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Pasteuria penetrans (ex Thorne, 1940) nom. rev., comb. n., sp. n., a Mycelial and Endospore-Forming Bacterium Parasitic in Plant-Parasitic Nematodes

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ABSTRACT: A spore-forming parasite of plant-parasitic nematodes, at first believed to be a sporozoan ("*Duboscqia penetrans*" Thorne, 1940), was later recognized to be a bacterium and was renamed "*Bacillus penetrans*" (Thorne, 1940) Mankau, 1975. Because "*Bacillus penetrans*" was not included in the 1980 "Approved Lists of Bacterial Names," it has no taxonomic standing. In effecting the formalities incident to reviving lapsed bacterial names, it became clear that "*Bacillus penetrans*" was misassigned to the bacterial genus *Bacillus* Cohn, 1872. Although the mode of formation and the structure of the endospore of the nematode parasite are similar to that described for members of the genus *Bacillus*, the organism differs from the description of that genus in cellular shape and size, motility, flagellation, sporangial shape and size, habitat, and nutritional requirements. The following traits of the nematode parasite suggest that it more properly belongs in the genus *Pasteuria* Metchnikoff, 1888: Primary vegetative colonies consist of a dichotomously branched, septate mycelium; daughter colonies, formed by fragmentation, gradually contain fewer but larger cells arranged predominantly in quartets; these larger vegetative cells differentiate into sporangia, arranged in quartets and doublets; eventually, single sporangia predominate in the nematode's pseudocoelom; the sporangium consists of a conical stem, a swollen middle cell, and an endogenous spore; the mature endospores, released from the remnants of the nematode, attach to the cuticles of other host nematodes and germinate; then, the parasitic cycle is repeated. A description of *Pasteuria penetrans* (ex Thorne, 1940) nom. rev., comb. n., sp. n., and an emended description of the genus *Pasteuria* are presented.

Archival Background

The first report of an organism resembling "*Bacillus penetrans*"[3] (Thorne, 1940) Mankau, 1975 was by Cobb (1906), who found numerous highly refractile spores infecting specimens of the nematode *Dorylaimus bulbiferous*. He erroneously viewed these spores as "perhaps monads" of a parasitic sporozoan. This error of placing in the protozoa an organism now known to be a bacterial parasite of nematodes was to persist for nearly 70 years. A more precise but still incorrect placement was suggested by Micoletzky (1925), who found a nematode parasite having spores similar in size and shape to those reported by Cobb; Micoletzky suggested that these spores belong to the genus *Duboscqia* Perez, 1908, again a sporozoan group (Perez, 1908). Later, Thorne (1940) described in detail a new parasite from *Pratylenchus pratensis* (de Man) Filipjev; on the assumption that this organism was similar to the nematode parasite described by Micoletzky, it also was assigned by Thorne (1940) to the protozoan genus *Duboscqia*, as *D. penetrans*.

Thorne's description and nomenclature were to persist until 1975, even though other investigators (Williams, 1960; Canning, 1973), who examined this nematode parasite in some detail, questioned this placement. It was not until the nematode parasite was reexamined using electron microscopy that its true affinities to the bacteria rather than to the protozoa were recognized and the name "*Bacillus penetrans*" (Thorne, 1940) Mankau, 1975 was applied to it (Mankau, 1975a, b; Mankau and Imbriani, 1975; Imbriani and Mankau, 1977).

A different set of incorrect conclusions have until now inhibited reassignment of "*Bacillus penetrans*" to the genus *Pasteuria* Metchnikoff, 1888, where we believe it properly belongs. The situation, stated briefly, is that Metchnikoff (1888) described an endospore-forming bacterial parasite of cladocerans; he named this bacterium *Pasteuria ramosa* Metchnikoff, 1888. Metchnikoff presented drawings and photomicrographs of the life stages of this parasite as they occurred in the

[3] We follow here the accepted practice in bacteriological nomenclature of enclosing illegitimate names within quotation marks.

hemolymph of the water fleas, *Daphnia pulex* Leydig and *D. magna* Strauss; he was, however, unable to culture the organism in vitro.

Subsequent workers (Henrici and Johnson, 1935; Hirsch, 1972; Staley, 1973), who were looking in cladocerans for Metchnikoff's unique bacterium, reported on a different bacterium with only superficial resemblance to certain life-stages of *P. ramosa.* Their investigations led to the axenic cultivation of a budding bacterium, which is occasionally found on the exterior surfaces of *Daphnia* sp. Unlike Metchnikoff's organism, this budding bacterium does not form endospores, it is not mycelial, its staining reaction is Gram-negative, and it is not an endoparasite of cladocerans. After searching for, but not finding in water fleas, the bacterial endoparasite as described by Metchnikoff, the erroneous conclusion was reached that this budding bacterium, which occurs on the surfaces of *Daphnia* sp., was the organism Metchnikoff had described. Culminating this chain of errors, a budding bacterium (strain ATCC 27377) was mistakenly designated (Staley, 1973) as the type culture for *Pasteuria ramosa* Metchnikoff, 1888, the type (and, then, sole) species of the genus *Pasteuria.*

This confusion between Metchnikoff's cladoceran parasite and the quite different budding bacterium was only recently resolved (Starr et al., 1983): The budding bacterium (with strain ATCC 27377 as its type culture) was named *Planctomyces staleyi* Starr, Sayre, and Schmidt, 1983 and conservation of the original description of *Pasteuria ramosa,* as updated, was recommended.

Using as a basis the conserved description of *Pasteuria ramosa* (i.e., Metchnikoff's, as updated), we present here data supporting our view that this bacterial parasite of nematodes, "*Bacillus penetrans*" (Thorne, 1940) Mankau, 1975, properly belongs in the genus *Pasteuria* rather than in the genus *Bacillus.* Its traits suggest that "*B. penetrans*" should be referred to a new species of the genus *Pasteuria.* "*Bacillus penetrans*" was not included in the "Approved Lists of Bacterial Names" (Skerman et al., 1980), nor subsequently legitimized; hence, it now has no standing under the current rules as given in the "International Code of Nomenclature of Bacteria" (Lapage et al., 1975). A formal description of this organism, a legitimate name, *Pasteuria penetrans* (ex Thorne, 1940) nom. rev., comb. n., sp. n., and an emended description of the genus *Pasteuria* Metchnikoff, 1888 are presented.

Materials and Methods

Bacterial specimens

The infectious spores of "*Bacillus penetrans*" (Fig. 1) were found adhering to the cuticles of larvae of *Meloidogyne incognita,* a sedentary nematode that parasitized roots of the ornamental pepper, *Capsicum annuum* Linn., grown in a greenhouse soil bench. All attempts at axenic cultivation of the vegetative stages or spores of "*B. penetrans*" have been unsuccessful to date. Hence, its life cycle was followed in nematode hosts reared on the roots of tomato seedlings (Sayre and Wergin, 1977). The specimens of *Pasteuria ramosa* were found in the hemolymph of cladocerans (*Moina rectirostris*) collected from a pond near Beltsville, Maryland. Axenic cultivation of *P. ramosa* has not yet been achieved; consequently, material derived from two-membered laboratory systems of bacteria plus cladocerans was used. Laboratory-reared cladocerans served as hosts for *P. ramosa*; procedures for cultivating cladocerans and inoculating them with *P. ramosa* were as previously described (Sayre et al., 1979).

Light microscopy

The developmental stages of "*Bacillus penetrans*" were obtained by macerating infested tomato roots in a 50% (v/v) solution of Pectinol (Rohm and Haas, Philadelphia). The macerated roots were shaken in water to dislodge nematode larvae and adults. The freed nematodes were transferred to glass slides and examined. Nematodes parasitized by "*B. penetrans*" were crushed on slides, air-dried, and stained by Gram's method. Cladocerans were filtered from an aquarium onto a 200-mesh screen, and then backwashed into a 150-mm plastic Petri dish provided with a counting grid. Excess water was removed with a pipette, leaving the cladocerans immobilized in a thin water film on the bottom of the dish. The dish, covered to prevent further evaporation of the water, was placed on the stage of a Leitz model 52 inverted microscope, and the contents of the cladocerans' coeloms were examined at 250× for the presence of *Pasteuria ramosa.*

Transmission electron microscopy

Root galls containing nematodes infected with "*Bacillus penetrans*" were placed in a solution of 3.0% (w/v) glutaraldehyde in 0.05 M phosphate buffer (pH 6.8). Galls were cut into 2–3-mm segments and transferred to glass vials containing the buffered fixative. In addition, mature, parasitized female nematodes, about 30 days old, were dislodged from roots with a scalpel, handpicked with a Pasteur pipette, and crushed in molten 3.0% (w/v) agar solution at 50°C; this procedure caused the sporangia to disperse in agar, making for easier observation. The agar, containing gall and nematode material, was allowed to solidify; it was then placed in 3.0% (w/v) glutaraldehyde for 1.5 hr, washed in six changes of buffer over a period of 1 hr, postfixed in 2.0% (w/v) osmium tetroxide for 2 hr, dehydrated in an acetone series that began with 10% acetone and

increased by 10% every 20 min until 100% acetone, and infiltrated with Spurr's low-viscosity resin mixture. Cladocerans parasitized by *Pasteuria ramosa* were fixed in 3.0% (w/v) glutaraldehyde for 24 hr, washed in six changes of 0.05 M potassium phosphate buffer (pH 6.8) over a period of 1 hr, postfixed in 2.0% (w/v) osmium tetroxide for 2 hr, and dehydrated in the acetone series. Following dehydration, the specimens were infiltrated with Spurr's low-viscosity resin mixture. The embedded material (root galls containing nematodes parasitized by "*Bacillus penetrans*"; cladocerans containing *Pasteuria ramosa*) were sectioned on a Sorvall MT-2 ultramicrotome with a diamond knife. Thin sections were mounted on uncoated copper grids (75 × 100-mesh), stained for 10 min with 2.0% (w/v) aqueous uranyl acetate, and then stained for 5 min with 2.66% (w/v) lead citrate. The sections were viewed with a Philips model 200 transmission electron microscope operating at 60 kV with 20-μm apertures.

Scanning electron microscopy

Second-stage larvae of the root-knot nematode encumbered with "*Bacillus penetrans*" spores were prepared for scanning electron microscopy by fixation in 3.0% (w/v) glutaraldehyde in 0.05 M phosphate buffer (pH 6.8) for 1.5 hr and dehydration in an ethanol series (20, 40, 60, 80, and 100% anhydrous ethanol), followed by critical-point drying from liquid CO_2. Such preparations, containing stages of the two parasites, were examined with a Hitachi model HHS-2R scanning electron microscope operated at 15 or 20 kV. Preparations of *Pasteuria ramosa* were made for scanning electron microscopy by crushing parasitized female cladocerans on aluminum stubs. The liberated sporangia were air-dried before coating and examination.

Results

When observed adhering to the cuticles of root-knot nematode larvae (Fig. 1) and parasitizing adult female nematodes, "*Bacillus penetrans*" is so distinctive in its morphology, ultrastructure, and life stages that these features alone served as reliable diagnostic traits.

Morphological characteristics of vegetative cells

Mycelial colonies of "*Bacillus penetrans*" up to approximately 8 μm in diameter are formed in the pseudocoelom, where they are observed after the parasitized larvae penetrate the plant roots (Fig. 2). The dichotomously branched mycelium comprising the colony is septate (Figs. 2, 3). Measurements of the diameters of hyphal cells vary from approximately 0.2–0.5 μm; because of the sinuous pattern of mycelial growth, estimates of cell length would not be meaningful. Cells are bounded by a compound wall, about 0.12 μm thick, composed of an outer and an inner membrane. The inner membrane of the wall forms the septation and delineates individual cells. In addition, this membrane is continuous with a membrane complex or mesosome frequently associated with the septum (Fig. 3).

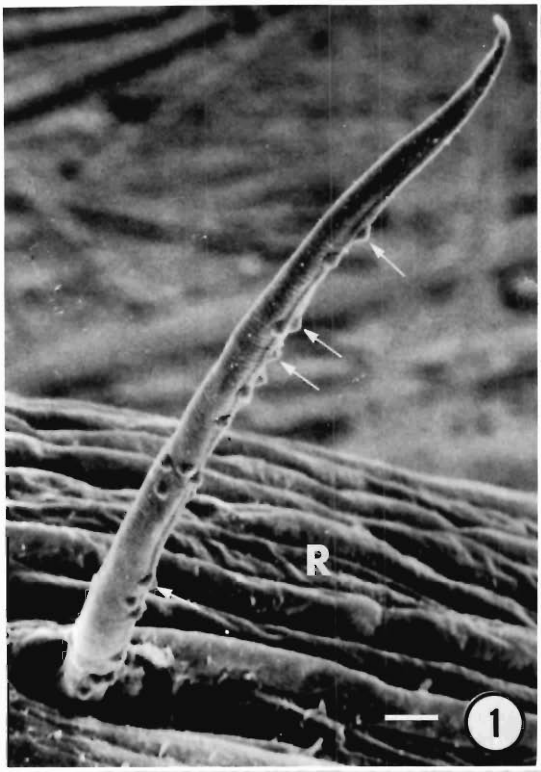
Sporulation of "*B. penetrans*" is a synchronously initiated process in the nematode host; it involves the terminal hyphal cells of the mycelium. As the process begins, the terminal cells bifurcate (Fig. 2) and enlarge from typical hyphal cells to ovate cells measuring about 2.0 by 4.0 μm. Structure and content of the cytoplast change from a granular matrix, which contains numerous ribosomes as found in the hyphal cells, to one that lacks particulate organelles. During these changes, the developing sporangia separate from their parental hyphae, which stop growing and eventually degenerate.

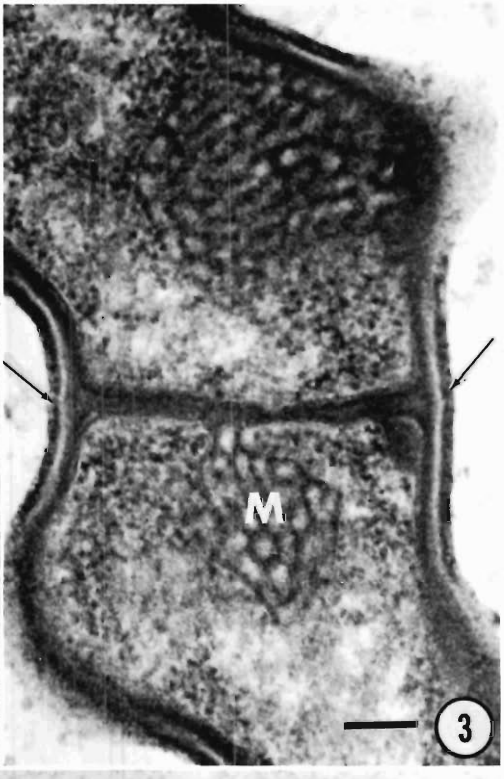
After these early structural alterations, a membrane forms within the sporangium and separates the upper third of the cell or forespore from its lower or parasporal portion (Fig. 4). The granular matrix confined within the membrane then condenses into an electron-opaque body, 0.6 μm in diameter, which eventually becomes encircled by a multilayered wall. The discrete structure that ensues is an endospore (Fig. 5).

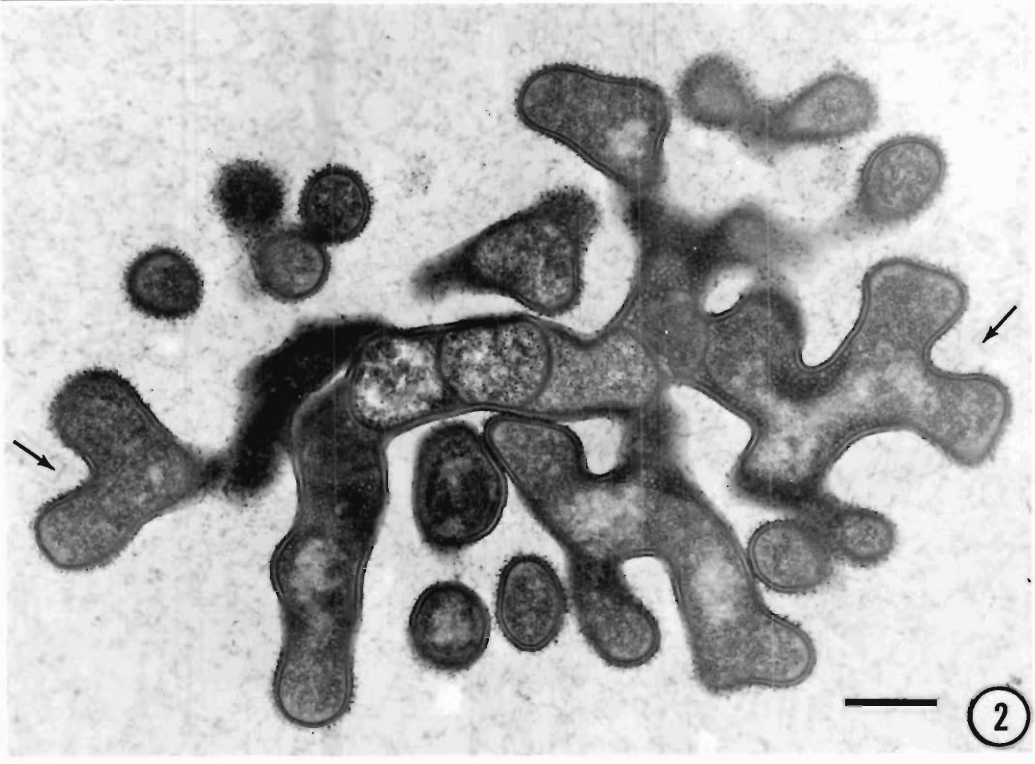
Morphological characteristics of sporangia and endospores

Spores of "*Bacillus penetrans*" that measure about 3.8 μm and adhere to the surface of root-knot nematode larvae are considered mature (Figs. 1, 6; Imbriani and Mankau, 1977; Sayre and Wergin, 1977). Two distinct forms of these spores can be observed with the scanning electron microscope (Fig. 6). The surface of one form appears as a wrinkled membrane that encompasses the entire spore. This "membrane" is actually the exosporium, which is generally sloughed prior to germination. In the absence of the exosporium, the second form of the spore can be resolved into two distinct components: a spherical central endospore, 2.3 μm in diameter, and a peripheral matrix, 1.85 μm wide, which surrounds the endospore (Fig. 6).

Cross sections viewed by transmission electron microscopy reveal (Imbriani and Mankau, 1977; Sayre and Wergin, 1977) that the "*B. penetrans*" endospore consists of a central, highly electron-opaque core surrounded by an inner and an outer wall composed of several distinct layers







(Fig. 7). When observed with the transmission electron microscope, the peripheral matrix of the spore is fibrillar. Fine microfibrillar strands, about 1.5 nm thick, extend outward and downward from the sides of the endospore to the cuticle of the nematode, where they become more electron-dense.

Morphological characteristics of parasporal structures

Coincident with the formation of an endospore in "*Bacillus penetrans*" is the emergence of the parasporal fibers. These fine fibers, which form around the base of the spore, differentiate from an electron-translucent, granular substance. They appear to connect with and radiate from the external layer of the wall of the endospore (Fig. 8). During development of the parasporal fibers, the formation of another membrane, the exosporium, isolates the newly formed endospore within the sporangium. At this later stage of spore development, the granular content of the parraspore becomes less dense, degenerates, and disappears. As a result, the mature sporangium contains a fully developed endospore enclosed within the exosporium (Fig. 8). The cell wall of the "*B. penetrans*" sporangium remains intact until the nematode is disrupted, after which event the endospores are released. The exosporium apparently remains associated with the endospore until contact is made with a new nematode and the infection cycle restarts.

Penetration of host

Larvae of root-knot nematodes belonging to the genus *Meloidogyne,* as well as other nematode species from plants, are susceptible to attack by this parasite (Table 3). Only vermiform stages of nematodes are encumbered by the parasite as they migrate through spore-infected soils. A mature spore of "*Bacillus penetrans*" attaches to the surface of a nematode so that a basal ring of wall material lies flatly against the cuticle. The orientation is such that a median section through the endospore and perpendicular to the surface of the nematode would bisect this basal ring. As a result, the ring appears as two protruding pegs that are continuous with the outer layer of the spore wall and rest on the cuticular surface of the nematode (Fig. 9).

The peripheral fibers of the spore also are closely associated with the cuticle of the nematode (Fig. 10). The fibers, which encircle the endospore, lie along the surface of the nematode and follow the irregularities of the cuticular annuli. They apparently secure the spore to the nematode, but do not appear to penetrate the cuticle.

The germ-tube of the "*B. penetrans*" endospore emerges through the central opening of the basal ring, penetrates the cuticle of the nematode, and enters the hypodermal tissue (Fig. 10). Hyphae were initially encountered beneath the cuticle of the nematode near the site of germ-tube penetration. From this site, they apparently penetrate the hypodermal and muscle tissues and enter the pseudocoelom.

Discussion

Now that the aforementioned problem involving the type strain of *Pasteuria ramosa* has been resolved (Starr et al., 1983), and the status of the genus *Pasteuria* has thereby been returned to something akin to the original concept of Metchnikoff (1888), the way is now clear to discuss assigning the nematode parasite to the genus *Pasteuria*—in our view, a much more fitting generic repository than *Bacillus,* as is demonstrated in what follows.

Morphological characteristics common to "*Bacillus penetrans*" and *Pasteuria ramosa*

A close relationship exists between "*Bacillus penetrans*" (Thorne, 1940) Mankau, 1975 and

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Figure 1. Scanning electron micrographs of many spores of *Pasteuria penetrans* (arrows) attached to a larva of a root-knot nematode that has partially penetrated a tomato root (R). The spores carried on the larva will germinate inside the plant and penetrate the developing nematode, completing their life cycles in synchrony with their host. On decay of the plant root in the soil, the complement of spores developed within the parasitized nematode will be released. Bar = 10 μm.

Figure 2. Cross section of a mycelial colony of *Pasteuria penetrans* in the pseudocoelom of the nematode. The septate hyphae appear to bifurcate (arrows) at the margins of the colony. Bar = 0.5 μm.

Figure 3. Hyphal cells of *Pasteuria penetrans* are bounded by a compound wall consisting of a double membrane (arrow). A mesosome (M) is associated with the septum. Bar = 0.1 μm.

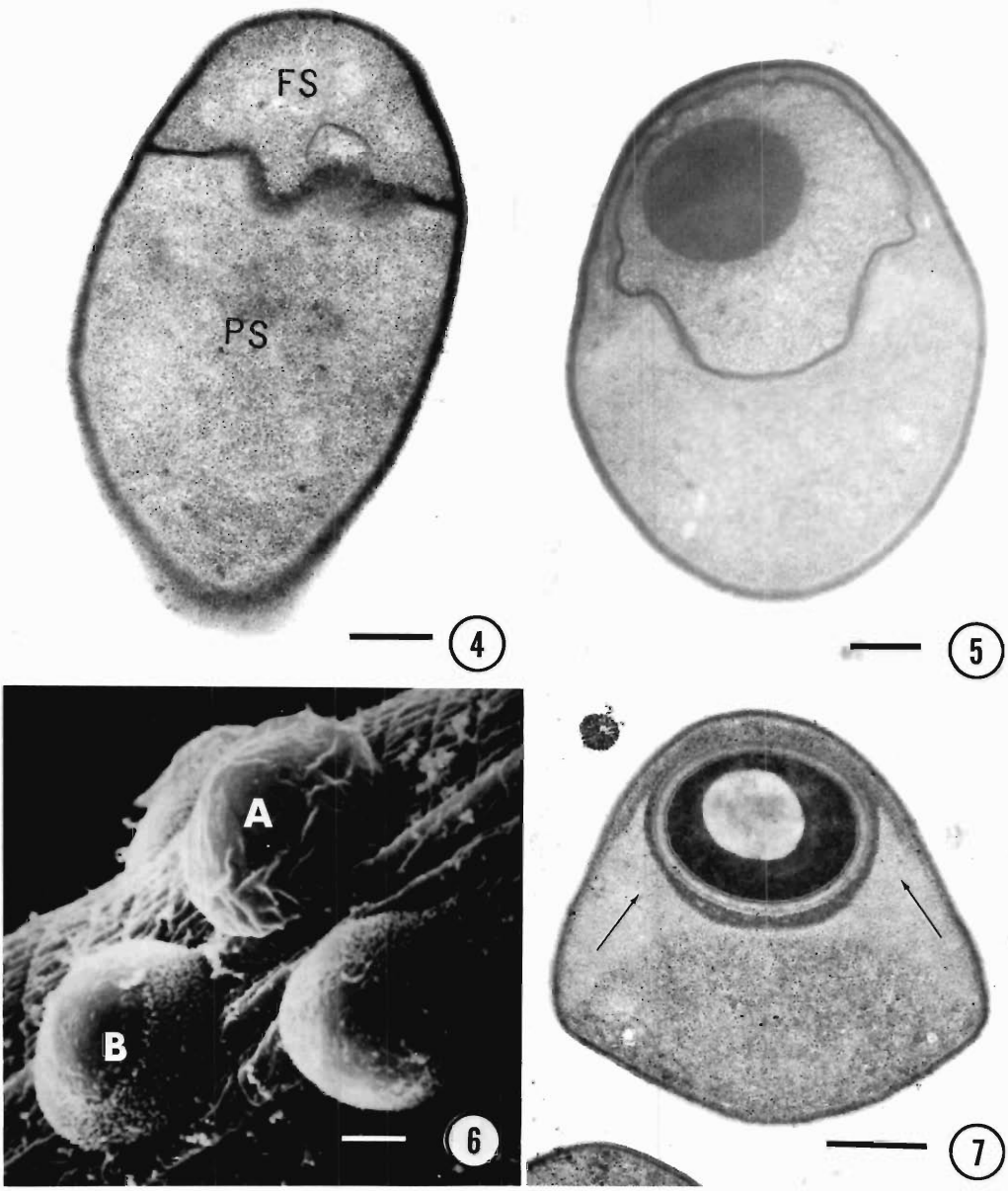


Figure 4. Section through a sporangium that has formed a membrane separating the anterior third of the spore or forespore (FS) from the parasporal segment (PS). Bar = 0.25 μm.

Figure 5. This median section through a sporangium illustrates an early stage of endospore development in *Pasteuria penetrans*. The electron-opaque body, which has formed within the forespore, is surrounded by membranes that will contribute to the multilayered wall of the mature endospore. Bar = 0.25 μm.

Figure 6. Scanning electron micrograph of endospores of *Pasteuria penetrans* attached to the cuticle along the lateral field of the larva of a root-knot nematode, *Meloidogyne incognita*. (A) Spore has retained its exosporium, resulting in the appearance of a crinkled or reticulated surface. (B) The exosporium has been sloughed, exposing the central dome of the endospore; the peripheral fibers can be distinguished. Bar = 0.5 μm.

Figure 7. Section through a sporangium of *Pasteuria penetrans* containing an almost developed endospore. The lateral regions (light areas marked by arrows) differentiate into parasporal fibers. Bar = 0.5 μm.

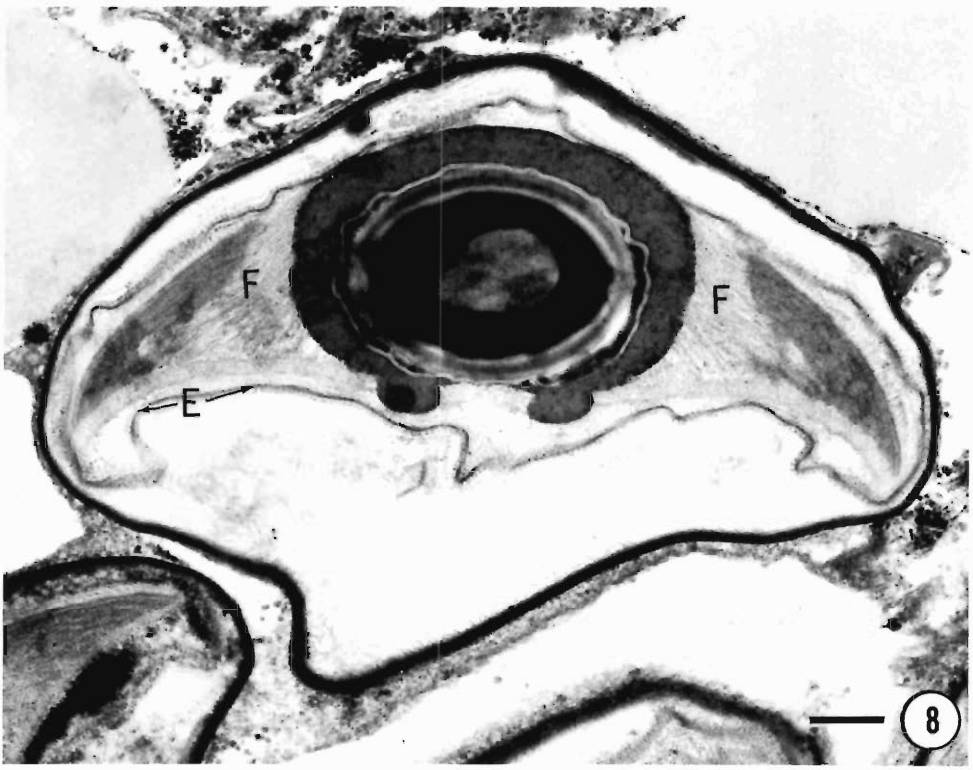


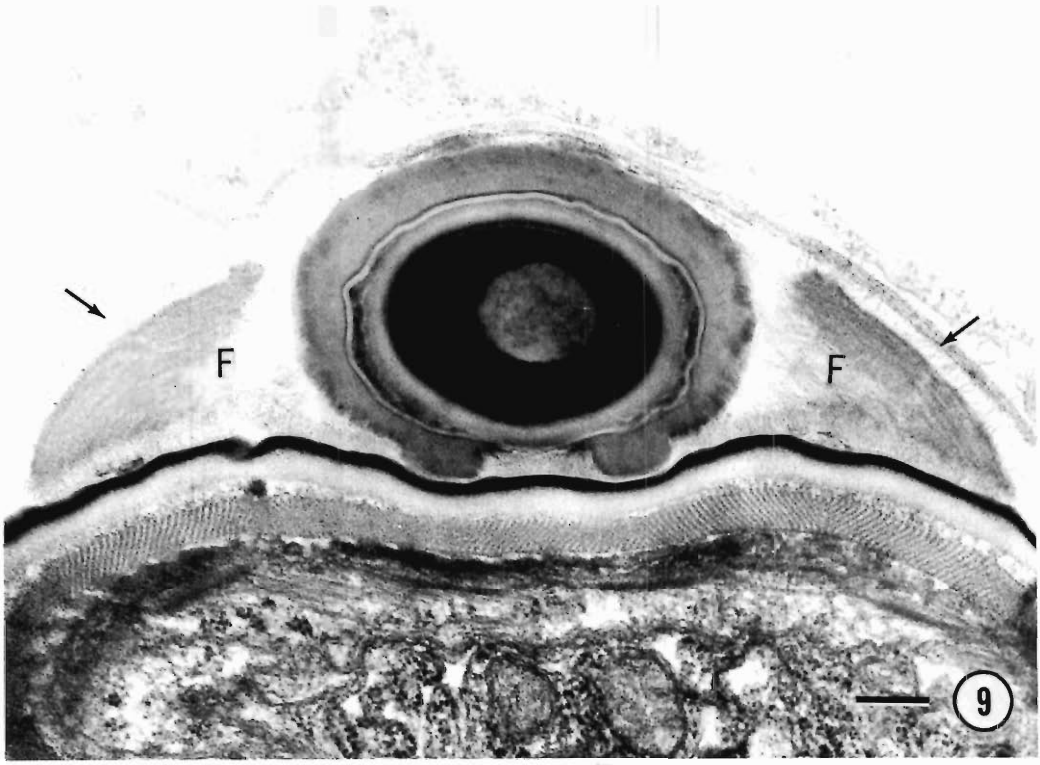
Figure 8. Median section through a sporangium of *Pasteuria penetrans* containing a fully developed endospore. The last stages of endospore differentiation include formation of an encircling membrane or exosporium (E) and emergence of parasporal fibers (F) within the granular material that lies laterally around the spore. Bar = 0.25 μm.

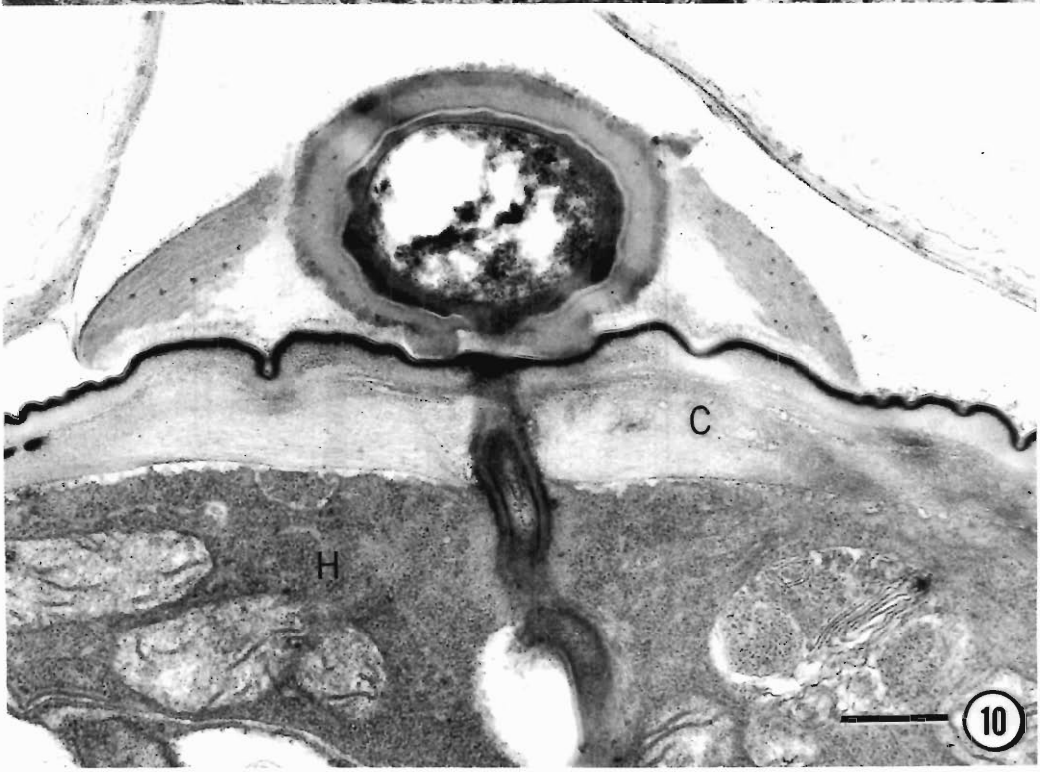
Pasteuria ramosa Metchnikoff, 1888, the type species of the genus *Pasteuria*. They share several distinctive morphological characteristics (Table 1), among which are the dichotomously branched mycelial microcolonies that give rise by fragmentation to sporangia arranged in quartets, in doublets, and eventually singly, and finally to endogenous spores formed within the old mother cell walls (Fig. 11B, C). Scanning and transmission electron microscopy reveal remarkable similarities at the ultrastructural level in the unique forms and sequences of life stages of the two organisms (Fig. 13).

Morphological characteristics of the genus *Bacillus* compared to "*Bacillus penetrans*"

The nematode parasite differs from the current concept of the genus *Bacillus* in many respects, particularly in cellular shape and size, motility, flagellation, sporangial shape and size, habitat, and nutritional requirements. The formation of a dichotomously branched mycelium by "*B. penetrans*" definitely excludes the nematode parasite from the family Bacillaceae Fischer, 1895 (we consider separately, below, its relationship to the mycelial actinomycetes). Most members of the genus *Bacillus* have flagella and are motile; by contrast, flagella and active motility have never been observed in "*B. penetrans*"; its microcolonies are carried passively by the currents of the hemolymph in the pseudocoelom of the nematode. *Bacillus* species are generally chemoheterotrophic and readily cultivated on laboratory media, whereas "*B. penetrans*" is a parasite that has resisted all efforts to culture it apart from the nematode host.

On the other hand, there is no doubt about the formation by the nematode parasite of endospores similar in origin, structure, and environmental resistance to the spores typical of mem-





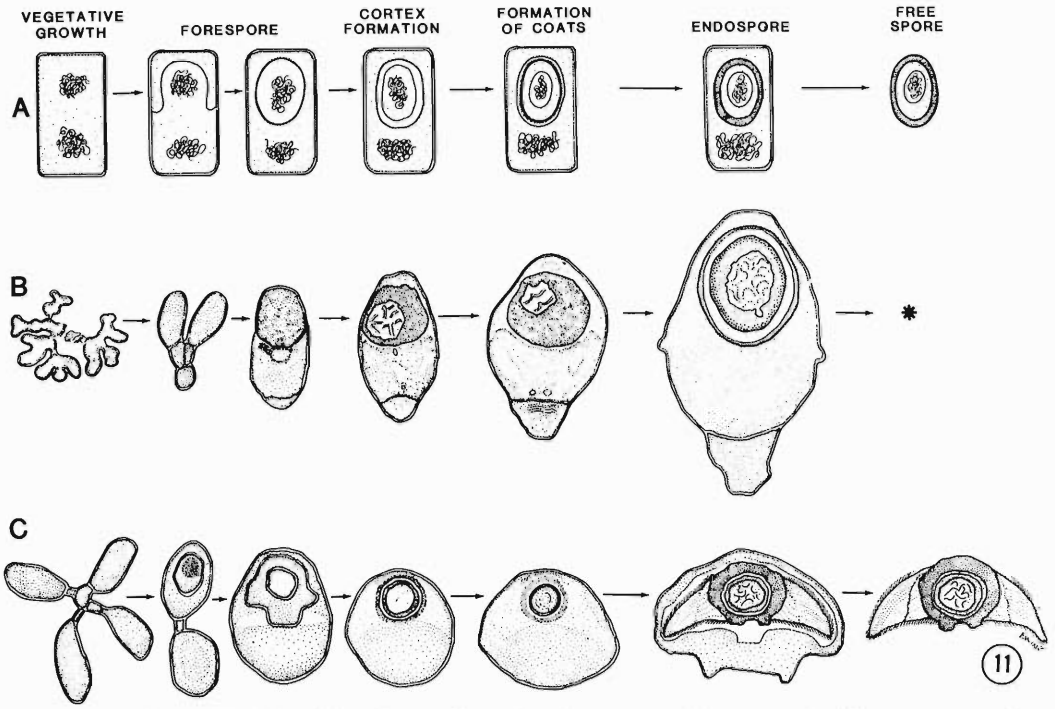


Figure 11. Generalized bacterial endospore formation (upper row; A) is compared with sporogenous stages of *Pasteuria ramosa* (middle row; B) and *Pasteuria penetrans* (lower row; C). Aside from the differences in parasporal structures, the stages of the two *Pasteuria* species result in spores having characteristics very similar to those of other endospore-forming bacteria. The asterisk (*) indicates that the free spore of that species has neither been observed, nor has its mode of penetrating and initiating infections in cladocerans been determined.

bers of the genus *Bacillus* (Fig. 11). The similarity extends to the following aspects: (i) septum formation in the anterior of the spore mother cell; (ii) condensation of a forespore from the anterior protoplast; (iii) formation of multilayered walls about the forespore; (iv) lysis of the old sporangial wall; and (v) release of an endospore that resists heat and desiccation and survives for long periods in storage. However, endospore formation is not an exclusive property of the Bacillaceae; it occurs also in the mycelial bacteria comprising the order Actinomycetales.

Affinities of the nematode parasite to the actinomycetes

Evidence has been presented (Cross, 1970; Cross and Unsworth, 1981) for true endospore formation in several genera of the Actinomycetales. "*Bacillus penetrans*" also has some characteristics consistent with placement in the Actinomycetales: (i) the Gram-positive staining characteristic; (ii) the slender vegetative cells, usually from 0.2 to 0.5 μm in diameter; (iii) the mycelial format, with terminal hyphae enlarging and then segmenting to yield club-shaped spo-

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Figure 9. Cross section through an endospore of *Pasteuria penetrans* on the surface of a nematode. Parasporal fibers (F) appear to radiate outward from the lower half of the spore to the cuticle of the nematode. Short "hairs" (arrows) project outward from the surface of the fibers to give the crinkled or reticulated surface appearance shown in Figure 6. Bar = 0.25 μm.

Figure 10. Cross section through a germinated spore of *Pasteuria penetrans*; the penetrating germ-tube follows a sinuous path as it traverses the cuticle (C) and hypodermis (H) of the nematode. Bar = 0.5 μm.

Table 1. Characteristics held in common by *Pasteuria ramosa* Metchnikoff, 1888 and *Pasteuria penetrans* (ex Thorne, 1940) nom. rev., comb. n., sp. n.

