 7 in single file. Body length 74–85 (80), maximum width 5.3–6.3 (5.7) near anterior extremity; width at level of Innenkörper 4.5–5.6 (5.2). Cephalic space 4.8–6.7 (5.7), with 4 large, ovoid, lightly staining nuclei, occasionally in transverse row. Nerve ring, excretory space not consistently evident. Innenkörper 38–48 (42), spheroid or slightly irregular in shape, hyaline with some staining or granularity at periphery, 1.9–2.4 (2.1) in diameter, occupying less than ½ (ca. 0.4) of body width. Anal space inconspicuous in most specimens.

MICROFILARIAE B, IN BLOOD FIXED IN 2% AQUEOUS FORMALIN (80 specimens, stained with azure II in glycerin; Fig. 13): Body widest near anterior extremity, cylindrical throughout most of length, gradually attenuated over posterior ⅓–¼. Tip of tail narrow, rounded. Sheath present. Cephalic extremity rounded to slightly constricted, apical cuticle thick, cephalic hook inconspicuous. Somatic nuclei as described above for specimens from vagina. Cuticle with fine transverse striae. Caudal nuclei 6 or 7 in single file. Body length 92–100 (97), maximum width 6.0–6.7 (6.5) near anterior extremity; width at level of Innenkörper 5.6–6.1 (5.8). Cephalic space 5.3–6.9 (6.0), nerve ring 19–26 (22), excretory pore 29–36 (32). Innenkörper 46–54 (50), spheroid to slightly irregular, hyaline with some staining or granularity at periphery, 2.0–2.6 (2.2) in diameter, occupying less than ½ (ca. 0.4) of body width. Anal space 66–76 (73).

In dried films of this same material stained with hematoxylin and eosin, dimensions (based on 40 specimens) as follows: body length 91–102 (97), maximum width 3.8–5.8 (4.9), cephalic space 4.8–6.9 (6.2), nerve ring 19–25 (23), excretory space 30–35 (32), Innenkörper 46–55 (51), anal space 69–85 (74).

MICROFILARIAE C, IN BLOOD FILM (40 specimens stained with hematoxylin and eosin; Fig. 14): Body widest near anterior extremity, cylindrical, gradually attenuated in posterior ¼; attitude gently curved to sinuous, seldom tightly coiled or looped. Sheath present. Cephalic extremity rounded; caudal extremity narrow but rounded. Somatic nuclei ovoid to angular, column 3 or 4 nuclei wide. Caudal nuclei ovoid, reaching tip of tail, posteriormost 6 or 7 in single file. Body length 60–74 (65), maximum width near anterior extremity 3.8–5.0 (4.6). Cephalic space 3.8–5.9 (4.9), containing 4 ovoid, lightly staining nuclei. Nerve ring 12–18 (16). Excretory space 20–25 (23), obvious. Innenkörper 32–40 (37), spheroid to ovoid, hyaline, occupying less than ½ diameter of body. Anal space 47–58 (53).

TYPE HOST: *Bufo marinus* Linnaeus, 1758, giant toad.

SITE OF INFECTION: Body cavity.

TYPE LOCALITY: Guatemala, Guatemala.

OTHER LOCALITY: Mexico, Chiapas, Huixtla.

SPECIMENS DEPOSITED: Holotype female, USNM Helm. Coll. No. 80274; allotype male, USNM Helm. Coll. No. 80275; paratypes, 4 females, 2 males, USNM Helm. Coll. No. 80276; microfilariae, 1 slide blood film, 2 slides blood fixed in 2% aqueous formalin, USNM Helm. Coll. No. 80276. Three other females (Caballero's specimens from Mexico) deposited in Helminth Collection, Instituto de Biología, Universidad Nacional Autónoma de México, México, D.F., Mexico, under augmented number IBUNM 106-4B.

ETYMOLOGY: This species is named for Dr. H. Figueroa-M. in Guatemala who provided the specimens described herein.

Remarks

The female of *O. figueroai* can be distinguished from most of the other species of *Ochoterenella* by the greater length of the midbody cuticular bosses (avg. 15–18), with shorter bosses being reported for *O. digitata* (7–10), *O. guyanensis* (5), *O. royi* (12 or less), *O. oumari* (10–12), *O. dufourae* (4–7), and *O. chiapensis* (10–12) (Bain and Prod'hon, 1974; Bain et al., 1979; Esslinger, 1986, 1988). The bosses of *O. nanolarvata* (8–15) approach those of the present species in length, but the bands are closer together in the former species (avg. 39 vs. 61) (Esslinger, 1987). The midbody

bosses of *O. albareti* are slightly longer (20–25), but the bands are only 18–20 apart (Bain et al., 1979). Both the length and arrangement of bosses of *O. caballeroi* are similar to *O. figueroai,* but the esophagus is much shorter in the former species (Esslinger, 1987). Although the lengths of the midbody bosses of the 3 species incompletely described by Travassos (1929) may fall within the range of those of the present species, the bosses are nearly twice the distance apart in *O. vellardi* and the tail is much longer than that of *O. figueroai. Ochoterenella scalaris,* recovered from the sublingual connective tissue of *Leptodactylus,* is reported as having the individual bosses only 8 apart, as well as closely approximated bands (5–6 apart). *Ochoterenella convoluta,* also from *Leptodactylus,* has bosses as short as 6, and the body is only about ½ the length of *O. figueroai.*

The size and shape of the microfilaria of *O. figueroai* readily distinguishes it from all the other species for which this stage has been described. Of those microfilariae of comparable length, *O. oumari* has a more abruptly attenuated tail with a more rounded tip; *O. caballeroi* is more slender with a filiform tail; and *O. chiapensis* has both abrupt cephalic and caudal attenuations.

The males of only 4 other species of *Ochoterenella* have been described. Three of these (*O. guyanensis, O. royi,* and *O. oumari*) can be separated from *O. figueroai* on the basis of the structure of the left spicule (calomus strongly cuticularized). The male of *O. figueroai,* however, closely resembles that of *O. digiticauda* in many respects, including spicular structure and most dimensions. The esophagus of *O. digiticauda* (avg. 1,603) tends to be shorter than that of the present species (avg. 2,199), but ranges observed overlap somewhat (1,287–1,832 vs. 1,796–2,604). The body bosses are slightly longer for *O. figueroai* (avg. 9.7) than for *O. digiticauda* (avg. 8.7). It should be noted that the lengths of the body bosses of the male of *O. figueroai* are not approximately the same as those of the female; this contrasts to the close correspondence observed with the other 4 species. Although the tail lengths of *O. figueroai* (132–170, avg. 154) and *O. digiticauda* (117–180, avg. 141) are comparable, the absolute and relative distance from the posteriormost caudal papillae to the tip of the tail is generally greater in the present species (66–93, avg. 76) than in *O. digiticauda* (55–69, avg. 61); the caudal extremity of *O. figueroai* appears correspondingly more elongate and digitiform. Another consistent difference between these 2 species is in the appearance of the median ventral preanal plaque in surficial view (cf. Figs. 18, 19), in which the components are separated into an anterior granular portion and a posterior hyaline portion in *O. figueroai,* but are superimposed in *O. digiticauda.*

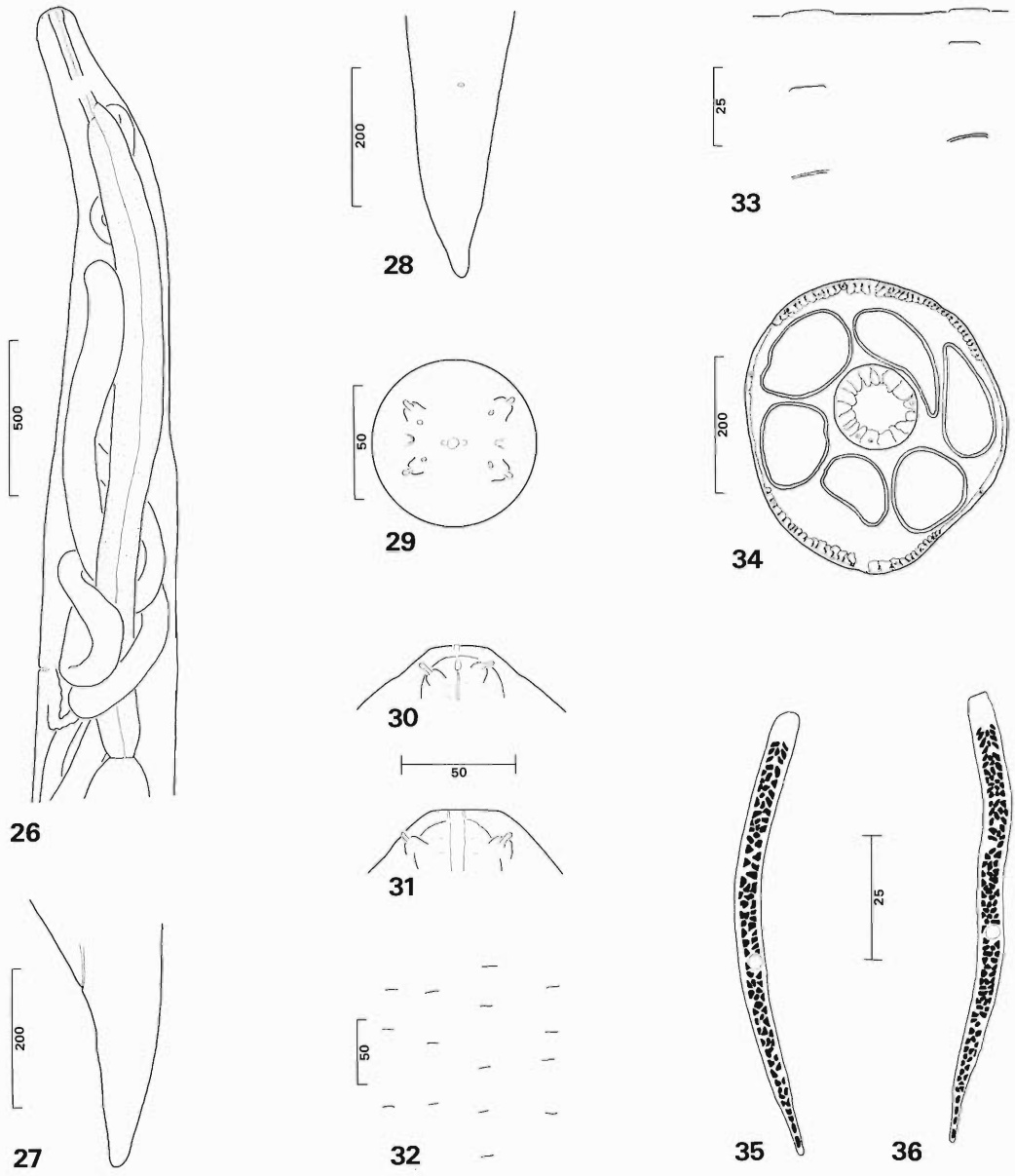
The close similarities between the males of *O. figueroai* and *O. digiticauda,* along with the disparity in boss length between the males and females of the present specimens, mandate some caution in designating the males as *O. figueroai.* However, additional supporting evidence, although not conclusive, that the present males correspond to the females of *O. figueroai* is the lack of either microfilariae or females of *O. digiticauda* (or any other species) in the particular hosts examined.

Ochoterenella lamothei sp. n. (Figs. 26–36)

Description

GENERAL: Onchocercidae (Leiper, 1911) Chabaud and Anderson, 1959; Waltonellinae Bain and Prod'hon, 1974; *Ochoterenella* Caballero, 1944 (sensu Esslinger, 1986). Male unknown. Female and microfilariae with characters of the genus.

FEMALE (based on 8 gravid specimens; dimensions of holotype specimen in brackets; Figs. 26–34): Body more robust throughout anterior ½, gradually becoming more slender posteriorly, widest just posterior to esophago–intestinal junction. Both extremities (Figs. 26–28) attenuated; anterior narrow to level of nerve ring. Cephalic extremity (Figs. 29–31) rounded, cephalic plate not distinctly salient; parastomal structures rounded to truncated distally, approximately 3 wide by 3 high; mouth 8–9 in diameter. Outer papillae salient, distal portion extending 4–5 beyond surface; inner papillae sessile. Cuticle in midbody region (Figs. 32, 33) with transverse bands of low, slender, longitudinally oriented bosses, discontinuous along lateral line. Body length 47–57 (52) [51] mm, maximum width 446–594 (531) [594]. Width of body at nerve ring 158–218 (194) [180], at junction of muscular and glandular portion of esophagus 178–252 (224) [235], at esophago–intestinal junction 337–500 (433) [495], at vulva 317–485 (418) [485]. Cephalic plate 32–38 (34) [36] by 46–58 (51) [50];



Figures 26–36. ***Ochoterenella lamothei*** **sp. n., gravid female. 26. Anterior end, lateral view. 27. Posterior end, lateral view. 28. Posterior end, ventral view. 29. Anterior extremity, en face. 30. Anterior extremity, lateral view. 31. Anterior extremity, dorsal view. 32. Midbody bosses. 33. Detail of midbody bosses; distance between bosses within band foreshortened by curvature of body. 34. Transverse section in midbody region; *contents of uteri* not shown. 35. Microfilaria ex vagina of fixed specimen. 36. Microfilaria in blood from toad preserved in 10% formalin.**

ratio length to width 1.4–1.7 (1.5) [1.4]. Esophagus total length 2,149–2,653 (2,440) [2,604]; muscular portion 297–406 (360) [396] long by 38–48 (44) [48] wide; glandular portion 1,832–2,297 (2,081) [2,208] long by 134–168 (150) [146] wide; ratio of length glandular to muscular 5.07–7.00 (5.83) [5.58]; ratio of width glandular to muscular 3.1–4.2 (3.5) [3.1]. Nerve ring 198–277 (236) [252] from anterior extremity. Vulva (Fig. 26) not salient, located just anterior to esophagointestinal junction, 1,554–2,277 (1,944) [2,203] from anterior extremity. Vagina vera directed

anteriad, often reaching base of muscular esophagus; vagina uterina usually forming loose folds or loops parallel to esophagus, not tightly coiled around glandular portion. Tail (Figs. 27, 28) 173–319 (268) [312] long, gradually attenuated, conical, with rounded tip; cuticular bosses lacking or extremely sparse and small (<1); anal region not salient; dorsoventral thickness at level of anus 122–149 (138) [139]; ratio of tail length to thickness at anus 1.42–2.29 (1.94) [2.24]. Midbody cuticular bosses as illustrated (Figs. 32, 33). Length individual bosses 7–18 with averages for each worm ranging from 10.8–12.9 (overall mean for 8 worms 12.2) [12.3]; distance between bosses within band with range of averages 28–42 (overall mean 34) [42]; distance between bands (center to center) with range of averages 48–59 (overall mean 51) [48]. In transverse section at level of midbody (Fig. 34), cuticle thin, lateral cords broad, very low, with demarcated portion at lateral line, each cord occupying slightly less than ¼ circumference of body; dorsal and ventral cords narrow; musculature confined to dorsal and ventral quadrants; intestine approximately ⅓ body diameter; uterine loops occupying most of body cavity.

MICROFILARIAE A, FROM VAGINA OF FIXED WORM (40 specimens, stained with azure II in glycerin; Fig. 35): Body cylindrical throughout anterior ⅔, posterior ⅓ gradually attenuated; tip of tail narrow, rounded. Sheath present. Cephalic extremity rounded to slightly truncated, cuticle at apex thickened and hyaline. Cephalic hook small, inconspicuous. Somatic nuclei ovoid to angular, those in anterior portion of body often directed anteromediad; column 3 or 4 nuclei wide, confined to central portion of body and giving appearance of being limited by thick (ca. 1.5) body wall. Cuticle with fine transverse striae. Caudal nuclei ovoid to elongate, nearly reaching tip of tail, posteriormost 4 or 5 (sometimes 6) in single file. Body length 88–96 (91), maximum width (in region of excretory space) 5.4–6.2 (5.8), width at level of Innenkörper 4.6–5.8 (5.3). Cephalic space 5.8–6.7 (6.3), with 4 large, ovoid, lightly staining nuclei. Nerve ring not consistently seen. Excretory space 28–35 (31), often difficult to recognize. Innenkörper 46–51 (49), spheroid, hyaline, without peripheral irregularity in shape or consistency, 2.9–3.4 in diameter, occupying greater than ½ (ca. 0.6) of body width. Anal space inconspicuous.

MICROFILARIAE B, FROM BLOOD OF HOST FIXED IN 10% FORMALIN (40 specimens, stained with azure II in glycerin; Fig. 36): Body cylindrical throughout anterior ⅔, posterior ⅓ gradually attenuated; tip of tail narrow, rounded. Attitude gently curved to obtusely bent near midbody. Sheath present. Cephalic extremity frequently truncated, cuticle at apex thickened and hyaline. Cephalic hook small, inconspicuous. Somatic nuclei, nuclear column as described for specimens from vagina. Cuticle with fine transverse striae. Tail often constricted in terminal region where 4–5 (sometimes 6) caudal nuclei form single file. Body length 85–98 (93), maximum width (anterior ¼) 5.9–6.8 (6.4), width at level of Innenkörper 4.8–6.0 (5.7). Cephalic space 5.0–7.2 (6.3). Nerve ring 21–26 (23). Excretory space 29–35 (32). Innenkörper 47–54 (50), spheroid to slightly ovoid with long axis transverse, entirely hyaline, 2.9–3.8 (3.2) in diameter, occupying greater than ½ (ca. 0.6) body width. Anal space 65–76 (73).

In dried films of this same material stained with hematoxylin and eosin, dimensions as follows: body length 82–96 (90), maximum width 4.0–4.8 (4.4), cephalic space 5.6–6.7 (6.0), nerve ring 18–23 (21), excretory space 27–34 (32), Innenkörper 44–52 (49) [2.7–3.5 (3.8) in diameter, occupying approximately 0.7–0.8 body width], anal space 64–74 (70).

TYPE HOST: *Bufo marinus* Linnaeus, 1758, giant toad.

SITE OF INFECTION: Body cavity.

TYPE LOCALITY: Mexico, Chiapas, Huixta.

SPECIMENS DEPOSITED: Holotype female, USNM Helm. Coll. No. 80277; paratypes (5 females), USNM Helm. Coll. No. 80278; microfilariae, 2 slides blood from host preserved in 10% formalin, USNM Helm. Coll. No. 80278.

ETYMOLOGY: This species is named for M. en C. Rafael Lamothe-A. of the Instituto de Biología at the Universidad Autónoma de México.

Remarks

On the basis of the length of midbody bosses alone, the female of *O. lamothei* (avg. 11–13) can be separated from *O. figueroai, O. albareti, O. scalaris, O. vellardi,* and *O. convoluta* that have longer bosses, and from *O. guyanensis, O. dufourae,* and *O. digiticauda* with smaller bosses. Adjacent bosses within a band are much closer together in *O. royi* (7–15 apart) than in *O. lamothei* (28–42 apart). *Ochoterenella figueroai, O. chiapensis,* and *O. oumari* have conspicuous cu-

ticular bosses on the dorsal surface of the tail; these are lacking in the present species. In *O. oumari* and *O. caballeroi* the vulva is located in the midesophageal region; it is near the esophago–intestinal junction in *O. lamothei.* The tail of *O. nanolarvata* is abruptly attenuated, in contrast to the slender conical appearance of the tail of the present species.

The microfilaria of *O. lamothei* most closely resembles that of *O. figueroai* but is readily distinguished from those of other species on the basis of its size and shape. These distinctions are the same as apply to *O. figueroai* (see previous Remarks).

Microfilariae of *O. lamothei* (88–96 ex vagina) are somewhat longer than those of *O. figueroai* (74–85 ex vagina), but this feature alone is of limited use. The appearance of the nuclear column differs between these 2 species. That of *O. figueroai* reaches the lateral margins, but that of *O. lamothei* is more narrow relative to the width of the body and gives the microfilaria the appearance of having a notably thick cuticle (body wall). The caudal extremity of *O. lamothei* characteristically has a single file of 4 or 5 nuclei, whereas 6 or 7 are usually seen in *O. figueroai.* The size and appearance of the Innenkörper afford the most consistent means of distinguishing these 2 species. That of *O. figueroai* occupies less than ½ the body width and is differentiated into central and peripheral regions, and that of *O. lamothei* occupies greater than ½ the width of the body and is homogeneously hyaline in appearance.

The addition of *O. figueroai* and *O. lamothei* brings the number of species in the genus *Ochoterenella* to 14, all reported from the Neotropical region. With the exceptions of *O. convoluta* and *O. scalaris,* both recovered from leptodactylid frogs (Travassos, 1929), all have been found only in the toad *Bufo marinus.*

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A New Kathlaniidae (Cosmocercoidea; Nematoda), *Megalobatrachonema* (*Chabaudgolvania*) *moraveci* sp. n. from the Intestine of the Rough-skinned Newt, *Taricha granulosa*

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ABSTRACT: *Megalobatrachonema moraveci* sp. n. is described from the intestine of the rough-skinned newt, *Taricha granulosa*. The new species most closely resembles *Megalobatrachonema waldeni* Richardson and Adamson, 1988, in having 3 small distinctly separate lips, a swelling at the posterior of the corpus, an elongated esophageal bulb, and lateral alae, but differs in that there are no cervical alae, cephalic papillae are raised on hypodermal peduncles, and the cuticle lining the inner surface of the lips is thickened. The new species further differs from *M. waldeni* in that the swelling at the base of the corpus is smaller, and the excretory pore and deirids are further posterior. *Megalobatrachonema moraveci* has 1 more postanal lateral and 3 more preanal papillae than *M. waldeni*. The spicules of *M. moraveci* are much more strongly alate, and the gubernaculum is hooklike. Female *M. moraveci* have more eggs in the uterus than those of *M. waldeni*.

KEY WORDS: Nematoda, Cosmocercoidea, Kathlaniidae, *Megalobatrachonema* (*Chabaudgolvania*) *moraveci* sp. n., newt, *Taricha granulosa*, taxonomy, morphology.

Moravec (1984) reported a nematode of the genus *Megalobatrachonema* in the intestine of *Taricha granulosa* from Vancouver Island, British Columbia. Because only a single female worm was found, the specimen was not given a species name. Recently, Richardson and Adamson (1988) described a species of *Megalobatrachonema* from *Ambystoma gracile* in the lower mainland of British Columbia. To determine whether Moravec's (1984) material was conspecific with that in *Ambystoma* we examined *Taricha* from the Nanaimo/Cambell River area. These were found to be infected with a new species that is described herein.

Materials and Methods

Twelve *Taricha granulosa* caught in the Nanaimo/Cambell River area between 4 to 8 May by Mr. Gordon Haas were donated to the authors. Specimens were examined on 11 May 1987. Parasites were fixed in hot alcohol/glycerine, preserved in 70% alcohol, and cleared and examined in lactophenol. Figures were drawn with the aid of a drawing tube. The single specimen found by Moravec (1984), and the other following specimens were examined: *Megalobatrachonema terdentatum* (syn. *M. campanae*) from the Paris National Museum ref. 620Q, and *Megalobatrachonema gigantica* (syn. *Aplectana gigantica*) #9054, *Megalobatrachonema elongata* (syn. *Falcaustra elongata*) #72190–72194, *Falcaustra mascula* (syn. *Spironoura mascula*) #72195–72198 from the U.S. National Museum Helminthological Collection. References used included those by Baird (1858), Chaubaud and Golvan (1957), Hartwich (1960), Skrjabin *et al.* (1976), and Baker (1980, 1986).

In the species description measurements are in micrometers unless otherwise specified; mean is given in parentheses.

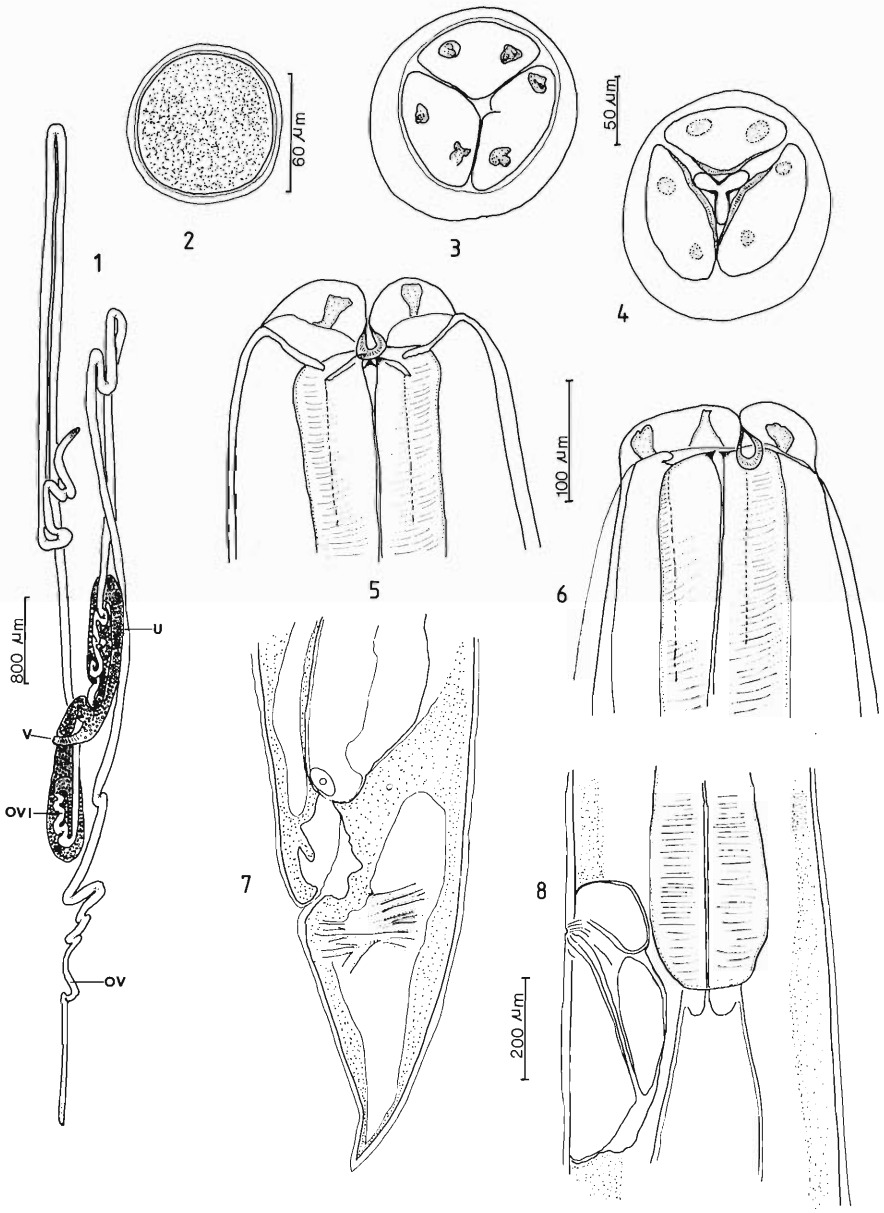
Results

Megalobatrachonema moraveci sp. n. (Figs. 1–14)

GENERAL: Oral opening triangular, surrounded by 3 distinctly separate lips. Cephalic sense organs located at end of hypodermal peduncles. Dorsal lip with 2 papillae; subventral lips each with single papilla and amphid. Inner papillae not observed. Cuticle lining inner surface of lips thickened. Anterior of esophagus bearing 3 onchia.

Esophagus long and narrow,with anterior pharyngeal portion and faintly separated corpus, isthmus, and bulb. Very slight swelling at posterior of corpus. Deirids at level of corpus swelling. Esophageal bulb lacking valves. Excretory pore located at level of esophageal bulb, opening into large sinus.

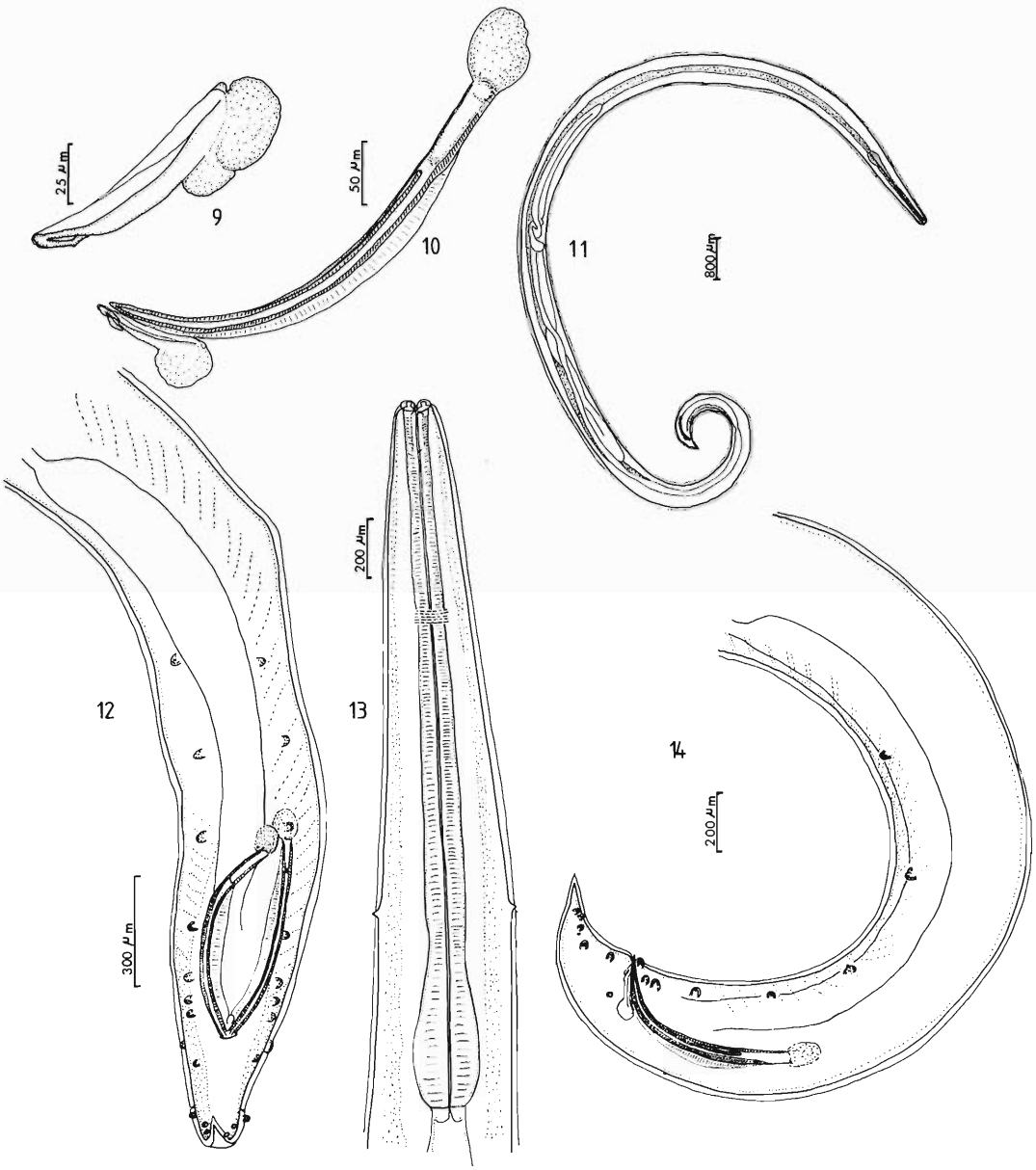
MALE: Caudal end curved ventrally. Preanal musculature consisting of 52–69 muscle pairs. Pseudosucker absent. Tail short. Caudal papillae arranged as follows: 7 pairs of preanal and median unpaired papillae on anterior anal lip, 5 pairs postanal (first and third pairs lateral). Holotype and 1 paratype showing the above papillae arrangement; 2 paratypes showing variation in papillae number. In these cases the fourth postanal pair (subventral) was absent in one specimen and represented by a single papilla in the other specimen. Phasmids between third (lateral) and fourth pairs of papillae. Spicules equal and broadly alate. Gubernaculum with rounded more dorsal end and crochet hook appearance on pointed ventral end.



Figures 1–8. ***Megalobatrachonema moraveci*** **sp. n. 1. Female reproductive tract. 2. Egg. 3. Cephalic extremity, apical view. 4. Cephalic extremity, optical section of apical view. 5. Cephalic extremity, ventral view. 6. Cephalic extremity, lateral view. 7. Caudal end of female, lateral view. 8. Excretory system, lateral view.**

FEMALE: Vulva in posterior third of body. Vagina muscular, anteriorly directed. Uteri amphidelphic. Ovary associated with posterior uterus originating in anterior of worm, running posteriorly, flexing anteriorly, then flexing posteriorly 15–22% body length behind esophageal bulb, and continuing to level of vulva before flexing anteriorly at oviduct. Oviduct emptying into uterus; uterus flexing anteriorly to join opposing uterus at vagina. Ovary associated with anterior uterus originating near rectum, extending anteriorly to within 1–13% body length from opposing ovary flexure, flexing posteriorly, coiling back on itself once then emptying into oviduct. Oviduct flexing anteriorly at junction with uterus; uterus flexing posteriorly and merging with opposite uterus at vagina.

MEASUREMENTS OF MALE (based on 4 speci-



Figures 9–14. ***Megalobatrachonema moraveci*** **sp. n. 9. Gubernaculum, lateral view. 10. Spicule and gubernaculum, lateral view. 11. Entire worm, male. 12. Caudal end of male, ventral view. 13. Ventral view showing position of nerve ring, deirids, narrowing of isthmus, and elongate esophageal bulb. 14. Caudal end of male, lateral view.**

mens): Length 21–31 (26) mm. Maximum width 619–715 (660). Esophagus 2,027–2,742 (2,529) including pharyngeal portion 130–200 (162). Bulb width 165–240 (215). Nerve ring 626–755 (709), deirids 1,612–1,985 (1,850), and excretory pore 1,717–2,491 (2,226) from anterior end. Tail 329–380 (355) long. Anterior flexure of testis 6,740–7,754 (7,277) posterior to esophageal bulb. Right spicule 600–646 (636) long by 42–50 (45) wide, left spicule 605–685 (649) long by 43–50 (48) wide; gubernaculum 128–160 (143) long by 27–32 (28) wide in lateral view.

MEASUREMENTS OF FEMALE (based on 4 specimens): Length 32–37 (34) mm. Width at vulva 832–890 (869). Esophagus 2,340–2,629 (2,489) including pharyngeal portion 165–172 (169). Bulb

width 230–280 (261). Nerve ring 680–752 (727), deirids 1,690–1,896 (1,813), and excretory pore 1,940–2,507 (2,245) from anterior end. Tail 676–820 (730) long. Vagina 440–560 (521) long. Vulva 8,861–10,100 (9,292) from posterior end. Distance from most anterior flexure of ovary to esophageal bulb 4,828–7,018 (5,702). Total egg number 767–1,325 (1,050), eggs 69–82 (76) long by 60–70 (67) wide.

HOST: *Taricha granulosa.*

SITE: Intestine.

LOCALITY: Brannen Lake, Nanaimo and unnamed lake, Cambell River (sometimes referred to as Quarry Lake, found at 50°15′ lat. by 125°40′ long.), Vancouver Island, British Columbia, Canada.

SPECIMENS DEPOSITED: U.S. National Museum Helm. Coll. holotype #79940 and 7 paratypes #79941; Czechoslovakia Institute of Parasitology 5 paratypes #N-244.

ETYMOLOGY: The new species is named in honor of Frantisek Moravec of the Czechoslovak Academy of Sciences, who was the first to signal the presence of a *Megalobatrachonema* sp. in *Taricha* from this area.

PREVALENCE AND INTENSITY DATA: Six out of 12 (50%) adult *Taricha granulosa* were infected with *M. moraveci.* Mean intensity of infection was 5.8 worms/host.

Discussion

Megalobatrachonema includes 5 species in addition to *M. moraveci* sp. n. These are divided between 2 subgenera on the basis of presence or absence of esophageal valves. According to this scheme, the new species can be referred to *M.* (*Chabaudgolvania*), which includes those species that lack esophageal valves. Other members of this subgenus are *M. elongata* (Baird, 1858) Baker, 1986, found in *Ambystoma* in Mexico, *M. terdentatum* (Linstow, 1890) Hartwich, 1960 (syn. *M. campanae* Chabaud and Golvan, 1957), found in *Triturus* spp. in Germany, Czechoslovakia, and France, and *M. waldeni* Richardson and Adamson, 1988, found in *Ambystoma gracile* in British Columbia, Canada. *Megalobatrachonema moraveci* resembles these species by its small lips, slender isthmus, and lack of valves in the esophageal bulb.

The present material agrees in all essential respects with that described by Moravec (1984). Moravec had at his disposal only a single female specimen and therefore could not give a complete description. He tentatively referred his material to *M. terdentatum.* However, *M. moraveci* differs from *M. terdentatum* by the distinct separation of the cephalic lips, presence of a labial cuticular thickening, more posterior position of the excretory pore, and slight swelling of the corpus. Male *M. moraveci* further differs from *M. terdentatum* in lacking a pseudosucker, in having 2 rather than 1 pair lateral postanal papillae, and in having 2 more preanal pairs.

Megalobatrachonema moraveci most closely resembles *M. waldeni* Richardson and Adamson, 1988, found in *Ambystoma gracile* of British Columbia. Both species have distinctly separate lips, a swelling at the base of the corpus, an elongate esophageal bulb, and lateral alae. *Megalobatrachonema moraveci* differs by its prominent hypodermal peduncles with raised cephalic papillae, labial cuticular thickening, and lack of cervical alae. Males of *M. moraveci* differ from *M. waldeni* by their hook-shaped gubernaculum, broadly alate spicules, and shorter tails. Males also have 1 more pair of postanal lateral papillae and 3 more preanal papillae than *M. waldeni.* Females differ in having more eggs than those of *M. waldeni.*

The labial cuticular thickening of *M. moraveci* is distinct from the cheilostomal ring found in members of *Falcaustra, Kathlania,* and *Tonaudia* (see Inglis, 1966), and in *Megalobatrachonema nipponicum* and *M. gigantica* according to Baker (1980). It lacks internal struts and tissue arcade characteristic of the cheilostomal ring but may be a convergent structure affording lip support.

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Report on the Brayton H. Ransom Memorial Trust Fund

The Brayton H. Ransom Memorial Trust Fund was established in 1936 to "encourage and promote the study and advance of the Science of Parasitology and related sciences." Income from the Trust currently provides token support of the *Proceedings of the Helminthological Society of Washington* and limited support for publication of meritorious manuscripts by authors lacking institutional or other backing. Contributions may be directed to the Secretary-Treasurer.

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55(2), 1988, pp. 160–164

Morphology of the Synlophe of *Nematodirus maculosus* (Trichostrongyloidea) with Comments on the Evolution of *Nematodirus* spp. Among the Caprinae (Artiodactyla)[1]

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ABSTRACT: The synlophe of male specimens of *Nematodirus maculosus* is characterized. The 16-ridge bilaterally symmetrical system in the cervical region appears to be unique when compared to those species of *Nematodirus* that have been studied in detail. *Nematodirus maculosus* appears most similar to those species from the Bovidae, characterized by long lateral ridges, a small number of ridges (generally 14) at the midbody, and short symmetrical spicule tips. Results of this study and previous investigations allowed alternative hypotheses to be developed for the evolution and historical biogeography of some *Nematodirus* spp. among the Caprinae (tribes Caprini and Rupicaprini) in the Holarctic.

KEY WORDS: *Nematodirus maculosus, Nematodirus* spp., Trichostrongyloidea, Bovidae, synlophe, coevolution, biogeography, cuticle.

The synlophe is a useful character for differentiation of genera (Durette-Desset, 1983) and related species of trichostrongyloid nematodes (Lichtenfels, 1983). In those studies 2 methodologies were generally employed: (1) a relatively narrow approach that stresses the number and axis of orientation of cuticular ridges at the midbody (Durette-Desset, 1983) or (2) a more synoptic examination of the number of ridges, their pattern in the cervical region, and the longitudinal extent of the synlophe (Lichtenfels and Pilitt, 1983a, b). Accurate evaluations of the synlophe were generally not included among early descriptions of trichostrongyloid nematodes, and relatively few species have been studied using modern techniques.

Detailed descriptions of the cuticular ridge systems for *Nematodirus* spp. from domestic ruminants in North America and Europe have been provided by Durette-Desset (1979), Lichtenfels and Pilitt (1983a), and Hoberg et al. (1986), but relatively little information is available on species parasitizing sylvatic hosts (Rossi, 1983). *Nematodirus maculosus* Becklund, 1965, is 1 of 4 species (*N. tarandi* Hadwin, 1922; *N. archari* Sokolova, 1948; and *N. odocoilei* Becklund and Walker, 1967) that parasitize sylvatic bovids and/or cervids in North America. At the time of the original description, from *Oreamnos americanus* (Blainville) (subfamily Caprinae), the synlophe had not been recognized as a significant character in nematode systematics. Although Becklund (1965) enumerated the ridges for both male and female specimens, an interpretation of the cervical synlophe was not provided. In the current study we present a detailed description of the synlophe of male specimens of *N. maculosus,* from the type host and *Ovis canadensis* Shaw, that will allow a comparison to ridge-patterns known for other species from ruminants, particularly bovids of the tribes Caprini and Rupicaprini in the Holarctic.

Materials and Methods

Specimens of *Nematodirus maculosus* were studied using interference-contrast light microscopy (Leitz) and scanning electron microscopy. Prior to examination, material for light microscopy was transferred to 70% ethanol/5% glycerine and cleared in glycerine by evaporation. Eight specimens were prepared as temporary whole mounts and 2 specimens were studied in transverse sections cut by hand with a scalpel blade or from paraffin-embedded material (latter provided by J. R. Lichtenfels). A single specimen was examined using scanning electron microscopy (stub deposited as part of USNM Helm. Coll. 58180).

The description of the synlophe is consistent with the methodology and terminology presented by Lichtenfels and Pilitt (1983a) for other species of *Nematodirus* from domestic ruminants. The current study was based solely on male specimens as females were not available. In the description all measurements are given in micrometers unless stated otherwise.

SPECIMENS EXAMINED: *N. maculosus* from *Oreamnos americanus,* USNM Helm. Coll. No. 58180 (5 males from Rattlesnake Creek, Montana), No. 58747 (1 male embedded in paraffin and sectioned, from Canada), and No. 66613 (4 males from South Dakota); and from

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Ovis canadensis, No. 66607 (1 male from Alberta, Canada).

Results

The synlophe of *Nematodirus maculosus* is composed of a bilaterally symmetrical system of 16 ridges, with an orientation parallel to the longitudinal body axis, in the cervical region (Fig. 1). Eight ridges extend to the base of the cephalic capsule: a pair of minute lateral ridges (unpaired and unnumbered) and dorsal–ventral ridges numbered 3–5 (paired) are continuous in the cervical zone. Four pairs of lateral ridges are discontinuous: ridges numbered 1 and 7 terminate anteriorly at the level of the cervical papillae; those numbered 2 and 6 end about 100–150 from the base of the cephalic capsule (Figs. 1, 2). The fourth ventral ridge is interrupted at the level of the excretory pore (Fig. 3). All lateral ridges are narrow and become diminished in height anteriad. Ventral–dorsal ridges are relatively robust with the fourth pair being finlike and hypertrophied. The spacing of the lateral ridges is narrow compared to those in the ventral and dorsal fields.

The number of ridges decreases extending posteriad from the cervical region. Minute lateralmost ridges terminate before the midbody. Lateral ridges numbered 1, 2, 7, and 6 are continuous; those numbered 1 and 7 are briefly interrupted for approximately 20 at a distance about 230 posterior to the cervical papillae. A reduction in height of the lateral ridges is evident toward the midbody (Figs. 4–6). The ventral and dorsal synlophe continues with a slight gradient in height (dorsal smaller than ventral) until the dorsal ridges become markedly attenuated 2–3 mm from the prebursal papillae. Robust ridges continue on the ventral surface to within 190 from the prebursal papillae where they become reduced in height. The synlophe extends from this region as a series of minute ridges, not discernible with the light microscope, that terminate 50 from the base of the copulatory bursa (Fig. 7).

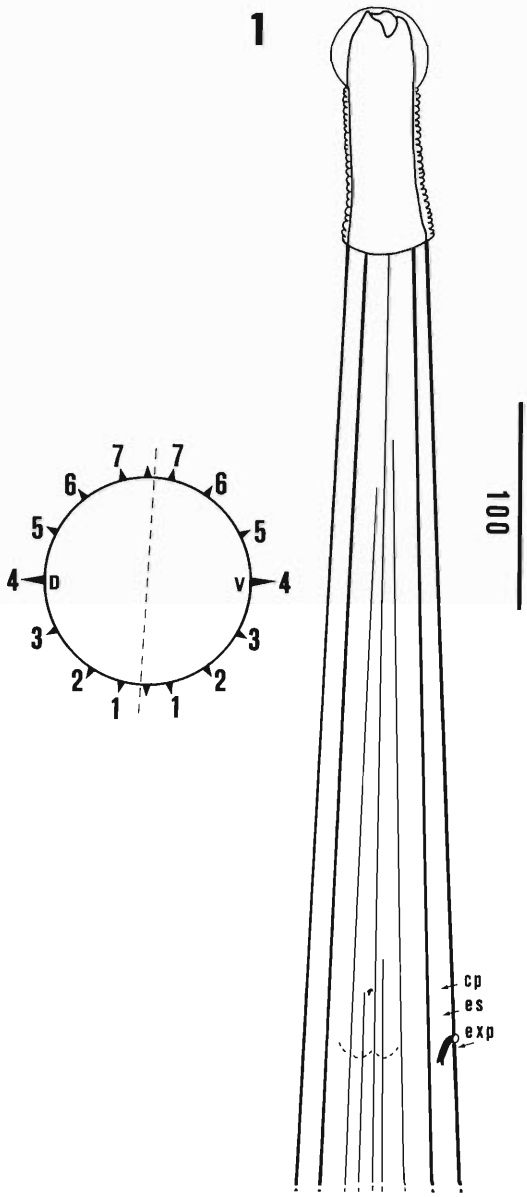
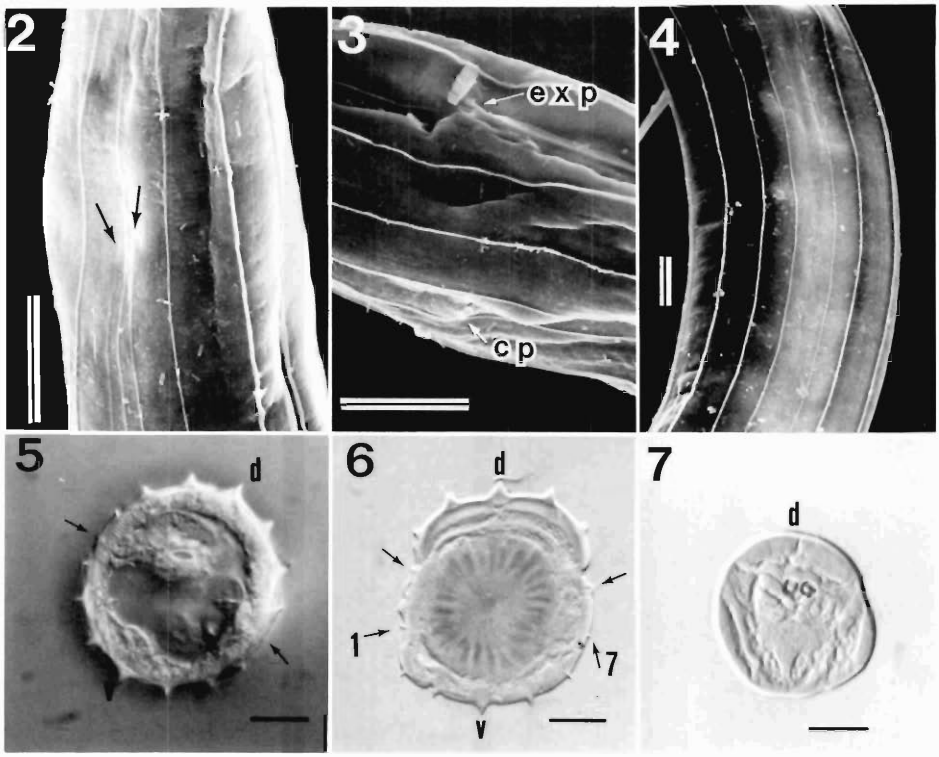


Figure 1. Synlophe in the cervical region of *Nematodirus maculosus* showing right lateral view (from camera lucida) and schematic of transverse section near the level of the excretory pore; D = dorsal, V = ventral.

Discussion

Observations from the present study in part confirm those of Becklund (1965) who reported 14 longitudinal ridges for male and female specimens of *N. maculosus*. Although 16 ridges were found in the cervical zone of male specimens, 14 were found at the midbody during the present study. Previous studies of *Nematodirus* spp. have indicated the similarity in the pattern of the cervical synlophe in male and female conspecifics (Lichtenfels and Pilitt, 1983a). Thus, it is anticipated that females of *N. maculosus* may be identified by the low number of cuticular ridges in the cervical region and by the unique structure of the synlophe anterior to the cervical papillae and excretory pore. Specimens of *N. maculosus* have fewer cervical ridges than any species of *Nematodirus* so far examined, although a rela-



Figures 2–7. Scanning electron microscopy and interference-contrast microscopy of the synlophe of *N. maculosus*. 2. Right lateral view in cervical region, anterior to cervical papilla, showing narrow lateralmost ridge and termination of ridge 2 (arrows). 3. Synlophe in cervical region at level of excretory pore (exp) and right cervical papilla (cp); anterior is to the left. 4. Synlophe in midbody, lateral view. 5. Transverse section in postcervical region showing 16 ridges; note minute lateralmost ridges (arrows). 6. Transverse section in midbody showing 14 ridges; note minute lateral ridges (arrows) numbered 1 and 7. 7. Posterior, toward distal end of spicules; ridges not discernible in transverse section with the light microscope. All scale bars = 20 μm. In Figures 5–7, v = ventral, d = dorsal.

tively great number are continuous to the cephalic capsule.

Criteria developed by Lichtenfels and Pilitt (1983a) suggested that *N. maculosus* was most similar to *N. filicollis* (Rudolphi, 1802) and *N. davtiani* Grigorian, 1949. These 3 species are characterized by the lateralmost pairs of ridges extending anteriorly more than ⅓ of the cervical distance, 14 ridges at the midbody, finlike dorsal and ventral ridges, a large copulatory bursa, and short symmetrical spicule tips. The characters of the synlophe have been postulated as synapomorphies for *N. filicollis* and *N. davtiani* (see Lichtenfels and Pilitt, 1983a), thus, the morphological similarity of *N. maculosus* could have a historical phylogenetic basis. Additionally, the minute, unpaired, discontinuous, lateralmost ridges in the cervical zone of *N. filicollis, N. davtiani,* and *N. maculosus* appear to be homologous. However, in specimens of *N. maculosus* these ridges are larger and extend posteriad beyond the cervical papillae, potentially representing a hypertrophied condition.

The cervical synlophe has been examined in detail for only 7 species of *Nematodirus* from domestic ruminants in North America (Lichtenfels and Pilitt, 1983a; Hoberg et al., 1986). However, data are available for the midbody region of 16 species from the Bovidae (Bovinae, Caprinae), Cervidae, and Camelidae in the Holarctic and Neotropics (Durette-Desset, 1978, 1979; Rossi, 1983). In addition to *N. maculosus, N. filicollis,* and *N. davtiani,* only 2 other species, viz. *N. hugonotae* Rossi, 1983, and *N. ibicis* Biocca, Balboa, and Costantini, 1982, were described with 14 ridges (all others have 18 or more). Preliminary studies of *N. archari* have also indicated the presence of 14 ridges in this species (Rickard and Lichtenfels, pers. comm.). Thus, there are at least 6 species, characterized by a postulated syn-

apomorphy (14 midbody ridges), that are also primarily parasites of the Caprinae (tribes Caprini and Rupicaprini).

The association between the Caprinae and *Nematodirus* spp. is thought to be of relatively recent origin in the Pleistocene (Rossi, 1983). Current hypotheses for the evolution and biogeography of this assemblage postulate multiple events of long-range dispersal and widespread colonization of the Caprini and Rupicaprini late in the history of these host groups. Both tribes of the Bovidae originated in the Palearctic during the Miocene and Pliocene. However *Nematodirus* (and Nematodirinae) is considered to be of Nearctic origin among the Camelidae (Durette-Desset and Chabaud, 1977; Rossi, 1983). Camels had dispersed across Beringia to Eurasia by the Pliocene, postdating the early evolution of the Caprinae, but became extinct in the Nearctic by the late Pleistocene (Kurtén and Anderson, 1980; Thenius, 1980). Rossi (1983) and Durette-Desset (1985) contended that *Nematodirus* spp. became parasites of the Caprinae via colonization from camelids only after the former host group had dispersed into North America during the Pleistocene. Thus, the widespread occurrence of *Nematodirus* spp. among some contemporary Caprinae in the Palearctic would have resulted from a subsequent event of dispersal (from the Nearctic) and broad colonization and speciation of nematodes among sylvatic sheep and antelope during the late Pleistocene. Current evidence however indicates that the primary movements of caprine bovids were from west to east across Beringia during the Wisconsin (and earlier during the Pleistocene), with little indication of dispersal into the Palearctic (Kurtén and Anderson, 1980; Geist, 1985).

Results from the present study and those provided by Lichtenfels and Pilitt (1983a) support alternative hypotheses for the evolution of *Nematodirus* among the Caprini and Rupicaprini. Two postulated synapomorphies may unite *N. maculosus* and other species (14 ridges at the midbody and anterior extent of the lateral cervical ridges), suggesting that some *Nematodirus* spp. have coevolved with these host groups. Species with a low number of midbody ridges that occur among the Caprini and Rupicaprini appear to be highly derived and otherwise do not share synapomorphic characters with species typical of camelids or other hosts. Such observations along with the biogeographic and evolutionary history of these ruminants (see Kurtén and Anderson, 1980; Geist, 1985, 1987) suggest that *Nematodirus* initially became parasites of the Caprinae in the Palearctic during the late Tertiary. Thus, only a single event of colonization of the Caprinae (from camelids) during the Pliocene is postulated. Dispersal from the Palearctic by precursors of *Ovis* spp. and *Oreamnos,* already parasitized by *Nematodirus,* into the Nearctic during the Pleistocene would then account for the contemporary distribution of some sylvatic hosts and their evolutionarily derived parasites in North America. This contention is further supported by the high diversity of *Nematodirus* spp. associated with sylvatic sheep, goats, and antelope in Eurasia (see Kulmamatov, 1974) and the paucity of nematodes with Holarctic distributions among these hosts. Additionally, the lack of endemic species of *Nematodirus* among bovids in Africa (Durette-Desset, 1979) and the history of bovid tribes (including the Caprini) in sub-Saharan Africa (see Vrba, 1985) suggests that the Caprinae were colonized in the Palearctic during the late Pliocene. Among other Nematodirinae, it has been postulated that host-switching from camelids to bovids by species of *Nematodirella* Yorke and Maplestone, 1926, also occurred in Eurasia prior to the Pleistocene (Lichtenfels and Pilitt, 1983b). Continued studies of *Nematodirus* spp. and eventual phylogenetic analyses will be required to fully evaluate these hypotheses.

Acknowledgments

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A History of Animal Parasitology in USDA

A chapter entitled "Animal Parasitology in the United States Department of Agriculture, 1886–1984," by the late Dr. John S. Andrews, is included in pages 113–166 of "*100 Years of Animal Health, 1884–1984,*" published as Journal of NAL Associates, Vol. 11(1–4), January/December 1986. This publication commemorates the centennial of the establishment of the Bureau of Animal Industry in USDA. It is available for $17.00 U.S. from: Associates of the National Agricultural Library, Inc., Room 203, 10301 Baltimore Boulevard, Beltsville, Maryland 20705, U.S.A.

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Cylicostephanus torbertae sp. n. (Nematoda: Strongyloidea) from *Equus caballus* with a Discussion of the Genera *Cylicostephanus, Petrovinema,* and *Skrjabinodentus*

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ABSTRACT: *Cylicostephanus torbertae* sp. n. is described. The new species differs from others in the genus in having esophageal teeth exceptionally deep in a long esophageal funnel and a vulva positioned at a greater distance from the anus than the length of the tail. The genera *Cylicostephanus, Petrovinema,* and *Skrjabinodentus* are discussed. *Cylicostephanus* (*Cylicostephanus*) is proposed to include *C.* (*C.*) *calicatus, C.* (*C.*) *longibursatus, C.* (*C.*) *minutus, C.* (*C.*) *hybridus, C.* (*C.*) *goldi* (=*C.* (*C.*) *ornatum*), *C.* (*C.*) *asymetricus,* and *C.* (*C.*) *bidentatus. Cylicostephanus* (*Skrjabinodentus*) is proposed to include *C.* (*S.*) *caragandicus, C.* (*S.*) *tshoijoi,* and *C.* (*S.*) *longiconus. Petrovinema* is *accepted* for *P. poculatus* and *P. skrjabini.*

KEY WORDS: helminth, horse, parasite, systematics, taxonomy, Nematoda, *Cylicostephanus* (*C.*) *torbertae* sp. n., *Cylicostephanus* (*C.*) *calicatus, Cylicostephanus* (*C.*) *longibursatus, Cylicostephanus* (*C.*) *minutus, Cylicostephanus* (*C.*) *hybridis, Cylicostephanus* (*C.*) *asymetricus, Cylicostephanus* (*C.*) *bidentatus, Cylicostephanus* (*C.*) *goldi* (=*C. ornatum*), *Cylicostephanus* (*Skrjabinodentus*) *caragandicus* comb. n., *Cylicostephanus* (*Skrjabinodentus*) *longiconus* comb. n., *Cylicostephanus* (*Skrjabinodentus*) *tshoijoi* comb. n., *Petrovinema poculatus, Petrovinema skrjabini,* Strongyloidea, *Equus caballus.*

Among the Cyathostominae collected from ponies in Louisiana (Torbet et al., 1986) was an undescribed species of the genus *Cylicostephanus* (Ihle, 1922). A decription of the new species is presented here.

The Cyathostominae are small strongylid nematodes parasitic, frequently in large numbers, in the large intestine of equids where they may cause considerable unthriftiness and loss of condition. Heavy infections may cause severe diarrhea (Ogbourne, 1978).

The genus *Cylicostephanus* was proposed by Ihle (1922) as a subgenus for 5 species of small strongyles with a depressed mouth collar including *C. calicatus* (Looss, 1900), *C. longibursatus* (Yorke and Macfie, 1918), *C. minutus* (Yorke and Macfie, 1918), *C. populatus* (Looss, 1900), and *C. hybridus* (Kotlan, 1920). Cram (1925) added 2 species, *C. asymetricus* (Theiler, 1923) and *C. bidentatus* (Ihle, 1925) and raised *Cylicostephanus* to generic level. Ershov (1943) removed *C. poculatus* to another genus, *Petrovinema,* but Lichtenfels (1975) returned *C. poculatus* to the genus *Cylicostephanus* and added *C. skrjabini* (Ershov, 1930), *C. goldi* (Boulenger, 1917), and *C. ornatum* (Kotlan, 1919). *Cylicostephanus longiconus* Scialdo-Krecek (1983) was described from the mountain zebra in southern Africa. Recently, Hartwich (1986), in a revision of the Cyathostominae, followed Ershov (1943) in placing *C. poculatus* and *C. skrjabini* in the genus *Petrovinema* and synonymized *C. ornatum* with *C. goldi.* Hartwich (1986) also provisionally placed the poorly described species, *C. caragandicus* (Funicova, 1939), in the genus *Cylicostephanus.* However, Dvoinos and Chartshenko (1986) studied newly collected specimens of *C. caragandicus* and retained it and a new species, *Skrjabinodentus tshoijoi,* in the genus *Skrjabinodentus* Tshoijo, 1957, because of the distinctly different character of the dorsal ray (only 4 branches). Dvoinos and Chartshenko (1986) also stated without further explanation that *C. longiconus* is a synonym of *C. caragandicus.* We do not accept this synonymy because of the much larger number of elements in the leaf crowns of *C. longiconus.* Thus, if *Petrovinema* is recognized and if *Skrjabinodentus* is accepted (tentatively) and *S. longiconus* is included in it as a third species, then *Cylicostephanus* includes 7 species from horses, including the new species described herein.

Materials and Methods

Hosts

Hosts and necropsy procedures were described by Torbert et al. (1986). Briefly, the ponies (*Equus caballus*) were collected in southern Louisiana and had a history of little or no anthelmintic treatment.

Nematodes

Specimens were fixed in formalin, cleared in phenol–alcohol (80 parts melted phenol crystals and 20 parts absolute ethanol), and studied with the aid of interference-contrast light microscopy. Drawings were prepared with the aid of a camera lucida. All measurements are given in millimeters. Type specimens were deposited in the U.S. National Museum Helminthological Collection, USDA, ARS, Beltsville, Maryland 20705.

Results

The new species was discovered among specimens of *Cylicodontophorus mettami* after specimens of that species from 3 hosts had been grouped in 1 lot. It was not possible, therefore, to determine whether more than 1 host was infected. The following generic diagnosis follows Lichtenfels (1975), but includes the modifications proposed by Hartwich (1986).

Diagnosis

Cylicostephanus

Strongyloidea, Strongylidae, Cyathostominae. Small to medium sized, 4–14 long. Mouth collar depressed. Lateral papillae (amphids) salient but not prominent. Submedian papillae prominent. Elements of external leaf-crown (ELC) longer, usually broader, and fewer than elements of internal leaf-crown (ILC), which are short pointed rods or triangular plates inserted close to the anterior, internal edge of the buccal capsule. Circular, sclerotized mouth collar support, usually small, cone-shaped in optical section, extends from outer anterior edge of buccal capsule toward mouth collar. Buccal capsule of varying thickness, usually with dorsal gutter. Buccal cavity usually slightly narrower anteriorly.

MALE: Dorsal rays of bursa split to level of proximal branch or to origin of externodorsal rays. Prebursal papillae not prominent. Spicules with pick-shaped or harpoon-shaped tip.

FEMALE: Vulva near anus.

Cylicostephanus torbertae sp. n. (Figs. 1–13; measurements in Table 1)

DESCRIPTION (based on 4 males and 7 females): With characteristics of the genus. Submedian cephalic papillae bipartite with proximal and distal segments of equal length. Lateral papillae (amphids) not elevated above surface of mouth collar. Buccal capsule cylindrical, wider than deep. Mouth collar support at top edge of buccal capsule elongate, triangular wedge in optical section; extends to internal surface of inflated mouth collar. External leaf-crown (ELC) of 30–38 elongate triangular elements that project beyond mouth collar for ½ of length of elements. Internal leaf-crown (ILC) extremely difficult to see, composed of triangular plates as broad at base as tall, 1.0–1.5 times as numerous as ELC elements. Dorsal gutter ½ as long as depth of buccal capsule. Nerve ring anterior to middle of esophagus. Excretory pore and elongate cervical papillae (deirids) slightly behind nerve ring. Esophagus of uniform thickness anterior to nerve ring, broadly claviform behind nerve ring. Esophageal–intestinal (E-I) valve short. Three teeth deep in esophageal funnel. Cuticle marked with annulation from region of buccal capsule to posterior end.

MALE: Trilobed copulatory bursa longer than broad in lateral view. Dorsal ray with median fissure to level of externodorsal ray; with 6 branches. Genital cone elongate, does not reach anterior margin of bursa. Gubernaculum massive with large proximal dorsal portion and notched ventral groove. Spicules with harpoon-shaped tip.

FEMALE: Vulva located farther from anus than length of tail. Vagina and vestibule short. Infundibula longer than sphincters. Eggs twice as long as wide.

HOST: *Equus caballus.*

SITE OF INFECTION: Large intestine.

LOCALITY: Southern Louisiana, U.S.A.

HOLOTYPE: Male. USNM Helm. Coll. No. 80205.

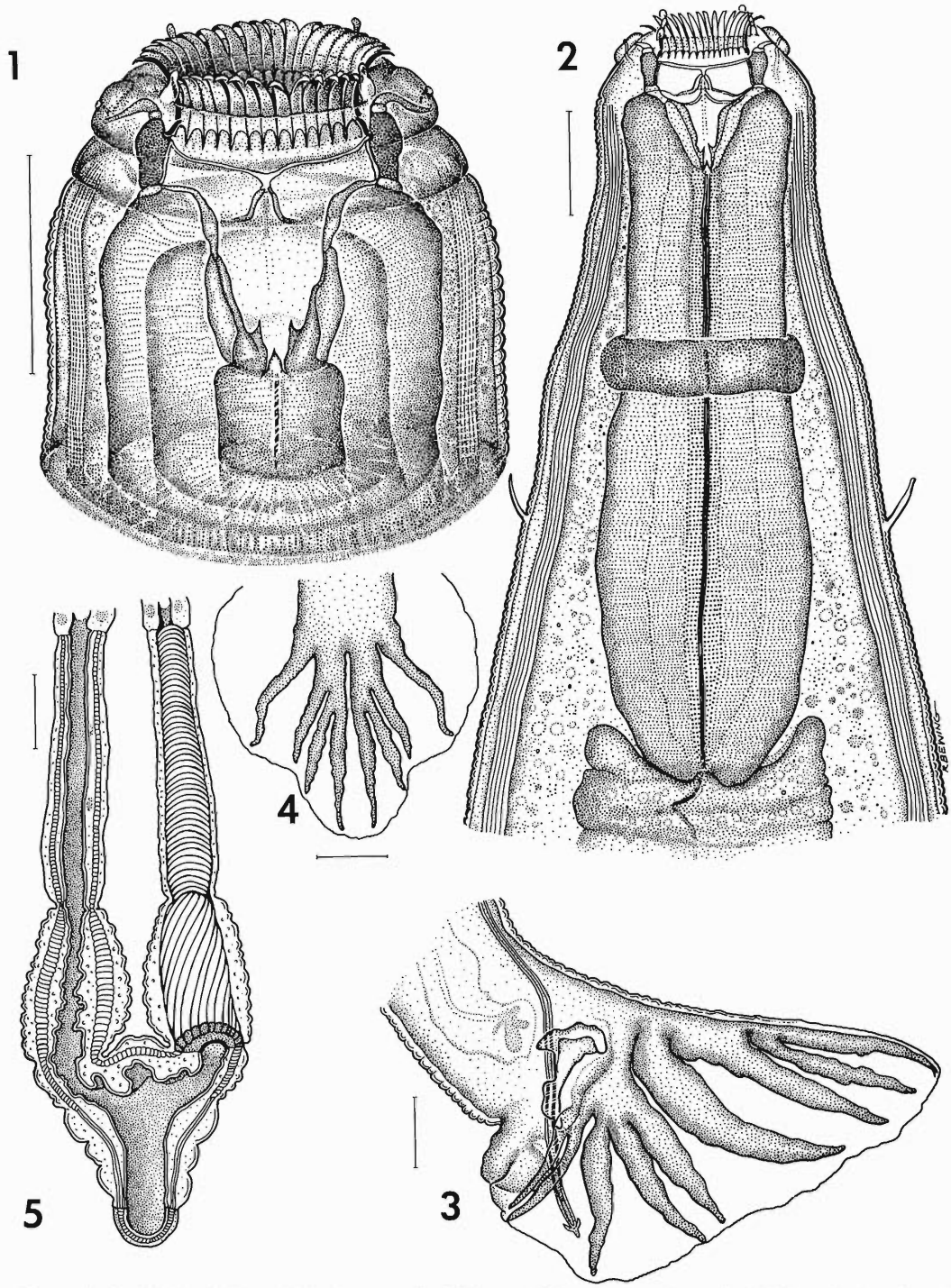
ALLOTYPE: Female. USNM Helm. Coll. No. 80206.

PARATYPES: Three males and 6 females, USNM Helm. Coll. No. 80207.

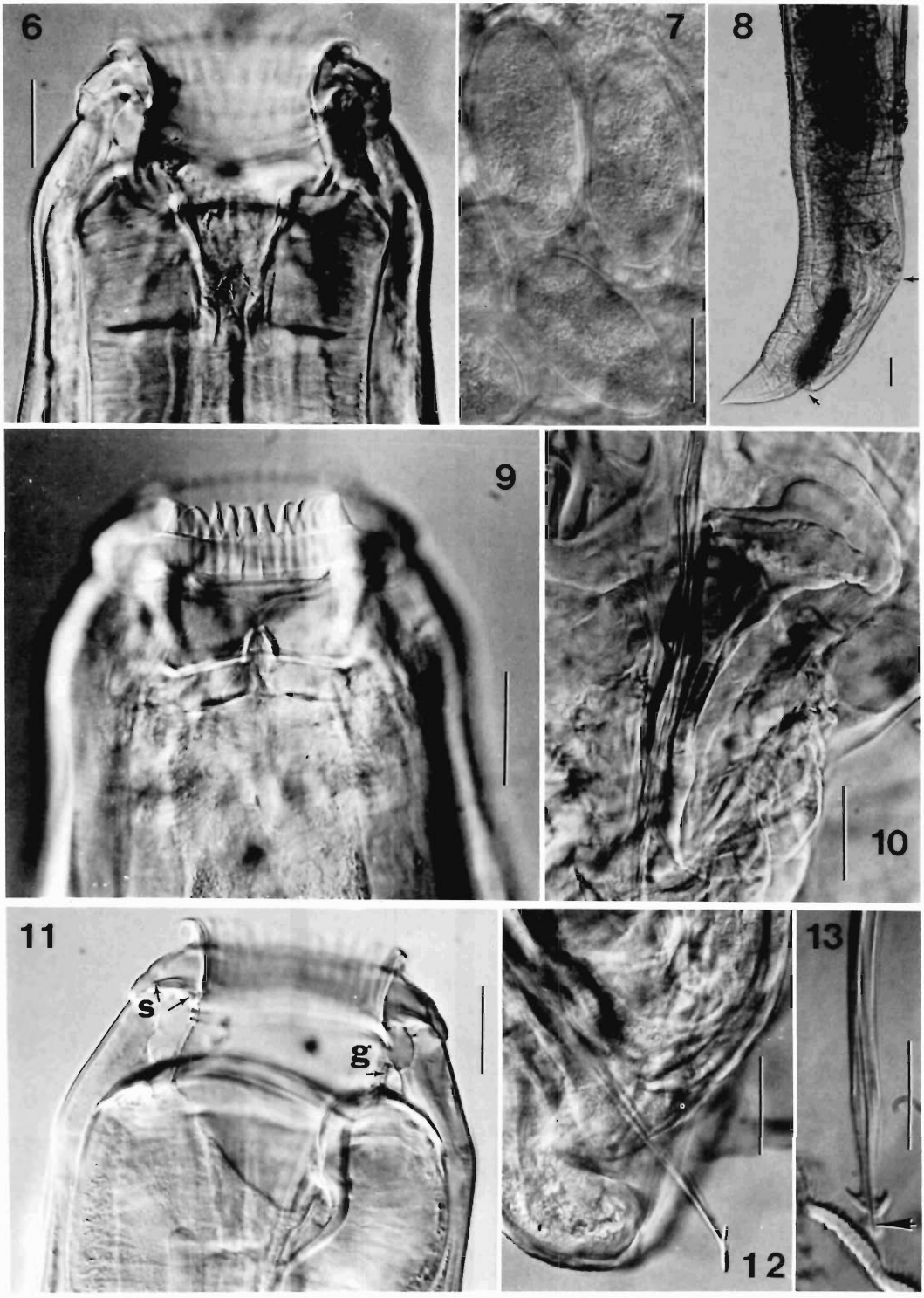
ETYMOLOGY: The species is named in honor of Mrs. Betty J. Torbert who recently retired from Louisiana State University after long and dedicated service in the Department of Veterinary Science.

Remarks

Both sexes of this species can be separated from all other species of the genus by 2 characteristics of the stoma and associated structures: (1) the exceptionally deep esophageal funnel and the deep location of the 3 esophageal teeth, and (2) the mouth collar support at the anterior external edge of the buccal capsule is longer than in any other species in the genus. In addition, the vulva position of *C. torbertae* sp. n. is more anteriorly placed than in any other species of the genus.



Figures 1–5. *Cylicostephanus torbertae* **sp. n. Scale bars = 100 μm. 1. Anterior end of allotype female, dorsal view. 2. Anterior end of allotype female, dorsal view, showing entire esophagus. 3. Ovijector, composite. 4. Dorsal ray of bursa, paratype. 5. Bursa of holotype male, lateral view.**



Figures 6–13. Interference-contrast photomicrographs of *Cylicostephanus torbertae* sp. n. 6. Anterior end of allotype, dorsoventral view, showing 3 teeth (arrows) deep in esophageal funnel. Scale bar = 50 μm. 7. Eggs in uterus of paratype. Scale bar = 50 μm. 8. Paratype female tail, vulva at upper arrow, anus at lower arrow. Scale

Table 1. Morphometrics (range followed by the mean in millimeters) of *Cylicostephanus torbertae* sp. n.

Character	Males	Females
Number of specimens	4	7
Body length	7.52–8.89 (7.98)	8.09–13.1 (10.6)
Diameter at E-I	0.296–0.385 (0.335)	0.385–0.451 (0.427)
Mouth collar height	0.030–0.034 (0.031)	0.027–0.042 (0.034)*
Number elements in ELC	30–34 (32.5)	30–38 (34.3)
Submedian cephalic papillae		
Proximal segment	0.007–0.010 (0.008)	0.008–0.012 (0.009)*
Distal segment	0.007–0.007 (0.007)	0.007–0.011 (0.009)*
Anterior end to:		
Nerve ring	0.304–0.342 (0.320)	0.300–0.392 (0.346)
Cervical papilla	0.399–0.520 (0.441)	0.312–0.555 (0.450)
Excretory pore	0.380–0.555 (0.455)†	0.342–0.518 (0.436)*
Junction, esophagus and intestine (E-I)	0.659–0.740 (0.690)	0.755–0.873 (0.796)
Width of cuticular annule at E-I	0.006–0.008 (0.007)	0.006–0.011 (0.008)
Buccal capsule width	0.110–0.125 (0.117)	0.125–0.163 (0.142)
Buccal capsule depth	0.034–0.041 (0.038)	0.030–0.049 (0.041)
Esophageal funnel depth	0.082–0.099 (0.090)	0.084–0.106 (0.091)
Egg (length × width)		0.114–0.133 × 0.057–0.065 (0.123 × 0.060)
Vulva to anus length		0.350–0.659 (0.526)
Vagina length		0.160–0.198 (0.176)
Vestibule length		0.084–0.152 (0.115)
Sphincter length		0.182–0.247 (0.203)
Infundibulum length		0.304–0.399 (0.362)
Spicule length	1.14–1.30 (1.23)†	
Gubernaculum length	0.190–0.274 (0.242)	
Tail length		0.236–0.414 (0.308)

* $N = 6$.
† $N = 3$.

Discussion

Hartwich's (1986) revision of the Cyathostominae made 2 significant changes in the genus *Cylicostephanus*. The most significant change was the recognition of a mouth collar support (termed "support" by Hartwich (1986) and labeled "s" in his figures) on the outer anterior edge of the buccal capsule wall. This structure, previously described only in the genus *Cyathostomum*, was named the "problematic structure in substance of mouth collar" by Looss (1902) and the "extrachitinous support" by Theiler (1923). Hartwich's (1986) use of "support" for this structure appears to us to be an improvement because it deletes chitinous from the name. However, we believe "mouth collar support" as used herein is a more precise and informative name for this structure. The second change proposed by Hartwich (1986) was the removal of *C. poculatus* and *C. skrjabini* to the genus *Petrovinema* as proposed earlier by Ershov (1943). Lichtenfels (1975) recognized that these 2 species were different, but because the generic definition used by Lichtenfels (1975) was broad enough, the 2 species were included in *Cylicostephanus*. We now agree with Hartwich (1986) that both *Cylicostephanus* and *Petrovinema* should be recognized. Both of these changes proposed by Hartwich (1986) are considered to be useful and to improve our understanding of the genus *Cylicostephanus*.

The acceptance of *Skrjabinodentus* is tentative because it is based solely on the 4-branched configuration of the dorsal ray. Lichtenfels (1980) reviewed the variability in branching of the dorsal ray in numerous genera of the Strongyloidea. Grouping the 3 species with only 4 branches on the dorsal ray (although *S. longiconus* shows a

←
bar = 100 μm. 9. Anterior end of paratype male, dorsal view, showing dorsal gutter, and elements of external leaf-crown and internal leaf-crown. Scale bar = 50 μm. 10. Gubernaculum of paratype. Scale bar = 50 μm. 11. Anterior end of holotype male, lateral view, showing dorsal gutter (g) and mouth collar supports(s) of the buccal capsule. Scale bar = 50 μm. 12. Tail of holotype male showing lateral view of spicule tip. Scale bar = 50 μm. 13. Dorsoventral view of holotype spicule tip. Scale bar = 50 μm.

distal bifurcation of the proximal branches (Scialdo-Krecek, 1983)) may help in understanding the evolution of this group of species. Because of the variation in this character among species of other genera, the use of *Skrjabinodentus* at the subgenus level may be more appropriate. Furthermore, we believe subgenus level recognition for *Skrjabinodentus* will call attention to the unique character of the dorsal rays of these 3 species and yet preserve their obvious relationship to the other 7 species in the genus *Cylicostephanus*. The suggested new combinations are as follows: *Cylicostephanus* (*Skrjabinodentus*) *tshoijoi* comb. n., *C.* (*S.*) *caragandicus* comb. n., and *C.* (*S.*) *longiconus* comb. n.

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Oversummer and Overwinter Survival of the Cattle Lungworm *Dictyocaulus viviparus* on Pasture in Louisiana (U.S.A.)[1]

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ABSTRACT: Two experiments were carried out during summer and winter conditions to determine if *Dictyocaulus viviparus* infective larvae could survive on pasture during these seasons in Louisiana. Pairs of tracer calves were grazed in August and September on a pasture that was heavily contaminated in May and then left free of cattle during June–July. These tracers were not infected with *D. viviparus,* indicating that the larvae did not survive summer conditions. However, a pair of tracer calves grazed in February on a pasture heavily contaminated into December and kept free of cattle until mid-February, was infected with lungworms, indicating that larvae could survive during this period on pasture. A second pair of tracers grazed on the overwintered pasture during March–April did not become infected with lungworm. Gastrointestinal nematodes, particularly intestinal species, did survive oversummer and overwinter conditions. *Ostertagia ostertagi,* however, did not survive the oversummer period. Temperature and rainfall patterns during the experiment differed only a little from those for 22-yr average conditions.

KEY WORDS: *Dictyocaulus viviparus,* trichostrongyles, oversummer, overwinter, pasture infectivity, survival of infective stages, transmission, *Ostertagia ostertagi.*

Little information is available on survival of *D. viviparus* on pasture in the U.S. (Lyons et al., 1981). Porter et al. (1941) and Porter (1942) in Florida and Alabama, respectively, did not observe infection in susceptible animals that were grazed on pastures held free of animals for 6 wk and 3 mo during winter. However, according to Lyons et al. (1981), lungworm larvae survived on a pasture in central Kentucky during the winter. Reports in other parts of the world are conflicting, because early investigations in the United Kingdom did not find evidence of larval survival during winter (Soliman, 1952; Michel and Shand, 1955). More recent observations in Canada, Ireland, and the United Kingdom have demonstrated that larvae can successfully overwinter (Gupta and Gibbs, 1970; Oakley, 1971; Downey, 1973).

The purpose of the present experiments was to determine the survival of *D. viviparus* infective larvae during summer and winter, under the subtropical climatic conditions of Louisiana.

Materials and Methods

The experiment was carried out on 2 pastures at the Department of Veterinary Science research farm in Baton Rouge, Louisiana. Climatological data were taken from the Ben Hur Experiment Station, roughly 4.8 km from the research site.

[1] Approved for publication by the Director of the Louisiana Agricultural Experiment Station as manuscript number 88-64-2108.

Oversummering experiment

A small pasture of 0.41 ha was contaminated by feces of 30 yearling cattle naturally infected with *D. viviparus* and GI nematodes for several weeks in spring (up to 19 May) 1986. From this time until 8 August (81 days), the pasture was maintained free of animals. Two groups of 2 Holstein tracer calves per group (4 mo old and reared free of parasites) were grazed on the pasture for 31 days during 8 August to 8 September and for 30 days during 16 September to 16 October, respectively.

In each case, at the end of the 2 tracer calf grazing periods, the animals were kept in a concrete-floored pen for 15 days and fed grass hay and a feed-protein supplement. At necropsy, the GI tract and lungs were examined for worm recovery, counting, and identification of parasites (Williams et al., 1988).

Overwintering experiment

A second small pasture of 0.41 ha was contaminated for several weeks in fall (up to 19 December) 1986 by 16 animals naturally infected with gastrointestinal nematodes and *D. viviparus.* This pasture was then maintained free of any animals until 2 February 1987 (45 days). Two groups of 2 Jersey tracer calves per group (4 mo old and reared free of parasites) were grazed on the pasture for 14 days during 2–16 February and during 24 March to 7 April, respectively. Tracer calf grazing periods during late winter–spring were of shorter duration than in late summer–fall because of expected greater levels of pasture contamination in the former period (Williams et al., 1983, 1987). In each case, at the end of the tracer calf grazing periods the animals were held in confinement for 15 days before slaughter as were the previous tracer calves.

Table 1. *Dictyocaulus viviparus* recovered from tracer calves used for testing oversummer and overwinter survival of L3.

Pasture vacant period	Tracer grazing period	No. of animals	Mean no. of worms (SD)*	Range	Percentage	
					Adult	Immature adult
Summer, 5/19–8/8/86	8/8–9/8/86	2	0	—†	0	0
	9/16–10/16/86	2	0	—	0	0
Winter, 12/19/86–1/30/87	2/2–2/16/87	2	68.5 (9.5)	59–78	100	0
	3/24–4/7/87	2	0	—	0	0

* Standard deviation.
† No data.

Results and Discussion

No clinical evidence of infection was observed or lungworms recovered from either of the groups of tracer calves that grazed the oversummered pasture in August or September (Table 1). However, as is shown in Table 2, gastrointestinal nematode L3, particularly those of the intestinal species, survived summer conditions and infected the 4 animals. *Ostertagia ostertagi* was the exception, because L3 of that species apparently did not survive the observed oversummer period, and the tracer calves were found free of this parasite. Even though rainfall was well above to near normal from June through August, normal high summer temperatures were apparently responsible for failure of L3 of *D. viviparus* and *O. ostertagia* to survive on pasture (Fig. 1).

A mean of 68.5 lungworms, with a standard deviation of 9.5 was found in the 2 tracer calves that grazed the overwintered pasture from 2 February to 16 February (Table 1). However, no clinical signs of infection or lungworms were observed in the 2 calves that grazed the overwintered pasture from 24 March to 7 April 1987 (Table 1).

Results obtained in the oversummer experiment indicated that lungworm infectivity on pasture did not persist through the summer pasture rest period. Apparently either the L3 or earlier larval stages did not survive conditions of summer.

In regard to overwintering of L3 of *D. viviparus* on pasture, results are not only controversial in Europe (Michel and Shand, 1955; Oakley, 1971), but also in North America. Porter et al. (1941) and Porter (1942) did not observe overwinter survival in Florida and in Alabama, respectively, but Lyons et al. (1981) found that lungworm larvae were capable of surviving overwinter in Kentucky. Results obtained in the present work indicated that susceptible calves were infected by L3 of *D. viviparus* that survived through the coldest portion of winter in Louisiana (to mid-February). Near continuous wet weather from October 1986 through March 1987 and only slightly lower than normal temperatures in December and January were apparently favorable to survival of lungworm L3 during winter (Fig. 1). Overwinter survival of gastrointestinal nematode larvae through this period was (Table 2) in accord with previous epidemiologial

Table 2. Mean gastrointestinal nematode counts recovered from tracer calves used for testing oversummer and overwinter survival.

Pasture vacant period	Tracer grazing period	No. of animals	*Ostertagia ostertagi*			*Trichostrongylus axei*	*Haemonchus* sp.	Small intestine‡	Large intestine‡	Total worm count
			Adults	DL*	EL_4†					
Summer, 5/19–8/8/86	8/8–9/8/86	2	0	0	0	50	818	1,800	50	2,718
	9/16–10/16/86	2	0	0	0	0	0	8,344	100	8,444
Winter, 12/19/86–1/30/87	2/2–2/16/87	2	6,381	141	674	770	806	5,947	150	14,869
	3/24–4/7/87	2§	0	43	11	0	0	2,007	0	2,061

* DL = developing fourth stage.
† EL_4 = early fourth stage.
‡ Primarily *Cooperia* spp. in small intestine; primarily *Oesophagostomum radiatum* in large intestine.
§ Abomasal worm count data on 1 animal only.

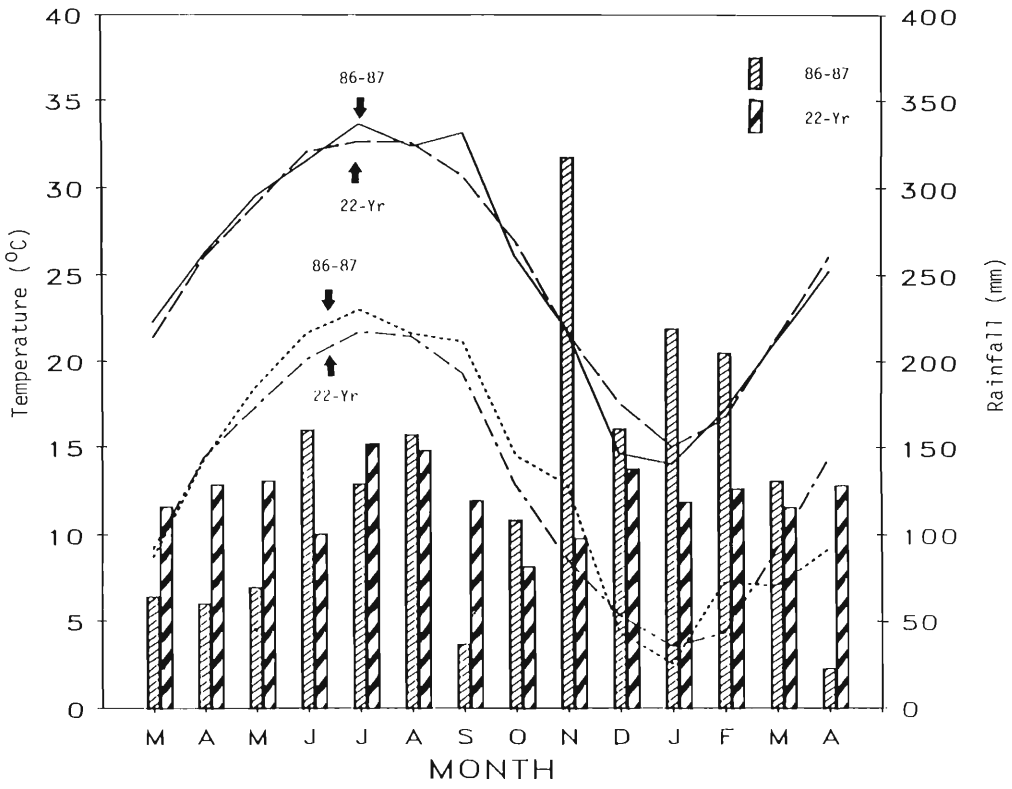

Figure 1. Line graph of monthly average maximum and minimum temperature (°C) and histogram of monthly rainfall from March 1986 to April 1987 compared to 22-yr averages.

observations in Louisiana (Williams et al., 1983, 1987). *Ostertagia ostertagi* was predominant in February, whereas intestinal species were more common in calves grazed during March–April.

Jørgensen (1980, 1981) in Denmark, found that few lungworm larvae survived overwinter and the number that did were not sufficient to produce clinical signs in calves. In our study, even though lungworm burdens were low in both tracers grazed on the overwinter pasture in February, clinical signs of coughing, anorexia, and weakness were obvious. However, neither of the 2 calves grazed during March–April acquired lungworm infection. Even though rainfall was minimal in April 1987, generally prevailing weather conditions of the spring would appear to have favored survival of lungworm L3 through the March–April tracer calf grazing period (Fig. 1). It may be that inherent limitation for lungworm L3 survival (depletion of stored energy reserve) was more important than weather factors in this case. When the second set of tracers grazed the overwinter pasture in March–April, the length of the overwinter period observed was similar to that reported by Porter et al. (1941), confirming their results, and suggesting that even under conditions when the larvae can overwinter until February, they may not survive until March–April.

