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Ultrastructure of the Esophagus of Larvae of the Soybean Cyst Nematode, *Heterodera glycines*

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ABSTRACT: The cell bodies of the stylet protractor muscles and of the tissue immediately surrounding the stylet shaft are located in the anterior procorpus alongside the cells of the procorpus proper. The protractor muscle cell bodies are in the dorsal and ventrosublateral sectors and those related to the shaft are in the dorsosublateral and ventral sectors. The dorsal gland duct ampulla lies in the anterior procorpus and from it, the sclerotized duct with its valve or end apparatus joins the esophageal lumen just behind the stylet knobs. Secondary muscle cells lie centripetal to the protractor muscle cells. Constraining muscles occur in the posterior region of the procorpus. The metacorpus consists of a pump chamber operated by a complex of muscle units with their perikaryons and innervation. The subventral gland processes end as ampullae from which the valved sclerotized ducts joint the esophageal lumen at the posterior triangular vestibule of the esophageal lumen. The isthmus is muscular anteriorly and the attenuated gland extensions are encircled by the nerve ring. The dorsal gland occupies most of the anterior of the gland lobe; the two subventral glands that appear as separate cells occupy the posterior region. The esophageal–intestinal valve is adjacent to the dorsal gland nucleus.

The soybean cyst nematode *Heterodera glycines* Ichinohe, 1952 is a major pest of soybeans. Host–parasite studies with emphasis on host responses (Endo, 1964, 1965; Gipson et al., 1971) have been augmented recently by ultrastructural studies on the morphology of males and larvae of the root-knot and soybean cyst nematodes (Baldwin and Hirschmann, 1973, 1975a, b, 1976; Endo and Wergin, 1973, 1977; Wergin and Endo, 1976; Baldwin et al., 1977; Endo, 1978, 1980). Observations and reviews of other tylenchid species (Bird, 1967, 1968, 1971; Wisse and Daems, 1968; Yuen, 1968b; Byers and Anderson, 1972; De Grisse et al., 1974; Anderson and Byers, 1975; De Grisse, 1977; Coomans, 1979b; Natasasmita, 1979; Shepherd et al., 1980; Baldwin, 1982) have provided a substantial base for further investigations of these and other major plant parasitic nematodes. Furthermore, in evaluating the ultrastructure of plant parasitic nematodes, it is essential that information gained on fundamental biology of the rhabditid nematode, *Caenorhabditis elegans,* and a wide range of animal parasitic species be applied to newly acquired data on plant parasitic species (Yuen, 1968a; Wright, 1974, 1980; Ward et al., 1975; Ware et al., 1975; Albertson and Thomson, 1976; McLaren, 1976; Sulston, 1976). New terminology proposed by Coomans (1979a) has been used to describe certain regions of the nematode that show well defined triradiate symmetry.

Previous ultrastructural observations of second-stage larvae of the soybean cyst nematode concentrated on the anterior neurosensory organs and the stomatal region of *H. glycines*. This paper described the ultrastructure of the esophagus with emphasis on the procorpus, metacorpus, and glandular organs. The general morphology of the nerve ring is also included.

Materials and Methods

Freshly hatched second-stage larvae of *Heterodera glycines* Ichinohe, 1952 were prepared for electron microscopic examination by using published procedures (Endo and Wergin, 1973; Wergin and Endo, 1976). Briefly, nematodes in a suspension of water were mixed with warm liquified 2% water–agar and the mixture was poured into small grooves in agar-filled petri dishes. The solidified agar, containing the nematodes, was diced into 2- to 3-mm blocks that were transferred to glass vials containing 3% glutaraldehyde in 0.05 M phosphate buffer (pH 6.8) at 22°C for chemical fixation of the larvae. Subsequent rinsing and postfixation in osmium tetroxide were also carried out in 0.05 M phosphate buffer (pH 6.8). The glutaraldehyde fixation (for 1.5 hr) was followed by washing in six changes of buffer over a period of 1 hr. The agar blocks were then postfixed in 2% osmium tetroxide for 2 hr at 22°C, dehydrated in an acetone series, and infiltrated with a low viscosity embedding medium (Spurr, 1969). Silver-gray sections of selected nematodes were cut with a diamond knife and mounted on uncoated 75 × 300-mesh copper grids. The sections were stained with uranyl acetate and lead citrate and viewed in a Philips 301 electron microscope[1] that was operated at 60 kV with a 20-μm aperture.

[1] Mention of a trademark, proprietary, product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products of vendors that may also be suitable.

Results

The anterior cells of the procorpus were closely associated with the stylet protractor muscles (Endo, 1983). The myofilamentous region of each muscle cell is continuous with the sarcoplasmic region that lies adjacent and posterior to the stylet knobs (Figs. 1–4). The cytoplasm around the zone of attachment to the knobs is largely electron translucent except for the tonofilaments. A region of abundant mitochondria lies behind this zone (Figs. 2–4).

Procorpus

Two protractor muscle cells, one with its nucleus in view (Fig. 2), are shown to be separated by another wedge-shaped cell—one of three such cells that have anterior extensions that traverse between the three stylet knobs. Their anterior extensions surround the shaft of the stylet (Figs. 1–4) and slightly posterior to the stylet knobs, these same cells appear perradial in cross section and extend from the lumen wall to the basement membrane of the procorpus (Fig. 5). Each of the three perradial cells (ShC) and the three interradial protractor muscle cells (PMC) contain numerous mitochondria and a nucleus. The sarcoplasm of the secondary stylet protractor muscles (ScC) lies between the arms of the perradial muscle cells (ShC) and is bordered on the outside by the protractor muscle cells (PMC) (Figs. 4, 5).

An integral part of the procorpus is the dorsally located branch of the esophageal lumen that ends in a tetraradiate valve within the ampulla of the dorsal esophageal gland (Figs. 1, 1c, 6, 6a, 6b, 7, 8). The valve is shown in a closed position in longitudinal-oblique view (Figs. 6, 6a) and in cross sections ranging from the highly sclerotized basal region (Figs. 6a, 7) to its distal regions (Figs. 6b and 8). In cross section, the distal parts of the closed valve form a cloverleaf pattern of multiple and single membranes (Fig. 6b). At the junction with the dorsal gland duct, the valve is continuous with the anterior wall of the dorsal gland ampulla (Fig. 6). Microtubules (Mt) that occur in the elongated cytoplasmic extension of the dorsal gland appear to converge and terminate at the base of the tetraradiate valve in the dorsal gland ampulla (Figs. 6, 6a, 9). In the vicinity of the valve, neurosecretion-laden nerve processes (NP) lie close to the dorsal surface of the ampulla (Fig. 8). Internally, the cytoplasm of the ampulla is interspersed with numerous dense secretory granules with closely adhering membranes (Figs. 6, 8).

The ampulla (Fig. 8) and the supporting dorsal gland extension (Fig. 9) lie in contact with cells that border the perimeter of the esophageal lumen wall. Thus, when the stylet is retracted, the dorsal gland process takes the same sinuous route through the procorpus as the lumen (Figs. 6, 9).

The multicellular procorpus contains six nuclei that lie in the extreme anterior region, three are associated with the stylet protractor muscles and three with the stylet shaft tissues. In addition, five nuclei occur in the midregion, and five in the narrow posterior region of the procorpus, just anterior to the metacorpus (Figs. 9, 10).

"Constraining muscles" (CM) are present in the posterior region of the procorpus. These multidirectional muscle elements are primarily circular in orientation with certain muscle elements directed anteriorly (Figs. 1, 9, 10, 12). These constraining muscles may affect the lateral limits of the dorsal gland extension as it passes from the metacorpus into the procorpus. The esophageal lumen (EL) is circular in cross section through this region and becomes triradiate at the metacorpus pump (Figs. 12–14, 17–19). In cross section, the constraining muscles are shown without hemidesmosomal contacts with the tubular lumen wall (Fig. 10a).

Metacorpus

Posterior to the constraining muscles of the procorpus, the lumen wall (ELW) is supported by multiple membranes (MM) and is surrounded by longitudinally oriented elements of the pump

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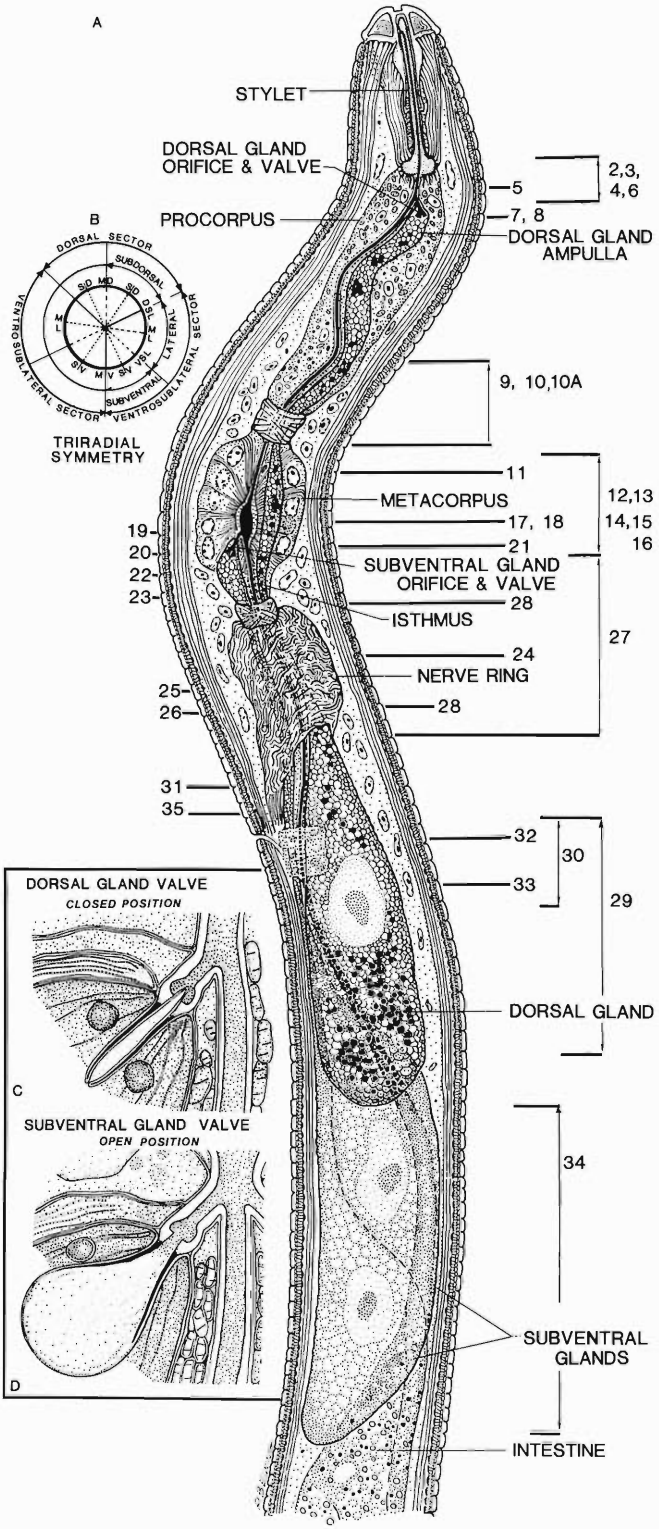
Figure 1. Diagrams of longitudinal views (LV) through the esophagus of *H. glycines*. (a) LV of the anterior region. Numbers of the diagram correspond to the micrographs in the text. (b) Terminology of body regions of nematodes with emphasis on triradiate symmetry of the esophagus (after Coomans, 1979a; Grootaert and Coomans, 1980). (c) LV showing a closed valve or end apparatus within a dorsal gland ampulla. Note the infolded valve membrane curves into the valve duct lumen. (d) LV showing the open valve or end apparatus within one of a pair of subventral gland ampullae.



muscle (Fig. 11). In this region, the dorsal gland extension (DGE) is bounded dorsally by a pair of nerve processes (NP) and neurosecretory cells (NSC), all of which appear to contain numerous neurosecretory granules (Fig. 11).

ANTERIOR REGION: Among the nine nuclei in this region, one large nucleus is slightly displaced posteriorly, and is in the mediolateral sector (Fig. 15). There are two each at the subdorsal radii (Fig. 11), the mediolateral radii, the ventrosublateral radii (Figs. 11–13) and the subventral radii (Fig. 11). The nuclei of the mediodorsal sector (Fig. 15) and of the ventrosublateral radii (Figs. 11, 13) appear to be neurons, whereas the remainder appear as parts of myoepithelial cell bodies of the metacorpus.

THE CENTRAL REGION: A second dorsal cell nucleus, which lies adjacent and immediately posterior to the nucleus in the anterior region of the metacorpus, extends posteriorly and is surrounded by the subdorsal pump chamber muscles of the metacorpus (Fig. 15). In each ventrosublateral (VSL) radius there are three nuclei that are aligned in a row (Fig. 13) such that the central one of each group lies in the same cross-sectional plane as the mediodorsal metacorpus nucleus (Fig. 17). The nuclei in the ventrosublateral radii are similar to each other and to other nuclei that lie anterior and posterior to them. At this same plane, a nucleus lies near each of the triradiate arms of the pump chamber (in Fig. 17 only two of three nuclei are seen). Thus 10 nuclei occur in the mid-region of the metacorpus, seven neural and three myoepithelial.

THE POSTERIOR REGION OF THE METACORPUS: One neural nucleus occurs in the dorsal section (Fig. 15), two neural nuclei in the ventrosublateral radii (Figs. 12, 13), and a single myoepithelial cell nucleus in the medioventral radius. The 10 nuclei in the ventrosublateral radii of the whole metacorpus are ovoid and are within cells that contain neurosecretory granules, similar to the

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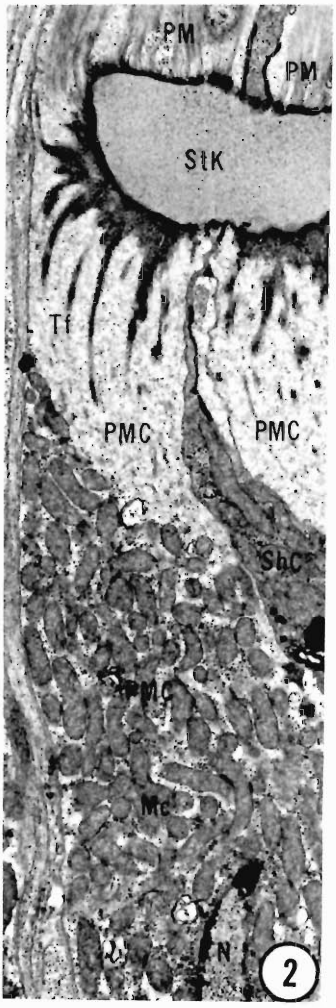
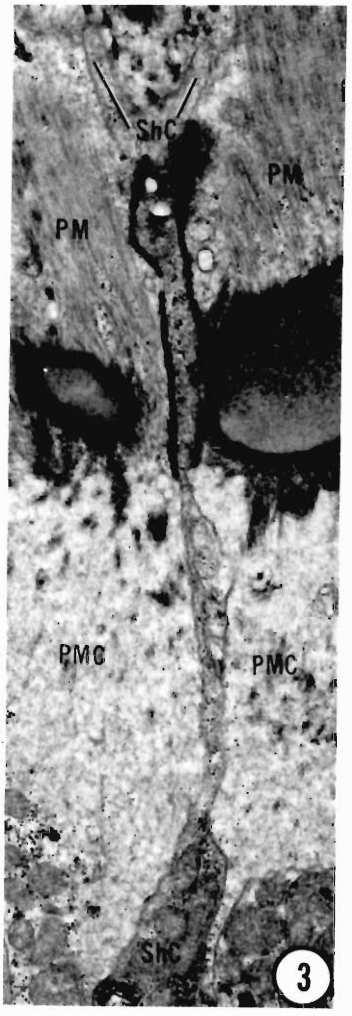
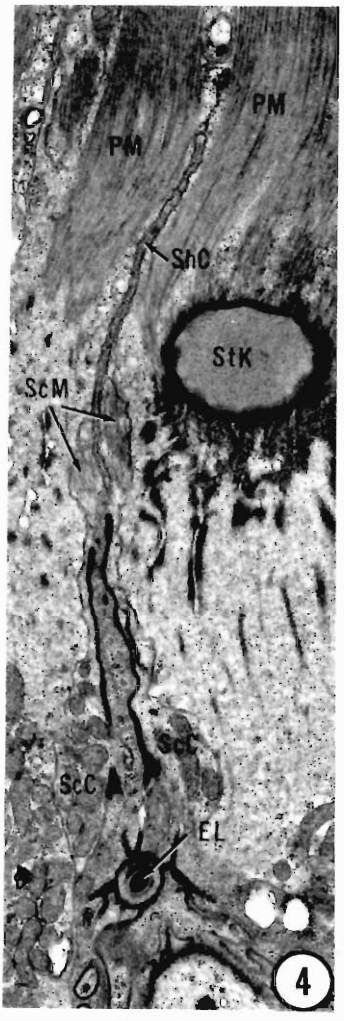
Figures 2–5. Longitudinal (LS) and transverse (TS) sections of the anterior procorpus region of esophagus. 2. LS shows the stylet protractor muscles (PM) and portions of their cell bodies (PMC). The tonofilamentous (Tf) region of the PMC's are separated by a delta-shaped, mitochondria-filled part of a stylet shaft supporting cell (ShC). 3. The stylet shaft cell (ShC) extends anteriorly and beyond the stylet knobs (StK). 4. Two of four secondary muscle (ScM) elements and their cell bodies (ScC) are located on either side of stylet shaft cell body (ShC). 5. Anterior procorpus just posterior to stylet knobs showing cell bodies of the stylet protractor muscles (PMC), the stylet shaft tissues (ShC), and the secondary muscle elements (ScC). C, cuticle; EL, esophageal lumen; Mc, mitochondria; N, nucleus; SM, somatic muscle; StK, stylet knob. Figure 2, ×30,040; Figure 3, ×49,320; Figure 4, ×37,600; Figure 5, ×31,150.

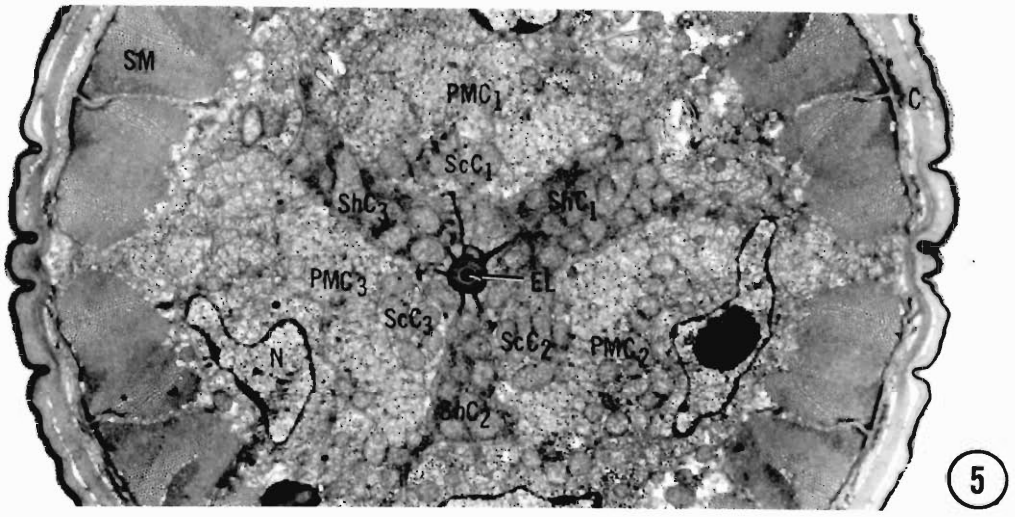
Figures 6–8. Longitudinal (LS) and transverse (TS) sections of the esophageal lumen (EL) and dorsal esophageal gland ampulla valve (DGAV). 6. LS through the stylet knobs (StK) and part of the lumen wall (ELW) with its supporting membranes (arrows). The lumen wall forms a short branch that ends in a membranous valve with cuticularized arms (DGAV) (see Fig. 8). The valve membrane is continuous with the anterior supporting membrane of the dorsal gland ampulla (DGA). Inset (a) Medial LS of the base (VB) of the DGA valve. Inset (b) TS of a distal section of a DGAV. Note that the microtubules (Mt) of the ampulla cytoplasm end at or near the base of the DGA valve (Figs. 6, 6a). 7. TS through the base of the valve (VB) of the dorsal gland ampulla (DGA). Procorpus cells are densely packed with mitochondria (Mc) and other organelles. 8. TS of the dorsal gland ampulla (DGA) showing the nearby esophageal lumen wall (ELW) and complex of cells and the support membranes that are continuous with the anterior ampulla membranes. The dorsal surface of the ampulla is apposed to an enlarged neural process (NP) that contains numerous neurosecretory granules. The cuticularized support arms (CSA) of the tetraradiate dorsal gland ampulla valve (DGAV) are shown closed. EL, esophageal lumen. Figure 6, ×15,050, (a) ×19,550; (b) ×26,130; Figure 7, ×32,400; Figure 8, ×22,100.

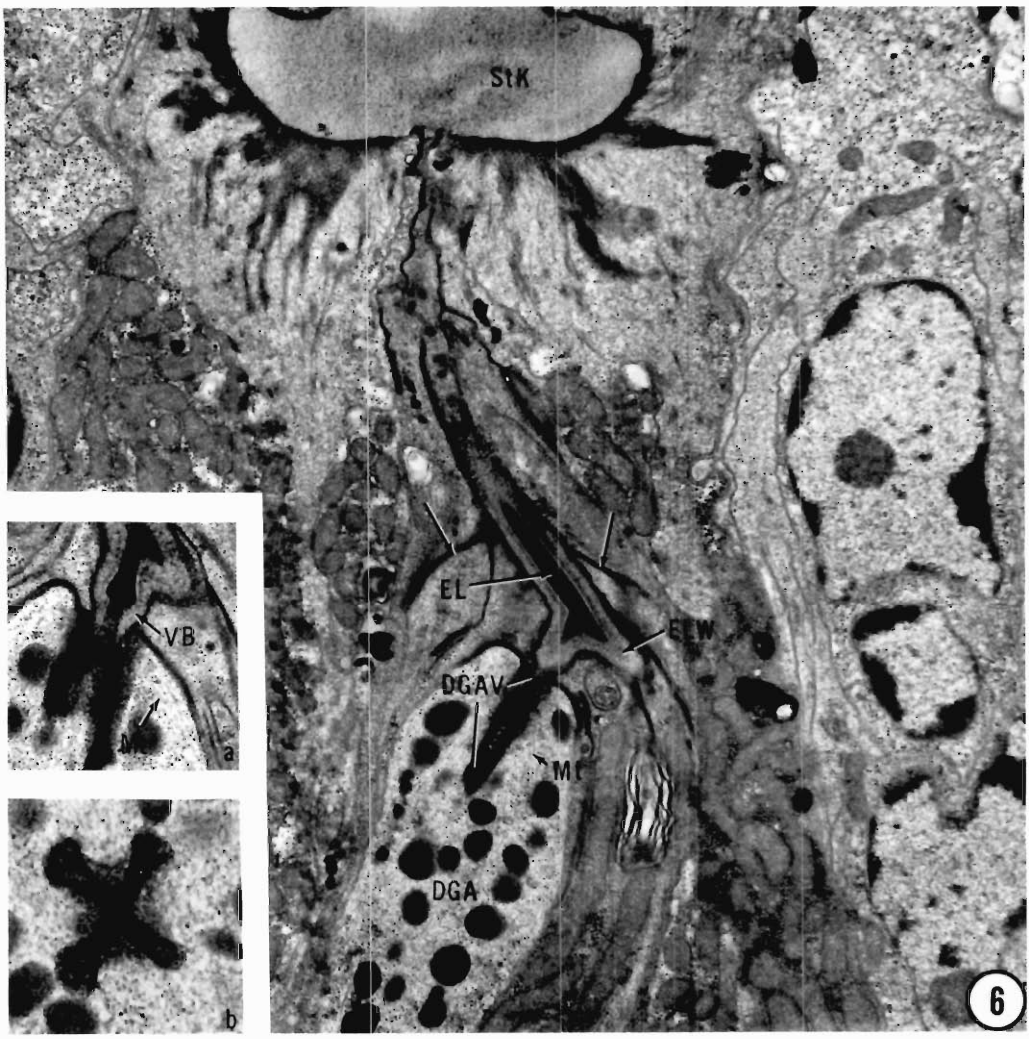
Figures 9–11. LS of the posterior procorpus and anterior metacorpus. 9. LS showing the dorsal gland extension (DGE) that traverses the procorpus (PC) and anterior metacorpus (MC), containing irregularly distributed groups of secretion granules (SG) and dilated, circular to elongate, vesicular rough endoplasmic reticulum (RER). The narrow posterior region of the procorpus consists of a collar of multidirectional constraining muscle (CM) elements. 10. A submedian LS of the junction between the procorpus and metacorpus showing a tangential view of a constraining muscle (CM) complex with some forward directed muscle elements (arrows). TS of the constraining muscles showing the lack of direct contact with the esophageal lumen wall (Fig. 10a). 11. TS of the anterior region of the metacorpus showing a membrane complex (MCX) attached to the lumen wall (ELW). Sections of five of the eight nuclei (N) in this plane of metacorpus are shown among muscle elments (MPM) that extend forward from the metacorpus pump wall. The dorsal region of the metacorpus is occupied by the dorsal gland extension (DGE) and three neural processes, two of which appear as axons (NP) and the other as a large neurosecretory cell (NSC). Figure 9, ×9,400; Figure 10, ×11,070, (a) ×9,650; Figure 11, ×41,800.

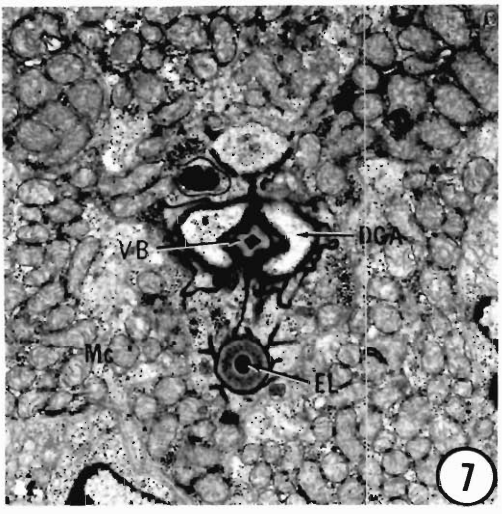


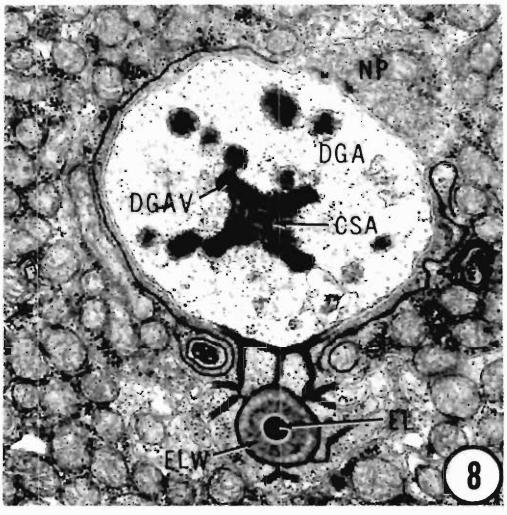


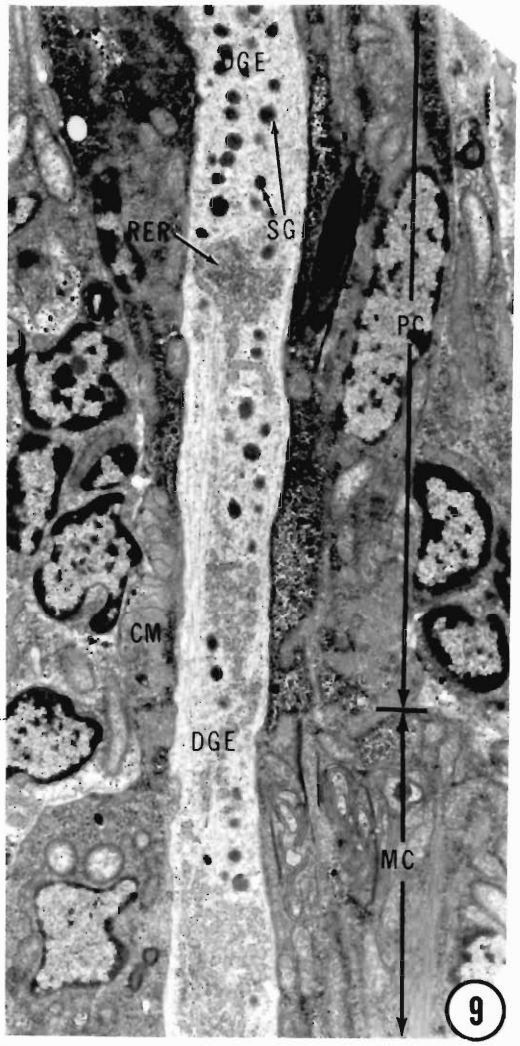


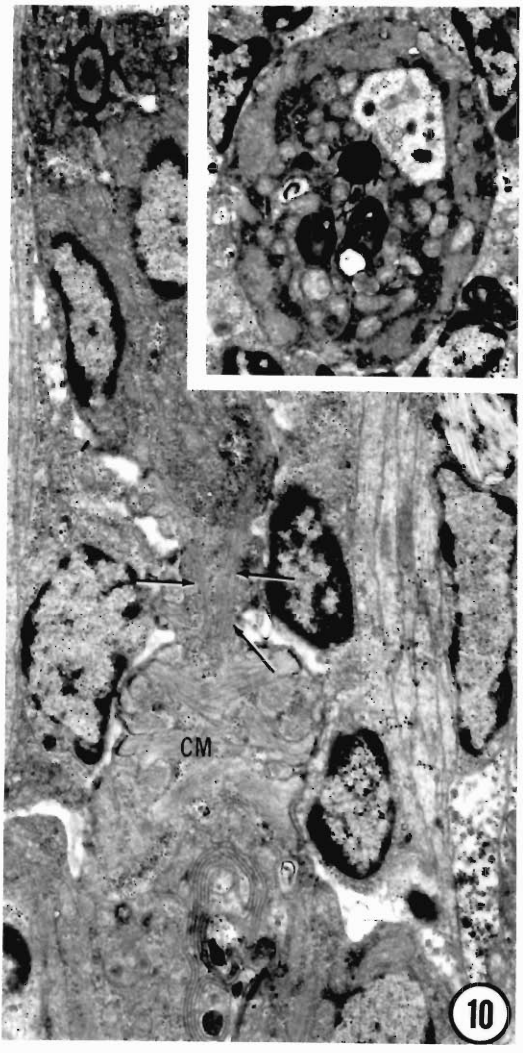


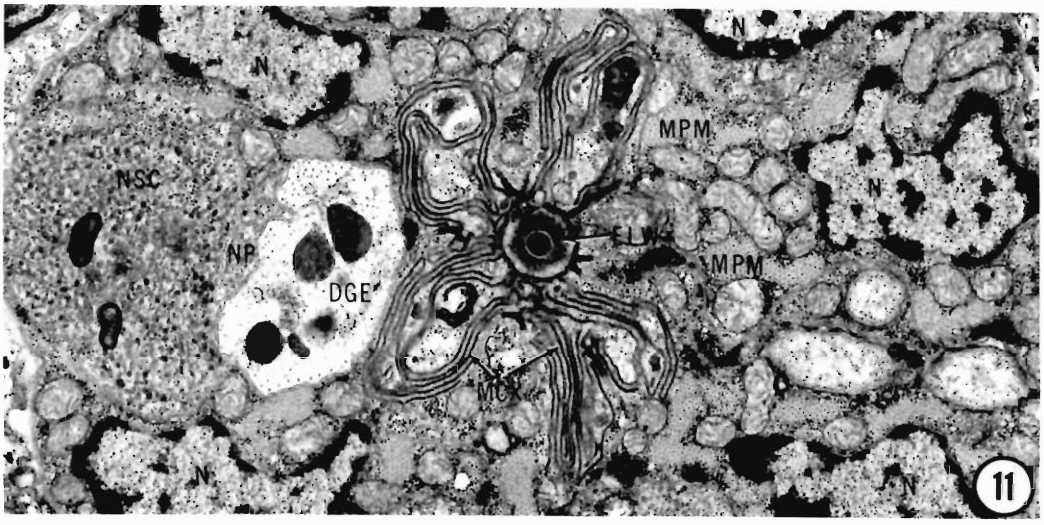












cells in the medial sector of the metacorpus (Figs. 12, 13, 15, 17). Thus, nine nuclei were found in the anterior, 10 in the central and four in the posterior regions of the metacorpus for a total of 23 nuclei; of these, 13 appear associated with neurons and 10 with myoepithelial cells. The number of nuclei and their morphological features were derived from a composite of several specimens.

Pump anatomy

In the relaxed, closed position, the lumen of the metacorpus pump is triradiate in cross section (Figs. 17, 18) and the thickened cuticle lining is cloverleaf-shaped. Bordering each arm of the pump are electron-translucent ridges (Fig. 14) that appear as spine-like projections (CR) in cross section (Fig. 18). Cell membranes attached below the ridges appear to be associated with the perradial nuclei of each arm of the lumen wall (Figs. 17, 18). The spine-like projections (Figs. 17, 18) when viewed in a longitudinal plane (Figs. 13, 14) are slightly below the crest of each arm of the pump wall.

Paired muscles are attached to the pump wall at the dorsal and the two ventrosublateral sectors by broad dense bands of hemidesmosomes (Fig. 17). The dorsal pump muscles with their adjacent membranes apposed, extend outward, separate, and move into the subdorsal sectors of the metacorpus (Figs. 17, 18). Similarly, each of the paired muscle elements of the ventrosublateral sectors extend outward to enter the lateral and subventral regions of the metacorpus (Figs. 17, 18). When viewed longitudinally, these muscle elements radiate outward and contact the boundary membrane of the metacorpus to show anterior, lateral, and slightly posterior projections of muscle ter-

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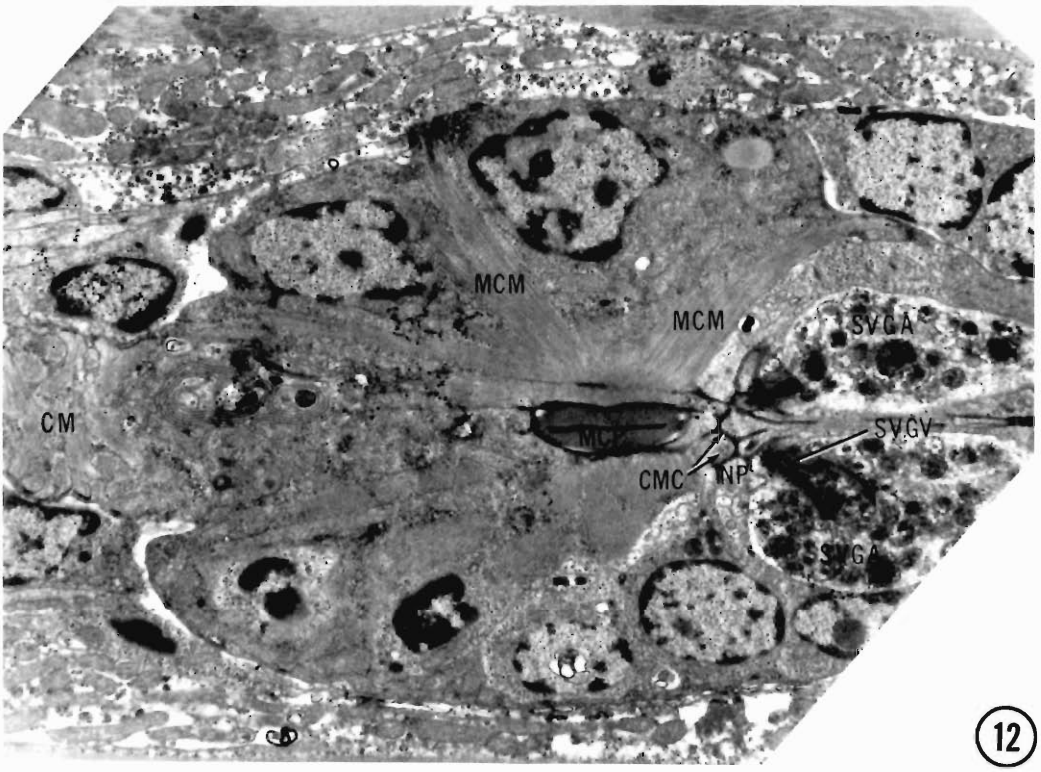
Figures 12–14. LS through the metacorpus showing the metacorpus pump (MCP) with associated muscles, nuclei and subventral esophageal gland ampullae and valves (SVGA, SVAV). 12. Sublateral section of the metacorpus showing the outline of the ventrally directed flange of the pump wall (MCP) with muscles (MCM) attaching to pump chamber lining and extending to various sectors of the metacorpus wall. The paired ampullae (SVGA) of the subventral esophageal gland extensions are supported by a cellular and membranous complex (CMC) just posterior to the pump. 13. A LS shows a row of five of the 10 nerve nuclei that occur in the ventrosublateral sectors of the metacorpus. Two arms of the triradiate pump chamber (MCP) are shown in TS and LS. 14. An enlargement of the metacorpus pump chamber (Fig. 13) shows the electron-translucent cuticular walls of the esophageal lumen branch (ELB) and the pump arms (PA), which are strengthened by a pair of electron-translucent cuticular ridges (CR). The lumen wall extends into the subventral gland ampulla (SVGA) and ends as slender electron-translucent supports in the membranous valve (SVGV). A cell complex (CMC) surrounds the base of the valve. Neurosecretory granules (NSG) are present in cells next to the SVG ampullae. Some secretion granules (SG) in an ampulla appear partially empty. The wall of the pump vestibule (PVe) narrows posteriorly to a triradiate closed lumen (TRL). In LS one arm of the TRL appears as a pair of electron-lucent cuticular walls separated by an electron-dense lumen. A similar region is shown in cross section (Fig. 20). Insert (a) of Figure 14. TS of a closed SVG valve in a second-stage larva at a feeding site. CM, constraining muscle; IM, isthmus muscle; N, nucleus; NP, neural process; SVG, subventral gland. Figure 12, ×9,370; Figure 13, ×7,950; Figure 14, ×19,000; (a) ×19,000.

Figure 15. Submedial LS showing nuclei of three neurons (Ne) with accumulations of neurosecretions lying dorsally in the metacorpus (also see Fig. 11). The adjacent dorsal gland extension (DGE) contains cytoplasm with prolific dilated cisternae of the rough endoplasmic reticulum (RER). Constraining muscles (CM) of the posterior procorpus are shown in tangential section. Figure 16. Oblique LS showing an open subventral gland ampulla valve (SVGV) supported by cuticularized arms (not shown, Fig. 14) and double and single membranes (M). The contents of the open valve (SVGV) are similar to the electron-dense contents of the secretion granules (SG) in the ampulla. Nerve processes (NP) of axons or dendrites surround the ampulla of the subventral gland. Muscle elements of the metacorpus pump (MPM) extend posteriorly and partially surround the ampullae and terminate near the muscular region of the isthmus (IM). MPM, pump muscle; SVGA, subventral gland ampulla. Figure 15, ×11,600; Figure 16, ×16,800.

Figure 17. TS of the metacorpus showing the triradiate pump chamber and muscles, neurons (NN) and somatic or sarcoplasmic cells. Figure 18. Enlargement of metacorpus pump of Figure 17 showing the attachment of the pump muscles (MPM) to the pump chamber wall. The pair of electron-translucent "ridges" on each arm of the pump wall were shown in Figures 12, 13, and 14 as spine-like ridges (CR) that follow the contour of the spindle-shaped valve. The paired muscle elements (MPM 1, 2) are attached to each other and to the interradial regions of the pump walls (PW). The dorsal esophageal gland extension (DGE) has cytoplasmic components that include secretion granules (SG), dilated rough endoplasmic reticulum (RER), and microtubules. Hd, hemidesmosomes. Figure 17, ×9,770; Figure 18, ×27,200.



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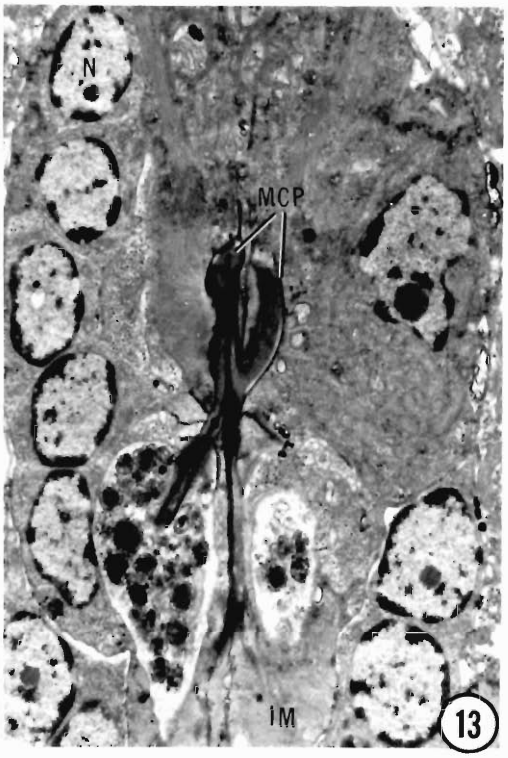

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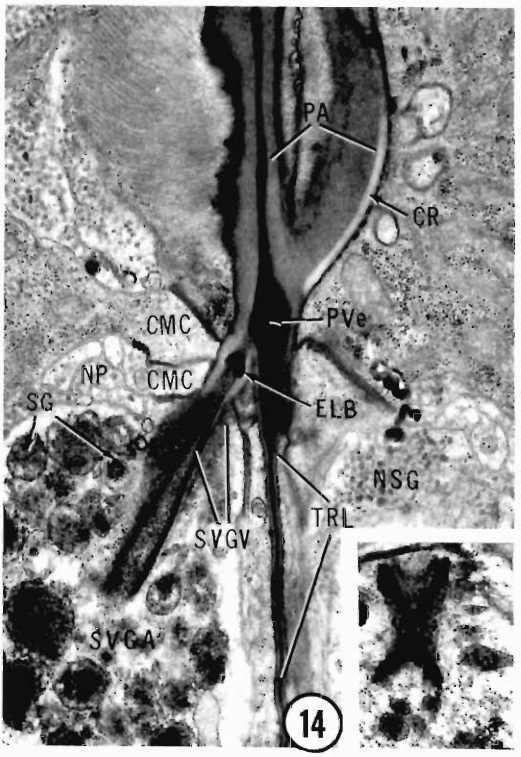

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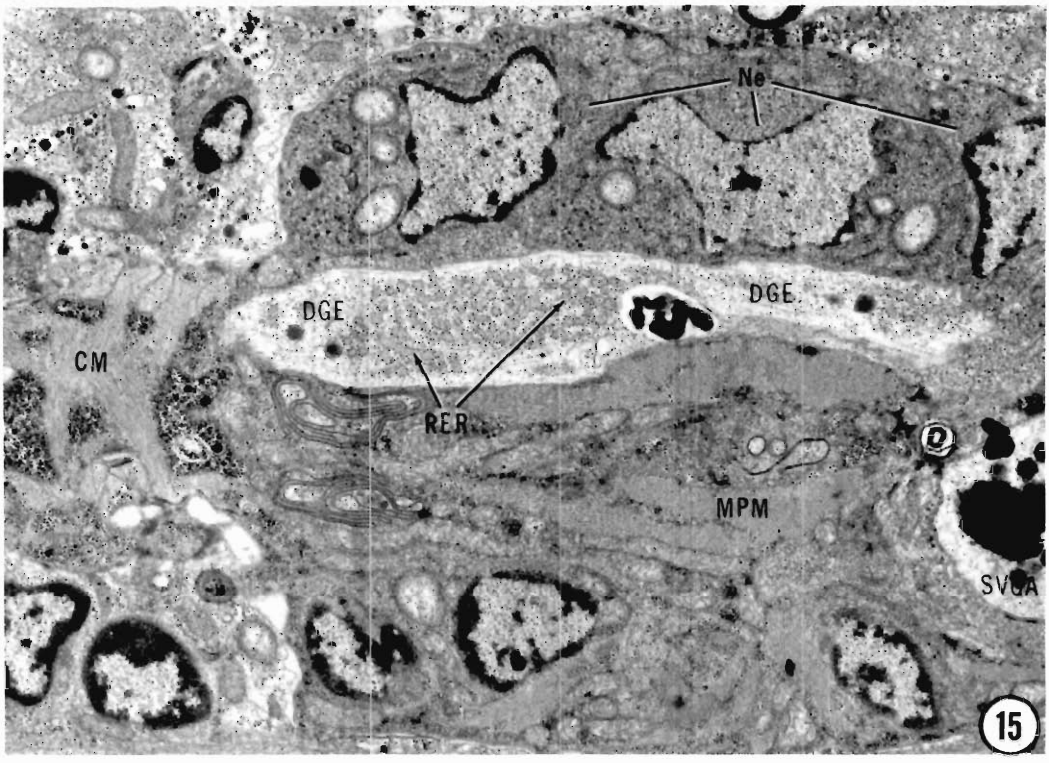

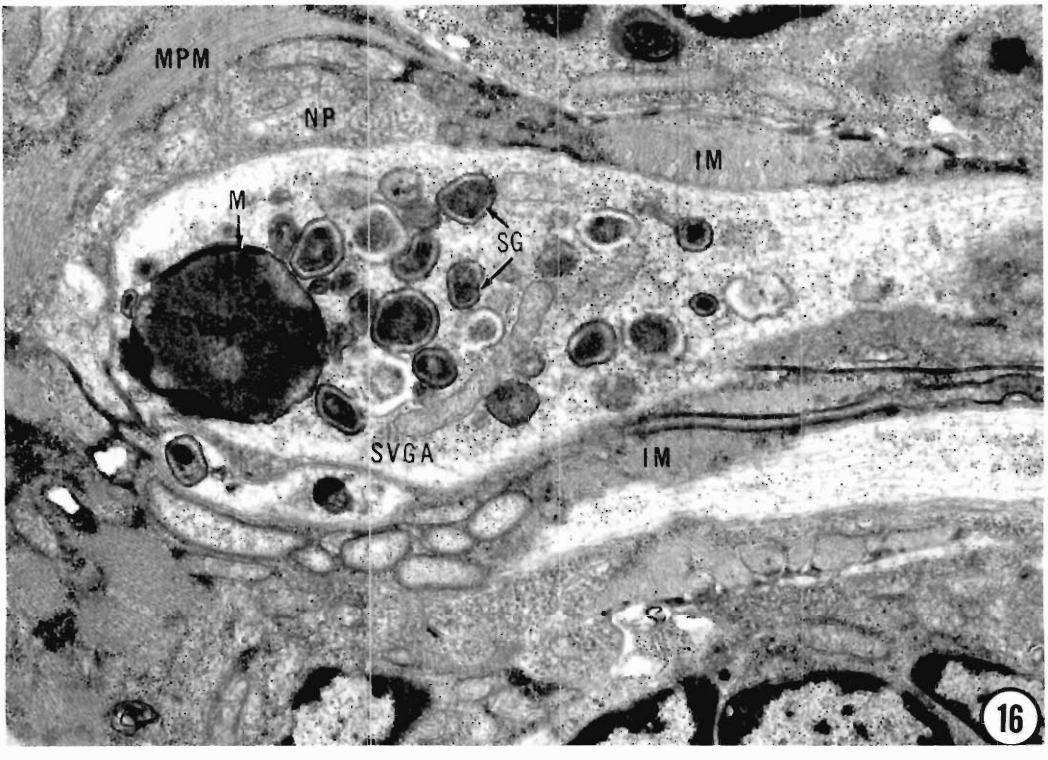

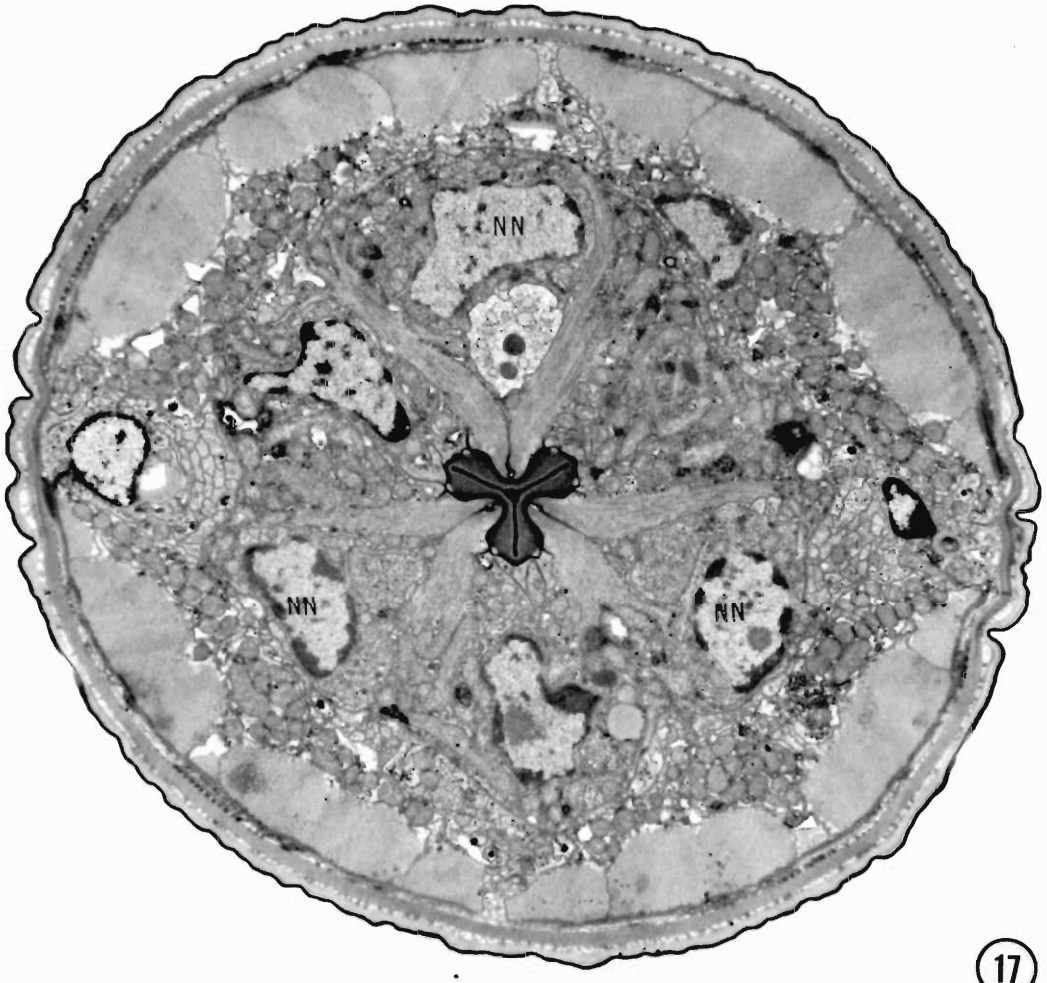

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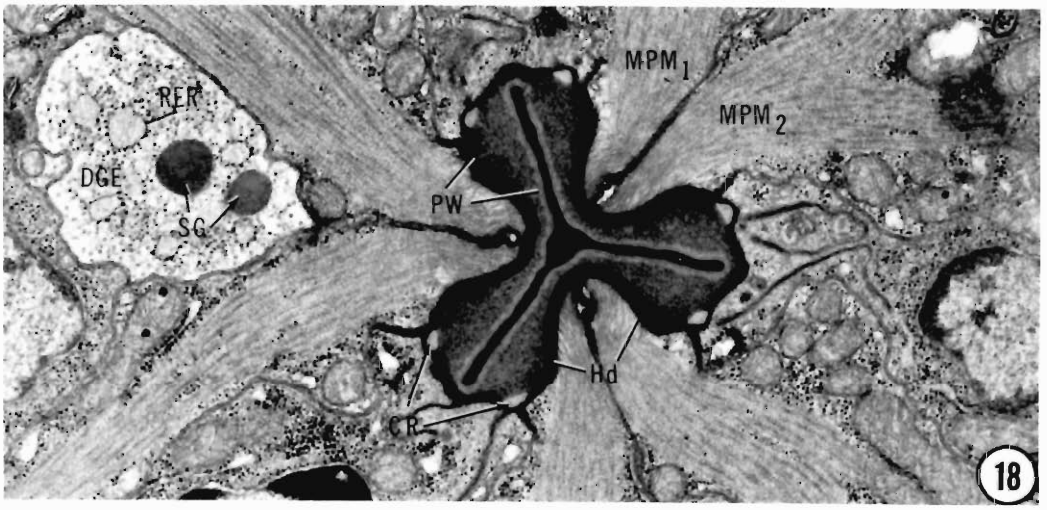

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minals. Numerous mitochondria and ribosomes occur throughout the sarcoplasm of these muscle cells (Figs. 15, 17, 18).

Subventral gland valves

The central lumen of the relaxed metacorpus pump chamber widens posteriorly into a triangular vestibule (Figs. 13, 14, 19). In each ventrosublateral sector, a branch adjoins the subventral gland ampulla to the central lumen of the vestibule (Figs. 12, 16, 19, 20). The lumen of the branch widens into a bulb-shaped cavity that narrows posteriorly when the valve is in a closed position (Figs. 13, 14). The lumen of the branch valve has a square cross-sectioned outline (Fig. 19). The valve, when closed, is tetraradiate in cross section (Fig. 14 inset), but when open, both the thickened membranes apposed to the cuticularized arms and the intermediate supporting membranes project outward to form circular outlines (Figs. 16, 20). The electron-dense material within the valves appears similar to the contents of adjacent secretory granules that are synthesized in the subventral glands (Figs. 29, 34, 35). The contents of the granules show various stages of opacity and are enclosed by membranes that have irregular surfaces or are discontinuous (Figs. 14, 16, 20). The cytoplasm of the subventral gland extension contains numerous microtubules, cisternae of rough endoplasmic reticulum, free ribosomes, and mitochondria (Fig. 12). The walls of the ampullae are surrounded by numerous nerve processes that are filled with neurosecretory granules (Figs. 14, 20). In cross section, the triangular pump vestibule (Fig. 19) narrows posteriorly into a triradiate lumen (Fig. 20). This lumen region is surrounded by attenuated cell processes, the dorsal gland extension and the subventral gland ampullae (Fig. 12).

Isthmus

The anterior region of the isthmus is muscular. The muscle elements appear similar to the constraining muscles that occur at the anterior of the

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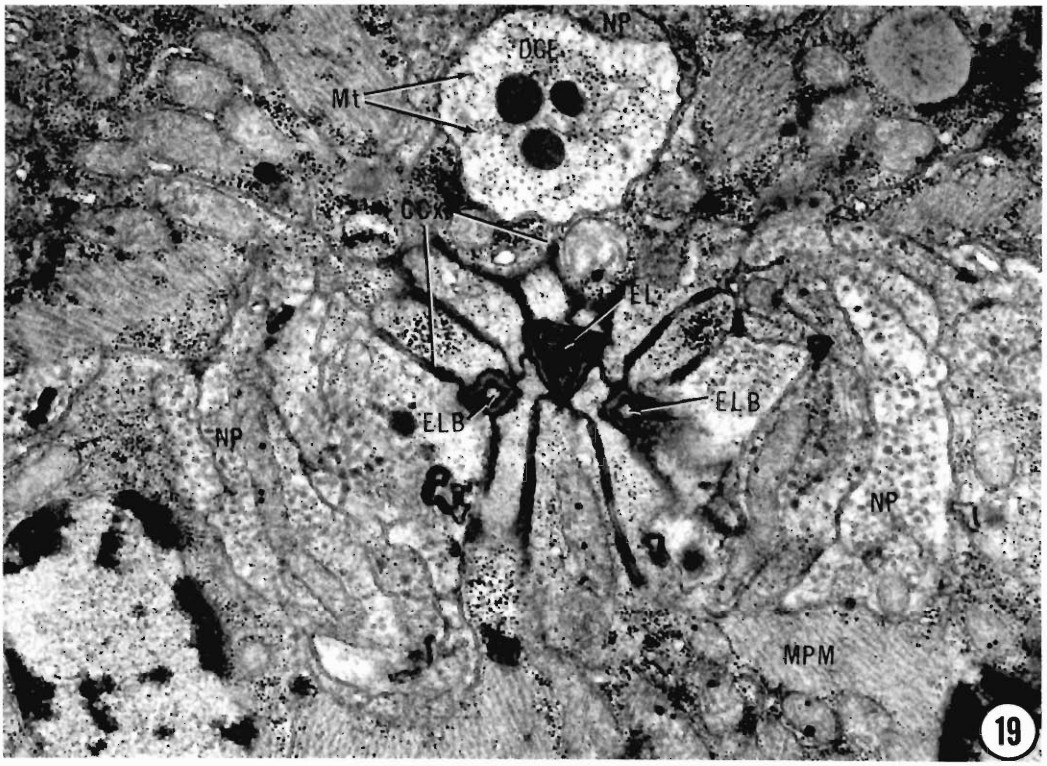
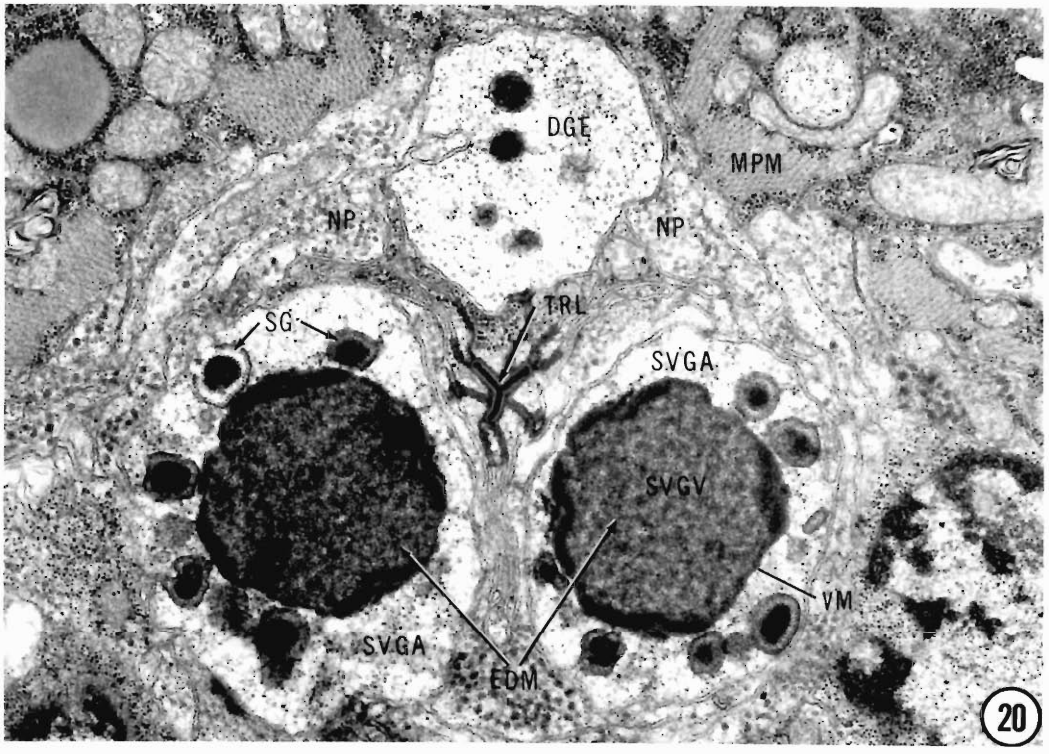
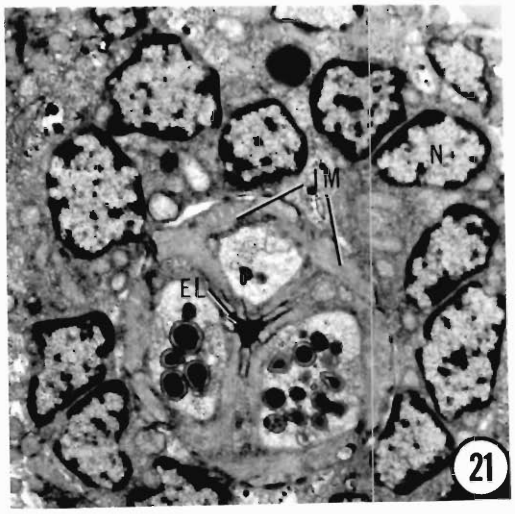
Figure 19. TS through posterior metacorpus showing the triangular shape of the central esophageal lumen (EL) and the square cross-sectional shape of its branches (ELB) that end in each of two subventral gland ampullae. These cuticular wall structures are connected with a complex of cells (CCx) and membrane junctions. Figure 20. TS of open valves (SVGV) within the subventral gland ampullae slightly posterior to that in Figure 19. The valve walls have characteristic single and membrane boundaries (VM) that confine the electron-dense material (EDM) which apparently accumulates from the secretory granules (SG). The closed triradiate esophageal lumen (TRL) extends from terminus of the metacorpus pump vestibule to the valve in the muscular anterior isthmus. The triradiate lumen is here not actuated by muscles but appears to be stabilized by membrane junctions and cells that are adjacent to and include the dorsal gland extension (DGE) and the subventral gland ampullae (SVGA). MPM, metacorpus pump muscle; Mt, microtubules; NP, nerve process. Figure 19, ×27,500; Figure 20, ×26,250.

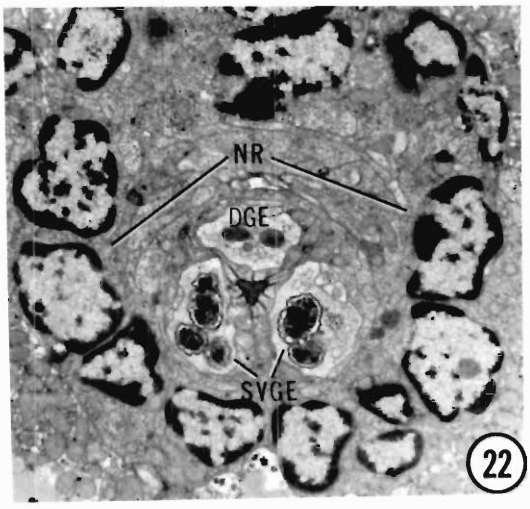
Figures 21–26. Series of TS through the isthmus and anterior gland lobe. 21. Partially open triradiate isthmus lumen (EL), with walls to which muscle elements are attached that interact with the peripheral muscle elements (IM). Isthmus is encircled by a cluster of nuclei (N) that lie between the metacorpus and the nerve ring. 22. The anterior of the nerve ring (NR) lies behind the muscular zone of the isthmus (Fig. 27) and is surrounded by the cluster of nuclei as shown in Figure 21. 23. The central region of the isthmus (Is) showing a triradiate lumen (EL) surrounded by the dorsal (DGE) and subventral gland extensions (SVGE). The nerve ring (NR) completely surrounds the isthmus with more neural processes in the dorsal region than in the ventral. 24. The dorsal esophageal gland (DG) dominates the anterior of the gland lobe where it is bordered laterally by nuclei and sublaterally by the subventral gland extensions (SVGE) that are filled with prominent secretion granules (SG). 25. Cross section of the irregularly shaped anterior glandular lobe (AGL). The lumen is encircled by slender supporting cells and outside these are the anterior portions of the dorsal (DG) and *subventral glands* (SVG). 26. A TS of the gland lobe showing different types of secretion granules (SG) in both the dorsal (DG) and subventral glands (SVG). VNP, ventral nerve processes. Figure 21, ×10,150; Figure 22, ×9,360; Figure 23, ×10,500; Figure 24, ×10,900; Figure 25, ×10,000; Figure 26, ×12,250.

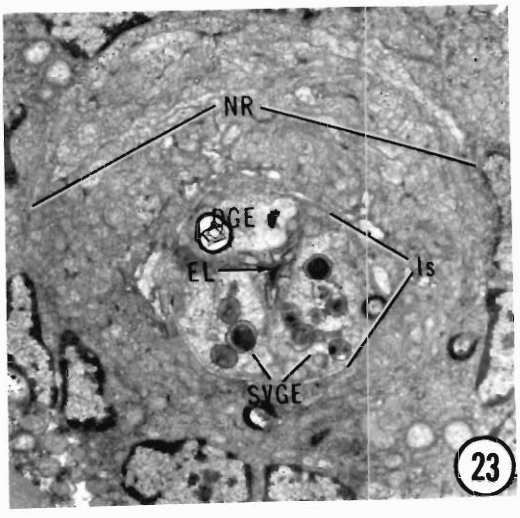
Figure 27. An oblique LS through the isthmus showing the dorsal gland extension (DGE) with nerve ring tissue (NR) on either side. The nuclei (N) adjacent to the nerve ring and the isthmus are part of the cluster that were shown to encircle this region in TS (see Figs. 21 and 22). Figure 28. A TS showing neurosecretions (NS) within nerve processes of the nerve ring (NR). Electron-dense sites (arrows) indicate axon–axon or axon–dendrite synapses. Secretory granules (SG) synthesized in the dorsal gland (DG) differ from those in the subventral glands (SVG), being more homogeneous and with less distinct limiting membranes (M). EL, esophageal lumen. Figure 27, ×13,900; Figure 28, ×34,800.

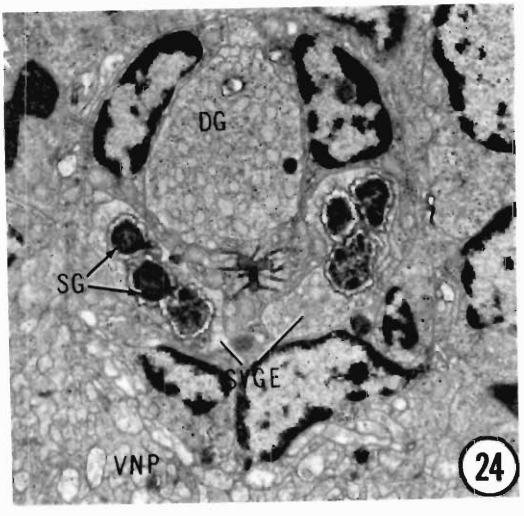


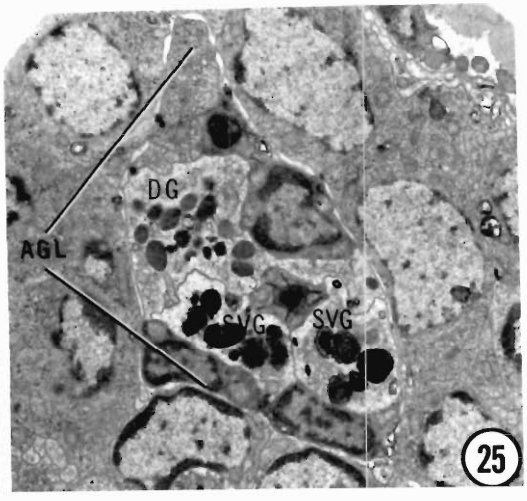


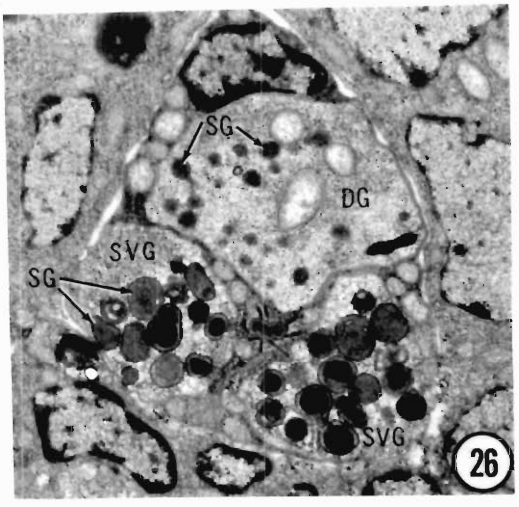


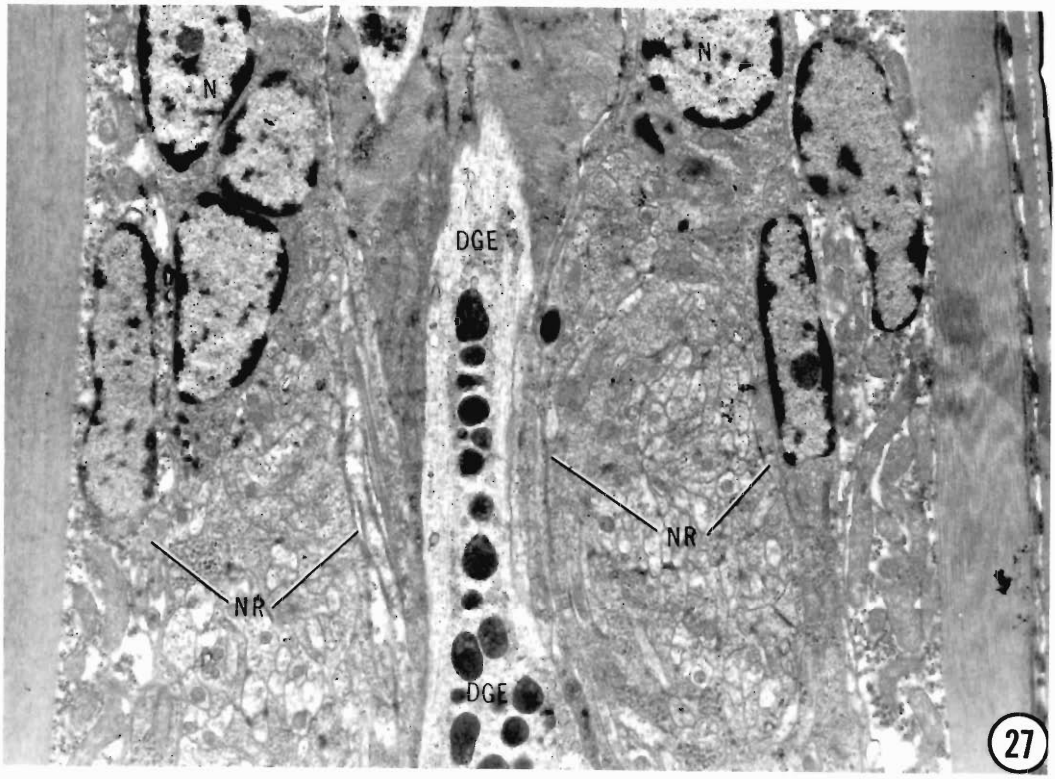


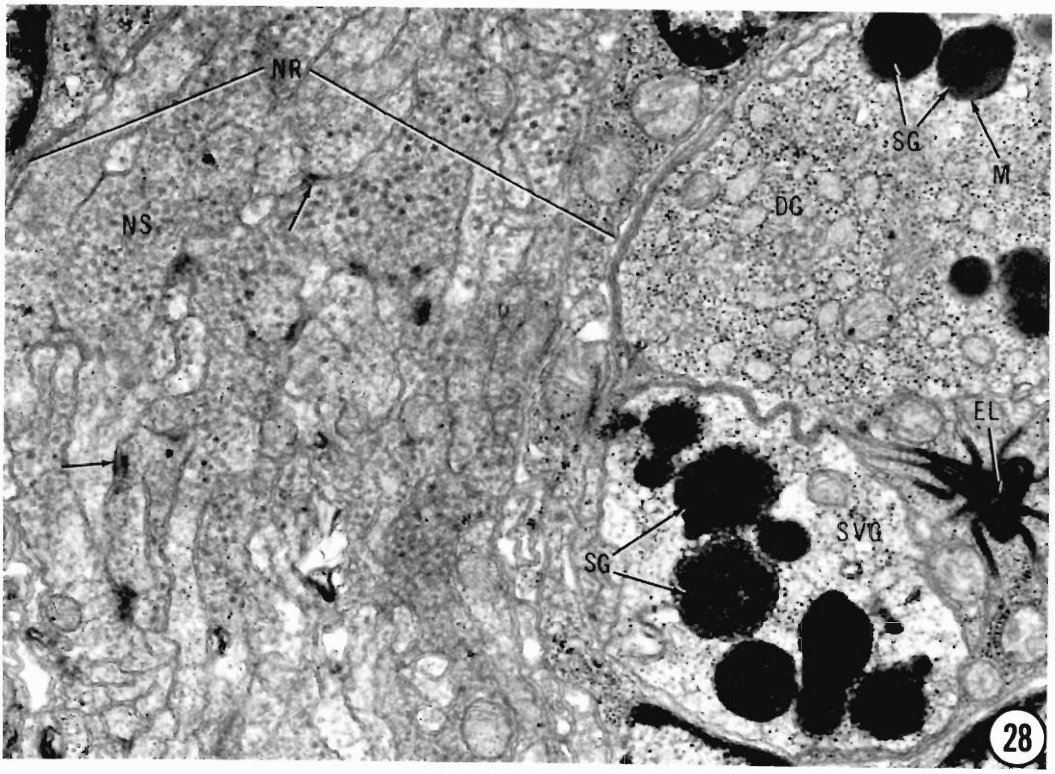












metacorpus. However, portions of the isthmus muscles also extend internally to attach to the wall of the triradiate esophageal lumen (Fig. 21). In longitudinal view, the isthmus muscle envelopes nearly one-third of the total length of the isthmus from the base of the metacorpus (Figs. 13, 16). The remainder of the isthmus includes the dorsal and two subventral gland extensions, the lumen wall with supporting membranes, nerve and somatic cells, and a supporting outer esophageal membrane (Figs. 22–25). The subventral gland extensions that traverse the isthmus contain irregular secretion granules. Some granules have a central electron-dense core surrounded by an electron-lucent perimeter with a clearly defined limiting membrane that occasionally has a wrinkled surface or appears discontinuous (Figs. 21–25). In contrast, the secretory granules of the dorsal gland process are somewhat more uniform in density and have a limiting membrane that is closely applied to the contents and is thus difficult to delineate (Fig. 22). Throughout the isthmus, the moderately thick lumen wall of the esophagus forms an open, triradiate lumen, which upon entering the gland lobe region becomes circular or flattened in cross section (Figs. 24–26).

The nerve ring encircles the isthmus and is regarded as the central nervous system of the nematode. It forms a circumesophageal commissure with an oblique orientation. The dorsal band of tightly arranged nerve processes are located more anteriad than the ventral band (Fig. 27). All views of the nerve ring show the complexity and omnidirectional pattern of the nerve processes that innervate specific cells, and show a high degree of interaction among the nerve processes through axon–axon and axon–dendrite synapses (Figs. 27, 28).

Gland lobe

Extending from the isthmus, the dorsal gland extension widens to form the anterior portion of the dorsal gland (Fig. 1) that contains densely packed cisternae of rough endoplasmic reticulum (RER) and moderate populations of secretion granules (Figs. 24–26, 28). The dorsal gland is bordered subdorsally by a pair of nuclei and ventrosublaterally by the subventral gland processes (Fig. 24). Further posteriorly, the adjacent limiting membranes of the dorsal and two subventral glands become apposed (Fig. 25). The glands surround a central complex of cells that support a cuticularized, thin-walled esophageal lumen (Figs. 25, 28). Sections through the narrow part of the gland lobe show that a central nonglandular complex of cells becomes progressively distended, thus separating the individual gland cells (Figs. 26).

The subventral glands (SVG) appear to have more abundant electron-dense secretion granules than the dorsal gland (DG), although some SVG granules have less uniform electron-dense contents than the DG granules (Figs. 26, 29, 31).

The terminus of the lumen of the esophagus is modified just anterior to the plane of the dorsal gland nucleus from an open, cuticularized structure to an esophageal–intestinal valve of closely apposed unlined cell membranes. This valve is continuous with the cuticle-lined lumen (Figs. 29, 30) and interacting apposing membranes as shown in tangential (Fig. 29), longitudinal (Fig. 30), and cross-sectional (Fig. 31) views. Ultimately, the contents of the lumen empty into a broad space surrounded by intestinal epithelial cells, seen in longitudinal and cross sections in Figures 32–34.

The large dorsal gland nucleus (Figs. 30, 33) and the pair of moderately large subventral gland nuclei (Fig. 34) are foci of secretory activity where Golgi apparatuses are abundant and where secretion granules are formed (Figs. 32, 33, 33a). The pair of subventral glands are readily discernible as individual units throughout the length of the isthmus and anterior part of the gland lobe (Figs. 22–26) and their individuality is confirmed by the presence of apposed membranes separating the cells well into the region of high secretory activity (Figs. 32, 35). Similarly, the discreteness of the dorsal gland cell is seen in Figures 32, 35.

Discussion

Anterior procorpus

The core of cells and membrane junctions that surround the lumen wall of the esophagus of *H. glycines* larvae was similar to that described for *H. glycines* males (Baldwin et al., 1977) and for several other tylenchid species (Chen and Wen, 1972; Wen and Chen, 1972; Anderson and Byers, 1975; Shepherd and Clark, 1976).

In larvae of *H. glycines,* the tissues referred to as marginal or perradial cells in the anterior procorpus of *T. dubius* (Anderson and Byers, 1975) and as interjacent cells of *H. glycines* males (Baldwin et al., 1977) are identified as stylet shaft cells. Slender cytoplasmic arms of these cells were found to extend from the procorpus, between the

stylet knobs, and to the tissues surrounding the stylet shaft. Although each of the cell bodies of stylet cells could not be followed to their respective terminations at the stylet shaft, the relationship is evident. Furthermore, the presence of circular membrane junctions at several vertical levels along the shaft (Endo, 1983) suggests tissues similar to arcade tissues described in nontylenchid species such as *Caenorhabditis elegans* (Wright and Thomson, 1981). Several cell nuclei occur in the central and posterior regions of the procorpus of *H. glycines* larvae. These nuclei could not be assigned to specific cells, but with others must relate to the secondary muscles and nerve processes of the procorpus. In contrast, an aphelenchid, *Aphelenchoides blastophthorus,* had no nuclei in the procorpus but the cell bodies of the procorpus cells and the stylet protractor muscle cells, with their nuclei, were located in the metacorpus (Shepherd et al., 1980). Like *H. glycines* larvae, other tylenchid species have procorpuses that are multinucleated (Yuen, 1968a; Anderson and Byers, 1975; Baldwin et al., 1977).

Dorsal gland ampulla and end apparatus

The duct at the anterior of the dorsal gland extension in larvae of *H. glycines* supports a membrane-bound valve with tetraradiate form when closed, like that described in other tylenchid species (Anderson and Byers, 1975; Baldwin et al., 1977). In the open position, the membrane-bound valve can become filled with material that looks like the contents of the secretion granules. A similar valve or end apparatus with a membrane boundary was shown closed in *T. dubius* (Anderson and Byers, 1975). On the basis of stereo images (not shown), the membrane of the closed valve of the dorsal gland ampulla extends into the vestibule of the sclerotized duct at the base of the valve (Fig. 6a).

Posterior procorpus and anterior metacorpus

The complex of muscles that encircles the posteriormost region of the procorpus in *H. glycines* appears similar in structure to the constraining muscles described for the metacorpus of *A. blastophthorus* (Shepherd et al., 1980) and may function as suggested for *A. blastophthorus,* by providing stability for the metacorpus during pump muscle action. However, in *H. glycines* these constraining muscles may also affect the movement and distribution of dorsal gland secretory granules as they pass through this muscular region. This would not apply to *A. blastophthorus* because the dorsal gland ampulla and valve join the esophageal lumen posterior to the constraining muscles. Constraining muscles were not described in the ultrastructural observations of *H. glycines* males (Baldwin et al., 1977) or of *Ditylenchus dipsaci* (Yuen, 1968b).

Central metacorpus

The lumen of the esophagus is circular in cross section anterior to the metacorpus pump. The closed pump chamber is triradiate in cross section and, except for a triangular vestibule behind the pump chamber, becomes triradiate through the isthmus. The complex of membranes from cells that surround the anterior circular esophageal lumen appears mainly structural. Most of the cytoplasm enclosed by these membranes appears to contain glycogen granules that are usual components of myoepithelial cells.