MORPHOLOGICAL SIMILARITIES AS OBSERVED BY LIGHT MICROSCOPY
Vegetative Cells
Dichotomously branched mycelium gives rise to microcolonies
Diameter of mycelial filaments similar (about 0.2–0.5 μm)
Mycelial filaments seen only in early stages in host tissues
Daughter microcolonies may be formed by lysis of intercalary "suicidal" cells, allowing mother colony to break internally
Nearly all vegetative cells lyse, leaving only sporangia
Stages of Sporogenesis
Only external or peripheral cells of the colony elongate and give rise to sporangia
A single spore produced in each sporangium
Spores are similar in size (about 4.0 μm)
Refractility of spores increases as spores mature
Staining Reaction
Gram-positive
ULTRASTRUCTURAL SIMILARITIES
Vegetative Cells
Mycelial cell walls are typical of Gram-positive bacteria
Mycelial filaments divided by septa
Double-layered cell walls
Mesosomes in both species are similar in appearance and seem to be associated with division and septum formation
Stages of Sporogenesis
Typical endogenous spore formation (Fig. 11)
Identical sequences of life stages in both organisms: (i) septa form within sporangia; (ii) sporangium cytoplast condenses to form forespore; (iii) spore walls form; (iv) final spore matures; and (v) light areas adjacent to spore give rise to extrasporal fibers
SIMILAR SEQUENCES OF LIFE STAGES (Fig. 13)
Microcolonies
Fragmentation of microcolonies
Quartets of sporangia
Doublets of sporangia
Single sporangia
Free endospores
HOST–BACTERIUM RELATIONSHIPS
Both parasitize invertebrates
Colonies first observed in the host are sedentary and located in the host's musculature
Growth in muscle tissue eventually leads to fragmentation and entry of microcolonies into the coelom or pseudocoelom of the respective host
Microcolonies carried passively by body fluids
Colonization of hemolymph or pseudocoelomic fluid by the parasite is extensive
Host ranges of the two bacteria are very narrow; moreover, *P. ramosa* occurs only in cladocerans and *P. penetrans* only in nematodes
Bacteria appear to develop in synchrony with their host which, although parasitized, continues its developmental cycle for some time
Host is completely utilized by the bacteria; in the end, the host becomes little more than a bag of bacterial endospores
SURVIVAL MECHANISMS
Survives in field soils and in muck soils at bottom of ponds
Resists desiccation
Moderately resistant to heating

rangia and eventually endospores; (iv) certain ultrastructural details, including the double-tracked membrane of the cell wall and hair-like projections of the outer membrane, are similar to those described by Slack and Gerencser (1975) in some species of actinomycetes; and (v) germination of the endospores of "*B. penetrans*" resembles the process found in the Actinomycetales (Fig. 12).

Although the aforementioned traits suggest that the genus *Pasteuria* (including the nematode parasite under consideration here) might possibly be placed in the Actinomycetales, further consideration of this point requires axenic cultures of these organisms and the usual phenotypic and genetic comparisons, one of our future research goals.

Table 2. Differences between *Pasteuria penetrans* (ex Thorne, 1940) nom. rev., comb. n., sp. n., and *Pasteuria ramosa* Metchnikoff, 1888.

Trait	*Pasteuria ramosa*	*Pasteuria penetrans*
Colony shape	Like cauliflower floret	Spherical, to cluster of elongated grapes
Sporangia		
Shape	Teardrop-shaped	Cup-shaped
Diameter (μm)	3.3–4.1	3.5–4.0
Height (μm)	4.8–5.7	2.2–2.9
Central spore, diameter (μm)	2.1–2.4	1.6–1.7
Host	Cladocerans	Nematodes
Location in host	Hemocoel and musculature; sometimes found attached to coelom walls	Pseudocoelom and musculature; no attachment observed
Attachment of spores on host	Spores not observed to attach or accumulate on surface of cladoceran	Spores accumulate in large numbers on cuticular surface
Mode of penetration of host	Not known; suspected to occur through gut wall	Direct penetration of nematode cuticle by hyphal strand
Source of host	Pond mud, freshwater	Soil, plants

Relationships of "*Bacillus penetrans*" with nematodes

Over the years, numerous reports (e.g., Kuiper, 1958; Esser and Sobers, 1964; Boosalis and Mankau, 1965; Williams, 1967; Prasad, 1971; Mankau and Prasad, 1977) have appeared listing—from various geographical localities in a score of countries—the nematodes parasitized by the organism under consideration here, usually under one of its earlier synonyms such as *Duboscqia penetrans* (Table 3). According to these reports, "*Bacillus penetrans*" parasitizes some 50 nematode species belonging to some 30 genera from widely separated families of the phylum Nematoda. These reports on the geographical occurrence of this bacterial parasite of nematodes suggest that any given isolate of "*B. penetrans*" might possibly be a nonspecific pathogen capable of parasitizing several nematode species at any one collection site. However, this is decidedly not the situation! For example, Dutky and Sayre (1978) have reported that the bacterial spores collected from *Pratylenchus brachyurus* attached to and parasitized only that nematode species and none of the other ten nematode species tested including *Pratylenchus penetrans*. Similarly, they found that spores of the bacterium from *Meloidogyne incognita* attached only to larvae of two additional species of root-knot nematodes but not to eight other nematode species, some of which had been reported as hosts of "*B. penetrans*". Mankau and Prasad (1977), working with "*B. penetrans*" material originating in a root-knot nematode population in California, reported that *Pratylenchus scribneri* and all tested species of *Meloidogyne* were infected, but not nematodes of several other *Pratylenchus* species or several plant-parasitic nematodes belonging to other genera. Prasad (1971), in a thorough review of reports on *Duboscqia penetrans* (="*Bacillus penetrans*"), concluded that this parasite was usually host specific. We agree with this assessment.

Considering this likely host specificity, together with the numerous reports of bacteria of this sort parasitizing several different species of nematodes, leads us to suggest that "*Bacillus penetrans*" is probably not a uniform species, but might be a complex of several taxa (at presently unknown categorial level) or pathotypes. There is already good evidence for at least two taxa, based on the two distinct ranges of endospore sizes discerned by Spaull (1981), who examined 13 species of parasitized nematodes and found that the sizes of the bacterial spore fell into two clusters with diameters of 2.9–4.4 μm and 4.3–6.6 μm. Arranging the reported sizes of spores as a function of the nematode host demonstrates interesting relationships (Table 4). As spore size increases, so does the size of the parasitized nematodes. Spore size also seems related to the feeding habits of the nematodes; the bacteria with the larger spore size are associated with the ectoparasitic nematodes, whereas those with the smaller spore size are associated with the en-

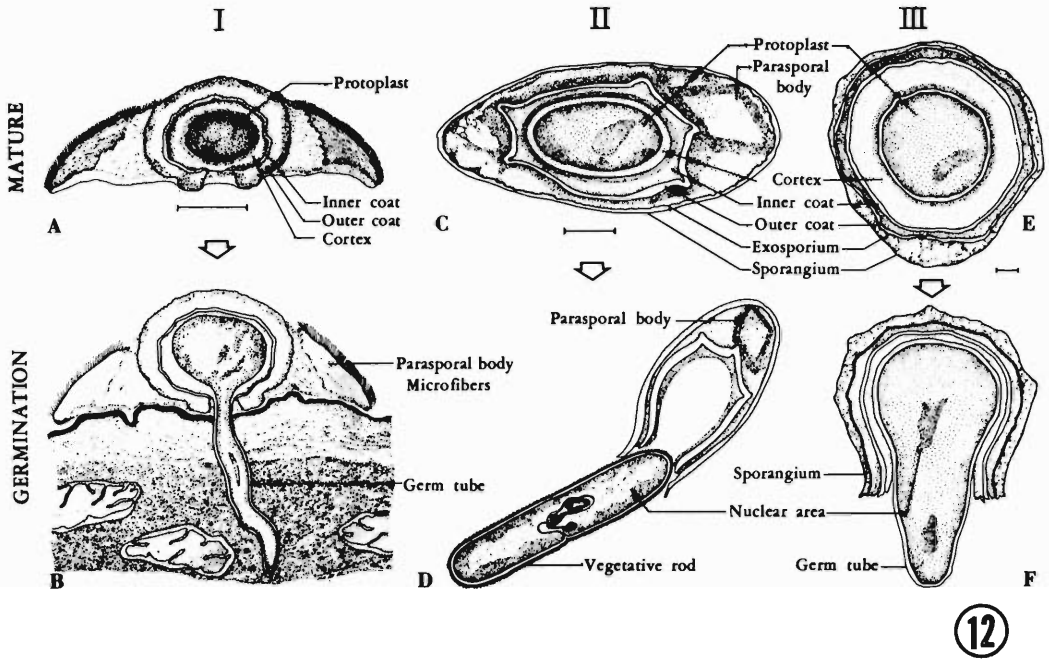

Figure 12. Comparison of the coat morphology and germination of endospores of *Pasteuria penetrans* (I; A, B), *Bacillus popillae* (II; C, D), and *Thermoactinomyces vulgaris* (III; E, F). Although *P. penetrans* (B) and *T. vulgaris* (F) differ in size, both form a filamentous tube on germination. *B. popillae* (D) germinates a vegetative rod. Bars = 1.0 μm (A, B, C, D) or 0.1 μm (E, F). After Sayre (1980).

doparasitic nematodes. The original description by Thorne (1940) dealt with *Duboscqia penetrans* on a *Pratylenchus* species and, because we intend the present taxonomic action to result in revival of Thorne's name, our description similarly deals with those bacteria having spores of the smaller diameter when attached to *Pratylenchus* or *Meloidogyne* species.

"*Bacillus penetrans*" has been reported to parasitize not only plant-parasitic nematodes but also free-living and/or predaceous nematodes belonging to several genera (Table 4). A report by Thorne (1927), read in the context of the belief held for some 70 years that this spore-forming bacterium was a sporozoan, deals with a "sporozoan" parasite of *Mononchus parabrachyurus* causing a decline in the field population of that predaceous nematode. Thorne theorized that the mononch had acquired the "sporozoan" by ingesting prey nematodes encumbered with spores or actively infected by the microorganism. In his drawing of an infected nematode, the parasite can be seen to differ from "*B. penetrans*" in that it lacks both the central refractile area and the cup-shaped profile. Thorne's critical attitude might profitably be extended to papers (e.g., Steiner, 1938) reporting the occurrence of "*B. penetrans*" in free-living nematodes. The short life cycle of only a few days and the four rapidly successive molts characteristic of most free-living nematodes would provide little opportunity for the bacterium to penetrate the nematodes through their cuticles, if penetration occurred in the same way as it does in the case of root-knot nematode infections. If something like "*Bacillus penetrans*" does parasitize predaceous or free-living nematodes, there is good reason to suspect these bacteria will be new species or pathotypes, because their modes of penetration and infection would necessarily differ from the typical model of "*B. penetrans*" parasitization.

Nomenclatural Formalities

As has been detailed in the foregoing, "*Bacillus penetrans*" differs in important respects from members of the genus *Bacillus*. It does share many

Table 3. Geographical and host ranges of *Pasteuria penetrans* (ex Thorne, 1940) nom. rev., comb. n., sp. n.*

Location	Nematode	Reference
United States		
California	*Dolichodorus obtusus, Meloidogyne hapla, Meloidogyne javanica, Meloidogyne incognita, Pratylenchus scribneri,* and *Hoplolaimus* sp.	Allen (1957), Boosalis and Mankau (1965), and Prasad and Mankau (1969)
Colorado	*Tylenchorhynchus* sp.	Prasad and Mankau (1969)
Florida	*Belonolaimus gracilis, Helicotylenchus microlobus, Hoplolaimus* sp., and *Pratylenchus* sp.	Esser and Sobers (1964)
Georgia	*Pratylenchus brachyurus*	Thorne (1940)
Hawaii	*Discolaimus bulbiferus*	Cobb (1906)
Illinois	*Pratylenchus* sp.	Boosalis and Mankau (1965)
Maryland	*Meloidogyne hapla, Meloidogyne incognita,* and *Pratylenchus brachyurus*	Dutky and Sayre (1978), and Sayre and Wergin (1977)
Oregon	*Pratylenchus* sp.	Prasad (1971)
South Carolina	*Pratylenchus brachyurus*	Thorne (1940)
South Dakota	*Aporcelaimus eurydorus, Laimydorus reversus, Nygolaimus parabrachyurus, Tylenchorhynchus nudus,* and *Xiphinema chambersi*	Thorne and Malek (1968), and Thorne (1974)
Utah	*Megadorus megadorus*	Allen (1941)
Australia	*Meloidogyne javanica*	Stirling and White (1982)
Belgium	*Tylenchorhynchus dubius* and *Tylenchorhynchus nanus*	Coomans (1962)
Ceylon	*Xiphinema americana*	Prasad (1971)
Congo	*Discolaimus* sp. and *Xiphinema* sp.	DeConinck (1962)
Germany	*Radopholus similis*	Thorne (1961)
India	*Hoplolaimus indicus, Meloidogyne javanica,* and *Paralongidorus sali*	Boosalis and Mankau (1965), and Siddiqi et al. (1963)
Italy	*Dorylaimellus virginianus, Dorylaimus* sp., and *Rotylenchus robustus*	Altherr (1954)
Japan	*Meloidogyne javanica*	Allen (1941)
Mauritius	*Meloidogyne incognita, Meloidogyne javanica,* and *Xiphinema elongatum*	Williams (1960, 1967)
Netherlands	*Hoplolaimus uniformis, Meloidogyne arenaria, Pratylenchus penetrans, Pratylenchus pratense, Rotylenchus robustus,* and *Tylenchorhynchus dubius*	Kuiper (1958)
Nigeria	*Helicotylenchus* sp., *Isolaimium nigeriense,* and *Scutellonema* sp.	Prasad (1971), and Timm (1969)
Scotland	*Eudorylaimus* sp., *Mononchus papillatus,* and *Tylenchorhynchus dubius*	Prasad (1971)
South Africa	*Discocriconemella mauritiensis, Helicotylenchus dihystera, Helicotylenchus krugeri, Histotylenchus histoides, Meloidogyne incognita, Pratylenchus zeae, Rotylenchus incultus, Rotylenchus unisexus, Scutellonema brachyurum, Scutellonema truncatum, Tylenchulus* sp., *Xiphinema elongatum,* and *Xiphinema* cf. *imitator*	Spaull (1981)
Sweden	*Ironus ignavus*	Allgen (1925)
Uganda	*Mumtazium mumtazae*	Siddiqi (1969)
Venezuela	*Eudorylaimus morbidus*	Loof (1964)

* As related in the text, *Pasteuria penetrans* was referred to in the earliest literature as a "sporozoan" belonging to the genus *Duboscqia*; in later literature, the name "*Bacillus penetrans*" was sometimes used.

Table 4. Variation in size of *Pasteuria penetrans* spores occurring within nematodes or adhering to their cuticles.*

Nematode host	Nematode size (μm)		Method of feeding†	Spore diam. (μm)	Reference
	Length	Diameter			
Pratylenchus brachyurus	450–750	23	ME	3.6	Dutky and Sayre (1978)
Pratylenchus zeae	360–580	14	ME	3.6‡	Spaull (1981)
Meloidogyne hapla	395–466	14	SE	3.7	Prasad (1971)
Pratylenchus sp.	—	—	ME	3.75	Prasad (1971)
Meloidogyne incognita	360–393	12	SE	3.8	Sayre and Wergin (1977)
Tylenchus sp.	—	—	ME	3.9	Spaull (1981)
Discocriconemella mauritiensis	—	—	EC	3.9	Spaull (1981)
Helicotylenchus dihystera	550–640	26	EC	3.9‡	Spaull (1981)
Meloidogyne incognita	360–393	12	SE	4.1‡	Spaull (1981)
Meloidogyne incognita	360–393	12	SE	4.27	Prasad (1971)
Meloidogyne javanica	340–400	14	SE	4.30	Prasad (1971)
Xiphinema elongata	2,090	43	EC	4.5‡	Williams (1967)
Mumtazium mumtazae	670–880	24	PRED	4.55	Prasad (1971)
Helicotylenchus krugeri	500–700	25	EC	4.7‡	Spaull (1981)
Rotylenchus unisexus	700–1,040	32	EC	4.8‡	Spaull (1981)
Scutellonema truncatum	610–750	27	EC	4.9‡	Spaull (1981)
Dolichodorus obtusus	1,900–2,700	57	EC	4.95	Prasad (1971)
Meloidogyne javanica	340–400	14	ES	5.0	Williams (1960)
Meloidogyne incognita	360–393	12	ES	5.0	Williams (1960)
Histotylenchus histoides	1,080–1,180	28	EC	5.1‡	Spaull (1981)
Scutellonema brachyurum	720–890	36	EC	5.2‡	Spaull (1981)
Isolaimium nigeriense	2,730–3,530	62	FL	5.3	Prasad (1971)
Xiphinema americanum	1,500–2,000	38	EC	5.3	Prasad (1971)
Scutellonema bradys	950–1,190	43	ME	5.5	Prasad (1971)
Hoplolaimus indicus	950–1,400	44	EC	5.65	Prasad (1971)
Xiphinema elongata	2,090	43	EC	5.7‡	Spaull (1981)
Xiphinema cf. *imitator*	2,220	39	EC	6.0‡	Spaull (1981)
Belonolaimus gracilis	2,150	43	EC	6.2	Prasad (1971)
Rotylenchus incultus	710–840	26	EC	6.2‡	Spaull (1981)
Xiphinema bakeri	4,050	63	EC	6.55	Prasad (1971)
Longidorus sp.	—	—	EC	6.55	Prasad (1971)
Paralongidorus sali	2,250–2,850	41	EC	7.0	Siddiqi et al. (1963)
Xiphinema sp.	—	—	EC	7.0	DeConinck (1962)
Discolaimus sp.	—	—	PRED	7.0	DeConinck (1962)

* Assembled from lists of Kuiper (1958), Williams (1967), Prasad (1971), Dutky and Sayre (1978), and Spaull (1981), with additions and corrections.

† ME = migratory endoparasite; SE = sedentary endoparasite; EC = ectoparasite; PRED = predator; and FL = free-living.

‡ Approximate mean of several determinations; variance usually was not over 0.5 μm.

morphological, ultrastructural, and ecological features with the type species of the genus *Pasteuria, Pasteuria ramosa* Metchnikoff, 1888 (Sayre and Wergin, 1977; Sayre et al., 1977, 1979, 1983; Table 1; Figs. 12, 13): Both are Gram-positive; both form a dichotomously branched, septate vegetative mycelium; both form endospores; neither has been cultivated axenically; both parasitize invertebrates. Despite the similarities, the two organisms are distinguishable on a number of grounds, including sporangial shape, host preferences, modes of host penetration, and sources (Table 2).

Because "*Bacillus penetrans*" was not included in the 1980 "Approved Lists of Bacterial Names" (Skerman et al., 1980), it presently has no taxonomic standing (Lapage et al., 1975). We now assign this organism to *Pasteuria penetrans* (ex Thorne, 1940) nom. rev., comb. n., sp. n. Under the "Code" (Lapage et al., 1975), "ex" indicates our belief that we are dealing here with Thorne's organism, "nom. rev." shows that we are reviv-

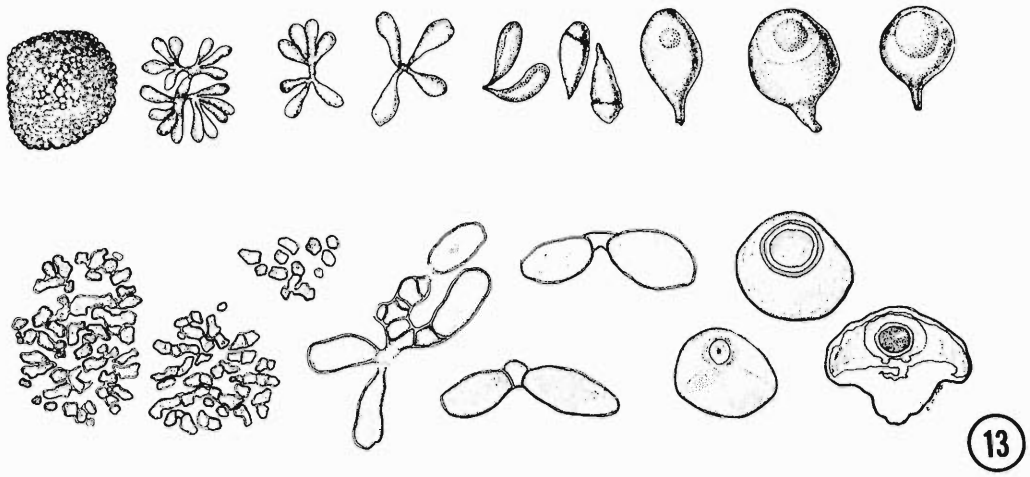

Figure 13. Drawings of the life stages of *Pasteuria penetrans* (bottom row) based on electron micrographs are compared with those of *Pasteuria ramosa* (top row) as drawn by Metchnikoff (1888). Starting at the far left of the bottom row, a vegetative colony of *P. penetrans* is followed by daughter colonies, quartets of sporangia, doublets of sporangia, single sporangia, and finally (at the far right) the mature endospore within the old sporangial wall. The corresponding drawings of *P. ramosa* in the top row are placed in order of their occurrence in the life cycle of the parasite as reported by Metchnikoff (1888).

ing a lapsed bacterial name, "comb. n." refers to placement of Thorne's species in a new genus, and "sp. n." indicates that it is a new species as mandated by the revised bacteriological nomenclatural system in effect since January 1, 1980. The formal description of *Pasteuria penetrans* (ex Thorne, 1940) nom. rev., comb. n., sp. n., and an emended description of the genus *Pasteuria* Metchnikoff, 1888 follow.

Genus *Pasteuria* (Metchnikoff, 1888) emend. mut. char.

Genus *Pasteuria* Metchnikoff, 1888, 166[AL], emend. mut. char. pas·teu'ri·a. M. L. gen. n. *Pasteuria* of Pasteur; named for Louis Pasteur, French savant and scientist. Synonyms: Not *Pasteuria* sensu Staley, 1973.

Gram-positive, dichotomously branching, septate mycelium, the terminal hyphae of which enlarge to form sporangia and eventually endospores. Maturing colonies are shaped like cauliflower florets or elongated grapes in clusters; daughter colonies are formed by fragmentation. The sporogenous cells at the periphery of the colonies are usually attached by narrow hyphae that lyse, causing arrangement of the developing sporangia in quartets, then in doublets, and finally as single, mature, teardrop- to cup-shaped sporangia. The rounded end of the sporangium encloses a single refractile endospore, 1.5–2.5 μm in diameter, slightly oval to spherical in shape, resistant to desiccation and elevated temperatures (one species has only limited heat tolerance). Nonmotile. Sporangia and microcolonies are parasitic in the bodies of freshwater, plant, and soil invertebrates. Has not been cultivated axenically, but can be grown in the laboratory with the invertebrate host.

TYPE SPECIES: *Pasteuria ramosa* Metchnikoff, 1888. Not *Pasteuria ramosa* Staley, 1973, a quite different bacterium (Gram-negative, nonmycelial, not endospore-forming, budding, nonprosthecately appendaged, not endoparasitic in cladocerans) belonging to the *Blastocaulis–Planctomyces* group (Starr et al., 1983). Modern descriptions of *P. ramosa* Metchnikoff, 1888 can be found in papers by Sayre et al. (1979, 1983) and Starr et al. (1983).

Pasteuria penetrans (ex Thorne, 1940) nom. rev., comb. n., sp. n.

Pasteuria penetrans (ex Thorne, 1940) nom. rev., comb. n., sp. n. pen'·e·trans. L. v. *penetro,* present participle *penetrans* to enter. Synonyms: "*Duboscqia penetrans*" Thorne, 1940; "*Bacillus penetrans*" (Thorne, 1940) Mankau, 1975.

Gram-positive vegetative cells. Mycelium is septate; hyphal strands, 0.2–0.5 μm in diameter,

branch dichotomously. Sporangia, formed by expansion of the hyphal tips, are pustule-like, about 1.6–2.5 μm by 3.7–4.3 μm, each divided into two unequal sections. The smaller proximal body is not as refractile as the larger, rounded, cup-shaped portion that encloses an endospore, 1.6–2.5 μm in diameter. Endospores seem to be of the kind typical of the genus *Bacillus*; they are resistant to both heat and desiccation. Sporangia and vegetative cells are found as parasites in pseudocoeloms of several species of plant-parasitic nematodes. Has not been cultivated axenically. Type descriptive material consists of the descriptions and illustrations in this paper and elsewhere (Sayre et al., 1979, 1983).

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Calliobothrium evani sp. n. (Tetraphyllidea: Onchobothriidae) from the Gulf of California, with a Redescription of the Hooks of *C. lintoni* and a Proposal for Onchobothriid Hook Terminology

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ABSTRACT: *Calliobothrium evani* sp. n. is described from the spiral valve of an unidentified carcharhinid shark from the Gulf of California. This species is most like *C. lintoni* Euzet, 1954, in that these two are the only species of *Calliobothrium* van Beneden, 1850 with an accessory piece between the axial hook bases. Unlike *C. lintoni,* the accessory piece is oval and broader posteriorly than anteriorly, the axial hooks are dissimilar, and the articular surfaces differ in position and shape. *Calliobothrium evani* also has a larger scolex (approximately twice as large), a more anterior genital pore, and a greater mean number of testes (46 as compared to 34). The hooks of *C. lintoni* are redescribed to include several features omitted from the original description. To facilitate the description of dissimilar hooks a revised hook terminology, applicable to all onchobothriids, is proposed.

A new species of *Calliobothrium* van Beneden, 1850 was collected from the spiral valves of two sharks caught in the Gulf of California near Puertecitos, México. To describe the dissimilar hooks of this species and to compare them with other species of *Calliobothrium,* onchobothriid hook terminology is revised and the hooks of *C. lintoni* Euzet, 1954 are redescribed.

Materials and Methods

The hosts were caught in the Gulf of California and purchased from fishermen at the shore in Puertecitos, México, in March 1982. Photographs of the hosts were taken for further identification, but the negatives were lost during processing. Four worms were collected alive from the spiral valves of two individual sharks and placed in AFA immediately after recovery. In the laboratory they were hydrated in a series of alcohols, stained with Ehrlich's hematoxylin, dehydrated in a series of alcohols, cleared with methyl benzoate and mounted in Canada balsam. Measurements are in micrometers unless otherwise stated, and ranges are given in the text. For each character measured Table 1 provides the mean, the standard deviation, the number of worms examined, and the total number of observations when more than one proglottid or structure per worm was examined. Illustrations were made with the aid of a drawing tube. One specimen, after being mounted in Canada balsam, was unmounted and soaked in xylene followed by several changes of 100% ethanol. It was subsequently prepared for scanning electron microscopy as given in Caira (1985). Measurements of bothridial spines were taken from scanning electron micrographs.

Terminology

In the onchobothriids there are either one or two pairs of bothridial hooks. If there is one pair, the hooks may be either single pronged or multipronged. If there are two pairs, the hooks are single pronged.

The simplest case is one pair of single-pronged hooks per bothridium such as those found in *Potamotrygonocestus* Brooks and Thorson, 1976; *Pachybothrium* Baer and Euzet, 1962; and some species of *Onchobothrium* Blainville, 1828. The hooks of such a pair have been referred to as *outer* and *inner* (Brooks et al., 1981 for *Potamotrygonocestus orinocoensis* Brooks, Mayes, and Thorson, 1981). In *Acanthobothroides* Brooks, 1977 there is one pair of hooks composed of one simple hook and one bipronged hook. Brooks (1977) referred to the hooks of the pair as *inner* and *outer* and also referred to the prongs of the bipronged hook as *inner* and *outer.* A single pair of bipronged hooks per bothridium is found in *Acanthobothrium* van Beneden, 1849; *Pedibothrium* Linton, 1909; *Platybothrium* Linton, 1890; *Uncibilocularis* Southwell, 1925; and some species of *Onchobothrium.* The hooks of a pair have been referred to as *inner* and *outer* (Yamaguti, 1952 for *Platybothrium auriculatum* Yamaguti, 1952); *proximal* and *distal* (Euzet, 1959 for *Platybothrium* spp.); or *larger* and *smaller* (Southwell, 1925 for *Onchobothrium farmeri* (Southwell, 1911) Southwell, 1925). The prongs of the hooks have been referred to as *inner* and *outer* (for example: Yamaguti, 1952; Goldstein, 1964; Campbell, 1969; Williams, 1969, for *Acanthobothrium* spp.; Linton, 1909 for *Pedibothrium* spp.); *small* and *large* (Southwell, 1925 for *Pedibothrium globicephalum* Linton, 1909); or *axial* and *lateral* (Euzet, 1959 for *Acanthobothrium* spp.). A single pair of three-pronged hooks per bothridium exists in *Phoreiobothrium*

Linton, 1889. In this case authors have referred to the prongs as *inner, middle,* and *outer* (Yamaguti, 1954; Campbell, 1975). Euzet (1959) referred to the hooks of a pair in this genus as *distal* and *proximal.*

In the case of two pairs of hooks per bothridium, as found in *Calliobothrium,* the hooks have been referred to as *inner* and *outer* (Yamaguti, 1952 for *C. verticillatum* (Rudolphi, 1819) van Beneden, 1850); *short* and *long* (Southwell, 1925 for *C. leuckartii* van Beneden, 1850); or *axial* and *lateral* (Euzet, 1959). No terminology has been used to distinguish between the hooks of a pair (for example between the two axial hooks) in this genus.

A consistent terminology is needed, particularly for describing onchobothriids with dissimilar hooks in a pair, because differences in size or shape may be of significant systematic value. It is critical in such cases that unambiguous reference to a particular hook be possible. The following terminology is proposed to permit consistent reference to an individual prong or hook for any onchobothriid. This terminology is based on the position of the hook or prong with reference to the axes of the bothridium and scolex.

Each bothridium can be divided into an axial area (along the axis of the bothridium) and two abaxial areas (away from the axis of the bothridium) (Fig. 1). In onchobothriids with two pairs of bothridial hooks such as *Calliobothrium,* the pair of hooks associated with the axis of the bothridium are the axial hooks, and the pair of hooks associated with the abaxial portions of the bothridium are the abaxial hooks (Fig. 1). This system retains the use of the term *axial* (Euzet, 1959) for those hooks associated with the axial portions of the bothridium, and replaces the term *lateral* (Euzet, 1959) with *abaxial* for hooks associated with the abaxial portions of the bothridium. The term *lateral* is reserved for reference to the entire scolex rather than for each bothridium.

The scolex can be divided into one medial area (along the midline of the scolex) and two lateral areas (away from the midline of the scolex) (Fig. 1). Thus, in members of *Calliobothrium,* the axial and abaxial hooks of each bothridium associated with the medial area of the scolex are termed the *medial axial* and *medial abaxial* hooks (collectively, the *medial* hooks), and the axial and abaxial hooks associated with each of the lateral areas of the scolex are termed the *lateral axial* and *lateral abaxial* hooks (collectively,

Table 1. Morphological characters of *Calliobothrium evani* sp. n.

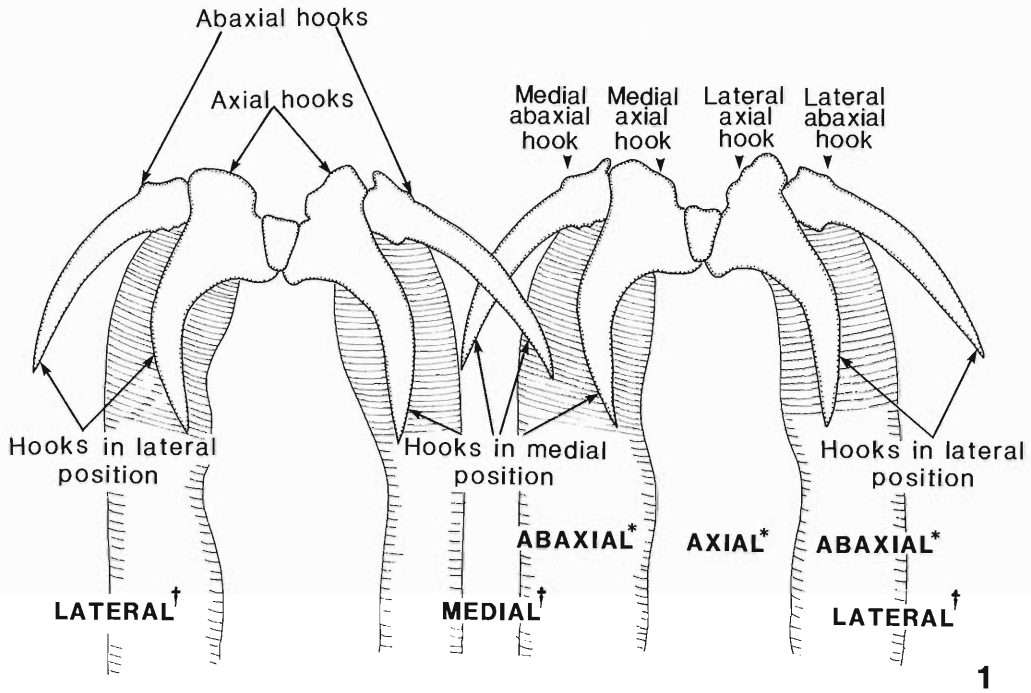
Character	$\bar{x}$	SD	N*	n†
Body:				
Length	10.7 mm	3.2 mm	4	4
Greatest width	770	120	3	3
No. of segments	22	4.9	4	4
Scolex:				
Length	836	49	4	4
Bothridium:				
Length	828	45	4	5
Width	289	43	4	7
Anterior loculus length	265	18.5	3	6
Second loculus length	144	2.5	3	6
Third loculus length	239	42	3	6
Spine length	3.25	0.27	1	10
Lateral axial hooks:				
A	137	18.3	4	11
B	190	11.5	4	12
C	128	5.5	3	10
F	95	6	4	11
Medial axial hooks:				
A′	144	11.3	4	11
B′	186	13	3	10
C′	78	6.5	3	7
F′	57	4.4	4	9
Accessory piece:				
Length	44	4.2	4	11
Width	23	1.7	3	10
Abaxial hooks:				
D,D′	190	12	4	22
E,E′	190	13.8	4	20
Hook tubercle:				
Length	46	3.2	3	11
Posterior proglottid:				
Length	1.29 mm	0.26	3	3
Width	468	87	4	4
No. of testes	46	5.1	3	13
Cirrus pouch:				
Length	247	34	2	4
Width	174	20	2	4
Position of genital pore from posterior end	44%	1.9%	4	11
Length of terminal dilation of cirrus	55	8.7	2	3
Length of vaginal sphincter	61	7.6	2	3

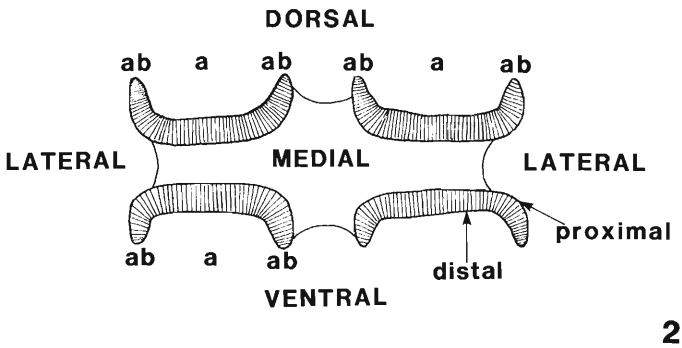
All measurements are in micrometers unless otherwise indicated.

* Number of worms examined.

† Total number of observations (greater than *N* when more than one structure or proglottid per worm was examined).

Letters for hook measurements refer to Figure 6.





Figures 1, 2. Schematic diagrams. 1. Dorsal or ventral view of hooks *in situ* on scolex of *Calliobothrium lintoni* Euzet, 1954 indicating bothridial (*) and scolex (†) axes. 2. Cross section through scolex; a, axial; ab, abaxial.

the *lateral* hooks) (Fig. 1). These terms have been established on a two bothridium basis as, although there are four bothridia per scolex, these bothridia tend to flatten with two positioned dorsally and two positioned ventrally; the terms given above pertain equally to the dorsal and ventral pairs of bothridia (Fig. 2).

For those onchobothriids possessing a pair of bipronged hooks, per bothridium, the hooks of a pair can be identified as medial and lateral, and the prongs of each hook as axial and abaxial. Because a description of the complex hooks of *Phoreiobothrium* involves homology as well as terminology, hook terminology for this genus is discussed in Caira (1985), but is consistent with the above system.

In *Acanthobothroides,* owing to the way the bothridia flatten, the simple hook of the pair is the medial hook, and the bipronged hook is the lateral hook. The prongs of the bipronged hook are axial and abaxial. For those species of onchobothriids with only one pair of simple hooks

per bothridium, such as *Potamotrygonocestus,* the terms axial and abaxial are not necessary; instead the hooks of a pair can be identified on an entire scolex basis as medial and lateral.

Calliobothrium evani sp. n. (Figs. 3-9)

DESCRIPTION (based on 4 specimens): Worms 7.25–14.5 mm long; greatest width 670–900 at anterior end of scolex; 18–29 proglottids per worm; apolytic. Scolex (Fig. 3) 775–880 long, carried on neck; surface of neck and proximal surfaces of bothridia densely covered with fine spines, 2.8–3.7 long, directed posteriorly. Bothridia four, 775–872 long by 220–343 wide; each with three loculi and one inconspicuous apical sucker. Anterior loculus 240–292 long; second loculus 136–144 long; third loculus 192–300 long.

Hooks (Figs. 4, 5) of each bothridium covered with thin layer of tissue. Axial hooks recurved. Lateral axial hooks: distance from point of hook to axial posterior extremity of base (A in Fig. 6) 112–172; distance from point to anterior abaxial extremity of base (B in Fig. 6) 164–204; distance from anterior abaxial extremity of base to posterior axial extremity of base (C in Fig. 6) 120–136; base 88–104 long (F in Fig. 6). Medial axial hooks: distance from point of hook to axial posterior extremity of base (A′ in Fig. 6) 128–164; distance from point to anterior abaxial extremity of base (B′ in Fig. 6) 164–204; distance from anterior abaxial extremity of base to posterior axial extremity (C′ in Fig. 6) 68–88; base 48–64 long (F′ in Fig. 6). Extended bases of axial hooks articulate with, and extend underneath oval accessory piece 40–52 long by 20–25 at widest point (near center) (Fig. 9c). Lateral and medial abaxial hooks of equal curvature and size; 164–208 from point of hook to rounded tubercle (D and D′ in Fig. 6) and 160–220 from point to notched axial extremities (E and E′ in Fig. 6). Notched axial extremities of abaxial hooks articulate with axial hooks on triangular tubercle on proximal side of axial hook base (Fig. 4). Tubercle 40–52 long. Abaxial hooks with rounded tubercle on distal side (Fig. 9a) articulating with anterior notch of axial hooks (Fig. 4). All hooks hollow with internal channel, granular in appearance, opening to outside via pore. In axial hooks channel extending from point of hook past pore into base farther in lateral axial hooks (Fig. 9b) than in medial axial hooks (Fig. 9d). In axial hooks channel opening to outside via pore at center of anterior portion of triangular tubercle. Abaxial hook channels extending from point of hook to anterior portion of hook, opening via pore at axial point of base of rounded tubercle (Fig. 9a). Accessory piece solid.

Proglottids acraspedote. Immature proglottids wider than long, mature proglottids longer than wide. Posterior proglottid mature, 1.08–1.59 mm long by 405–596 wide. Testes 35–53 in number, situated centrally between two lateral fields of ovary and vitellaria. Vas deferens coiled, median, preequatorial in second quarter of proglottid, entering base of cirrus pouch. Cirrus sac (Fig. 7) pyriform, 200–280 long by 148–188 at widest point; containing: coiled cirrus with terminal dilation 50–65 long, lined with spines 2–4 long; entire cirrus up to dilation enveloped in relatively thick cellular layer.

Ovary bilobed, at extreme posterior end of proglottid, poral lobe extending almost to cirrus sac, aporal lobe somewhat shorter. Vagina expanded at base, extending anteriorly to level of cirrus pouch, then laterally along anterior margin of cirrus pouch; small dilation lined with blunt protrusions 2–3 long at base of vaginal sphincter; sphincter 52–66 long, joining genital atrium (Fig. 7). Genital pore lateral 40–47% of proglottid length from posterior end (Fig. 8); pores alternating irregularly. Uterus extending anteriorly along median line from ovarian bridge to anterior third of proglottid; uterine pore not seen. Vitellaria indistinct, granular, extending laterally in narrow band from level of cirrus sac to anterior end of proglottid, somewhat more extensive on aporal side.

HOST: An unidentified shark of the family Carcharhinidae.

SITE OF INFECTION: Spiral valve.

LOCALITY: Gulf of California, vicinity of Puertecitos, Baja California Norte, México.