According to Oakley (1981), different results on overwinter survival of lungworm larvae on pasture may be due to climatic differences between regions and techniques used to detect the presence of the larvae. Although failure to detect larvae by tracer grazing experiments does not mean that they are absent, the use of young susceptible tracer calves grazed for 2–3 wk on a small paddock is obviously the most sensitive and quantitative means of testing availability.

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The African Species of *Quadriacanthus* with Proposal of *Quadriacanthoides* gen. n. (Monogenea: Dactylogyridae)

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ABSTRACT: Eight species of Dactylogyridae collected from the barrages of the Nile River near Cairo, Egypt, are reported from the external surfaces of *Clarias lazera* Cuvier and Valenciennes as follows: *Quadriacanthus clariadis* Paperna, 1961, *Q. aegypticus* El-Naggar and Serag, 1986, *Q. allobychowskiella* Paperna, 1979, *Q. bagrae* Paperna, 1979, *Q. papernai* sp. n., *Q. ashuri* sp. n., *Q. numidus* sp. n., and *Quadriacanthoides andersoni* gen. n., sp. n. *Quadriacanthus kearni* El-Naggar and Serag, 1985, is considered a junior subjective synonym of *Q. allobychowskiella*; *Q. allobychowskiella* and *Q. bagrae* are raised from subspecific to specific rank; the sclerotized structures of the haptor and copulatory complex of *Q. voltaensis* Paperna, 1965, are figured; 2 *Quadriacanthus*, previously recorded as *Q. c. clariadis* by Paperna (1979) from *Heterobranchus isopterus* Bleeker, are considered new and undescribed species; and *Q. clariadis* and *Q. bagrae* are redescribed. *Quadriacanthoides* gen. n. is proposed and is characterized by species possessing a modified distribution of haptoral hooks, a simple ventral bar, and a highly modified dorsal bar with an anterior shield and posterior T-shaped sclerite.

KEY WORDS: Monogenea, Egypt, Africa, *Clarias lazera, Quadriacanthus, Quadriacanthus clariadis, Quadriacanthus aegypticus, Quadriacanthus allobychowskiella, Quadriacanthus voltaenis, Quadriacanthus bagrae, Quadriacanthus papernai* sp. n., *Quadriacanthus ashuri* sp. n., *Quadriacanthus numidus* sp. n., *Quadriacanthoides* gen. n., *Quadriacanthoides andersoni* sp. n., *Quadriacanthus* spp. 1 and 2.

This study is the second in a series dealing with the revision of selected dactylogyrid genera comprising species infesting African freshwater fishes. Previously, *Characidotrema* Paperna and Thurston, 1968 (Dactylogyridae), was resurrected from synonymy with *Jainus* Mizelle, Kritsky, and Crane, 1968, and all included species were reviewed and figured by Kritsky et al. (1987). In this paper, *Quadriacanthus* Paperna, 1961, is reviewed and *Quadriacanthoides* gen. n. is proposed.

Materials and Methods

Seventeen *Clarias lazera* Cuvier and Valenciennes were collected by local fishermen from the barrages of the Nile River near Cairo, Egypt, in May 1984. Hosts were immediately placed in a container of 1:4,000 formalin/river water solution and left for several hours. The container was vigorously shaken several times during this period to dislodge parasites. Following the formalin treatment, fish were removed and the fluid was allowed to settle in order to concentrate parasites near the bottom. Decanting of the upper clear liquid and addition of formalin to approximately a 3–5% solution provided volumes suitable for shipment to Idaho. Hosts were identified by Dr. Ameen Ashur, Department of Zoology, Ain Shams University, prior to their disposal. The collection technique precluded exact determination of infestation site, which is herein considered the external surface and may include the gills, fins, skin, nares, or mouth. Methods of collection, mounting, illustration, and measurement of helminths are as described by Kritsky et al. (1987). All measurements are in micrometers; means are followed by ranges in parentheses.

Specimens collected during the present study were deposited in the helminthological collections of the U.S. National Museum (USNM), the University of Nebraska State Museum (HWML), the Instituto Nacional de Pesquisas da Amazônia (INPA), and the Musée Royal de l'Afrique Centrale (MRAC) as indicated in the respective descriptions. In addition, the following specimens from museums or private collections were examined: MRAC 35.521 (labeled as paratypes of *Quadriacanthus c. clariadis* from *Heterobranchus isopterus* Bleeker, 8 specimens); MRAC 35.941 (holotype, *Q. tilapiae*); MRAC 34.290 (holotype, paratypes, *Q. clariadis bagrae*, 7 specimens); MRAC 35.567, 35.568 (holotype, paratypes, respectively, *Q. voltaensis*, 3 specimens); *Bychowskyella gharhi* (2 specimens) and *B. pseudobagri* (12 specimens) from the collection of Dr. A. V. Gussev, U.S.S.R. Academy of Sciences, Leningrad; *B. pseudobagri* (4 specimens), *Clariotrema tchangi* (8 specimens), and *C. meridionalis* (7 specimens) from the collection of Dr. Zhang Jian-Ying, Department of Biology, South China Normal University; USNM 79901 (vouchers, *B. pseudobagri*, 2 specimens).

Results

Dactylogyridae Bychowsky, 1933

Ancyrocephalinae Bychowsky, 1937

Quadriacanthus Paperna, 1961

EMENDED DIAGNOSIS: Body divisible into cephalic region, trunk, peduncle, haptor. Tegument

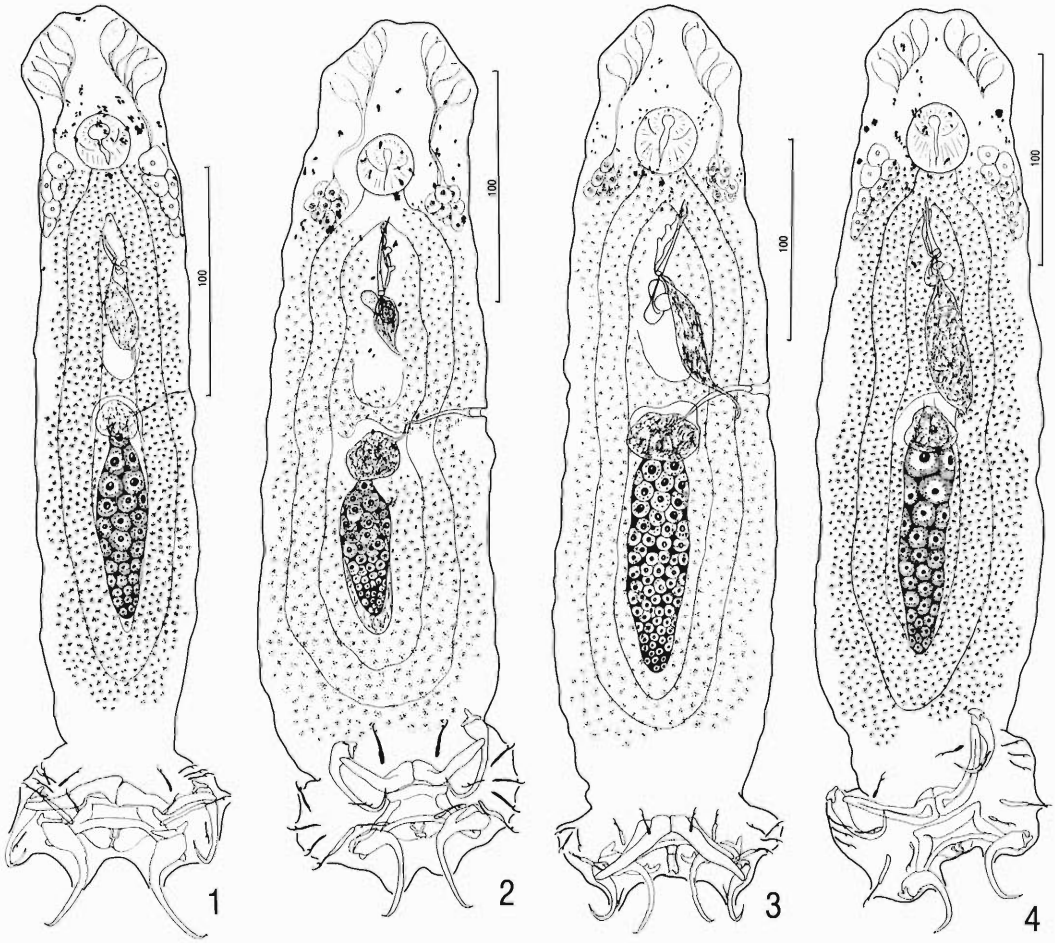
thin, smooth. Two bilateral, 2 terminal cephalic lobes; 4 pairs of head organs; cephalic glands unicellular, comprising 2 bilateral groups posterolateral to pharynx. Eyes present or absent; granules usually scattered in cephalic area, anterior trunk. Mouth subterminal, midventral; pharynx muscular, glandular; esophagus short; intestinal caeca 2, confluent posterior to gonads. Gonads intercecal, overlapping; testis dorsal to ovary. Vas deferens looping left intestinal caecum; seminal vesicle a dilation of vas deferens, lying diagonally to left of midline in anterior trunk; 2 prostatic reservoirs, one C-shaped wrapping around second. Copulatory complex comprising basally articulated cirrus, accessory piece. Oviduct short; uterus delicate; vagina sinistral; seminal receptacle intercecal, ventral to anterior end of ovary; vitellaria well developed, dispersed in 2 bilateral bands coextensive with intestinal caeca; common vitelline duct anterior to seminal receptacle. Haptor armed with dorsal and ventral pairs of anchors, dorsal and ventral bars, posterior muscular pad between bars, 14 pairs of hooks with ancyrocephaline distribution. Anchors with basal accessory sclerite; ventral bar comprising 2 components articulating medially; dorsal bar with bilateral arms, expanded midregion. Hook pair 6 with elongate dilated proximal shank; pairs 1, 7 usually with short proximal dilation of shank; pairs 2, 3, 4, 5 lacking dilated shank. Parasites of siluriform fishes of Africa and Asia.

TYPE SPECIES, HOST, AND LOCALITY: *Quadriacanthus clariadis* Paperna, 1961, from *Clarias lazera* Cuvier and Valenciennes, Lake Galilee, Israel.

OTHER SPECIES: *Quadriacanthus aegypticus* El-Nagger and Serag, 1986, from *Clarias lazera* (Egypt); *Q. allobychowskiella* Paperna, 1979, from *C. lazera* (Uganda, Egypt); *Q. bagrae* Paperna, 1979, from *Bagrus docmac* (Uganda), *B. bayad* (Uganda), *B. orientalis* (Tanzania), *Clarias lazera* (Egypt); *Q. gyrocirrus* Long So, 1981, from *Clarias batrachus* (China); *Q. kobiensis* Ha Ky, 1968, from *Clarias fuscus* (North Vietnam); *Q. tilapiae* Paperna, 1973, from *Tilapia esculenta* (Uganda); *Q. voltaensis* Paperna, 1965, from *C. lazera* (Ghana); and *Q. papernai* sp. n., *Q. ashuri,* sp. n., *Q. numidus* sp. n., all from *Clarias lazera* (Egypt).

REMARKS: Based on the anatomy of the internal organ systems, the genera *Quadriacanthus* Paperna, 1961, *Quadriacanthoides* gen. n., *Bychowskyella* Achmerow, 1952, and *Clariotrema* Long So, 1981, appear to be closely related groups. Species of these genera possess (1) overlapping gonads; (2) 2 prostatic reservoirs, the first of which is loosely wrapped around the second; (3) a simple tubular cirrus with basally articulated accessory piece; and (4) eyes poorly developed or absent. Generic separation is based solely on differences in the haptoral sclerites. *Quadriacanthus* is characterized by species having an accessory sclerite on both ventral and dorsal anchors and dorsal bar lacking an anterior shield. Accessory sclerites are lacking on the ventral anchors of species of *Bychowskyella* and *Clariotrema.* The species of *Quadriacanthoides* has a ventral bar not composed of 2 distinct components (present in *Quadriacanthus, Bychowskyella,* and *Clariotrema*) and a highly modified dorsal bar with anterior shield and posterior T-shaped sclerite. The only feature separating *Clariotrema* and *Bychowskyella* are the number of enlarged haptoral hooks, with *Clariotrema* possessing 1 pair and *Bychowskyella* 3 pairs (Long So, 1981).

Bychowsky and Nagibina (1978) elevated the Ancyrocephalinae to family status and Gussev (1961) proposed the Ancylodiscoidinae for ancyrocephalids occurring primarily on African and Eurasian siluriform fishes. Gussev (1978) included the following genera in the Ancylodiscoidinae: *Ancylodiscoides* Yamaguti, 1937; *Silurodiscoides* (Achmerow, 1964) Gussev, 1976; *Bychowskyella* Achmerow, 1952; *Protoancylodiscoides* Paperna, 1969; *Mizelleus* Jain, 1957; *Bifurcohaptor* Jain, 1958; *Quadriacanthus* Paperna, 1961; *Bagrobdella* Paperna, 1969; *Unilatus* Mizelle and Kritsky, 1967; and *Cornudiscoides* Kulkarni, 1969. He also provisionally placed *Schilbetrema* Paperna and Thurston, 1968, *Chauhanellus* Bychowsky and Nagibina, 1969, *Hargitrema* Tripathi, 1959, and *Urocleidoides* Mizelle and Price, 1964 (part) in the group. Based on the position of the reproductive organs, only *Schilbetrema* is similar to the group comprising *Quadriacanthus, Quadriacanthoides, Bychowskyella,* and *Clariotrema.* All of the remaining genera have tandem or slightly overlapping gonads, which suggests that the taxa proposed by Bychowsky and Nagibina (1978) and Gussev (1961) are polyphyletic. Phylogenetic analysis of all genera comprising the Ancyrocephalinae sensu Yamaguti (1963) would be necessary to determine evolutionary relationships of the generic



Figures 1–4. Whole-mount illustrations of *Quadriacanthus* species (ventral). 1. *Quadriacanthus clariadis*. 2. *Quadriacanthus aegypticus*. 3. *Quadriacanthus allobychowskiella*. 4. *Quadriacanthus bagrae*.

taxa. This, however, may not currently be possible because of the lack of information on many characters, particularly those of the internal organ systems, of many ancyrocephaline genera.

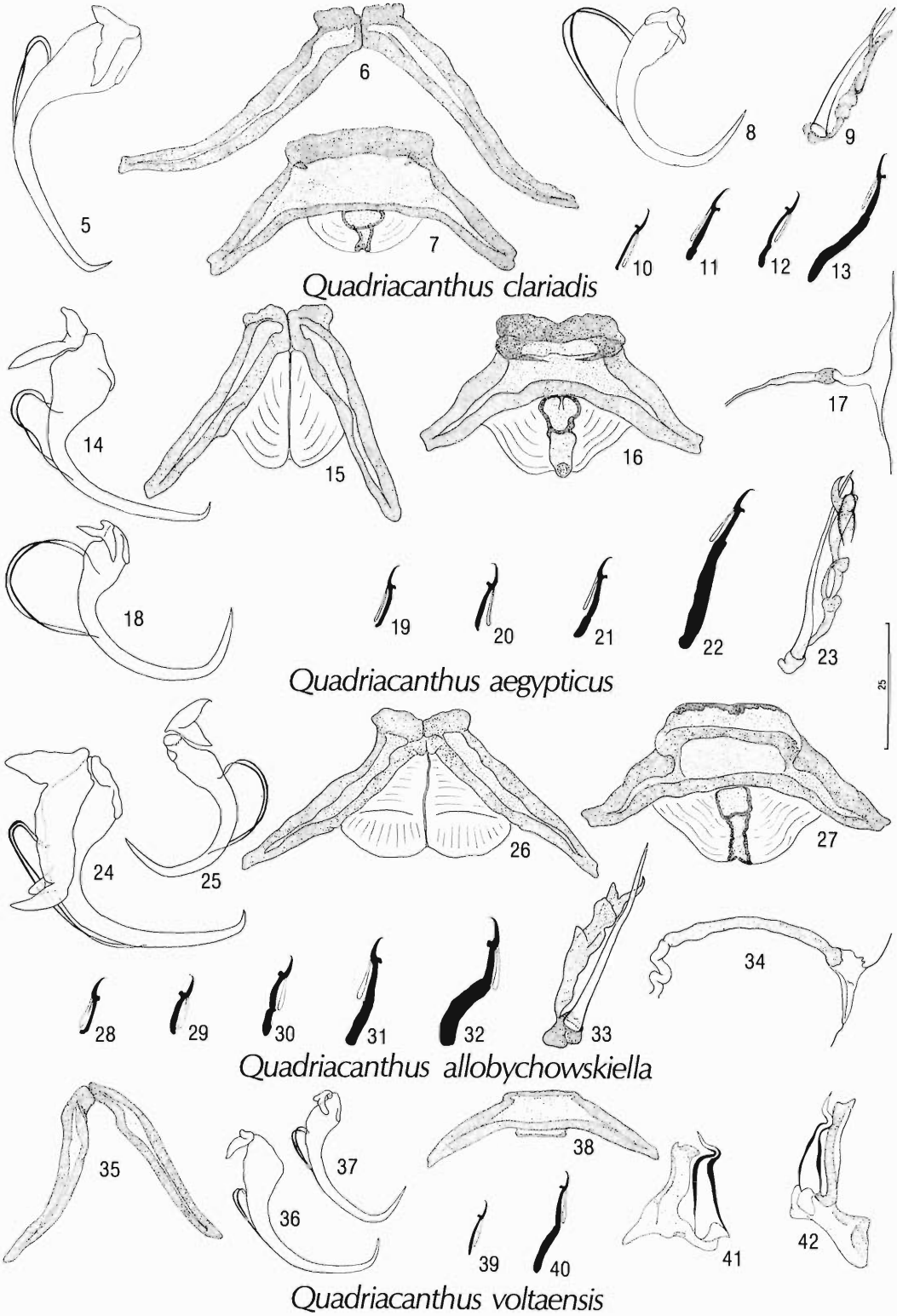
Quadriacanthus clariadis Paperna, 1961 (Figs. 1, 5–13)

SYNONYMS: *Quadriacanthus clariadis* of Molnar and Mossalam (1985), part; *Q. c. clariadis* Paperna, 1979, part.

HOST AND LOCALITIES: *Clarias lazera* Cuvier and Valenciennes, Lake Galilee, Israel (type locality) (Paperna, 1961); Bahr Mouis, Nile River near Zagazig, Egypt (Molnar and Mossalam, 1985); Manzala Lake and Demietta Branch, Nile River near Mansoura, Egypt (El-Naggar and Serag, 1985); Nile River near Cairo, Egypt (nobis).

SPECIMENS STUDIED: Vouchers (7); USNM 79896, HWML 20762, INPA PA 313-1, MRAC 37.160.

REDESCRIPTION (based on specimens from Cairo, Egypt): Body fusiform, 398 (343–444) long; greatest width 102 (71–134) at level of gonads. Eyes absent; granules variable in size, spherical to ovate. Pharynx spherical, 26 (22–31) in diameter. Peduncle broad, tapering posteriorly; haptor subhexagonal, 79 (74–91) long, 109 (98–120) wide. Ventral anchor 32 (29–34) long, with small base, short superficial root, curved shaft, elongate point; accessory sclerite small, with 2 subequal winglike processes; anchor base 10 (9–11) wide. Dorsal anchor 49 (47–51) long, with short roots, shaft bent proximally, short point; accessory sclerite with unequal wings; anchor base 14 (12–15) wide. Ventral bar component 53 (42–



65) long, elongate, delicate; dorsal bar 63 (52–72) long. Hook with delicate point, protruding thumb; pair 6: 35 (32–39) long; pairs 2, 3, 4, 5: 14 (13–15) long; pair 1—19 (18–20) long, with shank dilation comprising about ⅓ total length of hook; pair 7: 16–17 long, with small bulblike dilation of shank. FH loop about 0.8 length of distal portion of shank. Cirrus 25 (22–31) long, comprising straight tapered tube, small base; accessory piece 21 (17–26) long, with delicate terminal hook or spike. Testis not observed; ovary elongate ovate, 62 (53–74) × 21 (19–22). Vagina opening near midlength, comprising delicate sclerotized tube.

REMARKS: In previous reports, the epithet *Quadriacanthus clariadis* has been assigned to specimens that comprise several species from various hosts, with El-Naggar and Serag (1985) apparently being the first to correctly identify the species since its original description. Although having specimens of *Q. clariadis,* Molnar and Mossalam (1985) published photographs indicating that they did not distinguish between this species and *Q. aegypticus,* having assigned specimens of both to *Q. clariadis.* Similarly, Paperna and Thurston (1968), who reported *Q. clariadis* from Uganda, were likely dealing with *Q. aegypticus* based on their drawings of the accessory piece and vagina. Some records of *Q. clariadis* by Paperna (1979) are also questionable because his drawings of the haptoral and copulatory structures clearly were obtained from several different species. Finally, the reports of *Q. clariadis* from *Heterobranchus isopterus* by Paperna (1969, 1979) have been shown to be erroneous (see *Q.* sp. 1 and 2, nobis).

Unfortunately, the type specimens of *Q. clariadis* were lost in a fire that destroyed Dr. Paperna's laboratory in Eilat (Paperna, pers. comm.), and drawings presented with the original description of the species by Paperna (1961) are highly diagrammatic, both of which preclude absolute verification of the identification by El-Naggar and Serag (1985). However, Figures 19 and 22 of Paperna (1961) show elongate and delicate components of the ventral bar, a ventral anchor with a short curved shaft and long point, and a dorsal anchor with an elongate shaft and short point, all of which are consistent characters of specimens identified as *Q. clariadis* by El-Naggar and Serag (1985) and by us. In addition, Paperna's (1964) figure of the copulatory complex, which is based on the type specimens of *Q. clariadis,* approaches that of Egyptian specimens.

Quadriacanthus clariadis is distinguished from similar species found on *Clarias lazera* (*Q. aegypticus, Q. allobychowskiella,* and *Q. bagrae*) by having (1) a short curved ventral anchor shaft, (2) a delicate sinistral vagina, (3) a small accessory sclerite with unequal wings of the dorsal anchor, (4) a poorly developed terminal hook of the accessory piece, and (5) a short dorsal anchor point. In *Q. aegypticus,* the ventral anchor shaft is elongate, the vagina is heavily sclerotized, and the accessory piece possesses a distinct hooked termination. *Quadriacanthus allobychowskiella* is most easily separated from *Q. clariadis* by possessing a large accessory sclerite of the dorsal anchor. The dorsal anchor point of *Q. bagrae* is elongate and the vagina is unsclerotized; both features easily separate this species from *Q. clariadis.*

Quadriacanthus aegypticus El-Naggar and Serag, 1986 (Figs. 2, 14–23)

SYNONYM: *Quadriacanthus clariadis* of Molnar and Mossalam (1985), part; possibly *Q. clariadis* of Paperna and Thurston (1968).

HOST AND LOCALITIES: *Clarias lazera* Cuvier and Valenciennes, Manzala Lake and Demietta branch of the Nile River, Dakahlia Governorate, Egypt (type locality) (El-Naggar and Serag, 1986); Bahr Mouis, Nile River near Zagazig, Egypt (Molnar and Mossalam, 1985); Nile River near Cairo, Egypt (nobis).

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Figures 5–42. Sclerotized structures of *Quadriacanthus* species. Figures 5–13. *Quadriacanthus clariadis*. 5. Dorsal anchor. 6. Ventral bar. 7. Dorsal bar. 8. Ventral anchor. 9. Copulatory complex. 10. Hook (pairs 2–5). 11. Hook (pair 7). 12. Hook (pair 1). 13. Hook (pair 6). Figures 14–23. *Quadriacanthus aegypticus*. 14. Dorsal anchor. 15. Ventral bar. 16. Dorsal bar. 17. Vagina. 18. Ventral anchor. 19. Hook (pair 5). 20. Hook (pairs 2–4). 21. Hook (pairs 1, 7). 22. Hook (pair 6). 23. Copulatory complex. Figures 24–34. *Quadriacanthus allobychowskiella*. 24. Dorsal anchor. 25. Ventral anchor. 26. Ventral bar. 27. Dorsal bar. 28. Hook (pair 5). 29. Hook (pairs 2–4). 30. Hook (pair 1). 31. Hook (pair 7). 32. Hook (pair 6). 33. Copulatory complex. 34. Vagina. Figures 35–42. *Quadriacanthus voltaensis*. 35. Ventral bar. 36. Dorsal anchor. 37. Ventral anchor. 38. Dorsal bar. 39. Hook (pairs 1–5, 7). 40. Hook (pair 6). 41, 42. Copulatory complexes. All figures are to the 25-μm scale.

SPECIMENS STUDIED: Vouchers (26) from Cairo, Egypt; USNM 79900, HWML 20765, INPA PA315-1, 2, MRAC 37.163.

MEASUREMENTS: Body 394 (313–502) long; greatest width 93 (76–105) in posterior half of trunk. Pharyngeal diameter 24 (21–28). Haptor 76 (60–89) long, 98 (87–107) wide. Ventral anchor 38 (36–44) long, base width 10 (9–12); dorsal anchor 47 (43–51) long, base width 14 (13–18). Ventral bar component 47 (39–53) long; dorsal bar 57 (45–74) long. Hook pair 1: 18 (16–22); pairs 2–5: 14 (13–16); pair 6: 33 (31–36); pair 7: 20–21. Cirrus 43 (40–52) long; accessory piece 39 (33–49) long. Ovary 67 (59–75) × 21 (16–25).

REMARKS: This species was adequately described by El-Naggar and Serag (1986), and a photograph of the haptoral sclerites is presented in Figure 3a of Molnar and Mossalam (1985). The species closely resembles *Q. clariadis*; features distinguishing these species are presented in the remarks for *Q. clariadis*.

Quadriacanthus allobychowskiella Paperna, 1979 (Figs. 3, 24–34)

SYNONYMS: *Quadriacanthus clariadis allobychowskiella* Paperna, 1979; *Quadriacanthus kearni* El-Naggar and Serag, 1985.

HOST AND LOCALITIES: *Clarias lazera* Cuvier and Valenciennes, Lake George, Uganda (type locality) (Paperna, 1979); Manzala Lake and Demietta Branch, Nile River, near Mansoura, Dakahlia Governorate, Egypt (El-Naggar and Serag, 1985); Nile River near Cairo, Egypt (nobis).

SPECIMENS STUDIED: Vouchers (16); USNM 79899, HWML 20764, INPA PA314-1, 2, MRAC 37.162.

MEASUREMENTS: Body 438 (380–499) long; greatest width 104 (88–115) in posterior half of trunk. Pharyngeal diameter 32 (30–34). Haptor 73 (63–86) long, 104 (97–112) wide. Ventral anchor 29 (26–31) long, base width 11 (8–13); dorsal anchor 48 (44–51) long, base width 16 (15–18). Ventral bar component 50 (44–56) long; dorsal bar 63 (51–74) long. Hook pair 1: 18 (17–20) long, pairs 2–4: 14 (13–15) long, pair 5: 13 (12–14) long, pair 6: 29 (28–31) long, pair 7: 21 (19–23) long. Cirrus 45 (41–48) long; accessory piece 36 (30–43) long. Ovary 89 (75–104) × 25 (24–27).

REMARKS: This species was briefly described by Paperna (1979) as *Quadriacanthus clariadis allobychowskiella* from a single specimen. El-Naggar and Serag (1985) described *Q. kearni* from the same host in Egypt and differentiated it from *Q. c. allobychowskiella* by details of the copulatory complex, eyes, vagina, and the accessory sclerite of the dorsal anchor. Paperna (1979) did not mention the nature of the eyes or vagina, and his original drawings of the copulatory complex, anchors, and bars of *Q. c. allobychowskiella* are highly diagrammatic. Nonetheless, his Plate XLIV, Figures 1–3 indicate an accessory piece with a terminal hook and subterminal lobes, both features of which were described for *Q. kearni*. Although Paperna did not depict a bifid termination of the largest wing of the accessory sclerite of the dorsal anchor, we expect that both branches were present but not visible in the single specimen available to him. In some of our specimens, the bifid termination was difficult to discern either because of excessive clearing or because one branch was overlaid by the other. Thus, we consider *Q. kearni* El-Naggar and Serag, 1985, a junior subjective synonym of this species. No record could be found in the MRAC for the holotype of *Q. c. allobychowskiella*.

Quadriacanthus allobychowskiella is similar to *Q. clariadis*, differing from it primarily by the size and shape of the accessory sclerite of the dorsal anchor. Because *Q. allobychowskiella* and *Q. clariadis* occur simultaneously on the gills of *C. lazera* in Egypt, we consider the former to represent a distinct species. That no intermediate specimens were found further supports elevation to species rank.

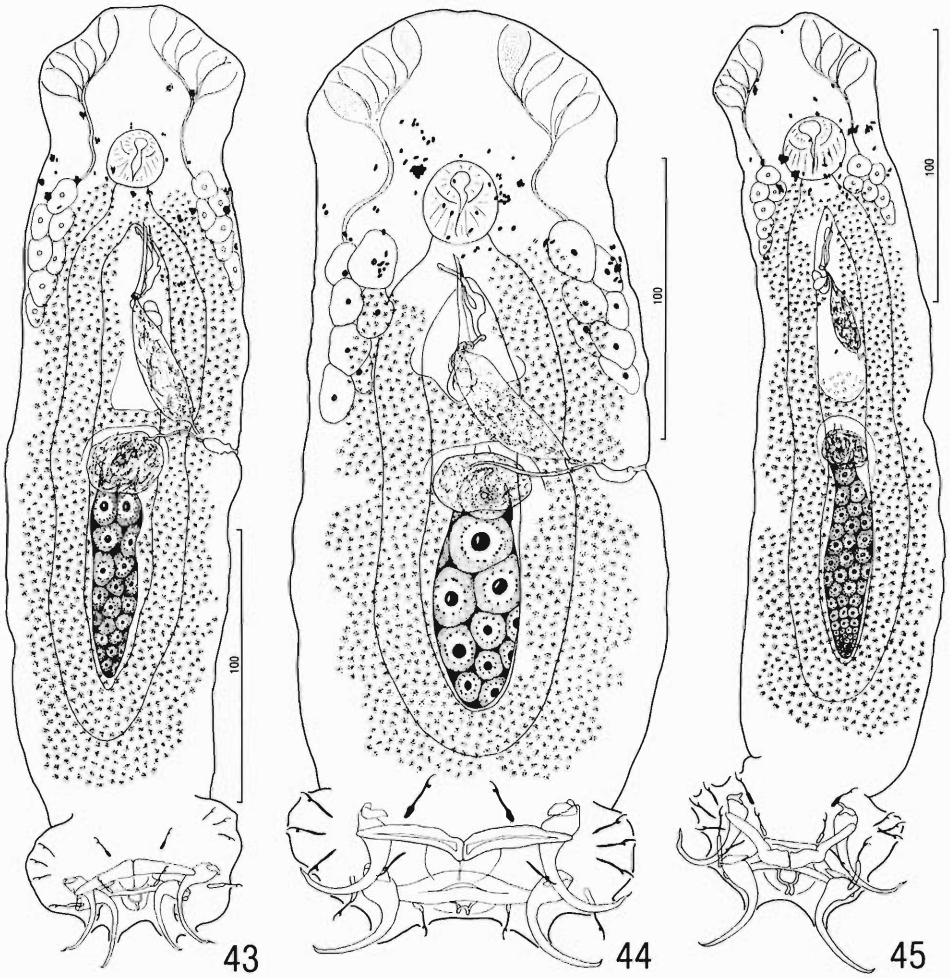
Quadriacanthus voltaensis Paperna, 1965 (Figs. 35–42)

HOST AND LOCALITY: *Clarias lazera* Cuvier and Valenciennes, stream near Kpotame, lower reaches of the Volta River, Ghana (Paperna, 1965).

SPECIMENS STUDIED: Holotype, MRAC 35.567; paratypes (2), MRAC 35.568.

MEASUREMENTS: Ventral anchor 27–28 long, base width 7 (6–8); dorsal anchor 34 (33–36) long, base width 9 (8–10). Ventral bar component 40 (38–42) long; dorsal bar 51 (48–53) long. Hook pairs 1–4, 7: 13 (12–14), pair 5: 10–11, pair 6: 22–23. Cirrus 22 (20–23) long; accessory piece 24 (20–31) long.

REMARKS: The slide containing the holotype was invaded by bubbles, which precluded its use in description. As a result, the figures and measurements are based on the 2 paratypes. All type



Figures 43–45. Whole-mount illustrations of new species of *Quadriacanthus* (ventral). 43. *Quadriacanthus papernai*. 44. *Quadriacanthus ashuri*. 45. *Quadriacanthus numidus*.

specimens were greatly flattened and cleared, which precluded study of soft body parts. Nonetheless, *Q. voltaensis* is easily recognized as a distinct species by its unique cirrus which comprises a broad tube rapidly tapering to a point. The anchors resemble those of *Q. bagrae* but are smaller and more delicate.

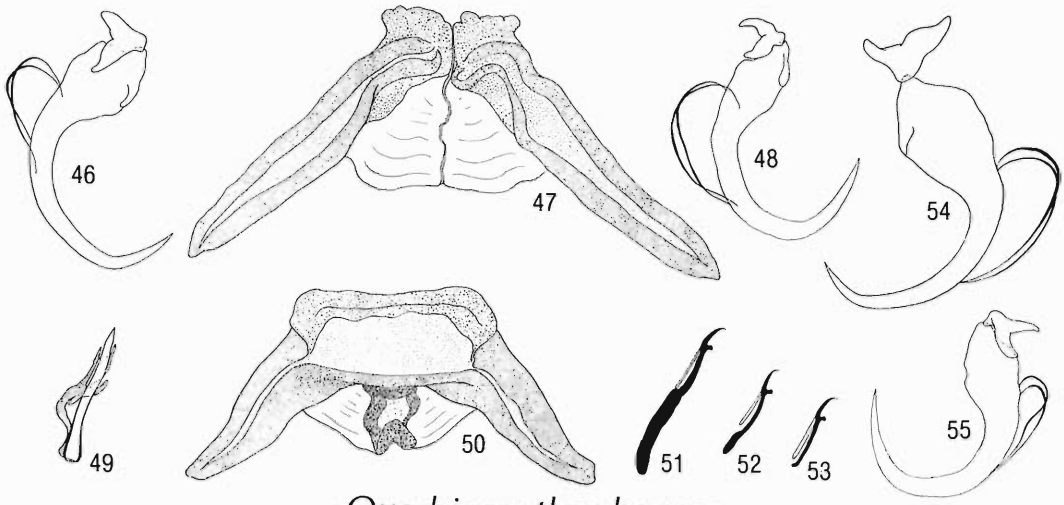
Quadriacanthus bagrae Paperna, 1979 (Figs. 4, 46–55)

SYNONYM: *Quadriacanthus clariadis bagrae* Paperna, 1979.

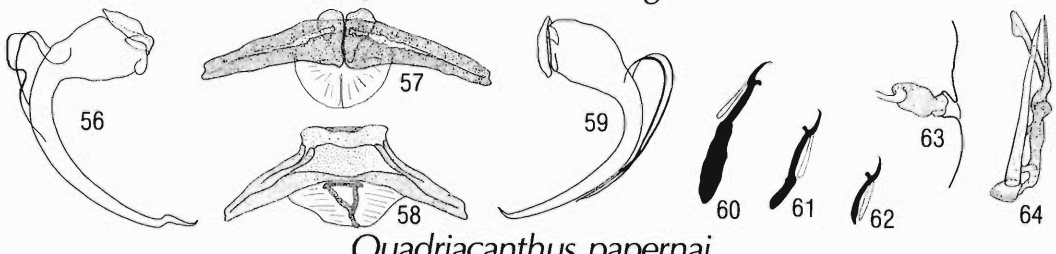
HOSTS AND LOCALITIES: *Bagrus docmac* (Forskål) (type host), Lake Victoria (type locality) and Albert-Nile near Chobe, Uganda (Paperna, 1979); *B. bayad* (Forskål). Lake Albert, Uganda (Paperna, 1979); *B. orientalis* (author?), Ruaha River, Tanzania (Paperna, 1979); *Clarias lazera* Cuvier and Valenciennes, Nile River near Cairo, Egypt (nobis).

SPECIMENS STUDIED: Types (8) of *Quadriacanthus clariadis bagrae* Paperna, 1979, MRAC 34290; vouchers (23), USNM 79891, HWML 20759, INPA PA310-1–3, MRAC 37.157.

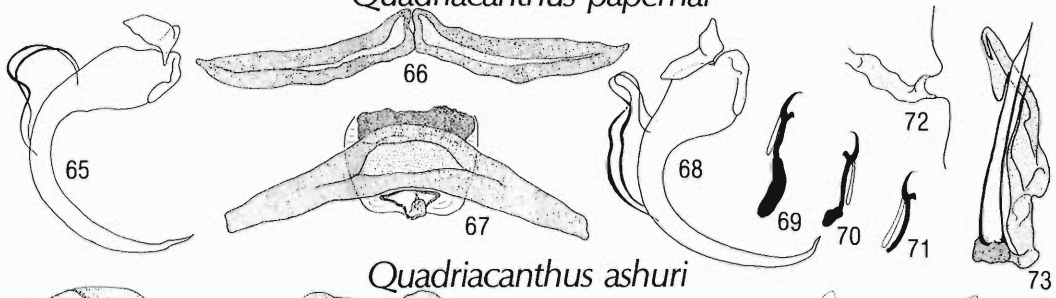
REDESCRIPTION (measurements of types follow those of Egyptian specimens in brackets): Body fusiform, slightly flattened dorsoventrally, 410 (287–481) long; greatest width 99 (80–126) near midlength. Eyes absent; granules small, diamond-shaped or ovate. Pharynx ovate, greatest diameter 26 (21–30). Peduncle broad; haptor subquadrate, 75 (65–93) long, 99 (83–126) wide.



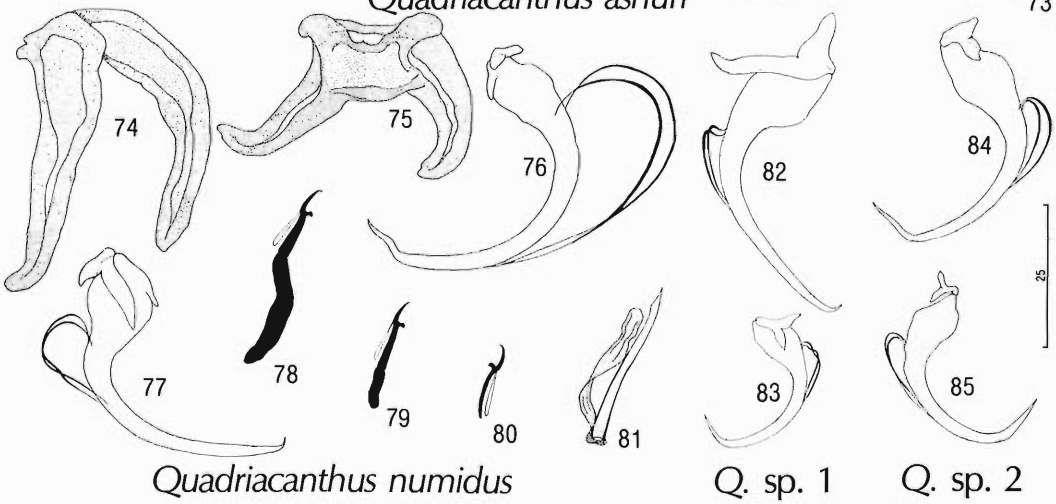
Quadriacanthus bagrae



Quadriacanthus papernai



Quadriacanthus ashuri



Quadriacanthus numidus *Q.* sp. 1 *Q.* sp. 2

Ventral anchor 32 (30–34) [31 (29–33)] long, with short superficial root, bent shaft, long point; accessory sclerite small, wings subequal; anchor base 10 (9–12) [12 (11–13)] wide. Dorsal anchor 39 (33–42) [41 (39–44)] long, with large base, short superficial root, curved shaft, long point; accessory sclerite small, wings unequal; anchor base 13 (12–16) [14 (13–17)] wide. Ventral bar robust, ventral bar component 52 (42–64) [54 (49–61)] long; dorsal bar 63 (51–78) [60 (56–66)] long. Hook with delicate point, protruding thumb; pair 1: 18 (17–19) long, with shank dilation comprising about ⅓ total hook length; pairs 2–5, 7: 14 (13–15) long, lacking inflation of shank; pair 5: 13 (12–14) long; pair 6: 27 (25–30) [23] long, with elongate dilation of shank. FH loop about equal to length of undilated portion of shank. Cirrus 25 (23–27) [31 (29–32)] long, comprising a straight tube with small base; accessory piece 19 (17–21) [24 (22–26)] long, variable, usually with 3 terminal projections. Testis not observed; ovary elongate, fusiform, 77 (60–104) × 20 (15–28). Vagina not observed.

REMARKS: Type specimens available for study were unstained and severely flattened, which precluded information on their internal anatomy; coverslip pressure had apparently distorted the anchor bases and copulatory complex. Although the slide (MRAC 34.290) is labeled as "holotype" of *Q. clariadis bagrae,* it was not possible to determine a holotype because all 8 specimens were present on the slide without designation of one as holotype. Further, the slide label indicates the worms were collected from *Bagrus docmac* from Kazi, Uganda, the locality of which differs from that reported for the species by Paperna (1979). Nonetheless, it is apparent that our specimens are conspecific with these types.

Quadriacanthus clariadis bagrae is elevated to specific rank because it is sympatric with *Q. clariadis* in Egypt. *Quadriacanthus bagrae* most closely resembles *Q. voltaensis* based on morphology of the dorsal and ventral anchors. These species are easily distinguished by comparative morphology of the copulatory complex.

Quadriacanthus tilapiae Paperna, 1973

HOST AND LOCALITY: *Tilapia esculenta* (author?), Jinja Bay, Lake Victoria, Uganda (Paperna, 1973).

SPECIMEN STUDIED: Holotype, MRAC 35.941.

REMARKS: This species is known from a single specimen and has not been reported since its original description by Paperna (1973). Examination of the holotype, which is unstained and flattened by coverslip pressure, showed that the species is similar to *Q. bagrae* based on the morphology of the anchor points and shafts. *Quadriacanthus tilapiae* may be a senior synonym of *Q. bagrae,* with the holotype representing a specimen with deformed anchor bases as a result of coverslip pressure. The finding of *Quadriacanthus tilapiae* on a cichlid host probably represents an accidental infestation of the host. However, we do not reduce *Q. bagrae* as a junior synonym because additional collections of siluriform and cichlid fishes from the type locality would be necessary for confirmation of the synonymy. The type locality for *Q. bagrae* is also Lake Victoria, Uganda; and measurements of the holotype of *Q. tilapiae* fall within corresponding ranges for *Q. bagrae.*

Quadriacanthus papernai sp. n. (Figs. 43, 56–64)

HOST AND LOCALITY: *Clarias lazera* Cuvier and Valenciennes, Nile River near Cairo, Egypt.

SPECIMENS STUDIED: Holotype, USNM 79892; paratypes (19), USNM 79893, HWML 20760, INPA PA311-1-3, MRAC 37.158.

DESCRIPTION: Body fusiform, slightly flattened dorsoventrally, 399 (329–461) long; greatest width 90 (78–108) near midlength. Eyes absent; granules diamond-shaped, relatively large,

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Figures 46–85. Sclerotized structures of *Quadriacanthus* species, cont. Figures 46–55. *Quadriacanthus bagrae.* 46. Dorsal anchor. 47. Ventral bar. 48. Ventral anchor. 49. Copulatory complex. 50. Dorsal bar. 51. Hook (pair 6). 52. Hook (pairs 1, 7). 53. Hook (pairs 2–5). 54. Dorsal anchor (from type specimen MT 34.290). 55. Ventral anchor (from type specimen MT 34.290). Figures 56–64. *Quadriacanthus papernai.* 56. Ventral anchor. 57. Ventral bar. 58. Dorsal bar. 59. Dorsal anchor. 60. Hook (pair 6). 61. Hook (pairs 1, 7). 62. Hook (pairs 2–5). 63. Vagina. 64. Copulatory complex. Figures 65–73. *Quadriacanthus ashuri.* 65. Ventral anchor. 66. Ventral bar. 67. Dorsal bar. 68. Dorsal anchor. 69. Hook (pair 6). 70. Hook (pairs 1, 7). 71. Hook (pairs 2–5). 72. Vagina. 73. Copulatory complex. Figures 74–81. *Quadriacanthus numidus.* 74. Ventral bar. 75. Dorsal bar. 76. Ventral anchor. 77. Dorsal anchor. 78. Hook (pair 6). 79. Hook (pairs 1, 7). 80. Hook (pairs 2–5). 81. Copulatory complex. Figures 82–83. *Quadriacanthus* sp. 1. 82. Dorsal anchor. 83. Ventral anchor. Figures 84–85. *Quadriacanthus* sp. 2. 84. Dorsal anchor. 85. Ventral anchor. All figures are drawn to the same scale (25 μm).

scattered from level of prostatic reservoirs anteriorly into cephalic area. Pharynx spherical, 24 (20–26) in diameter. Peduncle broad, haptor subquadrate, 67 (62–71) long, 87 (81–96) wide. Ventral anchor 37 (33–41) long, lacking roots, with curved shaft, point with strong double recurve; accessory sclerite small, with unequal wings; anchor base 10 (8–13) wide. Dorsal anchor 36 (34–37) long, lacking roots, with curved shaft, short point; accessory sclerite small, wings unequal; anchor base 11 (9–13) wide. Each ventral bar component rapidly tapering, 29 (24–33) long; dorsal bar 45 (40–49) long. Hooks with delicate point, protruding thumb; hook pair 1, 7 with dilated portion of shank about ⅓ total hook length, pair 1: 19 (17–21) long; pair 7: 16–17 long; pairs 2–5 lacking dilated shank portion, 14 (13–15) long; pair 6: 25 (23–27) long, with large, elongate dilation of proximal shank. FH loop about equal to length of undilated portion of shank. Cirrus 39 (35–43) long, comprising a straight tapered tube with small flange on base; accessory piece 33 (29–37) long, with spine arising near midlength. Testis not observed; ovary fusiform, 71 (59–90) × 21 (18–24). Vagina with terminal expansion opening into delicate sclerotized tube.

REMARKS: *Quadriacanthus papernai* is similar to *Q. ashuri* sp. n. as shown by the nature of the copulatory complex and doubly recurved point of the ventral anchor. They differ in body shape, the extent of the recurve of the ventral anchor point and size of the haptoral bars. This species is named for Dr. I. Paperna, Hebrew University of Jerusalem, in recognition of his pioneering work on African Monogenea.

Quadriacanthus ashuri sp. n. (Figs. 44, 65–73)

HOST AND LOCALITY: *Clarias lazera* Cuvier and Valenciennes, Nile River near Cairo, Egypt.

SPECIMENS STUDIED: Holotype, USNM 79894; paratypes (15), USNM 79895, HWML 20761, INPA PA312-1, 2, MRAC 37.159.

DESCRIPTION: Body robust, flattened dorsoventrally, 383 (340–431) long; greatest width 132 (101–165) usually at level of ovary. Eyes absent; granules relatively large, teardrop-shaped, occurring posteriorly as far as ovary, numerous in cephalic area and anterior trunk. Pharynx spherical, 30 (27–32) in diameter. Peduncle very broad; haptor subrectangular, 78 (65–84) long, 121 (111–140) wide. Ventral anchor 37 (35–38) long, lacking roots, with curved shaft, point with weak double recurve; accessory sclerite small, wings subequal; anchor base 14 (12–15) wide. Dorsal anchor 40 (37–42) long, with short superficial root, curved shaft, short point with nearly imperceptible recurve; accessory sclerite small, with unequal wings; anchor base 13 (12–14) wide. Ventral bar component 38 (33–45) long; dorsal bar 64 (56–75) long. Hook with delicate point, erect protruding thumb; pairs 1, 7: 16 (15–17) long, with small proximal shank enlargement; pairs 2–5: 14 (13–15) long, lacking shank enlargement; pair 6: 26–27 long, with basal shank dilation about ½ total hook length. FH loop about equal to length of undilated portion of shank. Cirrus 41 (38–43), a straight tapered tube with small base; accessory piece 34 (28–39) long, with small spine arising near midlength. Testis not observed; ovary ellipsoidal to fusiform, 69 (56–88) × 27 (23–31). Vagina with terminal dilation, delicate lightly sclerotized proximal tube.

REMARKS: This species is similar to *Q. papernai* sp. n. These species are easily distinguished by *Q. ashuri* being more robust and by the nature of the ventral anchor point. The species is named for Dr. Ameen Ashur, Department of Zoology, Ain Shams University, Cairo, Egypt.

Quadriacanthus numidus sp. n. (Figs. 45, 74–81)

HOST AND LOCALITY: *Clarias lazera* Cuvier and Valenciennes, Nile River near Cairo, Egypt.

SPECIMENS STUDIED: Holotype, USNM 79897; paratypes (3), USNM 79898, HWML 20763, MRAC 37.161.

DESCRIPTION: Body fusiform, 392 (346–432) long; greatest width 99 (80–121) at level of ovary. Eyes absent; granules relatively large, teardrop-shaped, occurring posteriorly to level of vitelline commissure, numerous in cephalic region and anterior trunk. Pharynx spherical, 24 (23–26) in diameter. Peduncle broad; haptor hexagonal, 75 (68–87) long, 89 (86–93) wide. Ventral anchor 42 (40–44) long, with small base, curved shaft, doubly recurved point; accessory sclerite small, wings subequal; anchor base 11 (10–13) wide. Dorsal anchor 45 (42–47) long, with broad base, curved shaft, short point; accessory sclerite moderately long, wings unequal; anchor base 14 (12–15) wide. Ventral bar component 46 (44–48) long; dorsal bar 46 (44–47) long. Hook with delicate point, protruding thumb; pairs 1, 7 with shank dilation about ⅔ total hook length, pair 1: 21

(20–22) long, pair 7: 18 (17–19) long; pairs 2–5: 14 (13–15) long, lacking shank dilation; pair 6: 32–33 long, with elongate dilation of shank. FH loop about equal to length of undilated portion of shank. Cirrus 30 (28–32) long, comprising straight tube with small base; accessory piece 22 (20–24) long, lamellate. Testis not observed; ovary fusiform, 66 (59–72) × 20 (19–21). Vagina not observed.

REMARKS: *Quadriacanthus numidus* resembles *Q. papernai* and *Q. ashuri* by possessing doubly recurved points of the ventral anchor. However, *Q. numidus* lacks a spine arising from the midlength of the accessory piece characteristic of the latter 2 species. The specific name is from Latin (*numid/o* = wandering) and refers to the ventral anchor point.

Quadriacanthus spp. 1 and 2 (Figs. 82–85)

HOST AND LOCALITY: *Heterobranchus isopterus* (Bleeker), Lake Bosomtwi, Ghana (Paperna, 1979).

SPECIMENS STUDIED: MRAC 35.521 containing 1 *Q.* sp. 1, 7 *Q.* sp. 2.

MEASUREMENTS: *Quadriacanthus* sp. 1: Ventral anchor 24–25 long, base 8–9 wide; dorsal anchor 43–44 long, base 12–13 wide; ventral bar component 30–31 long; dorsal bar 45–46 long; cirrus 38–39 long. *Quadriacanthus* sp. 2: Ventral anchor 28 (26–30) long, base 9 (8–10) wide; dorsal anchor 37 (36–38) long, base 9–10 wide; ventral bar component 38 (35–39) long; dorsal bar 46 (42–51) long; hook pair 6: 22–23 long; cirrus 23–24 long.

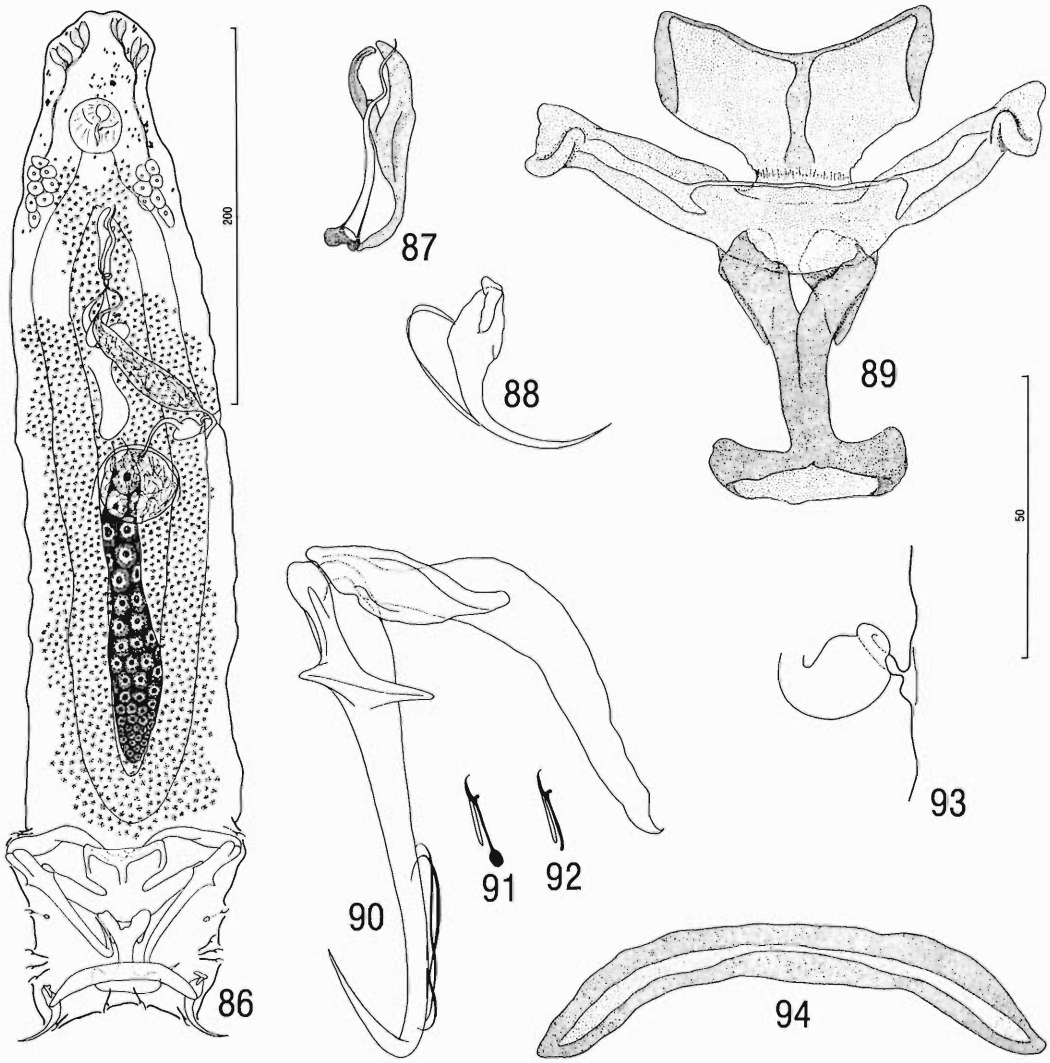
REMARKS: As a result of our attempt to locate the type specimens of *Quadriacanthus clariadis,* a slide (MRAC 35.521) deposited by Dr. Paperna in the Musée Royal de l'Afrique Centrale was located with a label indicating paratype specimens of *Q. c. clariadis* collected from *Heterobranchus isopterus.* Because *Q. clariadis* was originally described from *Clarias lazara* from Lake Galilee in Israel, the designation of the specimens on slide MRAC 35.521 as paratypes is obviously erroneous. Indeed, examination of the 8 worms on the slide indicates that none belong to this taxon, but rather that 2 undescribed species of *Quadriacanthus* parasitize *H. isopterus.* These species, designated herein as *Q.* species 1 and 2, are not described because information on internal anatomy and some sclerotized structures could not be determined satisfactorily from the unstained and flattened specimens. General haptoral morphology, however, clearly places them in *Quadriacanthus.* Species 1 resembles *Q. clariadis* in morphology of the anchors but differs from this species by possessing an accessory dorsal anchor sclerite with large wings. Species 2 is unique among described African species of the genus because of the slight double recurve of the elongate dorsal anchor point.

Quadriacanthoides gen. n.

DIAGNOSIS: Body divisible into cephalic region, trunk, haptor. Tegument thin, smooth. Two bilateral, 2 terminal cephalic lobes; 4 pairs of head organs; cephalic glands unicellular, comprising 2 bilateral groups posterolateral to pharynx. Eyes absent; granules scattered in cephalic area, anterior trunk. Mouth subterminal, midventral; pharynx muscular, glandular; esophagus present; intestinal caecae 2, confluent posterior to gonads. Gonads intercecal, apparently overlapping; testis dorsal to ovary. Vas deferens looping left intestinal caecum; seminal vesicle a dilation of vas deferens, lying diagonally to left of midline of anterior trunk; 2 prostatic reservoirs, one wrapped around second. Copulatory complex comprising basally articulated cirrus, accessory piece. Oviduct short; uterus delicate; vagina sinistral; seminal receptacle intercaecal, ventral to anterior extremity of ovary; vitellaria lying in 2 bilateral bands confluent posterior to ovary; vitelline commissure anterior to seminal receptacle. Haptor armed with ventral and dorsal pairs of anchors, dorsal and ventral bars, 14 hooks. Anchors with basal accessory sclerites. Ventral bar single; dorsal bar modified with anterior shield, posterior T-shaped process articulating with posteromedial surface of bar. Hooks displaced in a modified ancyrocephaline distribution (see Remarks); pair 6 with dilated shank. Parasites of siluriform fishes.

TYPE SPECIES, HOST, AND LOCALITY: *Quadriacanthoides andersoni* sp. n., from *Clarias lazera* Cuvier and Valenciennes, Nile River near Cairo, Egypt.

REMARKS: *Quadriacanthoides* is monotypic. The distribution of hooks in the haptor represents a modification of that normally occurring in ancyrocephaline species. Five pairs are ventral: pair 1 occurs near the posterior haptoral margin along the body midline; pairs 2, 3, 4 are placed laterally in the posterior half of the haptor; and pair 5 lies near the ventral anchor shafts on



Figures 86–94. ***Quadriacanthoides andersoni*. 86. Ventral view of holotype. 87. Copulatory complex. 88. Ventral anchor. 89. Dorsal bar. 90. Dorsal anchor. 91. Hook (pair 6). 92. Hook (pairs 1–5, 7). 93. Vagina. 94. Ventral bar. All drawings are to the 50-μm scale except Figure 86 (200 μm).**

the posterior haptoral margin. Two pairs are dorsal: pair 6 lies laterally about at haptoral midlength and anterior to the levels of ventral hooks and anchor/bar complex; and pair 7 occurs near the anterolateral corner of the haptor, anterior to all other sclerotized haptoral structures.

Quadriacanthoides andersoni sp. n. (Figs. 86–94)

HOST AND LOCALITY: *Clarias lazera* Cuvier and Valenciennes, Nile River near Cairo, Egypt.

SPECIMEN STUDIED: Holotype, USNM 79890.

DESCRIPTION: Body gently tapering anteriorly, 550 long; greatest width 120 in posterior trunk. Eye granules variable in shape and size, occurring from level of prostatic reservoirs into cephalic area, numerous anteriorly. Pharynx spherical, 28 in diameter. Haptor trapezoidal, 120 long, 138 wide. Ventral anchor 33 long, with delicate, evenly curved shaft and point, lacking roots; accessory sclerite small, wings unequal; anchor base 11 wide. Dorsal anchor 87 long, with hemicircular flange on superficial surface of base, straight shaft, long recurved point, base lacking roots; accessory sclerite large, with deep wing having hooked termination, superficial wing lamellate;

anchor base 14 wide. Ventral bar 88 long, broadly U-shaped, with tapering ends; dorsal bar 89 long, broadly V-shaped, with subrectangular anterior shield; T-shaped structure bifurcating anteriorly, articulating to posteromedial surface. Hooks with delicate point, shaft, protruding thumb; pairs 1–5, 7: 15 (14–16) long, lacking proximal shank enlargement; pair 6: 16–17 long, with ovate proximal enlargement of shank. FH loop about 0.8 length of undilated portion of shank. Cirrus 39 long, comprising loosely spiraled tube with small base; accessory piece chelate, 39 long. Margins of testis not observed; ovary elongate, spindle-shaped, 147 long, 24 wide. Vagina with subterminal bulb, lightly sclerotized.

REMARKS: This species is either rare on *Clarias lazera* or collection techniques were ineffective in removing the parasite from its site of attachment on the host. Although only 1 specimen was collected, the excellent condition of the specimen coupled with its distinctive features warranted the description of the species and proposal of the new genus. The species is named for Dr. Robert C. Anderson, Department of Biological Sciences, Idaho State University, who made the collections for this study while on a Fulbright Scholarship to Ain Shams University; Cairo, Egypt, during 1983–1984.

Acknowledgments

The authors are grateful for the following assistance: Drs. F. Puylaert (Musée Royal de l'Afrique Centrale, Tervuren, Belgium), A. V. Gussev (Zoological Institute, U.S.S.R. Academy of Sciences, Leningrad), Zhang Jian-Ying (Department of Biology, South China Normal University, Canton, China), and I. Paperna (Faculty of Agriculture, Hebrew University of Jerusalem) were instrumental in providing access to type and voucher specimens; Drs. R. C. Anderson (Department of Biological Sciences, Idaho State University) and A. Ashur (Department of Zoology, Ain Shams University, Cairo, Egypt) collected and identified hosts, respectively; and the Faculty and University Research Committees, Idaho State University, provided funds for collection and shipment of parasite specimens through grants 41, 556, and 596.

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Neotropical Monogenea. 12. Dactylogyridae from *Serrasalmus nattereri* (Cypriniformes, Serrasalmidae) and Aspects of Their Morphologic Variation and Distribution in the Brazilian Amazon

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ABSTRACT: The gills of *Serrasalmus nattereri* (Kner) were examined for Dactylogyridae from 2 distant locations in the Brazilian Amazon to determine species composition and morphologic variation. Three new genera are proposed. *Amphithecium* gen. n. is characterized in part by species possessing 2 dorsal bilateral vaginal pores. *Notothecium* gen. n. is distinguished by species having a vagina looping the left intestinal caecum and opening sinistrodorsally. The vagina in species of *Notozothecium* gen. n. loops the right intestinal caecum and opens on the dextrodorsal surface of the trunk. The following species are described: *Anacanthorus thatcheri* sp. n., *A. maltai* sp. n., *A. reginae* sp. n., *A. rondonensis* sp. n., *A.* sp., *Amphithecium calycinum* sp. n., *A. camelum* sp. n., *A. brachycirrum* sp. n., *A. falcatum* sp. n., *A. junki* sp. n., *A. catalaoensis* sp. n., *Notothecium mizellei* sp. n., *N. aegidatum* sp. n., *Notozothecium penetrarum* sp. n., and *N. minor* sp. n. Two morphologic forms of *Amphithecium camelum* (*A. camelum* forma amazonas and *A. camelum* forma rondonia) are reported from the Brazilian states of Amazonas and Rondônia, respectively. The community structure of the Dactylogyridae from *S. nattereri* differs somewhat with respect to species present between Amazonas and Rondônia, although some species occur at both locations. A hypothesis for the terminal phylogeny of species of *Amphithecium, Notothecium,* and *Notozothecium* is presented.

KEY WORDS: Dactylogyridae, Ancyrocephalinae, *Amphithecium* gen. n., *Nothothecium* gen. n., *Notozothecium* gen. n., *Anacanthorus thatcheri* sp. n., *Anacanthorus maltai* sp. n., *Anacanthorus reginae* sp. n., *Anacanthorus rondonensis* sp. n., *Amphithecium calycinum* sp. n., *Amphithecium camelum* sp. n., *Amphithecium brachycirrum* sp. n., *Amphithecium falcatum* sp. n., *Amphithecium junki* sp. n., *Amphithecium catalaoensis* sp. n., *Notothecium mizellei* sp. n., *Notothecium aegidatum* sp. n., *Notozothecium penetrarum* sp. n., *Notozothecium minor* sp. n., Cypriniformes, Serrasalmidae, *Serrasalmus nattereri,* piranha cajú, cladistics, phylogenetic analysis, morphologic variation, distribution, Brazilian Amazon.

The piranha cajú, *Serrasalmus nattereri* (Kner), is widely distributed in South America, being absent only west of the Andes, south of the 37th parallel, and in the small eastern coastal streams between the Rio São Francisco and the mouth of the Rio de la Plata (Fig. 1). The fish is considered the most dangerous of the piranhas and is the most filmed and written about in popular accounts. Despite its vicious reputation, the piranha cajú, like other piranhas, is an important food source especially to low-income families in Brazil (Braga, 1976).

Most studies on parasites of fishes from the Amazon are local or are based on specimens collected from hosts held in aquaria. However, evidence of geographic variation in ectoparasitic communities in the Amazon was provided by Thatcher and Boeger (1983, 1984), who observed distinct parasitic species of *Brasergasilus* (Copepoda) on the same host obtained from 2 distant Amazonian localities. In a broader study, Brooks et al. (1981), utilizing helminth species collected from the Magdalena, Maracaibo, Orinoco, Amazon, and Paraná-Rio de la Plata drainage systems, tested hypotheses of evolutionary origins of their hosts, freshwater stingrays (Potamotrygonidae), in South America. The purpose of the present study was to determine variation in monogenean communities occurring on the gills of *Serrasalmus nattereri* in ulterior Amazonian locations.

Materials and Methods

Fish hosts were collected during 1983–1984 from 6 sites (Fig. 1, Table 1). Methods for collection and preparation of Monogenea for study, measurement, and illustration are those described by Kritsky et al. (1986). Length of the accessory piece of the copulatory complex of *Amphithecium, Notothecium,* and *Notozothecium* species represents the interval between parallel lines on the respective drawings. Measurements are in micrometers; the mean is followed by the range in parentheses. Classification of Amazonian rivers (white

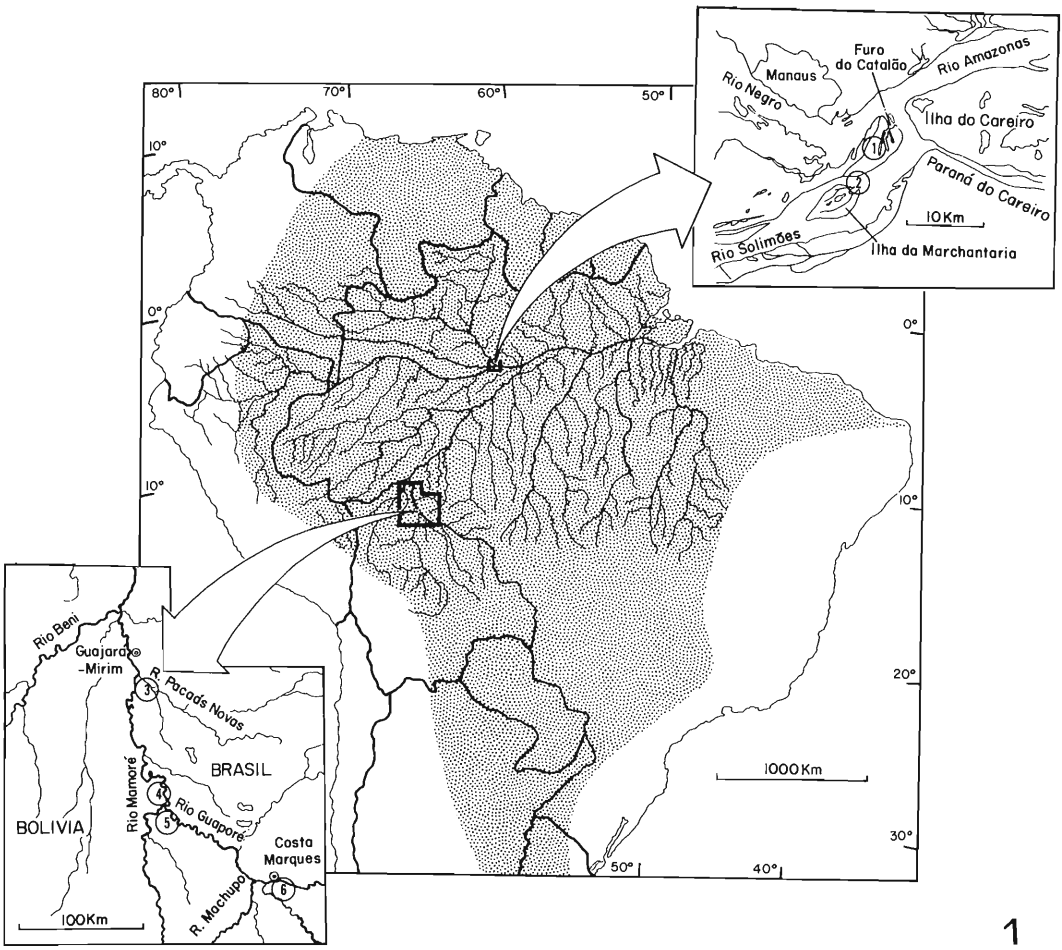


Figure 1. Map of northern South America showing collection sites (circled numbers) and distribution (modified from Braga, 1976) of *Serrasalmus nattereri* (shaded area). See Table 1 for specific identification of collection sites.

water, clear water, black water) follows that proposed by Geisler et al. (1973) and is based on respective physical and chemical characteristics.

Initial hypotheses on evolutionary relationships for species of *Amphithecium, Notothecium,* and *Notozothecium* were constructed using Hennigian argumentation (Hennig, 1966; Wiley, 1981) and tested with PAUP (D. L. Swofford, Illinois Natural History Survey, Champaign). Eighty character states comprising 36 homologous series were used in the analysis. Polarization within each series was determined by outgroup comparison (Wiley, 1981); functional outgroups as defined by Watrous and Wheeler (1981) were used when character states were entirely within the species group considered in the analysis. The dactylogyrid genus, *Urocleidoides* Mizelle and Price (sensu Kritsky et al., 1986) was used as the outgroup. Character states within homologous series represented by ratios were defined as groups of values with intervening distances greater than 0.15. Thus, some series utilizing ratios were not useful in the analysis because it was not possible to define more than 1 character state.

Homologous series utilized in the analysis are listed below; numbers listed in parentheses refer to the locations of change in character state in the cladogram (Fig. 125). Body length: plesiomorphy = <1 mm (1); apomorphy = >1 mm (44). Body shape: plesiomorphy = fusiform (2); apomorphies = strongly flattened (61), ventrally concave (82). Dorsal body hump: plesiomorphy = absent (3); apomorphy = present (83). Tegument: plesiomorphy = scaled (4); apomorphies = smooth (45, 76), papillated (84). Esophagus: plesiomorphy = absent (5); apomorphy = present (70). Eyes: plesiomorphy = present (6); apomorphy = absent (60). Number, position of vaginal apertures: plesiomorphy = 2, bilateral (7); apomorphies = 1, dextral (35), 1, sinistral (58). Sclerotized plate around vaginal pore: plesiomorphy = absent (8); apomorphy = present (41, 68). Vaginal duct: plesiomorphy = sclerotized (9); apomorphy = nonsclerotized (51). Seminal receptacle: ple-

Table 1. Localities from which collections of *Serrasalmus nattereri* (piranha cajú) were made during 1983–1984.

Locality number	Location	Dates of collection	No. fish	Water type
1	Furo do Catalão, near Manaus, Amazonas, Brazil	11/27/84 11/23/84	10	Mixed white/black water
2	Ilha da Marchantaria, Rio Solimões, near Manaus, Amazonas, Brazil	8/15/84 11/25/84	19	White water
3	Rio Pacaás-Novos, near Guajará-Mirim, Rondônia, Brazil	11/28/83	1	Clear water
4	Rio Mamoré, near Surpresa, Rondônia, Brazil	6/19/84	2	Mixed white/clear water*
5	Rio Guaporé, near Surpresa, Rondônia, Brazil	6/16/84	9	Clear water
6	Rio Guaporé, near Costa Marques, Rondônia, Brazil	11/22/83	3	Clear water

* Site 4 is at the confluence of the Rio Mamoré and Guaporé, therefore the water type is considered mixed.

siomorphy = present (10); apomorphy = absent (71). Ovary: plesiomorphy = with regular margins (11); apomorphy = with irregular margins (85). Vitellaria: plesiomorphy = randomly distributed (12); apomorphy = fimbriated (86). Prostatic reservoirs: plesiomorphy = 2 (13); apomorphy = 1 (46). Seminal vesicle: plesiomorphy = fusiform (14); apomorphy = C-shaped (62). Cirrus: plesiomorphy = coiled (15); apomorphy = straight (52). Number of cirral rami: plesiomorphy = 1 (16, 67); apomorphy = 2 (53). Relative length of cirral rami: plesiomorphy = subequal (54); apomorphy = unequal (57). Shape of cirral rami tips: plesiomorphy = pointed/pointed (50); apomorphies = pointed/funnel (75), pointed/reduced (56). Cirral aperture: plesiomorphy = terminal (17); apomorphy = diagonal (69). Cirral tip sclerotization: plesiomorphy = absent (18); apomorphy = present (80). Shape of accessory piece: plesiomorphy = T-shaped (19); apomorphy = otherwise (72, 77). Distal end of accessory piece: plesiomorphy = blunt (20); apomorphies = pointed (63), hooked (74), ornamented (36). Accessory piece flap: plesiomorphy = absent (21); apomorphy = present (64, 81). Size of accessory piece flap: plesiomorphy = about ½ length of distal portion of accessory piece (79); apomorphy = small (59). Ratio (hook pr. 5 length to cirral length): plesiomorphy = ≤0.1 (22); apomorphies = 0.2–0.5 (42, 73), 0.6–0.8 (55). Cleft on ventral bar: plesiomorphy = absent (23); apomorphy = present (87). Anteromedial projection on ventral bar: plesiomorphy = absent (24); apomorphy = present (34). Dorsal bar: plesiomorphy = projection absent (25); apomorphy = projection present (66). Anchor base: plesiomorphy = without proximal sclerotization (26); apomorphy = with proximal sclerotization (47). Ratio (ventral anchor length to dorsal anchor length): plesiomorphy = <1.2 (27); apomorphy = >1.4 (40, 88). Ratio (anchor point length to anchor shaft length): plesiomorphy = >0.2 (28); apomorphy = <0.2 (65, 78). Ratio (hook pr. 5 to hook pr. 2 length): plesiomorphy = 1.0 (29); apomorphy = <0.8 (37). Ratio (hook pr. 5 length to hook pr. 3 length): plesiomorphy = <0.8 (30); apomorphy = 1.0 (48). Ratio (hook pr. 5 length to hook pr. 4 length): plesiomorphy = 0.46–0.61 (31); apomorphies = 0.78 (43), 1.0 (38). Ratio (hook pr. 5 length to hook pr. 6 length): plesiomorphy = <0.85 (32); apomorphy = 1.0 (39). Ratio (hook pr. 5 length to hook pr. 7 length): plesiomorphy = <0.8 (33); apomorphy = 1.0 (49).

Type specimens are those collected from the type locality and were used in the description of species. Types and vouchers (specimens collected from other localities) were deposited in the helminth collections of the Instituto Nacional de Pesquisas da Amazônia (INPA), the U.S.N.M. Helm. Coll. (USNM), and the University of Nebraska State Museum (HWML). For comparative purposes, the following specimens of previously described species were examined: *Anacanthorus anacanthorus,* holotype (USNM 60459), paratype (HWML 21539); *Anacanthorus brazilensis,* holotype (USNM 60460), 6 paratypes (HWML 21538); *Anacanthorus neotropicalis,* holotype, paratype (USNM 60461), 2 paratypes (HWML 21537); *Cleidodiscus amazonensis,* holotype, paratype (USNM 60462), paratype (HWML 21289); *Cleidodiscus piranhus,* holotype (USNM 60463), paratype (HWML 21290); *Cleidodiscus serrasalmus,* holotype (USNM 60464); *Urocleidus crescentis,* holotype (USNM 60465); and *Urocleidus orthus,* holotype (USNM 60466).

Results

Dactylogyridae Bychowsky, 1933

Anacanthorinae Price, 1967

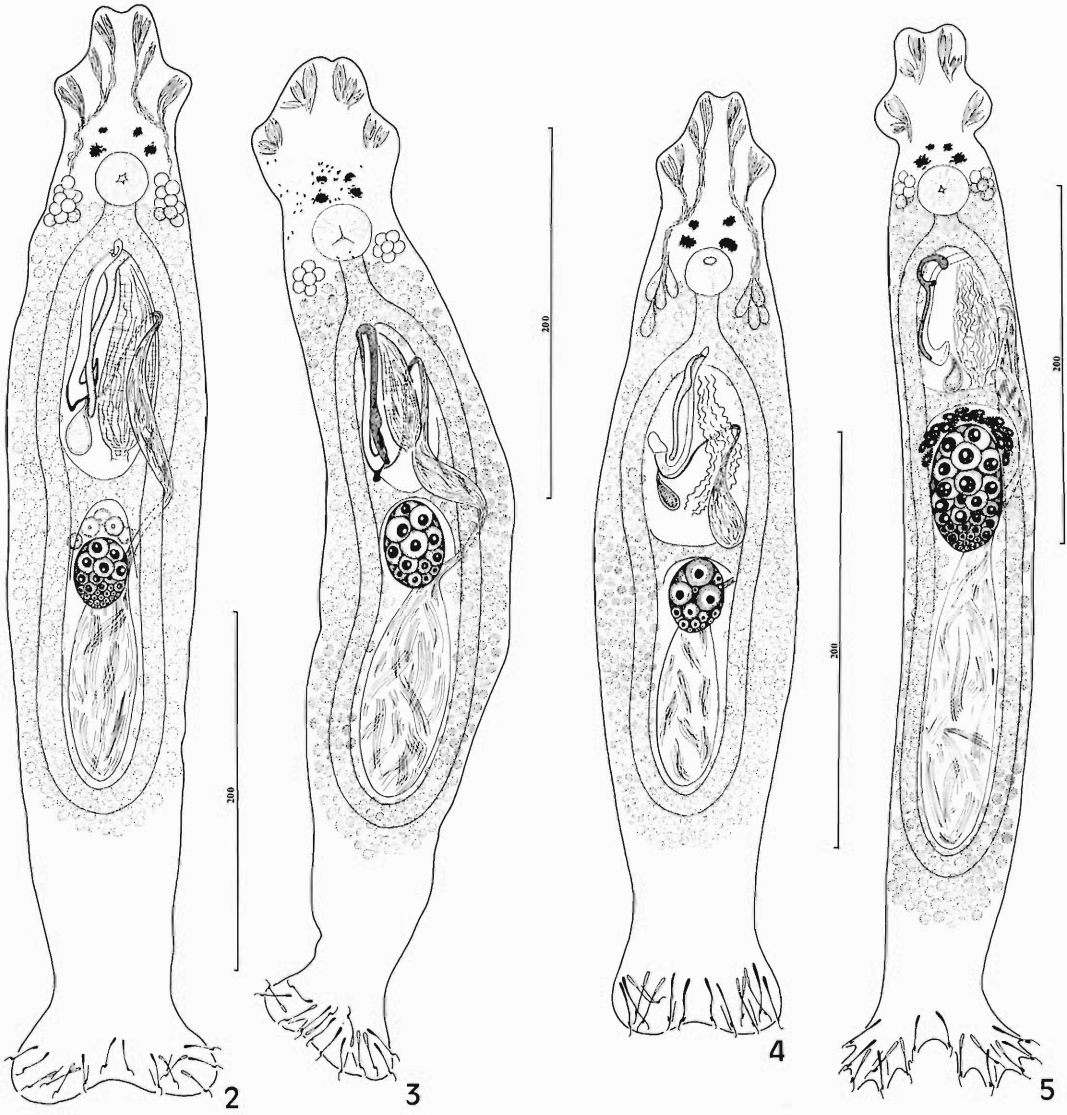
Anacanthorus thatcheri sp. n. (Figs. 2, 6–11)

TYPE LOCALITY: Rio Solimões, Ilha da Marchantaria near Manaus, Amazonas, Brazil (Locality 2).

OTHER RECORDS: Localities 1, 3, 4–6.

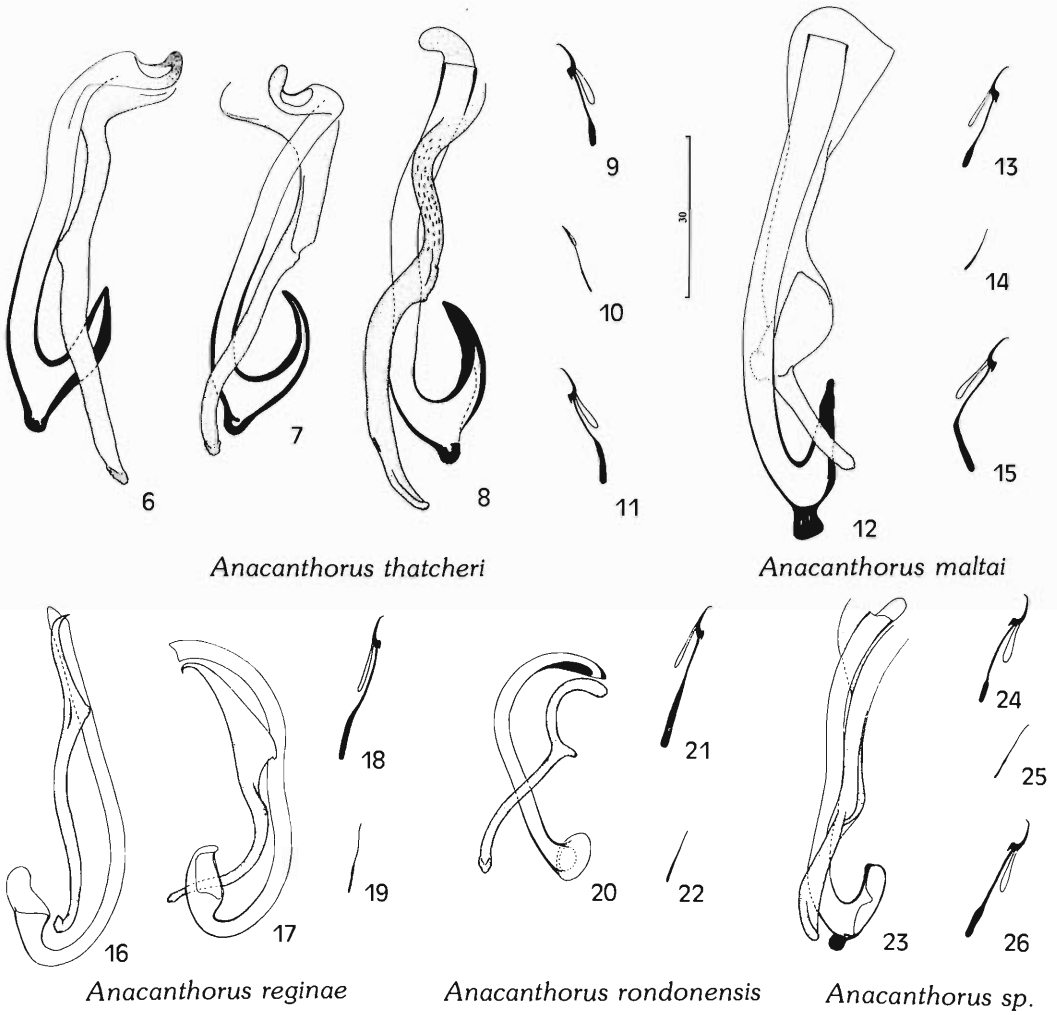
SPECIMENS DEPOSITED: Holotype, INPA PA300-1; paratypes, INPA PA300-2–10, USNM 79196, HWML 23370; vouchers, USNM 79203, 79213, 79219, 79223, 79237, 79286, HWML 23376, 23385, 23391, 23395.

DESCRIPTION (based on 65 specimens): Body fusiform, 684 (607–804) long; greatest width 126 (103–156) near midlength or in anterior trunk. Two terminal, 2 bilateral cephalic lobes well de-



Figures 2–5. Whole-mount illustrations (ventral) of *Anacanthorus* spp. 2. *Anacanthorus thatcheri* (holotype). 3. *Anacanthorus maltai* (holotype). 4. *Anacanthorus reginae* (holotype). 5. *Anacanthorus rondonensis* (holotype).

veloped; head organs large, generally 5 pairs lying in cephalic lobes and adjacent areas; cephalic glands lying near posterolateral margin of pharynx. Members of posterior pair of eyes larger, usually farther apart than members of anterior pair; granules of variable size, elongate ovate; accessory granules absent or few in proximity of eyes. Mouth subterminal; pharynx spherical, 36 (32–44) in diameter; esophagus short. Peduncle broad, moderately long; haptor bilobed, 57 (39–79) long, 128 (108–160) wide. Large hooks similar; each with short recurved point, depressed thumb, shank expanded proximally; hook 1–3, 6, 7—21 (19–23) long; hook 4, 5—24 (23–27) long; filamentous hook loop (FH loop) 0.5 shank length. Small hook splinter-like, 13 (11–15) long; FH loop 0.3 shank length. Testis ovate, 148 (120–178) long, 52 (30–67) wide; seminal vesicle elongate, a slight dilation of vas deferens; prostatic reservoir with thick wall. Cirrus 76 (68–83) long, J-shaped, base with anterior pointed process, cirrus tip with subterminal aperture and spathulate distal projection. Accessory piece 85 (80–94) long, not articulated to cirrus, with small expansion



Figures 6–26. Sclerotized structures of *Anacanthorus* spp. Figures 6–11. *Anacanthorus thatcheri*. 6–8. Copulatory complexes. 9. Hook 1, 3, 5–7. 10. Small hook. 11. Hook 2, 4. Figures 12–15. *Anacanthorus maltai*. 12. Copulatory complex. 13. Hook 1, 3, 5–7. 14. Small hook. 15. Hook 2, 4. Figures 16–19. *Anacanthorus reginae*. 16, 17. Copulatory complexes. 18. Large hook. 19. Small hook. Figures 20–22. *Anacanthorus rondonensis*. 20. Copulatory complex. 21. Large hook. 22. Small hook. Figures 23–26. *Anacanthorus* sp. 23. Copulatory complex. 24. Hook 1, 3, 5–7. 25. Small hook. 26. Hook 2, 4.

near midlength. Ovary ovate to subspherical, 73 (48–99) long, 43 (37–55) wide; ootype immediately anterior to ovary; uterus with moderately sclerotized distal wall; vagina, seminal receptacle absent. Vitellaria coextensive with caeca; vitelline commissure anterior to ootype.

ETYMOLOGY: This species is named for Dr. Vernon E. Thatcher, INPA, in recognition of his contributions in tropical parasitology.

REMARKS: Based on the morphology of the cirrus, this species resembles *A. anacanthorus* Mizelle and Price, 1965, and *A. maltai* sp. n. *Anacanthorus thatcheri* sp. n. is easily differentiated from these species by having a spathulate cirrus tip.

Anacanthorus maltai sp. n. (Figs. 3, 12–15)

TYPE LOCALITY: Rio Mamoré near Surpresa, Rondônia, Brazil (Locality 4).

OTHER RECORDS: Localities 3, 5, 6.

SPECIMENS DEPOSITED: Holotype, INPA PA301-1; paratypes, INPA PA301-2–5, USNM

79185, HWML 23359; vouchers, USNM 79210, 79224, 79246, HWML 23383.