In males of *H. glycines,* the two nerve processes that border the dorsal gland ampullae were traced to a pair of perikaryons that are located in the metacorpus (Baldwin et al., 1977). It was suggested that through intermembrane communication, these nerve processes produce neurosecretions that could modify dorsal gland products (Baldwin, 1973; Anderson and Byers, 1975). Similarly, the presence of secretory nerves in association with the ampulla in *T. dubius* led to the suggestion that ampullar contents are made fluid by neurosecretions or transmitters released during the salivation phase of feeding (Anderson and Byers, 1975). In second-stage infective larvae of *H. glycines,* dense accumulations of secretory granules lie along the length of these neuron processes with broad intermembrane contact with the dorsal gland extension. This close relationship is further indicated by synaptic junctions that occur between the gland extension and the neurons. It appears that neurosecretory products may enter the dorsal gland extension at the ampulla as reported by others (Baldwin, 1973; Anderson and Byers, 1975; Baldwin et al., 1977), but a similar contact zone also occurs in the anterior region of the metacorpus where the dorsal gland extension is bordered by a pair of axons and an enlarged neurosecretory cell.

The cytoplasm of the esophageal glands of males of *H. glycines* consists of dense spheres, microtubules, ribosomes, and a few mitochondria in a matrix of fine granules (Baldwin et al., 1977). In both males and larvae of *H. glycines,*

the granular appearance is due to irregular accumulations of dilated cisternae of rough endoplasmic reticulum (RER) densely packed around a prominent nucleus.

The metacorpus pump

In contrast to the irregular arrangement of the pump muscle elements in the metacorpus of *H. glycines* males (Baldwin et al., 1977), the pump muscle elements in the larvae were relatively symmetrical. The arrangement resembled that in *A. blastophthorus* which has the same triradiate symmetry as *H. glycines* but the muscle bands have more densely packed fibers (Shepherd et al., 1980). Other features of the metacorpus of the Aphelenchina that differ from that in *H. glycines* are the entrance of the dorsal esophageal gland duct into the isophageal lumen within the bulb just anterior to the pump chamber and the presence of a secondary triradiate valve in the circular lumen between the dorsal gland duct orifice and the pump chamber (Shepherd et al., 1980). In the Tylenchina, the dorsal gland orifice is just behind the stylet knobs and there is no secondary valve.

The electron-lucent regions of the outer layer of the pump wall were called "spines" in *A. blastophthorus* by Shepherd et al. (1980) and "tubercles" in *H. glycines* males by Baldwin et al. (1977). In *H. glycines* larvae, these regions are longitudinal thickenings that apparently provide support for the triradiate arms of the pump and maintain its shape as the interradial regions are pulled outward during the contraction of the pump muscle. The pump muscle terminals have uniform hemidesmosomal contacts along the wall of the pump which may result in coordinated muscle action that opens and closes the spindle-shaped lumen with a minimal displacement of surrounding cells.

Subventral esophageal gland duct and valve

The closed tetraradiate duct valves of the subventral and dorsal glands are similar to those occurring in several nematode species (Bird, 1969; Endo and Wergin, 1973; Anderson and Byers, 1975; Baldwin et al., 1977; Shepherd et al., 1980). In all cases, the valves (end apparatuses) were in closed positions. In *T. dubius,* the dorsal end apparatus was enclosed in a membranous sac-like region (end sac) with contents that differed from those of the surrounding ampulla (Anderson and Byers, 1975). In *H. glycines* larvae, the subventral gland valves with their four-sided sclerotized wall sections are shown open for the first time.

Membrane-bound secretory granules, synthesized in subventral glands, apparently are transported close to the surface of the membrane-bound end apparatus, perhaps by microtubules. Because the material in the expanded valve is homogeneous, it seems that only the contents of

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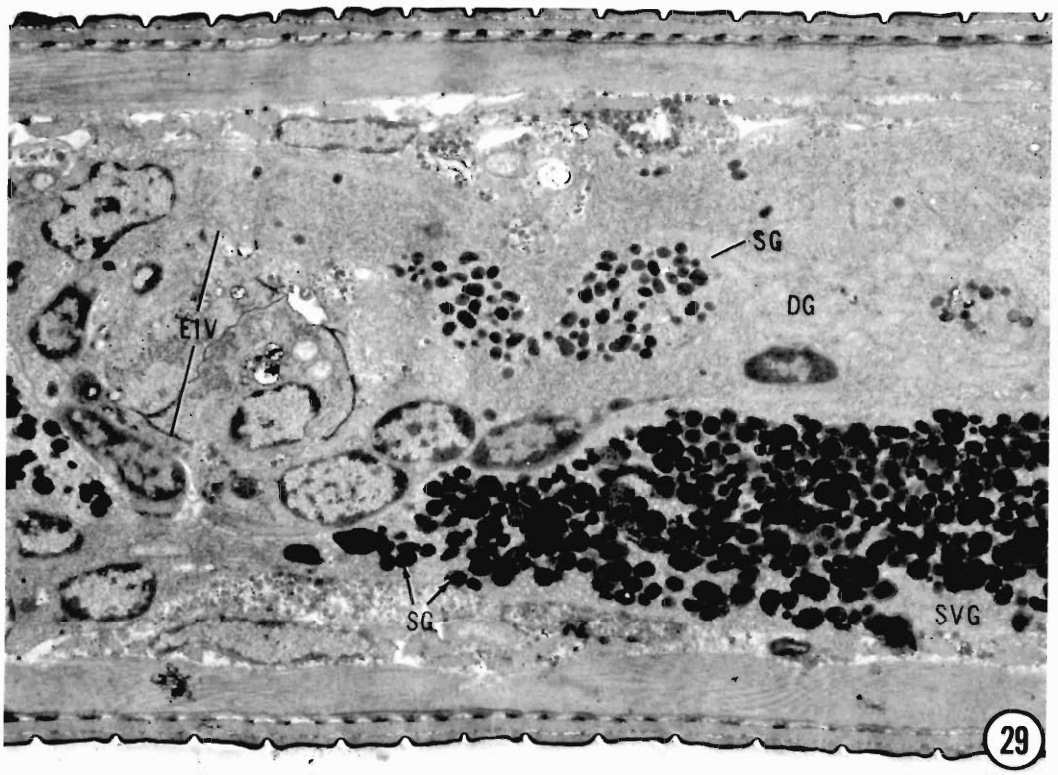
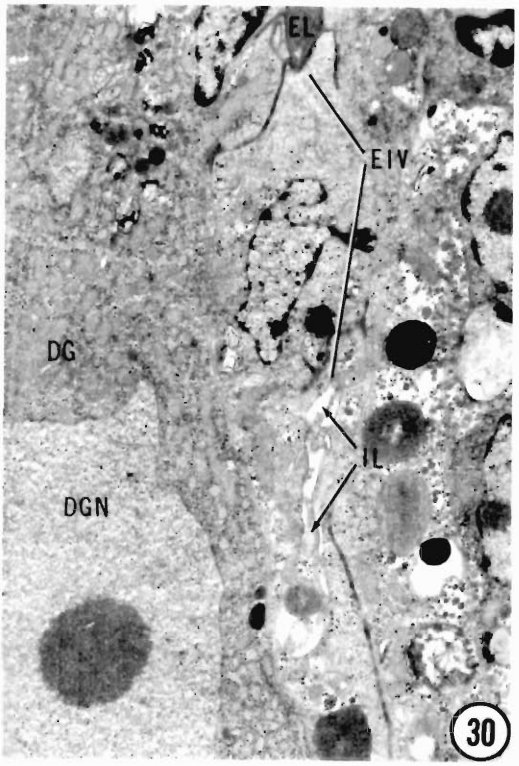
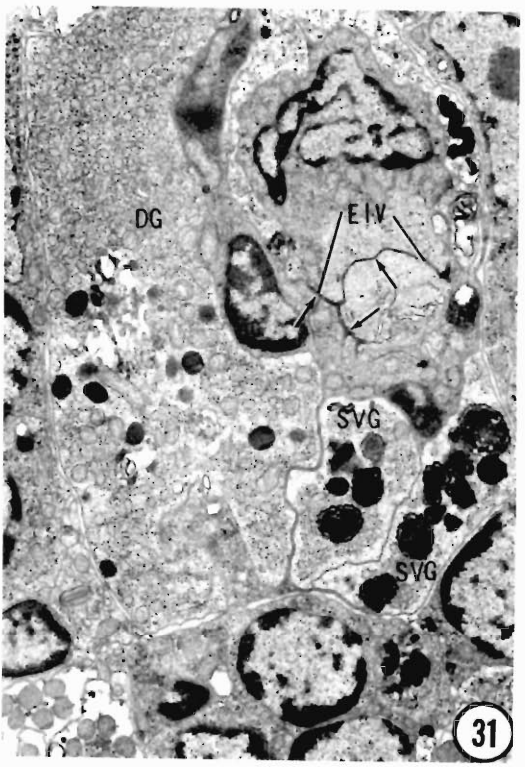
Figure 29. LS of the gland lobe showing portions of the esophageal–intestinal valve (EIV) and the secretion granules (SG) accumulated in the dorsal (DG) and subventral glands (SVG). Figure 30. LS of the gland lobe showing the terminus of the open cuticle-lined esophageal lumen (EL) and its relationship to the intestinal lumen (IL). Figure 31. TS slightly anterior to the dorsal gland nucleus showing the apposed membranes (arrows) of the esophageal–intestinal valve (EIV). The subventral gland (SVG) cells are slender and appear like discrete cells that lie adjacent to the esophageal–intestinal valve and the dorsal esophageal gland (DG). DGN, dorsal gland nucleus. Figure 29, ×7,220; Figure 30, ×8,480; Figure 31, ×11,550.

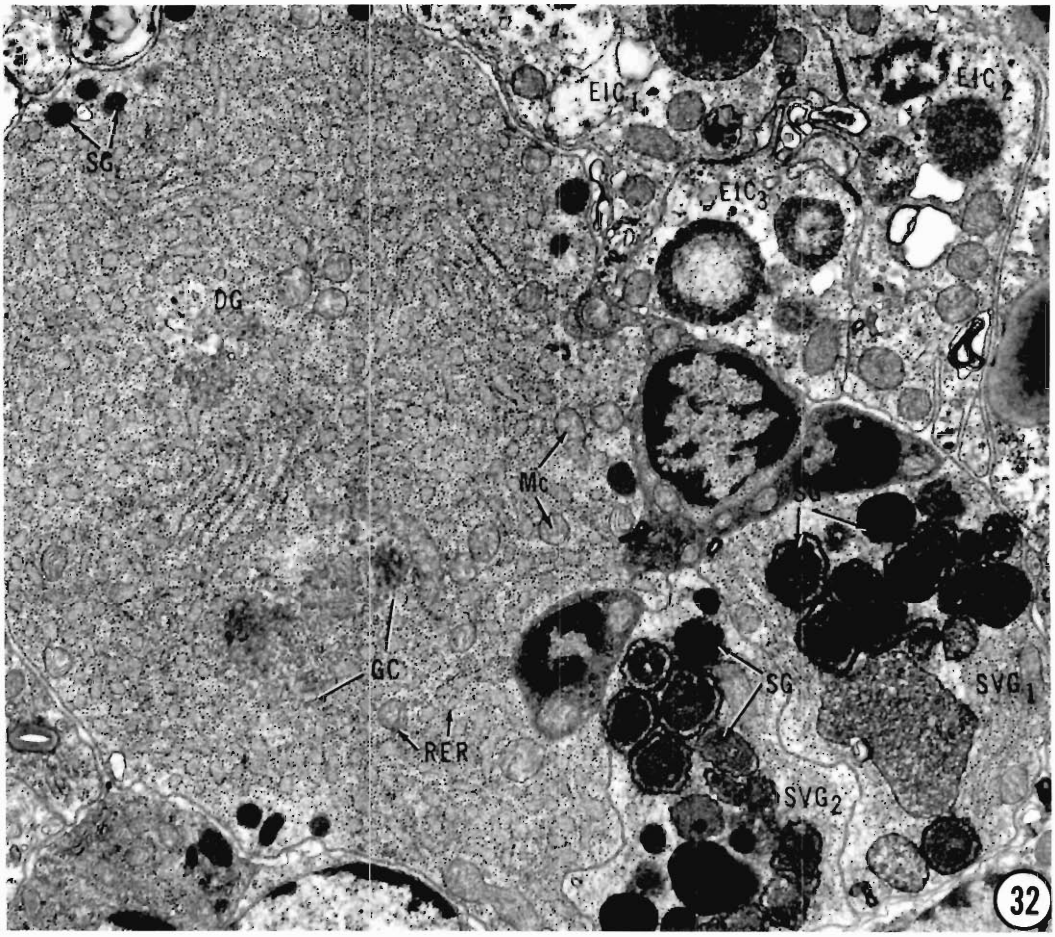
Figure 32. Transverse section of the central region of the dorsal esophageal gland (DG) filled with densely packed, dilated rough endoplasmic reticulum (RER), mitochondria (Mc), Golgi cisternae (GC), and some secretion granules (SG). The esophageal–intestinal tissue is composed of three cells (EIC 1, 2, 3) and their membrane junctions near the lumen. The two subventral glands (SVG_1, SVG_2) with prominent secretion granules are clearly separated from each other and the dorsal gland (DG) by well-defined membranes. Figure 33. A TS through the dorsal esophageal gland (DG) and nucleus (DGN) with adjacent intestinal lumen (IL) and intestinal epithelial cells (IC). Figure 33a. Golgi cisternae (GC) in early stages of formation of secretion granules (SG), some of which are shown in Figures 32 and 33. Figure 32, ×19,250; Figure 33, ×18,000; (a) ×28,000.

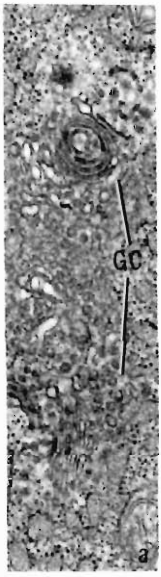
Figure 34. LS of the posterior gland lobe showing nuclei (N_1, N_2) and secretion granules (SG) of the paired subventral esophageal gland cells (SVG_1, SVG_2). The slender intestinal region (IR) broadens immediately posterior to the gland lobe. Figure 35. TS of the gland lobe showing many membrane-bound granules (SG) in the two subventral gland cells (SVG_1, SVG_2), with wide ranges of electron opacity, and the intercellular membranes (arrows) between the dorsal (DG) and subventral gland (SVG_1) cells. IL, intestinal lumen; SM, somatic muscles. Figure 34, ×4,950; Figure 35, ×16,900.

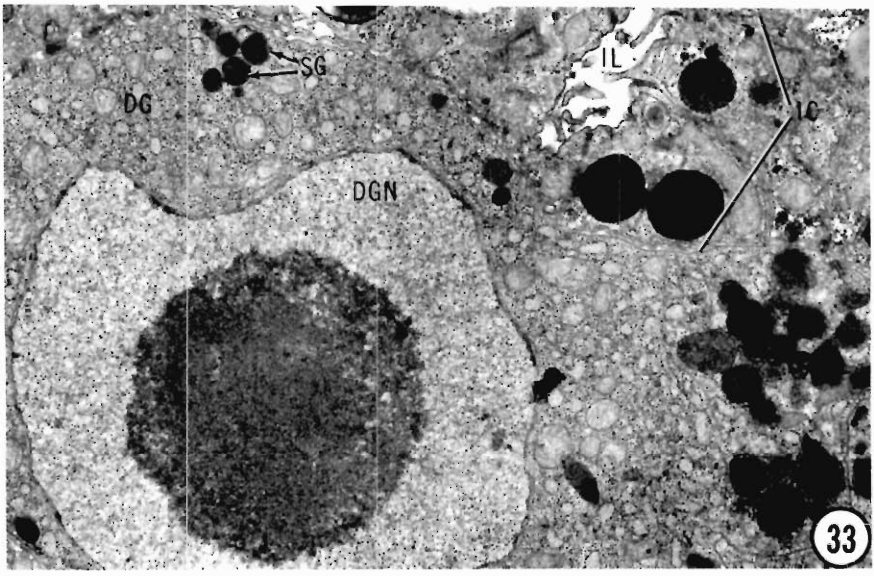


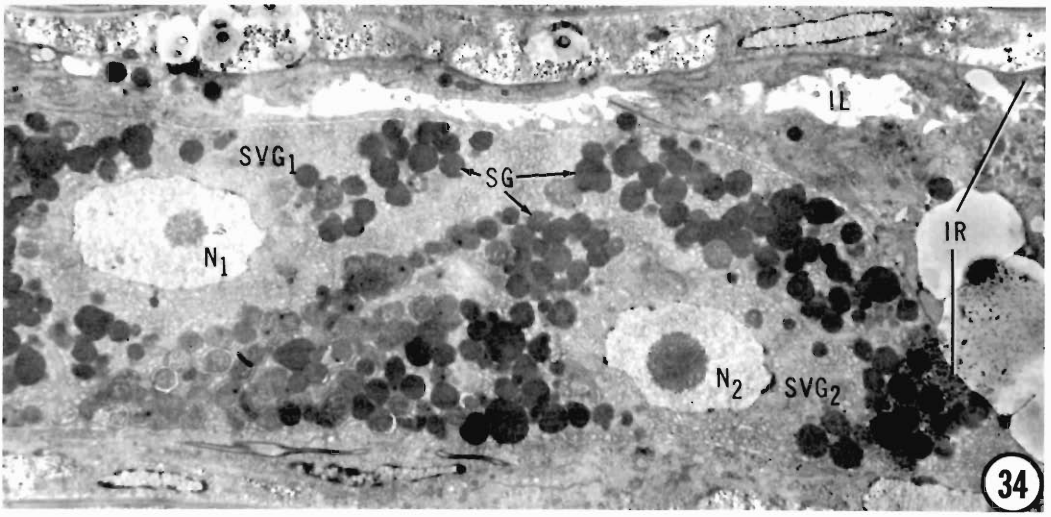


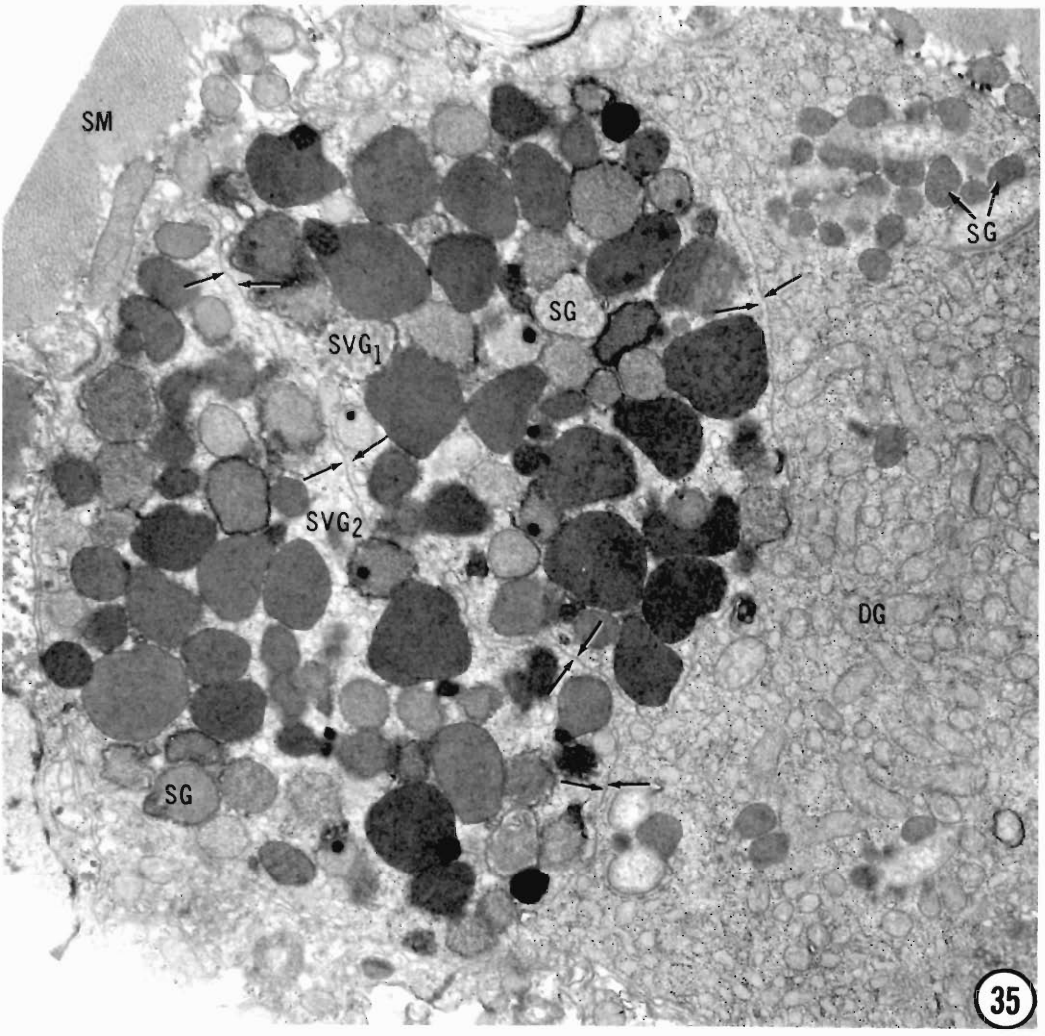












the secretion granules are transferred through the valve and then into the lumen of the esophagus. As in the dorsal gland extension and ampulla, the subventral gland ampullae contain the secretion granules, cisternae of rough endoplasmic reticulum, free ribosomes, mitochondria, and linearly oriented microtubules.

The mechanism controlling the end apparatus of the subventral gland ampulla appears similar to that described for the dorsal gland valve or end apparatus by Anderson and Byers (1975). Furthermore, certain nucleated cells that surround the sclerotized base of the valve may be involved in its operation. This may be especially pertinent if the sclerotized regions of these cells are resilient and can move the sclerotized valve arms and their attached membranes. Changes in turgor pressure of the ampulla contents of each subventral gland may also be involved in the valve operation.

Similar to the dorsal gland ampulla and the gland extension, the ampullae of the subventral glands are apposed to nerve cell processes, secretion granules of which may interact with the contents of the ampullae and in some way influence the movement and release of glandular secretions.

Posterior triradiate lumen of the metacorpus

The triradiate lumen of the closed metacorpus pump chamber leads to an elongated vestibule which is triangular in cross section (Fig. 19). This vestibule may be a collection point for a subsequent passage of subventral gland secretions sent forward or may be where ingested food mixes with gland secretions prior to movement into the posterior metacorpus lumen. Alternatively, as suggested by Bird (1968), the subventral glands and their secretions may be actively involved in the breakdown of egg shell and plant cell wall during hatching and initial host penetration, respectively. Further, studies on the mechanism and timing of secretory granule movement of esophageal glands await specialized technology such as the cine microscope procedures used by Doncaster (1966), Muller and Wyss (1981), and Wyss (1973, 1974) on tylenchid species and the cytochemical techniques used in studies of the pharyngeal pump of *Ascaris lumbricoides* and other species (Mapes, 1965a, b, 1966). The triradiate lumen between the pump chamber and the muscular posterior metacorpus region is usually closed. However, as food is ingested, materials may be pushed through the triradiate lumen by coordinated action between the central and posterior metacorporal pump muscles.

As the esophageal lumen extends through the muscular region of the isthmus, it becomes triangular in cross section and the lumen wall appears to be under outward tension. This region apparently functions as a valve and is controlled by some of the constraining muscles at the outer margin. Beyond this valve, the ingested materials pass through a lumen with an irregularly shaped cuticle-lined wall. Ultimately, these materials are regulated as they are transported between the unlined membranes of the esophageal–intestinal valve before entering the intestinal lumen as described by Seymour and Shepherd (1974).

Gland lobe

The dorsal and subventral esophageal glands constitute the major components of the gland lobe. However, several other cells and their nuclei occur within the gland lobe and are associated with the esophageal–intestinal valve and the esophagus. In *H. glycines* males, the subventral glands are not discrete and the pair is considered to be syncytial (Baldwin et al., 1977). However, in *H. glycines* larvae, the subventral gland appear intact. Thus, if the syncytial condition arises, as reported for males, cell membrane dissolution must occur in later developmental stages.

Near the site of synthesis, most of the secretion granules in subventral glands appear similar, but in the vicinity of the end apparatuses in the ampullae, many have a dense core graduating to a translucent periphery. Perhaps the granules, through membrane-to-membrane contact, transfer portions of their contents into the membrane-bound end apparatus. Alternatively, as reported by Baldwin (1973) and supported by Anderson and Byers (1975), the granules may release their contents into the ampulla through the action of neurosecretions released by adjacent neural processes. The modified gland secretions could then accumulate within the end apparatus and later pass into the central lumen of the esophagus. Whereas the secretion granules may differ in appearance in the dorsal and subventral glands, their chemical composition may be a more important factor in determining their functions. As an example of cytochemical tests, Bird (1968) showed that the secretions in the ampulla of the dorsal gland and the stylet exudations of root-knot nematode contained basic proteins. The

modification of these and related techniques adapted to ultrastructural studies (Bird, 1969) should be useful in determining differences between secretions from the dorsal and subventral glands of various stages of *H. glycines*.

Considerable effort is necessary to establish the relationships of nematode structure and function. With the utilization of existing and future technology, the functions of organs, especially the esophageal glands, that relate to nematode survival may be better defined. As shown in research on inhibitors of molting hormones in insects and other arthropods, inhibitors may be developed to interrupt or deter the feeding process. Very little is known about the synthesis of the stylet in the tylenchid plant parasitic nematodes. This is a part of the molting process that involves the tissues of the anterior esophagus. A detailed study of the molting stages of the nematode is required to clarify this site of cellular activity and this may provide clues for future control technologies.

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Comparison of Host Response of *Cryphodera utahensis* with Other Heteroderidae, and a Discussion of Phylogeny

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ABSTRACT: *Cryphodera utahensis* Baldwin et al., 1983 induces a single uninucleate giant cell (SUGC) in *Rosa* sp. The giant cell is similar to those associated with certain other heteroderids, including *Meloidodera* spp., *Hylonema* sp. and *Sarisodera* sp., but contrasts with the syncytia of *Atalodera* spp. and *Heterodera* spp. sensu lato. The wall of the SUGC of *C. utahensis* is unevenly thickened with the thickest region corresponding to the area penetrated by the stylet. The remainder of the wall includes numerous pit fields with plasmodesmata. Thorough examination of the cell wall with light and transmission electron microscopy indicates that wall ingrowths or protuberances are absent. The single nucleus is deeply invaginated with at least one nucleolus, and the cytoplasm includes abundant organelles. The pattern of host responses among Heteroderidae is congruent with existing hypotheses of phylogeny, and suggests that the syncytium of *Atalodera* spp. arose independently from that of *Heterodera* spp. sensu lato.

Heteroderidae modify their hosts in various ways to sustain nutrition during development as sedentary parasites. *Heterodera* sensu lato and *Atalodera* result in a syncytium (Mundo and Baldwin, 1983a), whereas *Hylonema* (Taylor et al., 1978), *Sarisodera* (Mundo and Baldwin, 1983b), and *Meloidodera* (Mundo and Baldwin, 1983c) induce a single uninucleate giant (hypertrophied) cell (SUGC). These two basic types of responses could be included with additional characters in a phylogenetic analysis of Heteroderidae (Mundo and Baldwin, 1983a, b, c).

The host response to *Cryphodera* has not been previously described; however, this genus shares a number of characters with *Meloidodera,* and on this basis we hypothesized that *Cryphodera* also induces a SUGC. In this paper we test the hypothesis by histological examination of roots of the type host, wild rose (*Rosa* sp. L.), infected with *Cryphodera utahensis* Baldwin et al., 1983.

Materials and Methods

Roots of rose infected with *C. utahensis* were collected at the type locality at Clear Creek Canyon, Sevier County, Utah (Baldwin et al., 1983). Root pieces containing mature females were prepared for histological examination including bright field and Nomarski interference light microscopy (LM), and transmission electron microscopy (TEM). Methods of processing tissue were generally as reported by Mundo and Baldwin (1983a, b, c). Roots were fixed for bright field LM in glutaraldehyde, embedded in Paraplast-Plus®, sectioned at 8 μm and stained with safranin and fast green. Additional material for examination with bright field LM was embedded in Spurr's resin, sectioned at about 2 μm and stained with methylene blue and azure II; other infected root pieces were stained with toluidine blue, which has been shown to be useful for detection of wall ingrowths. Some resin-embedded sections, which were examined by Nomarski interference LM, were not stained.

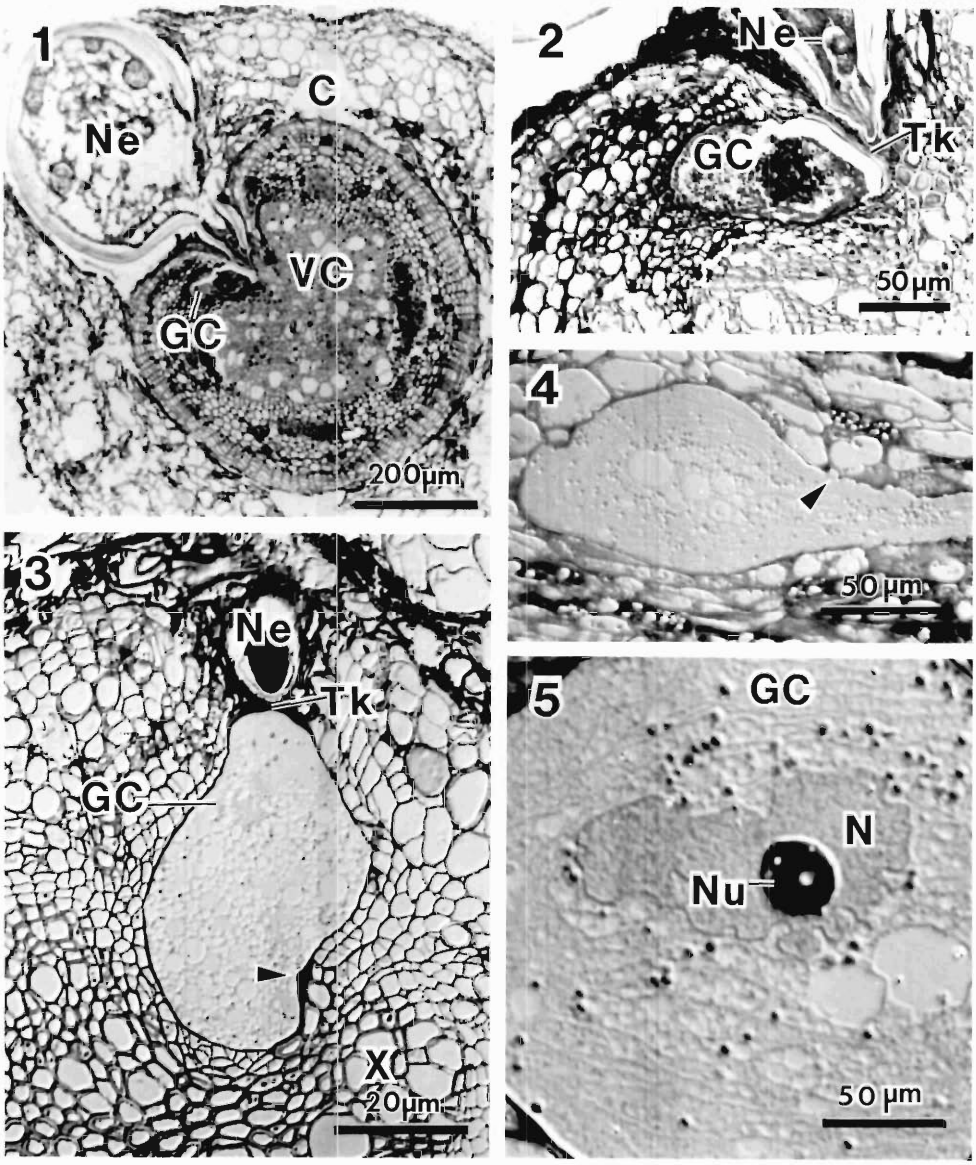
Root segments were fixed for observation with TEM in 3.0% glutaraldehyde, postfixed in 2.0% osmium tetroxide (OsO_4), embedded in Spurr's resin, thin sectioned, and stained with uranyl acetate and lead citrate.

Results

Rose infected with *C. utahensis* lacks external symptoms, but females partially protrude from roots (Fig. 1). The SUGC contacts cells of the vascular cylinder including phloem, vascular cambium and xylem (Figs. 1, 3). Surrounding cells were not hypertrophic, however, slight hyperplasia was often observed (Fig. 3). The SUGC varies in size and shape and averages 140 × 200 μm. Only one giant cell per nematode female was observed.

Cell walls of the SUGC induced by *C. utahensis* are unevenly thickened. The portion of wall closest to the nematode lip region is thickest (Figs. 2, 3, 11). The remaining area of wall has alternate thick and thin regions (Figs. 4, 12). However, in some areas where the SUGC wall contacts xylem elements, deposits of wall material are particularly heavy (Fig. 3). Thin portions of the wall correspond to the location of pit fields (Figs. 6, 12) which have a high frequency of plasmodesmata (Figs. 13–15). Plasmodesmata also characterize narrow portions of cells that sometimes occur adjacent to the SUGC (Fig. 13). Cell walls

[1] A portion of senior author's Ph.D. Thesis.



Figures 1–5. Transverse (unless otherwise indicated) sections of giant cells induced by *Cryphodera utahensis* in roots of *Rosa* sp. 1. Bright field light microscopy of female (Ne) in feeding position. C, cortex; GC, giant cell; VC, vascular cylinder. 2. Enlargement of the giant cell (GC) showing thick cell wall (Tk) in close proximity to nematode (Ne) lip region. 3. Giant cell (GC) showing wall thickening (Tk) in the area adjacent to the nematode head (Ne). Arrowhead indicates wall thickening in area adjacent to xylem (X). 4. Longitudinal view of a giant cell. Arrowhead indicates region of thin wall that characterizes pit field. 5. Giant cell (GC) showing nucleus (N) which includes a single nucleolus (Nu).

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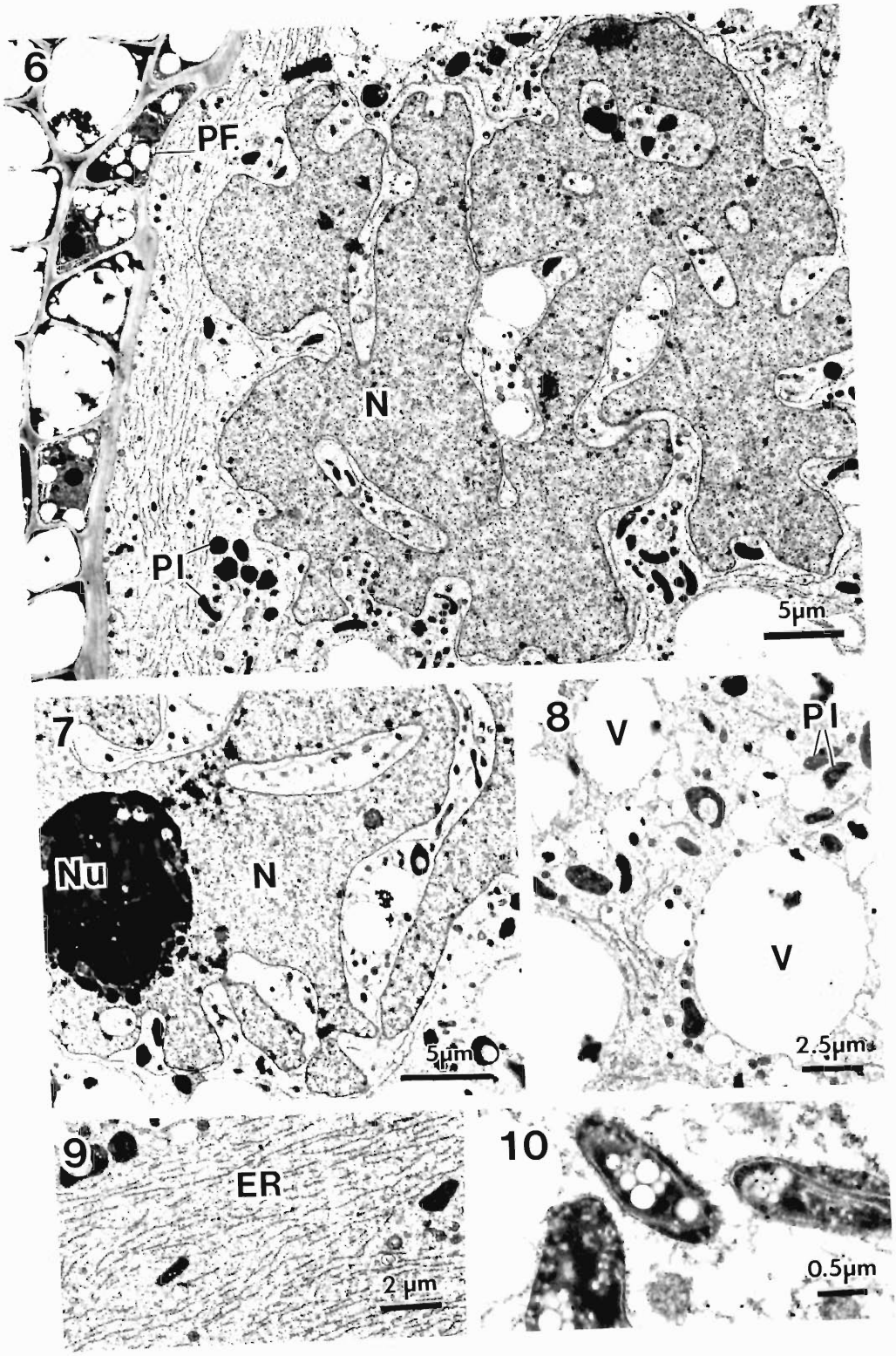
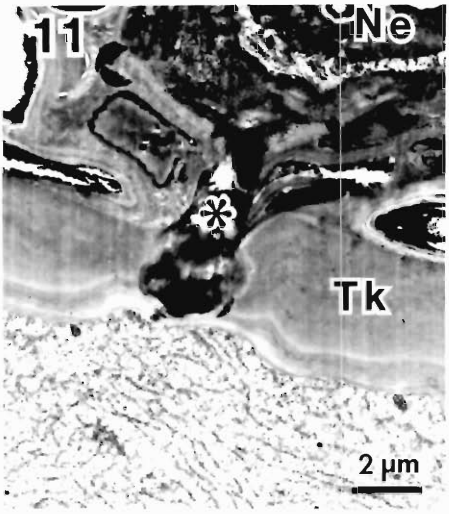
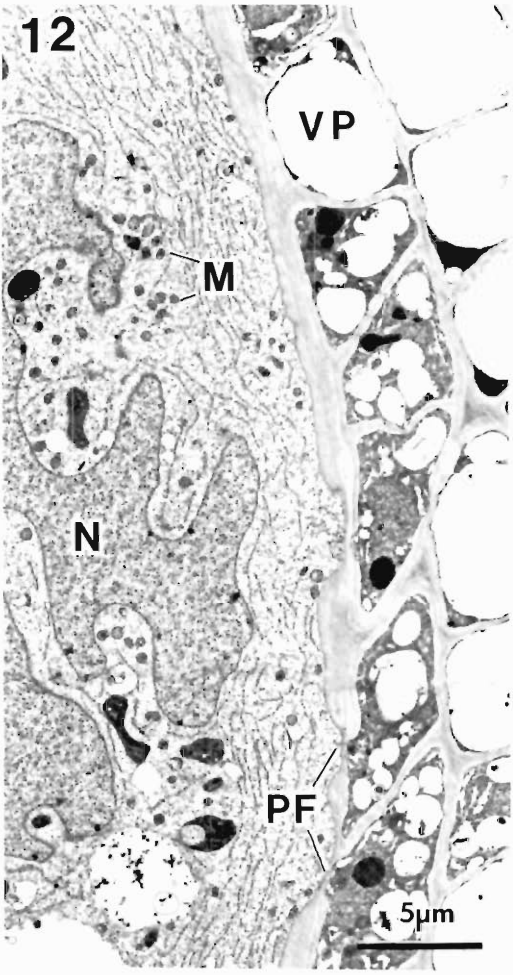
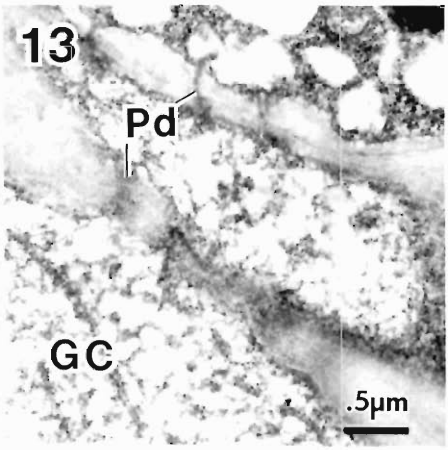
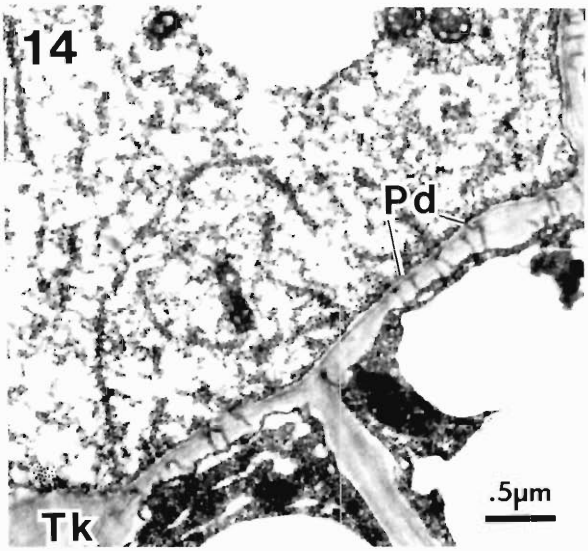
Figures 6–10. TEM of transverse sections of giant cells induced by *Cryphodera utahensis* in roots of *Rosa* sp. 6. Giant cell and adjacent cells showing pit fields (PF). Invaginated nucleus (N) surrounded by plastids (Pl). 7. Partial view of nucleus (N) and nucleolus (Nu). 8. Cytoplasm including vacuoles (V) and plastids (Pl). 9. Endoplasmic reticulum (ER). 10. Vacuolated plastids.











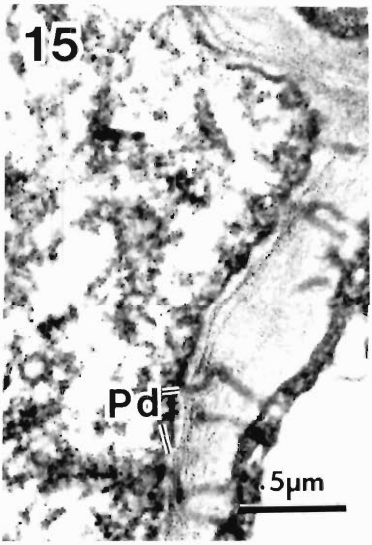


Table 1. Host responses induced by Heteroderidae

Genera	Host response	Cell wall ingrowths
Meloidodera floridensis	SUGC* (Ruehle, 1962; Mundo and Baldwin, 1983c)	Absent
M. charis	SUGC (Heald, 1978; Mundo and Baldwin, 1983c)	Absent
M. belli	SUGC (Mundo and Baldwin, 1983c)	Absent
Cryphodera utahensis	SUGC (Mundo and Baldwin)	Absent
Atalodera ucri	Syncytium (Mundo and Baldwin, 1983a)	Absent
A. lonicerae	Syncytium (Mundo and Baldwin, 1983a)	Absent
Thecavermiculatus gracililances	Syncytium (Mundo and Baldwin, unpublished)	Not reported
Hylonema ivorense	SUGC (Taylor et al., 1978)	Not reported
Sarisodera hydrophila	SUGC (Mundo and Baldwin, 1983b)	Absent
Heterodera spp.	Syncytium (e.g., Endo, 1964)	Present
Globodera rostochiensis	Syncytium (e.g., Feldmesser, 1953)	Present
Punctodera chalcoensis	Syncytium (Mundo and Baldwin, unpublished)	Not reported

* Single uninucleate giant cell.

of the SUGC induced by *C. utahensis* were thoroughly examined, particularly in regions adjacent to xylem; however, wall ingrowths or protuberances were not observed.

The single nucleus is deeply invaginated and has one, or sometimes more than one, nucleolus (Figs. 5–7). Often, numerous spherical lobes and shallow invaginations occur at the surface of the nucleolus (Fig. 7). The cytoplasm is granular and organelles include mitochondria, plastids, vacuoles, and abundant endoplasmic reticulum (Figs. 6, 8–10). Plastids are irregularly shaped and contain numerous small vacuoles (Fig. 10). In older giant cells the number of organelles is generally reduced, although small cytoplasmic vacuoles increase in number (Fig. 3) and eventually coalesce.

Discussion

The SUGC induced *C. utahensis* apparently originated from a cell in the pericycle as in many other heteroderids (Mundo and Baldwin, 1983c). The giant cell generally resembles that reported in association with *Hylonema ivorense, Sarisodera hydrophila* and *Meloidodera* spp. (Table 1), as well as certain nonheteroderids including *Rotylenchulus macrodoratus* (Cohn and Mordechai, 1977). The SUGC of *C. utahensis* is most specifically similar to that of *Meloidodera floridensis* with respect to the detailed morphology of the cell wall and nucleus (Mundo and Baldwin, 1983c).

The giant cell wall of *C. utahensis* is thickest adjacent to the nematode lip region, as is also the case for *S. hydrophila, Atalodera* spp., and *Meloidodera* spp.; this thickening probably occurs in response to penetration of the stylet (Mundo and Baldwin, 1983a, b, c). The remainder of the SUGC wall of *C. utahensis* is characterized by the absence of wall ingrowths and the presence of pit fields with abundant plasmodesmata. Similar morphology of the wall, in which ingrowths are absent, occurs in each case of SUGC examined among heteroderids, and of the syncytium induced by *Atalodera* spp. (Table 1). Although Taylor et al. (1978) did not observe a "discrete cell wall" in the SUGC of *H. ivorense,* we predict that examination with TEM will indicate cell walls that lack ingrowths. Wall ingrowths or abundant plasmodesmata apparently occur as alternate mechanisms for transport of large amounts of solutes in cells sustaining developing sedentary parasitic nematodes. This transport may be further increased with *C. utah-*

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Figures 11–15. TEM of transverse sections of giant cells induced by *Cryphodera utahensis* in roots of *Rosa* sp. 11. Thick region of the cell wall (Tk) adjacent to lip region of nematode (Ne). Asterisk indicates space previously occupied by protracted stylet. 12. Cell wall boundary of giant cell and vascular parenchyma (VP) showing pit fields (PF). M, mitochondria; N, nucleus. 13. Enlargement of narrow portion of cell adjacent to giant cell (GC) including plasmodesmata (Pd). 14. Region of the cell wall adjacent to the thick cell wall (Tk) showing plasmodesmata (Pd). 15. Enlargement of portion of Figure 14 showing plasmodesmata (Pd).

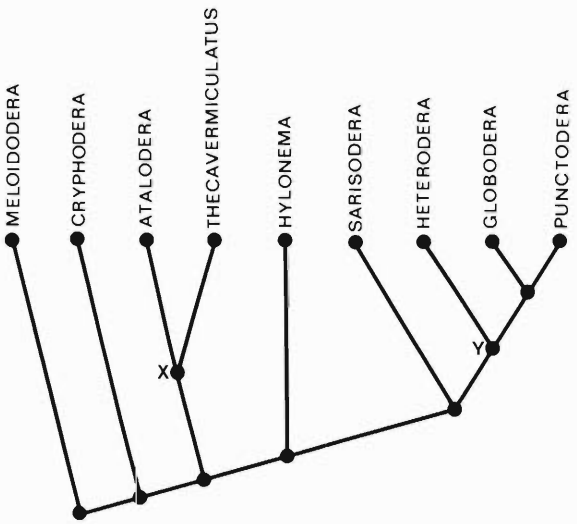

Figure 16. Cladogram of Heteroderidae (redrawn from Ferris, 1979). X, ancestor of *Atalodera* and *Thecavermiculatus*; Y, ancestor of *Heterodera* sensu lato.

ensis by numerous plasmodesmata in certain cells adjacent to the SUGC. Among heteroderids examined, wall ingrowths are limited to syncytia produced by species of *Heterodera* sensu lato (Jones and Dropkin, 1975; Gommers, 1981; Jones, 1981a, b); literature on ingrowths in nematode-induced transfer cells has been summarized by Mundo and Baldwin (1983c).

The nucleus of the SUGC of *C. utahensis* is deeply invaginated and is similar to that of *M. floridensis*. Conversely, nuclei of SUGC of *Meloidodera charis* and *M. belli* are composed of a cluster of interconnected spherical nuclear units (Mundo and Baldwin, 1983c). The resulting large area of nuclear membrane would facilitate a high rate of exchange at the nucleus–cytoplasm interface. In contrast, the nuclei of SUGC of *S. hydrophila* and *H. ivorense* are only slightly ameboid or oval (Taylor et al., 1978; Mundo and Baldwin, 1983b).

The abundance of organelles in cells that sustain sedentary heteroderids suggests a similar high rate of metabolism. Yet, specific occurrence, numbers and characteristics of organelles may vary with the nematode species. For example, plastids are abundant in the SUGC of *C. utahensis* but they were not observed in the SUGC of *Meloidodera* spp. Organelles also vary among three species of *Meloidodera* (Mundo and Baldwin, 1983c). Our fine structural observations have, however, been primarily of mature cells. Changes with respect to organelles probably occur throughout development of the host–parasite relationship, and specifically with the stage and age of the nematode. Furthermore, detailed characteristics might vary among hosts and specific sites of infection.

Two basic types of host responses induced by heteroderids are SUGC and syncytium. We have discussed evidence that these basic responses do not change depending on the host, and probably reflect more fundamental characteristics in the digestive enzymes of the nematodes (Baldwin et al., 1983; Mundo and Baldwin, 1983a, b, c). Furthermore, we suggest that "host response" is useful as a character for testing proposed phylogenies of Heteroderidae. Ferris (1979) hypothesized a phylogeny of Heteroderidae using a cladistic analysis based on designated polarities of eight characters. Host responses of at least one representative species of the nine genera included in the cladogram are presently known (Table 1); these include *C. utahensis* and our preliminary observations of *Thecavermiculatus gracililancea* Robbins, 1978 and *Punctodera chalcoensis* Stone et al., 1976. The pattern of response induced by these heteroderids is generally congruent with the cladogram if we assume SUGC is plesiomorphic (primitive) and syncytium is the apomorphic (derived) character state. However, *Atalodera* spp. and *Thecavermiculatus* sp., which induce a syncytium, appear to be an exception. In this case, the cladogram requires parallel evolution of the syncytium between the common ancestor (X) of *Atalodera* and *Thecavermiculatus* as well as in the common ancestor (Y) of *Heterodera* sensu lato (e.g., *Heterodera,* and *Globodera* + *Punctodera*). This explanation is plausible because ho-

moplasy might be suggested by the fundamentally distinct walls of the two types of syncytia. That is, the host response of descendants of X lack ingrowths and have abundant plasmodesmata; conversely, syncytia of descendants of Y have wall ingrowths. This hypothesized homoplasy can be further tested by principles discussed by Rieger and Tyler (1979), including, in this case, ultrastructure of the comparative development of the two types of syncytia.

Our studies have expanded knowledge of host responses of Heteroderidae including seven species in four genera, as well as preliminary observations on two additional genera (Table 1). These results indicate that the type of response induced by a given heteroderid can be useful in phylogenetic analysis. Additional information regarding host responses of other heteroderids is needed, particularly as new species and genera are described. Such information might be useful in testing monophyly, and could be combined with conventional characters in a phylogenetic analysis of Heteroderidae.

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Radopholus citrophilus sp. n. (Nematoda), a Sibling Species of *Radopholus similis*

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ABSTRACT: *Radopholus citrophilus* sp. n. is described and separated from its sibling species, *R. similis* by a number of biological characteristics. This new species is morphologically indistinguishable from *R. similis* by conventional means (i.e., morphometrics). However, the two closely related nematodes differ by chromosome number, behavioral differences, genetic variability in their proteins, and in their preference of hosts. The new species reproduces amphimictically and occasionally parthenogenetically and has a chromosome number of n = 5. In mating studies it will not copulate with *R. similis*. The assignment of the citrus race of *R. similis* to species is justified within the species concept. It is the first true sibling species to be described among the plant parasitic nematodes.

Two morphologically indistinguishable races of *Radopholus similis* (Cobb, 1893) Thorne, 1949 were first described by DuCharme and Birchfield (1956). One race was shown to parasitize banana, but not citrus, whereas the other race infects citrus and banana. The banana race has been identified from most banana growing regions of the world, whereas the citrus race is only known to occur in Florida, U.S.A. Both races have been reported to have rather wide host ranges; however, detailed studies of their respective host preferences have not been conducted (Poucher et al., 1976).

Since the two races are indistinguishable morphologically, the only means of their separation has been by host preference tests. Recently, however, a number of biological differences, including karyotype, isozymes, and pheromone-mediated behavior, have been detected between them (Huettel, 1979; Huettel and Dickson, 1981a, b). These differences led to the conclusion that the races of *R. similis* are reproductively isolated and that their biological status can be clarified by a change in their taxonomic status. Based on the biological differences between the citrus and banana races, the citrus race should be considered genetically separate. It is elevated to species rank and renamed *Radopholus citrophilus* sp. n. The original description of *R. similis* was based on specimens from banana and therefore the banana race will retain that original name. The use of racial designation is no longer necessary or appropriate.

Materials and Methods

Populations of the banana and the citrus races of *R. similis* were obtained from: 1) established greenhouse cultures maintained by the Florida Division of Plant Industries, Nematology Section, Gainesville, Florida; 2) the U.S.D.A. Horticultural Laboratory, Orlando, Florida; 3) infested citrus groves located in Polk County, and collected by the Florida State Department of Agriculture, Burrowing Nematode Laboratory, Winter Haven, Florida; and 4) infected *Anthurium* spp. from Hawaii. Stock cultures of *R. citrophilus* sp. n. were established in a temperature controlled greenhouse at 23–26°C on *Citrus aurantium* L. cv. "sour orange" and *Solanum nigrum* L. The soil type used in all experiments was Astatula fine sand (97% sand, 0% silt, 3% clay, and 0.13% O.M.). Nematodes were maintained also in culture on mung bean, alfalfa, or okra callus tissue and carrot disc (Huettel, 1982).

Host preference tests were conducted on sour orange in Astatula fine sand. Other host preferential tests were conducted on *Anthurium hookeri* and *A. clarinerivum*.

Procedures for karyological determinations, analysis of genetic variation, and copulatory behavior were described by Huettel and Dickson (1981a), and Huettel et al. (1982, 1983a).

Light microscope studies were conducted on freshly acquired male and female nematodes fixed in formalin-acetic acid (FAA) for a minimum of 48 hr (Goodey, 1963). The nematodes were placed on microscope slides and measured by an eye piece micrometer on an Olympus Vanox interference microscope. Measurements were recorded and the mean, range, and standard error including the a, b, and c Demanian ratios of males and females were calculated (Van Weerdt, 1958).

Species Description

FEMALES: Measurements of 30 females in FAA are listed in Table 1. *Measurements of holotype in glycerin.*—Body length: 628 μm; body width:

Table 1. Measurements of 30 females of *Radopholus citrophilus* sp. n.

Character	Range	Mean	Standard error of mean	Standard deviation
Linear (μm)				
Stylet length	18.0–20.0	19.1	±0.10	0.53
Stylet knob width	2.8–5.2	4.6	0.08	0.47
Stylet knob height	2.8–4.8	3.2	0.08	0.43
Head region height	3.2–5.2	4.5	0.07	0.42
Head region width	8.0–10.8	10.0	0.12	0.66
DGO	3.2–4.8	4.0	0.09	0.49
Metacorpus length	10.4–16.0	11.9	0.27	1.47
Metacorpus width	10.4–16.0	13.6	0.28	1.54
Metacorpus valve width	2.8–4.4	4.40	0.09	0.48
Metacorpus valve length	2.8–4.4	3.04	0.07	0.37
Excretory pore	64.0–96.0	78.02	1.41	7.69
Vulva to tail end	242.2–409.4	328.05	7.34	40.18
Tail end to anus	62.4–80.8	69.03	1.15	6.27
Body length	600.0–764.1	698.07	7.02	38.47
Greatest body width	21.6–30.4	27.08	0.38	2.07
Vulva %	46–58%			
Ratios				
a	21.4–31.7	28.00	0.47	2.59
c	8.7–12.2	10.00	0.18	0.97
Stylet width/length	0.6–2.0	1.05	0.04	0.23
Metacorpus length/width	0.8–1.5	1.02	0.03	0.12
Metacorpus valve length/width	0.9–1.7	1.04	0.03	0.19

23.1 μm; stylet length: 19.0 μm; stylet knob width: 2.8 μm; stylet knob height: 2.8 μm; head region height: 3.8 μm; head region width: 9.5 μm; DGO: 3.4 μm; metacorpus valve width: 3.1 μm; metacorpus valve length: 4.4 μm; metacorpus width: 11.8 μm; metacorpus length: 12.8 μm; excretory pore: 69.7 μm from anterior end; vulva to tail end: 300 μm; V% = 47%; and tail end to anus: 65.6 μm.

DESCRIPTION: Body slender, vermiform, tapering to rounded at end. Female labial (lip) region slightly or not set off, with 3 or 4 annules; head cap low. Six similar lips. Stylet knobs rounded. Vulva %, position of vulva from anterior end 46–58%; spermatheca with rod-like sperm. Tail tapering to a pointed terminus, exact shape varies with age.

MALES: Measurements of 30 males in FAA are listed in Table 2. *Measurements of allotype in glycerin.*—Body length: 600 μm; stylet length: 13.1 μm; stylet knob width: 1.3 μm; stylet knob height: 1.0 μm; head region height: 6.6 μm; head region width: 7.8 μm; excretory pore: 85.9 μm from anterior end; spicule length: 21.9 μm; length of bursa: 75.3 μm; width of bursa: 11.8 μm.

DESCRIPTION: Body slender, vermiform, tapering at both ends. Male head region offset, with high rounded lips, forming 4 distinct lobes on head. Tail tapering to rounded terminus, set off from lateral field. Spicules pointed, gubernaculum with titillae.

Since the juveniles, females, and males of *R. citrophilus* sp. n. are indistinguishable morphologically from those of *R. similis,* no drawings are included with this description. Illustrations of *R. similis* are available from Sher (1968), Van Weerdt (1958), Taylor (1969), and Thorne (1949).

Diagnosis

KARYOTYPE: The chromosome number of *R. citrophilus* sp. n. is n = 5, and is different from that of *R. similis* which has a chromosome number of n = 4. This difference in karyotype has been shown to be uniform within populations of each species from worldwide locations (Huettel and Dickson, 1981a; Huettel et al., 1983c).

DIAGNOSTIC PROTEINS: Analysis of enzyme-encoding loci by starch gel electrophoresis indicated seven loci were diagnostic in the identification of the two species (Huettel et al., 1983a). In comparisons of nonenzymatic proteins by polyacrylamide slab gels, a major protein band difference

Table 2. Measurements of 30 males of *Radopholus citrophilus* sp. n.

Character	Range	Mean	Standard error of mean	Standard deviation
Linear (m)				
Stylet length	11.6–16.0	14.8	0.26	1.41
Stylet knob width	1.2–2.0	1.6	0.07	0.40
Stylet knob height	0.8–2.0	1.4	0.06	0.31
Head region height	5.6–7.6	6.6	0.10	0.56
Head region width	7.2–9.6	8.3	0.12	0.65
Excretory pore	58.4–102.4	85.0	1.58	8.68
Phasmids to tail end	41.6–68.0	54.9	1.33	7.27
Spicule length	17.6–25.6	20.9	0.31	1.70
Length of bursa	58.4–89.6	71.8	1.47	8.03
Width of bursa	10.8–20.8	14.1	0.52	2.87
Body length	587.1–700.0	634.3	7.16	39.19
Ratios				
Overall length/excretory pore	5.7–10.8	7.5	0.17	0.91
Head width/height	1.1–1.7	1.3	0.03	0.14
Stylet knob width/height	0.7–2.0	1.4	0.07	0.39
Bursa length/width	3.8–7.6	5.2	0.15	0.84

was found in *R. citrophilus* sp. n. (Huettel et al., 1983b).

BEHAVIORAL COMPARISONS: In interspecific attraction studies, males of *R. similis* are attracted to, but do not copulate with, females of *R. citrophilus* sp. n. This behavioral difference may be caused by different sex pheromones in the two species (Huettel et al., 1982).

HOLOTYPE (female): Isolated from greenhouse cultures propagated on *Citrus aurantium,* and further propagated in carrot disc culture. Slide T-362t, U.S.D.A. Nematode Collection, Beltsville, Maryland.

ALLOTYPE (male): Same data as holotype. Slide T-363t, U.S.D.A. Nematode Collection, Beltsville, Maryland.

PARATYPES (females, males, second, third and fourth stage juveniles and eggs): Same data as holotype, USDANC, Beltsville, Maryland. University of California Nematode Survey Collection (USNSC), Davis, California, U.S.A.

TYPE HOST AND LOCALITY: Roots of *C. aurantium* cv. "sour orange" and *C. limon* cv. "rough lemon," Central Ridge area of Florida, U.S.A., and *Anthurium* sp., Hawaii.

Discussion

Sibling species have been described from many organisms (Bush, 1966; Christensen and Jensen, 1976; Carde and Roelofs, 1977). Many host races, after careful genetic and behavioral analyses, are shown to be sibling species (Bush, 1966, 1975). This appears to be the case for the newly described species, *R. citrophilus* sp. n.

No sibling species has been described among the plant parasitic nematodes. It was suggested that *Globodera rostochiensis* and *G. pallida* might be sibling species, but they were clearly morphologically distinguishable by juvenile and male measurements and female cyst color (Stone, 1972). These species, therefore, do not fit the definition of sibling species proposed by Mayr (1970) as "morphologically very similar or indistinguishable." Many other host races in plant parasitic nematodes, after careful genetic examination, may fit the description of sibling species.

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Response of the Nematode *Panagrellus silusiae* to Hypertonic Solutions

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ABSTRACT: Length measurements of *Panagrellus silusiae* incubated in hypertonic solution were used to assess responses to osmotic stress. Measurements obtained with six individuals/dish by standard projections of photomicrographs were reproducible. The responses of selected males, females and juveniles immersed in $NaNO_3$ solutions were substantially different thereby indicating a contribution by multiple mechanisms in varying degree depending upon the nematode stage.

Nematodes have been found in a wide range of aquatic habitats. Some of these habitats, e.g., brackish water, intertidal zones, and soil are normally subject to substantial fluctuations in the solute concentrations of the environmental media. The semi-permeability of the body wall of a number of nematodes has been well established (Lee and Atkinson, 1976; Wright and Newall, 1976). The mechanism by which nematodes can cope with the changing solute concentrations of their habitat has long been of interest. The permeability of nematodes by solutes at environmental concentrations is particularly difficult to study with the small forms; current technology requires large numbers at one stage for reliable results. Useful information, however, has been obtained by challenging individual nematodes with a variety of hypertonic solutions while using the nematode itself as a crude osmometer. The method has its limitations (Wright and Newall, 1976); nevertheless, it can be used to gain insight into the limiting constraints governing permeability (Croll and Viglierchio, 1969; Viglierchio, 1974). Volume change would be the measure of choice in assessing response when using the nematode as an osmometer. Nematode volumes have been estimated by considering the nematode as a cylinder (Overgaard Nielsen, 1949), a series of cylinders or by an empirical formula (Andrassy, 1956). These methods, depending upon precise measurements of body diameters, are unsuitable for active nematodes; therefore length measurements, notwithstanding their attendant limitations, were used as indicators of nematode response.

Materials and Methods

Panagrellus silusiae was reared on a commercially available protein amended baby gruel. Before use the nematodes were washed free of the nutrient and collected in water. From this stock individuals were selected by picking and placed in BPI dishes with water; normally six individuals per dish were used for tests. These nematodes were too small and too active to permit length measurements with an ocular micrometer; therefore, measurements were made from projections of photomicrographs taken with a Reichert Research microscope equipped with semi-automatic photographic accessories. After initial photographs in water, solute solutions replaced the water and photographs were taken at 60-min intervals for 6 hr. Solutes were then replaced with water and two additional photographs were taken at the same intervals. The solute, $NaNO_3$, was selected because previous studies (Viglierchio, 1974) had indicated it penetrated the body wall of other nematodes very well and was less toxic than equally active NH_4NO_3 or urea.

To expedite experimentation it was desirable to have as many individuals per BPI dish as possible consistent with ease and reliability of measurement. Preliminary experiments indicated six individuals per dish was a suitable number. Less than six substantially increased the number of experimental runs without a corresponding increase in reliability of measurements; more than six individuals per dish substantially increased the difficulty in maintaining all individuals within the microscopic field and in the plane of focus for photography. To establish a measure of reliability of the method using six individuals per dish comparative trials were run measuring individuals in six dishes, one per dish.

Preliminary trials using mixed stages (males, females, juveniles) of the same size demonstrated mixed responses with very large variability in length measurement. It was of interest, therefore, to compare the responses to hypertonic solutions of different nematode stages of the same size by selecting males, females not undergoing endotokia and juveniles.

Results

The photographic method for length measurements has limitations with very active nematodes. Keeping them within the microscopic field, from overlapping one another to conceal the ends,

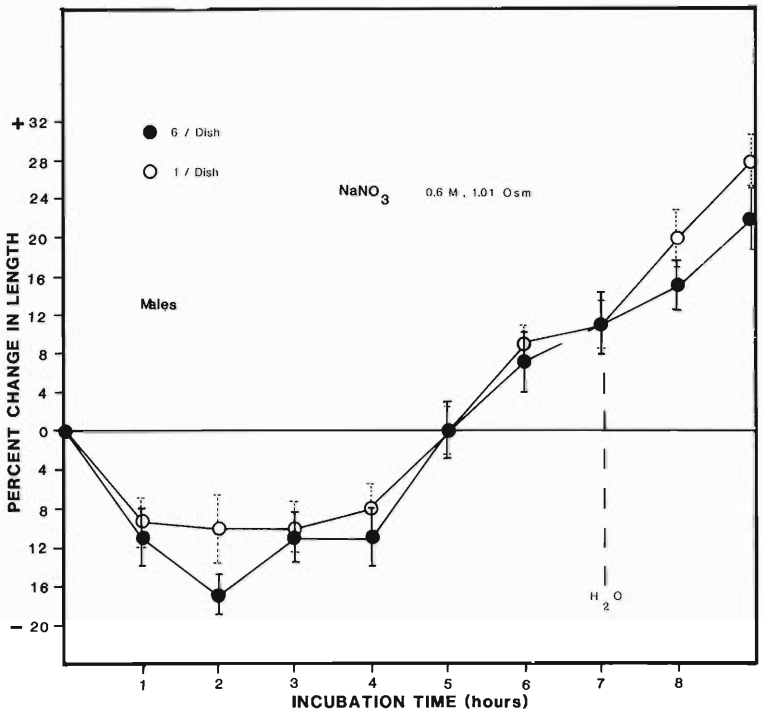

Figure 1. The percent change in length of males of *P. silusiae* as a function of incubation period in 0.6 M solution of $NaNO_3$ followed by transfer into water. One group of males was incubated six/dish and photographed hourly for subsequent length measurements; the second group of males, one/dish (six replicates), was also incubated and photographed for eventual measurement. The vertical bars indicate standard deviation.

and maintaining them reasonably within the depth of focus resulted in a somewhat higher variability than desired but nevertheless within practical limits; in water percent change = 0.0 ± 6.5. In hypertonic solution the high activity of the nematodes was reduced; correspondingly the variability diminished by one-half or more.

The data provided in Figure 1 comparing measurements of six nematodes in one dish and one individual in each of six dishes illustrate that the standard methodology utilizing six individuals per dish was acceptable within the limits of experimental error. Similar trials at other hypertonic concentrations confirm the results expressed in Figure 1.

When selections of individual stages (males, females or juveniles) were subjected to hypertonic solutions of varying strengths, reproducible behavioral curves were obtained (Figs. 2–4). From a superficial view the three stages behaved similarly; i.e., for an initial period internal water was lost as indicated by a negative change in length followed by a recovery period during which the negative change eventually increased to positive values (the normal response) as indicated by length measurements. There are, however, substantial differences in natures of the behavioral responses of the different stages, particularly at high osmolality (Figs. 2–4).

Discussion

Volume determinations would probably be one of the more effective means of assessing substantial solvent passage through a nematode body wall of individuals submersed in solutions of varying hypertonicity. For certain stages of some species which can be obtained in large numbers and a few very large nematodes, other methods are preferable. Most nematode species are small and poorly amenable to such methods. Volume estimates based upon calculations from length and one or more body diameter determinations are open to question. If, for purposes of discussion, *Euchromadora antarctica* (30 × 1,000 μM) is considered an average nematode (Cobb, 1914), then less than 75% of this organism can be con-

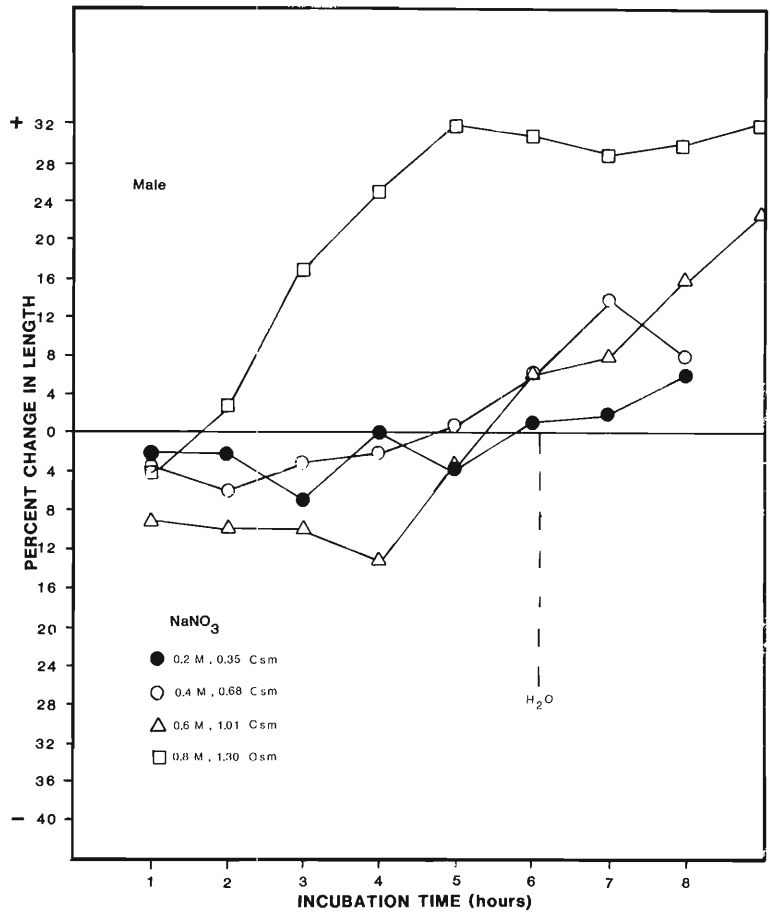

Figure 2. The percent change in length of males of *P. silusiae* as a function of incubation periods in different concentrations of $NaNO_3$ followed by transfer into water.

sidered approximately cylindrical. If the data of Wright and Newall (1976) can be interpolated for this nematode, a 10% change in length (100 μM) would be reflected in approximately a 20% change in volume and a change in diameter of 1.7 μM or 5.7%. If other factors are considered such as the limit of resolution of the optical microscope as 0.25 μM (Martin, 1977), the difficulties of mid-diameter focusing, the fuzziness of the cuticle edge determinations resulting from body wall curvature, and annulation and the movement of active nematodes, a body diameter differential determination in error by 50% is not excessive. Consequently the method established by Stephenson (1942) using the length of the animal body as an indicator remained a useful tool for assessing certain physiological responses of some nematodes.

That *P. silusiae* underwent a variation of the "normal response" already demonstrated by several other nematodes was not surprising. It was the individual responses of the different stages in solutions of varying hypertonicity that provided some insight into the nature of the controlling factors involved in this behavioral response. Figures 2, 3, and 4 show that the isotonic concentration of $NaNO_3$ is 0.2 M for males and juveniles but less than 0.2 M for females. In solutions of 0.68 Osm males demonstrated the "normal response" though remaining only slightly shortened in comparison to solutions of 0.35 Osm; males lengthened slightly over normal length on long exposure and additionally upon water immersion. Juveniles at 0.68 Osm solution showed the "normal response," initially shortening substantially, lengthening slightly with in-

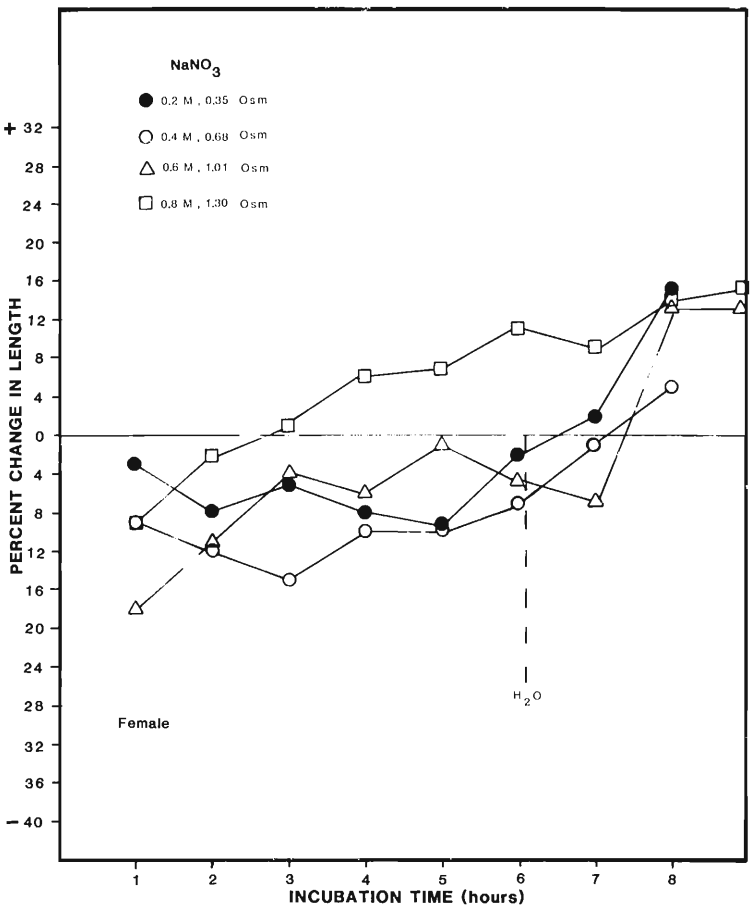

Figure 3. The percent change in length of females of *P. silusiae* as a function of incubation periods in different concentrations of $NaNO_3$ followed by transfer into water.

creasing incubation time until water immersion with rapid increase in length to greater than normal. Females shortened somewhat less than juveniles; both remained shortened until transfer into water whereupon juveniles showed a more rapid and proportionately a greater increase in length.

In 1.0 Osm solutions males shortened moderately and remained shortened for 4 hr before lengthening to more than normal at 6 hr and increasing even more after transfer into water. Juveniles, however, shortened much less than they did in 0.68 Osm solutions though the initial shortening intensified up to 4 hr before relaxing. Females in 1.0 Osm solution shortened substantially the first hour then lengthened by the third hour to slightly subnormal length, remaining at this extension for three additional hours; upon transfer into water they remained at the same extension in the first hour but in the second hour they rapidly increased in length to greater than normal. Plateaus in the curves or portions of curves, the presence of maxima and minima, and the lags in response upon immersion in water appeared not be explicable in terms of a simple passive process.

Immersion in 1.3 Osm solution revealed great differences in the responses of males, females, and juveniles. Juveniles that shortened drastically, on the order of 40% in the first 2 hr, relaxed to about a 27% decrease in length at 4 hr before shortening again to a 40% decrease. Immersion in water brought about an increase in length that by the second hour became approximately 14% greater than normal. Males shortened slightly by the first hour but thereafter increased in length

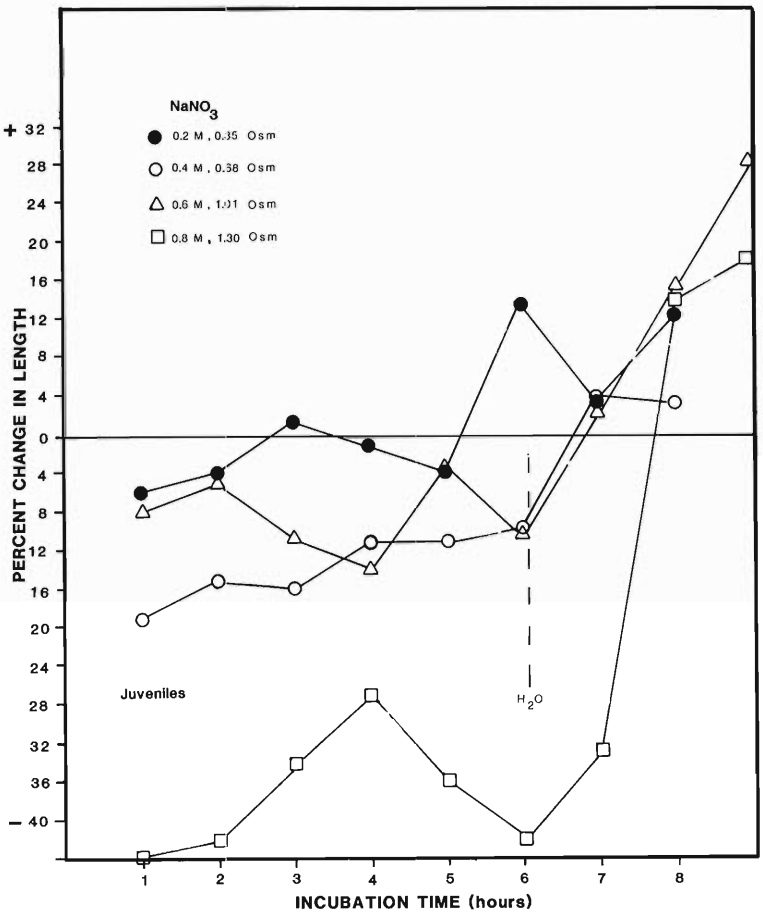

Figure 4. The percent change in length of juveniles of *P. silusiae* as a function of incubation periods in different concentrations of $NaNO_3$ followed by transfer into water.

sharply such that by the fifth hour lengths greater than 30% above normal were attained and remained so even after transfer into water. The female response was similar to that of males but much moderated.

The change in length of *P. silusiae* arises from a loss of solvent or uptake of solute and solvent. Multiple mechanisms combine to produce the resultant state, e.g., transport through the body wall, through the esophagus and intestine as the medium passes through the gut, and excretion. The rapid loss of water upon immersion into hypertonic solutions and the variable recovery in length indicates that the exchange takes place largely through the body wall. The shock of immersion is more likely to stop pumping activity that would transport medium through the gut, and in the shrunken condition the nematode would remain inactive.