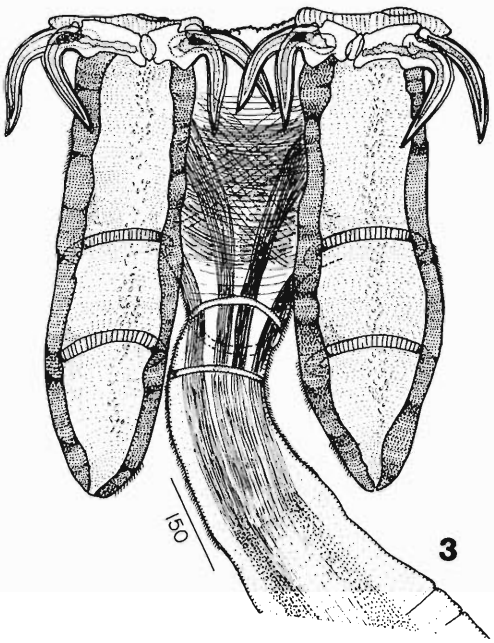
HOLOTYPE: USNM Helm. Coll. No. 78600.

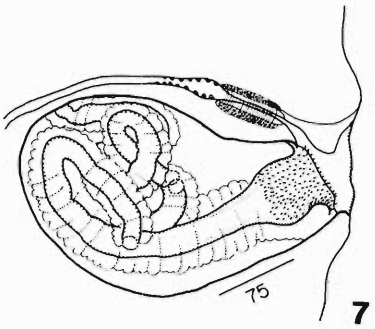
PARATYPES: USNM Helm. Coll. No. 78601, Univ. Neb. State Mus. HWML No. 22536.

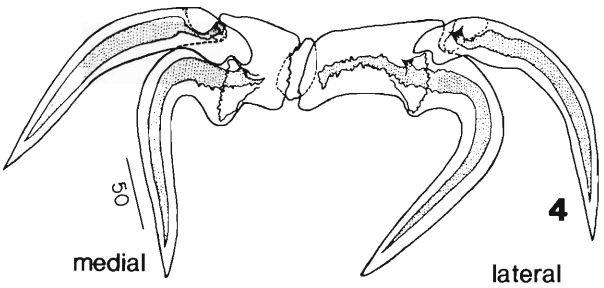
ETYMOLOGY: This species is named in honor of Dr. Evan C. Jolitz whose assistance and encouragement made the Mexican trip possible.

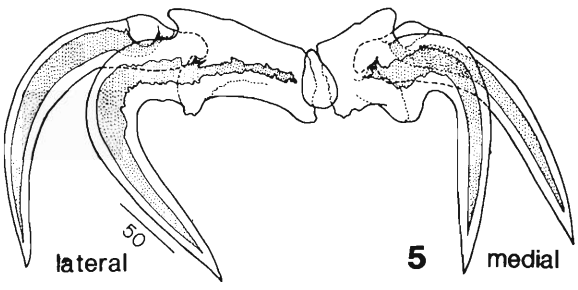
The Hooks of *Calliobothrium lintoni*

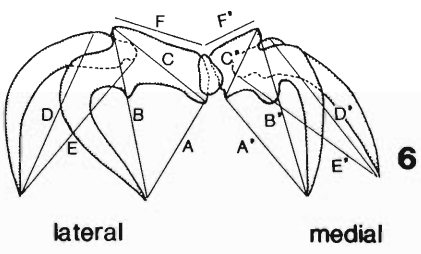
Calliobothrium evani most closely resembles *C. lintoni,* the type material of which was collected by Euzet from *Mustelus laevis* Risso at Sète, France. However, before a comparison of these two species is made, the hooks of *C. lintoni* are redescribed to include several features omitted from the original description. The author was

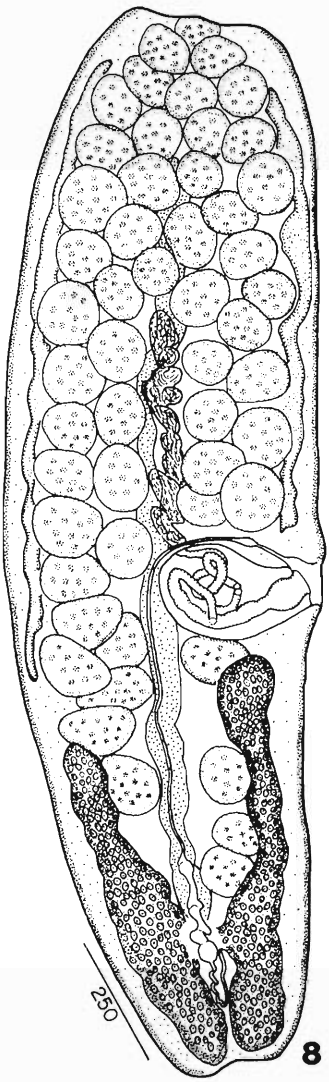












unsuccessful in obtaining the type material of *C. lintoni* from Professor Euzet; however, the following material was available for examination: five voucher specimens collected from *Mustelus canis* (Mitchill) at Wood's Hole, Massachusetts (HWML No. 20933 and USNM Helm. Coll. No. 34831); two voucher specimens collected from *M. canis* at Wood's Hole and identified by Linton (1924) as *Calliobothrium eschrichtii* van Beneden, 1850 (the same specimens that Euzet later [1954] recognized as a synonym of *C. lintoni*) (USNM Helm. Coll. No. 7680); the holotype specimen of *C. pellucidum* Riser, 1955 collected from *M. californicus* Gill in southern California (USNM Helm. Coll. No. 37419); and 15 specimens collected by the author from *M. henlei* Gill near Puertecitos, México (HWML No. 22537).

Euzet (1954, p. 76), at the time of his description of *C. lintoni,* examined his own type material from *M. laevis* in the Mediterranean in conjunction with Linton's specimens from Wood's Hole and wrote:

> . . . elle est identique au parasite de *Mustelus canis* et de *Galeorhinus* (*Mustelus*) *laevis* décrit sous le nom de *C. eschrichtii* par Linton en 1890 et 1924. Nous avons pu nous en assurer en examinant la préparation originale de Linton (U.S.N.M., Helm. Coll. No. 7680).

In addition Euzet (1959) considered *C. pellucidum* from *M. californicus* from the coast of southern California to be a synonym of *C. lintoni.*

Although there is a general similarity in overall morphology, several differences were noted between the specimens collected from Wood's Hole and those collected from California and Mexico. The latter specimens, in general, have smaller hooks and a proportionately smaller accessory piece: for most hook measurements the specimens from Wood's Hole occupy the upper end of the given size range and the specimens from Mexico and California occupy the lower end of the range. In addition, the axial hooks of the specimens from California and Mexico are slightly more recurved than those of the Wood's Hole specimens.

Table 2. Hook measurements of *Calliobothrium lintoni* Euzet, 1954 taken from voucher specimens.*

Character	$\bar{x}$	SD	N‡
Axial hooks:			
A,A′	96	31.3	23
B,B′	144	39.7	22
C,C′	71	20.4	22
Accessory piece:			
Length	25	10.2	23
Width	21	4.9	23
Abaxial hooks:			
D,D′	128	26.6	20
E,E′	127	30	23
Hook tubercle:			
Length	45	15.1	19

All measurements are in micrometers unless otherwise indicated.

* See text for details on specimens.

‡ Number of worms examined.

Letters refer to Figure 11.

Until the type material of *C. lintoni* can be reexamined and, despite the above-mentioned differences, the identity of specimens from Wood's Hole, California, and Mexico is accepted, and information from specimens from all three localities is used to supplement the original description of the hooks. The differences in hook morphology are perhaps attributable to interpopulation variability.

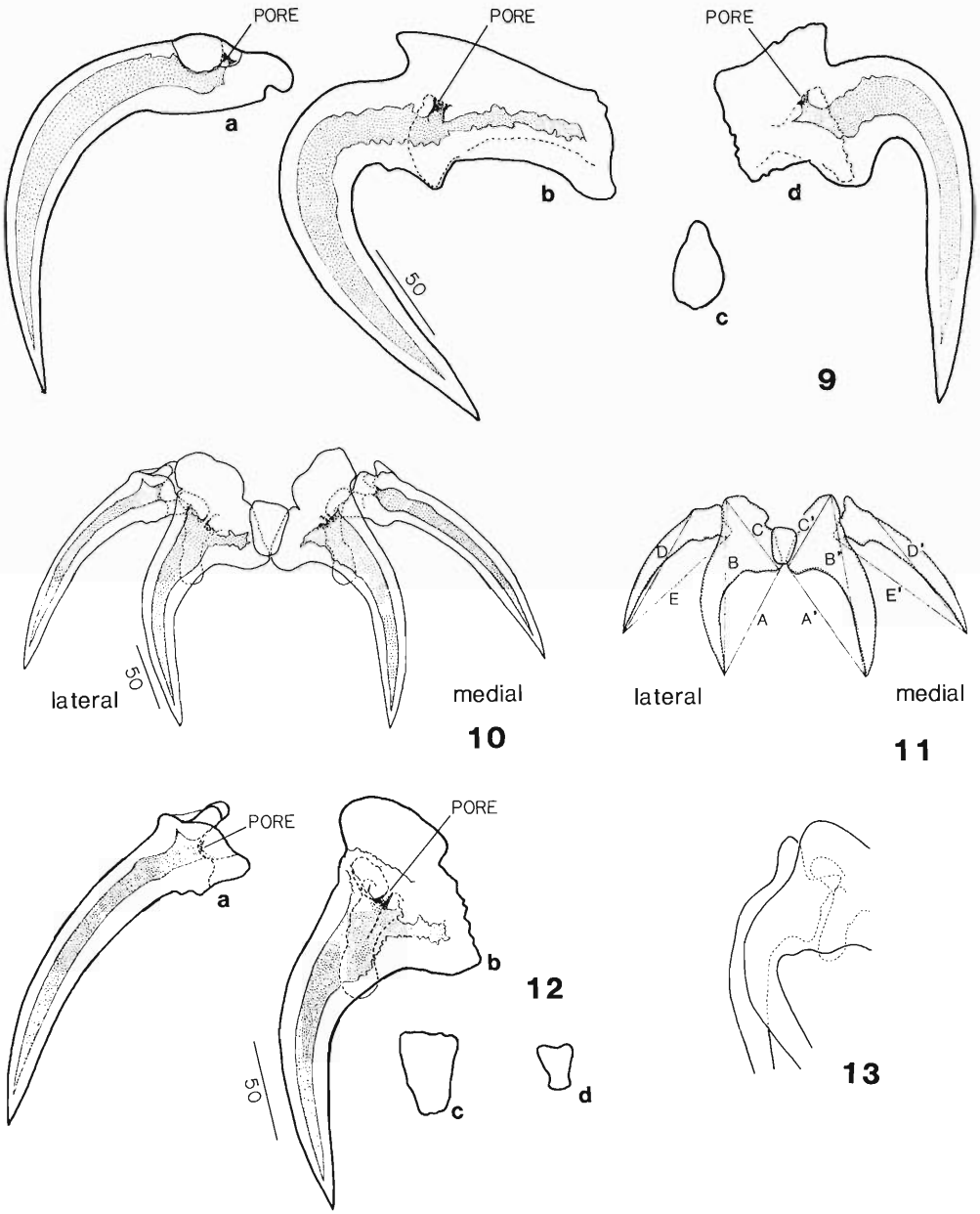
Measurements were taken from either the medial or lateral hooks as there appeared to be no differences between them. The mean, the standard deviation, the number of worms examined, and the total number of observations are given in Table 2.

Calliobothrium lintoni Euzet, 1954 emend. (Figs. 10–13)

The following information should supplement the description of *C. lintoni* Euzet (1954): each bothridium with two pairs of hooks (Fig. 10) covered with thin layer of tissue. Axial hooks recurved. Lateral and medial axial hooks of equal size, 52–155 from point of hook to posterior axial

←

Figures 3–8. ***Calliobothrium evani*** **sp. n. 3. Scolex of holotype indicating two of the four bothridia. 4. Proximal view of hooks; note articulation of abaxial hook with triangular tubercle of axial hook. 5. Distal view of hooks. 6. Hook measurements taken. 7. Terminal genitalia of paratype specimen. 8. Mature proglottid of paratype specimen.**



Figures 9-13. 9. ***Calliobothrium evani*** **sp. n. hook components separated; scale in Figure b applies to a, c, and d also. a. Abaxial hook, b. lateral axial hook, c. accessory piece, d. medial axial hook. 10-13.** ***Calliobothrium lintoni*** **Euzet, 1954. 10. Distal view of hooks of HWML No. 20933. 11. Hook measurements taken. 12. Hook components separated; scale in Figure b applies to a, c, and d also. a. Abaxial hook, b. axial hook, c. accessory piece of a Wood's Hole specimen (HWML No. 20933), d. accessory piece of a Mexican specimen (HWML No. 22537). 13. Distal view of abaxial and axial hook articulation in unflattened Mexican specimen (HWML No. 22537).**

extremity of base (A and A′ in Fig. 11) and 87–214 from point of hook to anterior abaxial extremity of base (B and B′ in Fig. 11). Length of diagonal from anterior abaxial extremity of base to posterior axial extremity of base 43–107 (C and C′ in Fig. 11). Axial hook bases articulate with and extend underneath trapezoidal accessory piece, 11–45 long by 14–30 at widest point (generally at anterior end) (Fig. 12c, d). Abaxial hook bases with four anterior knobs, number visible varying with degree of rotation of hook upon flattening (Fig. 12a). Lateral and medial abaxial hooks of equal curvature and size, 91–176 long from point to beginning of base on abaxial side (D and D′ in Fig. 11), and 80–182 from point of hook to axial extremity of base (E and E′ in Fig. 11). Axial extremity of abaxial hook base articulating with axial hook on elongated tubercle on proximal side of axial hook base (Fig. 13); tubercle 27–71 long. Anteriormost point of abaxial hook resting on notch on proximal side of axial hook tubercle. All hooks hollow, with internal channel granular in appearance, opening to outside via pore. In axial hooks channel extending from point into base to area of abaxial hook articulation and into axial extension of base; pore on proximal side of elongated tubercle (Fig. 12b). Abaxial hook channel extending from point to anterior portion of hook, opening via pore at end of channel (Fig. 12a). Accessory piece solid.

Discussion

The features that characterize *C. evani* are: bases of lateral axial hooks much longer than bases of medial axial hooks; accessory piece oval; articulation of hooks on triangular tubercle on proximal surface of axial hooks. Of the four other species in the genus, *C. evani* most closely resembles *C. lintoni* as they alone share the following features: presence of an accessory piece between bases of axial hooks; and internal channels extended into bases of axial hooks. In addition to the features listed above, *C. evani* differs from *C. lintoni* by possessing: a mean scolex length of 0.83 mm as compared to 0.5 mm; a mean scolex width of 0.77 mm as compared to 0.3 mm; a genital pore that is in the middle of the proglottid as compared to in the posterior one-third; a mean number of testes of 46 as compared to 34; and a total body length that is approximately twice as great.

In addition to the three features listed above, *C. evani* may be distinguished from *C. verticillatum* by its lack of scalloped proglottid overlaps, length of 7.25–14.5 mm as compared to 80–120 mm, 35–53 testes as compared to 110–130, and one accessory sucker per bothridium as compared to three. In addition, *C. evani* differs from *C. leuckartii* by its shorter length (as compared to 50–80 mm), and acraspedote as compared to weakly craspedote proglottids. More testes (as compared to 12–15) and acraspedote as compared to craspedote proglottids also distinguish *C. evani* from *C. eschrichtii* van Beneden, 1850.

It should be noted that the presence of internal channels and pores in hooks are not features limited in occurrence to *C. evani* and *C. lintoni.* These features are present in all species of *Calliobothrium,* and further investigation may reveal a widespread distribution of these traits among the hooked tetraphyllideans.

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I am grateful to Professor M. H. Pritchard of the H. W. Manter Laboratory, University of Nebraska State Museum, for many stimulating discussions as well as for her helpful criticisms of this manuscript. Special thanks are extended to Gordon Schneider of El Centro, California, who provided transportation down the difficult highway to Puertecitos, and whose knowledge of Spanish made possible the purchase of sharks from the Mexican fishermen. In addition I would like to thank Dr. J. R. Lichtenfels of the Animal Parasitology Institute, USDA, ARS, Beltsville, Maryland for lending specimens; and Scott W. Michaels for identifying the specimens of *M. henlei.*

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Distribution and Fine Structure of Tegumental Receptors in *Onchocleidus cyanellus* (Monogenea: Ancyrocephalinae)

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ABSTRACT: Scanning and transmission electron microscopy were utilized to determine the distribution and fine structure of uniciliate receptors of *Onchocleidus cyanellus* Mizelle, 1938. Receptors occur dorsally, ventrally, and laterally on the body surface. Most are distributed in the anterior region of the worm. Each receptor consists of a nerve bulb attached to the surrounding tegument by septate desmosomes. The bulb, which extends into the parenchyma, possesses a cilium with a 9+2 microtubular arrangement. The cilium arises from a closed-end basal body lacking a ciliary rootlet system.

Tegumental receptor fine structure has been examined in many major taxa of parasitic platyhelminths (Halton and Morris, 1969; Lyons, 1969, 1972, 1973a, b; Rohde, 1972; Webb and Davey, 1974; Richards and Arme, 1982). These receptors may be divided into two broad categories, ciliated and nonciliated. Lyons (1973a) has reported that both types are found in the Monogenea.

The present study focuses on the distribution and fine structure of uniciliate receptors of adult *Onchocleidus cyanellus* Mizelle, 1938 utilizing scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

Materials and Methods

SEM

Specimens of *Onchocleidus cyanellus* were collected from the gills of 20 freshly killed *Lepomis cyanellus* Rafinesque. The worms were fixed for 4 hr in 4% glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 7.2), washed twice for 2 hr each time in changes of the above buffer, dehydrated in a graded alcohol series, placed in 100% acetone, critical point dried with liquid CO_2, and coated with 200 Å of Au/Pd. Eighty specimens were then examined with a Hitachi S-500 scanning electron microscope to determine the receptor distribution.

TEM

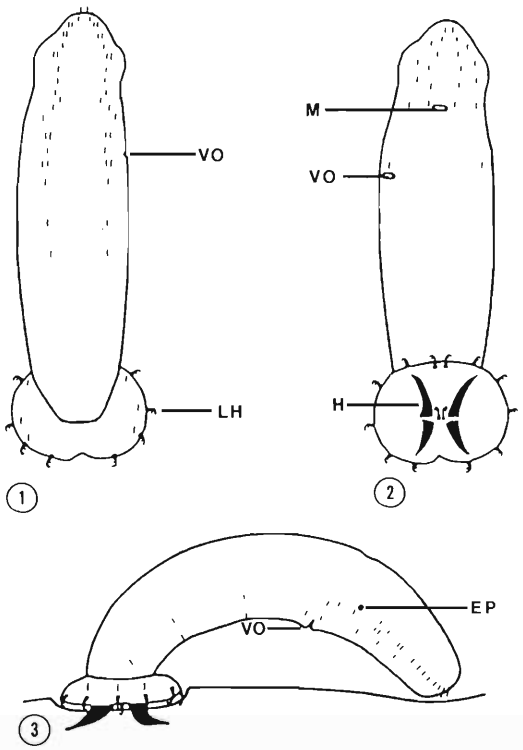
Specimens of *O. cyanellus* were fixed and washed as for SEM, but were then post-fixed for 1.5 hr in 1% osmium tetroxide in the above buffer, rinsed in that buffer, dehydrated in a graded alcohol series, and embedded in LR White medium grade acrylic resin. Ultrathin sections were stained with aqueous uranyl acetate followed by lead citrate and examined with a JEOL JEM 100CX transmission electron microscope. Six representative sections were selected for diagrammatic reconstruction.

Results

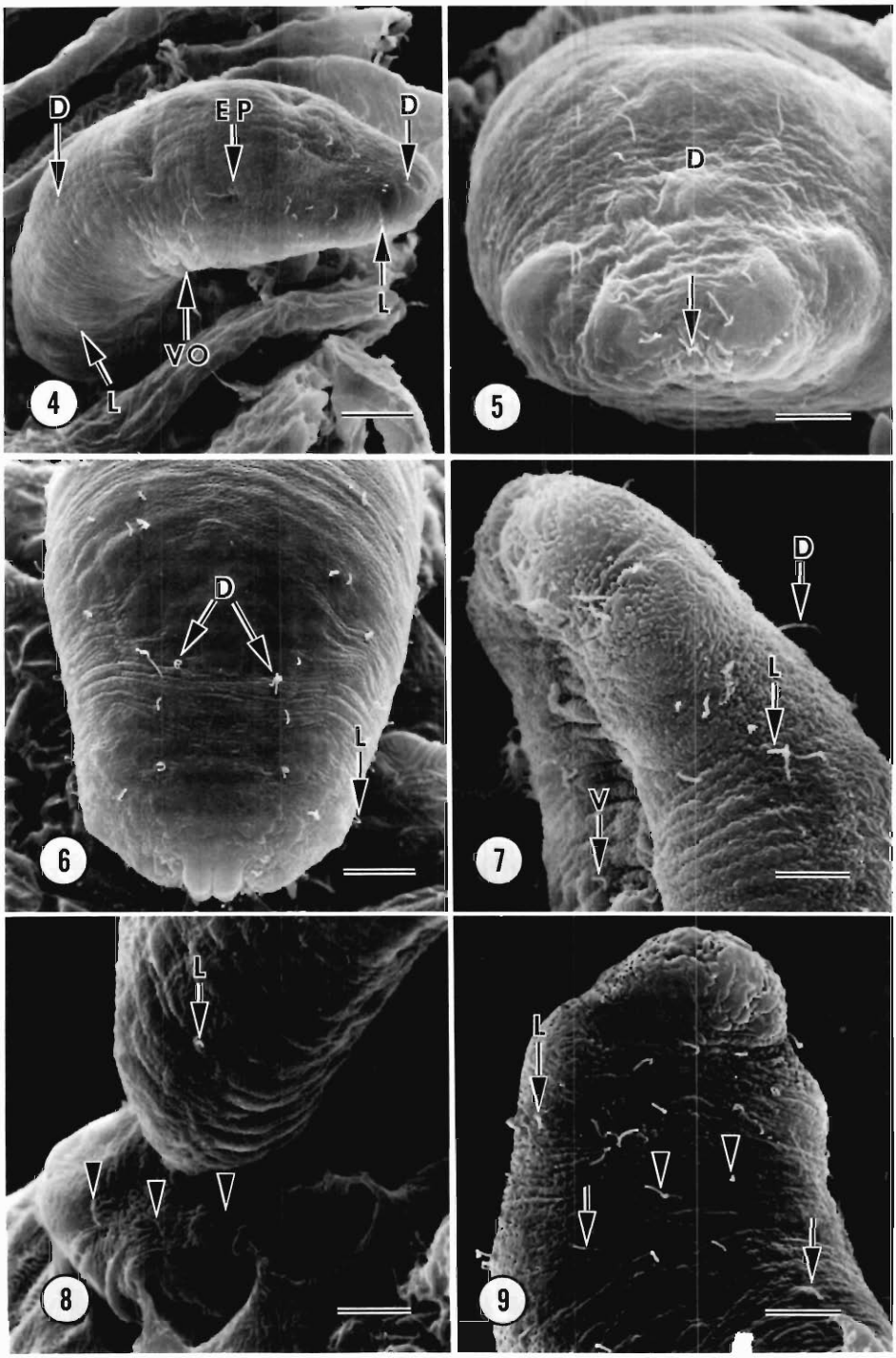
SEM—Receptor distribution

Uniciliate receptors are arranged bilaterally in dorsal, ventral, and lateral tegumentary tracts (Figs. 1–9). Each dorsal tract consists of 23 receptors, beginning at the anterior extremity of the worm and extending posteriorly ⅔ its length (Figs. 1, 4–6). In that tract, 10 receptors are in pairs and 13 occur singly.

Each ventral tract has 11 receptors that begin near the anterior extremity and extend to the level of the vaginal opening. Six are ventrolateral and five are medial (Figs. 2, 7, 9).



Figures 1–3. *Onchocleidus cyanellus*: diagrams of uniciliate receptor patterns of adult. 1. Dorsal surface. 2. Ventral surface. 3. Lateral surface. EP = excretory pore, H = hamulus, LH = larval hook on haptor, M = mouth, VO = vaginal opening.



Figures 4–9. ***Onchocleidus cyanellus*****: scanning electron micrographs of adult. 4. In situ dorsolateral view showing portions of the dorsal (D) and lateral (L) ciliated receptor tracts. EP = excretory pore, VO = vaginal opening. Bar = 14 μm. 5. En face view showing the point where the dorsal, ventral, and lateral tracts diverge (arrow). D = dorsal surface. Bar = 6.5 μm. 6. Anterodorsal view showing increasing distance between dorsal receptor tracts (D) from anterior to posterior. Also, portions of the lateral receptor tracts (L) are visible. Bar =**

Twenty-seven receptors are positioned on each side of the body from the anterior extremity to near the haptor (Figs. 3–9).

The dorsal surface of the haptor bears six receptors that are associated with larval hook pairs 4, 5, and 6. None is associated with the two pairs of hamuli nor the ventral surface of the haptor (Figs. 1–3, 8).

TEM—Receptor fine structure

A cilium exhibiting a 9+2 microtubular arrangement projects from a receptor nerve bulb that has an apical diameter of 0.46–0.56 μm lying 0.3–0.37 μm below the surface of the tegument. Distally, the bulb is attached to the adjacent tegument by septate desmosomes (Figs. 10–12).

Two electron-dense nerve collars encircle the nerve bulb. The distal one lies adjacent to the septate desmosomes and is separated from the second, more proximal collar, by a distance of approximately 0.11 μm. Within each nerve bulb is an electron-dense basal plate approximately 0.04 μm in thickness. Microtubules of the cilium extend to the basal plate, which is connected to a closed-end basal body, 0.50–0.56 μm long. Transitional fibers extend from the basal body to the distal nerve collar. The nerve bulb extends into the parenchyma as a process presumed to be continuous with the soma of a neuron (Figs. 10, 12–14). No striated rootlets nor mitochondria were observed.

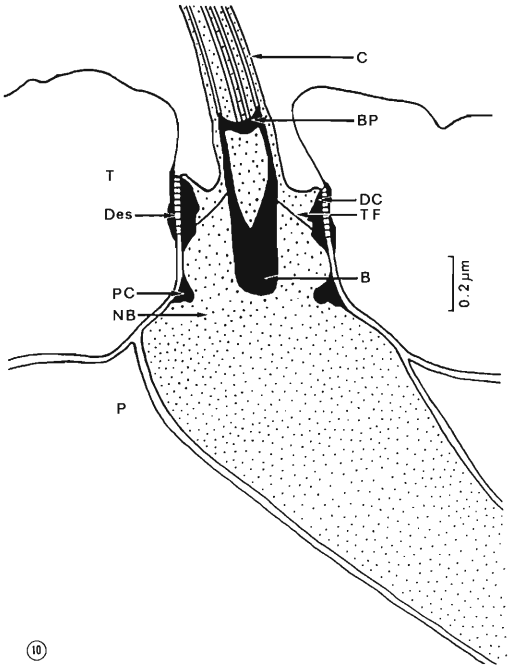


Figure 10. ***Onchocleidus cyanellus*****: diagram of a tegumental receptor. B = basal body, BP = basal plate, C = cilium, Des = septate desmosome, DC = distal nerve collar, NB = nerve bulb, P = parenchyma, PC = proximal nerve collar, T = tegument, TF = transitional fiber.**

Discussion

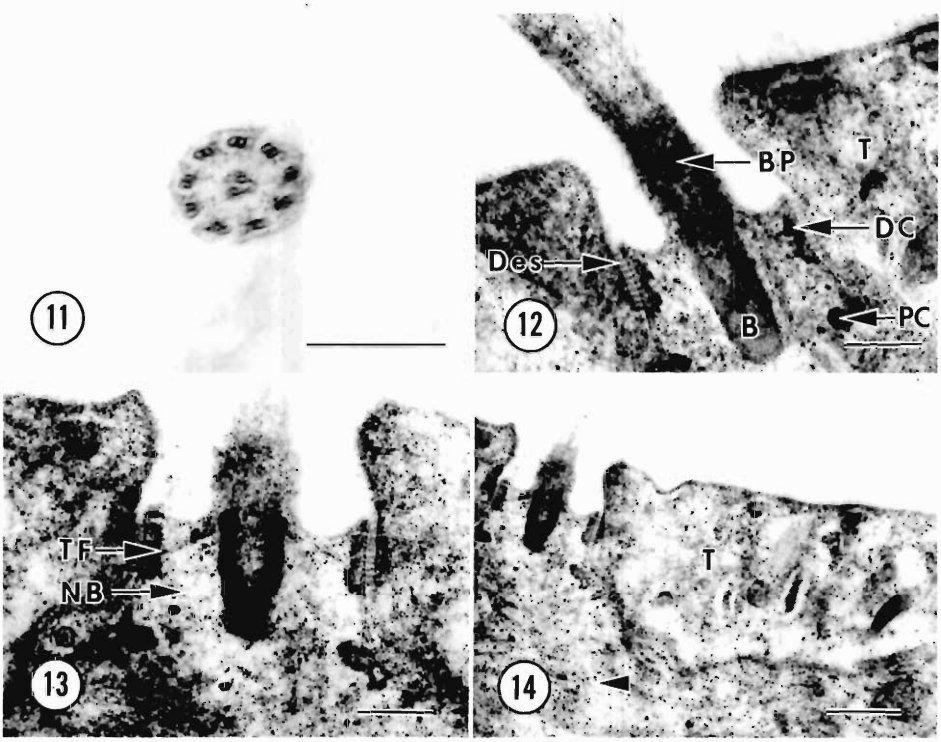
SEM—Receptor distribution

Although numerous studies of the surface topography of various adult Monogenea exist, few have concentrated on the overall distributional patterns of ciliated receptors. Harris (1983) used silver stain and SEM in observing uniciliate receptors in four transverse bands on adult *Oogyrodactylus farlowellae* Harris, 1983. Additional clusters occurred around the cephalic lobes, mouth, genital apertures, and haptor. Harris' figures showed that the receptors were elevated forms similar to ciliated papillae and that the majority occurred in the dorsoanterior region of the worm. In addition, five ciliated receptors shown on the ventral surface of the haptor of *O. farlowellae* clearly indicate a distributional pattern different from that of *O. cyanellus.*

The topography of the ventral surface of the monogenean haptor is variable from species to species. Cone and Beverley-Burton (1981) reported that ciliated receptors and papillae were absent from that surface of the haptor of *Benedenia* sp. On the other hand, Halton (1979) observed numerous elevated rosette- and dome-shaped structures on the ventral surface of the haptor of *Diclidophora merlangi* (Nordmann, 1832). He did not mention uniciliate receptors as being there although he did observe them on other areas of the body without discussing their

←

8 μm. 7. Ventrolateral view of the anterior region. Dorsal (D), lateral (L), and ventral (V) receptor tracts. Bar = 4 μm. 8. In situ dorsolateral view of haptor showing ciliary endings of receptors on the right side (arrowheads). Also note the most posterior ciliary ending of the right lateral receptor tract (L). Bar = 6 μm. 9. Ventral view showing inner (arrowheads) and outer (arrows) receptor tracts. Also note a portion of the lateral tracts (L). Bar = 5 μm.



Figures 11–14. ***Onchocleidus cyanellus*: transmission electron micrographs of uniciliate receptors. 11. Cross section of a receptor cilium showing a 9+2 microtubular arrangement. Bar = 0.2 μm. 12. Longitudinal section. B = basal body, BP = basal plate, Des = septate desmosomes, DC = distal nerve collar, PC = proximal nerve collar, T = tegument. Bar = 0.2 μm. 13. Longitudinal section showing transitional fiber (TF) in the nerve bulb (NB). Bar = 0.2 μm. 14. Longitudinal section showing a continuation of the receptor (arrowhead) below the level of the tegument (T). Bar = 0.4 μm.**

overall distributional pattern. Similarly, Lyons (1973b) reported numerous papillae on the ventral surface of the haptor of *Entobdella soleae* (van Beneden et Hesse, 1863) but gave no indication that uniciliate receptors were present. In the case of *O. cyanellus,* it more nearly resembles *Benedenia* sp. in that it lacks ciliated receptors and papillae on the ventral surface of the haptor.

TEM—Receptor fine structure

As revealed by TEM, the fine structure of tegumental receptors in *O. cyanellus* is markedly similar to that described by Lyons (1969) for another monogenean, *Gryodactylus* sp. Common to those receptors in both species are nerve collars, septate desmosomes, transitional fibers, and absence of ciliary rootlets.

Richards and Arme (1982) stated that the function of dense nerve collars in platyhelminth receptors is unclear, but their relationship to septate desmosomes suggests their function to be one of maintaining the shape of the distal region of the receptor during contraction or relaxation of the worm. Lyons (1973a) proposed that a thickened nerve collar is highly specialized and may, if not deformable, play a part in constraining cytoplasm affected by the shear force produced at the base of the cilium during, perhaps, mechanoreception. Webb and Davey (1974) further suggested that both nerve collars and rootlets are probably involved in support of the cilium, and the longer or more massive the cilium the greater is the degree of development of either the collar or rootlet system, or both. In *O. cyanellus,* as in *Gyrodactylus* sp., ciliary rootlets are lacking, therefore, the dense nerve collars would be the primary structures involved with constraint of the cytoplasm and ciliary support. In addition, the supportive function may be augmented by transitional fibers that interconnect the distal nerve collar and basal body.

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XVIIIth International Symposium of the European Society of Nematologists

The next International Symposium of the ESN, co-organized under the patronage of INRA and ORSTOM, will be held at the Conference Centre of Antibes, France, September 7–12, 1986.

It is planned to have four days of presented papers and colloquia. One or more special poster sessions with subsequent discussion meetings will be organized.

Official languages will be English and French.

A full day excursion, and a spouses' program are planned. A post-congress three-day tour of areas of interest in the south of France will be arranged.

For any further enquiries, please contact the President of the ESN: M. Ritter, INRA, Station de Recherche sur les Nématodes, B.P. 78, 06602 Antibes, France.

Proc. Helminthol. Soc. Wash.
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Levinseniella (*Monarrhenos*) *ophidea* sp. n. (Trematoda: Microphallidae) from the Western Garter Snake, *Thamnophis elegans* and the Bullfrog, *Rana catesbeiana*

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ABSTRACT: A new species of microphallid trematode, *Levinseniella* (*Monarrhenos*) *ophidea,* is described from the snake, *Thamnophis elegans,* and the bullfrog, *Rana catesbeiana,* from Willow Creek, Lassen County, northern California. It is distinguished from all other described members of the genus by its utilization of a snake and a bullfrog as definitive hosts and leeches as second intermediate hosts. It is most similar to *L.* (*M.*) *polydactyla* Deblock and Rosé, 1962, and *L.* (*M.*) species 2 and 3 (Heard *in* Deblock and Pearson, 1970), from which it differs by its larger size, the number of male pockets, presence of sclerotized structures in the pockets, and absence of oral sucker auricles.

The following species of leeches were collected from Willow Creek, Lassen County, California: *Glossiphonia complanata* (*L.*), *Helobdella stagnalis* (*L.*), *Erpobdella punctata* (Leidy) Moore, and *Dina parva* Moore. All harbored microphallid metacercariae later identified as *Levinseniella* Stiles and Hassall *in* Ward, 1901. Gravid adults were recovered from the intestines of the western garter snake, *Thamnophis elegans* Baird and Girard, and the bullfrog, *Rana catesbeiana* Shaw from the same stream. Morphology of the parasites, their host relationship, and experimental data show them to be a new species which is named *Levinseniella* (*Monarrhenos*) *ophidea* sp. n. and described below.

Materials and Methods

Study of material from natural infections was supplemented by feeding experiments involving laboratory-raised hamsters, chicks, and Mallard ducklings; *Thamnophis elegans* and the minnow, *Richardsonius egregius* (Girard), from Willow Creek; and *Rana catesbeiana* and *T. couchi* (Kennicott) from Shasta County, California. All vertebrates to be used in infection experiments were first determined to be free of trematodes using fecal flotation. Then, metacercariae dissected from leeches were given to vertebrates by stomach tube. In addition, *T. elegans* were placed with living, infected *Dina parva* leeches. Adult flukes were studied both alive and fixed. Specimens for whole mounts were heat-relaxed with warm saline, fixed in AFA with light coverslip pressure, and stained with Horn's trichrome stain; others were sectioned at 5 μm, and stained with hematoxylin and eosin. Drawings were made with the aid of a microprojector. The description is based on 100 whole mounts of gravid and nongravid adults, and on serial cross sections of three specimens. Measurements from 25 representative whole mounts are in micrometers, the range followed by the mean in parentheses, and the length stated first unless otherwise noted.

Results

Adult *Levinseniella* flukes of a previously undescribed species were obtained from naturally infected western garter snakes as well as from infection experiments; only western garter snake infection experiments were positive. Evidence that these hosts ingested leeches in nature was verified by finding four partially digested *D. parva* leeches in the stomach of a recently captured snake. The western garter snake readily consumed these leeches in the laboratory.

Levinseniella (*Monarrhenos*) *ophidea* sp. n. (Figs. 1, 2)

DESCRIPTION: With the characters of the genus. Body linguiform, 827–1,607 (1,109) long by 357–611 (412) in maximum width. Oral sucker terminal, 96–132 (110) long by 96–142 (125) wide. Acetabulum nearly round, 89–122 (101) by 84–118 (98). Sucker width ratio 1:1.3. Tegument spinose to level of acetabulum. Prepharynx 24–101 (70) long. Pharynx 41–58 (48) by 29–48 (36). Esophagus 168–379 (247) by 7–14 (10). Ceca 216–480 (298) by 19–36 (26), lined with prominent epithelium, terminating at or slightly posterior to anterior edge of acetabulum.

Testes transversely elongate, symmetrical, posterolateral to acetabulum, diffuse and obscure in more mature adults; right testis 24–86 (60) long by 96–221 (168) wide; left testis 48–96 (65) by 84–206 (134). Vasa efferentia dorsal; vas def-

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erens short, entering posterolateral margin of seminal vesicle. Seminal vesicle reniform, 103–228 (175) by 36–106 (77) at the greatest width, just anterior to acetabulum. Pars prostatica 49–118 (70) by 13–23 (18), surrounded by conspicuous prostatic cells within a thin, nonmuscular membrane. Genital pore a longitudinal slit immediately sinistral to acetabulum, 58–113 (73) long. Male pockets 18–23 (19) long, usually 10 or 12 but varying from 9–15, typically in two parallel rows on lateral atrial wall. Sclerotized tooth-like structures rarely seen; if present, only in one or two pockets. Male genital papilla muscular, 34–48 (41) long by 24–48 (31) wide, arising from anterodorsal wall of atrium and directed posterolaterally. Ejaculatory duct penetrating male papilla dorsolaterally, turning ventrolaterally, narrowing, and terminating in small opening at tip of papilla.

Ovary smooth, 84–235 (154) by 67–149 (108), dextral to acetabulum. Ootype, seminal receptacle (seen only in living specimens), and conspicuous Mehlis' gland intertesticular. Laurer's canal not observed. Metraterm 49–98 (75) long, originating between testicular level and posterior margin of acetabulum, entering genital atrium dorsally between male papilla and openings of male pockets. End of metraterm irregularly dilated with glandular lining. Vitellaria two lateral groups of 5–10 follicles, each 50–134 long by 36–113 wide, from testicular level almost to posterior end. Uterus filling most of hindbody, with lateral loops reaching ends of ceca. Female pouch absent. Eggs yellow-brown, operculate, 21–24 (23) by 12–14 (13). Excretory pore subterminal; bladder short, V- to U-shaped.

HOSTS: Western garter snake, *Thamnophis elegans* Baird and Girard, and the bullfrog, *Rana catesbeiana* Shaw.

LOCATION: Large intestine.

LOCALITY: Willow Creek, Lassen County, California.

TYPE SPECIMENS: National Parasite Collection, USDA, Beltsville, Maryland 20705.

Holotype: USNM Helm. Coll. No. 75986 (Ex *T. elegans*)

Paratype: USNM Helm. Coll. No. 75987 (Ex *T. elegans*)

THE METACERCARIA: Cysts spheroidal, 489–611 (543) in diameter; with double wall, inner wall 17 thick, outer 35. Excretory bladder of encysted metacercariae filled with elongate opaque granules discharged during excystment after which metacercariae differ little from ovigerous adults. Eggs are absent; testes are more rounded.