DESCRIPTION (based on 24 specimens): Body fusiform, 636 (461–815) long; greatest width 128 (57–152) near midlength. Cephalic lobes well developed, 2 terminal, 2 bilateral; head organs well developed, generally 5 pairs in cephalic lobes; cephalic glands lying near posterolateral margin of pharynx. Members of posterior pair of eyes farther apart, larger than those of anterior pair; accessory granules absent, few or numerous in cephalic region. Mouth subterminal; pharynx spherical, 34 (27–42) in diameter; esophagus elongate. Peduncle broad, elongate; haptor bilobed, 72 (42–104) long, 89 (55–144) wide. Large hooks similar; each with short recurved point, depressed thumb, tapered shank; hook 22 (19–27) long; FH loop about 0.5 shank length. Small hook splinter-like, 11 (10–12) long. Testis elongate ovate, 159 (112–202) long, 67 (37–90) wide; seminal vesicle a sigmoid dilation of vas deferens; prostatic reservoir not observed. Cirrus 93 (86–99) long, J-shaped, with elongate base possessing conspicuous posterior heel-like projection. Accessory piece 90 (85–95) long, not articulated to cirrus base, twisted near midlength, proximal portion rod-shaped, distal portion spathulate. Ovary ovate to subspherical, 69 (60–80) long, 43 (25–60) wide; ootype immediately anterior to ovary; uterus muscular, with sclerotized distal wall; vagina, seminal receptacle absent. Vitellaria dense, coextensive with caeca; vitelline commissure anterior to ovary.

ETYMOLOGY: *Anacanthorus maltai* sp. n. is named for José Celso de Oliveira Malta, who kindly provided the hosts from Rondônia.

REMARKS: The general morphology of the copulatory complex and hooks indicate a close relationship between *A. maltai* and *A. thatcheri*. *Anacanthorus maltai* differs from *A. thatcheri* by having a larger posterior heel-like projection on the cirrus base, a nonspathulate tip of the cirrus tube, and an elongate flattened distal portion on the accessory piece.

Anacanthorus reginae sp. n. (Figs. 4, 16–19)

TYPE LOCALITY: Rio Solimões, Ilha da Marchantaria near Manaus, Amazonas, Brazil (Locality 2).

OTHER RECORDS: Localities 1, 3–6.

SPECIMENS DEPOSITED: Holotype, INPA PA293-1; paratypes, INPA, PA293-2–5; USNM 79190, HWML 23364; vouchers, USNM 79204, 79209, 79220, 79221, 79236, HWML 23377, 23382, 23392, 23393, 23406.

DESCRIPTION (based on 35 specimens): Body fusiform, 443 (289–499) long; greatest width 107 (89–144) near midlength. Cephalic lobes well developed, 2 terminal, 2 bilateral; head organs large, generally 4 pairs lying in cephalic lobes; cephalic glands posterolateral to pharynx. Eyes equidistant, members of posterior pair larger; eye granules variable in size; accessory granules absent to numerous in cephalic area and anterior trunk. Mouth subterminal; pharynx spherical, 25 (22–32) in diameter; esophagus elongate. Peduncle broad; haptor bilobed, 48 (34–72) long, 81 (44–123) wide. Large hooks similar; each with short recurved point, depressed thumb, shank tapering distally; hook 28 (23–34) long; FH loop 0.5 shank length; small hook splinter-like, 11 (9–13) long. Testis ovate, 92 (73–120) long, 45 (34–62) wide; seminal vesicle a pyriform dilation of vas deferens; prostatic reservoir with thick wall. Cirrus 67 (57–76) long, J-shaped, with simple base. Accessory piece 59 (42–67) long, not articulated to cirrus base, rod-shaped, with variable subterminal expanded area (compare Figs. 16, 17). Ovary ovate to subspherical, 44 (31–55) long, 29 (25–37) wide; ootype inconspicuous; uterus with sclerotized distal wall; vagina, seminal receptacle absent. Vitellaria dense, coextensive with caeca; vitelline commissure anterior to ovary.

ETYMOLOGY: This species is named for Regina T. Boeger, wife of the senior author.

REMARKS: This species differs from all previously described species of *Anacanthorus* by possessing a rod-shaped accessory piece with a variable subterminal expansion. It is apparently related to *A. neotropicalis* Mizelle and Price, 1965, based on the comparative morphology of the large haptoral hooks.

Anacanthorus rondonensis sp. n. (Figs. 5, 20–22)

TYPE LOCALITY: Rio Mamoré near Surpresa, Rondônia, Brazil (Locality 4).

OTHER RECORDS: Localities 3, 5, 6.

SPECIMENS DEPOSITED: Holotype, INPA PA298-1; paratypes, INPA PA298-2–4, USNM 79194, HWML 23368; vouchers, USNM 79222, 79245, HWML 23394.

DESCRIPTION (based on 63 specimens): Body fusiform, 588 (497–692) long; greatest width 108 (78–173) near midlength.Cephalic lobes well de-

veloped, 2 terminal, 2 bilateral; head organs large, generally 4 pairs lying in cephalic lobes; cephalic glands unicellular, lying lateral to pharynx. Members of anterior pair of eyes smaller, usually closer together than those of posterior pair; accessory granules absent or few in proximity of eyes. Mouth subterminal; pharynx spherical, 29 (25–32) in diameter; esophagus short. Peduncle broad; haptor bilobed, 79 (41–83) long, 98 (63–132) wide. Large hook with short recurved point, depressed thumb, shank expanded proximally; hook 26 (23–31) long; FH loop 0.5 shank length; small hook 11 (10–13) long, splinter-like. Testis elongate ovate, 168 (110–212) long, 53 (42–65) wide; seminal vesicle an elongate fusiform dilation of vas deferens; prostatic reservoir with thick wall. Cirrus 44 (41–47) long, sickle-shaped, with subcircular base and thick-walled distal end. Accessory piece 42 (39–45) long, not articulating to cirrus base, with C-shaped distal portion. Ovary ovate, 57 (45–63) long, 32 (24–37) wide; ootype immediately anterior to ovary; uterus large with moderately sclerotized distal wall; vagina, seminal receptacle absent. Vitellaria dense, coextensive with caeca; vitelline commissure anterior to ovary.

ETYMOLOGY: The specific name reflects the Brazilian state from which the specimens were collected.

REMARKS: This species is similar to *Anacanthorus neotropicalis* Mizelle and Price, 1965, from which it differs by possessing an accessory piece with an elongate proximal arm and distinct C-shaped termination.

Anacanthorus sp. (Figs. 23–26)

RECORDS: Localities 1, 2.

SPECIMENS DEPOSITED: Vouchers, INPA PA305-1, 2; USNM 79197, HWML 23371.

REMARKS: The collections of this species were insufficient to provide a detailed description. Nonetheless, they apparently represent an undescribed species that is closely related to *Anacanthorus thatcheri* and *A. maltai*. The undescribed species lacks the acute anterior process on the base of the accessory piece, characteristic of the latter 2 species.

Ancyrocephalinae Bychowsky, 1937

Amphithecium gen. n.

DIAGNOSIS: Body divisible into cephalic region, trunk, peduncle, haptor. Tegument thin, smooth, scaled, or papillate. Two terminal, 2 bilateral cephalic lobes; head organs, unicellular cephalic glands present. Four eyes. Mouth subterminal, midventral; pharynx muscular, glandular; esophagus present; intestinal caeca 2, confluent posterior to testis, lacking diverticula. Gonads intercaecal, overlapping; testis dorsal to ovary. Vas deferens looping left caecum; seminal vesicle a simple dilation of vas deferens. Two prostatic reservoirs; prostates comprising 2 bilateral glandular areas lying dorsal to caeca. Genital pore midventral near level of caecal bifurcation. Copulatory complex comprising an accessory piece, biramous cirrus; accessory piece articulated to cirrus base. Two dorsal bilateral vaginae united medially by common nonsclerotized duct; seminal receptacle absent. Vitellaria coextensive with caeca, commissure anterior to ovary. Haptor armed with pairs of dorsal and ventral anchors, dorsal and ventral bars, 7 pairs of hooks with ancyrocephaline distribution. Hook with shank of 2 distinct parts. Parasites of gills of Serrasalmidae.

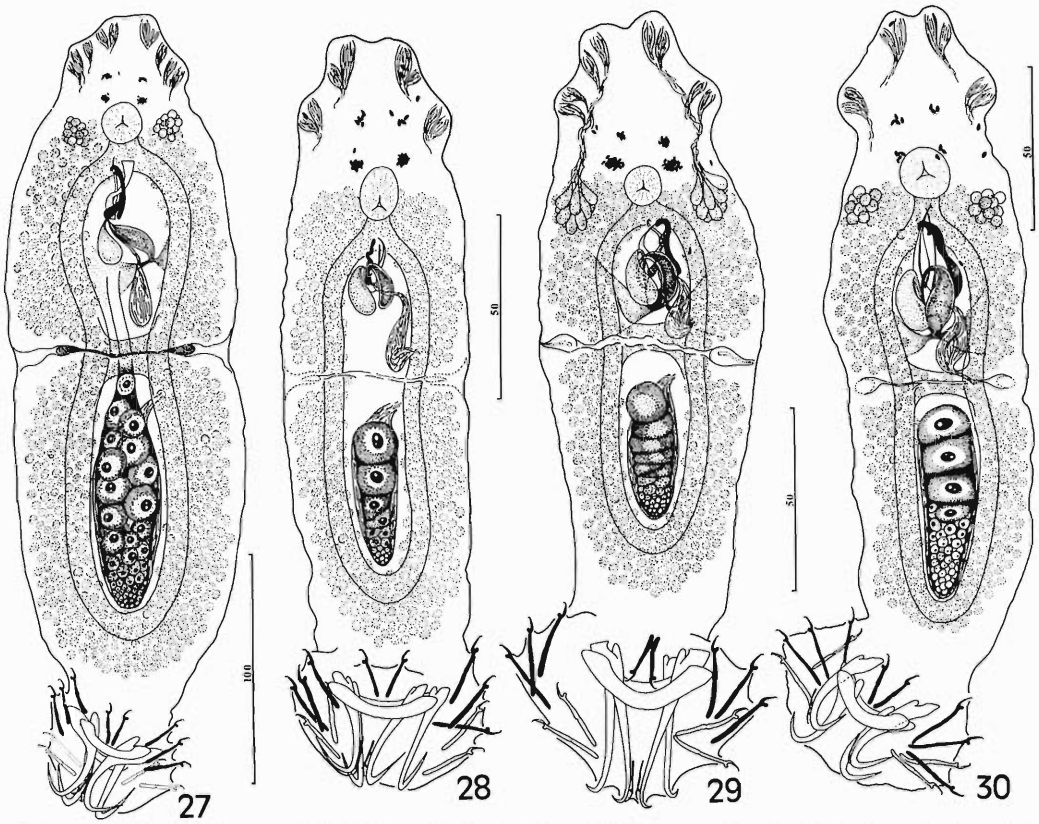
TYPE SPECIES: *Amphithecium calycinum* sp. n. from *Serrasalmus nattereri*.

OTHER SPECIES: *A. brachycirrum, A. camelum, A. catalaoensis, A. falcatum,* and *A. junki* spp. n., all from *Serrasalmus nattereri*.

OTHER POSSIBLE MEMBER: *Cleidodiscus amazonensis* Mizelle and Price, 1965, from *Serrasalmus nattereri*.

ETYMOLOGY: The generic name is from Greek (*amphis* = on both sides + *theke* = case) and refers to the dorsal bilateral vaginae.

REMARKS: *Amphithecium* gen. n. provides an example of the significance of the internal morphology in systematic studies of the Dactylogyridae. If the subsequent descriptions included details of only the sclerotized parts of the haptor and copulatory complex, all species, including those of the following 2 new genera, *Notothecium* and *Notozothecium,* would be included in the same generic taxon. Indeed, the placement of the previously described ancyrocephaline species from *Serrasalmus nattereri* by Mizelle and Price (1965) into *Cleidodiscus* and *Urocleidus* is likely a result of incomplete knowledge of internal anatomy. Although the haptoral structures of species of *Amphithecium* can be considered morphologically generalized for dactylogyrids, the new genus is unique in its character of the double unsclerotized vaginal apertures. Other combined features that characterize the genus are the bi-



Figures 27–30. Whole-mount illustrations (ventral) of *Amphithecium* spp. 27. *Amphithecium calycinum* (holotype). 28. *Amphithecium brachycirrum* (holotype). 29. *Amphithecium junki* (holotype). 30. *Amphithecium falcatum* (holotype).

ramous cirrus, overlapping gonads, accessory piece articulated to the cirrus base, and hooks with shanks of 2 distinct parts.

Gussev (1978) considered paired vaginae as an extraordinary character for dactylogyrideans and indicated *Neodactylodiscus* Kamegai, 1971, and *Dactylogyrus obscurus* Gussev, 1955 (=*Bivaginogyrus obscurus* (Gussev, 1955) Gussev and Gerasev, 1986), as the only taxa where this character is present. The functional relationship between the double vaginae and the biramous cirrus of *Amphithecium* species is not clear. In most of the specimens examined, the duct uniting the 2 vaginae is filled with sperm indicating that it functionally replaces the seminal receptacle.

Kritsky and Thatcher (1983) suggested that the previously described monogenes from *Serrasalmus nattereri* assigned by Mizelle and Price (1965) to *Cleidodiscus* and *Urocleidus* are members of unique and undefined Neotropical genera. Our examination of the holotypes and paratypes of these species indicated that one, *C. amazonensis,* may belong to *Amphithecium.* The cirrus of this species possesses 2 well-developed rami (Fig. 89), a characteristic of *Amphithecium.* However, because the internal morphology of this species could not be determined from the unstained and cleared types, it is provisionally retained in *Cleidodiscus* until recollected and its internal anatomy defined; the species is clearly not a member of *Cleidodiscus* as defined by Beverley-Burton (1984).

Amphithecium calycinum sp. n. (Figs. 27, 31–39)

TYPE LOCALITY: Rio Guaporé near Surpresa, Rondônia, Brazil (Locality 5).

OTHER RECORDS: Localities 1–4, 6.

SPECIMENS DEPOSITED: Holotype, INPA PA295-1; paratypes, INPA PA295-2–4, USNM 79192, HWML 23366; vouchers, INPA PA296-1, USNM 79198–79202, HWML 23372–23375.

DESCRIPTION (based on 44 specimens): Body fusiform, length 290 (250–341); greatest trunk width 110 (90–122) at level of gonads. Tegument smooth. Cephalic lobes well developed; usually 4 pairs of head organs, 1 additional pair occasionally present; cephalic glands lateral to pharynx. Members of anterior pair of eyes smaller, usually closer together than members of posterior pair; eye granules elongate ovate, variable in size; accessory granules absent or few in proximity of eyes. Pharynx spherical, 18 (17–21) in diameter; esophagus short. Peduncle broad. Haptor subhexagonal, 44 (35–50) long, 60 (50–73) wide. Anchors similar; each with elongate superficial root, evenly curved shaft, recurved point; ventral anchor 27 (25–30) long, base 13 (12–16) wide; dorsal anchor 28 (25–31) long, base 15 (14–16) wide. Ventral bar 26 (23–30) long, with subterminal expansions; dorsal bar 25 (24–28) long, undulating. Hook 2–4, 6, 7 similar; each with erect thumb, slightly curved shaft, short point, inflated shank; hook 1, 5 with slender shank; hook 1—14 (13–16) long; hook 2, 6—16 (15–18) long; hook 3, 4, 7—21 (19–24) long; hook 5—12 (11–14) long. FH loop 0.8 length of distal portion of shank. Cirrus 31 (26–40) long, with needle-like and funnel-shaped rami, base variable. Accessory piece 21 (18–26) long, with hooked distal process, flexible proximal process articulating to cirrus base. Testis elongate ovate, 64 (49–82) long, 28 (22–34) wide; seminal vesicle pyriform, small; prostates well developed; prostatic reservoirs with heavy walls. Ovary elongate ovate, 67 (51–88) long, 29 (18–48) wide; ootype consisting of large cells lying immediately anterior to common vaginal duct. Vitellaria dense.

ETYMOLOGY: The specific name is from Neolatin (*calycin/o* = cuplike) and refers to the shape of the largest cirral ramus.

REMARKS: *Amphithecium calycinum* is the type species for the genus.

Amphithecium brachycirrum sp. n. (Figs. 28, 40–47)

TYPE LOCALITY: Furo do Catalão near Manaus, Amazonas, Brazil (Locality 1).

OTHER RECORDS: Localities 2–6.

SPECIMENS DEPOSITED: Holotype, INPA PA302-1; paratypes, INPA PA302-2, USNM 79186, HWML 23360; vouchers, USNM 79206–79208, 79232, 79238, HWML 23379–23381, 23402.

DESCRIPTION (based on 10 specimens): Body fusiform, length 204 (196–217); greatest width 62 (54–72) near midlength. Tegument scaled on trunk, peduncle. Cephalic lobes well developed; generally 4 pairs of head organs. Members of anterior pair of eyes smaller, usually closer together than members of posterior pair; eye granules ovate; accessory granules usually present in cephalic region. Pharynx ovate, 12 (11–13) wide; esophagus short. Peduncle broad. Haptor subovate, 39 (37–45) long, 55 (53–57) wide. Anchors similar; each with well-developed roots, recurved point; ventral anchor 27 (25–28) long, with evenly curved shaft, base 12 (11–13) wide; dorsal anchor 27 (26–28) long, with straight shaft, base 11–12 wide. Ventral bar 30 (27–32) long, with subterminal anterior expansions; dorsal bar 29 (27–30) long, broadly U-shaped. Hook 1–4, 6, 7 with erect thumb, slightly curved shaft, short point, inflated shank; hook 5 with slender shank, small proximal inflation; hook 1, 5—13 (12–14) long; hook 2—19 (17–21) long; hook 3, 7—21 (20–23) long; hook 4—23 (21–24) long; hook 6—16 (15–17) long; FH loop 0.8 length of distal portion of shank. Cirral rami needle-like; cirrus 17 (14–21) long. Accessory piece 13 (9–16) long, variable. Testis elongate ovate, 32 (28–35) long, 11 (9–12) wide; seminal vesicle sigmoid. Prostates poorly developed; prostatic reservoirs with thick walls. Ovary elongate ovate, 36 (35–37) long, 13 (11–15) wide; ootype, uterus not observed. Vitellaria dense.

ETYMOLOGY: The specific name is from Greek (*brachys* = short) and refers to the small size of the copulatory complex.

REMARKS: Based on the morphology of the anchors, hooks, and bars, *Amphithecium brachycirrum* resembles the type species, *A. calycinum*. It is easily differentiated from this species by lacking a hook-shaped termination of the accessory piece.

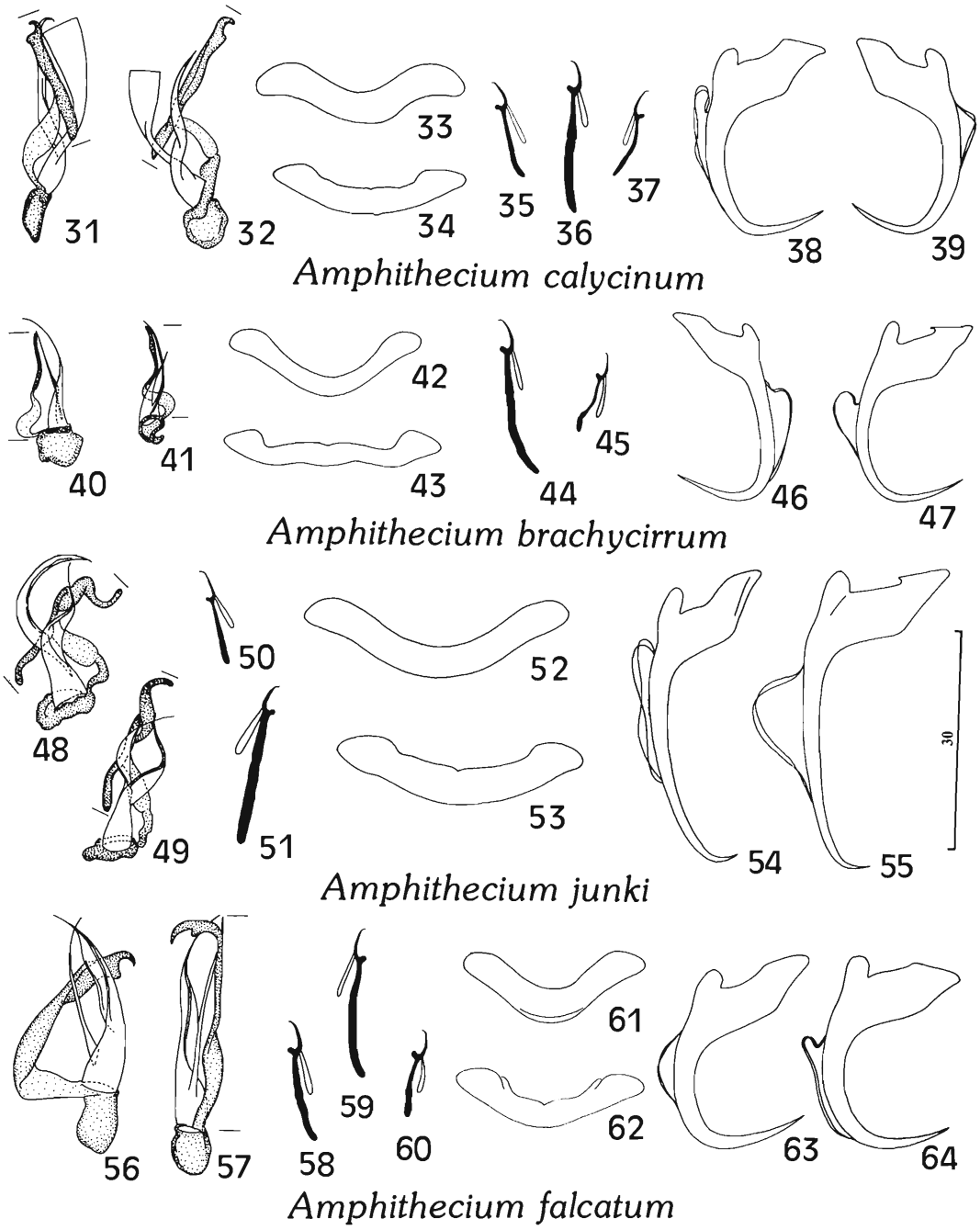
Amphithecium junki sp. n. (Figs. 29, 48–55)

TYPE LOCALITY: Furo do Catalão near Manaus, Amazonas, Brazil (Locality 1).

OTHER RECORDS: Localities 2–6.

SPECIMENS DEPOSITED: Holotype, INPA PA294-1; paratypes, INPA PA294-2–5, USNM 79191, HWML 23365; vouchers, USNM 79214, 79215, 79217, 79231, 79239, HWML 23386, 23387, 23389, 23401.

DESCRIPTION (based on 46 specimens): Body fusiform, length 228 (195–282); greatest width



Figures 31–64. Sclerotized structures of *Amphithecium* spp. Figures 31–39. *Amphithecium calycinum*. 31. Copulatory complex. 32. Copulatory complex (dorsal). 33. Dorsal bar. 34. Ventral bar. 35. Hook pair 1. 36. Hook 2–4, 6, 7. 37. Hook 5. 38. Dorsal anchor. 39. Ventral anchor. Figures 40–47. *Amphithecium brachycirrum*. 40, 41. Copulatory complexes (dorsal). 42. Dorsal bar. 43. Ventral bar. 44. Hook 1–4, 6, 7. 45. Hook 5. 46. Dorsal anchor. 47. Ventral anchor. Figures 48–55. *Amphithecium junki*. 48, 49. Copulatory complexes (dorsal). 50. Hook 5. 51. Hook 1–4, 6, 7. 52. Dorsal bar. 53. Ventral bar. 54. Dorsal anchor. 55. Ventral anchor. Figures 56–64. *Amphithecium falcatum*. 56. Copulatory complex. 57. Copulatory complex (dorsal). 58. Hook 1. 59. Hook 2–4, 6, 7. 60. Hook 5. 61. Dorsal bar. 62. Ventral bar. 63. Dorsal anchor. 64. Ventral anchor.

66 (46–81) near midlength or in anterior trunk. Tegument scaled on trunk, peduncle. Cephalic lobes well developed; usually 4 pairs of head organs; cephalic glands lying posterolateral to pharynx. Members of anterior pair of eyes smaller, usually closer together than members of posterior pair; eye granules ovate; accessory granules absent or few in proximity of eyes and anterior trunk. Pharynx spherical, 12 (9–14) in diameter; esophagus short. Peduncle broad. Haptor subpentagonal, 47 (38–59) long, 65 (54–81) wide. Anchors similar, each with robust superficial root, elongate shaft, short point; ventral anchor 42 (40–43) long, base 17 (14–19) wide; dorsal anchor 40 (38–43) long, base 15 (14–17) wide. Bars similar, broadly U-shaped; ventral bar 35 (34–36) long, dorsal bar 34 (30–37) long. Hook 1–4, 6, 7 with erect thumb, slightly curved shaft, inflated shank; hook 5 with shank tapering distally; hook 1, 2—20 (19–22) long; hook 3—24 (22–25) long; hook 4, 7—26 (24–28) long; hook 5—12 (11–14) long; hook 6—21 (17–22) long; FH loop 0.8 length of distal part of shank. Cirral rami bladelike, base variable; cirrus 26 (23–34) long. Accessory piece 20 (17–24) long, comprising blunt terminal, variable proximal portions. Testis elongate ovate, 36 (32–45) long, 16 (13–19) wide; seminal vesicle sigmoid. Prostates well developed; prostatic reservoirs with thick wall. Ovary elongate ovate, 42 (36–55) long, 15 (10–21) wide; oviduct, ootype, uterus not observed; distal portions of vaginae bulbous. Vitellaria dense.

ETYMOLOGY: This species is named for Dr. Wolfgang J. Junk, Max Planck Institute für Limnologie, in recognition of his contributions in aquatic ecology of the Amazon region.

REMARKS: The anchors of *Amphithecium junki* are similar to those of *A. catalaoensis* sp. n. *Amphithecium junki* differs from this species by possessing bladelike cirral rami and hooks with shorter shanks.

Amphithecium falcatum sp. n. (Figs. 30, 56–64)

TYPE LOCALITY: Furo do Catalão near Manaus, Amazonas, Brazil (Locality 1).

OTHER RECORDS: Localities 2–6.

SPECIMENS DEPOSITED: Holotype, INPA PA304-1; paratypes, INPA PA304-2, 3, USNM 79188, HWML 23362; vouchers, USNM 79211, 79212, 79216, 79218, 79241, HWML 23384, 23388, 23390.

DESCRIPTION (based on 18 specimens): Body fusiform, length 224 (193–238); greatest width 80 (54–93) near midlength or in anterior trunk. Tegument scaled on peduncle, trunk. Cephalic lobes well developed; usually 4 pairs of head organs lying in cephalic lobes; cephalic glands posterolateral to pharynx. Eyes equidistant, subequal, comprising few granules; eye granules ovate; accessory granules infrequent in cephalic area. Pharynx spherical, 15 (13–16) in diameter; esophagus short. Peduncle broad. Haptor subhexagonal, 40 (33–45) long, 63 (57–73) wide. Anchors similar, each with robust superficial root, short shaft, curved point; ventral anchor 28 (27–32) long, base 17 (15–18) wide; dorsal anchor 30 (27–35) long, base 17 (14–18) wide. Bars similar, broadly U-shaped; ventral bar 28 (27–29) long; dorsal bar 26–27 long. Hooks similar; each with erect thumb, slightly curved shaft, short point, shank with proximal portion of variable length among hook pairs; hook 1—17 (15–19) long; hook 2—21 (18–24) long; hook 3, 7—24 (21–26) long; hook 4—26 (24–28) long; hook 5—13–14 long; hook 6—19 (18–21) long; FH loop 0.8 length of distal portion of shank. Cirrus comprising 2 dissimilar rami: primary ramus bladelike, secondary ramus needle-like; cirrus 40 (30–48) long. Accessory piece 33 (25–39) long, with hooklike termination. Testis elongate ovate, 44 (39–47) long, 21 (17–23) wide; seminal vesicle sigmoid. Prostates well developed; prostatic reservoirs with thick walls. Ovary elongate ovate, 48 (40–64) long, 19 (15–22) wide; oviduct, ootype, uterus not observed; vitellaria dense.

ETYMOLOGY: The species name is from Latin (*falcatus* = sickle-shaped) and refers to the termination of the accessory piece.

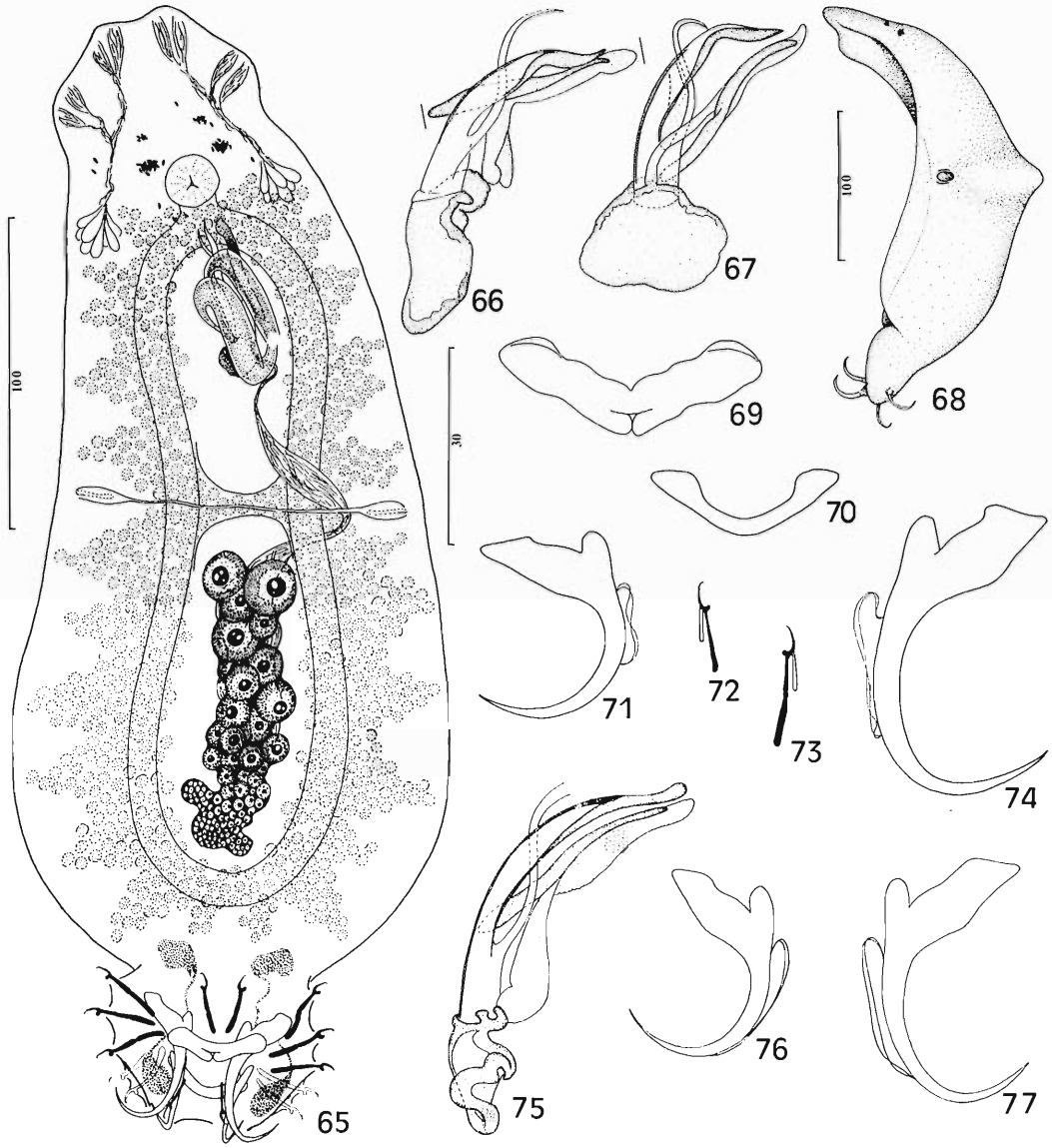
REMARKS: *Amphithecium falcatum* resembles *A. calycinum* in that the sclerotized structures of the haptor are similar and both possess hooklike terminations of the accessory piece. They differ most siginficantly by the accessory piece of *A. falcatum* lacking a posteriorly directed branch near its midlength.

Amphithecium camelum sp. n. (Figs. 65–77, 90, 91)

TYPE LOCALITY: Rio Solimões, Ilha da Marchantaria near Manaus, Amazonas, Brazil (Locality 2, forma amazonas).

OTHER RECORDS: Localities 1 (forma amazonas); 3–6 (forma rondonia).

SPECIMENS DEPOSITED: Holotype, INPA PA299-1; paratypes, INPA PA299-2–10, USNM



Figures 65–77. ***Amphithecium camelum.*** **Figures 65–74.** ***Amphithecium camelum*** **forma amazonas. 65. Holotype (ventral). 66. Copulatory complex (dorsolateral). 67. Copulatory complex. 68. Specimen (lateral view). 69. Ventral bar. 70. Dorsal bar. 71. Dorsal anchor. 72. Hook 5. 73. Hook 1–4, 6, 7. 74. Ventral anchor. Figures 75–77.** ***Amphithecium camelum*** **forma rondonia. 75. Copulatory complex. 76. Dorsal anchor. 77. Ventral anchor. All figures are to the same scale (30 μm) except Figures 65 and 68 (respective 100-μm scales).**

79195, HWML 23369; vouchers, USNM 79205, 79233–79235, 79242, HWML 23378, 23403–23405.

DESCRIPTION (based on 168 specimens): Body ovate, flattened dorsoventrally, ventral surface concave; mature specimens with dorsal hump-like protuberance near midlength (Figs. 68, 90). Tegument papillate (Fig. 91). Cephalic lobes well developed; generally 4 pairs of head organs lying in cephalic lobes; cephalic glands posterolateral to pharynx. Members of anterior pair of eyes smaller, usually closer together than those of posterior pair; eye granules ovate; accessory granules absent or distributed throughout cephalic region. Pharynx ovate; esophagus short. Peduncle short, broad. Haptor subhexagonal with 2 pairs of

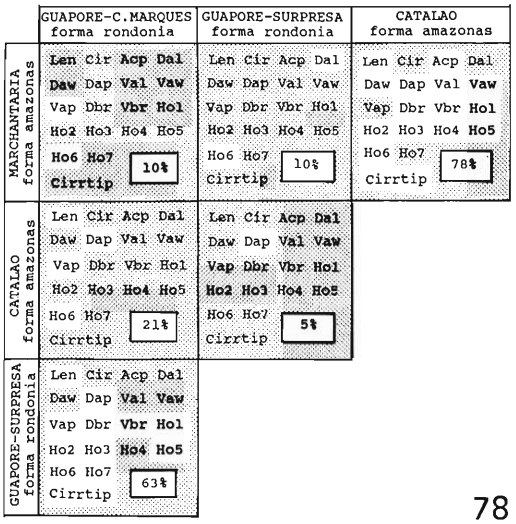


Figure 78. Comparison of characters between specimens of the 2 morphologic forms of *Amphithecium camelum* from 4 localities. Numerical values were compared by an ANOVA followed by the Tukey test. Characters significantly different ($P < 0.01$) appear shaded. Percent of similarity (number of characters not significantly different/total number of characters considered) is provided in the small squares at the bottom of each comparison. LEN = total body length; CIR = cirrus length; ACP = accessory piece length; DAL = dorsal length; DAW = dorsal anchor base width; DAP = DAL/DAW; VAL = ventral anchor length; VAW = ventral anchor base width; VAP = VAL/VAW; DBR = dorsal bar length; VBR = ventral bar length; H01–H07 = hook length of respective pairs; CIRRTIP = shape of the distal end of the primary ramus of the cirrus.

haptoral glands. Ventral anchor with well-developed roots, curved shaft, recurved point; dorsal anchor with evenly curved shaft and point, roots well developed. Ventral bar robust, broadly V-shaped, with expanded ends and mediopos-

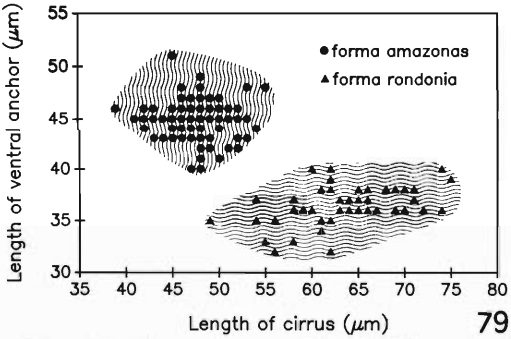


Figure 79. Scatter diagram of length of the ventral anchor versus length of the cirrus for the 2 forms of *Amphithecium camelum*.

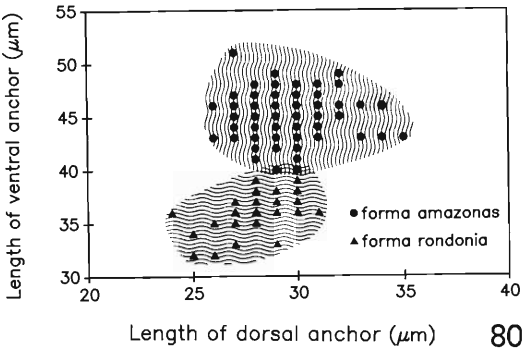


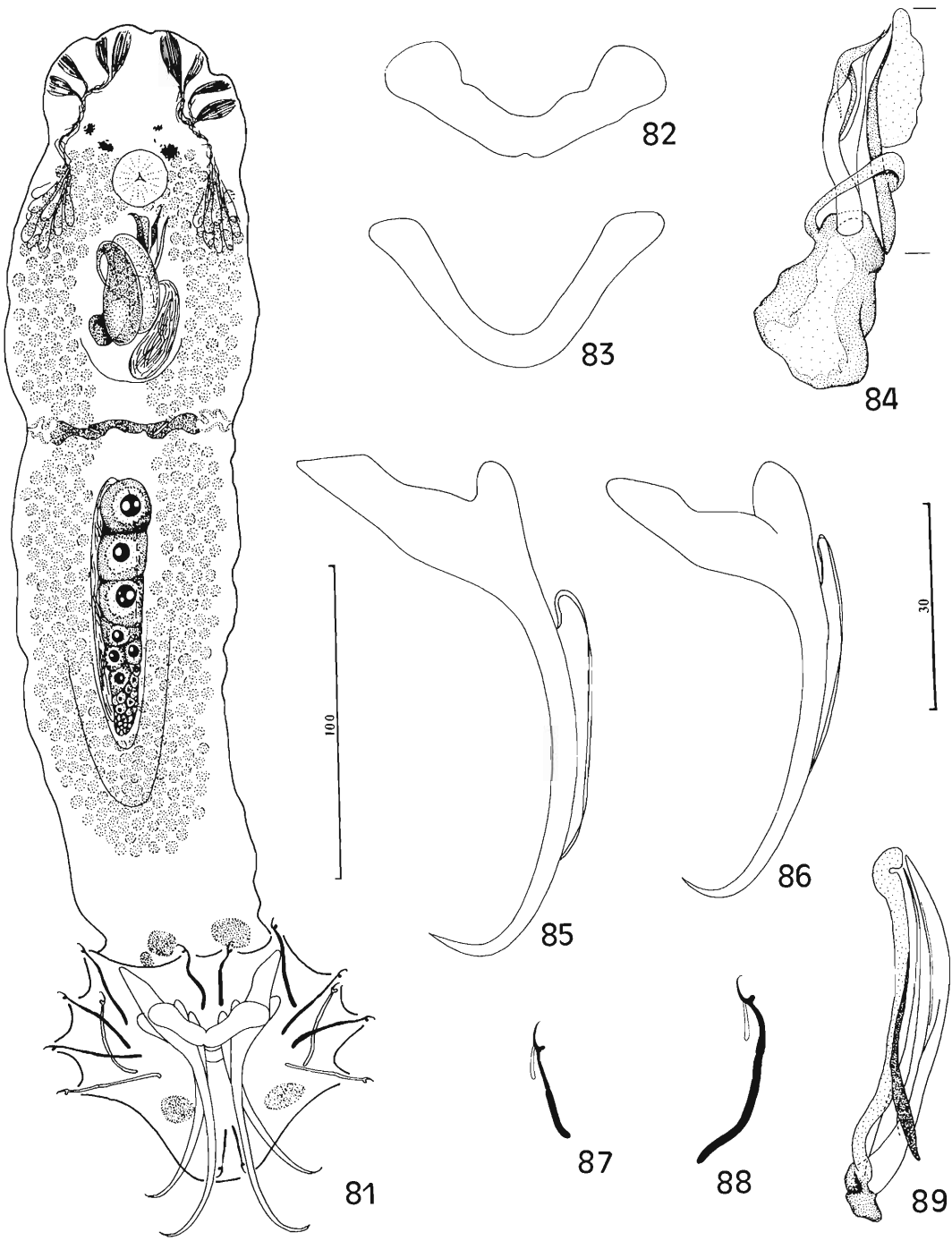
Figure 80. Scatter diagram of length of the ventral anchor versus length of the dorsal anchor for the 2 forms of *Amphithecium camelum*.

terior cleft. Dorsal bar with slightly expanded ends, broadly U-shaped. Hooks similar; each with erect thumb, slightly curved shaft, short point; FH loop 0.8 length of distal portion of shank. Primary cirral ramus heavily sclerotized, secondary ramus needle-like. Accessory piece comprising a distal rodlike portion with marginal distal flap, articulating to cirral base by variable proximal process. Testis elongate, irregular; seminal vesicle sigmoid. Prostatic reservoirs elongate, with thick walls; prostates not observed. Ovary irregular; oviduct, uterus not observed. Vitellaria dense, laterally fimbriated. Egg ovate with short filament.

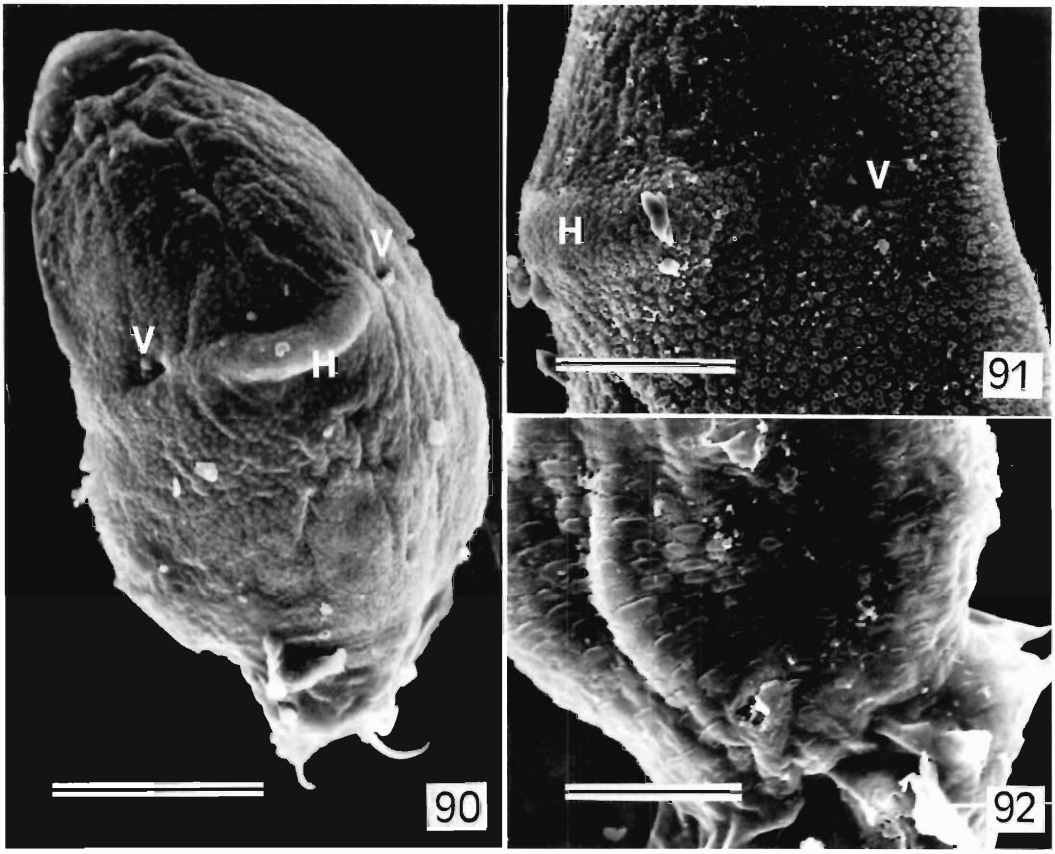
ETYMOLOGY: The specific name is from Greek (*kamelos* = camel) and refers to the presence of a dorsal hump in mature specimens.

REMARKS: *Amphithecium camelum* is the only known species of the genus possessing a dorsal trunk protuberance, gonads with irregular margins, anchors of noticeably different size, and laterally fimbriated vitellaria. The structure of the copulatory complex suggests a relationship to *A. catalaoensis* sp. n.

Two morphologic forms of *Amphithecium camelum* were recognized: *A. camelum* forma amazonas (Figs. 65–74) collected in the Central Amazon (Localities 1, 2) and *A. camelum* forma rondonia (Figs. 75–77) from the southwestern collection sites (Localities 3–6). Figures 65–80 and Table 2 provide the differentiation of the 2 forms: (1) the primary cirral ramus of *A. camelum* forma amazonas is acute, and in *A. camelum* forma rondonia it is blunt; (2) the copulatory complex is significantly longer in *A. camelum* forma rondonia, and the haptoral structures are generally smaller (Fig. 79); and (3) the ratio between the size of the dorsal and ven-



Figures 81–88. ***Amphithecium catalaoensis.*** **81. Holotype (ventral). 82. Ventral bar. 83. Dorsal bar. 84. Copulatory complex. 85. Ventral anchor. 86. Dorsal anchor. 87. Hook 5. 88. Hook 1–4, 6, 7. Figure 89.** ***Cleidodiscus amazonensis*** **Mizelle and Price, 1965. Copulatory complex from holotype. All figures are to the same scale (30 μm) except Figure 81 (100 μm).**



Figures 90–92. Scanning electron micrographs of new dactylogyrid species. 90. Specimen of *Amphithecium camelum* (dorsal) showing the double vaginal apertures (V) and the humplike protuberance (H). Scale = 100 μm. 91. Lateral view of *A. camelum* at the level of the right vaginal pore. Scale = 50 μm. 92. Dorsal view of peduncle of *Notothecium* sp. Scale = 15 μm.

tral anchor among species of the 2 forms shows substantial difference (Fig. 80).

Amphithecium camelum forma amazonas (Figs. 65–74, 90–91)

RECORDS: Localities 1, 2 (type).

DESCRIPTION: With characters of species. Measurements, based on 223 specimens, are provided in Table 2. Both cirral rami terminally acute. *Amphithecium camelum* forma amazonas is the type form for the species.

Amphithecium camelum forma rondonia (Figs. 75–77)

RECORDS: Localities 3–6.

DESCRIPTION: With the characters of species. Measurements, based on 75 specimens, are provided in Table 2. Primary ramus of cirrus terminally blunt; secondary ramus terminally truncate.

Amphithecium catalaoensis sp. n. (Figs. 81–88)

TYPE LOCALITY: Furo do Catalão near Manaus, Amazonas, Brazil (Locality 1).

SPECIMENS DEPOSITED: Holotype, INPA PA297-1; paratypes, USNM 79193, HWML 23367.

DESCRIPTION (based on 7 specimens): Body fusiform, length 383 (286–465); greatest width 90 (63–107) near midlength or in anterior trunk. Tegument scaled on trunk, peduncle. Cephalic lobes well to poorly developed; usually 4 pairs of head organs lying in cephalic lobes; cephalic glands posterolateral to pharynx. Distance between members of eye pairs variable, anterior pair smaller; eye granules ovate, small; accessory granules absent or few in proximity of eyes. Pharynx spherical, 19 (17–21) in diameter; caeca indistinct. Peduncle broad. Haptor subpentagonal, 75 (64–93) long, 90 (75–105) wide; 2 pairs of

Table 2. Measurements of *Amphithecium camelum* from 4 different localities.*

	Forma amazonas				Forma rondonia			
	Marchantaria		Catalão		Guaporé-Surpresa		Guaporé-C. Marques	
Length	387 (290–569)	(*N* = 71)	401 (338–518)	(*N* = 41)	521 (418–575)	(*N* = 14)	459 (362–502)	(*N* = 6)
Greatest width	136 (99–165)	(*N* = 10)	—		162 (152–181)	(*N* = 4)	—	
Cirrus length	47 (39–53)	(*N* = 110)	49 (45–55)	(*N* = 52)	64 (55–75)	(*N* = 45)	58 (49–64)	(*N* = 12)
Accessory piece length	31 (28–38)	(*N* = 100)	32 (30–37)	(*N* = 49)	41 (36–49)	(*N* = 43)	38 (34–43)	(*N* = 12)
Dorsal anchor length	29 (26–34)	(*N* = 100)	30 (27–35)	(*N* = 48)	29 (26–31)	(*N* = 42)	26 (24–28)	(*N* = 11)
Dorsal anchor base width	18 (16–20)	(*N* = 87)	18 (16–21)	(*N* = 47)	16 (15–19)	(*N* = 40)	15 (14–17)	(*N* = 11)
Ventral anchor length	45 (40–51)	(*N* = 127)	45 (40–49)	(*N* = 52)	37 (33–40)	(*N* = 47)	35 (32–37)	(*N* = 11)
Ventral anchor base width	21 (18–25)	(*N* = 126)	22 (19–25)	(*N* = 54)	20 (17–22)	(*N* = 44)	18 (17–19)	(*N* = 11)
Dorsal bar	34 (27–44)	(*N* = 67)	35 (31–42)	(*N* = 39)	31 (27–34)	(*N* = 15)	29 (25–31)	(*N* = 6)
Ventral bar	43 (35–49)	(*N* = 77)	44 (39–49)	(*N* = 46)	36 (33–38)	(*N* = 13)	34 (30–38)	(*N* = 9)
Hook 1	18 (16–20)	(*N* = 95)	18 (16–20)	(*N* = 45)	16 (15–18)	(*N* = 29)	15 (14–16)	(*N* = 6)
Hook 2	20 (17–22)	(*N* = 111)	20 (18–23)	(*N* = 50)	19 (17–20)	(*N* = 36)	18 (17–19)	(*N* = 10)
Hook 3	23 (20–28)	(*N* = 101)	23 (20–26)	(*N* = 43)	21 (19–23)	(*N* = 29)	20 (19–22)	(*N* = 11)
Hook 4	26 (23–29)	(*N* = 90)	26 (24–31)	(*N* = 45)	25 (22–27)	(*N* = 27)	23 (21–26)	(*N* = 11)
Hook 5	15 (14–19)	(*N* = 60)	16 (14–19)	(*N* = 27)	15 (14–16)	(*N* = 26)	14 (13–15)	(*N* = 6)
Hook 6	19 (15–21)	(*N* = 69)	19 (16–22)	(*N* = 29)	18 (16–20)	(*N* = 25)	18 (16–19)	(*N* = 10)
Hook 7	23 (22–27)	(*N* = 45)	25 (23–29)	(*N* = 21)	22 (20–24)	(*N* = 24)	21 (20–23)	(*N* = 9)
Pharynx diameter	22 (17–26)	(*N* = 10)	—		25 (22–28)	(*N* = 4)	—	
Haptor length	54 (42–66)	(*N* = 10)	—		52 (48–60)	(*N* = 4)	—	
Haptor width	81 (71–97)	(*N* = 10)	—		52 (48–60)	(*N* = 4)	—	
Testis length	67		—		—		—	
Testis width	36		—		—		—	
Ovary length	98 (67–116)	(*N* = 9)	—		170 (162–177)	(*N* = 3)	—	
Ovary width	30 (18–45)	(*N* = 9)	—		46 (41–50)	(*N* = 3)	—	
Egg length	65 (60–71)	(*N* = 2)	—		—		—	
Egg width	45 (44–47)	(*N* = 2)	—		—		—	

* The average is followed by the range and number of specimens measured, in separate parentheses.

haptoral glands. Anchors similar; each with elongate superficial root, long shaft, short point; ventral anchor 72 (71–74) long, base 34 (33–35) wide; dorsal anchor 61 (58–65) long, base 29 (28–30) wide. Ventral bar 45 (41–49) long, broadly U-shaped, with inflated ends; dorsal bar 44 (37–52) long, U-shaped. Hooks similar; each with erect thumb, slightly curved shaft, short point, variable shank; hook 1, 2—27 (25–28) long; hook 3, 7—29 (27–30) long; hook 4—31 (30–33) long; hook 5—16–17 long; hook 6—22 (20–24) long; FH loop 0.6 length of distal portion of shank. Cirrus comprising 2 dissimilar rami: primary ramus heavily sclerotized, secondary ramus needlelike; cirrus 54 (53–55) long. Accessory piece 36 (34–37) long, rodlike, with a marginal distal flap, articulated to cirrus base by variable proximal process. Testis elongate ovate, 57 (46–67) long, 27 (25–30) wide; seminal vesicle pyriform. Prostatic reservoirs with thick walls; prostates not observed. Ovary elongate ovate, 75 (51–96) long, 23 (15–33) wide; ootype, oviduct, uterus not observed. Vitellaria dense.

ETYMOLOGY: The specific name is derived from the type locality.

REMARKS: *Amphithecium catalaoensis* resembles *A. camelum* in the comparative morphology of the copulatory complex and *A. junki* by possessing elongate anchor shafts. It differs from *A. camelum* by lacking a conspicuously cleft ventral bar and by having an elongate hook shank in pairs 1–4, 6, and 7. It differs from *A. junki* by lacking bladelike cirral rami.

Notothecium gen. n.

DIAGNOSIS: Body divisible into cephalic region, trunk, peduncle, haptor. Tegument thin, scaled. Two terminal, 2 bilateral cephalic lobes;

head organs present; cephalic glands unicellular, lateral or posterolateral to pharynx. Eyes absent. Mouth subterminal, midventral; pharynx muscular, glandular; esophagus short or absent; intestinal caeca 2, confluent posterior to gonads, lacking diverticula. Gonads intercaecal, overlapping; testis dorsal to ovary. Vas deferens looping left caecum; seminal vesicle a dilation of vas deferens, C-shaped, looping dextrally; copulatory complex comprising articulated cirrus, accessory piece. Seminal receptacle present; vagina sinistrodorsal, dilated, looping left caecum; genital pore midventral near level of caecal bifurcation. Vitellaria coextensive with caeca. Haptor armed with pairs of dorsal and ventral anchors, dorsal and ventral bars, 7 pairs of hooks with ancyrocephaline distribution. Hooks with shank of 2 distinct parts. Parasites of gills of Serrasalmidae.

TYPE SPECIES: *Notothecium mizellei* sp. n. from *Serrasalmus nattereri.*

OTHER SPECIES: *Notothecium aegidatum* sp. n. from *Serrasalmus nattereri.*

ETYMOLOGY: The generic name is from Greek (*notos* = back + *theke* = case) and reflects the dorsal position of the vagina.

REMARKS: *Notothecium* includes species morphologically similar to those of *Amphithecium. Notothecium* is distinguished from all genera of Ancyrocephalinae by the combination of the following characters: (1) a single vagina opening sinistrodorsally and looping the left caecum internally; (2) overlapping gonads; and (3) a C-shaped seminal vesicle.

Notothecium mizellei sp. n. (Figs. 93–100)

TYPE LOCALITY: Furo do Catalão near Manaus, Amazonas, Brazil (Locality 1).

OTHER RECORDS: Localities 2–6.

SPECIMENS DEPOSITED: Holotype, INPA PA292-1; paratypes, INPA PA292-2–5, USNM 79189, HWML 23363; vouchers, USNM 79225–79228, 79244, HWML 23396–23398.

DESCRIPTION (based on 33 specimens): Body flattened dorsoventrally, length 230 (185–263); greatest width 77 (67–98) near midlength. Tegument scaled on trunk, peduncle (Fig. 92). Cephalic lobes well developed; generally 4 pairs of head organs lying in cephalic lobes; cephalic glands lateral to pharynx. Eyes absent; elongate ovate accessory granules infrequently present in cephalic region. Pharynx ovate, 13 (11–15) wide; esophagus short or absent. Peduncle broad, short. Haptor subpentagonal, 61 (53–67) long, 81 (58–105) wide. Anchors similar; each with well-developed roots, elongate shaft, short recurved point; ventral anchor 47 (45–49) long, base 20 (18–21) wide; dorsal anchor 45 (43–48) long, base 16 (15–17) wide. Bars similar, broadly V-shaped; ventral bar 40 (36–44) long; dorsal bar 35 (30–44) long, with short acute projection on posteromedial margin. Hook 1–4, 6, 7 similar; each with erect thumb, slightly curved shaft, short point, expanded shank; hook 5 reduced, with short shank slightly expanded; hook 1—21–22 long; hook 2—27 long; hook 3, 4—30 (29–32) long; hook 5—16 (15–17) long; hook 6—23 (22–24) long; hook 7—34 (30–37) long; FH loop about equal to length of distal portion of shank. Cirrus 24 (22–26) long, conical, curved, with incipient secondary ramus. Accessory piece 23 (21–24) long, rodlike, distally hooked, with subterminal flap, articulated to cirrus base by flexible proximal process. Testis elongate ovate, 45 (35–53) long, 22 (17–26) wide. Prostate representing a large mass of unicellular gland cells lying dorsal to copulatory complex immediately beneath the dorsal surface of trunk; prostatic reservoirs with thick walls. Ovary conical, 43 (30–60) long, 17 (12–25) in greatest width; oviduct, ootype, uterus not observed. Vagina nonsclerotized, greatly expanded proximally. Vitellaria dense; vitelline commissure usually not visible in mature specimens.

ETYMOLOGY: The species is named for Dr. J. D. Mizelle in recognition of his contributions in systematics of the Monogenea.

REMARKS: *Notothecium mizellei* is the type species for the genus.

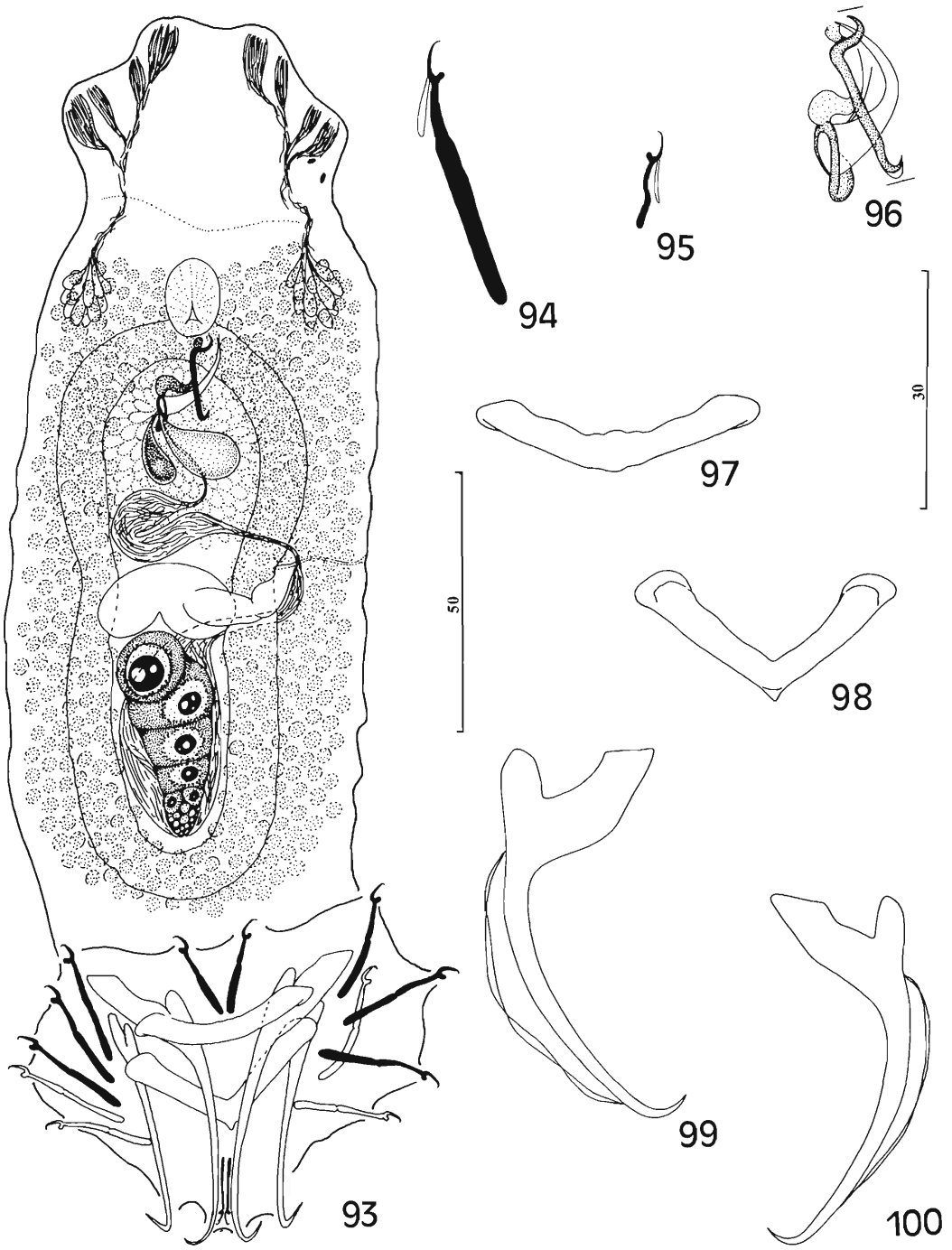
Notothecium aegidatum sp. n. (Figs. 101–109)

TYPE LOCALITY: Furo do Catalão near Manaus, Amazonas, Brazil (Locality 1).

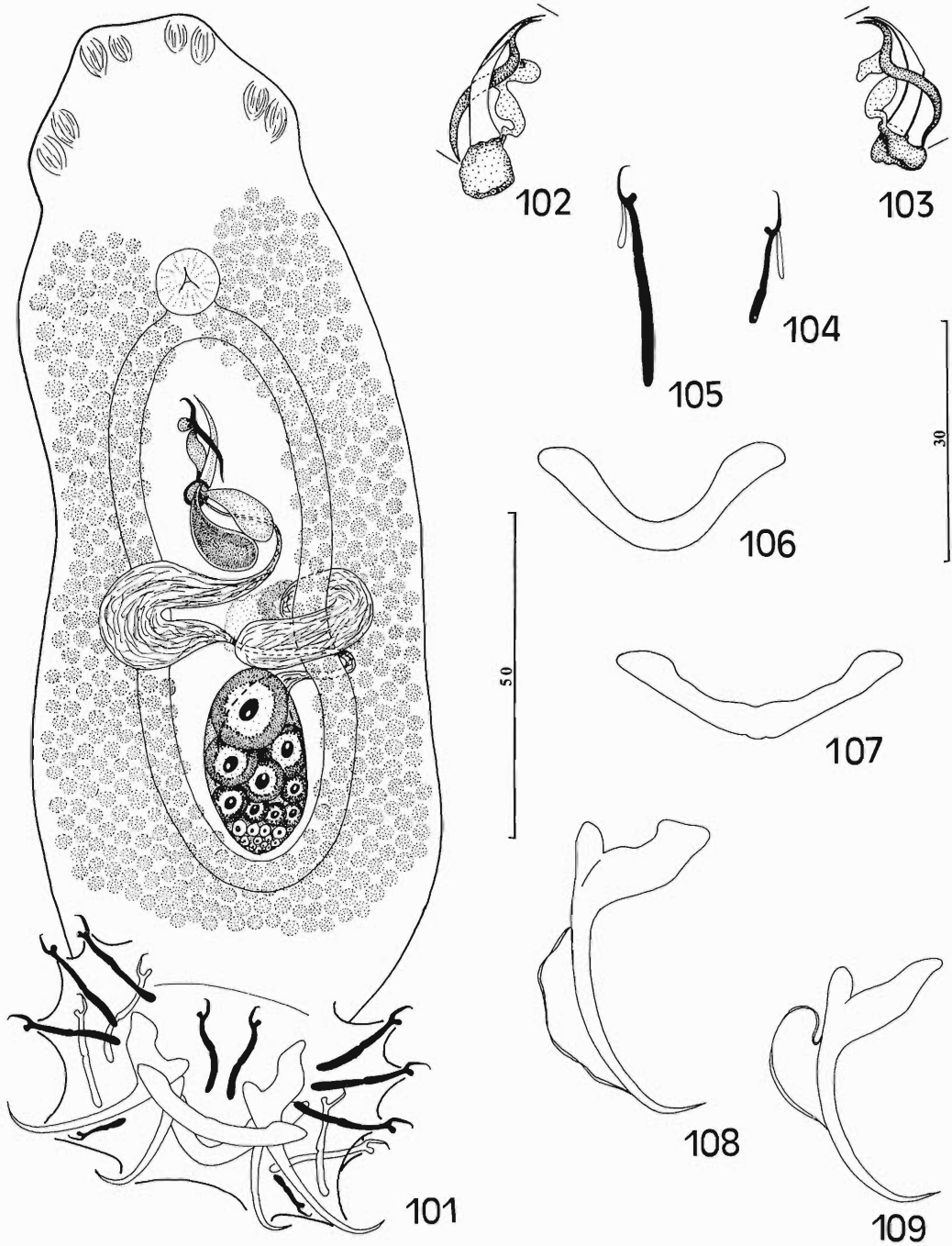
OTHER RECORDS: Localities 2, 4–6.

SPECIMENS DEPOSITED: Holotype, INPA PA303-1; paratypes, INPA PA303-2, 3, USNM 79187, HWML 23361; vouchers, USNM 79229, 79230, 79240, 79243, HWML 23399, 23400.

DESCRIPTION (based on 16 specimens): Body flattened dorsoventrally, length 213 (191–247); greatest width 78 (63–112) near midlength or in posterior trunk. Tegument scaled on trunk, peduncle (Fig. 92). Cephalic lobes well developed; generally 4 pairs of head organs lying in cephalic



Figures 93–100. *Notothecium mizellei.* **93. Holotype (ventral). 94. Hook 1–4, 6, 7. 95. Hook 5. 96. Copulatory complex. 97. Ventral bar. 98. Dorsal bar. 99. Ventral anchor. 100. Dorsal anchor. All figures are to the same scale (30 μm) except Figure 93 (50 μm).**



Figures 101–109. ***Notothecium aegidatum.*** **101. Holotype (ventral). 102. Copulatory complex (dorsal). 103. Copulatory complex. 104. Hook 1–4, 6, 7. 105. Hook 5. 106. Dorsal bar. 107. Ventral bar. 108. Ventral anchor. 109. Dorsal anchor. All figures are presented at the same scale (30 μm) except Figure 101 (50 μm).**

lobes; cephalic glands not observed. Eyes, eye granules absent. Pharynx ovate to spherical, 14 (12–19) in diameter; esophagus short. Peduncle broad. Haptor subquadrate, 48 (34–58) long, 87 (75–96) wide. Anchors similar; each with well-developed roots, long shaft, short curved point; ventral anchor 38 (32–42) long, base 17 (16–18) wide; dorsal anchor 34 (30–38) long, base 14 (13–15) wide. Bars similar, broadly U-shaped; ventral bar 39 (36–41) long; dorsal bar 39 (38–42) long. Hook 1–4, 6, 7 similar; each with erect thumb, slightly curved shaft, short point, expanded shank; hook 5 with short proximal portion of shank; hook 1—20 (18–21) long; hook 2—23 (22–24) long; hook 3, 4—28 (26–29) long; hook 5—15 (14–16) long; hook 6—21–22 long; hook 7—33 (32–35) long; FH loop 0.7 length of distal portion of shank. Cirral termination diagonally truncate, cirrus 21 (19–24) long; accessory piece 18 (16–20) long, spicular, with acute terminations, subterminal distal flap, articulated to cirrus base by flexible proximal process arising near midlength. Testis ovate, 32 (25–40) long, 20 (18–23) wide; seminal vesicle large. Prostate inconspicuous; prostatic reservoirs with thick walls. Ovary ovate, 32 (25–36) long, 18 (11–25) wide; oviduct, ootype, uterus not observed; vagina with terminal superficial sclerotization around margin of pore in mature specimens. Vitellaria dense; vitelline commissure usually not visible.

ETYMOLOGY: The specific name is from Latin (*aegidis* = a shield + *atus* = provided with) and refers to the sclerotization around the vaginal pore of adult specimens.

REMARKS: *Notothecium aegidatum* resembles *N. mizellei,* from which it differs by having: (1) a vaginal pore surrounded by a superficial sclerotization of the body surface, (2) a cirrus lacking a secondary ramus, (3) a more proximal subterminal flap of accessory piece, (4) a dorsal bar lacking a medioposterior pointed projection, and (5) smaller anchors and hooks.

Notozothecium gen. n.

DIAGNOSIS: Body divisible into cephalic region, trunk, peduncle, haptor. Tegument thin, smooth or scaled. Two terminal, 2 bilateral cephalic lobes; head organs present; cephalic glands unicellular, lateral or posterolateral to pharynx. Eyes present. Mouth subterminal, midventral; intestinal caeca 2, confluent posterior to gonads, lacking diverticula. Gonads intercaecal, overlapping; testis dorsal to ovary. Vas deferens looping left caecum; seminal vesicle a sigmoid dilation of vas deferens; copulatory complex comprising articulated cirrus, accessory piece. Cirrus coiled, with counterclockwise ring(s); accessory piece with distal ornate termination. Seminal receptacle present immediately anterior to ovary; vagina dextrodorsal, comprising a lightly sclerotized tube winding around right caecum; genital pore midventral near level of caecal bifurcation. Vitellaria coextensive with caeca. Haptor armed with pairs of dorsal and ventral anchors, dorsal and ventral bars, 7 pairs of hooks with ancyrocephaline distribution. Hook with shank of 2 distinct parts. Parasites of gills of Serrasalmidae.

TYPE SPECIES: *Notozothecium penetrarum* sp. n. from *Serrasalmus nattereri.*

OTHER SPECIES: *Notozothecium minor* sp. n. from *Serrasalmus nattereri.*

ETYMOLOGY: The generic name is derived from Greek (*notos* = back + *zotheke* = chamber) and refers to the position of the vaginal aperture.

REMARKS: *Notozothecium, Amphithecium,* and *Notothecium* are morphologically similar groups. Characteristics that distinguish *Notozothecium* from *Amphithecium* include the presence of a coiled cirrus and a single dorsal vaginal opening. The genus is most similar to *Notothecium,* from which it differs by possessing a vagina looping the right instead of the left intestinal caecum and by lacking a C-shaped seminal vesicle. It is emphasized that separation from the latter genus is not based on the position of the vaginal aperture but on the fact that the vaginal tube loops opposite caeca.

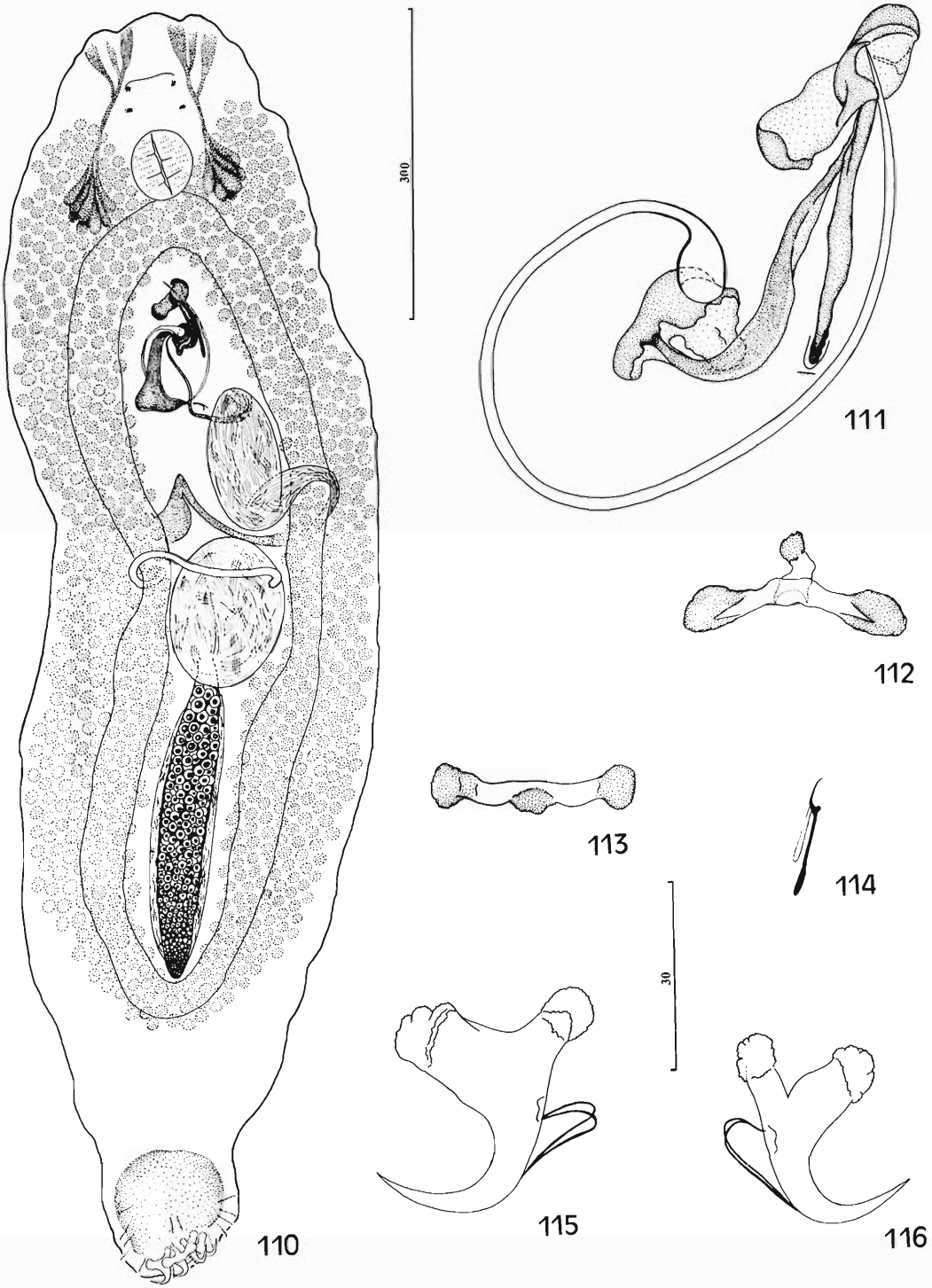
Notozothecium penetrarum sp. n. (Figs. 110–116)

TYPE LOCALITY: Rio Guaporé near Surpresa, Rondônia, Brazil (Locality 5).

OTHER RECORDS: Localities 1, 2, 4.

SPECIMENS DEPOSITED: Holotype, INPA PA306-1; paratypes, INPA PA306-2, USNM 79808, HWML 23666; vouchers, USNM 79809–79811, HWML 23665.

DESCRIPTION (based on 16 specimens): Body foliform, flattened dorsoventrally, length 1,250 (1,010–1,348); greatest width 343 (304–385) near midlength. Tegument smooth. Cephalic lobes poorly developed; 3 or 4 pairs of head organs; cephalic glands lateral to pharynx. Eyes subequal; members of anterior pair slightly closer together than those of posterior pair; granules



Figures 110–116. ***Notozothecium penetrarum.*** **110. Holotype (ventral). 111. Copulatory complex. 112. Ventral bar. 113. Dorsal bar. 114. Hook. 115. Ventral anchor. 116. Dorsal anchor. All drawings are to the 30-μm scale except Figure 110 (300 μm).**

small, ovate; accessory granules absent. Pharynx ovate, 68 (59–79) in greatest width; esophagus absent. Peduncle broad, tapered posteriorly; haptor globose, 133 (94–172) long, 127 (90–163) wide. Anchors similar; each with large roots provided with bulbous tips, short shaft, stout evenly curved point; ventral anchor 33 (31–36) long, base 28 (27–29) wide; dorsal anchor 32 (28–35) long, base 25 (23–28) wide. Ventral bar 37 (34–44) long, with enlarged ends, anteriorly directed process originating from posteromedial margin of bar; dorsal bar 35 (31–42) long, with expanded ends, posteromedial knob. Hooks similar, 18 (16–20) long; each with erect thumb, delicate shaft and point, shank with short proximal enlargement. FH loop extending to distal limit of proximal enlargement of shank. Cirrus 208–209 long, comprising a coil of about 1 ring, ring diameter 62 (55–72). Accessory piece 60 (53–68) long, π-shaped, with 1 basal arm articulating to cirral base. Testis elongate, fusiform, 162 (146–179) long, 40 (31–49) wide. Prostatic reservoir pyriform; seminal vesicle sigmoid, lying to left of midline. Ovary fusiform, 263 (183–336) long, 52 (35–80) wide; oviduct, ootype, uterus not observed; seminal receptacle large, lying on midline immediately anterior to ovary. Vitellaria dense; vitelline commissure immediately anterior to seminal receptacle.

ETYMOLOGY: This species is named for its means of attachment to the host's gill.

REMARKS: *Notozothecium penetrarum* is the type species for the genus. Adults appeared to have penetrated the host's tissue with the haptor, resulting in relatively permanent attachment to the gill.

Notozothecium minor sp. n. (Figs. 117–124)

TYPE LOCALITY: Furo do Catalão near Manaus, Amazonas, Brazil (Locality 1).

OTHER RECORDS: Localities 4, 5.

SPECIMENS DEPOSITED: Holotype, INPA PA307-1; paratypes, USNM 79805, HWML 23663; vouchers, USNM 79806, 79807, HWML 23662, 23664.

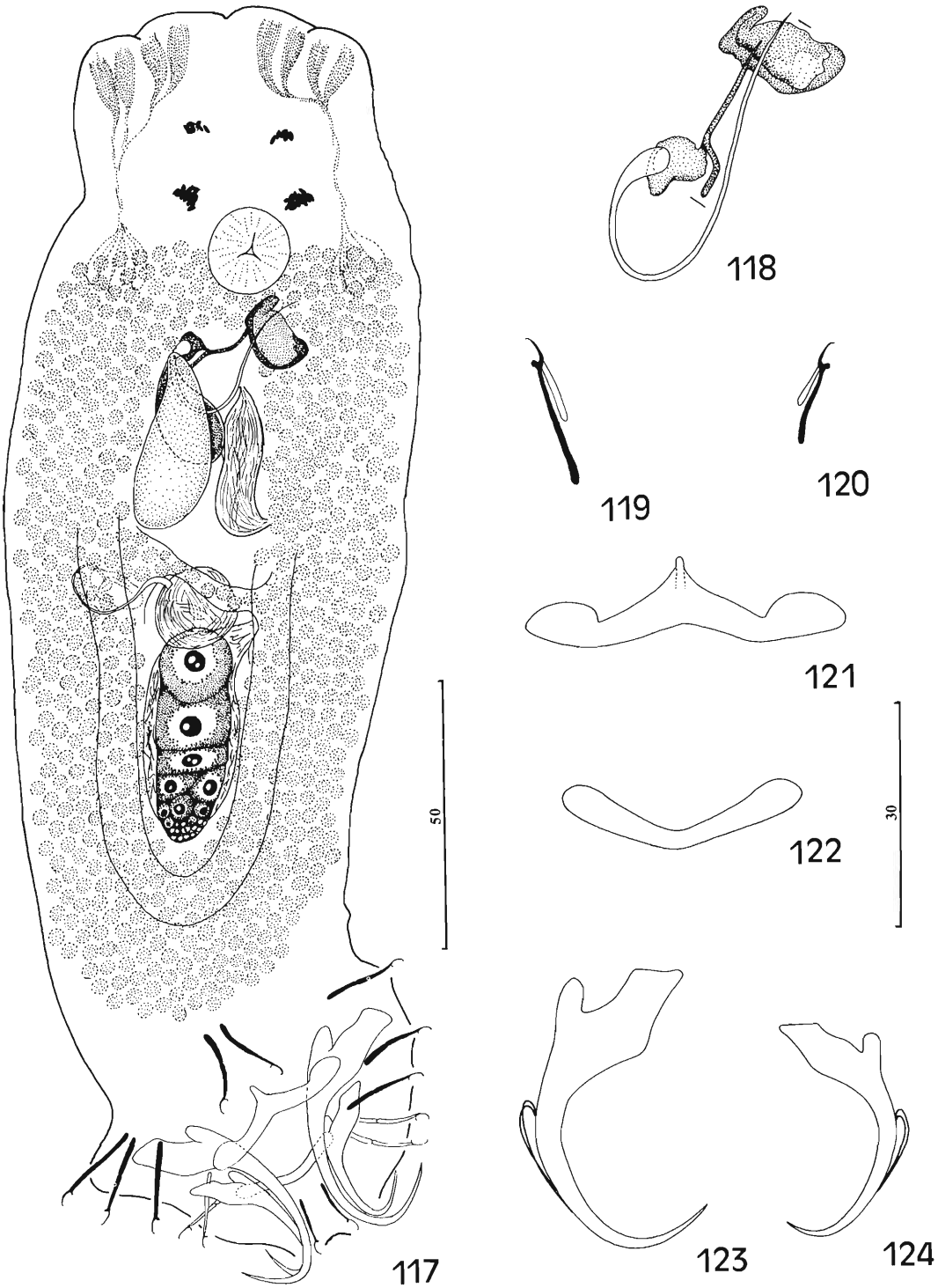
DESCRIPTION (based on 5 specimens): Body flattened dorsoventrally, length 217 (181–243); greatest width 79 (70–84) near midlength or in anterior half; cephalic area broad. Tegument scaled on peduncle, trunk. Cephalic lobes poorly developed; 4 pairs of head organs; cephalic glands lying lateral to pharynx. Members of posterior pair of eyes larger, farther apart than those of anterior pair; granules elongate ovate; accessory granules absent. Pharynx spherical, 15 (12–17) in diameter. Peduncle short, broad; haptor subpentagonal, 53 (45–66) long, 71 (63–78) wide. Anchors similar, each with large truncate superficial root, short deep root, evenly curved shaft and point; ventral anchor 45 (43–49) long, base 18 (16–20) wide; dorsal anchor 31–32 long, base 13–14 wide. Ventral bar 44 (43–45) long, with enlarged ends, short anteromedial process. Dorsal bar broadly V-shaped, 29 (28–30) long. Hooks similar, each with erect thumb, delicate shaft and point, shank slightly enlarged proximally; hook 1, 2, 5, 6—18 (16–20) long, hook 3, 4, 7—23 (21–26) long; FH loop extending to level of enlarged portion of shank. Cirrus comprising about 1 ring, 17 (16–18) in diameter; cirrus 74–75 long. Accessory piece T-shaped, 26–27 long, articulating to cirral base by short process originating about ⅓ the distance from proximal end. Testis elongate ovate, 42–43 long, 19–20 wide; seminal vesicle sigmoid; 2 large pyriform prostatic reservoirs. Ovary ovate, 43 (38–49) long, 18 (16–21) wide; oviduct, uterus, ootype not observed; vaginal pore lightly sclerotized, tube delicate; seminal receptacle spherical, lying immediately anterior to ovary; vitellaria dense; vitelline commissure lying ventral to seminal receptacle.

ETYMOLOGY: The specific name is from Latin (*minor* = smaller) and reflects the species' small size.

REMARKS: This species differs from the type species by being smaller, possessing more generalized anchors, lacking 2 well-developed proximal arms of the accessory piece, and having a scaled tegument.

Phylogenetic Analysis

Phylogenetic analysis of species of *Amphithecium, Notothecium,* and *Notozothecium* suggests that the 3 genera form a monophyletic group (Fig. 125). The cladogram has a consistency index of 83% indicating a low degree of homoplasy. The monophyly for this group of species is best defined by the following derived characters: dorsal vagina(e), T-shaped accessory piece, and scaled tegument. A double vagina is also considered a synapomorphy of the group even though placement of the character in the cladogram as a synapomorphy for *Amphithecium* spp. only is equally parsimonious. The dorsal vaginal aperture(s) and the vaginal tube(s) looping 1 or both of the



Figures 117–124. *Notozothecium minor.* **117. Holotype (ventral). 118. Copulatory complex. 119. Hook 1–4, 6, 7. 120. Hook 5. 121. Ventral bar. 122. Dorsal bar. 123. Ventral anchor. 124. Dorsal anchor. All figures of sclerotized structures are drawn to the 30-μm scale; Figure 117 to the 50-μm scale.**

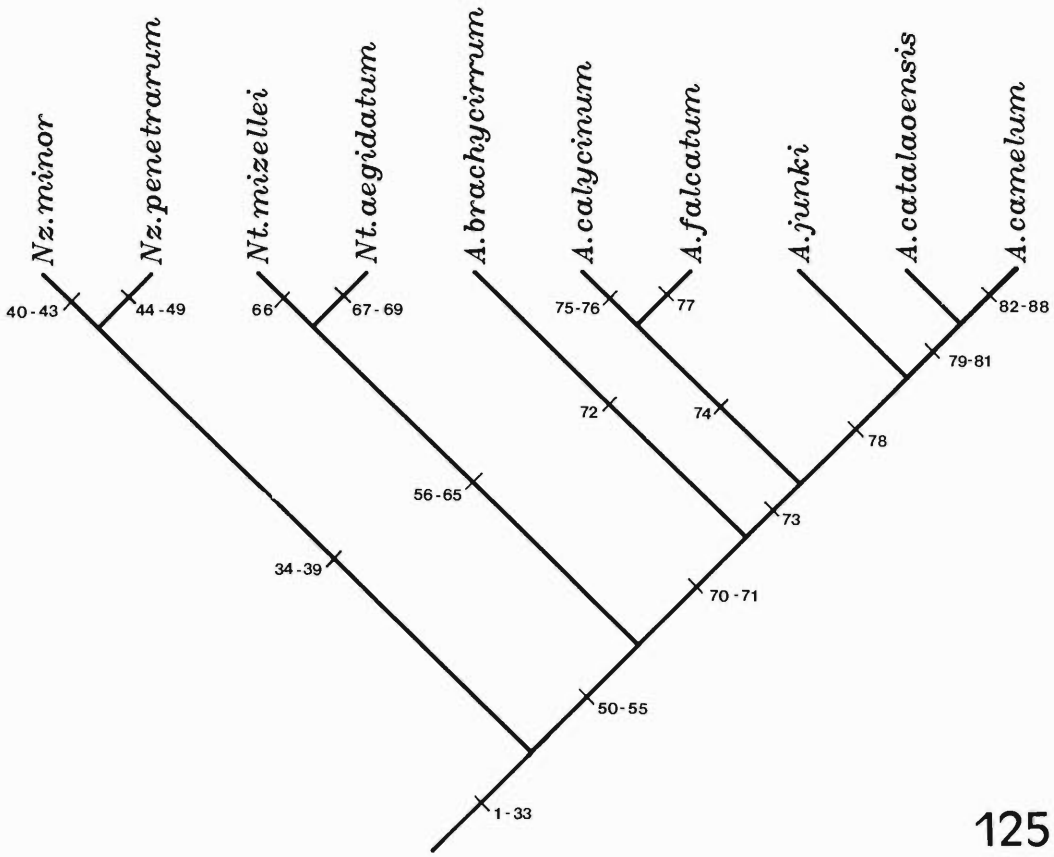


Figure 125. Cladogram showing relationships of the ancyrocephaline species infesting *Serrasalmus nattereri*. Characters are denoted by numbers and identified in the Materials and Methods.

intestinal caeca suggests that the conditions present in *Notothecium* and *Notozothecium* are derived states from the more primitive condition of double dorsal vaginal apertures exhibited in *Amphithecium*.

Synapomorphies for the 3 generic taxa are provided in Figure 125 (6 for *Notozothecium*, 10 for *Notothecium*, and 2 for *Amphithecium*). *Notothecium* and *Amphithecium* are defined as sister groups by 6 synapomorphies, of which the presence of double cirral rami is the most significant. In *Notothecium*, species exhibit secondary reduction or loss of 1 ramus. Further, the unusual seminal receptacle present in *Notothecium* spp. appears to be a derived state from the vaginal ducts of the common ancestor, which lacked a seminal receptacle. The latter condition remains expressed in *Amphithecium* spp. Thus, the absence of a well-defined seminal receptacle would also serve as a diagnostic synapomorphic character of the group comprising species of *Amphithecium* and *Notothecium*.