In this view the permeability of the body wall appeared to be a function of solute concentration and nematode developmental form. The varied behavioral responses observed in these experiments suggest that an explanation of solute transport may be based upon multiple mechanisms involving in part, passive and active transport, solute concentration dependent inhibition or promotion of transport, transient inhibition, and differences in membrane characteristics. Water transport appears not to be a limiting factor. Whether these mechanisms are specific to $NaNO_3$ or function for other solutes as well and thereby factor in the normal osmotic regulations of nematodes in nature remains to be established. The

nature of the mechanisms operating and their relative import now need more focused study for elucidation.

Acknowledgments

Participation by one of us (DRV) was supported in part by a grant of the Fulbright Commission. Acknowledgments are due to the Italian Ministry of Agriculture and the Osservatorio per le Malattie delle Piante per la Campania for facilitating this study.

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MEETING SCHEDULE

HELMINTHOLOGICAL SOCIETY OF WASHINGTON

Spring—1984

10 February	Naval Medical Research Institute/National Marine Fisheries Oxford Biological Laboratory Bethesda, Maryland
9 March	Walter Reed Army Institute of Research Washington, D.C.
13 April	School of Hygiene and Public Health Johns Hopkins University, Baltimore, Maryland
12 May	New Bolton Center, University of Pennsylvania Kennett Square, Pennsylvania

Proc. Helminthol. Soc. Wash.
51(1), 1984, pp. 42–48

Response of *Panagrellus silusiae* Males (Nematoda) to Hypertonic Solutions of Selected Solutes

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ABSTRACT: Male *Panagrellus silusiae* were immersed in solutions of various solutes in order to assess nematode response to the effects of osmotically active solutes. The solutes tested included sucrose, urea, KNO_3, $NaNO_3$, KCl, NaCl, Na_2SO_4, and $CaCl_2$. Length measurements were made both during exposure to solutes and after transfer to water. The effects of these various solutes on shortening and subsequent recovery are discussed. Only $NaNO_3$ allowed nematodes to recover to near normal dimensions.

As aquatic animals, nematodes possess a certain ability to cope with natural environmental changes. Like other organisms, the nematode is isolated from its micro-environment by a body wall incorporating semi-permeable membranes. The osmoregulatory characteristics of nematodes have been well reviewed (Lee and Atkinson, 1976; Wright and Newall, 1976, 1980). The response of nematodes to immersion in single solute hypertonic aqueous solutions has been useful in certain situations (Viglierchio et al., 1969) and a concern in other applications (Southey, 1970; Kermarrec and Scotto La Massesse, 1972; Coolen and D'Herde, 1977; Ayoub, 1977). Density flotation, a technique increasingly used for the extraction of nematodes from soil and tissue samples, involves the addition of dense aqueous solutions to a sample followed by mixing and centrifugation before collecting the supernatant containing the nematodes or counting or other use. The dense solutions normally used for this purpose are prepared with sugar or inorganic salts. The solutions prepared from such solutes are dense, a desirable property, and of high osmotic activity, an undesirable property. It is useful, therefore, to become more knowledgeable about the nature of nematode responses to solutions of osmotically active solutes. In the interest of developing baseline information, the responses of *Panagrellus silusiae* males were explored.

Materials and Methods

Males of *P. silusiae* selected from stocks maintained in the laboratory were immersed in solutions of various solutes in a series of concentrations where lengths were measured at half-hour intervals for some 6 hr then transferred to water and measured for an additional 2 hr. Solutes used for testing included sucrose, urea, KNO_3, $NaNO_3$, KCl, NaCl, Na_2SO_4, and $CaCl_2$. The details of the methodology and the desirability of using males have been described elsewhere (Prencepe et al., 1984).

Results

The primary effect observed was exosmosis; the water loss was reflected in shortening of the nematode. With certain solutes illustrated by sucrose (Fig. 1) the shortened condition remained until the nematodes were transferred into water after which they absorbed water to eventually reattain normal dimensions. With other solutes, KCl, Na_2SO_4, KNO_3, the nematode shrinkage was less pronounced depending upon the individual solute, however the curve patterns were similar. With another group of solutes illustrated by $CaCl_2$ (Fig. 2) the initial exosmosis of water resulting in nematode shortening was followed by gradual nematode lengthening throughout the remaining exposure period. Before transfer into water, lengths remained subnormal; however, in water, nematodes lengthened rapidly to lengths greater than normal. The curve pattern for NaCl was similar.

The behavior of *P. silusiae* males to immersion in $NaNO_3$ (Prencepe et al., 1984) was somewhat like that of $CaCl_2$ with exosmosis substantially reduced as was also the following lengthening rate. The response to urea (Fig. 3) indicated a slight decrease in length as a result of water loss followed by a rapid increase to supernormal lengths at the stronger hypertonicities and plateauing near the end of the exposure period. Upon transfer to water, nematodes exposed to the weaker hypertonic solutions continued to increase in length but those at the stronger hy-

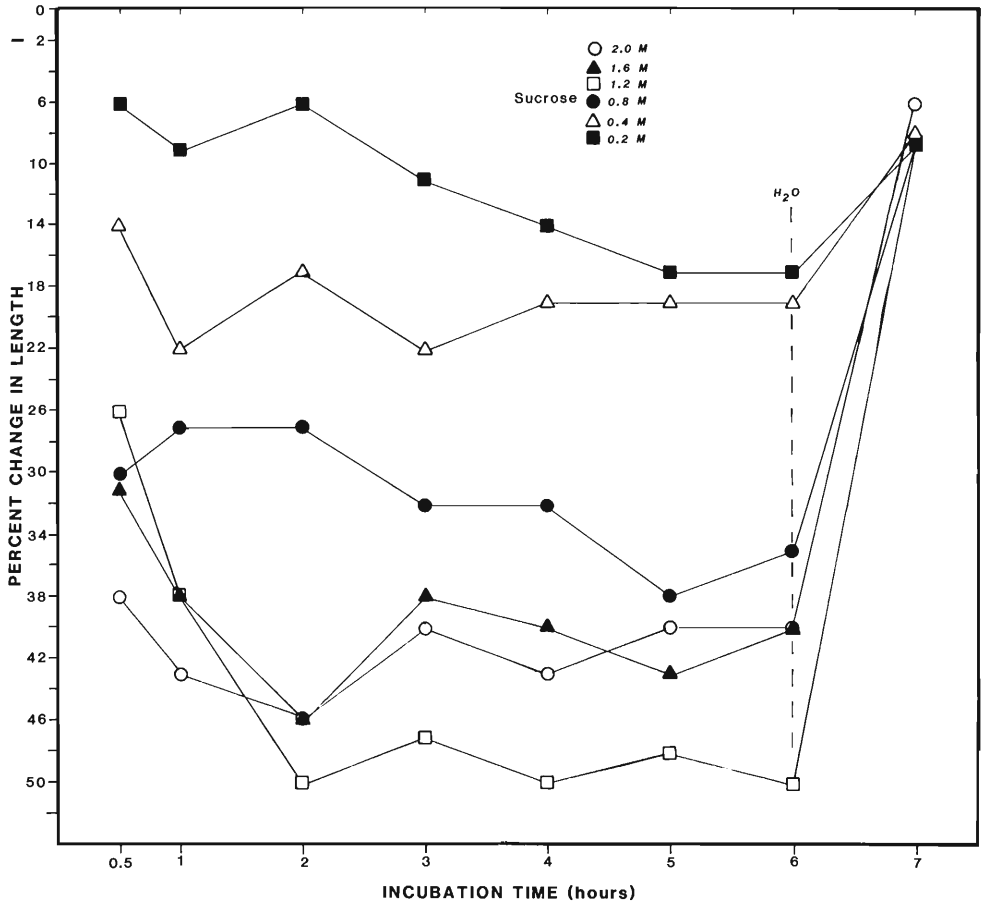

Figure 1. Percent change in length of *P. silusiae* males immersed in different aqueous concentrations of sucrose for varying immersion periods followed by transfer into water.

pertonic solutions shortened; all remained at normal or supernormal dimensions.

Discussion

The nature of the response curves suggested that secondary factors were operative. After the initial rapid loss of water, the outflux usually continued but at a reduced rate in the first hours until maximum shortening had been achieved. Factors other than osmotic pressure appeared to be involved. At modest osmolality (approximately 0.4 Osm) continued outflux of water after the initial rapid loss occurred only with certain solutes, recovery tended not to occur, and maximum shortening appeared to be a function of solute (Fig. 4). At a higher osmotic tension (approximately 1.1 Osm) the difference in solute characteristics became readily apparent (Fig. 5). Maximum shortening varied from approximately 10–30%. Recovery occurred, although with most solutes lengths remained below normal dimensions; however, with $NaNO_3$ normal dimensions were eventually achieved and in the case of urea a 20% length extension was observed during the exposure period. It appeared that only in those cases where some recovery occurred did lengthening to above normal dimensions take place after transfer into water. The above normal lengthening could be explainable in part most simply by solute uptake by the nematode followed by water uptake in an effort to establish osmotic equilibrium. The observations suggested that under the conditions of the experiment urea was highly permeable, sodium and nitrate

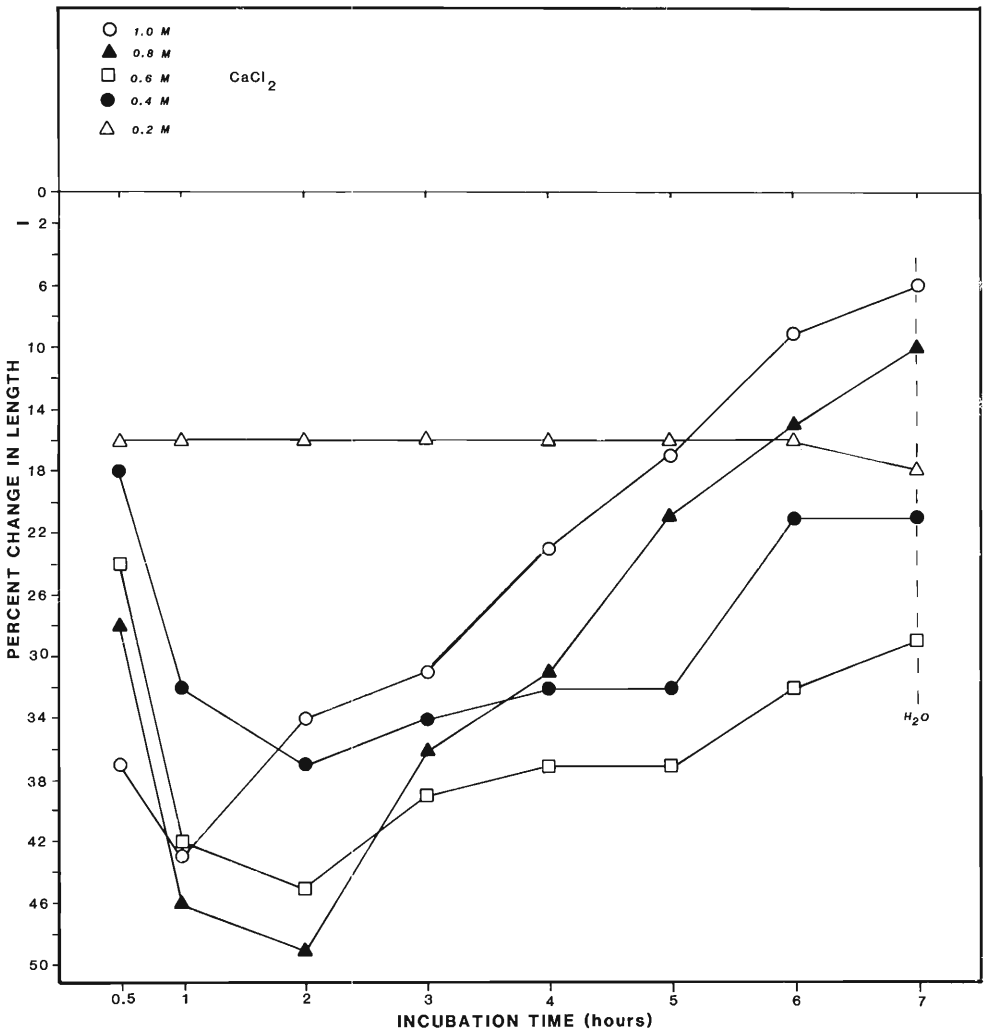

Figure 2. Percent change in length of *P. silusiae* males immersed in different aqueous concentrations of calcium chloride for varying immersion periods followed by transfer into water.

ions moderately permeable, calcium and chloride ions somewhat permeable with potassium ions less so. Sucrose and sulfate ions were impermeable. In no observation were controlling membranes destroyed, although membrane function may have been modified. At low osmotic values, as solute concentration was increased, nematodes responded with progressively more shortening; however, only with Na_2SO_4 did this progressive shortening continue to the highest concentration used. With all other solutes tested, this trend was reversed before the highest concentration was reached. This reversal appeared not to be a function of osmotic concentration for it occurred with KNO_3 at approximately 1.2 Osm, $NaNO_3$ at 1.0 Osm, urea at 0.4 Osm, sucrose at 1.9 Osm, KCl at 1.1 Osm, and $CaCl_2$ at 1.7 Osm. These observations support the view that membrane function may have been modified in part by the solute.

Despite the straightforward responses by *P. silusiae* males the nature of the membrane modification remains a matter of speculation. The slowing outflux of water seemed to be a reflection of reduced gradient; perhaps membrane condensation reduced water permeability. This expla-

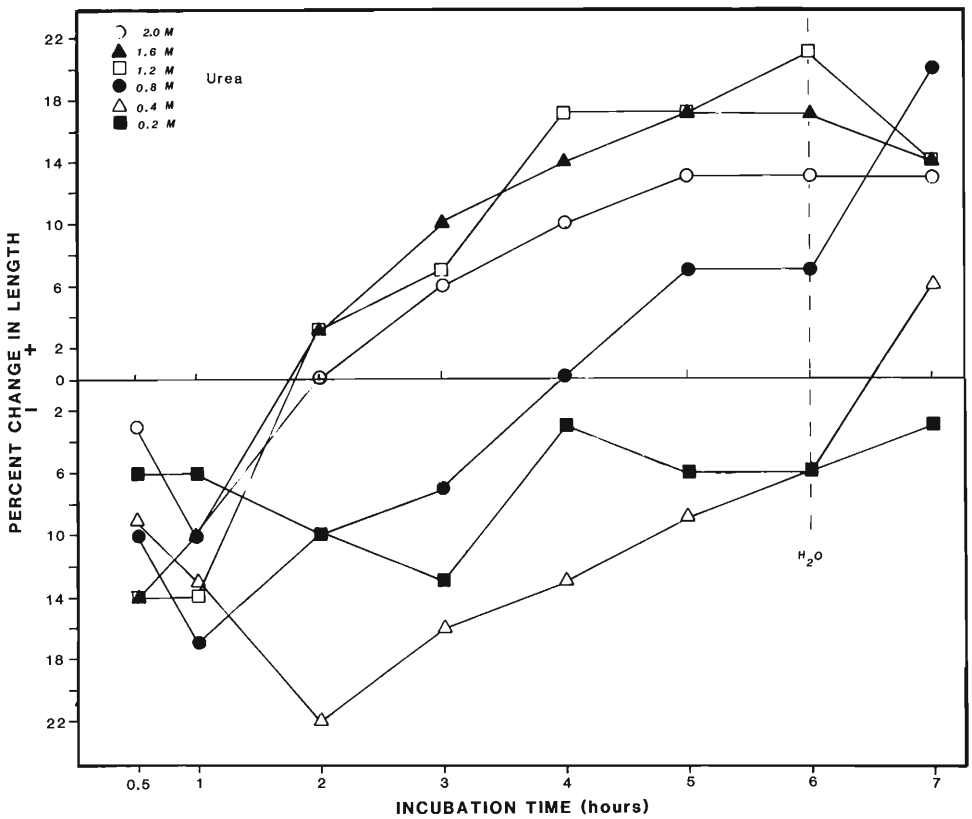

Figure 3. Percent change in length of *P. silusiae* males immersed in different aqueous concentrations of urea for varying immersion periods followed by transfer into water.

nation fails to explain the "reversal" of body shortening that takes place with different solutes of different osmolality. An altered ionic balance across the membrane, which affects the state of membrane lipids (Lee, 1975), combined with a condensed membrane may have affected a phase transition to initiate "reversal." Clearly an acceptable explanation relies upon an improved understanding of the parameters of membrane function including lipid aggregation and segregation, phase transitions, reversible or irreversible changes of protein components and the development of surface charges (Brewer and Passwater, 1974).

Although membrane integrity was not destroyed, in no case was normal activity observed after nematodes, exposed to hypertonic solutions, were transferred into water for several hours. In those solute solutions in which nematodes exhibited a recovery period, animals were inactive or extremely sluggish. With other solutes such as sucrose and Na_2SO_4, particularly at the low concentrations, modest activity would be found. Solute uptake during the recovery period appeared to establish high solute concentrations within the animal to obstruct normal physiological processes. Those nematodes exposed to solute solutions in which no uptake was observed appeared to have been injured as well. Though nematodes in nature survive desiccation (Viglierchio and Schmitt, 1981) nematodes desiccated rapidly do not (Crowe and Madin, 1975). The rapid and extreme loss of water in highly hypertonic solutions was likely to have been as injurious to nematodes as rapid desiccation in air.

Nematode extractions from soil or plant tissues involve osmotically active solutes at substantially higher concentrations (e.g., 1.5 M sucrose giving the normally used density of 1.2

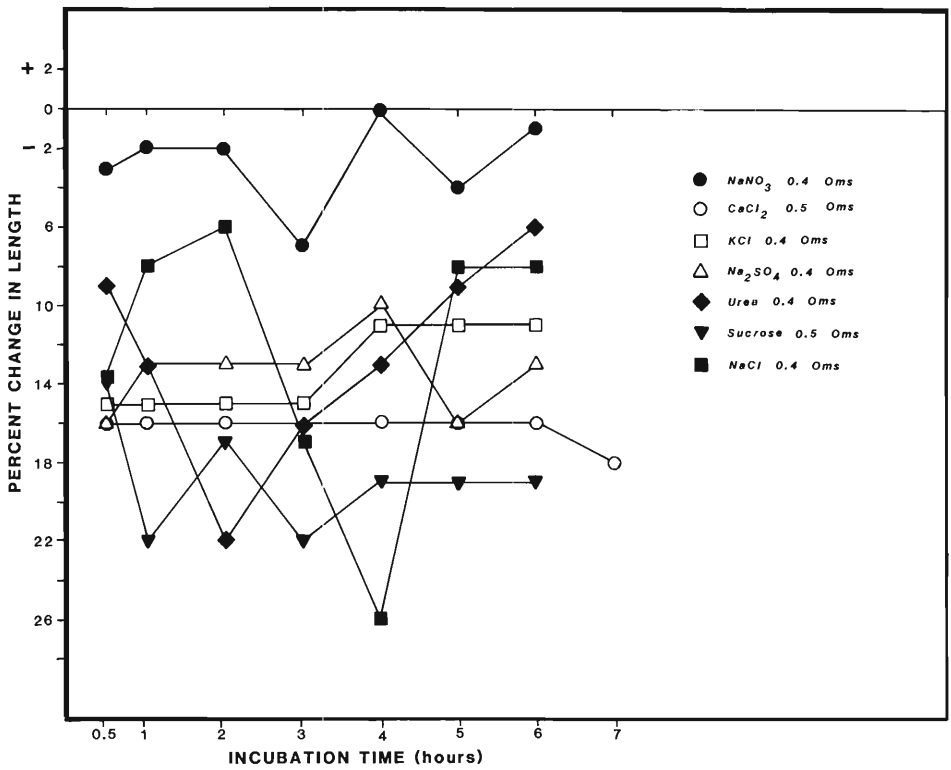

Figure 4. Percent change in length of *P. silusiae* males immersed in aqueous solutions of different solutes of approximately 0.40 osmolality.

[Price, 1982]) than those employed for these experiments and appear to be injurious. Shortening the exposure time reduces injury arising from long time exposure; however, the harmful effects occurring with the abrupt water loss upon immersion, remain. Although some solutes were less injurious than others, osmotically active solutions do not appear to be especially promising for nematode extraction technology.

Acknowledgments

Acknowledgments are due to the Italian Fulbright Commission for partial support of one of us (DRV).

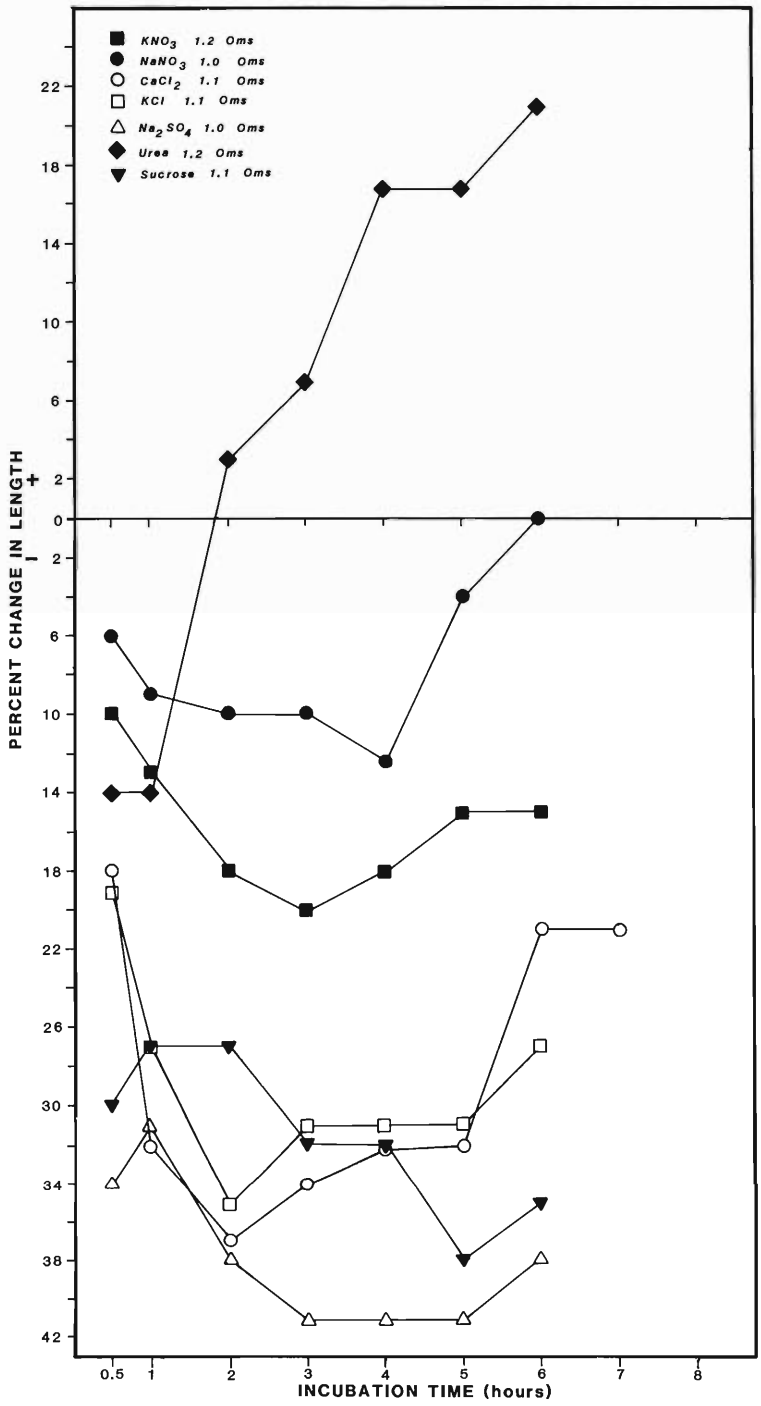

Figure 5. Percent change in length of *P. silusiae* males immersed in aqueous solutions of different solutes of approximately 1.1 osmolality.

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51(1), 1984, pp. 49–53

Loaina gen. n. (Filarioidea: Onchocercidae) for the Filariae Parasitic in Rabbits in North America

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ABSTRACT: A new genus, *Loaina,* is erected to accommodate the species of *Dirofilaria* parasitic in rabbits in North America. The genus is comprised of two species, *Loaina scapiceps* (Leidy, 1886) comb. n. and *Loaina uniformis* (Price, 1957) comb. n.; *L. uniformis* is designated as the type species. The genus *Loaina* is distinguished morphologically from *Dirofilaria* and other Dirofilariinae by a combination of characters, including an extremely short tail in both sexes, an undivided esophagus, a post-esophageal vulva, short, simple spicules, a small number of caudal papillae grouped at the posterior extremity of the body in the male, and a sheathed microfilaria. Two other species, *D. timidi* and *D. roemeri* are not regarded to be valid species of *Dirofilaria.* The genus *Dirofilaria* should be restricted strictly to those species having unsheathed microfilariae. Morphologically, the genus *Loaina* most closely resembles the genus *Loa.* The two genera are distinct, however, on the basis of host preference, size and cuticular ornamentation.

A review of the morphological features of the dirofilarias that parasitize Louisiana mammals revealed that there were marked morphological differences between those species of *Dirofilaria* Railliet and Henry, 1910 infecting rabbits in North America, i.e., *D. uniformis* Price, 1957 and *D. scapiceps* (Leidy, 1886), and other members of the genus. These differences were observed in both the adult worms and in the microfilariae. The distinctiveness of these morphological features warrants that a new genus, *Loaina,* which will accommodate the species *D. scapiceps* and *D. uniformis,* be erected.

The report that follows presents a description of the new genus, a discussion of its taxonomic relationships to other genera in the subfamily Dirofilariinae Sandground, 1921, as well as remarks on the above-mentioned species.

Materials and Methods

Nematodes in the authors' collections identified as *D. uniformis* were recovered at necropsy by skinning freshly killed rabbits and searching the subcutaneous and muscle tissues for worms, or, more commonly, worms were recovered after soaking the skin and carcass in physiological saline. Worms identified as *D. scapiceps* were recovered by blunt dissections of the tarsal bursae of the hind feet. Worms recovered in either manner were examined alive, then fixed in glacial acetic acid or hot (60°C) Bouin's solution, and stored in 70% alcohol to which 5% glycerine (by volume) had been added. Worms were studied microscopically after being cleared in glycerine. Specimens examined from other collections are listed under each species.

Microfilariae, obtained in cardiac blood, were fixed in 2% formalin. They were examined as wet preparations and as hematoxylin-stained smears. Measurements of anatomical features are from stained specimens.

Description

Loaina gen. n.
Dirofilaria Railliet and Henry, 1910, in part

General

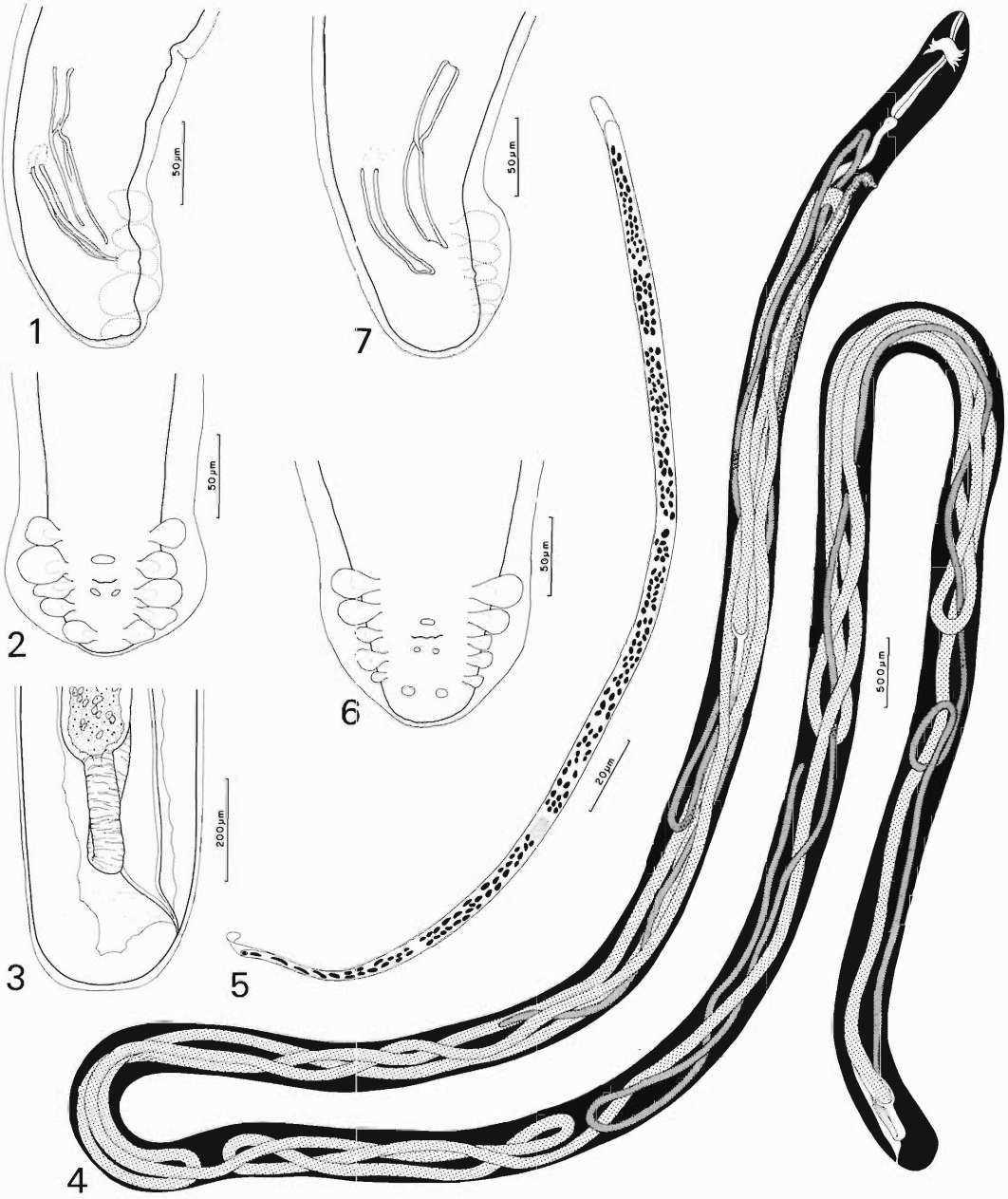
Onchocercidae (Leiper, 1911); Dirofilariinae Sandground, 1921. Anterior and posterior ends bluntly rounded in both sexes. Male one-half to one-third length of female. Cuticle without bosses, lateral alae sometimes present. Tail of both sexes extremely short, less than twice width of body at anus. Male tail not spirally coiled. Esophagus not divided, without long, sacculate, glandular part. Vulva postesophageal. Spicules short, simple. Caudal papillae large, paired, few in number, grouped at end of body. Microfilaria sheathed, nuclei to tip of tail; circulates in blood.

TYPE SPECIES: *Loaina uniformis* (Price, 1957) comb. n.

OTHER SPECIES: *Loaina scapiceps* (Leidy, 1886) comb. n.

Diagnosis

The genus *Loaina* is separated on morphological bases from other genera in the subfamily Dirofilariinae as outlined by Anderson and Bain (1976). The new genus is distinguished from *Bostrichodera* Sandground, 1938 and *Dirofilariaeformia* Lubimov, 1935 on the basis of the short male tail. *Loaina* lacks the long, sacculate, glandular esophagus, characteristic of *Edesonfilaria*



Figures 1–7. ***Loaina uniformis*** **comb. n. and** ***Loaina scapiceps*** **comb. n. 1.** ***L. uniformis*** **male tail, lateral view. 2.** ***L. uniformis*** **male tail, ventral view. 3.** ***L. uniformis,*** **female tail, lateral view. 4.** ***L. uniformis*** **female, lateral view. 5.** ***L. uniformis*** **microfilaria from 2% formalin concentration stained with hematoxylin. 6.** ***L. scapiceps*** **male tail, ventral view. 7.** ***L. scapiceps*** **male tail, lateral view.**

Yeh, 1960 and *Macacanema* Schad and Anderson, 1963. The short, simple spicules and small number of large, pedunculated caudal papillae grouped near the end of the body serve to distinguish *Loaina* from *Skrjabinodera* Gnedina and Ysevolodov, 1947, *Tawila* Khalil, 1932, and *Dirofilaria* Railliet and Henry, 1910. Also, the sheathed microfilaria with a blunt tail in which

the nuclei extend to the tip contrasts markedly with those of the genus *Dirofilaria* in which the microfilariae characteristically are unsheathed and possess thin, attenuated tails devoid of nuclei. The postesophageal position of the vulva separates *Loaina* from *Foleyella* Seraut, 1917 and *Pelicitus* Railliet and Henry, 1910. *Loaina* can be distinguished from *Loa* Stiles, 1905 by its smaller size, the absence of cuticular bosses, and spicule morphology. The genus *Loaina,* on the basis of present knowledge, appears to be restricted to the order Lagomorpha in North America.

Loaina uniformis (Price, 1957) comb. n.
Dirofilaria uniformis Price, 1957
(Figs. 1–5, 8, 10)

HOSTS: *Sylvilagus floridanus, S. palustris, S. aquaticus.*

SPECIMENS EXAMINED: USNM Helm. Coll. No. 38160, 73652, numerous specimens in authors' collections from Cameron, Orleans, and St. Tammany Parishes, Louisiana, and Dade County, Florida.

Diagnosis

Price (1957) provided an accurate and complete description of this species. However, he failed to recognize, at the generic level, significant morphological differences between the genus *Dirofilaria* and the specimens he described. Most notable among these are the short tail in both sexes (Figs. 1–4), the small number of large caudal papillae grouped at the end of the body and the short, simple spicules in the male (Figs. 1, 2), and the sheathed microfilaria. The illustration of the microfilaria furnished in the original species description is one of an unstained, wet-mount preparation. Consequently, the internal morphology and nuclear arrangement were not clearly evident. Because of the taxonomic significance, the microfilaria of *L. uniformis* has been illustrated by both a line drawing and a photomicrograph (Figs. 5, 8).

Loaina uniformis has been reported from subcutaneous tissues of rabbits in Maryland, South Carolina, Georgia, Florida, and Louisiana. The distribution may be more extensive than indicated by these reports.

Loaina uniformis is designated as the type species for several reasons, the most important being the availability of type specimens. Also,

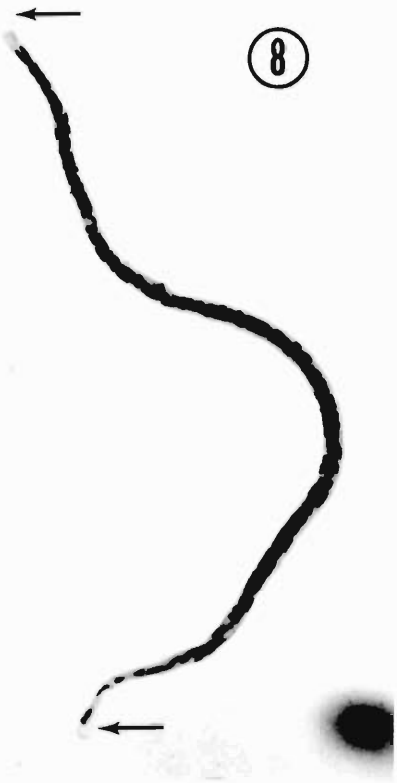


Figure 8. Formalin fixed, hematoxylin-stained microfilaria of *Loaina uniformis* comb. n., note sheath (arrows) (×425).

there is a more complete and accurate description, including illustrations of *L. uniformis.*

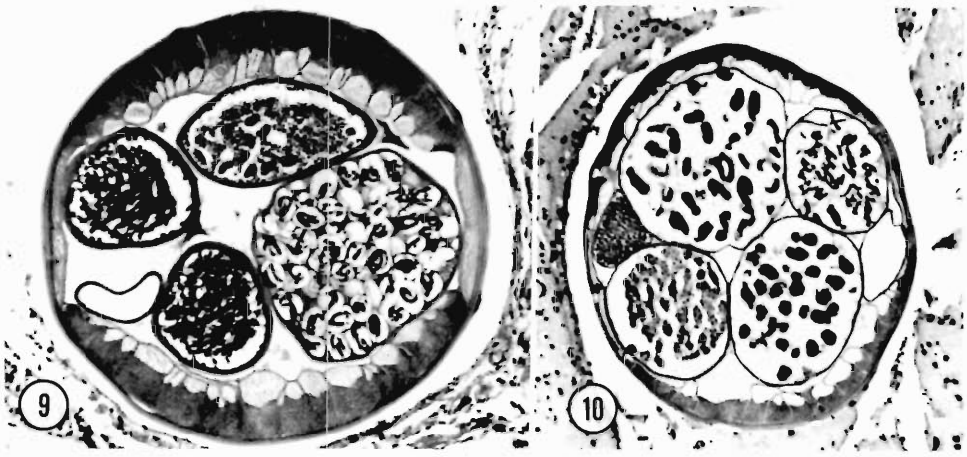
Loaina scapiceps (Leidy, 1886) comb. n.
Dirofilaria scapiceps (Leidy, 1886)
(Figs. 6–7)

HOSTS: *Sylvilagus floridanus, S. palustris, S. aquaticus, Lepus americanus, L. campestris, L. washingtonii.*

SPECIMENS EXAMINED: USNM Helm. Coll. Nos. 28647, 32016, 32621, 38066, 45326, 73651, and specimens in authors' collections from St. Tammany Parish, Lousiana.

Diagnosis

Leidy (1886) described in very cursory fashion *Filaria scapiceps,* noting the features of size, general body shape, and nature of the caudal papillae in the male. In 1916, Hall provided a more detailed and accurate description of the adult parasite, including measurements. However, the il-



Figures 9–10. Cross sections of female *Loa loa* and *Loaina uniformis* comb. n. at comparable levels. 9. *L. loa*, note various levels of uterus in same section and cuticular bosses (×110). 10. *L. uniformis*, note ovary and various levels of uterus in same section of worm (×110).

lustration of the male tail is misleading as it depicts the caudal papillae as being small and slender rather than large and bulbous (Figs. 6–7). Neither Leidy (1886) nor Hall (1916) provided a description of the microfilaria. Only Highby (1943), working on the life history of the parasite, has provided a substantial description of the microfilaria. He noted the size, internal anatomy, and the presence of a sheath. We have been able, in the present study, to examine microfilariae of *L. scapiceps*. We confirmed what was observed by Price (1957), that the microfilariae of *L. scapiceps* and *L. uniformis* are virtually identical in morphology. The only remarkable difference is in length; *L. scapiceps* averages 240 μm in length, whereas *L. uniformis* has a mean length of 270 μm.

Price (1957) noted also the close morphological similarity of the adults of *L. uniformis* and *L. scapiceps*; the two are distinguished most easily on body shape. *L. scapiceps* is coiled helically along the entire body length and the ends in both sexes are tapered, whereas the body of *L. uniformis* is not coiled and the ends are not tapered appreciably. Moreover, *L. scapiceps* has lateral alae, which are lacking in *D. uniformis*. The distal end of the right spicule in *L. scapiceps* is barbed, whereas in *L. uniformis* the distal end is smooth and pointed. There is also an additional pair of ventral, subterminal papillae near the tip of the male tail in *L. scapiceps*. In addition to the morphological features mentioned, adult *L. scapiceps* are characteristically found in the tarsal bursa of the hind feet, whereas adult *L. uniformis* are found in subcutaneous tissues.

Loaina scapiceps apparently has widespread geographical distribution, which includes: New Brunswick, Ontario, and British Columbia, Canada, and the northwestern, northeastern, eastern seaboard, and gulf coast regions of the United States.

Specimens deposited as USNM Helm. Coll. No. 32016 (three female worms), and identified as *D. scapiceps*, were recognized not to be that species. The specimens, although filaria, could not be identified to species. Likewise, specimens identified as *D. scapiceps* and deposited as USNM Helm. Coll. Nos. 42790 and 75430, are, in fact, specimens of *L. uniformis*.

Discussion

Taxonomically, *Loaina* appears to be most closely related to *Loa*. This is evidenced in several morphological features. The microfilariae, other than differences in size (*L. loa* 320 μm long, *L. uniformis* 270 μm long, *L. scapiceps* 240 μm long) are similar in morphological features, including arrangement of somatic nuclei in the tail. The shape and number of caudal papillae and small size of the spicules of the males are features common to both genera. It was noted in the present study that in female worms of both *L. scapiceps* and *L. uniformis*, one reproductive tube, including uterus, oviduct, and ovary, typically occupied the anterior half of the body and the second tube occupied the posterior half (Fig. 4).

This was noted by Eberhard and Orihel (1981) to be the case in *Loa* as well.

In general features, the shapes of the body in *L. loa* and *L. uniformis* are very similar. They both have bluntly rounded extremities, are more-or-less uniform in diameter throughout the body length, and the tail of the male is not coiled (the last feature is also shared by *L. scapiceps*). The two are remarkably similar at the microanatomical level, particularly in the structure of the body wall. Both possess a thick cuticle and prominent hypodermal tissue underlying the muscle cells. The muscle cells are few per quadrant, broad, and have a large cytoplasmic portion (Figs. 9–10). The feature of the muscles in *L. scapiceps* is similar except the cytoplasmic portion is less conspicuous. The character of the muscle cells further distinguishes *Loaina* from *Dirofilaria,* in which the muscle cells typically are many per quadrant, tall, and slender.

The two genera differ morphologically in size, *Loa* being considerably larger and by the absence of cuticular bosses in *Loaina.* The host preferences of the two genera also contrast markedly; *Loa* is a parasite of primates and *Loaina* parasitizes lagomorphs. Further, the arthropod vectors of *L. scapiceps* and *L. uniformis* have been demonstrated to be various species of mosquitoes (Highby, 1943; Price et al., 1963), whereas it is well documented that deerflies are the vectors of *Loa.*

A review of the genus *Dirofilaria* revealed two additional species which, based on morphological features, do not belong in this genus. *Dirofilaria timidi* has been described from rabbits in Russia (Gubanov and Fedorov, 1966), but study of the description of this species clearly indicates that it is not a *Dirofilaria,* nor is it related to either *L. uniformis* or *L. scapiceps.* Its morphological features suggest it is *Brugia*-like. Likewise, certain biological and morphological features of *Dirofilaria roemeri* have been noted to be unusual, including prominent lateral alae, a sheathed microfilaria and a tabanid intermediate host (Schacher, 1973; Spratt and Varughese, 1975). Anderson (1959) commented, when he placed it in the genus *Dirofilaria,* that *D. roemeri* was so distinct that it could be separated readily from other species in the genus. The features noted above, plus the small size of the spicules, are more characteristic of other genera, including the genus *Loaina,* than of *Dirofilaria.* However, it differs from *Loaina* and other Dirofilariinae in several features, among which the placement of the vulva and the number and distribution of caudal papillae are distinct. For these reasons, it is not possible at this time to accurately place *D. roemeri* in a proper generic group. However, we feel that the genus *Dirofilaria* should be restricted to those species that have unsheathed microfilariae.

Acknowledgments

The authors thank Dr. Ralph Lichtenfels, Animal Parasitology Institute, for loan of specimens. Dr. John Crites, Department of Zoology, Ohio State University, kindly supplied additional specimens of *L. scapiceps.* This study was supported by grant RR-00164 from the National Institute of Allergy and Infectious Diseases, NIH.

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51(1), 1984, pp. 54–61

Hexametra leidyi sp. n. (Nematoda: Ascarididae) from North American Pit Vipers (Reptilia: Viperidae)

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ABSTRACT: Specimens of *Hexametra* from six canebrake rattlesnakes, *Crotalus horridus atricaudatus,* and one North American water moccasin, *Agkistrodon piscivorus leucostoma,* collected in Louisiana are described as *Hexametra leidyi* sp. n. Observations based on 19 male and 21 female worms indicated that the specimens collected in Louisiana differed in several ways from the species reported from New World pit vipers. *H. leidyi* has lips significantly smaller ($P < 0.001$) than those of *H. boddaertii* from North American colubrid and viperid snakes. The dentigerous ridge of *H. leidyi* extends to the base of the lip and bears numerous small denticles, whereas in *H. boddaertii* the denticles are larger, fewer, and limited to the anterior margin of the lip. *H. leidyi* eggs are 90 μm by 81 μm (mean), whereas those of *H. boddaertii* are 80 μm by 73 μm (mean). Also, the egg of *H. leidyi* has fewer pits on its surface than does the egg of *H. boddaertii. H. leidyi* eggshells have an average of 58 pits per 80 μm, and in *H. boddaertii* the average is 90. Although previously synonymized with *H. boddaertii* sensu Sprent, 1978, *H. hexauterina* (Skrjabin, 1916) from a *Bothrops* sp. in Paraguay was described as having denticles extending to the base of its lips and eggs similar in size to those of *H. leidyi.* However, as the type specimens of *H. hexauterina* could not be found, and its description appears inadequate, it is considered to be in a position of subjudice.

Baird (1860) described *Ascaris boddaertii* from a single female specimen collected from a West Indian colubrine snake, *Mastigodryas* (=*Herpetodryas*) *boddaerti.* Baylis (1916, 1920) redescribed this worm and placed the species in the genus *Polydelphis* Dujardin, 1845, because the specimen had more than two uterine branches. Kreis (1944) later transferred the species to the genus *Hexametra* that Travassos (1920) had created for ascaridoids with six uterine branches. Until 1978, when Sprent reviewed the genus *Hexametra,* the worm described by Baird was the only known specimen of *H. boddaertii.*

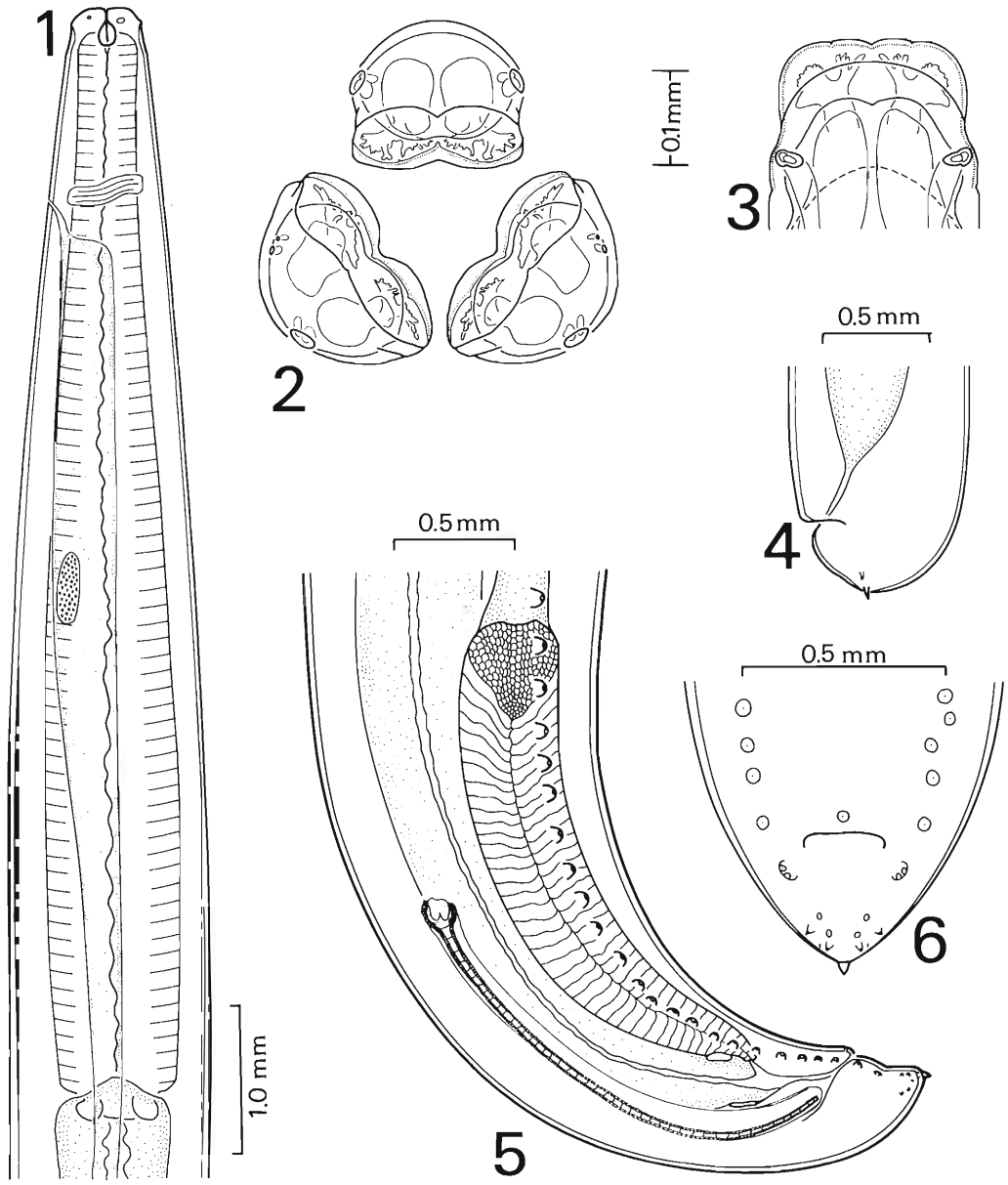
Araujo (1969) recovered worms from a Brazilian snake, *Crotalus durrissus terrificus,* and identified them as *H. quadricornis* (Wedl, 1861). However, Sprent (1978) concluded that the specimens examined by Araujo were *H. boddaertii,* not *H. quadricornis.* Sprent also placed two other species of *Hexametra* in synonymy with *H. boddaertii.*

One of the species that Sprent considered as synonymous with *H. boddaertii* was *Ascaris quadrangularis* Schneider, 1866. The type specimens of *A. quadrangularis* were collected from the same host and locality as the specimens examined by Araujo. However, Sprent found that the type collection was composed of a single *Hexametra* female mixed with several *Ophidascaris* specimens, and he felt that the female specimen was indistinguishable from *H. boddaertii,* which has taxonomic precedence. Sprent also indicated that *H. hexauterina* (Skrjabin, 1916) should be regarded as a synonym of *H. boddaertii.* The type specimens of *H. hexauterina* were collected from a snake, *Bothrops* sp., in Paraguay, and could not be found for examination. Although Sprent placed this species in synonymy with *H. boddaertii,* he did note that in Skrjabin's original description the denticles were shown to extend further posteriad on the lateral sides of the lips than they do in *H. boddaertii.*

Also, Sprent (1978) examined specimens of *Hexametra* from North American rattlesnakes in the collection of the U.S. National Parasite Collection and indicated that they were all specimens of *H. boddaertii.* He therefore concluded that all the specimens of *Hexametra* from the New World represented a single species *H. boddaertii.* Specimens collected recently from pit vipers in Louisiana were found to have characteristics that differentiate them from *H. boddaertii* sensu Sprent, 1978, and they are described herein as a new species.

Materials and Methods

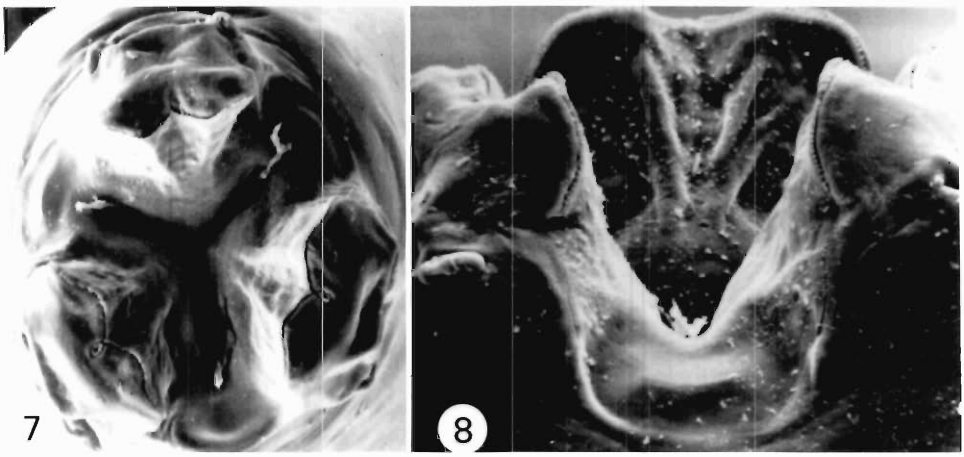
Adult worms were collected in Louisiana from the stomach and intestine of six naturally infected canebrake rattlesnakes, *Crotalus horridus atricaudatus,* and one naturally infected North American water moccasin, *Agkistrodon piscivorus leucostoma.* Specimens of *Hexametra boddaertii* (Wedl, 1861), three males and three females, were acquired through the kindness of Dr. P. Araujo (Departamento de Parasitologia do Instituto de Ciencias Biomedicas da Univ. Sao Paulo, Brasil). Also examined were the type specimen labelled



Figures 1–6. 1. Lateral view of left side of esophageal region of female. 2. En face view of male. 3. Dorsal view of dorsal lip of male. 4. Lateral view of female tail. 5. Lateral view of male tail showing spicule and ejaculatory duct. 6. Ventral view of male tail.

Ascaris boddaertii Baird, 1860 and the cotypes labelled *A. rotundicaudata* Linstow, 1904 on deposit in the British Museum of Natural History, London (BMNH 58.7.5.4 and BMNH 1946.12.31.4–7, resp.), the syntypes labelled *Ascaris quadrangularis* Schneider, 1866 from the Museum für Naturkunde der Humboldt Universität zu Berlin (Nr. 869), specimens of *Hexametra* from the U.S. National Parasite Collection (nos. 28248, 29862, 31646, 42175, 42658, 42736, and 57553) and specimens of a *Hexametra* sp. from a *Crotalus durissus* collected in Costa Rica by Dr. D. Pence (Dept. of Pathology, Texas Tech Univ., Health Sciences Center, Lubbock, Texas).

The specimens collected in Louisiana were fixed at room temperature in either 10% formalin, Carnoy's fixative, AFA (85 parts 50% ethanol, 10 parts form-



Figures 7–8. 7. Scanning electron micrograph showing en face view of female. ×90. 8. Scanning electron micrograph showing lateral side of dorsal lip and the extent of the dentigerous ridge. ×160.

aldehyde, and 5 parts glacial acetic acid), or at 60°C in 70% ethanol. For the observation of the internal morphological features, the specimens were passed through a graded series of ethanols ending with two changes of 100% ethanol and were then cleared in Beechwood creosote or liquified phenol. En face preparations were made by mounting the excised anterior extremity in glycerin jelly. For the observation of microanatomy, tissues were processed by routine histological procedures and stained with Ehrlich's hematoxylin and eosin. Drawings were made with the aid of a camera lucida. Material examined with the scanning electron microscope was postfixed in Zenker's fixative, placed in water, frozen in liquid nitrogen, lyophilized, gold plated, and examined with a Cambridge SEM.

Measurements, except on total body length, were made with the aid of an ocular micrometer. Total body length was measured by placing a hydrated worm between two panes of glass in front of a light source, tracing the worms on a piece of paper, and then measuring the length of the body outline. The small size of the pits in the shells of the eggs that were examined made it difficult to differentiate the individual pits at the circumference of the eggshell. Therefore to allow a comparison of the number of pits on the eggs, pits within an 80-μm area on the surface were counted under 1,250 magnifications. Ten eggs from the vagina and common uterus of each of the three Brazilian females, three Costa Rican females, and three specimens from three different collections made in Louisiana were examined in this manner, as well as 10 eggs from the feces of a canebrake rattlesnake experimentally infected with the species from Louisiana. The eggs of the type material and specimens from the U.S. National Parasite Collection were measured in utero under 500 magnifications, and because they were examined through the body wall, the pits could not be clearly differentiated. The female labelled as the cotype of *A. rotundicaudata* and the female labelled as the type of *A. quadrangularis* unfortunately did not have eggs.

Description

Hexametra leidyi sp. n. (Figs. 1–12)

GENERAL: Ascarididae Baird, 1853; Angusticaecinae Skrjabin and Karokhin, 1945; *Hexametra* Travassos, 1920. Head with 3 distinct lips of equal size, varying in shape from square to trapezoidal with small to prominent dip in center of anterior margin (Figs. 2, 3, 7, 8). Two labial pulps enter each lip; each pulp branches anteriorly into two lobes that then branch into smaller lobuli. First major branch of each labial pulp forming cleft into which fibers from small papilla on anterior margin penetrate. Denticles present, extending along inner surface of anterior margin and lateral edges of each lip to base (Figs. 3, 8). Esophagus 3.0% to 5.7% of total body length and without ventriculus or diverticulum (Fig. 1). Dorsal gland cell nucleus of esophagus spherical; 2 subventral gland cell nuclei spherical. Intestinal cecum occasionally present; cecum, when present, small, anteriorly directed, located on left side of esophageal–intestinal junction. Excretory pore slightly posterior to nerve ring. Excretory system with inverted "Y" shape; excretory duct extends posteriad to left lateral chord, then bifurcates when entering commissure of excretory cell. Excretory cell nucleus large, situated near posterior end of excretory cell commissure on left side. Deirids pit-like, lateral, slightly posterior to level of excretory pore. Lateral chords prominent, running length of body. Cuticle over lateral chords

Table 1. Measurements in mm of male specimens of *H. leidyi*.

Feature	Holotype	Male paratypes			
		N	$\bar{x}$	SD	Range
Body length	121	18	115	20.2	69–135
Dorsal lip:					
Length	0.209	18	0.186	0.032	0.115–0.235
Width at papillae	0.228	16	0.226	0.033	0.140–0.270
Width at base	0.223	16	0.240	0.035	0.145–0.294
No. denticles	190	16	172	18.7	141–204
Anterior end to:					
Nerve ring	1.02	18	0.999	0.199	0.606–1.32
Excretory pore	1.32	18	1.27	0.243	0.861–1.76
Deirid	1.68	9	1.60	0.260	1.17–1.88
Esophagus length	5.82	18	5.62	1.02	3.36–7.05
Cecum length	0.618	5	0.267	0.103	0.206–0.449
Body width at:					
Base of lips	0.401	12	0.433	0.054	0.330–0.504
Nerve ring	0.564	18	0.609	0.087	0.376–0.731
Excretory pore	0.616	18	0.655	0.104	0.396–0.762
Base esophagus	1.08	18	1.15	0.248	0.718–1.89
Maximum body width	1.67	17	1.75	0.276	1.05–2.04
Tail length	0.400	18	0.284	0.058	0.187–0.449
Tail width	0.410	17	0.377	0.069	0.234–0.475
Mucron length	0.028	16	0.022	0.008	0.005–0.033
Left spicule length	1.67	16	1.75	0.276	1.05–2.04
% Ejaculatory duct length	75.6%	16	82.5%	12.7%	107%–62.0%
Right spicule length	1.77	18	1.69	0.288	1.04–2.05
% Ejaculatory duct length	78.3%	18	82.3%	14.3%	109%–60.3%
Ejaculatory duct length	2.26	18	2.11	0.479	0.990–2.90
No. left preanal papillae	58	13	60	8.1	48–74
No. right preanal papillae	69	13	61	8.7	52–81
No. left postanal papillae	4	18	4		3–4
No. right postanal papillae	4	18	4		4–4

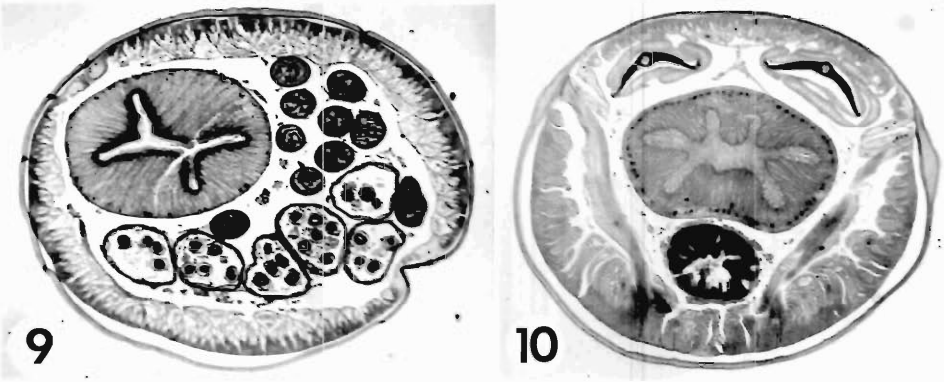
with cuticular bars beginning near nerve ring and extending to level of esophageal–intestinal junction. Cuticular bars each having shape of inverted "V" or flattened "M" when seen in transverse section.

MALE: Measurements as in Table 1. Testis single, beginning near midbody. Testis heavily coiled at midbody, then becoming straight and extending posteriad to about half of distance to tail where it joins vas efferens. Vas efferens continues posteriad, but then loops anteriad to about level of its junction with testis and joins seminal vesicle. Seminal vesicle extending posteriad to near tip of tail where it enters vas deferens proper that forms the ejaculatory duct.

Tail flexed ventrally. Spicules equal and alate. Ratio of spicule length to ejaculatory duct length, 0.60 to 1.09. Proximal portion of spicule an open cone of sclerotized cuticle; more distally becoming an elongate tubular shaft with lateral membranous wings (Figs. 5, 10). Wings short anteriad, becoming longer distally, and extending short distance beyond distal tip of sclerotized shaft.

Preanal papillae unpaired with different numbers of preanal papillae in different positions on each side; on individual worms, difference between number on each side small. Adanal papillae include ventral papilla on anterior lip of anus and ventrolateral double papilla on each side (Fig. 6). Postanal papillae 3 or 4 in number, usually 2 subventral and 2 subdorsal. Subdorsal papillae occasionally fusing to form a single double papilla or rarely having only one subdorsal single papilla and three subventral papillae. Phasmids lateral, usually posterior to all postanal papillae. Tail terminating in short conical mucron.

FEMALE: Measurements as in Table 2. Vulva near midbody; distance from anterior end to vulva highly correlated to total body length as shown by linear regression (df = 19, $r^2 = 0.9538$, y intercept = 2.11665, slope = 0.47569). Vagina vera



Figures 9–10. 9. Transverse section of female showing the six uterine branches. ×34. 10. Transverse section of male showing the expanded lateral wings of the spicules at about the level of mid shaft. ×85.

extending posteriad from vulva before joining vagina uterina. Vagina uterina extending to common uterus that divides posteriorly into 6 branches (Fig. 9). Each branch continuing posteriad forming seminal receptacle first and then oviduct. Oviduct reflexing anteriad to or beyond level of vulva. Ovary beginning at anterior extent of oviduct and continuing posteriad beyond end of uterine branches; here, all 6 ovaries forming tightly coiled mass.

Tail straight after fixation, short, and with small mucron; mucron occasionally withdrawn into body in large specimens. Phasmids lateral and slightly anterior to terminal mucron.

EGGS: Eggs spheroid to ovoid ($N = 40$) (Fig. 11); maximum diameter 90 μm (84–96 μm, SD = 0.003), minimum diameter 81 μm (74–89 μm, SD = 0.004). Shell approximately 5 μm thick; surface covered with fine pits (Fig. 12); 58 pits (45–73, SD = 7.8) within area of 80 μm.

Taxonomic Summary

DIAGNOSIS: Lips small in relation to body length; total body length 69–216 mm, dorsal lip length 115–291 μm, dorsal lip width at papillae 140–301 μm, dorsal lip width at base 145–326 μm. Dentigerous ridge with numerous, small denticles (141–216 per dorsal lip) extending to the base of the lip. Eggs 90 μm (84–96 μm) by 81 μm (74–89 μm); surface of egg with 58 (45–73) pits within a surface area of 80 μm².

SPECIMENS DEPOSITED (USNM Helminthological Collection, USDA, Beltsville, Maryland 20705): holotype (male), no. 77508; allotype (female), no. 77509; paratypes, nos. 77510–77516.

HOST: *Crotalus horridus atricaudatus,* canebrake rattlesnake (type host). Also found in *Agkistrodon piscivorus leucostoma,* water moccasin.

LOCALITY: Louisiana: Orleans Parish.

SITE OF INFECTION: Lumen of stomach and small intestine.

ETYMOLOGY: Named in honor of Dr. Joseph Leidy for his pioneering work in the field of parasitology.

Remarks

All *H. leidyi* examined differed in three major respects from *H. boddaertii* sensu Sprent (1978) and the specimens from Costa Rica that are considered here to also be *H. boddaertii.* The differences were in the size of the lips, the size, number, and distribution of the denticles on the lips, and the size of the eggs and the pits on their surfaces.

The lips of *H. leidyi* were found to be consistently smaller than on specimens of *H. boddaertii* of equal size. Because lip measurements are correlated with body length, the analysis of covariance test was used to compare the dimensions of the dorsal lips of *H. leidyi* and *H. boddaertii* specimens. Using this test, it was found that the differences in lip length and width at both the base of the lip and at the level of the double papillae were all significantly different in the two groups ($df = 1,51$; $P < 0.001$). The regression coefficients show that the length of the dorsal lip of *H. leidyi* is usually about 35 μm less, the width at the double papillae is usually about 60 μm less, and the width at the base of the lips is usually about 85 μm less than the respective dimensions on the dorsal lip of *H. boddaertii.*

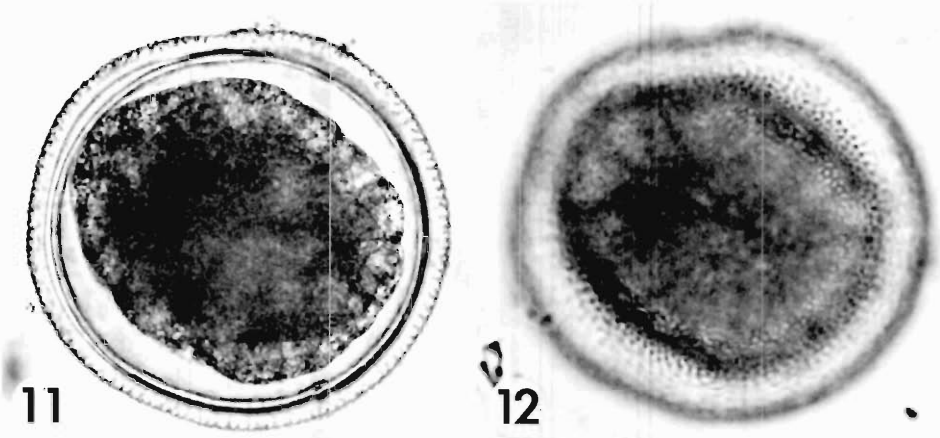
Table 2. Measurements in mm of female specimens of *H. leidyi*.

Feature	Allotype	Female paratypes			
		N	$\bar{x}$	SD	Range
Body length	194	20	167	34.8	107–216
Dorsal lip:					
Length	0.230	19	0.208	0.047	0.136–0.291
Width at papillae	0.266	19	0.254	0.038	0.190–0.301
Width at base	0.310	19	0.264	0.044	0.195–0.326
No. denticles	161	18	196	16.8	148–216
Anterior end to:					
Nerve ring	1.14	20	1.04	0.159	0.793–1.27
Excretory pore	1.53	19	1.25	0.182	0.973–1.60
Deirid	1.92	8	1.68	0.283	1.253–2.09
Vulva	91	20	81	17.0	53.5–109
% Body length	47.0%	20	48.8%	2.1%	53.0%–45.7%
Esophagus length	6.38	19	6.15	1.16	4.61–8.17
Cecum length	None	9	0.401	0.108	0.257–0.543
Body width at:					
Base of lips	0.422	17	0.477	0.064	0.371–0.577
Nerve ring	0.668	19	0.697	0.100	0.546–0.855
Excretory pore	0.793	19	0.751	0.107	0.567–0.948
Base esophagus	1.32	19	1.28	0.220	0.896–1.76
Vulva	1.74	20	1.93	0.346	1.32–2.82
Maximum body width	1.98	18	2.14	0.450	1.41–3.30
Tail length	0.295	19	0.320	0.081	0.206–0.484
Tail width	0.697	19	0.673	0.132	0.464–0.865
Mucron length	0.012	17	0.018	0.008	0.010–0.033
Vagina length	3.34	16	2.73	0.733	1.44–4.18
Common uterus length	5.74	16	4.78	1.03	3.60–7.43

In *H. leidyi,* the dentigerous ridge extends to the base of each side of the lip, whereas in *H. boddaertii* it extends only to the level of the anterior margin of the double papilla. Also, the denticles of *H. leidyi* are smaller than those of *H. boddaertii.* When the denticles along a distance of 55 μm were counted from the center to the right on dorsal lips of equal size specimens, the number in *H. leidyi* specimens was 22–28 denticles and only 11–15 in *H. boddaertii.* However, because the size of the denticles depends largely on the size of the worm, it was concluded that it would be better to use the total number of denticles on the dorsal lip for making comparisons between species rather than the number of denticles in a given length of the dentigerous ridge. Also, the total number of denticles on the dorsal lip of each specimen was found not to be related to the size of the worm examined.

When the total number of denticles was compared between the two species, *H. leidyi* was found to have more denticles per dorsal lip than *H. boddaertii.* The male specimens of *H. leidyi* were found to have a range of 141–204 total denticles on each of their dorsal lips, whereas two of the male specimens of *H. boddaertii* from Brazil had only 96 and 101 denticles on each of their dorsal lips. The female specimens of *H. leidyi* also had more numerous denticles on each of their dorsal lips with a range of 148–216 as compared with 79 and 83 for two female *H. boddaertii* from Brazil. The specimens of *H. boddaertii* from Costa Rica had denticles only on the anterior margin of their lips with the number of denticles ranging from 86 to 103. The denticles on the lips of the type specimen of *H. boddaertii* were not counted, but the dentigerous ridge did not extend beyond the anterior margin of the double papillae.

The eggs of *H. leidyi* are larger than those of *H. boddaertii.* Sprent (1980) reported egg sizes for *H. boddaertii* (78–88 μm by 62–86 μm) that are smaller than those reported in 1920 by Baylis (88–95 μm by 75–80 μm). The present study found measurements that were in agreement with those of Sprent rather than those of Baylis. The eggs of the type specimen of *H. boddaertii* measured 79 μm (74–85 μm, $N = 10$, SD = 3.3) by 71 μm (65–78 μm, $N = 10$, SD = 4.3), which when combined with measurements made on eggs



Figures 11–12. 11. Egg of *H. leidyi.* ×640. 11. Egg of *H. leidyi* showing the pits present on the eggshell. ×640.

from the Brazilian and Costa Rican specimens produced measurements of 80 μm (72–86 μm, $N = 70$, SD = 3.1) by 70 μm (65–82 μm, $N = 70$, SD = 3.8). The eggs of *H. leidyi,* on the other hand, were found to measure 90 μm (84–96 μm) by 81 μm (74–89 μm) showing that they are on the average about 10 μm larger in each dimension. Because the ranges overlap slightly, the means were compared using Student's *t*-test with pooled variances, and the difference was found to be significant ($P < 0.001$). It is believed that this difference in size clearly separates these two groups of eggs.

Also, when the number of pits on the eggs of *H. leidyi* was compared to the number present on the eggs of *H. boddaertii,* it was found that *H. boddaertii* had more pits within a defined area on the surface of its eggshell. The eggs from Brazilian and Costa Rican specimens each had a mean of 90 (75–114, $N = 60$, SD = 10.3) pits in an 80-μm^2 area of its surface. *H. leidyi* had a mean of about one-third less pits in the same area, i.e., 58 (45–73). Because the ranges did not overlap, it is apparent that this feature also separates *H. leidyi* from *H. boddaertii.*

Only two species of *Hexametra* have been previously described as having denticles extending to the base of the lips. One is *H. rotundicaudata* (Linstow, 1904) Mozgovoi, 1953 from lizards of the genus *Calotes* in Ceylon and India, the other is *H. hexauterina* (Skrjabin, 1916) Kreis, 1944 from a lance-headed viper, *Bothrops* sp., in Paraguay. Sprent (1978) indicated in a footnote that *H. rotundicaudata* is possibly an immature form of *H. quadricornis.* However, an examination of the type specimens indicated that the number and extent of the denticles were similar to those of *H. leidyi* rather than those of other *Hexametra* species, and for this reason, it is suggested that *H. rotundicaudata* be considered a separate species of *Hexametra* occurring in lizards in Asia. Sprent (1978) mentioned that the description of *H. hexauterina* by Skrjabin (1916) indicated that the only difference between this species and *H. boddaertii* was the extent of the denticles down the lateral sides of the lips, and he synonymized it with *H. boddaertii* which has precedence. *H. leidyi* appears similar to *H. hexauterina* in that it has numerous denticles extending far down the sides of the lips, and eggs similar in size to those described by Skrjabin (Skrjabin measured the eggs to be 0.09 mm by 0.07 mm). In light of the description of *H. leidyi,* the type specimens of *H. hexauterina* should be reexamined, but they could not be found for study. This lack of type material along with the inadequacy of the original description accompanying the illustration showing small denticles extending to the base of a subventral lip make it impossible to determine at this time whether or not *H. hexauterina* is the same species as *H. leidyi.* Therefore, until such time as the types are found or neotypes are collected from the same host and locality, it is felt that the species *H. hexauterina* should be considered in a position of subjudice.

Sprent (1978) reported that the specimens of *Hexametra* from North American rattlesnakes in the U.S. National Parasite Collection all ap-

peared to be *H. boddaertii,* but an examination of these worms disclosed one specimen that did not appear to be *H. boddaertii* sensu Sprent, 1978. This was a female (USNHC 28248) collected from a western rattlesnake, *Crotalus viridis.* This worm had denticles extending to the base of its lips with 189 denticles on the dorsal lip. Unfortunately, this specimen is in poor condition and many other features of this worm, including the size of the eggs, could not be determined. However, the extent and number of denticles on this specimen would suggest that it probably is *H. leidyi.*

The type specimen of *H. boddaertii* is from a colubrine snake, *Mastigodryas boddaerti,* collected in the West Indies. Sprent (1978) reported that *H. boddaertii* occurs in both colubrid and viperid snakes in Brazil, and the worms collected by Dr. Pence indicate that it is also found in viperids in Costa Rica. The range of *H. boddaertii* in North America, however, is difficult to determine because all but one of the specimens from rattlesnakes in the U.S. National Parasite Collection were from zoo animals. The only worm that was not from a captive host is a single female specimen (USNHC 57553) from a "rattlesnake" collected in central Florida. *H. leidyi,* on the other hand, appears to be a parasite of the canebrake rattlesnake, *Crotalus horridus atricaudatus,* in Louisiana that is now considered by most as synonymous with the timber rattlesnake, *Crotalus horridus,* with a range from southwestern Maine to northern Florida west to southwest Minnesota and central Texas. Finding *H. leidyi* in a water moccasin appears to be a very rare occurrence, because many water moccasins have been examined in this laboratory and not found to have *Hexametra* although they may have other ascaridoids. The specimen in the western rattlesnake, *Crotalus viridis,* that had been housed in a San Diego zoo may indicate that this parasite is present in snakes farther west than Louisiana.

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In Vitro Development of *Trichostrongylus colubriformis* from Fourth-Stage Larvae to Young Adults in a Chemically Defined Medium

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ABSTRACT: In vivo-grown fourth-stage (L4) and fourth-molt (4M) *Trichostrongylus colubriformis* larvae developed in large numbers to young adults, primarily females, in a roller culture system consisting of the defined medium RPMI 1640, with a gas phase of 85% nitrogen/5% oxygen/10% carbon dioxide at 39°C. In this system, 5-day-old L4, obtained from the intestines of experimentally infected guinea pigs, developed to 4M in 3 days and to young adult males and females in 6–7 days. Inocula of 8,000–34,000 produced yields of 61–73% 4M and 4–9% young adults in 7 days. When mixed populations of L4 and 4M larvae and young adults, obtained from guinea pigs and sheep 7–8 days after infection, were inoculated into culture medium, the 4M larvae completed ecdysis to young adults from days 0–3, and L4 advanced to 4M by day 3. Optimal development occurred in cultures of 34,000; maximal yields (21%) of young adults were obtained in 3 days. In vivo-grown 7–8-day-old young adult males and females did not grow or develop further in vitro.

Trichostrongylus colubriformis is a common, widely distributed, nematode parasite of the small intestine in ruminants. In previous attempts to grow advanced stages of *T. colubriformis* from infective larvae in vitro a few became young adult males and females (Douvres, 1980). This occurred in a two-step, roller culture system that consisted of complex cell-free media, RFN-2 (Step 1) and API-16, with appropriate additions of a reducing agent and pepsin (Step 2).

It has been shown that *Ascaris suum* third-stage larvae (L3) obtained from the lungs of experimentally infected animals can develop to fourth stage (L4) in vitro in defined media such as: Dulbecco's Modified Eagle's Medium (Urban and Douvres, 1981) and RPMI 1640 (Urban et al., 1983). In addition, metabolic products released by *A. suum* larvae during cultivation were immunogenic (Urban and Douvres, 1981; Urban and Romanowski, 1982; Urban et al., 1983).

The present report describes the use of a roller culture system for development of *T. colubriformis* larvae from L4, obtained from the intestines of sheep and guinea pigs, to young adults in the defined medium RPMI 1640.

Materials and Methods

Animal infection

Neutered Polled Dorset 2- to 4-month-old male sheep, raised helminth-free except for minimal infection with *Strongyloides papillosus*, and 300–400-g guinea pigs of both sexes were experimentally infected with *T. colubriformis* (RLS isolate). Advanced stages of *T. colubriformis* used for in vitro cultivation were obtained from the intestines of 80 guinea pigs that were each orally inoculated with 20,000 infective larvae and killed 5 and 7 days after infection (DAI); and from six sheep, each orally inoculated with 200,000 infective larvae and killed 7 and 8 DAI. The following developmental stages of *T. colubriformis* (Douvres, 1957) were recovered from the animal infections: At 5 DAI, from guinea pigs, larvae in mid and late L4, with the majority in mid-phase (Table 1); 7 and 8 DAI, from guinea pigs and sheep, mixed populations of larvae in late L4, fourth molt (4M), and young adults, with the majority in 4M (Table 2). The larval stages and young adults included both sexes in nearly equal numbers.

Preparation of inoculum for in vitro cultivation

The method of recovering *A. suum* larvae from the lungs of swine (Urban and Douvres, 1981) was adapted for recovering larval and adult stages of *T. colubriformis* from the intestines of guinea pigs and sheep. At slaughter the entire intestine of guinea pigs or anterior one-half of the small intestine of sheep was removed, opened longitudinally, and added to warm saline in a Baermann Apparatus (containing a wire screen insert to which a triple layer of fine cheesecloth was tied). The entire intestine of five guinea pigs, or one-fourth of intestine of a sheep was added to each apparatus. After 3–4 hr incubation at 37°C, nematodes that had settled to the bottom of the cone were collected into a tube. Although nematodes collected by this procedure were grossly free of host ingesta, they were washed four to six times with Earle's balanced salt solution containing antibiotics and an antimycotic agent (EBSSA) (Douvres and Malakatis, 1977), and centrifuged at 200 *g* for 2 min with no braking of the rotor (Urban and Douvres, 1981). Host ingesta remaining in the super-

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natant were removed by aspiration. Aliquots of 8,000, 25,000, and 34,000 washed larval and adult stages suspended in 1.0 ml of culture medium were used as inocula (Tables 1, 2).

Preparation of medium and cultures

RPMI 1640 medium was prepared as described by Morton (1970), except that phenol red was deleted, and the following antibiotics and antimycotic were added (per ml of medium): 1,000 units of penicillin G potassium, 1 mg of streptomycin sulfate, and 10 μg of amphotericin B (Fungizone). The pH was adjusted to 6.8 with 1 N HCl before use as the culture medium.

The culture system consisted of 20 ml of RPMI 1640 with a gas phase of 85% nitrogen/5% oxygen/10% carbon dioxide, in roller bottles (205 mm long × 15 mm diameter, supplied with teflon-lined screw caps, Bellco Glass, Inc., Vineland, New Jersey), that were rotated at 1 rev./1.5 min, at 39°C. The methods of Douvres and Malakatis (1977) were used to prepare and handle the roller cultures and to gas the cultures.