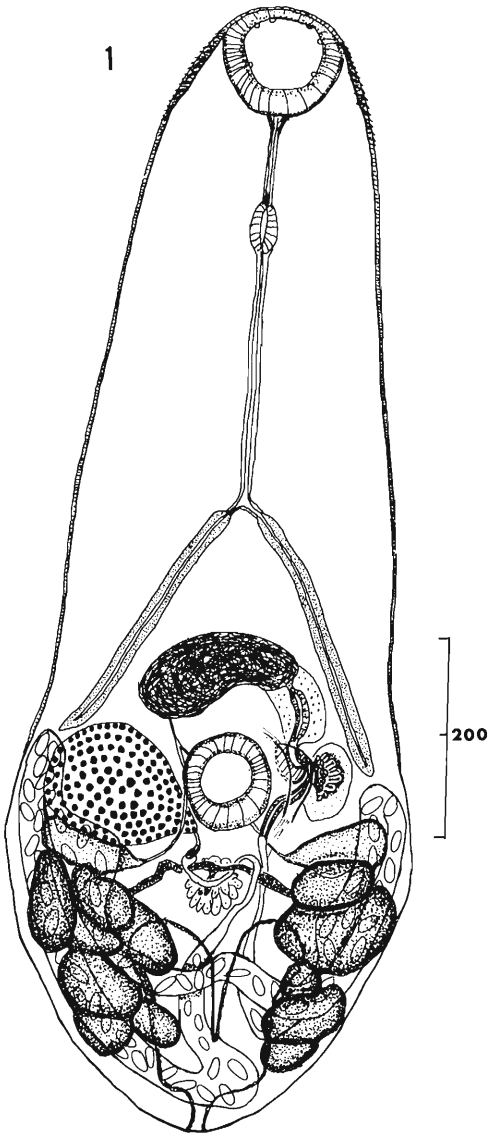

Figure 1. ***Levinseniella* (*M.*) *ophidea*, ventral view, from *Thamnophis elegans*.**

HOSTS: Leeches: *Dina parva* Moore, *Glossiphonia complanata* (L.), *Helobdella stagnalis* (L.), and *Erpobdella punctata* (Leidy) Moore.

LOCATION: Scattered in muscle layers and between internal organs.

LOCALITY: Willow Creek, Lassen County, California.

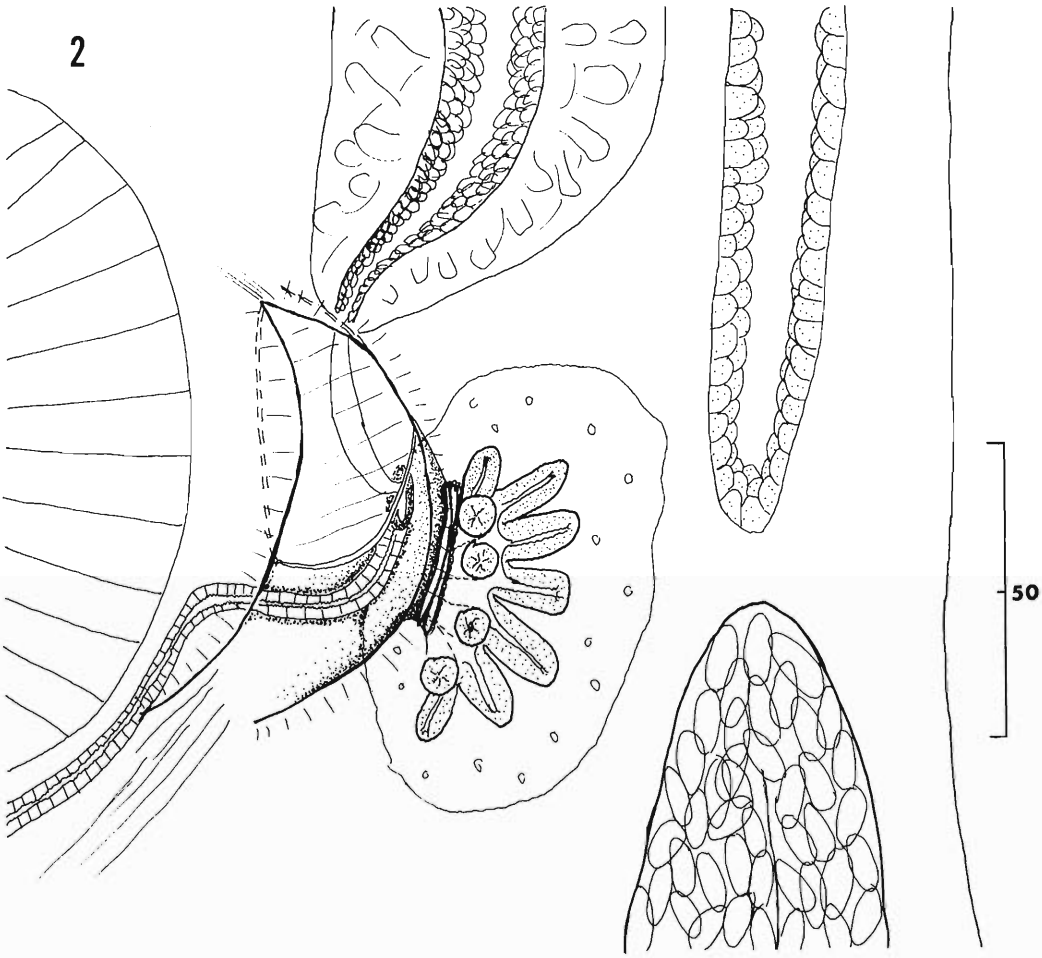

Figure 2. ***Levinseniella* (*M.*) *ophidea*, terminal genitalia, from *Thamnophis elegans*.**

Discussion

Heard (1968) divided the genus into four morphological groups based on the number of male pockets and the presence or absence of a female pouch. Deblock and Pearson (1970) proposed the subgenus *Monarrhenos* for those species lacking a female pouch. In Heard's scheme, *L.* (*M.*) *ophidea* is in Group IV, species with more than 5–7 pockets and no female pouch. This group contains *L.* (*M.*) *polydactyla* Deblock and Rosé, 1962, *L.* (*M.*) *hunteri* Heard, 1968, *L.* (*M.*) species 2 (Heard *in* Deblock and Pearson, 1970), *L.* (*M.*) species 3 (Heard *in* Deblock and Pearson, 1970), and *L.* (*M*) *capitanea* Overstreet and Perry, 1972.

Levinseniella (*M.*) *ophidea* most resembles *L.* (*M.*) *polydactyla,* and Heard's species 2 and 3. However, *L.* (*M.*) *ophidea* can be separated from them by its larger size (up to 700 longer), the number of male pockets, presence of sclerotized structures, and an oral sucker without auricles. *Levinseniella* (*M.*) *ophidea* possesses 9–15 male pockets whereas *L.* (*M.*) *polydactyla* and Heard's species 2 and 3 have 10–12, 8–10, and 7 male pockets, respectively. *Levinseniella* (*M.*) *ophidea* rarely contains sclerotized structures in the male pockets of the specimens examined, both living and fixed, whereas *L.* (*M.*) *polydactyla* and Heard's species 3 always possess sclerotized structures that are 10–13 long. When present in *L.* (*M.*) *ophidea,* the structures occur in only one or two pockets and are 18–23 (19) long. *Levinseniella* (*M.*) *ophidea* and *L.* (*M.*) *polydactyla* do

not possess oral sucker auricles, whereas auricles are sometimes seen in Heard's species 2 and always seen in his species 3.

Levinseniella (*M.*) *ophidea* also can be distinguished from all other members of the genus by its utilization of *T. elegans* and *R. catesbeiana* as definitive hosts. Most other species of *Levinseniella* utilize charadriiform, anseriform, and ralliform birds as definitive hosts (Deblock and Pearson, 1970). Overstreet (1978) mentions other possible hosts. Canaris (1971) reports *L. heardi* from the skink, *Ablepharus boutoni africanus* (Sternfeld), from East Africa. Leeches have not been previously reported as second intermediate hosts for *Levinseniella,* but other microphallids such as *Maritrema erpobdellicola* Timon-David have metacercariae within leeches (Timon-David, 1962).

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Proc. Helminthol. Soc. Wash.
52(2), 1985, pp. 184–195

Attempted Immunization of Mice Against *Schistosoma mansoni* by Inoculation with Purified Glycoprotein Antigens from Adult Worms[1]

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ABSTRACT: Eight-week-old Swiss albino mice, NIH/NMRI strain, were inoculated with one of three purified glycoprotein antigens from adult worms: FhConA, a concanavalin A-binding tegumental glycoprotein from *Fasciola hepatica*; SmConA, a concanavalin A-binding surface glycoprotein from *Schistosoma mansoni,* or SmFxI, a very large molecular weight glycoprotein extracted from lyophilized *S. mansoni.* Inoculation with FhConA produced a 26% reduction in challenge worm burden and a significant reduction in granuloma size when compared with controls. Granuloma size was also reduced following inoculation with SmConA. The other antigens were less effective in protecting mice against schistosomiasis. A striking inverse correlation was observed between the number of female worms present and the fecundity of each female, which suggests a crowding effect. There was also evidence for a change in frequency distribution or "overdispersion" of parasites following immunization. The implications of these findings for vaccine development are discussed.

Except for the partial immunity obtained following chronic infection, the most consistently successful means to protect animals against schistosomiasis has involved infection with irradiation-attenuated cercariae or schistosomula. Such treatment routinely produces a reduction in worm burden of up to 70% in inbred mice (Minard et al., 1978) and experiments with primates have been encouraging (Stek et al., 1981). However, there are a number of drawbacks to the development of an irradiation-attenuated vaccine. Production of the vaccine requires a reliable source of live material and sophisticated equipment for irradiation. Although irradiated schistosomula are still effective following cryopreservation (Murrell et al., 1979), freezing and storage become expensive procedures when one contemplates immunizing large populations in tropical climates. Even though they are attenuated, the irradiated cercariae or schistosomula are still live and may persist for several weeks after inoculation (von Lichtenberg and Byram, 1982). There are reports of Guillain-Barre syndrome associated with acute schistosomiasis mansoni (Neves et al., 1973). Although no significant lesions or permanent sequelae have been noted in experimentally vaccinated animals, perineural eosinophil infiltration is part of the local inflammatory response associated with a live vaccine (Byram et al., 1983). Thus, use of a live attenuated vaccine cannot be assumed to be free of untoward side effects.

Attempts to immunize mice using soluble extracts or metabolites of schistosomes have yielded equivocal results. In some studies partial protection was obtained, as measured by reduction in challenge worm burden (Watts, 1949; Sadun and Lin, 1959; Murrell and Clay, 1972) or increased survival of the host following a "lethal" infection (Levine and Kagan, 1960; DaSilva and Ferri, 1968), whereas in other studies inoculation of parasite extracts had no observed effect upon subsequent infection (Thompson, 1954; Ritchie et al., 1962; Sher et al.,1974). Furthermore, reduction in worm burden achieved in some experiments could not be reproduced when these

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The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Department of Defense or the Uniformed Services University of the Health Sciences.

The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Animal Resources, National Research Council, DHEW Pub. No. (NIH) 78-23.

experiments were repeated in the same laboratory (Murrell et al., 1975). This lack of consistency may be due, in part, to the extreme heterogeneity of most crude antigen preparations (Murrell et al., 1974). It is not known whether crude preparations contain protective antigens in sufficient quantities to be effective, or whether antigens are denatured by the isolation procedures. The injection of a broad spectrum of antigens may induce adverse antigenic competition, and some antigens present in complex extracts may induce counterproductive immune responses such as blocking factors and enhancing antibodies (Murrell et al., 1975).

In the present study we have attempted to immunize mice using well-defined, homogeneous antigens extracted from adult worms. A concanavalin A-binding surface glycoprotein (SmConA) and a 300,000 mol. wt. glycoprotein (SmFxI) from *Schistosoma mansoni* were chosen because of their reactivity with sera from infected humans (Hayunga et al., 1983). A concanavalin A-binding glycoprotein from *Fasciola hepatica* (FhConA) was chosen because it appears biochemically similar to SmConA, and because it has already been shown to be partially protective against murine schistosomiasis (Hillyer and Sagramoso, 1979).

Table 1. Experimental protocol for pre-exposure inoculation with purified glycoprotein antigens extracted from adult worms.

Group	Number of mice	Antigen*	Dosage per inoculation (μg)	Number of inoculations†	Total dosage (μg)
1	10	FhConA in saline	30	3	90
2	10	FhConA in adjuvant‡	30	3	90
3	10	FhConA in saline	30	2	60
4	10	FhConA in adjuvant‡	30	2	60
5	10	SmConA in saline	30	3	90
6	10	SmConA in adjuvant‡	30	3	90
7	10	SmFxI in saline	30	3	90
8	10	SmFxI in adjuvant‡	30	3	90
9	10	Adjuvant only‡	—	3	—
10	10	Intralipid (vehicle) only	—	3	—
11	10	Saline only	—	3	—

* Antigens: FhConA, a concanavalin A-binding glycoprotein from *Fasciola hepatica* prepared following Hillyer and Sagramoso (1979); SmConA, a concanavalin A-binding surface glycoprotein, and SmFxI, a 300,000 mol. wt. glycoprotein from *Schistosoma mansoni*, both prepared following Hayunga et al. (1983).

† Inoculations given i.m. at 4-week intervals.

‡ Adjuvant consists of Pfizer compound CP 20,961 prepared 5 mg/ml in Intralipid.

Materials and Methods

Antigens

Glycoprotein antigens from *Schistosoma mansoni* adult worms were extracted and purified as described previously (Hayunga et al., 1983). SmConA is a concanavalin A-binding surface antigen with apparent mol. wt. 58,000 and approx. pI value of 4.85. SmFxI is a large (>300,000) mol. wt. glycoprotein with approx. pI value of 4.27, obtained by gel filtration and isoelectric focusing of crude antigen. Both SmConA and SmFxI migrate as single peaks on ampholine columns and single bands on SDS-PAGE.

A concanavalin A-binding glycoprotein from *Fasciola hepatica* adult worms was prepared as described by Hillyer and Sagramoso (1979). *F. hepatica* adult worms, recovered from the bile ducts of condemned bovine livers, were washed twice in cold 0.85% NaCl to eliminate traces of bile and blood, homogenized in a Ten Broeck tissue grinder at 4°C, shell frozen, and lyophilized. The dry powder was resuspended in 0.01 M phosphate-buffered saline, pH 7.0, containing 0.03% SDS, and applied to a concanavalin A–Sepharose 4B column. Elution of bound material was done using 0.2 M α-D-methylglucoside. Separation on an Ampholine isoelectricfocusing column revealed the major component to have a pI of 4.0–4.4. This antigen was designated "FhConA."

FhConA and SmConA were lyophilized and reconstituted in saline prior to use. SmFxI was stored frozen at −40°C, then thawed and diluted in saline for immunization.

Immunization protocol

The adjuvant used for this study was Pfizer compound CP 20,961 prepared at a concentration of 5 mg/ml in Intralipid (a lipid amine dietary supplement used for intravenous therapy). This adjuvant was chosen instead of Freund's which would not be appropriate for subsequent human vaccine trials. Antigens were prepared at a final concentration of 30 μg/0.5 cc in either the adjuvant mixture or in saline. Controls consisted of adjuvant alone (Intralipid containing CP 20,961), Intralipid alone, and saline alone. Mice were arranged in groups of 10 animals as shown in Table 1; they were maintained five to a cage, and each mouse was marked for subsequent identification. Eight-week-old female Swiss albino mice, NIH/NMRI strain, were inoculated by intramuscular injection in the semimembranosus/semitendinosus group with 0.5 cc of the antigen preparation (approximately half in each leg). Inoculations were spaced at 4-week intervals. Eight weeks after the last inoculation, mice were bled for pre-challenge serology by tapping the periorbital artery, then challenged by percutaneous (tail) exposure to approximately 125 cercariae of a laboratory-maintained Puerto Rican strain of *S. mansoni*. All mice were challenged with the same batch of cercariae. Eight weeks after challenge mice were bled again, then perfused with citrated saline for recovery of adult worms. Livers were

Table 2. Number of *Schistosoma mansoni* adult worms recovered from mice 8 weeks after challenge infection with approximately 125 cercariae.

Group	Number of female worms*	Number of male worms*	Total number of worms*	% reduction†
1	7.60 (±5.08)	8.10 (±4.88)	15.70 (±9.59)	24.5
2	10.44 (±4.79)	9.22 (±4.23)	19.66 (±8.71)	5.5
3	10.00 (±4.69)	7.77 (±3.73)	17.77 (±7.63)	14.6
4	8.40 (±2.91)	7.00 (±2.36)‡	15.40 (±3.30)‡	25.9
5	8.40 (±2.95)	7.70 (±2.54)	16.10 (±4.72)	22.6
6	11.20 (±4.96)	10.60 (±3.30)	21.80 (±6.68)	−4.8
7	10.10 (±2.84)	11.00 (±4.00)	21.10 (±5.64)	−1.4
8	7.44 (±4.36)‡	8.44 (±6.06)	15.88 (±8.58)	23.6
9	9.50 (±2.77)	10.87 (±2.35)	20.37 (±3.96)	2.1
10	9.60 (±3.44)	10.80 (±3.88)	20.40 (±6.89)	1.9
11	10.40 (±3.95)	10.40 (±4.97)	20.80 (±8.65)	—

* Expressed as mean (±standard deviation).

† Percentage-reduction of total worm burden as compared with Group 11 (control).

‡ Significantly different from Group 11 (control) at $P < 0.05$.

cut in half, one sample used for KOH digestion (following Cheever, 1968a), the other fixed in 10% neutral formalin for histological examination.

Serology

Mouse IgG antibodies against parasite antigens were detected using an enzyme immunoassay (EIA) as described previously (Voller et al., 1978; Hayunga et al., 1983), using both crude antigen preparations and purified glycoproteins. One hundred microliters of antigen prepared at a concentration of 5 μg/ml in PBS incubation buffer, pH 8.0, was attached in solid phase to polystyrene microplates (96-well, NUNC) for a period of 2 hr at 37°C. The incubation buffer was removed and the plate washed once with PBS/Tween, pH 8.0. Next, 200 μl of blocking solution (1:15 dilution of 1% BSA in PBS) was added to each well and allowed to incubate for 1 hr at room temperature, to reduce background; plates were then washed with PBS/Tween as before. Serum dilutions, in 100-μl aliquots, in PBS were added to the test wells and incubated for 2 hr at room temperature; serum blanks and control positive and negative sera were run with each plate. Plates were washed three times with PBS/Tween. Conjugate, consisting of 100 μl peroxidase bound to goat anti-mouse IgG (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Maryland) at optimal dilution was added to each well. (The optimal conjugate dilution was determined by checkerboard titration of each batch of conjugate preparation.) The conjugate was allowed to incubate for 1 hr at room temperature, then plates were washed three times with PBS/Tween. To each well was added 100 μl peroxidase substrate solution consisting of 30% hydrogen peroxide and ABTS (2-2′ azino-di-[3 ethyl benzothiazolin sulfon-6], diazonium salt) freshly mixed in a 1:1 ratio. The plates were incubated with the substrate for 10–20 min, then analyzed spectrophotometrically at 414 nm using a Titer-Tek® microtiter reader (Flow Labs, McLean, Virginia).

The presence of mouse IgE antibodies was ascertained using a modification of the enzyme immunoassay. Microtiter plates were coated overnight with 1 μg protein A (Pharmacia). Serum samples, in 200-μl aliquots in PBS, were added to these wells and incubated for 2 hr at 4°C. One hundred microliters of supernatant (containing serum proteins that did not bind to protein A) were aspirated and added to antigen-coated wells as described above. The conjugate for the IgE assay consisted of peroxidase-labeled goat anti-mouse IgE (Miles Laboratories, Elkhart, Indiana).

Alternatively, 100-μl aliquots of undiluted sera were inoculated subcutaneously into the dorsal integument of 200-g female Sprague-Dawley rats. Rats were administered approximately 1 mg crude AFT-1 antigen in 1 ml 0.5% Evans Blue dye by intravenous injection through the tail vein, 48 hr after inoculation. Reactions were scored by measuring the diameter of the erythematous wheal. Controls consisted of subcutaneous inoculation of matched normal sera.

Analysis of data

Statistical significance of data was determined using a "two sample *t*-test for independent samples with unequal variances" as described by Rosner (1982). In cases where frequency distribution was over-dispersed and non-parametric, data were analyzed using a normal parametric statistical test (*t*-test) on rank transformed data, as described by Conover and Iman (1981). Granuloma volume was calculated from the measured diameter using the formula $\frac{1}{6}\pi D^3$. Ten granulomata were measured from each liver examined. Graphs shown in Figures 1, 2, 6 and 7 were prepared using a Hewlett-Packard Model 9862A Calculator Plotter. The "goodness of fit" of the regression lines shown in the figures was determined by the "*F* test for simple linear regression" as described by Rosner (1982).

Results

Worm burden

Analysis of worm counts revealed a statistically significant ($P < 0.05$) reduction in worm

Table 3. Hepatic egg burden 8 weeks after challenge infection. Data expressed as mean (±standard deviation).

Group	Number of eggs per mg liver	Granuloma diameter (in μm)	Granuloma volume (in 10^6 μm^3)
1	6.129 (±2.079)	596.96 (±26.92)	111.72 (±15.07)
2	6.357 (±1.627)	619.36 (±1.59)	124.39 (±0.95)
3	5.274 (±2.245)	623.84 (±99.68)	130.46 (±59.09)
4	4.901 (±2.007)	483.84 (±111.77)*	66.12 (±30.51)†
5	5.287 (±1.490)	588.01 (±71.23)	108.84 (±38.73)
6	6.766 (±2.235)	492.80 (±64.96)*	65.63 (±27.98)†
7	6.729 (±2.169)	631.68 (±43.23)	119.83 (±28.39)
8	5.622 (±2.587)	554.62 (±79.52)	94.33 (±40.05)
9	7.016 (±2.100)	619.36 (±42.76)	125.29 (±25.79)
10	6.775 (±2.262)	600.32 (±56.89)	114.81 (±32.32)
11	5.797 (±2.078)	547.23 (±38.50)	90.96 (±25.49)

* Significantly different from Group 11 (control) at $P < 0.002$.
† Significantly different from Group 11 (control) at $P < 0.05$.

burden for Experimental Group 4 (FhConA in adjuvant) as compared with the control group (Table 2). Worm burdens appear reduced for Group 1 (FhConA in saline), Group 5 (SmConA in saline), and Group 8 (SmFxI in adjuvant), but for these cases a significant difference was not proven. No consistent differences were found between groups receiving antigen in adjuvant and those receiving antigen in saline. Of particular interest is the close agreement between each of the three controls, Group 9 (complete adjuvant), Group 10 (Intralipid alone), and Group 11 (saline), which suggests that neither the adjuvant nor the vehicle (Intralipid) had an appreciable effect on protection.

Hepatic egg burden

The hepatic egg burden, calculated after potassium hydroxide digestion of host tissue, was expressed as the number of eggs per mg liver (Table 3). Although the egg burden appears lower in Group 4 (FhConA in adjuvant) than in Group 11, this difference was not statistically significant. In general, egg burden was characterized by extreme variation within each experimental group.

An attempt was made to correlate egg burden with worm burden by analyzing data from each mouse. As shown in Figure 1, the number of female worms present had little effect on the total number of eggs deposited in the liver. However, there is a very strong inverse correlation between the worm burden and the number of eggs deposited by each worm. Egg production per female is greatest in mice infected with only one female worm and decreases exponentially as the number of females increases (Fig. 2). The points on this graph describe an exponential curve, $y = a + b/x$, with $r = 0.841$, $F = 247.8$, $P < 0.005$. The one obvious point off the line (marked by ▲) represents a mouse from which two female but no male worms were recovered. If this point is excluded, the "degree of fit" (F) for the curve increases to 374.9, and $r = 0.886$.

Granuloma size

The size of hepatic granulomata was measured from histological sections in order to detect evidence of any modulation of the immune response to *S. mansoni* eggs. All tissue samples were taken at the same time (8 weeks post-infection). For uniformity, the sample measured was restricted to lesions that (a) were chronic, (b) were surrounded on all sides by healthy tissue, (c) contained an identifiable egg in the necrotic center, and (d) could be attributed to only one egg. In the case of lesions that were not round, an average was taken of the greatest and least diameters.

As shown in Table 3, granuloma size, expressed as either diameter or volume, was found to be markedly reduced in Group 4 (FhConA in adjuvant) and Group 6 (SmConA in adjuvant) as compared with the control group. Although there was variation within each experimental group, the reduction in size was statistically significant and striking (Fig. 3).

Serology

Serum samples were taken from each mouse after inoculation (but before challenge) and im-

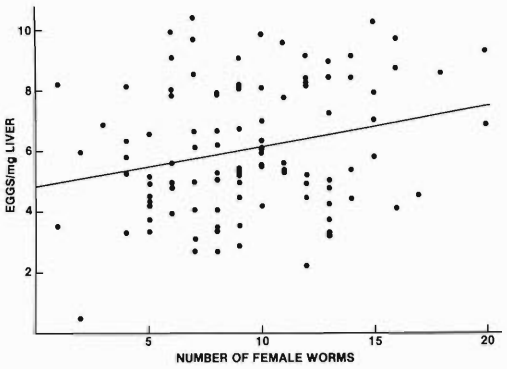

Figure 1. Hepatic egg burden in all mice, expressed as number of eggs per mg of liver. Note the high degree of scatter ($N = 105$; $r = 0.241$; $F = 6.379$; $P < 0.025$).

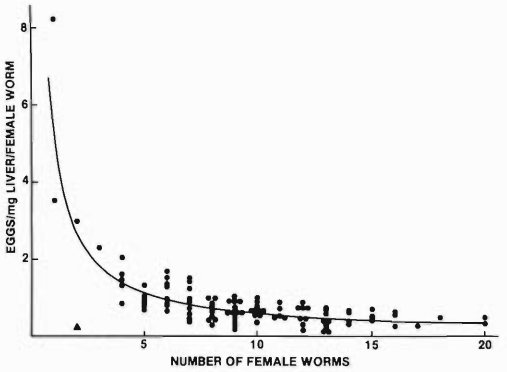

Figure 2. Average number of eggs deposited in the liver by each female worm. ▲ represents a mouse from which two females but no males were recovered; ● represents all other mice ($N = 105$; $r = -0.886$; $F = 374.9$; $P < 0.005$).

mediately prior to perfusion. These were tested by EIA for antibodies against the following antigens: SmConA, SmFxI, FhConA, crude *S. mansoni* adult freeze-thaw (AFT), crude *S. mansoni* cercarial freeze-thaw (CFT), and *S. mansoni* soluble egg antigen (SEA). The results are presented in Figures 4 and 5.

Pre-challenge antibody titers against the purified antigens reveal that the mice were definitely producing IgG antibodies to the antigens with which they had been inoculated (Fig. 4). The highest antibody levels against SmConA were found in Groups 5 and 6 which received that antigen, and in Groups 7 and 8 which received SmFxI. Antibody levels against FhConA are equally high in Groups 1 and 2 which received three inoculations of FhConA, and Groups 3 and 4 which received only two inoculations. Antibody levels against SmFxI were high in all but the control groups, indicating cross-reactivity between SmFxI and the other antigens. Antibody levels appeared slightly, but consistently, higher in animals given antigen prepared in adjuvant.

Pre-challenge antibody titers against the crude antigens also revealed evidence of cross-reactivity (Fig. 5). It is of interest that the highest antibody levels against adult antigen (AFT) were found in Groups 6, 7, and 8 which received *S. mansoni* antigens. Antibodies against egg antigen (SEA) were also greatest in Groups 6, 7, and 8. In contrast, the highest antibody levels against cercarial antigen (CFT) were found in Groups 2 and 4 which received *F. hepatica* antigen.

Pre-challenge antibody titer and subsequent worm burden were recorded for each individual mouse. As shown in Figure 6, there is an inverse correlation between worm burden and the pre-challenge IgG antibody titer against FhConA ($F = 3.91$, $r = -0.20$, $P < 0.05$). This correlation is even stronger ($F = 6.07$, $r = -0.24$, $P < 0.002$) when comparing antibody with the number of male worms (data not shown). No such correlation was found for antibody against other antigens. Nor was any correlation found between IgG antibody level and granuloma size (Fig. 7).

Attempts to demonstrate IgE antibodies, using either EIA or PCA in rats, were unsuccessful for both pre- and post-challenge sera. If antigen-specific IgE was present in these mice, levels were too low to be detected by these methods.

Discussion

Inoculation of mice with purified glycoprotein antigens resulted in a reduction in worm burden of 26% in the best group (Table 2). Such results are not at all impressive, and do not demonstrate any significant improvement over crude antigens used previously (Murrell et al., 1975; Stek et al., 1984). Furthermore, reduction in worm burden is not consistent, and variation is quite large in each experimental group. In previous experiments, comparable dosages of FhConA were far more efficacious, producing levels of protection as high as 68% (Hillyer and Sagramoso, 1979). The difference reported here may be attributed to different strains of both parasite and host, storage of antigen prior to inoculation, the use of different adjuvants, and slightly different immunization schedules. Use of the Pfizer adjuvant (Compound CP 20,961) appeared to be of little

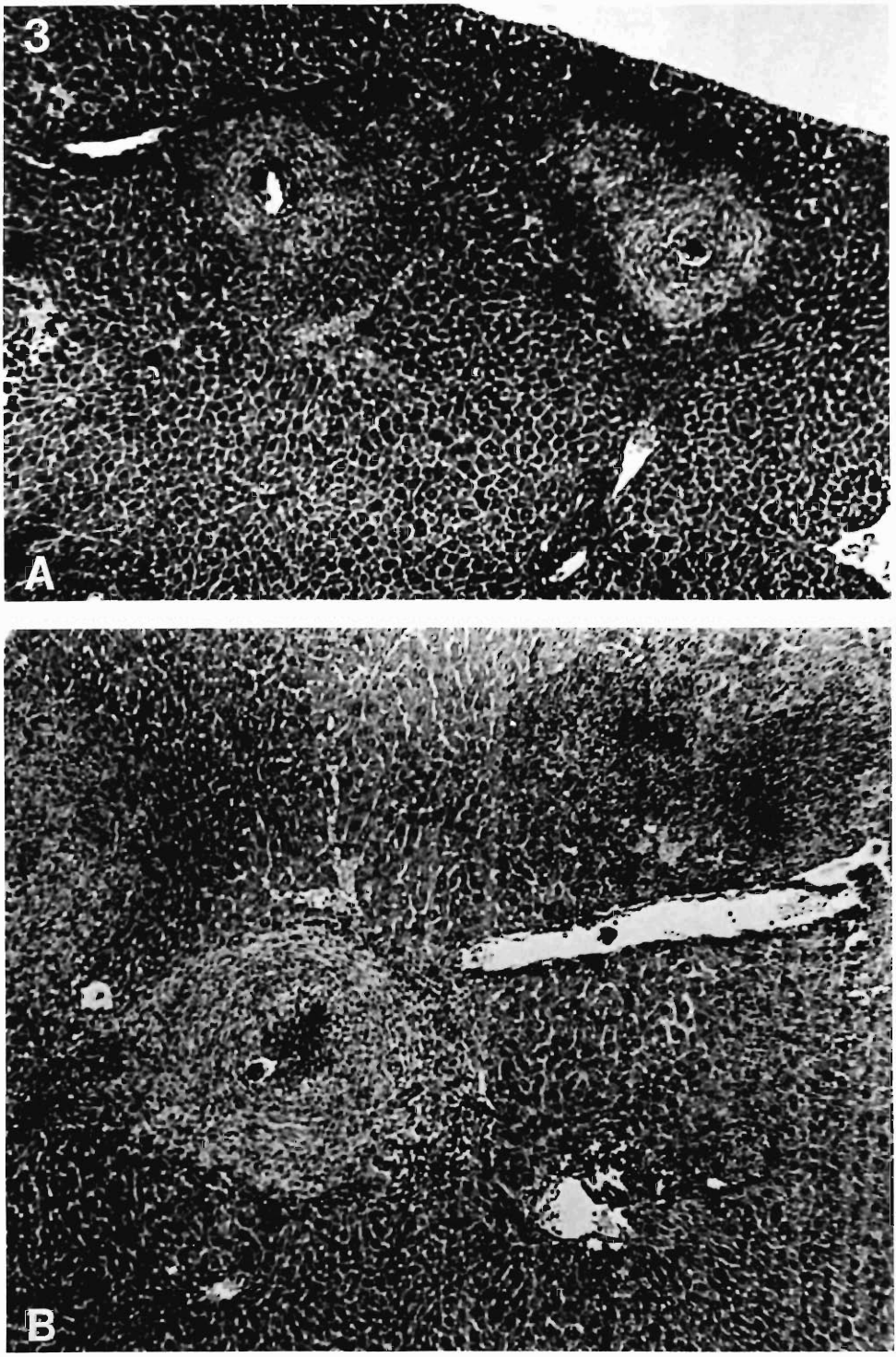

Figure 3. A. *Schistosoma mansoni*-induced egg granuloma in a mouse inoculated with FhConA antigen prior to exposure to approximately 125 cercariae. Hematoxylin and eosin, ×75. B. Hepatic granuloma in saline control group mouse. Identical magnification.

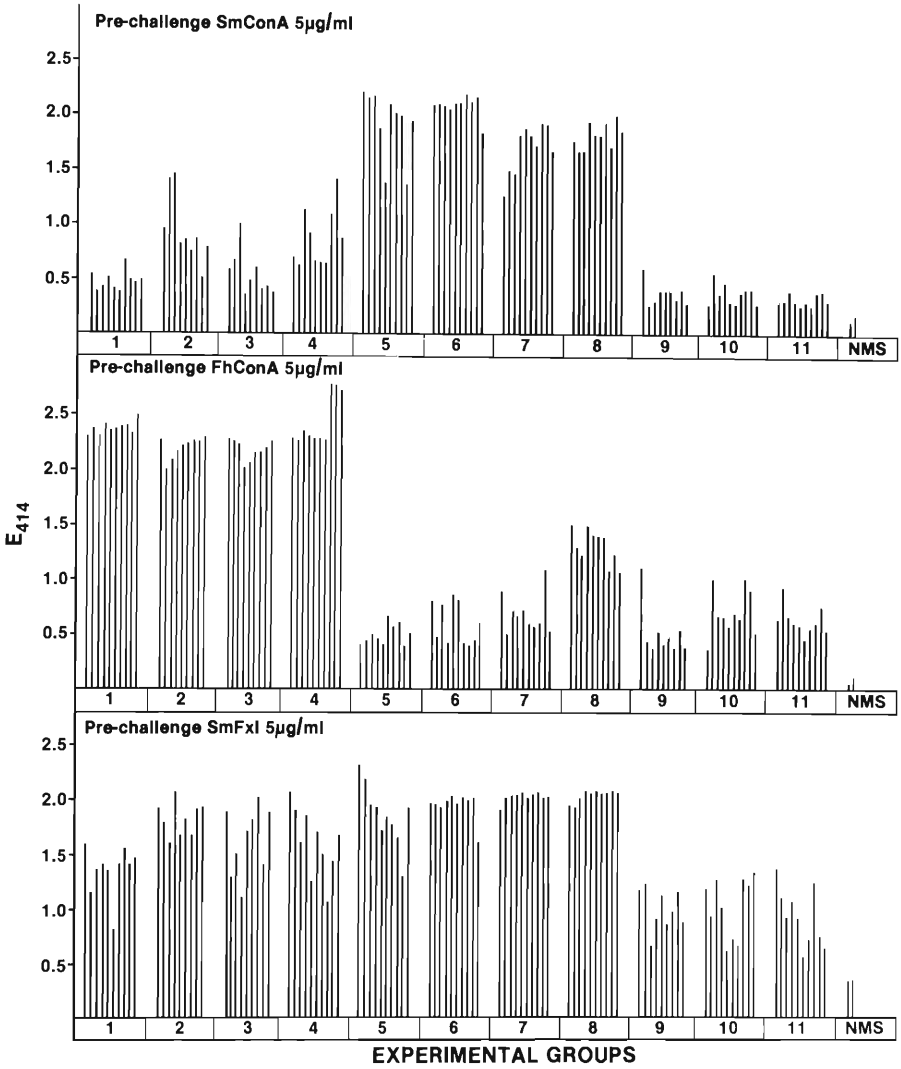

Figure 4. Serum antibody levels against purified antigens prior to challenge with cercariae. Data expressed as absorbance at 414 nm for each mouse tested, as determined by EIA.

consequence with regard to worm burden. In some cases, the antibody titer was increased for mice that received antigen mixed with the adjuvant (Figs. 4, 5). The greatest effect of adjuvant can be seen in Table 3, where granuloma size was reduced in mice receiving antigen in adjuvant (Groups 4 and 6) as compared to mice receiving the same antigen in saline (Groups 3 and 5).

Clearly, some very high IgG antibody levels were developed in the experimental groups prior to challenge (Figs. 4, 5). However, we do not know whether any of these correspond to the cytotoxic or opsonic antibodies that have been shown to kill schistosomula in vitro (Murrell and Clay, 1972; Dean et al., 1974; Dean, 1977). Failure to obtain protection despite high antibody titer may reflect the inability of these antigens to induce appropriate homocytotropic or reaginic antibodies (Ogilvie and Smithers, 1966; Sher et al., 1974; Murrell et al., 1975).

If a correlation between antibody titer prior to challenge and final worm burden exists, it would be very useful in terms of predicting resistance to infection before animals are sacrificed or measuring the immune status of a vaccinated indi-

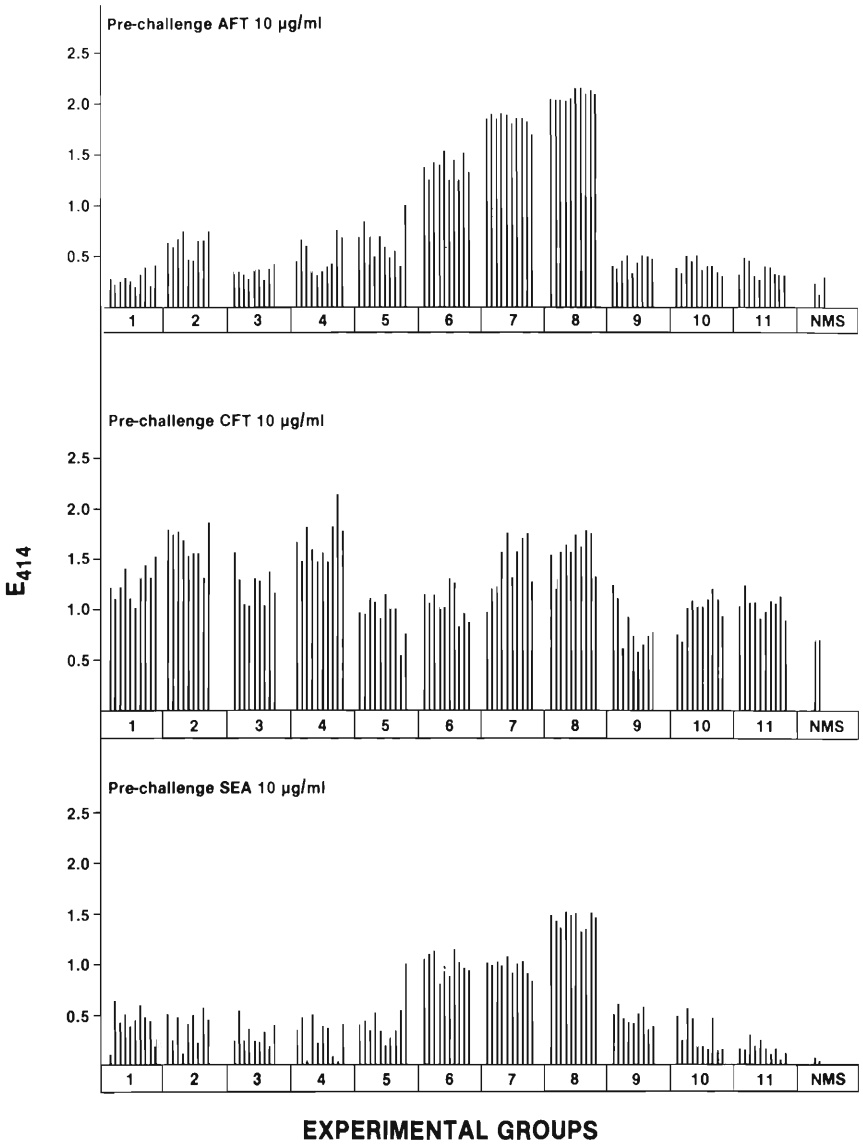

Figure 5. Serum antibody levels against crude antigens prior to challenge with cercariae. Data expressed as absorbance at 414 nm for each mouse tested, as determined by EIA.

vidual, in much the same way as hepatitis and rubella titers are used today. Although we did find a slight inverse correlation between IgG antibody titer against FhConA and worm burden (Fig. 6), the value of this correlation in predicting infection levels is dubious. Even at maximum antibody levels, as detected by EIA, worm burden was reduced by only 26%. This reinforces the notion that antibody is only one of several factors involved in immunity to schistosomiasis.

The inverse correlation between FhConA antibody titer and worm burden was greater for male worms than for females. This may simply reflect the fact that the male dorsal tegument is more exposed and, therefore, more susceptible to the effects of antibody. On the other hand, there may be significant biochemical differences between the antigenic faces presented by male and female worms as suggested by Brink (1977), Ruppel and Cioli (1977) and Podesta (pers.