The character with the lowest consistency index (C.I. = 0.33) in the analysis is the shape of the anchors as defined by the ratio between the lengths of the point and shaft. Wide differences in this character occur in species of many ancyrocephaline genera, and it is expected, therefore, that a high degree of homoplasy would occur within the species group studied as well as in other nonrelated genera.

Discussion

None of the monogenean species previously described from the gills of *Serrasalmus nattereri* by Mizelle and Price (1965) were found in our collections, and these workers did not specify a type locality within the Amazon Basin for their species. A high geographic variation of the composition of the monogenean community from

Serrasalmus nattereri could explain this finding. However, the data obtained in the present study indicate that despite some variation in community structure, a complete community replacement is unlikely. Eight of the 15 species reported herein were collected from all 6 localities in the Amazon Basin, representing intervening distances between extreme sites of greater than 1,000 km (air) and 1,600 km (river). Myers (1972) and Fink and Fink (1979) indicate that the classification of the piranhas is confused, with species identification often difficult. Based on the state of piranha classification during the 1960's and on the general high host specificity of the Dactylogyridae, it is possible that Mizelle and Price (1965) were working with a species of piranha other than *S. nattereri.*

The limited distribution of 4 of the 15 species reported in this study (*Anacanthorus* sp., *A. maltai, A. rondonensis,* and *Amphithecium catalaoensis*), and of the 2 morphologic forms of *Amphithecium camelum,* permit the clustering of the collection localities into 2 major areas: the central Amazon (Furo do Catalão and Ilha da Marchantaria) and southwestern Amazon (Rio Pacaás-Novos, Rio Mamoré, Rio Guaporé near Surpresa, and Rio Guaporé near Costa Marques). The sporadic occurrence of *Notozothecium penetrarum* (Localities 1, 2, 4, 5) and *N. minor* (Localities 1, 4, 5) is assumed to be the result of insufficient sampling. Therefore, these species are not considered important in defining major areas.

The monogenean community of *Serrasalmus nattereri* from the central Amazon is characterized by the presence of *Anacanthorus* sp. and *Amphithecium camelum* forma amazonas. *Anacanthorus maltai* and *A. rondonensis* are absent. In spite of sharing of these characteristics, the monogenean community of piranhas from the Furo do Catalão appears to bear some degree of uniqueness in that *Amphithecium catalaoensis* was collected only there. Signficant size differences ($P < 0.01$) were also found between specimens of *A. camelum* collected in these 2 localities (Table 2, Fig. 78). Whereas species of carnivorous piranhas are apparently rare or absent from blackwater rivers with low biologic productivity, as is the case for the Rio Negro (Goulding, 1980), it is unlikely that the specimens of piranha collected in the Furo do Catalão were originally members of populations inhabiting this river. The short distance (about 15 km) and the lack of an apparent physical barrier between Furo do Catalão and Ilha da Marchantaria suggest that the monogenean communities are not isolated. Therefore, the detected differences in the size of the anchors, cirrus, and hook 7 (Fig. 78) of the 2 forms of *A. camelum* are not genotypic. Also, *A. catalaoensis* is rare on piranhas from the Furo do Catalão, which may indicate that *S. nattereri* is a suitable but not a required host for this parasite. Its presence on this host could be the result of species exchange from parasitic communities from other piranhas, perhaps from the Rio Negro, because this species was not detected on piranha cajú from the Ilha da Marchantaria.

The monogenean community of piranha cajú from the southwestern Amazon is characterized by *Anacanthorus maltai, A. rondonensis,* and *Amphithecium camelum* forma rondonia. No compositional differences were observed between the collection sites of this area. The absence of *N. aegidatum* from the Rio Pacaás-Novos (Locality 3) cannot be considered significant because only 1 host specimen was available from this location.

Ectoparasitic Monogenea are in direct contact and consequently under constant influence of the environment during their entire life cycle. In the Amazon Basin, the distribution of aquatic organisms is primarily influenced by the seasonal variation of the water level, consequent changes in water quality, and the general hydrochemical characteristics of the rivers' water type (Fittkau et al., 1975; Junk et al., 1983). Our data do not indicate the affects of these factors on distribution of monogenean species from piranha cajú, although the presence or absence of certain species in the respective communities could be influenced by them. All collections from the southwestern Amazon represent primarily clearwater populations, whereas those of the central Amazon are white water. Also, the large distances between respective regions could be involved in the development of unique monogenean communities by providing a mechanism of isolation. This isolation may be enhanced by the behavioral characteristics of the host. By being nonmigratory and a fish of small horizontal displacement (Braga, 1976), the contact between neighboring groups of *Serrasalmus nattereri* is reduced.

Certainly, additional factors are involved in determining the species composition of the

monogenean community of *Serrasalmus nattereri*. Further research is needed on the biology and distribution of both host and parasites before the effect of these factors on the Monogenea is fully understandable.

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Dactylogyrus (Monogenea: Dactylogyridae) from Seven Species of *Notropis* (Pisces: Cyprinidae) from the Tennessee River Drainage: Descriptions of Four New Species and Remarks on Host Relationships

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ABSTRACT: Five previously described and 5 new species of *Dactylogyrus* are reported from *Notropis* species from the Tennessee River drainage: *D. bulbus* Mueller, 1938, *D. lepidus* Rogers, 1967, *D. luxili* Rogers, 1967, *D. manicatus* Rogers, 1967, and *D. perlus* Mueller, 1938, occurred on *N. chrysocephalus chrysocephalus*; *Dactylogyrus* sp. cf. *sceptici* Cloutman, 1980, occurred on *N. ariommus*; *D. circumflexus* sp. n. and *D. delicatus* sp. n. are described from *N. c. chrysocephalus*; *D. dolus* sp. n. is described from *N. coccogenis*; *D. spatulus* sp. n. is described from *N. telescopus* and was also found on *N. ariommus, N. coccogenis, N. leuciodus,* and *N. rubricroceus.* Based on examination of type specimens, *D. perlus* is considered here to be a senior synonym of *D. banghami* Mizelle and Donahue, 1944. No *Dactylogyrus* species were found on *N. rubellus.* Similarities in the *Dactylogyrus* species infesting *N. c. chrysocephalus, N. c. isolepis,* and *N. cornutus* corroborate ichthyological evidence that these hosts are very close relatives in the *N. cornutus* species group of *Notropis* (*Luxilus*). The presence of *D. dolus* and *D. spatulus* (close relatives of *D. manicatus* and *D. dubius* Mizelle and Klucka, 1953, respectively, that occur on certain members of the *N. cornutus* species group) on *N. coccogenis* indicate a not so close relationship between *N. coccogenis* and the *N. cornutus* species group within *Notropis* (*Luxilus*). The presence of *D. spatulus* on *N. ariommus, N. coccogenis, N. leuciodus, N. rubricroceus,* and *N. telescopus* indicates a possible phyletic link between these hosts, which has only in part been suggested through ichthyological studies. The presence of *Dactylogyrus* sp. cf. *sceptici* on *N. ariommus* corroborates ichthyological evidence of a close relationship between *N. ariommus* and *N. scepticus.*

KEY WORDS: Monogenea, Dactylogyridae, morphology, systematics, *Dactylogyrus circumflexus* sp. n., *Dactylogyrus delicatus* sp. n., *Dactylogyrus dolus* sp. n., *Dactylogyrus spatulus* sp. n., *Dactylogyrus banghami* as synonym, *Dactylogyrus bulbus, Dactylogyrus lepidus, Dactylogyrus luxili, Dactylogyrus manicatus, Dactylogyrus perlus, Notropis* spp., Cyprinidae, shiners, prevalence.

Twenty-seven species of *Dactylogyrus* Diesing, 1850, on 18 species of hosts have been reported from the Tennessee River drainage (Rogers, 1967; Chien, 1971, 1974a, b; Cloutman, 1987). This paper reports on 5 previously described and 5 new species of *Dactylogyrus* from 7 species of the cyprinid genus *Notropis* Rafinesque, bringing the total to 35 species of *Dactylogyrus* known from 22 species of hosts from the Tennessee River drainage. Also, evolutionary relationships of hosts based on infesting *Dactylogyrus* species are discussed.

Materials and Methods

The species and numbers of hosts examined are listed in Table 1. With the exception of museum specimens of *Notropis ariommus* (Cope), the hosts were placed in jars containing a 1:4,000 formalin solution immediately after capture; after approximately 1 hr, enough formalin was added to make a 10% solution (Putz and Hoffman, 1963). All parasites, collected from the gills of their hosts, were mounted in glycerin jelly, and observations were made with a Zeiss phase-contrast microscope. Drawings were made with the aid of a Zeiss drawing tube. Measurements, in micrometers, were made as presented by Mizelle and Klucka (1953); means are followed by ranges in parentheses. All type specimens of new species and representative specimens of previously described species were deposited in the helminthological collection of the National Museum of Natural History (USNM). Other nontype material is in the author's collection. For comparative purposes, all original descriptions and redescriptions of North American *Dactylogyrus* species and specimens of the following species from the USNM, the Harold W. Manter Laboratory, University of Nebraska State Museum (HWML), and the collection of Dr. Wilmer A. Rogers (WAR) were examined: *D. acus* Mueller, 1938, 5 syntypes (USNM 71445, 71446); *D. arcus* Rogers, 1967, holotype (USNM 61369), 6 paratypes (USNM 61370 [1 specimen] and WAR [5 specimens]); *D. banghami* Mizelle and Donahue, 1944, 3 syntypes (USNM 73552 [1 specimen] and HWML 21545 [2 specimens]), 4 nontype specimens (USNM 73161); *D. bulbus* Mueller, 1938, 16 syntypes (USNM 71454, 71457); *D. cornutus* Mueller, 1938, 6 syntypes (USNM 71454, 71457); *D. cursitans* Rogers, 1967, 1 paratype (USNM 61380); *D. dubius* Mizelle and Klucka, 1953, 4 nontype specimens (HWML 20535); *D. fulcrum* Mueller, 1938, 2 syntypes (USNM 71457); *D. lepidus* Rogers, 1967, holotype (USNM 61389) and 2 paratypes (USNM 61390); *D. luxili* Rogers, 1967, 1 paratype (USNM 61394); *D. manicatus* Rogers, 1967, 1 paratype (USNM 61397);

Table 1. Prevalence (% infestation), range, and relative density (total number of parasites/total number of hosts) of *Dactylogyrus* infesting 6 species of *Notropis* from the Tennessee River drainage. Numbers in parentheses represent the number of host specimens examined.

Species	Prevalence	Range	Relative density
Notropis ariommus (Cope), popeye shiner (21)			
Dactylogyrus sp. cf. *sceptici* Cloutman, 1980	9.5	0–5	0.3
Dactylogyrus spatulus sp. n.	5.0	0–7	0.3
Notropis (*Luxilus*) *chrysocephalus chrysocephalus* (Rafinesque), striped shiner (7)			
Dactylogyrus perlus Mueller, 1938	71.4	0–7	2.9
Dactylogyrus bulbus Mueller, 1938	71.4	0–9	3.0
Dactylogyrus circumflexus sp. n.	85.7	0–6	3.0
Dactylogyrus delicatus sp. n.	57.1	0–3	1.4
Dactylogyrus lepidus Rogers, 1967	57.1	0–2	0.7
Dactylogyrus luxili Rogers, 1967	100.0	1–3	1.9
Dactylogyrus manicatus Rogers, 1967	42.9	0–2	0.6
Notropis (*Luxilus*) *coccogenis* (Cope), warpaint shiner (9)			
Dactylogyrus dolus sp. n.	66.7	0–14	5.0
Dactylogyrus spatulus sp. n.	33.3	0–23	4.9
Notropis (*Hydrophlox*) *leuciodus* (Cope), Tennessee shiner (21)			
Dactylogyrus spatulus sp. n.	19.0	0–2	0.2
Notropis (*Hydrophlox*) *rubellus* (Agassiz), rosyface shiner (16)			
Notropis (*Hydrophlox*) *rubricroceus* (Cope), saffron shiner (25)			
Dactylogyrus spatulus sp. n.	8.0	0–16	0.8
Notropis telescopus (Cope), telescope shiner (10)			
Dactylogyrus spatulus sp. n.	90.0	0–22	8.2

D. perlus Mueller, 1938, 3 syntypes (USNM 71454, 71457), and 1 nontype specimen (HWML 21305).

Results

Dactylogyrus bulbus Mueller, 1938

HOST: *Notropis chrysocephalus chrysocephalus* (Rafinesque), striped shiner.

LOCALITIES: Tennessee: Blount Co., Little River near Waland (USNM 80175, 2 specimens); Little River, Hwy. 411 bridge (USNM 80176, 2 specimens).

REMARKS: *Dactylogyrus bulbus* is widely distributed on *Notropis chrysocephalus* and *N. cornutus* (Mitchill) in eastern North America (Mueller, 1938; Mizelle and Donahue, 1944; Mizelle and Klucka, 1953; Mizelle and Webb, 1953; Rogers, 1967; Molnar et al., 1974; Hanek et al., 1975). A record from *Notemigonus crysoleucas* (Mitchill) (Cone, 1980) is considered here to represent an abnormal infestation. *Dactylogyrus bulbus* appears to be specific for *N. c. chrysocephalus* in the Tennessee River drainage (Table 1; Rogers, 1967; Cloutman, 1987).

Dactylogyrus lepidus Rogers, 1967

HOST: *Notropis chrysocephalus chrysocephalus* (Rafinesque), striped shiner.

LOCALITIES: Tennessee: Blount Co., Little River near Waland (USNM 80177, 3 specimens); Little River, Hwy. 411 bridge.

REMARKS: This is the first report of *Dactylogyrus lepidus* since its original description from *Notropis chrysocephalus isolepis* Hubbs and Brown in Alabama (Rogers, 1967). *Dactylogyrus lepidus* appears to be restricted to the 2 subspecies of *N. chrysocephalus*. Its closest relative appears to be *D. fulcrum*, found on *N. cornutus* in New York (Mueller, 1938).

Dactylogyrus luxili Rogers, 1967

HOST: *Notropis chrysocephalus chrysocephalus* (Rafinesque), striped shiner.

LOCALITIES: Tennessee: Blount Co., Little River near Waland (USNM 80178, 4 specimens); Little River, Hwy. 411 bridge.

REMARKS: *Dactylogyrus luxili* has been reported on *Notropis chrysocephalus, N. cornutus,* and *N. pilsbryi* Fowler from Alabama, Ontario, and Arkansas, respectively (Rogers, 1967; Hanek et al., 1975). Thus, all known hosts are species of *Notropis* (*Luxilus*). However, *D. luxili* does not appear to infest all *Notropis* (*Luxilus*); for example, it has not been found on *N.* (*L.*) *coccogenis* (Rogers, 1967; present study).

Dactylogyrus manicatus Rogers, 1967

HOST: *Notropis chrysocephalus chrysocephalus* (Rafinesque), striped shiner.

LOCALITIES: Tennessee: Blount Co., Little River near Waland (USNM 80179, 2 specimens); Little River, Hwy. 411 bridge.

REMARKS: This is the first report of *Dactylogyrus manicatus* since its original description from *Notropis chrysocephalus isolepis* in Alabama (Rogers, 1967). *Dactylogyrus manicatus* appears to parasitize only the 2 subspecies of *N. chrysocephalus.*

Dactylogyrus perlus Mueller, 1938

HOST: *Notropis chrysocephalus chrysocephalus* (Rafinesque), striped shiner.

LOCALITIES: Tennessee: Blount Co., Little River near Waland (USNM 80180, 4 specimens); Little River, Hwy. 411 bridge.

REMARKS: The nominal form *Dactylogyrus perlus* has been previously reported from *Notropis cornutus* from New York (Mueller, 1938) and Ontario (Mizelle and Donahue, 1944). In the same paper that they reported *D. perlus* based on 1 specimen, Mizelle and Donahue (1944) described *D. banghami* based on 3 specimens. The 2 species were supposedly distinguished by the presence of an anteriorly directed process on the cirrus base of *D. banghami* and the lack of such a process in *D. perlus,* as interpreted from Mueller's (1938) drawing. Mizelle and Donahue (1944) noted, however, that the accessory pieces of these 2 species were of the same type. After examining the syntypes and Mizelle and Donahue's nontype specimen of *D. perlus,* syntypes of *D. banghami,* and 13 specimens from the Tennessee River drainage, I propose that *D. perlus* is a senior synonym of *D. banghami.* The specimens in Mueller's (1938) syntype series possess a distinct anteriorly directed process on the cirrus, and other sclerites are of the same general size and shape in both species.

Dactylogyrus perlus has been reported as *D. banghami* from several host species and localities in eastern North America (Mizelle and McDougal, 1970; Cloutman, 1974; Hanek et al., 1975; Beverley-Burton, 1984), but appears restricted to *N. chrysocephalus chrysocephalus* in the Tennessee River drainage. Hanek et al. (1975) noted that some specimens reported as *D. banghami* from various host species exhibit differences in the structure of the copulatory complexes and represent a species-group complex comprising several species. After reexamining specimens that I reported as *D. banghami* from *N. lutrensis* (Baird and Girard) and *N. stramineus* (Cope) (Cloutman, 1974), I believe they represent *D. beckeri* Cloutman, 1987, from *N. lutrensis* and an undescribed species from *N. stramineus.* Thus, I concur with the conclusion of Hanek et al. (1975) that many specimens reported as *D. banghami* represent a complex of several species. A thorough review of this complex is needed.

Dactylogyrus sp. cf. *sceptici* Cloutman, 1980

HOST: *Notropis ariommus* (Cope), popeye shiner.

LOCALITIES: Tennessee: Scott Co., North Fork Holston River, 0.5 km E of junction of Routes 614 and 778. Virginia: specific locality unknown, Powell River.

REMARKS: This undescribed member of the *Dactylogyrus perlus* species complex and its closest apparent relative, *D. sceptici,* have almost identically shaped sclerites. The 2 species differ most notably in the size of the copulatory apparatus: *Dactylogyrus* sp. cirrus length 45 (43–48), accessory piece length 31 (30–32); *D. sceptici* cirrus length 58 (53–61), accessory piece length 43 (37–44) (Cloutman, 1980). *Dactylogyrus* sp. will be named and described when enough specimens suitable for type material become available.

Dactylogyrus circumflexus sp. n. (Figs. 1–9)

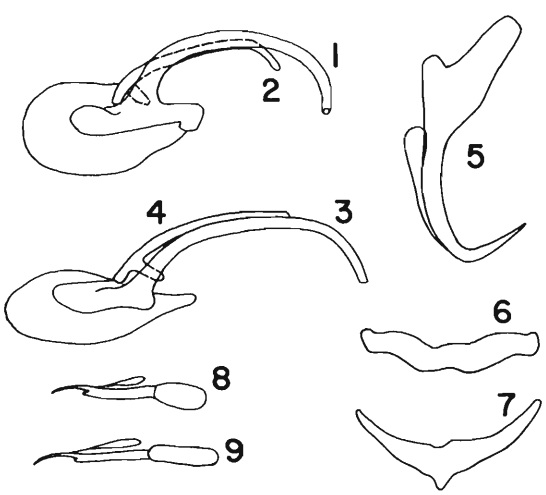
TYPE HOST: *Notropis chrysocephalus chrysocephalus* (Rafinesque), striped shiner.

TYPE LOCALITY: Tennessee: Blount Co., Little River near Waland.

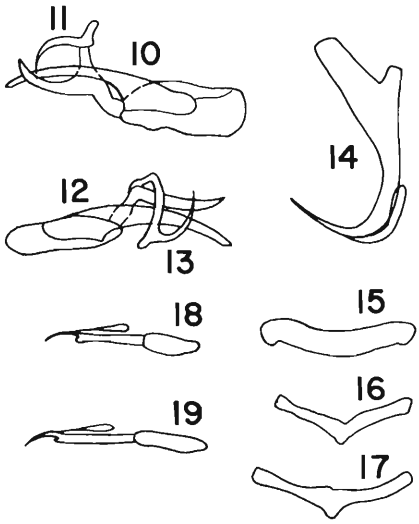
TYPE SPECIMENS: Holotype, USNM 80181; 10 paratypes, USNM 80182 (1 specimen), USNM 80183 (3 specimens), and USNM 80184 (6 specimens).

OTHER LOCALITY: Tennessee: Blount Co., Little River, Hwy. 411 bridge.

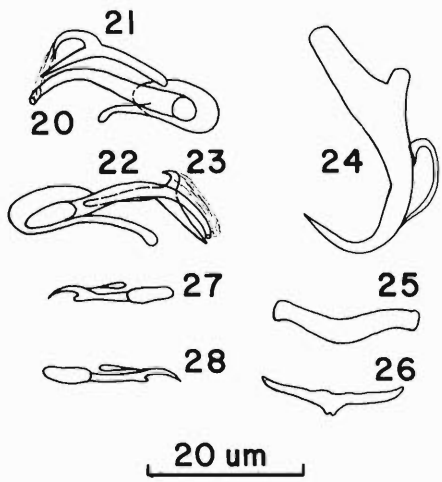
DESCRIPTION: With characters of the genus as emended by Mizelle and McDougal (1970). Body with thin tegument; length 287 (173–382), greatest width 71 (43–94). Two pairs of anterior cephalic lobes, lateral pair smaller than medial pair. Head organs not observed. Two pairs of eyes approximately equal in size, anterior pair usually farther apart than posterior pair. Pharynx cir-



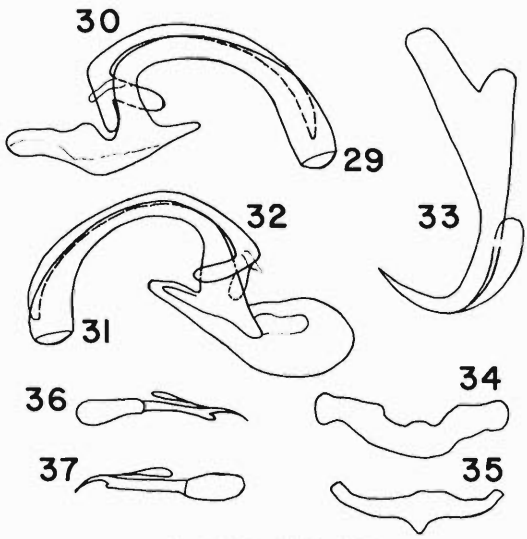
D. CIRCUMFLEXUS



D. DELICATUS



D. DOLUS



D. SPATULUS

Figures 1–37. Sclerotized parts of *Dactylogyrus* species (drawings are of holotypes unless otherwise specified). **1–9.** ***Dactylogyrus circumflexus*** **sp. n.: 1, 3 (USNM 80182), cirrus; 2, 4 (USNM 80182), accessory piece; 5, anchor; 6, dorsal bar; 7, ventral bar; 8, 9, hooks. 10–19.** ***Dactylogyrus delicatus*** **sp. n.: 10, 12 (USNM 80186), cirrus; 11, 13 (USNM 80186), accessory piece; 14, anchor; 15, dorsal bar; 16, 17 (USNM 80186), ventral bar; 18, 19, hooks. 20–28.** ***Dactylogyrus dolus*** **sp. n.: 20, 22 (USNM 80194), cirrus; 21, 23 (USNM 80194), accessory piece; 24, anchor; 25, dorsal bar; 26, ventral bar; 27, 28, hooks. 29–37.** ***Dactylogyrus spatulus*** **sp. n.: 29, 31 (USNM 80196), cirrus; 30, 32 (USNM 80196), accessory piece; 33, anchor; 34, dorsal bar; 35, ventral bar; 36, 37, hooks.**

cular (dorsal view), transverse diameter 21 (18–26), gut not observed.

Peduncle usually present, 38 (14–91) long, 31 (24–42) wide. Haptor 48 (36–70) long, 61 (49–70) wide. Single pair of dorsal anchors; each composed of solid base with short deep root, elongate superficial root, and solid shaft curving to a sharp point. Anchor length 30 (29–32), greatest width of base 13 (11-14). Dorsal bar length 21 (17–24). Vestigial ventral bar length 20 (17–22). Sixteen

hooks (8 pairs), similar in shape (except 4A), normal in arrangement (Mizelle and Crane, 1964). Each hook composed of solid base, solid slender shaft, and sickle-shaped termination provided with opposable piece (opposable piece lacking in 4A). Hook lengths: No. 1, 18 (16–19); 2, 19 (15–20); 3, 23 (17–25); 4, 21 (20–22); 4A, 6; 5, 20 (18–22); 6, 20 (17–22); 7, 20 (19–21). Cirrus an elongate arcuate tube with expanded base, length 43 (38–46). Accessory piece a solid slender rod arching closely alongside cirrus shaft to a position approximately ¼–⅓ of the length of the cirrus shaft from the terminus of the cirrus shaft, proximal end biramous, length 23 (20–27). Vagina not observed. Vitellaria usually moderate, usually distributed from pharynx to haptor.

REMARKS: *Dactylogyrus circumflexus* most closely resembles *D. arcus, D. dubius,* and *D. spatulus* sp. n. by possessing an elongate arcuate cirrus with the accessory piece arching closely alongside. However, *D. circumflexus* possesses a shorter accessory piece (extends approximately to ¼–⅓ of the length of the cirrus shaft from the terminus of the cirrus shaft) than *D. arcus, D. dubius, or D. spatulus* (extends approximately to terminus of cirrus shaft). The cirrus shaft of *D. arcus, D. circumflexus,* and *D. dubius* is approximately the same diameter for its entire length, whereas that of *D. spatulus* gradually broadens into a spatulate shape distally. Hanek et al. (1975) considered *D. arcus* to be a junior synonym of *D. dubius.* However, based on examination of nontype specimens conforming to the original description by Mizelle and Klucka (1953) and the redescription by Hanek et al. (1975) of *D. dubius* and type specimens of *D. arcus,* I consider *D. arcus* and *D. dubius* to be separate species. The proximal end of the accessory piece of *D. arcus* is biramous and not articulated with the cirrus base; the short ramus is sometimes not observed, the long ramus is bent at a right angle from the accessory piece shaft (see Rogers, 1967). The proximal end of the accessory piece of *D. dubius* is uniramous and articulated with the cirrus base, and a delicate ramus arises near the midlength of the accessory piece (see Mizelle and Klucka, 1953, and Hanek et al., 1975). I have been unable to locate the type specimen of *D. dubius,* which consists of a fragment containing the cirrus and accessory piece but lacking the haptor.

ETYMOLOGY: The species name is Latin (*circumflexus* = arched), referring to the arcuate cirrus shaft.

Dactylogyrus delicatus sp. n. (Figs. 10–19)

TYPE HOST: *Notropis chrysocephalus chrysocephalus* (Rafinesque), striped shiner.

TYPE LOCALITY: Tennessee: Blount Co., Little River near Waland.

TYPE SPECIMENS: Holotype, USNM 80185; 3 paratypes, USNM 80186 (1 specimen), USNM 80187 (1 specimen), and USNM 80188 (1 specimen).

OTHER LOCALITY: Tennessee: Blount Co., Little River, Hwy. 411 bridge.

DESCRIPTION: With characters of the genus as emended by Mizelle and McDougal (1970). Body with thin tegument; length 320 (252–425), greatest width 74 (50–94). Two pairs of anterior cephalic lobes, lateral pair smaller than medial pair. Head organs not observed. Two pairs of eyes approximately equal in size, anterior pair farther apart than posterior pair. Pharynx circular (dorsal view), transverse diameter 21, gut not observed. Peduncle 24 (14–33) long, 25 (28–42) wide. Haptor 39 (35–42) long, 60 (52–74) wide. Single pair of dorsal anchors; each composed of solid base with short deep root and elongate superficial root, and solid shaft curving to a sharp point. Anchor length 28 (27–28), greatest width of base 13 (11–14).

Dorsal bar length 21 (20–22). Vestigial ventral bar length 20 (18–21). Sixteen hooks (8 pairs), similar in shape (except 4A), normal in arrangement (Mizelle and Crane, 1964). Each hook composed of solid base, solid slender shaft, and sickle-shaped termination provided with opposable piece (opposable piece lacking in 4A). Hook lengths: No. 1, 18 (16–19); 2, 21 (20–21); 3, 23 (22–25); 4, 20 (18–21); 4A, 5; 5, 20 (18–22); 6, 20 (19–21); 7, 19 (18–20). Copulatory complex composed of cirrus and articulated accessory piece. Cirrus a curved tube with diagonally truncate point and expanded base, length 28 (26–29). Accessory piece bent near cirrus base and bent again before bifurcating; distal ramus curved, attenuated; medial ramus possessing short, blunt projection and long, thin, delicate, curved projection attenuating to sharp point; length 15 (13–16). Vagina not observed. Vitellaria moderate, distributed from pharynx to haptor.

REMARKS: *Dactylogyrus delicatus* most closely resembles *D. lepidus* in the shape of the cirrus, but the 2 species can be distinguished by the morphology of the accessory piece. The bifurcated accessory piece of *D. delicatus* possesses

a medial ramus with a short blunt projection and a long, delicate, sharply pointed distal ramus, whereas the accessory piece of *D. lepidus* is U-shaped (Rogers, 1967).

ETYMOLOGY: The species name is Latin (*delicatus* = delicate), referring to the delicate projection on the medial ramus of the accessory piece.

Dactylogyrus dolus sp. n. (Figs. 20–28)

TYPE HOST: *Notropis coccogenis* (Cope), warpaint shiner.

TYPE LOCALITY: Tennessee: Monroe Co., Citico Creek.

TYPE SPECIMENS: Holotype, USNM 80189; 15 paratypes, USNM 80190 (1 specimen), USNM 80191 (1 specimen), USNM 80192 (3 specimens), USNM 80193 (9 specimens), and USNM 80194 (1 specimen).

OTHER LOCALITIES: North Carolina: Yancey Co., South Toe River, Co. Rt. 1169 approximately 8 km E of Micaville. Tennessee: Blount Co., Little River near Waland; Little River, Hwy. 411 bridge.

DESCRIPTION: With characters of the genus *Dactylogyrus* as emended by Mizelle and McDougal (1970). Body with thin tegument; length 295 (173–396), greatest width 81 (43–115). Two pairs of anterior cephalic lobes, lateral pair smaller than medial pair. Head organs not observed. Two pairs of eyes approximately equal in size, posterior pair usually farther apart than anterior pair. Pharynx circular (dorsal view), transverse diameter 21 (15–25), gut not observed. Peduncle 32 (15–60) long, 33 (22-46) wide. Haptor 38 (32–43) long, 61 (35–71) wide. Single pair of dorsal anchors; each composed of solid base with short deep root and elongate superficial root, and solid shaft curving to a sharp point. Anchor length 29 (25–32), greatest width of base 13 (11-15). Dorsal bar length 20 (18–22). Vestigial ventral bar length 18 (17–21).

Sixteen hooks (8 pairs), similar in shape (except 4A), normal in arrangement (Mizelle and Crane, 1964). Each hook composed of solid base, solid slender shaft, and sickle-shaped termination provided with opposable piece (opposable piece lacking in 4A). Hook lengths: No. 1, 16 (15–18); 2, 18 (16–22); 3, 22 (18–25); 4, 18 (16–20); 4A, 6 (5–7); 5, 17 (14–19); 6, 17 (14–19); 7, 18 (15–20). Copulatory complex composed of cirrus and articulated accessory piece. Cirrus with rounded base, bearing a slender slightly curved process with a bulbous terminus, and curving tubular shaft arched near distal end and with blunt terminus, length 25 (22-29). Accessory piece bifurcate with almost straight dagger-shaped terminus and curved ramus arising near distal third of shaft; a nonsclerotized "sleeve" often observed attached to ramus and enveloping end of cirrus. Accessory piece length 18 (16–21). Vagina nonsclerotized, opening dextrally at body margin posterior to cirrus. Vitellaria usually moderate, distributed from pharynx to haptor.

REMARKS: The closest apparent relative of *Dactylogyrus dolus* is *D. manicatus*. Both species possess a "sleeve" attaching to the ramus of the accessory piece and enveloping the end of the cirrus. The major differences are: the cirrus shaft of *D. dolus* is arched most near the distal end, whereas the cirrus shaft of *D. manicatus* is highly arched at the basal end; the terminus of the basal process of *D. dolus* possesses a small bulb, whereas that of *D. manicatus* does not; the terminus of the accessory piece of *D. dolus* is almost straight and dagger-shaped, whereas that of *D. manicatus* is curved and attenuated to a point.

ETYMOLOGY: The name of this species is latinized Greek (*dolos* = dagger), referring to the dagger-shaped terminus of the accessory piece.

Dactylogyrus spatulus sp. n. (Figs. 29–37)

TYPE HOST: *Notropis telescopus* (Cope), telescope shiner.

TYPE LOCALITY: Tennessee: Blount Co., Little River near Waland.

TYPE SPECIMENS: Holotype, USNM 80195; 19 paratypes, USNM 80196 (1 specimen) and USNM 80197 (18 specimens).

OTHER HOSTS AND LOCALITIES: *Notropis ariommus* (Cope)—Tennessee: Claiborne Co., Powell River just upstream from Hwy. 25E bridge (USNM 80198, 2 specimens). *Notropis coccogenis* (Cope)—North Carolina: Yancey Co., South Toe River, Co. Rt. 1169 approximately 8 km E of Micaville. Tennessee: Blount Co., Little River near Waland; Little River, Hwy. 411 bridge (USNM 80199, 4 specimens); Monroe Co., Citico Creek. *Notropis leuciodus* (Cope)—Tennessee: Blount Co., Little River near Waland; Little River, Hwy. 411 bridge (USNM 80200, 1 specimen); Sevier Co., West Prong Little Pigeon River, 1.5 km downstream from Smoky Gap bridge between Gatlinburg and Pigeon Forge. *Notropis rubricroceus* (Cope)—Tennessee: Sevier Co., West Prong Little Pigeon River, 1.5 km downstream

from Smoky Gap bridge between Gatlinburg and Pigeon Forge (USNM 80201, 2 specimens). *Notropis telescopus*—Tennessee: Blount Co., Little River, Hwy. 411 bridge.

DESCRIPTION: With characters of the genus *Dactylogyrus* as emended by Mizelle and McDougal (1970). Body with thin tegument; length 308 (252–360), greatest width 106 (79–144). Two pairs of anterior cephalic lobes, lateral pair smaller than medial pair. Head organs not observed. Two pairs of eyes approximately equal in size, posterior pair usually farther apart than anterior pair. Pharynx circular (dorsal view), transverse diameter 24 (20–31), gut not observed. Peduncle usually present, 20 (7–28) long, 45 (27–73) wide. Haptor 53 (35–70) long, 67 (42–111) wide. Single pair of dorsal anchors; each composed of solid base with short deep root and elongate superficial root, and solid shaft curving to a sharp point. Anchor length 35 (28–39), greatest width of base 14 (11–18). Dorsal bar length 24 (21–27). Vestigial ventral bar length 20 (18–22). Sixteen hooks (8 pairs), similar in shape (except 4A), normal in arrangement (Mizelle and Crane, 1964). Each hook composed of solid base, solid slender shaft, and sickle-shaped termination provided with opposable piece (opposable piece lacking in 4A). Hook lengths: No. 1, 18 (14–21); 2, 20 (16–25); 3, 21 (18–24); 4, 20 (18–23); 4A, 6; 5, 20 (16–25); 6, 19 (15–22); 7, 19 (16–22). Copulatory complex composed of cirrus and accessory piece. Cirrus with expanded base and elongate arcuate shaft that gradually broadens toward distal end, length 42 (34–53). Accessory piece a solid slender rod arching closely alongside approximately the entire length of the cirrus shaft, proximal end biramous, length 30 (25–38). Vagina not observed. Vitellaria usually moderate, usually distributed from pharynx to haptor.

REMARKS: The closest apparent relatives of *Dactylogyrus spatulus* are *D. arcus, D. circumflexus,* and *D. dubius* (see Remarks for *D. circumflexus*). *Dactylogyrus arcus* was described from *Notropis chrysocephalus* and *N. coccogenis* by Rogers (1967). I examined the holotype and 3 paratypes from *N. chrysocephalus* and 3 paratypes from *N. coccogenis* and found that the specimens from *N. chrysocephalus* are *D. arcus,* but those from *N. coccogenis* are *D. spatulus. Dactylogyrus spatulus* exhibits a wider host range than most species of North American *Dactylogyrus* (Mizelle and McDougal, 1970). Comparative measurements of sclerites of *D. spatulus* from each host species are similar (Table 2).

ETYMOLOGY: The species name is Latin (*spatula* = shovel), referring to the broadened cirrus shaft.

Discussion

Six of the 7 species of *Notropis* examined harbored at least 1 species of *Dactylogyrus* (Table 1). *Notropis chrysocephalus chrysocephalus* was host for 7 species of *Dactylogyrus*. Although this is an unusually high number for most *Notropis* hosts, it is typical for the *N. cornutus* species group comprising *N. chrysocephalus* and *N. cornutus,* 2 very closely related species of *Notropis* (*Luxilus*) (Gilbert, 1964). *Notropis chrysocephalus* and *N. cornutus* have been reported previously as hosts for 8 and 11 species of *Dactylogyrus,* respectively (Mizelle and McDougal, 1970). In contrast, *N. rubellus* examined in this study was not infested, although it has been reported as the host of *D. orchis* Mueller, 1938, in New York (Mueller, 1938) and *D. rubellus* Mueller, 1938, in New York and Ontario (Mueller, 1938; Hanek et al., 1975).

The species of *Dactylogyrus* reported here, with the exception of *D. spatulus,* displayed high host specificity, parasitizing only 1 species of host in the Tennessee River drainage (Table 1). Although *D. perlus* (reported as *D. banghami*) has been reported from several host species over a wide geographical range (Mizelle and McDougal, 1970), I suspect that it normally parasitizes only the *N. cornutus* species group (see Remarks for *D. perlus*). Otherwise, considering the wide host range reported elsewhere, I would expect several of the species of *Notropis* examined here to harbor *D. perlus. Dactylogyrus bulbus* normally infests only *N. chrysocephalus* and *N. cornutus* elsewhere (Mizelle and McDougal, 1970). *Dactylogyrus circumflexus, D. delicatus, D. lepidus,* and *D. manicatus* appear to be strictly specific for *N. chrysocephalus,* and their closest apparent relatives are known only from other *Notropis* (*Luxilus*) (Mizelle and McDougal, 1970). *Dactylogyrus luxili* has been reported from *N. chrysocephalus, N. cornutus,* and *N. pilsbryi* (Fowler) (Mizelle and McDougal, 1970; Hanek et al., 1975), indicating specificity for *Notropis* (*Luxilus*). However, it does not appear to infest *N.* (*L.*) *coccogenis* (Table 1). *Dactylogyrus dolus* appears monoxenous for *N. coccogenis* but is phyletically

Table 2. Comparative measurements (μm) of sclerites of *Dactylogyrus spatulus* from 5 *Notropis* spp. from the Tennessee River drainage. A maximum of 20 specimens from each host species was measured.

	N. ariommus	*N. coccogenis*	*N. leuciodus*	*N. rubricroceus*	*N. telescopus*
Anchor length	37 (32–40)	32 (28–35)	34 (32–36)	33 (29–36)	35 (28–39)
Anchor width	13 (11–15)	13 (11–15)	14 (12–16)	13 (11–15)	14 (11–18)
Dorsal bar length	23 (20–25)	21 (16–25)	24 (23–24)	21 (18–24)	24 (21–27)
Ventral bar length	24 (20–26)	20 (16–22)	20 (19–20)	19 (15–22)	20 (18–22)
Hook 1	20 (18–22)	18 (16–19)	19 (18–19)	17 (16–18)	18 (14–21)
Hook 2	20 (18–21)	19 (18–21)	21 (16–25)	18 (17–20)	20 (16–25)
Hook 3	21 (19–22)	20 (17–25)	23 (22–23)	21 (18–24)	21 (18–24)
Hook 4	24 (20–28)	20 (18–24)	21 (19–23)	19 (16–22)	20 (18–23)
Hook 4A	7	—	6	6	6
Hook 5	22 (20–25)	19 (18–21)	20 (18–22)	19 (17–22)	20 (16–25)
Hook 6	20 (17–22)	19 (17–22)	20 (19–21)	19 (16–21)	19 (15–22)
Hook 7	20 (18–22)	18 (16–20)	20 (18–21)	18 (17–20)	19 (16–22)
Cirrus length	38 (28–48)	36 (32–46)	36 (31–44)	33 (29–38)	42 (34–53)
Accessory piece length	29 (20–39)	26 (22–31)	27 (23–32)	24 (22–29)	30 (25–38)
Number of specimens	7	20	5	19	20

linked to *D. manicatus,* which infests *N. chrysocephalus.*

The above data show, as I suggested previously (Cloutman, 1987), that species of *Dactylogyrus* parasitizing more than 1 host species usually infest closely related hosts. Furthermore, the closest relatives of the *Dactylogyrus* examined here infest closely related hosts, thus seeming to conform with the Fahrenholz rule, i.e., common ancestors of present-day parasites were themselves parasites of the common ancestors of present-day hosts (Noble and Noble, 1973). Therefore, these parasites should offer evidence for host relationships. These parasites especially indicate a very close phylogenetic relationship between *N. chrysocephalus* and *N. cornutus,* corroborating ichthyological evidence that these 2 host species are very close relatives (Gilbert, 1961, 1964). In fact, some ichthyologists regard these 2 species, at least in part, as conspecific (Menzel, 1976). *Dactylogyrus dolus* and *D. spatulus* indicate that *N. coccogenis* is phyletically linked, though not extremely closely, with other members of *Notropis* (*Luxilus*) because their closest apparent relatives were found on *N. chrysocephalus.* This phyletic link based on parasitological data supports Gilbert's (1964) ichthyological evidence that *N. coccogenis* is in a different species group within *Notropis* (*Luxilus*) than the *N. cornutus* species group, and that different species groups within *Notropis* (*Luxilus*) do not seem to be extremely closely related to one another.

Although *D. spatulus* appears to provide evidence of a phyletic link between *N. coccogenis* and other *Notropis* (*Luxilus*), it is also perplexing because it parasitizes species (*N. ariommus, N. leuciodus, N. rubricroceus,* and *N. telescopus*) that are not considered to be closely related to *N. coccogenis* or other *Notropis* (*Luxilus*). Either *D. spatulus* does not conform with the trend of a species of *Dactylogyrus* normally infesting only closely related hosts, or it may indicate that *N. coccogenis* is more closely related to these 4 hosts than is generally recognized. Although not compelling, there are ichthyological data providing some merit for the latter view. For example, the pattern of uniserial or biserial rows of nuptial tubercles with 2–6 points per tubercle on the pectoral fins in *Notropis* (*Luxilus*) was apparently derived from the uniserial condition found in *Notropis* (*Hydrophlox*), of which *N. leuciodus* and *N. rubricroceus* are members (Swift, 1970). Furthermore, species of *Notropis* (*Luxilus*) and *Notropis* (*Hydrophlox*) display similar reproductive behavior, i.e., they typically spawn over *Nocomis* Girard nests (Lee et al., 1980). In light of present classification, it is intriguing that *N. rubellus* is not infested with *D. spatulus,* as it is considered to be a member of *Notropis* (*Hydrophlox*) (Snelson, 1968; Swift, 1970). Perhaps these data are revealing that *N. rubellus* is not very closely related to other *Notropis* (*Hydrophlox*). Indeed, Swift (1970) placed *N. rubellus* in its own species group within the subgenus. Concerning *N. ariommus* and *N. telescopus,* Gilbert (1964) suggested a possible close relationship between *N. ariommus* (Cope) (=*N. telescopus* in part at that time) and species of *Notropis* (*Luxilus*) based on similarities in pharyngeal teeth, peritoneal pigmentation, eye size, position of dorsal fin, dorsal

striping, and ecological conditions. However, Snelson (1968) considered *N. ariommus* and *N. telescopus* as members of *Notropis* (*Notropis*), and Gilbert (1969) later argued against a close relationship between *Notropis* (*Luxilus*) and *N. ariommus* and *N. telescopus*. The presence of *D. spatulus* suggests that perhaps Gilbert (1964) was correct formerly in suggesting a relationship between *Notropis* (*Luxilus*) and *N. ariommus* and N. *telescopus*. The presence of *Dactylogyrus* sp. cf. *sceptici* on *N. ariommus* indicates a close phyletic link between *N. ariommus* and *N. scepticus* (Jordan and Gilbert), corroborating Gilbert's (1969) statement that *N. ariommus* most closely resembles *N. scepticus*.

These parasitological data should not be used alone to make inferences about host relationships. The possibility of host transfers that could invalidate inferences of close phyletic links between host species cannot be ignored. However, it also seems reasonable that transfers of parasite species from one host species to another are most likely to occur between closely related hosts. After a more thorough study of the taxonomy of *Dactylogyrus,* cladistic analysis such as that proposed by Brooks (1981) should provide more insight into the coevolution of *Dactylogyrus* and *Notropis*. Further ichthyological studies, including genetic and biochemical analyses, are needed to confirm or reject the host relationships suggested here.

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Light and Scanning Electron Microscope Studies of *Fundulotrema prolongis* (Monogenea: Gyrodactylidea) Parasitizing *Fundulus diaphanus* (Cyprinodontidae) in Nova Scotia, Canada, with an Emended Diagnosis of *Fundulotrema*

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ABSTRACT: The haptor and peduncle of *Fundulotrema prolongis* (Hargis, 1955) parasitizing the body surface of *Fundulus diaphanus* in Nova Scotia is examined with light and scanning electron microscopy. The study shows that this attachment organ is almost rectangular in ventral view, with marginal hooks I–III in 2 anterolateral groups set away from the posteriorly located hooks IV–VIII. The so-called peduncular bar is an apparently static, V-shaped sclerite positioned with its apex directed anteroventrally beyond the level of the surrounding tissue. The bar is covered by a smooth spineless tegument and may have its origin within this outer layer. In unextended animals the lateral regions of the bar can rest on the well-developed hamulus roots and may serve as a stabilizer or physical stop for the hamuli. The bar is not a supplementary organ of attachment and thus is functionally different from the prehensile squamodisc of diplectanid monogeneans. The study provides an emended diagnosis for the genus *Fundulotrema*.

KEY WORDS: SEM, Monogenea, *Fundulotrema prolongis, Fundulus diaphanus,* Gyrodactylidea, killifish.

Scanning electron microscopy (SEM) has been used successfully to study the haptor of species of *Gyrodactylus* Nordmann, 1832 (Monogenea: Gyrodactylidea) (Hawkes, 1977; Ergens, 1983; Kayton, 1983; Linnenbach and Hausmann, 1983; Malmberg, 1983; Cone and Odense, 1984; Cone and Cusack, 1988). In the present study we use the technique along with light microscopy for studying *Fundulotrema prolongis* (Hargis, 1955) Kritsky and Thatcher, 1977, the type species of a closely related but little-studied genus.

Materials and Methods

Samples of banded killifish (*Fundulus diaphanus*), 3–5 cm long and lightly infected with *F. prolongis*, were collected in June 1986 from Porters Lake, Nova Scotia (44°45′N, 63°18′W). Several hundred fish were held in a 200-liter aquarium supplied with 15°C dechlorinated tapwater. The fish were fed tropical fish food ad libitum. After 2 mo the intensities of *F. prolongis* increased in some cases to 500 worms per fish. Infected fish were frozen by immersion in liquid Freon 22 and freeze-dried overnight. Dried parasites were mounted ventral side up on SEM stubs prior to sputter coating with gold and examination with a JEOL 35 scanning electron microscope. Infected fish were also embedded in Paraplast for histological study; sections were stained in hematoxylin and eosin. Live parasites were fixed in 5% formalin and mounted unstained in glycerine jelly for light microscope studies. Live specimens were studied in wet mounts. Identification of the parasites was confirmed by examination of type material (USNM Helm. Coll. No. 49331). Numbering of the marginal hooks follows the system proposed by Llewellyn (1963) because of its consistency in an evolutionary context.

Results

The haptor of *F. prolongis* is almost rectangular in shape and constitutes ⅓ of the total body length in relaxed specimens (Fig. 1).

SEM enabled the relative positions of the sclerotized components, seen in flattened, glycerine-mounted preparations (Fig. 2), to be visualized in 3 dimensions (Fig. 3). The pair of hamuli are splayed laterally with the bare blades of each directed anteriorly and parallel to the ventral surface of the body. Two distinct muscular sheaths enclose the well-developed anteroventrally directed hamulus roots (Fig. 3). Full outlines of the ventral bar and associated anterolateral processes and posteriorly directed membrane are visible beneath a taut tegument surface (Fig. 3). The 16 marginal hooks are peripheral on the haptor with ventromedially directed sickle blades; pairs I–III are in 2 anterolateral groups; pairs IV–VIII form an evenly spaced posterior group (Figs. 1–3, 5).

An apparently static peduncular bar, covered by smooth tegument, is present immediately anterior to the haptor (Figs. 2, 3). Histological sections reveal that it is V-shaped in cross section (Fig. 4), and, like the ventral bar, is intensely eosinophilic. It appears to lie within the ventral tegument, protruding anteroventrally beyond the

surrounding tissue. In nonextended specimens the lateral regions of the bar are positioned immediately above or directly on the underlying hamulus roots.

Fundulotrema prolongis attaches to the host by means of the blades of the marginal hooks that impale epithelial cells on the skin surface. The 3 pairs of hooks grouped anteriorly maintain an anterior position when adhered to host tissue (Fig. 5). The skin beneath the haptor is compressed and occasionally pierced by the hamulus blades. Histological sections revealed that the peduncular bar does not pierce or touch the skin surface during normal attachment.

Discussion

The haptor of *F. prolongis* is extended anteriorly to accommodate the unusually long hamulus roots and thus takes on an almost rectangular outline in ventral view. This development appears to have left marginal hooks I–III in 2 anterolateral groups set away from the posterior ones.

The haptor has a distinct peduncular bar. This sclerite has been described as an anteriorly directed skirt with sclerotized points (Hargis, 1955; Beverley-Burton, 1984) and as a transverse peduncular bar with small oblong pits (Williams and Rogers, 1971). What is seen with light microscopy as a skirt is actually the outline of the V-shaped bar protruding anteroventrally beyond the surrounding tissue. The bar does not have spines but is pitted at its apex.

In unextended specimens the bar is positioned in such a way that its lateral regions could exert passive pressure on the roots of the hamuli. In this position it might stabilize these sclerites while allowing greater freedom of movement for the forebody. It might serve as a physical stop protecting the soft peduncular tissue from potential damage of any dorsally directed action of the well-developed hamulus roots. As a stop it might also facilitate removal of the hamulus blades from host tissue when required. The bar does not penetrate adjacent host skin and does not supplement permanent attachment to the host. It therefore differs functionally from the diplectanid squamodisc with which it might be confused (see Paling, 1966; Oliver, 1976).

In cross section (Fig. 4), however, the bar resembles the V-shaped spines present on the squamodisc of *Diplectanum aequans* (Wagener, 1857). Shaw (1981) showed that these spines are embedded in the tegument, thus supporting a possible tegumental origin (Kearn, 1968). Although ultrastructural studies are needed to confirm the relationship of the peduncular bar with the ventral tegument, our light microscopical study suggest that it lies within the tegument. If shown to be so, the bar may represent a sclerotized tegumental fold.

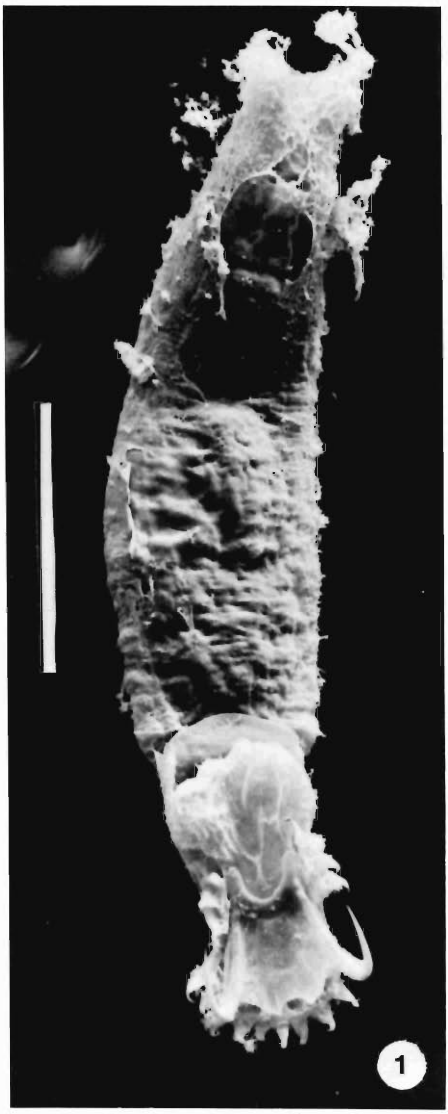
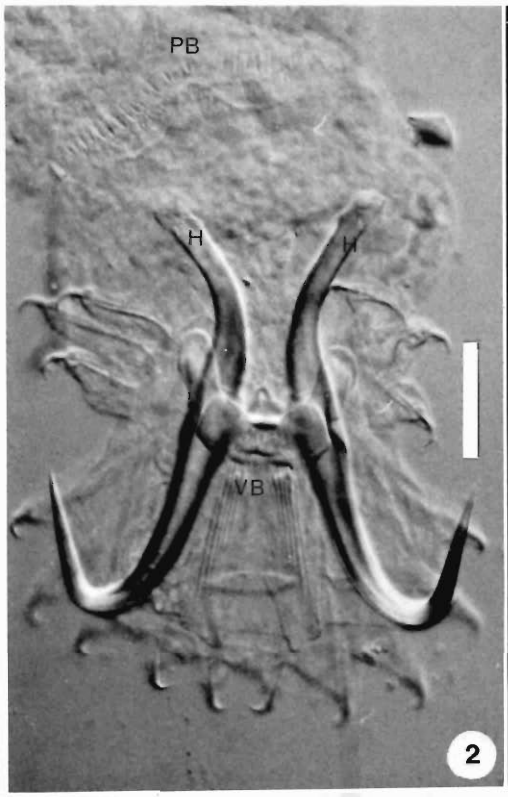
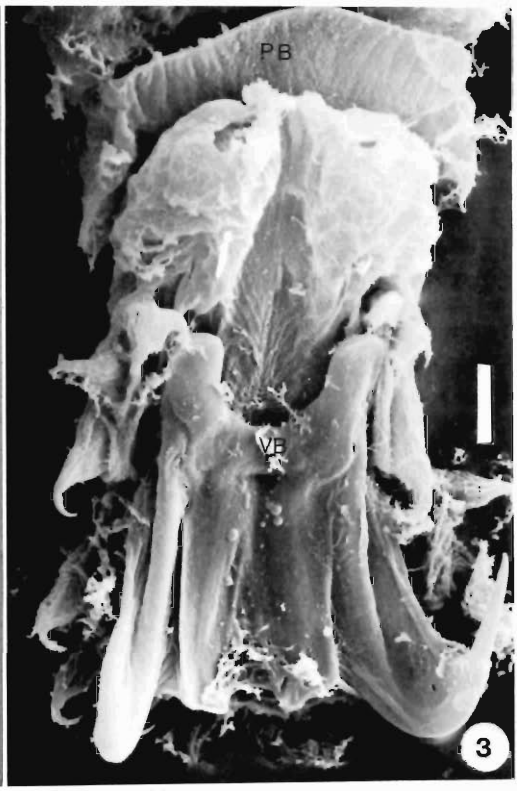
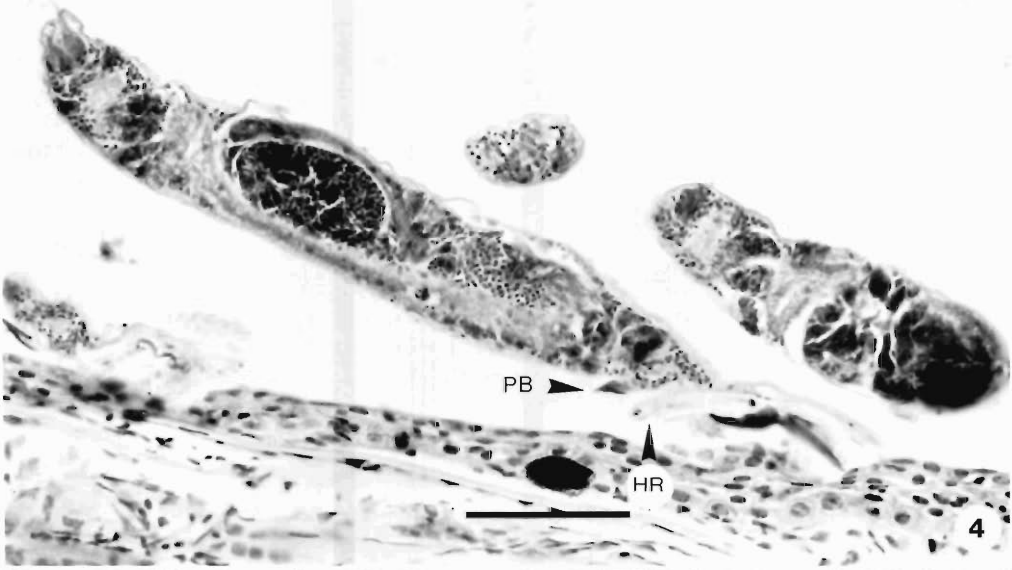


Figure 1. Scanning electron photomicrograph of *Fundulotrema prolongis* from the skin surface of *Fundulus diaphanus*. Ventral view. Scale bar is 100 μm.

Fundulotrema prolongis attaches to the host in a manner similar to *Gyrodactylus avalonia* Hanek and Threlfall, 1969, and *Gyrodactylus co-*







Figures 2–4. Photomicrographs of *Fundulotrema prolongis*. 2. Light photomicrograph of the haptor of *F. prolongis* showing the various sclerite components: peduncular bar (PB), hamuli (H), and ventral bar and membrane (VB). The marginal hooks are peripheral on the haptor, with 2 anterolateral groups set apart from the others. Ventral view. Glycerine-cleared specimen. Scale bar is 20 μm. 3. Scanning electron photomicrograph of the haptor of *F. prolongis,* showing the sclerites described in Figure 2 covered with a taut smooth tegument. Ventral view. Scale bar is 10 μm. 4. Longitudinal section through an attached *F. prolongis* showing the relative position of the peduncular bar (PB) and 1 of the hamulus roots (HR). Note the bar is V-shaped in cross section. 20 μm.

lemanensis Mizelle and Kritsky, 1967. All 3 species use the blade tips of the marginal hooks to pierce epithelial cells on the host surface and the hamulus blades for supplementary attachment (Cone and Odense, 1984; Cone and Cusack, 1988).

The genus *Fundulotrema* was established by Kritsky and Thatcher (1977) to accommodate species of Gyrodactylinae Monticelli, 1892, possessing a peduncular bar and a haptor with a ventral pair of hamuli and 2 support bars and 16 evenly spaced marginal hooks. The present study reveals that the marginal hooks in fact have an uneven distribution. Examination of type specimens of *Fundulotrema foxi* (Rawson, 1973), *Fundulotrema megacanthus* (Wellborn and Rogers, 1967), *Fundulotrema stableri* (Hathaway and Herlevich, 1973), and *Fundulotrema trematoclithrus* (Rogers, 1967) confirms that marginal hooks I–III are grouped anteriorly in all known members of the genus.

The above diagnostic correction does not jeopardize the taxonomic validity of *Fundulotrema* but simply ties the genus closer to *Polyclithrum* Rogers, 1967, and *Swingleus* Rogers, 1969. *Swingleus* has 2 bilateral winglike support plates, a peduncular bar, and the first 3 pairs of the 16 marginal hooks grouped anteriorly (Rogers, 1969). *Polyclithrum* has numerous ventral support bars, no peduncular bar, and the first 4 pairs of the 16 marginal hooks grouped anteriorly (Rogers, 1967).

New information gathered on the nature of the peduncular bar and on the distribution of the marginal hooks necessitates an emended diagnosis of *Fundulotrema.*

Fundulotrema (Hargis, 1955) Kritsky and Thatcher, 1977, emended diagnosis

GENERIC DIAGNOSIS: Gyrodactylidea, Gyrodactylinae; body divisible into cephalic region, trunk, peduncle, and haptor. Cuticle thin, smooth. Cephalic lobes (2) terminal, each containing portions or all of head organs. Cephalic glands present. Eyes absent. Pharynx composed of 2 subhemispherical bulbs; esophagus short; intestinal crura (2) without diverticulae, terminate blindly in posterior trunk. Gonads tandem; testis postovarian, intercecal. Penis (when present) ventral, submedian, situated at or immediately posterior to level of pharynx, armed with 1 spine and 1 to several spinelets distributed in single row. Ovary submedian, immediately postuterine, uterus

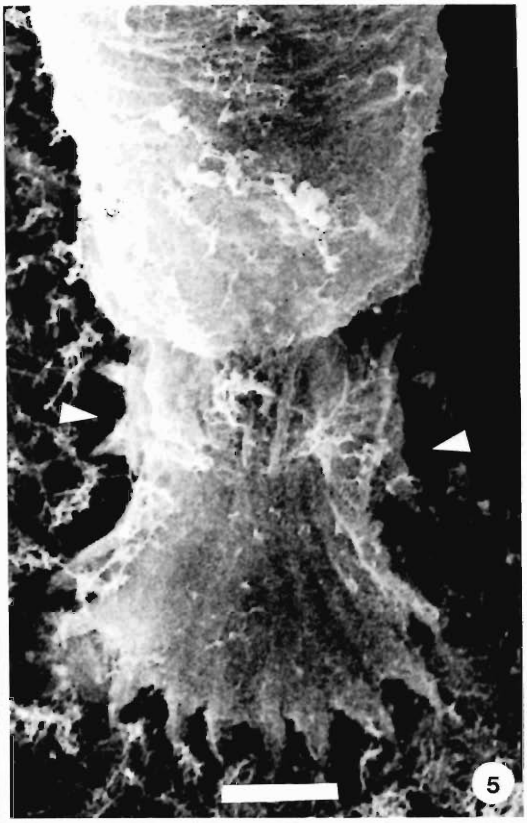


Figure 5. Dorsal view of the parasite attached in situ. Note that the 2 anterolateral groups of marginal hooks (arrows) retain their anterior position in attached parasites. Scale bar is 20 μm.

central or subcentral, usually containing an embryo that may contain an embryo or the next generation. Vitellaria, vagina, genitointestinal canal absent. Haptor gyrodactyloid, ventrally concave with pair of ventral hamuli supported by ventral and dorsal bar; 16 marginal hooks, similar in shape and size, and arranged in 2 anterolateral groups (hooks I–III) and a posterior group (hooks IV–VIII); ventral bar shield present. Peduncular bar present near ventral surface of peduncle. Parasitic on the external surfaces of fishes of the Cyprinodontidae.

Acknowledgments

We thank Dr. J. R. Lichtenfels for arranging the loan of the type specimens. The work was supported financially by an NSERC Operating Grant to DKC and by the National Research Council of Canada.

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Errata

In a recent issue of this journal, the following corrections should be made:

January 1988, 55(1):87–90, in the article by Boeger and Thatcher:

In the Discussion and Literature Cited *Gamidactylus jaraguensis* should be *G. jaraquensis.* In the Literature Cited *Semaprochilodus jaraguensis* should be *S. insignis.*

Proc. Helminthol. Soc. Wash.
55(2), 1988, pp. 229–245

Histofluorescent and Ultrastructural Identification of Aminergic Processes in the Opisthaptor of the Marine Monogene, *Microcotyle sebastis* (Polyopisthocotylea: Microcotylinae)

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ABSTRACT: The opisthaptoral clamp region of *Microcotyle sebastis* Goto, 1894, was examined for aminergic processes by histofluorescent and ultrastructural techniques. Glyoxylic acid-induced fluorescence, indicative of catecholamines, revealed blue/green varicosities and longitudinal processes directly associated with clamp elements. Ultrastructural examination of nerve processes in close histologic proximity to those found by fluorescence microscopy revealed numerous small dense and/or dense-core vesicles within the clamp musculature, as well as processes containing both electron-dense and lucent vesicles in the opisthaptoral neuropil. Two nerve bundles, surrounded by clamp basal lamina and epidermis within the clamp cavity, were found situated on either side of the median clamp sclerite. A nerve bundle was also found on the interior surface of the clamp cavity, approximately ⅔ the distance between the median clamp sclerite and the lateral sclerite elements. This bundle was serially sectioned and a single uniciliated receptor was found. Although it is speculated that these receptors exhibit bilateral symmetry, only 1 receptor was found. Other processes within the clamp musculature and in the opisthaptoral neuropil contained vesicle types consistent with cholinergic, aminergic, and peptidergic morphology. Nerve location and associated vesicle morphology within the clamp suggest a sensory/neuromodulatory role associated with sensory receptors. A role other than sensory is suggested for nerves containing small dense/dense-core vesicles and/or a combination of other vesicle types within nerves adjacent to or within the haptoral neuropil.

KEY WORDS: Monogenea, opisthaptor, histofluorescence, catecholamines, ultrastructure, aminergic processes, *Microcotyle sebastis*, TEM.

Biogenic monoamines have been considered to function as neurotransmitters, neuromodulators, and as neurohormones in many invertebrates (Lentz, 1967; Gerschenfeld, 1973; Kerkut, 1973; Gersch, 1975; Robertson and Juorio, 1976; Evans, 1980; Reuter et al., 1980; Webb and Orchard, 1980; Robertson, 1981; Barber, 1982; Orchard, 1982; Hoyle, 1985; Steinbusch et al., 1986); however, their location, structural morphology, and precise role(s) in the nervous system of parasitic helminths has not been completely resolved.

Biogenic monoamines in the nervous system of parasitic helminths have been studied in members of the Cestoda (Shield, 1971; Hariri, 1974; Webb and Davey, 1976; Lee et al., 1978; Gustafsson and Wikgren, 1981; Gustafsson, 1984, 1985; Gustafsson et al., 1985, 1986), Nematoda (Anya, 1973; Sulston et al., 1975—free-living; Goh and Davey, 1976a; Högger et al., 1978; Wright and Awan, 1978; Sharpe and Atkinson, 1980; Horvitz et al., 1982), and to a lesser extent, the Trematoda (Bennett et al., 1969; Bennett and Bueding, 1971; Chou et al., 1972; Machado et al., 1972; Bennett and Gianutsos, 1977; Mansour, 1984), and the Acanthocephala (Budziakowski et al., 1983, 1984; Budziakowski and Mettrick, 1985). In these studies, much of the work dealing with the identification of specific monoamines was conducted using biochemical and/or radioenzymatic techniques. Furthermore, green histofluorescence, indicative of the catecholamines dopamine, norepinephrine, and epinephrine, and the yellow fluorescence of the indolamine 5-hydroxytryptamine (5-HT), have been documented. Electron microscopic studies have, in some cases, yielded information about the ultrastructural morphology of amine production, storage, and release sites.

In the Monogenea, various studies have been conducted dealing with the ultrastructure of the epidermal sense organs (Halton and Morris, 1969; Lyons, 1969a, b, 1972, 1973a, b; Rohde, 1972, 1975) and various types of photoreceptors (Lyons, 1972; Kearn and Baker, 1973; Fournier, 1975; Fournier and Combes, 1978; Kearn, 1978). A brief description of central nervous system synapses (Rohde, 1972, 1975) and, in particular, an ultrastructural description of brain fine structure and synaptic morphology (Shaw, 1981, 1982) have also been published. Shaw (1979) briefly described the innervation of clamp musculature in *Gastrocotyle trachuri* and noted the presence

of electron-dense vesicles associated with the internal clamp musculature. Furthermore, Halton and Morris (1969) demonstrated cholinesterase activity in the clamp musculature of *Diclidophora merlangi,* but no mention was made of the ultrastructural morphology of clamp muscle innervation.

The occurrence and descriptive morphology of catecholamines and catecholaminergic processes in the opisthaptoral region of the Monogenea have not been previously reported. This study documents the presence of catecholamines using glyoxylic acid-induced fluorescence. The ultrastructural morphology of processes containing dense and/or dense-core vesicle storage sites are described and compared with histofluorescence findings associated with opisthaptoral clamps in the marine monogene, *Microcotyle sebastis* Goto, 1894. Areas of neuroactive substance storage and release, as well as other vesicle types and clamp morphology, are discussed.

Materials and Methods

Specimens of the marine monogene, *Microcotyle sebastis* Goto, 1894, were collected from the primary gill lamellae of black rockfish, *Sebastes melanops* (Girard), caught by fishermen on chartered vessels near Newport, Oregon, U.S.A. Specimens were either removed mechanically with the aid of microforceps and camel hair brush, or lamellae with attached specimens were removed en toto from the pharyngeal arch. In either case, specimens were placed in cold seawater, examined for movement at various intervals, and processed for examination as whole mounts for general morphology by light microscopy, examined for the presence of glyoxylic acid-induced histofluorescence of catecholamines by fluorescence microscopy, or were processed to epoxy resin, sectioned, and examined by light and transmission electron microscopy. Terminology of clamp element morphology was used according to Bonham and Guberlet (1937).

Light microscopy

Within 24 hr after collection, specimens were either fixed in alcohol–formaldehyde–acetic acid (AFA) or 10% buffered formalin with slight coverglass pressure to flatten and orient the opisthaptoral clamp region. They were stained with 1% Weigert's elastin stain and counterstained in 0.5% fast green to demonstrate internal muscle/clamp attachment.

Fluorescence microscopy

Living specimens, refrigerated in seawater for up to 3 days, were placed in 2°C 0.236 M potassium phosphate, monobasic, containing 600 mM sucrose and 2.0% glyoxylic acid (G.A.) at pH 7.2. They were reacted in G.A. for 5–7 min, placed on microscope slides, excess G.A. was blotted away, and the haptoral region was gently spread out with a camel hair brush to optimize clamp examination. Subsequently, specimens were gently dried for 5–7 min with warm air from a hair dryer, placed in a vacuum oven at 90°C for 5–7 min under a slight vacuum of between 15 and 20 cm Hg, removed from the oven, and while still warm, were mounted with nonfluorescent immersion oil and coverglassed. Control specimens were treated as above except that glyoxylic acid was omitted. Fluorescence was examined using a Zeiss Universal microscope equipped with a G 365 excitation filter and a Zeiss LP50 barrier filter in the epifluorescent mode under ultraviolet irradiation. Positive fluorescence for catecholamines was documented using Kodak Technical Pan film 2415 or Kodak Ektachrome 50 tungsten film.

Electron microscopy

Fixation of specimens employed 2 different methods: (I) cold 2.5% glutaraldehyde/1.5% paraformaldehyde, 0.005 M calcium chloride, 3% sodium chloride, 0.006 M sucrose, in a 0.2 M cacodylate buffer at pH 7.2; or (II) a modified Bouin fluid according to Stefanini et al. (1967) to which 3% sodium chloride was added. Immediately upon collection, specimens were fixed for 4 hr in one of the above fixatives, rinsed in cold 0.2 M cacodylate buffer for 45 min, either placed in 2% cacodylate-buffered osmium tetroxide for 1 hr at 4°C or placed in 2% cacodylate-buffered osmium tetroxide for 15 min, rinsed in buffer, placed in room temperature 1% cacodylate-buffered thiocarbohydrazide (TCH) or 1% buffered tannic acid for 15 min, and, in the method using TCH, were rinsed in buffer for 10 min and returned to 2% osmium tetroxide for 15 min. All specimens were rinsed in cold buffer for 45 min, dehydrated through graded alcohols to propylene oxide, and placed in a 2:1 mixture of propylene oxide/LX 112 overnight. They were then processed to fresh LX 112 and polymerized. Semithick sections were stained separately with 1% paraphenylenediamine and Stévenel's blue (del Cerro et al., 1980), 1% toluidine blue, or etched with a saturated solution of sodium ethoxide and stained with Protargol S per the Bodian's method (Luna, 1968). Thin sections were placed on naked grids, stained with uranyl acetate and lead citrate, and examined at 40 kV with an Elektros ETEM 101 electrostatic, transmission electron microscope.

Results

Light microscopy

Whole-mounted specimens stained with Weigert's stain showed a highly organized array of muscle fibers attaching to haptoral clamps.

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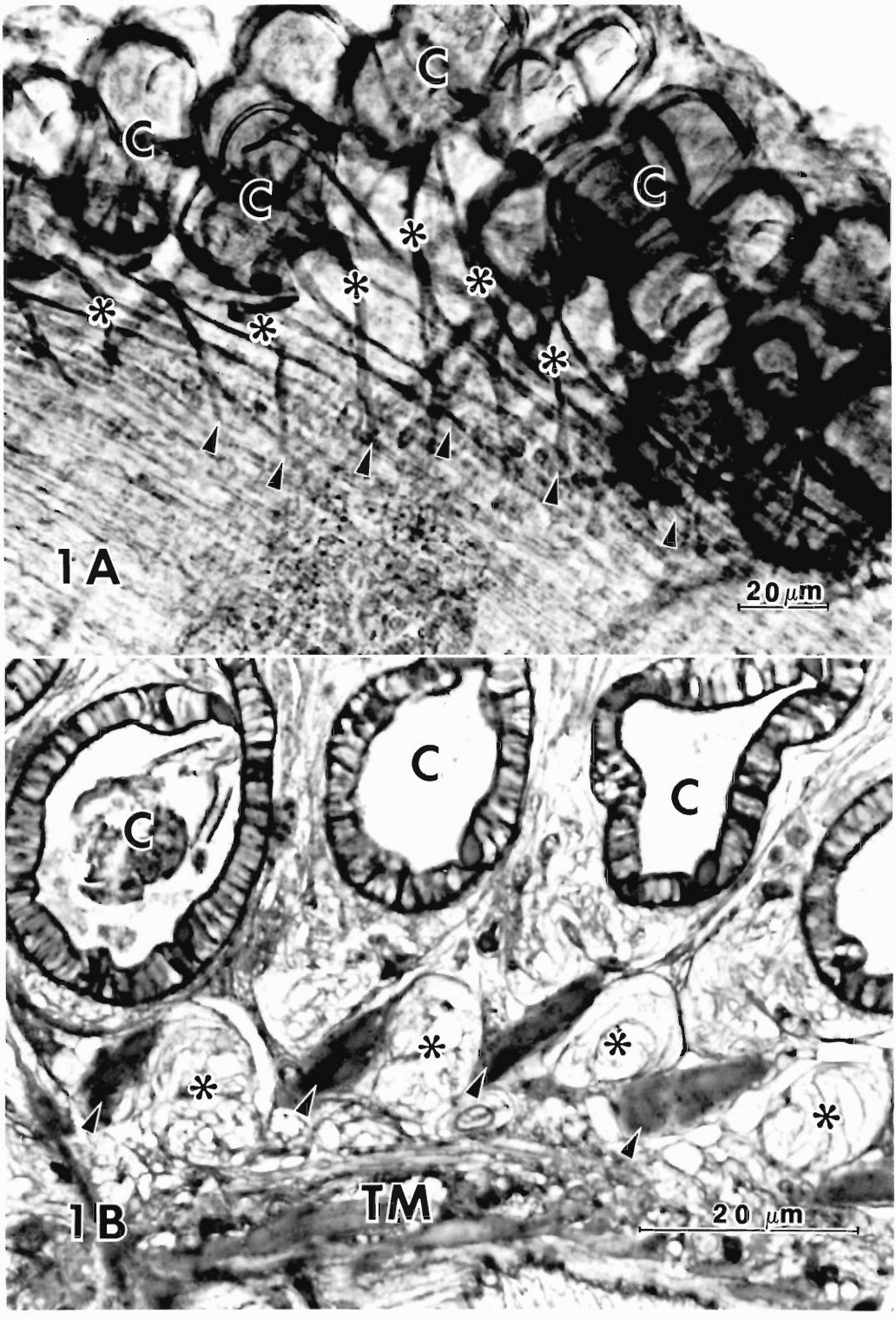
Figure 1A, B. Light photomicrographs of opisthaptoral clamp morphology of *Microcotyle sebastis*. 1A. Whole mount showing clamps (C) and muscle fibers (*) projecting medially from clamp attachment to merge with tegumental musculature (arrowheads). Weigert's elastin stain. 1B. Thick-sectioned epoxy-embedded specimen showing clamps (C), connecting clamp muscle fibers (arrowheads), tegumental musculature (TM), and nerve glomeruli (*). Bodian's silver stain.



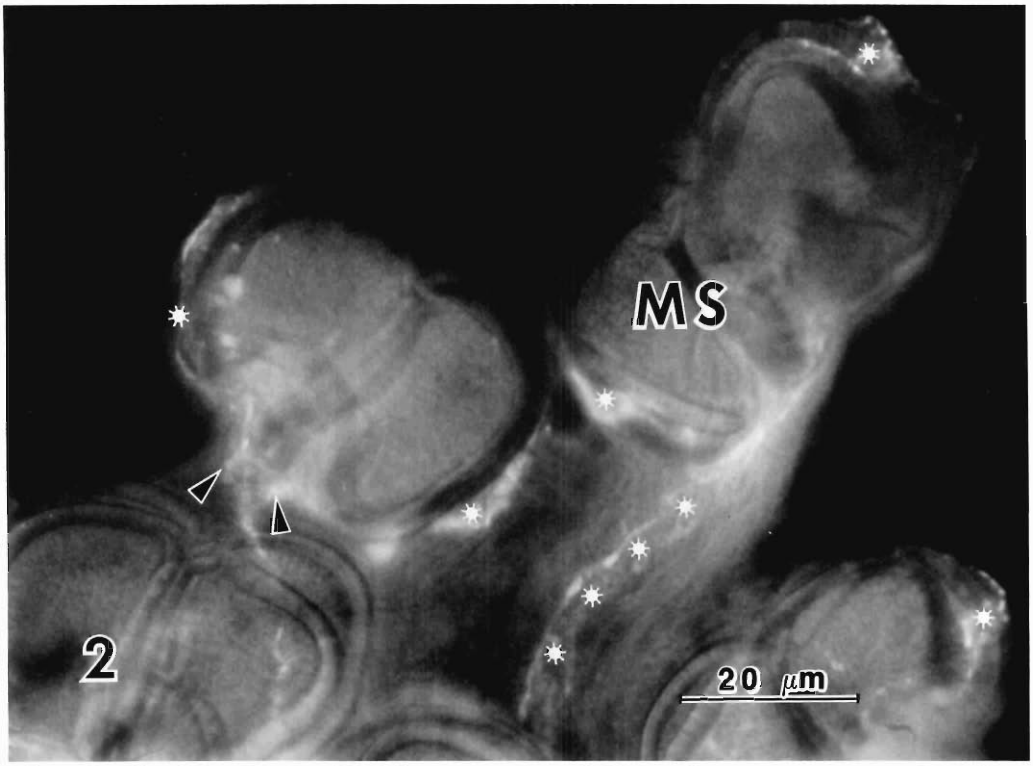


Figure 2. Glyoxylic acid-induced histofluorescence of catecholaminergic nerve processes associated with opisthaptoral clamps; fluorescent nerve processes (*), median clamp sclerite (MS), nerve bifurcation symmetrically situated on either side of the median clamp sclerite (arrowhead).

These fibers projected medially where they merged with tegumental muscle fibers (Fig. 1A).

Sections from plastic-embedded specimens showed evenly spaced muscle fibers which, in subsequent sections, attached to the anterior portion of the median and lateral clamp elements (Fig. 1B). In close association with the clamps and directly adjacent to these muscle fibers, evenly spaced nerve glomeruli were seen in cross section (Fig. 1B). In some sections, large vacant processes, cut in various planes, could be seen leading anteriorly from individual clamps.

Fluorescence microscopy

Specific blue/green fluorescence, indicative of catecholamines, was seen as varicosities and/or longitudinal processes in tissue directly associated with the anterior ½–⅔ aspect of the anterolateral, posterolateral, or anteromedial regions of clamp elements (Fig. 2). Fluorescent processes, symmetrically situated on either side of the median clamp sclerite and the lateral clamp elements, were also observed (Fig. 2). Nerve processes of similar location and symmetry were seen by electron microscopy (Fig. 4). Individual fluorescent nerves were seen extending anteromedially from many of the clamps (Fig. 2); however, these processes could not be traced to nerve somata. At higher magnifications, small processes were occasionally seen either branching from a major nerve or were seen lying separately in the neuropil.

The fluorescent presence of 5-hydroxytryptamine (5-HT), a fast-fading, yellow fluorescence, was not seen in the haptoral region of any of the specimens. Furthermore, control specimens, re-

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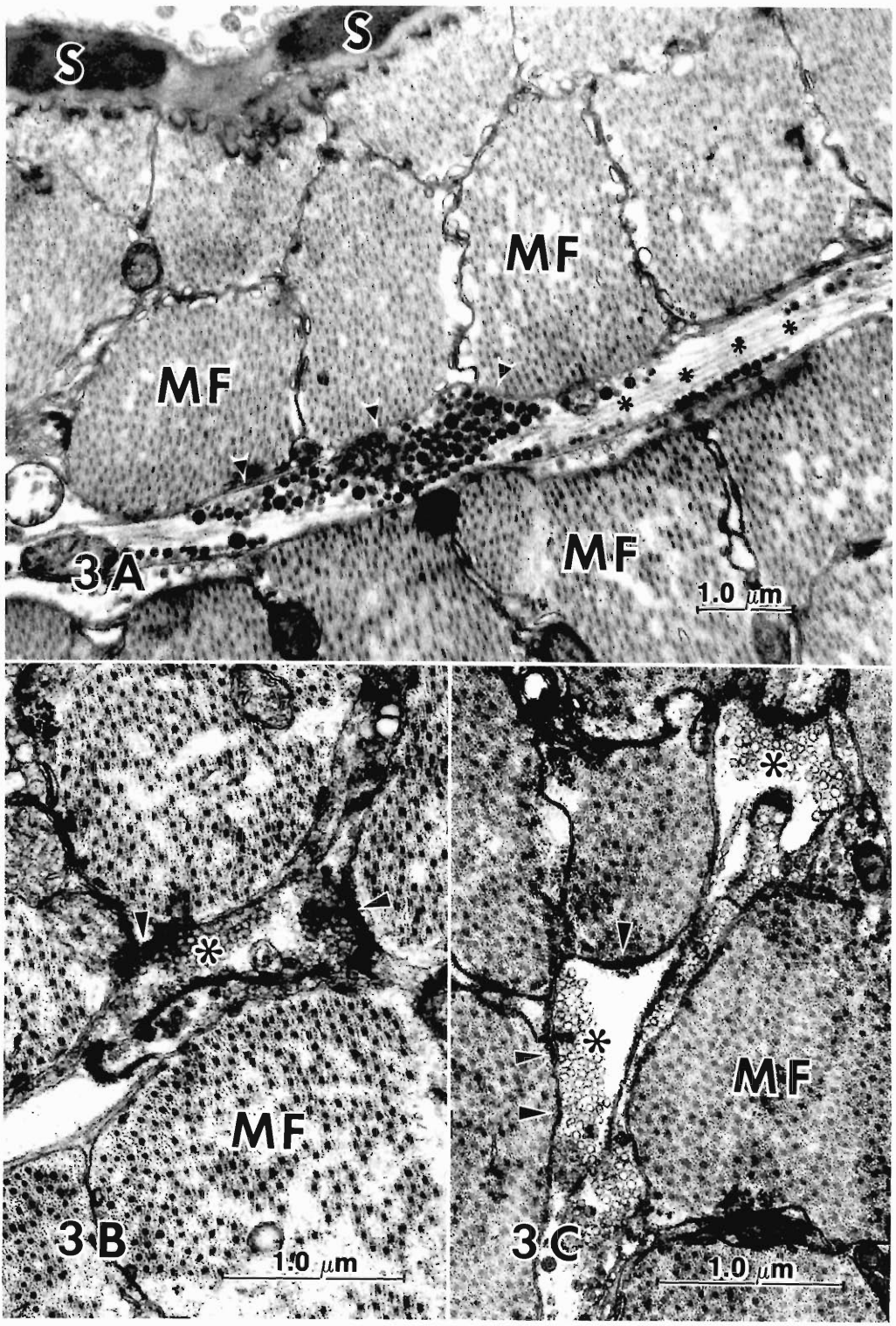
Figure 3A–C. Electron photomicrographs of internal clamp morphology: 3A, sclerotized "ribs" (S), musculature showing myofibrils (MF), nerve process containing dense/dense-core vesicles (arrowheads), and neurotubules (*); 3B, C, showing presumed cholinergic neuromuscular synapses and presynaptic and postsynaptic densities (arrowheads), lucent vesicles (*), and myofibrils (MF).



acted in the absence of glyoxylic acid, did not show the blue/green or yellow fluorescence.

Although there was positive blue/green fluorescence of some cell somata in the neuropil, the fluorescence was quite diffuse and consequently could not be photographed; however, large bodies were seen overlying individual clamps (Fig. 2).

Electron microscopy

Of the 2 fixatives used for preservation of ultrastructural detail, it should be noted that neither preserved all tissue equally. Method I preserved the muscle elements (myofibrils and myofilaments) much better than Method II, and Method II preserved the neuropil better than Method I. The addition of 1% thiocarbohydrazide or 1% tannic acid greatly improved preservation of osmiophilic structures and enhanced membrane contrast.

The clamp morphology consisted of densely packed, unsegmented myofibrils connected to the outer distal and inner proximal, amorphous clamp basal lamina. The myofilaments within individual myofibrils measured approximately 30 nm in diameter. Slender tubules lying adjacent to cisternae of sarcoplasmic reticulum, mitochondria, and small amounts of glycogen particles were observed. In some cases, nerves containing dense/dense-core vesicles were seen (Fig. 3A); neurotubules were prominent in these nerves containing dense/dense-core vesicles (Fig. 3A). In several instances, processes containing lucent vesicles were seen synapsing on what appeared to be clamp musculature (Fig. 3B, C).

The median clamp sclerite was made up of electron-dense material with open canals that sometimes contained vesicular material and/or debris. By light microscopy, these canals could be seen in whole-mounted specimens. In numerous places, the basal lamina and epidermis were supported by sclerotized "ribs" (Figs. 3A, 4) that were also seen by light microscopy. Two large nerve bundles were found symmetrically located, within the clamp cavity, on either side of the median clamp sclerite; these bundles corresponded with those seen by fluorescence microscopy (Fig. 2). They measured between 2.4 and 4.5 μm (N = 10 bundles, $\bar{x}$ = 3.8 μm) in diameter (Fig. 4). These bundles were ensheathed with the same semilucent amorphous basal lamina and a layer of epidermis, which also ensheathed the majority of the entire clamp. Each bundle was internally separated into individual processes by double membranes and contained mitochondria and numerous dense/dense-core vesicles ranging in measurement from 68.2 to 86.6 nm (N = 10 bundles, 20 vesicles/bundle, $\bar{x}$ = 82.0 nm) (Fig. 4). Upon subsequent sectioning, a nerve bundle of similar morphology to those found near the median clamp sclerite was found approximately ⅔ the distance between the median clamp sclerite and the distal portion of the lateral clamp sclerites (Fig. 5A). Serial sections of this bundle revealed the presence of a single uniciliated receptor (Fig. 5A, B). The morphology of this receptor was similar to those described in other helminths by previous investigators. Briefly, the distal portion of the receptor bulb was outlined by several apical "rings"; areas of zonula occludens or septate desmosomes were not seen in this section. A single tangentially sectioned cilium, basal body, and numerous microtubules were present; no ciliary rootlet was found (Fig. 5B). The proximal portion consisted of a bundle of several double membrane-bound processes that contained dense/dense-core vesicles and numerous mitochondria; a few vesicles with faint cores or inner membranes were also seen. The entire receptor was surrounded by basal lamina and epidermis. The epidermis consisted primarily of a multivacuolated matrix with numerous vesicles and occasional mitochondria. In several instances, large vesicles consisting of a thickened electron-dense outer membrane and a clear or slightly electron-dense center were observed within the epidermis. The size of these vesicles ranged from 155 to 266.3 nm (58 vesicles, $\bar{x}$ = 218 nm). The center portion of the large vesicles was often round, elongated, or divided into several compartments (Figs. 4, 5B). The exact nature of these vesicles is unknown; however, they were most often found on the externalmost membrane of the epidermis. Although none of these vesicles was seen associated with a synaptic cleft or in the process of exocytosis, they appear to serve a secretory function. Subsequent sections through the uniciliated receptor revealed the presence of a tapered elongation that may have entered the internal clamp musculature; this proposed site of entry was not found (Fig. 5C).