Eight cultivation trials were used to study the development of in vivo-grown larval and adult stages of *T. colubriformis* (Tables 1, 2). In trial 1, L4 obtained from guinea pigs 5 DAI were tested with inocula of 8,000, 25,000, and 34,000 in three or four cultures and handled as follows: the inocula were transferred to fresh medium and a new culture vessel on day 3, and the cultures were terminated on day 7. In trials 2 to 4, 7-day-old L4 to young adults from guinea pigs were tested with inocula of 8,000, 25,000, and 34,000 in three or four cultures/trial that were terminated on day 3. In the other trials, 7- and 8-day-old L4 to young adults recovered from sheep were tested with inocula of 25,000 in two to five cultures/trial that were terminated on day 3 (trial 5) or day 4 (trials 6–8). The methods of Douvres et al. (1966) were used to transfer cultures, and to examine and evaluate cultures. Cultures were judged free of contamination if there was no visible evidence of fungi or bacteria.

Results

In all test cultures, 5-day-old L4 developed to 4M in 3 days, and to young adult males and females in 6 or 7 days (Table 1). In all test cultures, 7–8-day-old L4 and 4M developed to 4M and young adult males and females, respectively, in 3 days. Larvae that were in 4M on day 0 completed ecdysis and advanced to young adults as early as day 1. In vivo-grown 7–8-day-old young adult males and females did not grow or develop further in vitro.

Morphogenesis and growth

Generally, morphogenesis of larval and young adult stages in vitro was identical to that obtained in sheep at 7–8 DAI. However, in all cultivation trials, the development to young adults was primarily attained by females (approximately 85% of adult populations). The development of 5-day-old L4 to young adults was 5 days slower than that obtained in vivo; growth in vitro was comparable to growth in guinea pigs at 7 DAI. Based on average measurements of 10 female specimens/stage, in vivo-grown L4 1.6 mm long developed to 4M and young adults that were 1.9 and 2.0 mm long, respectively. Growth of larval and young adult stages obtained from the development of 7–8-day-old L4 and 4M in vitro was not observed.

Table 1. *Trichostrongylus colubriformis* obtained from guinea pigs, 5 days after infection: survival and yield of advanced stages that developed from fourth-stage larvae in roller cultures using medium RPMI 1640.*

No. cultures	No. larvae per culture (×1,000)	Total inoculum alive (%)	Live worms (%) in stage			
			Mid fourth	Late fourth	Fourth molt	Young adult
At day 0	—	98	85	15	0	0
At day 3						
3	8	98	10	70	20	0
4	25	94	25	62	13	0
3	34	90	26	64	10	0
At day 7						
3	8	70	1	20	73	6
4	25	57	0	20	76	4
3	34	64	2	28	61	9

* Data based on sample count from one culture 3 days after infection and average of sample counts from two cultures 7 days after infection.

Survival rate and yields of advanced stages

Data on survival and yields of advanced stages obtained from the development of 5-day-old, in vivo-grown L4, with inocula of 8,000, 25,000, and 34,000 larvae, after 3 and 7 days are given in Table 1. These data show that survival rate and yields of advanced stages were best in cultures inoculated with 8,000 larvae. In these cultures, survival that was 98% on day 0 remained unchanged for the first 3 or 4 days, then decreased to 70% by day 7. Development beyond L4 by day 3 produced yields of 20% 4M and by day 7, yields of 73% 4M and 6% young adults. In cultures inoculated with 25,000 and 34,000 larvae survival was slightly lower and yields of advanced stages were slightly lower or higher (Table 1).

Data on survival and yield of advanced stages obtained from development of 7-day-old larvae

Table 2. *Trichostrongylus colubriformis* from guinea pigs and sheep: survival and yield of advanced stages that developed from larvae in fourth stage and fourth molt in a roller culture system using medium RPMI 1640.

Trial*	No. cultures	No. larvae per culture (×1,000)	No. days in culture	Total inoculum alive (%)	Live worms (%) in stage		
					Fourth	Fourth molt	Young adult
With inoculum from guinea pigs, 7 DAI†							
2	5	8	0	98	34	61	5
			3	78–90 (85)	0–1 (0.6)	77–84 (80)	14–23 (18)
3	4	25	0	97	13	87	0
			3	62–83 (76)	1–4 (2)	79–72 (86)	7–19 (12)
4	5	34	0	98	15	80	5
			3	89–98 (93)	0	75–92 (79)	8–25 (21)
With inoculum from sheep, 7 DAI							
5	3	25	0	97	1	90	9
			3	73–85 (78)	0	83–89 (85)	11–17 (15)
With inoculum from sheep, 8 DAI							
6	4	25	0	96	1	93	6
			3	69–90 (83)	0–2 (0.5)	78–88 (83)	12–22 (17)
7	2	25	0	97	12	83	5
			4	65–81 (73)	10–14 (12)	68–69 (69)	17–21 (19)
8	5	25	0	93	13	77	10
			4	61	2	58	40

* In trials 2–7, data based on ranges (and averages) of sample counts from cultures; and in trial 8, data based on sample count from pooled larvae and adults in five cultures.

† DAI = days after infection.

from guinea pigs after 3 days in culture and from development of 7–8-day-old larvae from sheep after 3 and 4 days in culture are given in Table 2. Survival rate and yields of advanced stages were best in cultures inoculated with 34,000 worms obtained from guinea pigs. In these cultures, survival that was 98% on day 0 decreased to 93% by day 3. All of the L4 advanced to 4M and there was a fourfold increase in the yield of young adults. In cultures inoculated with 8,000 and 25,000 worms obtained from guinea pigs and 25,000 worms from sheep, survival was lower (61–73%) and yields of L4 and young adults were slightly lower after 3 days.

Discussion

Although young adult male and female *T. colubriformis* have been grown previously in vitro in complex, cell-free media RFN-2 and API-16 (Douvres, 1980), the present study is the first to describe the successful use of a defined medium for this purpose. In addition, the present study shows that more efficient development of young adults was obtained. For the in vitro development of *T. colubriformis* from infective larvae, Douvres (1980) found that earliest development to L4, 4M and young adults occurred in 7, 13, and 16 days, and obtained maximal yields of less than 1% young adults in populations of about 100,000 in 16–28 days. In the present study, in cultures inoculated with 34,000 L4 obtained from the intestines of guinea pigs 5 DAI, larvae developed to 4M and young adults, in 3 and 7 days, respectively, and maximal yields of 9% young adults were obtained by day 7.

The defined medium RPMI 1640 has previously been used for growth of nematodes. Using stationary multi-well plate systems, Farrar and Klei (1981) found that infective larvae of the equine nematodes *Strongylus edentatus, S. vulgaris,* and *S. equinus,* developed to L4 in medium RPMI 1640 supplemented with 10% fetal calf serum. Urban et al. (1983) found that L3 of *A. suum,* recovered from the lungs of experimentally infected animals, developed to L4 in medium RPMI 1640, and that this development was enhanced when the medium was supplemented with cholesterol. The development of *A. suum* L3 to L4 in medium RPMI 1640 has been used by Urban (pers. comm.) to obtain larval-derived medium components that are immunogenic. In other work on the cultivation of *A.*

suum L3 to L4 in the defined medium, Dulbecco's Modified Eagle's Medium, Urban and Douvres (1981) found that larval-derived components were stimulators of porcine immune responses in vitro. Cultivation of *T. colubriformis* L4 to young adults in the present study extends the usefulness of medium RPMI 1640.

Applications for the use of media such as RPMI 1640 are many. For example, Dineen and Wagland (1966) and Herlich (1966) demonstrated that *T. colubriformis* L4 in guinea pigs were uniquely susceptible to immunologic attack. Rothwell and Love (1974) found that *T. colubriformis* L4 recovered from the intestines of sheep, and maintained in 0.85% NaCl for 6 hr released metabolic products with immunogenic properties. These investigators reported that a high level of protection against challenge infections of *T. colubriformis* in guinea pigs could be obtained by injections of the L4 metabolic products. Now, a roller culture system using medium RPMI 1640 provides a technique for the collection of large quantities of larval and adult metabolic products that may have immunogenic properties.

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Experimental Transmission of *Trichinella spiralis* to Swine by Infected Rats

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ABSTRACT: The role of rats in the transmission of *Trichinella spiralis* to swine is controversial because some experimental investigations have failed to show that swine will eat rat carcasses. Confinement-reared, grain-fed hogs were offered fresh rat carcasses harboring varying densities of muscle larvae. In all trials, rat carcasses were readily eaten in part or in their entirety, usually within a few hours. At necropsy these hogs were infected with *T. spiralis* muscle larvae. Hogs ate rat carcasses whether their regular feed was withheld or not. The initial portions of the carcass eaten were the naso-frontal section of the head (NF) and the tail. The consistent ingestion of the NF, including the tongue and facial muscles, is of significance since larval densities are usually high in these particular muscles. We conclude that rats must be considered a potential source of infection to swine until sufficient detailed epizootiological investigations are carried out to determine their actual importance on the farm.

Infected rats are commonly regarded as an important source of *Trichinella spiralis* for domestic swine (Hall, 1924); the evidence to support this is largely circumstantial, however, consisting mostly of prevalence data from farm rats (Moynihan and Musfeldt, 1949 a, b; Cironeanu, 1974; Smith, 1980; Ramisz and Balicka-Laurans, 1981). Others have discounted the significance of rats (Hall, 1938; Kozar, 1969; Merkushev, 1970; Madsen, 1974). Madsen (1974) argued that Zenker's (1871) original theory of "swine to swine" transmission has been unjustifiably ignored in favor of the "rat-swine-man" pattern proposed by Leuckart (1866). Cameron (1970) reported that domestic swine actually have a great aversion to rats and, hence, he doubted that rats play a significant part in infecting pigs.

This question is important in establishing the major routes of transmission in swine trichinosis in the United States and, therefore, we carried out the experiments described below. Frequently related anecdotes from hog farmers concerning the propensity of waste- and grain-fed hogs to eat dead rats provided an impetus for this work. Our experimental results unequivocably demonstrate that farm-raised hogs can acquire trichinosis through the ingestion of partial or whole infected rat carcasses.

Materials and Methods

Maintenance of *T. spiralis*, infection procedures, and recovery of parasites

Muscle larvae (L_1) were obtained from stock infections in TAC:SD rats (Taconic Farms, Germantown, New York) by digestion of muscle in 1% pepsin–1% HCl for 4 hr at 37°C. They were washed by sedimentation in warm water, counted and suspended to the desired concentration in water for oral inoculation into TAC:SD rats (150–200 g). The number of larvae/rat varied according to experimental design (see below).

Five to 6 weeks after infection, the rats were killed with CO_2, and placed in an isolation pen with the experimental hogs. The amount of rat carcass eaten was recorded at varying time intervals (see Results). Infected littermates of the rats fed to the hogs were sacrificed, skinned, eviscerated and divided into: naso-frontal portion of head (NF); tail, i.e., posterior two-thirds of tail; and body, the remaining portion of the carcass. These sections were digested separately and the number of L_1 present determined.

The experimental hogs were SPF animals farrowed and reared in confinement at the Animal Parasitology Institute. These hogs were routinely fed a grain ration once/day in the morning. Generally, the hogs were offered infected rat carcasses either in lieu of their grain ration (Exps. 1, 4, and 5), with their grain ration (Exp. 2) or after having their feed withheld for 24 hr (Exp. 3), which meant that they had received their previous ration 48 hr prior to exposure to the rat carcasses.

The hogs were sacrificed 5–6 wk after exposure to the rats, and the tongue and diaphragm were ground together; at least 100 g of the ground meat mix were digested and the number of larvae/g (LPG) was determined.

Results

The results of the rat-feeding experiments are summarized in Table 1. The particulars of each experiment are as follows:

EXPERIMENT 1: By the end of the first hour, the hogs had eaten the tails of both heavily infected rats. It was observed that both hogs masticated the carcasses, rendering the fur wet with saliva although the action did not resemble actual chewing; this suggested some distaste for the rat's

Table 1. Results of experimental attempts to transmit muscle larvae of *Trichinella spiralis* to hogs with infected rat carcasses.

Exp. no.	Pig no.	Wt. (lb)	Starved*	Portion of rat eaten	Time (hr) required†	Calculated number of muscle larvae ingested	Necropsy results LPG/hog
1	1115	220 (110 kg)	No	NF‡ and tail	3	18,000	776
	1116	240 (109 kg)	No	NF and tail	3	18,000	200
2	1094	250 (114 kg)	No (w/grain)	NF and tail	4	2,756	0.01
	1113	240 (109 kg)	No (w/grain)	NF and tail	4	2,756	0.03
3	P1	150	Yes	Complete carcass	6	11,733	388
	P2	150 (68 kg)	Yes	Complete carcass	6	11,733	110
4	P313	150 (68 kg)	No	Complete carcass	23	19,938	544
	P1153	75 (34 kg)	No	Complete carcass	23	19,938	236
5	XB-5	220 (100 kg)	No	NF and tail	0.5	1,320	8

* Previous feeding of grain ration was 48 hr before exposure to rat carcasses.
† Time required to consume amount of carcass indicated.
‡ NF = naso-frontal portion of head, including tongue and facial muscles.

fur. By the end of the third hour, the anterior or naso-frontal (NF) portions of the skulls were missing. Although grain was withheld from the hogs for a further 24 hr, no further consumption of the carcasses was observed. At necropsy, both hogs had substantial numbers of muscle larvae.

EXPERIMENT 2: In this trial, two lightly infected rats were offered to the hogs as a supplement to their normal grain ration. One hour later, the hogs were observed chewing the heads of the rats. After 4 hr, NF and tail portions were missing and the hogs were observed chewing on the body; this was accompanied by frequent dropping of the carcass. The rat carcass remains were removed after 24 hr by which time only the tails and NF had been consumed. At necropsy both hogs exhibited low LPG values.

EXPERIMENT 3: In this experiment, two lightly infected rats were offered; analysis of littermates indicated the LPG of the rats, based on whole carcass estimation, was about 103. Prior to offering the carcasses, feed was withheld from the test hogs for 24 hr (48 hr since last feeding). Two hours after the rat carcasses were presented to the hogs, they were thoroughly chewed and by 4 hr they were completely consumed. The hogs had high levels of LPG at necropsy.

EXPERIMENT 4: In this trial, in which two infected rats were offered in place of the regular grain ration, the experimental hogs ate the tails and NF portions of the carcasses within 5 hr, and had consumed the entire carcasses by 23 hr. The whole carcass L_1 levels of rat littermates were estimated to be 101 LPG (19,938 L_1 total). At necropsy both hogs had high LPG values.

EXPERIMENT 5: A single 220-lb grain-fed hog was offered a freshly killed rat approximately 1 hr before its normal feeding time. The rat had been inoculated 5 wk earlier with only 50 *T. spiralis* larvae; analysis of four similarly inoculated littermates indicated that the NF portion of the head weighed approximately 10 g and contained about 1,320 muscle larvae. After 30 min exposure to the hog, the rat was withdrawn and examined and the amount of carcass eaten recorded; by this time only the NF portion and about ¾ of the tail was eaten. The tails of the 4 infected littermates did not contain larvae. At necropsy, the hog's LPG for tongue and diaphragm mix was determined to be eight.

Discussion

Our results show unequivocably that confinement-reared, grain-fed hogs, without prior ex-

posure to rats, readily eat rat carcasses. In every instance, transmission of trichinae to the hogs occurred, even with lightly infected rats (Exp. 5). Prior starvation of the hogs was not necessary for the consumption of all or parts of the rat carcass (Exps. 1, 2, 4, and 5). A consistent observation was the tendency of the hogs to eat the naso-frontal (NF) and tail portions of the rats first, usually within a brief time after exposure to the carcass. This is significant because the facial muscles and tongue are among the richest muscles in terms of larval abundance. In lightly infected rats (inoculated with 50 larvae) the tails were devoid of encysted larvae, demonstrating that this muscle was of no consequence in transmission.

The claim that hogs have a strong aversion to rats (Cameron, 1970) is not supported by these results, nor by the observations of farmers and field investigators. Although the role of rodents in the transmission of sylvatic trichinosis is still subject to debate (Schad and Chowdhury, 1967), we conclude from our findings that rats cannot be discounted as an important source of infection for domestic swine, nor is the eating of rats by pigs predicated on diets deficient in protein as suggested by Hall (1938) and Cordero Del Campillo, et al. (1970) because the ration fed in these experiments contained 16% crude protein and an extensive mix of vitamins and minerals. Clarification of the major routes of transmission of *T. spiralis* to swine is of vital importance and the pursuit of this goal is hindered by the perpetuation of unsubstantiated claims regarding possible sources of swine infection. This is true also with regard to garbage feeding; the evidence bearing on its importance in swine trichinosis is essentially circumstantial and deserves careful study.

The sources of *T. spiralis* infection for rats trapped on hog farms also requires clarification. Although the prevalence of infection varies widely in rats associated with farms known to have infected hogs, its relation to the prevalence in swine is uncertain (Moynihan and Musfeldt, 1949a; Rothrock, 1965; Martin et al., 1968; Smith, 1980; Ramisz and Balicka-Laurans, 1981) and it could be argued that infected rats are of secondary importance rather than a direct source of infection to hogs. Hopefully, this problem will eventually be resolved by intensive investigation and a useful rationale for the control of swine trichinosis may result.

Acknowledgments

We wish to thank Mr. Lawrence Henson and Ms. Eleanor Moore for their important aid in carrying out these experiments.

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The Systematic Position of the Inglisonematinae Mawson, 1968 (Nematoda)

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ABSTRACT: New information concerning the female reproductive system, cephalic end, and esophagus supports the classification of the Inglisonematinae Mawson, 1968, in the Heterakoidea, rather than the Seuratoidea as originally proposed. Many primitive heterakoids (Heterakidae) possess a prominent vagina that is markedly elongated posteriorly and divided into distinct muscular and sac-like uterine portions. This arrangement does not occur in other Ascaridida, but it is observed in paratypes of *Inglisonema mawsonae* Schmidt and Kuntz, 1971 (Inglisonematinae). The esophagus and cephalic extremity of Inglisonematinae are morphologically similar to early fourth-stage larvae of Heterakidae (esophagus club-shaped and lacking valves, cephalic lips inconspicuous). In contrast in late fourth-stage and adult Heterakidae esophageal valves and three distinct cephalic lips are present. It is hypothesized that the Inglisonematinae evolved by paedomorphosis from heterakoids, with *Heterakis* (Heterakinae) as a possible ancestral group. It is proposed that Inglisonematinae be classified as a family in the Superfamily Heterakoidea.

The Subfamily Inglisonematinae Mawson, 1968 includes only three species in two genera: *Madelinema angelae* Schmidt and Kuntz, 1971; *Inglisonema typos* Mawson, 1968; *I. mawsonae* Schmidt and Kuntz, 1971. These species form a clearly homogeneous group restricted to birds of the Far East (Taiwan, Philippines) and Australia. The classification of the group to superfamily has been controversial because the caudal structures of males are typical of the Heterakoidea (Schmidt and Kuntz, 1971), whereas the cephalic and esophageal morphology are typical of the Seuratoidea (Chabaud, 1978).

Chabaud (1978) classified the Inglisonematinae in the Family Schneidernematidae (Seuratoidea) indicating that they represent a somewhat intermediate stage in the evolution of the Heterakoidea from Cosmocercoidea ancestors. However, other studies (i.e., Baker, 1981a) suggest that Heterakoidea most likely evolved directly from the Cosmocercoidea without seuratoid-like ancestors. In particular the presence of esophageal valves in the more primitive heterakoids (i.e., Heterakidae) and cosmocercoids, contrasted with their absence in seuratoids, is strong evidence that these three groups are not related in the evolutionary sequence Cosmocercoidea-Seuratoidea-Heterakoidea. It is unlikely that forms bearing esophageal valves (Cosmocercoidea) gave rise to forms lacking these structures (Seuratoidea) which in turn gave rise to forms bearing valves (Heterakoidea).

Schmidt and Kuntz (1971) pointed out that the male caudal ends of Inglisonematinae are markedly close to the Family Heterakidae. They also considered these groups closely related since in the Inglisonematinae the "reduced lips, lack of interlabia and oesophageal teeth are reminiscent of the Meteterakinae Inglis, 1958." This was considered sufficient grounds to place Inglisonematinae in the Heterakoidea with family rank and distinguished from Heterakidae by esophageal characters, and from Ascaridiidae by cephalic and male caudal characters. Although this correlation of cephalic structures between Inglisonematinae and Meteterakinae is not accepted (see below), the placement of the Inglisonematinae in the Heterakoidea is supported by new information concerning cephalic, esophageal, and female reproductive structures.

Material Examined

Paratype females of *Inglisonema mawsonae* (USNM Helm. Coll. No. 71750) were borrowed from the National Parasite Collection, U.S. Department of Agriculture, Beltsville, Maryland 20705.

Results

Female reproductive system

Female reproductive structures have not been considered of systematic value for the Heterakoidea and related groups. However, many Heterakoidea, including some of the most primitive representatives (Heterakidae), have evolved a morphologically unusual vagina that is unique for the Order Ascaridida and therefore may be a character indicating heterakoid affinities.

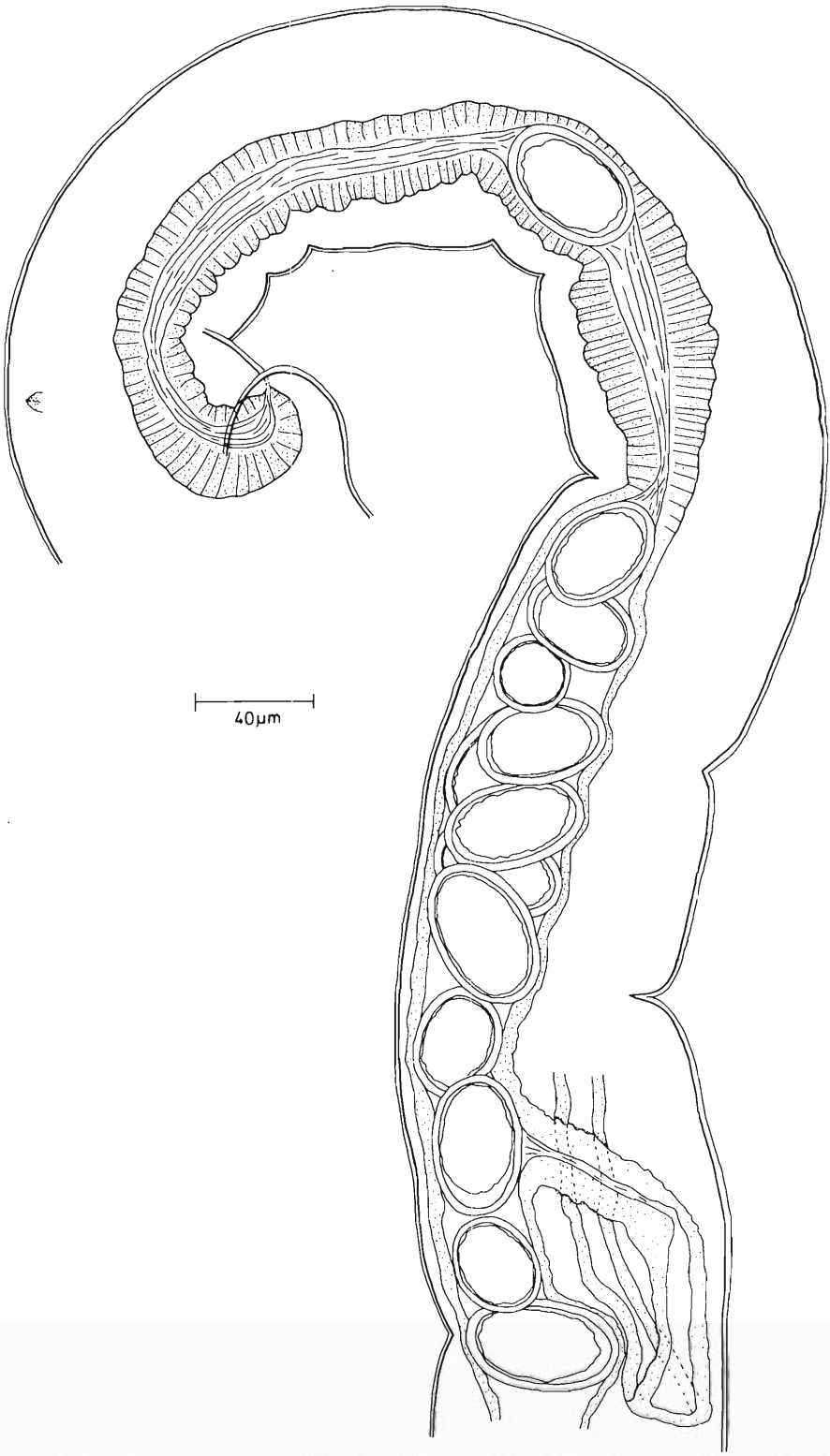

Figure 1. ***Inglisonema mawsonae*** **Schmidt and Kuntz, 1971. Vagina of paratype female, lateral view.**

Primitively, a short, muscular vagina gives rise to two opposed uteri near midbody. This is characteristic of most Cosmocercoidea and several species of *Heterakis* (Heterakinae). During evolution of heterakoids there has been a tendency toward the marked elongation of the vagina, which becomes directed posteriorly and divided into a muscular anterior portion and thin-walled sac-like posterior portion. This uterine portion may or may not be divided into two thin tubes. This arrangement is observed in several different species of Heterakidae, i.e., *Heterakis bosia* Lane, 1914, *Odonterakis fariae* (Travassos, 1913) (Heterakinae), *Africana chabaudi* Baker, 1981 (Spinicaudinae), and *Meteterakis* spp. (Meteterakinae) (see Lane, 1914; Travassos, 1945; Inglis, 1958; Baker, 1981b).

The female reproductive structures of Inglisonematinae have not previously been described. The vagina in a 2.8-mm-long gravid paratype female of *I. mawsonae* is 600 μm in length (Fig. 1). It is posteriorly directed from the vulva (located 1,100 μm from anterior extremity), muscular in the anterior 370 μm, and thin-walled in the posterior 230 μm. The uteri are opposed at their origin, with one ovary located near the anus, the other just posterior to the esophagus. This therefore corresponds closely to the elongated vagina of evolved heterakoids.

Cephalic and esophageal structures

The cephalic end and esophagus of the Inglisonematinae have been well described (Mawson, 1968; Schmidt and Kuntz, 1971). The esophagus is club-shaped, muscular, and lacking valves. The mouth is triangular and three inconspicuous lips lacking cuticular flanges are present. In contrast the esophagus of all Heterakidae bear large valves and the mouth is bordered by three distinct lips each of which bears a cuticular flange. These differences are of considerable systematic importance in the modern classification of the Order Ascaridida (Chabaud, 1978). However, when the cephalic and esophageal morphology of the few described fourth larval stage Heterakidae are compared to adult Inglisonematinae, a clear resemblance emerges. Early fourth-stage *Strongyluris brevicaudata* Mueller, 1849 (Spinicaudinae) and *Heterakis dispar* (Schrank, 1790) (=*Heterakis papillosa* (Bloch, 1782)) (Heterakinae) have club-shaped esophagi lacking valves and cephalic ends closely resembling adult Inglisonematinae (Uribe, 1922; Bain, 1970). The esophageal valves that are observed in adult *S. brevicaudata* and *H. dispar* develop only in the late fourth larval stage after considerable growth in body size. From these observations it is hypothesized that the Inglisonematinae evolved from the Heterakidae as a group that retained early fourth larval stage morphology of the anterior end into the adult sexually mature stage (paedomorphosis). The following observations support this hypothesis.

(1) Bain (1970) has noted that during the fourth larval stage of *S. brevicaudata* the body grows four times in size and there is an early development of the genital organs. This precocity of development is apparently true for the superfamily as a whole (Bain, 1970), indicating a possible predisposition to paedomorphosis.

(2) Adult Inglisonematinae are relatively small. For example, *Inglisonema typos,* the largest known species, reaches a maximum size of 3.7 mm for males and 4.4 mm for females (Mawson, 1968). This may be compared to Heterakidae, which are generally well over 5.0 mm in length. Thus, adult Inglisonematinae are comparable in size to fourth-stage Heterakidae.

If the Inglisonematinae indeed evolved by paedomorphosis, then it may be expected that in characters not affected by the paedomorphic development (i.e., sexual structures) a close resemblance may be found with the "parental" group. Thus, it is instructive to note the close resemblance between Inglisonematinae and the genus *Heterakis* (Heterakinae). In particular, the full array of male caudal characters (caudal alae, size and arrangement of caudal papillae, location of caudal sucker bearing a single papilla in its posterior rim, morphology of the spicules) is remarkably similar. *Heterakis* is a cosmopolitan genus that is also parasitic mainly in birds.

It is quite possible that the Inglisonematinae are a relatively recently evolved group since they occur in a restricted geographical range. The unique dispersive ability of birds has tended to produce a wide distribution for most bird parasites.

The classification of Inglisonematinae in the Heterakoidea is therefore supported. Although they are probably phylogenetically close to the Heterakinae, it is proposed that they hold the rank of family as suggested by Schmidt and Kuntz (1971). This is convenient from a diagnostic point of view as the Inglisonematidae can be distinguished easily from all other Heterakoidea except Ascaridiidae by their lack of esophageal valves. It is distinguished from the Ascaridiidae by the

cephalic morphology (lips inconspicuous as opposed to the presence of three massive lips).

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Chemotaxis of Male *Nippostrongylus brasiliensis* (Nematoda) to Some Biological Compounds

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ABSTRACT: Males of *Nippostrongylus brasiliensis* were significantly attracted to cyclic 3′,5′ adenosine monophosphate by in vitro assay. Other cyclic nucleotides caused no response or repelled the males. No detectable c-AMP was found in female incubates by ion exchange chromatography. Helminths were attracted also to several biogenic amines, which included p-tyrosine, dopamine, 5-hydroxytryptamine, L-DOPA, octopamine and histamine. Other compounds elicited no response. Extraction and chromatography of female incubate revealed no identifiable levels of catecholamines or phenolic amines, according to authentic compounds. Bioassay of male responses to aliphatic amines showed no significant attraction and indicated repellency. Chromatography of female incubate revealed a compound that was similar to amylamine, but other identifiable compounds were not present, based on standards. Tested sugars and amino acids failed to elicit any significant male responses. However, dilutions of intestinal chyme from the host were attractive to male nematodes whereas certain concentrations of host serum were repellent.

Helminths respond to a variety of chemosensory stimuli. Ward (1973) showed that the nematode *Caenorhabditis elegans* was attracted to cyclic AMP and GMP. Maximal responses were obtained at a 4×10^{-3} M concentration during in vitro assay, but other nucleotides had no effect. Larvae of *Neoaplectana carpocapsae* aggregate also in c-AMP gradients (Pye and Burman, 1981).

The free-living nematode *Panagrellus redivivus* contains c-AMP and c-GMP (Willett, 1980). Cyclic AMP levels increase with age whereas c-GMP concentrations are greater than c-AMP. However, the regulatory functions of these messengers remain unstudied.

The occurrence of biological amines in nematodes has been reviewed by Willett (1980), but studies of behavioral responses by nematodes to biological amines are scant. Kelley et al. (1974) found that intraduodenal injection of histamine had no effect on the expulsion of *Nippostrongylus brasiliensis* from the rat, whereas Croll (1975) stated that serotonin, 5-hydroxytryptophan and epinephrine caused muscle contractions in vitro. Mettrick and Cho (1981) have demonstrated the dosage-dependent movement of the tapeworm *Hymenolepis diminuta* to serotonin gradients in the intestine.

Cyclic nucleotides and biological amines are ubiquitous as intra- and inter-cellular messengers whereas aliphatic amines may modify facets of the helminth's microenvironment (Castro et al., 1973). Other substances, such as sugars, amino acids, or host compounds, may represent important nutritive signals for helminth feeding. Accordingly, the orientation of male *Nippostrongylus brasiliensis* to these compounds was evaluated to broaden our understanding of the chemosensory behavior of this nematode.

Materials and Methods

Nippostrongylus brasiliensis was maintained in white mice and larval culture as described previously (Bone et al., 1977). Seven-day-old adult males were obtained for bioassay of their orientation response according to procedures for pheromone responsiveness (Bone et al., 1978). A dosage of 20 μl was used in each bioassay chamber.

Cyclic nucleotides were tested over a concentration range of 1×10^{-5} to 1×10^{-2} M in Tyrode's solution. Cyclic nucleotides evaluated were adenosine 2′,3′ cyclic monophosphoric acid, adenosine 3′,5′ cyclic monophosphoric acid, cytidine 3′,5′ cyclic monophosphate, guanosine 3′,5′ cyclic monophosphoric acid, inosine 3′,5′ cyclic monophosphoric acid, thymidine 3′,5′ cyclic monophosphoric acid, and uridine 3′,5′ cyclic monophosphate (Sigma).

Additionally, tissue levels of c-AMP and c-GMP were determined in female *N. brasiliensis*. Nucleotides were extracted according to Willett and Rahim (1978). Ion exchange chromatography was conducted with Dowex-50 (Sigma) for comparison to standards by detection at 210 nm (Schultz et al., 1974). Separations were performed also with worm incubate.

Biological amines were tested also and included L-β-3,4-dihydroxyphenylalanine (L-DOPA), L-epinephrine, histamine (diphosphate), DL-β-hydroxyphenylethanolamine, 5-hydroxytryptamine (creatinine sulfate complex), 3-hydroxytyramine (HCl), L-norepinephrine, DL-octopamine (HCl), L-phenylalanine, β-phenylethylamine, tyramine (HCl), and L-tyrosine

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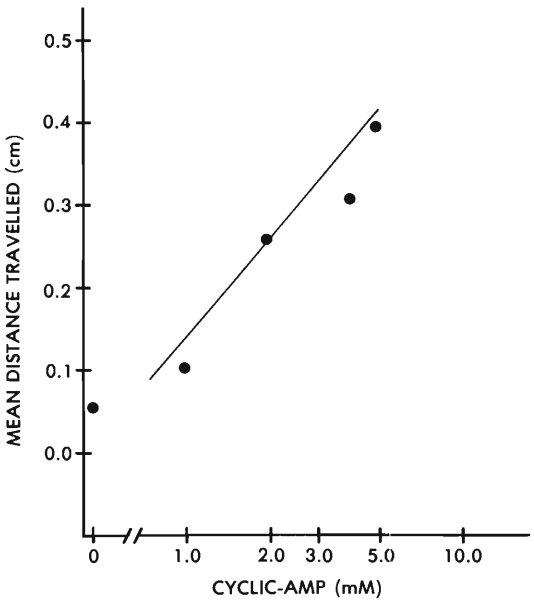

Figure 1. Responses of male *N. brasiliensis* to dosages of c-AMP ($r = 0.94$; MSE = 0.19) by in vitro assay.

(HCl) (Sigma). The above procedures were used for bioassay of these compounds over a range of 10^{-6} to 10^{-2} M. Some compounds were prepared as acidified solutions to reduce oxidation. Any release of these substances by female *N. brasiliensis* into an incubate solution was evaluated by extraction and separation procedures for phenolic amines (Kakimoto and Armstrong, 1962) and catecholamines (Hallman et al., 1978).

Additionally, nematode responses to aliphatic amines were examined. These included 2-aminoethanol, n-amylamine, cadaverine (dihydrochloride), ethylamine, ethylene diamine (dihydrochloride), methylamine (hydrochloride), propylamine, n-heptylamine, and n-hexylamine (Sigma). Isopropylamine, n-butylamine, and sec-butylamine (Aldrich) were assayed also. A range of doses from 10^{-6}–10^{-2} M were used. Any release of these compounds by female helminths was determined by thin-layer chromatography of incubate according to Castro et al. (1973).

Likewise, orientation responses to amino acids were tested. The above procedures were used over a dosage range of 10^{-6}–10^{-3}. Amino acids examined included alanine, aspartic acid, glycine, glutamic acid, leucine, lysine, methionine, proline, serine, threonine, and valine (Merck). L- or DL-isomers were used.

Additionally, several sugars were evaluated as described above. These included L-arabinose, D-fructose, D-galactose, D-glucose, lactose, D-mannitose, L-sorbose, sucrose, and D-xylose (Merck).

Study of the male's response to host chyme was done also. Intestinal contents from uninfected animals were obtained by gentle scraping of the anterior 50% of the pyloric–caecal length. Then, 1 mg of chyme was suspended in a 1-ml volume of Tyrode's solution. After dilution, a range of chyme concentrations from 10–100% were assayed according to previous procedures.

Male's responses to host serum were determined similarly. Blood was obtained from animals by cardiac puncture. After centrifugation, dosages of serum from 0.1–100% were tested after dilution with Tyrode's solution. Previous procedures were followed.

Data were evaluated by linear regression or analysis of variance. The 0.05 probability level was considered significant. Data are presented as a single point, based on the regression mean, for comparison of the multiple lines from some studies. Twenty replicates were done for each dose and at least five dosages were examined for each substance.

Table 1. Response of male *N. brasiliensis* to biological amines.

Compound	Mean distance traveled (cm) (±MSE)*	r
p-Tyrosine	+1.15 (0.20)	0.90
Dopamine	+0.67 (0.18)	0.98
5-Hydroxytryptamine	+0.5 (0.14)	0.85
Histamine	+0.34 (0.054)	0.80
L-DOPA	+0.33 (0.19)	0.94
Octopamine	+0.17 (1.16)	0.91

* As predicted by regression analysis for a 1×10^{-5} M dosage.

Results

Cyclic 3′,5′AMP was the only nucleotide that attracted male *N. brasiliensis* by in vitro bioassay (Fig. 1). A significant, dosage-dependent response was found from $1–5 \times 10^{-3}$ M ($r = 0.94$). No response was elicited by 2′,3′ c-AMP at similar concentrations. Thus, *N. brasiliensis* may respond to some structural feature of c-AMP.

Other nucleotides yielded variable results. Cyclic UMP, IMP, and CMP showed no obvious attractant or repellent activity during assay. Dosages of c-GMP at 1×10^{-5} to 5×10^{-3} M and c-TMP at 3×10^{-6} to 1×10^{-2} M caused repulsion of the nematodes, although no dosage-dependency was evident in these negative responses.

Ion exchange chromatography of worm incubate revealed no discernible c-AMP or c-GMP, relative to the standards. Tissue extraction yielded a c-AMP level of 95.3 pmole/female, based on the 210-nm absorbance of authentic c-AMP. However, no material that co-eluted with c-GMP was detected in either incubates or extracts of female *N. brasiliensis*.

The response of male *N. brasiliensis* to the biological amines is given in Table 1 for selected

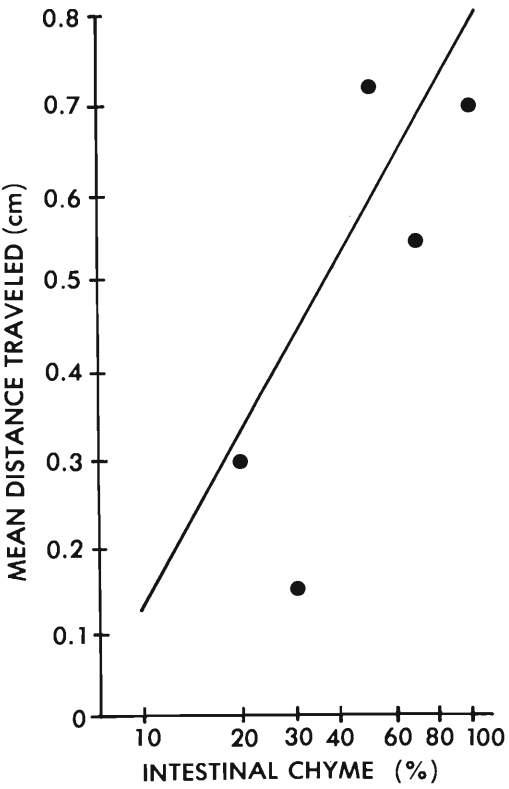

Figure 2. Responses of male *N. brasiliensis* to dilutions of host chyme (1 mg/ml) ($r = 0.81$; MSE = 0.11) by in vitro assay.

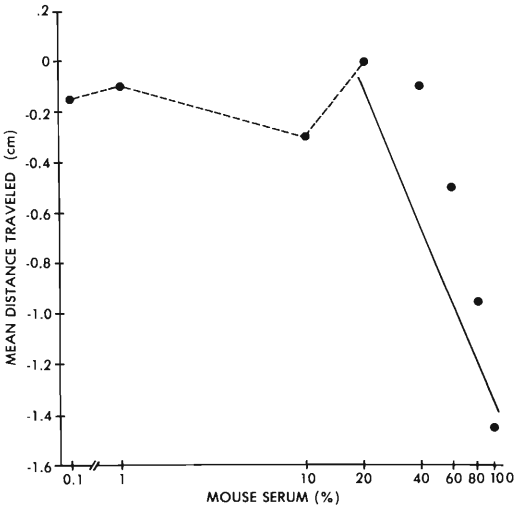

Figure 3. Responses of male *N. brasiliensis* to dilutions of host serum ($r = 0.98$; MSE = 0.24) by in vitro assay.

compounds that elicited a significant response. Five of the 12 compounds caused a dosage-dependent response. The most significant response was elicited by p-tyrosine. Octopamine elicited only slight attractancy at 1×10^{-5} M, but caused a +1.28-cm response at 1×10^{-2} M. Other compounds were intermediate in their effect on locomotion by *N. brasiliensis*.

Extraction and chromatography of female incubate revealed only minor amounts of three compounds that were isolated as catecholamine-like material. However, these components did not coincide with any authentic compound.

Isolation of phenolic amines from female incubate revealed seven components in the first chromatographic dimension. Separation in the second dimension resolved an eighth compound. However, none of the worm-derived materials coincided with the tested standards.

None of the aliphatic amines that were tested caused a significant attraction. However, amylamine, cadaverine, and heptylamine significantly repelled the nematodes whereas others caused no significant positive or negative movement according to dose-response analyses.

Thin-layer chromatography of a 22,000 female-hour incubate revealed the presence of four presumptive aliphatic amines. A spot at R_f 0.75 was comparable to amylamine as a standard, but the remaining compounds did not correlate with female-derived materials.

Examination of the male's response to various amino acids and sugars revealed no apparent effect. Their movement to a 1,000-fold range of concentrations showed no dosage-dependency. Thus, these compounds may have only a minor chemosensory significance to male *N. brasiliensis* at the tested levels.

Males responded also to compounds of host origin. Assay of various dilutions of host chyme caused attraction in a dose-dependent manner ($r = 0.81$). A maximal response of +0.8 cm was elicited by the 100% concentration of chyme (1 mg/ml), according to regression analysis (Fig. 2).

In contrast, mouse serum repelled male *N. brasiliensis* at the higher doses ($r = 0.98$). Little responsiveness was seen at or below a 20-μl dosage of 20% serum. However, greater concentrations caused male movement away from the chemical source (Fig. 3).

Discussion

Chemotactic responses of adult zooparasitic nematodes are largely unexplored, although these

stimuli may have paramount importance to the organism. Croll (1976) investigated the role of host feeding as a cause of longitudinal orientation of *N. brasiliensis* in the intestine. However, defined chemicals were not determined. Adult *N. brasiliensis* also were attracted to host serum (Cunningham, 1956). Our results support the attraction of at least males to host food, but conflict with the role of host serum as a chemoattractant. Bioassay differences may account partially for this discrepancy.

In contrast, Dusenbery (1980) has reviewed a number of chemical stimuli that elicit responses in free-living *C. elegans*. Although these defined compounds may cause attractancy and repellancy during bioassay, the biological rationale remains unknown for the chemical stimuli, although bacterial feeding has been suggested.

Similarly, the role of the known biological compounds that were tested in this study is not clear. Although c-AMP acts as an aggregation pheromone in slime molds (Konijn et al., 1967), release of this chemical at a threshold level by female *N. brasiliensis* appears unlikely, based on chromatographic procedures and bioassay data. Our study of this facet was stimulated initially by the co-elution of c-AMP with the Kav 1.0 pheromone of female *N. brasiliensis* during gel filtration (Bone et al., 1979). Thus, speculation on the chemical composition of helminth natural products, based solely on chromatography, appears tenuous without spectral analyses.

The responses of *N. brasiliensis* to biological amines revealed no discernible pattern, according to functional chemical groups. Six of the examined compounds are considered as putative neurotransmitters in various invertebrate groups. Interestingly, worms exhibited little response to these, except for the moderate activity of octopamine and dopamine. This tendency suggests that any biological role may represent site-selection in the host as in *H. diminuta* rather than interhelminth communication. The general repellency of the aliphatic amines seemingly indicates a role in population dispersal if a biological function is truly present. Castro et al. (1973) suggested these compounds may modify the worm's microenvironment. However, resolution of the behavioral significance of any known or host chemical for nematodes awaits additional research.

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).

Helminths and Protozoans

U.S. National Parasite Collection
Dr. J. Ralph Lichtenfels, Curator
USDA, ARS, BARC-East No. 1180
Beltsville, Maryland 20705
(Phone: 301-344-2444)

Protozoans

International Protozoan Type Slide Collection
Dr. Klaus Ruetzler, Curator
Department of Invertebrate Zoology
National Museum of Natural History, W-325
Smithsonian Institution
Washington, D.C. 20560
(Phone: 202-357-2486)

Mites

USDA and USNM Acari Collections
Dr. E. W. Baker, Curator
USDA, ARS, BARC-West No. 004
Beltsville, Maryland 20705
(Phone: 301-344-3890)

Ticks

Dr. James E. Keirans, Curator
Museum Support Center
Smithsonian Institution
Washington, D.C. 20560
(Phone: 301-423-1085)

Leeches

Hirudinea Collection
Department of Invertebrate Zoology
National Museum of Natural History
Smithsonian Institution
Washington, D.C. 20560
(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
Lincoln, NE 68588
(Phone: 402-472-3334)

Proc. Helminthol. Soc. Wash.
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Camallanid and other Nematode Parasites of Lake Fishes in Southeastern Wisconsin

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ABSTRACT: Nine species in eight families of nematodes are reported from 18 species of fish collected from two inland lakes in southeastern Wisconsin between 1976 and 1982. New host records include *Camallanus oxycephalus* Ward and Magath, 1916 from *Esox americanus, Capillaria catenata* Van Cleave and Mueller, 1932 from *Umbra limi, Dorylaimus* sp. Dujardin, 1845 from *Culaea inconstans,* and *Spiroxys contorta* (Rud., 1819) from *Lepomis gibbosus, Micropterus salmoides,* and *Fundulus notti.* All records except that of *Dorylaimus* sp. are new to southeast Wisconsin. The taxonomic problem posed by the discovery of female *C. oxycephalus* exceeding the maximum length of 25.0 mm (redescription by Stromberg et al., 1973) and reaching 51.0 mm was resolved by using the tail length and distance between vulva and posterior end as percentages of body length. In that species, seasonal reproduction and recruitment occur in the summer with minimal growth and maturation until spring. Females live longer than one year. Maturation occurs in only a few among the larger number of reservoir hosts. Infections are initially established in anterior intestinal locations during the summer but are later confined to the rectum. Males are fewer than females but the ratio undergoes seasonal changes, and the prevalence of infection shows a definite relationship to host size but not sex.

This is the first report of nematode parasites of lake fishes in southeastern Wisconsin. Some records are new, others were previously reported by Pearse (1924a, b), Bangham (1946), or Fischthal (1953) from elsewhere in Wisconsin. As in recent reports on leech, crustacean, trematode, etc. parasites of fishes from the same lakes by the author, morphological–taxonomic observations and ecological associations of the more common species of nematodes recovered are discussed.

Materials and Methods

The fishes examined were from Silver Lake, a 200-ha eutrophic land-locked lake in Kenosha County, and from Tichigan Lake (Racine County), a 108-ha lake in an advanced state of eutrophication on the Fox River (a tributary of the Mississippi River). Seasonal collections were made from both lakes during the spring (April), summer (June, July, and early August), and autumn (late October and November) between 1977 and 1982, and from Silver Lake during the summer of 1976. A total of 1,812 fishes representing 32 species and 10 families were captured by electroshocking from both lakes. An additional 1,543 fishes representing 27 species and 11 families were seined or minnow trapped in a channel draining the swampy western area of Tichigan Lake during 1978, 1979, and 1981.

Fish were systematically dissected shortly after capture. Recovered specimens were refrigerated in distilled water overnight, fixed in cold AFA, stained in Mayer's acid carmine, cleared in terpineol, and mounted in Canada balsam. Representative specimens were deposited in the U.S. National Museum Helminthological Collection (USNM Helm. Coll.) and in the Milwaukee Public Museum Collection (MPM Coll.).

Results and Discussion

Nine species of nematodes representing eight families were recovered from 18 species of fish from Wisconsin as discussed below. Fish species that were negative for any nematode infections from Silver Lake (S), Tichigan Lake (T), or Tichigan Canal (TC) and numbers examined are Amiidae: *Amia calva* Linn. (14,S; 37,TC); Catostomidae: *Carpiodes carpio* (Raf.) (3,T), *C. cyprinus* (LeSueur) (19,T), *Catostomus commersoni* (Lacépède) (10,S; 54,T; 11,TC), *Erimyzon sucetta* (Lacépède) (94,S; 1,TC), *Moxostoma anisurum* (Raf.) (4,T), *M. carinatum* (Cope) (3,T), *M. erythrurum* (Raf.) (8,T; 10,TC); Centrarchidae: *Ambloplites rupestris* (Raf.) (2,T), *Chaenobryttus gulosus* (Cuvier) (1,S), *Lepomis cyanellus* Raf. (18,T; 22,TC), *L. gibbosus* (Linn.) (14,TC), *L. macrochirus* Raf. (121,TC), *Micropterus salmoides* (Lacépède) (3,TC), *Pomoxis annularis* (Raf.) (3,TC), *P. nigromaculatus* (LeSueur) (10,TC); Cyprinidae: *Cyprinus carpio* (Linn.) (52,S; 66,T), *Notropis cornutus* (Mitchill) (107,TC), *N. umbratilis* (Girard) (16,S; 17,T), *Pimephalus* sp. (765,TC); Cyprinodontidae: *Fundulus notatus* (Raf.) (19,TC); Esocidae: *Esox americanus* LeSueur (2,TC), *E. lucius* Linn. (2,TC); Ictaluridae: *Ictalurus melas* (Raf.) (1,S; 8,T), *I. natalis* (LeSueur) (4,S; 5,TC), *I. nebulosus* (LeSueur) (3,S; 10,T; 3,TC), *I. punctatus* (Raf.) (35,T; 2,TC), *Schilbeodes gyrinus* (Jordan and Gilbert) (2,TC); Lepisosteidae: *Lepisosteus osseus* (Linn.) (14,S; 9,T); Percidae: *Perca fla-*

Table 1. Distribution of juvenile *Camallanus oxycephalus* in fishes from Tichigan Lake, 1977–1981.

Fish species	Summer	Autumn	Spring	Total
Amiidae				
Amia calva	0,0/5(0,0)*	0,0/5(0.0)	2,1/13(0.15,8)	2,1/23(0.09,4)
Centrarchidae				
Lepomis gibbosus	0,0/32(0,0)	0,0/13(0,0)	1,1/15(0.07,7)	1,1/60(0.02,2)
L. macrochirus	0,0/74(0,0)	4,4/87(0.05,5)	0,0/51(0,0)	4,4/212(0.02,2)
Micropterus salmoides	0,0/19(0,0)	182,13/23(7.91,56)	9,1/2(4.50,50)	191,14/44(4.34,32)
M. dolomieui	0,0/10(0,0)	0,0/2(0,0)	1,1/6(0.17,17)	1,1/18(0.06,6)
Pomoxis annularis	13,3/5(2.60,60)	0,0/3(0,0)	6,1/7(0.86,14)	19,4/15(1.27,27)
P. nigramaculatus	19,2/33(0.58,6)	77,29/59(1.30,49)	42,14/70(0.60,20)	138,45/162(0.85,28)
Esocidae				
Esox lucius	0,0/7(0,0)	1,1/7(0.14,14)	3,2/8(0.37,25)	4,3/22(0.18,14)
Ictaluridae				
Ictalurus natalis	1,1/1(1.0,100)	0,0/0(0,0)	1,1/7(0.14,14)	2,2/8(0.25,25)
Percidae				
Perca flavescens	0,0/3(0,0)	0,0/17(0,0)	1,1/57(0.02,2)	1,1/77(0.01,1)
Stizostedion vitreum	0,0/20(0,0)	2,1/28(0.07,4)	198,3/4(49.50,75)	200,4/52(3.85,8)
Serranidae				
Roccus chrysops	0,0/2(0,0)	1,1/12(0.08,8)	0,0/7(0,0)	1,1/21(0.05,5)
Total	33,6/211(0.16,3)	267,52/256(1.04,20)	264,26/247(1.07,10)	564,81/714(0.79,11)

* Number of nematodes recovered, number of fish infected/number of fish examined (mean per examined fish, percent fish infected).

vescens (Mitchill) (67,S; 2,TC), *Stizostedion vitreum* (Mitchill) (3,TC); Salmonidae: *Salmo gairdneri* Richardson (1,T), *S. trutta* Linn. (1,T); Serranidae: *Roccus chrysops* (Raf.) (2,TC), and *R. mississippiensis* (Jordan and Eigenmann) (1,T).

Family Camallanidae Railliet and Henry, 1915 *Camallanus oxycephalus* Ward and Magath, 1916

Juvenile *C. oxycephalus* (immature adults) of both sexes were recovered from 12 and nine fish species of six and three families from Tichigan (564 worms) and Silver (250) lakes, respectively (Tables 1, 2). Gravid adults (only females) were rarely encountered; four from three *Lepomis macrochirus* during the autumn from Silver Lake, and eight from three *Pomoxis nigromaculatus* and two from one *L. macrochirus* during the summer and three from one *L. macrochirus* during the autumn from Tichigan Lake. A miscellaneous collection of one large spawning female *P. annularis* measuring 32 cm in total length during June 1982 from Tichigan Lake yielded one juvenile and 39 gravid females (16–27 mm long); all other gravid females (above) were 26–51 mm long. Three larval *C. oxycephalus* were found in the intestinal mesenteries of one *P. nigromaculatus* during the spring from Silver Lake and one *L. gibbosus* during the autumn from Tichigan Lake. The record from *Esox americanus* (Table 2) is a new host record.

The discovery in this study of gravid females exceeding the maximum length of 25.0 mm (redescription by Stromberg et al., 1973) and reaching 51.0 mm poses a taxonomic problem. Also note differences in tail length and the distance between vulva and posterior end (Table 3). These worms were, however, classified as *C. oxycephalus* because of comparable similarities to that species in all other respects. Taxonomically important characteristics were compared among Wisconsin females (3–7, 16–25, >25 mm long females) and those reported by Stromberg et al. (1973) (16–25 mm) (Table 3). Juveniles are described here for the first time. All structures clearly grow in size with maturity. The dimensions of buccal capsule and the length of muscular and glandular esophagus and the distance between the nerve ring and the anterior end do not appreciably change in gravid worms above or below 25.0 mm in length; such raw measurements may thus be retained in the description. Tail length and the distance between the vulva and the pos-

Table 2. Distribution of juvenile *Camallanus oxycephalus* in fishes from Silver Lake, 1976–1981.

Fish species	Summer	Autumn	Spring	Total
Centrarchidae				
Ambloplites rupestris	0,0/8(0,0)*	4,3/13(0.31,23)	3,1/4(0.75,25)	7,4/25(0.28,16)
Lepomis cyanellus	0,0/13(0,0)	0,0/0(0,0)	1,1/3(0.33,33)	1,1/16(0.06,6)
L. macrochirus	0,0/98(0,0)	2,2/141(0.01,1)	6,2/62(0.10,3)	8,4/301(0.03,1)
Micropterus salmoides	0,0/38(0,0)	0,0/6(0,0)	9,7/28(0.32,25)	9,7/72(0.12,10)
Pomoxis annularis	0,0/0(0,0)	0,0/0(0,0)	1,1/1(1.00,100)	1,1/1(1.00,100)
P. nigromaculatus	0,0/4(0,0)	21,5/18(1.17,28)	13,9/25(0.52,36)	34,14/47(0.72,30)
Esocidae				
Esox americanus	0,0/1(0,0)	2,1/2(1.00,50)	0,0/0(0,0)	2,1/3(0.67,33)
E. lucius	0,0/0(0,0)	10,3/15(0.67,20)	8,2/5(1.60,40)	18,5/20(0.90,25)
Percidae				
Stizostedion vitreum	0,0/10	21,5/23(0.91,22)	149,11/21(7.09,52)	170,16/54(3.15,30)
Total	0,0/172(0,0)	60,19/218(0.27,9)	190,34/149(1.27,23)	250,53/539(0.46,10)

* Number of nematodes recovered, number of fish infected/number of fish examined (mean per examined fish, percent fish infected).

terior end, however, greatly increase in females >25.0 mm but their ratio relative to body length (%) remains stable and should be included in the description. The fact that the latter ratios remain consistent even in juveniles supports their reliability and precludes taxonomic difficulties resulting from the discovery of forms of sizes not available to the investigator during the reporting of the original work. Graphic presentation, using five worm length classes, shows that the tail and the distance between the vulva and the posterior end grow at about the same rate as body length; all other structures grow at a lesser rate. This figure is repetitious of data already in Table 3 and is thus not included.

Infections with juvenile *C. oxycephalus* were more common and relatively more heavy in Tichigan Lake fishes, particularly *Micropterus salmoides* and *Stizostedion vitreum* (Table 1), than in Silver Lake where the latter species was more

Table 3. Morphometric comparisons among female *Camallanus oxycephalus* of various size classes.

	Juveniles	Gravid adults		
	3–7 mm long Wisconsin (N = 31)	16–25 mm long Wisconsin (N = 31)	16–25 mm long Redescription (N = 10)*	25 mm long Wisconsin (N = 9)
Body length (BL); mm	3.4–7.0 (5.0)†	16.2–23.1 (21.0)	15.9–25.0 (18.2)	26.0–51.0 (33.5)
Body width (BW); mm	0.12–0.18 (0.14)	0.24–0.44 (0.33)	0.21–0.28 (0.24)	0.36–0.52 (0.46)
BW/BL (%)	1.9–3.7 (2.8)	1.3–2.1 (1.6)	(1.3)	1.0–1.6 (1.3)
Buccal capsule length	98–126 (115)	126–140 (131)	128–142 (137)	126–140 (135)
% of body length	1.7–3.3 (2.4)	0.5–0.8 (0.6)	(0.9)	0.3–0.5 (0.4)
Buccal capsule width	98–119 (108)	112–140 (126)	136–165 (151)	112–168 (132)
Muscular esophagus length	392–490 (438)	476–574 (524)	483–666 (569)	490–616 (558)
% of body length	6.4–13.1 (9.0)	2.2–3.1 (2.5)	(3.1)	1.1–2.0 (1.9)
Glandular esophagus length	420–518 (489)	560–700 (625)	558–748 (652)	658–742 (698)
% of body length	6.5–13.6 (9.8)	2.6–3.9 (3.0)	(3.6)	1.4–2.7 (2.3)
Nerve ring—ant. end	168–224 (207)	210–266 (236)	222–300 (262)	224–280 (238)
% of body length	3.2–5.5 (4.3)	1.0–1.5 (1.1)	(1.4)	0.5–1.0 (0.9)
Tail length; mm	0.35–0.77 (0.54)	2.00–2.68 (2.37)	1.53–2.21 (1.87)	2.28–8.4 (3.2)
% of body length	8.1–13.7 (11.0)	9.8–13.1 (11.3)	(10.2)	8.4–16.5 (11.7)
Vulva—post. end; mm	0.46–1.04 (0.76)	2.80–3.76 (3.27)	2.24–3.11 (2.69)	2.8–9.3 (4.1)
% of body length	11.6–19.0 (15.3)	13.5–18.5 (15.4)	(14.8)	10.4–18.6 (15.0)

* From Stromberg et al. (1973); percentages are calculated from mean values.
† Range (mean); measurements are in micrometers unless otherwise specified.

Table 4. Seasonal distribution of juvenile *Camallanus oxycephalus* of both sexes in the gut of all infected hosts from Tichigan Lake (Table 1) and Silver Lake (Table 2).

	Tichigan Lake				Silver Lake			
Season	Rectum (%)	Other (%)	*N*	Males (%)	Rectum (%)	Other (%)	*N*	Males (%)
Summer	3	97	33	30	—	—	0	—
Autumn	86	14	267	18	78	22	60	18
Spring	95	5	264	22	91	9	190	30

heavily infected. The connection between Tichigan Lake and the Mississippi River might have affected these differences. Rare or lack of infections with juveniles in the summer, their marked and progressive increase in frequency and intensity in the autumn and spring, and the recovery of gravid adults only during the (following) summer and autumn suggests a generation cycle starting with seasonal reproduction and recruitment in the summer with minimal growth and maturation until the spring. This is in general agreement with Stromberg and Crites' (1975) findings, from *Morone chrysops* in western Lake Erie, who also reported a life span of one year and worm die-off during the summer. The fact that my two largest gravid females (50.0 and 51.0 mm long) were obtained in November from *L. macrochirus* in Tichigan Lake suggests that female *C. oxycephalus* live more than one year and continue to grow and reproduce at least through the following autumn.

Three fish species, *M. salmoides, S. vitreum,* and possibly *P. nigromaculatus* appear to be important local reservoirs of the infective stages of *C. oxycephalus* (Table 1). The fact that gravid adults were recovered from only *L. macrochirus, P. annularis,* and *P. nigromaculatus* suggests that *C. oxycephalus* matures in only a few among the larger number of reservoir fish species (Tables 1, 2). Stromberg and Crites (1974) reported a high prevalence of larval *C. oxycephalus* in young *Dorosoma cepedianum,* an important reservoir host in western Lake Erie, but no gravid females were found, and they suggest that no final molt to adult occurs in this host.

The seasonal distribution of juveniles in the intestinal tract of infected hosts (Table 4) supports the above seasonal pattern. Early in the

Table 5. The relationship between the sex and size of three host species and the prevalence of infection with juvenile *Camallanus oxycephalus*.

Pomoxis nigromaculatus			*Micropterus salmoides*			*Stizostedion vitreum*		
	Tichigan Lake	Silver Lake		Tichigan Lake	Silver Lake		Tichigan Lake	Silver Lake
Relationship to host length (cm):								
No. infected/no. examined (%):								
(10–14)	1/9 (11)	0/3 (0)	(11–20)	1/11 (9)	0/7 (0)	(15–24)	0/26 (0)	0/2 (0)
(15–19)	16/59 (27)	3/12 (25)	(21–30)	9/19 (47)	2/26 (8)	(25–34)	0/13 (0)	5/24 (21)
(20–24)	24/83 (29)	8/28 (29)	(31–40)	2/11 (18)	4/28 (14)	(35–44)	1/8 (12)	6/20 (30)
(25+)	4/11 (36)	3/4 (75)	(41–50)	2/3 (67)	1/11 (9)	(45+)	3/5 (60)	5/8 (62)
Relationship to host sex:								
No. males infected/no. examined (%):								
	20/73 (27)	4/25 (16)		4/16 (25)	2/29 (7)		1/20 (5)	9/29 (31)
No. females infected/no. examined (%):								
	25/89 (28)	10/22 (45)		10/28 (36)	5/43 (12)		3/32 (9)	7/25 (28)
Mean fish length in cm (range):								
Males	19.2 (10–28)	21.1 (13–31)		24.3 (11–36)	29.5 (18–46)		30.1 (13–47)	35.6 (16–54)
Females	20.6 (15–30)	19.9 (13–25)		28.0 (13–50)	33.1 (12–48)		26.8 (15–53)	38.0 (25–54)

infectious cycle (summer) worms appear to be initially established in the anterior portions of the gut but subsequently move posteriorly and become primarily confined to the rectum by the spring. Most gravid females were found at least partially protruding from the anus.

Juvenile males were invariably less frequent than females from both lakes in all seasons and their percent in the population was lowest in the autumn (Table 4). Stromberg and Crites (1975) reported a 2:1 ratio of new males : new females during August that changed to 1:1 from September until the following July. They related the initial sex ratio of 2:1 to the more rapid recruitment of males into the adult population because of their shorter molting period compared to females; "when all larvae had molted . . . , the ratio became 1:1." This probably accounts for the higher proportion of males in the population from Tichigan Lake during the summer (30%) than during the autumn (18%) and the spring (22%) (Table 4).

The prevalence of infections with juvenile *C. oxycephalus* showed a definite relationship with size of the three common hosts, *P. nigromaculatus, M. salmoides,* and *S. vitreum,* from both lakes (Table 5). Such a relationship was not as pronounced in *M. chrysops* from western Lake Erie (Stromberg and Crites, 1975). Host sex did not appear to influence the prevalence of infection except when host size was involved. For example, the larger female *M. salmoides* were more frequently infected than the smaller males from both lakes. The one exception in *P. nigromaculatus* from Silver Lake can not be explained.

DEPOSITED SPECIMENS: Twenty-six gravid females from Tichigan Lake and 57 juveniles from Silver Lake in USNM Helm. Coll. Nos. 77429–77434; and six gravid females and 331 juveniles from Tichigan Lake in MPM Coll. No. IZ454a–d.

Family Rhabdochonidae Skrjabin, 1946
Spinitectus corolini Holl, 1928

Fifty-nine adult females were recovered from *L. gibbosus, L. macrochirus, M. salmoides,* and *M. dolomieui* in Silver Lake (41 specimens) and from *L. macrochirus* from Tichigan Lake (18 specimens), as well as one larva from the intestinal mesenteries of *M. salmoides* in Silver Lake during the spring. Adults conformed to the description given by Mueller and Van Cleave (1932) except that the worms were about 10.0 mm long and the ratio of muscular to glandular esophagus reached 1:9 compared to a supposed 7–8 mm and 1:5 to 1:6, respectively. All Tichigan Lake females were gravid. Those from Silver Lake were not gravid in the spring, 90% gravid in the summer, and 58% gravid in the autumn. Worms were more anteriorly located in their host intestine during the summer than during other seasons. Infection parameters were, however, too low in both lakes throughout the year to allow any meaningful discussion of seasonal trends.

DEPOSITED SPECIMENS: Twenty-four males and females from *L. macrochirus*; nine from Tichigan Lake in USNM Helm. Coll. No. 77437 and 15 from Silver Lake in MPM Coll. No. IZ454e.

Family Heterocheilidae Railliet and Henry, 1915
Hysterothylacium brachyurum (Ward and Magath, 1916)

Ten adults (five males and five mostly gravid females) were recovered from one *L. cyanellus* during the summer and one encysted immature from the body cavity of *P. nigromaculatus* during the autumn from Silver Lake. The worms closely agreed with the description given by Van Cleave and Mueller (1934) except that the eggs were somewhat smaller—42 μm in diameter.

DEPOSITED SPECIMENS: Eight worms from *L. cyanellus* in USNM Helm. Coll. No. 77438.

Family Haplonematidae Sudarikov and Ryzhikov, 1952
Haplonema immutatum Ward and Magath, 1916

Four male and five mostly gravid female *H. immutatum* were recovered from the intestine (one from the stomach) of three *Amia calva* from Tichigan Lake. Eight worms were recovered from two hosts during the summer and one in the autumn. Worms compared well with those described by Arthur and Margolis (1975).

DEPOSITED SPECIMENS: Two males and four females in USNM Helm. Coll. No. 77439.

Family Trichuridae Railliet, 1915
Capillaria catenata Van Cleave and Mueller, 1932

One 6.6-mm-long juvenile female was obtained from the intestine of *Umbra limi* from Tichigan Lake Canal during May 1978; a total

of 86 mud minnows were examined. This is a new host record. The specimen had longer than wide esophageal cells as described by Van Cleave and Mueller (1932) but their number was 28 instead of the "about forty" reported by the above authors. Whether the number of these cells increases with maturity is unknown. In the other North American species, *C. catostomi,* 187 wider than long cells are present (Pearse, 1924b).

DEPOSITED SPECIMENS: One worm in USNM Helm. Coll. No. 77440.

Family Dorylaimidae (de Man, 1876) Thorne, 1934
Dorylaimus sp. Dujardin, 1845

Seven 3.2–5.2-mm-long adults (one male, six gravid females) were recovered from the intestine of three *Culaea inconstans* from the Tichigan Lake Canal during May 1979; a total of 182 sticklebacks were examined. This is a new "host" record. *Dorylaimus* are free-living mud-dwelling nematodes that are occasionally observed accidently "infecting" bottom-feeding fish. They were first reported in Wisconsin by Amin (1974) from Pike River *Catostomus commersoni* in large numbers.

DEPOSITED SPECIMENS: One male and one female in USNM Helm. Coll. No. 77441 and four females in MPM Coll. No. IZ454f.

Family Philometridae Baylis and Daubney, 1926
Philometra cylindracea Ward and Magath, 1916
Philometroides nodulosa Thomas, 1929

Two females, one of each species, were recovered from *Micropterus salmoides* in Silver Lake. The liver surface of one host was infected with *P. cylindracea* in the autumn, and the inside of the cheek of the other bass had a *P. nodulosa* cyst during the spring. In a miscellaneous collection of *C. commersoni* from the Pike River, Kenosha County during May 1983, two *P. nodulosa* cysts were recovered from the caudal fin of two suckers, the usual hosts of this nematode.

DEPOSITED SPECIMENS: One *P. cylindracea* and one *P. nodulosa* in USNM Helm. Coll. Nos. 77443 and 77442, respectively.

Family Spiruridae Oerley, 1885
Spiroxys contorta (Rud., 1819)

Twelve *S. contorta* larvae were found encysted in the mesenteries of four fish species from both lakes. In Silver Lake two *L. gibbosus* were each infected with one larva during the autumn and one *M. salmoides* with two during the summer. One *U. limi* (86 were examined) was infected with seven larvae and one *Fundulus notti* (six were examined) with one during the summer in the Tichigan Lake Canal. The records from *L. gibbosus, M. salmoides,* and *F. notti* are new host records. *Spiroxys* sp. was previously reported from *L. gibbosus* and *M. salmoides* in Wisconsin by Fischthal (1947).

DEPOSITED SPECIMENS: Six larva from *U. limi* in USNM Helm. Coll. No. 77444.

Acknowledgments

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Scanning Electron Microscope Studies of Adults, First- and Third-Stage Larvae of *Angiostrongylus cantonensis* (Nematoda: Metastrongyloidea)

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ABSTRACT: The surfaces of larval and adult *Angiostrongylus cantonensis,* causative agent of human eosinophilic meningitis, were studied by the use of scanning electron microscopy. Annular striae were observed on the surface of larvae and adults. Differences in the appearance of alae in larval stages, and in the shape of the tail at different stages of development, are described and illustrated.

Angiostrongylus cantonensis is a nematode parasite of rats and also the etiologic agent of human eosinophilic meningitis, which is prevalent in many Pacific Islands, Southeast Asia and Cuba. Adult worms were first discovered and described by Chen from the lungs and heart of wild rats in Canton, China. Later, Chen (1935) gave a more detailed description of the adult worm.

The life cycle of *Angiostrongylus* was elucidated by Mackerras and Sandars (1955) in Australia. They also gave the first morphological description of the larval stages. Subsequently, Alicata and Jindrak (1970) gave details of the morphology of the adult worms and various larval stages. However, due to the limitations of light microscopy, some of the fine structure of the adult parasite and the larva could not be recognized.

The present paper describes the morphology of the adult worms and the first- and third-stage larvae by using scanning electron microscopy.

Materials and Methods

Adult worms were obtained from the hearts and pulmonary blood vessels of experimentally infected white rats. First-stage larvae were obtained from the feces of infected rats. Third-stage larvae were obtained from freshwater snails, *Biomphalaria pfeifferi,* that had been experimentally infected with first-stage larvae.

The materials used for SEM observation were prepared following the instructions of Voge et al. (1978).

An ETEC autoscan was used for scanning.

Results

Larval stages

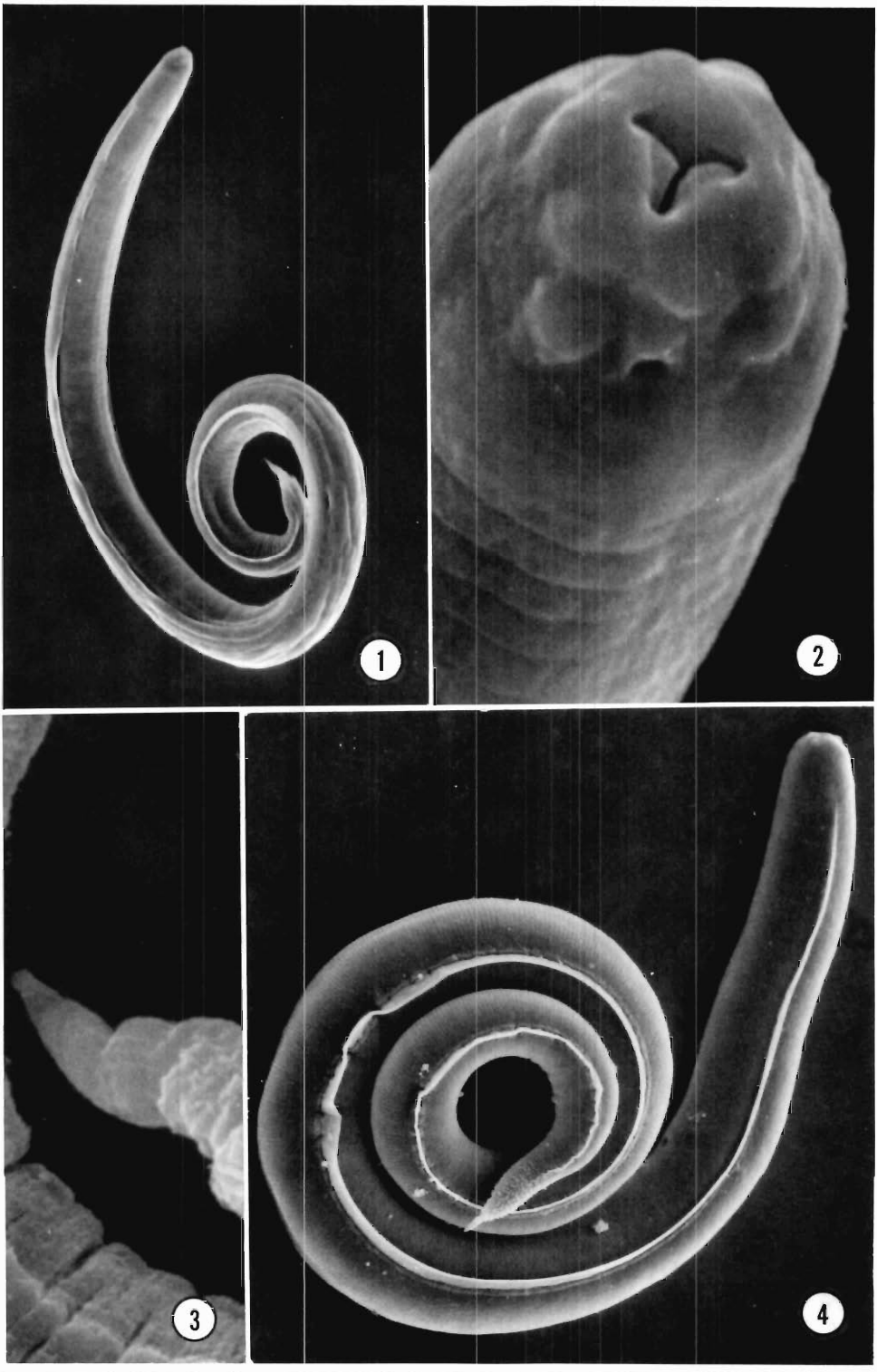
FIRST-STAGE LARVAE: Cylindrical in shape, the posterior portion usually curved ventrad. A long and broad membranous ala occurs on each side of the body beginning near the anterior end and extending posteriorly to a point short of the end of the tail (Fig. 1). It divides the whole body into distinct dorsal and ventral portions. The body surface, except for the anterior end and the tip of the tail, is covered by a transversely striated cuticle. At the anterior end there is a mouth opening within which the triangular lumen of the esophagus is apparent (Fig. 2). The margin of the mouth is thickened and the cephalic papillae are arranged in two circles, of six papillae each. The opening of what is most likely the amphidial pore can be distinguished at the external border of one of the outer papillae (Fig. 2). The tip of the tail is pointed and has a rough surface with transverse striations (Fig. 3).

THIRD-STAGE LARVAE: Compared to the first-stage larvae, the third-stage larvae are larger and thicker (Fig. 4). The shape of the body is similar except that the posterior portion of the body is more coiled. The lateral alae (Fig. 6) are less broad and appear thicker. The body surface is covered by a circular striated cuticle, with a short longitudinal ridge. The two circles of cephalic papillae (Fig. 5) are not as closely spaced as in first-stage larvae; only four groups could be seen distinctly. The amphidial pore is discernible as a small slit-like opening between two large cephalic papillae (Fig. 5). The excretory pore and details of the body surface are illustrated in Figure 8.

The surface of the tail (Fig. 7) bears many, tiny bleb-like structures, arranged in transverse rows. The tip of the tail terminates in a spear-like end.

The adult worm

Body surfaces of both sexes are similar. With the exception of the head and tail regions, the cuticle on the body surface has two different appearances (Fig. 9). Dorsally (Fig. 10), one ob-



Figures 1–4. 1. First-stage larva. 1,120×. 2. Head of first-stage larva. 16,800×. 3. Tail of first-stage larva. 11,200×. 4. Third-stage larva. 1,120×.

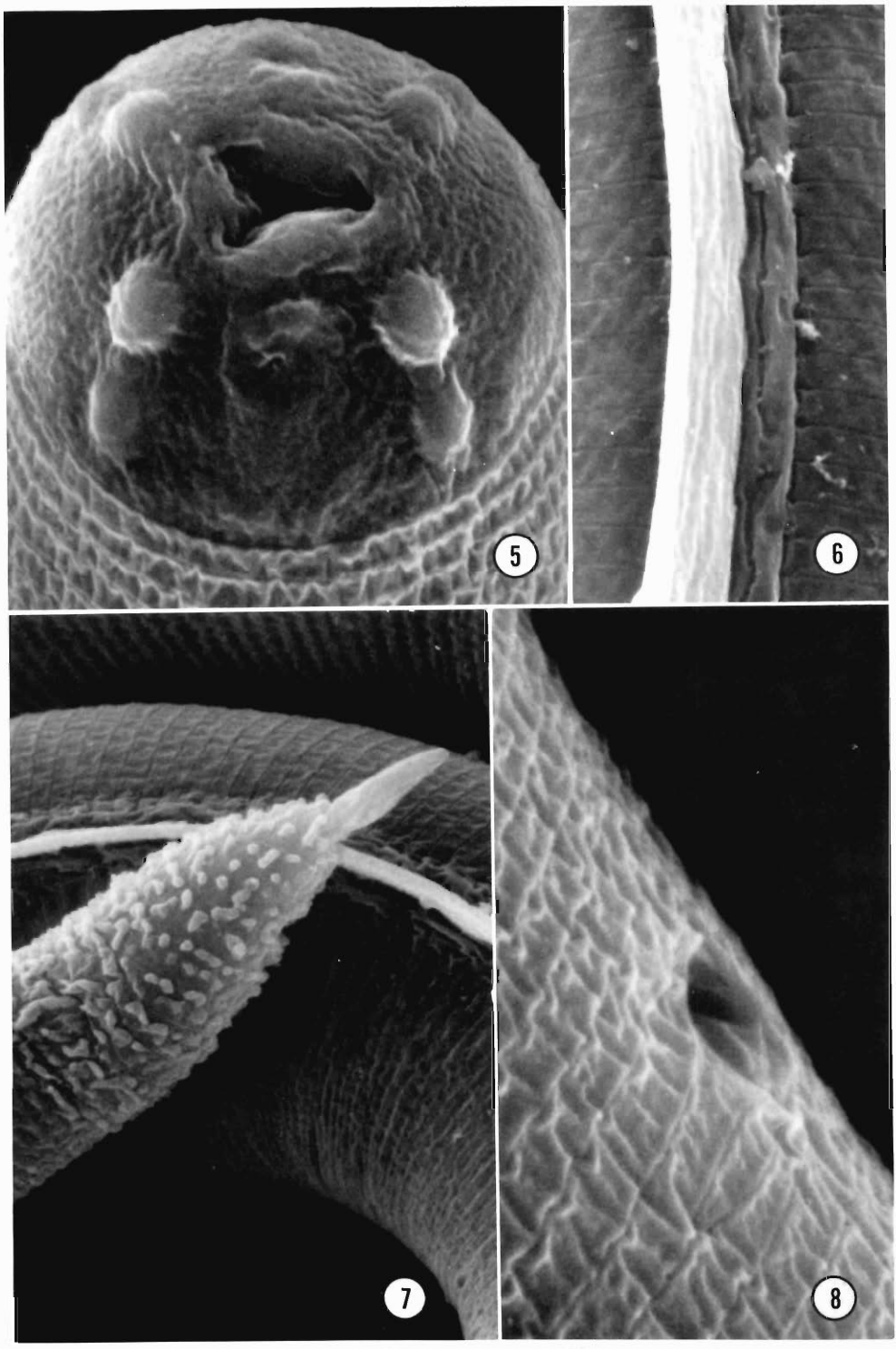


Figure 5–8. 5. Head of third-stage larva. Note opening to amphid between two large cephalic papillae. 11,200×. 6. Lateral surface of third-stage larva. 8,400×. 7. Posterior end of third-stage larva. 5,600×. 8. Excretory pore of third-stage larva, located on ventral surface, posterior to nerve ring. 16,800×.