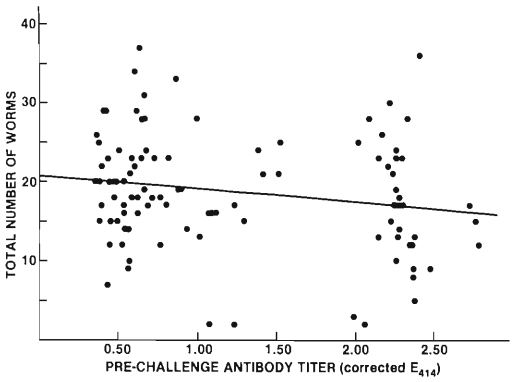

Figure 6. Correlation between worm burden and pre-challenge antibody titer against FhConA ($N = 105$; $r = -0.20$; $F = 3.91$; $P < 0.05$).

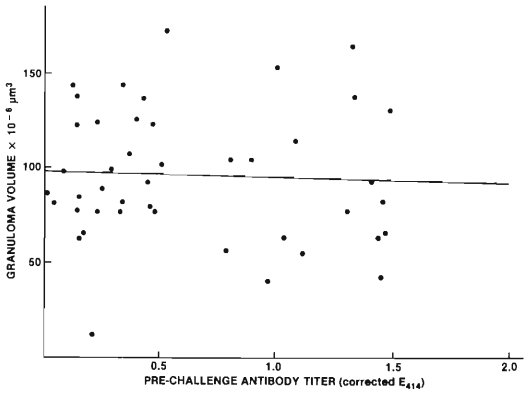

Figure 7. Correlation between granuloma size and pre-challenge antibody titer against SEA ($N = 44$; $r = -0.054$; $F = 0.1216$; $P =$ not significant).

comm.). Although SmConA and FhConA are similar in terms of molecular weight, pI value, affinity for concanavalin A and reactivity with antisera, FhConA proved to be a more effective immunogen. This suggests that subtle differences between these two fractions may be important immunologically. It is possible that the protein moiety of the glycoprotein is the "relevant" antigen, whereas the ubiquitous terminal carbohydrates have a limited functional role in immunity. In this regard, there is evidence to suggest that the immune response can be enhanced by immunization with peptide fragments instead of intact native antigens (Lerner, 1982).

The inability to bring about a major reduction in worm burden by inoculation of glycoprotein antigens may not necessarily represent a failure, because pathology in schistosomiasis is associated primarily with egg deposition. Comparison of worm burden and egg burden revealed little correlation between the two (Fig. 1). However, there was a striking inverse correlation between the number of female worms and the number of eggs deposited in the liver by each female (Fig. 2). If, as we assume, hepatic egg burden in acute infections is representative of total egg output, then we have observed decreased fecundity associated with increased worm burden.

A similar decrease in egg production has been documented for the intestinal nematodes *Ascaris lumbricoides* (Croll et al., 1982), *Necator americanus* (Hill, 1926) and *Ancylostoma duodenale* (Anderson and May, 1982). In the tapeworm *Hymenolepis diminuta* crowding is evidenced by a decrease in total length and number of gravid proglottids (Read, 1951; Roberts, 1961), which implies reduced fecundity. Although a crowding effect has been demonstrated for cercarial production in snails (MacDonald, 1965), the evidence for crowding of adult *Schistosoma mansoni* has been contradictory. Whereas an inverse relationship between worm length and intensity of infection has been reported in some instances (Radke et al., 1957; Grimaldo and Kershaw, 1961), Lennox and Schiller (1972) were unable to demonstrate a crowding effect. They speculated instead that "because of the extreme pathology that accompanies heavy worm burdens in schistosomiasis, it is likely that the weight and physiological condition of the parasites are influenced more by the poor physical condition of the host than by some intrinsic density-dependent factor" (Lennox and Schiller, 1972, p. 493). Data from human autopsies (Cheever, 1968b; Cheever et al., 1977; Anderson and May, 1982) and from experimental infections of the grivet monkey *Cercopithecus aethiops aethiops* (Cheever and Duvall, 1974) indicated decreased oviposition in heavier infections. However, interpretation of data from these studies is complicated by changes in fecundity in older female worms and by destruction of tissue eggs in chronic infections. In chronic infection, too, the immune response of the host may play an important role in limiting both growth and reproduction of the parasites. Our findings in acute, experimentally infected mice show clear evidence of reduced fecundity as the intensity of infection increases, and thus support the observations of Harrison et al. (1982) who also found evidence of a crowding effect in mice in early infections.

Whether due to a crowding effect or some other

"negative feedback" mechanism, a decrease in fecundity serves to regulate population size and is a fairly common strategy used by many parasites (Kennedy, 1975; Anderson, 1982). Crowding results from competition for a limited resource such as space or nutrients (perhaps a particular amino acid may be essential for egg shell formation). Negative feedback mechanisms include such things as the immune response of the host to large numbers of parasites or large quantities of parasite secretions. Regardless of the mechanisms involved, our findings suggest that even had the "vaccine" been successful in reducing worm burden, the egg burden would have remained unacceptably high. For this reason, we feel that worm burden alone should not be the sole criterion for vaccine evaluation.

Although neither worm burden nor egg burden were diminished to an appreciable extent in this study, we did observe a marked reduction in granuloma size, presumably as a result of immunization. At the one point in time that we examined (8 weeks after infection) egg-induced hepatic granulomata were significantly smaller in two groups of "immunized" animals than in the controls. In some mice, the reduction was striking (Fig. 3). We do not know whether this finding represents an absolute reduction of granuloma size throughout the course of infection, or whether it merely reflects an acceleration in immunized animals of the normal attenuation of lesions that occurs in time. The mechanisms involved in granuloma modulation remain obscure, as we found no correlation between granuloma size and IgG antibody levels either before (Fig. 7) or after challenge, which is consistent with previous reports that antibody-mediated reactions play little or no role in granuloma formation (Boros and Warren, 1970). Nevertheless, the ability to diminish one of the most serious pathological sequelae in this disease by immunization would represent a major breakthrough. Indeed, such an approach was suggested years ago by Warren (1972). If our results are found to be routinely reproducible, then the concanavalin A-binding glycoproteins from either *S. mansoni* or *F. hepatica* may prove to be excellent candidates for an anti-granuloma killed vaccine.

Perhaps the most significant finding of this study involves neither granuloma attenuation nor reduction in the intensity of infection, but rather the change in distribution or "dispersion" of parasites in "immunized" animals. In examining the worm burden data in Table 2, it can be seen

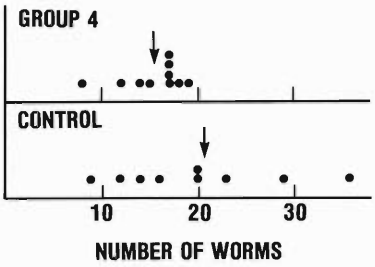

Figure 8. Frequency distribution of parasites in mice "immunized" with FhConA antigen (Group 4) as compared to the control group. Arrows indicate the arithmetic means of each group.

that in the control (Group 11) the variance (s^2 = 74.8) far exceeds the mean ($\bar{x}$ = 20.8) giving a variance to mean ratio ($s^2/\bar{x}$) of 3.6, which suggests other than normal frequency distribution. In contrast, worm burden in the "immunized" Group 4 clearly follows a normal Poisson distribution. Thus, although the means of these groups do not differ greatly, there is a major difference in the frequency distribution between the two groups (Fig. 8). In the "immunized" group, one does not find individual mice with very heavy infections as one does in the control group.

The kind of non-random frequency distribution or "overdispersion" found in the control group is typical of most parasite populations in both natural (Kennedy, 1975) and experimental (Kennedy, 1974) infections. In an overdispersed distribution, a large number of parasites are concentrated into relatively few hosts. Overdispersion implies that "the flow of parasites through all the hosts in a population may not be similar . . . and the *mean* number of parasites per host may obscure important differences and be misleading. The *single* very heavily infected *individual* may be the important animal as far as survival and transmission of the parasite to the next host is concerned" (Kennedy, 1975, p. 69). This suggests a radically different approach to the control of parasitic diseases. If complete control cannot be achieved, an alternative strategy might be aimed at reducing morbidity in the population by reducing the number of hosts with massive infections. In this regard, selective treatment by chemotherapy targeted at the most heavily infected individuals has been proposed as an effective means of reducing the abundance of the parasite and, hence, the frequency of symptoms and transmission of disease (Anderson and

May, 1982). By analogy, in the absence of complete protection, a "vaccine" that prevents massive infection might offer an acceptable compromise in hyperendemic areas.

In all fairness, our data supporting such a hypothesis are weak, being derived from only a single experiment. The frequency distribution in Group 11, although suggestive of overdispersion, does not fit closely the negative binomial model of Bliss and Fisher (1953). Furthermore, the apparent shift in distribution was not found in all treatment groups. However, we feel that the phenomenon observed may be an important one, and that the population dynamics of helminths should receive attention in future immunological studies.

Acknowledgments

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Larval Didymozoids (Trematoda) in Fishes from Moreton Bay, Australia

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ABSTRACT: Nine species of immature didymozoids (Trematoda, Didymozoidae) were found in small intertidal fishes belonging to 13 families from Moreton Bay, Queensland, Australia. Most of them are previously undescribed.

The larvae occurred either between muscle fibers, or encapsulated in the wall of the alimentary tract or mesentery, or free in the stomach. One was found in a copepod that had been ingested by a goby.

The small fishes probably function as third intermediate hosts, being infected by feeding on crustacean second intermediate hosts or invertebrate transport hosts. The first intermediate hosts, probably molluscs, are unknown. Larger predatory fishes act as final hosts. It was not possible to correlate the larval didymozoids with adults from local predatory fishes.

The nine species could be differentiated from each other by the structure of the digestive tract, particularly the number of cecal chambers, which varied from 5 to 16. One species had one short and one long cecum, and one species had prominent eyespots.

Each of five species occurred in one fish species only. The remaining four species were not host-specific as they were found in fishes belonging to from 3 to 12 families.

Fischthal and Kuntz (1964), Fischthal and Thomas (1968), Nikolaeva (1965, 1970) and Kurochkin and Nikolaeva (1978) have reviewed known larval didymozoids from fishes and described new forms. Studies of small fishes from Moreton Bay, Queensland, Australia, revealed several species of larval didymozoids, most of which were previously undescribed. An attempt to elucidate a didymozoid life-cycle in the area was unsuccessful. The immature didymozoids are compared with previously described immature didymozoids from other fishes and from invertebrates.

Material and Methods

The fishes were caught in December 1983 and January 1984 using a beach seine. Most fishes were caught at Wellington Pt., central Moreton Bay. A small number were caught at Caloundra at the extreme northern part of Moreton Bay, and some were caught south of Moreton Bay close to the border between Queensland and New South Wales.

During dissection the alimentary tract was examined and the body musculature teased apart. Larval didymozoids were fixed in glacial acetic acid:40% formaldehyde (19:1) and stored in 70% alcohol. Some were mounted unstained in poly-vinyl lacto-phenol, others were stained using carmalum, then dehydrated and mounted in Canada balsam. Specimens with eyespots were transferred directly from the fixative to poly-vinyl lacto-phenol as the eye pigment disappeared in alcohol. Measurements in μm are based on stained specimens (whole mounts).

Results

Fishes belonging to more than 15 families were examined. Infected fishes, which included both adults and juveniles, measured between 3 and 10 cm in total length. None of the examined fishes longer than 10 cm was found infected. Where different size groups of one species were examined, e.g., of *Sillago maculata* and gobies, more didymozoid larvae were recovered from the smaller size group than the larger. Whereas most small fishes from Moreton Bay harbored one or more species of immature didymozoids (Table 1), most fishes from Caloundra were uninfected, and the infected ones were less heavily infected and had only species that were common in the fishes caught in Moreton Bay. None of the small fishes from the oceanic coast of southern Queensland were infected, although most belonged to species that were infected in Moreton Bay. This suggests that the small fishes (Table 1) become infected in Moreton Bay and that the molluscan hosts live in this area. Examination of a large number of intertidal molluscs did not reveal didymozoid cercariae, suggesting that the molluscan hosts occur subtidally. A didymozoid metacercaria (species 1) was found in a copepod that had been ingested by a goby.

Didymozoidae Poche, 1907

GENERAL FEATURES OF SPECIES 1–9: Body subcylindrical with rounded extremities. Thick, unarmed tegument. Oral sucker terminal with terminal mouth. Pharynx spherical, immediately posterior to oral sucker. Preequatorial muscular acetabulum. Preacetabular cecal bifurcation, posterior to mid-forebody. Ceca terminating close to posterior end of body (except the short right

Table 1. Larval didymozoids in fish (3-10 cm long) from Moreton Bay.

Number examined	Host	Species 1	2	3	4	5	6	7	8	9
	Engraulidae									
30	*Stolephorus bataviensis* Hardenberg	x		x				xxx	x	
	Atherinidae									
4	*Pranesus ogilbyi* Whitley	x				(x)		xxx		
	Ambassidae									
4	*Velambassis jacksoniensis* (MacLay)	x	x					xx		
	Scorpaenidae									
30	*Centropogon marmoratus* (Gunther)	x								
	Theraponidae									
40	*Pelates quadrilineatus* (Bloch)	x	x					x		
	Sparidae									
20	*Acanthopagrus australis* (Gunther)	x								
	Gerridae									
30	*Gerres ovatus* Gunther	x								
	Sillaginidae									
30	*Sillago maculata* Quoy and Gaimard	xx–xxx	x–xx				x	x		x
	Mullidae									
3	*Upeneus tragula* Richardson				x					
	Gobiidae									
40	Unidentified specimens	xx–xxx						x		
	Canthigasteridae									
20	*Spheroides hamiltoni* (Gray and Richardson)	x				(xxx)		x		
	Aluteridae									
10	*Scobinichthys granulatus* (Shaw)	x						x		
10	*Monacanthus chinensis* (Osbeck)									
	Bothidae									
6	*Pseudorhombus jenynsii* (Bleeker)	xx–xxx	x			x–xx		x		

x: Rare, maximum of one specimen in each fish.
xx: Relatively common, most often 2–10 specimens in each fish.
xxx: Common, most often more than 10 specimens in each fish.
( ): One fish, data of Mr. P. W. Shield (pers. comm.).

cecum of species 8). Cecal chambers (except species 9) jointed by tubular canals. Excretory vesicle postcecal; pore terminal. One median ventral excretory duct. No eyespot or eyespot pigment (except species 7). Cerebral commissure between pharynx and cecal bifurcation. No reproductive rudiment.

SPECIMENS DEPOSITED: Zoological Museum, Copenhagen, Denmark.

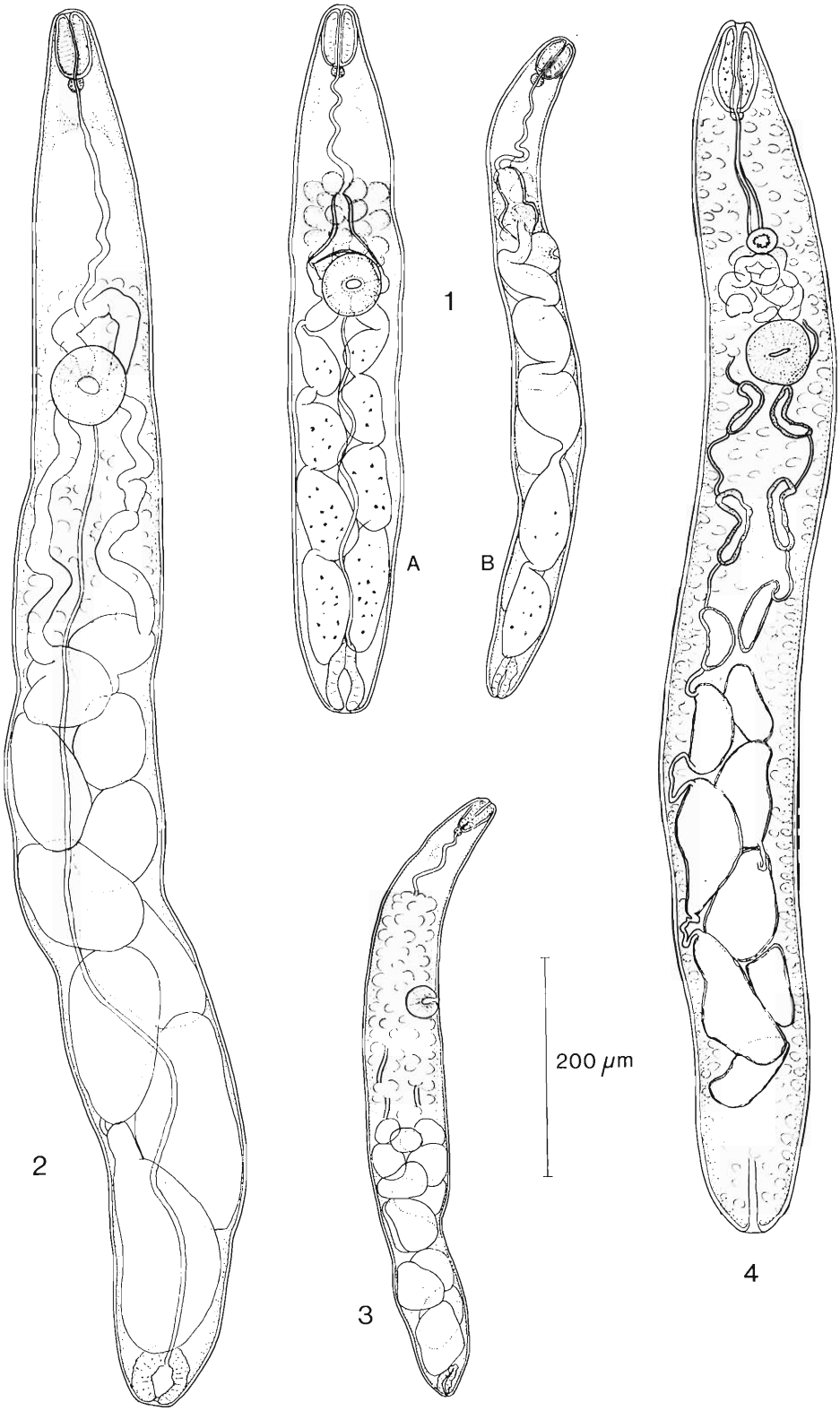
Species 1
(Fig. 1A, B)

HOSTS: See Table 1.

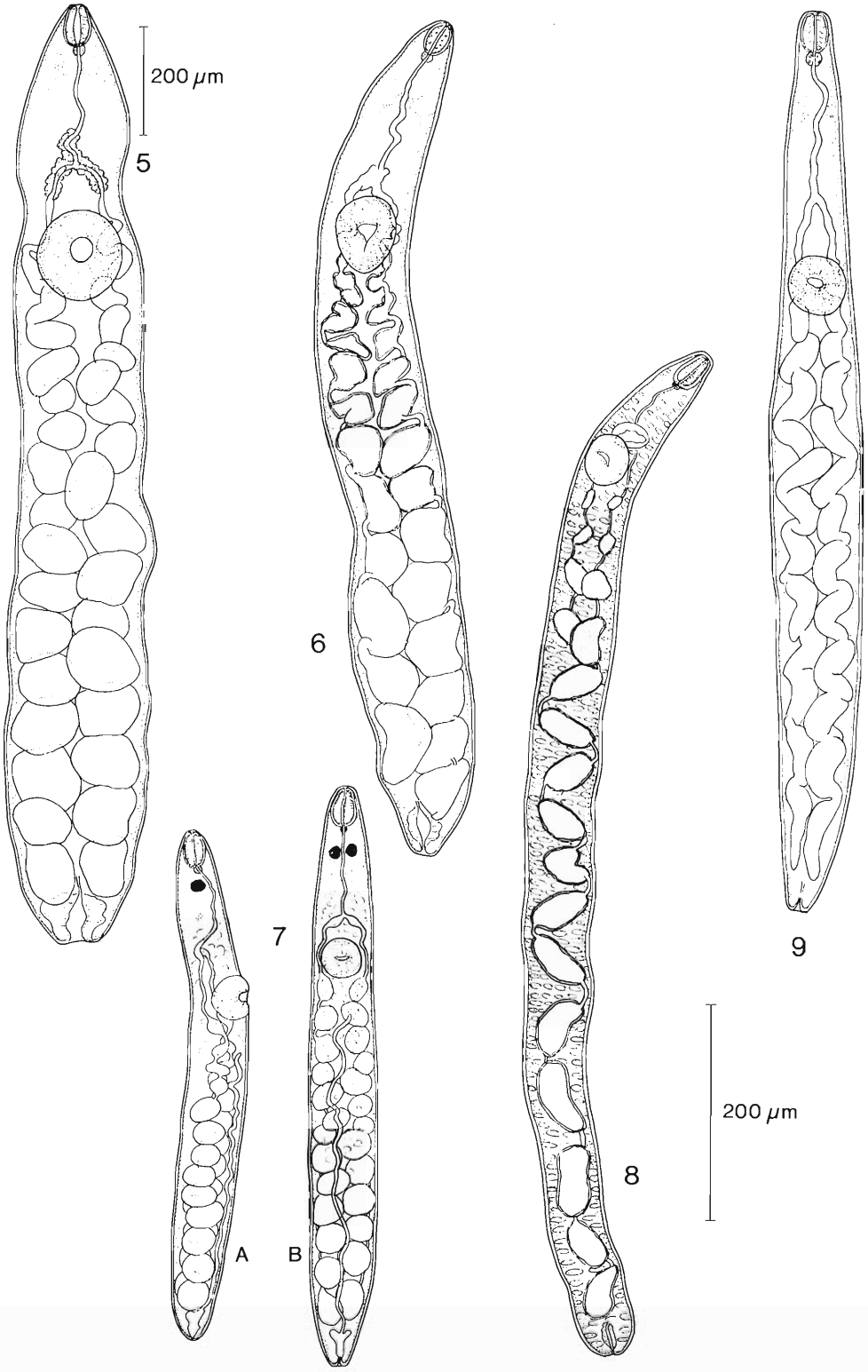
SITES: Encapsulated in external wall of alimentary tract and mesentery and free in stomach. On two occasions a specimen was found in the body cavity of a copepod from the stomach of a goby.

DESCRIPTION (based on 20 specimens from different hosts): Body 200–800 (450) long by 28–100 (70) wide at widest level. Oral sucker 20–60 (40) by 10–40 (26) composed of outer thin layer of longitudinal and inner thick layer of circular muscles. Pharynx 4–12 (8). Acetabulum 18–56 (36) in diameter. Length of forebody (anterior border of acetabulum to anterior tip of body) 72–260 (154), its percentage of body length 33–36 (34). Esophagus, thin-walled, sinuous, lumen gradually enlarging distally. "Stomach," thick-walled, surrounded by numerous gland cells. Cecal bifurcation at anteriormost acetabular level. Each cecum composed of anterior tubular part and five dilated, thin-walled chambers with viscous fluid containing granular material. Excretory vesicle thick-walled.

REMARKS: This species was illustrated by Les-



Figures 1–4. Larval didymozoids, ventral views except Figure 1B. All to same scale. 1A. Species 1, ventral view. 1B. Species 1, lateral view. 2. Species 2. 3. Species 3. 4. Species 4.



Figures 5–9. Larval didymozoids, ventral views except Figure 7A. All to same scale except Figure 5. 5. Species 5. 6. Species 6. 7A. Species 7, lateral view. 7B. Species 7, ventral view. 8. Species 8. 9. Species 9.

ter (1980). Other larval didymozoids that also contain both a "stomach" and moniliform ceca have been reported from a variety of fishes (Nikolaeva, 1965, 1970; Fischthal, 1982, and others). This species differs from them by having a smaller number of cecal chambers. It is most similar to a didymozoid metacercaria from a copepod (Madhavi, 1968), but the latter has a slightly larger acetabulum and no pharynx.

Species 2
(Fig. 2)

HOSTS: See Table 1.

SITES: Body muscles and mesentery.

DESCRIPTION (based on 10 specimens from different hosts): Body 310–1,280 (785) by 65–140 (103). Oral sucker 35–70 (50) by 24–46 (36), entirely muscular (as species 1). Pharynx 8–16 (12). Acetabulum 40–72 (60) in diameter. Length of forebody 130–300 (204), its percentage of body length 23–42 (26). Esophagus narrow, thin-walled. Ceca sinuous, surrounded by gland cells, continuing in posterior half of body as five pairs of oval chambers; gland cells extending about one acetabular length anterior to acetabulum and two to three lengths posterior to it. Chambers thin-walled. Excretory vesicle thick-walled.

REMARKS: This species mostly resembles *Distomum* sp. described by Linton (1905, fig. 214) and *Torticaecum nipponicum* Yamaguti, 1942. It differs from the former by a different arrangement of the glands and larger acetabulum. It differs from *T. nipponicum* by having a pharynx (Yamaguti, 1942, interpreted the muscular oral sucker as a pharynx).

Species 3
(Fig. 3)

HOSTS: *Stolephorus bataviensis* Hardenberg.

SITES: Body muscles.

DESCRIPTION (based on three specimens): Body 330, 530, and 570 by 36, 70, and 70. Oral sucker 16, 38, and 38 by 8, 20, and 20, muscular, pyriform, widest anteriorly. Pharynx 4, 8, and 8. Acetabulum 20, 28, and 28 in diameter. Length of forebody 114, 175, and 190, its percentage of body length 35, 33, and 33. Esophagus narrow, sinuous. Cecal bifurcation not seen due to numerous large glandular cells occupying posterior half of forebody and anterior third of hindbody. Ceca narrow, tubular in anterior third of hindbody, each continuing posteriorly into seven thin-walled spherical to oval chambers.

REMARKS: This species differs from all previously described immature didymozoids by its arrangement of gland cells and its narrow, tubular postacetabular ceca.

Species 4
(Fig. 4)

HOST: *Upeneus tragula* Richardson.

SITE: Body muscles.

DESCRIPTION (based on single specimen): Body 1,150 by 130, filled with vesicular parenchyma. Oral sucker 82 by 44, pyriform, widest posteriorly, composed of outer thick layer of longitudinal muscles and inner non-muscular part. Pharynx 14, acetabulum 60 in diameter. Length of forebody 290, its percentage of body length 25. Esophagus with increasing lumen and thicker walls posteriorly, ending in muscular dilation. Remaining preacetabular digestive system concealed by glands. Ceca posterior to acetabulum form seven chambers connected by long tubular ducts, chambers larger and more thin-walled posteriad until the second and third from the posterior end.

REMARKS: This species differs from all known immature didymozoids by the shape of the intestinal chambers.

Species 5
(Fig. 5)

HOSTS: See Table 1.

SITES: Body muscles and mesentery.

DESCRIPTION (based on one large [ ] and five smaller specimens, all from *P. jenynsii*): Body [1,720 by 250], 700–1,230 (962) by 110–210 (148). Oral sucker [68 by 54] 40–55 (46) by 22–35 (30), entirely muscular. Pharynx [22] 12–16 (14). Acetabulum [165] 70–130 (94) in diameter. Length of forebody [380] 190–290 (236), its percentage of body length [22] 22–28 (25). Esophagus sinuous, thin-walled. Gland cells line distal part of esophagus and proximal parts of ceca. Ceca tubular to midacetabular level, continuing into dilated winding, thin-walled ducts which postacetabularly form [15] 11–12 spherical to oval chambers. Excretory vesicle thick-walled.

REMARKS: This species appears very similar to or identical with immature Didymozoid E described by Fischthal and Thomas (1968).

Species 6
(Fig. 6)

HOST: *Sillago maculata* Quoy and Gaimard.

SITE: Body muscles.

DESCRIPTION (based on single specimen): Body

800 by 100. Oral sucker 38 by 22, composed of outer longitudinal muscles and inner non-muscular part. Pharynx 7. Acetabulum 70 by 58. Length of forebody 180, its percentage of body length 23. Esophagus sinuous with dilation at bifurcation area. Ceca at acetabular level tubular, winding, continuing posteriorly into 11 and 13 chambers, which sequentially become more thin-walled and larger.

REMARKS: Species 6 appears very similar to or identical with immature Didymozoid A described by Fischthal and Kuntz (1964). Superficially it also resembles metacercaria Didymozoidae gen. sp. larvae V Nikolaeva, 1962 (see Nikolaeva, 1965), though this apparently comprises more than one species.

Species 7
(Fig. 7A, B)

HOSTS: See Table 1.

SITES: Body muscles and free in stomach.

DESCRIPTION (based on 20 specimens from different hosts): Body 190–560 (335) by 27–70 (46), filled with large vesicular cells. Oral sucker 15–36 (28) by 8–25 (14), entirely muscular (as species 1). Pharynx 5–8 (6). Acetabulum 16–36 (25) in diameter. Length of forebody 70–130 (100), its percentage of body length 23–37 (31). Eyespot 3–12 (7) in diameter, irregular in shape, often slightly crescent-shaped when seen ventrally, but oval and 4–15 (10) wide when seen laterally. The red eye pigment disappears in alcohol. Esophagus sinuous. Proximal preacetabular ceca slightly dilated. Ceca tubular at acetabular level. Each cecum continues postacetabularly into 13 oval to spherical thin-walled chambers. Anterior 3–4 chambers filled with granular material that may be found also in the narrow cecal branches and esophagus. Excretory duct terminating posterior to acetabulum without bifurcating.

REMARKS: This species most closely resembles a metacercaria found in a coelenterate from the Mediterranean Sea (Dollfus, 1963). The latter had no eyespots but Dollfus suggested that granules immediately behind the oral sucker were pigmented. Larval didymozoids with red eyespots have previously been described by Nikolaeva (1965) in fishes from the Mediterranean Sea.

The eyespots of species 7 are larger in large individuals than in small individuals. In other digeneans cercarial eyespots gradually disappear in later developmental stages. Thus the function of the eyespots of this didymozoid may differ from that of other digeneans. It is conceivable that they are used during the migration inside the final host, i.e., if the final site is close to the surface where some light may penetrate. No remains of eye pigment have been described in any adult didymozoids. Linton (1907) drew what appear to be eyespots in *Distomum tomex* (=*Gonapodasmius tomex* (Linton, 1907) Yamaguti, 1971), although these were not mentioned in the text.

Species 8
(Fig. 8)

HOST: *Stolephorus bataviensis* Hardenberg.

SITE: Body muscles.

DESCRIPTION (based on five specimens): Body 1,000–1,530 (1,200) by 70–100 (82). Pseudosegments closely arranged throughout body, all filled with large vesicular cells. Oral sucker 35–48 (40) by 20–30 (24), entirely muscular (as species 1), pyriform, widest posteriorly, often fixed partially protruded. Pharynx 5–8 (7). Acetabulum 40–60 (50) in diameter. Length of forebody 105–144 (124), its percentage of body length 9–11 (10). Esophagus, thin-walled, narrow. Muscular "stomach" overlapping anteriormost part of acetabulum. Ceca thin-walled and tubular at acetabular level. Right cecum with four (in one worm five) oval, thick-walled chambers, left cecum with 16 chambers.

REMARKS: This species differs from all known immature didymozoids in having one short and one long cecum. The only known didymozoid with one cecum, *Angionematobothrium jugulare* Yamaguti, 1970 (Nematobothriinae), differs from species 8 in that the esophagus of the former leads directly into the single intestine, and in the absence of an acetabulum.

Species 9
(Fig. 9)

HOST: *Sillago maculata* Quoy and Gaimard.

SITE: Body muscles.

DESCRIPTION (based on single specimen): Body 850 by 88. Oral sucker 40 by 28, entirely muscular. Pharynx 14, inner part non-muscular, composed of vesicular cells. Acetabulum 58 in diameter. Length of forebody 230, its percentage of body length 27. Esophagus sinuous. Ceca tubular at preacetabular and acetabular levels, dilated postacetabularly, descending in zigzag fashion, with nearly uniform diameter and not forming discrete chambers.

REMARKS: This species is most similar to *Dis-*

tomum fenestratum Linton, 1907, but the latter had no pharynx.

Discussion

No didymozoid life-cycle has been worked out experimentally. It is likely that the didymozoid life-cycles show the same patterns as those of Hemiuridae (see Cable, 1956; Nikolaeva, 1965). Two cystophorous cercariae developing in unrelated snails have been thought to belong to Didymozoidae because cercariae still within the snails had developed into typical didymozoid larvae (Manter, 1932; Cable, 1956). This is an unusual development, as normally the cystophorous cercariae must emerge from the snail host to complete its development. Didymozoid metacercariae have been found in pelagic copepods (Madhavi, 1968; Reimer et al., 1971; present study) and in *Lepas* sp. (Cable and Nahhas, 1962) indicating that the cystophorous cercariae are eaten by crustaceans as described for hemiurids (Køie, 1979). Didymozoid metacercariae have also been found in chaetognaths, coelenterates, ctenophores and a polychaete (e.g., by Reimer et al., 1971, 1975). These planktonic invertebrates presumably become infected by eating the crustacean host (the second intermediate host) and thus may function as transport hosts as occurs in some hemiurids (Køie, 1983). Larval didymozoids have also been recorded from cephalopods (Overstreet and Hochberg, 1975).

The small fishes presumably acquire their infection by ingesting crustacean second intermediate hosts or invertebrate transport hosts. The larval didymozoids grow in the small fishes, but they were never found mature in these hosts. The small fishes may act as third intermediate host in an obligatory four-host life-cycle. Larger predatory fishes act as final hosts. Similar life-cycles have been described for hemiurids (Chabaud and Campana-Rouget, 1959). Three-host life-cycles also occur in the Didymozoidae, e.g., mollusc–copepod–flying fish (Nikolaeva, 1981) but they may not be as common as the four-host cycles.

The small fishes are well-adapted intermediate hosts. Most larval didymozoids occurred in the body muscles parallel with the muscle fibers. There was no evidence of host tissue reaction. Specimens belonging to species 1 occurred on the external wall of the digestive tract and in the mesentery, and were always encapsulated in a sheath of host connective tissue (Lester, 1980). No dead worms were found.

It was impossible to correlate the larval didymozoids with mature specimens as most of the adult didymozoids in fishes from south-eastern Queensland have not been studied.

The planktophagous *S. bataviensis* had two species that were not found in the other fishes, and the benthophagous *U. tragula* had a species that only occurred in this fish (Table 1). Species 7 dominated in planktophagous fishes as *S. bataviensis* and *P. ogilbyi,* whereas species 1 dominated in fishes as *S. maculata,* gobies, and *P. jenynsii* with a mainly benthophagous habit. Species 1 and 7 were found in most families (Table 1). It is not known whether the different occurrence in different fish families is due to host-specificity or is due to different feeding habits. The fact that larval didymozoids do not accumulate in mainly piscivorous fishes such as *C. marmoratus* indicates that a transfer from one fish to another does not or rarely take place, in accordance with the observations by Lester (1980), although this may also be explained by the presence of some host-specificity.

The final hosts of species 3, 7, and 8, which were common in pelagic fishes, may be pelagic piscivorous such as tuna, whereas species 1, 4, and 5, which were common in benthic fishes may mature in demersal piscivorous such as platycephalids.

The concentration of infected third intermediate hosts in Moreton Bay is difficult to explain. The didymozoid fauna of the Pacific Ocean is rich and varied. This ocean is apparently the center of origin of the didymozoids (Nikolaeva, 1981). About ⅔ of the 212 species of adult didymozoids known until 1981 (Nikolaeva, 1981) occur in the Pacific Ocean, and most of these species have been found in Hawaiian fishes (Yamaguti, 1970). Many didymozoids still remain to be described so the total number of didymozoids should probably be doubled or more.

As most didymozoids probably have a four-host life-cycle several hundred species of larval didymozoids must be expected to occur in fishes. At the moment it is impossible to correlate these immature developmental stages with mature specimens.

The classification of larval didymozoids in fishes has been discussed by Nikolaeva (1965), Yamaguti (1970, 1975), and Kurochkin and Nikolaeva (1978). Yamaguti (1942) described *Monilicaecum* sp. and *Torticaecum* sp. from Japanese fishes. They mainly differed by the shape of the intestine (hence the names) and by the presence or absence of a "stomach." Yamaguti (1970, 1975) suggested the names as collective group names, which should include all larval di-

dymozoids. Nikolaeva (1965) differentiated seven immature didymozoids from each other in body size, the body length to width ratio, the distance between suckers, and in parasitizing different hosts. Fischthal and Kuntz (1964) and Yamaguti (1970, 1975) doubted the feasibility of this classification as the characters used are extremely variable and change with age. Kurochkin and Nikolaeva (1978) suggested a temporary system based on the presence or absence of acetabulum, pharynx, "stomach" and gland cells, and on the relative position of the cecal bifurcation and the acetabulum. They differentiated 11 "genera" from each other. However, the "stomach" is poorly defined, and it always disappears with age. Examination of younger developmental stages often reveals both a pharynx and an acetabulum. These structures are therefore unsuitable for classification.

With the present knowledge it is impossible to classify the larval didymozoids to generic or higher taxonomic levels, and the larval didymozoids should not be given Latin names to avoid a double taxonomic system, one for the larval and one for the mature didymozoids.

Acknowledgments

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The Effects of Parasitism and Long Term Cutaneous Respiration Upon the Survival and Egg Production of the Lymnaeid Snail, *Stagnicola bulimoides techella*

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ABSTRACT: Twenty *Stagnicola bulimoides techella* were maintained underwater without access to air, and an equal number of snails were maintained as controls with access to air. Ten snails from each group were infected with the liver fluke *Fasciola hepatica*. The life spans of uninfected and infected snails were not significantly shortened by the lack of atmospheric oxygen. Cercariae were shed approximately 6 weeks postexposure from snails with or without access to air. None of the infected snails from either group produced eggs. All 10 uninfected snails with access to air produced eggs whereas 9 of 10 uninfected snails maintained underwater did not.

Pulmonate lymnaeid snails, despite their ability to utilize atmospheric oxygen through their pulmonary sacs, are capable of shifting from pulmonary to cutaneous respiration and may complete their entire life cycle underwater without access to air as demonstrated by Noland and Reichel (1943) for *Lymnaea stagnalis*. The purpose of this investigation was to determine the effects of parasitism (with *Fasciola hepatica*) and long term cutaneous respiration upon the life span of *Stagnicola bulimoides techella*.

Materials and Methods

Twenty immature (less than 5 weeks old) snails were separated into two groups of 10 snails for each of two experiments performed 6 months apart. One group was infected with *F. hepatica* (two miracidia per snail), the other group was not exposed. Five snails from each group were maintained individually in underwater chambers, and the remaining snails were maintained as controls with access to air in individual fingerbowls.

The underwater chambers were composed of fiberglass netting (mesh size 0.36 mm²) capped at either end with small glass dishes. Two underwater chambers were submerged in each 7.5-liter battery tank which contained 4.5 liters of spring water. An airstone in the center of the tank (powered by an aquarium pump) kept the water circulating and well oxygenated, but was far enough from the chambers to prevent air bubbles from entering. Twice a week approximately ⅓ of the water was siphoned off and fresh water added. A Winkler analysis was performed weekly to ensure the water was well oxygenated (approximately 8 ppm). Snails were fed boiled lettuce and tadpole food (Carolina Supply Company). The air bubble present in the snail's pulmonary sac was forced out by placing a blunt probe in the aperture of the shell before placing the snail in the chamber. Once a week the probe was used to verify that the snail had not acquired another bubble.

Results

The survival time (in days) for each snail is recorded in Table 1. The mean survival time was longer for uninfected snails than infected snails in both experiments. Combining the data from both experiments showed that the average life span for uninfected snails was 1 week longer (120.0 days underwater, 137.9 days with access to air) than infected snails (113.4 days underwater, 131.4 days with access to air), however a one-way ANOVA showed this difference was not statistically significant at $P \leq 0.05$. The lack of atmospheric oxygen did not appear to interfere with the development of the parasite, as snails

Table 1. Comparison of the life spans (days) of infected and uninfected *Stagnicola bulimoides techella* maintained with and without access to atmospheric oxygen.

	Infected		Uninfected	
	Underwater	In air	Underwater	In air
Experiment I				
	134	127	152	148†
	144	140	157†	173†
	97	161	144	133†
	112	119	129	142†
	121	123	34	128†
Experiment II				
	135	108	156	75†
	150	120	56	141†
	57*	122	105	143†
	42*	149	116	139†
	142	145	151	157†
Overall	—	—	—	—
Sample size	10	10	10	10
Mean	113.4	131.4	120.0	137.9
Standard error	11.83	5.22	13.76	8.05

* Died before shedding.
† Produced eggs.

maintained underwater shed cercariae at approximately the same time as those snails with access to air (within 13 days of each other).