The internal anteroproximal aspect of the clamp element was surrounded by muscle elements and/or neuropil. Nerves in this region, observed in various planes, contained lucent, dense/dense-core, or a mixture of lucent and electron-dense vesicles. Two major types of electron-dense/dense-core vesicles were observed in

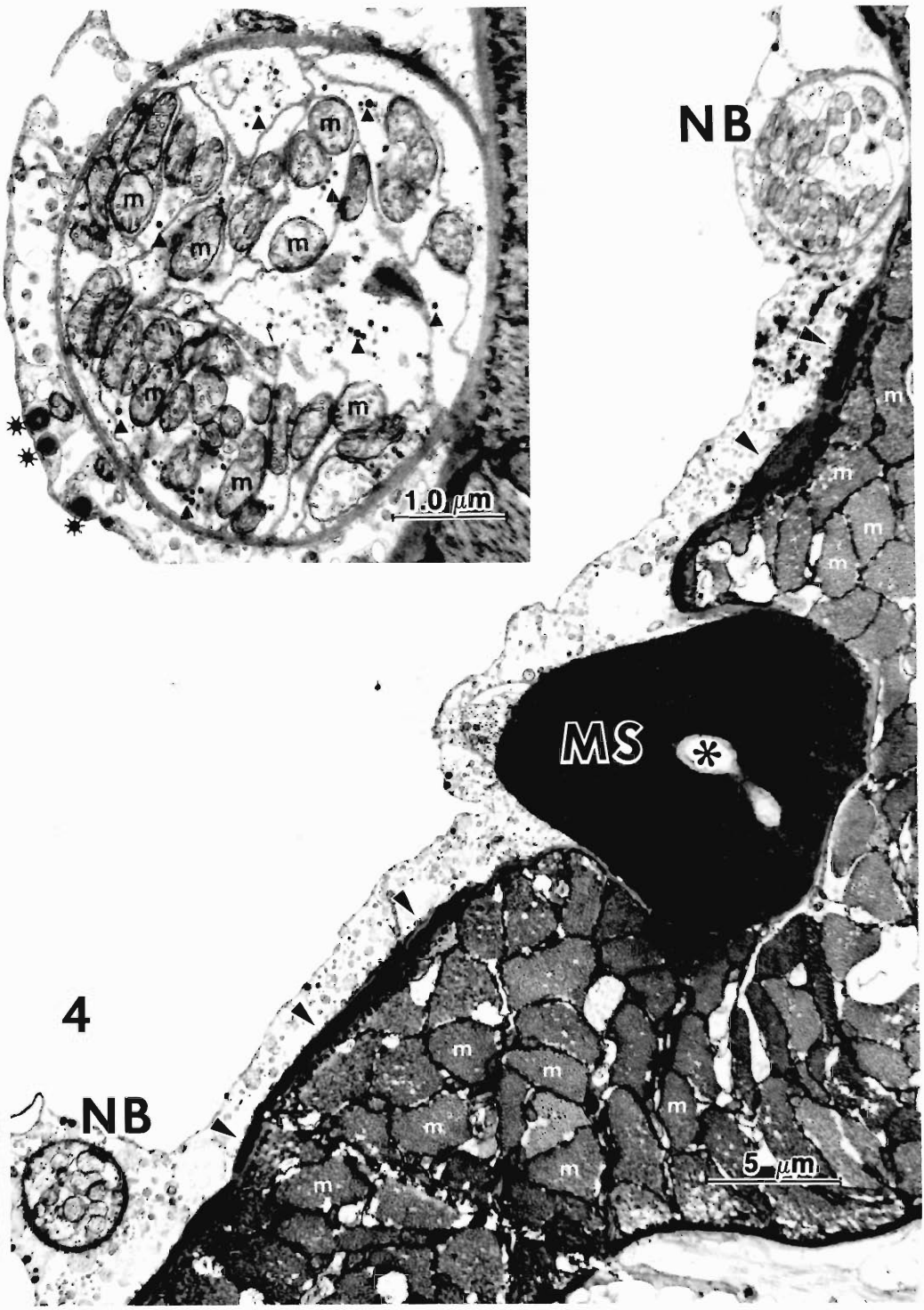
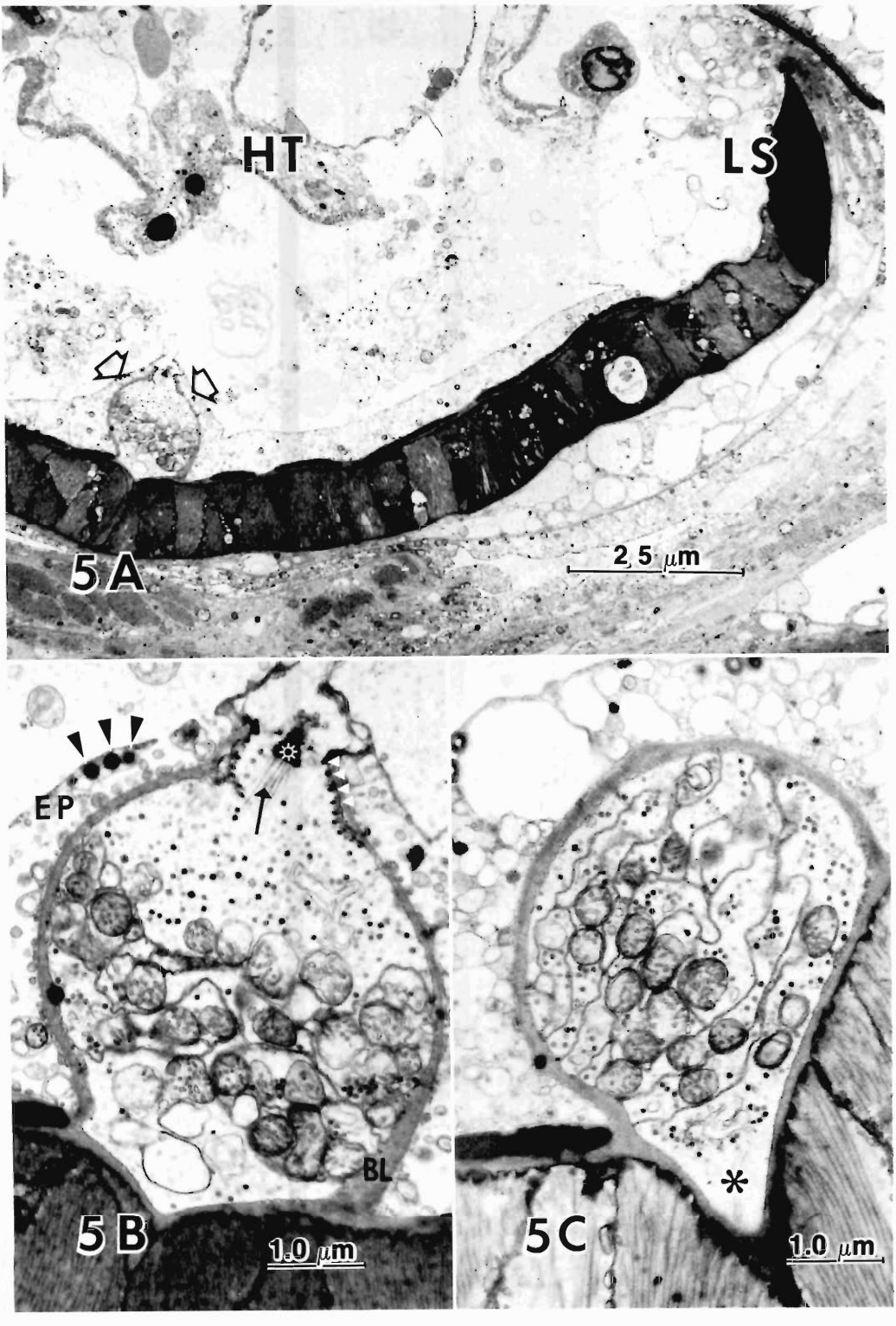


Figure 4. Electron photomicrograph of proximal portion of clamp showing myofibrils (M), median clamp sclerite (MS) with open canals (*), sclerotized "ribs" (arrowheads), and 2 large nerve bundles ensheathed in external basal lamina and epidermis (NB). Insert: ensheathed nerve bundle showing separate nerve processes containing numerous mitochondria (M), dense/dense-core vesicles (arrowheads), and large epidermal vesicles (*).



processes found in the neuropil directly adjacent to or anteromedial to the clamp elements. The small dense and/or dense-core vesicles, ranging from 66 to 135 nm in diameter (N = 221 dense and/or dense-core vesicles from 26 axons, $\bar{x}$ = 81 nm), were consistent in morphology with aminergic vesicles. In some instances, longitudinal processes were found that contained small dense/dense-core vesicles (Fig. 6A, B); these processes may correspond to those seen by fluorescence microscopy (Fig. 2). A few questionable synoptoid sites were found associated with processes containing the small dense/dense-core vesicles (Fig. 6B). The large dense and/or dense-core vesicles, consistent in morphology with peptidergic vesicles, were not extensively examined; however, of the 20 vesicles examined, their size ranged from 154.8 to 346 nm ($\bar{x}$ = 231 nm) in diameter. In one instance, large electron-dense vesicles were found to be in the process of exocytosis of the vesicle content into the adjacent process (Fig. 7A). In a few instances, large lucent vesicles were found in close association with the clamps or distributed throughout the haptoral neuropil (Fig. 7B). Nerves that contained small lucent vesicles, consistent in morphology with cholinergic vesicles, were also not extensively studied. Processes that contained lucent vesicles often showed pre- and postsynaptic clefts. Furthermore, in nerve processes containing both lucent and electron-dense vesicles, the lucent vesicles were often seen lining up along the presynaptic cleft (Fig. 7C).

Discussion

Catecholamine-specific histofluorescence has been observed in numerous parasitic helminths (Bennett and Bueding, 1971; Sulston et al., 1975; Goh and Davey, 1976a—free living; Bennett and Gianutsos, 1977; Högger et al., 1978; Sharpe and Atkinson, 1980; Horvitz et al., 1982; Budziakowski et al., 1983). Moreover, dopamine has been found to play a prominent role as a neurotransmitter substance in many invertebrates (Gerschenfeld, 1973; Kerkut, 1973).

Previous studies have, in some cases, identified dopamine as the predominant monoamine in members of the Trematoda and Acanthocephala (Bennett et al., 1969; Bennett and Bueding, 1971; Chou et al., 1972; Shishov et al., 1974; Bennett and Gianutsos, 1977; Budziakowski et al., 1983). Bennett and Bueding (1971) suggested that catecholamines in *Schistosoma mansoni* may act as interneuronal transmitters and/or induce the release of 5-HT from its storage sites, thus transmitting nerve impulses to effector organs and cells. It was further speculated (Bennett and Gianutsos, 1977) that dopamine in *Fasciola hepatica* may act as a sensory transmitter in "sensory papillae" located on the oral sucker and dorsal surface or as an inhibitory transmitter in the head region and anterior border of *F. hepatica*. Budziakowski et al. (1983) also found dopamine to be associated with the "sensory bulbs" and the anterior nervous system of *Moniliformis dubius*. She (1983) stated that dopamine may act as a sensory transmitter in the Acanthocephala.

Although the extrapolation of results from one invertebrate to another must be treated with caution, it has been speculated that dopamine may function as a sensory neurotransmitter that modulates the rhythmic contractile activity of the earthworm (Bieger and Hornykiewicz, 1972; Gardner and Cashin, 1975). Furthermore, Goh and Davey (1976a) suggested that the fluorescent catecholaminergic neurons observed in the ventral nerve cord of the nematode *Phocanema decipiens* may play a role in the control of somatic muscular activity.

In order to compare the relevance between the role of neuroactive substances and the function of sclerotized or keratinized structures, the results from several studies involving the finding of dopaminergic and/or cholinergic processes associated with nematode spicule innervation were examined. Sulston et al. (1975) reported dopaminergic fluorescent cell somata, preanal ganglion, and lateral ganglion in the posteroventral tail region of male *Caenorhabditis elegans*. However, mutant male *C. elegans*, which did not ex-

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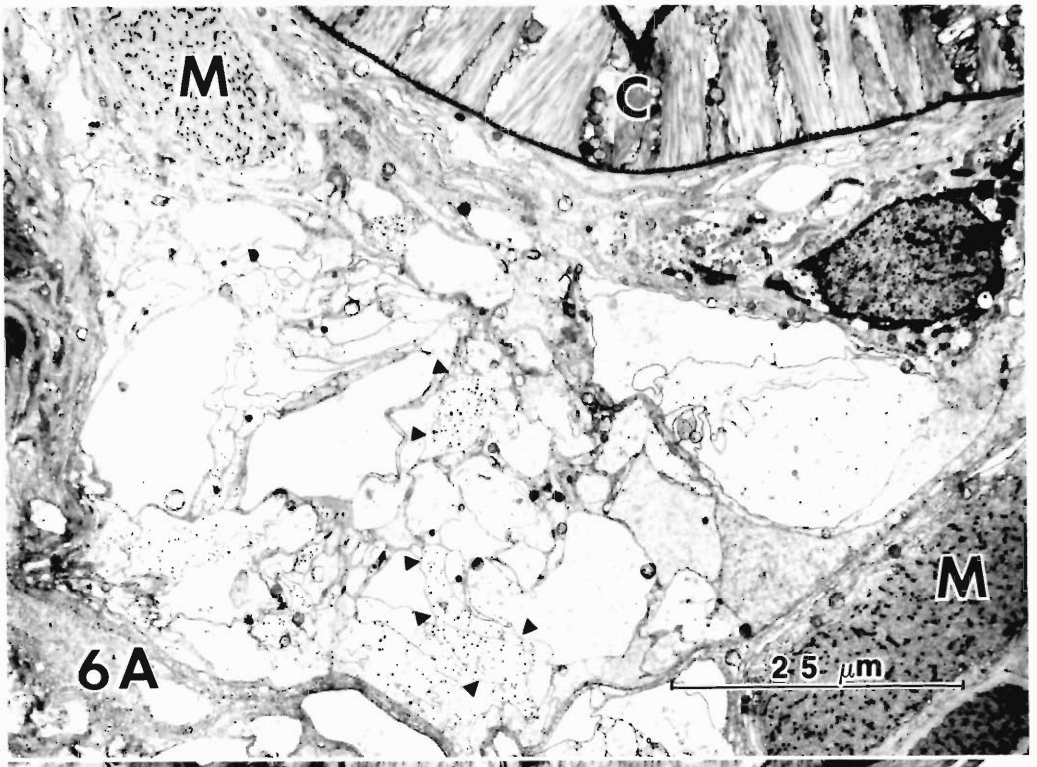
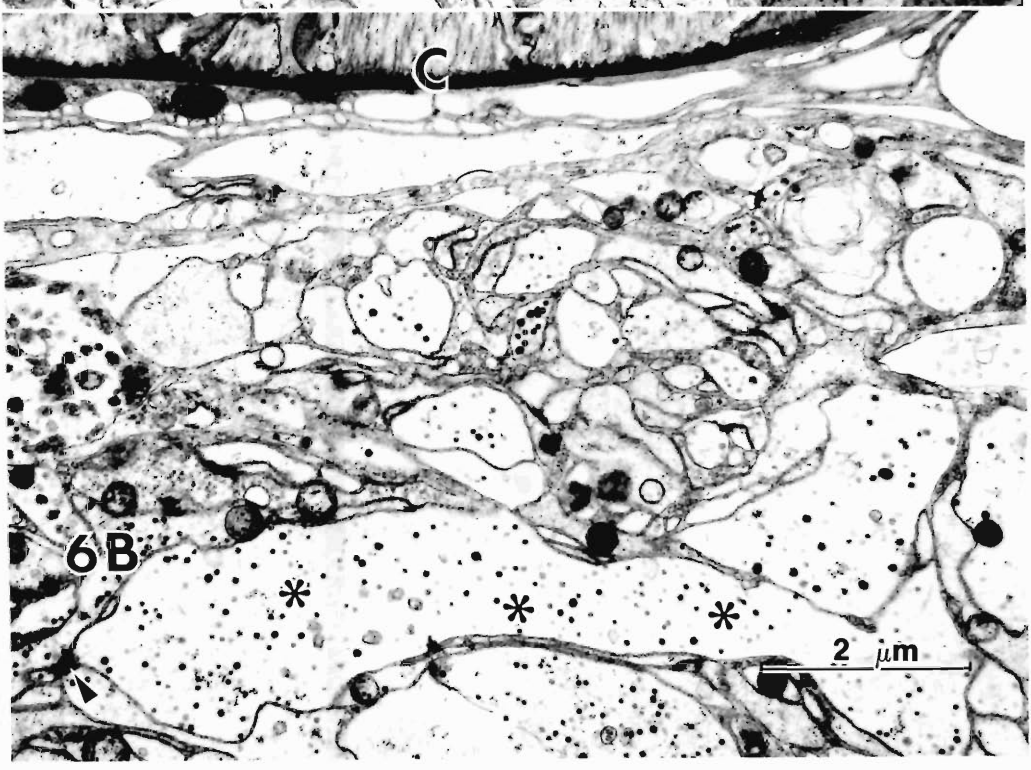
Figure 5A–C. Electron photomicrograph of distal portion of lateral clamp element showing morphology of element, uniciliate receptor, and possible nerve bundle entry into the external basal lamina: 5A, lateral sclerite (LS), uniciliated receptor (arrowheads), and host tissue (HT); 5B, uniciliated receptor showing receptor bulb containing cilium and basal body (*), cross sections of dense apical rings (arrowheads), microtubules (arrow), basal lamina (BL), epidermis (EP), and large epidermal vesicles (black arrowheads); and 5C, a possible site of nerve bundle entry into the clamp intramusculature. Note the elongated nerve bundle ensheathed by basal lamina extending into clamp musculature.





hibit these fluorescent structures, were capable of mating. They (1975) suggested that these fluorescent structures were mechanosensory but, based on the absence of fluorescent structures in mutant males, concluded that alternative mechanosensory elements may be present. Dopaminergic processes associated with spicule innervation in *Nematospiroides dubius* and *Panagrellus redivivus* have been reported (Sharpe and Atkinson, 1980); these dopaminergic processes were considered to be mechanosensory. Ultrastructural examination of spicule innervation was studied by neither Sulston et al. (1975) nor Sharpe and Atkinson (1980); however, Lee (1973) examined the tail region of male *Heterakis gallinarum* and *Nippostrongylus brasiliensis* stained for cholinesterase and prepared for electron microscopy. By light microscopy, Lee (1973) reported positive results for cholinesterase activity and reported ultrastructural evidence of cholinergic nerves running through the spicules of both species. Lee (1973) suggested that these nerves may serve a tactile function. Although no mention was made of spicule nerve innervation in the surrounding tissue, it is interesting to note that there appear to be dense-core vesicles associated with the spicule nerve processes (see Lee, 1973). The finding of intramuscular nerves containing small dense/dense-core vesicles within the clamps of *M. sebastis,* the lack of synaptic densities associated with these nerves, and the presence of a uniciliate receptor in this region strongly suggest a neuromodulatory role for muscle activity. Moreover, the presence of lucent vesicles synapsing on clamp myofibrils and the close association between processes containing these vesicles and those that contain dense/dense-core vesicles would further support a speculated neuromodulatory role between neuroactive substances.

Numerous nerve processes containing a mixture of lucent and dense/dense-core vesicles were found in the haptoral region of *M. sebastis*; these lucent vesicles were often found lining the presynaptic cleft. The combination of different vesicle types within the same process is not uncommon (Reger, 1965; Goh and Davey, 1976b; Webb and Davey, 1976; Shaw, 1982). Goh and Davey (1976b) found both small lucent vesicles and dense/dense-core vesicles within the nerve processes of *Phocanema decipiens.* In light of the cholinergic link hypothesis, as proposed by Burn and Rand (1959, 1965) and expanded by Koelle (1971), Goh and Davey (1976b) suggested that although extremely speculative, but nonetheless a possibility, acetylcholine may be initially released to mediate the release of other neurotransmitters within the same process. Whether lucent vesicles mediate other neurotransmitter release in *M. sebastis* or in other helminths remains to be answered.

Shaw (1979) described the general ultrastructure of clamp innervation in *Gastrocotyle trachuri.* He (1979) described a single nerve bundle that bifurcated just prior to entering the clamp at an opening in the clamp internal basal lamina. These bundles further branched and spread throughout the clamp and contained mainly electron-dense, membrane-bound vesicles. In *M. sebastis,* a fluorescent process or nerve bundle was seen bifurcating either just anterior to or on either side of the median clamp sclerite. These nerve bundles projected posteriorly, overlying the interior and exterior clamp element surfaces, and terminated near the skeletal sclerites. Two nerve bundles were found situated on either side of the median clamp sclerite by electron microscopy of the surface of the clamp cavity interior; this finding was not reported by either Shaw (1979) in *Gastrocotyle trachuri* or by Halton and Morris (1969) in *Diclidophora merlangi.* The site of nerve bundle entry through the internal basal lamina was not found nor were the bifurcated nerves branching throughout the clamp as described by Shaw (1979). A possible site of nerve entry into the external basal lamina was seen; thus, it can be speculated that the uniciliated receptor found associated with this nerve bundle could in fact modulate intramuscular clamp activity.

In *M. sebastis,* a large number of nerve processes within the clamp musculature, the nerve bundles in the clamp cavity interior, and the nerve bundles and bulb area of the uniciliated receptor contained small dense/dense-core vesicles, pre-

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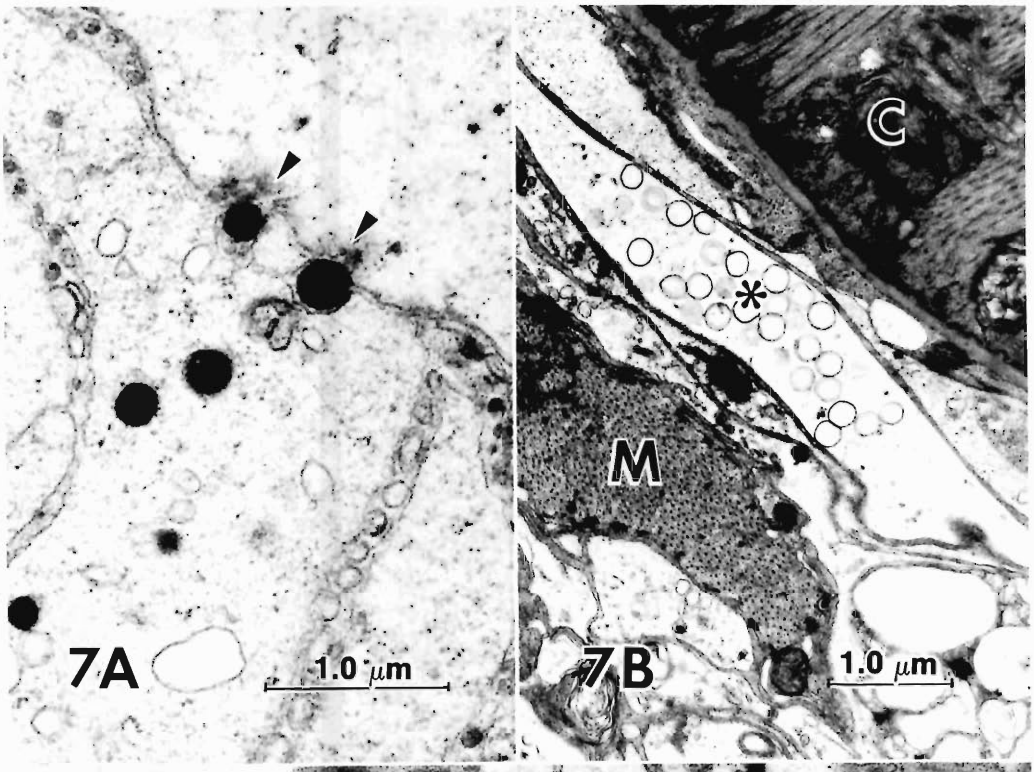
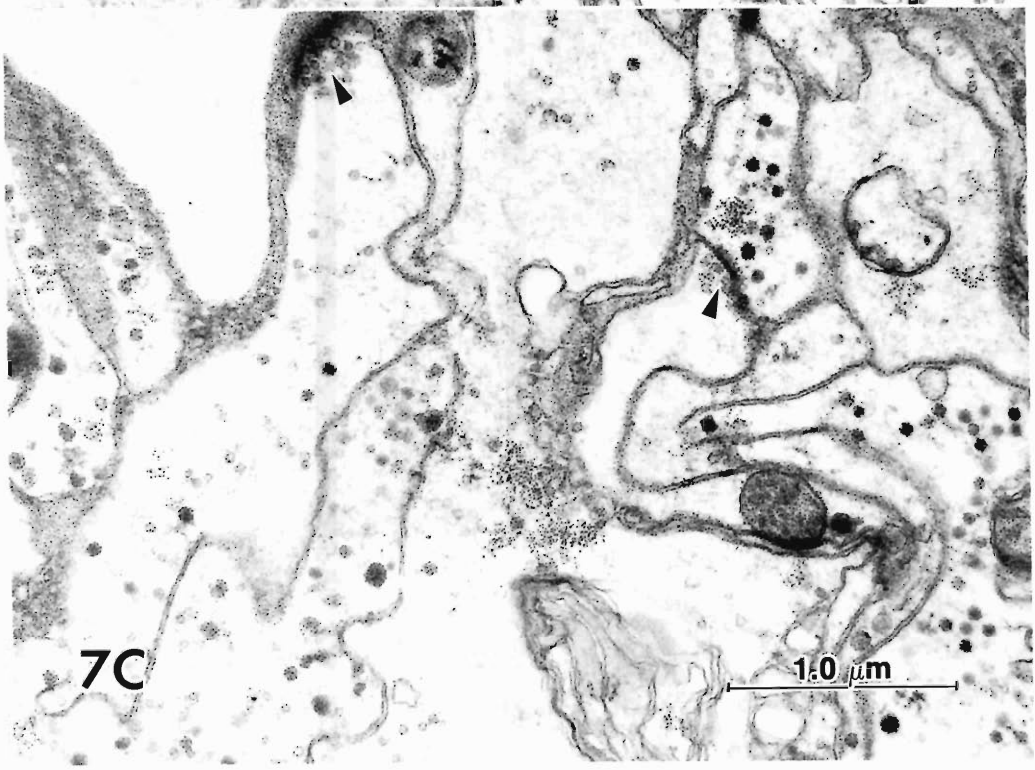
Figure 6A, B. Electron photomicrographs of neuropil directly associated with haptoral clamps: 6A, showing nerve processes, projecting anteriorly, which contain dense vesicles (arrowheads) and 2 muscle fibers (M); and 6B, showing a short, longitudinally sectioned nerve process containing dense, dense-core, and faintly electron-dense vesicles. A synapse is present at one end of the longitudinal process (arrowhead). Other processes contain dense/dense-core and a mixture of electron-dense and lucent vesicles.





sumably aminergic; small lucent vesicles or vesicles with a faint core were also occasionally found. Other processes within the clamp musculature contained lucent vesicles, presumably cholinergic; neuromuscular synapses were common. Halton and Morris (1969) demonstrated cholinesterase activity in the clamp musculature of *Diclidophora merlangi* but did not illustrate ultrastructural evidence of cholinergic nerve processes in the clamps. Shaw (1979) did not find specialized synapses, similar to neuromuscular junctions reported in other helminths (Dixon and Mercer, 1965; Morseth, 1967; Ward et al., 1975, 1986; Ware et al., 1975; Goh and Davey, 1976b), in the intramuscular clamp region of *Gastrocotyle.* In *M. sebastis,* no easily discerned synaptic densities were found associated with intramuscular clamp nerve processes containing electron-dense vesicles; however, nonspecialized synapses or synaptoid release sites, which superficially resemble conventional synapses, have been reported (Myhrberg, 1972; Webb, 1976; Reuter and Lindroos, 1979; Reuter, 1981; Shaw, 1979).

As demonstrated by electron microscopy, the uniciliated receptor located near the skeletal element appeared to be located at the point of fluorescent termination. Neither Halton and Morris (1969) nor Shaw (1979) described the presence of a uniciliated receptor(s) within the interior clamp cavity of *D. merlangi* and *G. trachuri.* However, numerous investigators have reported uniciliate receptors in a variety of helminths (Dixon and Mercer, 1965; Morris and Threadgold, 1967; Morseth, 1967; Halton and Morris, 1969; Lyons, 1969a, 1972, 1973b; Rohde, 1972; Hockley, 1973; Bennett, 1975; Kritsky and Kruidenier, 1976; Edwards et al., 1977; Allison, 1980; Fairweather and Threadgold, 1983; Rohde and Garlick, 1985a, b). The presence of a uniciliated receptor or others does not fully explain the discrepancy between the large number of neuronal processes directly adjacent to the clamps or processes containing small dense/dense-core vesicles within the haptoral neuropil and the total number of fluorescent processes. This difference may be due to the seemingly dubious path these processes take through the haptoral neuropil. In a given plane of section, most nerves could be followed for only short intervals. The presence of a single longitudinal nerve process or bundle, as seen by fluorescence microscopy, and the lack of neurons in the neuropil may indicate the origin of neuronal axons somewhere "outside" or adjacent to the haptoral neuropil.

Bovet (1967) and Knowles (1972), as discussed in Shaw (1979), described a modified "muscle-tendon-fair-lead-hinged-jaw" system in *Diplozoon paradoxum, Axine belones,* and *Microcotyle donavini.* Both investigators concluded that suction pressure within the clamp cavity was produced by the contraction of the clamp wall musculature. Shaw (1979) suggested that clamp myofiber contractions in *Gastrocotyle trachuri* caused suction or cross-bridging between the thick and thin myofilaments causing clamp rigidity. In order to mediate these suggested activities a sensory receptor(s), located near the skeletal sclerites or elsewhere within the clamp cavity, may be necessary to "inform" or mediate the clamp musculature when contact has been made with host tissue. Thus, this proposed function of the uniciliated receptor would support the speculation that other receptors exist within the clamp cavity.

A suggested function or even a term to describe the receptor found in the clamp cavity interior of *M. sebastis* would be tentative. Moreover, numerous speculations regarding the function of uniciliate receptors exist in the literature; however, all speculations are based on anatomic morphology and not anatomic physiology (Lyons, 1969a, 1972; Halton and Lyness, 1971; Webb and Davey, 1975; Edwards et al., 1977; Hess and Guggenheim, 1977; Allison, 1980; Hoole and Mitchell, 1981; Rohde and Garlick, 1985a, b).

Although the vesicle morphology in a variety of helminths has been described by numerous investigators, 3 main types exist: (1) small dense-core vesicles, presumed to be aminergic; (2) small, regularly shaped lucent vesicles, presumed to be cholinergic; (3) large dense vesicles, presumed to be peptidergic. In the present study, nerve processes in the opisthaptoral neuropil exhibited

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Figure 7A–C. Electron photomicrographs of 3 vesicle types in processes in haptoral neuropil: 7A, showing large, possibly peptidergic, dense vesicles in the process of exocytosis (arrowheads); 7B, showing large lucent vesicles of unknown function adjacent to clamp internal basal lamina; and 7C, showing numerous processes containing a mixture of dense/dense-core and lucent vesicles. Lucent vesicles are present aggregated at sites of synaptic release (arrowheads).

these 3 vesicle types. Furthermore, vesicles exhibiting varying degrees of opacity and density were observed within the same process. Based on the type(s) of fixative(s) used to preserve catecholaminergic vesicle morphology, the content and/or number of vesicles present will fluctuate (Livett, 1973). It was found in the present study that the introduction of tannic acid or thiocarbohydrazide during specimen processing improved general ultrastructural morphology and contrast; morphology and contrast of dense/dense-core vesicles also improved.

Understanding the role of the nerve processes in *M. sebastis* is complicated by the presence of nerve glomeruli and processes exhibiting a great degree of diverse vesicle morphology, e.g., density and opacity. The occurrences of large lucent and dense/dense-core vesicles were occasionally seen in the neuropil. The presence of large dense vesicular bodies in the epidermis and large lucent vesicles in the opisthaptoral neuropil represent vesicles of unknown function. In such instances, pharmacologic depletion studies, loading studies, biochemical and/or radioenzymatic studies, catabolic inhibition, and immunocytochemical labeling studies could aid in further identifying vesicle contents and may advance our understanding of the function(s) and role(s) of neuroactive substances associated with sensory receptors, sensory papillae, spicules, clamps, and hooks in other helminth species.

Based on the fluorescence evidence presented in this study, a neurosynaptic function associated with catecholamines could be assumed. The fluorescent processes and varicosities associated with the opisthaptoral clamps of *M. sebastis,* as well as the presence of processes containing small dense/dense-core vesicles, representing sites of potential synaptic release, would support this assumption. However, the scarcity of well-defined pre- or postsynaptic densities associated with processes containing dense/dense-core vesicles may indicate the contrary. Catecholamines may function as sensory transmitters as well as neuromodulators in conjunction with other vesicle types in the opisthaptor of *M. sebastis.* Continued studies are necessary to ascertain the role of catecholamines and other neuroactive substances in *M. sebastis.*

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Monthly Length Class Frequencies of *Microcotyle spinicirrus* (Monogenea: Microcotylidae) from the Freshwater Drum, *Aplodinotus grunniens,* in West Virginia

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ABSTRACT: The gills of 173 freshwater drum, *Aplodinotus grunniens* Rafinesque, taken from 2 locations on the Kanawha River, West Virginia, were examined for *Microcotyle spinicirrus* MacCallum. A prevalence of 81.0% was recorded for hosts at Winfield, and 75.4% at Marmet. Intensity of infection, expressed as mean number of *M. spinicirrus* individuals per infected host, for drum at these 2 locations was 8.5 and 8.7, respectively. Larger hosts carried significantly more *M. spinicirrus* individuals than smaller ones. Large (>5.0 mm) *M. spinicirrus* individuals dominated the May sample, whereas smaller length classes of this monogenean were dominant from June through October of 1986.

KEY WORDS: *Microcotyle spinicirrus,* population dynamics of monogeneans, *Aplodinotus grunniens,* gill parasites.

Microcotyle spinicirrus MacCallum has been reported on freshwater drum from New York (MacCallum, 1918), Mississippi (Simer, 1929), Lake Erie (Bangham and Hunter, 1936), Tennessee (Bangham and Venard, 1942), and Iowa (Linton, 1940; Remley, 1942). This paper constitutes the first report of this monogenean species from West Virginia.

The goal of this study was to determine relative monthly abundance of different *M. spinicirrus* length classes present from May through October. Information on prevalence rates, intensity of infection, and the relationship between numbers of *M. spinicirrus* individuals to host length was also sought.

Materials and Methods

From May through October 1986 the West Virginia Department of Natural Resources conducted a creel survey (fish census) at 2 sites on the Kanawha River—Winfield Locks and Dam (KRW) at mile point 31.1, and Marmet Locks and Dam (KRM) at mile point 67.7. Public fishing access areas have been constructed on the downstream side of each dam by the American Electric Power Co. as a public service.

With the exception of the October sample at KRW, live drum, *Aplodinotus grunniens* Rafinesque, were obtained from anglers fishing from the access areas, then placed on ice. Each fish was measured for total length within 2 hr of capture. After measuring, all gill material was removed from each fish at the field site and placed in separate, appropriately labeled jars (i.e., 1 set of gills per jar) with a solution of 1 part 10% buffered formalin acetate and 1 part distilled water. This relatively simple field procedure resulted in minimal distortion to monogenean specimens (Fig. 4). Drum representing the KRW October collection were obtained from a rotenone application to the small lock chamber. In all other respects, KRW October fish and gill material were handled and examined as previously described. Some worms were recovered from the bottom of collection jars, but most had to be removed from the gill filaments with jewelers forceps. Recovered specimens were stored in 10% buffered formalin acetate. All worms were counted and measured while in formalin under a stereomicroscope equipped with a calibrated ocular micrometer. After measuring, *M. spinicirrus* specimens were assigned to 1 of 4 length classes: ≦1.0 mm (I); >1.0–3.0 mm (II); >3.0–5.0 mm (III); or >5.0 mm (IV) (Fig. 4). Admittedly, length classes were selected as much for convenience as for any particular standard procedure although the smallest class corresponds to the "first attachment form" described by Remley (1942, Figs. 11, 13, p. 148), whereas Class II was roughly equivalent to his "second and third attachment forms."

Statistical analyses follow the procedures outlined by Clayton (1984). Correlation between variables was determined by the Pearson product-moment correlation. Critical values for r, at $P < 0.05$ for a 1-tailed test, were extrapolated from Clayton's Table C (1984, p. 355).

Results

A total of 173 freshwater drum—116 and 57 from Winfield and Marmet, respectively—were captured from the Kanawha River from May through October 1986. Prevalence was 81.0% (94 of 116) at KRW, and 75.4% (43 of 57) at KRM (Fig. 1A, B). Overall intensity of infection, expressed as mean number of *M. spinicirrus* individuals per infected host, was nearly the same at both river locations (8.52 at KRW; 8.74 at KRM). Variation from both combined means was considerable (Fig. 1A, B), but no particular month contributed unduly to this overall variation (i.e., wide variation from mean intensity was a consistent monthly phenomenon). Larger drum at both study sites carried significantly more

M. spinicirrus individuals than their smaller counterparts (Fig. 2A, B).

Large (Class IV) *M. spinicirrus* individuals were dominant in May, but made up a relatively small percentage of the population for the remainder of the study period (Fig. 3A, B). Length Class II clearly dominated the June sample but shared dominance with Class III individuals for the remainder of the study period.

Discussion

There are few detailed studies with which to compare present findings of *Microcotyle spinicirrus* infections on *Aplodinotus grunniens*. MacCallum's original description of this monogenean in 1918 was based upon specimens from a single "large" heavily infected drum from the New York Aquarium and not from material collected as part of a comprehensive survey. Simer (1929) noted briefly that *M. spinicurrus* was recovered from over half of 41 drum examined, but he did not include intensity data. Bangham and Hunter (1936) reported that in a sample of 45 drum taken from Lake Erie, ". . . five were found carrying a total of 9 trematodes of the genus *Microcotyle* . . . ." These authors described a new species, *M. eriensis,* as well as provided a redescription of *M. spinicirrus*. Unfortunately, there was no indication of the number of individuals belonging to each species, or which of the 5 infected fish harbored them. Still, it is a minor point because even the combined prevalence (5 of 45) and mean intensity (1.8) could be considered low. Linton (1940) examined a small number of *M. spinicirrus* specimens sent to him by Dr. Coker from Iowa but included no information pertaining to prevalence or intensity of infection. Bangham and Venard (1942) recovered 4–26 *M. spinicirrus* individuals from 4 (of 13 examined) drum, whereas Remley (1942) noted only that several hundred *M. spinicirrus* individuals were recovered from approximately 130 drum.

French investigators have provided prevalence and intensity data on marine species of *Microcotyle* (Euzet and Marc, 1963; Noisy and Maillard, 1980; Oliver, 1984). Although extrapolating their data to the present study may be questionable because of host and habitat differences, some generalizations seem appropriate. Oliver (1984) found that prevalence rates of *M. chrysophrii* on *Sparus aurata* in several coastal ponds were highly variable (1.3–62.0%) depend-

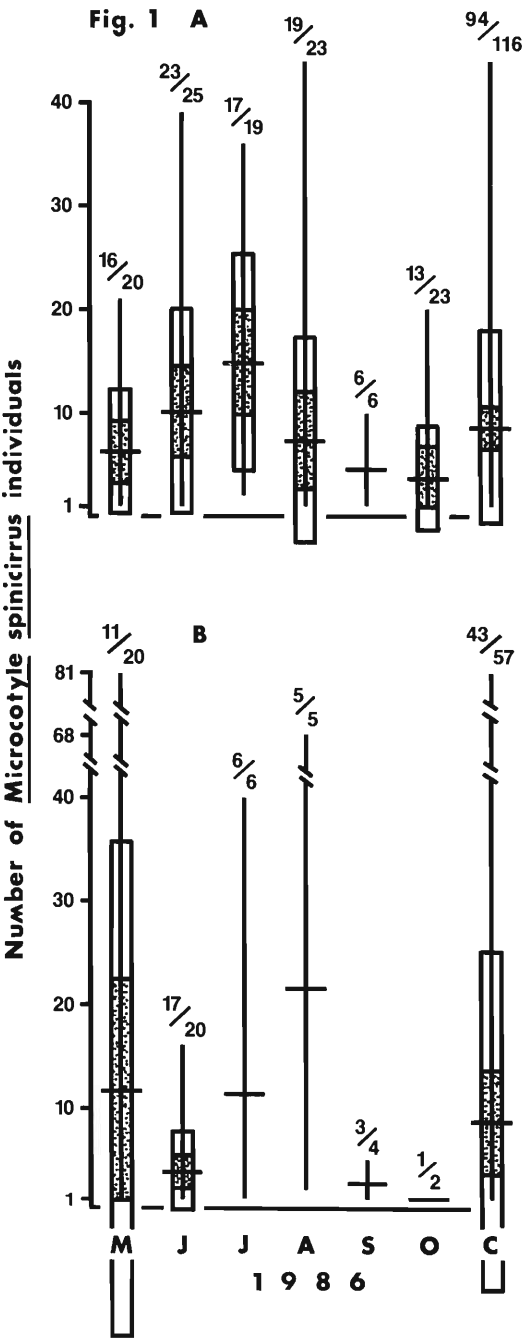
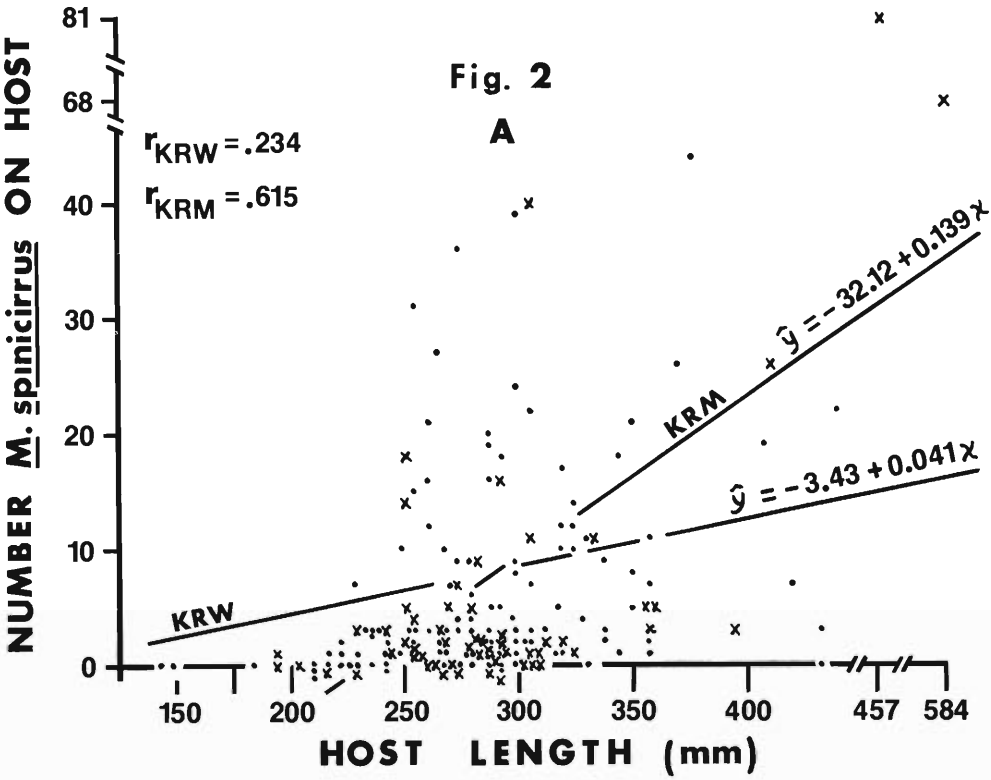
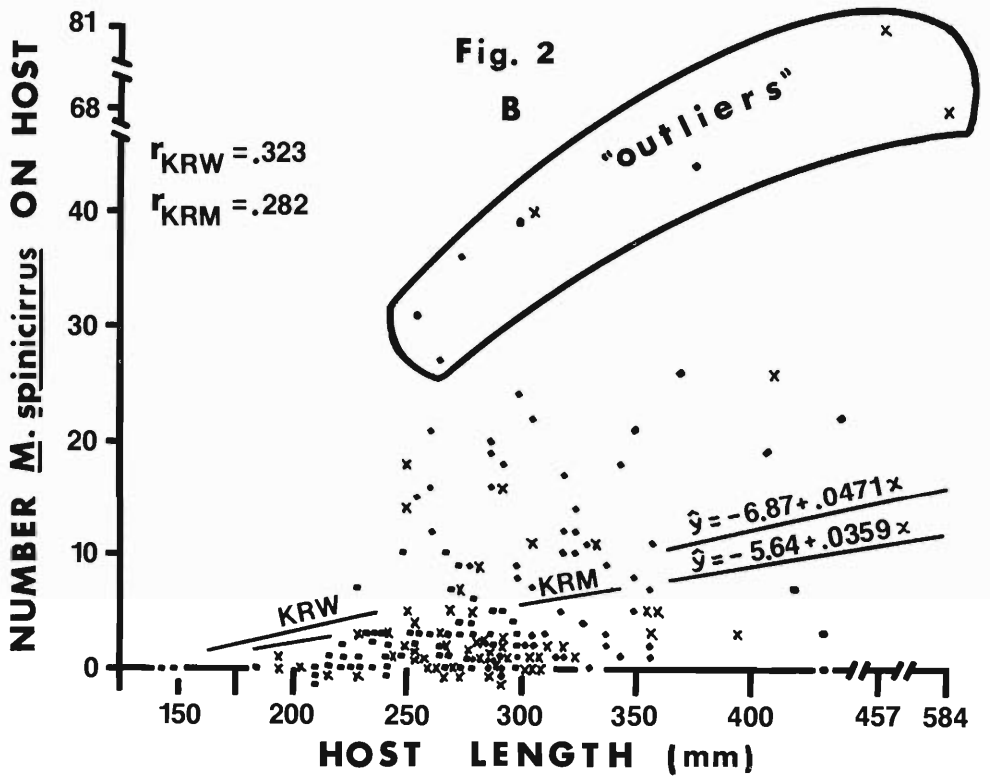


Figure 1. Intensity of *M. spinicirrus* infections in freshwater drum from the Winfield Dam (A), and Marmet Dam (B), Kanawha River, West Virginia. Horizontal lines = means; vertical lines = ranges. Vertical open bars = standard deviations; vertical stippled bars = 2 standard errors from the mean. C = combined data. Prevalence shown as fraction above range line with denominator representing host sample size, and numerator the number of infected hosts in that sample.





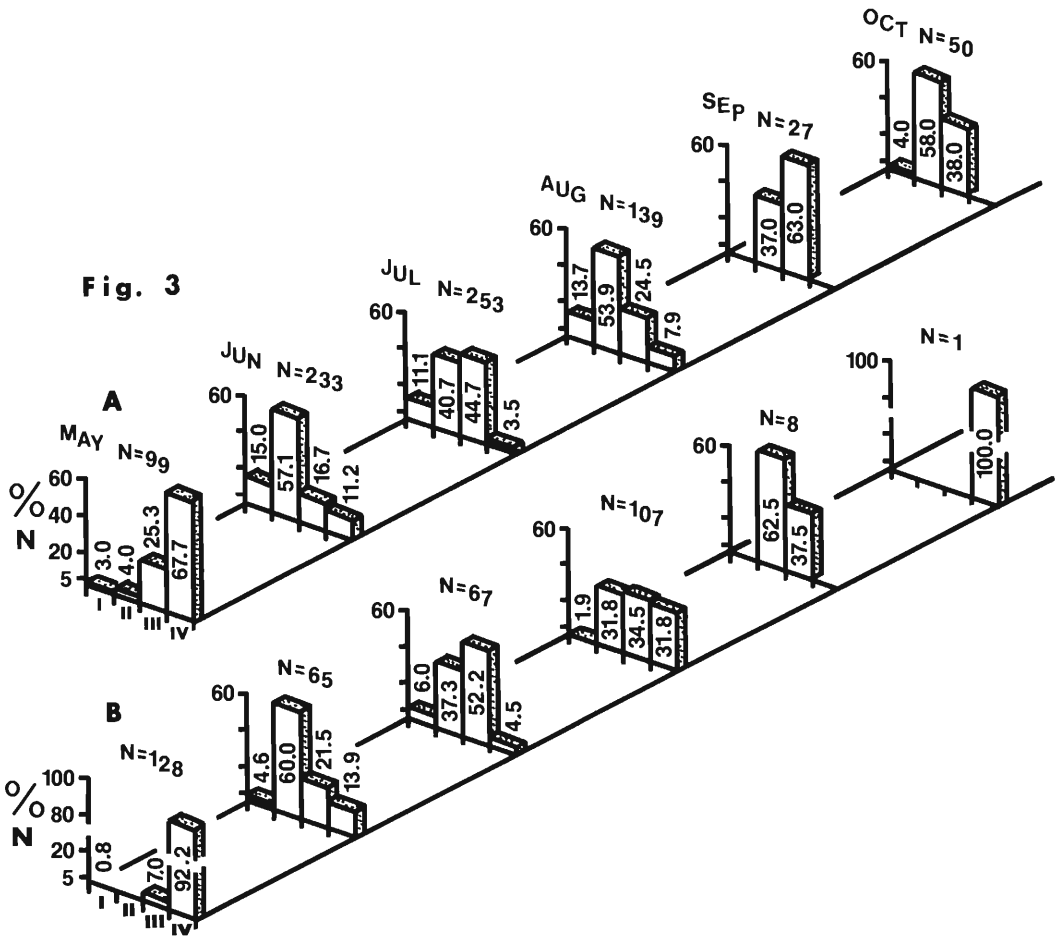


Figure 3. Percent frequency distributions of body lengths (length classes I–IV) of *M. spinicirrus* individuals from freshwater drum at Winfield Dam (A) and Marmet Dam (B) on the Kanawha River, West Virginia by month of collection. *N* = number of *M. spinicirrus* individuals recovered. See Figure 1 for monthly host sample sizes and prevalence of infection. *Microcotyle spinicirrus* length classes: I, 1.0 mm or less; II, >1.0–3.0 mm; III, >3.0–5.0 mm; and IV, >5.0 mm.

ing upon geographical location and year of sampling. Intensity of infections similarly were varied with means generally lower than recorded for the present study. Even though the present study was confined to the same year there was little difference in prevalence and intensity for drum sampled from the 2 Kanawha River collection sites separated by a distance of nearly 37 mi. A somewhat higher prevalence of 85.1% (103 of 121) and mean intensity (10.9) was reported by Noisy and Maillard (1980) for the same microcotylid/host association studied by Oliver, indicating that the infection dynamics of marine microcotylids can be comparable to freshwater forms.

References to correlations between number of

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Figure 2A, B. Scatter diagrams depicting number of *M. spinicirrus* individuals (y-axis) plotted against length of host (x-axis). Closed circles and x's represent drum taken from Winfield (KRW) and Marmet (KRM) dams, Kanawha River, West Virginia, respectively. Each point, whether closed circle or x, represents a single host. All months combined. A. Calculated *r*-values of 0.234 (KRW) and 0.615 (KRM) are significant because critical values for Pearson's *r* at $P < 0.05$ for a 1-tailed test are ≅0.170 with 92 df (KRW) and ≅0.254 with 41 df (KRM). B. With "outliers" excluded from calculations *r*-values were 0.323 (KRW) and 0.282 (KRM). Again, both calculated *r*-values are significant because critical values for Pearson's *r* at $P < 0.05$ for a 1-tailed test are ≅0.176 with 87 df (KRW) and ≅0.261 with 38 df (KRM).

Figure 4. ***Microcotyle spinicirrus*** **individuals, stained with Meyer's acid carmine, representing the four length classes presented in this study. Vertical line to the right of each specimen = 0.5 mm. A voucher specimen for each length class has been deposited under USNM Helm. Coll. No. 79939.**

microcotylids present and host size do appear in the literature. For example, Remley (1942) found that none of the 25 yearling drum in his sample were infected. He added that older fish had more parasites with 1 large drum (25 pounds) harboring, ". . . approximately 125 specimens in all stages of development." Similarly, Euzet and Marc (1963) found that smaller hosts (*Labrus bergylta*) (<12 cm) were not infected, whereas larger hosts (≧12 cm) carried a mean of 9.0 *M. donavini* individuals. Such positive correlations are not universal, because Noisy and Maillard (1980) found mean intensity levels of 12.4 and 6.4 for smaller (<25 cm) and larger (25–47 cm) hosts, respectively.

In the present study the number of *M. spinicirrus* individuals was positively correlated with host length (Fig. 2A, B). Both *r*-values in Figure 2A were significantly different from 0. This is so because calculated *r*-values of 0.234 (KRW) and 0.615 (KRM) exceeded critical *r*-values (at $P < 0.05$ for a 1-tailed test) of ≅0.170 with 92 df (KRW) and ≅0.254 with 41 df (KRM). It was obvious, however, that a few points deviated greatly from their respective means and likely served as sources for distortions of *r*-values and regression lines. So coefficients of correlation and regression lines were recalculated after excluding the "outliers" (Fig. 2B). The resulting *r*-values, compared to their respective critical values with appropriate degrees of freedom at $P < 0.05$, were still significantly different from 0 (Fig. 2B), strengthening the contention that number of *M. spinicirrus* individuals was indeed positively correlated with increasing host length.

Length classes of *M. spinicirrus* have been described from a developmental standpoint (Remley, 1942), but not from the ecological perspective of following cohorts through a single growing season. For the purpose of this discussion, May through October is being defined as the growing season for *M. spinicirrus,* because interviews with frequent anglers at both collection sites confirmed that drum are rarely, if ever, caught in the Kanawha River from November through April. Apparently drum migrate up the Kanawha from the larger Ohio River during the spring (Dr. Don Tarter, pers. comm.). Large Class IV (Plate I) *M. spinicirrus* individuals clearly dominated the May population (Fig. 3A, B), an indication that attainment of this size occurred during the winter or early spring, while their hosts were still in the Ohio or en route up the Kanawha. That this size class does not achieve dominance in

subsequent months (discounting October at KRM where $N = 1$) is evidence that only 1 generation of this gill parasite occurs per year on drum in the Kanawha River. Progeny of these large individuals show up in June and July when the *M. spinicirrus* population is dominated by Classes II and III, respectively (Figs. 3A, B, 4). It is difficult to speculate why the pattern of dominance alternates for classes II and III from August through October, but a small sample number of hosts may be a factor. Presumably, Class I never achieves dominance because the individuals simply "grow through" it so quickly.

In May, when large *M. spinicirrus* individuals dominated the monogenean population, they were well dispersed throughout the host sample population—15 of the 20 hosts carried Class IV individuals at KRW, and 6 of 11 infected hosts were similarly parasitized by these large individuals at KRM. Conversely, in August at KRM, when Class IV individuals made up a noticeable proportion (31.8%) of the total *M. spinicirrus* population, only 1 large host (584 mm) carried all the Class IV worms. The same was true for the KRW collection in August, where only 2 of 19 infected drum carried all Class IV individuals. Although it is quite speculative, one cannot ignore the possibility that a relatively few large drum serve as reservoirs of infection throughout the year. Remley (1942) may have observed this same phenomenon with his 1 large drum carrying many *M. spinicirrus* individuals, "... in all stages of development." Perhaps this opens new avenues of research on population dynamics—of any monogenean species, not just microcotylids—to be addressed by other investigators.

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Acanthocephala from Lake Fishes in Wisconsin: On the Ecology of *Leptorhynchoides thecatus* (Rhadinorhynchidae)

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ABSTRACT: *Leptorhynchoides thecatus* was recovered from the ceca and/or other intestinal sites of *Micropterus salmoides* and 11 other fish species from 2 SE Wisconsin lakes. Infections were moderate in *M. salmoides* and gravid worms were recovered throughout the year with maximum recruitment in the autumn and maturity and reproduction in spring and summer. Males and larger fishes were more frequently and heavily infected than females and smaller ones. Larval *L. thecatus* utilized 5 fish species as transport hosts.

KEY WORDS: Acanthocephala, *Leptorhynchoides thecatus*, ecology, Wisconsin lakes, seasonality, distribution, carrying capacity, transport hosts.

Fishes were collected from 2 southeastern Wisconsin lakes, Silver Lake (Kenosha County) and Tichigan Lake (Racine County). The 188-ha Silver Lake has a maximum depth of 13.4 m. It is a eutrophic landlocked lake of glacial origin lying within the lateral moraine of the Lake Michigan lobe of the Wisconsin glacier. A small outlet historically permitted the discharge of overflow waters (until dammed in 1932) into the Fox River, a tributary of the Illinois River (Mississippi River drainage system). The size of the more eutrophic Tichigan Lake, originally a natural lake on the same Fox River, increased to 458 ha as a result of an impoundment of the Fox River in 1830; it has a maximum depth of 19.2 m. A canal draining the western swamp of Tichigan Lake was also surveyed. Systematic collections yielding *Leptorhynchoides thecatus* (Linton, 1891) Kostylev, 1924, were made during the autumn (Au; late October, November), spring (Sp; April), and summer (Su; June, July, early August). Mean parasites per host (relative density) is the number of worms collected per number of fishes examined, unless otherwise stated. Worms are classified as either sexually mature adults or juveniles with incompletely developed reproductive system (Table 1).

Lake distribution

A total of 257 adult *L. thecatus* infected 12 species of fish in 6 families from both lakes. Fishes from the landlocked Silver Lake were markedly more frequently (9%) and heavily (0.29) infected than those from the river-connected Tichigan Lake (4%, 0.10) (Table 1). This difference was similar to that noted in various species of *Neoechinorhynchus* (see Amin, 1986) but not in *Pomphorhynchus bulbocolli* Linkins *in* Van Cleave, 1919 (see Amin, 1987). Factors other than the composition of the invertebrate fauna in both lakes are probably involved, because amphipods (*Gammarus* spp. and *Hyalella azteca*) serve as intermediate hosts for both *L. thecatus* and *P. bulbocolli*.

Host and site distribution

Leptorhynchoides thecatus has been reported in the intestine of many species of fish (see Hoffman, 1967) with particular preference to the pyloric ceca of centrarchids (DeGiusti, 1949; Venard and Warfel, 1953; Uznanski and Nickol, 1982). Worms were also reasonably well represented in the ceca of centrarchids other than *Micropterus salmoides* (Table 1). Uznanski and Nickol (1982) demonstrated the loss of all intestinal worms in *Lepomis cyanellus* infections older than 1 wk. The situation in *M. salmoides* appears to vary from that in *L. cyanellus* because many of the intestinal worms there were sexually mature (Table 2) clearly representing infections older than 1 wk. DeGiusti (1949) has shown that at least 4 wk are necessary for the maturation of *L. thecatus* in *Ambloplites rupestris*.

Carrying capacity

The levels of *L. thecatus* infections are usually low. A carrying capacity of 10–15 worms demonstrated for *L. cyanellus* was apparently determined by the amount of cecal space available, thus causing the loss of excess intestinal worms—a density-dependent factor (Uznanski and Nickol, 1982). This limitation appears to vary by fish species. Although the levels of infection in this study were generally low, up to 29 *L. thecatus*

Table 1. A comparison of *Leptorhynchoides thecatus* infections in fishes from Silver and Tichigan lakes, 1976–1978.

Fish species	Silver Lake					Tichigan Lake*				
			No. worms					No. worms		
	No. exam.	No. (%) infected, season	Adults	Juve-niles	Mean (max.)	No. exam.	No. (%) infected, season	Adults	Juve-niles	Mean (max.)
Amiidae										
Amia calva	18	2 (11) Sp, Su	1	1	0.11 (1)	23	1 (4) Su	1	0	0.04 (1)
Catostomidae										
Erimyzon sucetta	116	1 (1) Sp	1	0	0.01 (1)	0	0	0	0	–
Centrarchidae										
Ambloplites rupestris	25	3 (12) Sp, Su	1	3	0.16 (2)	2	0	0	0	–
Lepomis gibbosus	16	0	0	0	–	60	2 (3) Au, Su	3	0	0.05 (2)
Lepomis macrochirus	301	3 (1) Au, Sp	3	2	0.02 (2)	212	0	0	0	–
Micropterus dolomieui	4	2 (50) Sp, Su	0	3	0.75 (2)	18	4 (22) Sp	5	0	0.28 (2)
Micropterus salmoides	72	38 (53) Au, Sp, Su	138	26	2.28 (29)	44	17 (39) Au, Sp, Su	50	0	1.14 (10)
Poxomis nigromaculatus	47	4 (9) Sp	4	1	0.11 (2)	162	0	0	0	–
Esocidae										
Esox lucius	20	2 (10) Sp	0	3	0.15 (2)	22	0	0	0	–
Ictaluridae										
Ictaluris natalis	4	1 (25) Sp	0	1	0.25 (1)	8	1 (12) Sp	1	0	0.12 (1)
Ictaluris nebulosus	3	0	0	0	–	10	1 (10) Au	1	0	0.10 (1)
Percidae										
Stizostedion vitreum	54	5 (9) Au, Sp	0	8	0.15 (4)	52	0	0	0	–
Total	680	61 (9) Au, Sp, Su	148	48	0.29 (29)	613	26 (4)	61	0	0.10 (10)

* In Tichigan Lake canal, 1 of 37 *A. calva* was infected with 1 adult worm in the summer, 1 of 14 *L. gibbosus* with 1 juvenile in the spring, and 2 of 121 *L. macrochirus* with 2 larvae (cystacanths in intestinal mesenteries) in May and June. Mesenteric cystacanths were also found in 2 *M. salmoides* (summer) (Tichigan Lake proper), and in 5 *A. rupestris* (autumn), 1 *L. gibbosus* (spring), 2 *M. dolomieui* (spring), and 4 *M. salmoides* (autumn, spring, summer) (Silver Lake).

Table 2. Seasonal and intestinal distribution of *Leptorhynchoides thecatus* in *Micropterus salmoides* from Silver and Tichigan lakes, 1976–1978.

Lake	Season	No. fish infected/ examined (%)	Worms collected					No. (%) in intestinal regions					
			N (mean)	Males	Females	% juveniles	% females with eggs	Ceca	Stomach	Small intestine*		Large intestine†	
									A	B_1	B_2	C_1	C_2
Silver Lake	Autumn	2/6 (33)	2 (0.33)	1	1	100	0	—	1 (50)	—	—	—	1 (50)
	Spring	18/28 (64)	88 (2.31)	32	56	26	68	42 (48)	4 (4)	35 (40)	6 (7)	1 (1)	—
	Summer	18/38 (47)	74 (2.64)	29	45	1	60	44 (59)	12 (16)	6 (8)	8 (11)	2 (3)	2 (3)
Tichigan Lake	Autumn	7/23 (30)	29 (1.26)	11	18	0	61	10 (34)	5 (17)	8 (28)	5 (17)	—	1 (3)
	Spring	2/2 (100)	2 (1.00)	1	1	0	100	1 (50)	—	1 (50)	—	—	—
	Summer	8/19 (42)	19 (1.00)	8	11	0	64	9 (47)	4 (21)	4 (21)	2 (11)	—	—

* B_1, B_2 = first and second limbs of small intestine.
† C_1 = first limb of large intestine. C_2 = second limb of large intestine leading to vent.

were recovered from 1 *M. salmoides* in Silver Lake (Table 1). In other studies *M. salmoides* also appears to be the more common host among the centrarchids but its levels of infection with *L. thecatus* are usually low. For example, Samuel et al. (1976) reported up to 5 *L. thecatus* per infected bass in Nebraska. The above understandings regarding host and site preferences and levels of infections were challenged by the finding that the intestine of 93% of 176 slimy sculpins, *Cottus cognatus,* from eastern Lake Michigan was infected with as many as 132 *L. thecatus* (none in the ceca); the mean was 17 and 10 in male and female fishes (Heufelder and Schneeberger, 1980), respectively.

Transport hosts

The recovery of larval *L. thecatus* from the body cavity of *A. rupestris, Lepomis gibbosus, Lepomis macrochirus, Micropterus dolomieui,* and *M. salmoides* (footnote, Table 1) and of many other fish species by various authors (see Hoffman, 1967) demonstrates the existence of an alternate pathway for the completion of the *L. thecatus* life cycle utilizing fish as transport hosts. DeGiusti (1949) related the mesenteric sites to the inability of incompletely developed *L. thecatus* larvae (in *H. azteca*) to survive in the intestine of *A. rupestris.* Uznanski and Nickol (1982), however, attributed the same phenomenon in *L. cyanellus* to behavioral differences.

Seasonality

Only data from *M. salmoides* were adequate to use. Egg presence, worm recruitment, and maturation appear to occur throughout the year in both lakes with maximum recruitment in the autumn and maturity and reproduction in spring and summer (Table 2). These findings agree with those reported by Pearse (1924) in *Perca flavescens* and by DeGiusti (1949) in *A. rupestris.* The frequency of infection in the pyloric ceca was highest in the oldest (summer) infections in both lakes (Table 2). This agrees with Uznanski and Nickol's (1982) observations except for the persistence of additional worms in other intestinal locations.

Host size and sex

Data from *M. salmoides* in both lakes were considered (Table 3). Larger fish were more heavily and frequently infected than smaller fish (except for a relatively lower mean in the 40–50-

Table 3. The relationship between the size and sex of *Micropterus salmoides* examined from Silver and Tichigan lakes and their infection with *Leptorhynchoides thecatus*.

Fish size class (total length, cm)	No. examined	Percent infected	Mean/ infected fish	Maximum/ fish
11–19	19	16	1.33	2
20–29	44	52	3.13	7
30–39	38	53	5.70	29
40–50	15	53	3.12	10
Males	46	57	4.58	29
Females	70	40	3.28	16

cm-long bass). Males also had higher levels of infection than females (Table 3). The last observation was unrelated to size, because the average length of male bass examined from Silver and Tichigan lakes was smaller (29.5 and 24.3 cm) than that of females (33.1 and 28.0). In *Perca flavescens* from Ontario, the frequency and density of *L. thecatus* infections were higher in larger fish and in females (Cannon, 1973). Cannon (1973) related this pattern to dietary habits associated with size, i.e., larger fish ate more infected invertebrate hosts and female perch were larger than males. My results from *M. salmoides* (Table 3) totally agree with those reported from *C. cognatus* by Heufelder and Schneeberger (1980) who related the lighter infection in the mostly gravid female sculpins to flattening of their intestinal lumen as a result of pressure from their overly developed ovaries.

Deposited specimens

USNM Helm. Coll. Nos. 79823 and 79824 and HWML (University of Nebraska State Museum, H. W. Manter Laboratory) Coll. Nos. 20790 and 20791 from Silver and Tichigan lakes.

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Eimeria lineri sp. n. (Apicomplexa: Eimeriidae) from the Mediterranean Gecko, *Hemidactylus turcicus* (Sauria: Gekkonidae), in Louisiana and Texas

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ABSTRACT: *Eimeria lineri* sp. n. (Apicomplexa: Eimeriidae) is described from the feces of 24 of 47 (51.1%) Mediterranean geckos, *Hemidactylus turcicus turcicus* (Linnaeus, 1758), in Houston, Texas, and Houma, Louisiana, U.S.A. Oocysts of this new eimerian are ellipsoid, 24.8 × 19.5 (21.6–28.0 × 18.4–21.6) μm, with a smooth bilayered wall, ca. 1.5 μm thick; shape index 1.3 (1.1–1.5) μm. One (rarely 2 or 3) polar granule(s) is (are) usually present, but a micropyle and oocyst residuum are absent. Sporocysts are ellipsoid, 9.0 × 7.8 (8.2–9.6 × 7.2–8.8) μm, with a smooth thin wall and faint sutures; shape index 1.2 (1.1–1.3) μm. Stieda and substieda bodies absent; sporocyst residuum present, 4.1 × 4.4 (2.5–5.6 × 3.6–5.6) μm, composed of numerous granules in a spherical or ellipsoid mass. Sporozoites vermiform, 11.6 × 2.4 (8.8–13.6 × 2.0–2.8) μm, and arranged head-to-tail within the sporocyst. Each sporozoite contains a single spherical anterior refractile body, a central nucleus, and a spherical or ovoid posterior refractile body. In addition to the new species, 8 (17.0%) of the geckos harbored *E. turcicus* Upton, McAllister, and Freed, 1988. Observations on prevalence and comparisons with similar species are included.

KEY WORDS: *Eimeria lineri* sp. n., *Eimeria turcicus,* coccidia, Gekkonidae, Mediterranean gecko, *Hemidactylus turcicus turcicus,* Texas, Louisiana, prevalence, Apicomplexa, Eimeriidae.

In the latest reviews of the coccidian parasites of lizards of the family Gekkonidae, Matuschka and Bannert (1986a, b) listed a total of 15 named and 4 unnamed species of *Eimeria* and 6 named and 2 unnamed species of *Isospora* from 11 and 5 taxa of geckos, respectively. The vast majority of these hosts, and likewise their coccidia, occur in the Old World. Recently, Upton and Barnard (1987) and Upton et al. (1988) added 3 additional species of *Eimeria* to the list, bringing the total number of named eimerians to 18.

Upton et al. (1988) examined 38 Mediterranean geckos, *Hemidactylus turcicus turcicus* from the Houston Zoological Gardens in Houston, Harris County, Texas. These geckos disperse freely and are not part of the captive fauna housed at the Zoo. Mediterranean geckos range naturally over portions of the Old World and, nearly a century ago, were introduced into the New World inadvertently by way of port cities (McCoy, 1970). Today, *H. turcicus* occurs throughout much of the Gulf coastal states from Florida to Texas and south into Mexico (Conant, 1975). Therefore, it is possible that coccidian parasites of this gecko may actually represent Old World species introduced into the New World along with the host (Upton et al., 1988).

We had the opportunity to collect and examine additional *H. t. turcicus* from the Houston Zoo, from another Houston locality, and also from a locale in coastal Louisiana. Fecal samples from some of these geckos were positive for oocysts of a previously undescribed species of *Eimeria,* which was not observed in our earlier study (see Upton et al., 1988). Herein, we provide a description of the new form that we observed, along with prevalence data and comparisons with similar species of *Eimeria* from gekkonid hosts.

Materials and Methods

From March through June 1987, a total of 47 immature and adult *H. t. turcicus* ($\bar{x} \pm$ SD snout–vent length = 45.7 ± 8.3, range = 28–56 mm) were collected on the walls of St. Anne's Catholic Church in Harris County ($N = 21$), from within the reptile and amphibian facility of the Houston Zoo ($N = 18$), and at a private residence in Houma, Terrabonne Parish, Louisiana ($N = 7$). Within 48 hr, geckos were killed with an overdose of sodium pentobarbital. Their slit intestines, along with portions of feces from the rectum, were placed in individual vials of 2.5% (w/v) aqueous potassium dichromate ($K_2Cr_2O_7$). Individual samples

were screened by flotation (see below) for the presence of coccidian oocysts, and positive samples containing unsporulated oocysts were mailed to Kansas State University and stored at 4°C prior to further examination. In addition, squash preparations were made of intestinal tissues from some of the geckos and examined for parasite developmental stages. Samples were sporulated at room temperature (ca. 23°C) in petri dishes containing a thin layer of 2.5% $K_2Cr_2O_7$. Sporulated oocysts were concentrated by centrifugation–flotation in Sheather's sugar solution (specific gravity, 1.18) and examined and photographed using Nomarski interference-contrast microscopy. Thirty oocysts were measured using a calibrated ocular micrometer and are reported in micrometers, with the means followed by the ranges in parentheses.

Results

Eimeria lineri sp. n. (Apicomplexa: Eimeriidae) (Figs. 1, 2)

DESCRIPTION: Oocysts ellipsoid, 24.8 × 19.5 (21.6–28.0 × 18.4–21.6); shape index (length/width) 1.3 (1.1–1.5). Wall smooth, about 1.5 thick, with a thick outer layer about 1.0 and a thin inner layer about 0.5 thick. Micropyle and oocyst residuum absent; one (rarely 2 or 3) polar granule(s) usually present. Sporocysts ellipsoid, 9.0 × 7.8 (8.2–9.6 × 7.2–8.8), with a smooth thin wall about 0.5 thick; shape index 1.2 (1.1–1.3). Sometimes faint sutures can be seen in the sporocyst wall revealing the sporocyst to be composed of plates. Stieda and substieda bodies absent. Sporocyst residuum present, composed of numerous granules in a spherical or ovoid mass, 4.1 × 4.4 (2.5–5.6 × 3.6–5.6). Sporozoites vermiform, 11.6 × 2.4 (8.8–13.6 × 2.0–2.8) in situ, arranged head-to-tail within the sporocyst. Sometimes faint striations can be observed at the anterior ends of the sporozoites. Each sporozoite contains a spherical anterior refractile body, 1.9 (1.6–2.4), and a spherical or ovoid posterior refractile body, 2.2 × 2.8 (1.8–2.8 × 1.8–3.2). A nucleus can sometimes be observed between the refractile bodies.

TYPE SPECIMENS: Syntypes (oocysts in 10% formalin) are deposited in the U.S. National Helminthological Collection, Animal Parasitology Institute, U.S. Department of Agriculture, Beltsville, Maryland 20705, as USNM Helm. Coll. No. 80260.

TYPE HOST: *Hemidactylus turcicus turcicus* (Linnaeus, 1758) (Sauria: Gekkonidae), Mediterranean gecko. Voucher specimens are deposited in the Arkansas State University Museum of Zoology, State University, Arkansas (ASUMZ 8535–8541, 8649–8665, 8667–8673).

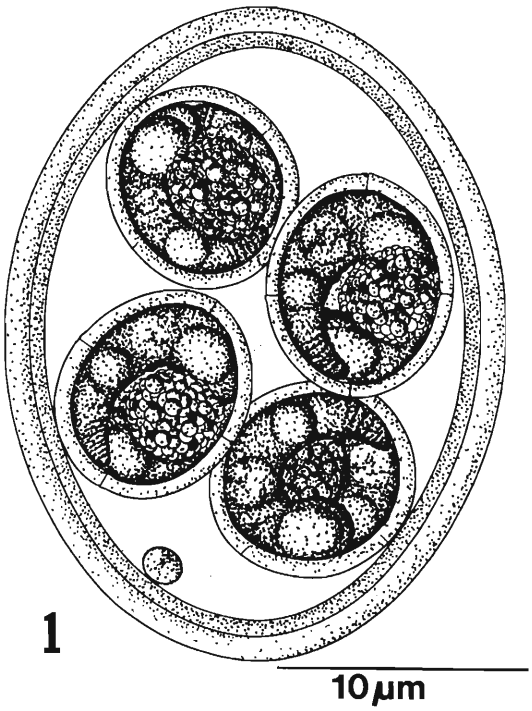

Figure 1. Line drawing of sporulated oocyst of *Eimeria lineri* sp. n.

TYPE LOCALITY: Houston, Harris County, Texas, U.S.A.

OTHER LOCALITIES: Houma, Terrabonne Parish, Louisiana, U.S.A.

SITE OF INFECTION: Anterior ½ of small intestine.

SPORULATION: Exogenous. Oocysts recovered from the feces were unsporulated and became fully sporulated within 7 days at ca. 23°C in 2.5% aqueous $K_2Cr_2O_7$.

PREVALENCE: 24/47 (51.1%) of the geckos were passing the new species at the time they were examined; 17/22 (77.3%) from St. Anne's Catholic Church, 4/18 (22.2%) from the Houston Zoo, and 3/7 (42.9%) from Houma, Louisiana.

ETYMOLOGY: Named in honor of Ernest A. Liner, in recognition of his numerous contributions to neotropical herpetology.

REMARKS: Of the hundreds of oocysts observed from the geckos from Houston, Texas, all but 1 of the oocysts possessed a polar granule. In specimens observed from *H. t. turcicus* from Louisiana, however, most (but not all) failed to possess this structure. Although it is possible that

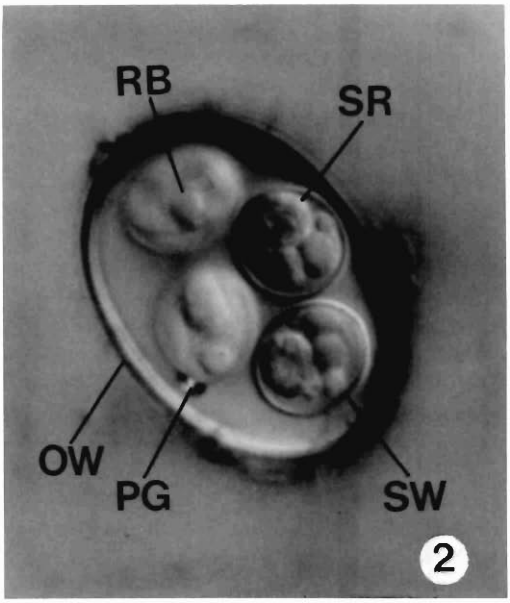

Figure 2. Nomarski interference-contrast photomicrograph of sporulated oocyst of *Eimeria lineri*: OW (oocyst wall), PG (polar granule), RB (refractile body), SR (sporocyst residuum), SW (sporocyst wall). ×2,100.

the form from Louisiana actually represents a species separate from that described herein, all other measurements appeared identical to *Eimeria lineri* sp. n., and thus, we consider it unwise to describe the form from Louisiana as another new species.

Discussion

Eimeria lineri sp. n. most closely resembles *Eimeria cicaki* Else and Colley, 1975, from the Pacific gecko, *Gehyra mutilata,* and house gecko, *Hemidactylus frenatus,* and *Eimeria telfordi* Bovee, 1971, from *G. mutilata* in Japan. However, the species we report herein has more elongate oocysts, shorter and stouter sporocysts, and fewer polar granules than reported for *E. cicaki.* Although *E. lineri* sp. n. has slightly more elongate oocysts and somewhat larger sporocysts than *E. telfordi,* the latter species is reported never to possess a polar granule (see Bovee, 1971; Else and Colley, 1975). The new species does not resemble any of the remaining species of gekkonid or other lizard *Eimeria* (see Matuschka and Bannert, 1986a, b, 1987; Upton and Barnard, 1987; Upton et al., 1988).

In addition to the new species, we also observed oocysts of *Eimeria turcicus* Upton, McAllister, and Freed, 1988, in 8/47 (17.0%) of the geckos (2/22 [9.1%] from St. Anne's Church and 6/18 [33.3%] from the Houston Zoo). None of the *H. t. turcicus* from Houma, Louisiana, harbored *E. turcicus.* Further, on 1 occasion a single adult female gecko from St. Anne's Church had a polyinfection of both *E. lineri* and *E. turcicus.*

When a Chi-square analysis was performed on the prevalence data, no significant differences based on sex were noted (12/23 [52.2%] males versus 12/24 [50.0%] females). However, when prevalence is examined on the basis of differences in age (measured in snout–vent length, adults being >44 mm) between *E. turcicus* and *E. lineri,* contrasting results are obtained. More of the younger geckos (hatchlings and juveniles) and fewer adults harbored *E. lineri* (41.9 ± 8.3, range 29–54 mm), whereas only the older, more mature adults (52.0 ± 3.2, range 47–56 mm) were infected with *E. turcicus.* It is unknown whether these trends are a seasonal or ecological phenomenon, the result of differential immunological or humoral responses, or whether they are related to differences in sporulation and site of infection between the 2 species (i.e., *E. lineri* sporulates exogenously and obviously develops in the intestine, whereas *E. turcicus* sporulates endogenously and infects the gall bladder). However, we have noted that reptiles harboring coccidia known to infect the gall bladder tend to pass oocysts more or less continually, as opposed to intestinal forms that eventually stop passing oocysts (unpubl. obs.).

Acknowledgments

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MEETING SCHEDULE
HELMINTHOLOGICAL SOCIETY OF WASHINGTON
1988–1989

(Wed) 12 Oct 1988	"USUHS Overseas Research and Teaching Programs," Uniformed Services University of the Health Sciences, Bethesda, MD
(Wed) 16 Nov 1988	"Novel Forms of Biological Control of Parasitic Infections," Animal Parasitology Unit, United States Department of Agriculture, Agricultural Research Service, Beltsville, MD
(Wed) 7 Dec 1988	"Natural Regulators of Parasites," Plant Protection Institute, United States Department of Agriculture, Beltsville, MD
(Wed) 11 Jan 1989	"Interactions Between Parasites and Their Hosts," Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD
(Wed) 15 Feb 1989	"Malaria Vaccine Development," Department of Immunoparasitology, Naval Medical Research Institute, Bethesda, MD
(Wed) 15 Mar 1989	"To Be Announced," Division of Experimental Therapeutics, Walter Reed Institute of Research, Washington, DC and Armed Forces Institute of Pathology, Washington, DC
(Wed) 12 Apr 1989	"To Be Announced," School of Public Health, Johns Hopkins University, Baltimore, MD and Medical School, University of Maryland, Baltimore, MD
(Sat) 13 May 1989	"To Be Announced," Department of Pathobiology, Veterinary School, University of Pennsylvania, New Bolton, PA; Joint Meeting with Royal Society of Tropical Medicine and Hygiene and New Jersey Society for Parasitology

Members that are in the area and could attend meetings who do not receive announcements by mail should contact Dr. John Cross, Department of Preventive Medicine, Uniformed Services University of Health Sciences, Bethesda, MD 20705.

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Trypanosoma (*Megatrypanum*) sp. from a Mountain Goat (*Oreamnos americanus*)[1]

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ABSTRACT: Eighty-nine trypanosomes were recovered in conventional thin blood films prepared on slides from a single male mountain goat that originated in Montana and was held at the Jennings Center for Zoological Medicine, Zoological Society of San Diego, San Diego, California. The trypanosomes were photomicrographed and measurements were made for morphometric comparison with trypanosomes reported from other North American ruminant hosts. The trypanosomes appeared most like *Trypanosoma cervi* from the Cervidae in North America, were unlike *T. theileri* from cattle, and were least like *T. melophagium* from sheep. Identification to species must await recovery of live trypanosomes and results of cross-transmission trials. Trypanosomes have not been reported previously from mountain goat.

KEY WORDS: Protozoa, *Trypanosoma* (*Megatrypanum*) sp., *Oreamnos americanus,* mountain goat, morphology, mensural values.

Stercorarian trypanosomes have been reported from North American ruminants in sheep (Swingle, 1909), cattle (Glaser, 1922), mountain sheep (*Ovis dalli*) (Bequaert, 1942), white-tailed deer (*Odocoileus virginianus*) (Kistner and Hanson, 1969), mule deer (*Odocoileus hemionus*) (Clark, 1972), elk (*Cervus elaphus*) (Kingston and Morton, 1973), moose (*Alces alces*) (Kingston et al., 1981), pronghorn (*Antilocapra americana*) (Kingston et al., 1981), reindeer (*Rangifer tarandus*) (Kingston et al., 1982), bison (*Bison bison*) (Kingston et al., 1986), and musk ox (*Ovibos moschatus*) (Kingston, unpubl. data).

Trypanosomes from cattle and bison have been identified as *Trypanosoma theileri* Laveran, 1902 (Matthews et al., 1979; Kingston et al., 1986). Trypanosomes from musk ox are considered to be *T. theileri* (Kingston, pers. obs., 1986) also. Trypanosomes from sheep have been identified as *Trypanosoma melophagium* (Flu, 1908) Noller, 1917 by Hoare (1972). Trypanosomes from North American cervid hosts have been identified as *Trypanosoma cervi* Kingston and Morton, 1975 (Kingston and Morton, 1975; Kingston and Crum, 1977; Matthews et al., 1977; Kingston et al., 1981, 1982, 1985). Trypanosomes from pronghorn and mountain sheep are as yet unidentified.

Recently we received blood films prepared from the blood of a mountain goat (*Oreamnos americanus*) from the Jennings Center for Zoological Medicine, Zoological Society of San Diego, San Diego, California. This animal had been recently critically injured in Montana and shipped to San Diego for veterinary care. The specimens found on these slides are described morphologically

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Table 1. Summary of measurements of trypanosomes recovered from mountain goat (*Oreamnos americanus*) (μm).

$N = 89$	PK*	KN	PN	NA	BL	FF	L
$\bar{x}$	13.47	8.41	21.53	26.33	47.69	7.82	55.16
SD	3.56	2.36	3.73	3.94	5.81	3.18	5.87
SE	0.38	0.25	0.40	0.42	0.62	0.36	0.67
Min.–max.	5.0–23.0	3.0–15.0	13.0–30.0	15.0–35.0	33.0–58.0	3.0–20.0	40.0–66.0
95% C.I. of mean†	12.71–14.23	7.91–8.92	20.74–22.31	25.50–27.16	46.47–48.92	7.10–8.54	53.82–56.49

* PK = posterior end-to-kinetoplast distance, KN = kinetoplast-to-nucleus distance, PN = posterior end-to-nucleus distance, BL = body length, FF = length of free flagellum, L = overall length, W = width at level of nucleus, FF:BL = body length-to-flagellum index, NI = PN/NA, KI = PN/KN.

† Two standard errors on each side of mean.

Table 2. Mensural values for trypanosomes from mountain goat, Bovidae, and Cervidae (μm).*

Species		PK†	KN	PN	NA	BL	FF	L	W	FF:BL	NI	KI
Mountain goat	$\bar{x}$	13.47	8.41	21.53	26.33	47.69	7.82	55.16	3.52	7.17	0.84	2.70
(N = 89)	SD	3.56	2.36	3.73	3.94	5.81	3.18	5.87	0.94	3.28	0.20	0.99
All deer	$\bar{x}$	11.53‡	7.05‡	18.53‡	23.33‡	41.99‡	8.24	50.08‡	5.45‡	6.16‡	0.81	2.74
(N = 174)	SD	5.60	2.10	6.34	7.30	12.44	3.24	13.64	2.48	3.74	0.22	0.96
All bovines	$\bar{x}$	7.23‡	8.68	15.86‡	19.83‡	35.60‡	14.0‡	49.56‡	3.30	2.81‡	0.83	1.87‡
(N = 336)	SD	3.24	2.60	5.0	6.19	10.38	4.46	12.56	1.97	2.20	0.22	0.41

* All values calculated from raw data (N. Kingston, Dept. of Veterinary Sciences, University of Wyoming).

† PK = posterior end-to-kinetoplast distance, KN = kinetoplast-to-nucleus distance, PN = posterior end-to-nucleus distance, BL = body length, FF = length of free flagellum, L = overall length, W = width at level of nucleus, FF:BL = body length-to-flagellum index, NI = PN/NA, KI = PN/KN.

‡ Denotes significantly different from mountain goat form using the Duncan's multiple range test ($P \leq 0.05$).

herein and this description is compared with trypanosomes from other North American ruminant hosts.

Materials and Methods

Twenty-four conventional thin blood films prepared on slides from blood from a single male mountain goat and stained with Giemsa were examined. The slides were coated with a thin layer of immersion oil and scanned under a light microscope at 100× magnification (10× ocular, 10× objective). When a trypanosome was located, photomicrographs (color transparencies) were taken at 1,000× (both light and phase-contrast microscopy) magnification (10× ocular, 100× objective) and projected at a standard distance on a sheet of tracing paper. Eighty-nine specimens were adequate for further study. Outline tracings were made to determine size and shape with emphasis given to nucleus and kinetoplast positions and lengths of the free flagellum. In addition, a transparency (1,000×) of a stage micrometer was traced at the bottom corner of the paper for calibration of the measuring device. Drawn specimens were measured using an Alvin 1112 curvimeter. Descriptive statistics were obtained using the SPSS subprogram Condescriptive (Statistical Packages for the Social Sciences [SPSS] Version 8). Representative trypanosome specimens have been deposited in the United States National Museum (USNM) Helminthological Collection (No. 79903), Beltsville, Maryland.

Comparisons of mensural values from mountain goat trypanosomes and pooled data for *T. cervi* from cervid hosts and *T. theileri* from bovine hosts were made using the Duncan's multiple range test option in the SPSS subprogram Oneway (Kingston et al., 1985).