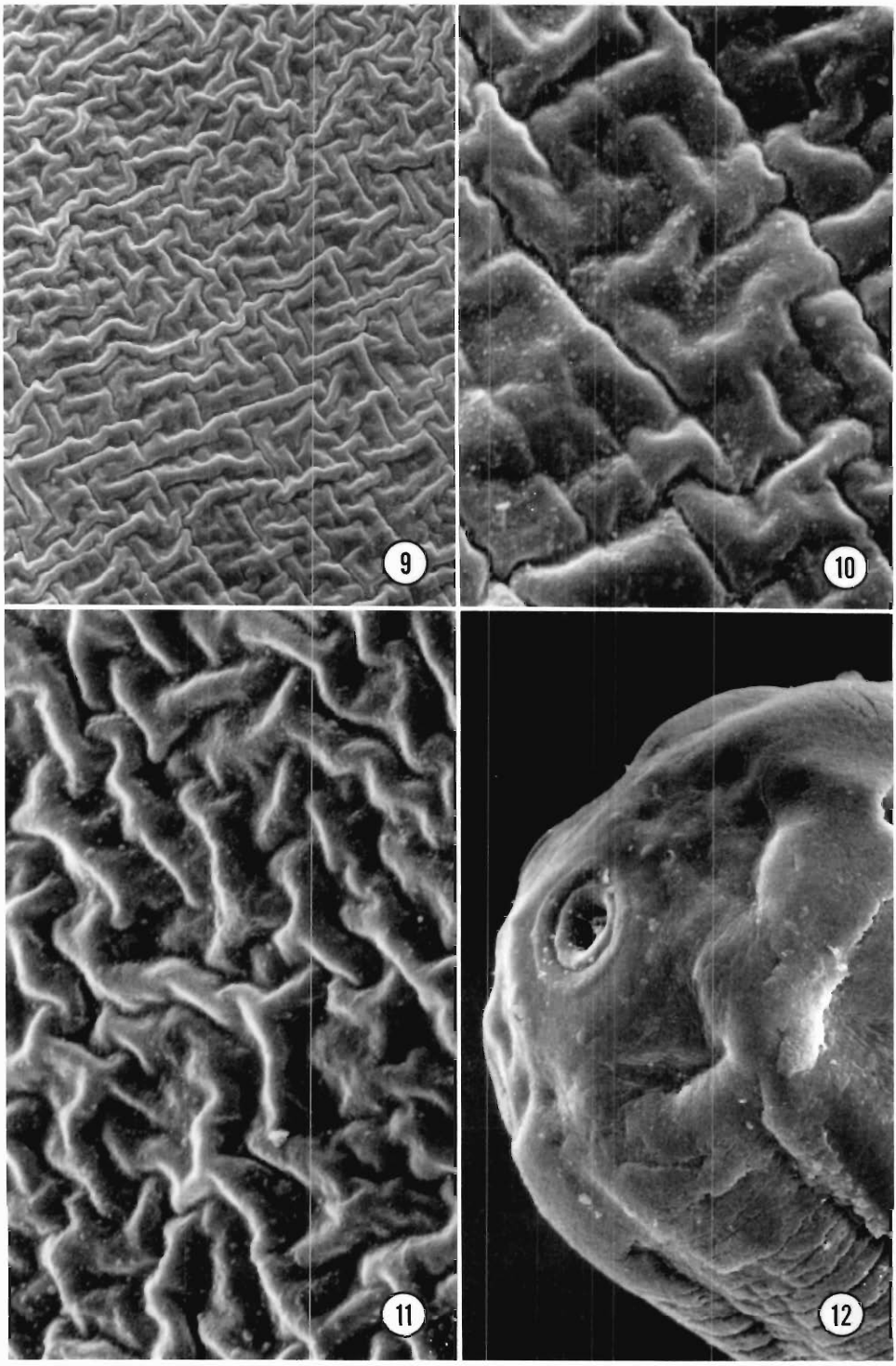
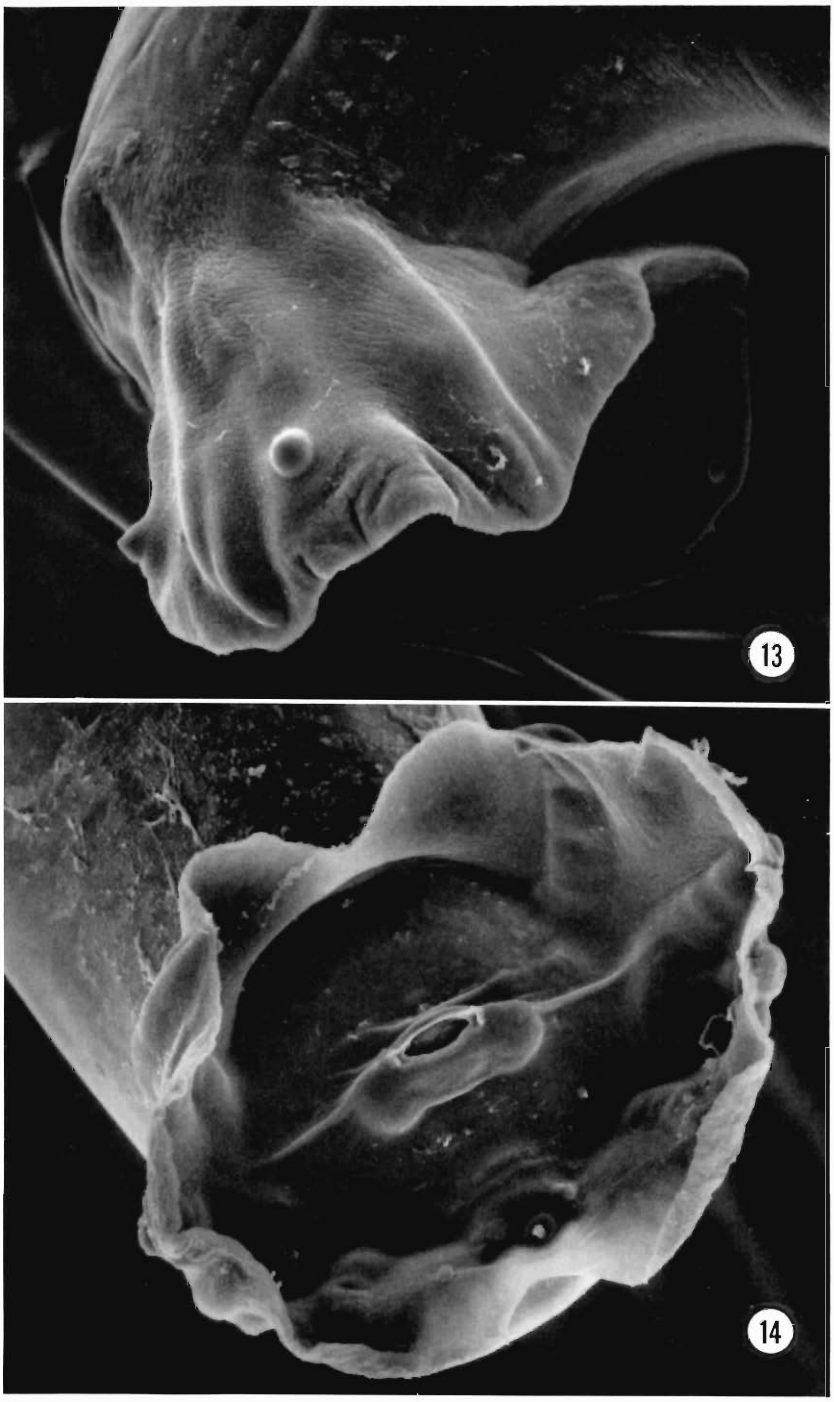
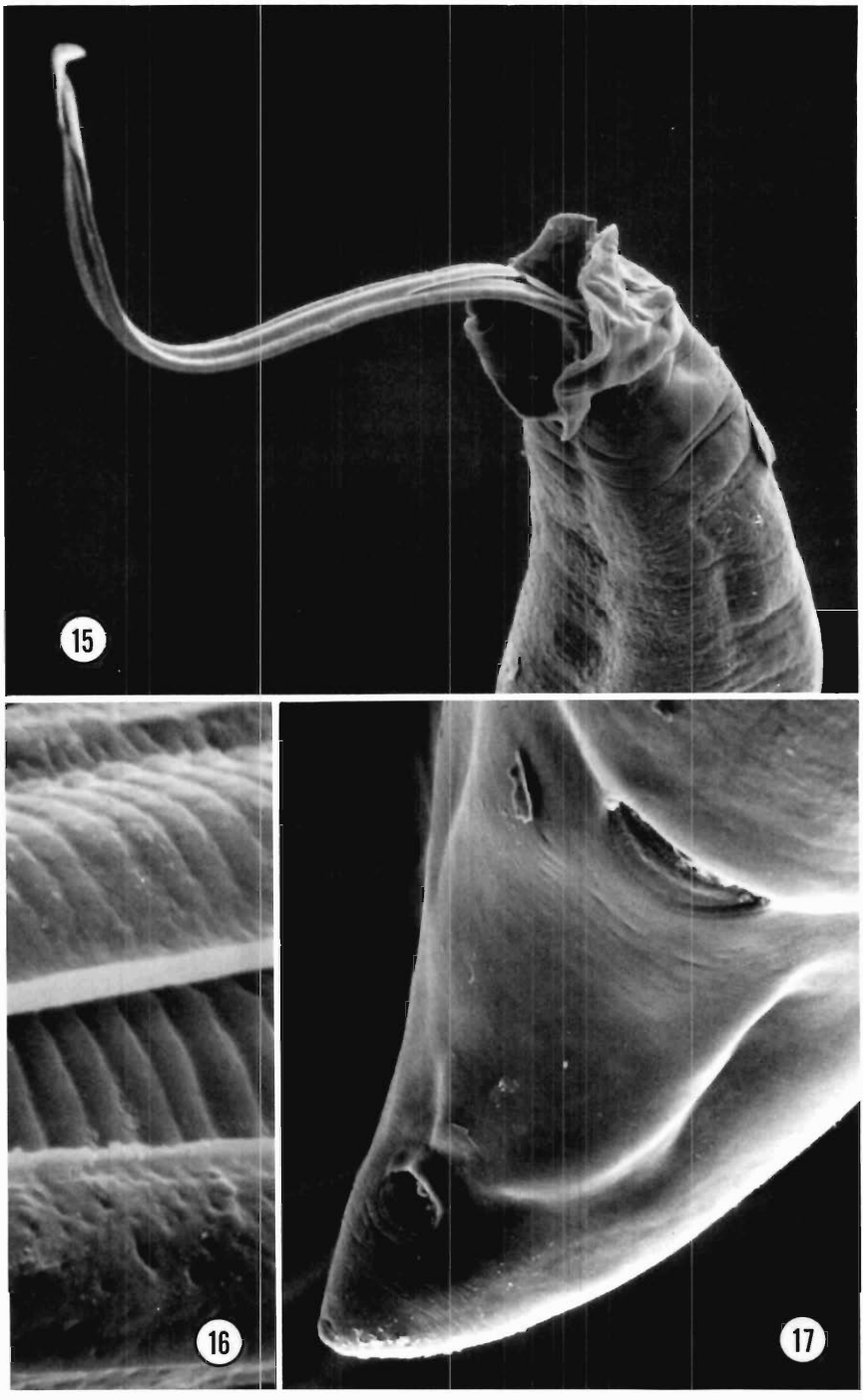


Figure 9–12. 9. Body surface of adult, showing transition between dorsal (below) and ventral surface (above). 1,680×. 10. Dorsal body surface of adult. 5,600×. 11. Ventral body surface of adult. 5,600×. 12. Anterior end of adult worm. 1,680×.



Figures 13, 14. **13. Posterior end of male, lateral view. 840×. 14. Ventral view of male bursa. 840×.**



Figures 15–17. 15. Posterior end of male with spicule extended. 280×. 16. Enlargement of the shaft of the spicule. 5,600×. 17. Posterior end of female, ventral view. 560×.

serves transverse striae that fade into an irregular pattern on the ventral surface (Fig. 11). The anterior end is rounded (Fig. 12) and has six small sensory papillae equidistantly placed in a single row around the orifice.

MALE: The posterior end shows a well developed caudal bursa supported by bursal rays (Figs. 13, 14). The outer surface of the bursa bears 4–6 small, rounded papillae that are situated near the edge of the bursa. The cloacal opening has an ovoid shape, with papillae flanking the opening. Long copulatory spicules are often found extending from the cloacal opening (Fig. 15). The spicules are composed of a broad ventral portion with slight curved edges (Fig. 16) that are fused together at the tip to form a beak-like structure (Fig. 15).

FEMALE: The tail portion is shaped somewhat like the head of a shark (Fig. 17). The larger opening of the vulva is situated anteriorly to the smaller, semicircular anal opening. Phasmids were not seen.

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Observations on Two Acanthocephalan Species Infecting the Central Mudminnow, *Umbra limi,* in a Michigan River

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ABSTRACT: Four hundred eighty-one central mudminnows, *Umbra limi,* were collected in 23 months over a 35-month period, beginning in September 1979, and examined for intestinal helminths. The study area was the Looking Glass River, located in the south-central portion of the lower peninsula of Michigan. The acanthocephalan species found were *Neoechinorhynchus limi* Muzzall and Buckner, 1982 (Acanthocephala: Neoechinorhynchidae) and *Fessisentis tichiganensis* Amin, 1980 (Acanthocephala: Fessisentidae). Both species were present in mudminnows in the spring and absent in the summer and fall. These seasonal patterns of infection can be related to the movements of mudminnows into and out of the sampling area, changes in water temperature, the life span of the acanthocephalans, and possibly, in the case of *N. limi,* to a change in the diet of mudminnows.

Neoechinorhynchus limi occurred throughout the intestine of mudminnows, whereas *F. tichiganensis* was attached in the anterior portion of the intestine or in the pyloric ceca. The prevalence and the mean numbers of these worms did not significantly change as mudminnow length increased. Gravid *N. limi* and *F. tichiganensis* occurred only in the mudminnow in the Looking Glass River. Immature *N. limi* were, however, occasionally found in other species of fish. *F. tichiganensis* is reported for the first time from Michigan.

Although there are various reports of parasites infecting the central mudminnow, *Umbra limi* (Kirtland), scattered throughout the literature, there has been no extensive study on the intestinal helminths of this piscine species. Meyer (1953) examined 118 mudminnows from several geographical locations in Ohio and found 11 genera of parasites. Peckham and Dineen (1957) reported that the common parasites of 167 mudminnows from Indiana were *Clinostomum complanatum, Gyrodactylus limi* and *Phyllodistomum brevicecum.* Recently, Amin (1980) and Muzzall and Buckner (1982) described *Fessisentis tichiganensis* (Acanthocephala: Fessisentidae) and *Neoechinorhynchus limi* (Acanthocephala: Neoechinorhynchidae) from mudminnows collected in Wisconsin and Michigan, respectively. While surveying the parasites of fishes in south-central Michigan, these acanthocephalan species were found in a population of mudminnows. This report presents data regarding the seasonal occurrence and host–parasite relationships of *N. limi* and *F. tichiganensis* infecting the central mudminnow.

Materials and Methods

Although the collection period occurred from September 1979 through July 1982 (35 months), mudminnows were collected by seining (¼-inch mesh minnow seine) and electrofishing from the Looking Glass River, Woodbury Avenue, Shiawassee County, Michigan, only during September through November 1979, May through November 1980, March through November 1981, and March through July 1982. The collecting area has an average width of 5 m, and the bottom consists of silt and mud. Water depth varies seasonally from 0.5 to more than 3 m. The aquatic plant *Ludwigia palustris* L. var. *americana* (Family: Onagraceae) is abundant along the banks and is the favorite habitat of the mudminnow.

Fish were taken to the laboratory alive, measured, sexed, and necropsied within 36 hr of capture. The entire alimentary canal (from the esophagus to the vent) was removed. The position, number, degree of development, and sex of the acanthocephalans were recorded. Female acanthocephalans were classified as immature, or those with ovarian balls; and gravid, or those with ovarian balls and eggs, as well as those with eggs only. Acanthocephalans were fixed and stained using standard techniques. Prevalence is the percentage of fish infected in a given sample, and mean number is the number of worms per host in a given sample. The value following a mean is the standard error unless otherwise stated. Statistical procedures used were from Sokal and Rohlf (1969).

Results

Neoechinorhynchus limi Muzzall and Buckner, 1982 (Acanthocephala: Neoechinorhynchidae)

Eighty (16.6%) of the 481 mudminnows examined from the Looking Glass River were infected with *Neoechinorhynchus limi. N. limi* exhibited a definite seasonal occurrence in prevalence and numbers in mudminnows (Table 1). It occurred in mudminnows in May and June 1980, March through June 1981, and April through June 1982. *N. limi* did not infect mud-

Table 1. Prevalence, mean number, and maturation of *Neoechinorhynchus limi*.

Month	No. examined	No. infected (%)	Mean number ± SE*	No. of parasites present	Mean length of parasites	% gravid worms	Mean length (mm) of infected fish	Mean length (mm) of examined fish
Sept (79)	4	0	—	—	—	—	—	58 ± 5.8
Oct	24	0	—	—	—	—	—	59 ± 4.1
Nov	6	0	—	—	—	—	—	61 ± 1.0
May (80)	22	12 (55)	6.5 ± 1.9	77	2.2 ± 0.10	44	68 ± 3.2	68 ± 2.2
June	30	11 (37)	2.1 ± 0.5	23	2.9 ± 0.20	59	67 ± 1.5	69 ± 1.6
July	28	0	—	—	—	—	—	72 ± 1.1
Aug	25	0	—	—	—	—	—	69 ± 2.3
Sept	26	0	—	—	—	—	—	56 ± 2.9
Oct	25	0	—	—	—	—	—	60 ± 4.3
Nov	3	0	—	—	—	—	—	68 ± 12.2
Mar (81)	5	3 (60)	2.0 ± 0.6	6	1.5 ± 0.12	0	56 ± 8.3	52 ± 5.1
Apr	18	5 (28)	2.4 ± 0.7	12	2.2 ± 0.10	25	56 ± 3.2	63 ± 2.6
May	17	11 (65)	4.3 ± 1.2	47	2.3 ± 0.10	30	58 ± 1.2	59 ± 3.0
June	26	6 (23)	1.3 ± 0.2	8	3.0 ± 0.33	50	60 ± 5.3	61 ± 3.0
July	31	0	—	—	—	—	—	47 ± 2.7
Aug	22	0	—	—	—	—	—	43 ± 4.1
Sept	24	0	—	—	—	—	—	57 ± 2.7
Oct	21	0	—	—	—	—	—	56 ± 1.9
Nov	4	0	—	—	—	—	—	64 ± 3.9
Apr (82)	8	5 (63)	6.0 ± 1.3	30	1.6 ± 0.10	0	60 ± 7.2	58 ± 4.6
May	29	13 (45)	3.9 ± 1.4	51	2.1 ± 0.10	9	60 ± 2.7	56 ± 1.9
June	47	14 (30)	3.1 ± 0.5	44	2.8 ± 0.10	57	74 ± 1.7	69 ± 1.8
July	36	0	—	—	—	—	—	66 ± 2.1

* Standard error.

minnows during September through November 1979, July through November 1980, 1981, and July 1982.

The mean number of worms for the entire sampling period was 3.8 ± 0.45. There was no significant difference in the mean number of *N. limi* found in mudminnows between May through June 1980 (4.3 ± 1.10), March through June 1981 (3.0 ± 0.70), and April through June 1982 (3.9 ± 0.64) ($F = 0.86$, $P > 0.50$). Also, the percentages of fish infected with *N. limi* during these respective periods were not significantly different ($\chi^2 = 1.67$, $P > 0.50$). The mean length of the parasites as well as the percentage of gravid females increased from May through June 1980, March through June 1981, and April through June 1982. The maximum number of worms was 21, which occurred in a male mudminnow, 73 mm long. Infected fish had a significantly smaller mean length in March through June 1981 than did infected fish in May through June 1980 or April through June 1982 ($F = 7.62$, $P < 0.01$). There was no significant difference between the number of male (42) and female (38) mudminnows infected with *N. limi* ($\chi^2 = 0.20$, $P > 0.90$) or in their mean numbers (4.0 ± 0.72, 3.5 ± 0.55, respectively; $t = 0.41$, $t > 0.90$).

Neoechinorhynchus limi infected fish in all length classes (Table 2). Prevalence varied from 21 to 45 percent and the mean number of worms was not significantly different between host length classes ($F = 2.21$, $P > 0.10$). Fish 49 mm in length or less had the largest mean number of worms; the maximum number of worms found was 88 in the 60–69 mm length class. There were no significant differences in the prevalence or mean number of *N. limi* between male and female fish of each length class. *N. limi* was attached throughout the gut of mudminnows, ranging from the anterior portion of the intestine to the rectum. Of the 300 *N. limi* collected, 285 (95%) were sexed and measured; 118 (41%) were males, 73 (26%) were immature females, and 94 (33%) were gravid females.

Gravid *N. limi* were found only in mudminnows. Four immature specimens of *N. limi* were collected from four of 39 brook sticklebacks, *Culaea inconstans,* one immature *N. limi* from one of two redhorses, *Moxostoma* sp., and one immature *N. limi* from one of the 18 bluegills *Le-*

Table 2. Prevalence and mean number of *N. limi* collected from 202 mudminnows and *F. tichiganensis* collected from 172 mudminnows of arbitrary length classes (mm), examined in those months when mudminnows were infected.

	N. limi				*F. tichiganensis*			
Length class	No. examined	No. infected (%)	No. of parasites present	Mean no. of worms ± SE*	No. examined	No. infected (%)	No. of parasites present	Mean no. of worms ± SE
<50	14	3 (21)	25	8.3 ± 4.5	15	3 (20)	4	1.3 ± 0.3
50–59	64	27 (42)	78	2.9 ± 0.6	62	10 (16)	13	1.3 ± 0.2
60–69	59	24 (41)	88	3.7 ± 0.7	45	6 (13)	16	2.7 ± 1.7
70–79	42	19 (45)	69	3.6 ± 1.1	29	9 (31)	15	1.7 ± 0.4
>79	23	7 (30)	40	5.7 ± 1.6	21	7 (33)	11	1.6 ± 0.5

* Standard error.

pomis macrochirus, examined. The following piscine species (number collected) representing a total of 130 fishes from 16 species of six families examined, during the months when mudminnows were infected, were not infected: Catostomidae—*Catostomus commersoni* (16), *Erimyzon* sp. (5), *Minytrema melanops* (5); Centrarchidae—*Ambloplites rupestris* (3), *Lepomis cyanellus* (3), *L. gibbosus* (9), *Pomoxis nigromaculatus* (9); Cyprinidae—*Notropis cornutus* (17), *Notropis* sp. (2), *Pimephales promelas* (12), *Semotilus atromaculatus* (4); Esocidae—*Esox americanus* (5); Ictaluridae—*Ictalurus melas* (15); Percidae—*Etheostoma nigrum* (2), *Perca flavescens* (8), *Percina maculata* (15).

Fessisentis tichiganensis Amin, 1980 (Acanthocephala: Fessisentidae)

Although *Fessisentis tichiganensis* infected only 35 (7.3%) of the mudminnows examined, a seasonal occurrence was exhibited by this species in Looking Glass River mudminnows (Table 3). It occurred in mudminnows in May 1980, March through June 1981, and April through June 1982. The mean length of parasites increased from March through June 1981 and April through June 1982; gravid females occurred in hosts in May 1980, April through June 1981, and June 1982. Mudminnows were not found infected during September through November 1979, June through November 1980, July through November 1981, and July 1982.

The mean number of *F. tichiganensis* for the entire sampling period was 1.7 ± 0.31. There was no significant difference in the number of *F. tichiganensis* found between March through June 1981 (1.6 ± 0.34) and April through June 1982 (1.2 ± 0.20) ($t = 0.73$, $P > 0.50$); nor was there a significant difference in the length of infected fish between May 1980, March through June 1981, and April through June 1982 ($F = 0.52$, $P > 0.50$). The maximum number recovered from a single mudminnow was 11 in May 1980. *F. tichiganensis* was always attached to the anterior intestine or pyloric ceca of mudminnows. All 59 *F. tichiganensis* found were sexed; the population in mudminnows consisted of 26 (44%) males, 15 (25%) females with ovarian balls, and 18 (31%) gravid females. No significant difference existed between the number of male (15) and female (20) mudminnows infected with *F. tichiganensis* ($\chi^2 = 0.71$, $P > 0.50$). Also, the mean number of *F. tichiganensis* in male (1.4 ± 0.10) and female (2.0 ± 0.75) mudminnows was not significantly different ($t = 1.23$, $P > 0.10$).

The prevalence of *F. tichiganensis* in the five length classes of fish varied from 16 to 33% (Table 2). Although fish 60–69 mm long had the largest mean number of worms, a significant difference did not exist in the mean number of worms among hosts in the different length classes ($F = 0.49$, $P > 0.50$). Correlation coefficients between host length and the numbers of *F. tichiganensis* ($r = 0.63$) and *N. limi* ($r = 0.68$) were not significant.

Twelve (five males, seven females) of the 481 mudminnows were simultaneously infected with *N. limi* and *F. tichiganensis.* In concurrent infections, the mean numbers of *N. limi* and *F. tichiganensis* were 2.8 ± 0.90 and 1.2 ± 0.40, respectively. The mean length of infected hosts was 65.3 ± 2.9 mm.

Discussion

Mudminnows, *Umbra limi,* were not present throughout the year in the Looking Glass River

Table 3. Prevalence, mean number, and maturation of *Fessisentis tichiganensis*.

Month	No. examined	No. infected (%)	Mean number ± SE*	No. of parasites present	Mean length of parasites	% gravid worms	Mean length (mm) of infected fish	Mean length (mm) of examined fish
Sept (79)	4	0	—	—	—	—	—	58 ± 5.8
Oct	24	0	—	—	—	—	—	59 ± 4.1
Nov	6	0	—	—	—	—	—	61 ± 1.0
May (80)	22	1 (5)	11.0	11	13.4 ± 0.87	55	68	68 ± 2.2
June	30	0	—	—	—	—	—	69 ± 1.6
July	28	0	—	—	—	—	—	72 ± 1.1
Aug	25	0	—	—	—	—	—	69 ± 2.3
Sept	26	0	—	—	—	—	—	56 ± 2.9
Oct	25	0	—	—	—	—	—	60 ± 4.3
Nov	3	0	—	—	—	—	—	68 ± 12.2
Mar (81)	5	2 (40)	2.0	2	13.7 ± 2.32	0	61 ± 11.5	52 ± 5.1
Apr	18	8 (44)	2.0 ± 0.6	16	16.1 ± 0.68	37	71 ± 3.9	63 ± 2.6
May	17	2 (12)	2.0	2	17.4 ± 1.96	50	56 ± 0.5	59 ± 3.0
June	26	4 (15)	1.5 ± 0.3	6	18.9 ± 1.93	50	77 ± 4.3	61 ± 3.0
July	31	0	—	—	—	—	—	47 ± 2.7
Aug	22	0	—	—	—	—	—	43 ± 4.1
Sept	24	0	—	—	—	—	—	57 ± 2.7
Oct	21	0	—	—	—	—	—	56 ± 1.9
Nov	4	0	—	—	—	—	—	64 ± 3.9
Apr (82)	8	3 (38)	1.3 ± 0.6	4	14.0 ± 1.01	0	62 ± 11.0	58 ± 4.6
May	29	10 (35)	1.3 ± 0.5	13	13.6 ± 0.89	0	57 ± 8.9	56 ± 1.9
June	47	5 (11)	1.0	5	20.9 ± 2.84	40	86 ± 14.5	69 ± 1.8
July	36	0	—	—	—	—	—	66 ± 2.1

* Standard error.

sampling area. Although many hours were spent electrofishing and seining, few or no mudminnows were collected in November and December 1979 and 1980, and in February, March, November, and December 1981. Two suggestions can be made about the absence of mudminnows. One is that mudminnows "hibernate" in the mud during the colder months of the year as reported by Abbott (1870). However, during December 1980 and 1981, the bottom of the collecting area was stirred vigorously with a rake, but no mudminnows could be found. It is also possible that mudminnows moved into and out of the Looking Glass River collecting area seasonally. The absence of mudminnows in the mud supports this latter suggestion. Peckham and Dineen (1957) reported that migration of mudminnows was limited to lateral movements from the main stream into flooded areas following heavy rains and that there was no evidence of hibernation. Based on these results, it appears that mudminnows exhibit different activity and movements depending on geographical location.

The seasonal occurrence of mudminnows and of *Neoechinorhynchus limi* in the Looking Glass River collecting area appear to be influenced by the movements of mudminnows, an increase in water temperature, the presence of *Ludwigia palustris* L. var. *americana,* and possibly to a seasonal change in mudminnow diet. *L. p. americana* is used by mudminnows as cover to avoid predators and as a cover for spawning. It does not start to grow in the area until late April or early May. As the plant becomes more abundant, so do the mudminnows. The sampling of mudminnows in the beginning of 1980, 1981, and 1982 varied (Table 1) and depended on water level and flow; in March 1980 and 1982, water was deeper than 3 m and the flow was fast, making sampling difficult. However, when fish were first sampled in each year, some were infected with *N. limi.* The mudminnows may, therefore, become infected with *N. limi* before they enter the sampling area. However, transmission of the parasite must also occur in the sampling area because of the small mean length of parasites that occurred in fish in March 1981 and April 1982, followed by the growth of parasites that occurred afterwards. Also, the number of parasites increased to its maximum in May 1981 and 1982, suggesting that recruitment of *N. limi* into the mudminnows occurred in the collecting area.

Although the number of parasites decreased in June, the worms increased in length and matured sexually from May through June 1980, March through June 1981, and April through June 1982; the largest percentage of gravid worms also occurred in June. Water temperature increased from 5°C or less in March to its maximum of 24°C or higher in July or August 1980, 1981, 1982. This increase in water temperature was accompanied by growth and maturation of the worms followed by the loss of *N. limi* from mudminnows in July through November 1980, 1981, and July 1982. In July or August, water was shallow and much of the *L. p. americana* was not submerged.

Mudminnows spawned in the collecting area during the early part of May through June. In the latter part of May and in June, young-of-the-year mudminnows (<25 mm in length) were present. In general, mudminnows increased in length during summer and fall and their diet varied seasonally. Small crustaceans (Cladocera, Copepoda, and Ostracoda) were commonly eaten during the spring and early summer. During the summer and early fall, as mudminnows increased in length, snails, small crayfish, and amphipods (*Hyalella azteca*) were commonly eaten. As this general change in diet occurred, and as mudminnows grew and water temperature increased, there was a loss of *N. limi*. Moreover, recruitment also stopped.

Others who have reported seasonal occurrences of neoechinorhynchids in North America are: McDaniel and Bailey (1974) and Eure (1976) for *N. cylindratus* in centrarchids; Van Cleave (1916) and Jilek (1978) for *Gracilisentis gracilisentis* and *Tanaorhamphus longirostris* in gizzard shad, *Dorosoma cepedianum*, from lentic environments; and Muzzall (1980) for *N. cristatus* in white suckers, *Catostomus commersoni*, from a lotic environment. Muzzall and Bullock (1978) and Muzzall (1980) reported that *N. saginatus* in fallfish, *Semotilus corporalis*, and *Octospinifer macilentus* in white suckers, respectively, did not exhibit seasonal differences of occurrence in lotic environments.

Differences in the seasonal occurrence of *F. tichiganensis* can also be explained by the movement of mudminnows into and out of the collecting area, the presence of *L. p. americana*, an increase in water temperature, and the loss of spent worms. *F. tichiganensis* was first found in fish in March. The length of *F. tichiganensis*, as well as the percentage of gravid females, increased from March through June 1981 and April through June 1982. During July 1981 and 1982, mudminnows were not found infected when water temperature was high and water level low. The collection of most *F. tichiganensis* in the spring corresponds to the result of Amin (1980), who, working in a lentic habitat, collected 85% of the worms he found during the spring. Conversely, Fried et al. (1965) found that white suckers from a lotic habitat were infected with *F. friedi* in October through December and not in May.

Muzzall (1980) suggested that the movements of white suckers into and out of a sampling area was involved in the seasonal periodicity of *Pomphorhynchus bulbocolli* in a lotic environment. Aho et al. (1982) and Camp et al. (1982), following the long-term changes in prevalence and density of *Diplostomulum scheuringi* and *Ornithodiplostomum ptychocheilus*, respectively, in populations of *Gambusia affinis* demonstrated that the life history characteristics of the mosquitofish, along with temperature, play a major role in the patterns of infection exhibited by these trematodes. It appears that the patterns exhibited by *N. limi* and *F. tichiganensis* in Looking Glass River mudminnows are, in part, a consequence of the combined actions of the movements of mudminnows and temperature. The movements of mudminnows into the Looking Glass River sampling area initiated the infection cycle of both *N. limi* and *F. tichiganensis*. These worms have a life span of less than one year in the mudminnows because they were absent from them for at least five months. The termination of the infection cycle of *N. limi* and *F. tichiganensis* in June 1980, 1981, 1982 was due to the growth, maturation, and the loss of spent worms as temperature increased; gravid individuals of both species were found unattached in the posterior portion of the intestine or rectum in late May and June 1981. Although mudminnows were collected in 23 months over only a 35-month period, the prevalence and mean number of *N. limi* and *F. tichiganensis* remained consistent during May through June 1980, March through June 1981, and April through June 1982, suggesting that ecosystem stability plays a role in these similar yearly patterns of infection as discussed by Camp et al. (1982).

Amin (1980) described *F. tichiganensis* from the mudminnow and 10 other fish species of seven families from southeastern Wisconsin. He (pers. comm.) has also collected *F. tichiganensis* from the black bullhead, *Ictalurus melas* from

SW Lake Michigan. The suggestion of Amin (1980) that the mudminnow serves as a transport host for *F. tichiganensis* is an interesting one; however, in the present study there was no evidence to support this notion. *F. tichiganensis* was not found in the body cavity of mudminnows or in the body cavity or digestive tract of any other fish species examined. Gravid *F. tichiganensis* occurred only in mudminnows, suggesting that in the Looking Glass River, *F. tichiganensis* is specific for mudminnows. The collection of *F. tichiganensis* from mudminnows in Michigan represents a new state record and extends the known range of *F. tichiganensis* eastward.

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Posterior Dorsal and Lateral Medial Nerves of *Macracanthorhynchus hirudinaceus* (Acanthocephala)

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ABSTRACT: Two of the major nerves associated with the cerebral ganglion of *Macracanthorhynchus hirudinaceus* have been reevaluated using wax as well as thin sections (1.5 μm) of Epon-embedded material. Each of the large paired (22–23 neurites) lateral posterior nerves was observed to provide two neurites—one of which services the dorsal receptacle flexor muscle and the other joins the dorsal receptacle protrusor muscle. These neurites, previously called the posterior dorsal nerve, separate from the lateral posterior nerve immediately upon its penetration of the proboscis sheath and are never enclosed by the retinacular muscle which accompanies the remaining neurites to the body wall musculature.

The paired lateral medial nerves each consist of a pair of neurites. After penetration of the proboscis sheath, they extend posteriorly to the posterior level of the cerebral ganglion before reversing their direction and turning anteriorly. They then parallel the descending loop and continue anterior to the cerebral ganglion where they join a larger group of neurites from the anterior ventral and anterior lateral nerve. The lateral medial nerve services the anterior proboscis musculature.

Examination of the literature published on the nervous system of Acanthocephala indicates that it is somewhat more complex in male worms than in females. In male *Moniliformis dubius* three ganglia have been described, each with an assortment of associated nerve tracts. Nerves originating from the cerebral (cephalic) ganglion are the most often cited, having a long list of investigators beginning with von Siebold in 1848 (according to Kaiser, 1893) and continuing sporadically until now.

In the last 50 years published descriptions on major parts of the nervous system of Acanthocephala have appeared by: Harada (1931) for *Bolbosoma turbinella,* Kilian (1932) for *Oligacanthorhynchus microcephala,* Dunagan and Miller (1976, 1977, 1978) for *Moniliformis moniliformis* (=*M. dubius*), Makhanbetov (1972, 1974, 1975) for *Polymorphus phippsi,* Ivanova and Makhanbetov (1975) for *Polymorphus phippsi,* and Dunagan and Miller (1981) for *Oligacanthorhynchus tortuosa.* Reviews of the earlier works were made by Rauther (1930), Hyman (1951), Bullock and Horridge (1965) and Nicholas (1967). It is obvious from this list that only a few of the larger species have been studied regarding anatomical details of the nervous system.

The purposes of this study were: (1) to provide evidence that the lateral medial nerve does not divide upon leaving the proboscis sheath but forms a posterior loop before going anteriorly; (2) to show that the dorsal receptacle muscles are serviced by neurites from the lateral posterior nerves collectively termed posterior dorsal nerves.

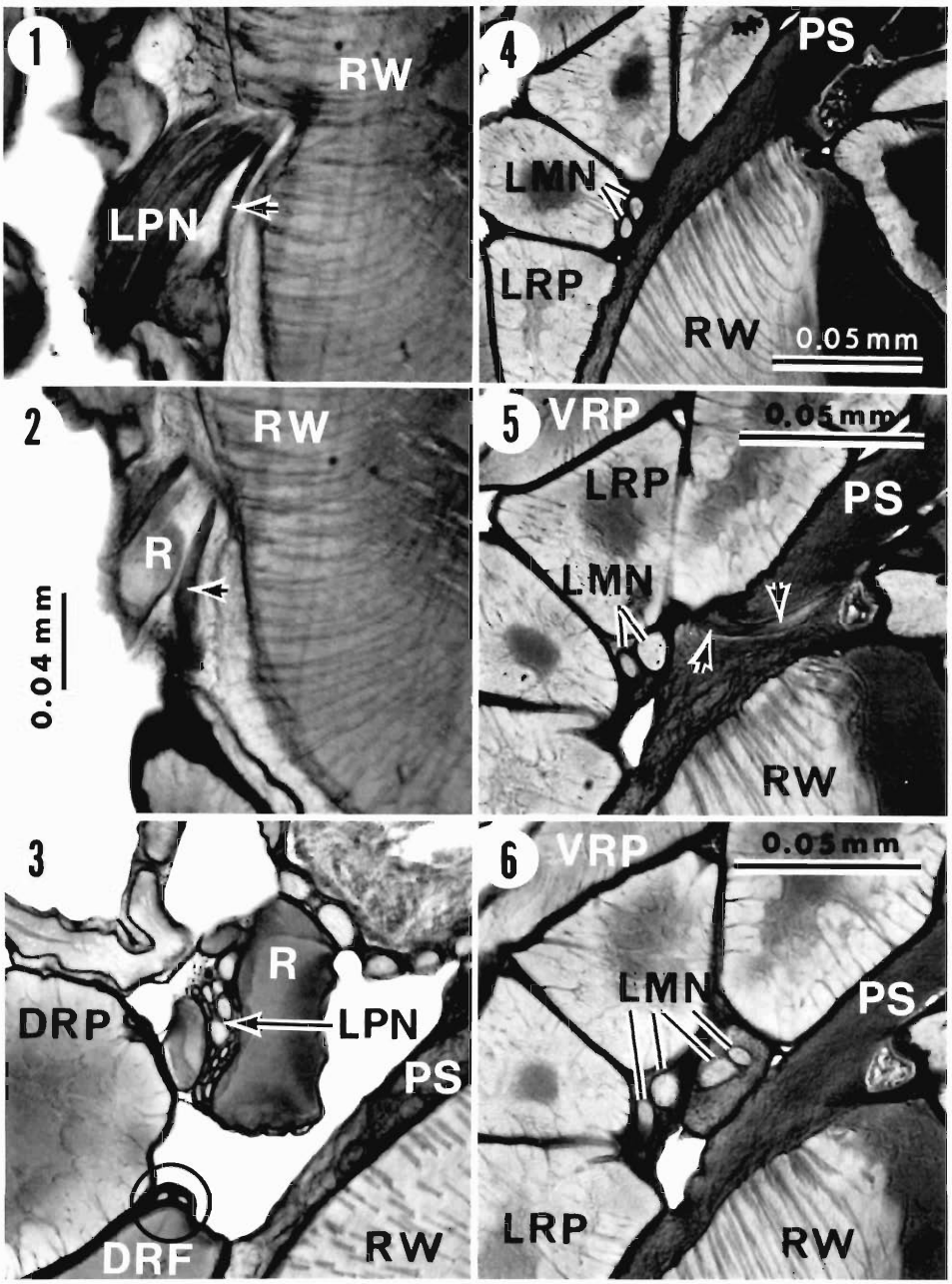
Materials and Methods

Macracanthorhynchus hirudinaceus was obtained through the courtesy of Swift Fresh Meats Company in East St. Louis, Illinois. Following removal, the worms were transported to the laboratory in Dewar flasks containing gut contents. Female worms were washed and cleaned in 30% artificial seawater prepared from Instant Ocean synthetic sea salts. Specimens were prepared for routine paraffin embedding by the technique previously described by Dunagan and Miller (1970). Thick (8 μm) sections were prepared and stained by standard methods for H&E and PAS-hematoxylin. Thin sections (1.5 μm) were prepared by fixing specimens in 3% glutaraldehyde in 0.05 M s-collidine buffer at pH 7.2 for 2 hr at 4°C. Osmotic pressure was controlled by completing this process in 4.5% sucrose. The tissue was postfixed at 4°C with 1% osmium tetroxide in 0.05 M s-collidine buffer (pH 7.2) and 0.27 M sucrose. Samples were dehydrated through a series of alcohols and embedded in Epon 812 according to Luft (1961). Serial sections were cut on a Reichert OMU-2 microtome and stained with combinations of PAS and toluidine blue. Sections were studied and photographed using a Leitz light microscope.

The ventral surface was determined according to the position of the cerebral ganglion (Hyman, 1951). The photographs in Figures 1–6 have the ventral surface toward the top of each figure.

Results

Figure 1 shows the separation of the first of two neurites of the posterior dorsal nerve from the lateral posterior nerve (LPN) as the latter exits the receptacle wall muscle and proboscis sheath. Figure 2 shows the exit of the second



Figures 1–6. Cross sections of the praesoma of *Macracanthorhynchus hirudinaceus*. Top of each photograph is toward the ventral surface. 1–2. Arrows identify first and second neurite of posterior dorsal nerve. 3. Circle encloses both neurites of posterior dorsal nerve after their association with DRP and DRF. 4. Position of LMN anterior to cerebral ganglion. 5. Exit of LMN from cerebral ganglion and ascending loop of LMN. 6. Ascending and descending loops of LMN near posterior terminus of cerebral ganglion. Abbreviations: DRF, dorsal receptacle flexor muscle; DRP, dorsal receptacle protrusor muscle; LMN, lateral medial nerve; LPN, lateral posterior nerve; LRP, lateral receptacle protrusor muscle; PS, proboscis sheath; R, retinacular muscle; RW, receptacle wall muscle; VRP, ventral receptacle protrusor muscle. Scale for 1 and 3 is the same as for 2.

neurite from the LPN. Notice that the two fibers exit posterior to the attachment and remain adjacent to the proboscis sheath (PS) a short distance before they join (Fig. 3) the dorsal receptacle flexor muscle (DRF) and the dorsal receptacle protrusor muscle (DRP). As the neurites continue posteriorly, they separate and each one is restricted to one of the above mentioned muscles. These nerves do not appear to have additional major branches.

Figure 4 shows one of the pair of lateral medial nerves (LMN) adjacent to the proboscis sheath as viewed anterior to the cerebral ganglion. These nerves, which consist of two neurites each, are always in this position and easily identified at least to the level where they are joined by other nerves from the cerebral ganglion. Figure 5 shows the exit (arrows) of the LMN to the outside of the proboscis sheath along the lateral margin of the cerebral ganglion 50–100 μm posterior to its anterior terminus. The exit of the same nerve on the opposite margin was always slightly offset from its counterpart. Once outside the proboscis sheath this nerve makes a long posterior loop that turns in an anterior direction near the posterior terminus of the cerebral ganglion. The LMN observed in cross section in Figures 4 and 5 are the ascending neurites as are those furthest from the PS in Figure 6. Figure 6 shows both the descending and ascending pairs of nerves. The descending pair are adjacent to the PS. This nerve moves anteriorly along the medial surface of the ventral receptacle protrusor muscles and adjacent to the proboscis sheath. Some distance anterior to the ganglion this pair of nerves is joined by neurons from the anterior ventral nerve and the anterior lateral nerve, and together they proceed to the level of the lateral sensory organ. As these additional components are added, it becomes increasingly difficult to maintain the identity of any one component throughout its length. However, it appears that the LMN does not service the lateral sensory organ but continues anteriorly to the proboscis musculature.

Discussion

Reviews of the nervous system of Acanthocephala (Hyman, 1951; Bullock and Horridge, 1965) indicated that *M. hirudinaceus* was one of the most extensively studied. Nevertheless, the literature on the nervous system of this worm consists of less than a dozen articles published in the last half century and not many more than that in the century before 1930. The earlier literature tended to lean more heavily on rhetoric rather than graphic descriptions thus making comparative interpretations of the system most difficult.

Brandes (1899) is the most quoted reference on the nervous sytem of *M. hirudinaceus* but we have found the extensive work of Kaiser (1893) to be more useful. In that study, Kaiser reported that two nerve fibers separated from the lateral posterior nerve soon after it penetrated the proboscis sheath. His figure 1 (plate 1) identified this pair of neurites as "nervus dorsalis posterior." They correspond to the pair of neurites that we have circled in Figure 3. The arrows in Figures 1 and 2 clearly show each of these neurites separating from the main bundle as the lateral posterior nerve penetrates the PS, an event not depicted by Kaiser or others. Kaiser (plate 1, fig. 8) also illustrated one of the neurites of this pair in a section anterior to the cerebral ganglion as being on the outside dorsal surface of the dorsal receptacle protrusor muscle. This suggests that one of the neurites in each pair either divides with a branch anterior and posterior or that one turns anteriorly shortly after exiting the PS. We believe that neither of these occurs. Sections that we have examined show these two neurites associating with the dorsal receptacle muscles. They follow either the DRP or DRF posteriorly and disappear near the posterior terminus of the praesoma. We have not been able to find evidence that they service any ventral protrusor muscle as stated by Kaiser (1893, p. 11).

The lateral medial nerve has been identified by several different names. Fortunately, this paired nerve can easily be located in the vicinity of the ganglion and when illustrated, it can readily be recognized. Kaiser (1893) most frequently used the term "nervus lateralis anterior" for this nerve but also used "vordere Seitennerven." Rauther (1930) used "vordere Seiten-Nerven" and Brandes (1899) used "Nervilaterales mediales" which Hyman (1951) translated as lateral medial nerve. Dunagan and Miller (1970) also used lateral medial nerve and indicated that this nerve appears from the lateral edge of the ganglion. That all of these terms refer to the same nerve seems reasonably certain when one compares the illustrated material. However, it does raise a question of what Kaiser meant his "mittlere Seitennerven" to represent which he stated were poorly developed. Bullock and Horridge (1965) presented Brandes' (1899) drawing of the nervous system of *M. hirudinaceus*. This draw-

ing depicted the lateral medial nerve as an anterior branch of the lateral posterior nerve. We believe the illustrator made a simple mistake by not extending the identifying line to the proper nerve originating from the ganglion. We appreciate how easily these mistakes can occur.

Figures 4–6 clearly show the general position of the LMN in relation to surrounding structures. The extent of the loop may reflect the position of the praesoma or the state of contraction of the proboscis. There has always been a posterior loop in the material we have examined. As the LMN extends anteriorly, it is joined by the sensory support cell (SSC) duct and neurites from the anterior lateral and anterior ventral nerve. This association makes a large collection of 11 neurites and one SSC duct. Maintaining the identity of each neurite as it proceeds anteriorly cannot always be done with assurance. However, we believe that one and perhaps both of the neurites in each LMN extend beyond the lateral sensory organ to service the anterior proboscis musculature.

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Anterior Lateral and Anterior Ventral Nerves from the Cerebral Ganglion of *Macracanthorhynchus hirudinaceus* (Acanthocephala)

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ABSTRACT: The paired anterior lateral nerves and the anterior ventral nerves exit the ventral surface of the cerebral ganglion of *Macracanthorhynchus hirudinaceus* near its anterior terminus. Each anterior ventral nerve contains seven neurites that proceed anteriorly from the ganglion along the medial surface of the proboscis sheath. These nerves penetrate the proboscis sheath at the level where the anterior ducts of the sensory support-cell reach the lateral extremity of the midventral longitudinal receptacle muscle. Once outside the sheath they follow the sensory support-cell ducts to the lateral sensory organ. Most or all of the seven neurites enter the sensory organ and service this receptor. Each of the paired anterior lateral nerves consists of a large and small neurite. These originate posterior to the anterior ventral nerves. They penetrate the proboscis sheath as well as the midventral longitudinal receptacle muscle before coming to lie medial to the sensory support-cell body. They move to the anterior lateral margins of this cell and eventually follow its anterior extensions to the level of the lateral sensory organ. The smaller neurite of the anterior lateral nerve is frequently flattened and difficult to see in sectioned material.

Kaiser (1893) reviewed the early literature regarding major nerves associated with the cerebral ganglion of *Macracanthorhynchus hirudinaceus*. He described the following nerves: "Vonderer Mediannerv, Nervus ventralis anterior, vorderer Seitennerven (Nervus lateralis anterior), mittlere Seitennerven, hinteren Seitennerven." Rauther (1930) listed this same group of nerves for *M. hirudinaceus* but called the "Nervus ventralis anterior" the "Ventral nerve." Dunagan and Miller (1970) called this paired nerve the anterior ventral nerve and so labeled it in figure 3 indicating in the text that each nerve consisted of seven neurites. Unfortunately, they switched labels for the anterior lateral and anterior ventral (or ventral anterior) nerve in figure 5. Kaiser (1893) illustrated (plate 1, figs. 5, 8, 18) these two separate but paired nerves under the same label—"nervus ventralis anterior."

The purpose of this paper is to clarify the status of these two paired nerves by showing that they originate from separate sites in the cerebral ganglion as well as differ in their initial movement in relation to surrounding tissue.

Materials and Methods

Macracanthorhynchus hirudinaceus was obtained through the courtesy of Swift Fresh Meats Company in East Saint Louis, Illinois. Following removal, the worms were transported to the laboratory in Dewar flasks containing gut contents. Female worms were washed and cleaned in 30% artificial seawater. Serial sections (8 μm) were prepared after routine paraffin embedding as previously described by Dunagan and Miller (1970). Staining of these sections was accomplished by standard methods for H&E and PAS-hematoxylin. Serial sections (1.5 μm) were also prepared by fixing specimens in 3% glutaraldehyde in 0.05 M s-collidine buffer at pH 7.5 for 2 hr at 4°C. Osmotic pressure was controlled by completing this process in 4.5% sucrose. The tissue was post-fixed at 4°C with 1% osmium tetroxide in 0.5 M s-collidine buffer (pH 7.5) and 0.27 M sucrose. Tissues were dehydrated through a series of alcohols and embedded in Epon 812 according to Luft (1961). Serial sections were cut on a Reichert OMU-2 microtome and stained with combinations of PAS and toluidine blue.

The ventral surface has been determined according to Hyman (1951). That surface closest to the cerebral ganglion has therefore been designated as the ventral surface. In all figures the ventral surface is toward the top.

Results

Figures 1–26 are a series of drawings made from photographs of serial sections of the praesoma of *M. hirudinaceus*. They show the special relationship of the anterior lateral (ALN) and anterior ventral nerve (AVN) to the proboscis sheath (PS), midventral longitudinal receptacle

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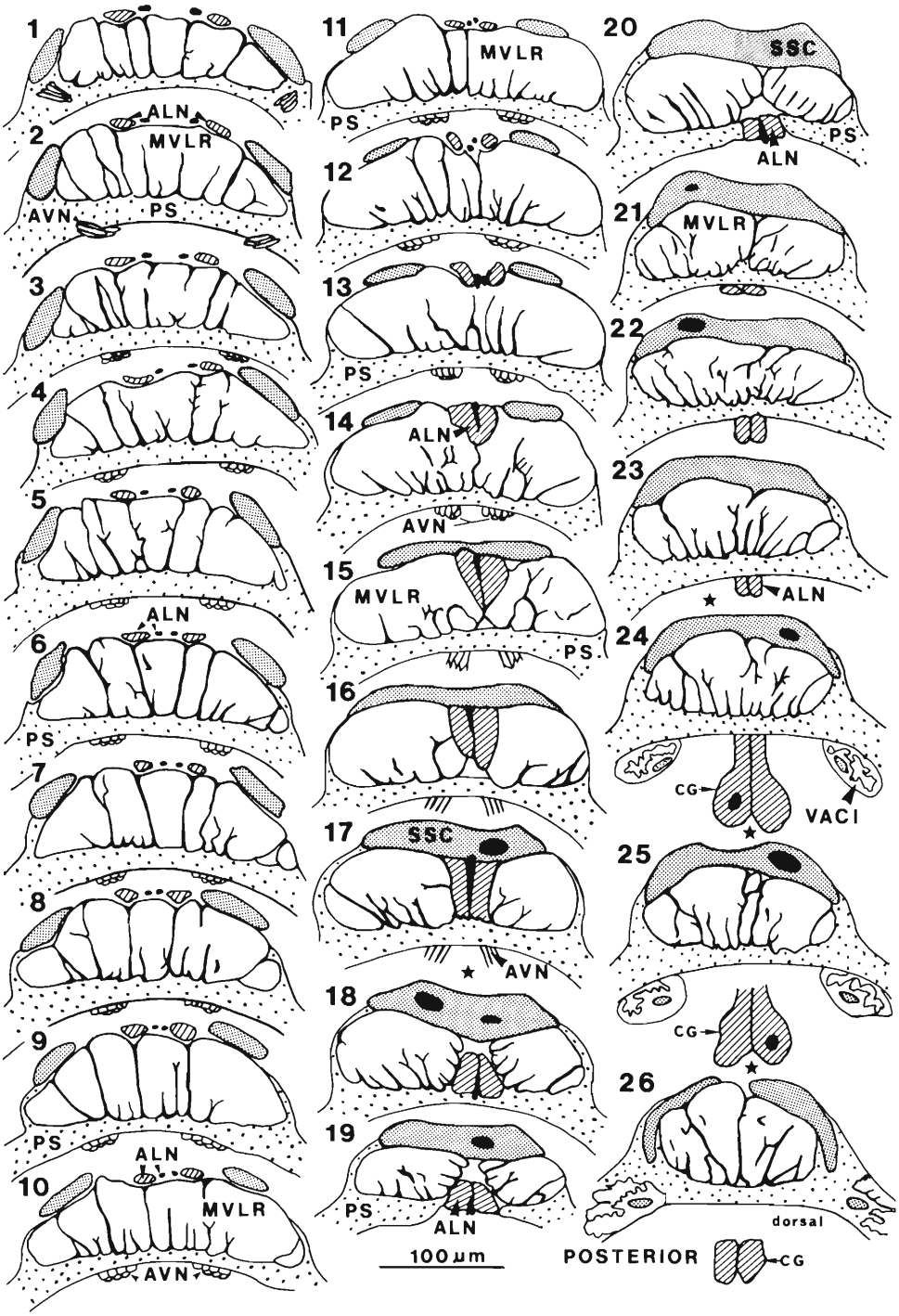
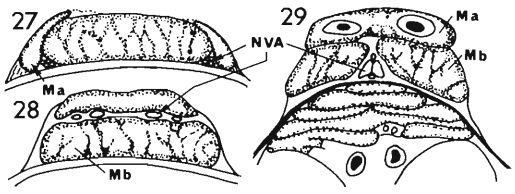
Figures 1–26. Diagrams of 8 μm cross sections of a portion of the praesoma beginning at the level of the anterior cerebral ganglion and extending anteriorly in *M. hirudinaceus*. The ventral surface is toward the top of



each figure. 1, 2. Anterior ventral nerve (AVN) penetrating proboscis sheath along lateral margins of midventral longitudinal receptacle muscle (MVLR). 1–12. Movement of anterior lateral nerve (ALN) toward midventral plane. 13–20. Penetration of MVLR by ALN. The AVN leaves the cerebral ganglion (CG) and moves to the proboscis sheath (PS) in Figure 17. 18–20. ALN penetrating the PS. 21–26. Posterior movement of larger neurite of ALN into an appropriate cell of CG. VACI, ventral apical cone invertor muscles; star, a deleted section in the sequence.



Figures 27–29. Figures redrawn from Kaiser, 1893. 27. Represents plate 1—figure 18. 28. Represents plate 1—figure 16. 29. Represents plate 1—figure 8. Notice that Kaiser depicted both the larger and the smaller neurite in each of the paired anterior lateral nerves that he designated as NVA. Note also that he identified the anterior ventral nerve by the same designation. Ma, Aeusserer Deckmuskel des Receptaculum (sensory support-cell body); Mb, Innerer Deckmuskel des Receptaculum (midventral longitudinal receptacle muscle).

muscle (MVLR), and sensory support-cell (SSC). Other muscles occurring in the area have been deleted in order to conserve space. Note that the ALN forms adjacent to the PS but more posteriorly than the AVN. The ALN penetrates both PS and MVLR along the midventral plane whereas the AVN never penetrates the MVLR and penetrates the PS lateral to the midventral plane.

Figures 1–10 show a gradual movement of the ALN over the ventral surface of the sensory support-cell and toward the midventral plane. Each ALN is clearly composed of one large and one small neurite with the smaller neurite reaching the midventral point first. This movement over the surface of the MVLR is in concert with that of the anterior ducts of the sensory support-cell. Figures 1, 2, and 31 show the initial penetration of the PS by the AVN. More anteriorly than Figure 1, the AVN will complete its penetration and come to lie outside the PS but medial to the sensory support-cell duct. This can be clearly followed in Figures 30–32. There are seven neurites in each of the AVN. Two of these neurites seem to move more ventrally than the remainder and come to lie along the lateral ventral surface of the MVLR (Fig. 30). However, they all eventually follow the same course to the lateral sensory organ.

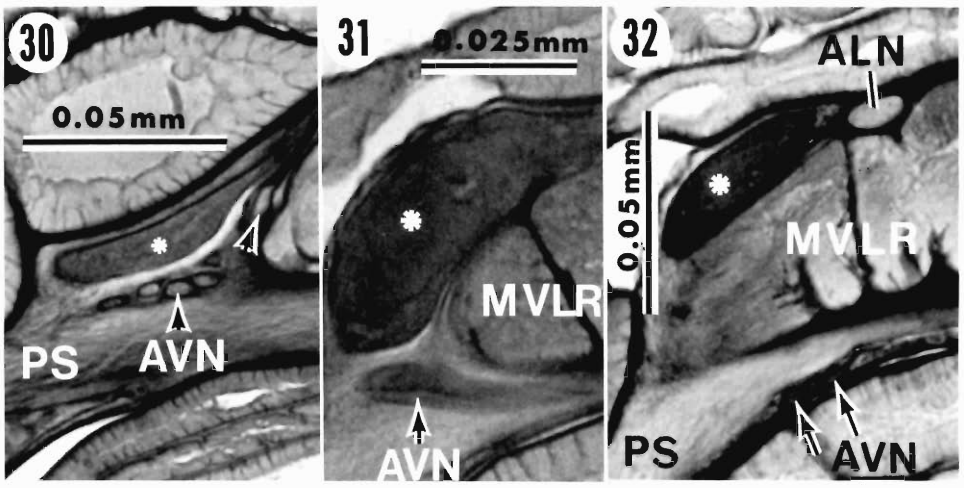
Figures 11–19 show the penetration of the MVLR by the ALN. The smaller neurite frequently blends so well with its larger counterpart that they are difficult to distinguish as separate components. Note that complete penetration does not occur until after the ducts of the anterior sensory support-cell have fused into the SSC body. These sections also show that the AVN follows the midventral medial surface of the PS posteriorly until they enter the cerebral ganglion (Figs. 16–17). Note that the ALN has moved away from the PS prior to the AVN's entry into the PS.

Figures 19 and 20 show the entry and penetration of the PS by the ALN. We have been unable to discern clearly the course taken by the smaller components once they are inside the PS. Indeed, they do not appear in Figures 21–26. Figures 24–26 show the very large cerebral ganglion cells in which the larger neurites of the ALN terminate. Notice that in this specimen these cells are located at the posterior terminus of the SSC body.

Figures 27–29 are redrawn after Kaiser (1893) corresponding as follows: 27, plate 1—figure 18; 28, plate 1—figure 16; 29, plate 1—figure 8. Note that Kaiser labeled both the ALN and AVN as NVA (Nervus ventralis anterior).

Discussion

Kaiser (1893) illustrated the paired "Nervus ventralis anterior" (NVA; Fig. 27) as adjacent to the proboscis sheath (Sarcolemmahüllmembran des Receptaculum) and between the "Aeusserer und Innerer Deckmuskel des Receptaculum." It seems obvious from this drawing that there are several fibers in each of these nerves. Kaiser also illustrated this same nerve (NVA; Figs. 28, 29) as a paired nerve of two fibers each consisting of one large and one small neurite. Since Figure 29 is closer to the cerebral ganglion (CG) than Figure 28, Kaiser was showing the penetration of the "Innerer Deckmuskel des Receptaculum" (see also his statement on p. 9) and the subsequent movement of this nerve between the inner and outer muscle group. Kaiser also stated (p. 9) that the NVA repeatedly divided so that it eventually formed 10–15 fibers. We believe that Kaiser combined two different nerves under the designation NVA. Hyman (1951) used Brandes (1899) as a guide and called this the ventral anterior nerve. Dunagan and Miller (1970) designated the nerve penetrating the midventral longitudinal receptacle muscles (MVLR) (Innerer Deckmuskel des Receptaculum) as the anterior lateral nerve (ALN) but made no attempt to clarify Kaiser's descriptions further. Figures 1–26 clearly show that Kaiser was dealing with two groups of nerves. The ALN originates more posteriorly on the ganglion than does the anterior ventral nerve (AVN) (Nervus ventralis anterior, Ventral anterior nerve) and moves only a short distance before obliquely



Figures 30–32. Photographs of sections from the praesoma of *M. hirudinaceus* showing the penetration of the proboscis sheath (PS) by the anterior ventral nerve (AVN). Note that this nerve does not penetrate the midventral longitudinal receptacle muscle (MVLR). 30. AVN medial to sensory support-cell duct but outside PS. 31. Penetration of PS by AVN. 32. AVN inside PS just prior to its penetration of this sheath.

penetrating the proboscis sheath and then the MVLR. The AVN originates more anteriorly on the CG and continues anteriorly along the medial surface of the PS for a distance of 100 or more micrometers before penetrating the PS (Figs. 30–32). This nerve never penetrates the MVLR. After moving outside the PS the seven neurites of the AVN come to lie between the anterior ducts of the sensory support-cell (Aeusserer Deckmuskel des Receptaculum) and the MVLR. The seven neurites of the AVN and the two neurites of the ALN follow the SSC duct along the outside of the PS. At this level this group can easily be interpreted as 10 neurites belonging to a single nerve. Kaiser apparently overlooked the penetration of the PS (Fig. 31) by the AVN and explained the large number of neurites anterior to this penetration as the result of division of the "NVA." Interestingly, the lateral medial nerve also joins this group more anteriorly forming a group of 11 neurites and one SSC duct. We believe that all of the AVN neurites service the lateral sensory organs. The larger of the two neurites from each ALN divides prior to reaching the level of the lateral sensory organ and disappears into muscle. The destination of the smaller neurite is unclear.

The terminology used for muscles has been based on Dunagan and Miller (1974); however, the change in designation of the "Aeusserer Deckmuskel des Receptaculum" is based on our recent discovery (Miller and Dunagan, 1983) that this tissue was not muscle but a multinucleated cell that we designated the sensory support-cell. The anterior lateral extensions of this cell go to the lateral sensory organs and give them their general form. It is these extensions that are followed by the AVN and ALN.

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Angularella audubonensis sp. n. (Dilepididae) and Other Cestodes of Cliff Swallows in Colorado

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ABSTRACT: One hundred forty-five cliff swallows, *Petrochelidon pyrrhonota,* were examined for cestodes in two separate surveys in Weld County, Colorado. Seven species representing four genera were found. *Angularella audubonensis* sp. n. is differentiated by the small size of the rostellar hooks (8.4–13.5 μm) and the medial relationship of the cirrus pouch to the osmoregulatory canals. Other species collected were *Angularella beema, Anonchotaenia globata, Mayhewia ababili, Vitta magniuncinata, Vitta parvirostris* and *Vitta riparia.* Seven new hosts and geographic distribution records were established for Colorado, and six new records were determined for North America.

Only one paper (Kayton and Schmidt, 1975) has been published on helminths of the cliff swallow, *Petrochelidon pyrrhonota.* That study, however, omitted cestodes. In order to determine a more complete picture of parasitism of this bird in Colorado, we examined 145 swallows for cestodes and report the results herein.

Materials and Methods

Cestodes were relaxed in tap water, fixed in AFA or 10% formalin, and stained with acetocarmine.

Illustrations were completed with the aid of a camera lucida. All measurements are in micrometers unless otherwise indicated.

Seven species of cestodes were identified: *Angularella audubonensis* sp. n., *Angularella beema* (Clerc, 1906) Strand, 1928; *Anonchotaenia globata* Linstow, 1879; *Mayhewia ababili* (Singh, 1952) Yamaguti, 1956; *Vitta magniuncinata* Burt, 1938; *Vitta parvirostris* (Krabbe, 1869) Baer, 1959; and *Vitta riparia* (Dubinina, 1953) Spasskaya, 1966. All were new host records for the cliff swallow, and Colorado, and all except *A. globata* are new for North America. One species of *Angularella* Strand, 1928 is new to science and forms the basis of the following description.

Angularella audubonensis sp. n. (Figs. 1–5)

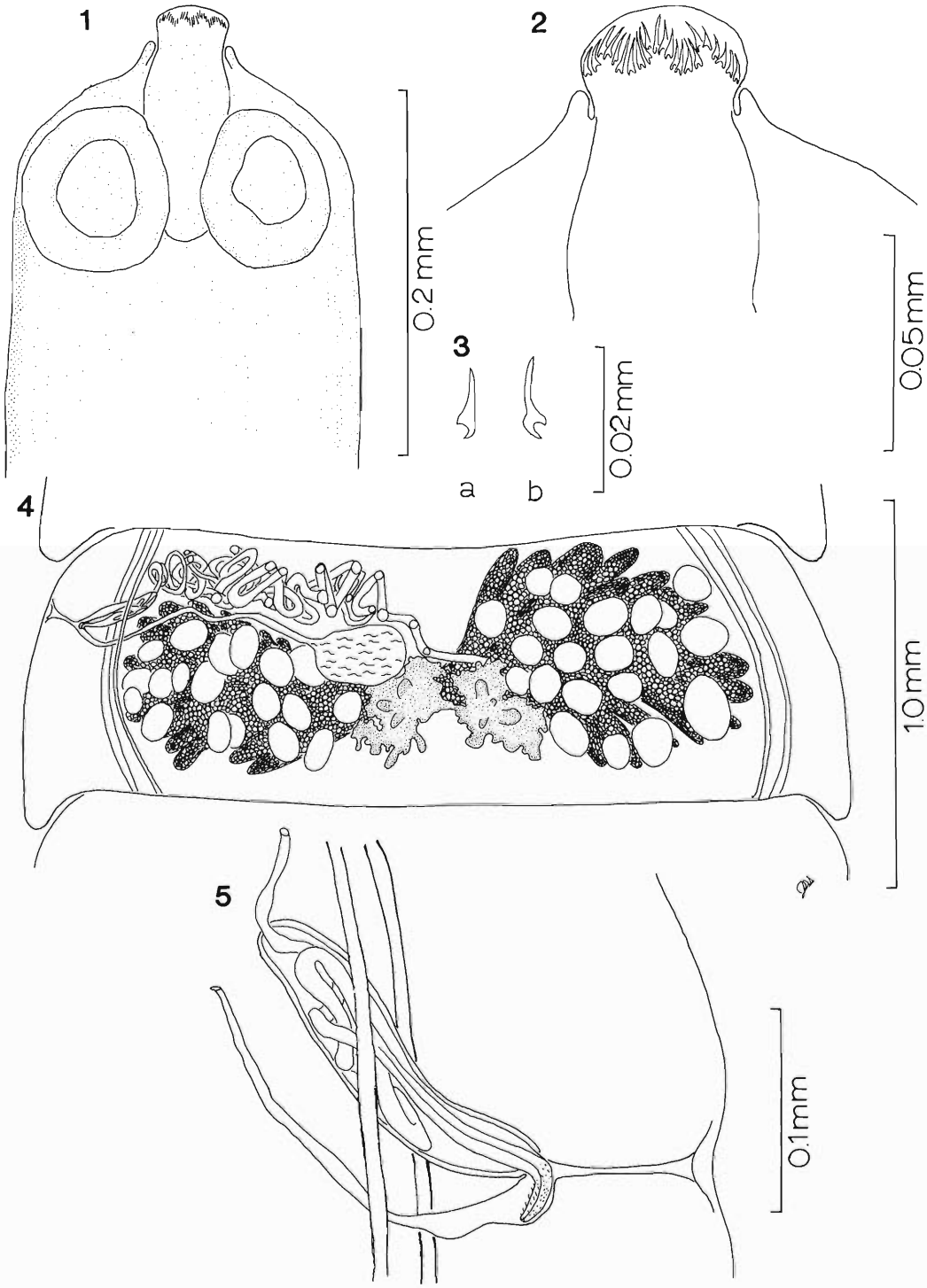
Five complete specimens and several fragments were recovered from the small intestines of seven of 145 cliff swallows collected from May–August 1978, 1979, 1980.

DESCRIPTION: Dilepididae, Dilepidinae. Length of strobila about 44 mm long, 196 wide at base of scolex and reaching a maximum width of about 1,800 ($N = 5$) in the posterior gravid proglottids. Proglottids craspedote, wider than long. Scolex (Fig. 1) 186–210 ($N = 5$) at level of suckers. Four muscular, aspinose suckers measure 76–102 long by 72–115 wide ($N = 14$); circular to oval in shape. Rostellum (Fig. 2) 39–41 wide at apex, 93–110 long ($N = 5$), armed with 52–56 hooks ($N = 2$) arranged in zigzag formation in a single row. Hooks (Fig. 3) 8.4–13.5 ($N = 60$) long with the longer hooks at the posterior position of the zigzag and shorter hooks at the anterior position. Undifferentiated neck region measuring 50–104 ($N = 5$) from base of rostellar pouch to first segmentation (Fig. 4). Genital pores alternate irregularly, in anterior third of proglottid. Cirrus pouch (Fig. 5) muscular, double-walled, 165–200 long, 40 ($N = 65$) maximum width. Cirrus pouch passes between osmoregulatory canals at approximately its midpoint. Cirrus armed with minute spinules less than 1 in length. Vas deferens highly convoluted, extending to midline of anterior third of proglottid. Testes number from 26–47; ovoid, 37–40 wide by 60–70 ($N = 170$) long and are interspersed throughout proglottid, rarely anterior to ovary or vagina. Vagina posterior to cirrus pouch and vas deferens, 100–116 ($N = 70$) long, about 5 wide, curving slightly posteriad between dorsal and ventral osmoregulatory canals. Vagina expands into prominent, oblong seminal receptacle 210–211 long, 79–86 ($N = 70$) wide. Seminal receptacle slightly poral and medial, anterior to vitellarium. Ovary bilobed, often occupying most of mature proglottid, sometimes with fingerlike projections extending past the osmoregulatory canals. Vitellarium lobated, 277–313 wide, 116–121 ($N = 70$) long, posterior to seminal receptacle between lobes of ovary. Uterus sac-like, developing quickly, occupying entire proglottid when gravid. Eggs 23–31 long by 14–23 ($N = 70$) wide.

HOST: *Petrochelidon pyrrhonota* (Veillot, 1817) (Passeriformes: Hirundinidae).

LOCALITY: Weld County, Colorado.

HABITAT: Duodenum.



Figures 1–5. ***Angularella audubonensis*** **sp. n. from** ***Petrochelidon pyrrhonota.*** **1. Scolex, dorsoventral view. 2. Rostellum with hooks. 3. Hooks. a—small hook; b—large hook. 4. Mature proglottid. Large oval structures are testes. 5. Close-up of cirrus pouch and vagina from mature proglottid.**

Table 1. Characteristics of *Angularella* spp. (data after original authors).

Characteristic	*A. beema* (Clerc, 1906) Strand, 1928	*A. hirundina* (Fuhrmann, 1907) Spasskaya and Spasskii, 1971	*A. urbica* Spasskaya and Spasskii, 1971	*A. ripariae* Yamaguti, 1940	*A. audubonensis* sp. n.
Number of hooks	56–66	56	48	40	52–56
Length of hooks	22–29	19–20	18–23	27	8.4–13.5
Number of testes	20–42	28	45	32–45	26–47
Cirrus pouch	133–175 × 39	117–140 × 28	247 × 33	75–90 × 33–63	165–200 × 40
Uterus	Sac-like, lobated	Lobated	Lobated margins	Sac-like, filling entire segment	Sac-like, filling entire segment

TYPE SPECIMENS: USNM Helm. Coll. holotype no. 77136, paratype no. 77137.

ETYMOLOGY: The species is named in honor of the Greeley, Colorado Audubon Society, who encouraged and helped fund this research.

REMARKS: *Angularella audubonensis* differs from four species, *A. beema* (Clerc, 1906) Strand, 1928; *A. hirundina* (Fuhrmann, 1907) Spasskaya and Spasskii, 1971; *A. urbica* Spasskaya and Spasskii, 1971; and *A. ripariae* Yamaguti, 1940, by the size of its rostellar hooks, those being much smaller (8.4–13.5 μm) than any reported from the other species (Table 1). The cirrus pouch passes between the dorsal and ventral osmoregulatory canals and extends medially past them, whereas those of all the other species are completely poral to the canals. *Angularella audubonensis* most closely resembles *Angularella beema* in the number and arrangement of hooks, number of testes, and the highly convoluted nature of the vas deferens.

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Biology of *Archigetes* (Caryophyllaeidae) in *Limnodrilus hoffmeisteri* (Tubificidae)

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ABSTRACT: The progenetic caryophyllid *Archigetes sieboldi* cycles in the annelid *Limnodrilus hoffmeisteri* in the Kinnickinnic River in Wisconsin. A high mortality rate occurred in procercoids experimentally reared in the oligochaete host. Of 221 infected hosts, 160 (72%) lost their infections. Only seven (0.9%) of 821 parasites survived between 81 and 111 days which was long enough to produce eggs. Maximum survival of any infected annelid was 168 days. Early survival of procercoids in this self-fertilized species is compared to that of *Archigetes iowensis,* presumed to undergo cross-fertilization in the carp host.

High population densities of *L. hoffmeisteri* may be required to support *A. sieboldi* in nature. Populations of this annelid in the Kinnickinnic River averaged 13,023/sq. m. Three rivers where *A. sieboldi* was not found had the following *L. hoffmeisteri* populations: Iowa, 815/sq. m, Mississippi 419/sq. m and Red Cedar, 368/sq. m. A progenetic population of *A. iowensis* is reported from the Kinnickinnic River.

The caryophyllid cestode *Archigetes sieboldi* Leuckart occurs in Asia, Europe, South America and in North America, from the Kinnickinnic River in Wisconsin. This progenetic species apparently depends primarily upon oligochaete annelids for egg production and is either absent or rare in fish (Wisniewski, 1930; Nybelin, 1962; Calentine and DeLong, 1966).

Archigetes iowensis Calentine is known from carp (*Cyprinus carpio* L.) and oligochaetes (*Limnodrilus hoffmeisteri* Claparède) from the Iowa River (Calentine, 1964) and the Red Cedar River in Wisconsin (Williams, 1979 and personal communication regarding its presence in carp). Calentine (1964) suggested the occurrence of two strains of *A. iowensis,* one utilizing both annelid and carp hosts, with the second strain producing eggs in oligochaetes, but not infecting fish. Kennedy (1965a, b) in Britain, studied *A. limnodrili* (Yamaguti) in oligochaetes, but could not find this parasite in fishes. Mackiewicz (1981) discussed evolution and classification of caryophyllid cestodes with considerable emphasis on the biology of *Archigetes.* The present report involves host–parasite interactions and aspects of population biology for *Archigetes* and the annelid *L. hoffmeisteri.*

Materials and Methods

The lower impoundment of the Kinnickinnic River no longer supports an annelid population of *A. sieboldi.* Sufficient specimens for this study were recovered from *L. hoffmeisteri* in the upper impoundment of this river (Pierce Co., corporate limits, River Falls, Wisconsin) during the springs of 1979 and 1980. Other Tubificidae present, but not infected, were *Limnodrilus udekemianus* Claparède, *Tubifex tubifex* Müller, *Ilyodrilus templetoni* (Southern), and *Peloscolex multisetosus* Smith.

Annelids were also collected from rivers with similar habitats: Red Cedar (Colfax, Wisconsin), Iowa (Alden, Iowa) and Mississippi (Diamond Bluff, Wisconsin and 4.8 km upstream). No *A. sieboldi* were found in these sites.

Annelids for population density studies were collected with a 10-cm (Kinnickinnic upper impoundment) or 15-cm (all other sites) diameter can pushed 15 cm into the substrate in water 30–40 cm in depth. Contents were washed through a 60-mesh brass screen. Annelids were sorted while alive and identified with a compound or dissecting microscope. Results are reported per square meter of substrate. Immature *Ilyodrilus* and *Tubifex* cannot be differentiated. In the upper Kinnickinnic impoundment, immature Tubificidae with hair setae are reported as 87% *T. tubifex* and 13% *I. templetoni* since these were the percentages of sexually mature forms found.

Laboratory-reared oligochaetes, 4–8 mm in length, were exposed to embryonated cestode eggs in autoclaved mud medium for 24 hr. All annelids were then removed and examined individually with 100× of a compound microscope with slight coverslip pressure. The number of parasites present was determined and all hosts containing the same number of parasites were cultured in separate containers. Annelids were examined at approximately 14-day intervals to determine survival rates of parasites and hosts.