Discussion

Noland and Reichel (1943) showed that *L. stagnalis* raised without access to atmospheric oxygen took several months longer to mature and produce eggs than those raised with access to air. It should be noted, however, that unlike *L. stagnalis* studied by these authors, *S. b. techella* tends to be more amphibious and may therefore be more susceptible to stress when raised in the absence of atmospheric oxygen. Whereas the lack of atmospheric oxygen does not significantly shorten the life span of *S. b. techella* or interfere with the development of *F. hepatica* it does appear to cause some metabolic stress as only one uninfected snail maintained underwater produced eggs, and all 10 uninfected snails with access to air produced eggs. None of the infected snails produced eggs. It is possible that the energy drain from the energy budget of this snail host by the asexual reproductive effort of *F. hepatica* is sufficient to reduce and even prevent egg production in the snail. However, the effects of parasitism on snail reproduction should be more fully studied. No attempt was made to weigh or measure the snails (in order to minimize stress), so no conclusions could be drawn as to the effects on growth rate.

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Genital Ganglion and Associated Structures in Male *Neoechinorhynchus cylindratus* (Acanthocephala)

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ABSTRACT: The genital ganglion of male *Neoechinorhynchus cylindratus* consists of two 18- or 19-cell accumulations connected by a large ventral and smaller dorsal commissure. An additional midventral cell is located in the ventral commissure. No binucleate cells occur. Each cell has an ovoid to round centrally located nucleus with well-developed nucleoli. Perinuclear rings are common. The pair of ganglia are located at the junction of the anterior bursal musculature with Saefftigen's pouch. Each ganglion measures approximately 0.050 mm long by 0.055 mm at its widest point. The relationship of the cement gland ducts, vas deferens, and bursa musculaure is illustrated in relation to the ganglia.

Although earlier writers (Kaiser, 1893; Kilian, 1932) had discussed the peripheral nervous system, the general organization of the male genital ganglion into two accumulations of cells connected by commissures was first discovered by Schneider (1868) in his study of *Macracanthorhynchus hirudinaceus*. Using this same species, Kaiser (1893) enlarged on Schneider's description by identifying individual nerves as well as what they innervate. Thus, by 1900 a general description of the male genital ganglion was known which Kaiser (1893, p. 12) stated was also connected to the cerebral ganglion by way of nerve pathways in the longitudinal muscles.

At present, several species have had some information published on the number of cells associated with the genital ganglion. The only species in the order Neoechinorhynchida studied has been *Gracilisentis gracilisentis*. Van Cleave (1914) reported that in this species each lateral accumulation had 18 cell bodies. However, he did not further describe either the cells or their organization.

This paper identifies the cells in the genital ganglion of *Neoechinorhynchus cylindratus* and illustrates relationships between ganglion, adjacent musculature and duct work.

Materials and Methods

Neoechinorhynchus cylindratus were removed from largemouth bass, *Micropterus salmoides*, collected by angling from several Southern Illinois lakes located in Jackson County. Following removal, worms were cleaned of adhering debris and preserved overnight at 4°C in 3% glutaraldehyde buffered with 0.1 M cacodylate (pH 7.2). No attempt was made to control osmotic pressure during fixation. Samples were dehydrated through a graded ethanol series and embedded in JB-4 (Polysciences). Sections were cut at 4 μm with glass knives on an LKB pyramitome and stained with H&E.

The dorsal–ventral orientation was based on the position of Saefftigen's pouch which has been described (Kaiser, 1893) as occurring nearest the ventral surface when viewed in worms examined in cross section. This also follows our (Dunagan and Miller, 1978b) previous designation for *Moniliformis moniliformis* and our (Dunagan and Miller, 1979) orientation of *M. hirudinaceus*.

Results

Figures 1–15 are a collection of illustrations constructed from photographs of a set of serial cross sections of a portion of the reproductive apparatus of male *N. cylindratus* beginning anteriorly with the dorsal commissure of the genital ganglion (Fig. 1) and terminating near the posterior margin of this ganglion (Fig. 15). The tegument does not appear in these illustrations but all structures medial to the circular muscles are included.

The genital ganglion consists of two accumulations of 18 or 19 cells connected by a dorsal commissure (Figs. 1–3) originating from the anterior margin of each ganglion and a ventral commissure near the posterior terminus (Figs. 8–11). The dorsal commissure is the smaller of the two passing over the outer surface of the genital sheath near its posterior termination. The larger ventral commissure passes on the outside surface of Saefftigen's pouch (Figs. 6–12). A single bipolar cell is located along its midventral margin (Figs. 7, 8). Each ganglion measures 0.046–0.053 mm long by 0.050–0.058 mm at its widest point and tapers at each end. Considerable variation was observed in these measurements as a result of

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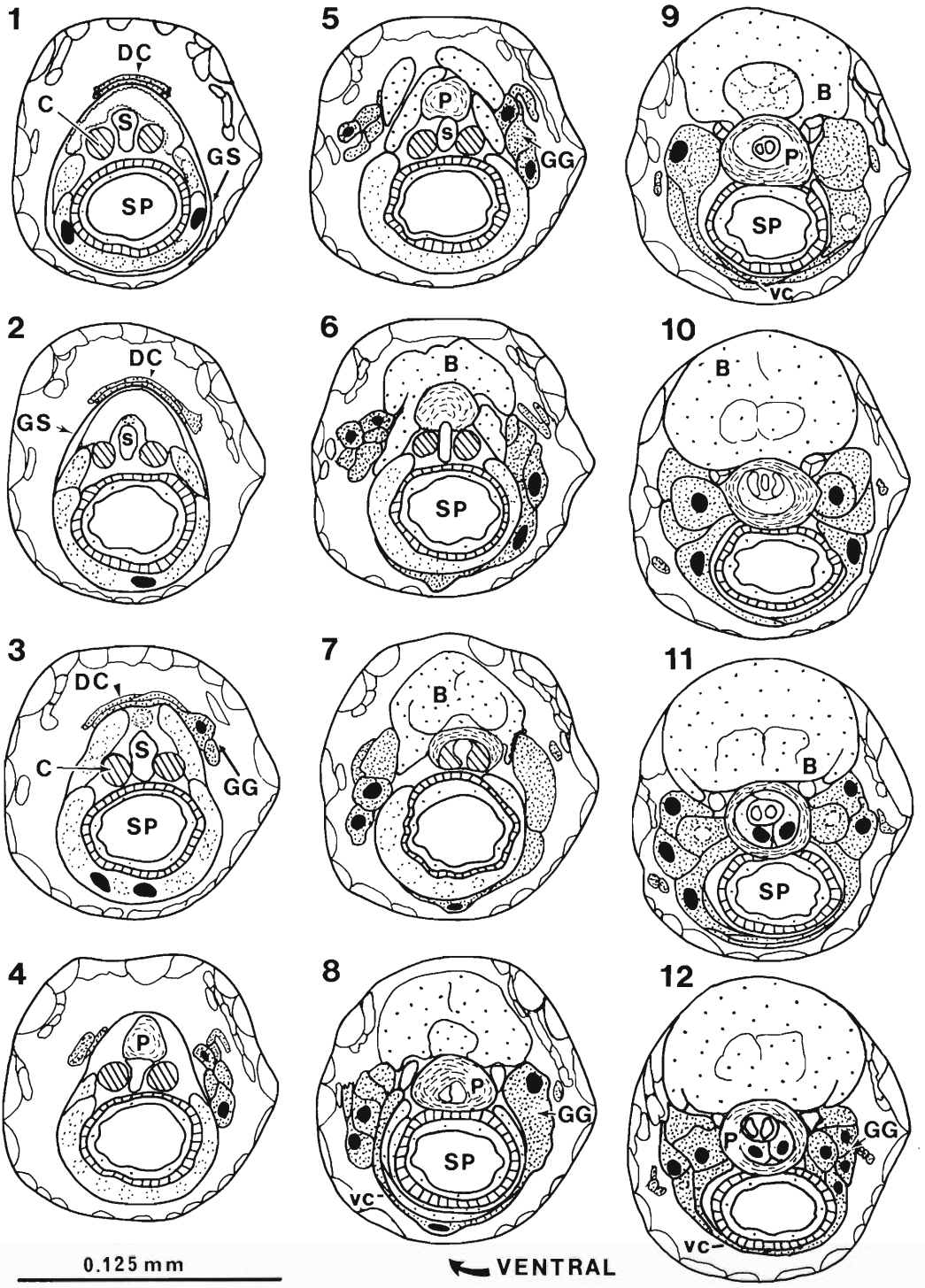
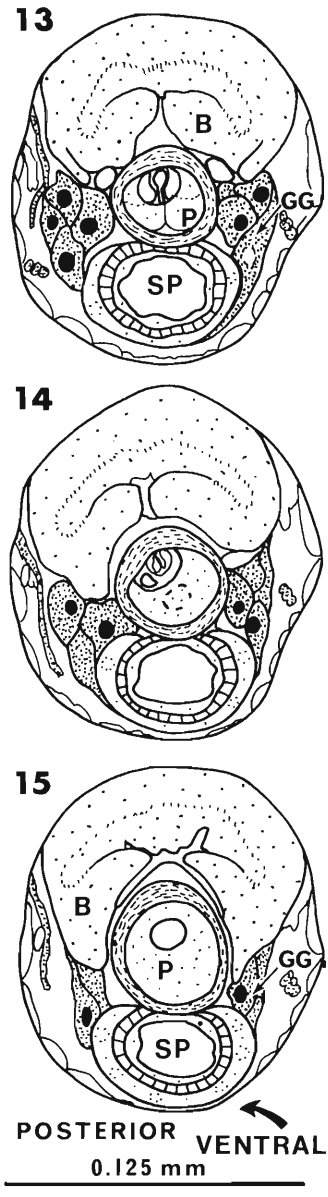


Figures 1-12. Serial cross sections (0.4 μm) of genital ganglion (GG) of *N. cylindratus*. Sections sequenced anterior to posterior. 1-3. Dorsal commissure (DC) at posterior end of genital sheath (GS). 4, 5. Anterior beginning of ganglion cells. 6-12. Appearance of ventral commissure (VC). Notice relationship of cement gland ducts (C) to vas deferens (S) and their entry into penis (P) (Figs. 6-8). Saefftigen's pouch (SP) prominent throughout. Bursa (B) evident in Figures 5-12.



Figures 13–15. Serial cross sections (0.4 μm) of genital ganglion of *N. cylindratus*. Ganglion cells (GG) are adjacent to sheath of penis (P) and Saefftigen's pouch (SP). Bursa (B) prominent.

the fixation procedures and state of muscle contraction. Cell position seems to be similar in each ganglion. This is best seen in Figure 10. There are no binucleate cells but existing nuclei are prominent with well-formed nucleoli. The nuclei typically have perinuclear rings but this along with granulation will vary depending on the cell; however, because the cells are matched between each ganglion, the characteristics observed in one cell are also found in its counterpart. The number of axons from each cell as well as their destination were not determined.

Saefftigen's pouch is muscular and prominent throughout the area of the genital ganglia and enters the bursal musculature posterior to Figure 15. The penis first appears in Figures 3–6 and the anterior bursal musculature in Figures 5 and 6. Figures 14 and 15 show the beginning penetration of the penis through the bursal musculature. The paired cement gland ducts (Figs. 1–6) from the cement gland reservoir enter the anterior margin of the penis (Figs. 6, 7). Throughout these sections they parallel and are adjacent to the lateral surfaces of the vas deferens. Once both structures are enclosed by the thick musculature of the penis (Fig. 8) their channels are not so clearly separated for a short distance (Figs. 9–13). Only one channel is observed at the point of penetration of the bursal musculature by the penis (Fig. 15). This channel continues the remaining distance to penis tip.

Discussion

Seven species of Acanthocephala have had their genital ganglia examined to the extent that the number of cell bodies has been reported. However, only five of these: *Acanthocephalus ranae* (15), *Oligacanthorhynchus microcephala* (30), *Macracanthorhynchus hirudinaceus* (19 or 20), *Moniliformis moniliformis* (19) and *Bolbosoma turbinella* (14 or 15) have been examined extensively. The number of cell bodies reported in each ganglion is indicated in parenthesis. For a review of the early history of these developments see Dunagan and Miller (1978a, 1979) and Miller and Dunagan (1985).

The general organization of the ganglia of *N. cylindratus* is similar to all other species studied; namely, two accumulations of large cell bodies along the posterior lateral margin of the genital sheath adjacent to the origin of the bursa musculature. Staining characteristics of cell bodies indicate similarities between this and larger worms such as *M. hirudinaceus*. Because we know these differences reflect the presence of different neurotransmitters (Wang, 1976) in *M. hirudinaceus*, it seems probable that *N. cylindratus* also possesses several different neurotransmitters. Each accumulation is connected with the other

by a dorsal and ventral commissure although we have had difficulty confirming the latter in certain species (Dunagan and Miller, 1979).

Ivanova and Makhanbetov (1975) described the commissures in *P. phippsi* as originating from opposite ends of the ganglion. Harada (1931) indicated that in *B. turbinella* they originated from the same general area of the ganglion. In *N. cylindratus* the ventral commissure is the most anterior whereas the dorsal commissure originates near the posterior subterminal margin. *M. moniliformis* and *M. hirudinaceus* have dorsal commissures originating from the anterior lateral margins (Dunagan and Miller, 1978a, 1979). These descriptions would indicate that the point of origin of the commissure varies considerably among the different species but tends to be terminal or near terminal. In larger species the dorsal commissure is medial to the ejaculatory duct and Saefftigen's pouch but external to the bursa; however, in this study both dorsal and ventral commissures are external to the ducts and muscles of the reproductive apparatus.

The cytoarchitecture of individual cells differs little from that previously described for the genital ganglion (Harada, 1931; Dunagan and Miller, 1978a, 1979) with matched cells in each of the paired ganglion having the same morphology. Nuclei are large with no cell being double nucleate although such is illlustrated by Harada (1931, p. 185, fig. E) for *B. turbinella*. Perinuclear rings are common and nucleoli are prominent in all cells. A neuropile is absent but bipolar cells can be identified and multipolarity may be common. Harada (1931) described a bipolar cell in the central region of the dorsal commissure of *B. turbinella*. His illustration (fig. 21, p. 197) does not show enough additional anatomy to enable us to judge if the bipolar cell located in the midventral part of the ventral commissure of *N. cylindratus* is identical with this description. Our differences in location may only be based on improper interpretation of the dorsal surface by one of us.

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Hosts and Geographic Distribution of *Acanthocephalus* (Acanthocephala: Echinorhynchidae) from North American Freshwater Fishes, with a Discussion of Species Relationships

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ABSTRACT: Populations of the three known species of the genus *Acanthocephalus* from North American freshwater fishes are found in the Mississippi River drainage system, or waters previously connected to it, in the Mobile Bay drainage, Great Lakes, and in New England. *Acanthocephalus dirus* (Van Cleave, 1931) Van Cleave and Townsend, 1936 has the widest host and geographical distribution and exhibits the greatest morphological variability compared to *A. tahlequahensis* Oetinger and Buckner, 1976 and *A. alabamensis* Amin and Williams, 1983. The Wisconsin–Lake Michigan population of *A. dirus* was geographically isolated from Mississippi River *A. dirus* less than 15,000 years ago. The New England population of *A. dirus* may have originated in a like manner. Given sufficient time and continued isolation, the Wisconsin–Lake Michigan and New England populations may diverge enough to achieve a more distinct taxonomic status. The proposed associations provide satisfactory explanation of present geographical distribution, host relationships, and degree of morphological diversification.

A critical appraisal of the taxonomic affinities and associations within *Acanthocephalus* from North American freshwater fishes may only be attained through detailed studies of intraspecific variability as well as ecological and host associations (Amin, 1975). The subject matter of this paper could not have been explored until after the intraspecific variability of the most variable species, *Acanthocephalus dirus,* had been studied, the species redescribed (Amin, 1984), and the intra- and interspecific variability in all three species analyzed (Amin and Huffman, 1984). Additionally, knowledge of host and geographical distribution and geological history, made the description of the intra- and interspecific relationships of this group of acanthocephalans possible.

Materials and Methods

The characteristics of the three species of *Acanthocephalus* discussed in this report are based on the studies by Amin (1984) and Amin and Huffman (1984). Sources of each of the host and geographic distributional records used in the map (Fig. 1) and Table 1 are footnoted in the legend to the map and given the same numbers.

Results and Discussion

Three species of *Acanthocephalus* (*A. dirus, A. tahlequahensis, A. alabamensis*) are so far known from North American freshwater fishes. These appear to fall into two presumably natural groups distinguished primarily by size. *Acanthocephalus dirus,* includes larger forms compared to the smaller individuals of the two southern species, *A. tahlequahensis* and *A. alabamensis.* It is herein proposed that the present distribution of the Wisconsin–Lake Michigan population of *A. dirus,* and possibly that from New England, occurred through dispersal from a Mississippi River source similar to *A. dirus* followed by geographical isolation.

1. Geographical distribution

Acanthocephalus dirus has the widest range of geographical distribution in North America compared to the two other species. Practically all its Mississippi River populations studied and/or reported in Amin (1984 and this paper) were taken from tributaries of the Mississippi River drainage system in 10 states of the United States. Only those collections from Ohio and Lake Erie (Nos. 17–19, Fig. 1) are from waters not presently connected to the Mississippi River but which were connected via the Wabash River to glacial Lake Maumee in the Erie basin during the late Woodfordian Substage of the Wisconsinan glaciation about 14,000 B.P. (before the present) (Hough, 1958; Goldthwait et al., 1965; Fullerton, 1980). The two northern populations of *A. dirus* add three more states to that species: New Hampshire, Massachusetts, and Michigan. The Michigan material was obtained by Muzzall (pers. comm., 1982) from the Rogue River, a tributary of the Grand River which flows into Lake Michigan.

Compared to *A. dirus,* the distribution of the

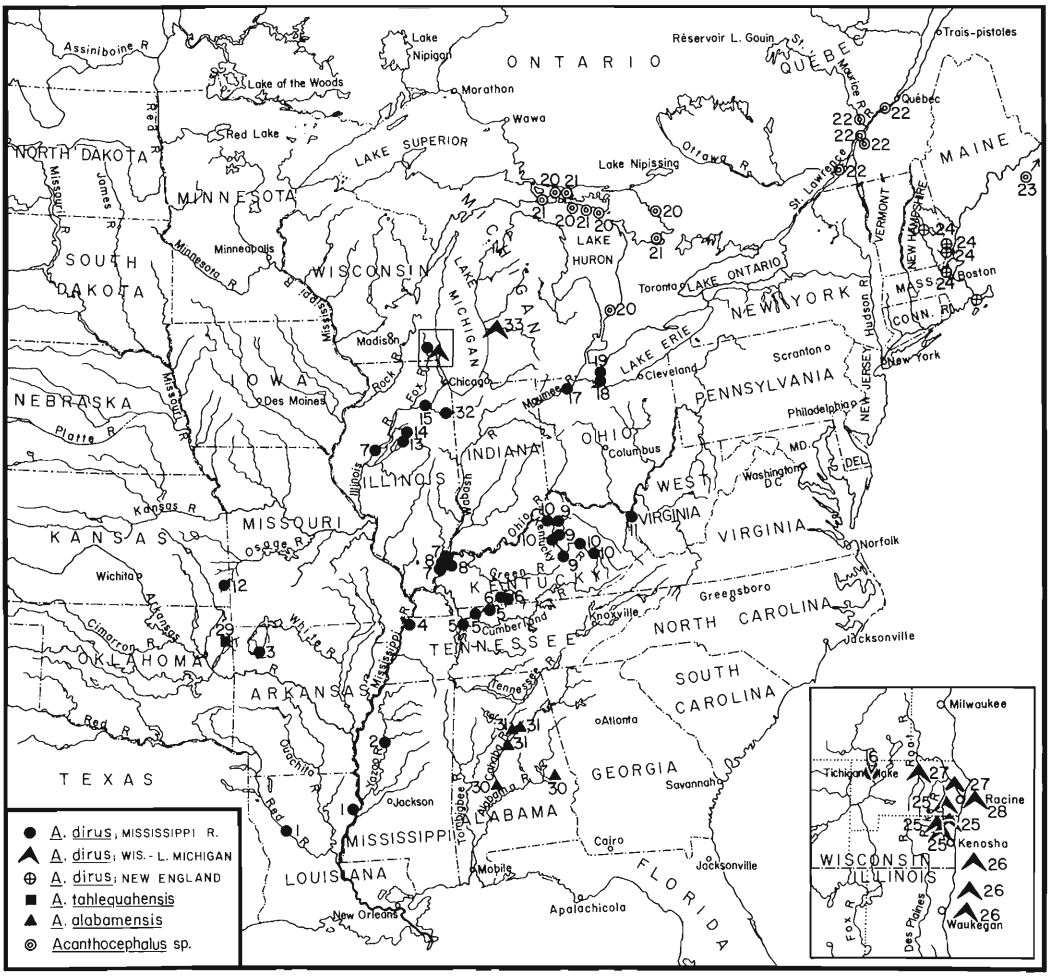

Figure 1. Map of eastern United States showing the present distribution of *Acanthocephalus* spp. from freshwater fish in the Mississippi River drainage system, Great Lakes and the St. Lawrence watershed as well as from New England, Nova Scotia, and Alabama. Numbers refer to the following collections: 1: Arnold et al. (1967, 1968); 2: Van Cleave (1931) (specimens from *Ictiobus* sp. in USNM Helm. Coll. No. 37600); 3: Oetinger and Nickol (1981) (host species undisclosed); 4: Bangham and Venard (1942); 5: Ewell (1953); 6: Gleason (pers. comm., 1982), McDonough and Gleason (1981), Talton and Gleason (1978); 7: Van Cleave and Townsend (1936); 8: Page (1974); 9: White (1974, pers. comm., 1981), White and Harley (1973, 1974); 10: Combs et al. (1977); 11: Huffman (pers. comm., 1981); 12: Gash et al. (1972), R. Gash (pers. comm., 1982); 13: Camp (1977, pers. comm., 1981), Camp and Huizinga (1980); 14: Seidenberg (1973); 15: Schmidt et al. (1974); 16: Amin (this paper); 17: Muzzall and Rabalais (1975a, b), Muzzall (pers. comm., 1982); 18: Bangham (1972); 19: Venderland (1968); 20: Collins and Dechtiar (1974); 21: Dechtiar and Berst (1978), Dechtiar (pers. comm., 1983); 22: Frechette et al. (1978); 23: Wiles (1975); 24: Bullock (1962, 1963, pers. comm., 1982–1984); 25: Amin (1975); 26: Amin (1977a, unpubl.), Amin and Burrows (1977); 27: Amin (1977b); 28: same as for *E. salmonis* in Amin (1978a); 29: Oetinger and Buckner (1976); 30: Amin and Williams (1983), Williams and Rogers (1982), Williams (1974a, b); 31: Burns (1970, 1971); 32: Skelly (pers. comm., 1982); 33: Muzzall (pers. comm., 1982). All specimens from Canada (20–23) are regarded as *Acanthocephalus* sp. until further study.

Table 1. Comparative distribution of adult *Acanthocephalus* spp. in North American freshwater fish hosts.

Fish host*	Species of *Acanthocephalus* and host records (reference numbers correspond to those in map, Fig. 1)					
	Acanthocephalus dirus			*Acantho-cephalus tahle-quahensis*	*Acantho-cephalus alaba-mensis*	*Acantho-cephalus* sp.†
	Mississippi River	Wisconsin–Lake Michigan	New England			
Amiidae						
*Amia calva**	(16, 18)					
Anguillidae						
Anguilla rostrata			24			
Catostomidae						
*Catostomus commersoni**	(9, 16, 17)	(25, 26, 27, 33)	(24)			20, 21
Hypentelium etowanum					30	
*H. nigricans**	(6, 17)					21
Ictiobus sp.*	(2)					
Minyatrema melanops	10					
Moxostoma duquesnei	6					
*M. erythrurum**	(6, 10, 32)					
M. macrolepidotum	32					
M. poecilurum					30	
Centrarchidae						
Ambloplites rupestris	16, 17					
*Lepomis cyanellus**	(3, 16, 17)	(25, 27)				
*L. gibbosus**	(16, 17)		24			
*L. macrochirus**	(1, 7, 16, 17)	(25)				
L. megalotis	6					1
L. microlophus	1					
Micropterus coosae					30	
*M. salmoides**	(7)	(25)				
*Pomoxis nigromaculatus**	(16)					
Clupeidae						
Alosa pseudoharengus		26				20, 21
Cottidae						
*Cottus bairdi**	(5, 15)					
*C. cognatus**		(26)				21
Cyprinidae						
Campostoma anomalum	17					
*Carassius auratus**	(17)					
*Cyprinus carpio**	(16, 17)	(26)			30	
Hybopsis sp.	14					
Nocomis asper				29		
*Notemigonus crysoleucas**	(17)	(25)	24			
*Notropis cornutus**	(11)					
N. crysocephalus	17					
N. hudsonius	17					
N. pilsbryi				29		
N. spilopterus	17					
*N. umbratilis**	(17)					
Pimephalus notatus	14					
*P. promelas**		(25)				
*Semotilus atromaculatus**	(6, 16, 13, 17)	(25)	24			
S. margarita		25				
Cyprinodontidae						
Fundulus diaphanus						23
Esocidae						
Esox sp.			(24)			

Table 1. Continued.

Fish host*	Species of *Acanthocephalus* and host records (reference numbers correspond to those in map, Fig. 1)					
	Acanthocephalus dirus			*Acantho-cephalus tahle-quahensis*	*Acantho-cephalus alaba-mensis*	*Acantho-cephalus* sp.†
	Mississippi River	Wisconsin–Lake Michigan	New England			
*E. americanus**	(11)		(24)			
*E. lucius**	(16)					
E. niger			24			
Gadidae						
Lota lota		26				
Microgadus tomcod			24			
Gasterosteidae						
Culaea inconstans		25				
Ictaluridae						
*Ictalurus melas**	16	(25)				
*I. natalis**	(12, 16)					
I. nebulosus			(24)			22
I. punctatus	7, 16					
Osmeridae						
*Osmerus mordax**		(26)				20
Percidae						
Etheostoma bellum	6					
E. blennoides	6, 17					
E. coeruleum	6					
*E. punctulatum**				(29)		
E. spectabile				29		
E. squamiceps	6, 8					
E. stigmaeum	6				31	
E. whipplei					31	
E. zonale	6					
*Perca flavescens**	(16)		(24)			20
Percina caprodes	6					
Stizostedion vitreum	16					
Salmonidae						
Coregonus clupeaformis						20
C. hoyi		26				
*Onchorhynchus kisutch**		(26)				
O. nerka						20
*O. tshawytscha**		(26, 28)				
*Salmo gairdneri**		(25, 26, 33)	(24)			
*S. irideus**	(5)					
S. trutta		26, 33	24			
*Salvelinus fontinalis**		33	(24)			
S. fontinalis × *S. namaycush*					20, 21	
*S. namaycush**		(26)				
Sciaenidae						
*Aplodinotus grunniens**	(2, 4, 7, 19)					
Umbridae						
*Umbra limi**	(16)					
No. of host species (families)	46 (11)	22 (10)	14 (9)	4 (2)	6 (4)	11 (9)

* Fishes marked with asterisks are those from which gravid worms were reported from populations/species with parenthesized reference numbers.

† All specimens from Canada (20–23) are regarded as *Acanthocephalus* sp. pending examination.

Table 2. Geographical variation in the number of proboscis hooks per row in *Acanthocephalus dirus*.*

Species	Population	No. hooks/row (mean) *N*	
		Males	Females
A. dirus (MISS)	Mississippi (Van Cleave)	11–13 (?)	11–13 (?)
	Mississippi (type, Amin)	11–12 (11.5) 3	11–13 (12.3) 5
	Kentucky (Gleason, White)	7–12 (9.5) 21	8–14 (10.9) 59
	West Virginia (Huffman)	6–11 (8.6) 7	8–11 (9.1) 15
	Illinois (Camp, Seidenberg, Schmidt)	9–12 (10.5) 25	9–13 (10.3) 53
	Ohio (Muzzall)	7–11 (9.3) 18	8–11 (9.2) 17
	Wisconsin (Amin)	7–12 (10.3) 39	9–13 (10.8) 83
	All *A. dirus* (MISS)	6–13 (9.9) 113	8–14 (10.6) 232
A. dirus (WI–LM)	Wisconsin (Amin)	8–11 (9.3) 142	8–11 (9.6) 206
A. dirus (N-ENG)	New England (Bullock)	7–10 (8.3) 167	7–10 (8.9) 130

* Listed in order of northward distribution.

other two species is restricted to waters of one state each; *A. tahlequahensis* in Oklahoma and *A. alabamensis* in Alabama.

2. Host distribution

A. dirus has been reported from more species (65) and families (16) of fishes than the other two. Of these, 46 species and 11 families are reported as hosts of the Mississippi River population, 22 and 10 of the Wisconsin–Lake Michigan population, and 14 and 9 of the New England population. By comparison, *A. tahlequahensis* and *A. alabamensis* were reported from only four and six species and two and four families, in the same order (Table 1). Only two host species of *A. dirus* (4%) (*C. carpio* and *E. stigmaeum*) were also infected with *A. alabamensis*, none with *A. tahlequahensis*.

3. Variability

Analysis of interspecific morphometric variability discussed by Amin and Huffman (1984) shows that *A. dirus* exhibits the widest range of morphological variation among the species considered. One of the most important taxonomic characteristics expressing such variation is the number of proboscis hooks per row, being 6–13 in males and 7–14 in females. Within that range, a northward reduction in the number of hooks per row in the Mississippi River population is observed from 11–13 in Mississippi to 7–12 (males) and 9–13 (females) in Wisconsin and 7–11 and 8–11 in Ohio. The same character was 8–11 in the Wisconsin–Lake Michigan population and 7–10 in the New England population (Table 2). This probably represents a form of clinal variability that may correspond with some climatic factors, e.g., temperature gradient. A similar and a more definite clinal pattern was also observed in hook size, another important taxonomic characteristic. Largest proboscis hooks were characteristically and progressively longer in the more northern locations (Table 3). Clinal variations in hook number and size were also observed in other helminth parasites of fish including *Leptorhynchoides thecatus* (Linton, 1891) by Lincicome and Van Cleave (1949) and *Triaenophorus nodulosus* Pallas (1781) by Kuperman (1973).

The adaptability of *A. dirus* to the largest number of host species spread throughout its extensive geographical range from Mississippi to the Great Lakes and its widest range of anatomical variability may be construed as providing hypothetical support to its antiquity and to its possible role as the present representative of the ancestral stock from which the other species may have diverged. Alternatively, *A. dirus* could be interpreted as a successful adaptable new species with the other two species exhibiting relictual distributions. These issues will be explored in a separate publication.

Origin and Dispersal of *A. dirus*

The present distribution of *A. dirus* may be explained as suggested below. It is proposed that the early *A. dirus* ancestors were probably found in fishes of the Mississippi River basin before the Wisconsinan glaciation; the host and geographical distributions of the more variable *A.*

Table 3. Geographical variation in the length of the largest proboscis hook in *Acanthocephalus dirus*.*

Species	Population	Length of largest hook in micrometers (mean) *N*	
		Males	Females
A. dirus (MISS)	Mississippi (type, Amin)	45–51 (48) 3	54–70 (64) 6
	Kentucky (Gleason, White)	35–54 (43) 27	42–70 (52) 67
	West Virginia (Huffman)	42–54 (49) 14	51–74 (62) 19
	Illinois (Camp, Seidenberg, Schmidt)	45–61 (53) 76	58–80 (68) 74
	Ohio (Muzzall)	42–64 (53) 21	61–80 (70) 19
	Wisconsin (Amin)	45–74 (58) 93	58–90 (74) 112
	All *A. dirus*	35–74 (54) 234	42–90 (66) 297
A. dirus (WI–LM)	Wisconsin (Amin)	42–64 (52) 133	54–77 (66) 197
A. dirus (N-ENG)	New England (Bullock)	41–84 (66) 153	55–103 (83) 144

* Listed in order of northward distribution.

dirus population from the Mississippi River are considerably wider than those of other *A. dirus* populations from Wisconsin–Lake Michigan and New England. Its northward dispersal into the Great Lakes region would have occurred through the southerly discharge outlet during glacial retreat. The glacial connections of the Mississippi River to glacial Great Lakes were direct through its tributaries with glacial lakes in the Superior, Michigan, and Erie basins and through connections of these lakes with glacial and modern lakes in the Huron and Ontario basins. The *Acanthocephalus* specimens reported from Lake Erie by Venderland (1968) and Bangham (1972) and from the Maumee River tributary of the Lake Erie basin by Muzzall and Rabalais (1975a, b) decidedly belong to the Mississippi River population of *A. dirus*. The synonymy of *A. jacksoni* with *A. dirus* (see Amin, 1984) would place the "*A. jacksoni*" specimens reported from Lake Huron by Collins and Dechtiar (1974) and Dechtiar and Berst (1978) in *A. dirus*. The common occurrence of this acanthocephalan in other fish species from Lake Huron was indicated by Dechtiar (pers. comm., 1982). The outlet of the Great Lakes through the St. Lawrence basin appears to provide an opportunity for farther eastward dispersal of this acanthocephalan. This is supported by the recovery of *Acanthocephalus* sp. from various sites along the St. Lawrence River by Frechette et al. (1978). These Canadian materials, as well as *Acanthocephalus* sp. from Nova Scotia (Wiles, 1975) were unavilable for examination but are tentatively regarded as *Acanthocephalus* sp. (Table 1, Fig. 1) until their identity is determined.

The proposed events in the origin and dispersal of *A. dirus* parallel those known of its type host, the freshwater drum, *Aplodinotus grunniens* Rafinesque from which *A. dirus* has been reported throughout most of its range between Mississippi (Van Cleave, 1931) and Lake Erie (Venderland, 1968). *Aplodinotus grunniens,* the only freshwater representative of Sciaenidae, apparently originated in the Gulf of Mexico before the Wisconsinan glaciation (Barney, 1926). After establishing itself in the Mississippi River, its northward movement carried it into the Pleistocene Great Lakes and Lake Agassiz through discharge outlets. Further movement in glacial (and recent) times has established the fish in the St. Lawrence, the Champlain, and the Ottawa river basins (Barney, 1926; Priegel, 1967). The above parallelism may be regarded as suggestive of the possible route through which *A. dirus* may have attained its present distribution. Other widely distributed common host genera of *A. dirus* that might have contributed to its spread throughout the range include *Lepomis, Semotilus,* and *Catostomus*. For distributional maps see Trautman (1957).

It is clear that the distribution of the isopod intermediate host(s) into waters being colonized by adult *A. dirus,* among others, is a primary prerequisite for the establishment and persistence of the species endemic populations. Undoubtedly, this factor has played (and continues to play) a major role in limiting or extending the geographical distribution of all acanthocephalans. In the presence of well-established isopod populations, the distribution of the fish definitive hosts, particularly those in which propagation of

the species can be attained, becomes the most critical factor determining its ultimate geographical range. Consequently, hosts in which worms can sexually mature and breed are especially significant. Where known, these are noted (with asterisks) in Table 1.

Acanthocephalus dirus appears to be capable of breeding also in amphibians. Of seven mudpuppies, *Necturus maculosus* Rafinesque (six gravid females and one male), examined from Wind Lake in April 1979, three females were infected with 18 *A. dirus* (nine males and nine females). One host was infected with two males and one gravid female. One *N. maculosus* examined from nearby Waubeesee Lake in June 1979 was also infected with one *A. dirus*. Both lakes are connected to the Fox River (a Mississippi River tributary) near Tichigan Lake, Racine County, Wisconsin. The acanthocephalans were similar to those previously reported from Tichigan Lake (Amin, 1984) but some exhibited extreme morphological variations. No fishes were examined from either Wind or Waubeesee lakes and mudpuppies were not found in Tichigan Lake.

The possibility that the present fauna of *Acanthocephalus* of North American freshwater fishes may have resulted from recent accidental introduction of related or "ancestral" forms via fish (or amphibian) sources is unlikely. The relationship between the *A. dirus* populations from Wisconsin–Lake Michigan and those from the Mississippi River alone (next section) indicates a common ancestry which must have predated their present distribution 5,000–15,000 years B.P.

Population Dispersal and Diversification

The *Acanthocephalus dirus* population from Wisconsin-Lake Michigan

This population is known from a few streams in southeastern Wisconsin (Pike and Root rivers) as well as from southwestern Lake Michigan into which those streams drain (inset, Fig. 1). Streams like the Pike and Root rivers are post-glacial that formed after the withdrawal of the Lake Michigan Lobe of the Wisconsinan ice sheet from the area between 15,000 and 12,000 years B.P. The colonization of the two rivers with their present fauna must have taken place with Mississippi River elements from the Des Plaines–Illinois River system (tributaries of the Mississippi River) (1) by ponding of the Fox River (a tributary of the Des Plaines River) and subsequent diversion of the drainage eastward towards the Lake Michigan shoreline. This could have taken place about 15,000 years ago (Schneider, pers. comm., 1981–1982) and/or (2) via the Des Plaines River and the Chicago outlet into the southwestern part of the Lake Michigan basin. The last connection between the Des Plaines River and the Lake Michigan basin occurred during the Nipissing Great Lakes Stage which began about 5,000 years B.P. (Schneider et al., 1979; Cowan, 1978) and probably lasted for less than 2,000 years (Frye et al., 1965). It should be noted that, like the other acanthocephalans considered herein, this *A. dirus* population is primarily a riverine rather than a lake population. Its major isopod intermediate host, *Caecidotea militaris,* is only well established in its river habitats (Amin et al., 1980). Although its Lake Michigan amphipod intermediate host *Pontoporeia affinis* is apparently a less effective host (Amin, 1978b), it may become sufficiently infected to help extend the range of this Wisconsin–Lake Michigan population into streams associated with Lake Michigan elsewhere. The recent discovery of worms belonging to this population by Muzzall (pers. comm., 1982) from the Rogue River in Michigan which drains into eastern Lake Michigan supports this assumption but does not exclude other possibilities yet to be identified.

It is proposed that since the introduction of ancestral forms into Lake Michigan and its associated streams, its populations have been geographically and reproductively isolated from those of the Mississippi River *A. dirus* for 15,000 years or less. During this period, the Wisconsin–Lake Michigan population typically retained the body form and comparative size of male reproductive system characteristic of its Mississippi River sources but varied in certain other traits discussed by Amin (1984) and Amin and Huffman (1984). Most notably, the clinal trait of reduced number of proboscis hooks per row and their narrower range of variation in northern *A. dirus* populations (Table 2) appear to have become genetically locked in the present Wisconsin–Lake Michigan population as the opportunity of its interbreeding with its Mississippi River sources ceased to exist. Similar "variations in proboscis hook pattern between Canadian and United States" *L. thecatus* were "considered as probably genetic variations indicative of trends toward subspeciation" by Lincicome and Van Cleave (1949). In Palaeacanthocephala the magnitude of individual variation in proboscis char-

acters expressing genetic differences presents opportune conditions for the operation of natural selection. Barriers "to free interbreeding within a variable species may result in certain features becoming fixed in an isolated portion of the population . . . resulting ultimately in the establishment of features which may be recognized as distinctive of a new species" (Van Cleave, 1952). If variation from the Mississippi River population of *A. dirus* continues to be enhanced by isolation, the Wisconsin–Lake Michigan population may in time achieve a higher taxonomic rank.

The *Acanthocephalus dirus* population from New England

The origin of this population cannot be determined. Its present distribution in the coastal streams of New England does not appear to be related to possible dispersal from a source that might have been present in the St. Lawrence watershed during the later episodes of the Wisconsinan glaciation. No direct connection between the two areas appears to have existed (Koteff, pers. comm., 1982).

The possibility that more recent introductions might explain the origin and/or distribution of this population in New England is explored but considered unlikely. Two of the common hosts of the New England population are not native to New England. The rainbow trout, *Salmo gairdneri,* was introduced from western United States where no *Acanthocephalus* worms have been reported, and the brown trout, *Salmo trutta,* from Europe where it is a host for *A. lucii* (Bullock, pers. comm., 1982) as well as some other acanthocephalans, e.g., *Echinorhynchus truttae* (see Awachie, 1965). A phylogenetic relationship between *A. lucii,* with its triangularly shaped proboscis hook roots, would have to be documented. Based on morphological evidence alone, the New England population appears to have also originated from a Mississippi River *A. dirus*-like source. This evidence includes similar anatomy, and a good fit along the clinal gradients of the number and size of proboscis hooks characteristic of the more northern *Acanthocephalus* populations (Tables 2, 3). In addition, host specificity shows considerable overlap with that of *A. dirus* from the Mississippi River and Wisconsin–Lake Michigan; eight species of its 14 reported fish hosts (57%, Table 1) are shared with acanthocephalans of either one or both of the other two populations. Five of the remaining six host species are either of essentially more northern distribution that would not be available for infection with the more southern *A. dirus* populations, e.g., *Microgadus tomcod,* or hosts not identified to species, e.g., *Esox* sp. The New England population is believed to have diversified to its present form in North America; this might have occurred through a process similar to that proposed for the Wisconsin–Lake Michigan population.