Table 1. Continued.

W	FF:BL	NI	KI
3.52	7.17	0.84	2.70
0.94	3.28	0.20	0.99
0.10	0.37	0.02	0.11
3.0–8.0	1.90–17.67	0.54–1.4	0.0–6.0
3.32–3.72	6.42–7.91	0.79–0.88	2.49–2.91

Results and Discussion

Descriptive statistics for the 89 trypanosomes recovered from the mountain goat are presented in Table 1. The specimens had a body length (BL) that averaged 47.69 μm with pointed anterior and posterior ends. The nucleus usually appeared as a band across the organism or occasionally was oval in shape and was located slightly posteriorly; the average nuclear index (PN/NA) equaled 0.84. The darkly staining kinetoplast was located in the posterior half of the organism closer to the nucleus than to the posterior end; the kinetoplast index (PN/KN) averaged 2.7. The flagellum originated near the level of the kinetoplast, followed the border of the undulating membrane to the anterior tip, and extended beyond the body as a free flagellum; average free flagellar length (FF) equaled 7.82. A flagellar pocket was observed only rarely. All specimens were typical *T.* (*Megatrypanum*) trypanosomes: (1) all were large, (2) all had a kinetoplast nearer the nucleus than the posterior end, and (3) all had a sharply pointed posterior end (Hoare, 1972).

Results of the Duncan's multiple range test for similarities of means between the mountain goat trypanosomes and pooled data for *T. cervi* from all North American cervid hosts and *T. theileri* from all bovid hosts are presented in Table 2. Comparisons between mountain goat forms and mensural values from pooled data for cervid trypanosomes (Table 2) indicated that values for

Table 3. Comparison between trypanosomes from mountain goat and those from bovids, cervids, and sheep based on Mayr's 75% rule for subspecificity.

Hosts	Trypanosome characteristic*	Standard population			No. goats excluded/N	% goats excluded
		−2 SD	$\bar{x}$	+2 SD		
Cervids†	PK	0.33	11.53	22.73	1/87	1.1
(N = 174)	KN	2.85	7.05	11.25	7/87	8.0
	PN	5.85	18.53	31.21	0/89	0.0
	NA	8.78	23.33	37.93	0/89	0.0
	BL	17.11	41.99	66.87	0/89	0.0
	FF	1.76	8.24	14.72	4/77	5.1
	L	22.80	50.08	77.36	0/77	0.0
	W	0.49	5.45	10.41	0/89	0.0
	FF:BL	−1.32	6.16	13.64	4/77	5.2
	NI	0.37	0.88	1.25	6/89	6.7
	KI	0.82	2.74	4.66	4/87	4.6
Bovids†	PK	0.75	7.23	13.71	32/87	36.0
(N = 336)	KN	3.48	8.68	19.08	2/87	2.2
	PN	5.86	15.86	25.86	9/89	10.0
	NA	7.45	19.83	32.21	3/89	3.3
	BL	14.84	35.60	56.36	5/89	5.6
	FF	5.08	14.0	22.92	27/77	35.1
	L	24.44	49.56	74.68	0/77	0.0
	W	−0.64	3.30	7.24	1/89	1.1
	FF:BL	−1.59	2.81	7.21	29/77	37.6
	NI	0.39	0.83	1.27	6/89	6.7
	KI	1.05	1.87	2.69	34/87	39.1
Sheep‡	PK	8.9	14.7	20.5	10/87	11.5
(N = 111)	KN	2.9	5.1	7.3	69/87	79.3
	PN	12.8	19.8	26.8	8/89	8.9
	NA	15.7	19.5	23.3	67/89	75.2
	BL		[39.3]§			
	FF	2.8	6.0	9.2	19/77	24.7
	L	37.1	45.3	53.5	45/77	58.4
	FF:BL		[6.55]§			
	W‖	2.1		4.6	18/89	20.2
	NI‖	0.9		1.2	75/89	84.3
	KI‖	3.3		4.9	73/87	83.9

* Abbreviations explained in Tables 1 and 2.
† All values calculated from raw data (N. Kingston, Dept. of Veterinary Sciences, University of Wyoming).
‡ Values reported by Buscher and Friedhoff (1984).
§ Value calculated from reported ranges.
‖ Only range for central 70% of sample reported.

FF, NI, and KI were similar at the 0.05 level of significance and additionally for FF:BL at the 0.01 level. Mountain goat and cervid values were not similar for PK, KN, PN, NA, BL, L, and W. Comparisons with mensural values from pooled bovine data showed mountain goat trypanosomes to be similar for KN, W, and NI at both the 0.05 and 0.01 levels of significance and not similar for PK, PN, NA, BL, L, FF:BL, and KI.

A second comparison was done using a technique adapted by Kingston et al. (1985) from Mayr's 75% rule for subspecificity (Mayr et al., 1953). This rule suggests that "A population is subspecifically distinct if 75 per cent of its individuals differ from a "standard population" . . . ." Although this procedure cannot be strictly applied in an analysis of mountain goat trypanosomes and trypanosomes from other North American hosts, it does demonstrate some obvious differences that separate these forms. This procedure was of particular value when examining differences between the mountain goat form and *T. melophagium* from sheep for which only means and standard deviations for normally distributed parameters (PN, NA, KN, PK, L, BL, W) and medians and ranges of the central 70% of values for nonnormally distributed parameters (NI, KI) were available (Buscher and Friedhoff, 1984). Following the method used by Kingston et al. (1985), a standard population was

determined for each parameter for *T. cervi, T. theileri,* and *T. melophagium* by taking the mean ±2 SD, thus encompassing 95.45% of the population. The recorded measurements for each parameter for the mountain goat form were compared against ranges for parameters for the other species. The number of mountain goat measurements outside the range divided by the total number of measurements recorded gives a per cent excluded value for that parameter. An excluded value of 25% or greater was considered to indicate a significant difference for that parameter between the mountain goat form and the species with which it was compared.

Results of this examination (Table 3) confirmed results obtained by use of the Duncan's test for both *T. cervi* and *T. theileri* when compared with the mountain goat form. Trypanosomes from mountain goats were found to differ most from *T. cervi* in KN (8%) and the ranges of excluded values for PN, NA, BL, L, and W spanned from 0 to 8%. For the indices FF:BL, NI, and KI, excluded values ranged from only 4.6 to 6.7%. When mountain goat trypanosomes were compared with *T. theileri* from bovids, pronounced differences were found for PK (36% excluded), FF (35.1%), FF:BL (37.6%), and KI (39.1%). Values ranged from 0 (L) to 39.1%. Of the 3 indices, only NI showed a low excluded value (6.7%). For comparisons with *T. melophagium* from sheep, differences in mountain goat trypanosomes were seen in KN (79.3% excluded), NA (75.2%), FF (24.7%), and L (58.4%) for values for which a standard population could be determined, and differences were seen in NI (84.3%) and KI (83.9%) for values for which only the medians and ranges of central 70% of measurements were available. These results indicate that, of the 3 species of trypanosomes compared with the mountain goat form, *T. cervi* is the form showing the most morphologic similarity, whereas *T. melophagium* is the form most different from mountain goat forms.

Results of the comparisons reported here indicate that the trypanosomes described from mountain goats are most similar to those reported from North American cervids based on similarities in FF and the indices FF:BL, NI, and KI. Differences seen between these 2 forms exist only in absolute measurements (PK, KN, PN, NA, BL, L, W), and these absolute measures or linear measurements are all functions of body size. Kingston et al. (1985) have argued that it is unreasonable to separate trypanosomes from a given cervid host from other cervid trypanosomes based on the criterion of absolute measurements as these will vary within a normal population. Accepting this, the trypanosomes from mountain goats can be said to be *T. cervi*-like morphologically. However, in addition to morphologic similarity, separation at the species level of ruminant trypanosomes in the subgenus *Megatrypanum* demonstrates certain host restriction qualities. *Trypanosoma cervi* is found in members of the family Cervidae, *T. theileri* in the Bovidae, tribe Bovini, and *T. melophagium* in the bovid tribe Ovini. The mountain goat is placed in the Rubicaprini, and, based on a consideration of host restriction, it might be expected to harbor its own species of *Megatrypanum.* However, due to the morphologic similarity of the mountain goat form to *T. cervi,* identification of the rubicaprid species prudently must await recovery of live material and results of cross-transmission trials.

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Establishment, Survival, and Fecundity in *Echinostoma caproni* (Trematoda) Infections in NMRI Mice

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ABSTRACT: The population regulation (establishment, survival, fecundity) of *Echinostoma caproni* was studied in 6 and 25 metacercariae/mouse infections in outbred 8-wk-old female NMRI mice. Establishment, pattern and rate of growth, and initial pattern of intestinal distribution were comparable at the 2 infection levels, thus exhibiting infection-dose independency. Decreased susceptibility to infection with increasing age was demonstrated in experiments comprising 8-, 10-, 16-, and 24-wk-old mice. The pattern of worm expulsion was negatively infection-dose dependent with persistence of infections with 25 metacercariae for a period of at least 112 days and with a high proportion of mice harboring infections with 6 metacercariae turning negative for infection by day 28 following infection. This was paralleled by a more marked anterior migration of worms in infections with 6 metacercariae than of worms in infections with 25 metacercariae. An infection-dose independency of worm fecundity expressed by eggs per gram of feces per worm combined with the negatively infection-dose-dependent expulsion pattern resulted in the overall reproductive capacity per worm from infections with 25 metacercariae exceeding markedly that from infections with 6 metacercariae. It is concluded that the *E. caproni*/NMRI mouse system represents a useful model for studying the population regulation in intestinal trematode infections.

KEY WORDS: Trematoda, *Echinostoma*/mouse model, *Echinostoma caproni* (=*E. revolutum*, of some authors), population regulation, establishment, survival, fecundity, *Echinostoma revolutum*, taxonomy, mice.

Recent studies have demonstrated that the *Echinostoma*/mouse model is a highly suitable system for studying the immunological responsiveness of experimental hosts to infection with intestinal trematodes. Thus, interesting findings, among others, include concomitant immunity and rapid expulsion of superimposed infections (Christensen et al., 1986), a negative dose-dependent pattern of expulsion (Christensen et al., 1981), and interference with the immunological reactivity in response to concurrent helminth or protozoan infections (Christensen et al., 1984, 1985). However, detailed information concerning more basic aspects of the *Echinostoma*/rodent model such as establishment, growth, development, and distribution appears limited to infection with *E. revolutum* in Swiss Webster and ICR mice (Hosier and Fried, 1986), with *E. revolutum* in the golden hamster (Franco et al., 1986), and with *E. malayanum* in the rat (Mohandas and Nadakal, 1978). The aim of the present study is to contribute to the knowledge available concerning the *Echinostoma*/mouse model by examining basic aspects of the host–parasite relationship and the population regulation (establishment, survival, fecundity) in *E. caproni* infections in outbred female NMRI mice. Such information provides an essential background for further development of the *E. caproni*/mouse system as an epidemiological model for the transmission of infections with intestinal trematodes in man and domestic stock.

Materials and Methods

In experiment 1, 8-wk-old outbred female albino NMRI mice (Gl. Bomholtgård, Ry, Denmark) weighing 20–25 g were used. In experiment 2, similar but 8-, 10-, 16-, and 24-wk-old mice were used. Metacercariae of *E. caproni* (Egyptian strain) were obtained from *Biomphalaria glabrata* as described by Christensen et al. (1980), and infection of mice with metacercariae was made with a stomach tube. The echinostome population used is identical to the population named *E. revolutum* in previous work on echinostomes by Christensen et al. (1981, 1984, 1985, 1986). However, taxonomic revision of the genus *Echinostoma* by Kanev (1985) shows that the correct name of the population is *E. caproni*.

In experiment 1, groups of mice (42 mice/group) were inoculated with either 6 or 25 metacercariae/mouse. The time pattern of expulsion was determined by weekly examination for eggs in feces using the direct smear technique. At regular intervals, 3–4 mice from each group were randomly selected from among those found positive for infection. Feces from individual mice were collected over a 12-hr period according to the method described by Keymer and Hiorns (1986). Eggs per gram of feces were determined according to Brindley and Dobson (1981). Worm burden and worm localization in the individual mouse were subsequently

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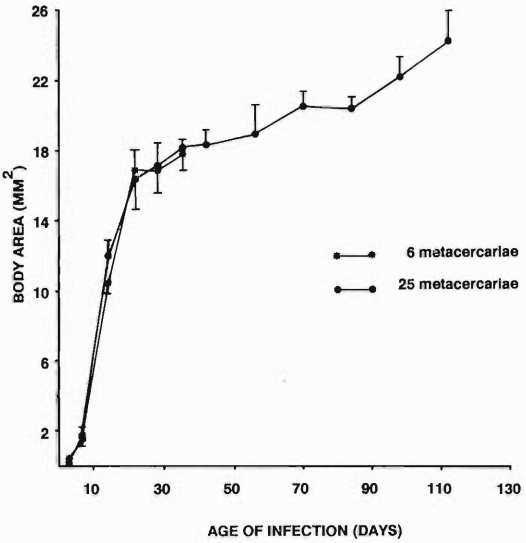

Figure 1. Body area (mm², $\bar{x} \pm 2$ SE) of *Echinostoma caproni* at increasing age (days) with infections of 6 and 25 metacercariae in female albino NMRI mice (10 worms from each infection level at each recording).

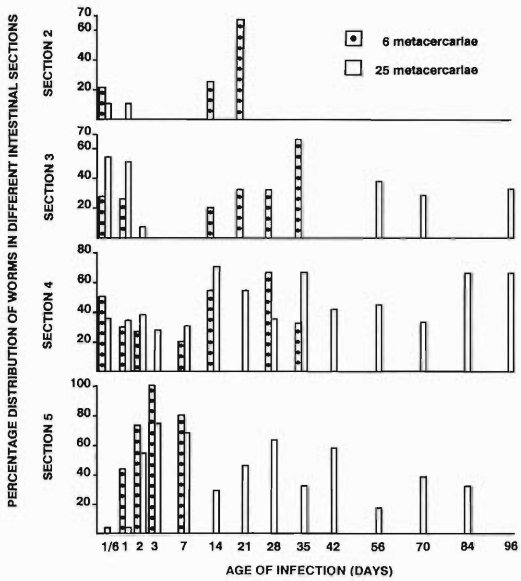

Figure 2. Percentage distribution of *Echinostoma caproni* in the small intestine at increasing age (days) with infections of 6 and 25 metacercariae in female albino NMRI mice. (Successive 20% sections numbered from the pylorus. Results based on 3–4 mice at each infection level at each recording.)

determined. Recovery of worms was conducted according to the procedure described by Christensen et al. (1986). To determine worm localization, the small intestine was divided into 20% sections (sections 1–5) starting from the pylorus. Body area measurements (maximum width × length) were conducted on 10 randomly selected worms from each group of mice at each recording. Measurements were done using a calibrated ocular micrometer on worms relaxed in 32% acetic acid and fixed in alcohol–formalin–acetic acid (AFA) for 24 hr. Uterine egg counts were determined by dissection of an additional 10 randomly selected worms from each group of mice at each recording.

In experiment 2, 4 groups of mice aged 8, 10, 16, and 24 wk, respectively (10 mice/group), were inoculated with 12 metacercariae/mouse. Worm establishment was recorded at necropsy on day 8 following infection.

The statistical tests used were the Student's *t*-test and analyses of variance, and a value of $P > 0.05$ was considered nonsignificant.

Results

Initial worm establishment, as measured on days 3–7 following infection, was infection-dose independent. The percentage of *E. caproni* establishment in NMRI mice following the administration of 6 and 25 metacercariae/mouse was 93.6% (83–100%) and 84.7% (64–100%), respectively ($P > 0.05$). Initial growth of *E. caproni* using body area as the criterion was also infection-dose independent. At no time, prior to the expulsion of infections with 6 metacercariae, was there a significant difference in parasite body area between infections with 6 and 25 metacercariae ($P > 0.05$). The increase in the body area at both levels of infections was most marked during the second and third week of infection (Fig. 1). However, growth continued until expulsion in infections with 6 metacercariae and throughout the period of observation in infections with 25 metacercariae (Fig. 1). Thus, the body area in infections with 25 metacercariae on day 112 exceeded that on day 42 following infection ($P < 0.05$) (Fig. 1). Data on the distribution of *E. caproni* in the small intestine are given in Figure 2. For up to 24 hr following infection, worms were found throughout the small intestine from sections 2 to 5. Subsequently, worms at both infection levels migrated posteriorly with worms establishing, on days 3 and 7, in sections 4 and 5 only. Worms in infections with 6 metacercariae subsequently migrated anteriorly with worm establishment in sections 2–4 on days 14 and 21. In contrast, worms in infections with 25 metacercariae remained in sections 4 and 5 for up to at least 42 days following infection (Fig. 2).

All mice harboring infections with 25 metacercariae remained positive for infection, as is evident by the presence of eggs in feces throughout the observation period of 112 days. The percent worm recovery in infections with 25 meta-

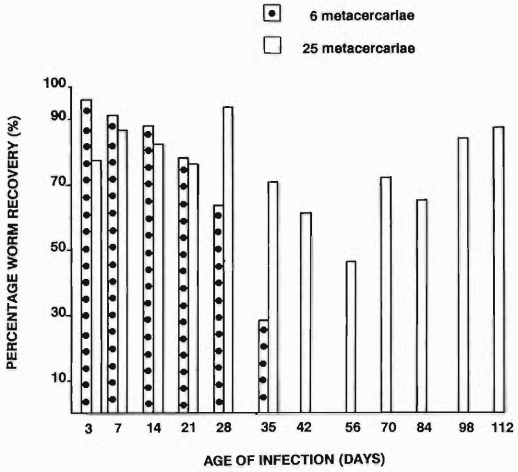

Figure 3. Percentage *Echinostoma caproni* worm recovery at increasing age (days) with infections of 6 and 25 metacercariae in female albino NMRI mice. (Results based on 3–4 mice at each infection level at each recording.)

cercariae remained unchanged throughout (F-test, $P > 0.05$). In contrast, worm numbers in individual mice in infections with 6 metacercariae became markedly reduced by day 28 of infection (F-test, $P < 0.05$) (see Fig. 3). All 6 metacercariae infections were expelled prior to day 42 following metacercarial inoculation (Fig. 3). The worm loss in individual mice was of a gradual nature.

Initial *E. caproni* worm establishment declined markedly with increasing age of the mouse host, with mean establishment in 16- and 24-wk-old mice being only 50 and 57%, respectively, of that in 8-wk-old mice (Fig. 4). There was a significant difference ($P < 0.05$) between mean worm establishment in 8-wk-old mice and 16- or 24-wk-old mice. The marked increase in the variance-to-mean ratio of worm establishment with increasing age of the mouse host reflects that the reduced mean establishment is a result of a part of the older mice developing innate resistance to infection.

Amounts of feces produced per mouse per time unit were strictly comparable at the 2 infection levels (data not presented). Figure 5 shows that the initial egg production per gram feces per worm was also comparable ($P > 0.05$) in infections with 6 and 25 metacercariae, reaching figures of 2.5 and 2.6 $\times$ 10^{-3}, respectively, by day 28 of infection. The egg production capacity per worm in infections with 6 metacercariae remained constant until expulsion ($P > 0.05$). However, the reproductive capacity per worm in infections with

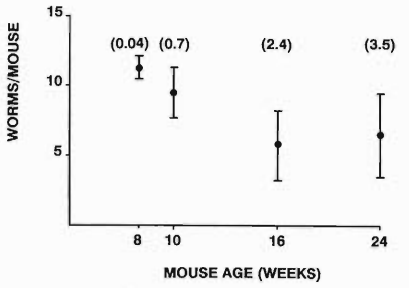

Figure 4. *Echinostoma caproni* establishment ($\bar{x} \pm 2$ SE; variance/mean ratio [figure in parentheses]) in female albino NMRI mice aged 8, 10, 16, and 24 wk, respectively (10 mice/group; 12 metacercariae/mouse; necropsy day 8 following infection).

25 metacercariae increased with the mean eggs per gram feces per worm from day 35 onwards exceeding that at days 14, 21, and 28 ($P < 0.05$) (Fig. 5). The prolonged persistence of 25 meta-

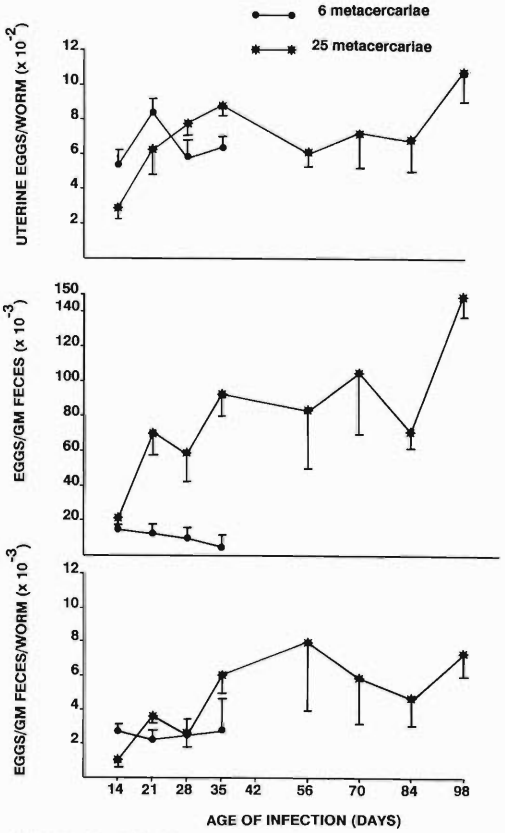

Figure 5. Uterine egg counts per worm, eggs per gram feces, and eggs per gram feces per worm ($\bar{x} \pm 2$ SE) of *Echinostoma caproni* at increasing age (days) in infections of 6 and 25 metacercariae in female albino NMRI mice. (Results based on 3–4 mice at each infection level at each recording.)

cercariae infections as compared with that of 6 metacercariae infections thus results in the overall reproductive potential per established fluke in 25 metacercariae infections markedly exceeding that in 6 metacercariae infections (Fig. 5). Figure 5 also shows that the uterine egg counts during early infection are comparable in infections of 6 and 25 metacercariae ($P > 0.05$). The uterine egg counts per worm and number of eggs per gram feces per worm were highly correlated ($r = 0.89$).

Discussion

The results from the present study show a high level of compatibility between *E. caproni* (Egyptian strain) and female albino NMRI mice using the criterion of initial worm establishment. The *E. caproni*/NMRI mouse relationship is furthermore characterized by a pattern of worm growth corresponding to that described in other *Echinostoma*/rodent models (i.e., Mohandas and Nadakal, 1978; Franco et al., 1986) and by reduced initial worm establishment with increasing age of the mouse host. Such age-related susceptibility to infection is also known from other intestinal trematode/rodent models (Molan and James, 1984). However, the causative mechanism(s) remains to be demonstrated. Worm establishment, growth, and uterine egg counts in *E. caproni* infections in NMRI mice in the present study exceeded markedly that in infections with the closely related *E. revolutum* in Swiss Webster and ICR mice (Hosier and Fried, 1986).

The negatively dose-dependent pattern of expulsion of *E. caproni* in NMRI mice with persistence of infections with 25 metacercariae and expulsion of infections with 6 metacercariae is suggested to have been induced by the heavier *E. caproni* infections interfering with the ability of the host to mount an effective immune response at the intestinal level. Interestingly, the negatively dose-dependent pattern of expulsion is paralleled, as shown in the present study, by a more marked anterior migration in infections of 6 than in infections of 25 metacercariae. The expulsion of *E. caproni* in NMRI mice comprised a gradual loss of worms.

The pattern of expulsion of *E. caproni* infections in NMRI mice deviated markedly from that of *E. revolutum* in Swiss Webster and ICR mice (Hosier and Fried, 1986). Thus, worms from infections of 25 metacercariae with *E. revolutum* in Swiss Webster and ICR mice are expelled at a rate even exceeding that of worms from infections of 6 *E. caproni* metacercariae in NMRI mice. Whether such basic differences in the host–parasite relationship reflect mouse strain-dependent differences in response to echinostome infection or fundamental biological differences between morphologically similar *Echinostoma* species remains to be elucidated. Interestingly, however, NMRI mice have been shown to be refractory (as judged by worm recovery 7 days following infection) to infection with the *E. revolutum* isolate used in the experiments conducted by Hosier and Fried (1986) (Odaibo, unpubl.).

No density-dependent constraint on parasite fecundity was observed in these studies. The uterine egg counts and initial fecal egg counts per gram feces per worm were comparable in infections of 6 and 25 *E. caproni* metacercariae. The use of higher infection levels appears biologically irrelevant due to associated heavy intestinal pathology. The density-dependent constraint on parasite fecundity described in a number of other helminth–host combinations (Keymer, 1982; Kino, 1984; Anderson and Schad, 1985) may therefore play no major role in the *E. caproni*/NMRI mouse system.

The absence of density-dependent constraints on egg production capacity per *E. caproni* worm results in the overall reproductive performance of the *E. caproni* population being determined primarily by population size and worm survival. This fact, combined with the negatively dose-dependent pattern of expulsion, means that the overall *E. caproni* reproductive potential in NMRI mice becomes favored by an overdispersed distribution of parasites in the host population. Thus, the negatively dose-dependent pattern of expulsion may be biologically very important in terms of parasite fecundity.

The present study has provided a firm background for further studies on the population regulation in intestinal trematode infections and for the use of the *E. caproni*/mouse system as an epidemiological model for the transmission of infections with intestinal trematodes in man and domestic stock.

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A Survey of Helminth Parasites of Chiropterans from Indiana

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ABSTRACT: From 1980 to 1982, 888 bats representing 9 host species were collected from Indiana and examined for helminth parasites. Sixty-three percent of the bats were infected with 1 or more species of parasite. Six nematode species, 4 cestode species, and 20 digenean species were recovered. *Paralecithodendrium transversum* and *Ochoterenatrema diminutum* were the most commonly occurring parasite species, being found in high intensities or prevalence in the most bat species. This is the first survey of chiropteran helminths in Indiana.

KEY WORDS: Nematoda, Cestoda, Trematoda, Chiropterans, bats, survey, new host records.

Chiropteran helminths have been recorded from the early 19th century to the present. Prior to 1930, only 5 species of chiropteran helminths had been reported from the United States: *Acanthatrium nycteridis* from *Lasiurus borealis* (red bat) (Faust, 1919); *Distoma* sp. from *L. cinereus* (hoary bat) (Stiles and Hassall, 1894); and *Lecithodendrium anticum, L. posticum,* and *Plagiorchis vespertilionis* from *Myotis leibii* (small-footed *Myotis*) (Stafford, 1905). Reports since that time concerning helminth faunas of bats have primarily been species descriptions or new host or locality records (e.g., Macy, 1931; Nickel and Hansen, 1967; Kunz, 1968; Lotz and Font, 1983), although Lotz and Font (1985) have described the community structure of helminths in big brown bats (*Eptesicus fuscus*) in Wisconsin and Minnesota. Prior to the present report, only 1 species of helminth had been reported from any chiropteran host in Indiana. Seamster and Stevens (1948) described *Paralecithodendrium macnabi* from the big brown bat in northern Indiana. The present study surveys the helminths from 9 of the 12 species of bats that have been reported from Indiana.

Materials and Methods

Bats were collected from November 1980 to December 1982 from 65 of the 92 counties (71%) of the state using mist nets, a shotgun, or taken by hand at roost sites. In addition, specimens of *Myotis sodalis* (Indiana bat) were obtained from the Indiana State Board of Health, which had received them for rabies testing. Bats were killed and necropsied. The lungs, liver, gall bladder, heart, stomach, and intestines were examined for helminths. Trematodes and cestodes were fixed in AFA, stained in Semichon's acetocarmine, and mounted in Permount. Nematodes were killed in 70% ethanol, cleared in glycerol, and examined as temporary mounts. The use of prevalence, mean intensity, and range of intensity follows the definitions of Margolis et al. (1982). Representative specimens are deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705 (Nos. 80046–80122).

Results and Discussion

A total of 888 bats, representing 9 species, was collected and examined. Helminths were found only in the stomach and the small and large intestines. Of the total bats, 63% (559) were infected with 1 or more helminths. Nematodes and cestodes were found in 5 of the species and trematodes in all 9 (Table 1). The numbers of nematodes and cestodes were low, in most cases with mean intensities of less than 10. However, trematodes were abundant, ranging from 1 to 753 per host (Table 1). The highest numbers occurred in the Indiana bat, the little brown bat (*M. lucifugus*), and the silver-haired bat (*Lasionycteris noctivagans*), and the lowest in the evening bat (*Nycticeius humeralis*) and the hoary bat, although sample sizes were small in these latter 2 species (Table 1). Prevalence of trematodes was highest in the Indiana bat and the evening bat and lowest in the 2 species of *Lasiurus* (Table 1). There were no significant differences of prevalence or mean intensities between male and females of any species ($P > 0.05$ Mann Whitney U-test [MWU] and $\chi^2 > 0.05$).

New host records established for helminths from Indiana bats include: *Hymenolepis lasionycteridis, Acanthatrium eptesici, A. microcanthum, Paralecithodendrium chilostomum,* and *P. naviculum* from the little brown bat; *Rictularia lucifugus* and *H. lasionycteridis* from Keen's bat; *R. lucifugus, H. lasionycteridis, A. eptesici, A. oligacanthum, Glyptoporus noctophilus,* and *Plagiorchis vespertilionis* from the Indiana bat; *A. eptesici, A. microcanthum, Allassogonoporus marginalis,* and *Ochoterenatrema diminutum*

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Table 1. Mean intensities and prevalence of helminths of 9 species of bats from Indiana.

Species	Host number examined	Host number infected	Trematoda			Host number infected	Nematoda			Host number infected	Cestoda		
			Total	Mean intensity	*P* (%)		Total	Mean intensity	*P* (%)		Total	Mean intensity	*P* (%)
Eptesicus fuscus	464	317	7,811	25.3	68	32	90	2.8	7	37	251	6.8	8
Lasionycteris noctivagans	6	5	224	45.1	86	—	—	—	—	—	—	—	—
Lasiurus borealis	90	24	245	10.2	27	—	—	—	—	5	8	1.6	6
Lasiurus cinereus	18	3	9	3.0	17	—	—	—	—	—	—	—	—
Myotis keenii	30	26	424	16.7	87	1	3	3.0	3	3	3	1.0	10
Myotis lucifugus	191	128	5,802	45.5	67	19	60	3.2	10	8	16	2.0	4
Myotis sodalis	17	17	783	46.8	100	1	2	2.0	6	3	6	2.0	19
Nycticeius humeralis	1	1	2	2.0	100	1	12	12.0	100	—	—	—	—
Pipistrellus subflavus	71	38	396	10.2	54	—	—	—	—	—	—	—	—

from the eastern pipistrelle; *Acanthatrium eptesici, Limatulum gastroides,* and *P. vespertilionis* from the silver-haired bat; and *A. eptesici* and *A. oligacanthum* from the evening bat.

Urotrematulum attenuatum in the silver-haired bat was placed into synonymy with *Urotrema scabridum* by Caballero (1942). He examined specimens of *U. scabridum, U. shillingeri, U. minuta, U. lasiurensis,* and *Urotrematulum attenuatum* and discussed differences between *U. scabridum* and the other *Urotrema* species. However, he did not discuss specific differences between *U. scabridum* and *Urotrematulum attenuatum.* The specimens I collected were similar to those described by Macy (1933). These similarities included the characteristics used to differentiate *Urotrematulum* from *Urotrema*: the lobed testes, the length between the acetabulum and ovary, the length of the esophagus, and the narrowed anterior region of the body. I did not see any differences as Macy did in the size and development of the seminal receptacle. Based upon these similarities and the lack of any discussion by Caballero (1942) on the noted differences I do not agree with this synonymy and have used the original name (Macy, 1933).

The number of helminth species in individual bats ranged from 1 to 7 (Table 2). The number of species of helminths found in each bat species ranged from 1 in the hoary bat to 17 in the big brown bat (Table 3). The common species of helminths (those found in at least 10% of the bats) generally varied between bat species (only those bat species with samples of at least 10 individuals were considered) (Table 3). In the big brown bat there were 4: *Paralecithodendrium transversum, Ochoterenatrema diminutum, Acanthatrium microcanthum,* and *Plagiorchis vespertilionis.* In the little brown bat there were 3: *O. diminutum, A. eptesici,* and *Allassogonoporus marginalis.* The red bat and the eastern pipistrelle (*Pipistrellus subflavus*) had 1 each: *P. transversum* and *Acanthatrium pipistrelli,* respectively. The common species in Keen's bat (*Myotis keenii*) were *Hymenolepis lasionycteridis, O. diminutum,* and *P. transversum*; *O. diminutum* and *P. vespertilionis* were considered common because of the small sample size.

The mean intensity and prevalence of common helminth species varied between bat species (Table 3). In the big brown bat the mean intensity of *O. diminutum* was significantly higher ($P < 0.05$ MWU) than that of any other species, whereas *P. transversum* was found more often ($\chi^2 < 0.05$) (Table 3). In the little brown bat the mean intensity and prevalence of *O. diminutum* was significantly higher ($P < 0.05$ MWU and $\chi^2 < 0.01$) than that of *A. eptesici* and *A. marginalis* (Table 3). A similar result was noted for *P. transversum* in Keen's bat, the Indiana bat, and the red bat and for *A. pipistrelli* in the eastern

Table 2. Mean number (±SE) of helminth species infecting 9 species of bats from Indiana.

Host	Mean number	SE	Range
Eptesicus fuscus	1.7	1.0	1–7
Lasionycteris noctivagans	2.2	0.8	2–3
Lasiurus borealis	1.2	0.4	1–2
Lasiurus cinereus	1.0	0.0	1
Myotis keenii	1.3	0.5	1–4
Myotis lucifugus	1.8	1.1	1–7
Myotis sodalis	1.8	0.8	1–3
Nycticeius humeralis	2.0	0.0	2
Pipistrellus subflavus	1.3	0.5	1–3

Table 3. Summary of the helminths of 9 species of Indiana bats.

Parasite	Host	Prevalence %	Intensity	
			Mean	Range
Nematoda				
Rictularioidea				
Rictularia lucifugus	EF*	2.8	2.6	1–12
	ML	8.4	2.0	1–4
	MK†	3.3	3.0	3
	MS†	6.0	2.0	2
Trichostrongyloidea				
Allintoshius travassosi	EF	1.0	2.3	1–3
	NH	100.0	12.0	12
Trichuroidea				
Capillaria palmata	EF	1.7	2.3	1–4
	ML	2.6	2.8	1–5
Capillaria sp.	ML	1.0	8.0	8
Platyhelminthes				
Cestoda				
Hymenolepis roudabushi	EF	5.2	8.5	1–34
	ML	1.0	1.0	1
Hymenolepis christensoni	EF†	1.7	2.5	1–5
	ML	1.0	1.5	1–2
Hymenolepis lasionycteridis	MK†	10.0	1.0	1
	ML†	0.5	7.0	7
	MS†	29.4	1.8	1–4
Cycloskrjabinia taborensis	LB	7.8	1.6	1–3
Trematoda				
Lecithodendriidae				
Acanthatrium alicatai	EF	0.2	11.0	1
Acanthatrium eptesici	EF	2.2	2.5	1–6
	LN†	16.7	13.0	13
	LB	1.1	29.0	29
	MK	3.3	7.0	7
	ML†	11.5	5.6	1–26
	MS†	11.8	8.5	5–12
	NH†	100.0	1.0	1
	PS†	2.8	4.5	1–7
Acanthatrium microcanthum	EF	9.7	9.9	1–123
	ML†	1.0	3.5	1–5
	PS†	1.4	1.0	1
Acanthatrium nycteridis	ML	0.5	1.0	1
Acanthatrium oligacanthum	EF	3.7	6.4	1–27
	MK	6.7	5.0	2–8
	ML	8.9	5.8	1–22
	MS†	11.8	8.0	5–11
	NH†	100.0	1.0	1
Acanthatrium pipistrelli	EF	8.4	6.4	1–33
	ML	0.5	1.0	1
	PS	43.7	10.0	1–72
Allassogonoporus marginalis	EF	0.2	1.0	1
	ML	9.9	4.8	1–31
	PS†	1.4	1.0	1
Glyptoporus noctophilus	ML	3.7	155.0	1–467
	MS†	5.9	1.0	1
Limatulum gastroides	EF	0.2	2.0	2
	LN	33.3	37.0	20–54
	ML	9.4	22.9	1–140
Ochoterenatrema breckenridgei	EF	0.6	186.0	2–286
	PS	7.0	13.2	1–44
Ochoterenatrema diminutum	EF	11.9	49.5	1–680
	MK	13.3	6.8	2–12

Table 3. Continued.

Parasite	Host	Prevalence %	Intensity Mean	Intensity Range
	ML	35.1	50.8	1–508
	MS†	29.4	21.2	1–33
	PS†	1.4	1.0	1
Paralecithodendrium chilostomum	EF	1.1	7.2	1–16
	ML†	0.5	1.0	1
Paralecithodendrium macnabi	EF	1.9	10.0	1–69
	LN	66.7	17.8	5–28
Paralecithodendrium naviculum	EF	5.8	44.1	1–753
	ML†	5.2	30.3	2–64
	MS	23.5	8.0	2–13
Paralecithodendrium nokomis	EF	1.3	4.2	1–7
Paralecithodendrium swansoni	EF	3.2	3.6	1–14
	MK	3.3	2.0	2
	ML	1.0	13.0	1–25
Paralecithodendrium transversum	EF	42.2	7.7	1–157
	LB	24.4	9.2	2–5
	MK	86.7	14.5	2–51
	ML†	8.9	6.8	1–32
	MS	52.9	59.2	2–301
	PS†	2.8	2.5	2–3
Plagiorchiidae				
Plagiorchis vespertilionis	EF	18.8	5.4	1–83
	LN†	16.7	6.0	6
	LB	3.3	1.0	1
	LC	22.2	2.0	1–3
	MK	3.3	1.0	1
	ML	8.4	4.5	1–20
	MS†	35.3	12.8	2–49
	PS	7.0	1.4	1–2
Urotrematidae				
Urotrema scabridum	EF	0.2	1.0	1
	LB	2.2	4.0	2–6
	ML	6.3	8.5	1–16
Urotrematulum attenuatum	LN	50.0	20.0	3–44

* EF—*Eptesicus fuscus*, LN—*Lasionycteris noctivagans*, LB—*Lasiurus borealis*, LC—*L. cinereus*, MK—*Myotis keenii*, ML—*M. lucifugus*, MS—*M. sodalis*, NH—*Nycteceius humeralis*, and PS—*Pipistrellus subflavus*.
† New host record.

pipistrelle ($P < 0.05$ MWU and $\chi^2 < 0.01$) (Table 3).

The survey demonstrates that bats in Indiana are infected with a complex and often abundant helminth fauna and there are differences in combinations or abundance of helminth species for each bat. These may be a result of differences in food habits, immunological responses of the host, interspecific interactions of the parasites, or in the geographic distribution of the helminths. In addition, the high intensity of helminth infection in bats from Indiana is consistent with other reports of bats from various areas (e.g., Kunz, 1968, in Iowa; Coggins et al., 1982, in Wisconsin; and Lotz and Font, 1983, 1985, in Wisconsin and Minnesota) and suggests that bats are excellent hosts for a variety of helminth species.

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Comparison of Helminths of Rice Rats, *Oryzomys palustris,* from Freshwater and Saltwater Marshes in Florida[1]

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ABSTRACT: Examination of 288 rice rats, *Oryzomys palustris,* collected in Florida over a 2-yr period revealed 45 species of helminth parasites: 4 cestodes, 21 trematodes, and 20 nematodes. Rats from a freshwater marsh harbored 20 species (3 cestodes, 2 trematodes, 15 nematodes), whereas those from a saltwater marsh had 40 species (3 cestodes, 20 trematodes, 17 nematodes), with 15 species shared between marshes (2 cestodes, 1 trematode, 12 nematodes). Microphallid trematodes (10 species) dominated the fauna in the saltwater marsh, and nematodes (15 species) dominated in the freshwater marsh. Prevalence and intensity of infection of shared nematode species were reduced greatly in rats from the saltwater marsh. The fauna of the omnivorous rice rat was characterized by heteroxenous species. Known life cycles and feeding experiments indicated that rice rats ate tadpoles, frogs, insects, and land snails in the freshwater marsh, and crabs, fish, aquatic snails, and insects in the salt marsh. The rice rat shared helminth species with the cotton rat (*Sigmodon hispidus*), round-tailed muskrat (*Neofiber alleni*), opossum (*Didelphis virginiana*), raccoon (*Procyon lotor*), and clapper rail (*Rallus longirostris*).

KEY WORDS: rice rat, *Oryzomys palustris,* survey, Florida, helminths, freshwater marsh, salt marsh, intermediate hosts, prevalence, intensity.

Rice rats, *Oryzomys palustris* (Harlan), inhabit both inland freshwater marshes and coastal salt marshes of the southeastern United States. They occupy an unusual trophic niche for a rodent, feeding on insects, snails, crabs, and fish (Negus et al., 1961; Sharp, 1967), as well as preying on the eggs and nestlings of marsh birds (Post, 1981). Because the literature on the helminth fauna of the rice rat consisted only of scattered records, a study was initiated in Florida in April 1970.

As a result of this study, 6 new species of nematodes (*Parastrongylus schmidti, Hassalstrongylus forresteri, Hassalstrongylus lichtenfelsi, Litomosoides scotti, Syphacia oryzomyos, Capillaria forresteri*), and 1 new species of trematode (*Lyperosomum intermedium*) have already been described (see Kinsella, 1971; Denton and Kinsella, 1972; Quentin and Kinsella, 1972; Forrester and Kinsella, 1973; Durette-Desset, 1974; Kinsella and Pence, 1987). In addition, the rice rat was found to be the first known natural definitive host for 2 other trematodes (*Stictodora cursitans, Catatropis johnstoni*), and the life cycles of these trematodes have been described (see Bush and Kinsella, 1972; Kinsella and Heard, 1974).

The purpose of the present paper is to compare the helminth fauna of rice rats from freshwater and saltwater marshes in Florida.

Materials and Methods

Rats were live-trapped in Sherman traps at 3-mo intervals between April 1970 and May 1972. Ages were determined on the basis of weight and pelage. The ecosystem of the freshwater marsh, Paynes Prairie, near Gainesville, Alachua County, has been described extensively by Birkenholz (1963). A typical *Juncus-Spartina* saltwater marsh on Waccasassa Bay near Cedar Key, Levy County, was also trapped. The ecosystem of this area was described by Post (1981).

Rats were brought to the laboratory and killed immediately before examination. Methods of collecting, fixing, and staining helminths have been described by Kinsella (1974). When trematodes were very abundant, their numbers were estimated by mixing thoroughly with 250 ml of water and counting a 10-ml aliquot.

In order to determine the intermediate hosts of some trematodes in the salt marsh, various invertebrates were fed to laboratory-born rice rats obtained from pregnant females captured on Paynes Prairie. Later these rats were killed and examined for trematodes by the same methods. Intermediate hosts from Cedar Key were also dissected and examined for metacercariae.

Chi-square and Mann-Whitney *U*-tests were used to determine significant differences ($P < 0.05$) in prevalence and intensity, respectively, with regard to sex, age, and locality of hosts.

Results

Forty-five species of helminths (4 cestodes, 21 trematodes, 20 nematodes) were found. Table 1 lists the site, prevalence, and intensity of infection of each species. Rats from Paynes Prairie

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Table 1. Helminth parasites of *Oryzomys palustris* from freshwater and saltwater marshes in Florida.

	Fresh water (N = 178)			Salt water (N = 110)		
	Prevalence (%)	Intensity		Prevalence (%)	Intensity	
		Mean	(Range)		Mean	(Range)
Cestoda						
Hymenolepis diminuta (Rudolphi, 1819) (3)*	19	4	(1–15)	1	1	(1)
Taenia rileyi Loewen, 1929 (8)	0	—	—	1	2	(2)
Taenia mustelae Gmelin, 1790 (8)	0.5	†	†	0	—	—
Cladotaenia circi Yamaguti, 1935 (8)	0.5	†	†	1	†	†
Trematoda						
Microphallus basodactylophallus (Bridgman, 1969) (3)	0	—	—	94	‡	‡
Microphallus nicolli (Cable and Hunninen, 1938) (3)	0	—	—	9	‡	‡
Microphallus sp. (3)	0	—	—	10	‡	‡
Maritrema prosthrometra Deblock and Heard, 1969 (3)	0	—	—	5	‡	‡
Maritrema sp. I (3)	0	—	—	69	‡	‡
Maritrema sp. II (3)	0	—	—	19	‡	‡
Probolocoryphe glandulosa (Coil, 1955) (3)	0	—	—	56	‡	‡
Gynaecotyla adunca (Linton, 1905) (3)	0		—	15	‡	‡
Levinseniella sp. (3)	0	—	—	49	‡	‡
Odhneria odhneri Travassos, 1921 (3)	0	—	—	6	‡	‡
Ascocotyle angrense Travassos, 1916 (3)	0	—	—	25	‡	‡
Ascocotyle mollienisicola (Sogandares-Bernal and Bridgman, 1960) (3)	0	—	—	9	‡	‡
Stictodora cursitans (Holliman, 1961) (3)	0	—	—	52	36	(1–100)
Parvatrema sp. (3)	0	—	—	26	‡	‡
Lyperosomum intermedium Denton and Kinsella, 1972 (6)	0	—	—	45	20	(1–50)
Zonorchis komareki (McIntosh, 1939) (7)	0	—	—	1	1	(1)
Echinochasmus schwartzi Price, 1931 (3)	0	—	—	19	4	(1–15)
Catatropis johnstoni Martin, 1956 (4)	0	—	—	30	91	(1–500)
Urotrema scabridum (Braun, 1900) (3)	0	—	—	23	8	(1–25)
Brachylaeme virginianum (Dickerson, 1930) (3)	15	5	(1–23)	0	—	—
Fibricola lucida (LaRue and Bosma, 1927) (3)	67	143	(1–1,975)	11	17	(1–65)
Nematoda						
Capillaria hepatica (Bancroft, 1893) (5)	8	†	†	6	†	†
Capillaria gastrica Baylis, 1926 (1)	4	1	(1–2)	6	4	(1–10)
Capillaria forresteri Kinsella and Pence, 1987 (2)	46	10	(1–50)	1	1	(1)
Strongyloides sp. (3)	30	4	(1–30)	0	—	—
Monodontus sp. (3)	0	—	—	1	1	(1)
Hassalstrongylus spp.§ (3)	92	99	(1–515)	9	11	(1–75)
Trichostrongylus sigmodontis Baylis, 1945 (3)	8	4	(1–15)	3	2	(1–4)
Trichostrongylus affinis Graybill, 1924 (4)	14	3	(1–14)	6	7	(1–19)
Parastrongylus schmidti (Kinsella, 1971) (9)	7	4	(1–20)	3	3	(1–8)
Syphacia oryzomyos Quentin and Kinsella, 1972 (4, 5)	42	34	(1–275)	0	—	—
Physaloptera hispida Schell, 1952 (2)	35	14	(1–92)	0	—	—
Physaloptera sp. (2)	0	—	—	4	2	(1–2)
Mastophorus muris (Gmelin, 1790) (2)	36	6	(1–70)	1	1	(1)
Pterygodermatites spp.‖ (3)	20	3	(1–9)	5	3	(1–12)
Skrjabinoclava thapari deFrietas, 1953 (2)	0	—	—	28	4	(1–10)
Larval spirurid (2, 3, 4)	0	—	—	5	2	(1–5)
Litomosomoides scotti Forrester and Kinsella, 1973 (10, 11)	0	—	—	57	12	(1–104)

* Location in host: (1) gastric mucosa, (2) stomach cavity, (3) small intestine, (4) cecum, (5) large intestine, (6) pancreatic duct, (7) bile duct, (8) liver mucosa, (9) pulmonary arteries, (10) abdominal cavity, (11) pleural cavity.

† Not counted.

‡ Individual counts of microphallids, gymnophallids, and heterophyids not made. Total numbers ranged from 40 to 30,000/rat (mean 4,050).

§ A complex of 3 species, *H. musculi* (Dikmans, 1935), *H. forresteri* Durette-Desset, 1974, and *H. lichtenfelsi* Durette-Desset, 1974. Females cannot be distinguished so data were combined.

‖ Two species, *P. ondatrae* (Chandler, 1941) and *P.* sp. Females cannot be distinguished so data were combined.

were infected with 3 cestodes, 2 trematodes, and 15 nematodes, whereas those from Cedar Key were infected with 3 cestodes, 20 trematodes, and 17 nematodes. Fifteen of the 45 species were shared between rats from the 2 marshes (2 cestodes, 1 trematode, 12 nematodes).

The number of helminth species per infected host in the freshwater marsh varied from 1 to 11 (mean 6.1, median 6). In the salt marsh, the number of species varied from 2 to 16 (mean 8.4, median 8). No rice rat was helminth free. Representative specimens were deposited in the U.S. National Parasite Collection in Beltsville, Maryland: Nos. 71579 (*P. schmidti*), 63079 (*S. oryzomyos*), 72288 (*L. scotti*), 72799 (*S. cursitans*), 73424 (*C. forresteri*), 79871 (*Hassalstrongylus* spp.), and 79872 (mixed microphallids and heterophyids).

Cestodes

The only adult tapeworm encountered was *Hymenolepis diminuta,* a common parasite of rodents in the United States (Doran, 1954). Prevalence was high on Paynes Prairie, but only 1 rat was infected at Cedar Key with a single worm. Individual infections were found of larval *Taenia rileyi,* a parasite of bobcats; *Taenia mustelae,* a parasite of skunks and other mustelids; and *Cladotaenia circi,* a parasite of hawks.

Trematodes

Only 1 trematode, *Fibricola lucida,* was recorded from rats in both types of marsh. Both prevalence and intensity of infection were significantly higher on Paynes Prairie. The genus *Fibricola* uses tadpoles and frogs as the infective intermediate hosts (Chandler, 1942). *Brachylaeme virginianum* was found only on Paynes Prairie and uses land snails as second intermediate hosts (Krull, 1934).

The remaining 19 species of trematodes were found only in rats from Cedar Key. Ten of these species belong to the family Microphallidae, a group possessing precocious metacercariae that may already be producing eggs in the intermediate host. These minute trematodes show little host specificity and are common parasites of various marine mammals and shore birds. *Microphallus* sp. and *Maritrema* sp. I are the same undescribed species reported by Heard (1970) in clapper rails (*Rallus longirostris*) from the Atlantic and Gulf Coasts. *Maritrema* sp. II and *Levinseniella* sp. apparently are undescribed species.

Feeding experiments established the following as intermediate hosts of microphallids at Cedar Key: *Uca rapax* (fiddler crab)—*Gynaecotyla adunca, Probolocoryphe glandulosa, Microphallus* sp.; *Uca pugilator* (fiddler crab)—*Maritrema prosthrometra, Maritrema* sp. I. and II, *Levinseniella* sp.; *Callinectes sapidus* (blue crab)—*Microphallus basodactylophallus*; *Eurytium limosum* (stone crab)—*Microphallus nicolli.* Up to 30,000 microphallids were present in individual rats, with *M. basodactylophallus* and *Maritrema* sp. I and II predominating over other species by a ratio of 20 to 1.

An undescribed gymnophallid, *Parvatrema* sp., was present in small numbers. This was confirmed as the *Parvatrema* sp. II reported by Heard (1970) in clapper rails (Heard, pers. comm.). One rice rat fed several snails, *Melampus coffeus,* from Cedar Key was found infected with 125 flukes of this species.

The life cycle of *Stictodora cursitans* at Cedar Key was reported by Kinsella and Heard (1974). Killifish, *Fundulus* spp., were found infected with the metacercariae. *Fundulus* spp. have also been reported as the intermediate hosts of *Ascocotyle angrense* and *Echinochasmus schwartzi* (Sogandares-Bernal and Lumsden, 1963; Heard, 1970), recorded here for the first time from rice rats. The intermediate hosts of *Ascocotyle mollienisicola* are small fish called mollies, of the genus *Mollienisia* (Sogandares-Bernal and Bridgman, 1960).

Bush and Kinsella (1972) found the intermediate host of *Catatropis johnstoni* at Cedar Key to be the snail, *Cerithidea scalariformis.* Although the cercariae can encyst on vegetation, they showed an affinity for encysting on the snail's operculum, so the rice rat may be infected by eating the snail rather than vegetation.

Urotrema scabridum has been reported from bats (*Molossus* sp.) in Brazil and muskrats (*Ondatra zibethicus*) in Maryland (Penner, 1941) and commonly infected the rice rat at Cedar Key. An insect intermediate host may be the link between infections in these 2 disparate groups of mammals.

Nematodes

Although more species of nematodes (17) were found in rice rats at Cedar Key than at Paynes Prairie (15), prevalence and intensity of infection in 8 of 12 of the shared species were significantly lower in the salt marsh (see Table 1). Three species (*Strongyloides* sp., *Syphacia oryzomyos, Physa-*

Table 2. Prevalence and intensity of nematode infections with regard to sex and age of host.

Locality and species	Juveniles		Subadults and adults		Males		Females	
	Prevalence (%)	Intensity mean	Prevalence (%)	Intensity mean	Prevalence (%)	Intensity mean	Prevalence (%)	Intensity mean
Freshwater marsh								
Capillaria forresteri	4	1	52*	10*	45	10	46	9
Parastrongylus schmidti	0	—	8*	7*	5	6	10	2
Pterygodermatites spp.	4	1	23*	3	21	3	18	3
Mastophorus muris	4	5	25*	6	40	17	25*	5*
Physaloptera hispida	7	1	41*	14*	33	6	40	7
Syphacia oryzomyos	37	7	43	33*	53	37	24*	21*
Saltwater marsh								
Pterygodermatites spp.	0	—	7*	3*	4	5	7	1
Litomosoides scotti	44	11	73*	13	66	11	44	16

* Significant difference ($P < 0.05$).

loptera hispida) were found only at Paynes Prairie, and 4 species were restricted to the salt marsh (*Monodontus* sp., *Skrjabinoclava thapari, Litomosoides scotti,* and in an unidentified larval spirurid). The larval spirurid was found in dissections of fiddler crabs, *Uca rapax* and *Uca pugilator,* and may be a parasite of birds that does not mature in rice rats. Larvae thought to be the infective stages of *S. thapari* were found in the same 2 species of crabs, but their identity was not confirmed by infecting laboratory-born rats.

Only nematodes showed significant differences in prevalence or intensity of infection according to sex or age of the host (Table 2). On Paynes Prairie, 3 nematodes (*Capillaria forresteri, Parastrongylus schmidti, P. hispida*) were higher in prevalence and intensity in adults and subadults than in juveniles, 3 (2 species of *Pterygodermatites* and *Mastophorus muris*) were higher in prevalence but not in intensity, and 1 (*S. oryzomyos*) was higher in intensity, but not in prevalence. At Cedar Key, *Pterygodermatites* spp. were found only in adults, whereas *L. scotti* was higher in prevalence, but not intensity, in adults. *Mastophorus muris* and *S. oryzomyos* had a significantly higher prevalence and intensity in male rats.

Discussion

The presence of 45 species of helminths, and, more specifically, 21 species of trematodes in a rodent is unprecedented and certainly is related to both the diversity of habitat and unique food habits of the rice rat. The trematode fauna especially reflected the omnivorous diet. Intermediate hosts confirmed here by dissection or feeding experiments included fiddler crabs, blue crabs, stone crabs, killifish, mollies, and aquatic snails. Other food items indicated from known life cycles of trematodes are tadpoles, frogs, and land snails.

Prevalence and intensity of infection of trematodes in rats from the salt marsh indicated that virtually 100% of rice rats have a carnivorous diet. In the freshwater marsh, the high prevalence of *F. lucida* showed that tadpoles and frogs are an important component of the diet, and the prevalence of 4 nematodes (*P. hispida, M. muris, Pterygodermatites* spp.) and 1 cestode (*H. diminuta*) indicated that insects may take the place in the diet of crabs in rats from the salt marsh.

The fauna of the rice rat in the freshwater marsh was dominated by nematodes, both in number of species present and total helminth burden. Monoxenous nematodes (*Strongyloides* sp., *Hassalstrongylus* spp., *Trichostrongylus sigmodontis, T. affinis*) were absent or greatly reduced in prevalence in the salt marsh, where the brackish environment may affect their larval development. Although the pinworm, *S. oryzomyos,* presumably is transmitted directly from host to host by the egg, infections were absent in rice rats from the salt marsh. Paradoxically, *Syphacia sigmodontis,* collected from the cotton rat (*Sigmodon hispidus*) from the same 2 marshes, had a much higher prevalence and intensity of infection in the salt marsh (Kinsella, 1974).

The acquisition of nematodes by young rice

rats showed that monoxenous species (*Strongyloides* sp., *Hassalstrongylus* spp., *Trichostrongylus* spp.) were acquired earliest, whereas heteroxenous species (*P. hispida, M. muris, Pterygodermatites* spp., *P. schmidti*) were acquired later. The prevalence of *L. scotti,* which is transmitted by a mite vector, was shown to increase directly with age (Forrester and Kinsella, 1973). Intensity of infection with *S. oryzomyos* also increased with age, although prevalence was not significantly different.

The pattern of acquisition of trematodes was less clear-cut. Because microphallids, gymnophallids, and heterophyids are precocious, and require very little development time within the definitive host, even the youngest rats collected in the salt marsh were infected with 1–4 species of trematodes. This indicated that their carnivorous diet may begin as soon as they leave the nest. Also, the time these trematodes live within the host is relatively short, so intensity of infection does not necessarily increase with age, but varies seasonally with availability of intermediate hosts (Heard, 1970).

The only other rodents trapped at Paynes Prairie were the cotton rat and the round-tailed muskrat (*Neofiber alleni*). At Cedar Key, the cotton rat was the only other rodent present. The rice rat shared 6 helminth species (1 cestode, 5 nematodes) with the cotton rat at Paynes Prairie, and 9 species (4 trematodes, 5 nematodes) at Cedar Key; and only 3 nematode species with the round-tailed muskrat (Kinsella, 1974; Forrester et al., 1987).

The rice rat also shared helminths with ecological associates such as the opossum, *Didelphis virginiana* (7 species), the raccoon, *Procyon lotor* (7 species), and the clapper rail (7 species) (see Harkema and Miller, 1964; Heard, 1970; Kinsella and Heard, 1974; Kinsella, unpubl. data), again primarily reflecting the lack of host specificity of the salt marsh trematodes.

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Helminths of Rainbow Smelt, *Osmerus mordax*, from Five Localities in Lake Huron and Lake Michigan, with Emphasis on *Diplostomum spathaceum*

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ABSTRACT: A total of 428 smelt, *Osmerus mordax* (family: Osmeridae), was collected from 4 Michigan localities associated with Lake Huron and from 1 Indiana locality in southern Lake Michigan. Seven helminth species (1 Digenea, 3 Cestoda, 2 Nematoda, 1 Acanthocephala) were found in spawning smelt sampled in April 1984, 1985, and 1986. At least 97% of the smelt from each locality was infected with 1 or more helminths. *Diplostomum spathaceum, Eubothrium* sp., *Cystidicola* sp., and *Echinorhynchus salmonis* infected smelt from all localities. *Cyathocephalus truncatus* is reported from smelt for the first time. The helminth faunas of smelt from all localities were numerically dominated by *D. spathaceum* and *E. salmonis*. The lens of the right eye of smelt from all localities had a higher mean intensity of *D. spathaceum* than did the left lens; these right lens–left lens differences were statistically significant from 2 localities. *Diplostomum spathaceum* exhibited significant asymmetry between lenses in 21 (15%) female and 29 (10%) male smelt.

KEY WORDS: helminth parasites, smelt, *Osmerus mordax, Diplostomum spathaceum, Cyathocephalus truncatus, Eubothrium* sp., *Proteocephalus* sp., *Capillaria* sp., *Cystidicola* sp., *Echinorhynchus salmonis*, Great Lakes, prevalence, mean intensity.

There have been relatively few studies on the parasites and diseases of smelt in the Great Lakes (Lake Michigan: Amin and Burrows, 1977; Lake Superior: Fischthal, 1952; Lankester and Smith, 1980; Lake Huron: Collins and Dechtiar, 1974; Lake Erie: Dechtiar, 1965). Parasitological studies on smelt in Michigan waters have not, hitherto, been published except for the brief information in Allison et al. (1977) and Muzzall (1984). The present study reports on helminths of smelt from 5 Great Lakes localities.

Materials and Methods

Three hundred forty-two smelt were collected by dip net in April from 4 Michigan localities associated with Lake Huron: 51 from the Carp River, Mackinaw County, 1984; 91 from a drainage ditch near Hessel, Mackinaw County, 1985; 100 from the pier at De Tour Village, Chippewa County, 1986; and 100 from offshore at Point Lookout, Arenac County, 1986. Smelt were spawning in lotic waters and along the lake edge. The first 3 sites are in the eastern part of Michigan's Upper Peninsula. Point Lookout, located in the Lower Peninsula, extends into the northwest portion of Saginaw Bay. An additional 86 smelt were collected in April 1986 by trawl in Indiana waters of southern Lake Michigan. The mean length ± 1 SD (range in parentheses) of the smelt from each locality was: Carp River, 140 ± 11.6 mm (105–165); Hessel drainage ditch, 141 ± 11.3 mm (116–165); De Tour pier, 121 ± 12.4 mm (103–154); Point Lookout, 146 ± 16.8 mm (112–177); and southern Lake Michigan, 128 ± 20.5 mm (62–212).

Smelt were fixed in 15% formalin at the site. Sex and total length to the nearest millimeter were recorded when the fish were necropsied. All organs and the left or right half of the body musculature of at least 40 smelt and the gills of 50 smelt from each locality were examined. Helminths found in preserved fish were processed using conventional parasitological techniques. The terms prevalence and mean intensity follow definitions given by Margolis et al. (1982). The value following a mean is the standard deviation. Student's *t*-test and analysis of variance followed by 95% confidence intervals were used to determine if the mean intensity of *Diplostomum spathaceum* and *Echinorhynchus salmonis* differed between female and male fish and between localities. Voucher specimens of the following helminths (USNM Helminthological Collection No.) have been deposited in the USNM Helminthological Collection: *Diplostomum spathaceum* (80128), *Cyathocephalus truncatus* (80129), *Eubothrium* sp. (80130), *Proteocephalus* sp. (80131), *Capillaria* sp. (80132), *Cystidicola* sp. (80133), *Echinorhynchus salmonis* (80134).

Results

At least 97% of the smelt examined from each locality were infected with 1 or more helminths. The prevalence, total number of individuals found, and mean intensity for the 7 helminth species (1 Digenea, 3 Cestoda, 2 Nematoda, 1 Acanthocephala) are in Table 1. The number of helminth species in smelt from the different localities ranged from 4 to 6. The helminth fauna of smelt collected in April 1984, 1985, 1986 consisted of 1 species of metacercaria, mature stages of 1 cestode, and 1 acanthocephalan species, and

Table 1. Prevalence, total, and mean intensity of helminths found in *Osmerus mordax* from 5 localities of Lake Huron and Lake Michigan.

Parasite	Carp River			De Tour pier			Hessel drainage ditch
	Prevalence (N = 51)*	No. found (% of community)	Mean intensity ± 1 SD (range)	Prevalence (N = 100)	No. found (% of community)	Mean intensity ± 1 SD (range)	Prevalence (N = 91)
Digenea							
Diplostomum spathaceum† (Rudolphi, 1819)							
Both lenses	45 (88)#	495 (69)	11.0 ± 8.6 (1–43)	93 (93)	990 (72.4)	10.6 ± 8.6 (1–43)	89 (98)
Left lens	37 (73)	226 (46)‖	6.1 ± 4.5 (1–19)	86 (86)	407 (41)	4.7 ± 3.4 (1–12)	85 (93)
Right lens	40 (78)	269 (54)	6.7 ± 4.6 (1–24)	87 (87)	583 (59)	6.7 ± 5.9 (1–34)	82 (90)
Cestoda							
Cyathocephalus truncatus‡ (Pallus, 1781)	—	—	—	4 (4)	10 (0.7)	2.5 ± 3.0 (1–7)	—
Eubothrium sp.§	1 (2)	1 (0.1)	1.0	10 (10)	24 (1.8)	2.4 ± 2.6 (1–9)	7 (77)
Proteocephalus sp.§	—	—	—	—	—	—	—
Nematoda							
Capillaria sp.§	1 (2)	2 (0.2)	2.0	—	—	—	—
Cystidicola sp.§	2 (4)	2 (0.2)	1.0	5 (5)	5 (0.4)	1.0	2 (2)
Acanthocephala							
Echinorhynchus salmonis‡ Müller, 1784							
Intestine	39 (77)	207 (29)	5.3 ± 4.3 (1–17)	73 (73)	317 (23.1)	4.3 ± 3.3 (1–15)	80 (88)
Nonintestinal sites	6 (12)	10 (1.4)	1.7 ± 0.8 (1–3)	12 (12)	21 (1.5)	1.8 ± 0.9 (1–13)	9 (10)

* N = number of smelt examined.
† Metacercariae.
# Number infected (% infected).
‡ Adult parasites.
§ Immature parasites.
‖ Percent *D. spathaceum* in left or right lens.

immature stages of 2 cestode and 2 nematode species. *Diplostomum spathaceum, Eubothrium* sp., *Cystidicola* sp., and *Echinorhynchus salmonis* infected smelt from all localities. *Cyathocephalus truncatus* is reported from smelt for the first time. *Diplostomum spathaceum* occurred unencysted in the lens of the eye. *Capillaria* sp. and *Cyathocephalus truncatus* were found in the anterior intestine and pyloric ceca. *Eubothrium* sp., *Proteocephalus* sp., and *E. salmonis* occurred in the posterior intestine and rectum. A total of 98 acanthocephalans, all presumably *E. salmonis,* were encapsulated in the mesenteries of the digestive tract and on the outside of the liver, gonads, and swim bladder. Although many of the acanthocephalans were enclosed within envelopes of undetermined origin and appeared to be in a degenerate condition, 6 individuals contained eggs. *Cystidicola* sp. infected the swim bladder. Parasites were not found on the gills or in the muscle of smelt. No significant differences by Chi-square analyses occurred in the prevalence of helminth species between localities or between female and male fish. The mean intensities of *D. spathaceum* and *E. salmonis* were not significantly different between female and male fish.

Diplostomum spathaceum had the highest prevalence and mean intensity of the helminths found in 4 localities, followed by *E. salmonis.* At Point Lookout, *E. salmonis* had the highest prevalence and mean intensity, followed by *D. spathaceum.* Smelt from Point Lookout had a significantly higher mean intensity of *E. salmonis* in the intestine than did smelt from De Tour pier, Hessel drainage ditch, and southern Lake Michigan ($F = 8.86$, $P < 0.001$). The intensities of *D. spathaceum* had significant, but low, correlations with host length at the Carp River ($r = 0.29$, $P < 0.05$) and De Tour pier ($r = 0.55$, $P < 0.01$). The numbers of *E. salmonis* had significant low correlations with host length from De Tour pier ($r = 0.35$, $P < 0.01$), Point Lookout ($r = 0.21$, $P < 0.05$), and Southern Lake Michigan ($r = 0.46$, $P < 0.01$). Together, *D. spathaceum* and *E. salmonis* made up at least 97% of

Table 1. Continued.

Hessel drainage ditch		Point Lookout			Southern Lake Michigan		
No. found (% of community)	Mean intensity ± 1 SD (range)	Prevalence (*N* = 100)	No. found (% of community)	Mean intensity ± 1 SD (range)	Prevalence (*N* = 86)	No. found (% of community)	Mean intensity ± 1 SD (range)
1,264 (75.3)	14.2 ± 11.0 (1–56)	84 (84)	580 (44.2)	6.9 ± 6.2 (1–33)	80 (93)	1,219 (83.4)	15.2 ± 15.7 (1–69)
524 (41)	6.2 ± 5.4 (1–30)	63 (63)	235 (41)	3.7 ± 2.7 (1–11)	73 (85)	543 (45)	7.4 ± 7.5 (1–38)
751 (59)	9.0 ± 6.6 (1–31)	79 (79)	345 (59)	4.4 ± 4.0 (1–22)	67 (78)	676 (55)	10.1 ± 9.3 (1–46)
—	—	11 (11)	13 (1.0)	1.2 ± 0.4 (1–2)	—	—	—
13 (0.8)	1.9 ± 1.5 (1–5)	3 (3)	8 (0.6)	2.7 ± 1.5 (1–4)	1 (1)	1 (0.1)	1.0
—	—	1 (1)	1 (0.1)	1.0	—	—	—
—	—	—	—	—	—	—	—
2 (0.1)	1.0	14 (4)	17 (1.3)	1.2 ± 0.4 (1–2)	2 (2)	3 (0.2)	1.5 ± 0.7 (1–2)
386 (23.0)	4.8 ± 3.5 (1–22)	87 (87)	659 (50.2)	7.6 ± 5.3 (1–31)	51 (59)	219 (15)	4.3 ± 3.2 (1–4)
13 (0.8)	1.4 ± 0.7 (1–3)	26 (26)	34 (2.6)	1.3 ± 0.7 (1–4)	6 (7)	20 (1.3)	3.3 ± 1.9 (1–6)

the helminths from each locality. The other helminth species occurred infrequently.

Diplostomum spathaceum infected at least 84% of the smelt from each locality. Smelt from Point Lookout had a significantly lower mean intensity of *D. spathaceum* than did smelt from the other localities ($F = 8.03$, $P < 0.001$). The right lens of the eye of smelt from all localities had a higher mean intensity of *D. spathaceum* than the left lens; these differences in smelt from De Tour pier ($t = 2.6$, $P < 0.01$) and Hessel drainage ditch ($t = 3.2$, $P < 0.01$) were significant. By Chi-square analysis, *D. spathaceum* showed significant asymmetry between lenses in 21 (15%) female and 29 (10%) male smelt. When significant asymmetry of *D. spathaceum* was compared between lenses, 42 cases favored the right lens and 8 favored the left lens.

Discussion

Margolis and Arthur (1979) listed 24 parasite genera (2 Protozoa, 6 Trematoda, 2 Cestoda, 8 Nematoda, 5 Acanthocephala, 1 Copepoda) infecting smelt from Canada. Collins and Dechtiar (1974) found 9 parasite species in smelt from Lake Huron, and Fischthal (1952) and Muzzall (1984) reported 5 and 3 helminth species infecting smelt from Lake Superior and the St. Marys River, Michigan, respectively. None of the helminths in the present study have been reported from smelt in marine and brackish water. The absence of gill parasites has been observed in other parasitological studies of smelt. In our study, the muscle did not harbor larval helminths that could be transmitted to animals that eat smelt. Dechtiar (1965) reported on the occurrence of *Glugea hertwigi,* a pathogenic protozoan known to cause mortality in smelt from Lake Erie. Results of the present study as well as other studies (Fischthal, 1952; Bangham, 1955; Collins and Dechtiar, 1974; Muzzall, 1984) indicate that *G. hertwigi* does not occur in smelt from Lake Michigan, Lake Huron, and the St. Marys River.

Bangham (1955) reported that 11 of 37 smelt had more than 10 *Diplostomum* in the lenses of the eyes, but precise mean intensity values of *D.*

spathaceum in smelt were not found in the literature. Muzzall and Peebles (1987) reported that the mean intensities of *D. spathaceum* in the lenses of emerald shiners, *Notropis atherinoides,* were 6.9 and 6.7 from 2 localities in the St. Marys River. Two percent of Lake Munoscong emerald shiners and 5% of Raber Bay emerald shiners showed significant asymmetry between lenses when numbers of *D. spathaceum* were compared. Generally, the mean intensities and asymmetry percentages of *D. spathaceum* in the lenses of smelt were higher than those for emerald shiners (Muzzall and Peebles, 1987) and for *Diplostomum* in lake whitefish, *Coregonus clupeaformis* (see Rau et al., 1979). Ching (1985) reported that *D. baeri bucculentum* infecting the eyes exhibited asymmetry in 50% of lake whitefish; 32% of mountain whitefish, *Prosopium williamsoni*; 31% of rainbow trout, *Salmo gairdneri*; and 23% of chinook salmon, *Oncorhynchus tshawytscha.*

The mean intensities of *D. spathaceum* were higher in the right lens than in the left lens of smelt from all localities in the present study; in smelt from De Tour pier and Hessel drainage ditch, these mean intensities were statistically significant. Muzzall and Peebles (1987) also found that the mean intensity of *D. spathaceum* was highest in the right lens of emerald shiners from 2 localities. Published studies comparing the infection rates of *Diplostomum* spp. between left and right eyes (lenses) of fishes were not found except for Palmieri et al. (1977) and Rau et al. (1979), who found no significant differences.

It is generally accepted that after strigeid trematodes penetrate fish, they move to the site of development either via the bloodstream (Davis, 1936; Ferguson, 1943; Johnson, 1971) or by burrowing through connective tissues (Erasmus, 1959). It has been demonstrated experimentally that the factors that orient *D. flexicaudum* to the eyes are associated with eye tissues (Ferguson, 1943). Rau et al. (1979), discussing metacercarial accumulation in lake whitefish, stated (p. 293) "... a natural asymmetry in the blood supply would favor one eye consistently and this is not the case." A possible explanation for these consistent right lens–left lens differences of *D. spathaceum* in smelt may involve differences in the movement of blood to the right and left eye. Studies on the circulatory system of smelt were not found in the literature. However, based on studies of other teleosts (Anderson and Mitchum, 1974; Grizzle and Rogers, 1976; Groman, 1982), differences in the circulatory system between the right and left sides of the head or in the vessels leading to the eyes do not occur. Rau et al. (1979) suggested that the invading cercariae may cause an asymmetry in the ocular blood supply resulting in asymmetrical eye infection, but found that departure favored neither the left nor the right eye or lens. However, in smelt in the present study, 42 of the 50 significant asymmetries favored the right lens. When smelt that exhibited significant *D. spathaceum* asymmetry were removed from the analyses, the mean intensity was always higher in the right lens when compared to the left lens and the difference remained statistically significant in smelt from De Tour pier and Hessel drainage ditch. A definite explanation for the right lens–left lens differences cannot be presented.

Lankester and Smith (1980) found that *Cystidicola farionis* infected 95% of the smelt examined from Lake Superior with a mean intensity of 26. These infection values are much higher than those of *Cystidicola* sp. in the present study. However, the mean length (175 mm) of the smelt they examined was much larger than the mean lengths of smelt we examined. Six percent of the *C. farionis* that Lankester and Smith found were mature, whereas none of the *Cystidicola* sp. found in the present study were mature.

Amin and Burrows (1977) reported that *E. salmonis* infected 75% of the smelt examined from southwestern Lake Michigan with a mean intensity of 3.1. Generally, the prevalences of *E. salmonis* in smelt in the present study are similar to those reported by Amin and Burrows, but the mean intensities of *E. salmonis* from the 5 localities are all higher. However, the mean length of smelt examined by Amin and Burrows was greater than the mean lengths of smelt in the present study.

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Ultrastructure of the Second-stage Juvenile of the Root-knot Nematode, *Meloidogyne incognita*

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ABSTRACT: Juveniles of the root-knot nematode were examined to elucidate the fine-structural details and interrelationships of the feeding, digestive, excretory, and nervous systems. This study follows the continuity of the lumens of the stylet, esophageal canal, intestine, and excretory pore. Passage of material through these systems is controlled by the triradiate metacorpus pump and the esophago–intestinal valve, while secretory activities of accessory glands, namely a dorsal and 2 subventrals, are mediated by tetraradiate valves. A muscle system, which is structured to control stylet and valve movements during an active feeding process, is attached to the stylet knobs, cephalic framework, and median valve via hemidesmosomes. The nervous system of the juvenile consists of 6 inner labial and 2 lateral amphidial sensory receptors that are exposed to the external environment through cuticular openings. In addition, 4 outer labial, 4 cephalic, and several accessory receptors lie beneath the cuticle within the cephalic framework. The receptors can be traced posteriorly to the nerve ring, which has its dorsal region slightly anteriad to the ventral region. A ventral ganglion extends from the nerve ring posteriorly toward the tail of the juvenile.

KEY WORDS: esophagus, excretory gland, dorsal gland, hemizonid, *Meloidogyne incognita* infective juveniles, metacorpus, procorpus, root-knot nematode, sensory organs, stylet, subventral glands.

The root-knot nematodes (*Meloidogyne* spp.) are among the most important plant-parasitic nematodes that cause extensive damage to crop plants. Tissue responses to nematode feeding and development have been extensively studied and reviewed (Dropkin, 1969; Bird, 1974; Endo, 1975). The features of interest in the host–parasite interactions have included the secretions produced by these nematodes (Bird, 1968) and the morphology of the salivary glands during preparasitic and parasitic stages of development (Bird, 1967). Considerable interest was also placed on the cellular changes of host tissues and the mechanism(s) by which giant cells were formed (Huang and Maggenti, 1969). In addition, investigators focused on the wall alterations of giant cells as they interacted with adjacent plant tissues (Huang and Maggenti, 1969; Jones and Northcote, 1972; Wergin and Orion, 1981). Only recently have observations been made of the fine-structure of nematode secretions and their accumulations within the giant cell (Rumpenhorst, 1984; Sundermann and Hussey, 1988).

Early studies on the ultrastructure of the root-knot nematode body focused on the anterior sensory organs and the esophagus of males. Studies on root-knot and cyst nematodes emphasized comparative morphology specifically on the taxonomic and phylogenetic differences during development (Baldwin and Hirschmann, 1973, 1975, 1976). At this time, studies were also conducted on the anterior sensory organs of preparasitic or infective J2 juveniles of root-knot nematode (Wergin and Endo, 1976; Endo and Wergin, 1977).

Other studies, which were not directly related to host–parasite interactions of root-knot nematodes and their hosts, examined the ultrastructure of the esophagus of males of root-knot nematodes. The studies documented the absence of the dorsal gland and supported the concept that males do not feed in the same manner as females, which do derive nutrients from the host giant cells (Baldwin et al., 1977). A fine-structural study of root-knot nematode, *Meloidogyne incognita,* in clover roots showed cytological changes in host cells stimulated by juveniles as they migrated between cells during penetration (Endo and Wergin, 1973). Although this study emphasized the host reactions, the esophageal glands appeared to be active in that secretion granules accumulated in the dorsal and subventral gland ampullae. During these observations, attention of the investigators was drawn to an unusual sensory organ that was later termed a nerve process and found to be a component of the amphidial gland (Wergin and Endo, 1976). The amphidial gland, which is a prominent sensory organ in a wide range of nematodes, was intensely studied by Ward et al. (1975) in *Cae-*

norhabditis elegans. Focus on the sensory organs of animal parasitic nematodes was reviewed by McLaren (1976). These sensory organs had their counterparts among a wide range of plant-parasitic genera (Baldwin and Hirschmann, 1973, 1975; Endo and Wergin, 1973; Wergin and Endo, 1976; De Grisse, 1977; Coomans, 1979; Natasasmita, 1979; Endo, 1980).

Although the esophagus of males of *M. incognita* and of J2 juveniles penetrating host cells has been examined, a lack of information exists on the fine-structure of the esophagus of infective juveniles of this species. Ultrastructural studies of the esophagus of several species of plant-parasitic nematodes have provided leads into the explanation of structure–function relationships in various organs of the anterior portion of the root-knot nematode (Endo, 1980; Shepherd et al., 1980). To increase our understanding of the feeding and digestive functions in nematodes this report examines and describes the ultrastructure of the esophageal region of the root-knot nematode with emphasis on the stomatal region, procorpus, metacorpus, glandular organs, esophago–intestinal valve, and the excretory gland.

Materials and Methods

Samples of nematodes of *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949, used in these observations were processed in the same manner as those described by Endo (1984, 1987) and Endo and Wergin (1973) for previous ultrastructural studies of infective and parasitic juveniles of *H. glycines* Ichinohe, 1952, in the soybean host, *Glycine max* (L.) Merr.

Results

Stomatal region

At the anterior region of the second-stage juvenile, the cuticular cephalic framework provides for support and guidance of the stylet (Fig. 1A, B). This framework, which exhibits bilateral symmetry, not only accommodates the stylet but also the amphidial and inner labial receptors and several other chemo- and tacto-receptors that terminate beneath the cuticle in the cephalic region. The internal cephalic framework supports and is continuous with the outer somatic cuticle. At the lip region, the cuticle has a central opening for the stylet that is surrounded by 6 inner labial pores and 2 lateral amphidial pores. Each of these openings has a cuticular lining that is continuous with the somatic cuticle but extends internally into the cephalic region (Figs. 1B, 2).

The stomatal wall extends posteriad from the surface cuticle, through the cephalic framework and terminates about ⅔ of the length of the retracted stylet (Figs. 1B, C, 2–8). The posteriad region of the stomatal wall folds inward and anteriad to connect with the perimeter of the base of the stylet cone (Figs. 8, 9).

The stomatal wall, which is moderately thick anteriorly (Figs. 1C, 3, 4), thickens in the midregion and thins posteriorly (Figs. 6–8) before it contacts the perimeter of the stylet. The midregion of the stomatal wall has contacts with portions of the stylet protractor muscles as they extend from the cephalic region to the stylet knobs (Figs. 1C, 4). The internal surface of the stomatal wall is smooth; the outer surface is irregular and has an electron-lucent layer of fibrillar material. This layer is bounded by a convoluted membrane that extends the full length of the stomatal wall. However, the membranes appear more prominent in the region posteriad from where the protractor muscle contacts the thickened stomatal wall (Figs. 3–8). The stylet protractor muscles consist of 10 muscle elements when viewed in cross section just posteriad from the cephalic framework (Fig. 4). These 10 elements eventually merge to form 3 primary muscle units, each of which attaches to 1 of the tripartite stylet knobs (Figs. 4–11). A set of 3 secondary muscle units lies between the primary units (Fig. 6). The sarcoplasm of these muscles is located in the anterior region of the procorpus (Fig. 1E).

The stylet tip, which is cone-shaped, has a ventrally located, subterminal lumen orifice. The lumen of the stylet cone is bordered by a sinus that in cross section is crescent-shaped anteriad (Fig. 5) but forms a ring posteriad to completely encircle the lumen at its base (Fig. 8).

The lumen of the stylet may exceed ½ the diameter of the anterior portion of the stylet cone (Fig. 3). However, posteriad, the lumen of the stylet narrows to less than ½ of the diameter of the stylet shaft and continues through the stylet knob region (Figs. 9–14). The stylet lumen is continuous with the esophageal lumen that is supported by a cuticular wall (Figs. 1A, 15–17).

A cuticularized canal extends from the dorsal gland ampulla to join the esophageal lumen posteriad to the stylet base (Fig. 1A). This branch canal terminates with a single, tetraradiate valve (Figs. 1A, D, 15). The main tubular canal supporting the lumen of the esophagus sinuously traverses the procorpus (Figs. 1A, E, 15–17) and becomes surrounded by sphincter muscles at the

anterior of the metacorpus (Fig. 18). Nuclei within the procorpus are cellular components of the protractor muscles and somatic cells of the procorpus (Figs. 1A, E, 17).

The anterior of the procorpus has membrane junctions that provide support for the lumen wall and the components of the procorpus, including the dorsal gland extension and the valvular mechanism that is located in the ampulla (Figs. 1A, D, 15). The secretion granules within the dorsal gland extension are electron dense and smaller than those in the subventral gland extensions where larger granules have dense centers and electron-lucent peripheral areas (Figs. 1A, D, E, G, 17, 26, 27).

The procorpus–metacorporal valve

Between the procorpus and the metacorpus lies a sphincter valve that consists of multiple-oriented muscle fibers through which the alimentary canal and the dorsal gland extension traverse (Figs. 18, 19). The esophageal lumen, as part of the alimentary canal, is circular in cross section (Fig. 20). The lumen is formed by a thick wall that changes from circular to triradiate within the relaxed valve of the metacorpus pump (Figs. 1F, 18, 22, 23).

Parallel arrays of electron-dense membranes occur at the anterior of the metacorpus (Figs. 18–21). The membranes are associated with muscle elements that are part of the metacorpus pump. The muscle elements attach to regions along the sclerotized walls of the pump valve and extend outward in a radiating pattern to the basal membrane of the lateral walls of the metacorpus and to regions anterior and posterior to the pump valve. At these equidistant locations the sclerotized ribs on the valve wall are attached with membrane junctions to adjacent supporting cells. Between these attachments, a pair of muscle elements are attached to the pump valve wall by hemidesmosomes (Figs. 18, 22, 23). The distal ends of each muscle element are attached to the basal lamella of the metacorpus via hemidesmosomes (Figs. 22, 23). The 3 inflexible attachments between the ribs and adjacent wall cause the bulb to form a triradiate structure when the intervening muscle elements are relaxed (Fig. 23).

The metacorpus is multicellular and contains nuclei, mitochondria, ribosomes, endoplasmic reticulum, and Golgi. Longitudinal sections through the metacorpus illustrate the muscle-to-lumen wall attachments and show the anteriad and posteriad orientation of the muscle elements (Figs. 18, 22). Extending posteriad from the pump chamber, the lumen of the esophagus branches into 2 canals that enter the ampullae of the subventral gland extensions (Figs. 22, 24, 25). Access to the glands is mediated by the subventral gland valves, which are tetraradiate in cross section when closed but form a bulbous shape when open (Figs. 22, 24).

The posterior region of the metacorpus includes the metacorpus–isthmus sphincter valve that consists of 3 sectors of muscles through which the dorsal and 2 subventral gland extensions traverse (Figs. 1G, 22, 25, 26). In cross section, muscle fibers, some of which contact the wall of the triradiate esophageal lumen, surround each gland extension.

Midway between the sphincter and nerve ring, the subventral gland extensions are distended and filled with enlarged secretion granules (Figs. 25, 25A, 27). Alternatively, the dorsal gland extension is narrow and contains small electron-dense secretion granules (Figs. 25, 26). The subventral and dorsal gland extensions are moderately restricted as they traverse through the nerve ring (Figs. 30, 31). However, beyond the nerve ring, the gland extensions are unrestricted and aligned parallel to each other before merging with their respective gland cells.