Thirty-eight carp were obtained by seining the Kinnickinnic River lower impoundment in June 1980. *Atractolytocestus huronensis* Anthony and *Khawia iowensis* Calentine and Ulmer were common, but only one fish harbored *A. sieboldi.* Ten mature and gravid cestodes were found free in the intestinal contents of this one fish. This area was drained in March 1981 (for the entire summer) and only 12 carp (5–10 cm in length) were obtained. All of these fish were negative for *A. sieboldi.* The use of seines and baited hoop nets failed to yield carp in the upper impoundment in May 1982.

Twenty-seven carp were obtained with hoop nets and with bow and arrow from the Mississippi River

at Diamond Bluff, Wisconsin and near Hastings, Minnesota (11–24 km downstream from the mouth of the Kinnickinnic River) during three collections in May 1982. Again, *A. huronensis* and *K. iowensis* were relatively common, but no *Archigetes* were present.

Eggs of *A. iowensis* were recovered from 63 cestodes present in one of four carp seined from the Iowa River (Alden, Iowa) in June 1982, and from *L. hoffmeisteri* in the Kinnickinnic River during August 1982.

Egg counts on gravid *A. iowensis* were conducted by dissecting individual specimens in a depression slide and transferring this material to three or four grid slides prepared with mm-ruled graph paper.

Results and Discussion

Archigetes sieboldi

Eight feeding experiments conducted with *A. sieboldi* resulted in 821 parasites (24 hr post-exposure) in 221 *L. hoffmeisteri*. Parasite burden ranged from 1–18 worms/host at that time. Sixty-two (28%) were single infections. Of these 221 annelids, 160 (72%) lost their infections by death of the developing procercoids. Death of most larvae occurred in the first 50 days. In five cultures examined at 30 days, 106 (64%) of 157 hosts had lost their parasites. Only seven (0.9%) of the 821 parasites survived between 81 and 111 days which was long enough to produce eggs. The seven gravid procercoids occurred in three infected hosts, each of which had five or more parasites at the beginning of the experiment. No parasite in single infections ever became gravid. Maximum survival of any infected annelid was 168 days. Forty-seven (21%) of the infected hosts perished during the experiments (168 days). However, death of uninfected annelids in control cultures was normally about 50% by 213 days.

At one day postfeeding, most oncospheres occurred in the coelom of the annelid in the posterior one-third of the body. By 39 days, most surviving larvae had migrated anteriorly, localizing in the posterior seminal vesicle. After this time, procercoids were found in the coelom only if the seminal vesicles were occupied by other parasites. No parasite ever matured in the coelom.

Visible damage to the oligochaete became evident as the parasites neared maturity. The anterior of the body swelled and body wall tissues weakened. Coelomocytes commonly accumulated in great numbers and destruction of the host's reproductive organs occurred if mature or gravid procercoids were present. Death of the host invariably took place as the tapeworms became mature or gravid by rupture of the weakened body wall, resulting in release of the cestodes to the environment. This is similar to Kennedy's (1965a) results with *A. limnodrili*. He found that multiple infections usually resulted in the death of most procercoids, but that some oligochaetes survived rupture of the body wall as the parasites became mature and gravid.

Archigetes sieboldi eggs would likely not be viable if the cestodes depended upon natural death of the annelid. Oligochaetes over 30 days old are usually refractile to infection. Caryophyllid eggs remain viable only 80–90 days at 20°C. Some *L. hoffmeisteri* have survived over 2 yr in laboratory cultures. Death of the annelid host as the parasite becomes gravid is apparently necessary for the survival of this species. The scarcity of *A. sieboldi* in carp seems to indicate that the few specimens occasionally found in carp are simply parasites of annelids eaten by the fish. It is not known whether these cestodes remain in carp long enough for eggs to be produced by cross-fertilization. Bottom feeding fishes probably disseminate *Archigetes* eggs.

The cause of the high procercoid mortality of *A. sieboldi* has not yet been identified. *L. hoffmeisteri* exhibited no successful humoral or cellular response to six other caryophyllid species where eggs hatched and oncospheres penetrated the intestinal wall into the coelom (Calentine et al., 1970). No cellular response was evident in early stages of *A. sieboldi* infections. A humoral response is apparently present.

Four caryophyllid species are known to produce eggs by parthenogenesis (Mackiewicz, 1981). Species of *Archigetes* producing eggs in annelids apparently undergo self-fertilization. A layer of larval tegument covers the genital pore preventing the entrance of spermatozoa from another worm. Abundant sperm were evident in the vas deferens, seminal vesicle and vaginal pathway of sectioned material of both *A. sieboldi* and *A. iowensis* from annelids. Motile sperm were observed in the seminal receptacle of progenetic *A. iowensis*.

A. iowensis

The host–parasite relationships between *A. iowensis* (from fish hosts) and *L. hoffmeisteri* are quite different from those of *A. sieboldi*. Two feeding experiments (cestode eggs derived from parasites of Iowa River carp) resulted in 192 parasites in 98 annelid hosts. After 30 days, only six (6%) of the 98 hosts had lost their infections. Calentine (1964) found 40 (38%) of 115 annelids infected with *A. iowensis* survived at least 200

Table 1. Tubificid population data.

	Mean number of oligochaetes/sq. m		
	Total Tubificidae	*T. tubifex*	*L. hoffmeisteri*
Kinnickinnic River			
Upper impoundment, *A. sieboldi* site, 4/81 through 5/82, $N = 13$*	22,999	8,754	13,023
Upstream channel, *A. iowensis* site, $N = 3$	10,701	†	4,204
Lower impoundment, $N = 2$	331	‡	276
Red Cedar River, $N = 3$	404	‡	368
Mississippi River, $N = 5$	739	‡	419
Iowa River, $N = 5$	883	0	815

* N = number of samples.
† A single immature specimen which could be either *Ilyodrilus* or *Tubifex*.
‡ All Tubificidae with hair setae were immature.

days and some as long as 788 days. None of these procercoids produced eggs during this time. Since carp are infected with *A. iowensis* only during April through June, it is important that the annelid host survive until the following spring when fish acquire their infections.

Archigetes iowensis was recovered from *L. hoffmeisteri* in backwater channel areas of the Kinnickinnic River during the present study. In August 1982, six (0.4%) of 1,476 *L. hoffmeisteri* were infected. Of 24 cestodes recovered from additional annelids, 22 (92%) were gravid. Five cestodes in which the eggs filled the body parenchyma to the scolex region had a mean egg count of 792 (485–1,342). After 25 days in water, 167 (43%) of 388 eggs were viable, containing developed oncospheres. In one experiment with these eggs, 33 of 80 immature *L. hoffmeisteri* acquired infections. At 107 days, 22 (67%) of the annelids still harbored infections. At this time, 24 (71%) of the 35 procercoids present were gravid. Immature and mature procercoids occurred only in multiple infections.

The above data definitely indicate the presence of two strains of *A. iowensis,* one cycling from annelid to annelid only while the other requires a carp host to produce eggs. Both strains co-exist in the Iowa and Red Cedar rivers, but only the progenetic form is present in the Kinnickinnic River.

Archigetes and *Limnodrilus hoffmeisteri* populations

As indicated by Mackiewicz (1981) no information is available regarding comparative quantities of eggs produced by *Archigetes* in fish and annelid hosts. Eggs produced by progenetic procercoids in oligochaete hosts would seemingly be fewer than in fish. The annelid host rapidly succumbs as *A. sieboldi* becomes gravid and eggs of *A. iowensis* accumulate within the body parenchyma, causing destruction of the internal organs.

The limited egg production (especially coupled with the extremely high procercoid mortality in *A. sieboldi*) would likely require high annelid host densities to support these parasites in nature. This is supported by quantitative annelid counts in the study areas (Table 1). The mean population estimate for *L. hoffmeisteri* during 13 monthly samples was 13,023/sq. m. The population varied from a low of 7,516 in April 1981 to a high of 32,866 in July 1981. The population mean for *L. hoffmeisteri* in the Kinnickinnic River was 16 times greater than its population in the Iowa River (815), 35 times its population in the Red Cedar River (368), and 31 times that of its population in the Mississippi River (419).

The annelid samples in the Iowa and Red Cedar rivers were taken at sites where gravid *A. iowensis* have been recovered from both carp and annelids. The population of *L. hoffmeisteri* in the upstream channel area of the Kinnickinnic River (where *A. iowensis* has been found only in annelids) was 4,204/sq. m (five times greater than the Iowa River and 11 times greater than the Red Cedar River). Howmiller (1974) found an average of 239 (77–596) *L. hoffmeisteri*/sq. m (with his immature "probables") in 12 inland Wisconsin lakes, including Lake Pepin (174), a

part of the Mississippi River near Diamond Bluff, Wisconsin. Other population estimates for *L. hoffmeisteri* include 923 for Lake Ontario (Hiltenen, 1969) and 172 in central Lake Michigan (Howmiller and Beeton, 1970). Kennedy (1965a) did not indicate population densities of *Limnodrilus* spp. in collection sites for *A. limnodrili.*

Routine annelid collections in 1981 and extensive collections during 1982 have failed to produce a single annelid infected with *A. sieboldi* in the upper impoundment of the Kinnickinnic River. A possible explanation for the apparent disappearance of *A. sieboldi* may be related to a reduced population of *L. hoffmeisteri.* Although quantitative studies were not conducted prior to 1981, a significant decrease seems to have taken place, based upon collections made solely for the recovery of infected specimens. Studies are being continued on the annelid cycle of *A. iowensis* and population densities of *L. hoffmeisteri* in regional waters.

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Studies on the Development of *Dioecocestus acotylus* (Cestoda) with Emphasis on the Scanning Electron Microscopy of Embryogenesis[1]

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ABSTRACT: The embryogenesis of the tapeworm *Dioecocestus acotylus* from the oocyte to the oncosphere is followed by means of scanning electron microscopy, histochemistry, and light microscopy using both glycol methacrylate and paraffin sections. Inferences are drawn from these techniques concerning: (1) the early cleavage and the movement of shell granules with the macromeres, (2) the source of the outer capsule and the formation of the inner capsule (embryophore), (3) the formation and delamination of the oncospheral membrane.

Dioecocestus acotylus Fuhrmann, 1904 is a parasite of the Least Grebe (*Podiceps dominicus*), a host which extends its range northward into South Texas. This worm exhibits the following characteristics that are unusual in the cyclophyllidean cestodes: (1) the dioecious condition with sex organs *completely* separated into two different strobila, (2) the worms occur in pairs—one male and one female in each host, (3) the scolex lacks both suckers and an armed rostellum, (4) the female reproductive system lacks a typical vagina.

In addition, these worms are large (up to 19 cm), ensuring, in the continuum of development from oocyte to oncosphere, numerous representatives of the various stages of development. The embryogenesis of *Dioecocestus* shows no striking deviations from the central theme seen in the other Cyclophyllidea studied, but there are interesting variations.

Earlier studies on the embryogenesis of dioecious tapeworms were undertaken by me in an attempt to show natural relationships between what seemed to be disparate families. These early studies yielded limited success in this regard. Preliminary work was done on *D. acotylus* (Coil, 1970) and the present study is a continuation of that work.

Materials and Methods

Host animals were shot in the field (San Patricio Co., Texas) and the worms were removed from the small intestine within 15 min after the death of the host. Worms were rinsed in saline and placed in Hanks' BSS until used. Relaxation of the worm was accomplished by: (1) swishing the worm in saline until relaxed and then swishing the worm in fixative until contractions ceased, (2) placing the worm on a damp paper towel and gently brushing it with dilute fixative until it was fully extended.

Specimens for scanning electron microscopy (SEM) were relaxed as in (2) above and then fixed on ice using 3% glutaraldehyde buffered to pH 7.4 with cacodylate with 3% sucrose for 1 hr. The worms were rinsed and stored in the same buffer with 5% sucrose. Six days later the worms were post-fixed in 1% osmium tetroxide. Ruthenium red was used for "osmium intensification" according to the method of Feria-Velasco and Arauz-Coutreras (1981).

Specimens used in ethanol cryofracture were dehydrated in a series of six dilutions of ethanol, placed in a Parafilm tube in 100% ethanol, quenched in liquid nitrogen, fractured in liquid nitrogen, and then returned to room temperature ethanol for critical point drying. Critical point drying was carried out in CO_2. Worm fragments were attached to aluminum stubs by means of silver paste and they were coated with a thin layer of gold–palladium. Scanning electron photomicrographs were taken on a Philips 501 microscope. Additional data concerning the processing of specimens for SEM are given in the explanation for some of the respective photomicrographs.

Certain specimens were collected in Hidalgo Co., Texas in June 1969. These worms were fixed in San Felice's fixative (chromic acid, 80 ml 1%; formalin 40 ml; glacial acetic acid 5 ml) for 6 hr and they were stored for the intervening period (1969–1982) in 70% alcohol. Ethanol freeze fracture preparations from these worms (Figs. 1, 4) clearly show extraction of certain organelles (compare Fig. 1 with Fig. 6). The use of compounds to extract or the use of methods to ash cellular components differentially in conjunction with SEM is clearly an area replete with opportunity for productive exploration in the helminths. The use of coagulative fixatives might be useful as well.

The terminology used in this paper has been developed over the past 25 years by various students of tapeworm embryology including Ogren, Rybicka, and Coil.

Results

SCOLEX: All other species in the genus *Dioecocestus* have scolices that function as holdfast organs by the use of suckers and armed rostella. The absence of attachment organs in *D. acotylus*

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(Fig. 5) poses a problem in our usual concepts of how a tapeworm maintains a certain position in a region of the intestine. In most species, the scolex serves to hold the anterior end of immature worms in place, but in large, robust worms the function is less clear. What is apparent is that the neck can be broken during periods of intense intestinal activity (diarrhea, e.g.). An attractive hypothesis for the maintenance of position in *D. acotylus* is that the worms move up the intestine (by continuous muscular activity) in response to the downward movement of the chyme. In one host collected for this study, both worms moved out of the host's gut within 10 min of death of the host. Presumably this reaction was in response to the traumatic disruption of the worms' niche.

OOCYTE: Measurements taken from SEM micrographs indicate the following approximate sizes: uterine oocyte 18.0 μm, nucleus 9.0 μm, nucleolus 2 μm, oocyte "shell granules" 0.6–1.0 μm. Both the nucleoplasm and the cytoplasm contain small granules metachromatic to toluidine blue. The "shell granules" are seen early as a clump of material adjacent to the nucleus with a reniform appearance. As the oocyte develops, the granules become more discrete and enlarge to the size above, and they appear to separate, making the amount of material appear greater (Figs. 1, 4).

EARLY CLEAVAGE: Division of the zygote begins in the uterus after fertilization and the completion of the maturation divisions. This process is carried out within the delicate first "shell" that is deposited around the oocyte in the ootype (Figs. 1, 2, 4, 5). The oocyte divides and produces two cells, one (called a macromere) with the "shell granules" and a nucleus about 6 μm in diameter and the other cell, a micromere, has a nucleus about 3 μm and no "shell granules." Thus, the first cleavage is total and unequal. The second division results in another cell, a mesomere, with a nucleus about 4 μm in diameter. The macromere with the "shell granules" divides equally when the embryo has reached about the 15-cell stage; the shell granules separate and approximately half of the original amount is found in each macromere.

The outer capsule (OC) (sometimes called shell for all or part of this layer) originates in the ootype as an exceedingly thin layer (about 0.2 μm) and later it is seen in the uterus where it thickens (Fig. 2). The OC fits loosely, sometimes contingent with the oocyte, but spaces up to 3 μm are seen with stellate connectives reaching from the oocyte to the OC. Granules (about 0.5 μm) and thickenings on the OC give the same histochemical reactions as the OC (positive for PAS, bromophenol blue, and malachite green). In one SEM micrograph of the OC, the cross-section area was 452 μm^2 of which the oocyte occupied 143 μm^2 of this cross section. The OC is in close contact with the outer capsules of other embryos or with the uterine epithelium (Fig. 3) as well.

After early cleavage is completed, the embryo grows and differentiates into the oncosphere, which is about twice the diameter of the oocyte. During the growth of the larva, there is a large increase in the overall size of the egg due to the growth of the inner and outer envelopes that surround the developing embryo. In the mature oncosphere, the OC is almost 1 μm thick and it has a diameter of about 35 μm. In some cases the OC appears to consist of two layers that have separated slightly (Fig. 10). The OC is positive for PAS, bromophenol blue, and malachite green. During early cleavage, numerous secretory granules (0.2–0.5 μm) are found on both the inner and outer surfaces of the OC (Fig. 2). It is assumed that this material is added to both sides of this template during the process of growth and thickening of this capsule.

OUTER ENVELOPE (OE): The OE is composed of the cytoplasm of two macromeres (Figs. 7–9) that surround the inner envelope in an eccentric layer. Early in development these macromeres contain "shell granules." The macromere nuclei are 8 μm in diameter (the size given here may be a function of the preparation).

The OE has numerous PAS-positive granules

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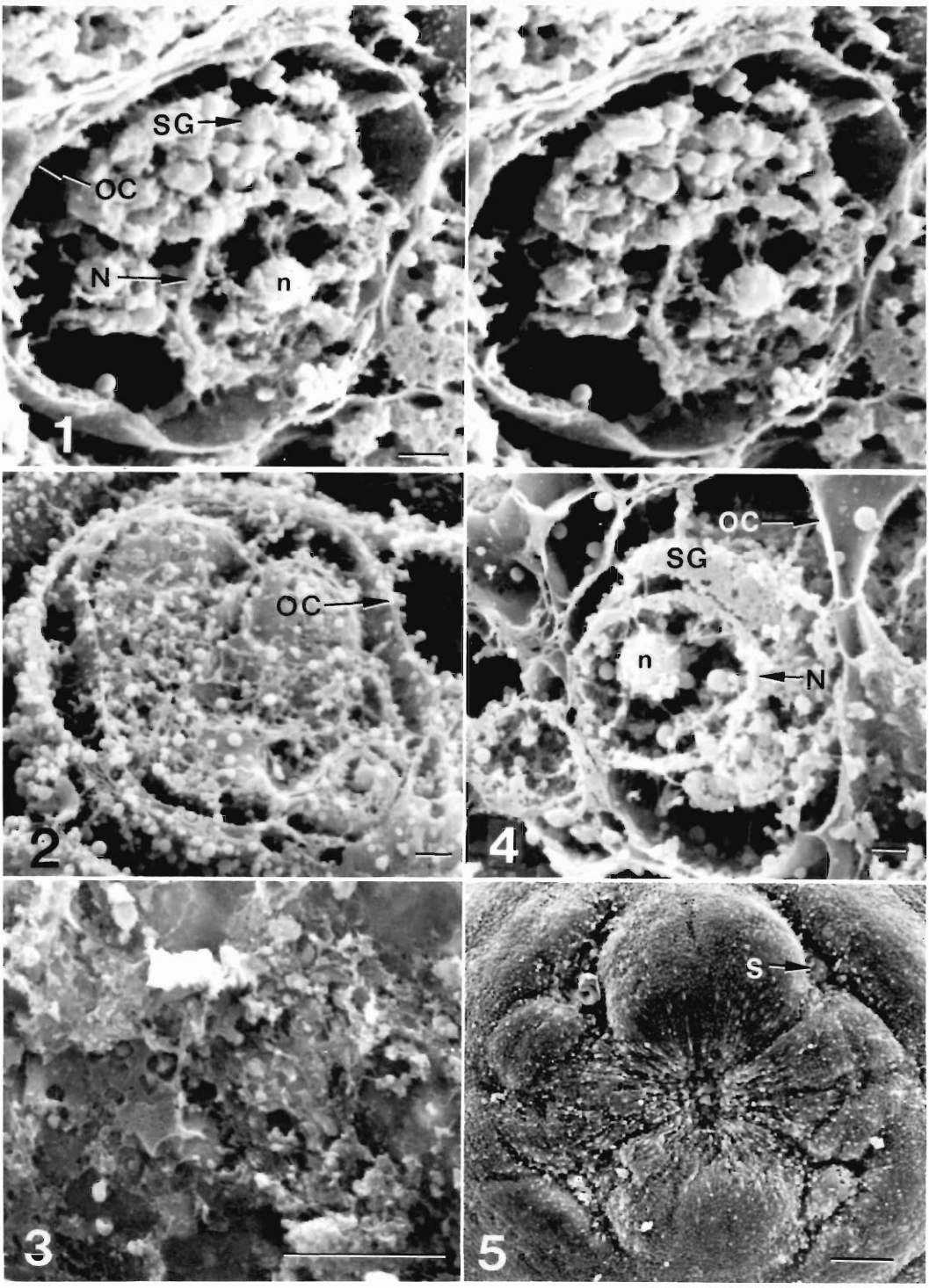
Figures 1–5. ***Dioecocestus acotylus.*** **Labeled structures include nucleus (N), nucleolus (n), outer capsule (OC), shell granules (SG), and vestigial sucker (S). 1. Stereo pair of SEM of ethanol cryofracture of a uterine oocyte showing shell granules. Scale bar 1 μm. 2. SEM of ethanol cryofracture of the early embryo. Note the presence of what are assumed to be secretory granules on both the inside and the outside of the early outer capsule and also on the surface of the embryo. Scale bar 1 μm. 3. SEM of ethanol cryofracture of the uterine epithelium. Note the absence of secretory granules in this part of the uterus. Scale bar 10 μm. 4. SEM of ethanol cryofracture**



of an early uterine oocyte. This oocyte is judged to be younger than Figure 1 due to the presence of compact shell granules and the thinness of the outer capsule. Scale bar 1 μm. 5. SEM of en face view of the scolex showing the vestigial suckers and the retracted rostellum. Scale bar 30 μm.

and it is strongly metachromatic with thionin. The thickness varies from virtually no thickness to 15 μm (Fig. 9).

The ephemeral nature of the OE can be seen in the high point of synthetic activity (inferred by the presence of the Golgi complex) and the later loss of thickness as the secretory activities of this envelope are completed.

INNER ENVELOPE (IE): The inner envelope is formed from the cytoplasm of three mesomeres that cleave off after the macromeres have started the formation of the OE. Mesomere nuclei in light microscope preparations measure about 6–7 μm. The IE forms later than the OE (Figs. 6, 8) and it persists longer (Fig. 10). It is similar to the OE in that after its secretory activities are completed, i.e., the formation of the inner capsule and oncospheral membrane, the IE disintegrates leaving behind certain organelles as remnants (Figs. 10–12).

The IE varies in thickness from 3–14 μm at the height of its synthetic activity.

EMBRYOPHORE (inner capsule, EP): The formation of the inner capsule begins at the cell membrane interface of the OE and IE (Fig. 7) at a time before hook formation. With maturation, the EP shrinks in size, constricting toward the surface of the oncosphere and trapping the three mesomere nuclei inside, but apparently allowing certain organelles to pass through the IE (Fig. 10). The final result is a capsule <1 μm thick with very little, if any, cytoplasm from the IE on the inside of the EP (Figs. 11, 12).

The EP is positive for PAS and alkaline phosphatase.

ONCOSPHERE MEMBRANE (OM): The OM is a delicate envelope (Fig. 11) found in the space between the oncosphere and the inner envelope (i.e., after the senescence of the IE). Although the complete details of OM formation could not be discerned here, certain aspects could be seen with both SEM and light microscopy. The OM can be seen stripping away from the IE at the time the early EP is near the IE–OE interface (Fig. 7). Complete delamination of the OM from the IE occurs before the EP is complete (Fig. 11). The OM is positive for toluidine blue.

Discussion

OOCYTE: At least three different kinds of oocytes occur in the cyclophyllidean tapeworms: (1) oocytes with large shell granules that are carried into the macromeres and released later [*Moniezia* (Rybicka, 1964); *Cittotaenia* (Coil, 1979); *Dioecocestus* (Coil, 1970); and *Schistotaenia* (Coil, unpublished)]; (2) oocytes with small (visible with LM) granules that are released in the ootype [*Hymenolepis* (Löser, 1965); *Diplophallus* (Coil, 1967); *Infula* (Coil, 1968 and unpublished TEM micrographs); and *Mesocestoides* (Ogren, 1956)]; (3) oocytes with small (visible with LM) granules that are carried into the uterus, but change form during the process of cleavage [*Gyrocoelia* (Coil, 1972)].

Clearly, the contribution of oocyte cytoplasm to the formation of the outer capsule is a process of basic interest to tapeworm development and it warrants intensive effort with TEM on widely separated taxa.

CLEAVAGE: The first cleavage is total and unequal in *D. acotylus,* a characteristic found in a number of other cestodes [*Infula macrophallus* (Coil, 1968); *Diplophallus polymorphus* (Coil, 1967); *Gyrocoelia pagollae* (Coil, 1972); *Shipleya inermis* (Coil, 1970); *Baerietta diana* (Douglas, 1963); and *Diorchis inflata* (Spätlich, 1925)]. Examples of first cleavage that is total, but equal, resulting in two similar macromeres are: *Mesocestoides corti* (Ogren, 1956); and *Oochoristica symmetrica* (Ogren, 1957) and several others.

OUTER CAPSULE (OC): The start of the OC begins in the ootype as a delicate envelope seen clearly around the oocyte in the uterus. The early OC apparently results from vitelline products released in the ootype (Coil, 1970). The Mehlis gland is small [a fact not reported by Fuhrmann (1904)] and probably plays no great role in early capsule formation. The IC steadily thickens and it enlarges to accommodate the increased size of

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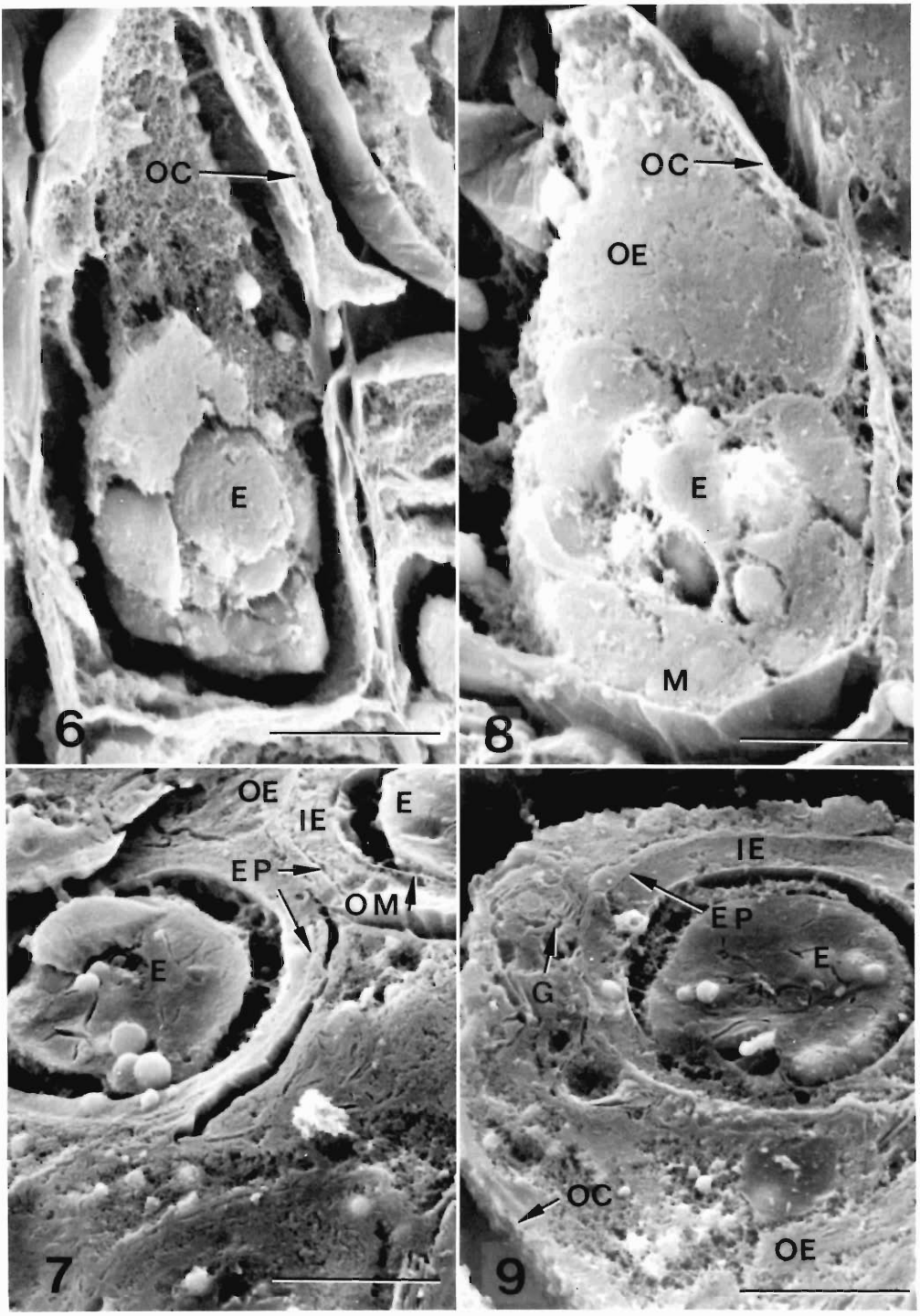
Figures 6–9. ***Dioecocestus acotylus.*** **Scale bars 10 μm. Labeled structures include embryo (E), embryophore (EP), Golgi (G), inner envelope (IE), mesomere (M), outer capsule (OC), outer envelope (OE), and oncosphere membrane (OM). 6. SEM of ethanol cryofracture of early embryo before the formation of the envelopes. 7. SEM of ethanol cryofracture of embryo showing the early formation of the embryophore and the partial delamination of the oncospheral membrane. The white spheres are assumed to be the same osmiophilic granules seen in the**



light microscope. 8. SEM of ethanol cryofracture of early embryo in which the outer envelope is forming and the mesomeres that will form the inner envelope are on the edge of the embryo. 9. SEM of ethanol cryofracture of embryo showing the formation of the early embryophore at the interface of the outer and inner envelopes. Note what appears to be a Golgi complex in the outer envelope.

the whole embryo. It should be emphasized that it is the production of envelopes which accounts for the great increase in overall size of the egg, the mature oncosphere generally being very little larger than the original oocyte.

It is clear that the early OC serves as a template surface on which shell material is deposited on both sides, thus the early OC becomes incorporated into the OC as additional shell material is added. However, the amounts of material contributed by the various sources (uterine epithelium, macromere granules, and the outer envelope) to the formation of the OC is not clear.

There are some adaptations of the OC that reflect the nature of the tapeworm life cycle: the resistance of the OC to proteolytic enzyme digestion (Coil, 1967, 1968, 1970; Gönnert et al., 1967; Lethbridge, 1971, and others) affords protection against digestion in the definitive host. Also, the need to remove the OC by mechanical means (chewing, e.g.) gives some protection to the intermediate host against fatal overinfections. In addition, the development of OC mimicry with organisms of food (Jarecka, 1961), OC flotation (Coil, 1977) are intriguing aspects of tapeworm life cycles about which we know little.

OUTER ENVELOPE: The OE in *D. acotylus* is generally located in an eccentric position giving the early embryo a "tear-drop" shape. Thus, the term "envelope" is not exactly appropriate, but the function appears to be similar to that found in other cyclophyllidean tapeworms. The ephemeral nature of the OE (growth, secretion, senescence, and disintegration) and the lack of precision in identifying these stages makes study difficult.

The secretory nature of the OE and its contribution to the OC are well documented from both histochemistry and TEM (Coil, 1967, 1968, 1977, 1979; Fairweather and Threadgold, 1981); the presence of Golgi, numerous mitochondria, and secretory granules all are organelles that lend evidence to the secretory function of the OE.

INNER ENVELOPE: The IE arises in a well-known way from the mesomeres, one of which arises from a cleavage of the macromere. The other two arise at an unknown time later. The contribution of the IE in the formation of the embryophore is well known in other tapeworms and this one appears to fit that pattern. In addition, the IE is similar to the OE in its growth and senescence and also virtually nothing is known concerning the mechanisms of these events.

EMBRYOPHORE: Our knowledge of embryophore formation is preliminary and fragmentary, but certain patterns seem to exist resulting in a capsule that is proteolytic-enzyme labile and which is sometimes unique for certain taxa (at the family level), but there are exceptions. At the light microscope level, the embryophore of *D. acotylus* appears to be of the generalized type seen, for example, in *Hymenolepis diminuta.*

The formation of the EP in the outer part of the IE envelope appears to be typical for many cyclophyllideans, but the process of formation, seemingly simple, belies the great complexity seen in the final product of such diverse embryophores as seen in *Taenia, Infula, Shipleya,* and *Cittotaenia.*

Many cestodes have a process of constriction or shrinkage of the embryophore from the position on the interface of the OE and IE down to a size slightly larger than the oncosphere. During constriction, the IE cytoplasm passes through the early embryophore (with the exception of the nuclei) until most of the IE cytoplasm is found outside the embryophore. An exception to our present knowledge of this process is in *Hymenolepis nana* in which the EP persists as a thin capsule in the periphery of the IE (Fairweather and Threadgold, 1981).

ONCOSPHERAL MEMBRANE: The OM is a loose-fitting membrane of unknown function that encloses the embryophore. Generally, it is considered to be so delicate that histochemical reactions cannot be seen; however, in this species it is robust enough to be seen with the light microscope in glycomethacrylate sections. The tim-

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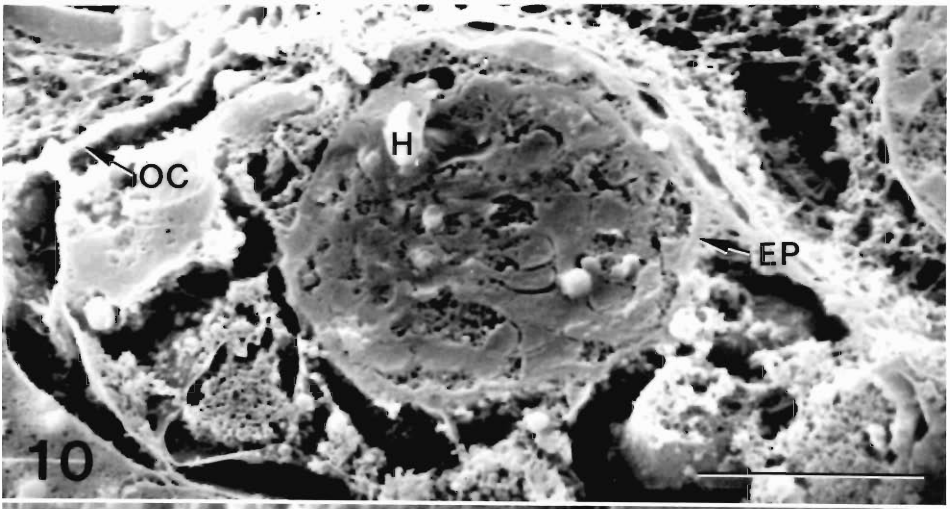
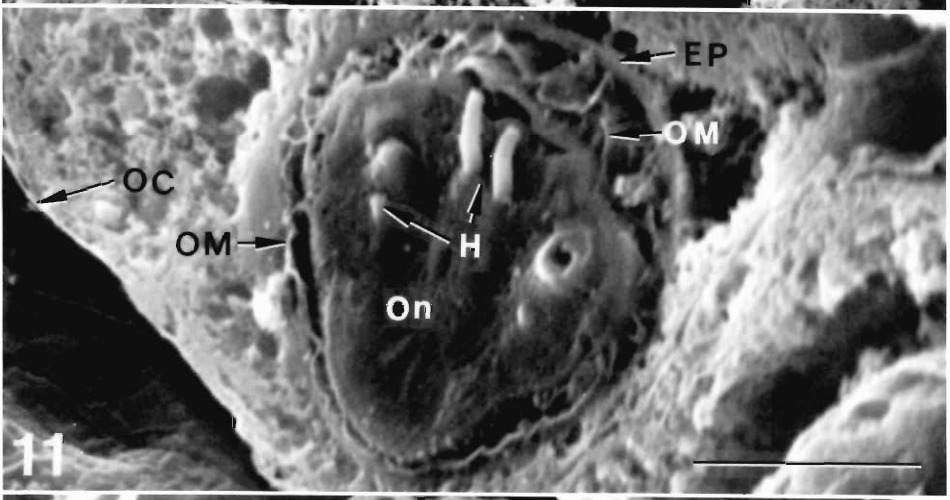
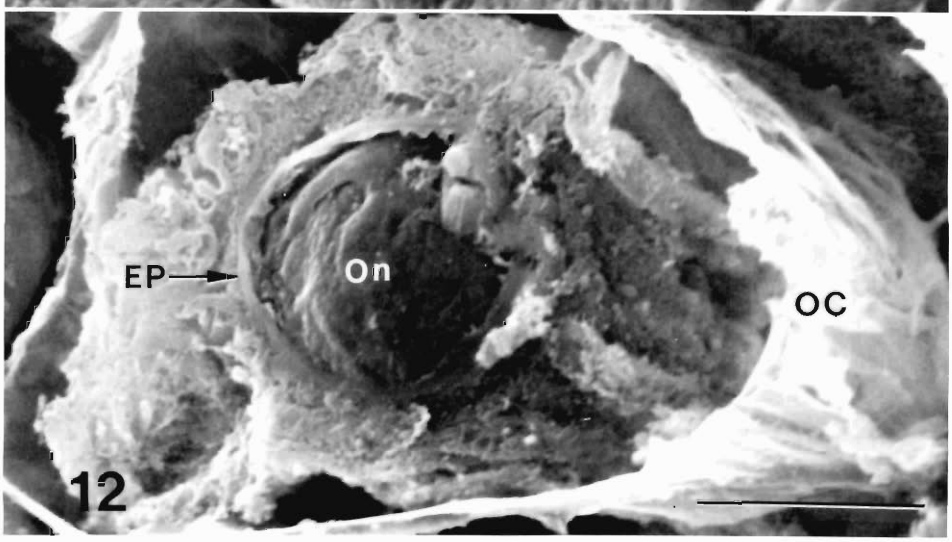
Figures 10–12. ***Dioecocestus acotylus.*** **Scale bars 10 μm. Labeled structures include embryophore (EP), hook (H), outer capsule (OC), oncosphere membrane (OM), and oncosphere (On). 10. SEM of ethanol cryofracture of nearly mature oncosphere. Maturity can be judged by the thickness of the embryophore, the senescence of the envelopes, and the constriction of the embryophore around the oncosphere. 11. SEM of ethanol cryofracture of oncosphere. Note the size of oncosphere and the embryophore. 12. SEM of ethanol cryofracture of oncosphere. Note the thickness of embryophore and its close fit next to the oncosphere.**







ing of the separation may well be a function of the senescence of the IE, i.e., when the integrity of the cellular organization of the IE begins to break down, the OM begins to delaminate from it.

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Tylocephalum Linton, 1890 (Cestoda: Lecanicephalidea) from the Cownose Ray, *Rhinoptera bonasus* (Mitchill, 1815) with a Discussion of Its Validity and Systematic Relationships[1]

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ABSTRACT: The first generic diagnosis of *Tylocephalum,* based upon Linton's type species, *T. pingue,* from the type host and locality is presented. *Tylocephalum pingue* is most common in cownose rays, *Rhinoptera bonasus,* from Chesapeake Bay but is occasionally found in summer migrants to southern New England waters. The description of *T. marsupium* Linton, 1916 is expanded based upon information contained in the original publication and careful examination of serial sections of Linton's type material. A new species, *T. bonasum,* from *Rhinoptera bonasus,* is described and critically compared with *T. pingue.* Histology and reconstruction of the internal anatomy of *T. pingue* and *T. bonasum* show that scolex variability can resemble either *Hexacanalis* Perrenoud, 1931 or *Cephalobothrium* Shipley and Hornell, 1906. *Tylocephalum* is diagnosed by its fungiform metoporhynchus and globose posterior portion of the scolex, herein termed the pars basalis, marginal genital pore, preovarian testes, conspicuous external seminal vesicle, unarmed cirrus, bilobed ovary and circumcortical vitellaria. Species having lateral bands of vitellaria but otherwise similar to *Tylocephalum* are referred to *Cephalobothrium. Tetragonocephalum* Shipley and Hornell, 1905, recognized by its external and internal seminal vesicles and lateral bands of vitellaria continuing into the postovarian space, is considered a lecanicephalid most closely related to *Cephalobothrium.* The systematic relationships of *Tylocephalum* to other lecanicephalans are discussed. *Spinocephalum* Deshmukh, 1980 is considered a junior synonym of *Tylocephalum* Linton, 1890. *Hexacanalis* Perrenoud, 1931 is considered a junior synonym of *Cephalobothrium* Shipley and Hornell, 1906.

Historically, the taxonomy of certain of the lecanicephalans has been in a state of confusion because of the inadequacy of old descriptions and the lack of specimens for study. Linton's (1890) *Tylocephalum,* and *Cephalobothrium* of Shipley and Hornell (1906) are among the genera that have never been completely described and their true systematic status clarified. *Tylocephalum pingue* Linton, 1890, the type species of the genus, was never described beyond scolex morphology nor were any type specimens deposited in museum collections. MacCallum (1921) deposited specimens he believed to be *T. pingue* from hosts taken in the Atlantic and Pacific oceans but they are unsatisfactory for studying internal anatomy. Southwell (1925) emended *Tylocephalum* using the anatomy of *Tetragonocephalum* Shipley and Hornell, 1905, which he and Shipley and Hornell (1906) considered to be synonymous with *Tylocephalum.* The continued lack of knowledge about *T. pingue* led to confusion involving the validity of *Tetragonocephalum.* Yamaguti (1959) gave an expanded diagnosis of *Tylocephalum,* based upon descriptions of species he considered to be congeneric, and created a new family, Tetragonocephalidae, to contain *Tetragonocephalum.* Euzet and Combes (1963) were unable to find *T. pingue* in cownose rays, *Rhinoptera bonasus* (Mitchill, 1815), from the Woods Hole area where Linton (1890) had discovered it and concluded that *Tylocephalum* should be considered a junior synonym of *Tetragonocephalum.* More recently, Shinde (1976) described a species of *Tylocephalum* he called *T. pingue* from a ray in Indian waters but it is not conspecific with Linton's species. We have found that *Tylocephalum pingue* occurs in cownose rays that enter New England coastal waters during summer months but is more common in hosts taken in the warmer waters of Chesapeake Bay. The description of *T. pingue* given herein is the first complete description based upon specimens taken from the type host in the type locality.

Cephalobothrium Shipley and Hornell, 1906, like *Tylocephalum,* was never sufficiently described to justify creation of any suprageneric taxa but Pintner (1928) created the family Cephalobothriidae for it and Perrenoud (1931) placed a second genus, *Hexacanalis,* in it. Yamaguti (1959) tentatively accepted the family until detailed description of the internal anatomy proved it distinct from the Lecanicephalidae Braun, 1900. We have found specimens representing a new

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species of *Tylocephalum* in cownose rays taken from southern New England coastal waters that allows critical comparison with these cephalobothriid genera and assessment of the taxonomic status of the family Cephalobothriidae.

Materials and Methods

Living worms were obtained from rays taken in commercial fish traps at Sakonnet Point, Rhode Island and studied in the laboratory, in sea water, prior to fixation. Other specimens, including those from Chesapeake Bay, were fixed in situ by immersion in 10% formalin. Whole mounts and sections of free and attached specimens were prepared using standard procedures. Internal anatomy was reconstructed from serial sections cut in frontal and transverse views. Drawings were made with the aid of a drawing tube and microprojector. Measurements include the mean and the range in parentheses. The standard deviation is given for some characters. All measurements are expressed as length by width and are in micrometers unless otherwise indicated. Specimens were compared with *Tylocephalum pingue* (USNM Helm. Coll. Nos. 35865 and 36053) deposited by MacCallum and *T. marsupium* Linton, 1906 (USNM 8990). Preserved specimens, whole mounts, and sectioned material obtained during this study have been deposited in the National Parasite Collection, USDA, Beltsville, Maryland 20705.

Tylocephalum pingue Linton, 1890 (Figs. 1–3, 8–14, 21, 22)

DESCRIPTION (based on 145 specimens; 24 measured): Strobila ($N = 24$), 23–46 mm by 180–820, rounded in cross section, craspedote, hyperapolytic. Number of segments ($N = 24$), 175 (127–235). Scolex bipartite, acorn-like; anterior region consists of metoporhynchus and basal collar, metoporhynchus rounded ($N = 24$), 248 (176–300) by 320 (180–460); posterior region subglobular ($N = 24$), 477 (300–640) by 957 (600–1.2 mm), bearing four accessory suckers ($N = 48$), 92 (57–114) in diameter. Neck short, concealed by posterior half of scolex. Immature segments wider than long ($N = 48$), 121 (80–272) by 540 (304–820); mature segments longer than wide ($N = 48$), 1.02 mm (504–1.3 mm) by 636 (420–820). Genital pore marginal, in anterior ⅓ of segment, irregularly alternating. Cirrus sac subspherical ($N = 20$), 199 ± 35 (150–261) by 157 ± 22 (126–197), extending approximately to midline. Cirrus muscular, aspinose, sparsely covered by gland cells. Vas efferentia converge near ovarian isthmus to form vas deferens. Vas deferens ascends for short distance in midline, expanding to form voluminous external seminal vesicle as segments mature. Seminal vesicle joins cirrus sac on anteromedial surface. Testes per segment ($N = 24$), 58 ± 3 (53–62), enlarging, then degenerating as segments mature. Testes distribution: prevaginal ($N = 24$), 12 ± 1 (11–13); postvaginal ($N = 24$), 16 ± 2 (14–18); antiporal ($N = 24$), 29 ± 2 (27–32). Vagina originating at genital atrium dorsolateral to cirrus sac, traversing to midline in parallel with cirrus sac, turning posteriorly and gradually spiralling ventrally beneath seminal vesicle, enlarging to form seminal receptacle near ovary; surrounded by dark staining cells throughout most of its length. Ovary divided into two masses of digitiform lobes; bilobed in dorsoventral view, and in cross section. Ovarian lobes subequal ($N = 20$), 304 ± 24 (247–399) by 241 ± 38 (228–304), subdivided into digitiform lobules radiating from ovarian isthmus. Mehlis' gland compact, subspherical, 72–88 by 136–152, immediately posterior to ovarian isthmus. Uterine duct coiled, anterior to ovarian isthmus, ascending in midline to join uterus at level of cirrus sac. Uterus ventral, surrounded by dark staining gland cells, irregular in shape, displaced laterally by enlarged seminal vesicle, terminating near anterior extremity. Uterine pore develops midventrally at level of cirrus sac. Vitelline follicles circumcortical, continuous anterior to ovary, none postovarian ($N = 40$), 47 (38–57) by 33 (19–49). Dorsal and ventral pairs of osmoregulatory ducts present, dorsal pair largest.

HOST: *Rhinoptera bonasus* (Mitchill, 1815); cownose ray (Rhinopteridae).

LOCALITIES: Sakonnet Point, Rhode Island (41°26′N, 71°14′W; this study) and Chesapeake Bay, Virginia (this study); Woods Hole, Massachusetts (Linton, 1890; type locality; 41°30′N, 70°40′W).

LOCATION: Spiral valve (concentrated in middle one-third).

SPECIMENS DEPOSITED: One whole mount, USNM Helm. Coll. 77631 (neotype); two whole mounts, six vial specimens, and four slides of serial sections, USNM Helm. Coll. 77632 (paraneotypes); two additional paraneotypes deposited in the British Museum (Natural History); one paraneotype in the Harold W. Manter Laboratory, University of Nebraska State Museum. Paraneotype is used as defined by the Smithsonian Institution.

Designation of a neotype

Linton (1890) did not indicate deposition of the type specimen of *Tylocephalum* in any museum collection nor have other authors deposited

types in substitution for Linton's original material. In order to complete this revisory work on the parental genus of the group of genera (*Tylocephalum, Tetragonocephalum, Hexacanalis, Cephalobothrium*) that were supposedly differentiated from *Tylocephalum* at their description, we consider it essential to establish a neotype to resolve this complex problem and to establish a basis for future comparisons and taxonomic stability. The decision to designate a neotype was not made as a matter of convenience or curatorial routine and *Tylocephalum* is a valid name and not a synonym of any existing genus. We acknowledge the advice and assistance of Dr. J. Ralph Lichtenfels, Curator, National Parasite Collection who has also advised us that all type specimens of *Tylocephalum pingue* Linton, 1890 are untraceable at the United States National Museum and the Smithsonian Institution. The following persons have verified that all type material is untraceable at: American Museum of Natural History, New York, E. Kirsteuer and H. Feinberg; University of Pennsylvania, G. Schad and D. Muncey; Academy of Natural Sciences of Philadelphia, R. Robertson and R. Gore. Our specimens are described from the type host, *Rhinoptera bonasus* (Mitchill, 1815), taken near Sakonnet Point, Rhode Island as near to the type locality as practicable. We regard the characteristics given in the emended generic diagnosis herein as distinctive of *Tylocephalum* and the description and data provided are sufficient to recognize *T. pingue* Linton, 1890 for which the neotype is designated. The neotype is consistent with that of Linton (1890) for *Tylocephalum,* and *T. pingue* as based upon an immature specimen from a cownose ray taken at Woods Hole, his recognition of the genus in describing *T. marsupium* Linton, 1916, and the use of the name *Tylocephalum* in the recent literature (Cheng, 1966, 1975; Shinde, 1976; Wolf, 1976; Cake and Menzel, 1977; Cake, 1978; Stephen, 1978; and others). These steps have been taken to satisfy the requirements of Article 75 of the International Code of Zoological Nomenclature.

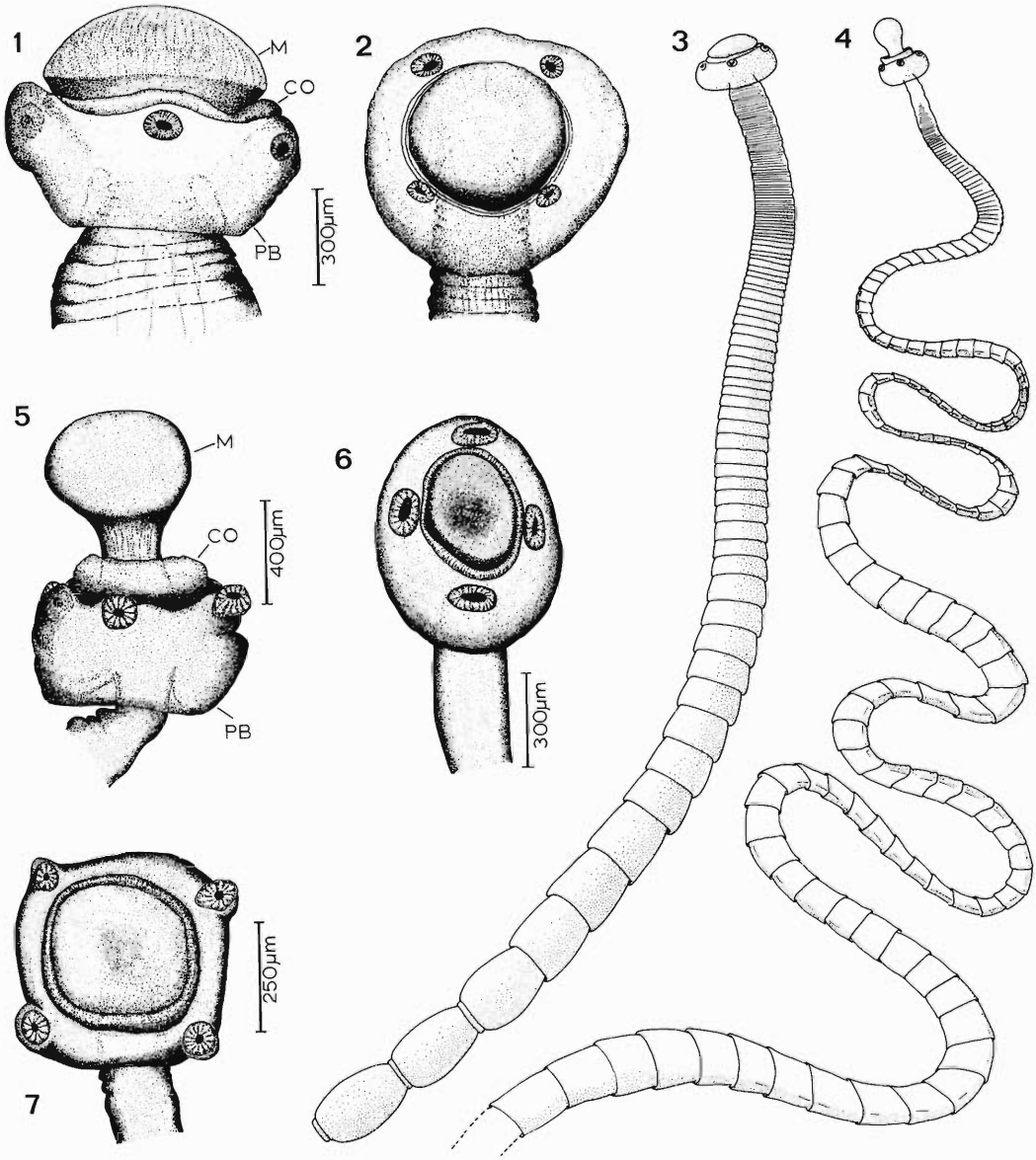
Morphology

Whether viewed whole or in section, the scolex and segments of *Tylocephalum pingue* are unusual. The most conspicuous feature of the scolex is the fungiform "metoporhynchus" (Euzet and Combes, 1963), which, in our observations of *T. pingue,* always protrudes from the posterior region of the scolex and is never retracted into it (Fig. 21). In situ the metoporhynchus provides only a superficial attachment (Fig. 22) and has not been found to penetrate the mucous membrane, become embedded deep within it, or cause significant changes in its appearance (Fig. 22; compare with *T. bonasum* Figs. 5, 6, 23, 24). A permanent collar-like fold of the tegument lies between the base of the metoporhynchus and its origin in the posterior portion of the scolex. This is apparent in whole mounts (Fig. 21) and sections (Fig. 22). Thus, the scolex consists of three discernible parts, i.e., the metoporhynchus, a collar around the base of the metoporhynchus near its origin, and the globose, posterior portion bearing suckers (see pars basalis below; Figs. 1, 5).

The posterior portion of the scolex is fleshy, round on cross section and bears four suckers spaced equidistant on its anterior edge. The posterior region resembles a parabaloid with a concavity in the anterior and posterior surfaces. The anterior concavity is a shallow depression from which the metoporhynchus protudes. However, the posteror cavity is deep and funnel-shaped, thus concealing the short neck of the worm (Fig. 1). We find it unnecessarily awkward to refer to the "posterior region of the scolex" by lengthy phrases and suggest the term pars basalis to refer to that region in all lecanicephalans.

Internally, the tegument and musculature of the two scolex regions also differ. Under light microscopy the cellular detail of the tegument over the two regions appears to be very similar, but that of the pars basalis is denser and more deeply staining like the tegument of the strobila. The musculature of the metoporhynchus contains many small fascicles in parallel that run longitudinally (Fig. 22). Fascicles in the metoporhynchus originate along a well-defined boundary that divides the metoporhynchus and pars basalis (Fig. 22). This boundary is apparent in drawings of *Tetragonocephalum trygonis* Shipley and Hornell, 1905 by Southwell (1925; Fig. 66) and *Tylocephalum uarnak* Shipley and Hornell, 1906 by Euzet and Combes (1963, Fig. 3). Posterior to this boundary is the collar-like portion of the scolex containing numerous longitudinal and circular fascicles that radiate from the origin of the neck through its interior.

The strobila is noticeably round in cross section, very muscular and rather rigid compared to other cestodes. The terminal segment is typ-



Figures 1–7. ***Tylocephalum*** **from the cownose ray. Figures 1–3.** ***Tylocephalum pingue*** **Linton, 1890. 1. Scolex, dorsoventral view. 2. Scolex, apical view. 3. Entire worm. Note concealed neck; most of strobila is round in cross section except for oldest segments. Figures 4–7.** ***Tylocephalum bonasum*** **sp. n. 4. Entire worm. Note presence of neck; early mature segments are rectangular in cross section, older segments oval in cross section. 5. Scolex, dorsoventral view (holotype). 6. Scolex, apical view (paratype). 7. Scolex, apical view (paratype). Note similarity to** ***Hexacanalis*** **Perrenoud, 1931. Abbreviations: M, metoporhynchus; CO, collar; PB, pars basalis.**

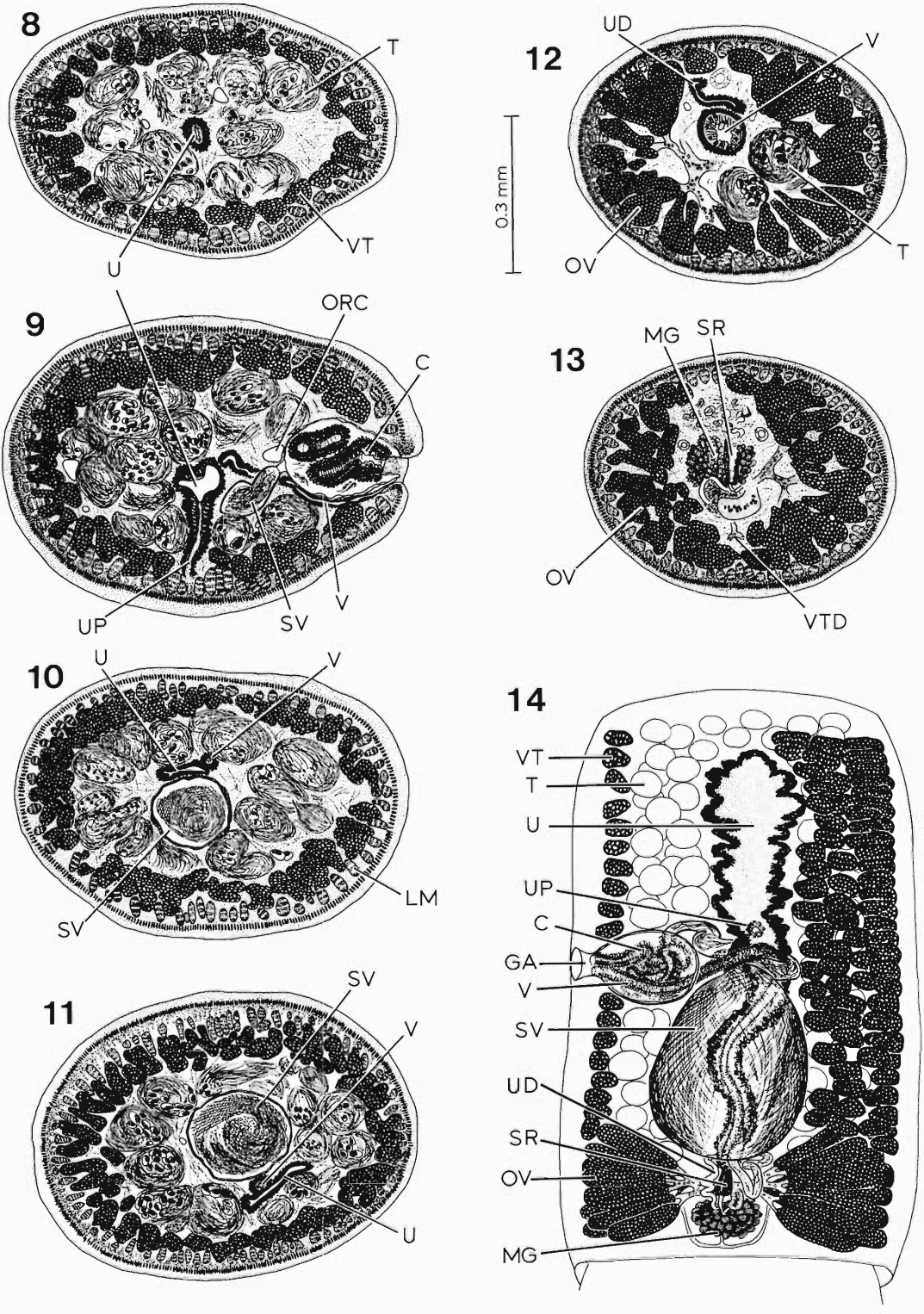
ically rotund and rather conical, thus giving the end of the strobila a pointed appearance. Occasionally a specimen is found in which the last few segments have a droplet-shape and are oval in cross section. Such segments are apparently about to be shed.

In sectioned mature segments the most conspicuous features of the male reproductive system are the cirrus sac, expanded vas deferens and voluminous external seminal vesicle (Fig. 14). The testes encircle the vas deferens, vagina, and uterus in the preovarian space (Fig. 10). A few

testes are found between the ovarian lobes anterior to the isthmus (Fig. 12). In fully mature segments the testes are layered three to five deep and are no longer evident as solid bodies but exist as enlarged spherical masses of spermatozoa with long tails. Clusters of sperm heads are evident in both the testes and within the seminal vesicle (Fig. 11). The proximal end of the vas deferens is of small diameter and loops anteriad from the region of the ovarian isthmus to expand into an enormous seminal vesicle that often occupies the median one-third of the segment between the ovary and the cirrus sac (Fig. 14). In whole mounts and frontal sections the seminal vesicle appears to be the seminal receptacle connecting in the vicinity of the ootype, but cross sections clearly show that this is not the vagina (compare Figs. 9–13). This fact is even more obvious when compared with *T. bonasum* sp. n. described herein (Figs. 15–20). Striations of muscle fibers can be seen in the walls of the seminal vesicle and the interior is packed with whorls of sperm and the crisscrossing of their tails forms the "herring bone pattern" referred to in earlier descriptions of related worms (Figs. 10, 11). Initially the seminal vesicle lies dorsal to the uterus and vagina (Fig. 11) but just posterior to the level of the cirrus pouch the vesicle decreases in diameter and twists around the uterus and vagina displacing them dorsally (Fig. 10). Thus, the distal portion of the seminal vesicle lies ventral to the uterus and vagina but the position of the three tubes is exactly reversed in the posterior region of the segment (compare Figs. 9–11, 14). The twisting and transposition of these three tubes occurs within the space of six consecutive 10-μm transverse sections. The seminal vesicle joins the cirrus sac on its anterior surface (Fig. 14). The cirrus pouch contains a long, coiled, muscular, unarmed cirrus surrounded by gland cells (Fig. 9), but there is no internal seminal vesicle. The genital atrium is well developed. The female reproductive system, traced from the genital atrium, originates as a narrow glandular duct that parallels the ventral surface of the cirrus pouch and turns posteriad immediately above the uterus (Figs. 9, 10). The vaginal duct twists around the uterus and seminal vesicle on the antiporal side then proceeds posteriorly in the midline between the seminal vesicle and ventrally situated uterus (Fig. 11). The vaginal duct maintains a narrow diameter and small lumen until it reaches the ovary where it suddenly expands to form a seminal receptacle. Externally the seminal receptacle is surrounded by gland cells; internally it is lined with columnar cells (Fig. 12). Distally the seminal receptacle lacks gland cells externally, has a thick wall and smooth lining, and tapers into a short sperm duct that joins the oviduct (Fig. 13). The ovary consists of numerous lobules that radiate from primary and secondary branches of the central isthmus (Fig. 12). The seminal receptacle passes dorsal to the ovarian isthmus (Figs. 12–13). An elongated oocapt arises from the isthmus and curves posteriorly to give rise to a short oviduct. Large vitelline follicles form a dense circumcortical layer anterior to the ovary. The main vitelline ducts join the oviduct as it enters the cluster of Mehlis' gland cells surrounding the ootype. Mehlis' gland is large and situated posterior to the ovarian isthmus. The proximal portion of the uterine duct forms several coils dorsal to the ovarian isthmus and then ascends in the median line to join the uterus just anterior to the ovary. An external coat of gland cells surrounds the distal portion of the uterine duct and continues over the surface of the uterus. The proximal portion of the uterus ascends in the midline near the ventral surface and is displaced dorsally near the level of the cirrus sac by the gradual spiralling of the vagina and seminal vesicle (Figs. 10, 11). A duct leads ventrally from the uterus to the developing uterine pore at the level of the cirrus sac (Fig. 9). Anterior to the cirrus sac the uterus continues in the center of the medulla and terminates near the anterior margin of the segment. Numerous diverticula appear in the uterus as it continues to develop. Several pairs of osmoregulatory ducts, both large and small, are present. The exact number is difficult to determine but a large pair pass dorsal to the vagina and a smaller pair pass ventrally at the level of the cirrus sac. The longitudinal muscle bundles remain prominent in all attached segments and do not show signs of atrophy.

Systematics

Tylocephalum pingue was described by Linton (1890) from an immature specimen. He never found additional specimens. The scolex morphology, upon which the genus *Tylocephalum* was based, was accurately figured (Linton's figs. 5–9) and described. However, he did not deposit any specimens in the helminth collection of the United States National Museum. Shipley and Hornell (1906) placed *Tetragonocephalum* Shipley and Hornell, 1905 in synonymy with *Tylocephalum* on the basis of scolex morphology.



MacCallum (1921) deposited several specimens in the USNM (35865, 56053) from a cownose ray taken by the New York Aquarium (dated 1914) and from the spotted eagle ray, *Aetobatis narinari,* taken in the Pacific at Singapore (dated 1916). The immature specimens from the cownose ray (USNM 35865) cannot be identified but the spherical scolex and swollen neck region are more suggestive of *Glyphobothrium zwerneri* Williams and Campbell, 1977, also from *Rhinoptera bonasus,* than of *T. pingue.* MacCallum questioned the identity of these specimens in writing found on the original slide labels. His specimens labelled *T. pingue* (USNM 36053) from *Aetobatus narinari* from Singapore are similar to *T. pingue* in scolex morphology and testes number (comparison with our specimens from *R. bonasus*) but differ in the following ways: (1) the strobila is distinctly flattened in cross section instead of being round to oval; (2) both mature and gravid segments have the shape of elongated rectangles instead of gradually elongating; (3) all segments overlap to such a degree that they resemble a laciniated condition instead of having minimal overlap; and, (4) vitelline follicles form narrow lateral bands instead of being circumcortical in distribution. Actually, MacCallum's specimens from *A. narinari* are closer to *Tylocephalum elongatum* Subhapradha, 1955 than Linton's *T. pingue.*

Southwell (1925) suppressed *Tylocephalum pingue* as type species because it had not been described but Euzet and Combes (1963) rejected the genus in favor of *Tetragonocephalum* because it had not been found since Linton's original report. Southwell (1925, p. 268) stated,

> "As the anatomy of *T. pingue* Linton has not been described, it is impossible to identify Linton's species and it therefore cannot be compared with any of the species described below; it may, or may not, be identical with any of them. *T. trygonis* Shipley and Hornell, 1905, was the next species to be described and it therefore becomes the type (species) of the genus."

Euzet and Combes (1963, p. 104) stated,

> "Nous faisons nôtres les conclusions de J. G. Baer: A notre avis considérant la confusion qui règne dans ce groupe, il est parfaitment inutile de conserver l'espèce *T. pingue* qu'il n'est past possible de reconnaître et qui n'a jamais été retrouvé depuis sa découverte en 1887. D'ailleurs rien ne permet de supposer qu'un Ver semblable, retrouvé chez un hôte identique et au même endroit, soit le *T. pingue* de Linton . . . . D'aprés J. G. Baer l'echantillon type est perdu. Ce cestode ne paraît jamais avoir été retrouvé dans *Rhinoptera* sur les côtes Américaines de l'Atlantique Nord."

On the contrary, we have found that *T. pingue* is a common parasite of *Rhinoptera bonasus* in Chesapeake Bay and occurs seasonally in southern New England waters with the summer migrations of its host. The fish and *T. pingue* are only incidental visitors to these waters at the extreme northern limits of the host's migratory range. In any case, frequency of occurrence of a species has no bearing on its validity and this description of *T. pingue* from the type host and locality reaffirms the validity of this genus and species in accordance with Article 23 (Law of Priority) and Article 24 (the Law of Priority applies when any part of an animal is named before the whole animal) of the International Code of Zoological Nomenclature (1964). We choose not to make *Tegragonocephalum* Shipley and Hornell, 1905 a junior synonym because *Tetragonocephalum uarnak* as described by Euzet and Combes (1963) has both external and internal seminal vesicles, some postovarian vitellaria and two ovarian lobes arched in the form of a circle. Yamaguti (1959) created a new family, Tetragonocephalidae, to receive *Tetragonocephalum* but presented a rather confused diagnosis which states that members of the family would lack

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Figures 8–14. Reproductive system of *Tylocephalum pingue* Linton, 1890. Figures 8–13. Transverse sections of mature segment. All sections drawn to same scale. 8. Anterior to cirrus pouch. 9. At level of genital atrium. 10. Just posterior to cirrus pouch. 11. Just anterior to ovary. 12. Just anterior to ovarian isthmus. 13. Through ootype complex. 14. Schematic diagram of reproductive system (dorsal view) reconstructed from frontal and transverse serial sections. Abbreviations: C, cirrus; GA, genital atrium; LM, longitudinal muscle; MG, Mehlis' gland; ORC, osmoregulatory canal; OV, ovary; SR, seminal receptacle; SV, seminal vesicle; T, testis; U, uterus; UD, uterine duct; UP, uterine pore; V, vagina; VT, vitellaria; VTD, vitelline duct.

suckers on the posterior region of the scolex (p. 94) but possess them by definition (p. 102), lack a seminal vesicle, have bilobed postovarian vitellaria and a bilobed ovary. This diagnosis is inaccurate and we do not feel there is anything in it to justify the inclusion of *Tetragonocephalum* in any family except the Lecanicephalidae. The description of Euzet and Combes (1963) should be referred to until *Tetragonocephalum uarnak* and the type species, *T. trygonis* Shipley and Hornell, 1905, are studied from the type host and locality to confirm that the type species of *Tetragonocephalum* is not synonymous with *Tylocephalum*.

Linton (1916) described a second species of *Tylocephalum, T. marsupium,* from the spotted eagle ray, *Aetobatis narinari,* taken at Tortugas, Florida. *Tylocephalum marsupium* Linton, 1916 was not adequately described by either Linton (1916) or Southwell (1925) but serial sections are available for study (USNM 8990). Southwell (1925) considered *T. marsupium* a synonym of *Adelobothrium aetiobatidis* Shipley, 1900 because Linton's description of the segments did not include details such as testes number. Linton's description of the scolex does not fit *Adelobothrium* (pars basalis membranous) and the segments of *A. aetiobatidis* contain about twice the testes number (130–150) of *T. marsupium.* Additional information pertinent to the recognition of *T. marsupium* and its relationship to *T. pingue* was obtained by studying Linton's serial sections. The following description is provided and includes characters we feel are significant additions to those extant in the original description.

Tylocephalum marsupium Linton, 1916

DESCRIPTION (portions from Linton italicized; other observations from USNM 8990): Scolex acorn-like, metoporhynchus rather pointed *160 by 210,* pars basalis rounded *300 by 690,* not membranous, bearing 4 suckers. *Neck present, 160 wide.* Segments numerous, markedly craspedote, overlapping 75% of following segment in young segments with testes. First segments narrow, *14 to 50 by 180 to 240,* with large extensions on posterolateral margins. *Mature segments 460 to 560 by 280 to 380; terminal segment vase-shaped, 840 by 560.* Laciniated margin comprises 12% of segment length in terminal segment. Numerous oval vacuolated spaces (calcareous corpuscles?) in laciniated margins. Internally, longitudinal muscle bundles divided into a single inner layer of about 40 large fascicles surrounding internal organs. Genital pore marginal, approximately equatorial, irregularly alternating. Testes preovarian, subspherical, arranged in two layers or in parallel depending upon the distribution of the other organs. Testes number 60–74 per segment, distributed as: 22 prevaginal, 12 postvaginal, and 40 antiporal; dimensions 57–87 by 23–65. Seminal vesicle sinuous anteriorly, voluminous posteriorly, occupying space between cirrus sac and ovary on poral side of median line, typical "herring bone" pattern evident in interior. Cirrus sac oval, 96 by 80. Ovary divided into two masses of digitiform lobules. Lobules radiate laterally and posteriorly from ovarian isthmus, tapering to enlarged rounded ends, maximum dimensions 160 by 24. Uterus median, terminating about 20% of segment length from anterior margin. Vitelline follicles 15–34 by 8–11, circumcortical in spaces between inner layer of longitudinal muscles. Ventral osmoregulatory ducts paired, large, antiporal duct distinctly larger.

Tylocephalum Linton, 1890

EMENDED DIAGNOSIS: Lecanicephalidea, Lecanicephalidae. Scolex divided into two parts: metoporhynchus fungiform; pars basalis fleshy and globose, bearing four suckers. Strobila robust, rounded or flattened, inner longitudinal muscle bundles well developed. Segments distinct, craspedote. Genital pore marginal. Cirrus sac extends into medulla, cirrus unarmed. Conspicuous saccular external seminal vesicle present. Testes occupy available preovarian space. Vagina opens ventrolateral to cirrus sac. Seminal receptacle present. Ovary subdivided into two groups of digitiform lobules. Uterus ventral, median, with preformed aperture. Vitelline follicles circumcortical. Dorsal and ventral osmoregulatory ducts well developed or multiple small ducts present.

TYPE SPECIES: *Tylocephalum pingue* Linton, 1890.

TYPE HOST: *Rhinoptera bonasus* (Mitchill, 1815); cownose ray (Rhinopteridae).

LOCALITIES: Woods Hole, Massachusetts (type locality); Sakonnet Point, Rhode Island and Chesapeake Bay, Virginia (this study).

Tylocephalum bonasum sp. n. (Figs. 4–7, 15–20, 23, 24)

DESCRIPTION (based on 7 specimens; 6 measured): Strobila 4.6–25 cm by 160–1.72 mm, serrated, craspedote, muscular, hyperapolytic. Number of segments ($N = 5$) 262 (83–493). Scolex bipartite; anterior portion a bulbous, collared metoporhynchus, variable in appearance, highly evaginable or invaginable into pars basalis; pars basalis quadrangular or subglobular ($N = 6$), 417 (296–560) by 682 (496–840), cuplike, bearing 4 suckers anteriorly. Suckers ($N = 17$) 113 (88–152) by 99 (80–122). Evaginated metoporhynchus ($N = 5$) 514 (232–786) by 410 (141–500); may be invaginated into suckerlike aperture of pars basalis, aperture 440 by 300. Neck ($N = 6$) 269 (125–480) by 160 (141–200). Immature segments wider than long ($N = 20$), 82 (24–160) by 278 (140–400), distinctly rectangular in cross section; mature segments longer than wide ($N = 20$), 1 mm (272–1.72 mm) by 887 (256–2 mm), oval in cross section. Gravid segments not observed. Genital atrium shallow. Genital pores marginal, in anterior ½ of segment, irregularly alternate. Cirrus pouch subspherical ($N = 12$), 367 ± 32 (328–416) by 210 ± 14 (192–224). Cirrus muscular, aspinose. Vas deferens dorsomedial to uterus forming a sinuous external seminal vesicle, entering cirrus pouch on anteromedial surface. Seminal vesicle enlarging as segments mature. Testes subspherical or irregular in shape ($N = 12$), 73 (49–84) by 41 (30–57), forming 3–5 layers, approximately 82 (68–93) per segment (based on sections and whole mounts); degenerating in segments with fully developed ovaries. Testes distribution: antiporal 55 ± 7 (44–63); prevaginal 9 ± 1 (8–10); postvaginal 19 ± 2 (14–20). Vagina entering genital atrium ventrolateral to cirrus, glandular, enlarging to form a seminal receptacle anterior to ovarian isthmus. Ovary bilobed, each lobe subdivided into dendritic processes radiating from ends of ovarian isthmus. Ovarian lobes about equal ($N = 20$), 178 (152–198) by 144 (129–156). Oocapt conspicuous, Mehlis' gland complex, well developed, occupying most of available space posterior to ovarian isthmus. Uterus glandular, ventromedian, somewhat sinuous, terminating near anterior margin. Vitelline follicles irregular ($N = 20$), 63 (46–80) by 33 (27–41), numerous, interspersed between longitudinal muscles, encircling internal organs anterior to ovary. Osmoregulatory ducts multiple, small, interspersed among testes and vitellaria; exact number and distribution not determined.

HOST: *Rhinoptera bonasus* (Mitchill, 1815); cownose ray.

LOCALITY: Sakonnet Point, Rhode Island.

TYPE SPECIMENS: USNM Helm. Coll. Nos. 77633 (holotype) and 77634 (two paratypes).

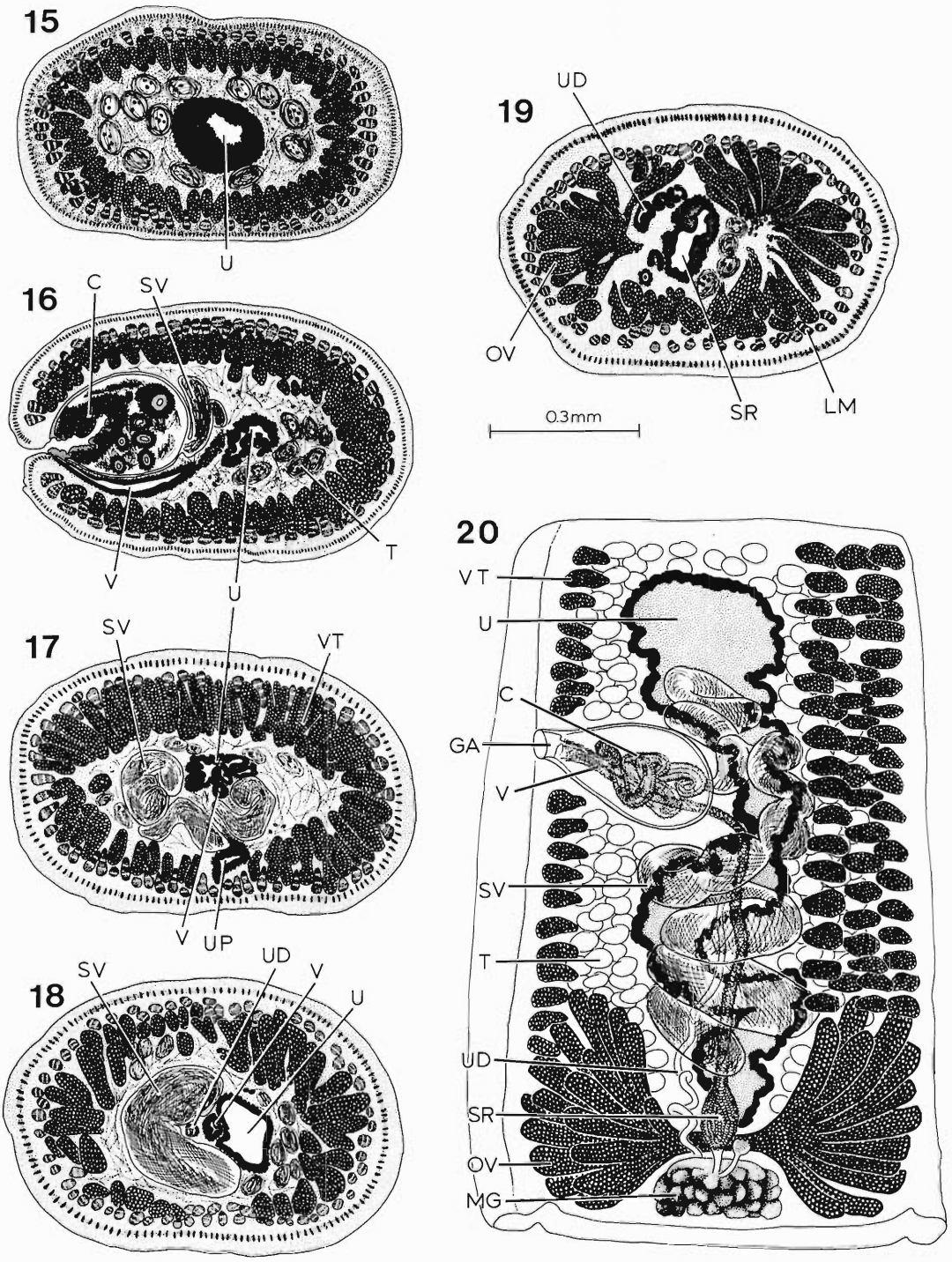
ETYMOLOGY: The species is named after its host.