As with the Wisconsin–Lake Michigan population, if variation and isolation continue, the New England population might in time achieve a higher taxonomic rank.

Acanthocephalus tahlequahensis

This southern species has a limited distribution in an Oklahoma tributary of the Mississippi River. It infects only a few hosts, mostly certain species of *Etheostoma,* not parasitized by other species of *Acanthocephalus.* "The high degree of host specificity exhibited by *A. tahlequahensis* for *E. punctulatum*" in particular (the only host from which gravid worms were found) "suggests that its distribution may be limited to the distribution of this host" (Oetinger and Buckner, 1976). *Campostoma anomalum, Cottus carolinae, Gambusia affinis, Lepomis macrochirus, Noturus exilis, Phoxinus erythrogaster,* and *Semotilus atromaculatus* were also examined by the same authors but were found negative for *A. tahlequahensis* infections. Two of these species, i.e., *L. macrochirus* and *S. atromaculatus* are common hosts of *A. dirus* (Table 1).

Acanthocephalus alabamensis

This acanthocephalan is primarily found in a distinct set of host species including species of *Etheostoma* in the Mobile Bay drainage system, Alabama. The common hosts of *A. alabamensis* appear to be *Etheostoma stigmaeum* and *E. whipplei* (see Amin and Williams, 1983). As with *A. tahlequahensis,* many fish species (145 species in 38 families) were examined, some of which are usual hosts of *A. dirus* elsewhere, that were negative for *A. alabamensis* infections (Amin and Williams, 1983). According to Ramsey (1982, pers. comm.), actual geologic traces of past connections have been occasionally reported between the Mobile Bay and the Mississippi River drainages, chiefly as minor headwater piracies between the Tennessee and Coosa basins. Other large aquatic continuations have been hypothesized because of obvious faunistic similarities. Cladistic relations, however, along with clues to

recent dispersal, show Mississippi elements to have entered the Mobile basin in several waves. More precisely, barriers to recent dispersal have risen since the faunas were continuous (Ramsey, pers. comm., 1982).

Conclusions

Except for the *A. dirus* population from New England, all populations of *Acanthocephalus* from North American freshwater fishes are known only from the Mississippi River drainage system or waters which have been previously connected to it including the Mobile Bay drainage and the Great Lakes.

Based on its broad geographical and host distribution and its great morphological varaibility, *A. dirus* is hypothesized to represent a successful persistent general ancestor whereas *A. tahlequahensis* and *A. alabamensis* seem to exhibit new restricted distributions. *A. dirus* may also be interpreted as a successful adaptable new species with the other two species exhibiting relictual distributions. Some combination of these two interpretations may also be theorized. These issues will be explored within a cladistic context in a subsequent publication.

Geological and morphological evidence support the proposition that the Wisconsin–Lake Michigan population of *A. dirus* dispersed from an early Mississippi River-based source and then became geographically isolated less than 15,000 years ago. Given sufficient time and continued isolation, this situation would be a classical example of Van Cleave's (1952) earlier proposition that in Palaeacanthocephala "isolation might allow the normal extremes in a highly variable species to become segregated as distinct species." Morphological and clinal variations suggest a similar process for the New England population even though the origin of its source remains to be identified.

The discovery of additional species of *Acanthocephalus* in new hosts from the Mississippi River drainage system which might have evolved through host isolation is not unlikely. The involvement of geographical isolation in the potential evolution and discovery of new species in waters previously connected to these drainage systems is also not improbable. In all incidences, a relationship to Mississippi River-like forms should prove demonstrable.

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New Format for Citations

Following the lead of several parasitology journals, the Proceedings will require full titles of journals cited (no abbreviations) both in Literature Cited lists in full papers and in the text of Research Notes beginning with the January 1986 issue.

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Joseph H. Howard, Director, National Agricultural Library

Since 1892 the U.S. Department of Agriculture has prepared and arranged for the issuance of the Index-Catalogue of Medical and Veterinary Zoology (ICMVZ). In 1983 ICMVZ data collection became fully automated and part of the system used by the National Agricultural Library (NAL) to produce the AGRICOLA online database. Responsibility for publication of the ICMVZ itself was relinquished to the private sector (Orynx Press) at that time. In 1984 the Agricultural Research Service found it impossible to continue funding the ICMVZ. Arrangements made with NAL will insure that this important portion of the agricultural literature continues to be covered in AGRICOLA. NAL will index the animal parasitology (nonhuman) literature using indexing terms adapted from the thesaurus (authority list) of the Commonwealth Agricultural Bureaux. This will not provide the in-depth parasite–host linkages ICMVZ users have become accustomed to, but we trust the ability to access the data in the online environment of AGRICOLA will help provide the necessary information access. Access to human parasitology is available through NLM's MEDLINE database. The AGRICOLA online database is available through two commercial services in the United States:

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New Aspects of Acanthocephalan Lacunar System as Revealed in Anatomical Modeling by Corrosion Cast Method

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ABSTRACT: In order to study the acanthocephalan lacunar system we previously constructed a series of two-dimensional representations utilizing india ink injection, glycerol clearing, and transmission photography. Three-dimensional models were then produced by stereological extrapolation of the photographic images. We now describe a technique that produces a permanent three-dimensional model. The lacunar system is injected with partially polymerized colored butyrate monomer which is allowed to fully polymerize in situ. The tissues are then disintegrated with potassium hydroxide leaving a pliable replica of the original lacunar system that is suitable for photography. New aspects of the acanthocephalan lacunar system, which have not previously been reported, have been revealed by these corrosion cast models. We conclude from this study that the lacunar system is a very effective fluid transport system and possibly serves as a hydrostatic skeleton.

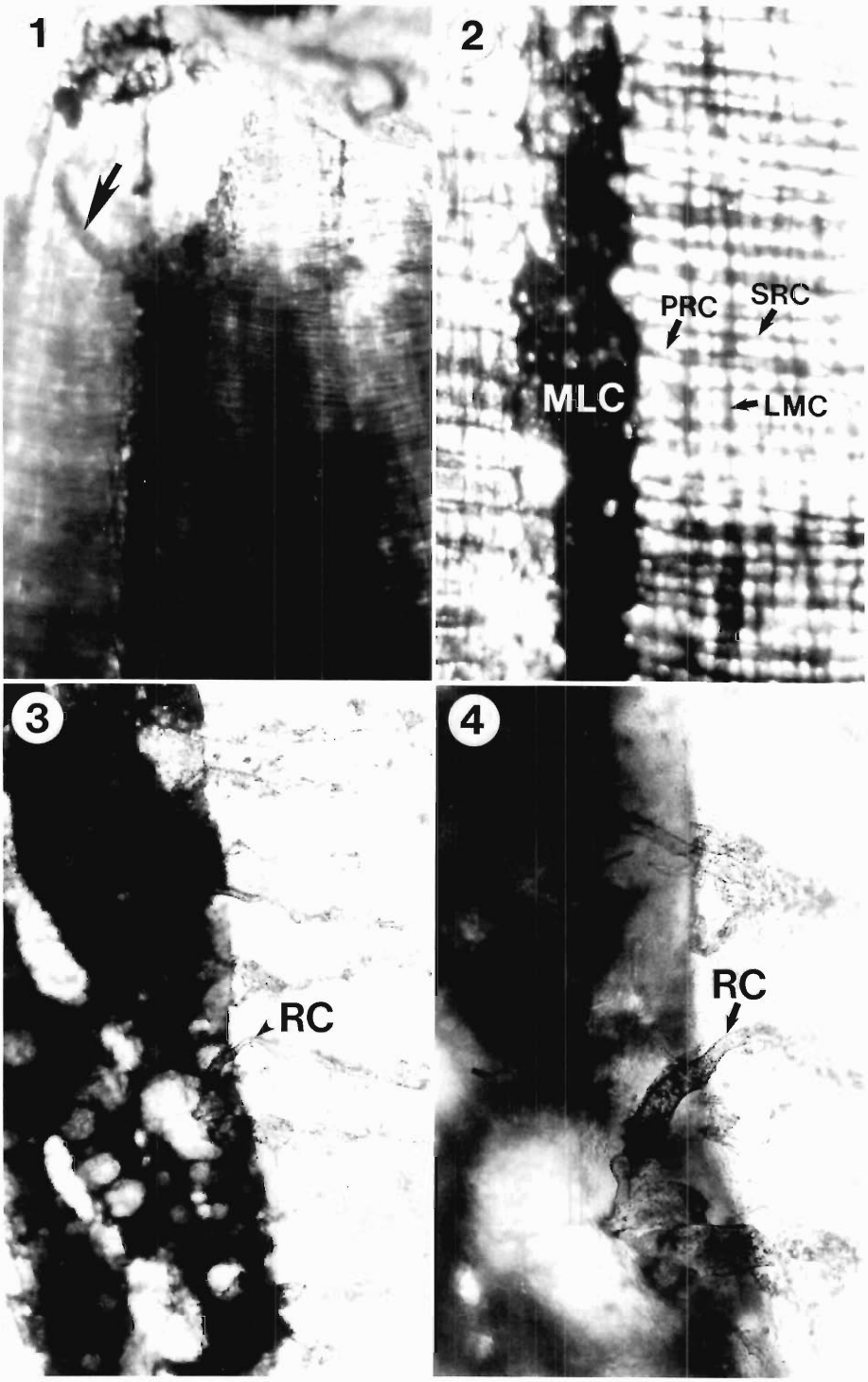
Early attempts (Miller and Dunagan, 1977) to examine the electrophysiology of acanthocephalan muscle cells revealed their unique tubular nature as part of the lacunar system. Previously (Miller and Dunagan, 1976, 1978), models were made of the lacunar system utilizing india ink injection, glycerol clearing and transmission photography. Interpretation of these produced three-dimensional representations of the lacunar canalicular system in *Macracanthorhynchus hirudinaceus* and *Oligacanthorhynchus tortuosa.* Preparations upon which these models were based were not permanent and left much for interpretation. Nevertheless, they did indicate that the lacunar system of the Acanthocephala was a vastly more complex system than had previously been described. We now report the outlining of the lacunar system by means of corrosion modeling (Batson, 1935). This method has allowed construction of permanent models of the system, which may be colored (Narat et al., 1936) to add clarity to interpretation. The flow of injected material also indicates patterns of circulation around the system, and has disclosed outlines of additional features not previously described in the lacunar system.

Materials and Methods

Male and female Acanthocephala, *Macracanthorhynchus hirudinaceus* were obtained from Swift Fresh Meats Company, East St. Louis, Illinois, and transported to the laboratory in Dewar flasks containing a small amount of gut contents. Afterwards, the worms were transferred into a physiological saline solution (Denbo and Miller, 1975). The injectate was a partially polymerized monomer, Batson's #17 kit obtained from Polysciences Inc., to which coloring dyes (10 mg/200 ml), promoter (2 drops/200 ml), and catalyst (1 ml/200 ml) were added. The injection was performed with disposable 5-ml syringes and #26 hypodermic needles. After injection, manual pressure was applied at the injection site to offset contractile activity of the worm and to keep the plastic inside until polymerization occurred. Once the plastic had polymerized, parasite tissues were macerated at 50°C using technical potassium hydroxide (340 g to 1,000 ml of water). Tissues were removed from the solution every 5 min and rinsed in water. Dissolution of tegument occurred in the first 15 or 20 min leaving the muscle tissue intact. Fibrous connective tissues were the last to be macerated and required 12–24 hr of processing. Photography was accomplished using a Leitz Orthomat photomicroscope.

Results

OVERALL SYSTEM: Monomer injected into any channel eventually flowed into all connecting channels and canals (Figs. 1, 2). However, contractions of the worm tended to squeeze the monomer back out through the injection hole. Therefore, it was necessary to hold pressure on the area of injection until the plastic polymerized. In addition, it was noted that when plastic monomer was injected into the medial longitudinal channel, flow in the posterior direction was much easier than in the anterior direction. Attempts to drain the worm of lacunar fluid one day prior to injection did not produce satisfactory results. Apparently, the collapsed channels tended to fuse as a result of the drainage. This was also the case if we removed most of the fluid osmotically by putting worms in a very concentrated brine solution prior to injection. However, a secondary opening of the lacunar system that allowed fluid to drain and reduce back pressure, did expedite the injection process.



Figures 1–4. Photomicrographs of injected preparations. 1. Photomicrograph near the anterior end of the worm. Note branching of medial longitudinal channel into two limbs (×40). 2. Photomicrograph of a corrosion model in which the medial longitudinal channel has been injected and the partially polymerized monomer has

Observations of contractile activity of fresh worms indicated that there was alternate constriction of longitudinal and circular muscle sets interrupted by relaxation phases. This action tended to move lacunar fluid alternatively into the circular and then longitudinal channels of the lacunar system. Indeed, stretching the worm after injection of colored monomer tended to move material preferentially into tegumental canals. The channels identified by this technique corresponded for the most part but not entirely to previous findings (Miller and Dunagan, 1976, 1977, 1978).

MUSCULAR CHANNELS: Models produced when monomer was injected into the medial longitudinal channel agreed with previous scanning electron microscope studies (Miller and Dunagan, 1978): channels in the longitudinal, circular, and rete muscles were patent and irregularly interconnected (Figs. 1, 2). Radial connections to longitudinal muscles alternate sides (Figs. 3, 4).

MEDIAL LONGITUDINAL CHANNELS: Corrosion modeling has demonstrated that the medial longitudinal channel alternates its connections with the inner muscular layer of the body wall. Within these connection areas, radial canals arise leading to both muscle and more externally to primary ring canals. At each end of the worm the medial longitudinal channels branch and invade the body wall (Fig. 1). After they pass through the body wall they form smaller longitudinal channels that double back in the tegument and are termed secondary longitudinal channels. These channels interconnect with other channels in the tegument by way of the primary ring canals.

SECONDARY RING CANALS: In agreement with a previous study of injected dyes (Miller and Dunagan, 1976) secondary ring canals were discerned, but with this technique these canals were discovered to be entirely within the tegument. They, like the primary ring canals, also connect with the medial longitudinal channel by means of short radial canals (Figs. 3–5), however radial canals to secondary ring canals do not alternate and they outnumber the primary ring canal connections an average of four to one.

HYPODERMAL CANALS: Photomicrographs of lacunar models demonstrated an extensive invasion of outer tegument layers by the lacunar tubular system that reached to within microns of the surface (Figs. 6–10). Interestingly enough, the three-dimensional structure of these connections, which extend from one secondary ring canal to another, is not that of a tube but rather that of a tortuous irregular sinus (Figs. 9, 11) whose morphology varies with the stretching of the body wall. We previously termed these connections hypodermal canals (Miller and Dunagan, 1976). When the longitudinal muscles are contracted, these hypodermal canals are very tortuous whereas when the same muscles are relaxed they appear straightened.

HYPODERMAL DUCTS: This name was applied to the small outward extensions observed in previous work (Miller and Dunagan, 1976). Our results provide evidence that hypodermal ducts are small projections on the hypodermal canals' periphery which reach through the radial layer of the tegument (Figs. 6–10). In high magnification

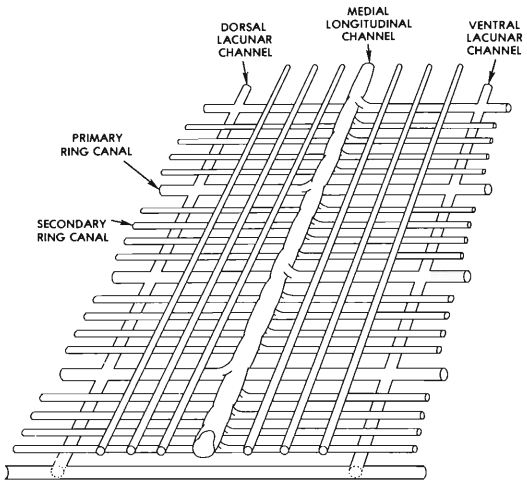


Figure 5. Schematic diagram outlining relationship of medial longitudinal channel to primary and secondary ring canals. Note that radial canals to primary ring canals alternate whereas those to secondary ring canals do not, and the ratio of secondary to primary ring canals is approximately 4:1.

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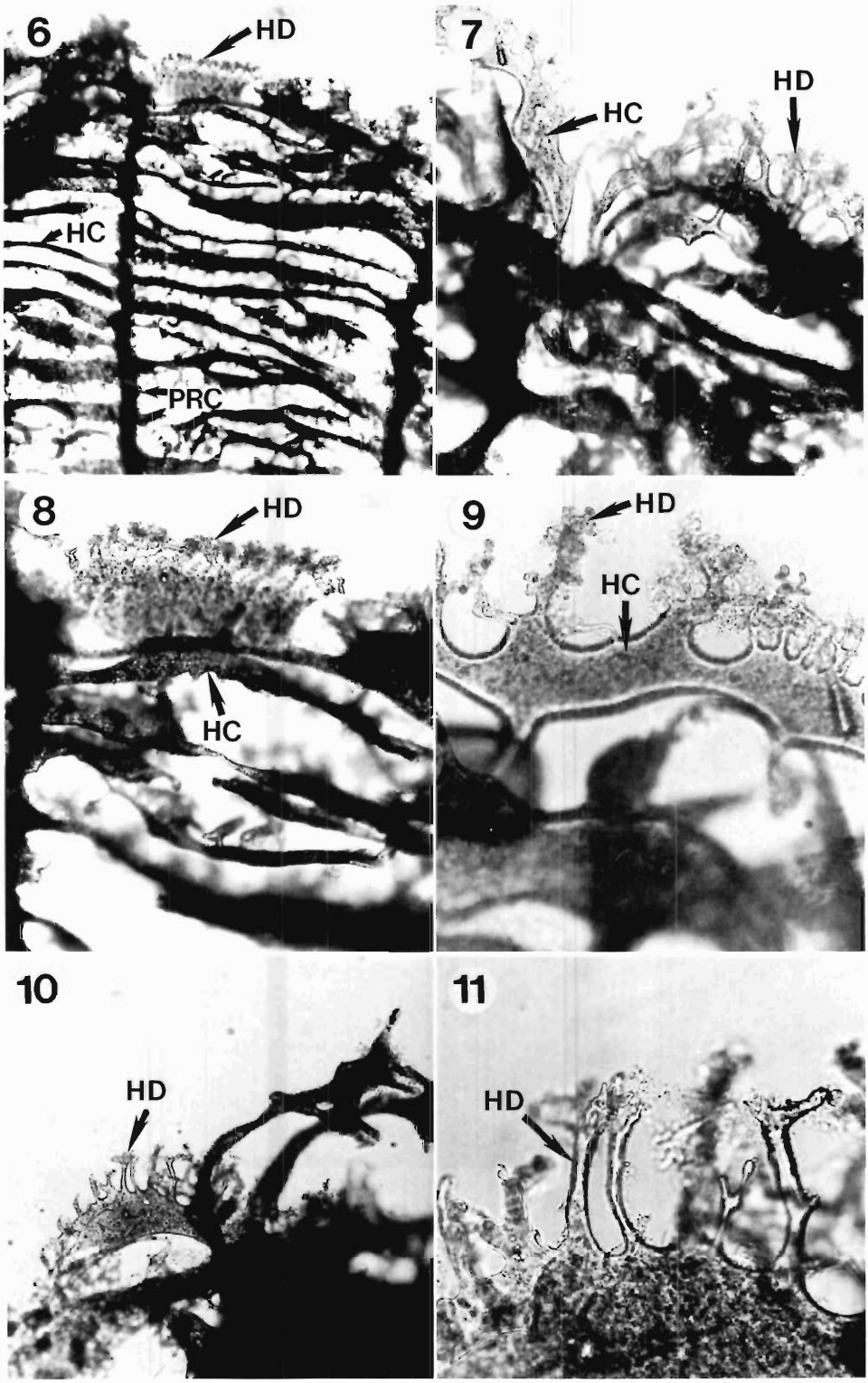
flowed into other tubules of the lacunar system. MLC = Medial longitudinal channel, PRC = primary ring canal, SRC = secondary ring canal, and LMC = longitudinal muscle channel (×100). 3. Enlargement of medial longitudinal channel area from Figure 2 detailing the numerous radial canals that conduct fluid to primary and secondary ring canals (×400). 4. Further enlargement of medial longitudinal channel and radial canals (×1,000).



micrographs (Fig. 10) they appear to be fine, sometimes branched projections (or tufts) from the hypodermal canals which extend to just under the tegument surface.

Discussion

A FLUID TRANSPORT SYSTEM: We consider the lacunar system of the body wall of the acanthocephalan which we have modeled and described herein to be a well-defined "fluid transport system." A fluid transport system (LaBarbera and Vogel, 1982) has been defined as "any system in which internal bulk convection of a fluid functions to reduce diffusion distances between points within an organism or between a point within the organism and the external environment." The definition of a "fluid transport system" has been accepted rather than the use of any variant of "circulatory system" because the latter may imply the presence of a mesodermal lining. In previous studies on the tegument utilizing transmission electron microscopy, lacunar canals were found to be surrounded by membranes in *Polymorphus minutus* (Crompton and Lee, 1965), but were not in *Moniliformis dubius* (Nicholas and Mercer, 1965). This important point needs clarification. If all the canalicular elements of the lacunar system are membrane limited, then perhaps there is no difference between a canal and a channel. But if certain parts in the tegument are not membrane limited, the terminology should be changed accordingly.

Energy for movement of fluids within the acanthocephalan body results from contraction of muscle sets. Acanthocephalans thus have a distributed macropump system which is closed. As such, it follows the usual rules for such systems as previously defined by LaBarbera and Vogel (1982): "(1) While diffusion is always used for short distance transport, it is augmented by bulk flow of fluid for any long distance transport. (2) Transport systems use both large and small pipes—small pipes at exchange sites and large pipes for moving fluid from one exchange site to another. (3) Total cross sectional area of smaller pipes greatly exceeds that of larger pipes, so that velocity in smaller pipes is less than that in larger ones."

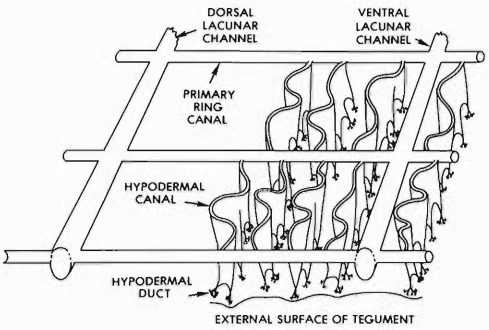


Figure 12. Schematic diagram of lacunar system within the tegument. Hypodermal canals appear straighter when the worm is extended. Hypodermal canals are much more numerous than shown in diagram.

A HYDROSTATIC SKELETON: Acanthocephalans drained of their lacunar fluid are very flaccid and lose their general contractile activity. Thus the lacunar system clearly functions as a hydrostatic skeleton for the worm. Also, we conclude that lacunar channels tend to fuse (i.e., become harder to inject) whenever lacunar fluid is removed or the worm is dehydrated. This and the fact that fixation of the worm usually results in drastic muscle contraction would account for difficulties in studying this system of canals with normal histological techniques.

CIRCULATION DYNAMICS: As diagrammed in Figure 13 the structure of the system allows fluid communication between the outermost tegmental tufts and the innermost rete system and medial longitudinal channel.

In summary, this study has clarified the form and location of some of the previously named canals of the lacunar system, pointed out the

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Figures 6-11. Photomicrographs of a corrosion model of the tegumental system. 6. Low magnification photomicrograph of the model contains representations of primary ring canals (PRC), hypodermal canals (HC), and barely visible are hypodermal ducts (HD) (×100). 7. Enlargement of tegumental model taken from a side angle at the edge. At this magnification hypodermal ducts are more apparent on top of the hypodermal canals (×250). 8. Further enlargement of tegumental model taken from a side angle at the edge. At this magnification hypodermal ducts are evident on top of the hypodermal canals (×160). 9. Close-up photomicrograph of a single hypodermal canal and the hypodermal duct extensions from it (×400). 10. Side view of hypodermal canal and ducts (×160). 11. High power magnification of hypodermal duct extensions (×1,000).

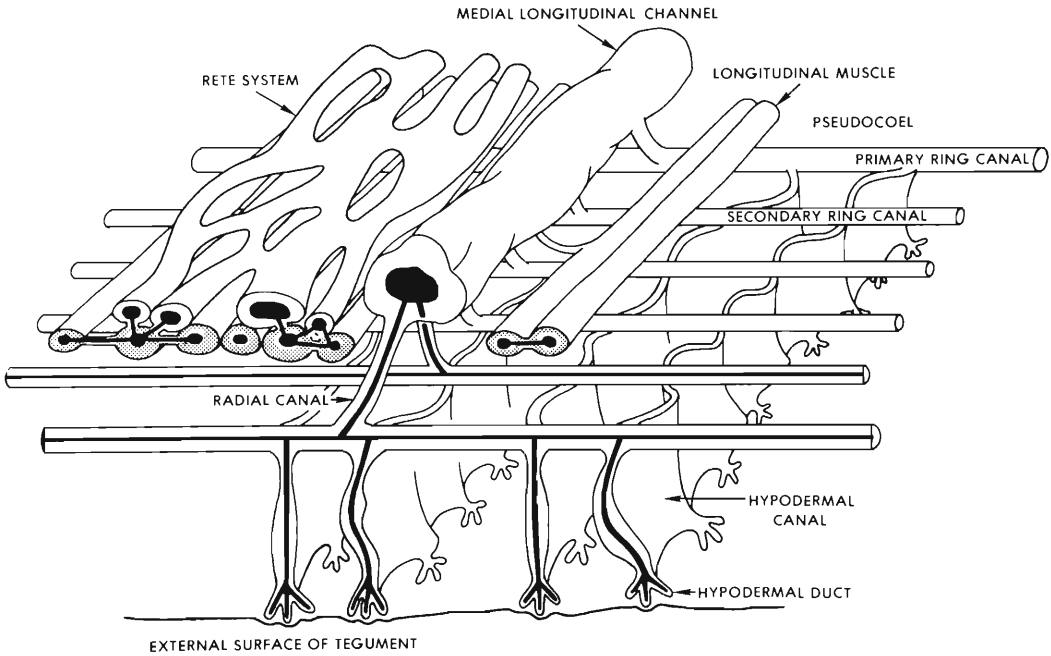

Figure 13. Diagrammatic stereogram of body wall lacunar system showing interconnection of most of the canals and channels that allow for circulation of lacunar fluids throughout body wall. This stereogram is a composite of Figures 5 and 12. Rete system and most longitudinal muscles have been eliminated from right side of the medial longitudinal channel to allow showing the presence of hypodermal canals and ducts underneath in the tegument. Dark lines and spaces indicate interconnections within lacunar system.

extensive interconnection between all of the canalicular elements, identified the possible circular nature of flow within the system, clarified the functional nature of the lacunar system within the acanthocephalan, and demonstrated the use of corrosion modeling in an endoparasitic worm. We speculate that the injection technique described would work for the study of transport systems that must exist within other large, relatively fast-growing endoparasites (e.g., tapeworms), which by virtue of their size would be diffusion limited in their growth.

Acknowledgments

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Phylogenetic Relationships of Some Strongylate Nematodes of Primates

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ABSTRACT: Numerical phylogenetic analysis of 12 species of nematodes in the genus *Oesophagostomum* inhabiting primarily primates is performed. Based on 22 homologous series, treated as 25 numerical characters, the subgenera *Ihlea, Lerouxiella,* and *Conoweberia* are considered a single monophyletic group, the subgenus *Conoweberia.* Host relationships support an interpretation predominantly of coevolution, with little host switching. Biogeographic relationships implied by the phylogenetic tree support an interpretation of African origin, with subsequent dispersal to Southeast Asia, followed by secondary re-invasion of Africa by these helminths and their hosts.

As part of a study on the parasitological evidence pertaining to the phylogeny of the Great Apes, we have analyzed the phylogenetic relationships of the members of the nematode genus *Oesophagostomum* Molin, 1861, which inhabit primates. *Oesophagostomum* is a member of the superfamily Strongyloidea. This superfamily is almost exclusively parasitic in mammals, and is usually characterized by having a high degree of host specificity. Although a number of classifications of the Strongyloidea exist (Popova, 1955; Yamaguti, 1961; Chabaud, 1965), we have relied heavily on the most recent attempt (Lichtenfels, 1980). One of the major problems with earlier classifications is that too much emphasis was placed on too few characters.

> Authors of earlier classifications of the Strongyloidea relied heavily on the morphology of buccal capsules. Separation of families and subfamilies was difficult and arbitrary and there was little indication of the relation between parasite and host evolution below the superfamily level. (Lichtenfels, 1980:1)

Lichtenfels proposed two additional characters in an attempt to make the classification more reflective of the evolutionary process; (1) the shape of the female ovejector and (2) the arrangement of rays associated with the male copulatory bursa. Lichtenfels used these two characters to separate two of the four families of Strongyloidea. Following this scheme, *Oesophagostomum* belongs to the family Chabertiidae (Table 1). Lichtenfels relied on the shape of the buccal capsule to indicate subfamilies. For example, *Oesophagostomum* belongs in the Oesophagostominae, a subfamily characterized by small cylindrical buccal capsules (except those in Australian marsupials), and it is likely that at this level a number of groups are either para- or polyphyletic. The Oesophagostominae contains three tribes, two of which, Bourgelatioidinea and Oesophagostominea, possess a well-developed cervical groove. In the Bourgelatioidinea the groove encompasses the entire body and in the Oesophagostominea it is restricted to the ventral surface. Two genera are placed in the Oesophagostominea, *Daubneyia* (Le Roux, 1940), found in African suids, and *Oesophagostomum,* reported from a number of mammals including primates.

Within *Oesophagostomum* a number of subgenera have been proposed (Table 2), and this splitting has been widely criticized. Thornton's (1924) criticisms stemmed from the fact that the splitting up of the genus *Oesophagostomum* was based primarily on host records, and not on characteristics of the parasites themselves. Yamaguti (1961) supported these criticisms as follows:

> I agree with Goodey (1924), Thornton (1924), and Baylis and Daubney (1926) in that the division of the genus into four subgenera (*Oesophagostomum, Proteracrum* and *Hysteracrum* of Railliet and Henry, 1913; and *Conoweberia* of Ihle, 1922) is unnecessary, and so are the additional subgenera *Bosicola* (Sanground, 1929), *Ihleia* [sic] of Travassos and Vogelsang, 1932, *Hydsonia,* Le Roux, 1940 and *Pukuia* Le Roux, 1940. (p. 394)

We examined in detail three of these subgenera, *Lerouxiella, Ihlea,* and *Conoweberia.* All species

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Table 1. Partial classification of the Strongyloidea according to Lichtenfels (1980).

Superfamily	Family	Subfamily	Tribe
Strongyloidea	Strongylidae		
	Deletrocephalidae		
	Syngamidae		
	Chabertiidae	Chabertiinae	
		Cloacininae	
		Oesophagostominae	Bourgelatioidinea
			Oesophagostominea
			Bourgelatiinea

in these subgenera have well-developed esophageal funnels, consisting of three sclerotized plates, each with a denticle that may project into the posterior portion of the buccal capsule. The subgenus *Ihlea* is characterized by the presence of six denticles. The degree of development of this character is unique to these three subgenera, and it separates them from other members of the genus *Oesophagostomum.*

Materials and Methods

Specimens belonging to the subgenera *Lerouxiella, Conoweberia,* and *Ihlea* were borrowed from various parasite collections. Table 2 lists the species examined, and the museums from which specimens were obtained. The specimens were stored in a mixture of 70% ethanol and 5% glycerine, and cleared in lactophenol before examination. A drawing tube was used to prepare Figures 1–4.

Specimens of *O. susannae, O. raillieti, O. zukowskyi,* and *O. ventri* were not available. For these species original descriptions and diagrams were used. Papers in which additional characters and measurements were proposed were also used (for example, Travassos and Vogelsang, 1932). Although more than 20 nominal species have been assigned to *Ihlea, Conoweberia,* and *Lerouxiella,* only 12 species are recognized as valid in this analysis. This agrees with the most recent key for these subgenera, in which 11 species were recognized (Chabaud and Durette-Desset, 1973). The only species added is *O. brumpti.* On the basis of its overall length, shape of the buccal capsule, and size of its spicules it is distinct from other members of the study group. However, only two specimens were available, and ideally more specimens are required to more accurately establish the ranges of some characters.

The systematic technique used in this analysis was cladistics or phylogenetic systematics (Hennig, 1966; Wiley, 1981). A number of precis of this methodology have been published (Brooks et al., 1984, 1985), and we will add only statements that refer specifically to the present study. Polarization of character transformations was based on the outgroup criterion. These character transformations were summarized in a numerically coded data matrix (Table 4). Analysis of the polarized character transformations was aided by use of the PHYSYS computer systematics system, developed by Dr. J. S. Farris (State University of New York, Stony Brook).

Character analysis

A total of twenty-two characters was used in this analysis. For a summary of oesophagostomine morphology, see Chabaud and Durette-Desset (1973). Some characters, such as the shape of the external elements, form fairly simple binary characters, whereas others, such as the number of denticles, form more complex multi-state characters. All twenty-two characters and their character states are summarized in Table 3. Some characters, for example, the number of elements in the external corona, are not sufficiently explained in the table, and the following sub-sections provide further explanation.

1) Number of elements in the external crown: The only way to clearly establish the number of external elements is to do en face examination of the anterior

Table 2. *Oesophagostomum* species and specimen location.

Subgenus	Species	Museums A	B	C
Conoweberia	*blanchardi* Travassos and Vogelsang, 1932	X	X	
	aculeatum Linstow, 1879		X	
	bifurcum Creplin, 1849	X	X	X
	ovatum Linstow, 1906	X		
	pachycephalum Molin, 1861	X	X	X
	raillieti Travassos and Vogelsang, 1932			
	zukowskyi Travassos and Vogelsang, 1932			
	brumpti Raillieti and Henry, 1905	X		
Ihlea	*stephanostomum* Stossich, 1904	X	X	X
	ventri Thornton, 1924			
Lerouxiella	*xeri* Ortlepp, 1922	X		
	suzannae LeRoux, 1940			

Museums: A—British Museum of Natural History (London); B—United States National Museum (Beltsville); C–Museum National D'Histoire Naturelle (Paris).

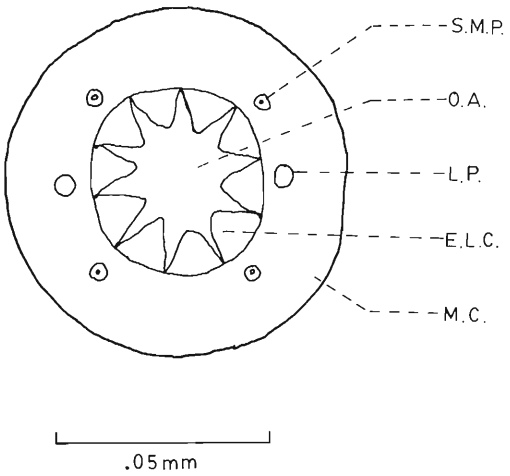

Figure 1. ***Oesophagostomum bifurcum*** **en face view. Abbreviations: S.M.P., sub-medial papillae; O.A., oral aperture; L.P., lateral papillae; E.L.C., external leaf crown; M.C., mouth collar.**

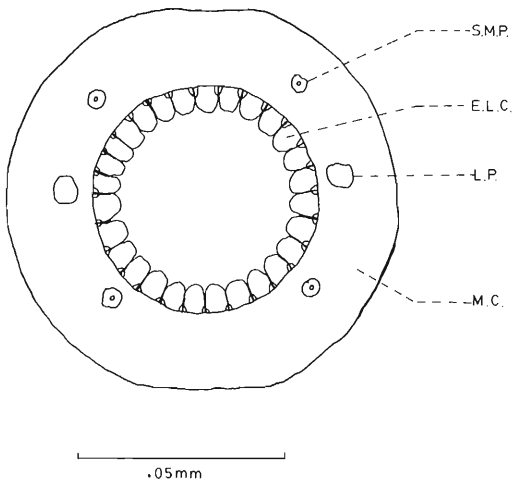

Figure 2. ***Oesophagostomum stephanostomum*** **en face view. Abbreviations: S.M.P., sub-medial papillae; E.L.C., external leaf crown; L.P., lateral papillae; M.C., mouth collar.**

ends of worms. Many descriptions however, have been based on whole mounts, and the number of elements have usually been placed at ten. In a few studies en face views have been examined, and in some of these the number of elements have been reported at 12–16, and in three species the number reported was numerous (30–40 elements). Preparation of en face views of all the species would have been useful in resolving this dispute. Unfortunately, however, not enough material was available to permit sectioning. Regardless of whether 10–16 elements are present, there are at least two clearly defined character states in the study group, those species with few elements (10–16), and those with many elements (30–40).

2) PATTERN OF ELEMENTS OF THE EXTERNAL CORONA—EN FACE VIEW: There are two states evident for this character. For most members of the subgenus *Conoweberia* the external elements converge towards the center of the oral aperture (Fig. 1). In contrast, those in *O. stephanostomum* and *O. ventri* do not converge (Fig. 2).

3) PATTERN OF ESOPHAGEAL SCLEROTIZATION: All the species in the study group have a well-developed esophageal funnel. In most species the funnel is described as cup- or goblet-shaped, the sides being curved. In members of the subgenus *Ihlea* and in *O. pachycephalum* the funnel is V-shaped, the sides being straighter.

4) SHAPE OF THE ELEMENTS OF THE EXTERNAL CORONA: Two states are evident for this character. For most members of the subgenus *Conoweberia* the elements are pointed and triangular in shape. In those species with more than 30 external elements, the elements are more rounded and cylindrical in shape.

5) NUMBER OF DENTICLES IN THE ESOPHAGEAL FUNNEL: The pattern and the degree of development of the esophageal denticles is unique to the study group. In members of the subgenera *Conoweberia* and *Leroux-iella* there are three denticles projecting into the lumen of the funnel, and in *Ihlea* there are six denticles present. As with the number of external elements, it is very difficult to count the number of denticles when looking at a lateral section of a whole mount. A serial section through the funnel is necessary to accurately establish the number of denticles. To the best of our knowledge serial sections of *O. pachycephalum* have not been made, but unfortunately not enough material was available to permit sectioning. These would be particularly interesting in light of its large number of external elements and the shape (V) of the esophageal funnel.

Only shared derived or synapomorphic traits are capable of indicating natural groups. Because of this, determining which characters are derived (apomorphic) and which are primitive (plesiomorphic) is very important. The most general method for determining character polarity is the outgroup method (Lundberg, 1972; Wiley, 1981). This method is based on the following concept:

> Given two characters that are homologues and found within a single monophyletic group, the character that is also found in the sister group is the plesiomorphic character whereas the character found within the monophyletic group is the apomorphic character. (Wiley, 1981, p. 139)

That is, the character state present in at least one member of the study group (or ingroup), and in some species outside the ingroup (the outgroup), will be primitive. Usually the most suitable outgroup is the sister group, or a group that is closely related to the ingroup. In order to determine which members of the genus *Oesophagostomum* are most suitable as outgroups, the phylogenetic relationships of the various subgenera have been partially reconstructed (Fig. 3). Information for this cladogram was obtained from keys published by Chabaud and Durette-Desset (1973) and Lichtenfels (1980). The cladogram suggests that the subgenera *O.*

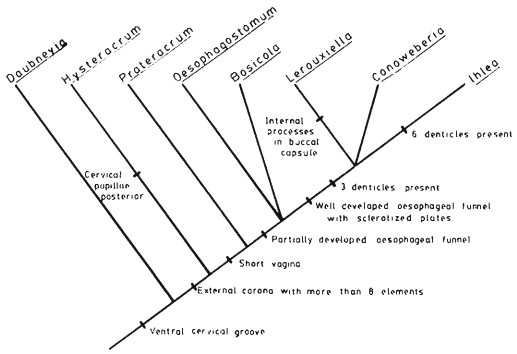

Figure 3. Partial phylogenetic reconstruction of the genus *Oesophagostomum*. Terminal taxa are subgenera.

Bosicola and *O. Oesophagostomum* are the most appropriate outgroups.

For two types of characters, however, the above outgroups were not sufficient for completely determining the polarity of transformation series. In these cases the functional outgroup method outlined by Watrous and Wheeler (1981) was used. This method is based on the idea that, in those instances where the outgroup is unable to determine the polarity of a transformation, it is best to use the most plesiomorphic members of the ingroup as a functional outgroup. The "outgroup comparison need not be constrained by nomenclatural rank or Linnaean hierarchical structures" (Watrous and Wheeler, 1981). Thus, determining the most plesiomorphic members of the ingroup is based on characters polarized using the initial outgroup (the subgenera *Bosicola* and *Oesophagostomum*). An example of this method involves the esophageal funnel. All members of the study group have well-developed sclerotized funnels with projecting denticles. This character is absent in the outgroup subgenera, and consequently these outgroups are unable to determine which character state, cup-shaped or V-shaped, is primitive. However, on the basis of other characters, *O. zukowskyi, O. bifurcum, O. brumpti,* and *O. aculeatum,* are the most plesiomorphic members of the ingroup, and because all these species have a cup-shaped funnel, this character state is hypothesized to be primitive. The same reasoning was used to polarize the transformation series for the character involving the length of the esophageal funnel.