Nerve ring

The nerve ring, or the circumesophageal commissure, forms a collar-like band in the isthmus region of the nematode (Figs. 1A, 30–33). The dorsal region of the nerve ring is slanted obliquely and anteriad when viewed in longitudinal section (Fig. 32). The nerve ring consists of a myriad of nerve endings in the form of axons and dendrites filled with neurosecretory granules. Neurons have membrane-to-membrane contacts and synapses with one another (Fig. 32). The ventral portion of the nerve ring extends posteriad to form the ventral ganglion (Figs. 32, 34, 35). A portion of the ventral ganglion merges with lateral nerve fibers that traverse between the subventral somatic muscles and cuticle. These nerve fibers form a lateroventral commissure that is observable as a multiple layer of fibers adjacent to the cuticle when the nematode is viewed in cross section (Fig. 35). In a tangentially oriented longitudinal section through the ventrosublateral regions of the nematode, these neurons form a

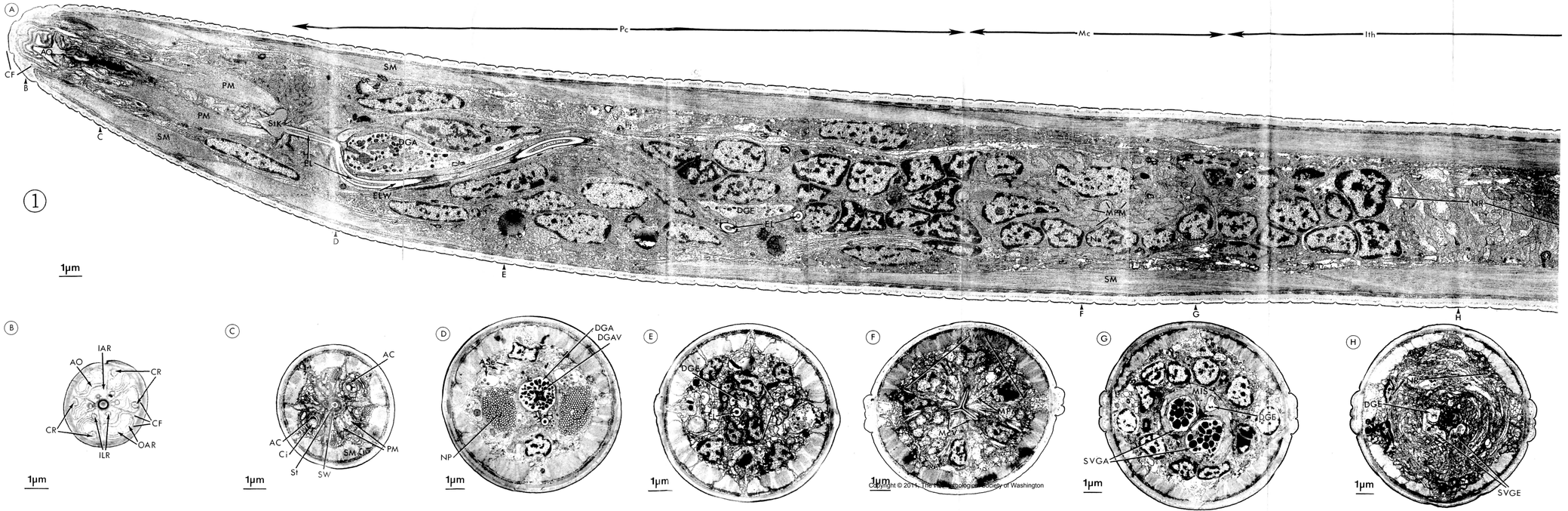

Figure 1 (foldout). Longitudinal and selected cross-sectional views of the anterior region of an infective J2 of *Meloidogyne incognita*. A. Longitudinal section of a submedian lateral view of the nematode showing parts of the cephalic framework (CF), amphidial opening (AO), somatic muscles (SM), stylet protractor muscles (PM), and stylet knobs (StK). The cuticularized lumen of the esophagus, which is branched just below the stylet knob, forms a tetraradiate valve that leads to the ampulla (DGA) of the dorsal gland extension (DGE). Beyond the branch, the cuticularized well (ELW) of the esophageal lumen (EL) extends sinuously through the length of the procorpus (Pc) and opens into the triradiate lumen of the metacorpus valve. Features of the metacorpus (Mc) include the pump muscles (MPM) and nuclei (Nu). Nuclei near the isthmus (Ith) are bordered by nerve fibers of the nerve ring (NR). B. Cross section slightly below the cephalic framework (CF) shows sensory organs that include the inner labial receptors (ILR), the cephalic receptors (CR), inner accessory receptors (IAR), amphidial openings (AO), and components of the outer accessory receptors (OAR). C. Section through the anterior part of the stylet cone (St) showing the stomatal wall (SW), stylet protractor muscles (PM), somatic muscles (SM), and amphidial canal (AC), each of which contain cilia (Ci). D. Section through the dorsal gland ampulla (DGA) showing the closed tetraradiate valve (DGAV) of the esophageal lumen branch, axons of the amphidial sensillae (ASe), and nerve processes (NP). E. Section through the procorpus (Pc) showing a cross section of the esophageal lumen (EL), dorsal gland extension (DGE), and associated nuclei. F. Section near the center of the metacorpus (Mc). Because the section is not medial through the pump, the arms of the triradiate valve (McV) are unequal in length. Muscle fibers (MPM), attached to the outer cuticular wall of the pump, extend radially to the basal lamella of the metacorpus. Similar pump muscles are shown in cross section and longitudinal sections in Figure 1A. G. Section through the posterior region of the metacorpus showing ampullae of the subventral glands (SVGA) and dorsal extensions (DGE) that are enclosed by the metacorporeal–isthmus sphincter muscles (MIS). H. Section through the nerve ring (NR) showing the narrow channels of the dorsal (DGE) and subventral gland extensions (SVGE) that are completely surrounded by axons and dendrites of neurons that are filled with neurosecretory granules.

commissure of tightly arranged nerve fibers just under the cuticle anteriad to the cuticular pore of the excretory gland (Fig. 33). A midlongitudinal section shows the lenticular shape of the lateroventral commissure, usually termed a hemizonid (Fig. 34).

The excretory gland

The excretory gland, which is a cone-shaped body, has a median excretory duct that lies in the ventral region of the nematode (Fig. 37). The base of the excretory gland is located posteriad to the nerve ring (Fig. 34). The anterior of the excretory gland is broad near the outlet of the duct (Fig. 37). As it extends posteriad, the gland tapers and shifts from a centralized ventral position to a median-lateral site adjacent to the dorsal esophageal gland extensions. The median excretory duct is part of the invaginated cuticle that forms the excretory pore or pit (Figs. 37, 37A, 39). As shown in other nematode species (Narang, 1972), the cuticular layers of the excretory pore and the duct are in reverse order to the layers of the body cuticle from which they are perceived to be derived. The pore of the excretory duct is formed and supported by layers of the cuticle and membrane junctions related to the hypodermis (Figs. 37, 37A, 39). The excretory duct, which extends medially through the length of the excretory gland (Fig. 37), differs from the cuticle that lines the excretory pore (Fig. 37A). The cuticle of the pore wall is relatively uniform and continuous with several layers of the body cuticle, whereas the cuticular wall of the median duct is porous and is more electron dense (Fig. 37A). As the excretory duct extends into the excretory gland, the duct wall has a distinct radially oriented porocity throughout its length (Fig. 37, 37B, C). From the point at which the excretory duct enters the cone-shaped base of the excretory gland, membranes of apparent hypodermal origin invaginate into the excretory gland. These hypodermal membranes are closely associated with the excretory duct surface by direct contact and by evaginations into the surrounding cytoplasm of the excretory gland (Fig. 37C). The membranes along the duct surface evaginate to form tubular and spherical membrane complexes throughout the cytoplasm of the excretory gland (Fig. 37, 37C). These complexes greatly increase the surface areas of the hypodermal membranes.

Esophago–intestinal valve

The triradiate esophageal lumen extending from the metacorpus pump valve (Fig. 24) becomes a flattened tubular channel within the confines of the nerve ring of the nematode (Figs. 27–29). Each end of the flattened cuticular esophageal canal is attached to membranes of adjoining epithelial cells (Fig. 30). More posteriad, the cuticular lumen wall is replaced by apposed membranes of 2 esophageal cells that form the esophago–intestinal valve. The lateral boundaries of these cells are supported by membrane junctions. Thus, the esophago–intestinal valve consists of noncuticularized membranes of 2 adjacent esophageal cells. During food ingestion, these noncuticularized membranes apparently separate (Fig. 30). The valve cells lie adjacent to and are supported laterally by cells of the intestinal epithelium (Figs. 27, 31).

Intestine

Posteriad from the esophago–intestinal valve, the intestinal epithelium forms a dilated irregular lumen. In cross section, the intestinal epithelium consists of 3 cells that are joined at their lateral surfaces by membrane junctions (Figs. 31, 40). The membranes between junctions constitute the boundary of the intestinal lumen. The nucleated cells of the anterior portion of the intestinal epithelium contain moderate numbers of mitochondria, ribosomes, and endoplasmic reticulum. A cross section through the dorsal and subventral gland extensions shows areas of the intestine where 1 of the epithelial cells is filled with lipid droplets, as well as other cellular components (Fig. 40). Lipid droplets in this region tend to be electron lucent; in some cross sections of the epithelium, they appear to occupy most of the space of the epithelial cells. The intestinal lumen surface of these J2 infective juveniles is smooth and without microvilli (Figs. 37, 40). The cell bodies of the esophageal glands consist of a dorsal and 2 subventral glands that are distinct and separated by intact membranes (Figs. 40, 41). The dorsal gland has an enlarged nucleus surrounded by numerous clusters of Golgi apparatus dispersed among parallel arrays of rough endoplasmic reticulum. This cell contains scattered small electron-dense secretion products (Figs. 41, 42). The subventral glands that lie posteriad and partially overlapping with the dorsal gland have organelles comparable to those in the dorsal gland but differ in that the nuclei are not

as prominent and that the secretion granules are larger and more numerous (Fig. 43). The relative density and sizes of the secretion granules within the cell bodies of the 2 types of gland cells are consistent with the secretion granule population observed in their corresponding gland extensions and ampullae (Figs. 30, 31, 37).

Discussion

Previous observations of the anterior sensory organs of *Meloidogyne incognita* juveniles and males provided information on the anatomy of the cephalic and stylet region of root-knot nematodes and related tylenchid species (Baldwin and Hirschmann, 1973; Wergin and Endo, 1976; De Grisse, 1977; Endo and Wergin, 1977). Ultrastructure of the cephalic and stylet regions of *M. incognita* juveniles was reported during the study of the anterior sensory organs (Wergin and Endo, 1976; Endo and Wergin, 1977); however, this study emphasized the cephalic and stylet regions in conjunction with that of the esophagus.

Stylet

The subterminal, ventrally located pore of the stylet is the anteriormost opening of the nema-

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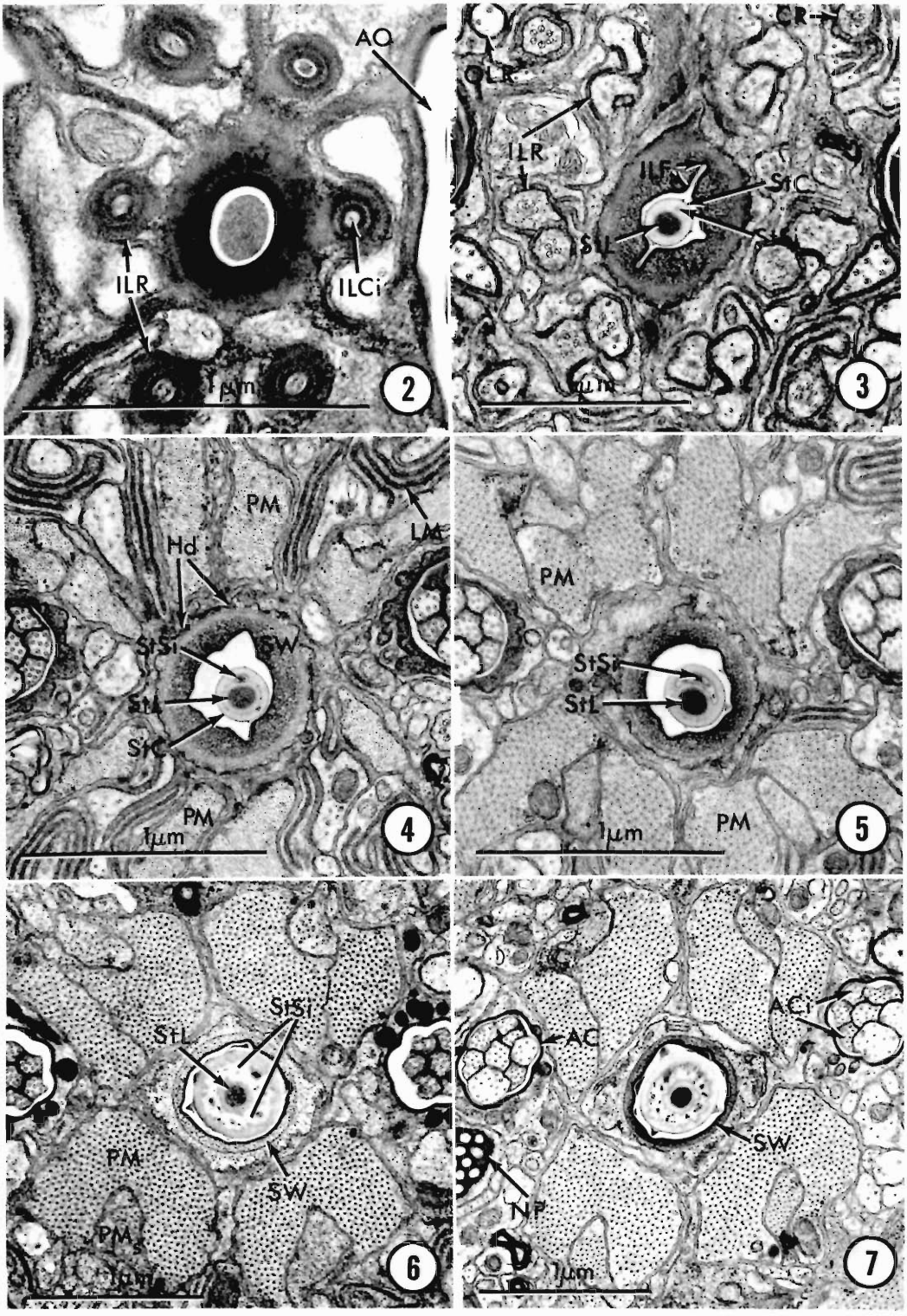
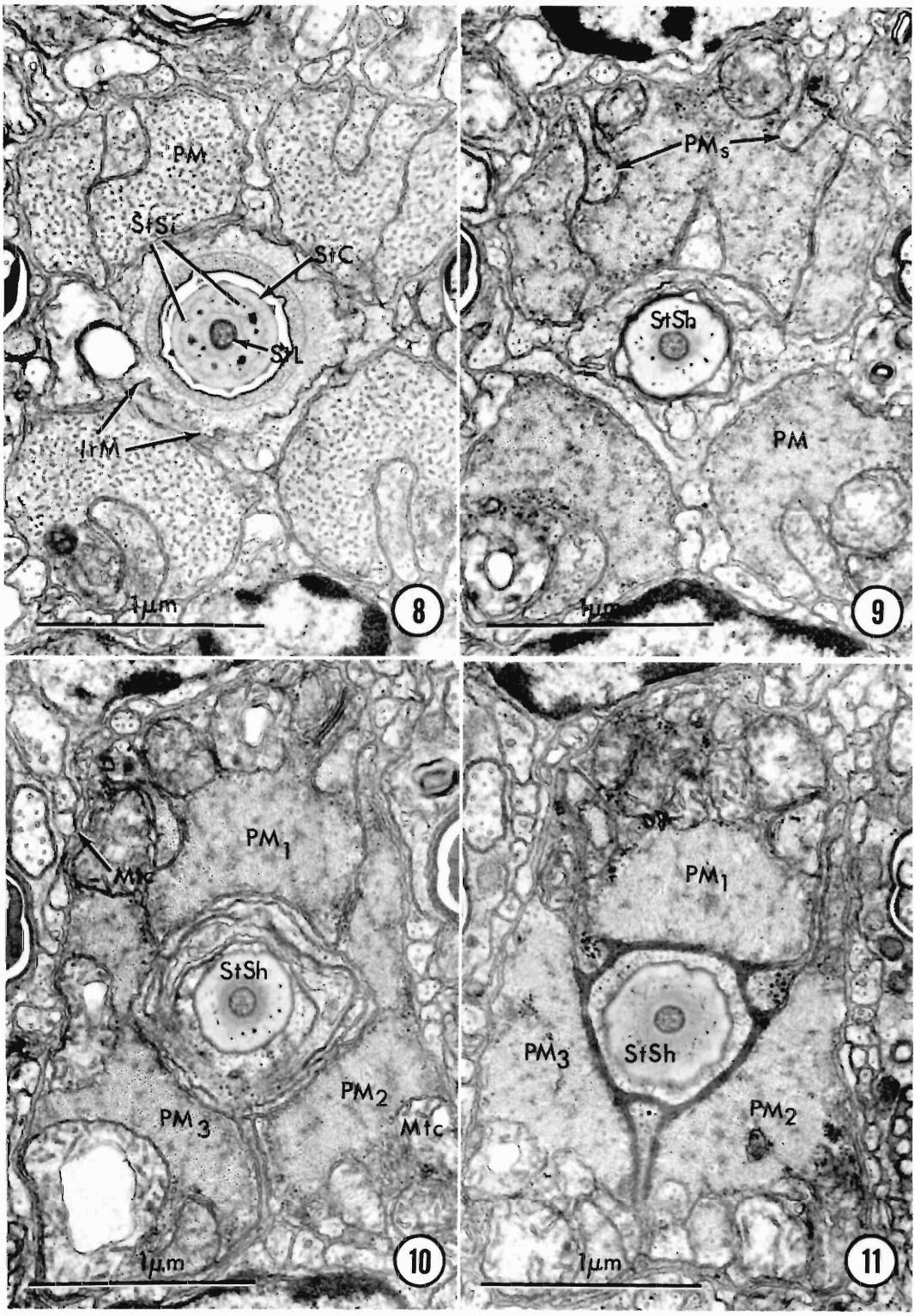
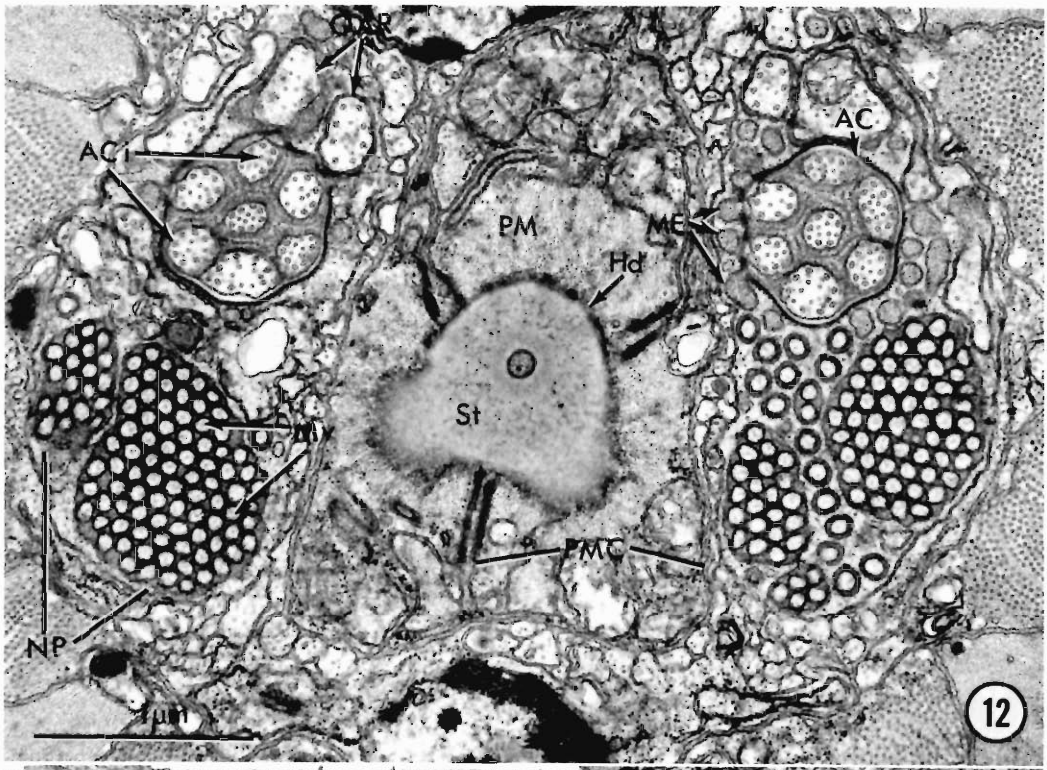
Figures 2–7. Cross sections of infective J2 *Meloidogyne incognita* show relationships among anterior sensory receptors, stomatal wall, stylet, and stylet protractor muscles. 2. Cross section shows the terminus of the stomatal wall (SW) just anterior to the retracted stylet. Six inner labial receptors (ILR) are bordered laterally by the amphidial opening (AO). ILCi, inner labial receptor cilium. 3. Section showing the stylet cone (StC) with a large lumen (StL) and a vestige of the sinus (StSi). The stomatal wall (SW) has invaginated longitudinal folds (ILF) that apparently allow for stomatal wall expansion during stylet projection. Basal body regions of cilia of the inner labial receptors (ILR), outer labial receptors (OLR), and cephalic receptors (CR) are shown. 4. Section below the cephalic framework shows the stomatal wall (SW) with a broad aperture and a stylet cone (StC). The stylet lumen (StL) has a large diameter as compared to total diameter of the stylet cone and the sinus (StSi). Stylet protractor muscles (PM) have lateral membrane contacts with the cylinder shaped stomatal wall through hemidesmosomes (Hd). These same muscle elements spread anteriad to contact the cephalic framework and the somatic muscles. Lamellar membranes (LM) associated with ciliated neurons occur in the intermuscular spaces. 5. Section through the stylet cone showing the crescent-shaped sinus (StSi) and circular stylet lumen (StL). Protractor muscle (PM) elements, dorsal and ventral to the stomatal wall, have apposed membranes. The 10 muscle elements in this cross section extend posteriad, merge into 3 groups, and attach to the stylet knobs. 6. Section through the stylet shaft shows a narrow stylet lumen (StL) and a broad sinus (StSi) that encircles the stylet lumen wall. Stomatal wall (SW) is reduced in thickness. The stylet protractor muscle (PM) elements are reduced to 6 major units plus secondary elements (PMs). 7. Section similar to Figure 6 showing reduced stomatal wall (SW) thickness and irregular boundary of supporting material. The anterior boundary of the nerve process (NP) is shown ventral to an amphidial canal (AC). ACi, amphidial cilia.

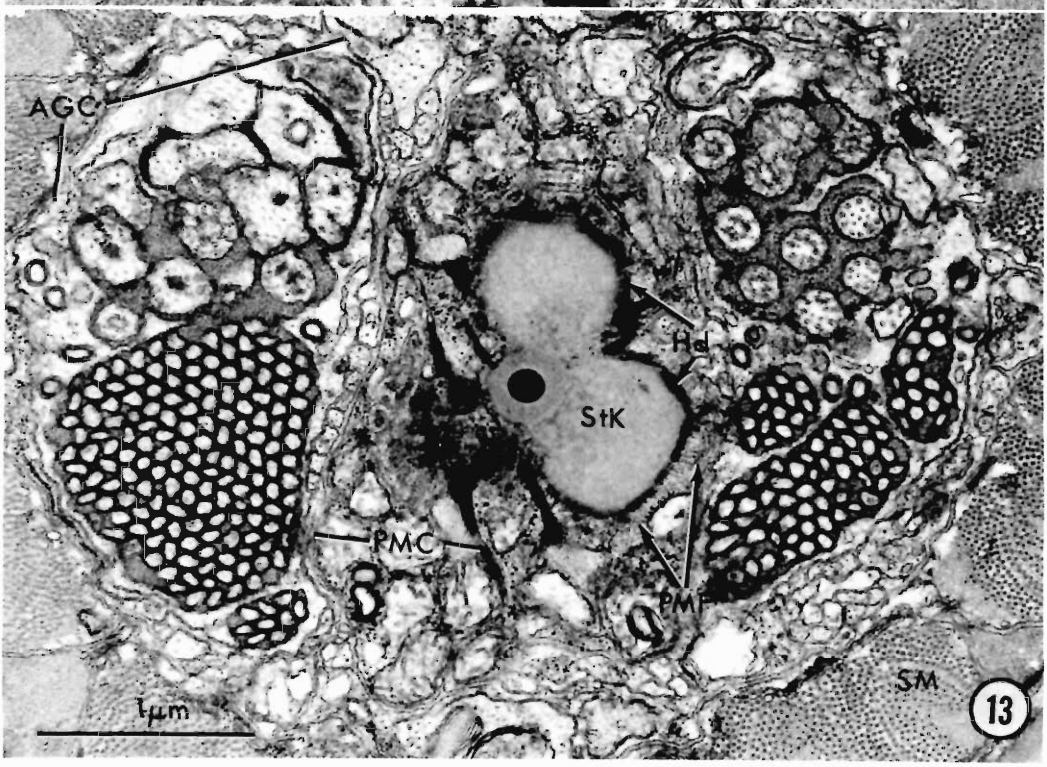
Figures 8–11. Cross sections of J2 *M. incognita* show transition of stylet cone to stylet shaft and the merging of stylet muscle units to form the tripartite muscles located anteriad to the stylet knobs. 8. Section showing enlarged sinus (StSi) of the stylet cone (StC) and the stylet central lumen (StL). The stomatal wall is thin and completely surrounded by a region of moderate electron density, which is supported by an irregular membrane (IrM). PM, protractor muscle. 9. Section near the stylet knobs below the sinus region. The shaft (StSh) is no longer surrounded by the stomatal wall. The 4 protractor muscle (PM) elements dorsal to the stylet begin to merge and the 2 subventral muscle elements retain their integrity. Secondary muscle (PM's) fibers occur in each muscle sector. 10. Section through the stylet shaft (StSh) showing multiple membranes that encircle the stylet shaft. Protractor muscles (PM) show a tripartite arrangement with groupings at a dorsal and 2 subventral positions. The numerous mitochondria (Mtc) at the periphery of the muscle elements are part of the sarcoplasm of these muscles. 11. Section shows the membrane junctions that support the tripartite protractor muscles (PM) surrounding the stylet shaft (StSh). Membrane junctions with intermembrane spaces separate the muscle elements as they extend posteriad to the surfaces of each of the 3 knobs of the stylet.

Figures 12, 13. Sections show the structure of stylet knobs and adjacent sensory cilia and microvilli within the amphidial gland of J2 *M. incognita*. 12. Section of stylet (St) showing the hemidesmosomal (Hd) contact of the protractor muscles with the base of the stylet shaft and its transition to stylet knobs. Membrane junctions and intermuscular spaces show well-defined boundaries between the 3 muscle elements supporting each sector of the tripartite stylet knobs. Amphidial canals (AC), each with 7 cilia (ACi), lie adjacent and lateral to the protractor muscle cells (PMC). Outer accessory receptors (OAR) and microvilli (Mv) of the nerve processes (NP) lie dorsal and ventral, respectively, to the amphidial canal. The amphidial canal shows membranous evaginations (ME) into the amphidial gland cell. 13. Section through stylet knobs (StK) shows the hemidesmosomes (Hd) that connect the stylet knob surfaces and the protractor muscle fibers (PMF). Amphidial gland cells (AGC) enclose the basal region of the amphidial and outer accessory receptor cilia and the nerve processes. SM, somatic muscles; PMC, protractor muscle cell.









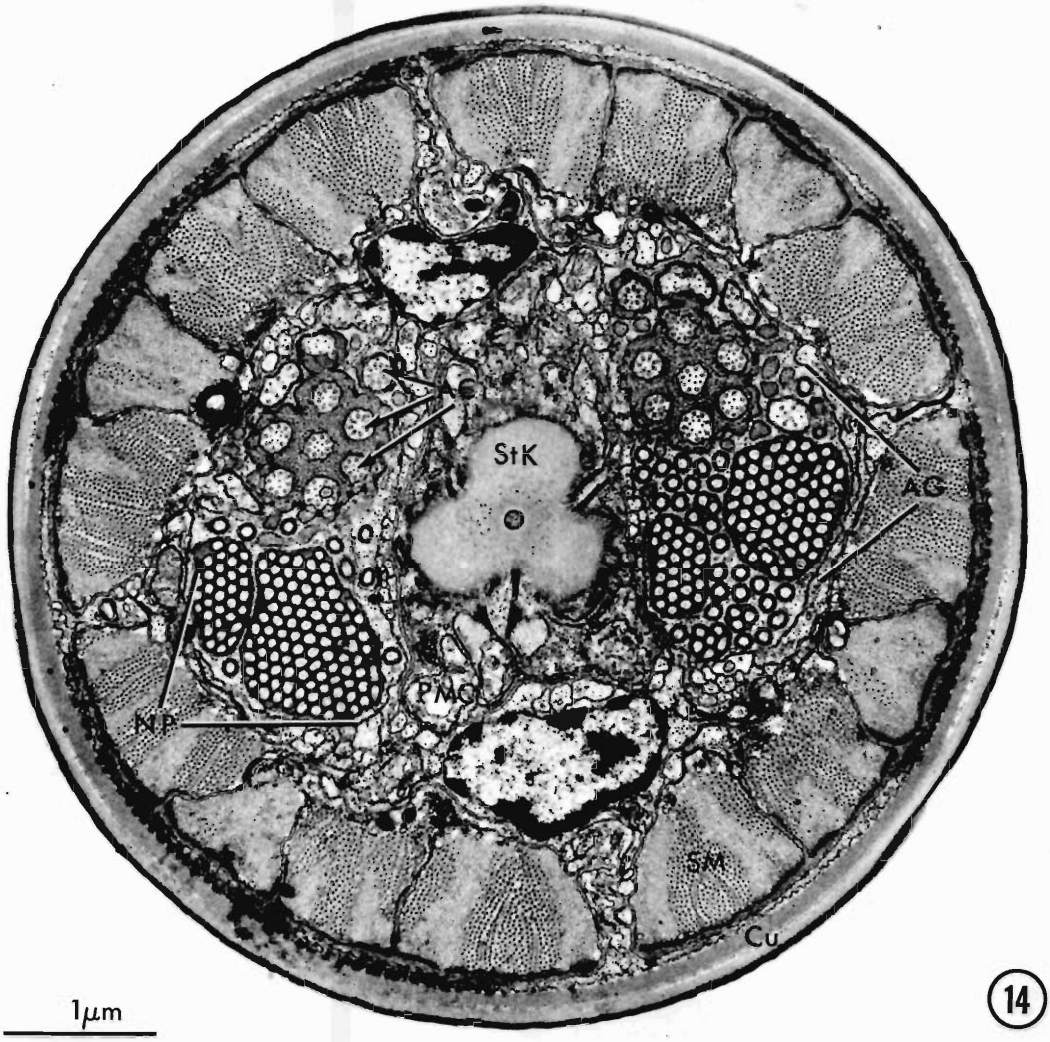


Figure 14. Cross section of J2 juvenile *M. incognita* showing the stylet knobs (StK) and amphidial gland (AG) with cilia (ACi), nerve processes (NP), and the somatic musculature (SM). This figure shows a section about midway between sections shown in Figures 12 and 13. Cu, cuticle; PMC, protractor muscle cell.

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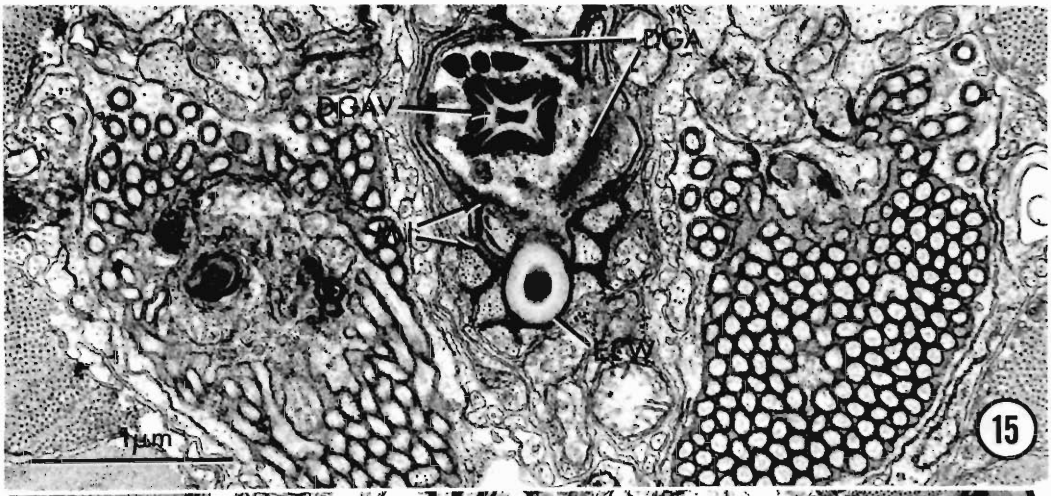
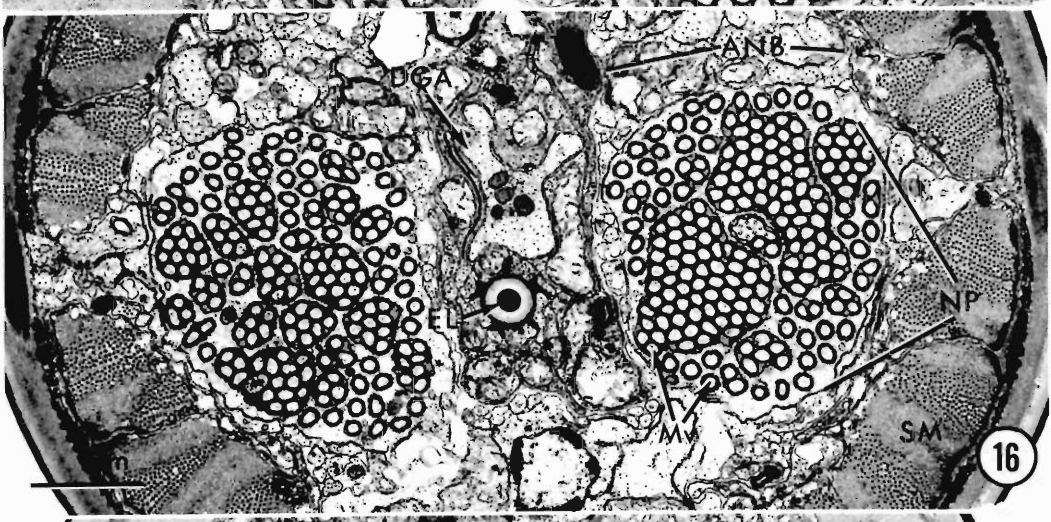
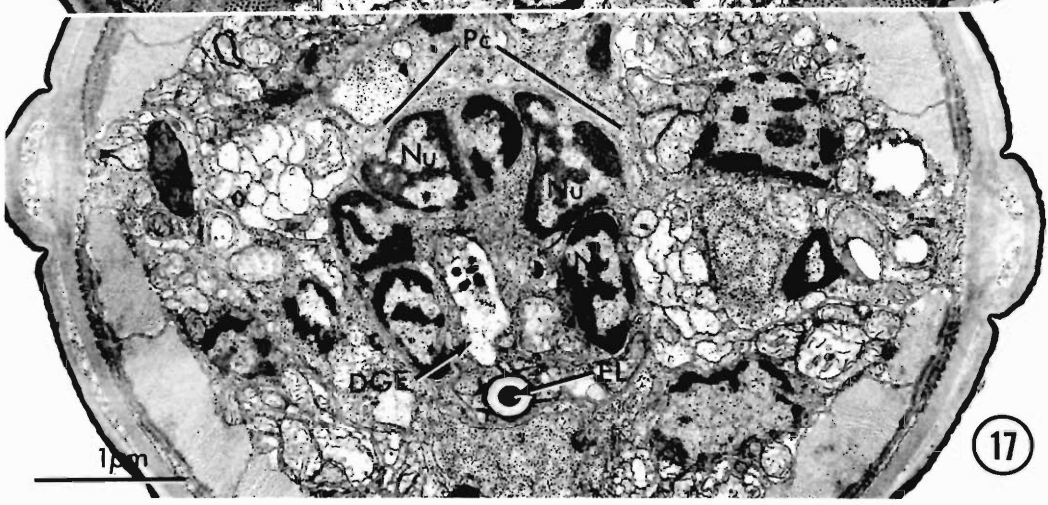
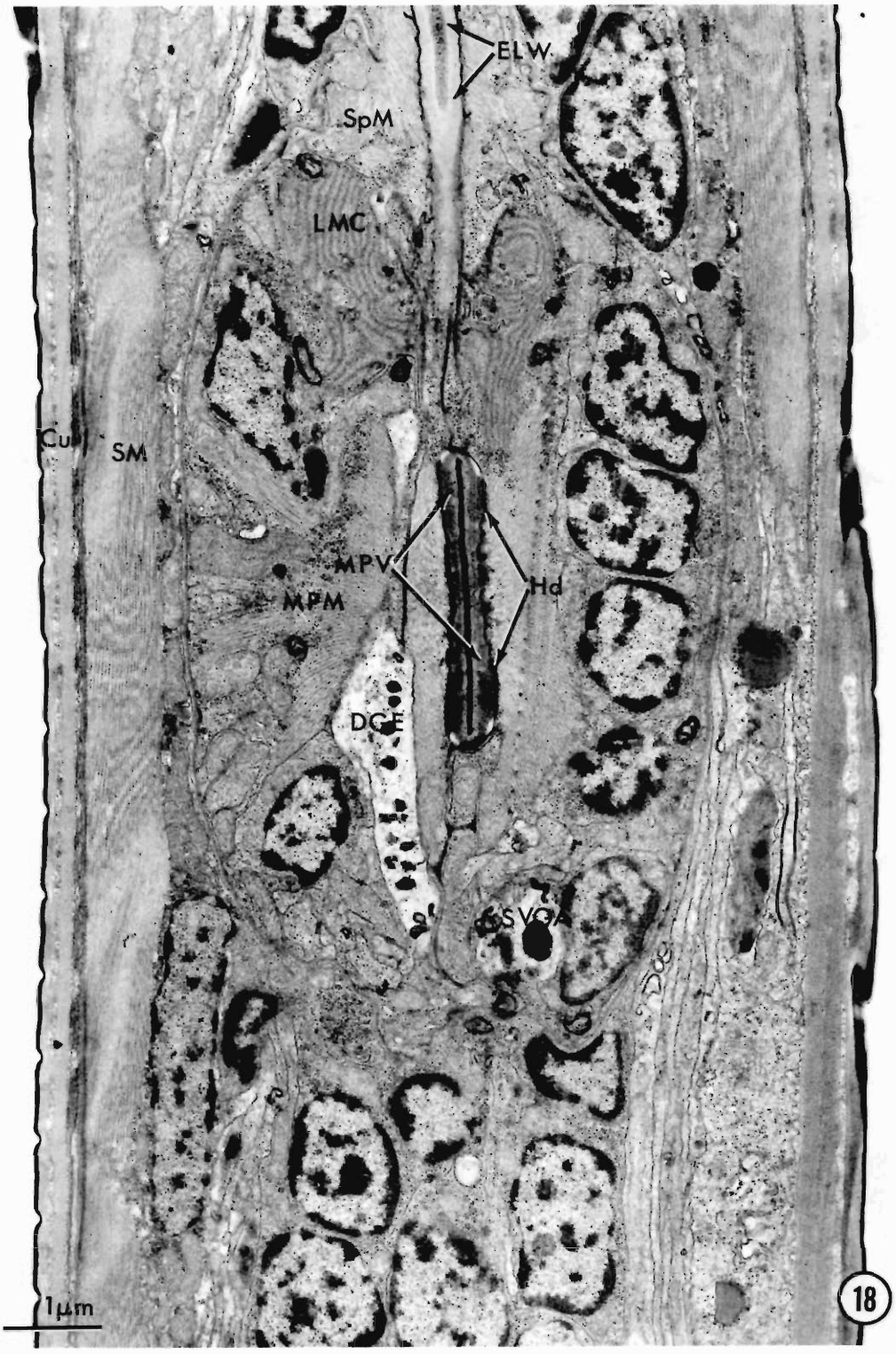
Figures 15–17. Cross sections of J2 *M. incognita* showing the dorsal gland ampulla with the tetraradiate valve and related sections through the gland extension and procorpus. 15. Section through the ampulla (DGA) of the dorsal gland extension showing the tetraradiate esophageal gland valve (DGAV). The ampulla is bordered on either side by the nerve bundles that support the amphidial and outer accessory receptors. Membrane junction (MJ) connects the dorsal gland extension to the esophageal lumen wall (ELW). 16. Section through the ampulla (DGA) of the dorsal gland extension. The microvilli (Mv) of the nerve processes (NP) lie ventral to the amphidial nerve bundle (ANB). EL, esophageal lumen; SM, somatic muscles. 17. Section through the procorpus (Pc) shows the dorsal gland extension (DGE) and clusters of nuclei (Nu) within the procorpus tissue. EL, esophageal lumen.









tode through which glandular secretions are emitted and food is ingested. The stylet lumen of the J2 of *M. incognita* is wider than that of the J2 of *H. glycines*: the approximate ratio of the lumen diameter to the overall stylet diameter as shown by Endo and Wergin (1977) is nearly 1:2 for *M. incognita,* whereas the ratio of the same dimensions for *H. glycines* is 1:8 (Endo, 1983). A sinus lies dorsal to the stylet lumen and extends along most of the stylet length. This sinus is similar to that found in the cone and shaft of the stylet of *Pratylenchus penetrans.* The shaft and knob components of the stylet of *P. penetrans* were designated as living tissue, whereas the tooth was considered a nonliving, sclerotized component of the stylet (Chen and Wen, 1972).

The stylets of *M. incognita* juveniles are supported by protractor muscles attached anteriorly at the base of the cephalic framework with 10 muscle fibers and by related secondary muscles that converge posteriad and attach to the tripartite stylet knobs (Endo and Wergin, 1977). The stylet of the J2 of *M. incognita* is relatively slender and lacks the robust appearance of the stylet of the J2 of *H. glycines.* For example, the approximate ratios of the average diameter of the stylet, cone, and shaft in relation to the overall length of the stylet was 1:32 for *M. incognita* and 1:18 for *H. glycines* reported by Endo and Wergin (1973) and Endo (1980). Although the number of protractor muscle fibers are comparable in both nematode species, considerable space occurs between the various protractor muscle units in *M. incognita.* In contrast, the spaces between the protractor muscle elements of J2 *H. glycines* is minimal (Endo, 1983). Muscle attachments via hemidesmosomes of protractor muscles are similar among *M. incognita* and *H. glycines* J2 and males (Baldwin and Hirschmann, 1976; Endo, 1983). Contacts are made anteriorly to the cephalic framework, laterally for support between somatic muscles, central and anteriorly for contact with the stomatal wall, and posteriorly at the stylet knob surface. Although the muscle to the stylet knob is similar between J2 of *M. incognita* and *H. glycines,* the surface interactions of the muscle to the stylet knobs of *M. incognita* are not as distinct as in *H. glycines* where extensive hemidesmosomes occur (Endo, 1983).

Procorpus

The anteriormost region of the procorpus is occupied by the ampulla of the dorsal gland extension. In contrast to males and females of *M. incognita,* the branch of the esophageal lumen in J2's forms as a single channel and terminates as a single tetraradiate valve within the dorsal gland ampulla (Baldwin et al., 1977; Eisenback et al., 1980). In males of *M. incognita,* the dorsal gland duct has a row of 3 channels that are dorsal to the esophageal lumen. Slightly posteriad, they are arranged in a triangle and within the ampulla, the channels separate and are terminated with 3 tetraradiate branches or valves (Baldwin et al., 1977). With this apparent increased capacity for flow of secretions from the ampulla of the dorsal gland, the male of *M. incognita* might undergo extensive feeding. Alternatively, males were shown to have gland lobes consisting only of 2 subventral glands and no dorsal gland. Furthermore, males show only a vestige of a dorsal gland in the anterior region of the procorpus (Baldwin et al., 1977). However, these qualifications concerning the structure-to-function relations of males would not be appropriate in describing females of root-knot nematodes. The report of females with a 3-branched channel leading from the dorsal gland extension to the lumen of the esophagus coincides with the extensive feeding requirements of a developing female (Eisenback et al., 1980).

The main lumen of the procorpus of the J2 of *M. incognita* is typical among tylenchids. The body of the procorpus is cellular and supports the protractor muscles and the procorpus. The secretion granules within the dorsal gland extension of the J2 of *M. incognita* are similar in size and density to granules observed in J2 of *H. glycines* prior to feeding (Endo, 1984).

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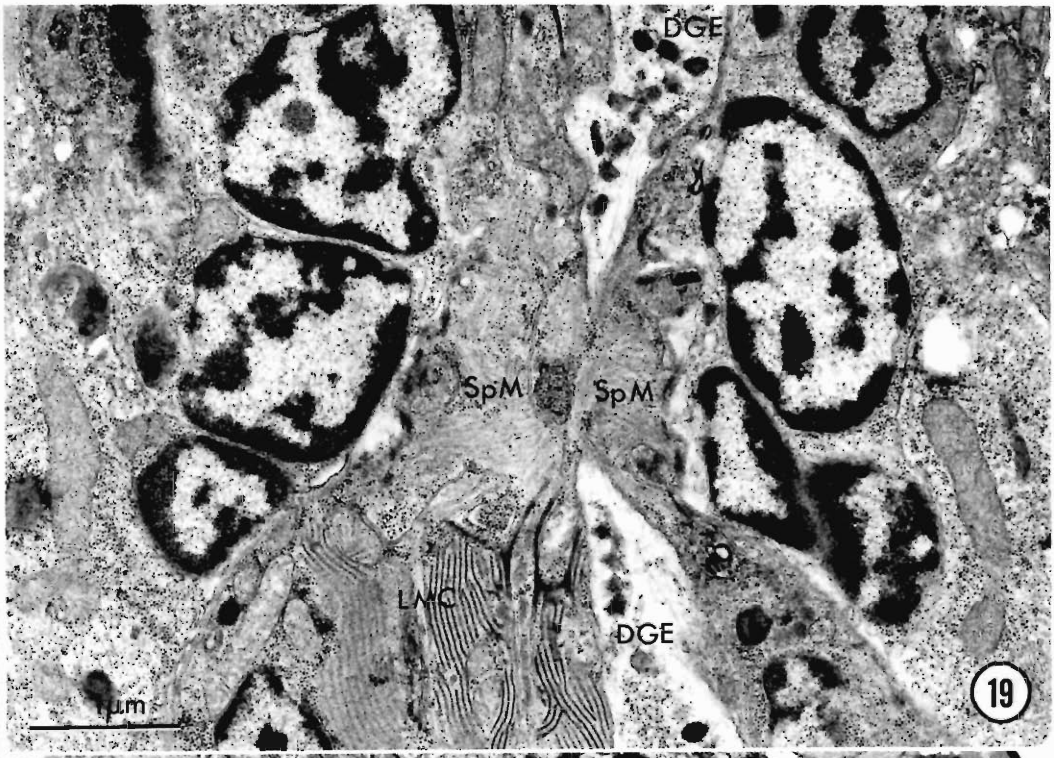
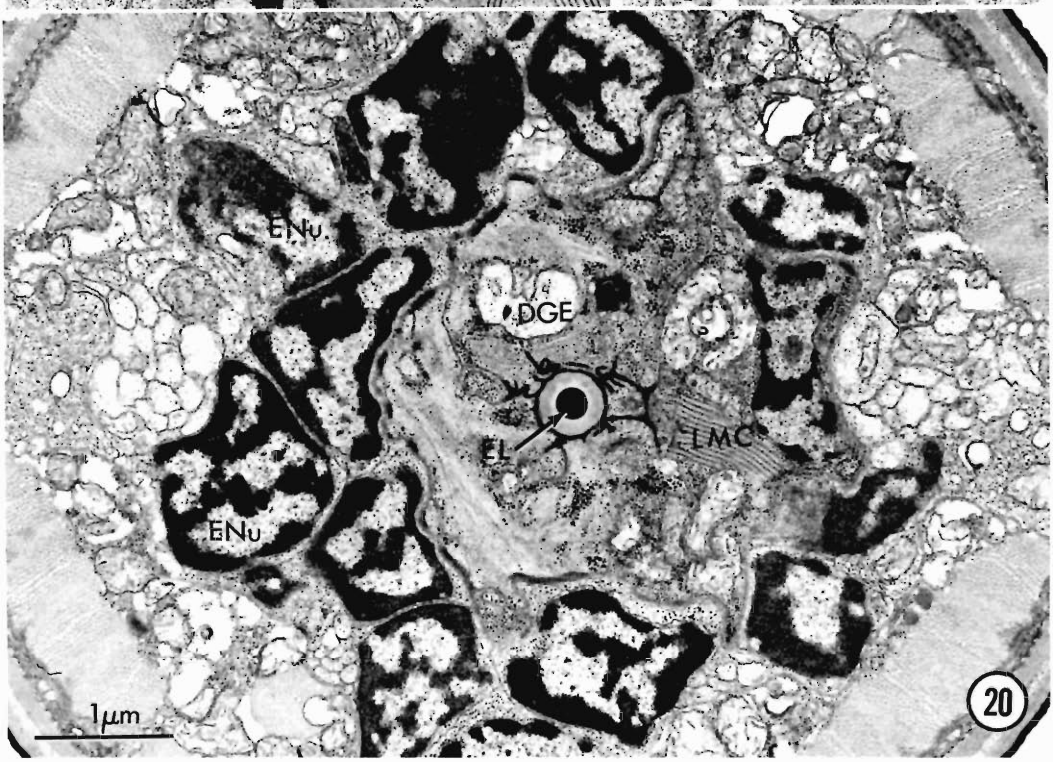
Figure 18. A submedian longitudinal section of J2 *M. incognita* through the metacorpus shows the multidirectional muscle fibers of the sphincter muscles (SpM) located at the anterior of the metacorpus. The lamellar membrane complex (LMC) apparently functions to support the metacorpus pump muscles (MPM). The wall of the valve (MPV) is attached to pump muscles through hemidesmosomes (Hd). The flow of the secretion granules of the dorsal gland extension (DGE) is controlled by the anterior sphincter and the posterior sphincter muscles of the metacorpus. Cu, cuticle; ELW, esophageal lumen wall; SM, somatic muscles; SVGA, subventral gland ampulla.





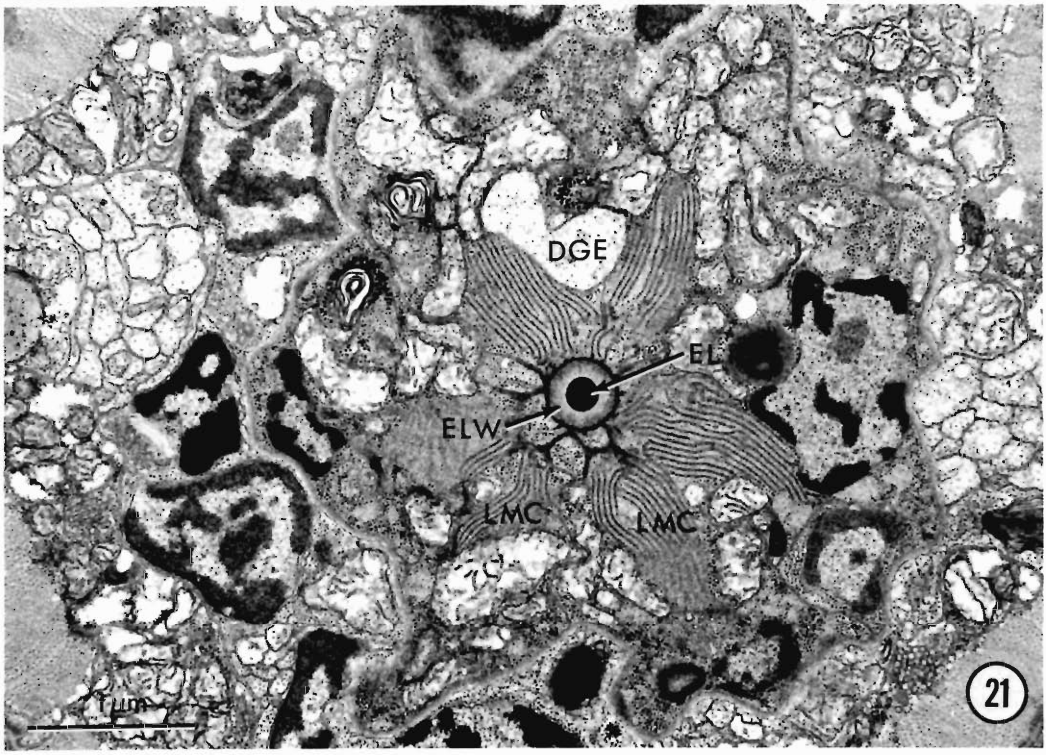


Figure 21. Cross section through the anterior region of the metacorpus in J2 *M. incognita* showing the connections between the cuticular wall (ELW) of the esophageal lumen (EL) and the bands of lamellar membrane complexes (LMC) that in turn are integrated with the metacorpus pump muscles. DGE, dorsal gland extension.

Anterior metacorpus

The sphincter muscles at the anterior of the metacorpus of *M. incognita* are similar to those described for *H. glycines* juveniles (Endo, 1984) and to the constraining muscles reported by Shepherd et al. (1980) in the esophagus of *Aphelenchoides blastophthorus*. The alignment and tight grouping of the cytoplasmic microtubules enclosed by the sphincter is not as distinct as that of *H. glycines* juveniles (Endo, 1984); however, the sphincter muscles apparently can change the aperture of the dorsal gland extension and modify the flow of secretion granules. These granules are synthesized in the dorsal gland cell and accumulate in the ampulla of the gland extension.

The metacorpus pump

The anterior region of the metacorpus consists of parallel arrays of lamellar membranes that are attached to the outer wall of the esophageal lumen. These membranes apparently function as supporting elements of the metacorpus muscles. Similar lamellar membranes were shown to be attached to the esophageal lumen of *Heterodera glycines* J2 and *Ditylenchus dipsaci* reported by Endo (1984) and Shepherd and Clark (1983).

A transverse section of the J2 of *M. incognita* shows that the valve arms of the metacorpus pump are long and slender as compared to those in the J2 of the soybean cyst nematode (Endo, 1984). Furthermore, the relative size of the

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Figures 19, 20. Longitudinal and cross sections through the anterior region of the metacorpus in J2 *M. incognita*. 19. Longitudinal submedian section through the anterior sphincter muscles is bordered posteriad with a lamellar membrane complex (LMC). The sphincter muscles (SpM) delineate sections of the dorsal gland extension (DGE). 20. Cross section through the anterior metacorpus just below the sphincter muscle. Lamellar membranes (LMC) are closely allied to membranes supporting the cuticular wall of the esophageal lumen (EL). Esophageal nuclei (ENu) that are shown as a circular cluster around the anterior metacorpus are compared to the nuclei shown in Figure 19. DGE, dorsal gland extension.

metacorpus pump of *M. incognita* is greater than that of *H. glycines*. For example, in the closed mode, the ratio of the pump valve arm diameter to total body diameter of a preparasitic J2 was 1:3 for *M. incognita* and 1:8 for *H. glycines*. Although the metacorpus valve was not observed in a fully open position, contraction of the metacorpus muscles apparently causes the triradiate pump to enlarge into a spherical chamber.

The ampullae of subventral esophageal glands are located at the subventral positions of the metacorpus just posteriad from the metacorpus valve. The tetraradiate valves, which could be observed in their closed or open positions, have morphology similar to that described for *M. incognita* males and *H. glycines* J2 (Baldwin et al., 1977; Endo, 1984).

Although the structure of *M. incognita* may differ slightly from cyst nematodes, results from the video-enhanced light microscope study of the feeding mechanism of *H. schachtii* are probably similar to those of *M. incognita*. The translocation of secretion granules in the dorsal and subventral esophageal glands were observed in *H. schachtii* J2 (Wyss and Zunke, 1985) and in *H. glycines* (unpubl.). However, video-enhanced light microscopic observations of *H. schachtii* provided opportunities for observing the opening and closing of glandular valves in conjunction with feeding of radish roots (Wyss and Zunke, 1985, 1986). After 1 hr of feeding, the sphincter muscle of the posterior metacorpus contracted and stopped the anteriad flow of secretion granules. Subsequently, the contents of the swollen ampullae valves of the subventral gland extensions were released, a process that is visible through video-enhanced light microscopy.

Presumably, the gland valve contents flowed into the esophageal lumen and the digestive process began. The third phase of feeding consisted of salivation during which time feeding tubes were secreted into the host cell. It is assumed that food ingestion takes place via these feeding tubes (Wyss and Zunke, 1985). In another study of endoparasitic nematodes, the feeding tubes were interpreted as a drainage system for the secretion of the nurse cells; nematodes were thought to feed on these secretions rather than on the cytoplasm of nurse cells (Rumpenhorst, 1984).

The presence of numerous enlarged secretion granules in the subventral glands of root-knot nematodes is consistent with earlier reports by Bird (1967) that the glands were most active during egg hatch and host penetration. In contrast, parasitic juveniles observed 1–2 days after inoculation had subventral glands with fewer numbers of secretion granules. Similarly, secretory granules of the subventral glands of infective J2 of *H. glycines* are relatively large and numerous, whereas secretion granules in the dorsal glands are much smaller (Endo, 1983). Conversely, the dorsal glands and their extensions in parasitic juveniles of *H. glycines* contain numerous enlarged secretion granules (Endo, 1987).

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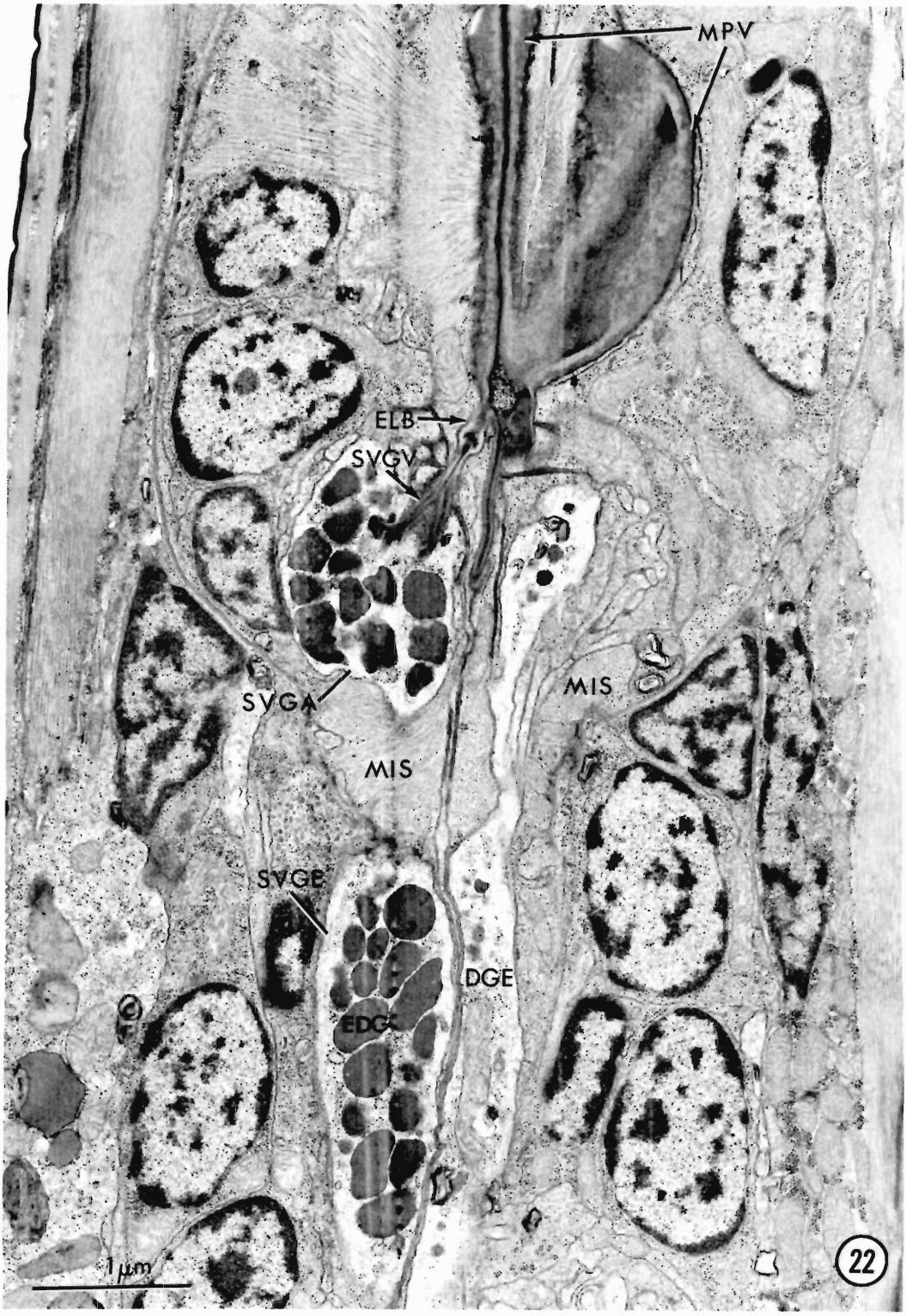
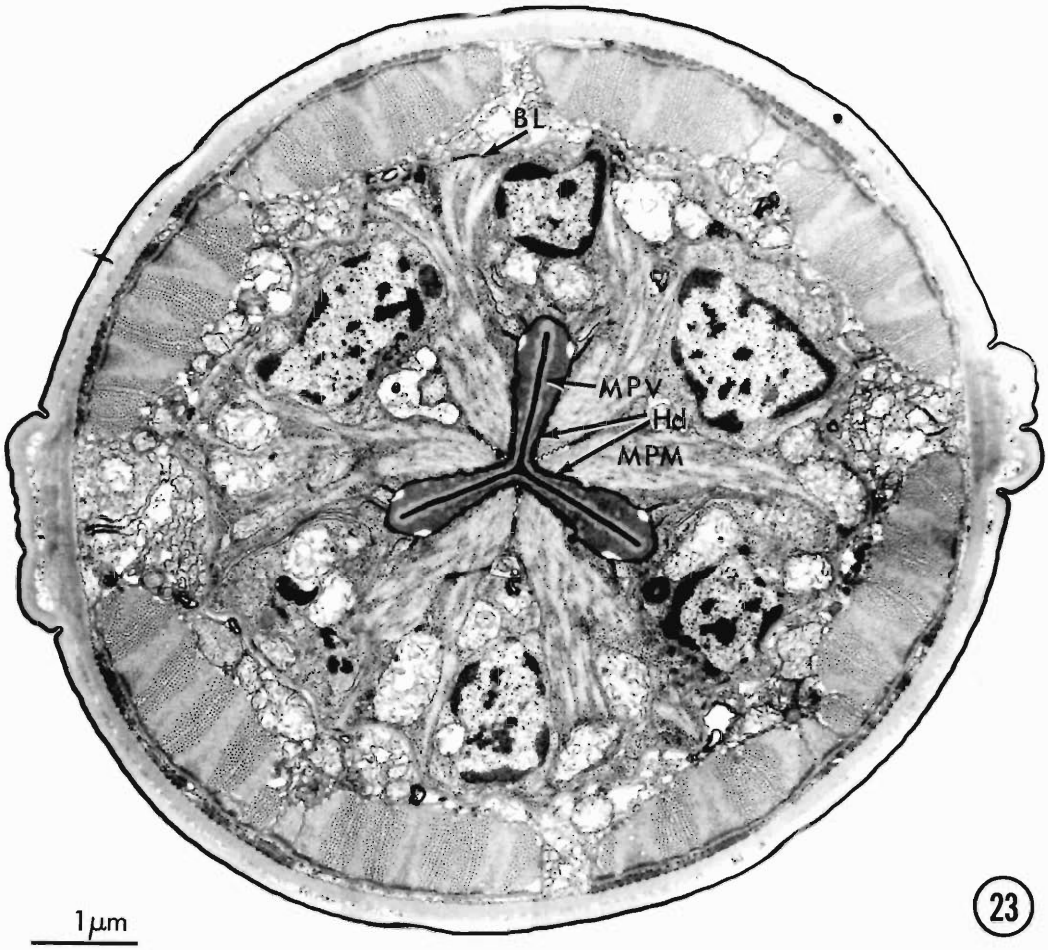
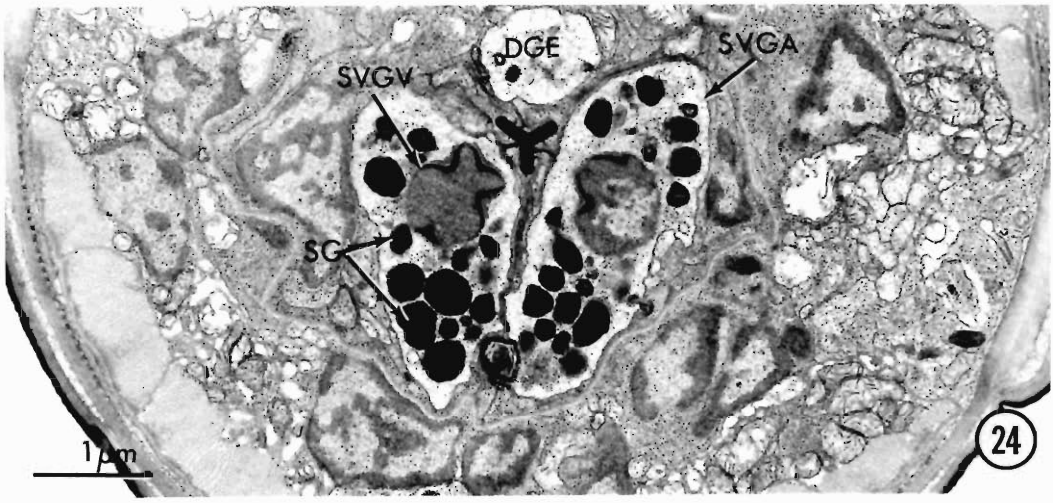
Figure 22. Longitudinal section through the metacorpus pump of J2 *M. incognita* shows the relation between the pump valve (MPV) in a closed position and the circular contour of 1 of 3 sections of the valve wall. The central lumen has 2 branches (ELB), one of which is shown as a cuticularized channel that opens as a terminal valve into an ampulla (SVGA) of a subventral gland extension (SVGE). The subventral gland valve (SVGV) is shown in a closed position. The large electron-dense granules (EDG) of the gland extension (SVGE) can be restricted to the ampulla by the metacorpus–isthmus sphincter muscle (MIS). Similarly, the sphincter muscle also surrounds the dorsal gland extension (DGE) where a slight constriction is shown.

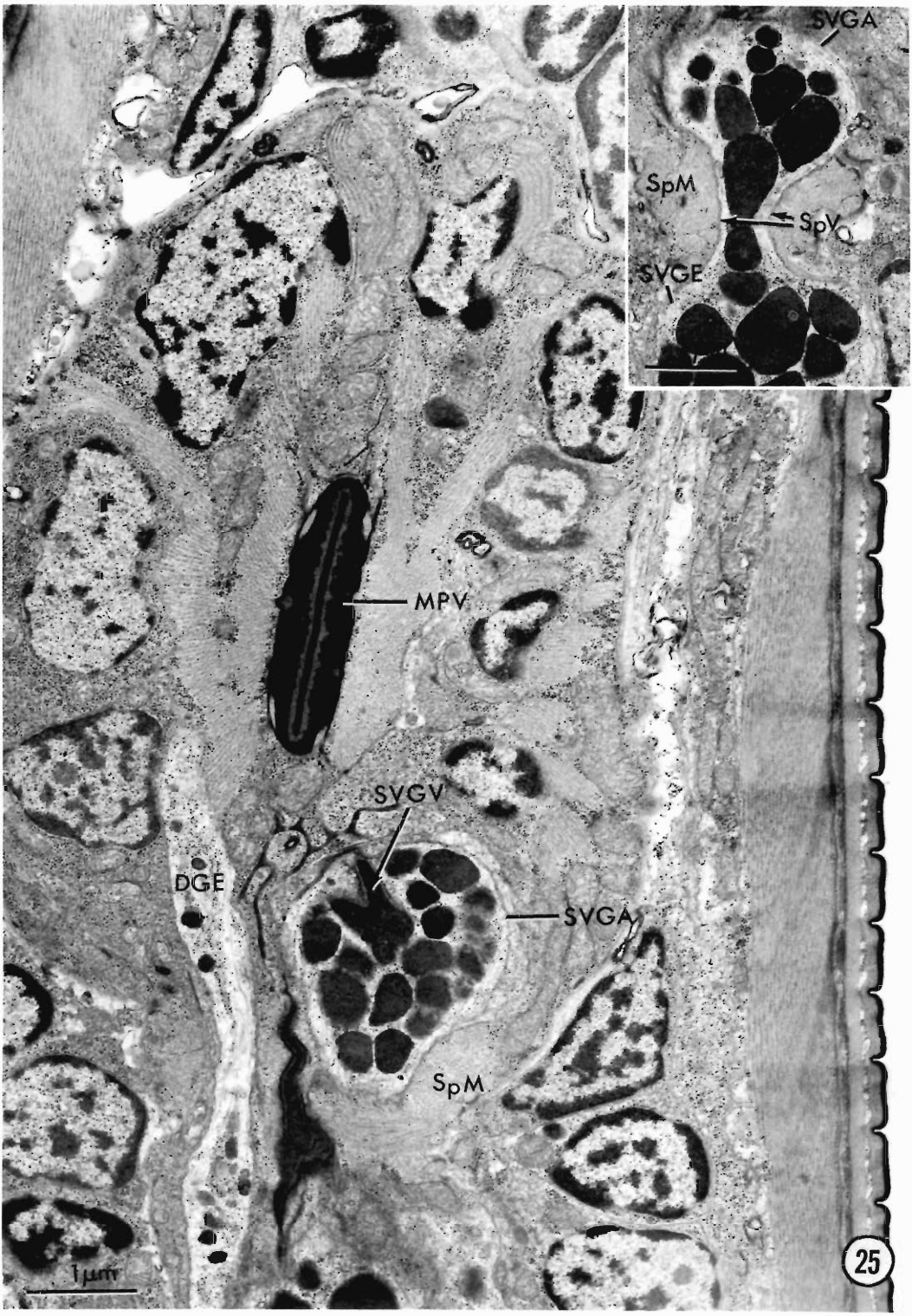
Figures 23, 24. Cross sections of the metacorpus with views of the metacorpus and the subventral gland valves in J2 *M. incognita*. 23. Cross section through the metacorpus showing the triradiate valve of the metacorporeal pump. The cuticular wall of the pump valve (MPV) is attached by hemidesmosomes (Hd) to 6 groups of muscles (MPM), 2 per sector of the triradiate valve. These muscles extend to the basal lamina (BL) and other peripheral regions of the metacorpus. 24. Cross section through the ampulla (SVGA) of the subventral gland extensions showing the gland valves (SVGV) in open positions and containing products of the secretion granules (SG) of the subventral glands. DGE, dorsal gland extension.

Figure 25. Longitudinal tangential section of the metacorpus in J2 *M. incognita* showing a cross section of 1 arm of the triradiate pump valve (MPV) with muscle attachments. The dorsal gland extension (DGE) is only slightly restricted and lies adjacent to an ampulla of a subventral gland (SVGA). The subventral gland valve (SVGV) is in a partially open position. The inset shows a median longitudinal section of a sphincter valve (SpV) that restricts the movement of secretion granules accumulating in the ampulla (SVGA) and the subventral gland extension (SVGE). Inset and Figure 25 are from the same specimen. SpM, sphincter muscle.









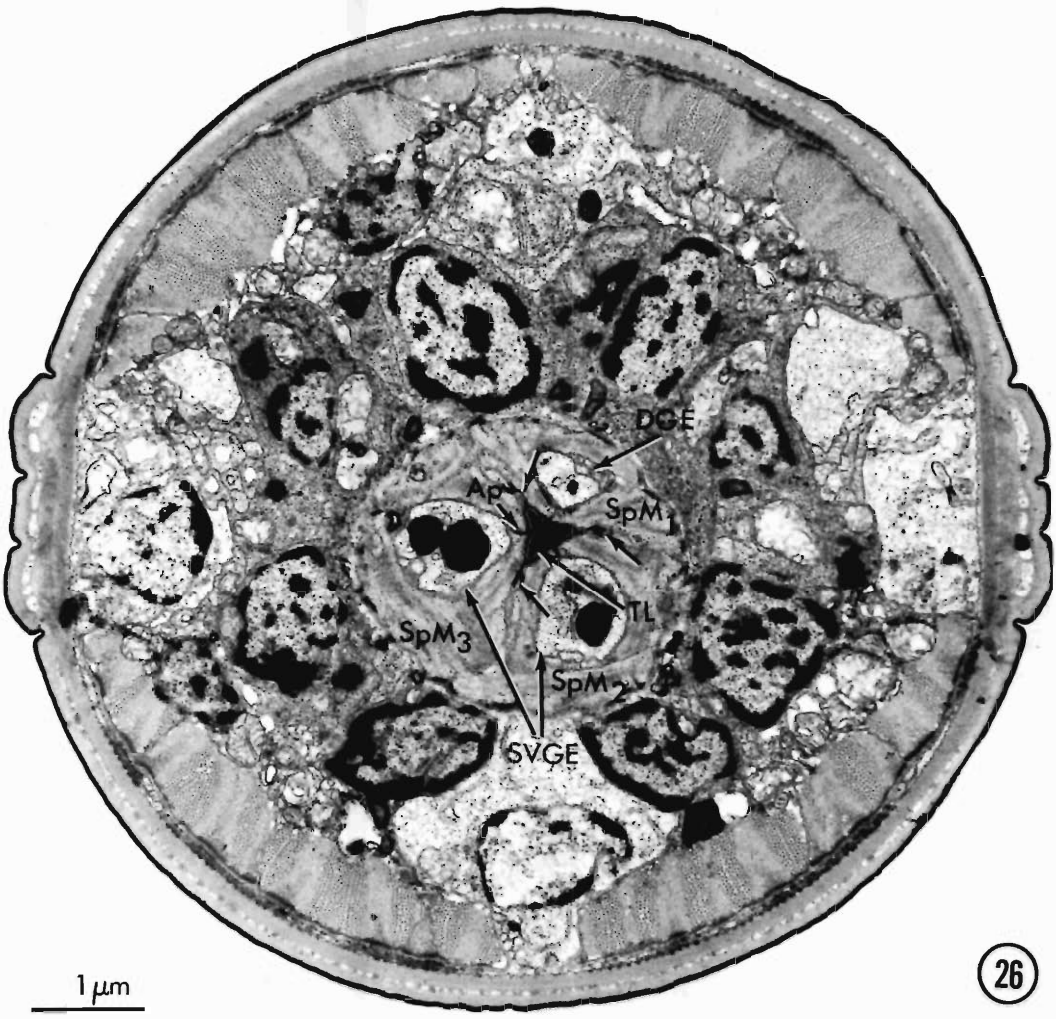


Figure 26. Cross section of the metacorporal-isthmus sphincter in J2 *M. incognita* shows criss-cross muscle fibers that encircle each of the dorsal (DGE) and subventral gland extensions (SVGE). The base of each sphincter muscle (SpM) is attached to the wall of the triradiate lumen (TL). Membrane junctions (arrows) occur between adjacent cells for a short distance away from each apex of the triradiate lumen. Distally, these cell membranes separate and show 3 sets of sphincter muscles, 1 for each gland extension. Each of these muscles are further divided at their apices (Ap) by membrane junctions that join the gland extension membranes to the interapex positions of the wall of the esophageal lumen.

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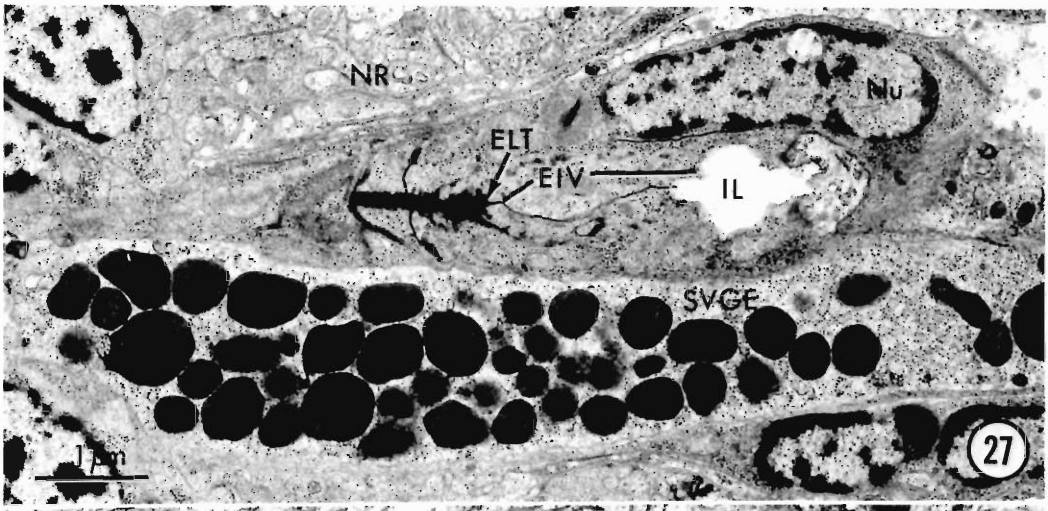
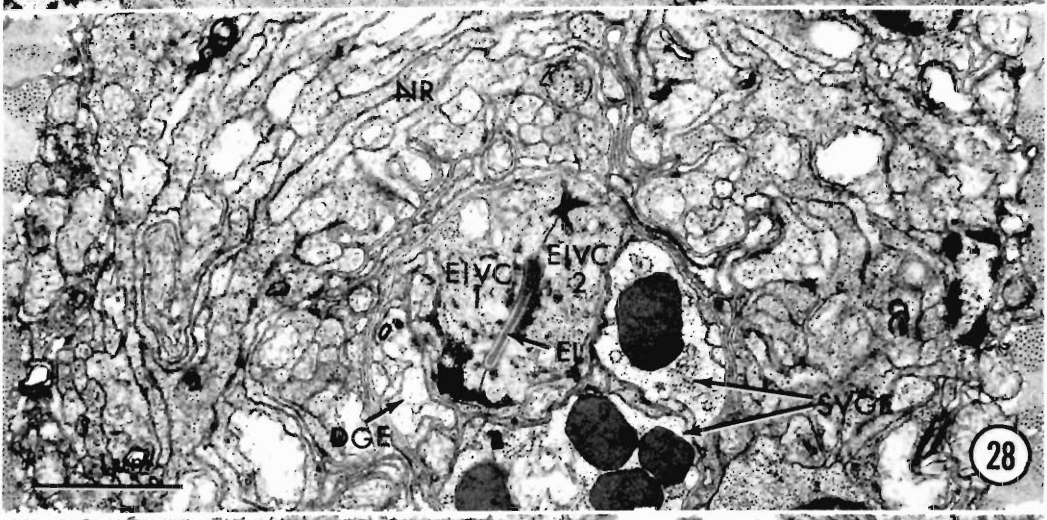
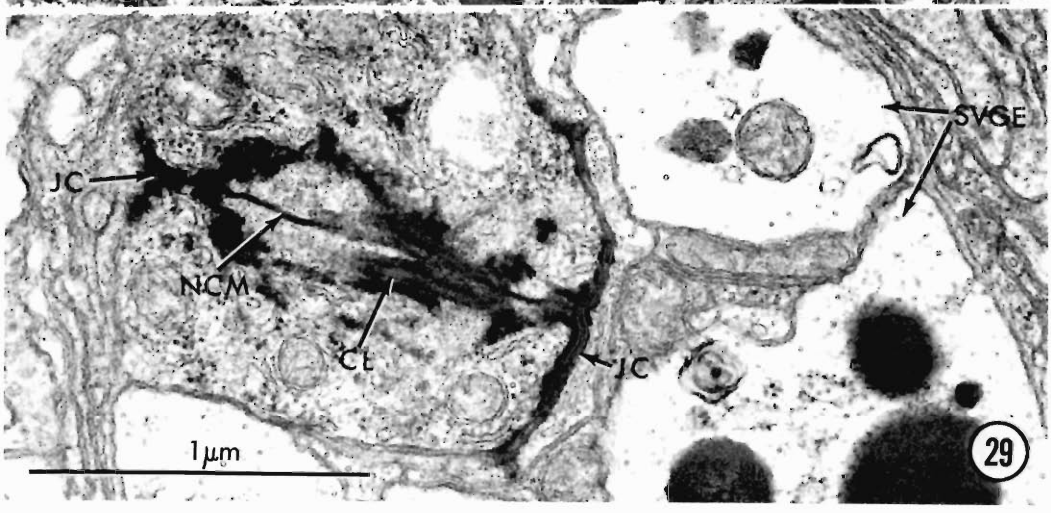
Figures 27–29. Longitudinal and cross sections with emphasis on the structure of the esophago–intestinal valve in J2 *M. incognita.* **27.** Longitudinal section showing the cuticularized terminus of the esophageal lumen (ELT) and membranes associated with the esophago–intestinal valve (EIV). The nucleus (Nu) of one of a pair of cells that comprise the EIV lies adjacent to the anterior intestinal lumen (IL). The EIV is bordered on one side with part of the nerve ring (NR) and on the other side with the subventral gland extension (SVGE). **28.** Cross section of slit-shaped lumen (EL) of the esophagus shows the transition from a cuticle-lined lumen to apposed membranes of 2 esophago–intestinal valve cells ($EIVC_1$, $EIVC_2$). The valve is surrounded by extensions of 2 subventral glands (SVGE) and a dorsal gland (DGE). Dorsally, the EIV is partially surrounded by the nerve ring (NR), consisting of membranes of axons and dendrites. **29.** Cross section at the transition between the cuticularized wall (CL) of the esophagus and the noncuticularized, apposed apical membranes (NCM) of 2 cells of the esophagus that form the EIV. Junctional complexes (JC) occur at each end of the valve. SVGE, subventral gland extensions.







Sphincter muscles

In *M. incognita,* as well as other tylenchids (Endo, 1984), the sphincter muscles at the base of the metacorpus surround the subventral and the dorsal gland extensions. This sphincter muscle, viewed in cross section, consists of 3 well-defined muscles, 1 for the dorsal gland extension and 2 for each of the subventral gland extensions. These sphincter muscles are attached to the outer wall of the triradiate esophageal lumen.

The closure of sphincter muscles described in *H. schachtii* affected the passage of secretion granules in the dorsal and subventral gland extensions (Wyss and Zunke, 1985). The localization of secretion granules in the gland extensions of *M. incognita* suggest that similar muscle action controls the flow of secretions in this nematode.

The esophago–intestinal valve

The esophago–intestinal valve in J2 of *M. incognita* is similar in morphology and location to the valve reported for males of this species (Baldwin et al., 1977). The valve is located close to the base of the metacorpus and is encircled by the nerve ring. In contrast, the esophago–intestinal valve of the J2 and males of *H. glycines* are located considerably more posteriad and are not encircled by the nerve ring (Baldwin et al., 1977; Endo, 1984). Instead, the esophago–intestinal valve lies adjacent to the dorsal gland cell nucleus. The valve mechanism of the esophago–intestinal valve for *M. incognita* is similar to the valve described for J2 of *H. glycines* and other tylenchid species. The esophago–intestinal valve has been the subject of intensive study to determine its structure and function (Seymour and Shepherd, 1974; Shepherd and Clark, 1976; Shepherd et al., 1980), and it has been suggested that the extensive desmosomal complexes between cells or cell processes are related to cell movement. However, the mechanism by which closely apposed membranes of the lumen lining of the esophago–intestinal valve are separated (unzipped) and brought together during food ingestion is not clear (Shepherd et al., 1980).

In various nematode species, the pressure of food ingestion in the lumen at pump closure could force the esophago–intestinal valve open and allow the passage of food into the intestine (Seymour and Shepherd, 1974). This concept appears reasonable for *M. incognita.* The anterior intestinal epithelium is distinctly tripartite in cross section. Cell junctions join the lateral membranes of epithelial cells, thereby sealing their boundaries and forming a lumen with their apical cell membranes. In contrast to a previous report that microvilli border the lumen of the

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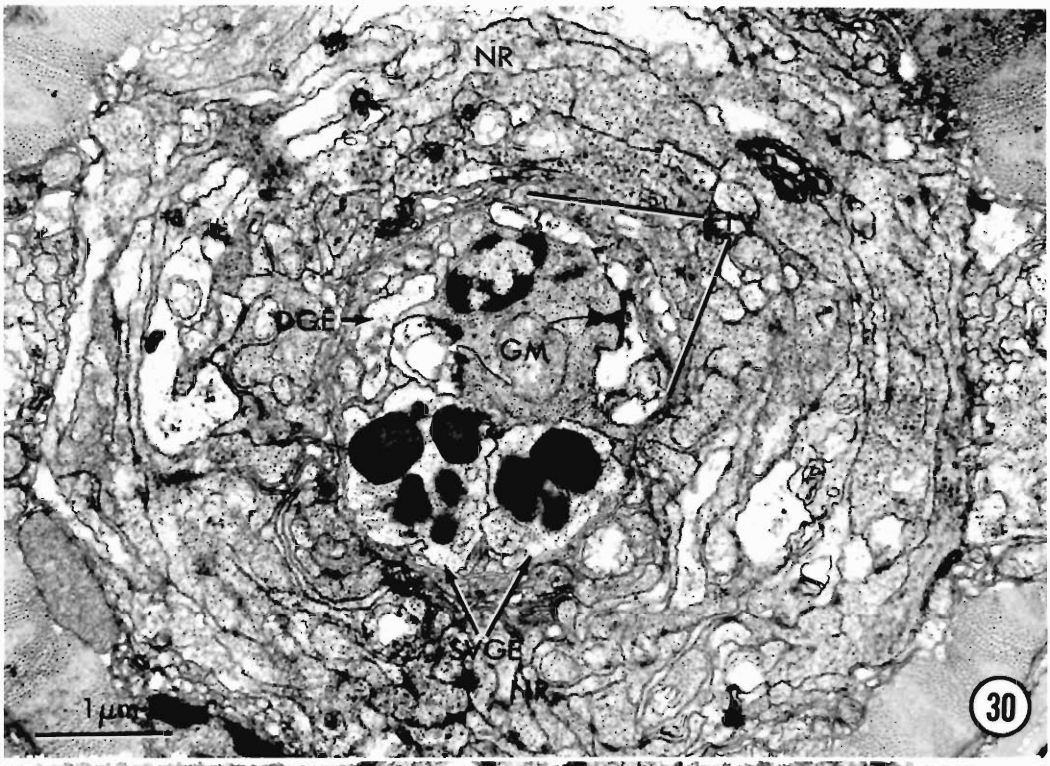
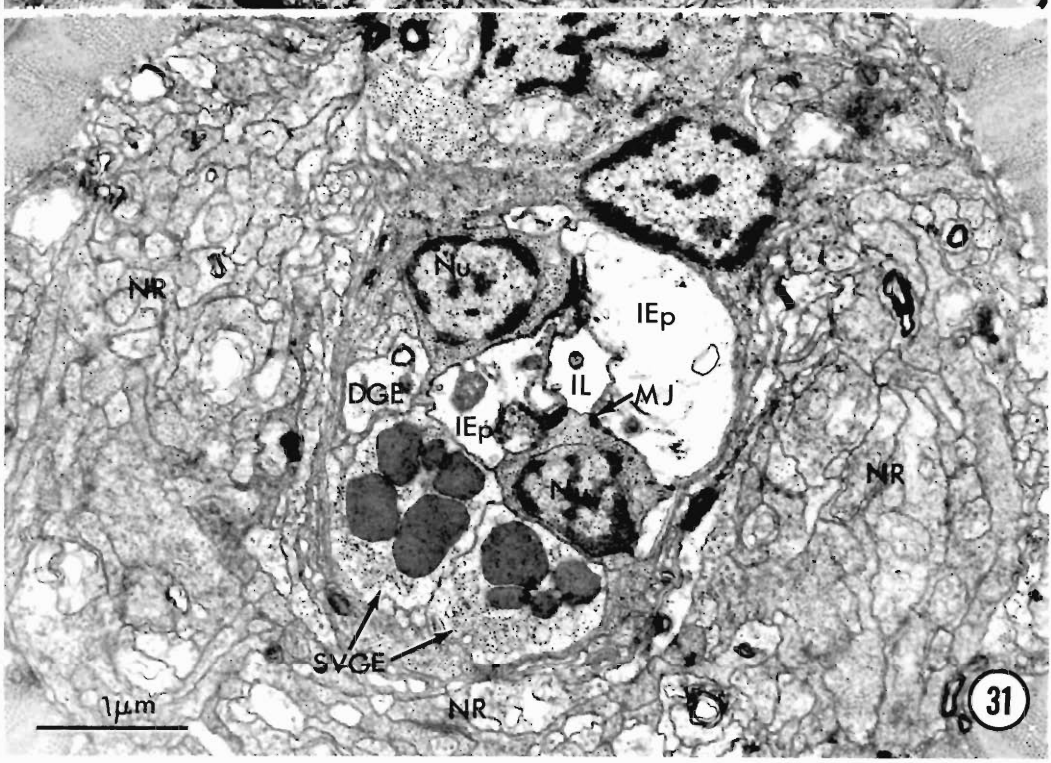
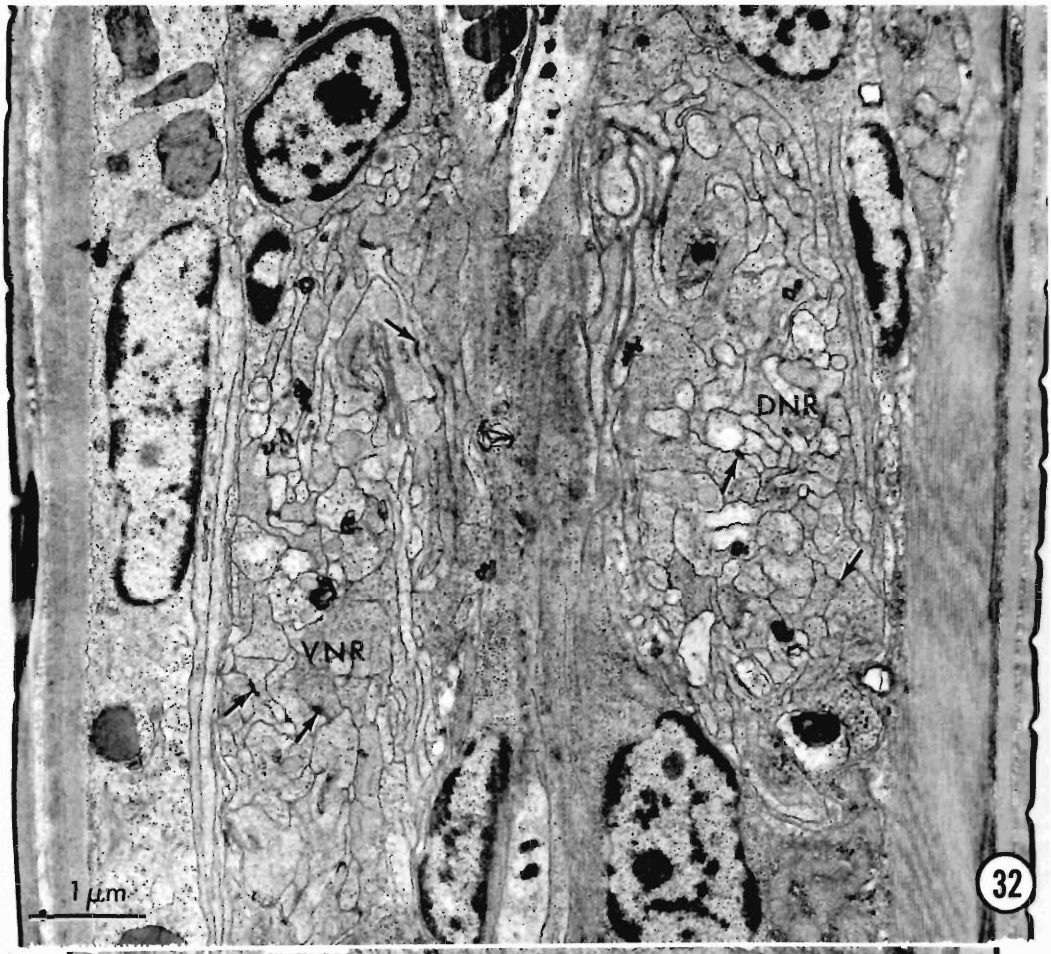
Figures 30, 31. Cross sections through the nerve ring of J2 *M. incognita* show a view of the esophago-intestinal valve (EIV) and anterior intestinal epithelium (IEp). 30. Cross section through isthmus of esophagus shows the EIV, and the extensions of the dorsal (DGE) and subventral gland extensions (SVGE). They are entirely surrounded by a circumferential nerve tissue, the nerve ring (NR). The EIV is in an apparent functional mode in that the apical membranes of the valve cells are separated and displaced by a large globular mass (GM) of material. 31. Section through the posteriad region of the nerve ring (NR) within the isthmus of the esophagus. This region is just posteriad from the EIV and shows 2 electron-dense cells with nuclei (Nu) that are cell bodies of the EIV. The adjacent electron-lucent cells are cells of the intestinal epithelium (IEp). The intestinal lumen (IL) is formed by the apical membranes of epithelial cells that are joined at their lateral wall surfaces by membrane junctions (MJ). DGE, dorsal gland extension; SVGE, subventral gland extension.