Remarks

Unlike *T. pingue* the metoporhynchus of *T. bonasum* is very protrusible and is used for penetration into the submucosa (Figs. 23, 24). Destruction of the mucosa is evident at the attachment site (Fig. 24). When evaginated, the metoporhynchus has the shape of a light bulb (Figs. 5, 23) but it may also be withdrawn well into the pars basalis (Figs. 6, 7). Like *Tylocephalum,* there is a collar-like ring at the base of the metoporhynchus (Figs. 23, 24) that creates the sucker-like rim around the metoporhynchus when it is withdrawn (Figs. 6, 7). The tegument over the metoporhynchus does not stain as intensely as that over the posterior portion of the scolex and strobila (Fig. 24). Large muscle fascicles extend from the posterior region of the scolex directly into the metoporhynchus. The pars basalis bears four suckers on its anterior rim and is deeply recessed on its posterior surface to receive the neck of the strobila. The scolex is usually oval in cross section (Fig. 6) but can be quadrangular (Fig. 7) like Perrenoud's (1931) *Hexacanalis.*

The strobila is long, flexible and distinctly rectangular in cross section for most of its length. Older segments gradually become more oval in cross section. Dense layers of muscle and vitelline follicles around the medulla prevent easy viewing of the reproductive system in whole mounts.

Details of the reproductive system can be seen in frontal or transverse serial sections of mature segments. Numerous testes, layered three to five deep, fill all available space in the medullary region from the anterior extremity of the segment posterior to the ovarian isthmus. An elongated, coiled external seminal vesicle ascends in the midline dorsal to the uterus (Figs. 17, 20). Occasionally a coil of the proximal portion of the seminal vesicle displaces the uterus laterally (Fig.



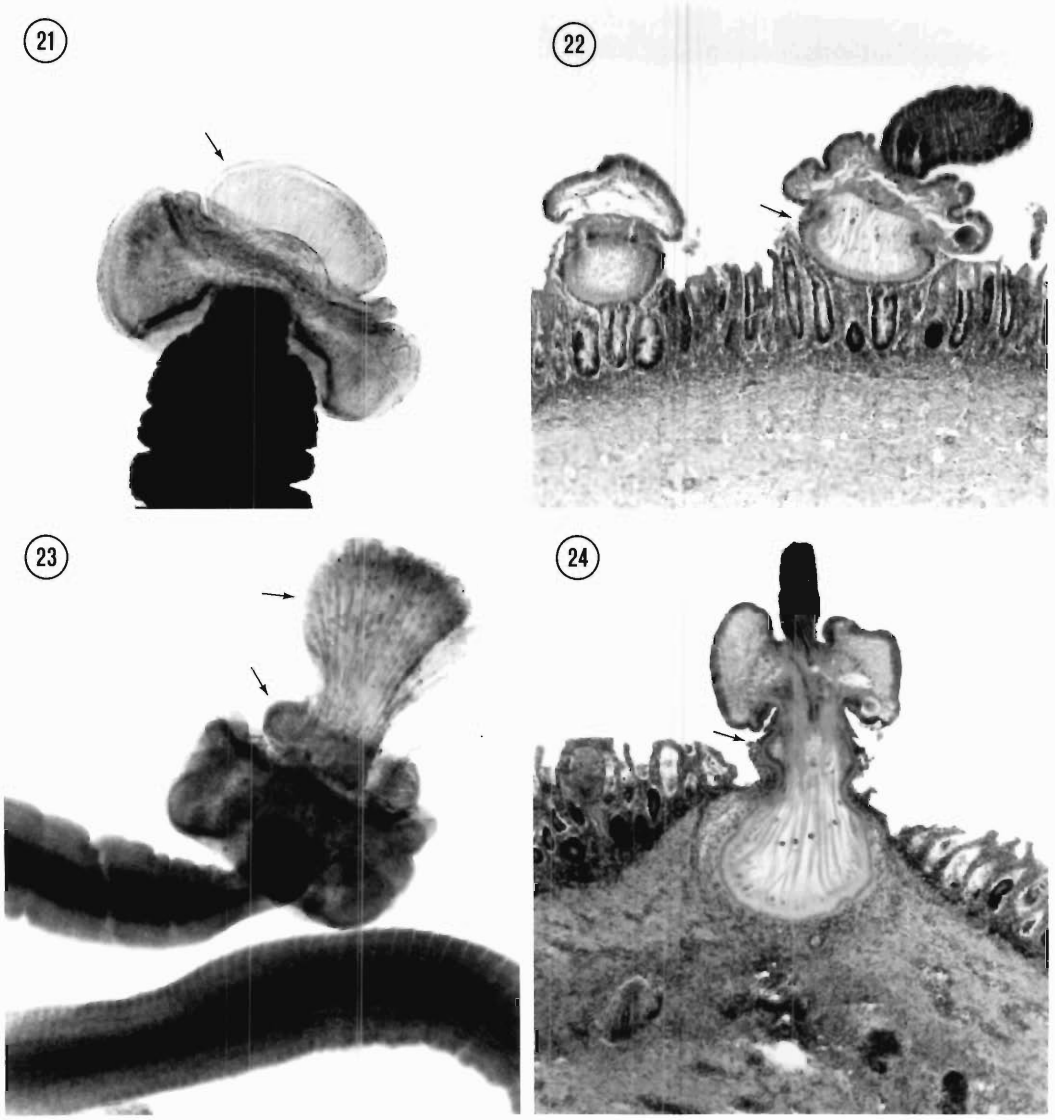
Figures 15–20. Reproductive system of *Tylocephalum bonasum* sp. n. Figures 15–19. Transverse sections of mature segment. All sections drawn to same scale. 15. Anterior to cirrus sac. 16. Through genital atrium. 17. Just posterior to cirrus sac. 18. Through proximal end of uterus. 19. Through ovary. 20. Schematic diagram of reproductive system (dorsal view) reconstructed with overlays of frontal and transverse serial sections.

18). A few coils of the seminal vesicle extend anterior to the cirrus pouch (Fig. 20). The seminal vesicle joins the cirrus pouch at its medial end but there is no internal seminal vesicle (Fig. 16). A long, coiled, unarmed cirrus surrounded by dark staining cells occupies the interior of the cirrus pouch. The cirrus can be everted through the shallow genital atrium. The vagina originates as a narrow tube at the genital atrium and parallels the cirrus pouch on its ventrolateral surface. At the midline the vaginal duct turns posteriad and follows a direct course in the median line to the ovary where it expands to form a small seminal receptacle (Fig. 19). The position of the vagina relative to the uterus varies with the displacement of the uterus by the large coils of the seminal vesicle (Figs. 17, 18). Both the vagina and seminal receptacle are surrounded by dark staining cells. The seminal receptacle terminates in a sperm duct that passes over the ovarian isthmus and joins the oviduct at the ootype (Fig. 20). The ovary (Figs. 19, 20) is divided into two masses of lobules that radiate from the ends of a distinct ovarian isthmus. A large Mehlis' gland complex is located immediately posterior to the isthmus. The glandular and coiled uterine duct emerges from the ootype, passes dorsal to the ovarian isthmus (Fig. 19), and parallels the vagina until it joins the uterus (Figs. 18, 20). The uterine duct joins the uterus about half-way between the ovary and cirrus pouch. The uterus, surrounded by a thick layer of dark staining "gland" cells, follows a sinuous course beginning just anterior to the ovarian isthmus and ends near the anterior margin of the segment (Fig. 20). It occupies the center of the segment (Fig. 15) and expands to its greatest non-gravid dimension (Fig. 20) anterior to the cirrus pouch. Posterior to the cirrus pouch the uterus is displaced vertically (Fig. 17) or laterally (Fig. 18) by the coils of the seminal vesicle. A duct connecting the developing uterine pore with the ventral surface of the uterus is found just posterior to the level of the cirrus pouch. All of the osmoregulatory ducts are small and their exact number and distribution are obscured by the large vitelline follicles that occupy so much of the cortical region of the segments.

Systematics

The scolex and strobilar form of *Tylocephalum bonasum* are remarkably similar to the descriptions given for *Cephalobothrium* Shipley and Hornell, 1906 and *Hexacanalis* Perrenoud, 1931. However, detailed comparison of *T. bonasum* and *T. pingue,* as described herein, show that neither external or internal anatomy warrants placement of *T. bonasum* in any genus except Linton's *Tylocephalum. Tylocephalum bonasum* may be differentiated from all other species by its scolex, strobila, testes number, circumcortical vitellaria, form of the seminal vesicle, and osmoregulatory system.

Cephalobothrium was created by Shipley and Hornell (1906) for a single specimen from *Aetobatus narinari* (Euphrasen, 1790) which they named *C. aetobatidis.* Southwell (1925) accepted *Tylocephalum* and *Cephalobothrium* by making the distinction (p. 249) that the myzorhynchus is normally evaginated but in *Cephalobothrium* it is normally invaginated (note his key p. 250 states "myzorhynchus absent" in *Cephalobothrium*). Perrenoud (1931) created the genus *Hexacanalis* after examining sections of worms designated as *C. abruptum* Southwell, 1911. It should be noted that (1) *Hexacanalis* was created without examining whole mounts or live specimens, (2) the internal anatomy of *Cephalobothrium* was unknown at that time, and still is not, because Perrenoud (1931) did not examine Shipley and Hornell's type species. Despite the fact that Perrenoud had not seen *C. aetobatidis,* he used the following reasons for creating *Hexacanalis* and making *C. abruptum* Southwell, 1911 type species: (1) different hosts for *C. aetobatidis* Shipley and Hornell, 1906 and *C. abruptum*; (2) differences in segmentation (*C. aetobatidis,* strongly craspedote; *C. abruptum,* slightly craspedote); (3) scolex shape and histology (*C. aetobatidis* is rounded in cross section; *C. abruptum* non-glandular and quadrangular in cross section); and (6) osmoregulatory system (*C. aetobatidis* has an unknown osmoregulatory system and *C. abruptum* has six main osmoregulatory ducts). These characteristics did not warrant the creation of a new genus because (1) the internal anatomy of *C. aetobatidis* was unknown, (2) different elasmobranch host species usually harbor different species of cestodes, and (3) use of the quadrangular scolex shape as a generic character is questionable because it was probably a consequence of fixation. Variation in scolex shape from square to round is readily apparent in our specimens of *Tylocephalum bonasum* from the cownose ray (Figs. 6, 7). A non-glandular scolex and six osmoregulatory ducts



Figures 21–24. Scolex attachment of *Tylocephalum*. 21. Scolex, *T. pingue*. Note metoporhynchus (arrow). 22. Sagittal section of two *T. pingue* scolices in situ. Note surficial attachment. 23. Scolex, *T. bonasum*. Note everted metoporhynchus and basal collar (arrows). 24. Sagittal section of *T. bonasum* in situ. Note embedded metoporhynchus and basal collar (arrow).

did not justify creation of a new genus when histology and internal anatomy were unknown for *Cephalobothrium*. The significance and interpretation of the number of osmoregulatory ducts is questionable for as Euzet and Combes (1963) noted for *Tetragonocephalum uarnak*,

"Sur coupes transversales on distingue 4 vaisseaux principaux de diamètre sensiblement égal, placés en face des côtes du tétragone. Mais ces vaisseaux (2 dorsaux et 2 ventraux) sont entrêment contournés et ramifiés, de telle sorte que sur coupe transversale on compte souvent 5 ou 6 canaux que semblent être principaux.

On distingue en outre dans le parenchyme médullaire de très nombreux canalicules. Nous n'avons pas observé d'ouverture à l'extérieur de ces canalicules. Ces pore excréteurs secon-

daires ont été signalés par Perrenoud chez *Hexacanalis abruptum* (Southwell, 1911).

The genus *Hexacanalis,* therefore, should be considered a synonym of the enigmatic *Cephalobothrium.* Furthermore, the invagination of the metoporhynchus into a large terminal sucker is the only character upon which *Cephalobothrium* was based. We do not consider that character unique in view of the variability of the two species we have described herein and see no practical value in attempting to use it. Southwell (1925, p. 271) stressed the variability of the scolex of *T. dierama* (also see Wardle and McLeod, 1952), which includes total evagination and the sucker-like appearance of the metoporhynchus upon retraction. Therefore, we feel that there is no valid basis for retaining either *Cephalobothrium* or *Hexacanalis* except that we consider it of practical value and less disruptive to the literature to retain *Cephalobothrium* for species similar to *Tylocephalum* but having vitellaria in lateral bands. We consider it a lecanicephalid. *Tetragonocephalum,* as described by Euzet and Combes (1963), creates no conflict with this scheme because it contains an internal seminal vesicle.

Thus, a key differentiating these lecanicephalan genera would read as follows:

Metoporhynchus fungiform; pars basalis globose, bearing four suckers.

1a. Internal and external seminal vesicles present ..... *Tetragonocephalum*

1b. External seminal vesicle present; internal seminal vesicle absent 2

2a. Vitellaria in lateral bands ..... *Cephalobothrium*

2b. Vitellaria circumcortical ..... *Tylocephalum*

Discussion

Previous descriptions of *Tylocephalum* and *Cephalobothrium* have repeatedly referred to the enormous "seminal receptacle" of species in these genera. Descriptions by Subhapradha (1957) and Euzet and Combes (1963) are the only authors to note the presence of a large seminal vesicle. We have reconstructed the internal anatomy of *T. pingue* and *T. bonasum* and, after careful examination, have found that this "seminal receptacle" is part of the male system, not an enlargement of the vagina or any part of it, and is therefore an external seminal vesicle. The vagina, vas deferens and seminal vesicle can be easily traced because they are histologically distinct and there is no question that this structure has been repeatedly misinterpreted (see Figs. 8–19). The expansion of the seminal vesicle in older segments forces the proximal end of the vas deferens into the vicinity of the ovarian isthmus and creates the illusion that the two are connected. Transverse sections show that they are not.

The inadequacy of old descriptions, the often poor condition of type specimens or their unavailability hinders progress in resolving taxonomic problems and establishment of meaningful systematic relationships. Many of the genera of the Lecanicephalidea and Tetraphyllidea that must be considered incertae sedis were discovered in Ceylonese waters but they are unusable until completely redescribed. Numerous synonymies may result from such studies but unless adequate diagnoses and descriptions are provided only further confusion will result from their use. For example, *Glyphobothrium* Williams and Campbell, 1977 may be a synonym of *Tiarabothrium* Shipley and Hornell, 1906 despite the fact that Southwell (1925, p. 211) considered *Tiarabothrium* to be "clearly synonymous with *Echeneibothrium,*" and *Tetragonocephalum* Shipley and Hornell, 1905 to be a synonym of *Tylocephalum* Linton, 1890. We conclude that *Hexacanalis* Perrenoud (1931) is a synonym of *Cephalobothrium* for the reasons discussed previously. We also consider that some recently created genera are synonyms of "older" genera. The most important characteristic of *Spinocephalum* Deshmukh, 1980 separating it from *Tylocephalum* is the spination of the metoporhynchus. Tegumental spination is variously developed among many cestode genera and is characteristic of *T. yorkei* Southwell, 1925 and the posterior portion of the scolex of *T. ludificans* Jameson, 1912 bears "tricuspid denticles." In any case, we consider such a characteristic to have only specific value and see no reason why *Spinocephalum* should not be considered a junior synonym of *Tylocephalum.* Likewise, we find nothing in the description of *Flapocephalus saurashtri* Shinde and Deshmukh, 1979 that separates it from *Lecanicephalum* Linton, 1890. The scolex of *F. saurashtri* is compressed (see fig. A of Shinde and Deshmukh, 1979) and the presence of two semicircular flaps, the most important generic characteristic and the only character making the genus distinct from *Lecanicephalum,* is not evident

in the figure. Even the type species, *F. trygonis* Deshmukh, 1979, is questionable because the type specimens are not accessible to disprove that compression of the scolex has not created the "flaps" described for the type species.

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Elytrophallus carettae sp. n. (Digenea: Hemiuridae) from the Stomach of Loggerhead Turtles (*Caretta caretta* (L.)) from Australia

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ABSTRACT: *Elytrophallus carettae* sp. n. is described from the stomach of loggerhead turtles (*Caretta caretta* (L.)) from Queensland and Western Australia. The new species is distinguished from other members of the genus by the following characters; large size of body and seminal vesicle, tegumental papillae on ventral sucker and around oral opening, testes almost opposite and well separated from one another, uterus not extending posterior to vitellaria.

The species described herein is based upon nine specimens from loggerhead turtles taken in Pacific and Indian Ocean coastal waters of Australia. Five specimens came from the stomach of a female turtle (66 kg, 83 cm curved carapace length) taken at Shark Bay, Western Australia on July 5, 1979. These worms, which were alive when collected, were killed with hot (70°C) water, manipulated on a piece of moist filter paper so as to lie straight, and fixed by overlaying a second piece of filter paper soaked in 10% formalin. In this way the worms could be held straight during initial fixation without undue pressure being exerted on them. After 1 hr they were transferred to a vial of 10% formalin. Four of the five specimens from Western Australia were stained with Gower's carmine and mounted in Canada balsam. The fifth was prepared as serial transverse sections. The four specimens from the Pacific coast were from the stomach of a subadult turtle in poor condition taken at Heron Island, Queensland on May 15, 1975. These worms, which were sent to the author in a vial of 70% alcohol, were very contracted and were prepared as serial sagittal sections.

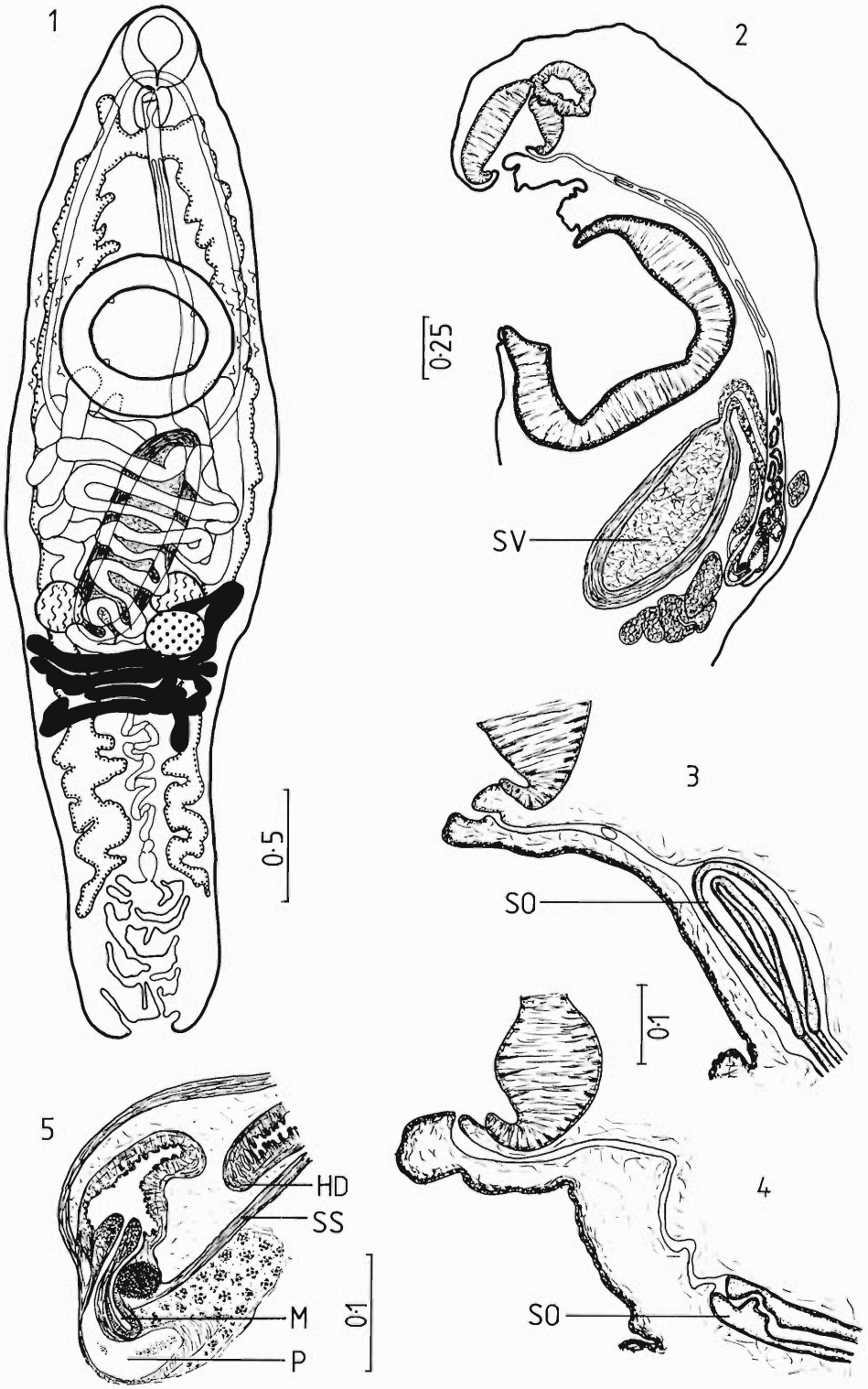
Terminology used throughout this paper follows Gibson and Bray (1979). Measurements (in mm) are from the four whole mounts and are given as a range, followed by the mean in parentheses.

Elytrophallus carettae sp. n. (Figs. 1–5)

DESCRIPTION: Body thick, 3.8–4.6 (4.15) long, 0.93–1.26 (1.08) wide a short distance behind ventral sucker; more or less tapering anteriorly, bluntly truncated posteriorly; ecsoma withdrawn in all specimens. Preoral lobe inconspicuous or absent. Presomatic pit absent. Tegument smooth, thicker dorsally than ventrally; with lateral and ventral papillae in region of ventral sucker including four on its inner margin. Other papillae occur around oral opening but their number and distribution not determined. Oral sucker subterminal, 0.32–0.36 (0.34) in diameter. Ventral sucker 0.74–0.84 (0.79) in diameter in second quarter of body. Sucker ratio 1:2.25–2.4. Prepharynx absent; pharynx 0.19–0.2 (0.2) long, 0.16–0.18 (0.16) wide. Esophagus absent. Arch of each cecum sometimes having a short anterior diverticulum that differs histologically from rest of cecum but not markedly glandular as in "Drüsenmagen" (Gibson and Bray, 1979). Ceca irregular in width along most of their length, probably entering ecsoma when this protruded.

Testes 2, rounded or irregularly oval, subequal, 0.19–0.38 (0.25) in diameter, opposite or slightly oblique, in third quarter of body. Seminal vesicle conspicuous, spindle shaped, thick walled, 0.74–1.04 (0.88) long, 0.25–0.35 (0.32) wide, median or obliquely placed between testes and ventral sucker. Pars prostatica tubular, surrounded along most of its length by delimited gland cells, originating at anterior end of seminal vesicle and running posteriorly to join base of sinus sac near mid-length of seminal vesicle. Sinus sac muscular, its wall thinning slightly as sac tapers toward junction with genital atrium about midway between suckers. Hermaphroditic duct formed by union of pars prostatica and metraterm within base of sinus sac; duct muscular proximally, convoluted when sinus organ withdrawn, terminating as a permanent amuscular sinus organ. Genital atrium thin walled, tubular, dilated proximally when sinus organ everted. Genital opening midventral, immediately posterior to oral sucker.

Ovary rounded to oval, 0.16–0.22 (0.19) long, 0.23–0.32 (0.27) wide, dextral or sinistral and immediately posterior to testis on that side. Ovi-



duct arises posteriorly and receives vitelline duct as it enters Mehlis' gland posterior to ovary. Laurer's canal ends in a well-developed Juel's organ, with inner vesicle sometimes bipartite, anterodorsal to Mehlis' gland. Vitellarium consists of seven long tubular lobes, arising ventrally at posterior edge of ovary, often passing dorsally external to other organs (sometimes intercecal). Vitelline reservoir absent. Uterus initially coiled at posterior end of Mehlis' gland but never extending posterior to vitellarium, distended as a uterine seminal receptacle before turning anteriorly and ascending ventral to seminal vesicle. Uterine loops eventually occupy much of space between testes and ventral sucker. Metraterm short, muscular. Intrauterine eggs (10 measured) 0.016–0.02 (0.018) by 0.009–0.011 (0.01).

Excretory ducts extend posteriorly from commissure dorsal to oral sucker to unite posterior to ventral sucker, forming convoluted median common duct slightly dilated before opening into cavity formed by withdrawn ecsoma (=tip of ecsoma when this protruded).

HOST: *Caretta caretta* (Linnaeus, 1758).

SITE IN HOST: Stomach.

TYPE SPECIMENS: Holotype: Whole mount from Western Australia, in South Australian Museum, Adelaide, S.A. 5000 (No. V3385). Paratypes: One (whole mount) (No. V3386) from Western Australia and one (serial sagittal sections) (No. V3387) from Queensland, in South Australian Museum. One (whole mount) (No. 77458) from Western Australia and one (serial sagittal sections) (No. 77459) from Queensland in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705, USA.

ETYMOLOGY: The species is named after its host.

Discussion

The genus *Elytrophallus* belongs in the subfamily Elytrophallinae (following Gibson and Bray, 1979). Within this subfamily, *Elytrophallus* is closest to *Johniophyllum* Skrjabin and Guschanskaja, 1954 and especially to *Elytrophalloides* Szidat, 1955. The latter genus bears plications on the body surface, a character often regarded as of generic value, and the principal distinguishing feature between *Elytrophallus* and *Elytrophalloides* (Gibson, 1976). It should be noted that extended representatives of species with a plicate tegument sometimes fail to exhibit this feature (Gibson, 1976). Prudhoe and Bray (1973) reported that in some specimens of the type species of *Elytrophalloides, E. oatesi* (Leiper and Atkinson, 1914) Szidat and Graefe, 1967, the plications are not apparent. Such specimens could be difficult to distinguish from members of *Elytrophallus,* and especially from *E. carettae.* However, in *Elytrophalloides oatesi* the uterus extends posterior to the vitellarium whereas it does not in *Elytrophallus carettae.*

Gibson and Bray (1979) noted that *Johniophyllum johnii* (Yamaguti, 1938) Skrjabin and Guschanskaja, 1954, although inadequately described, keyed out with *Elytrophallus* in their key to the subfamily Elytrophallinae. This species would seem to be excluded from *Elytrophallus* by its short sinus sac that does not extend into the hind-body. It can be further distinguished from *E. carettae* by its small size and uterine loops posterior to the vitellaria. Ichihara (1968) figured *J. johnii* but his drawing provided little morphological information.

In addition to the type, *Elytrophallus mexicanus,* Yamaguti (1971) recognized seven species within *Elytrophallus.* These were *E. australis* (Woolcock, 1935) Yamaguti, 1971; *E. chloroscombri* (Siddiqi and Cable, 1960) Yamaguti, 1971; *E. decapteri* Yamaguti, 1970; *E. fistulariae* Yamaguti, 1970; *E. holocentri* Yamaguti, 1970; *E. lovettiae* (Crowcroft, 1947) Yamaguti, 1971; and *E. mulloidichthydis* Yamaguti, 1970.

Of these, two species, *E. australis* and *E. lovettiae* do not appear to belong to *Elytrophallus.* The presence of plications on their tegument, the arrangement of the testes in tandem and of the vitellarium in two lobed masses argue in favor of their return to *Parahemiurus* (subfamily Hemiurinae), the genus in which they were orig-

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Figure 1. *Elytrophallus carettae* sp. n. composite wholemount in ventral view (based mainly on holotype). Figures 2–5. Terminal genitalia and associated structures of *Elytrophallus carettae* in sagittal section. 2. Terminal genitalia. 3, 4. Distal terminal genitalia of two specimens showing sinus organ extended (3) and withdrawn (4). 5. Proximal end of sinus sac showing entrance of genital ducts. HD, hermaphroditic duct; M, metraterm; P, pars prostatica; SO, sinus organ; SS, sinus sac; SV, seminal vesicle.

inally placed by Woolcock (1935) and Crowcroft (1947). However, the form of the seminal vesicle and the terminal genitalia suggests that these two species could belong in the Elytrophallinae. Prudhoe and Bray (1973) and Gibson (1976) suggested that they belong in *Elytrophalloides*. Indeed, Gibson (1976) stated that "it seems very likely that these two species are conspecific with *Elytrophalloides oatesi*." Reimer (1981), apparently independently of Prudhoe and Bray (1973) and Gibson (1976), has synonymized these three species under the name of *Elytrophallus oatesi*. His choice of genus appears to be based on his acceptance of the inclusion of *E. australis* and *E. lovettiae* in *Elytrophallus* by Yamaguti (1971).

Elytrophallus chloroscombri was originally placed in *Parectenurus* by Siddiqi and Cable (1960). This genus was reduced to a synonym of *Ectenurus* by Manter and Pritchard (1960b). The original description, based on a single specimen of *E. chloroscombri*, is not very detailed, especially with regard to the terminal genitalia. Fischthal and Thomas (1971) have re-examined the holotype and considered the species synonymous with *Ectenurus lepidus* Looss, 1907. No further discussion of this species is required here.

It remains to distinguish between *Elytrophallus carettae* and the five remaining species of the genus. *E. carettae* is much larger than *E. mexicanus*, there being no overlap between the two in any dimensions. *E. carettae* has papillae on and around the ventral and oral suckers (papillae present only on pre-oral lobe in *E. mexicanus*), testes that are almost opposite and well separated from one another (oblique and contiguous in *E. mexicanus*), uterus that does not extend posterior to the vitellarium (may enter the ecsoma in *E. mexicanus*) and an enormous thick-walled seminal vesicle (smaller and sometimes indistinctly tripartite in *E. mexicanus*). Manter (1940) reported a very small seminal receptacle embedded in the Mehlis' gland of *E. mexicanus* (this is probably a Juel's organ) and made no mention of a uterine seminal receptacle such as is seen in *E. carettae*. From the descriptions by Manter (1940), Manter and Pritchard (1960a) and comment by Yamaguti (1958) it seems that the terminal genitalia of *E. mexicanus* and *E. carettae* closely resemble one another. However, the genital atrium of *E. mexicanus* is muscular, except at its distal end, whereas in *E. carettae* it is not very muscular and is not differentiated into regions.

The suite of characters used above to distinguish between *E. carettae* and *E. mexicanus* largely serves to separate the former from the four species described by Yamaguti (1970). Three of the species (*E. fistulariae, E. holocentri, E. mulloidichthydis*) are substantially smaller than *E. carettae*. Only *E. decapteri* bears papillae on the tegument. In all four species, the uterus is coiled posterior to the vitellarium and the testes are generally contiguous.

Elytrophallus carettae appears to be only the second hemiurid reported from a chelonian. Dwivedi (1967) described *Lecithochirium chauhani* from the intestine of a freshwater tortoise, *Kachuga intermedia* from north-central India.

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Histochemical Localization of Hydrolytic Enzymes in the Cercaria and Excysted Metacercaria of *Echinostoma revolutum* (Trematoda)

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ABSTRACT: Histochemical methods for acid (AcP) and alkaline phosphatases (AlP), leucine aminopeptidase (LAP), non-specific esterase (NSE), and acetylcholinesterase (AChE) were applied to whole cercariae and excysted metacercariae of *Echinostoma revolutum*. All of these enzymes were active in the larvae. The activity was of particular importance in localizing the digestive, excretory and nervous systems in these larvae. In general, enzyme activity decreased in the metacercaria compared to the cercaria, suggesting that these enzymes are involved in the complex processes of encystment and excystment in *E. revolutum*. Some decrease in enzyme activity in metacercariae might be the result of the end of requirements for them as the active cercariae become less active metacercariae.

As reviewed by LeFlore (1979), several studies have described the histochemical localization of hydrolytic enzymes in cercariae. Little information is available on these enzymes in chemically excysted metacercariae. Fried and Butler (1978) described chemical excystation of the metacercaria of *Echinostoma revolutum*. In the U.S.A., rediae and cercariae of this trematode occur in the fresh water planorbid snail, *Helisoma trivolvis*, and encysted metacercariae are in the kidney of physid and lymnaeid snails (Fried and Weaver, 1969). In the wild, waterfowl and mammals serve as definitive hosts (Beaver, 1937), and in the laboratory the domestic chick is a convenient host (Fried and Weaver, 1969).

There is no information on hydrolytic enzymes either in freshwater echinostome cercariae or excysted echinostome metacercariae. The purpose of this study was to use histochemical methods to show the localization of various hydrolytic enzymes in cercariae compared to excysted metacercariae of *E. revolutum*.

Materials and Methods

Cercariae of *E. revolutum* emitted from naturally infected *H. trivolvis* snails maintained in finger bowls containing pond water (Fried and Weaver, 1969) were used within 2 hr postemission. Encysted metacercariae from the kidney of experimentally infected *Physa heterostropha* snails (Fried and Weaver, 1969) were excysted in an alkaline trypsin-bile medium (Fried and Butler, 1978); excysted metacercariae were rinsed in saline, and used within 15 min postexcystation.

Cercariae and excysted metacercariae were fixed in 10% neutral buffered formalin at 1°C for 5–30 min and then flattened with gentle coverslip pressure. To remove the fixative, cold distilled water was passed between the slide and the coverslip and larvae were then treated by: (1) the naphthyl AS-BI phosphate method for acid phosphatase (AcP) (Burstone, 1958); (2) the β-glycerophosphate calcium salt method for alkaline phosphatase (AlP) (Gomori, 1952); (3) the L-leucyl-β-naphthylamide hydrochloride method for leucine aminopeptidase (LAP) (Burstone and Folk, 1956); (4) the 5-bromoindoxyl acetate method for non-specific esterase (NSE) (Holt and Withers, 1952); (5) the acetylthiocholine iodide method for acetylcholinesterase (AChE) (Gomori, 1952). With the AcP and LAP methods, cercariae and metacercariae were pretreated with a graded series of acetone at 1°C to remove lipids. Controls consisted of specimens heat inactivated at 90°C for 5 min prior to incubation and omission of specific substrates from the incubation medium. Additional controls for the NSE and AChE methods consisted of incubation of larvae in medium containing 10^{-4} and 10^{-3} M eserine. Following incubation, larvae were washed in distilled water and mounted in glycerol jelly.

Results

A strongly positive reaction for AcP was obtained in the esophagus and intestine of the cercaria; moderate reactions occurred in the oral sucker, cerebral ganglia, and excretory bladder (Fig. 1). Weak reactions were seen in the pharynx, excretory ducts, acetabulum, and tail. In the metacercaria, AcP was localized only in the esophagus and intestine. Reactions were abolished in all control larvae exposed at 90°C for 5 min, and did not occur in controls incubated in the absence of substrate.

A strongly positive reaction for AlP was seen in the excretory concretions anterior to the acetabulum, the excretory bladder, and the tail nuclei of the cercaria (Figs. 2, 3). The reaction was moderate in the oral sucker and acetabulum. In

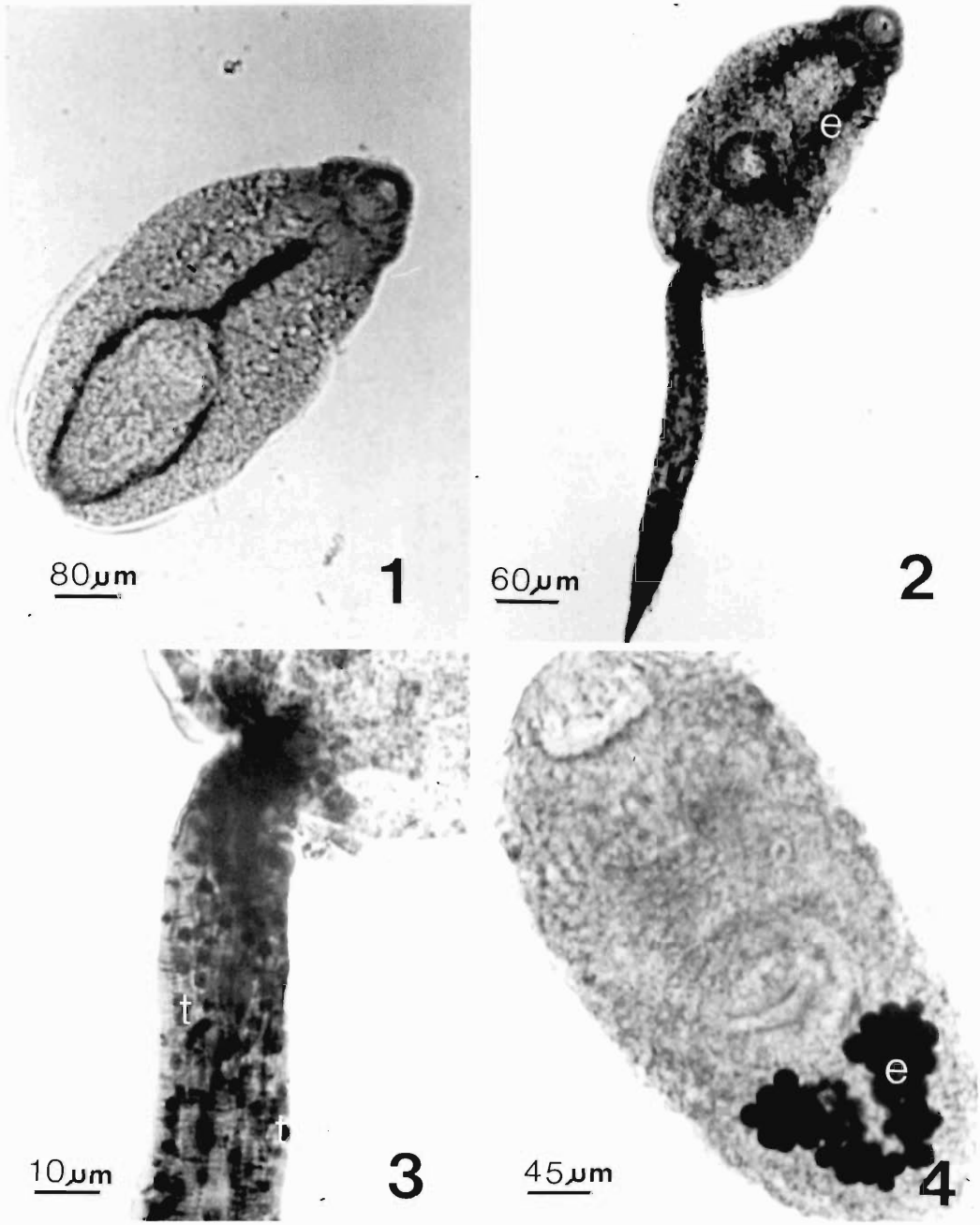

Figure 1. Cercaria treated by the naphthyl AS-BI phosphate method for AcP. Cercarial tail is missing. **Figure 2.** Cercaria treated by the β-glycerophosphate calcium salt method for AlP. e = excretory concretions. **Figure 3.** Tail nuclei of a cercaria treated as in Figure 2. t = tail nuclei. **Figure 4.** Excysted metacercaria treated as in Figure 2. e = excretory concretions.

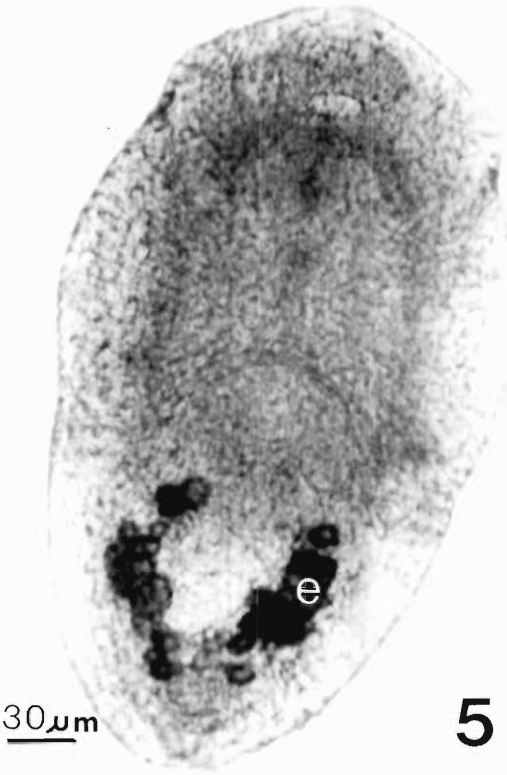

Figure 5. Excysted metacercaria treated by the L-leucyl-β-naphthylamide hydrochloride method for LAP. e = excretory concretions. Figure 6. Excysted metacercaria treated by the 5-bromoindoxyl acetate method for NSE.

the metacercaria, the AlP reaction occurred in the flame cells and concretions in the posterior excretory ducts (Fig. 4). No reactions occurred in the controls.

In the cercaria a reaction for LAP was observed in muscle. There was an especially strong reaction in the excretory concretions anterior to the acetabulum; moderate reactions occurred in the esophagus, intestine, and tail. In the metacercaria, wherever localization occurred, it was more intense than in the cercaria. The excretory concretions posterior to the acetabulum stained heavily (Fig. 5). The intestine and musculature were also reactive. No reactions occurred in controls.

Both NSE and AChE were strongly visualized in the oral sucker, acetabulum, and nervous system of the cercaria. As in other cercariae (Bettendorf, 1897; Bruckner and Voge, 1974; LeFlore, 1979; LeFlore et al., 1980), the nervous system of *E. revolutum* is composed of cerebral ganglia connected by a commissure with lateral, ventral and dorsal trunks passing forward to the oral sucker. Three pairs of longitudinal trunks (lateral, ventral, and dorsal) pass posteriorly to the end of the body. Cross commissures connect these trunks throughout their length. NSE was also visualized in the excretory bladder, flame cells, esophagus, and intestine of the cercaria. In the metacercaria these enzymes localized mainly in the nervous system (Fig. 6), although a faint reaction was seen in the intestine. Inclusion of 10^{-4} and 10^{-3} M eserine abolished the reaction in the cercarial nerve trunks. However, a strong reaction persisted in the esophagus, intestine, excretory bladder, flame cells, and a faint reaction in the cerebral ganglia of the cercaria. These same inhibitors completely blocked the reaction within the metacercarial nervous system, and a faint reaction persisted in the esophagus and intestine.

Discussion

The histochemical localization of hydrolytic enzymes in several whole cercariae has been de-

scribed (Fripp, 1967; Jennings and LeFlore, 1972; LeFlore, 1979; LeFlore et al., 1980). Such studies allow for the visualization of various cercarial systems, particularly that of the excretory, digestive, and nervous. Our studies show that this methodology is also suitable to visualize organ systems in chemically excysted metacercariae.

Our studies show that cercariae and metacercariae of *E. revolutum* localize AcP, AlP, NSE, and AChE, and are in general accord with that of LeFlore (1979) on the cercaria of *Plagiorchis elegans* and LeFlore et al. (1980) on the cercaria of *Cloacitrema michiganensis.* Although LAP was not visualized in the cercaria of *P. elegans* or *C. michiganensis,* it was clearly localized in the cercaria and excysted metacercaria of *E. revolutum.* The probable physiological functions of hydrolytic enzymes in cercariae have been discussed by LeFlore (1979) and LeFlore et al. (1980).

Excysted metacercariae of *E. revolutum* contain less AcP, AlP, NSE, and AChE than cercariae, suggesting that some of these enzymes are used during the complex processes of encystment and excystment. An intense AlP activity in the excretory concretions of the metacercaria of *E. revolutum* suggests that this enzyme may be involved in the excystation process. During excystation of *E. revolutum,* concretions are released (see Fig. 3 in Fried and Butler, 1978), and are visible in the inner cyst. In trematodes, concretions are released by metacercariae and are usually not found in adults (Erasmus, 1967; Fried and Butler, 1979). Lackie (1975) has suggested that enzymes of parasite origin (intrinsic enzymes) are involved in the excystation of metacercarial cysts. The inner cyst wall of *E. revolutum* is proteinaceous (Gulka and Fried, 1978). The concretions in *E. revolutum* contain LAP and this enzyme may be involved in breeching the inner cyst wall during excystation.

NSE and AChE were present in the nervous system of the cercaria and excysted metacercaria of *E. revolutum.* The nervous system visualized in the metacercaria was essentially similar to that of the cercaria. A detailed discussion on the cercarial nervous system based on the localization of NSE and AChE has been presented by LeFlore et al. (1980).

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Immunization in Murine Schistosomiasis Using Soluble *Schistosoma mansoni* Antigens and Irradiation-attenuated Vaccines

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ABSTRACT: Single inoculations of heterologous (*Fasciola hepatica*) and homologous (*Schistosoma mansoni*) soluble trematode antigenic extracts were compared with cesium-137 irradiation-attenuated *S. mansoni* schistosomula and cercariae as to their induction of immunity to challenge infection in murine schistosomiasis mansoni. The live-attenuated vaccines produced greater than twice the protection afforded by the single inoculation of either trematode antigenic extracts. Both heterologous and homologous extracts resulted in similar reductions in worm burdens. Combining the trematode antigens with attenuated cercariae in sequential immunizations improved protection as compared with use of the live vaccine alone suggesting the presence of common or cross-reacting worm antigens with cercariae.

Several approaches have been used to attempt to immunize against schistosomiasis. The most consistently successful has been the use of irradiation-attenuated cercariae or schistosomula (reviewed in Taylor et al., 1977; Minard et al., 1978; Bickle et al., 1979; Hsü et al., 1980). Irradiation-attenuated vaccines have been shown effective in homologous systems (immunization and challenge with the same species) and to some extent in heterologous systems (immunization and challenge with different species). A second immunization tactic has employed non-trematode vaccines such as Bacillus Calmette-Guerin (Capron and Lesoin, 1969; Bout et al., 1977; Civil et al., 1978), *Corynebacterium parvum* (Maddison et al., 1978), *Toxoplasma gondii* (Mahmoud et al., 1976), and cord factor (Olds et al., 1980). A third approach has used both schistosomal (Murrell et al., 1975; Maddison et al., 1978) and non-schistosomal (Hillyer et al., 1975, 1977; Hillyer, 1976, 1979, 1980; Hillyer and Sagramoso de Ateca, 1979) trematode extracts. The rationale for the latter is supported by the fact that at least five common antigens are shared between *Schistosoma mansoni* and *Fasciola hepatica* (Capron et al., 1968; Pelley and Hillyer, 1978; Hillyer, 1979).

Studies designed to compare the efficacy of live attenuated vaccines with trematode antigenic extracts in immunization experiments are lacking. In addition, the use of antigenic extracts as primers or boosters of immunity have not been evaluated. We, therefore, decided to compare the protective effects of single doses of *F. hepatica* and *S. mansoni* soluble whole worm extracts (FhWW and SmWW, respectively) with irradiation-attenuated *S. mansoni* cercariae (Cerc*) and schistosomula (Som*) in mice subsequently challenged with normal non-attenuated *S. mansoni* cercariae and to assess the effects of these trematode antigenic extracts in combination with the attenuated cercariae vaccine.

Materials and Methods

TEST ANIMALS: Female inbred NMRI (NIH/Nmri CV) mice were used as test animals. The mice for each experiment came from the same lot and were 6 wk of age at the start of each experiment.

SOLUBLE TREMATODE ANTIGENIC EXTRACTS: Soluble trematode antigenic extracts used in these experiments were prepared from *F. hepatica* and *S. mansoni* adult worms. *F. hepatica* worms were obtained from infected bovine livers, washed repeatedly in normal saline, homogenized in a tissue grinder, and lyophilized (Hillyer et al., 1975). This preparation was stored at −20°C until use (up to 6 mo). *S. mansoni* (Puerto Rican strain) adult worms were obtained from heavily infected Swiss albino mice by hepatic-portal perfusion (Yolles et al., 1947). These worms were then washed repeatedly in normal saline, homogenized in a tissue grinder, lyophilized and stored as above. At the time of the experiments, both lyophilized worm preparations were reconstituted in Hanks' balanced salt solution (BSS), sonicated, and centrifuged at 3000 *g* at 4°C for 30 min to obtain the soluble portions. The soluble antigenic extracts of *F. hepatica* worms (FhWW) and *S. mansoni* (SmWW) were adjusted to 10 mg/ml of protein (Lowry) in Hanks' BSS. For immunization, 0.4 ml of these

Table 1. Comparison of soluble trematode extracts and irradiation-attenuated *S. mansoni* schistosomula and cercariae as vaccines in murine schistosomiasis.

Group	No. mice	Vaccine	Route	Worm burden $\bar{x} \pm$ SD	% reduction of worm burden	*t*-test *P* values
A	10	FhWW	SC	61.4 ± 13.8	22.4	<0.01
B	10	FhWW	IM	59.1 ± 14.6	25.3	<0.01
C	10	SmWW	SC	55.7 ± 14.2	29.6	<0.01
D	10	SmWW	IM	57.4 ± 12.6	27.4	<0.01
E	10	Som	SC	26.7 ± 10.9	66.2	<0.01
F	10	Som	IM	29.8 ± 13.0	62.3	<0.01
G	10	Cerc	PC	21.3 ± 11.0	73.0	<0.01
H	10			79.1 ± 12.4		

Vaccines: FhWW = *F. hepatica* soluble worm extract (4 mg/mouse); SmWW = *S. mansoni* soluble worm extract (4 mg/mouse); Som = irradiation-attenuated *S. mansoni* schistosomula (400 attenuated schistosomula/mouse); and Cerc = irradiation-attenuated *S. mansoni* cercariae (370 attenuated cercariae/mouse).

Routes of immunization: SC = subcutaneous; IM = intramuscular; and PC = percutaneous.

Challenge and perfusion: All mice were challenged with 200 normal non-attenuated cercariae 4 wk after immunization and subsequently perfused 6 wk post-challenge to determine the worm burdens.

preparations without adjuvant were injected in equally divided doses at two different sites for a total of 4 mg of protein per mouse.

LIVE-ATTENUATED VACCINES: Attenuated cercariae and schistosomula for immunization were prepared from pooled *S. mansoni* (Puerto Rican strain) cercariae shed from *Biomphalaria glabrata* snails and utilized within 3 hr of shedding. The cercariae, suspended in dechlorinated tap water (pH 7.4), were attenuated with a total of 45 kilorad (krad) of gamma ionizing irradiation from a cesium-137 source at 1.8 krad/min (Gammator M gamma-emitter, Isomedix Inc.). Three separate batches of cercariae were attenuated using the same irradiation methods. Half of the first batch of cercariae, after attenuation, were artificially transformed into schistosomula (Bickle et al., 1979) and utilized for subcutaneous and intramuscular immunizations in the first experiment. Suspensions of these schistosomula in Earle's medium were adjusted to 400 schistosomula/ml. Injections of 1 ml through 21G needles were administered either subcutaneously or intramuscularly in equally divided doses at two sites to mice in the irradiated schistosomula groups. The other half of the attenuated cercariae from the first cercarial batch were used to percutaneously immunize mice in the irradiated cercariae groups of the first experiment. Subsequently, two other batches of cercariae were shed 3 wk apart, irradiation attenuated, and employed as matched vaccines in the second experiment. Cercarial suspensions were adjusted to administer 400 irradiated cercariae per mouse percutaneously in the irradiated cercariae groups by 1 hr tail immersion. After the exposure period, the number of nonpenetrants per mouse were counted to determine the percent penetration and thus the actual immunization dose.

CHALLENGE AND PERFUSION: In both experiments, mice (immunized and nonimmunized) were challenged with 200 cercariae 4 wk after the last immunization date. Cercariae for challenge infections were obtained from separate snail sheddings for each of the two experiments. Six weeks after challenge, the mice were perfused (Yolles et al., 1947) to obtain worm load counts. The percent reduction of the worm burden was calculated for each group receiving immunization by comparison with the nonimmunized control group in each of the experiments using the following formula:

$$\% \text{ reduction} = (C - I)/C \times 100$$

where C = mean number of worms in the control group, and I = mean number of worms in the immunized group.

EXPERIMENT 1: In the first experiment the prophylactic effects of the two trematode (heterologous and homologous species) antigenic extracts were compared with each other and with the two live attenuated preparations (schistosomula and cercariae). In addition, the effects of varying the routes of administration (subcutaneous and intramuscular) were investigated for the trematode antigenic extracts and the irradiated schistosomula vaccine. The irradiated cercariae were administered percutaneously. Ten mice were allotted to each of eight groups. Group A was immunized subcutaneously and group B intramuscularly with FhWW; group C was immunized subcutaneously and group D intramuscularly with SmWW; group E was immunized subcutaneously and group F intramuscularly with irradiation-attenuated schistomula; group G was immunized percutaneously with attenuated cercariae; and group H was held as the nonimmunized control group (Table 1).

EXPERIMENT 2: In the second experiment the effects of combining the trematode antigenic extracts as immunogens administered intramuscularly with the irradiation-attenuated cercariae vaccine administered percutaneously in two immunization sequencing schemes were studied. In the first scheme, 10 mice each in groups A, B, and C initially received FhWW, SmWW, and Hank's BSS, respectively, as priming immunizations followed in 3 wk by the administration of irradiation-attenuated cercariae for all three of these groups.

Table 2. Effects of soluble trematode extracts on irradiation-attenuated *S. mansoni* cercariae in murine schistosomiasis immunization.

Group	No. mice	Vaccines 1	Vaccines 2	Worm burden $\bar{x} \pm$ SD	% reduction of worm burden	*t*-test *P* values
A	10	FhWW	Cerc	15.6 ± 8.8	77.6	<0.01
B	10	SmWW	Cerc	18.6 ± 9.0	73.3	<0.01
C	10		Cerc	21.5 ± 13.3	69.2	<0.01
D	10	Cerc	FhWW	21.5 ± 13.2	69.2	<0.01
E	10	Cerc	SmWW	20.2 ± 11.4	71.0	<0.01
F	10	Cerc		26.1 ± 13.7	62.6	<0.01
G	10			69.7 ± 12.1		

Vaccines: FhWW = *F. hepatica* soluble worm extract (4 mg/mouse); SmWW = *S. mansoni* soluble worm extract (4 mg/mouse); and Cerc = irradiation-attenuated *S. mansoni* cercariae (1 = 365 attenuated cercariae/mouse; 2 = 335 attenuated cercariae/mouse).

Immunizations: Immunizations were spaced 3 wk apart. The antigenic extracts were administered intramuscularly and the irradiated cercariae were administered percutaneously.

Challenge and perfusion: All mice were challenged with 200 normal non-attenuated cercariae 4 wk after the last immunization and subsequently perfused 6 wk post-challenge to determine the worm burdens.

In the second scheme, 10 mice each in groups D, E, and F were initially immunized with irradiation-attenuated cercariae followed in 3 wk by immunization with FhWW, SmWW, and Hanks' BSS, respectively, as booster immunizations. In the nonimmunized control group G, 10 mice were given Hanks' BSS intramuscularly 3 wk apart at the two immunization periods used for groups A through F (Table 2).

STATISTICAL ANALYSIS: *t*-tests were used to compare the separate immunized groups with the controls in each experiment. The Student-Newman-Keuls multiple range test was used to define statistically significant homologous subsets of groups for the two experiments.

Results

The mean worm burdens and the corresponding percentages of worm reductions in groups of mice receiving single immunizations of the soluble trematode antigenic extracts (FhWW or SmWW) and the irradiation-attenuated *S. mansoni* schistosomula (Som*) and cercariae (Cerc*) in experiment 1 are given in Table 1. Using the *t*-test, each of the immunized groups (A through G) showed a significant reduction in worm burdens over the control group H (Table 1). As determined by the Student-Newman-Keuls multiple range test, mice immunized with irradiation-attenuated schistosomula (groups E and F) had significantly higher reductions in worm burdens than did mice immunized with any of the soluble trematode extracts (groups A through D).

Varying the route of immunization (IM or SC) for either the trematode antigenic extracts or the attenuated schistosomula did not result in any significant differences in worm burden reductions. Since the penetration rate for the irradiation-attenuated cercariae in experiment 1 was 93%, the mice in that group (group G) received approximately 370 cercariae while mice in the schistosomula vaccine groups (groups E and F) received 400 schistosomula. Therefore, the antigenic dose of the immunizing cercariae was somewhat less than the immunizing schistosomula. However, the greatest degree of protection was achieved with the percutaneously administered attenuated cercariae (group G).

All of the immunized groups (A through F) in experiment 2 presented mean worm burdens and corresponding percentages of reduction that were significantly different from the nonimmunized control group G as determined by the *t*-test (Table 2). Immunization using the soluble trematode extracts in tandem with the irradiation-attenuated cercariae (groups A/B and D/E) produced improvement over the levels of protection obtained with the matched attenuated cercariae vaccine alone (groups C and F, respectively). For the mice in which the trematode extract preceded the attenuated cercariae vaccine (groups A and B), an increase of 8.4% worm burden reduction was recorded using FhWW and 4.1% with SmWW when compared with the percent reduction obtained with irradiated cercariae alone (group C). For the mice in which the trematode extract followed the attenuated cercariae (groups D and E), an increase of 7.6% was recorded using FhWW and 8.4% with SmWW when compared with the percent reduction obtained in the irradiated cercariae alone (group F). These differences, however, were not found to be sta-

tistically significant using the Student-Newman-Keuls multiple range test.

The percent penetration of attenuated cercariae in groups A, B, and C (91.2%) was greater than that in groups D, E, and F (83.7%) indicating that the antigenic dose for the former was somewhat higher. Groups A through C received approximately 365 attenuated cercariae while groups D through F received approximately 335 attenuated cercariae per mouse.

Discussion

These studies demonstrated that single inoculations (SC or IM) of soluble *F. hepatica* and *S. mansoni* in the absence of adjuvant extracts could induce significant although low levels of acquired resistance to *S. mansoni* challenge infection. Such levels of protection may not, however, be a demonstration of specific immunity (Mahmoud et al., 1976). In addition, the levels of protection derived from either heterologous or homologous trematode extracts were significantly lower than obtained with attenuated schistosomula and cercariae. The greatest degree of immunity was attained with the percutaneously administered irradiated cercariae suggesting that the percutaneous route was an important factor in establishing an optimal immune response.

In previous work (Hillyer et al., 1975, 1977, 1979; Hillyer, 1976, 1979), Freund's adjuvant was used with the *F. hepatica* extracts. However, this adjuvant was not employed in this study because it is unacceptable for human use and data on the sole use of the trematode extracts were desired.

Since partial resistance was acquired with the trematode antigenic preparations, these extracts were combined with the live-attenuated cercarial vaccine in an effort to augment the immune response. Improved levels of resistance were noted when either extract was employed before or after attenuated cercarial immunization when compared with the irradiated cercariae alone groups. Although these differences were not statistically significant, the data suggest that the extracts were able to influence both the induction and maintenance of immunity. It is possible that two separate mechanisms for the acquisition of immunity to schistosomiasis were thus stimulated as suggested by Olds et al. (1980). Therefore, it may be possible to combine the use of trematode antigenic extracts with live-attenuated cercariae to immuno-potentiate both the induction and memory phases in schistosomiasis immunization. Attempts to establish an optimal sequence of tandem immunization based on these data alone are hampered primarily because of differences in batches of irradiated cercariae. Optimal dosage, sequencing of vaccines, and the use of purified immunogens are under investigation.

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In Vitro Excystment of the Metacercaria of *Acanthoparyphium spinulosum* (Trematoda: Echinostomatidae)

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ABSTRACT: In vitro excystment studies on the metacercaria of *Acanthoparyphium spinulosum* indicated that optimal excystment occurred when the cysts were incubated in either a complete medium containing trypsin and bile salts, or bile salts alone following acid pretreatment, and the reductant (sodium dithionite) at a temperature of 42°C and a pH of 7.8. A relatively lower percentage of excystment was obtained following pretreatment with acidified pepsin or Hanks' BSS, the reductant, and incubation in trypsin at a pH of 7.8. Worms that excysted in any medium containing trypsin became sluggish after 1 hr in comparison to worms that excysted in bile salts, indicating that trypsin may have an inhibitory effect. Worms excysted in bile salts medium after pretreatment in acid and the reductant were more active, suggesting that bile salts may act as a muscular stimulant. Pretreatment with acidified pepsin followed by incubation in the complete medium, in the absence of the reductant, resulted in a low percentage of excystment; whereas, pretreatment with acidified Hanks' BSS followed by incubation in the complete medium in the absence of the reductant resulted in no excystment. The in vitro excystment process was initiated by acid pretreatment that resulted in vigorous muscular activity of the enclosed larva that eventually produced the escape aperture for emergence. A synergistic effect between trypsin and bile salts does not appear to occur in the excystment of this parasite.

Several reports have been concerned with the in vitro excystment of metacercarial cysts of echinostomes. Howell (1970) studied the conditions necessary for the excystment of *Echinoparyphium serratum*; Fried and Grigo (1975) reported on *E. flexum*; Fried and Butler (1978) observed the excystment of *Echinostoma revolutum*; Kirschner and Bacha (1980) described factors involved in the excystment of *Himasthla quissetensis*; and LeFlore and Bass (1982) investigated the conditions responsible for the excystment of *H. rhigedana*. There have been no similar studies on the metacercaria of *Acanthoparyphium spinulosum*. The first and second intermediate host of *A. spinulosum* is the brackish-water snail *Cerithidea hegewischi californica*. Cercariae that develop in daughter rediae excyst in the radular mass of the snail. The natural adult hosts are the black-bellied plover, *Pluvialis squaturola*, and the American avocet, *Recurvirostra americana*, which acquire the parasite by eating infected snails. Adults have been grown experimentally in the domestic chick by Martin and Adams (1961). The present study was undertaken to examine the factors that bring about excystment of *A. spinulosum* in vitro and to describe the behavior of the metacercaria during excystment.

Materials and Methods

Metacercariae of *Acanthoparyphium spinulosum* were obtained from the mudflat snail *Cerithidea californica* purchased from Jones Biomedicals and Laboratory, Long Beach, California, and Pacific Bio-Marines Laboratories, Venice, California. Several snails were placed in a finger bowl containing 50 ml of artificial seawater and maintained at room temperature. To obtain metacercariae, snails were crushed and the radular mass was removed and washed in artificial seawater. Cysts were dissected from the radular tissue and then washed in 0.85% saline prior to use.

Experiments were done in 2 ml of test medium in 3.5-cm-diameter culture dishes placed in a water bath at 42°C. The treatment groups were as follows: 1) pretreatment with 0.5% pepsin (1:10,000) in Hanks' BSS adjusted to pH 2.0 with 6 N HCl (acidified pepsin) for 1 hr and incubation in 0.2% sodium taurocholate (crude ox bile) and 0.5% trypsin (1:250) in Hanks' BSS adjusted to pH 7.8 with 7.8% $NaHCO_3$ (complete medium); 2) pretreatment with acidified pepsin for 1 hr, the reductant 0.015 M sodium diothionite ($Na_2S_2O_4$) for 10 min, and incubation in the complete medium; 3) pretreatment with acidified pepsin for 1 hr, the reductant for 10 min, and incubation in 0.2% ox bile and Hanks' BSS, pH 7.8; 4) pretreatment with acidified pepsin for 1 hr, the reductant for 10 min, and incubation in 0.5% trypsin in Hanks' BSS, pH 7.8; 5) pretreatment with acidified pepsin for 1 hr, the reductant for 10 min, and incubation in Hanks' BSS, pH 7.8; 6) pretreatment with the reductant for 10 min and incubation in the complete medium; 7) incubation in the complete medium; 8) pretreatment with Hanks' BSS, pH 2.0 (acidified Hanks') instead of acidified pepsin for 1 hr and incubation in the complete medium; 9) pretreatment with acidified Hanks' for 1 hr, the reductant for 10 min, and incubation in the complete medium; 10) pretreatment with acidified Hanks' BSS for 1 hr, the reductant for 10 min, and incubation in 0.2% ox bile and Hanks' BSS, pH 7.8; 11) pretreatment with acidified Hanks' for 1 hr, the reductant for 10

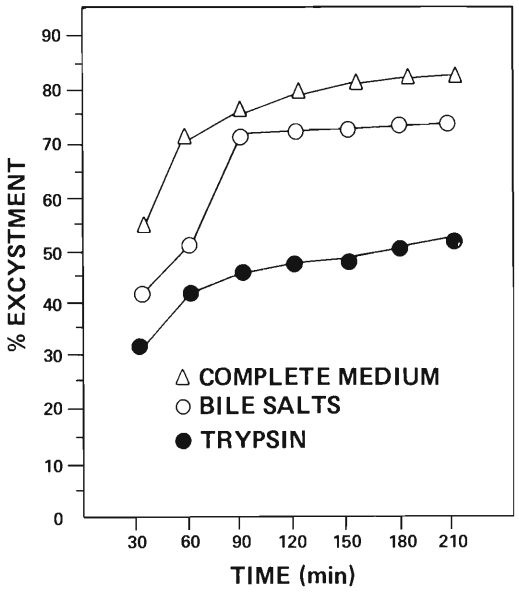


Figure 1. Excystment of *Acanthoparyphium spinulosum* metacercaria after pretreatment in acid pepsin and the reductant followed by incubation in either the complete medium (△), bile salts alone (○), or trypsin alone (●).

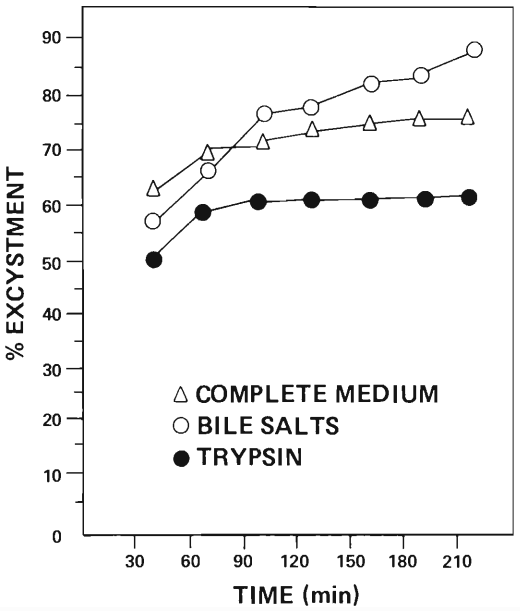


Figure 2. Excystment of *Acanthoparyphium spinulosum* metacercaria after pretreatment in acid Hanks' and the reductant followed by incubation in either the complete medium (△), bile salts alone (○), or trypsin alone (●).

min, and incubation in 0.5% trypsin and Hanks' BSS, pH 7.8; 12) pretreatment with the reductant for 10 min and incubation in Hanks' BSS, pH 7.8; 13) incubation in Hanks' BSS, pH 7.8 in the absence of any pretreatment; 14) pretreatment with acidified Hanks' BSS for 1 hr, the reductant for 10 min, and incubation in Hanks' BSS, pH 7.8; and 15) incubation in warm 0.85% physiological saline. All tests were performed in triplicate on 20 cysts and observations were made at 30-min intervals up to 3.5 hr. Excystment was scored when the larval worm was completely freed of cyst membranes.

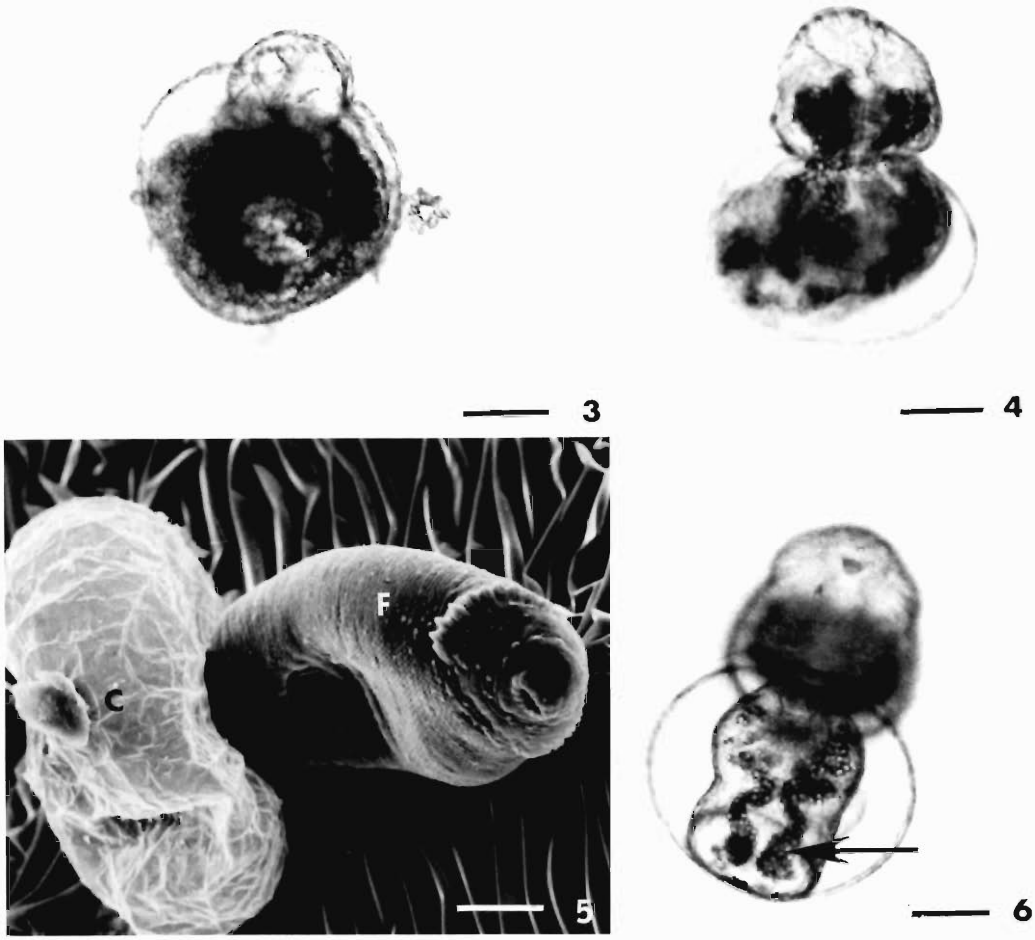
During excystment some metacercarial cysts were fixed for SEM in a freshly prepared mixture of 4% glutaraldehyde and 1% osmium tetroxide in distilled water, dehydrated through ethanol to amyl acetate and dried in a Ladd critical point drying apparatus using CO_2. Specimens were mounted on stubs, with Scotch double stick tape, gold-palladium coated in a Filmvar sputter coating unit and examined in a Perkin-Elmer ETEC Scanning Electron Microscope at 19.9 kV.

Results

Effects of treatments

Maximal excystment of *Acanthoparyphium spinulosum* occurred in the complete medium or in 0.2% bile salts following pretreatment with acidified pepsin or acidified Hanks' BSS for 1 hr, and the reductant for 10 min. Figure 1 shows that the highest rate and percentage was obtained using the complete medium, whereas, either bile salts or trypsin alone produced lower percentages after pretreatment with acidified pepsin and the reductant. Figure 2 shows that after pretreatment with acidified Hanks' BSS and the reductant, the highest rate and percentage resulted when bile salts alone were used, whereas, the complete medium and trypsin alone produced lower percentages.

Pretreatment with either acidified pepsin or Hanks' BSS and incubation in the complete medium in the absence of the reductant resulted in 1% and 0% excystment, respectively. When cysts were pretreated with acidified pepsin for 1 hr, the reductant for 10 min followed by Hanks' BSS at pH of 7.8, 35% of the larvae excysted, but when similar experiments were repeated using acidified Hanks' instead of acidified pepsin, no excystment occurred. If cysts were exposed to the reductant for 10 min and incubated in either the complete excystment medium or alkaline Hanks' BSS only 11% and 0% excysted, respectively. No excystment was observed when cysts were incubated in either the complete medium, alkaline Hanks' BSS, and 0.85% physiological saline.



Figures 3-6. Excystment of *Acanthoparyphium spinulosum* metacercaria. 3. Metacercaria partially emerged after brief treatment with the complete medium. Scale bar = 0.025 mm. 4. Anterior portion of metacercaria partially emerged through the cyst wall. Scale bar = 0.025 mm. 5. Scanning electron micrograph of a partially excysted metacercaria. Note forebody (F) and cyst wall (C). Scale bar = 78 μm. 6. Encased posterior portion of metacercaria with concretions (arrow) in the excretory system. Scale bar = 0.025 mm.

Excystment

The untreated metacercarial cyst wall is composed of two layers, the outer rough and irregular, the inner smooth and ovoid. Both layers are transparent, enabling direct observation of the enclosed larva. The cyst wall show no distinct region through which the metacercaria could emerge during excystment. Excystment was initiated during acid pretreatment at which time the concretions within the excretory system moved back and forth and the larva swelled within the cyst wall. When the metacercaria was exposed to the reductant, the outer cyst membrane became delicate and sticky. The worm then developed an opaque color. In the complete medium the worm slowly rotated until its oral sucker was aligned against the inner cyst membrane. Then the worm vigorously pushed against the inner cyst wall, with its collar eventually producing an escape aperture (Fig. 3). Once the outer cyst membrane was broken, the larva rapidly exited to the area of the acetabulum (Fig. 4) and then beyond that area almost freeing itself (Fig. 5). The emerged anterior portion of the worm

moved back and forth and the enclosed posterior portion pushed itself against the cyst membrane (Fig. 6) until the parasite completely freed itself. The excystment process was usually completed within 1–3 min in the complete medium. Worms that excysted in bile salts alone were very active for up to 3.5 hr compared to those that excysted in media that contained trypsin. Those worms became sluggish after 1 hr postexcystment.

Discussion

The results of the present investigation reveal that maximal excystment of *Acanthoparyphium spinulosum* occurs at 42°C and pH 7.8 as a result of acid pretreatment, followed by incubation in the reductant, sodium dithionite, and exposure to either bile salts alone or a complete medium consisting of bile salts and trypsin. Although acid pretreatment appears to be necessary, acidified pepsin increased the rate of excystment when compared with pretreatment with acidified Hanks'. This observation is in accord with that of Howell (1970) on *Echinoparyphium serratum* cysts. Acid pretreatment and reducing conditions are prerequisites for the successful excystment of *E. serratum* and *Himasthla quissetensis* (Howell, 1970; Kirschner and Bacha, 1980). These two echinostomes are similar to *A. spinulosum* in that they also require a second intermediate host. Moreover, our results are similar to those of Kirschner and Bacha (1980) with *H. quissetensis* in that pretreatment with either acidified pepsin or Hanks' and the reductant are required for maximal excystment. Acid pretreatment and reducing conditions are important in achieving a high percentage of excystment of other trematodes, notably *Fasciola hepatica* and *Zygocotyle lunata* (Dixon, 1966; Fried et al., 1978; Fried and Butler, 1979). However, the excystment pattern of *A. spinulosum* differs from the observation of LeFlore and Bass (1982) for *H. rhigedana* in that acid pretreatment and the reductant are not required for excystment, although both enhanced the rate and percentage of excystment. This may be explained because of the difference in encystment of these two echinostomes. The cercaria of *H. rhigedana* encysts in the open and does not require a second intermediate host.

Excystment in a trypsin-bile salt medium with a synergistic effect has been reported for *E. serratum* by Howell (1970), *Parorchis acanthus* by Fried and Roth (1974), *E. flexum* by Fried and Grigo (1975), *H. rhigedana* by LeFlore and Bass (1982) and *Cloacitrema michiganensis* by LeFlore and Bass (1983a). Maximum excystment of *A. spinulosum* can be achieved with a trypsin-bile salt medium; however, a medium with bile salts alone increased the rate and percentage of excystment. Therefore, a synergistic effect between these two substances does not seem to occur in this case. Bile salts appeared to play a significant role in stimulating muscular contraction and rate of emergence of *A. spinulosum*. The metacercariae, of a number of other trematodes have been shown to be stimulated by the addition of bile salts, resulting in increased muscular activity and rate of emergence (Erasmus and Bennet, 1965; Dixon, 1966; Howell, 1970; Fried and Butler, 1978; Mitchell et al., 1978; Johnston and Halton, 1981). Lackie (1975) suggested that bile salts stimulate increased muscular activity of metacercaria, and this increased activity may be assumed to assist the larva in pushing its way out of the cyst membrane.

As the excystment process of the metacercaria of *A. spinulosum* was initiated during pretreatment, the concretions within the excretory system began moving. The function of these structures is unknown; however, Smyth (1969) suggested that they play a very important part in phosphorylation and energy transfer mechanisms. Concretions may be seen in adult trematodes of a few marine families, but are probably expelled during excystment of most other adults or shortly thereafter (Erasmus, 1967; Fried and Butler, 1978). LeFlore and Bass (1983b) observed that concretions within the excretory system of the excysted metacercaria of *H. rhigedana* histochemically localized alkaline phosphatase.

Incubation in any medium containing trypsin resulted in a low percentage of excystment, producing worms that became sluggish within 1 hr. Apparently, trypsin has a detrimental effect on the excysted larva of *A. spinulosum*. Johnston and Halton (1981) reported an inhibitory effect by trypsin on the excystment of *Bucephaloides gracilescens*. Various percentages of excystment have been obtained in media containing trypsin for *Sphaeridotrema globulus* and *H. quissetensis* (Macy et al., 1968; Kirschner and Bacha, 1980; Fried and Huffman, 1982).

Acknowledgments

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Isospora suis: Development in Cultured Cells with Some Cytological Observations

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ABSTRACT: Oocysts of *Isospora suis* obtained from experimentally infected pigs were sporulated and cleaned of fecal debris. Sporozoites were excysted in vitro and inoculated into Leighton tube cultures of Mardin-Darby bovine kidney cells, embryonic bovine trachea cells, bovine colon cells, and porcine kidney cells. Sporozoites entered and developed in all cell types. Progeny were formed within the host cell by endodyogeny as with *Toxoplasma, Hammondia, Besnoitia,* and *Hepatozoon* grown in vitro. Schizogony as it occurs in *Eimeria* was not observed. Polysaccharide, observed as periodic acid-Schiff stained granules, appeared to be utilized during excystation and cell penetration and to be reformed intracellularly by sporozoites and in progeny. Polysaccharide was depleted in sporozoites incubated for 120 min before inoculation into cell cultures. When such sporozoites were inoculated into cell cultures they did not become intracellular.

Five *Isospora* species from avian, feline, and canine hosts have developed in a variety of cultured cells inoculated with the excysted sporozoite stage (Turner and Box, 1970; Fayer, 1972; Fayer and Kocan, 1972; Fayer and Mahrt, 1972; Fayer and Thompson, 1974). *Toxoplasma gondii, Hammondia hammondi,* and *Isospora bigemina* (small form), which actually may have been either of the former two species, are feline coccidia with isosporoid oocysts that have also developed in a variety of cultured cells inoculated with excysted sporozoites (Sheffield and Melton, 1970; Shibalova and Petrenko, 1972; Sheffield et al., 1976). The numerous cell types used for cultivation of these species are reviewed by Doran (1982). With the exception of an isosporan from English sparrows (Turner and Box, 1970), all of the above species were reported to have multiplied in culture by endodyogeny. Because the economically important parasite *Isospora suis* was reported to multiply by endodyogeny during part of its endogenous development in swine (Lindsay et al., 1980; Matuschka, 1982) it appeared to be a good candidate for the present study to determine its ability to enter cells and develop intracellularly in vitro. Included in this cultivation study are observations of changes in the quantity of carbohydrate in sporozoites and in progeny.

Materials and Methods

Oocysts of *Isospora suis* were obtained from feces of experimentally infected piglets; no other species of coccidia were found during examination of feces.