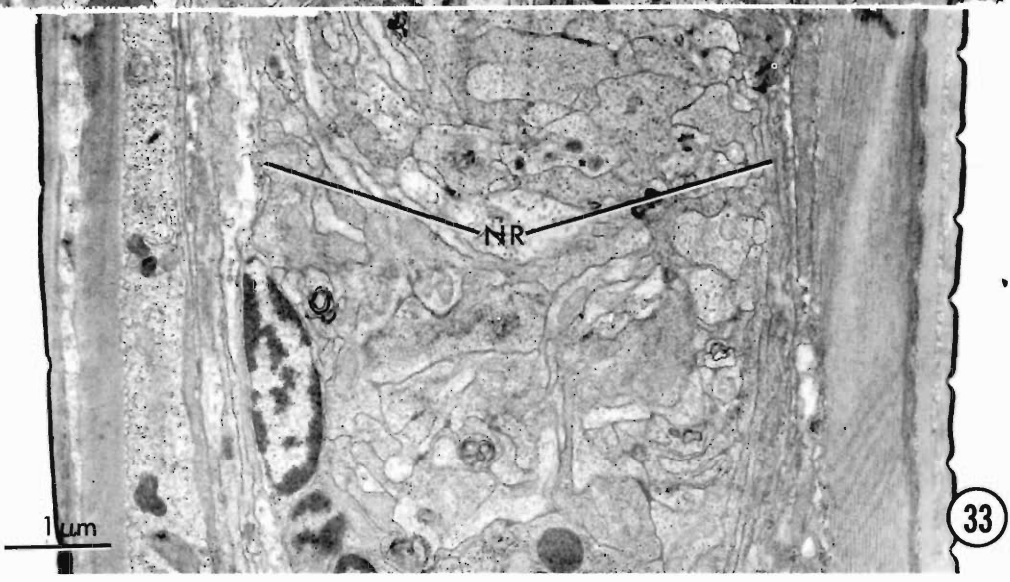
Figures 32, 33. Median and tangential longitudinal sections through the nerve ring of J2 *M. incognita.* 32. Lateral longitudinal submedian section through the nematode shows the slightly anteriad position of the dorsal part of the nerve ring (DNR) and correspondingly the posteriad position of the ventral (VNR) sector of the nerve ring. The nerve ring consists of axons and dendrites with a wide range of shapes and dimensions. Interaction between the neurons is observable by the numerous points of synapses (arrows) between the axons and dendrites. 33. A lateral longitudinal tangential section shows the nerve ring (Nr) with axons and dendrites in various configurations. This section is taken from the same nematode shown in Figure 32.

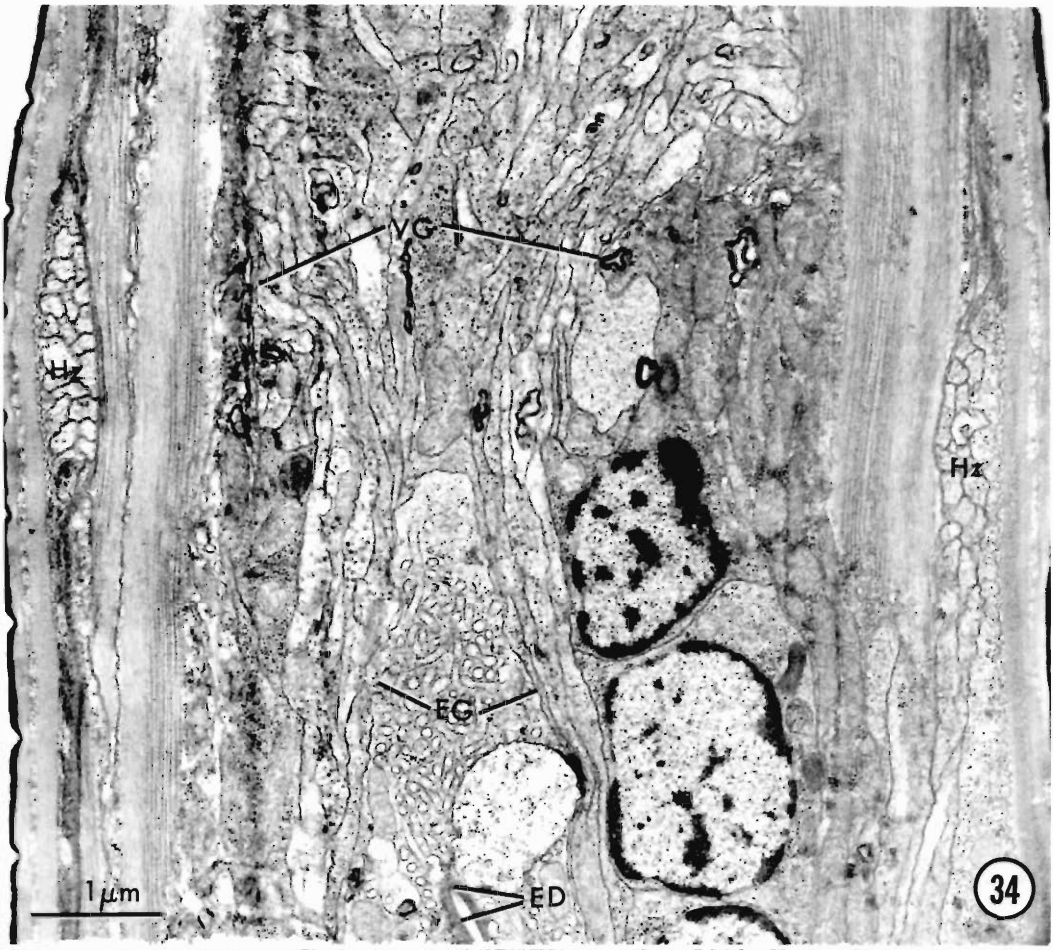
Figures 34, 35. Longitudinal and cross sections of J2 *M. incognita* show nerve fibers of nerve ring, hemizonids, and ventral ganglion. 34. A submedian longitudinal section near the ventral ganglion (VG) shows the close relation between this nerve tissue and the anterior portion of the excretory canal (EG). Certain nerve fibers traverse along the lateral cord and form a tight grouping of lenticular nerve tissue called a hemizonid (Hz). These nerve fibers extend posteriad and circumferentially to merge with the ventral ganglion. ED, excretory duct. 35. Cross section of the ventral region showing the orientation of the nerve fibers of the hemizonid (Hz). Nerve fibers appear as parallel arrays of neurons that lie between the cuticle (Cu) and the somatic muscles (SM) and extend from the lateral cord (LC) to the ventral ganglion (VG). SVGE, subventral gland extension.

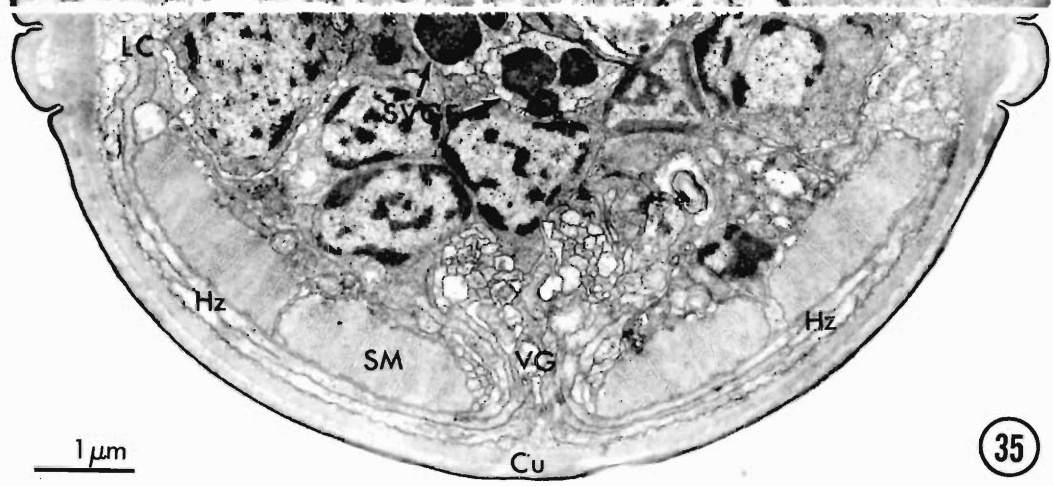












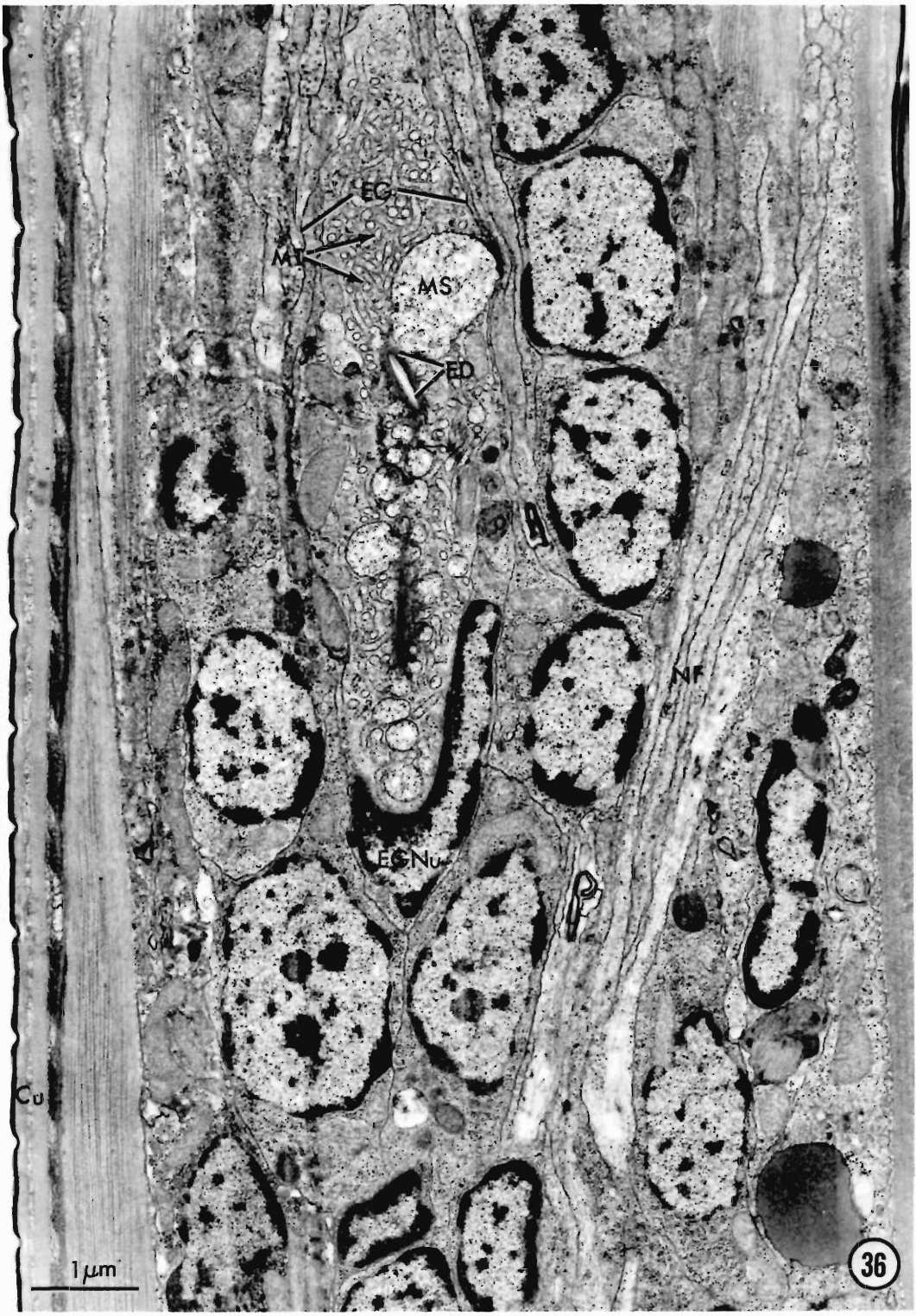


Figure 36. Longitudinal section through the broad region of the excretory canal (EG) in J2 *M. incognita.* Short sections of the duct (ED) are surrounded by an intertwining of membranous tubules (MT) and spherical modifications (MS) of these tubules, all of which appear related to the duct wall. A single nucleus (EGNu) with a contour modified to the outline of the duct cell appears to be part of the gland cell. NF, nerve fibers; Cu, cuticle.

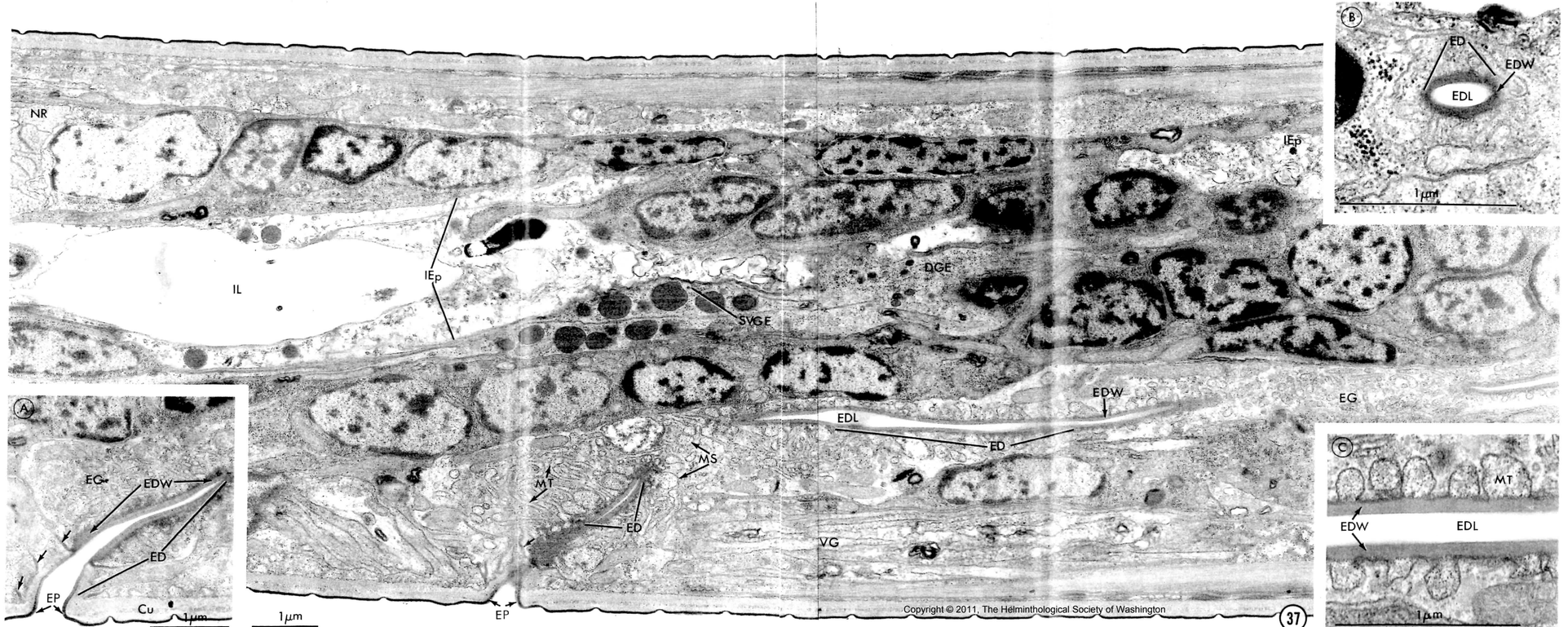
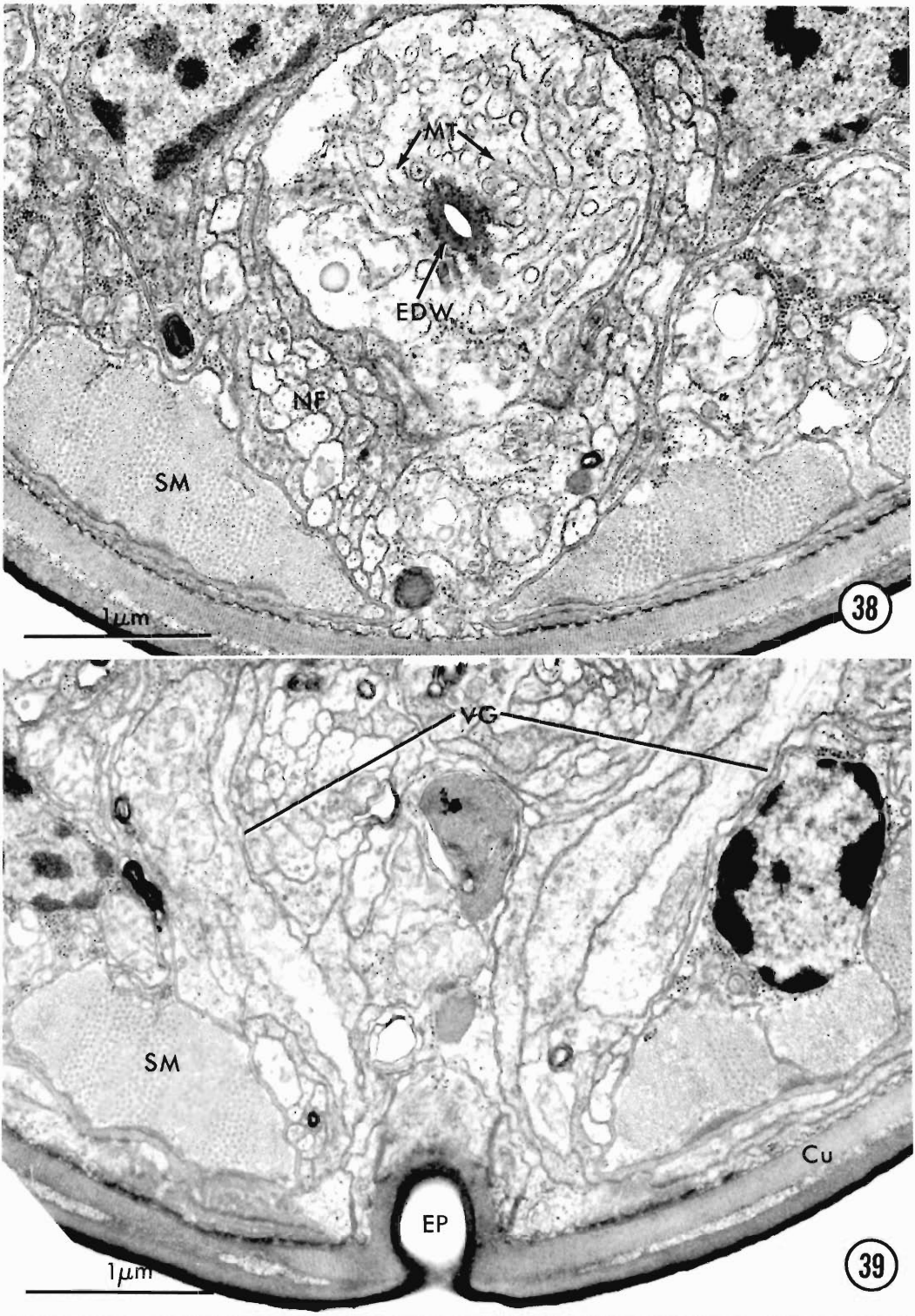


Figure 37 (foldout). A longitudinal section of the excretory gland (EG) in J2 *M. incognita* shows its ventral position in relation to the dorsal (DGE) and subventral gland (SVGE) extensions, and the intestinal epithelium (IEp). This cuticular excretory pore (EP) (median sections of Fig. 37, 37A) is supported by an invaginated cuticle and membrane junctions (arrows) that are part of hypodermal membranes. The excretory duct (ED) is shown as a porous-appearing tube (posteriad region of a different specimen, Fig. 37B) extending through a major length of the nematode. Invaginated hypodermal membranes form tubules (MT) and spheres (MS) while retaining contact with the wall of the duct (EDW) (Fig. 37, 37C). Cu, cuticle; EDL, excretory duct lumen; IL, intestinal lumen; NR, nerve ring; VG, ventral ganglion.



Figures 38, 39. Cross sections show the excretory gland and the ventrally located duct pore in J2 *M. incognita*. **38.** A cross section through the broad region of the excretory canal showing tubular membrane (MT) attachments to the median duct wall (EDW). NF, nerve fibers; SM, somatic muscle. **39.** Cross section through the terminus of the cuticle-lined excretory pore (EP). The duct region is surrounded by nerve fibers of the ventral ganglion (VG). Cu, cuticle; SM, somatic muscles.

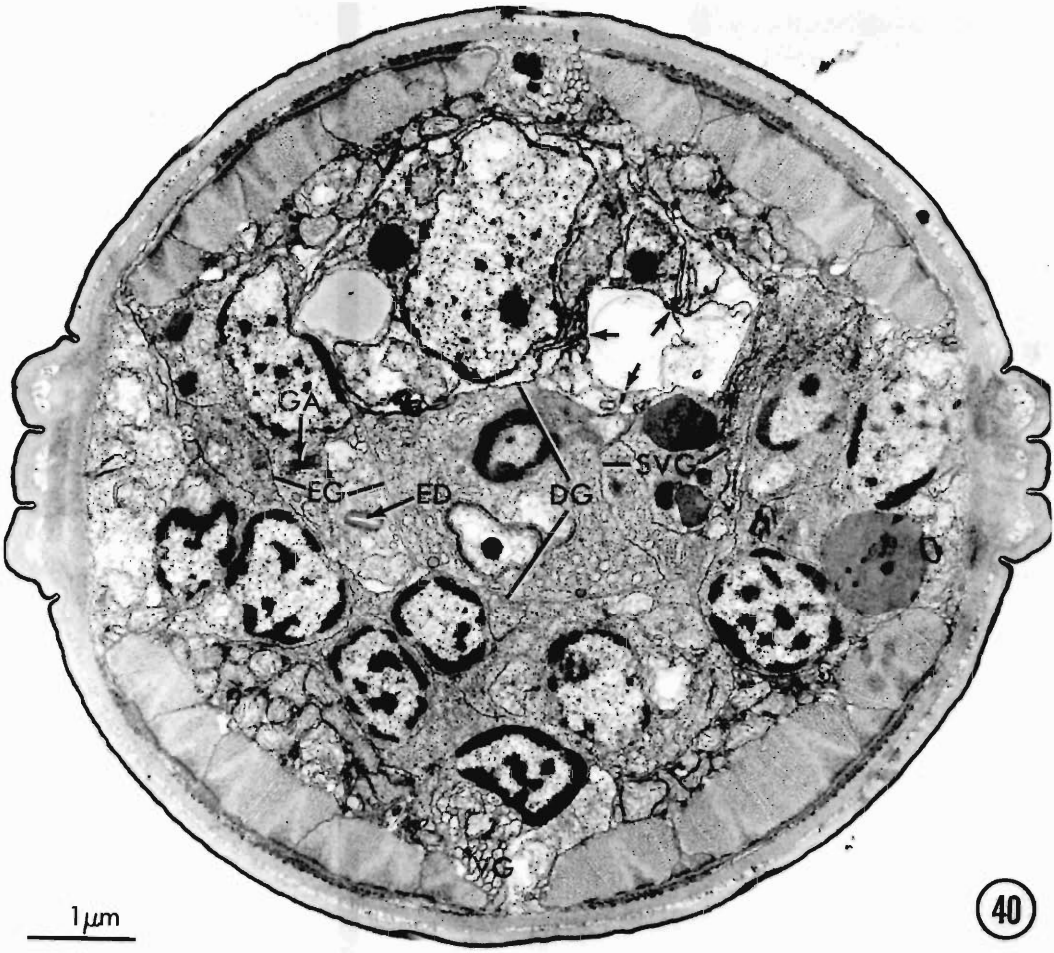


Figure 40. Cross section of the anterior glandular region of the dorsal (DG) and subventral glands (SVG) in J2 *M. incognita*. Excretory gland duct (ED) and excretory gland (EG) with prominent Golgi apparatus (GA) lie lateral to the dorsal esophageal glands. The intestinal lumen formed by apical surfaces of epithelial cells lacks microvilli-like evaginations. Cells of the intestinal epithelium are joined by distinct membrane junctions (arrows) near the lumen wall surface. VG, ventral ganglion.

intestine of *M. hapla* second-stage juveniles (Ibrahim, 1971), microvilli were not observed in the lumen of the intestine of J2 of *M. incognita*. Instead, the entire intestinal surface is devoid of microvilli at this stage of development. Further observations are required to determine the extent of lumen wall proliferation at later stages of nematode development.

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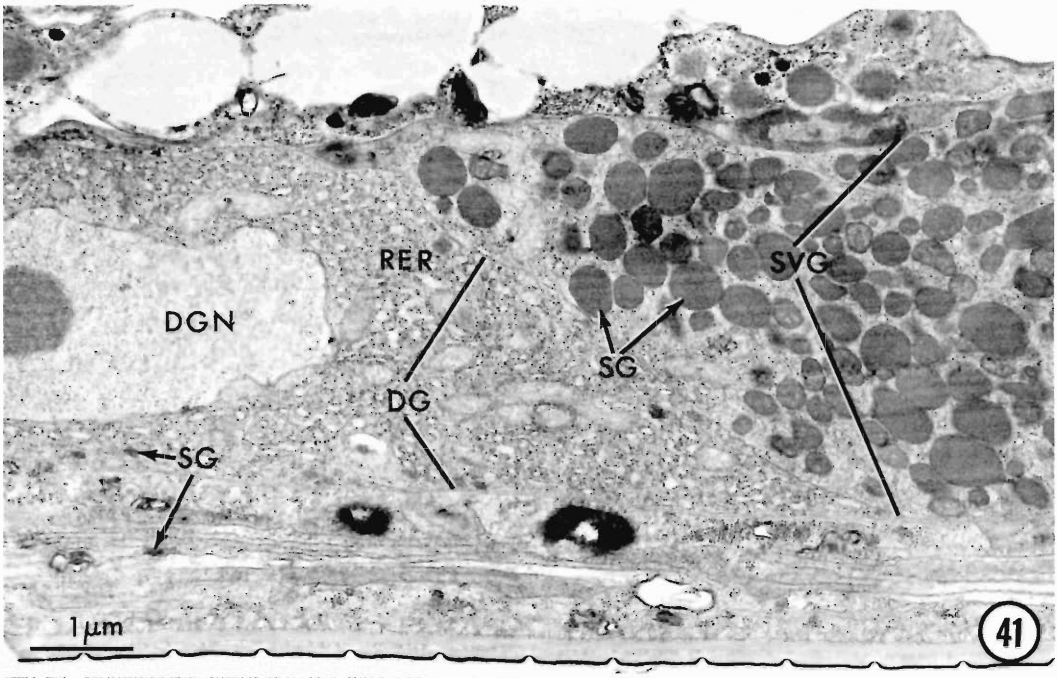
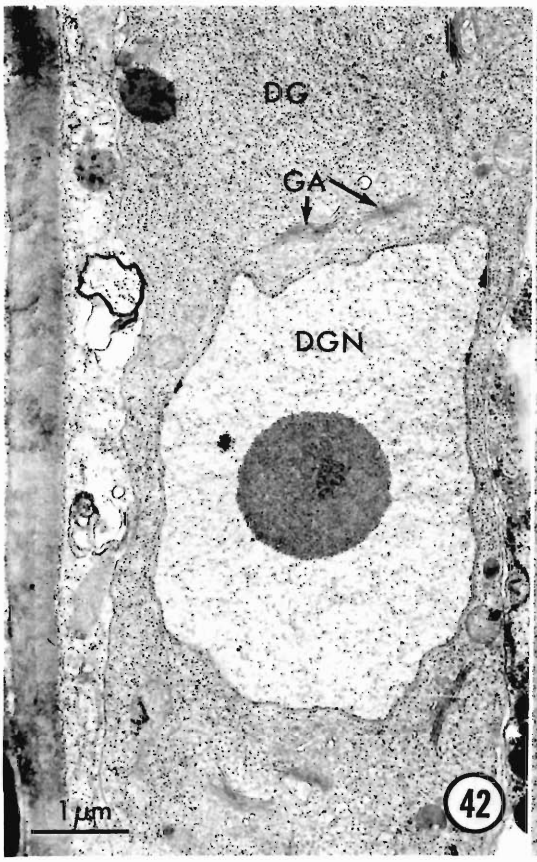
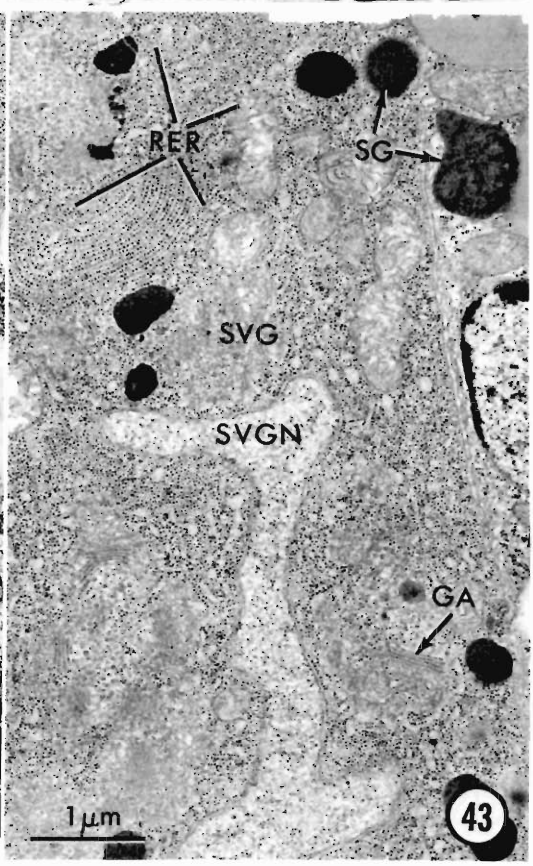
Figures 41–43. Longitudinal sections of J2 *M. incognita* show organelles in the dorsal and subventral glands and related secretory products. 41. Longitudinal section through portions of the dorsal (DG) and subventral glands (SVG). The dorsal gland is shown with a prominent nucleus (DGN) within a matrix of rough endoplasmic reticulum (RER) within which are scattered small electron-dense secretion granules (SG). Adjacent and posteriad to the dorsal gland is a subventral gland cell with large electron-dense secretion granules (SG). Nuclei of the 2 subventral gland cells are located posteriad from this region. 42. Median longitudinal section shows the enlarged dorsal gland nucleus (DGN) surrounded by several Golgi apparatus (GA). DG, dorsal gland. 43. A longitudinal section through the subventral gland (SVG) showing the enlarged electron-dense secretion granules (SG) that are synthesized within the cell. Ribosomes occur throughout the cell as oriented stacks of rough endoplasmic reticulum (RER), some of which appear as cross sections of dilated RER. GA, Golgi apparatus; SVGN, subventral gland nucleus.







The isthmus of the root-knot nematode consists of the dorsal and 2 subventral gland extensions, and the anterior region of the intestine that overlaps the cells forming the esophago–intestinal valve. This region is surrounded by the nerve ring as the circumesophageal ganglion.

The nerve ring

According to Ware et al. (1975), the nerve ring in *Caenorhabditis elegans* is its central nervous system. The ring receives sensory input from the anterior region of the nematode via 6 nerve bundles with all nerve fibers having centrally located cell bodies. The authors reported that the anterior sensory structures are divided into 2 types, the papillary and the amphidial. The papillary fibers were found to enter directly into the nerve ring, whereas the amphidial fibers entered the neural ganglion, which is a posterior extension of the nerve ring. Among the papillary nerve fibers, 22 were identified to terminate as easily recognizable sensory structures. Fourteen terminated distally but could not be assigned functions based on comparative morphology (Ware et al., 1975). The nerve ring and ventral ganglion of *M. incognita* appears similar to *C. elegans* in general morphology. However, in the absence of detailed studies showing dendrite-to-nerve cell continuity as was done with *C. elegans,* one can only assume similar structural and functional relationships.

A bundle of nerve fibers of apparent amphidial function in *M. incognita* was observed along the lateral cord. This nerve bundle merged with nerve fibers that traversed between the cuticle and the underlying somatic muscles and terminated in the ventral ganglion. This circumferentially oriented subcuticular nerve fiber has been observed and identified by morphologists and taxonomists as a hemizonid. It has also been described as a ventrolateral commissure of the nervous system (Bird, 1971).

The excretory gland

The excretory gland of *M. incognita* is a relatively simple, single tubular structure (Hirschmann, 1971). However, further studies may reveal a more complex structure similar to that of *Caenorhabditis elegans* described by Nelson et al. (1983). For example, reconstruction of serial sections of electron micrographs showed that juveniles of *C. elegans* have an excretory system consisting of 4 cells including: (1) the pore cell that encloses the terminal ⅓ of the excretory duct, which in turn leads to the ventrally located secretory pore; (2) the duct cell that surrounds the excretory duct with a lamellar membrane from the origin of the duct to the pore cell boundary; (3) the large H-shaped excretory cell with bilateral canals that extend anteriorly and posteriorly for most of the length of the nematode; and (4) the H-shaped, binucleate gland cell that extends bilateral processes anteriorly from their cell bodies to just posteriad of the esophagus.

The excretory system of *M. incognita* consists of an excretory gland with a medial excretory duct, which extends posteriad from the ventrally located duct pore for a considerable length of the nematode body. Supporting membranes occur along the invaginated cuticle forming the excretory pore. This duct pore region may be comparable to the pore cell described by Nelson et al. (1983) in *C. elegans.* The nucleus of the excretory gland was not clearly defined. However, longitudinal sections showed an excretory gland with a nucleus and cytoplasm having Golgi activity. A characteristic feature of the excretory gland is the presence of numerous membranous tubules that intertwine among themselves and are an integral part of the hypodermal membranes adhering to the excretory duct wall. Furthermore, some tubules appear to give rise to spherical terminals. Because the duct wall appears porous, this interaction of the tubules and spheres probably provides the mechanism for the accumulation of secretion products and their release to the outside of the nematode via the excretory pore. Energy for this interaction can be derived from mitochondria that occur throughout the canal cytoplasm.

The esophageal glands

Although the dorsal gland is associated with gland extensions containing relatively small dense secretory granules, the potential for producing secretions is reflected in the presence of numerous Golgi and an abundance of endoplasmic reticulum throughout the gland. Alternatively, the subventral glands and their extensions in infective J2 juveniles have secretion granules that are large and electron dense. Golgi are quite numerous and are in a relatively active stage of secretion granule production. The observations of J2 infective juveniles of *M. incognita* are similar to reports by Endo (1984) of the same phase of *M. glycines* development. However, the rel-

ative size and morphology of the secretory granules may differ widely during the parasitic stage as was demonstrated for early stages of *H. glycines*. Apparently, the function of the glands changed: when the nematode began to feed, the dorsal gland and its extensions were filled with enlarged secretory granules. During a similar stage of development and salivation, feeding by *Heterodera schachtii* in cultivars of *Raphanus sativus* was observed using video-enhanced light microscopy (Wyss and Zunke, 1985). A similar buildup of secretory products in the esophageal glands and extensions apparently occurs in other tylenchid species, including *M. incognita*.

The intestine

The intestine of J2 infective stages of *M. incognita* is readily recognized by the presence of lipid droplets throughout the epithelium. Other reports (Ibrahim, 1971) showed the presence of microvilli on the inner surfaces of the intestinal epithelium; no characteristic microvilli were observed in the intestinal lumen of *M. incognita*.

In the anterior region of the intestine, the intestinal epithelium appears to be 3-celled in cross section; each of the cells was joined laterally, near the lumen surface, with membrane junctions. Cross sections through the anterior intestine just posteriad of the esophago–intestinal valve show the distinct nucleated cells of the esophago–intestinal valve surrounded by the cells of the intestinal epithelium.

Further studies are required to elucidate the relationship of structure and function among root-knot nematodes. Although considerable progress has shown how hosts respond to nematode infections, we are only beginning to see how the ultrastructure of the nematode changes during feeding and development.

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Hapalorhynchus brooksi sp. n. (Trematoda: Spirorchiidae) from the Snapping Turtle (*Chelydra serpentina*), with Notes on *H. gracilis* and *H. stunkardi*[1]

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ABSTRACT: *Hapalorhynchus brooksi* is described as a new species from the snapping turtle, *Chelydra serpentina*, and is conspecific with *H. stunkardi* of Brooks and Mayes (1976; nec Byrd, 1939). The new species differs from other North American species, with the exception of *H. albertoi* Lamothe-Argumedo, 1978, in the possession of a distinct cirrus and cirrus sac. Reexamination of type specimens of *H. gracilis* Stunkard, 1922, *H. foliorchis* Brooks and Mayes, 1975, and *H. stunkardi* Byrd, 1939, revealed they do not possess a cirrus sac. Structures previously described as a cirrus, or weakly muscular ejaculatory duct (=cirrus), were found to be the pars prostatica. The presence of a cirrus in these species could not be determined. The synonymy of *Coeuritrema* Mehra, 1933, and *Tremarhynchus* Thapar, 1933, with *Hapalorhynchus* Stunkard, 1922, is provisionally supported.

KEY WORDS: *Hapalorhynchus brooksi* sp. n., *Hapalorhynchus gracilis, Hapalorhynchus stunkardi, Hapalorhynchus albertoi, Coeuritrema, Tremarhynchus, Chelydra serpentina,* taxonomy, morphology, snapping turtles, North America.

The genus *Hapalorhynchus* Stunkard, 1922, as currently defined (Brooks and Mayes, 1976), contains 6 species occurring in North America. Three species, including the type, *H. gracilis* Stunkard, 1922, have been reported from the snapping turtle, *Chelydra serpentina.* Brooks and Mayes (1975, 1976) reported *H. foliorchis* Brooks and Mayes, 1975, and *H. stunkardi* Byrd, 1939, from this same host in Nebraska.

Examination of snapping turtles from Westhampton Lake on the University of Richmond (Richmond, Virginia) campus yielded 2 species of the genus: *H. gracilis,* and a new species, which is herein designated as *Hapalorhynchus brooksi.*

Materials and Methods

Turtles were captured during a 2-yr period beginning June 1979 and examined within 2 days. For necropsy they were injected intraperitoneally with a saturated solution of choral hydrate before removal of the plastron. The digestive tract, with the mesenteries intact, was removed and the blood vessels were examined with a dissecting microscope. Worms seen in the vessels were removed and placed in 0.7% saline. The remaining blood was pooled in citrated (1.0%) saline and examined microscopically.The other organs were removed and placed in separate petri dishes for examination. Worms were killed and fixed with cold AFA under light coverglass pressure. Whole mounts prepared in this way were stained with Semichon's acetic-carmine. A single specimen was prepared as serial sections, cut at 7 μm, and stained in hematoxylin and eosin. Drawings were made with the aid of a drawing tube. Measurements are in micrometers with the mean followed by the range in parentheses.

Type and voucher specimens were examined as follows: *Hapalorhynchus albertoi* (paratypes), Universidad Nacional Autonoma de Mexico No. 229-20; *H. gracilis* (holotype), American Museum of Natural History No. 125; *H. foliorchis* (paratypes), USNM Coll. No. 73821; *H. reelfooti* (holotype), USNM Coll. No. 9226; *H. stunkardi* (holotype), USNM Coll. No. 9225; and *H. stunkardi,* of Brooks and Mayes (1976), University of Nebraska State Museum, Harold W. Manter Laboratory No. 20217.

Observations

Hapalorhynchus gracilis Stunkard, 1922

TYPE HOST: *Chelydra serpentina* (Linneaus), snapping turtle.

TYPE LOCALITY: Hammond, Indiana.

VOUCHER SPECIMENS: USNM Helm. Coll. No. 80371; Univ. Neb. State Mus., Harold W. Manter Lab., No. 20731.

COMMENTS: Stunkard (1922) described *H. gracilis* from snapping turtles collected in Hammond, Indiana, and it has since been reported on 3 additional occasions (Table 1). The specimens in my study closely conform to the type specimen (Table 2) and the original description. The wider testes and less extensive vitellaria in my specimens (Figs. 1, 2) concern characters that may vary with age and handling of material. The male genitalia and the hindbody as a percentage of the total body length are sufficiently similar to be considered part of a single species. Eggs in

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Table 1. Reports of *Hapalorhynchus* spp. from North America.

Species	Host	Location	Source
H. gracilis	*Chelydra serpentina*	Indiana	Stunkard (1922)
H. gracilis	*Chelydra serpentina*	Tennessee	Byrd (1939)
H. gracilis	*Chelydra serpentina*	Wisconsin	Guilford (1959)
H. gracilis	*Chelydra serpentina*	Virginia	Present study
H. foliorchis	*Chelydra serpentina*	Nebraska	Brooks and Mayes (1975)
H. brooksi	*Chelydra serpentina*	Nebraska	Brooks and Mayes (1976)
H. brooksi	*Chelydra serpentina*	Virginia	Present study
H. albertoi	*Kinosternon leucostomum*	Mexico	Lamothe-Argumedo (1978)
H. evaginatus	*Trionyx spiniferus*	Tennessee	Byrd (1939)
H. reelfooti	*Sternotherus odoratus*	Tennessee	Byrd (1939)
H. stunkardi	*Sternotherus carinatus*	Georgia	Byrd (1939)

my specimens are tricornuate, as previously described. In my opinion these specimens are conspecific. Although Stunkard (1922, 1923) described the seminal receptacle as branching from the oviduct, examination of the type specimen and my material clearly show the seminal receptacle to be an expansion of the oviduct (Fig. 3). This arrangement is similar to that described for the genus *Spirorchis* MacCallum, 1918 (e.g., see Stunkard, 1923).

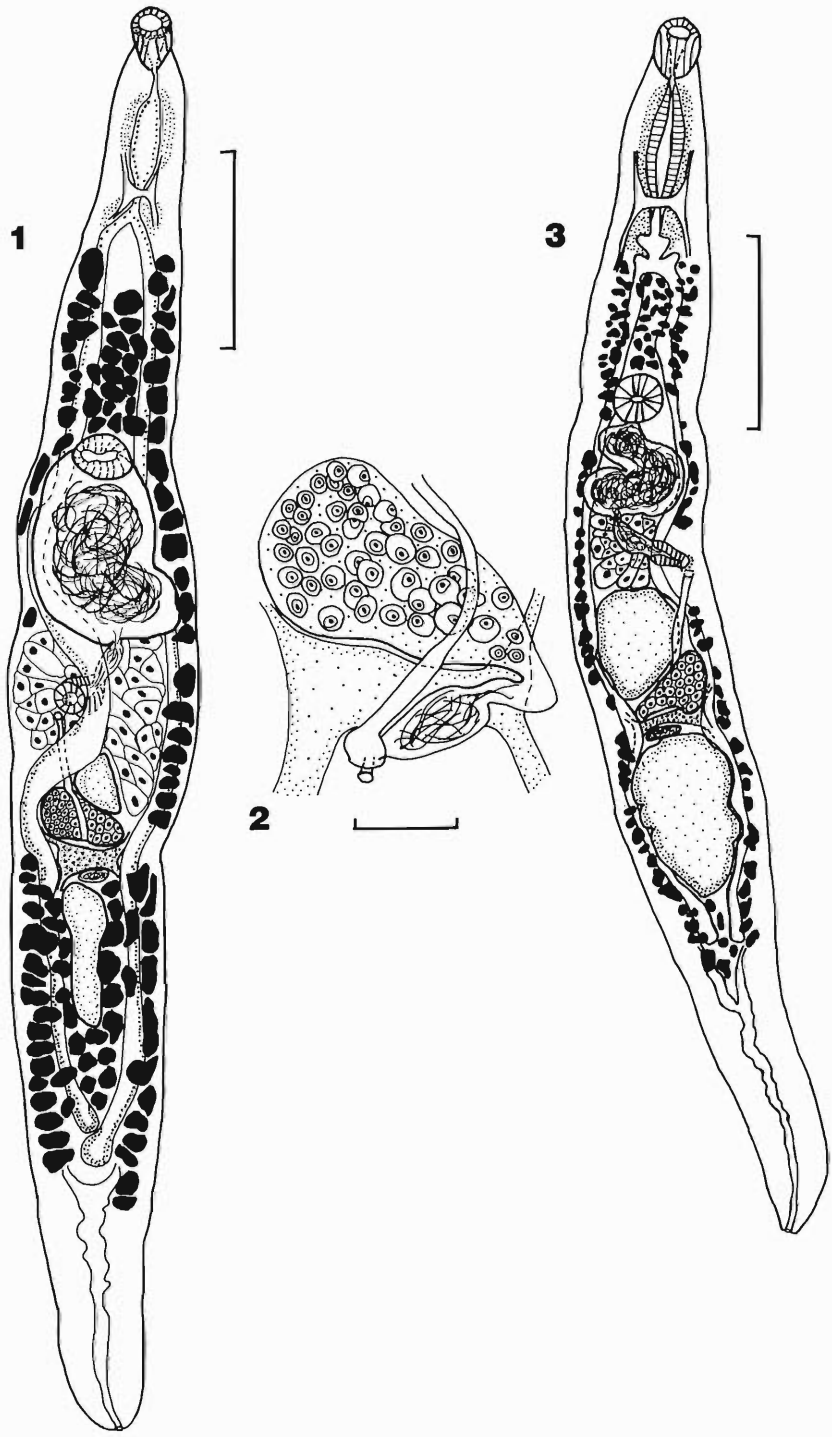
Hapalorhynchus stunkardi Byrd, 1939

TYPE HOST: *Sternotherus carinatus* (Gray).
SITE: Blood vessels of the lung.
LOCALITY: Athens, Georgia (U.S.A.).
COMMENTS: The holotype of *H. stunkardi* is consistent with the original description in most respects. Byrd (1939) differentiated *H. stunkardi* from other species of the genus based on the presence of "hair-like" spines on the body, the

Table 2. Comparative measurements of *Hapalorhynchus* spp.

	H. gracilis			*H. stunkardi*	*H. brooksi*		
	Holotype	Current material		holotype	Holotype	Type series*	
N	1	14		1	1	11	
Total length	1,789	1,412	(1,094–1,723)†	1,442	1,990	1,748	(1,337–2,430)
Maximum width	234	156	(96–219)	350	300	323	(267–416)
Oral sucker							
Length	75	79	(50–106)	90	132	136	(110–163)
Width	58	52	(32–70)	80	94	96	(72–113)
Acetabulum							
Length	63	55	(40–74)	134	140	138	(104–175)
Width	75	58	(50–72)	130	154	149	(112–179)
Ovary							
Length	65	57	(28–84)	160	82	105	(54–163)
Width	115	90	(56–120)	144	144	159	(102–221)
Anterior testis							
Length	100	106	(50–204)	112	175	158	(100–200)
Width	60	80	(42–134)	150	142	140	(83–175)
Posterior testis							
Length	178	166	(100–284)	174	229	183	(121–248)
Width	38	85	(44–150)	150	142	140	(83–175)
Hindbody (% of total length)	19.4	23.0	(20.9–36)	8.2	13.0	13.9	(12.9–15.4)
Sucker ratio‡	1.29	1.15	(1.1–1.56)	1.60	1.64	1.57	(1.21–2.09)

* This includes the holotype and 2 specimens reported as *H. stunkardi* by Brooks and Mayes (1976).
† Mean (range).
‡ Acetabulum width/oral sucker width.



Figures 1–3. ***Hapalorhynchus gracilis.*** **1. Dorsal view of holotype. 2. Female genital complex of holotypoe (dorsal). 3. Ventral view of specimen from Virgina. (Scale bar = 250 μm, Figs. 1, 3; 50 μm, Fig. 2.)**

nature of the cirrus sac, the nature and extent of the ceca, the shape of the body, and the egg. Examination of the type specimen (Fig. 4) revealed the presence of plications or folds in the tegument (fixation artifacts?), but no spines. The male genitalia are similar to those in *H. gracilis,* i.e., there is no cirrus sac. *Hapalorhynchus stunkardi* has a much shorter hindbody than *H. gracilis* (Table 2), although the posterior end of the specimen appears contracted (Fig. 4). The 2 species can be distinguished on the basis of a difference in sucker ratio (Table 2) and the oval versus tricornuate egg in *H. stunkardi* and *H. gracilis,* respectively. The seminal receptacle is formed as an expansion of the oviduct (Fig. 5), similar to that described for *H. gracilis* (see above), contrary to the original description.

Brooks and Mayes (1976) reported *H. stunkardi* from snapping turtles in Nebraska. Examination of voucher specimens revealed that they are not consistent with Byrd's description. This material is conspecific with specimens collected in Virginia and are described below as a new species.

Hapalorhynchus brooksi sp. n. (Figs. 6, 7)

DESCRIPTION (based upon measurements of 11 whole mounts and 1 sectioned specimen): Spirorchiidae, with characteristics of the genus. Tegument unspined; body spatulate, weakly muscled, tapering at both ends with a slight constriction at acetabulum; length 1,749 (1,337–2,430), maximum width 323 (267–416) at level of genital openings. Oral sucker terminal 96 (72–113) in diameter, 136 (110–163) long. Esophagus with thick cellular lining anterior to cerebral ganglia and dilated immediately preceding cecal bifurcation, surrounded by secretory cells for entire length; diverticula absent. Intestinal bifurcation 159 (93–240) anterior to acetabulum, 1 pair of anterolateral diverticula present. Ceca sinuous, ending 245 (179–360) from posterior end; hindbody 14% (12.9–15.4%) of total body length. Left cecum slightly indented at level of genital pore. Acetabulum 138 (104–175) long by 149 (112–179) wide, in second quarter of body 587 (429–800) from anterior end. Oral sucker to acetabulum width ratio 1:1.57 (1:1.21–2.08). Testes lobed, in third quarter of body, separated by female complex. Anterior testis 159 (100–200) long by 140 (83–175) wide, immediately posterior to cirrus sac. Posterior testis 182 (121–248) long by 149 (112–193) wide. Seminal vesicle immediately postacetabular; ejaculatory duct immediately enters cirrus sac. Cirrus sac 165 (124–225) long by 74 (62–82) wide proceeds posterosinistral, crosses left cecum ventrally, terminating in dorsal genital pore. Prostatic cells numerous, surrounding conspicuous pars prostatica, 89 (70–114) long by 43 (30–58), leading to a short cirrus. Genital pore 827 (689–972) from anterior end. Ovary intertesticular, 105 (54–163) long sinistrally, tapering dextrally, 160 (102–221) wide. Oviduct reflexed, expanding to form a seminal receptacle mesiad and posterior to the ovary. Vitelline reservoir ventral to seminal receptacle; vitelline duct joining oviduct dorsal and mesiad to ovary. Mehlis' gland and Laurer's canal not observed. Uterus short, passing dorsal to ovary and left of anterior testis, crossing left cecum ventrally; terminating dorsally in female pore, immediately posterior to male pore. Eggs with 1 filament on each end, not tricornuate. Vitelline follicles abundant, beginning at intestinal bifurcation, terminating at ends of ceca; lateral to ceca and filling intercecal space anterior to acetabulum and behind posterior testis. Excretory vesicle irregularly branched, Y-shaped, bifurcating at level of cecal tips.

SYNONYM: *Hapalorhynchus stunkardi* of Brooks and Mayes, 1976 (nec Byrd, 1939).

TYPE HOST: *Chelydra serpentina* (Linneaus), snapping turtle.

TYPE LOCALITY: Westhampton Lake, Richmond, Virginia (campus of the University of Richmond).

OTHER LOCALITIES: Missouri River, 1.5 mi south of Brownsville, Nebraska.

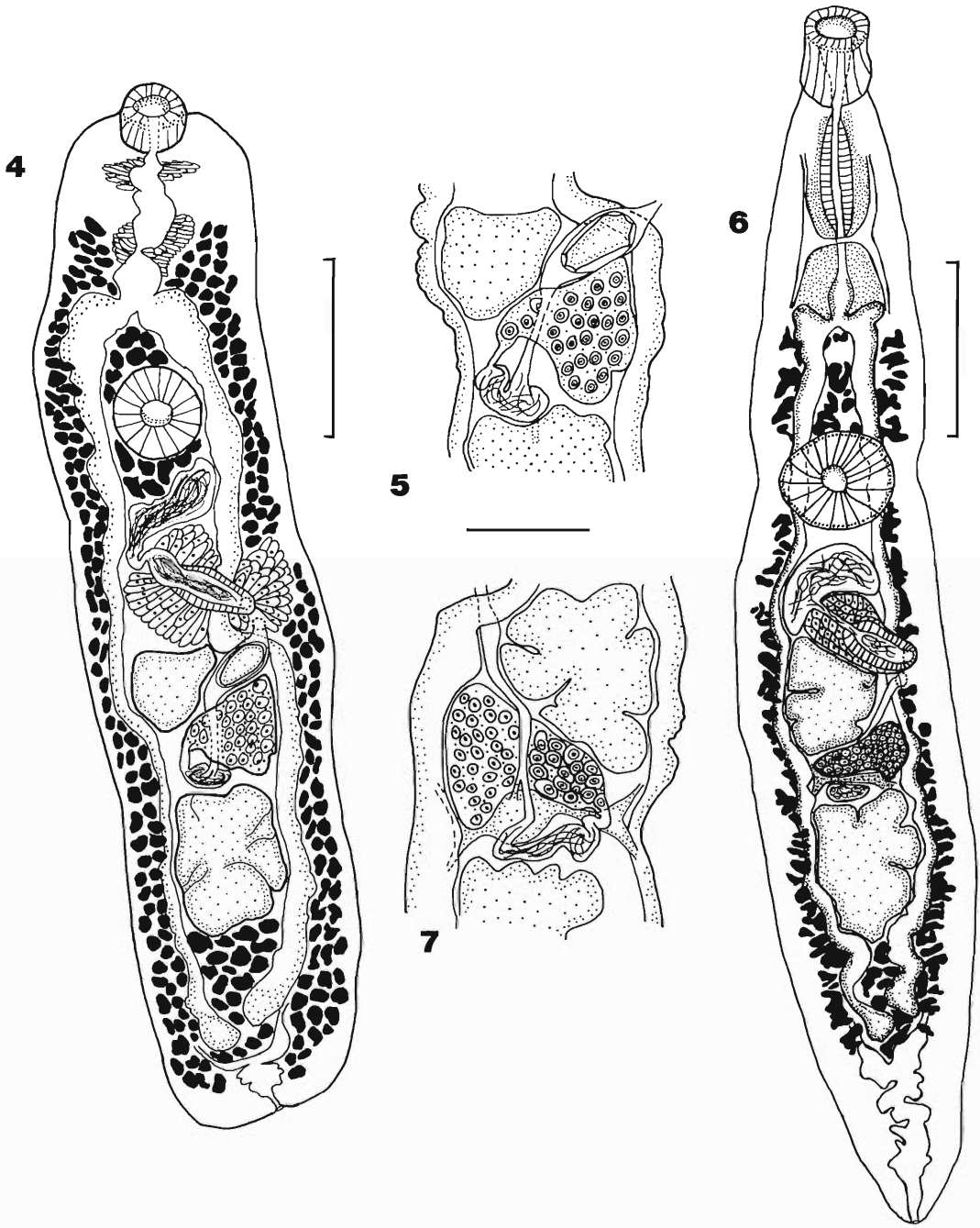
SITE: Mesenteric blood vessels, spleen.

TYPE SPECIMENS: Holotype and 1 paratype USNM Helm. Coll. Nos. 80369 and 80370. Additional paratypes, Univ. Neb. State. Mus., H. W. Manter Lab. No. 20217 (see Brooks and Mayes, 1976).

ETYMOLOGY: This species is named in honor of Dr. Daniel R. Brooks for his outstanding contributions to helminth taxonomy and systematics.

Remarks

Hapalorhynchus brooksi sp. n. is most similar to *H. stunkardi, H. gracilis,* and *H. foliorchis,* but differs from these species by the possession of a cirrus sac. The egg of *H. stunkardi* is oval, lacking polar filaments, but the egg in *H. brooksi* is oval



Figures 4–7. ***Hapalorhynchus*** **holotypes. 4, 5.** ***Hapalorhynchus stunkardi.*** **4. Ventral view of holotype. 5. Female genital complex, holotype (ventral). 6, 7.** ***Hapalorhynchus brooksi*** **sp. n. 6. Ventral view of holotype. 7. Female genital complex, dorsal (paratype). (Scale bar = 250 μm, Figs. 4, 6; 50 μm, Figs. 5, 7.)**

with bipolar filaments. The vitellaria begin at the middle of the esophagus in *H. stunkardi* and at the level of the cecal bifurcation in *H. brooksi.*

The new species differs from *H. gracilis* in body shape and has a much smaller postcecal space, 14% vs. 23%, respectively. The egg is not tricornuate as in *H. gracilis.* The urinary bladder in *H. brooksi* is highly evaginated and is either straight or only slightly sinuous in *H. gracilis.* Sucker ratios (acetabular width/oral sucker width) in the 2 species are different, averaging 1:1.57 for *H. brooksi* and 1:1.15 for *H. gracilis.*

Hapalorhynchus foliorchis differs from *H. brooksi* in having a smaller sucker ratio (1:1.16) and the ovary in *H. foliorchis* is small, ovoid, and restricted to the extreme left side of the intercecal space. As a result the testes are contiguous. The ovary of *H. brooksi* is large and pyriform, separating the testes into anterior and posterior fields. *Hapalorhynchus foliorchis* also lacks the paired anterior cecal diverticula present in the new species.

Hapalorhynchus albertoi Lamothe-Argumedo, 1978, is the only other North American species with a cirrus sac. It differs from *H. brooksi* in having a smaller sucker ratio (<1.0) and 5–8 esophageal evaginations.

Discussion

Stunkard (1922) erected the genus *Hapalorhynchus* for distomate blood flukes with 2 testes separated by the female genital complex, separate dorsal genital pores, and lack of a cirrus sac. Since that time 6 additional species have been described from North American turtles, including 3 species from the snapping turtle, *Chelydra serpentina* (Table 1).

There has been some question as to the form of the terminal male genitalia in this genus. Stunkard (1923) provided histological evidence for the absence of a cirrus sac in *H. gracilis.* Byrd (1939) described 3 new species, reporting a cirrus sac in 2; *H. stunkardi* and *H. reelfooti.* Genitalia of the third species, *H. evaginatus,* were not described. He also reported the presence of a cirrus sac in *H. gracilis* but provided neither an illustration nor information regarding the deposition of specimens. Brooks and Mayes (1976) reported a well-developed cirrus in *H. stunkardi* (=*H. brooksi*), a weakly muscular ductus ejaculatorius in *H. foliorchis,* and comparable morphology in the type specimen of *H. gracilis.* The structure referred to by these authors (i.e., cirrus, weakly muscular ejaculatory duct) is the pars prostatica. Brooks and Mayes (1976) argued that a weakly muscular ejaculatory duct is equivalent to a poorly developed cirrus, and thus supported the synonymy of *Coeuritrema* Mehra, 1933, and *Tremarhynchus* Thapar, 1933 (both of which possess a cirrus sac), with *Hapalorhynchus,* as proposed by Price (1934) and Byrd (1939).

The species examined in this study exhibit elements of a common morphology in the male genitalia. The ejaculatory duct arises at the base of the seminal vesicle and forms a large conspicuous pars prostatica. The prostate cells of *H. gracilis, H. foliorchis,* and *H. stunkardi* are not bound by a limiting membrane and appear irregularly scattered in the surrounding parenchyma. The prostatic complex of *H. brooksi* and *H. albertoi,* however, is bound by a limiting membrane. Histological sections clearly showed such a structure in *H. brooksi,* and the distal portion of the ejaculatory duct is eversible in both species. Whether the first 3 species possess an eversible ejaculatory duct, i.e., a cirrus, could not be determined. They clearly do not possess a cirrus sac. The distal portion of the ejaculatory duct in the type specimen of *H. reelfooti* is obscured and could not be observed.

The presence of a cirrus sac is a synapomorphy for the subclass Digenea (Brooks et al., 1985) and cannot be used to delimit a distinct genealogical entity at a lower level of universality (Wiley, 1981). Therefore, the synonymy proposed by earlier workers is supported and the diagnosis of *Hapalorhynchus* should be emended to include species with "cirrus sac present or absent."

The presence of an oviducal seminal receptacle has been reported previously in *Hapalorhynchus albertoi* Lamothe-Argumedo, 1978, and was confirmed by my examination of the paratypes. Stunkard (1922, 1923) stated that the seminal receptacle arose from the oviduct near the margin of the posterior testis, prior to the origin of Laurer's canal and crossed anterosinistrally to the genital pore. Byrd (1939) described a similar situation for *H. stunkardi.* Examination of the type specimens of both species revealed a sperm-filled expansion of the oviduct before the origin of Laurer's canal and no indication of a separate seminal receptacle. This may be the common condition for *Hapalorhynchus* and should be investigated in other species.

At the present time, species of *Hapalorhynchus* appear to be host specific, however, reports of

these worms in North America have been rare (Table 1). There is little doubt that they are more widely distributed than current records indicate and may show a broader range of coaccommodation than is now evident.

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55(2), 1988, pp. 324–325

Isospora robini sp. n., a New Coccidian Parasite (Apicomplexa: Eimeriidae) from the American Robin (*Turdus migratorius*)

THOMAS E. MCQUISTION AND BLYTHE B. HOLMES
Millikin University, Department of Biology, Decatur, Illinois 62522

ABSTRACT: *Isospora robini* sp. n. was found in the fecal contents of 4 of 12 American robins (*Turdus migratorius*) that were housed at 3 wildlife rehabilitation centers in central Illinois. The oocysts are ovoidal to ellipsoidal with a single-layer, smooth, brownish wall ~1.0 μm thick, 20 × 23 μm (range: 16–22 × 20–28 μm) and a mean L:W ratio of 1.15. The sporulated oocysts have no micropyle or residuum but contain 1, round to ovoidal, polar granule. The sporocysts are ovoidal, 9.0 × 13.8 μm (range: 7–12 × 10–17 μm) with a mean L:W ratio of 1.5, a protruding nipple-like Stieda body, a prominent substieda body, and a granular, smoothly contoured, but irregularly shaped, residuum. The sporozoites are sausage-shaped and generally lie lengthwise in the sporocysts.

KEY WORDS: Coccidia, Apicomplexa, Eimeriidae, *Isospora robini* sp. n., *Turdus migratorius,* American robin, prevalence.

No coccidian parasite has been described from the American robin (*Turdus migratorius*), yet Welte and Kirkpatrick (1986) reported the prevalence of unsporulated oocysts in 23.8% of the robins examined for intestinal parasites. The present study describes *Isospora robini* sp. n., a new coccidian found in American robins captured in central Illinois.

Materials and Methods

Fecal samples were obtained from 12 birds housed at 3 wildlife rehabilitation centers in Illinois during August and September 1987. The fecal samples were placed in vials of 2.5% $K_2Cr_2O_7$ and sent to our laboratory for processing and examination according to the method of McQuistion and Wilson (1988).

The oocysts were examined after flotation in Sheather's sugar solution. Drawings and photographs were made of the oocysts at a magnification of 1,250× with a Nikon microscope with planapochromatic objectives and a calibrated ocular micrometer. All measurements are in micrometers with size ranges in parentheses following the mean. Thirty-eight sporulated oocysts and sporocysts were measured.

Results

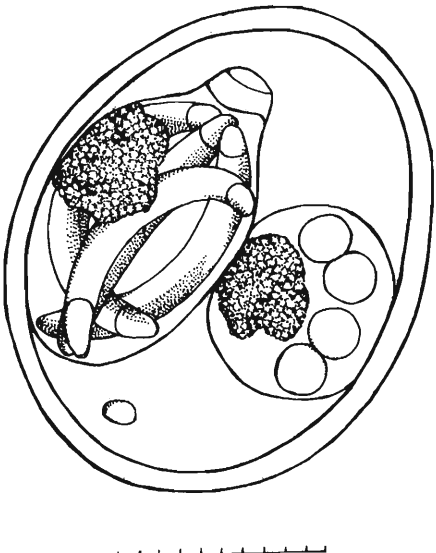
DESCRIPTION: The oocysts are ovoidal to ellipsoidal (Figs. 1, 2) with a single-layer, smooth, brownish wall about 1.0 thick, 20 × 23 (16–22 × 20–28) and a mean L:W ratio of 1.15. The sporulated oocysts have no micropyle or residuum but contain a large prominent, round to ovoid, polar granule. The sporocysts are ovoidal, 9.0 × 13.8 (7–12 × 10–17) with a mean L:W ratio of 1.5, a protruding, nipple-like Stieda body, a prominent substieda body, and a granular smoothly contoured, but irregularly shaped, residuum. The sporozoites are sausage-shaped with refractile bodies at each end and generally lie lengthwise in the sporocysts.

PREVALENCE: Of the 12 birds in which fecal samples were obtained, 6 were female, 4 were male, and 2 were fledglings. Four robins were infected with *I. robini* (33%), 2 females, 1 male, and 1 fledgling. All of the fecal samples that were positive for *I. robini* contained between 5 and 15 oocysts.

Discussion

In describing a new species of coccidium, researchers generally have accepted the principle that oocysts of identical structure from closely related hosts, especially within the same genus, are members of a single species (Marquardt, 1973; Levine, 1982). Conversely oocysts of similar structure from hosts that are widely separated taxonomically are considered to be different species.

The possibility that *I. robini* sp. n. has been described previously from a different host species seems remote for 2 reasons: (1) The American robin is a migratory bird with potential for oocyst transmission from a large number of avian species in its range. However, the American robin is the only member of the genus *Turdus* in its range of habitation. Other birds in the American robin's range that were reported to have isosporan infections did not have oocysts similar in structure to that of *I. robini* and/or belonged to families other than the Turdidae. (2) *Isospora turdi* was reported from the blackbird (*Turdus merula*) and



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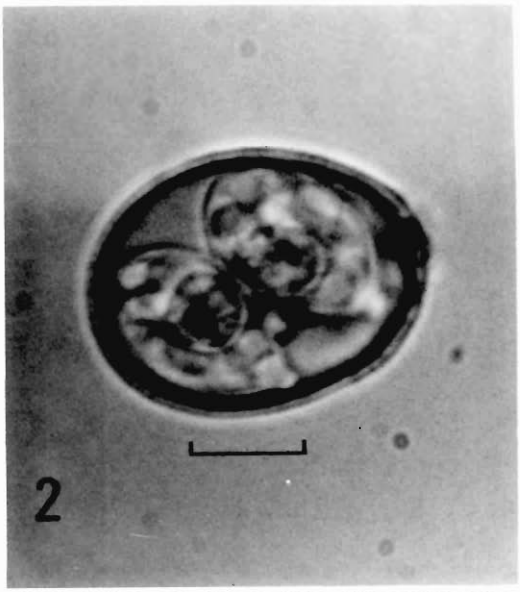

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Figures 1, 2. ***Isospora robini.*** **1. A composite drawing of *I. robini* sp. n. Bar = 10 μm. 2. A photomicrograph of *I. robini* sp. n. magnified 1,250×. Bar = 10 μm**

the fieldfare (*Turdus pilaris*) by Schwalbach (1959). These birds resemble the American robin but are found only in Europe and Asia and have different plumage. The description of *I. turdi* is incomplete but its oocysts are smaller (mean 16.2 × 18.9) than those of *I. robini.*

Acknowledgments

We thank Dr. Norman Levine, University of Illinois, for his assistance in the preparation of the manuscript and Mr. Jay Helton for the line drawing.

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IN MEMORIAM

EDNA MARIE BUHRER
8 December 1898–29 February 1988

The long, illustrious, and dedicated career of Edna Buhrer was ended by death on 29 February 1988. Though in poor health in recent years, her active career spanned more than 50 yr, serving as scientist, teacher, friend, and colleague in the scientific communities of both nematology and parasitology.

She was born in Tiffin, Ohio, and entered Goucher College in 1917 where she earned her B.A. degree in 1921 with a major in Biology. During the summer of 1920 she held a scholarship for study at the Woods Hole Marine Biological Laboratory. In 1921, she was appointed Junior Nematologist in the U.S. Department of Agriculture and advanced through the ranks to Nematologist. Her professional career was spent with the Nematology Division, USDA, from which she retired on 31 December 1962.

During her career, she published about 40 scientific papers on various aspects of plant nematodes. She was well recognized for her early nematode host lists; and later, her publication on common names for many plant-parasitic nematodes served a highly useful and stabilizing effect in nematology and set a pattern for other workers to follow. Among her other published papers, her 1949 publication proved to be a most widely used paper in nematology, detailing the techniques for beheading and en face examination of nematodes and similar animal forms. She worked closely with Steiner in particular but was also associated with Cobb, Christie, Thorne, Taylor, and many other workers within and outside the USDA. She was highly knowledgeable in the various facets of nematology and was most meticulous in using the many specialized techniques and procedures necessary in working with plant and soil nematodes. These qualities elevated her over the years to a pinnacle as a teacher of nematology in the Nematology Laboratory for large numbers of students and scientists from the U.S. and foreign countries visiting the Laboratory to study, work, and gain knowledge on nematodes.

She was active in and highly honored by 2 scientific societies. In February 1933 she was elected a member of the Helminthological Society of Washington; and late in 1934 was elected Corresponding Secretary-Treasurer of this Society. She served in this capacity through 1971, an unprecedented 37 years; and during this time she also functioned, unofficially, as Business Manager of the Proceedings. She was elected President of this Society for 1972. In 1960 she was selected by the Awards Committee to be the first recipient of the Anniversary Award of the Helminthological Society of Washington; in 1963 she was elected Life Member in the Society; and in 1980, at the Society's 70th Anniversary Dinner, she was presented a Special Service Award for her long record of outstanding contributions to the Society. She served as a Trustee of the Brayton H. Ransom Memorial Trust Fund from 1956 to 1975 and then continued as Trustee Emeritus for the remainder of her life. She was a Charter Member of the Society of Nematolo-

Edna M. Buhrer.

gists, and in 1968 was elected Honorary member for her service to the Society and her many contributions to Nematology.

For those who had the privilege and pleasure of knowing her personally, Edna Buhrer's sterling character and delightful personality will be a treasured memory; and for everyone, her scientific contributions and dedicated service to 2 societies will serve as an inspiration to strive for the standards of excellence so common in our departed friend and colleague.

(This tribute will also be published in the Nematology Newsletter [Vol. 34] of the Society of Nematologists.)

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IN MEMORIAM

Albert Lee Taylor
1901–1988

A. L. Taylor passed away March 11, 1988 at Shands Hospital in Gainesville, Florida at the age of 87. Al was born in Florence, Colorado.

Al spent his early years as a printer. His interest in archery indirectly led to meeting the late Gerald Thorne, who also had a similar interest. It was through this and subsequent meetings that Al developed his lifetime career in nematology.

Al attended George Washington University where he graduated in 1935. He established the fourth nematology field laboratory for the United States Department of Agriculture at Tifton, Georgia in July 1935. There, in association with C. W. McBeth, he made many outstanding contributions in the areas of nematode taxonomy, host interactions, and control. His pioneering work on chemical control of nematodes provided the basis for establishing agricultural nematology as a recognized discipline. From October 1946 to November 1949 he was employed as a technologist by the Shell Chemical Corporation to develop the use of DD in the eastern United States. In December 1949 he returned to the USDA as a senior nematologist. He was appointed Head of the Nematology Section on May 1, 1956. He remained in this supervisory and leadership position until March 1964, at which time he accepted an appointment with the Food and Agricultural Organization of the United Nations. While with FAO, he conducted nematode surveys, established nematology laboratories, and initiated research programs in Cyprus and Thailand. He returned to the USDA in March 1966 to take up a new research assignment at the United States Horticultural Research Laboratory at Orlando, Florida. He retired from the USDA on September 29, 1967 after nearly 30 years of Federal Service.

Al immediately took up a new assignment with FAO in Fiji, where he conducted research until 1968. In mid-1968 he returned to Bangkok, Thailand as Project Manager of the Rice Protection and Plant Protection Projects. He returned to Gainesville, Florida in July, 1972.

Al's nematological career was far from over as in November, 1972, he accepted a position as Chief of the Bureau of Nematology, at the Division of Plant Industry, Florida Department of Agriculture and Consumer Services. He remained in that position until December 30, 1975. From 1976–1983 Al was closely associated with Dr. Joe Sasser as a consultant in the International *Meloidogyne* Project during part of each year. The remainder of the year he spent studying and working with graduate students and staff in the Department of Entomology and Nematology at the University of Florida in Gainesville. Since 1983, Al had been devoting his energies towards studying root-knot nematodes at the Bureau of Nematology, Division of Plant Industry, in Gainesville. He continued to maintain a close association with faculty and students at the University of Florida. Al was active right up until his death. His recent publication "Identification and Estimation of Root-Knot Nematode Species in Mixed Populations", published in late 1987, is a testimonial to Al's devotion to the science of nematology. He published more than 90 scientific publications dealing with a wide variety of subjects.

Al's many other contributions are well known to his colleagues from whom he received international acclaim over the years. Al was a man of action and a pragmatic scientist. During his more than 50 year career in nematology he and Josephine, his wife of nearly 60 years, travelled widely, making friends wherever they went. In his quiet unassuming manner, Al was truly a goodwill ambassador for nematology.

Al was a Charter Member and an Honorary Member of the Society of Nematologists. He was also a Life Member of the Helminthological Society of Washington and served as a member of the Editorial Board of the Proceedings of this Society from 1952 to 1963.

Prepared by J. H. O'Bannon and R. P. Esser
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Florida Department of Agriculture and Consumer Services
Gainesville, FL 32605

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MINUTES

Five Hundred Eighty-Ninth Through Five Hundred Ninety-Sixth Meetings

589th Meeting: Uniformed Services University of the Health Sciences, Bethesda, MD, Cosponsor Food and Drug Administration, Washington, DC, 14 October 1987. Patricia A. Pilitt presided over the business meeting and Everett L. Schiller was presented the Anniversary Award. A slate of nominees for officers was announced and further recommendations were to be sent to the Recording Secretary. Bryce C. Redington presided over the scientific program. John H. Cross discussed angiostrongyliasis and its spread; Tom K. Sawyer, the emerging patterns of free-living amebae in the pathogenesis of human disease; and Peter L. Perine, the effects on parasites in Africa due to HIV infection.

590th Meeting: Animal Parasitology Institute, USDA, Beltsville, MD, 18 November 1987. Patricia A. Pilitt presided over the meeting and the election of new officers for 1988: Robin N. Huettel, President; Jeffrey W. Bier, Vice President; Michael D. Ruff, Corresponding Secretary-Treasurer; John H. Cross, Recording Secretary; and David J. Chitwood, Assistant Corresponding Secretary-Treasurer. Dr. Tom K. Sawyer presented the Life Membership Award to Dr. Harry Herlich, Dr. Herlich's wife and daughter unveiled the memorial portrait by Robert Ewing. The scientific program was presented by Ronald Fayer who introduced the speakers. The focus was on protective immunity in parasite infections. Chris Davies discussed the effect of the major bovine histocompatibility complex and susceptibility to helminth parasites; Huyn Lillehoj presented a paper on cell-mediated immunity of the host against Coccidia and implication for the development of vaccines against Coccidia and discussed the role of the mast cell in swine intestinal immunity to parasitic infections. The president announced executive committee action to elect the following to membership in the Society: Larry S. Roberts, Theodor W. Ramp, Stephen G. Kays, Steven A. Borne, Lisa Schoor, and John Bridgman.

591st Meeting: Plant Protection Institute, USDA, Beltsville, MD, 9 December 1987. Patricia A. Pilitt presided over the business meeting and the new officers were installed. Robin Huettel presided over the scientific program and William Fisher discussed molluscan internal defense mechanisms; Susan Fricke Meyer, the biological control of plant parasitic nematodes by fungi; and Len Francl, the interaction of fungi and plant parasitic nematodes on potatoes. President Pilitt turned the presidency over to Robin Huettel. The executive counsel elected the following to membership: Glenn A. Bristow, Dominic A. Strohlein, Edmund J. Washuta, Kimberly Thayer Pearlstein, Mark Jenkins, Susan L. F. Meyer, and Leonard Francl.

592nd Meeting: National Institutes of Health, Bethesda, MD, 13 January 1988. Robin Huettel presided over the meeting and announced the election of new members to the Society: Lester R. G. Cannon, Carol A. Nielsen, Patricia R. Komuniecki, Gary L. Uglem, Santiago Mas-Coma, Frank F. Jaszcz, Kim J. Hamann, Francis R. Ragsdale, James C. Parker, Sun Huh, Franz Schulte, David Abraham, and Barry G. Campbell. Frank Neva presided over the scientific session where the following papers were presented: Compliment receptors mediating entry of *Leishmania* promastigotes into human macrophages, by Rosangela da Silva; Genetic cross between chloroquin susceptible and chloroquin resistant *Plasmodium falciparum,* by Lindsey Panton; T cells and immunity to malaria sporozoites, by Walter Weiss; and In-vitro assessment of neovascularization in chronic schistosomiasis, by David Freedman.

593rd Meeting: Naval Medical Research Institute, Bethesda, MD, 17 February 1988. President Robin Huettel presided over the meeting and announced the resignation of the editor of the Proceedings, J. Ralph Lichtenfels, and the selection of a search committee for a new editor. The proposed budget for 1988 was presented ($36,869) and accepted. New memberships were announced: Gary W. Long and Bill Chobotar. The scientific meeting was presided over by Richard Beaudoin and the following papers were

presented: Malaria vaccine trials with a vaccinia construct of the full length CS gene of *Plasmodium yoelii,* by Martha Sedegah; *Plasmodium falciparum* blood stage antigens: allelic forms of gp195 are also found in the live stages, by Ana Szarfman; Inhibitory activity of IFN- on the development of *Plasmodium falciparum* in human hepatocyte cultures, by Sylvie Mellouk. Posters were also displayed: Use of vaccinia construct expressing the circumsporozoite protein in the analysis of protective immunity to *Plasmodium yoelii,* by R. L. Beaudoin et al.; Human lymphocyte proliferative response to a circumsporozoite protein derived peptide correlates with susceptibility to falciparum malaria, by S. L. Hoffman et al.; and Malaria—20th Century Plague Persists.

594th Meeting: Walter Reed Army Institute of Research Forest Glen Annex, Silver Spring, MD, 16 March 1988. Robin Huettel presided over the meeting and the following new members were accepted: James C. Hall, John A. Griesbac, William S. Fisher, Carl C. Bursey, Hussein S. Hussein, Martha W. Runey, Gerardo Guajardo-Martinae, William H. Patton, and Larry D. Brown. The deaths of the following members of the Society were announced with a moment of silence: Edna Buhrer, John S. Andrews, and Albert Taylor. The scientific session was introduced by Willis Reid and the following papers were presented: Detection of leishmanial drug resistance and new drug screening using an in vitro microprocedure, by Joan E. Jackson and J. D. Tally; Resistance modulators: novel adjunct to antimalarial chemotherapy, by Dennis E. Kyle, Wilbur Milhous, and Ayo M. J. Oduola; Characteristics of drug resistance in leishmania, by Max Grogl, Dennis E. Kyle, Wilbur K. Milhous, and Ayo M. J. Oduola; Metabolic profiling of parasitic infections, by J. E. Hall, Robert E. Miller, Matthew A. Ward, Karl R. Witter, and Max Grogl.

595th Meeting: Johns Hopkins University School of Hygiene and Public Health, held jointly with the Tropical Medicine of Baltimore Dinner Club, Baltimore, MD, 13 April 1988. Robin Huettel presided over the business meeting and Michael Gottlieb presided over the scientific session. The following papers were presented: Transmission dynamics of malaria in a small rural village in southern Mexico, by Chris Frederickson; Molecular approach to the identification of surface proteins of *Onchocerca volvulus* microfilariae, by John Dinman; and Control of mosquitoes with *Bacillus thuringiensis* and larviparous fish *Tilapia nilotica* in rice fields in Liberia, West Africa, by Fatorma Bolay.

596th Meeting: New Bolton Center, University of Pennsylvania, Kennett Square, PA, 7 May 1988. Robin Huettel presided over the business meeting and announced the following new member: Rodrigo Ponce de Leon of Uruguay. Tom Sawyer announced the selection of new Life Members: Robert E. Kuntz, Glenn L. Hoffman, and Raymond V. Rebois. G. A. Schad presided over the scientific session on opportunistic parasitism in man and animals. The following papers were presented: Opportunistic protozoan parasites in animals, by J. P. Dubey; Opportunistic protozoan parasites in man, by Pearl Ma; and Opportunistic helminth parasites, by R. Genta.

Respectfully submitted,

John H. Cross
Recording Secretary

AUTHOR INDEX FOR VOLUME 55

KEY WORD AND SUBJECT INDEX FOR VOLUME 55

The Helminthological Society of Washington

Application for Membership

Any person interested in parasitology or related fields is eligible for membership. Subscription to the Society's Proceedings is included in the dues. Members are privileged to publish therein at reduced rates. The annual dues are payable on notification of election. Send this completed form to:

The Recording Secretary
Helminthological Society of Washington
Post Office Box 368
Lawrence, Kansas 66044

Print name: _____ Date of birth: _____

Mailing address: _____
_____
_____

Degree and year received: _____

Present position: _____

Field of interest: _____
_____

Signature of applicant: _____ Date: _____

Signature of sponsor: _____
(a member)

ANNIVERSARY AWARD RECIPIENTS

* Edna M. Buhrer	1960	David R. Lincicome	1975
Mildred A. Doss	1961	Margaret A. Stirewalt	1975
* Allen McIntosh	1962	* Leo A. Jachowski, Jr.	1976
* Jesse R. Christie	1964	Horace W. Stunkard	1977
Gilbert F. Otto	1965	Kenneth C. Kates	1978
* George R. LaRue	1966	* Everett E. Wehr	1979
* William W. Cort	1966	O. Wilford Olsen	1980
* Gerard Dikmans	1967	Frank D. Enzie	1981
* Benjamin Schwartz	1969	Lloyd E. Rozeboom	1982
* Willard H. Wright	1969	Leon Jacobs	1983
Aurel O. Foster	1970	Harley G. Sheffield	1984
Carlton M. Herman	1971	A. Morgan Golden	1985
May Belle Chitwood	1972	Louis S. Diamond	1986
* Elvio H. Sadun	1973	Everett L. Schiller	1987
E. J. Lawson Soulsby	1974		

HONORARY MEMBERS

* George R. LaRue	1959	Justus F. Mueller	1978
Vladimir S. Ershov	1962	John F. A. Sprent	1979
* Norman R. Stoll	1976	Bernard Bezubik	1980
Horace W. Stunkard	1977	Hugh M. Gordon	1981

CHARTER MEMBERS 1910

* W. E. Chambers	* Philip E. Garrison	* Maurice C. Hall	* Charles A. Pfender
* Nathan A. Cobb	* Joseph Goldberger	* Albert Hassall	* Brayton H. Ransom
* Howard Crawley	* Henry W. Graybill	* George F. Leonard	* Charles W. Stiles
* Winthrop D. Foster			

LIFE MEMBERS

* Maurice C. Hall	1931	* Albert L. Taylor	1975
* Albert Hassall	1931	David R. Lincicome	1976
* Charles W. Stiles	1931	Margaret A. Stirewalt	1976
* Paul Bartsch	1937	* Willard H. Wright	1976
* Henry E. Ewing	1945	* Benjamin Schwartz	1976
* William W. Cort	1952	Mildred A. Doss	1977
* Gerard Dikmans	1953	* Everett E. Wehr	1977
* Jesse R. Christie	1956	Marion M. Farr	1979
* Gotthold Steiner	1956	John T. Lucker, Jr.	1979
* Emmett W. Price	1956	George W. Luttermoser	1979
* Eloise B. Cram	1956	* John S. Andrews	1980
* Gerald Thorne	1961	* Leo A. Jachowski, Jr.	1981
* Allen McIntosh	1963	Kenneth C. Kates	1981
* Edna M. Buhrer	1963	Francis G. Tromba	1983
* Benjamin G. Chitwood	1968	A. James Haley	1984
Aurel O. Foster	1972	Paul C. Beaver	1986
Gilbert F. Otto	1972	Raymond M. Cable	1986
* Theodor von Brand	1975	Harry Herlich	1987
May Belle Chitwood	1975	Glenn L. Hoffman	1988
Carlton M. Herman	1975	Robert E. Kuntz	1988
Lloyd E. Rozeboom	1975	Raymond V. Rebois	1988

* Deceased.

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ANNOUNCEMENTS

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