Oocysts were sporulated in 2.5% potassium dichromate at room temperature for 2 days and stored for less than one month at 5°C. For cultivation, oocysts were rinsed free of dichromate by repeated washings with tap water followed by centrifugation and then cleaned of fecal debris by sugar flotation and centrifugation through 40% Percoll (Sigma). Sporocysts, obtained by rupturing oocysts in a teflon tissue grinder, were suspended in a mixture of 0.75% sodium taurocholate and 0.25% trypsin for 30–120 min at 37°C. Excysted sporozoites were concentrated and rinsed free of trypsin-bile solution by centrifugation at 550 *g* for 5 min. They were resuspended in the culture medium appropriate for the cell type into which the sporozoites were to be inoculated.

All cells were grown on 10.5 × 35-mm coverslips within Leighton tubes with loosened screw-type caps at 37°C with a 5% CO_2 atmosphere. Cells included embryonic bovine trachea (EBTr), Madin-Darby bovine kidney (MDBK), porcine kidney (PK), and bovine colon (BC). The medium used for growing the first three cell types was Eagle's minimal essential medium with Earle's balanced salts + 1% nonessential amino acids + 10% new born calf serum + 50 units penicillin G and 50 μg dihydrostreptomycin/ml (PS). The medium used for growing the fourth cell type was F12K + 10% fetal calf serum + PS. All media contained phenol red indicator. For each cell type 47,000–65,000 cells were suspended in 1 ml of medium that was pipetted into each culture tube and incubated for 18–24 hr before inoculation.

A 1-ml suspension containing 50,000 sporozoites (Exps. 1–7) or 25,000 sporozoites (Exps. 8–13) was inoculated into each tube. After 60–120 min incubation at 37°C the inoculation medium was aspirated and replaced with 1 ml of fresh culture medium. Coverslips were removed from culture tubes at intervals shown in Table 1 and examined by phase-contrast microscopy or fixed and stained with special procedures. For evidence of entry into cells, development, and methods of reproduction, cells were fixed with methanol, air dried and stained with Giemsa stain (Exps. 1–7, 10–13). For refractile body changes (Exp. 8), cells were fixed with Carnoy's fluid and stained with Whipf's polychrome (Vetterling and Thompson, 1972). For

Table 1. Materials and methods used to study development and cytochemical changes of *Isospora suis* in cultured cells.

Exp. no.	Cell type*	No. of tubes inoculated	Time of inoculation after initiating excystation (min)	Time of examination or fixation (hr after inoculation)	Procedures
1	MDBK	7	120	24, 48, 72	Phase contrast,† Giemsa
2	EBTr	7	120	24, 48, 72	Phase contrast, Giemsa
3	BC	7	120	24, 48, 72	Phase contrast, Giemsa
4	MDBK	3	30	24, 48	Phase contrast, Giemsa, quantitation‡
5	EBTr	3	30	24, 48, 120	Phase contrast, Giemsa, quantitation
6	BC	3	30	24, 48	Phase contrast, Giemsa, quantitation
7	PK	3	30	24, 48	Phase contrast, Giemsa, quantitation
8	MDBK	6	30	1, 24, 48, 120, 144, 168	Whipf's polychrome
9	EBTr	6	30	1, 24, 48, 120, 144, 168	Periodic acid-Schiff stain
10	BC	6	30	24, 48, 96, 120, 144, 168	Phase contrast, Giemsa, morphometric§
11	PK	6	30	24, 48, 96, 120, 144, 168	Phase contrast, Giemsa, morphometric
12	MDBK	1	120	1	Giemsa, quantitation
13	EBTr	1	120	1	Giemsa, quantitation

* MDBK = Madin-Darby bovine kidney cells, EBTr = embryonic bovine trachea cells, BC = bovine colon cells and PK = porcine kidney cells.

† Live specimens examined with phase-contrast microscopy.

‡ Quantitation = count of number of stages.

§ Morphometric = measurement of sizes of stages.

carbohydrate changes (Exp. 9), cells were fixed with Carnoy's fluid and stained by the periodic acid-Schiff (PAS) reaction (Thompson, 1966). A quantitative determination of multiplication (Exps. 4–7) was made by differential count of the first 50 intracellular organisms in selected cultures at sequential time intervals (Table 1).

In five experiments, sporozoites were incubated for 120 min from initiation of excystation until inoculation into cell cultures to determine if this longer time period (compared with 30 min for all other experiments) affected penetration or development. In experiments 1–3 sporozoites were held in excystation fluid at 37°C for 120 min before they were resuspended in culture medium and inoculated into cell cultures; in experiments 12 and 13 sporozoites were held in excystation fluid at 37°C for 30 min then resuspended in culture medium and incubated for an additional 90 min before inoculation in cell cultures.

For comparison with intracellular sporozoites and to explain effects of prolonged incubation before inoculation into cell cultures, sporozoites suspended in inoculation medium were smeared on glass slides coated with air-dried bovine serum and fixed with methanol at 30 min after initiating excystation (time of inoculation into cell cultures) and at 120 min after

Table 2. Quantitation of multiplication of *Isospora suis*.

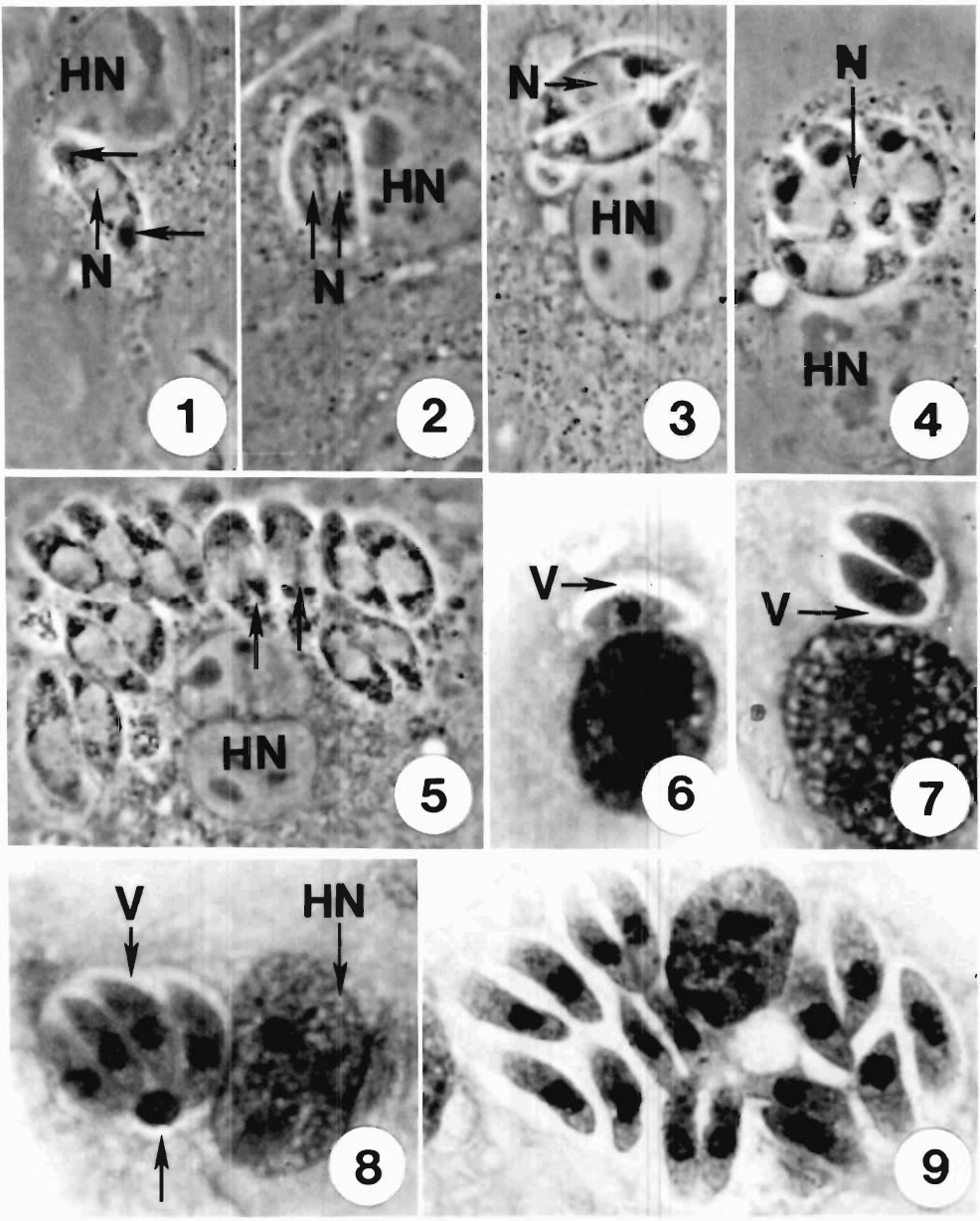
		Hours after inoculation											
		24				48				120			
Exp. no.	Cell type*	sp†	Pairs‡ 1	2	≥3	sp	Pairs 1	2	≥3	sp	Pairs 1	2	≥3
4	MDBK	50	0	0	0	17	28	5	0	ND			
5	EBTr	50	0	0	0	17	21	12	0	9	22	7	12§
6	BC	50	0	0	0	19	26	5	0	ND			
7	PK	50	0	0	0	18	22	10	0	ND			

* MDBK = Madin-Darby bovine kidney cells, EBTr = embryonic bovine trachea cells, BC = bovine colon cells, and PK = porcine kidney cells. ND = not done.

† sp = single sporozoites.

‡ Pairs = no. of paired daughter zoites in a single parasitophorous vacuole.

§ As many as 20 zoites (10 pairs) were observed in a single cell.



Figures 1–5. Phase contrast photomicrographs of intracellular *Isospora suis* in cultured cells. Magnified ×1,800. Abbreviations: Host cell nucleus, HN; parasite nucleus, N. 1. Sporozoite adjacent to nucleus of bovine colon cell at 24 hr after inoculation. Refractile bodies are anterior and posterior to the parasite nucleus (horizontal arrows). 2. Sporozoite adjacent to nucleus of Madin-Darby bovine kidney host cell at 48 hr after inoculation. Sporozoite contains two developing progeny. 3. Two first-generation progeny adjacent to nucleus of embryonic bovine trachea host cell at 48 hr after inoculation. Each progeny has two distinct refractile bodies, anterior and posterior to the nucleus. Remains of the parent sporozoite are not evident. 4. Two generations of progeny within the same Madin-Darby bovine kidney host cell at 48 hr after inoculation. Anterior and posterior refractile bodies are still present. 5. Fourteen zoites within a binucleate embryonic bovine trachea host cell at 120 hr after inoculation. Two of the zoites each contain two developing progeny (arrows). Figures 6–9. Bright field photomicrographs of Giemsa-stained intracellular *I. suis* in cultured cells. Magnified ×1,800. Abbreviations: Host cell nucleus, HN; parasitophorous vacuole, V. 6. Sporozoite adjacent to nucleus of bovine colon host cell at 24

Table 3. Average size (μm) of 10 intracellular *Isospora suis* in bovine colon cells observed by phase-contrast microscopy (Exp. 10).

Stage of development	Hours after inoculation into cultures				
	24	48	96	120	144
Sporozoite	4.4 × 10.9	4.7 × 12.4	5.6 × 16.7*	5.6 × 16.7*	3.7 × 10.2†
Progeny	—	3.8 × 14.2	4.1 × 15.2	4.5 × 13.5	4.4 × 12.7‡

* $N = 1$.
† $N = 2$.
‡ $N = 7$.

initiating excystation. These slides were stained by the PAS reaction.

Results

In the eight experiments (Exps. 4–11) in which sporozoites were inoculated into Leighton tube cultures of MDBK, EBTr, BC, and PK cells, sporozoites entered and developed in all cell types. Sporozoites were intracellular within 1 hr after inoculation in both MDBK and EBTr cells. At 24 hr sporozoites were intracellular in all cell types, but development was not observed (Table 2). Intracellular sporozoites were located adjacent to the host cell nucleus and were surrounded by a parasitophorous vacuole (Fig. 6). Sporozoites contained a single, large, central nucleus with a prominent eccentric nucleolus (Fig. 1). Refractile bodies, also referred to as paranuclear bodies, were found anterior and posterior to the nucleus (Fig. 1). At 48 hr, 62–66% of the intracellular parasites had undergone asexual development by endodyogeny (Table 2). Two progeny formed within the parent organism (Figs. 2, 5) and grew until only the progeny remained (Figs. 3, 7). This process was repeated within the first 48 hr in culture so that a second generation (4 zoites) was found (Figs. 4, 8) in all of the cell types examined and constituted 10–24% of the parasites present (Table 2). Morphologically, the first, second and any successive generations of progeny, greatly resembled the sporozoite. At 72, 96, 120, 144, and 168 hr after inoculation, single sporozoites and pairs or multiple pairs of zoites were found in all cell types examined. As many as 8 and 10 pairs of zoites were found in EBTr cells at 120 hr (Fig. 9). Endodyogeny was the only form of multiplication observed. Development of zoites into sexual stages was not observed.

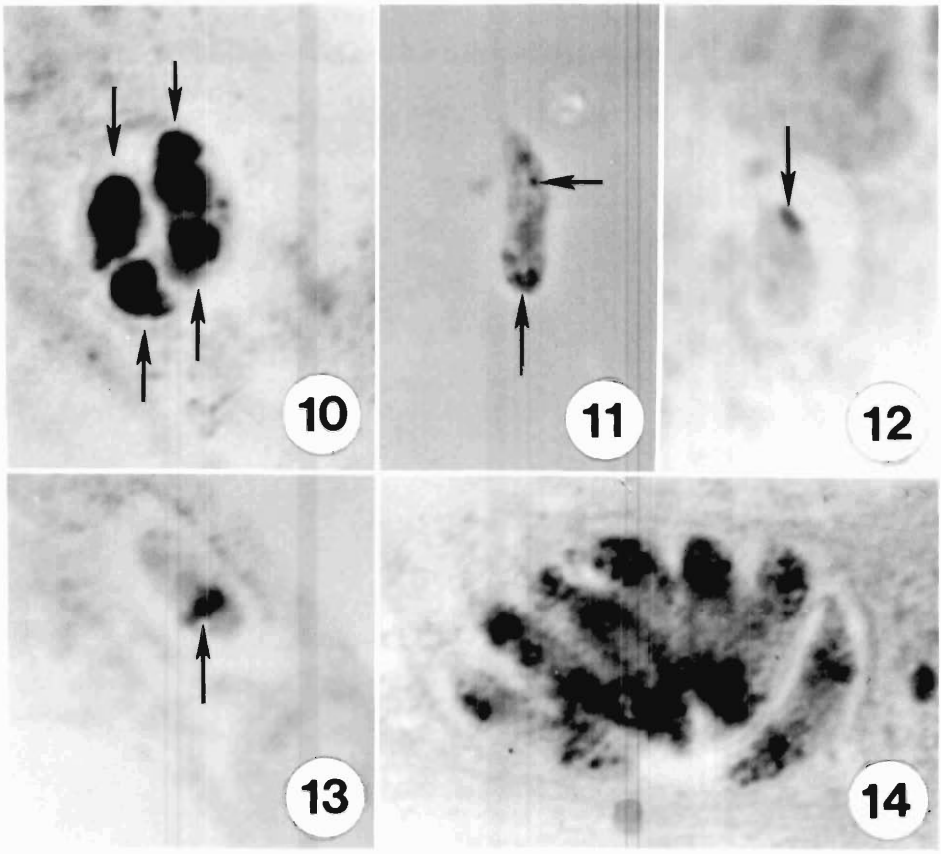
Morphometric data obtained in experiment 10 are tabulated for 24, 48, 96, 120, and 144 hr after inoculation (Table 3). The number of intracellular sporozoites observed at 96 hr and later was small. The number of progeny observed at 144 hr was small. Parasites were not found at 168 hr. Morphometric data were not obtained in experiment 7 because the highly granular cytoplasm of the porcine kidney cells obscured the intracellular parasites.

Staining of MDBK cell cultures with Whipf's polychrome stain (Exp. 8) confirmed the presence of refractile bodies in sporozoites and progeny as observed with phase contrast microscopy (Figs. 1, 3, 4).

The periodic acid-Schiff (PAS) reaction was utilized to study the location and quantity of polysaccharide in sporozoites and progeny. Before excystation, sporozoites in sporocysts contained large quantities of PAS-positive material (Fig. 10). Specific location and structure could not be identified because of the staining intensity. Within ½ hr after excystation extracellular sporozoites contained numerous discrete PAS-positive granules anterior and posterior to the nucleus (Fig. 11). Although some extracellular sporozoites still contained PAS-positive granules

←

hr after inoculation. 7. First generation progeny adjacent to nucleus of embryonic bovine trachea host cell at 48 hr after inoculation. 8. Four zoites in a single parasitophorous vacuole in an embryonic bovine trachea host cell at 48 hr after inoculation. The posterior ends of the zoites appear to be attached to a common residuum. 9. Sixteen zoites within a single embryonic bovine trachea host cell at 120 hr after inoculation. Several zoites appear to contain two developing progeny.



Figures 10–14. Phase-contrast and bright field photomicrographs of periodic acid-Schiff (PAS) stained stages of *Isospora suis*. Magnified ×2,200. 10. Extracellular sporocyst containing four intensely stained sporozoites (arrows) in an embryonic bovine trachea cell culture 96 hr after inoculation. Bright field microscopy. 11. Extracellular sporozoite in a smear one-half hour after beginning excystation. PAS-positive granules (arrows) at anterior and posterior ends. Phase contrast microscopy. 12. Intracellular sporozoite in an embryonic bovine trachea host cell 1 hr after inoculation. Only two indistinct PAS-positive granules (arrow) are present. Phase contrast microscopy. 13. Intracellular sporozoite in an embryonic bovine trachea host cell 24 hr after inoculation. A cluster of PAS-positive granules (arrow) are present at one end. Phase contrast microscopy. 14. Seven zoites in an embryonic bovine trachea host cell 96 hr after inoculation. Numerous PAS-positive granules at anterior and posterior ends. Bright field microscopy.

at 120 min after excystation, others were completely lacking such granules. At 60 min after inoculation into cell culture intracellular sporozoites contained relatively few PAS-positive granules (Fig. 12). The number of granules increased at 24 hr (Fig. 13) and were numerous in all progeny (Fig. 14).

In the five experiments (Exps. 1–3, 12, 13) in which sporozoites were incubated at 37°C for 120 min after beginning excystation and before inoculation in Leighton tube cultures of MDBK, EBTr and BC cells, sporozoites did not enter any cells. Sporozoites observed with phase-contrast microscopy at the time of inoculation and at 24-hr intervals after inoculation appeared normal in shape, highly birefringent, and extracellular.

Discussion

Sporozoites of *I. suis* became intracellular and underwent asexual development in cells of bovine and porcine origin grown in vitro. Asexual development was by endodyogeny, the method of development reported for other species of *Isospora* as well as *Besnoitia, Hammondia, Hepatozoon,* and *Toxoplasma* in cell cultures (Doran, 1982). Neither *I. suis* nor other *Isospora,* nor other genera mentioned above have developed

gamonts in vitro. The only coccidian genera to do so are *Eimeria, Klossia,* and *Sarcocystis* (Doran, 1982). Although *I. suis* developed only by endodyogeny in vitro, in the pig endodyogeny was followed by schizogony and then gametogony (Lindsay et al., 1980; Matuschka, 1982).

The number of intracellular *I. suis* appeared to decrease after 5 days in culture. Similarly, the number of *I. canis* usually declined after 8 days in culture (Fayer and Mahrt, 1972). The number of *Hammondia* and *Toxoplasma* also declined with time in culture but could be maintained for weeks and for months, respectively, via subculturing (Chernin and Weller, 1954; Sheffield et al., 1976).

The failure of sporozoites to become intracellular after 120 min incubation in excystation fluid or culture medium may be related to the concurrent depletion of PAS-positive granules representing loss of carbohydrate reserves. Progressive depletion of PAS-positive granules was observed, beginning with sporozoites that contained large amounts of PAS-positive material while still within sporocysts, to free sporozoites with moderate amounts of PAS-positive material after 30 min of excystation, to free sporozoites nearly devoid of PAS-positive material after 120 min. Sporozoites of *Eimeria acervulina, E. necatrix,* and *E. meleagrimitis* were also examined for carbohydrate content before and after excystation and after entry into cells (Vetterling and Doran, 1969). Because of the decline in the quantity of carbohydrate after excystation and further decline after cell entry these authors concluded that carbohydrate was the energy source of sporozoites for excystation and cell penetration. They further concluded that when the carbohydrate content fell below a certain level, sporozoites lacked sufficient energy to infect cells.

An increase in the amount of PAS-positive material in intracellular *I. suis* sporozoites from 1–24 hr after inoculation into cell cultures and moderate quantities in progeny suggests replacement of carbohydrate. Similarly, carbohydrate identified by PAS-positive granules disappeared and later reappeared during in vitro development of *I. felis* and *E. tenella* (Hare and Strout, 1972; Fayer and Thompson, 1974). It is suggested that carbohydrate was utilized for energy in the development of progeny and then resynthesized in progeny for further energy requirements.

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Research Note

Infection of Captive Adult Collared Peccaries, *Dicotyles tajacu* (Woodburne, 1968), with the Nematode, *Ascaris suum* (Goeze, 1782)

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The collared peccary (*Dicotyles tajacu*) is one of three species in the family Tayassuidae, ranging from Argentina to the southwestern United States. Seven species of nematodes have been reported in parasitological surveys of free-ranging populations of this host (Table 1). Sprent (1982, J. Helminthol. 56:275–295) has noted the only ascaridoid infection of a wild collared peccary (Table 1). Harwell et al. (1977, J. Wildl. Dis. 13:445–447) discovered two adult *Ascaris suum* in the jejunum of a captive juvenile collared peccary while investigating the effects of an experimental infection with *Stephanurus dentatus*. Results presented herein show the adult collared peccary to be an acceptable host for adult *A. suum*.

Twenty-five adult collared peccaries, trapped in Zavala County, Texas, were released in an outdoor enclosure (30 m × 30 m) located on the Texas A&M University campus in March 1982. Approximately 3 mo after their introduction to the enclosure, some of the animals exhibited signs of respiratory distress and moderate amounts of coughing. Hematological profiles of the animals revealed marked leukocytosis (25–70 × 10^3 WBC/mm^3) with elevated eosinophil counts (3–8% WBC), similar to pathological values seen in ascarid-infected domestic swine (Weide and Twiehaus, 1959, Am. J. Vet. Res. 20:562–567). Necropsy of an adult female collared peccary that died of undetermined causes revealed a single adult *A. suum* infecting the bile duct. No other gross pathological evidence was seen in lung or liver sections.

Based on these observations, the penned animals were given monthly anthelmetic treatments with levamisole hydrochloride. This eliminated the coughing symptoms and returned the leukocyte profile to normal (12–13 × 10^3 WBC/mm^3). Following each monthly treatment, an occasional adult *A. suum* was defecated by the penned animals.

Infection appeared to be at low to moderate levels throughout the summer and fall, but reached considerably higher levels during the winter after placement of the female peccaries in individual pens (2 m × 3 m). Within three days following an anthelmetic treatment in February 1983, 44 adult *A. suum* were collected from the voided feces of 21 female collared peccaries. Fourteen individuals (66.7%) were infected at a mean intensity of 3.14 (range 1–6) parasites per host individual.

The most plausible explanation for the *A. suum* infection is egg concentration in the soil by surface runoff from a swine research facility located about 100 m uphill from the collared peccary enclosure. The elevated burdens observed during the winter could be attributed to restriction of the animals to small penned areas.

The adult collared peccary is apparently an acceptable host for adult *A. suum*; however, the significance of this observation is uncertain. We have necropsied 26 collared peccaries collected from the wild in Zavala and LaSalle counties.

Table 1. Species and locality of nematode infections in the collared peccary (*Dicotyles tajacu*).

Nematode	Locality*
Dirofilaria acutiuscula	Texas[a]; Brazil[b]
Gongylonema baylisi	Texas[a]; Brazil[c]
Parabronema pecarie	Texas[adf]; New Mexico[de]
Parostertagia heterospiculum	Texas[af]
Texicospirura turki	Texas[ad]; New Mexico[d]
Physocephalus sp.	Texas[a]
Physocephalus sexalatus	Texas[f]
Toxocara alienta	Surinam[g]

* [a] Samuel and Low, 1970, J. Wildl. Dis. 6:16–23; [b] Lent and Teixerira de Freitas, 1937, Mem. Inst. Oswaldo Cruz 32:37–54; [c] Teixerira de Freitas and Lent, 1937, Mem. Inst. Oswaldo Cruz 32:299–304; [d] Chitwood and Cordero de Campillo, 1966, J. Parasitol. 52:307–310; [e] Samson and Donaldson, 1968, Bull. Wildl. Dis. Assoc. 4:131; [f] Schwartz and Alicata, 1933, Proc. U.S. Nat. Mus. 82:1–6; [g] Sprent, 1982, J. Helminthol. 56:275–295.

Most of these have been free of endoparasites, as had been observed in other surveys (Table 1), and none have harbored *A. suum*.

Feral swine (*Sus scrofa*), which occur sympatrically with collared peccaries in south Texas and have been reported to be extremely common in Zavala County (Anonymous, 1982, Southeast. Coop. Wildl. Dis. Study, 1 p.), are known to harbor *A. suum* (Hanson and Karstad, 1959, J. Wildl. Manage. 23:64–74; Coombs and Springer, 1974, J. Wildl. Dis. 10:436–441). Coombs and Springer (1974, loc. cit.) reported burdens ranging from one to three adult worms per infected pig from southern Texas. The feeding behavior of feral swine overlaps with that of collared peccaries, which, although primarily browsers on prickly pear (*Opuntia* sp.), are known to root for underground plant parts and invertebrates (Eddy, 1961, J. Wildl. Manage. 25:248–257; Sowls, 1978, pp. 191–205 *in* Schmidt and Gilbert, eds., Big Game of North America. Stackpole Books, Harrisburg, Pennsylvania). Thus, the possibility for parasite transmission between these two hosts exists. However, the mechanism of geographical dilution of ascarid ova acting to maintain low levels of infection in wild swine populations suggested by Coombs and Springer (1974, loc. cit.) may be operating in collared peccary herds as well. Because of the ecological and behavioral similarities of these two suiforms, further investigations into the transmission of *A. suum* between these species is warranted.

We would like to thank T. M. Craig for identifying the *A. suum* specimens and reviewing the manuscript, and K. McBee for reviewing the manuscript. Specimens have been deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705 (No. 77612).

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Research Note

Trichuris spp. (Nematoda: Trichuridae) in Sika Deer (*Cervus nippon*) in Texas

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Trichuris skrjabini was described by Baskakov from camels in Turkestan, USSR (1924, Trudy Gosudarstv. Inst. Eksper. Vet. 2:92–105). Skrjabin (1957, Osnovy Nematodologii, Vol. 6, Moscow) reported it also from domestic cattle, sheep, and goats, and in feral goral (*Nemorrhaedus raddeanus*), roe deer (*Capreolus pygargus*), Persian gazelle (*Gazella subgutturosa*), fallow deer (*Cervus dama*), elk (*Alces alces*), axis deer (*Pseudaxis hortulorum*, syn: *Cervus nippon*[1]), Asiatic ibex (*Capra sibirica*), and saiga (*Saiga tatarica*). Knight redescribed *T. skrjabini* from Nebraska sheep (1971, J. Parasitol. 57:302–310) and subsequently reported it generally throughout the U.S. in sheep (1972, Proc. Helminthol. Soc. Wash. 39:242–244). No reports exist for *T. skrjabini* in other ruminants in North America.

In 1981, three sika deer (*Cervus nippon*) that had grazed pastures with sheep on Real Ranch, Kerr County, Texas were killed by Mr. B. Wiley and examined for parasites. The deer aged 3 mo, 6 mo, and 3 yr harbored 5♂ and 2♀, 3♂, and 1 whipworm, respectively. The single worm from the 3-yr-old deer consisted of only an anterior portion, and therefore was unidentifiable to species.

Examination of the worms showed all anterior portions missing in part, but the posterior portions of the worms generally were in good condition. The worms were cleared in lacto-phenol for microscopic study, and selected morphological characters were measured by ocular micrometer to make identification.

Measurements obtained from seven male specimens are presented in Table 1, and agree with those described for *T. skrjabini*; one male specimen from the 6-mo-old deer was identified as *T. ovis* (Abildgaard, 1795), which was reported from *C. nippon* by Skrjabin (idem.). One

[1] Feldhamer, 1980, Mammalian Species 128:1–7.

Table 1. Average measurements (range) of *T. skrjabini* found in sika deer (*Cervus nippon*) in Texas (all measurements in mm).

Character	Texas specimens	*T. skrjabini**
Posterior length	11.1 (7.9–14.0)	11.3 (7.9–14.6)
Spicule length	1.01 (0.96–1.12)	1.12 (0.83–1.58)
Spicule width	0.012 (0.011–0.013)	0.012 (0.011–0.014)
Ejaculatory duct	6.07 (3.27–8.40)	7.90 (4.80–10.92)
Vas deferens	3.38 (2.25–4.38)	4.59 (2.10–7.27)
Cloaca	1.03 (0.77–1.32)	1.50 (1.0–2.0)
Ratio ejaculatory duct/vas deferens	1:1.76	1:1.81

* Knight, 1971, J. Parasitol. 57:302–310; 1972, Proc. Helminthol. Soc. Wash. 39:242–244.

male appeared to be immature and two females had no eggs present in the uteri. The females were nearly straight, had small vulval spines, and ovary tips as described by Knight for *T. skrjabini* (1971, idem.).

This represents the first report of *T. skrjabini* in a ruminant other than sheep in North America, and the first report of this species from *Cervus nippon* in the United States. The finding of *T. skrjabini* and *T. ovis* in other deer sharing pastures grazed by sheep in the United States may be predicted. Conversely, whipworms normally associated with and found in deer may be acquired by sheep through such mutual grazing of pasture.

The specimens have been deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705 (Nos. 66995 and 69492 for *T. skrjabini* and 66994 for *T. ovis*).

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Research Note

The Occurrence of *Pterygodermatites nycticebi* (Nematoda: Rictulariidae) in a Captive Slow Loris, *Nycticebus coucang*

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Adult and immature rictulariid nematodes were recovered at necropsy from the small intestine of an adult slow loris, *Nycticebus coucang*, from the Milwaukee County Zoo in Wisconsin. The lumen of the entire small intestine was packed with more than 100 nematodes, the intestinal wall appeared thickened and the mucosal surface contained numerous petechial hemorrhagic foci. The cause of death was diagnosed as a septicemia and possible lupus erythematosis.

Nematodes collected were fixed in 10% formalin or AFA and cleared in glycerine alcohol. Intestinal tissue was fixed in 10% formalin sectioned using standard histological procedures at 5μm and stained with H&E.

The nematodes were later identified as *Pterygodermatites* (*Mesopectines*) *nycticebi* (Monnig, 1920). A brief description of eight males and eight females is as follows:

Buccal capsule opening dorsally with a single row of perioral denticles present in the upper rim of the capsule and three large teeth in the bottom.

An inner circle of six large rounded papillae and an outer circle of eight smaller papillae are present. The inner circle consists of two pairs of dorsal labial papillae on the head and one pair of dorsal labial papillae beneath the rim of the dorsal lip. Amphids are external and slightly posterior to the lateralmost labial papillae. The outer circle consists of one pair of laterodorsal papillae on each side of the buccal capsule and two lateroventral pairs just posterior to the crest of the head. Nerve ring at level of sixth pair of cuticular processes, excretory pore at level of eighth pair. Esophagus muscular anteriorly, glandular posteriorly, muscular portion one-eighth total length.

MALES: 9–12 mm in length, 0.4–0.9 mm in width; 68–70 pairs of ventral cuticular processes; three caudal ventral cuticular fans present; 10 pairs of caudal papillae; spicules equal, 82–85 μm in length; gubernaculum absent.

FEMALES: 22–28 mm in length, 0.5–1.2 mm in width; 90–92 pairs of ventral cuticular processes; 42–43 pairs prevulvular; vulva anterior to posterior end of esophageal-intestinal junction; eggs, embryonated, 42.5 by 27.5 μm.

A histological examination of the small intestine showed that the lamina propria was moderately infiltrated with mononuclear leucocytes and a portion of the attached mesentery was heavily infiltrated with inflammatory cells including neutrophils. Several cross sections of nematodes were observed adjacent to areas where the heavy infiltration of cells occurred.

Mönnig (1920, Zentrabl. Bakteriol. Parasitenk. Infektioskr., Abt. I, Orig. 85:216–221) first described *P. nycticebi* females from the intestine of *Nycticebus tardigrada*. The males, also obtained from a slow loris, were later described by Quentin and Krishnosamy (1979, Ann. Parasitol. (Paris) 54:527–532). In comparison, the nematodes reported here differ from the above descriptions in size only. Representative specimens of *P. nycticebi* were deposited in the USNM Helminthological Collection (No. 77360). This is the first report of this species from a captive slow loris in North America.

Other members of the Rictulariidae that parasitize primates have been associated with intestinal intussusception (Lindquist et al., 1980, Proc. Helminthol. Soc. Wash. 47:224–227). The pathologic significance of *P. nycticebi* in this case is uncertain because of the accompanying septicemia. Other documentation of pathology associated with this species is unavailable primarily because of the rarity of its occurrence. However, the thickened intestinal wall, mucosal hemorrhagic foci and mononuclear leucocyte infiltration may have all been attributed to high burdens of this nematode species.

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Research Note

A Unique Bioassay: *Haemonchus contortus* Adult Male Spicule Sheaths Attract Conspecific Larvae

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Bioassays for attractants or pheromones produced by either free-living or parasitic nematodes commonly use adults as test responders (Stringfellow, 1978, Proc. Helminthol. Soc. Wash. 45:261–263; Bone, 1980, Proc. Helminthol. Soc. Wash. 47:228–234). However, adult gastrointestinal nematodes of agricultural importance are too large to bioassay for the presence of attractants with the methods currently used. Stringfellow (1981, J. Parasitol. 67:124–126; 1983, Proc. Helminthol. Soc. Wash. In press) reported that substances found in the abomasal contents repelled infective larvae of *Ostertagia ostertagi*. Pilot studies indicated that the posterior end of males contained an unknown substance that attracted infective larvae of *Haemonchus contortus*

Table 1. Response of conspecific infective larvae to test agar blocks with spicule sheaths of *Haemonchus contortus* compared with control agar blocks.

	No. petri plates	Number of larvae*	
		Control agar block	Test agar block
Baseline†	28	0 (400)	0 (380)†
In vivo	53	1 (974)	120 (1,009)
In vitro‡	20	0 (301)	0 (290)

* The numbers in parentheses were the total number of larvae counted within the agar blocks. The numbers not in parentheses were the total number of larvae counted on top of the agar blocks.

† Did not have spicule sheaths.

‡ Adults in vitro 18 hr.

infective larvae (unpubl. data). Successful use of infective larvae in those studies provided the impetus for further developing the present technique using infective larvae of *Haemonchus contortus* as responders in the bioassay reported herein.

The present bioassay was run on 2% purified agar petri plates at pH 6–7 with infective larvae of *H. contortus* (BPL strain). The larval inoculum was baermanized from either sheep or cattle feces mixed with sphagnum peat moss. The spicule sheaths were dissected from live adult male worms. The worms were recovered from the abomasum of experimentally infected cattle or sheep, washed free of debris with sterile saline (5 washes each with 500 ml), and resuspended in saline in plant industry dishes (10 × 27 mm). The spicule sheaths were then removed under the low power of the dissecting microscope. The cuticle was cut with a number 11 scalpel on each side slightly anterior to the spicule sheaths. The worm was grasped anteriorly with a small forceps while a blunt fine instrument was placed anterior to the bursae. The forceps were then gently pulled so that about 95% of the nematode was pulled away leaving the bursae with the spicule sheaths. The remaining bursal lobes and as much muscle as possible were removed leaving a preparation used in the bioassay. When three preparations were made, they were either picked up with insect pins and placed on the test agar block, or they were pipetted onto the test agar block. The 15 × 100-mm agar petri plates were prepared in the standard manner and then stored at 4°C until used. Two dots were marked on the bottom of each petri plate equidistant from the center and 6 cm from one another. One thousand fresh, infective larvae were pipetted midway between a control and a test agar block. This larval inoculum was dried on a slide warmer at 37–40°C until the larvae began to migrate over the agar. Both control and test agar blocks (0.5 cm^2), cut from an agar plate, were placed on the agar above the dot marked on the bottom of the plate at the proper moment of dryness. The control block was simply agar. Three spicule sheaths dissected from adult male worms were placed on top of the test agar block. The petri plates were incubated with every other position reversed relative to its odd numbered plate. Twenty-four hours after the larvae had been allowed to migrate over the agar, the number of larvae both on top as well as inside each agar block was counted with the aid of a dissecting microscope.

An initial test using 28 petri plates was run in which no spicule sheaths were placed on the test agar blocks. Larvae were allowed to migrate across the agar plates for 24 hr to determine if their movement was at random. The number of larvae both on top as well as those that penetrated each agar block were counted at the end of this time. A second test using 53 petri plates was run in which spicule sheaths dissected from adult male worms harvested directly from the abomasum of either cattle or sheep were placed on the test agar blocks. A third test using 20 petri plates was run to determine if the larvae could detect the attractant produced from spicule sheaths of males maintained in vitro. Adult male worms were obtained from sheep infected with 10,000 infective larvae of *H. contortus*. They were washed five times with saline and EBSS plus antibiotics in a 40-ml centrifuge tube. They were then subcultured in 40 ml of cell free API-1 medium at pH 6.4 for about 18 hr. The overlying gas phase was 85% N_2 : 5% O_2 : 10% CO_2 (Stringfellow and Douvres, 1982, The ASB Bulletin 29:85–86). They were removed from culture, washed twice with saline in a 40-ml centrifuge tube, and tested for the presence of the attractant with the bioassay and methods described above. The data were tested with Chi square at the 0.05 level of significance at one degree of freedom.

The data obtained from the three experiments are presented in Table 1. They are interpreted as follows: (1) The difference between the number of larvae within all of the control and the test agar blocks was not significant as shown by the numbers in parentheses. No larvae were found

on top of either the control or test agar blocks. These data established a baseline and demonstrated that the larvae spread across the agar petri plates randomly. (2) The difference between the number of larvae on top of the control agar block versus the number on top of the agar blocks with spicule sheaths obtained from adult males taken directly from the abomasum was significant (in vivo). Only one-tenth of the one percent of those larvae that entered the control agar blocks reached the top of the blocks. About 11% of those larvae that entered the test agar blocks were present on top of the blocks. Many of these larvae were curled up in the vicinity of the test preparations. (3) The difference between the number of larvae on top of the control and test agar blocks with spicule sheaths dissected from adult male worms maintained for 18 hr in vitro was not significant.

The spicules and their sheaths as well as associated debris that could not be dissected away with the scalpel was called a spicule sheath in the present paper. Each male of *H. contortus* had two long spicules that could be extruded from the cloacal opening. Each spicule had a sheath attached to its head end that contained a fluid. There was continuity between both the lumen of the sheath and that of the spicule, which also had a cytoplasmic core. The bioassay described herein demonstrated that an attractant was present in the spicule sheath of adult male *H. contortus* grown in vivo. There was no attempt to determine if it was a general or specific attractant, such as a sex pheromone; however, adult male worms maintained for 18 hr in vitro lost the capacity to attract the larvae. Furthermore, the present study corroborated previous work (1981, loc. cit.; 1983, loc. cit.) which indicated that nematode receptors had the ability to receive specific chemical stimuli even as early as the infective larval stage.

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Research Note

Cloacal Drop Inoculation of Trematode Larvae, a Mechanical Aspect

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The cloacal drop method of inoculating chicks with larval trematodes has been used by Allison (1943, Trans. Amer. Microscp. Soc. 62:127–128); Fried and Harris (1971, J. Parasitol. 57:866–868); Fried and Pucci (1976, Int. J. Parasitol. 6:479–482); Herman and Bacha (1978, J. Parasitol. 64: 827–830); and Fried and Schnier (1981, Proc. Helminthol. Soc. Wash. 48:83–86) among others. A suspension of free (unencysted) metacercariae or cercariae is transferred by pipette to the cloacal lips. The mouthlike movements of the stimulated lips draw the suspension into the cloaca.

Fried and Schnier (1982, Proc. Helminthol. Soc. Wash. 49:151–153) have suggested that free sterol from the bursal mucosa might serve as a chemoattractant for *Leucochloridiomorpha constantiae* following per cloaca inoculation of chicks. The present study was undertaken to determine if entry into the bursa might also be accounted for, at least in part, by muscular movements of the cloaca.

A drop of suspension containing 50–60 living cercariae, metacercariae, or heat-killed cercariae of the marine avian trematode *Himasthla quissetensis* was placed upon the cloacal lips of 10-day-old white leghorn chicks anesthetized with nembutal (0.03 mg/g body wt., intramuscular). Suspensions were in seawater (metacercariae) or a 50/50 mixture of seawater and Locke's solution for poikilotherms. In each instance the inoculum was quickly ingested by the movements of the cloacal lips and worms were recovered from the bursa at necropsy 1 min postinoculation. The results (Table 1) clearly indicate that movements of the cloacal lips and adjacent musculature serve to mechanically move an inoculum into the bursa as neither metacercariae nor dead cercariae

Table 1. Larval trematodes recovered from the bursa of Fabricius and last 10 cm of intestine of 18 chicks killed with ether and necropsied 1 min after per cloaca inoculation with 50–60 living cercariae, dead cercariae or metacercariae of *Himasthla quissetensis*.

	Number of larvae recovered								
	Living cercariae			Dead cercariae			Metacercariae		
		Intestine			Intestine			Intestine	
Bird	Bursa	A*	B†	Bursa	A	B	Bursa	A	B
1	45	7	0	7	1	0	13	7	0
2	55	0	0	14	0	0	28	17	0
3	56	1	0	19	2	0	34	8	0
4	58	0	0	5	1	0	15	7	0
5	76‡	1	0	9	0	0	22	4	0
6	91‡	1	0	1§	0	0	8	3	0

* A = Last 5 cm of intestine.
† B = Penultimate 5 cm of intestine.
‡ Bird overdosed with more than 50–60 cercariae.
§ Bird defecated shortly after inoculum was applied.

are capable of locomotion. The possibility that living worms migrated into the bursa may have contributed to the larger numbers recovered from this group. However, differences in worm recovery from all three groups may have simply resulted from variations in body size, shape, or consistency of the larval stages. For example, dead cercariae are elongate and flaccid whereas encysted metacercariae are spherical. Living cercariae contract when disturbed becoming somewhat rounded. The effectiveness of the mechanical forces responsible for propelling the worms following inoculation may vary with such characteristics.

The fate of those worms not recovered from the bursa or intestine was not determined.

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Research Note

Occurrence of *Leucochloridiomorpha constantiae* and *Amblosoma pojmanskae* Metacercariae in the Southeastern United States

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Leucochloridiomorphid metacercariae parasitize viviparid snails in Europe, Asia, and North America. Adults of *Leucochloridiomorpha* and *Amblosoma* species occur in waterfowl, which may account for the broad geographic distribution of these parasites. However, all reports of their metacercariae in North America are from northeastern and north-central states. This study was made to determine whether leucochloridiomorphid metacercariae also occur in the southeastern United States.

From September 1981 to May 1982, 465 snails from four localities in Louisiana, one in Mississippi, and 12 in Florida were examined. They included 11 species in the genera *Campeloma, Viviparus,* and *Lioplax.* All were negative for leucochloridiomorphid metacercariae except six specimens of *V. georgianus* from a single locality; one harbored a single specimen of *Leucochloridiomorpha constantiae,* five harbored a total of seven metacercariae of *Amblosoma pojmanskae* (Table 1). In contrast, examination of 25 *Campeloma decisum* and 25 *C. integrum* from Wisconsin showed all specimens of both snail species to be infected with 19–224 metacercariae of *L. constantiae* per snail. Each of 12 *C. decisum* harbored 1–10 metacercariae of *A. suwaense,* and each of four *C. integrum* contained one or two metacercariae of that species. All but one specimen of *A. pojmanskae* from Florida exceeded the maximum length of 1,600 μm reported by Fischthal for *A. pojmanskae* metacercariae in New York (1974, Acta Parasitol. Pol. 22:165–169). The specimens from Florida were also

Table 1. Viviparid snails from Louisiana, Mississippi, Florida, and Wisconsin examined for leucochloridiomorphid metacercariae.

Locality	Species	No. snails examined	Snail voucher number*
Tchefuncte R. at LA. Hwy 40, Tangipahoa Par., LA	*Campeloma exilis*	50	UF 37650
Bayou Grosse Tete, 1 mi N Rosedale Iberville Par., LA	*Viviparus subpurpureus*	10	UF 37627
Bayou Manchac at US Hwy 61, East Baton Rouge Par., LA	*Viviparus subpurpureus*	50	UF 37646
	Viviparus intertextus	2	UF 37647
Pond, LA Hwy 22, 4 mi NE Head of Island, Livingston Par., LA	*Viviparus intertextus*	5	UF 37645
Backwater, Pascagoula R., MS Hwy 614, 2 mi W Wade, Jackson Co., MS	*Campeloma exilis*	25	
Yellow R. at U.S. Hwy 90, Okaloosa Co., FL	*Lioplax pilsbryi choctawatchensis*	30	UF 37636
	Campeloma geniculum	10	UF 37635
Rocky Cr. at FL Hwy 71, Washington Co., FL	*Campeloma* cf. *limum*	20	UF 37641
Chipola R. at FL Hwy 20, Calhoun Co., FL	*Viviparus georgianus goodrichi*	15	UF 37637
	Lioplax p. pilsbryi	10	UF 37625
Oklawaha R. at FL Hwy 40, Marion Co., FL	*Vivparus georgianus*	15	UF 37630
Alexander Springs, Ocala National Forest, Lake Co., FL	*Campeloma floridense*	5	UF 37632
	Vivparus georgianus	5	UF 37631
DeLeon Springs, Volusia Co., FL	*Viviparus georgianus*	5	UF 37634
Lake Monroe, Enterprise, Volusia Co., FL	*Viviparus georgianus*	3	
Wekiva R. at FL Hwy 46, Seminole Co., FL	*Viviparus georgianus*	20	UF 37633
St. John's R. at FL Hwy 415, Seminole Co., FL	*Viviparus georgianus*†	80	UF 37643
Withlacooche R. at FL Hwy 48, Sumter Co., FL	*Viviparus georgianus*	30	UF 37626
Suwanee R. at U.S. Hwy 98, Levy Co., FL	*Campeloma geniculum*	25	UF 37642
Lake Talquin at Coe's Landing, FL Hwy 20, Wakulla Co., FL	*Lioplax pilsbryi choctawhatchensis*	5	UF 37638
	Campeloma cf. *limum*	5	UF 37648
	Viviparus georgianus	10	UF 37629
Ochlockonee R., at U.S. Hwy 90, Gadsden Co., FL	*Campeloma geniculum*	30	UF 37640
Moose Ear Creek, Barron Co., WI	*Campeloma decisum*	25	UF 40232
O'Neil Creek, Chippewa Co., WI	*Campeloma integrum*	25	UF 40016

* Florida State Museum, University of Florida, Gainesville.

† Voucher specimens: *Leucochloridiomorpha constantiae* USNM Helm. Coll. No. 77412; *Amblosoma pojmanskae* USNM Helm. Coll. No. 77413.

morphologically similar to metacercariae of *A. suwaense* metacercariae from *Campeloma* spp. in Wisconsin. Metacercariae of North American species of *Amblosoma,* therefore, are more similar morphometrically than previously thought. Adult specimens of *A. pojmanskae* have not been described, and adults of *A. suwaense,* unknown from natural hosts, have been described only from gravid specimens grown in ovo (Shimazu, 1974, Jap. J. Parasitol. 23:100–105).

It is difficult to explain the contrasting distributional pattern of these metacercariae in northern and southern states. Possibly, southern species of viviparid snails are less suitable as hosts. More likely, migration of waterfowl definitive hosts is an important determinant of parasite distribution. Adult specimens of *Leucochloridiomorpha* and *Amblosoma* inhabit the bursa of Fabricius of ducks, an organ that is resorbed as the birds mature. Possibly, only young waterfowl in their northern breeding grounds harbor these parasites, and infections are lost prior to arrival of birds in the southern feeding range. A more complete understanding of the life histories and host specificity of these parasites is required before we can determine the reasons responsible for this pattern of geographic distribution.

I thank Dr. F. G. Thompson for identifying snails in this study. Drs. K. C. Corkum, R. M. Overstreet, R. W. Heard, and Mr. A. Blinkman assisted in collection of snails. Dr. Overstreet generously provided laboratory facilities at Gulf Coast Research Laboratory, Ocean Springs, Mississippi. Mrs. L. Glenna typed the manuscript. This research was funded by a grant from the University of Wisconsin–Eau Claire.

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Research Note

Prevalence of the Ancyrocephalinae (Monogenea) on Largemouth and Spotted Basses in Beech Fork Lake, West Virginia

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Prevalence of *Actinocleidus fusiformis* (Mueller, 1934) Mueller, 1937; *Clavunculus bursatus* (Mueller, 1936) Mizelle, 1956; *Urocleidus furcatus* (Mueller, 1937) Mizelle and Hughes, 1938; and *U. helicis* (Mueller, 1936) Mizelle and Hughes, 1938; on largemouth and spotted basses (*Micropterus salmoides* and *M. punctulatus*) from Beech Fork Lake, West Virginia, was studied monthly from May through October 1981. Seven species of Ancyrocephalinae are known from largemouth bass; however, Mizelle and Crane (1964, Trans. Am. Microsc. Soc. 83:343–348) noted that no more than four of the seven species occurred at any one locality. Rawson and Rogers (1972, Proc. Helminthol. Soc. Wash. 39:159–162) felt this may be because of competition within species groups or the difficulty encountered in separating species (e.g., *C. bursatus* from *C. unguis*; *U. principalis* from *U. helicis* and *U. furcatus* from *U. dispar*).

Fish were captured by electroshocking or rotenone spraying, transported to the laboratory on ice, sexed, and measured for total length. Fish <100 mm were considered immature. All gills were removed from the fish within 3 hr of capture, placed in individual jars with a small amount of water and subsequently frozen. Gills were thawed and examined, along with the water in each jar, for monogeneans within 48 hr. Monogenetic trematodes were studied in water, polyvinyl alcohol, or glycerine jelly mounts. Although specific numbers of monogeneans per host were not recorded, there were seldom more than 25 individuals (all four species combined) per host. Thus parasite loads were similar to those found in largemouth bass in Puerto Rico (Pomales and Williams, 1980, J. Parasitol. 66:81), but smaller than reported by Rawson and Rogers (1972, loc. cit.) and Cloutman (1975, Trans. Am. Fish. Soc. 104:277–283).

Eighty-eight percent of the largemouth bass and 61.9% of the spotted bass were infested by at least one species of monogenetic trematode (Tables 1, 2). *Urocleidus helicis* was the most frequent monogenean encountered on both species of basses. *Clavunculus bursatus* was the least frequent on largemouth (Table 1), while sharing the least frequent category with *U. furcatus* in spotted bass (Table 2). Mizelle and Crane (1964, loc. cit.), working on largemouth bass in California, reported prevalence levels of *U. principalis* and *U. furcatus* comparable to those of

Table 1. Prevalence of monogenetic trematodes on largemouth bass from Beech Fork Lake, West Virginia in 1981.

Month	Number in sample	Prevalence	Prevalence by species			
			Actinocleidus fusiformis	*Clavunculus bursatus*	*Urocleidus furcatus*	*Urocleidus helicis*
May	3♂	3/3	3/3	0/3	2/3	3/3
	5♀	5/5	2/5	1/5	2/5	5/5
	2○	2/2	0/2	0/2	2/2	2/2
Jun	7♂	6/7	2/7	2/7	3/7	6/7
	5♀	4/5	0/5	1/5	0/5	4/5
	0○	0/0	0/0	0/0	0/0	0/0
Jul	10♂	10/10	5/10	3/10	7/10	10/10
	6♀	5/6	3/6	2/6	2/6	5/6
	4○	4/4	2/4	1/4	2/4	3/4
Aug	2♂	1/2	1/2	0/2	1/2	1/2
	1♀	1/1	1/1	1/1	0/1	1/1
	11○	9/11	5/11	5/11	3/11	6/11
Sep	3♂	3/3	3/3	1/3	3/3	3/3
	0♀	0/0	0/0	0/0	0/0	0/0
	10○	10/13	4/13	6/13	1/13	7/13
Oct	0♂	0/0	0/0	0/0	0/0	0/0
	1♀	1/1	1/1	0/1	1/1	1/1
	2○	2/2	2/2	0/2	1/2	0/2
Totals	25♂	23/25	14/25	6/25	16/25	23/25
	18♀	16/18	7/18	5/18	5/18	16/18
	32○	27/32	11/32	12/32	9/32	18/32
	75	66/75	32/75	23/75	30/75	57/75

Table 2. Prevalence of monogenetic trematodes on spotted bass from Beech Fork Lake, West Virginia in 1981.

Month	Number in sample	Prevalence	Prevalence by species			
			Actinocleidus fusiformis	*Clavunculus bursatus*	***Urocleidus furcatus***	*Urocleidus helicis*
May	2♂	2/2	0/2	0/2	2/2	1/2
	2♀	1/2	0/2	0/2	0/2	1/2
	1○	1/1	0/1	0/1	0/1	1/1
Jun	4♂	3/4	1/4	0/4	0/4	3/4
	0♀	0/0	0/0	0/0	0/0	0/0
	0○	0/0	0/0	0/0	0/0	0/0
Jul	5♂	3/5	0/5	0/5	0/5	3/5
	6♀	5/6	0/6	0/6	0/6	5/6
	1○	0/1	0/1	0/1	0/1	0/1
Aug	1♂	1/1	1/1	0/1	1/1	1/1
	0♀	0/0	0/0	0/0	0/0	0/0
	5○	3/5	2/5	1/5	1/5	1/5
Sep	1♂	1/1	0/1	0/1	0/1	1/1
	7♀	4/7	1/7	3/7	1/7	4/7
	2○	0/2	0/2	0/2	0/2	0/2
Oct	2♂	1/2	1/2	0/2	0/2	0/2
	2♀	1/2	1/2	1/2	0/2	1/2
	1○	0/1	0/1	0/1	0/1	0/1
Totals	15♂	11/15	3/15	0/15	3/15	9/15
	17♀	11/17	2/17	4/17	1/17	11/17
	10○	4/10	2/10	1/10	1/10	2/10
	42	26/42	7/42	5/42	5/42	22/42

Table 3. Chi-square contingency values for monogenetic trematodes associated with largemouth and spotted bass in Beech Fork Lake.

	M. salmoides vs. *M. punctulatus*	*M. salmoides*		*M. punctulatus*	
		♂ vs. ♀	Adults vs. immatures	♂ vs. ♀	Adults vs. immatures
Actinocleidus fusiformis	8.19*	1.23	1.57	0.28	0.11
Clavunculus bursatus	5.20†	0.08	1.23	0.91	0.05
Urocleidus furcatus	10.14*	5.50*	3.28	1.45	0.05
Urocleidus helicis	6.85*	1.20	11.94*	0.08	7.00*

* $P = 0.01$.
† $P = 0.05$.

U. helicis and *U. furcatus* in the present study. However, they reported levels of *A. fusiformis* considerably lower than in Beech Fork largemouth bass. Cloutman (1975, loc. cit.), working on largemouth bass in Arkansas, and Rawson and Rogers (1972, loc. cit.), examining largemouth from Alabama, emphasized abundance of monogeneans rather than prevalence. It is interesting that both Cloutman and Rawson and Rogers also reported *U. principalis* as the dominant gill parasite on largemouth bass (in terms of individuals per host) and *C. bursatus* as the least abundant. Thus their parasite loads exhibited some similarity to the prevalence levels in the present study.

A Chi-square contingency test with one degree of freedom was used to compare the prevalence of monogeneans between host species, sex within each host species, and mature versus immature hosts (Table 3). Calculations followed the procedure of Croxton (1951, Elementary Statistics with Applications in Medicine and the Biological Sciences, Dover Publications, Inc., New York, pp. 271–273). Prevalence levels recorded for each species of monogenetic trematode on largemouth bass were significantly higher than recorded for spotted bass (Table 3), indicating that *M. salmoides* was the "preferred" host for each trematode species.

With one exception—the occurrence of *U. furcatus* on male largemouth at a significantly higher prevalence rate than females—the prevalence of infestation on male basses was not significantly different from that found on females (Table 3). Generally, there was no difference in prevalence levels of monogenetic trematodes when comparing adult basses with immatures. There was an exception. Adults of both largemouth and spotted basses were infested with significantly higher prevalence levels of *U. helicis* than were immature basses (Table 3).

I would like to thank Dr. Wilmer Rogers, Auburn University, for confirming my identification of *A. fusiformis* and assisting me with the identifications and questions of synonymy of the other monogeneans.

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Research Note

Effect of Concanavalin A-Binding Sites on Invasion of Cultured Cells by *Eimeria tenella* Sporozoites

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Cinemicrographic studies of sporozoite invasion (Speer et al., 1971, J. Protozool. 18 [Suppl.]: 11) suggest that some interaction occurs between *Eimeria* sporozoites and the eventual host cell. A sporozoite may glide past several potential host cells without reacting and suddenly "recognize" a cell, passing by several others to penetrate it. Carbohydrate moieties make up large units of the cell membranes (Marchesi et al., 1972, Fed. Proc. 32:1833–1837) and differ in number, size, and distribution between cell types (Ozanne and Sambrook, 1971, Nature New Biol. 232:156–160). Therefore, they were a logical membrane component to examine in determining the nature of possible host cell recognition sites or areas for penetration by eimerian sporozoites. Okada and Kim (1972, Virology 50:507–515) discovered that treatment of animal cells with concanavalin A (Con A), a tetrameric protein that binds to α-D-mannopyranosyl moieties of cell membranes (Lis and Sharon, 1973, Ann. Rev. Biochem. 42:541–574) inhibited plaque formation by several viruses. Lonberg-Holm (1975, J. Gen. Virol. 28: 313–327) found that HeLa cells treated with Con A were not invaded by human rhinovirus or poliovirus. The object of the present experiment was to determine if Con A interactions with host cell membrane receptors likewise inhibited invasion of cells by *Eimeria tenella* sporozoites.

Primary chick kidney cell cultures (PCK) were prepared from kidneys of 3-wk-old chicks (Doran, 1971, J. Parasitol. 57:891–900) and grown in Leighton tubes in a medium consisting of Hanks' balanced salt solution (HBSS), plus 10% lactalbumin hydrolysate and 10% fetal calf serum. Bovine embryonic kidney (BEK) and Madin-Darby bovine kidney (MDBK) cell cultures were prepared from frozen ampules of cells and established in Lab-Tek, four-compartment chamber/slides using basal medium Eagle (BME) containing HBSS and 10% fetal calf serum.

Concanavalin A (Sigma Chemical Co., St. Louis, Missouri) in concentrations of 0.5, 1.0, 5.0, 10.0, 25.0, 50.0, 100.0, and 200.0 μg/ml, was added to the cell cultures 24 or 1 hr before inoculation of sporozoites and, in some procedures, was included in the inoculation media with the sporozoites. After pretreatment, the cultures were washed twice with HBSS or BME media and then inoculated with freshly excysted (Doran and Vetterling, 1967, Proc. Helminthol. Soc. Wash. 34:59–65) *E. tenella* sporozoites. Inoculated cultures were incubated for 4 hr; the cell layers were fixed and then stained with hematoxylin and eosin or examined unstained with phase contrast microscopy. The number of intracellular sporozoites in Leighton tubes was estimated by counting 240 microscopic fields (4.6% of the coverslip under 625× magnification). All intracellular sporozoites in the chamber slides were counted. Each experiment was replicated three times. To examine binding of Con A, cultured cells and sporozoites were treated with fluorescein-conjugated Con A (FITC-Con A) (Sigma Chemical Co., St. Louis). To test for saturation of Con A sites, PCK cultures were treated first with 0 or 25 μg/ml Con A and then with 25 μg/ml of FITC-Con A. All cultures treated with FITC-Con A were then examined with a UV microscope.

Results of the studies are found in Tables 1 and 2. Values for the highest concentrations of

Table 1. Effect of pretreating cultured cells with 25 μg/ml concanavalin A for 24 or 1 hr on invasion of the cells by *Eimeria tenella* sporozoites.

Cell type	Con A (μg/ml)	Sporozoites/10 mm² cells*	
		24 hr	1 hr
PCK†	0	3.72 ± 0.32	3.10 ± 0.27
	25	3.87 ± 0.34	3.78 ± 0.57
BEK†	0	4.12 ± 0.37	3.99 ± 0.37
	25	4.17 ± 0.38	4.09 ± 0.39
MDBK†	0	2.87 ± 0.32	2.65 ± 0.37
	25	2.66 ± 0.32	2.70 ± 0.32

* Means ± standard error of the means.

† PCK = primary chick kidney; BEK = bovine embryonic kidney; MDBK = Madin-Darby bovine kidney.

Table 2. Effect of including concanavalin A in the inoculation medium on invasion of cultured cells by *Eimeria tenella* sporozoites.

Cell type	Con A (μg/ml)	Sporozoites/10 mm² cells* 4 hr incubation
PCK†	0	3.83 ± 0.33
	10	4.01 ± 0.58
BEK†	0	4.00 ± 0.39
	25	4.27 ± 0.39
MDBK†	0	3.04 ± 0.28
	25	2.78 ± 0.24

* Means ± standard error of the means.
† PCK = primary chick kidney; BEK = bovine embryonic kidney; MDBK = Madin-Darby bovine kidney.

Con A that were not toxic to the cell cultures are shown. There were no statistically significant differences in the numbers of sporozoites that penetrated Con A-treated and untreated host cells in any of the experimental procedures (Table 1). Concentrations of Con A in excess of 25 μg/ml were toxic to all three cell types. However, when small patches of cells were found still adhering to the coverslip, intracellular sporozoites were occasionally seen even at 200 μg/ml. Inclusion of Con A in the inoculation medium also had no effect on invasion (Table 2). At 25 μg/ml, FITC-Con A bound to the cultured cells. If the cultures were first treated with 25 μg/ml Con A and then with FITC-Con A, there was a fourfold reduction in the fluorescence, indicating that ~75% of the binding sites were saturated by 25 μg/ml of Con A. Con A did not agglutinate or cause lysis of the sporozoites at any of the concentrations used. At concentrations up to 200 μg/ml, no binding of FITC-Con A to the sporozoites was seen.

In summary, binding of ~75% of the α-D-mannopyranosyl moieties on the PCK cultures by Con A did not inhibit invasion of the cells by *E. tenella* sporozoites. Therefore, it seems logical to conclude that these carbohydrate moieties do not have a major function in the invasion of cultured cells by *E. tenella* sporozoites.

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Research Note

Anatomical Anomalies in a Leech, *Cystobranchus meyeri* (Hirudinea: Piscicolidae), Infesting the White Sucker, *Catostomus commersoni* Lacépède

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Cystobranchus meyeri has been reported from only one population of white suckers in the vicinity of Albany, New York (Hayunga and Grey, 1976, J. Parasitol. 62:621–627). Leeches are usually attached to the distal end of the pectoral fin. Although the parasites are frequently found in large numbers, damage to host tissue appears limited to localized ecchymoses associated with feeding (Fig. 1).

Approximately 95 leeches were collected from 1974 through 1976. Specimens were examined live and as formalin-fixed whole mounts stained with Mayer's paracarmine. Two different anatomical anomalies were observed. One leech was found to have only 11 testes instead of the usual 12 (Fig. 2); another lacked ocelli on the posterior sucker (Fig. 3). The frequency of both anomalies seems relatively high in this sample (>1%). It is tempting to speculate that such anomalies in either parasite or fish host may serve as indicators of chemical pollution, as suggested by Hayunga (1980, J. Fish. Dis. 3:167–172). However, it is perhaps just as likely that this particular species simply exhibits a high rate of spontaneous morphological variation.

Atrophy or aplasia of a single testis ought to be easily recognized as an abnormality and, therefore, is of little taxonomic consequence. In contrast, the number of posterior ocelli is one of several key characters used in identifying *C.*

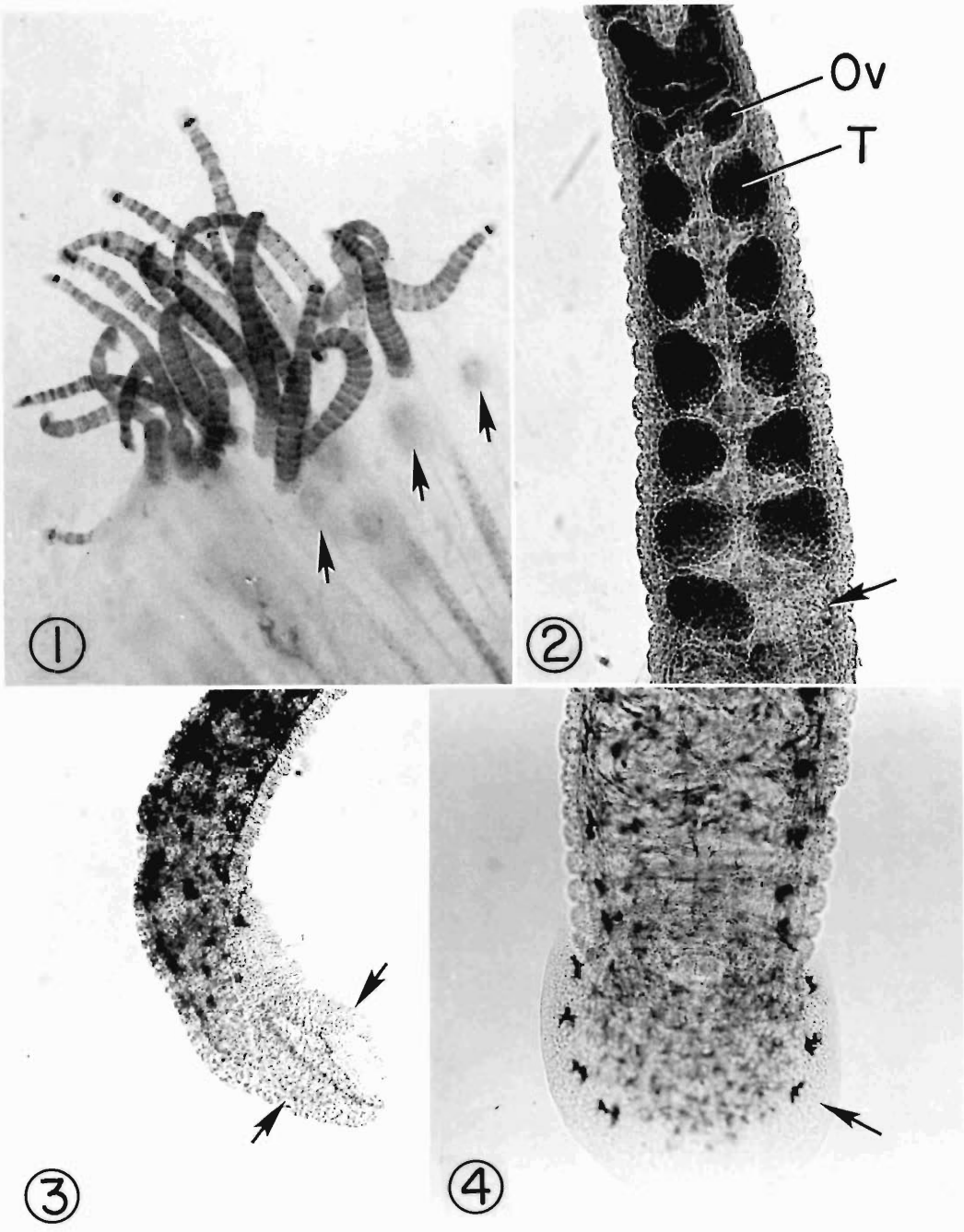

Figure 1. Pectoral fin of *Catostomus commersoni* Lacépède to which are attached 20 leeches. Note localized ecchymoses (arrows) that appear to result from the feeding activity of the parasites. Living specimens, ×7. Figure 2. *Cystobranchus meyeri* missing one testis in somite XVIII (arrow); ovary (Ov), testis (T). Stained whole mount, ×30. Figure 3. *C. meyeri* with no ocelli on the posterior sucker (arrows). Unstained whole mount, ×25. Figure 4. Typical *C. meyeri* with eight posterior ocelli (arrow). Unstained whole mount, ×40.

meyeri (Fig. 4). Intraspecific variation has been reported for external markings (Meyer, 1946, J. Parasitol. 32:467–476), ratio of body and sucker diameter to length (Moore and Meyer, 1951, Wasmann J. Biol. 9:11–77), and annulation (Meyer and Roberts, 1977, Univ. Nacional Mex. Inst. Biol. Publ. Esp. 4:513–519). Interpretation of such variable or "changeable" features in these soft-bodied animals is further complicated by fixation artifact, as improper preservation may cause gross distortion of specimens or fading of pigmentation (Meyer, 1965, Atlantide Report No. 8:237–245, Danish Scient. Press, Ltd., Copenhagen). For these reasons, size or external markings should not be the sole criteria for classification of leeches. Such characters should be used with caution and observations made on a representative sample of the population rather than single specimens.

Recognizing that internal organs are less susceptible to fixation artifact, Johansson (1898, Zool. Anz. 21:581–595) suggested a classification of the Piscicolidae based primarily upon internal anatomy. However, since the internal anatomy of many species has not been fully described, a comprehensive revision of the family on this basis is not possible. Likewise, the promising techniques of karyotype analysis and isoenzyme electrophoresis are limited in practical application because they generally require fresh material. Although very subtle interspecific differences may be detected by these methods, data can be obtained only from newly collected specimens. Thus, at present, taxonomists have no alternative but to rely upon characters of external morphology despite their obvious shortcomings.

Specimens were collected and examined in collaboration with Dr. John S. Mackiewicz and Dr. Anthony J. Grey, State University of New York at Albany. Dr. Marvin C. Meyer, University of Maine at Orono, provided assistance with the original species description and shared with me his extensive knowledge of the literature. This work was supported, in part, by Grant No. 20-A022-A from the Research Foundation of the State University of New York to Dr. Mackiewicz, and by a Grant-in-Aid-of-Research from the Society of the Sigma Xi to the author.

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Research Note

SEM of Tapeworm Flame Cells

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Parasitologists have been intrigued by the nature of the platyhelminth flame cell—the morphology, the taxonomic significance of the "flame cell formula," the possible function in osmoregulation and/or in excretion, etc. This interest is illustrated by numerous literature citations, but the landmark papers are those of: Kümmel (1958, Z. Naturf. 136:677–679), Wilson (1969, Parasitology 59:461–467), Howells (1969, Parasitology 59:449–459) and Wilson and Webster (1974, Biol. Rev. 49:127–160). More recent contributions are those of Gambrion (1981, Thèse, Montpellier, France) and Rohde (1982, Prog. and Abstr., Fifth Int. Cong. Parasitol., Toronto, Vol. II, p. 99). All of the above references are based on TEM studies.

The data presented here are the result of scanning electron microscope studies on *Dioecocestus acotylus* and *Schistotaenia tenuicirrus* that were prepared by ethanol cryofracture for embryological studies (Coil, 1979, Z. Parasitenk. 59:151–159).

Basically, the terminal organ consists of a cell body that extends from the base of a parenchymal crater (Fig. 1). Internally, and not seen here, the cell body bears a tuft of typical, but fused cilia that project into the tubule. The distal end of the tubule is composed of two parts: (1) an

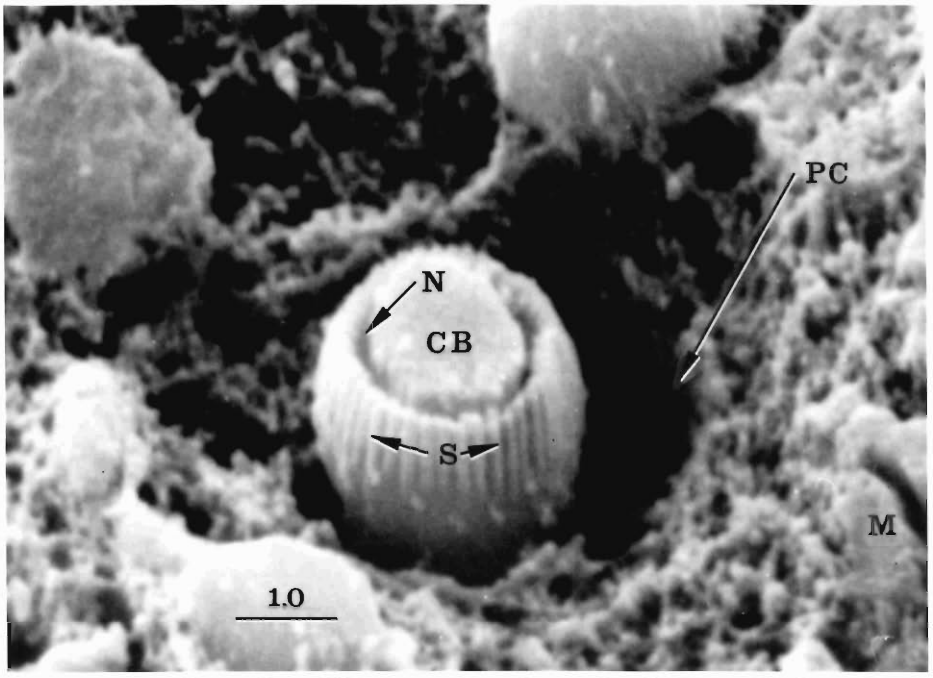

Figure 1. ***Schistotaenia tenuicirrus.*** **SEM of ethanol cryofracture showing flame cell body and distal tubule. Terminal part of cell body was lost during cleavage. Scale bar equals 1 μm. CB—cell body, M—muscle, N—nephrostome, PC—parenchymal crater, and S—slits.**

external, corrugated barrel that is derived from the tubule, (2) an internal series of bars that are derived from the flame cell body (Wilson, 1969, loc. cit.). The internal bars form a covering for the slits that are seen around the circumference of the barrel. The barrel extends over the cell body forming a cytoplasmic fold beneath which lies the nephrostome (Howells' term, 1969, loc. cit.).

The cleavage of worm tissue to reveal flame cells is a fortuitous event and the subsequent viewing of the terminal organ is a matter of chance. External leptotriches as reported by Kümmel (1964, loc. cit.) in *Fasciola* and by Lumdsen (1981, *Hymenolepis diminuta,* Academic Press) were not seen on the bars between the slits in the species studied here. The structures are not highly developed in the cestodes, but at the magnifications used here they would be visible. The number of slits is close to 40 based on extrapolations; in no case were all the slits visible in one flame cell.

It should be noted that the genera studied here (*Dioecocestus* and *Schistotaenia*) must be considered as bizarre tapeworms (unusual, at least) with a number of attributes not ordinarily seen in "typical" tapeworms. For example: (1) they lack vaginae, (2) both infect primitive hosts (grebes), (3) one is dioecious, etc. (Coil, 1970, Z. Parasitenk. 33:314–328; Boertje, 1974, Proc. La. Acad. Sci. 37:89–103). On the basis of only a few studies on tapeworm flame cells, it would be premature to conclude that the flame cells here represent the primitive (plesiomorphic) condition by lacking the leptotrichs, but this is an interesting idea that may warrant further study. This is especially true since Rohde (1980, Angew. Parasitol. 21:32–48; 1982, loc. cit.) has proposed that relationships among the platyhelminths can be discerned by a study of the morphology of the flame cells.

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PRESENTATION

1983 Anniversary Award of the Helminthological Society of Washington

According to the Bylaws of the Society, the Awards Committee is charged with recommending, to the Executive Committee, a candidate for the Anniversary Award which may be given annually or less frequently at the discretion of the Committee. The Award is made to a present or past member of the Society who is honored for one or more of the following achievements: (a) outstanding contributions to parasitology or related sciences that bring honor and credit to the Society, (b) an exceptional paper read at a meeting of the Society or published in its Proceedings, (c) outstanding service to the Society, and (d) other achievement or contribution of distinction that warrants highest and special recognition by the Society. This year the Executive Committee has supported the recommendation of the Awards Committee and I am very pleased to announce that the recipient of the 1983 Anniversary Award is Dr. Leon Jacobs.

Dr. Jacobs was born in Brooklyn, N.Y. and received his early education there getting the Bachelor of Arts degree from Brooklyn College. He then moved to Washington, D.C. and, except for a period of service with the Army during the Second World War, continued onward to completion of his doctorate at George Washington University in 1947. He began his career with the National Institutes of Health in 1937 and, except for two years as Deputy Assistant for Science at the Department of Health, Education and Welfare, he remained at the NIH until his retirement in 1979. Among the many positions held at the NIH were Chief of the Laboratory of Parasitic Diseases, Scientific Director of the Division of Biological Standards, Associate Director of Collaborative Research, and Director of the Fogarty International Center. At the present time Dr. Jacobs is the president and chairman of the Board of Directors of the Gorgas Memorial Institute of Tropical and Preventive Medicine.

Although Dr. Jacobs was highly successful in his administrative endeavors he was best known to most of us as an accomplished, internationally

Dr. Sheffield (left) presenting Anniversary Award to Dr. Jacobs.

known scientist. Early in his research career, he worked with Dr. Benjamin Chitwood on chemistry of nematodes. (Dr. Jacobs claims that he got his start in the laboratory of Dr. Gotthold Steiner as an office boy with a microscope.) Soon he began to broaden his interests and carried out studies on cultivation of *Entamoeba histolytica.* Later, in 1950, he initiated studies on toxoplasmosis which became his major area of expertise. Although his contributions to the knowledge of the biology of *Toxoplasma gondii* and the disease caused by it were many, his development of a model of toxoplasmic uveitis in rabbits is considered to be one of the most significant. As he became more involved in administrative duties, Dr. Jacobs continued to be active in the field of toxoplasmosis as a consultant and author of numerous review articles. Dr. Jacobs has been the recipient of many awards, one of which was the Henry Baldwin Ward Medal of the American Society of Parasitologists.

Dr. Jacobs became a member of the Helminthological Society of Washington in the mid-'40s. He served on the Executive Committee and as Vice President and President of the Society. During his long term of membership and service he presented or co-authored many papers at the Society's meetings.

I have personally known Dr. Jacobs since 1962 at which time I came to the NIH to work for him in the Laboratory of Parasitic Diseases. Since that time I have developed the highest respect for him as a scientist, teacher, and friend. I feel very honored to have the opportunity to convey the admiration and respect of the members of this Society by presenting him with the 1983 Anniversary Award.

Harley G. Sheffield,
Chairman, Awards Committee

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:

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Helminthological Society of Washington
Post Office Box 368
Lawrence, Kansas 66044

Print name: _____ Date of birth: _____

Mailing address: _____
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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	May Belle Chitwood	1972
Mildred A. Doss	1961	* Elvio H. Sadun	1973
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* Jesse R. Christie	1964	David R. Lincicome	1975
Gilbert F. Otto	1965	Margaret A. Stirewalt	1975
* George R. LaRue	1966	* Leo A. Jachowski, Jr.	1976
* William W. Cort	1966	Horace W. Stunkard	1977
* Gerard Dikmans	1967	Kenneth C. Kates	1978
* Benjamin Schwartz	1969	* Everett E. Wehr	1979
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Aurel O. Foster	1970	Frank D. Enzie	1981
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* Jesse R. Christie	1956	* Benjamin Schwartz	1976
* Gotthold Steiner	1956	Mildred A. Doss	1977
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* Allen McIntosh	1963	George W. Luttermoser	1979
Edna M. Buhrer	1963	John S. Andrews	1980
* Benjamin G. Chitwood	1968	* Leo A. Jachowski, Jr.	1981
Aurel O. Foster	1972	Kenneth C. Kates	1981
Gilbert F. Otto	1972	Francis G. Tromba	1983
* Theodor von Brand	1975		

* Deceased.

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