The other type of character that required a functional outgroup was that in which there was considerable variation in the outgroup. For example, in the subgenus *O. Bosicola* the cervical papillae are situated close to the ventral groove, and in the subgenus *Oesophagostomum* they are more posterior, being closer to the esophageal swelling. Within the ingroup the papillae occur between the above two extremes. Without fully resolving the relationships between the subgenera *Bosicola* and *Oesophagostomum* it is impossible to tell whether the trend in the ingroup is towards the papillae moving anteriorly (from that state inthe subgenus *Oesophagostomum*), or posteriorly (from that state in the subgenus *Bosicola*). Appealing to a functional outgroup suggests that primitively the papillae are close to the ventral groove.

Character states designated "0" are the plesiomorphic states, and positive whole numbers indicate the derived states. Reversing this convention (for binary characters) would not affect the analysis so long as the same convention was followed for an entire data set. The coded values and their corresponding character states are summarized in Table 3. The character states found in each species of the ingroup are summarized in Table 4. A value of "9" was entered for missing data; there are two reasons for such entries. For some species, for example, *O. raillieti,* specimens were not available, and the original descriptions were not sufficiently detailed to establish the nature of all characters and character states used in this analysis. Also, sectioning of specimens was not possible and consequently some character states in a few species could not be determined.

One of the statistics used to measure the fit of particular characters to a hypothesized phylogeny is the consistency (c) index (Farris, 1970). This value is calculated by dividing the minimum possible number of character transformations by the actual number of transformations hypothesized from the phylogeny. For example, a c-index of 1.0 (100%) for a particular character suggests that there is no postulated homoplasy for that character. Rather than list the c-index for each character, an overall c-index is usually listed. This value represents an average fit of all the characters used to the hypothesized tree topology.

Three characters in this analysis were split into two transformation series each. The reason for this was that leaving them as a single transformation series resulted in artificially low c-indices. For example, there are four character state ranges for male body length: 6–13 mm, 13–18 mm, 18–25 mm, and less than 6 mm. The range in the outgroup is 6–13 mm and this state was coded as 0. This suggests two trends, one of increasing length to 13–18 mm and 18–25 mm, and one of decreasing length to less than 6 mm. If the four character states are coded as: 6–13 mm = 0, 13–18 mm = 1, 18–25 mm = 2, and <6 mm = 3, a c-index of 0.60 is calculated. This reflects the fact that PHYSYS reads the transformation series as

$$0-1-2-3$$

which suggests that there is an increase in length followed by a secondary decrease. However, if the transformation is coded as

$$\begin{array}{l} 0-1-2 \\ \quad\ \ \backslash 3 \end{array}$$

a c-index of 1.00 is calculated. In order to code for this second transformation series the character *male body length* was split into two transformation series. The other two characters treated in this manner were *female body length* and *male body width.*

Results

Twenty-two characters were studied and used to reconstruct the phylogeny of the 12 species in the subgenera *Conoweberia, Ihlea,* and *Leroux-*

Table 3. Summary of characters and coded character states.

Character	Character states	Coding
Male body length:		
Increasing transformation	6–13 mm	0
	13–18 mm	1
	18–25 mm	2
Decreasing transformation	6–13 mm	0
	<6 mm	1
Female body length:		
Increasing transformation	7–13 mm	0
	13–20 mm	1
	20–30 mm	2
Decreasing transformation	7–13 mm	0
	<7 mm	1
Number of elements in the external corona	8–14	0
	30–40	1
Pattern of external elements—en face section	Figure 1	0
	Figure 2	1
Pattern of esophageal sclerotization	Absent	0
	Cup-shaped	1
	V-shaped	2
Shape of external elements	Triangular	0
	Rounded	1
Number of esophageal teeth	Absent	0
	3	1
	6	2
Distance of ventral groove from anterior end	0.16–0.23 mm	0
	0.23–0.30 mm	1
	0.30–0.45 mm	2
Width of cephalic distension	0.12–0.25 mm	0
	0.25–0.32 mm	1
Distance of cephalic papillae from anterior end	0.30–0.40 mm	0
	0.40–0.50 mm	1
	0.50–0.60 mm	2
Length of esophagus	0.30–0.40 mm	0
	0.70–1.0 mm	1
	1.0–1.4 mm	2
Width of esophagus	0.09–0.16 mm	0
	0.16–0.20 mm	1
	0.20–0.30 mm	2
Length of external elements	0.025–0.040 mm	0
	0.012–0.025 mm	1
Ratio of buccal capsule length to width	1/1.3–1/1.5	0
	1/2.5–1/3.2	1
	1/3.6–1/4.1	2
Width of buccal capsule	0.020–0.050 mm	0
	0.050–0.070 mm	1
	0.080–0.140 mm	2
Length of esophageal funnel	0.024–0.040 mm	0
	0.040–0.10 mm	1
Length of spicule	0.70–1.25 mm	0
	0.50–0.70 mm	1
	1.25–2.00 mm	2
Length of female tail	0.20–0.40 mm	0
	0.13–0.20 mm	1
Transverse processes	Absent	0
	Present	1
Distance of vulva from posterior end	0.50–0.75 mm	0
	0.35–0.50 mm	1
Maximum female body width	0.30–0.50 mm	0
	0.50–0.65 mm	1
	0.65–1.10 mm	2
Maximum male body width:		
Increasing transformation	0.30–0.60 mm	0
	0.60–1.20 mm	1
Decreasing transformation	0.30–0.60 mm	0
	<0.30 mm	1

iella. Figure 4 represents the most parsimonious phylogeny for these taxa and characters. The character transformations used in this analysis have been mapped on the cladogram. Those characters postulated to exhibit some homoplasy are marked with a star. The overall consistency index for this cladogram is 80%. This figure represents an average measure of fit for all characters used in the hypothesized tree topology. The individual c-indices for all the characters are listed in Table 5. A c-index of 80% is a high consistency, representing a good fit of the data to the tree topology.

A number of characters in this analysis are used as absolute lengths. That is, they were not converted to ratios. These include such characters as esophageal length, female tail length, and length and width of the cephalic distension. In a few descriptions and diagnoses these characters have been presented as ratios. During the present study these characters were coded as ratios (proportional to overall body length and width), and it was found that doing this did not affect the topology of the tree but did decrease the c-index. For this reason these characters were left as absolute lengths. Furthermore, removing these characters had little effect on the cladogram. Of the 11 nodes, two collapsed resulting in one polytomy. The rest of the tree remained identical with the fully resolved tree shown in Figure 4.

The monophyletic nature of the 12 species in this study is supported by three characters. Two of these involve the esophageal funnel. All members in the study group have well-developed sclerotized funnels that are either cup- or

Table 4. Data for *Oesophagostomum* analysis.

	Characters																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Outgroup	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
blanchardi	1	0	1	0	0	0	1	0	1	1	0	2	1	1	1	1	1	1	2	0	0	0	1	0	0
aculeatum	0	0	1	0	0	0	1	0	1	1	0	0	0	0	1	1	0	1	0	1	1	0	0	0	0
bifurcum	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	1	0	0	0	0	9	0	0	0	0
brumpti	0	0	0	0	0	9	1	0	1	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0	0
ovatum	1	0	1	0	0	9	1	0	1	2	1	2	9	1	1	2	2	1	2	9	9	0	9	1	0
pachycephalum	2	0	2	0	1	9	2	1	1	2	1	2	2	2	1	2	2	1	2	0	0	0	2	1	0
raillieti	1	0	1	0	0	9	1	0	1	2	9	9	2	9	1	1	2	1	2	0	0	0	2	1	0
susannae	0	0	1	0	0	9	1	0	1	1	0	1	0	0	1	1	0	1	2	1	1	1	1	0	0
ventri	2	0	2	0	1	1	2	1	2	2	1	2	2	2	1	2	2	1	2	0	0	0	2	1	0
xeri	0	0	1	0	0	0	1	0	1	1	0	1	0	0	1	1	0	1	2	1	1	1	1	0	0
zukowskyi	0	1	0	1	0	0	1	0	1	0	0	1	0	0	1	0	1	0	1	0	0	0	0	0	1
stephanostomum	2	0	2	0	1	1	2	1	2	2	1	2	2	2	1	2	2	1	2	0	0	0	2	1	0

V-shaped. Also, projecting into the lumen of the esophageal funnel are three (or six) denticles. The third character unique to the study group involves the size of the elements of the external corona. In all species they are in the range of 0.012–0.025 mm.

The parasite phylogeny presented in this study suggests that two of the subgenera studied (*Ihlea* and *Lerouxiella*) are monophyletic, and one (*Conoweberia*) is paraphyletic. A paraphyletic group is a group that includes a common ancestor and some but not all its descendants (Farris, 1974). The monophyly of *Lerouxiella* is supported by the presence of transverse processes in the buccal capsule, and the monophyly of *Ihlea* by the presence of six esophageal denticles, and the en face pattern of the elements of the external corona. Traditionally two species (*O. stephanostomum* and *O. ventri*) have been placed in the subgenus *Ihlea,* and one of the major diagnostic features of this subgenus has been the presence of 30–40 external elements. The *O. pachycephalum* specimens examined in this study also had 30–40 elements. This suggests that this species belongs in the subgenus *Ihlea* and not *Conoweberia.* In order to clarify this point the type specimens should be examined. Also, serial sections and an en face view are necessary to establish the number of esophageal denticles and the pattern of the external elements. Because the number of *O. pachycephalum* specimens available was limited it was not possible to get permission to section this species.

Although this analysis recognizes that *Ihlea* and *Lerouxiella* are monophyletic, we do not recommend that these two subgenera be maintained, but that they be considered junior synonyms of the subgenus *Conoweberia.* The reason for this is that if *Ihlea* and *Lerouxiella* are recognized, there is no way to group the remaining eight species in a manner consistent with their genealogy without assigning each species to its

Table 5. Consistency indices of characters used in the analysis of *Oesophagostomum*.

No.	Character	Consistency index
1	Increasing male body length	100.00
2	Decreasing male body length	100.00
3	Increasing female body length	100.00
4	Decreasing female body length	100.00
5	Number of external elements	100.00
6	Pattern of external elements en face view	100.00
7	Pattern of funnel sclerotization	100.00
8	Shape of external elements	100.00
9	Number of esophageal teeth	100.00
10	Length of cephalic distension	100.00
11	Width of cephalic distension	100.00
12	Position of cephalic papillae	66.67
13	Length of esophagus	100.00
14	Width of esophagus	100.00
15	Length of external elements	100.00
16	Buccal capsule ratio	100.00
17	Buccal capsule width	66.67
18	Length of esophageal funnel	100.00
19	Length of spicules	100.00
20	Length of tail	50.00
21	Position of vulva	50.00
22	Presence of transverse processes	100.00
23	Female body width	100.00
24	Increasing male body width	100.00
25	Decreasing male body width	100.00

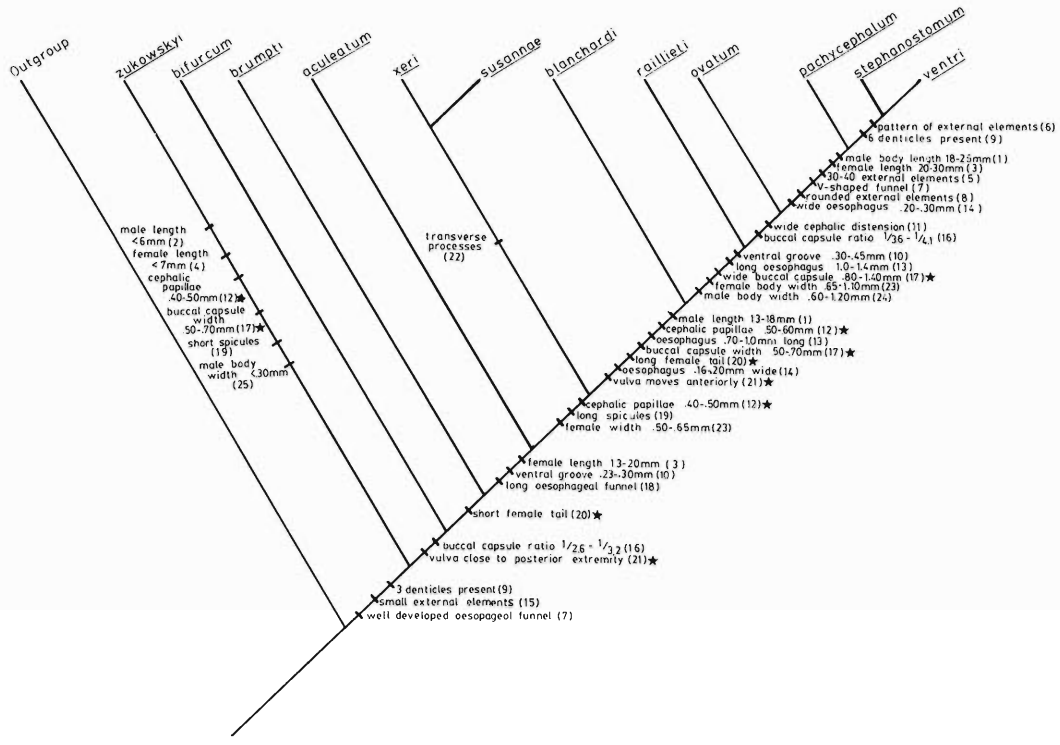

Figure 4. Phylogeny of *Oesophagostomum* (*Conoweberia*). Each slash mark designates synapomorphic occurrence of trait listed beside it. Stars indicate homoplasious characters.

own subgenus. Ten subgenera would be required for the 12 species in the study group. This would considerably complicate the existing classification. By assigning all 12 species to the subgenus *Conoweberia,* the existing classification is disrupted least. Thus, it is proposed that all those *Oesophagostomum* species with a well-developed esophageal funnel with denticles (three or six), and small elements of the external corona be assigned to the subgenus *Conoweberia.*

Discussion

The existence of a particular parasite in a particular host may be explained in terms of either a historical coevolutionary association, or a more recent host transfer. In the case of coevolution the parasite or its close relatives are postulated to have evolved with the host lineage. In contrast, hypotheses of host transfers are required when a parasite is reported from a host and there appears no congruence between the parasite phylogeny and the host phylogeny. In order to distinguish between those associations that have resulted from host/parasite coevolution, and those from more recent host transfers, phylogenies of the parasites and the hosts are required.

The phylogeny of the *Oesophagostomum* species studied in this analysis suggest a pattern that can be explained predominantly in terms of coevolution. Table 6 presents a list of the 12 parasite species analyzed and the hosts in which they are found. Of these 12, all but three are present in primates.

The primate relationships that require postulating the fewest host transfers for the *Oesophagostomum* species in the study group is shown in Figure 5. According to this host phylogeny seven host transfers are necessary to explain the host records. These transfers are mapped onto the parasite phylogeny (Fig. 6). The first of these host transfers involved the infection of primates by the common ancestor of all *Conoweberia* species. Postulating this as a transfer is supported by the occurrence of the outgruop subgenera *Bosicola* and *Oesophagostomum* in ruminants and suids respectively, rather than New World monkeys, lemurs, or insectivores. The four most plesiomorphic parasite species, *O. zukowskyi, O.*

Table 6. Host records and distribution of species in the study group.

Parasite species	Host genera	Distribution
O. zukowskyi	*Papio*	Africa
O. bifurcum	*Cercopithecus, Papio, Macacus, Cercocebus, Cynomolgus Homo, Pan*	Africa, S.E. Asia
O. brumpti	*Macacus, Homo, Pongo*	Africa
O. aculeatum	*Macacus, Cebus, Cynomolgus*	S.E. Asia
O. susannae	*Pedetes*	S. Africa
O. xeri	*Xerus*	S. Africa
O. blanchardi	*Hylobates, Pongo*	S.E. Asia
O. raillieti	*Hylobates*	S.E. Asia
O. ovatum	*Hylobates*	S.E. Asia
O. pachycephalum	*Cercopithecus*	Africa
O. stephanostomum	*Homo, Pan, Gorilla*	Africa, S. America
O. ventri	"Cat"	S. America

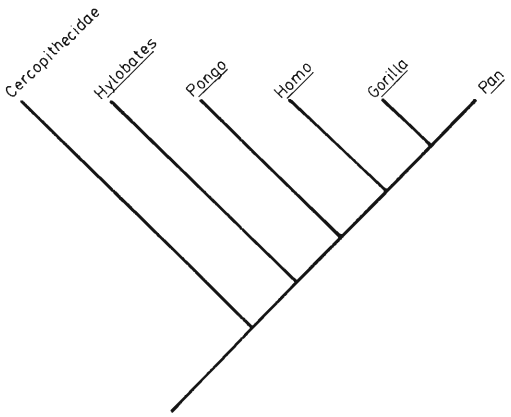

Figure 5. Primate phylogeny that best fits *Oesophagostomum* (*Conoweberia*) phylogeny. This phylogeny is consistent with the one presented by Schwartz et al. (1978).

bifurcum, O. brumpti, and *O. aculeatum,* are all reported from Old World monkeys (family Cercopithecidae), and these associations are consistent with a coevolutionary hypothesis. Two of these four parasite species have secondarily transferred to additional primate hosts. *O. bifurcum* has infected humans and chimpanzees, and *O. brumpti* has infected humans. Two parasite species in the study group, *O. xeri* and *O. susannae,* are reported from South African rodents (ground-squirrels and springhares, respectively); and their presence in these hosts is clearly the result of a host transfer. A second transfer is likely to have occurred from ground-squirrels to springhares, or vice-versa, but because it has no bearing on the relationships with primates it has not been included in Figure 6. According to Le Roux (1940) these two hosts live in close proximity, thus facilitating this host transfer. The three *Oesophagostomum* species from gibbons are also consistent with a coevolutionary hypothesis. One parasite species from gibbons (*O. blanchardi*) has also been reported from orangutans. Because *O. blanchardi* is the most plesiomorphic of the species in gibbons, its occurrence in orangutans is postulated to be the result of a host transfer. The presence of *O. pachycephalum* in monkeys is also postulated to be the result of a host transfer. *Oesophagostomum stephanostomum* has been reported from humans, chimpanzees, and gorillas. Finally, *O. ventri* was described from a wild cat (the scientific name of the wild cat was not given in the original description) in Brazil (Thornton, 1924). If this species is valid its relationship with cats is clearly the result of a host transfer. Of the 12 parasite species studied in this analysis, eight are present in a host for which a coevolutionary hypothesis is sufficient. The only parasites for which hypotheses of host transfers are necessary are *O. xeri* and *O. susannae* from rodents, *O. pachycephalum* from monkeys, and *O. ventri* from cats. Of those species in primates only *O. pachycephalum* does not fit into a coevolutionary framework. Furthermore, of the eight species that appear to have coevolved with primates, three have secondarily transferred to additional primate hosts. These include the infection of humans and chimpanzees by *O. bifurcum,* humans by *O. brumpti,* and orangutans by *O. blanchardi.*

The geographic distributions of the parasite species studied in this analysis are listed in Table 6. Most of these distributions are listed as general distribution, for example, Africa and Southeast Asia. One of the reasons for this is that for some primates, the parasites are removed in zoos and research settings that may be far from the original host habitat. Consequently, the exact locations of primates are not always specified. Also, the collections from primates are uneven and incomplete, and accurate geographic distributions can only be speculative.

One of the most important steps in discussing the biogeography of a group of organisms is the evolutionary history or phylogeny of that group

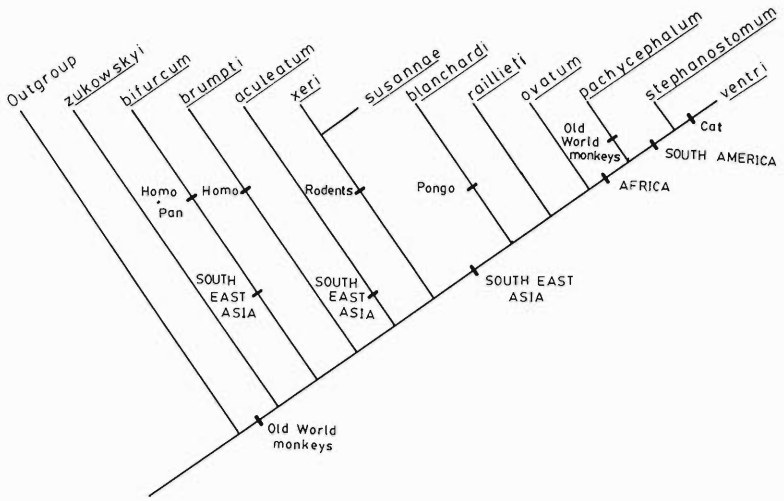

Figure 6. Phylogeny of *Oesophagostomum* (*Conoweberia*) with host and geographic changes.

of organisms. The biogeographic scenario developed in this thesis is dependent on the parasite phylogeny proposed herein. In Figure 6 the minimum number of major geographic changes are mapped onto the parasite cladogram. The outgroups used in this analysis were not useful in establishing the plesiomorphic geographic range of the *Oesophagostomum* species in primates, because both the subgenera *Bosicola* and *Oesophagostomum* are ubiquitous. However, the occurrence of *O. zukowskyi, O. bifurcum,* and *O. brumpti* in Africa suggest that Africa is the plesiomorphic range for *Oesophagostomum* species in primates. This suggests that *O. bifurcum* has secondarily dispersed to Southeast Asia. The existence of *O. aculeatum, O. blanchardi, O. raillieti,* and *O. ovatum* in Southeast Asian primates and *O. xeri* and *O. susannae* in South African rodents suggest either of the following two scenarios, each of which require hypothesizing two geographic changes. In the first scenario, the common ancestor of *O. aculeatum* dispersed to Southeast Asia, and those species in rodents have dispersed back to Africa. In the second scenario there have been two dispersal events to Southeast Asia, the first involving *O. aculeatum,* and the second the common ancestor of *O. blanchardi* and the rest of the subgenus. In terms of the number of geographic changes both these scenarios are equally parsimonious. The occurrence of *O. pachycephalum* and *O. stephanostomum* in Africa suggest that there has been a dispersal event returning these parasites to the plesiomorphic range. Thus, there appear to be two major geographic shifts affecting the *Oesophagostomum* species in primates, a dispersal from Africa to Southeast Asia followed by a return from Southeast Asia to Africa. Finally, there has been a dispersal event to South America (Brazil) by the ancestor of *O. stephanostomum* and *O. ventri.* Because *O. stephanostomum* is present in humans, chimpanzees, and gorillas (and the latter two hosts do not appear to disperse very much) it is very likely that humans have transmitted this parasite to South America. The indigenous South Americans are hypothesized to have come from Asia, so it is possible that these species were transmitted with the African slave trade.

Summary

A well-corroborated phylogenetic tree of members of the genus *Oesophagostomum* inhabiting primates supports the hypothesis that the parasite species form a monophyletic group, the subgenus *Conoweberia.* The parasite species exhibit host relationships that are predominantly coevolutionary, with relatively few cases of host switching. The geographic history implied by the phylogenetic tree suggests an African origin, with dispersal to Southeast Asia, followed by re-invasion of Africa.

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Scanning Electron Microscopy of Adult *Parascaris equorum* (Nematoda)

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ABSTRACT: The scanning electron microscope was used to illustrate the microtopographic features of adult *Parascaris equorum*. The shapes of the labia of *P. equorum* were distinctive. The papillae on the three labia comprised an external ring of four large double papillae consisting of two dorsolaterals on the dorsal labium and one ventrolateral on each subventral labium. Also on each subventral labium was an externolateral papilla and an amphid. The six internal labial papillae consisted of small pits. The shape and distribution of the denticles were similar to descriptions for other ascaridoid nematodes. The preanal papillae of the male were numerous and there were five pairs of postanal papillae that were distributed as follows: the first pair just posterior to the anus being double papillae with the remaining four pairs more closely associated in a group near the tail end; the second pair also being double papillae, and the last three pairs of papillae were single. The fourth pair of caudal papillae were the phasmids (between the third and fifth pairs of papillae) that were situated more laterally and appeared closer to the third pair of postanal papillae. The caudal end of the female lacked papillae except for the phasmids, which were approximately one-third the distance from the tip of the tail to the anus. Several of these findings represent new information concerning the adult structure of *P. equorum*.

Parascaris equorum is an ascarid that occurs in the small intestine of the horse, ass, mule, zebra, and other equids throughout the world. Infection with this parasite is very common in foals and young horses and when present in large numbers may kill their hosts (Levine, 1980).

Yorke and Maplestone (1926) orignally described this ascarid with the light microscope and a few of the current texts in veterinary parasitology refer to the original description given by these authors. Lichtenfels (1975) further described this ascarid with the aid of the light microscope. Ansel et al. (1974) described the cephalic end of adult *Parascaris* and Pilitt et al. (1979) described the cephalic structures of fourth- and early fifth-stage *Parascaris* with the aid of the scanning electron microscope (SEM).

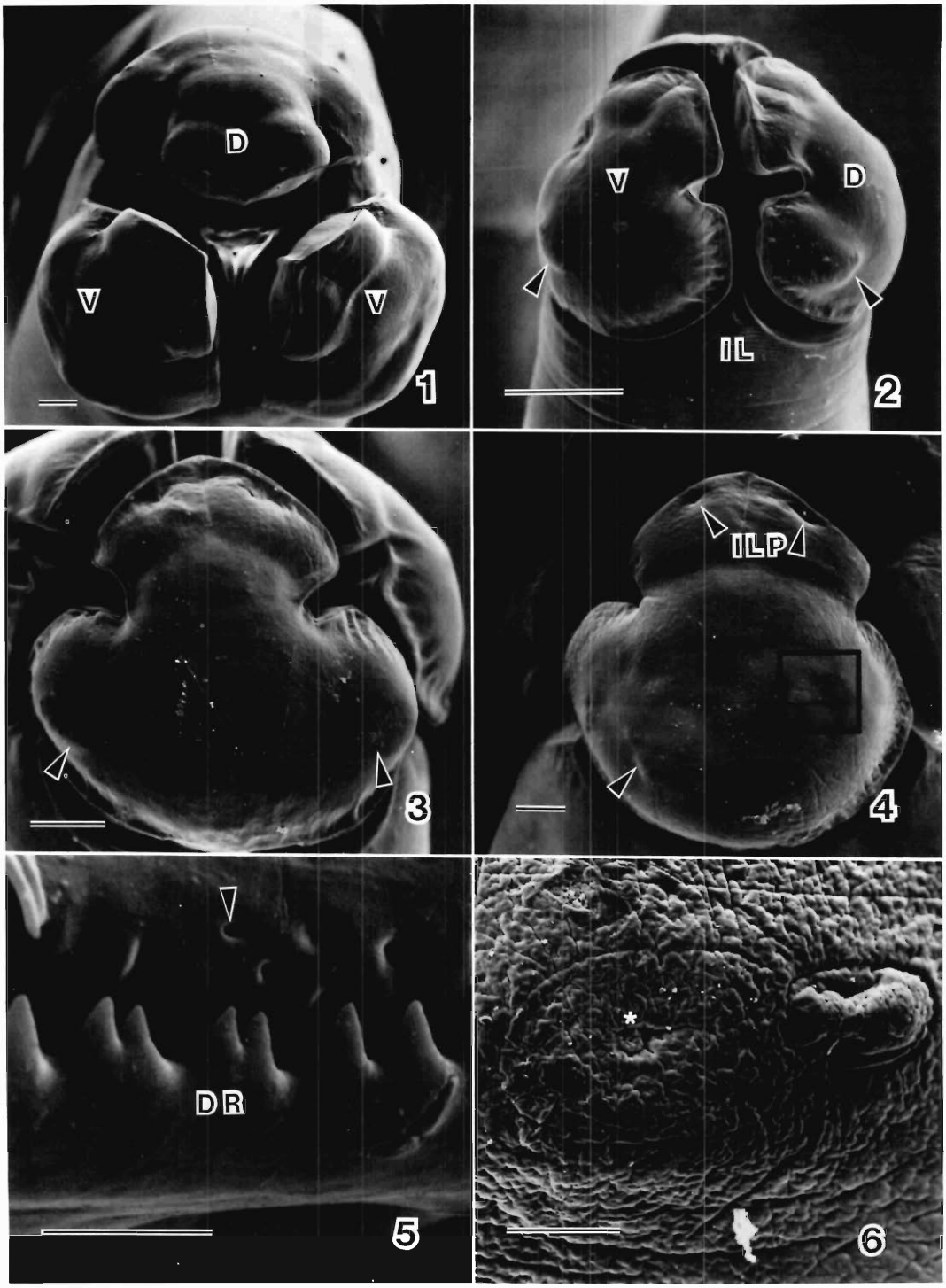
Within the last 10–15 years the SEM has proven to be an important aid in observing the microtopographic features of various nematode species (Madden et al., 1970; Uni and Takada, 1975; Wong and Brummer, 1978; Tiekotter, 1981). It is extremely important when one is working with a particular parasite to have a thorough working knowledge of its taxonomic characteristics. The purpose of this paper is to present detailed information on the microtopographic features of the cephalic and caudal ends of adult *Parascaris equorum* with the aid of the SEM.

Materials and Methods

Adult *Parascaris equorum* were recovered from the small intestines of young horses necropsied at the University of Illinois, College of Veterinary Medicine. Initially, the worms were washed several times in physiologic saline and finally in 10% acetic acid in order to remove any mucus or host debris. Five to 10 mm of the cephalic and caudal ends of individual worms were cut away with a razor blade and fixed in 3.0% phosphate-buffered glutaraldehyde (pH 7.3) for 18–24 hr. Fixed specimens were then washed several times over an hour in 0.1 M phosphate buffer (pH 7.3) and sonically cleaned for 2 min. The specimens were then post-fixed for one hour in 1.0% osmium tetroxide in 0.1 M phosphate buffer (pH 7.3) at room temperature. The specimens were then washed several additional times in buffer and dehydrated in standard dilutions of ethanol up to 70% ethanol–5% glycerol and stored until final processing. Worms taken from 70% ethanol–5% glycerol were dehydrated through 100% ethanol and critical-point dried in CO_2 with a Tousimis Samdri®-790 critical-point drying system (Tousimis Research Corp., Rockville, Maryland). Dried pieces of worms were attached to aluminum SEM stubs with the aid of a mixture of equal parts of Duco® Cement (Dupont Co., Wilmington, Delaware) and isopropyl colloidal graphite (Ted Pella, Inc., Tustin, California). The stubs with attached worms were placed in a SPI sputter coater® (SPI Supplies, West Chester, Pennsylvania) and coated with a thin layer of gold. Coated specimens were stored in a desiccator jar charged with calcium chloride until observed at the Center for Electron Microscopy, University of Illinois, with the aid of an ISI-DS-130 scanning electron microscope operating at an accelerating voltage of 10 keV. Micrographs were made with either Polaroid P/N 55 (Polaroid Corp., Cambridge, Massachusetts) or Kodak 4127 graphic arts film (Eastman Kodak Co., Rochester, New York). The observations were made on 25–30 specimens of each sex.

Results

Scanning electron micrographs illustrating the characteristic microtopographic features of adult



Figures 1–6. Scanning electron microscopy of labia and associated structures of *Parascaris equorum*. 1. En face view showing dorsal labium (D), ventral labia (V), and stoma. ×65; bar = 100 μm. 2. Lateral view of dorsal labium (D) with dorsolateral double papilla (arrowhead) and ventral labium with ventrolateral double papilla (arrowhead); interlabium (IL) and deep marginal horizontal labial grooves are also shown. ×65; bar = 250 μm.

Parascaris equorum are represented in Figures 1–14.

At low magnification, the anterior end of *P. equorum* had three prominent lips typical of the ascaridoid nematodes, one dorsal and two subventral surrounding the central stoma (Fig. 1). The organization of the male and female labia were similar except for the larger size of the females. The three large, broad-based labia when viewed laterally had the shape of a spade (a suit in playing cards), with a deep horizontal groove on each margin almost midway between the apex and base of each lip (Figs. 2, 3). The labia were set off from the rest of the body by a deep postlabial constriction, giving the appearance of the head extending over the anterior extremity of the body (Fig. 2). En face, the triradiate esophageal opening was seen at the base of the stoma (Fig. 1). Situated between and at the level of the base of each lip were small expansions of the cuticle, often referred to as interlabia (Fig. 2). Lateral cervical alae were not seen. A single row of denticles were present at the inner surface of each lip just below the anterior margin. The denticles were generally evenly spaced, and their shape triangular in outline. Most of the denticles were unicuspid; however, a few bicuspid denticles were noted (Fig. 5). Small round holes were noted in some of the specimens directly above the pointed ends of individual denticles, suggesting that as the pointed ends of the denticles contact the cuticle of the lip, these depressions are formed.

The papillae on the three labia comprised an external ring of four large double papillae consisting of two dorsolaterals on the dorsal lip (Fig. 3) and one ventrolateral on each subventral lip (Fig. 4). Also on each subventral lip, anterior and lateral to the ventrolateral double papillae, were an externolateral papilla and an amphid (Fig. 4). The double papillae on the dorsal and subventral labia were divided and consisted of small round to oval, slightly raised medial elevations without central pores and larger oval to round flattened lateral elevations with central pores (Fig. 6). The cuticle of the smaller medial elevation was generally smooth whereas the larger lateral elevation contained several small slits and depressions on its surface. Each externolateral papilla was sculptured, with numerous slits and creases over its entire surface, and also contained a central pore-like opening (Fig. 7). The amphids, slightly lateral and anterior to the externolateral papillae appeared as small domed areas with central pores (Fig. 7). The internal ring of papillae consisted of two small distinct pits on each labium, at approximately 11 o'clock and 1 o'clock in position (Fig. 4).

The tail of the male was relatively long, smoothly attenuated and often had a small button-like termination (Fig. 8). The preanal papillae were numerous, consisting generally of 4 longitudinal rows, with the two more medial rows containing fewer papillae and not extending as close to the anal opening as the two lateral rows of preanal papillae (Fig. 8). Just anterior to the anus was a double medioventral papilla (Figs. 9, 10). There were five pairs of postanal papillae (Fig. 11) that were distributed as follows: the first pair just posterior to the anus being double papillae with the remaining four pairs more closely associated in a group near the tail end, the second pair also being double papillae, and the last three pairs of papillae were single. The fourth pair of caudal papillae were the phasmids (between the third and fifth pairs of papillae) that were situated more laterally and appeared closer to the third pair of postanal papillae (Figs. 8, 11). The phasmids appeared either as tear-drop in shape or oval (Figs. 11, 12). In the center of each phasmid was a ringed pore-like opening (Fig. 12). Caudal alae were not seen.

The tail of the female was conical and slightly attenuated at its distal one-third. No caudal papillae were seen except for the ventrolateral phasmids, which were approximately one-third the distance from the tip of the tail to the anus (Fig.

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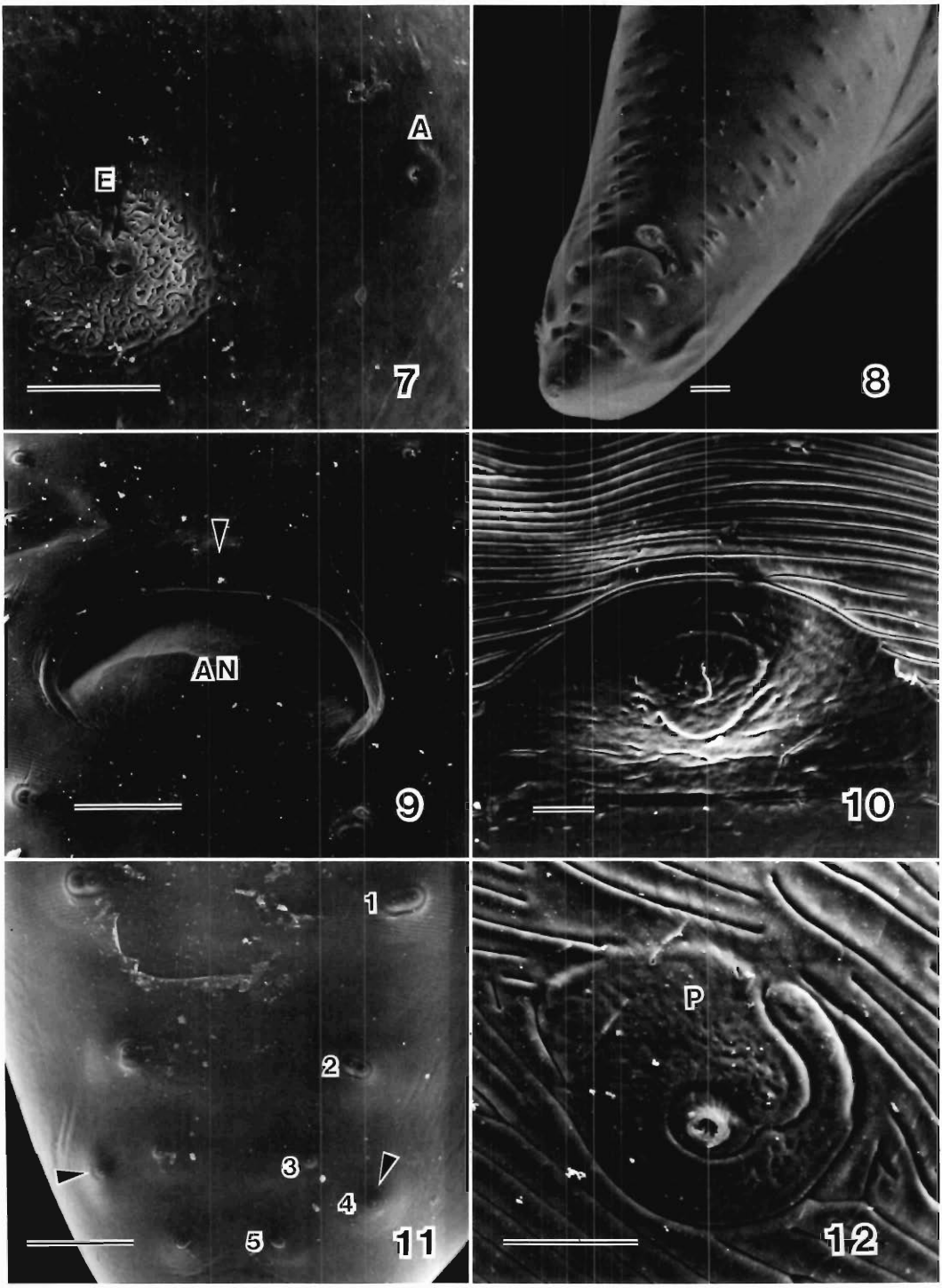
3. Lateral view of dorsal labium showing two dorsolateral double papillae (arrowheads); higher magnification of double papilla is shown in Figure 6. ×90; bar = 100 μm. 4. Lateral view of ventral labium showing ventrolateral double papilla (arrowhead); an externolateral papilla and amphid are enclosed in box which is magnified in Figure 7; internal labial papillae (ILP) are also shown. ×70; bar = 100 μm. 5. Dentigerous ridge (DR) showing unicuspid and bicuspid denticles and small pits (arrowhead) in cuticle. ×4,660; bar = 5 μm. 6. Higher magnification view of a double labial papilla showing large pored (* = pore) and small unpored constituent papilla; there is some shrinkage of the cuticle surrounding and covering the papilla in this figure. ×680; bar = 25 μm.



Figures 7–12. Scanning electron microscopy of labial structures and male caudal end of *Parascaris equorum*. 7. Higher magnification of externolateral papilla (E) and amphid (A). ×700; bar = 25 μm. 8. Ventrocaudal end of male showing arrangement of pre- and postanal papillae. ×55; bar = 100 μm. 9. Perianal area of male showing preanal central papilla (arrowhead) and anus (AN). ×160; bar = 100 μm. 10. Higher magnification of preanal

13). The female phasmids were similar in appearance to those seen on the male caudal end (Fig. 14).

Discussion

The position and arrangement of the labial papillae as described above agree somewhat with previous light and scanning electron microscope studies on *P. equorum*. Yorke and Maplestone (1926) and Ansel et al. (1974) did not describe the externolateral papillae and amphids on the subventral labia or the internal ring of papillae on each labium. The double labial papillae as described herein for *P. equorum* are similar to previous reports for ascaridoid nematodes from other animals (Kazacos and Turek, 1982; Snyder, 1983).

The large labial papillae of these ascaridoid nematodes have traditionally been thought to function as mechanoreceptors (McLaren, 1976; Wright, 1980). However, as Kazacos and Turek (1982) discussed, these double labial papillae, which are characteristic of *P. equorum* and several other ascaridoid nematodes, may have a dual function; the smaller unpored medial elevation of the double papilla may be a mechanoreceptor whereas the larger pored lateral elevation of the double papilla may be a chemoreceptor. As Kazacos and Turek noted, confirmation of these proposed dual functions for these double labial papillae must wait until detailed light and transmission electron microscope (TEM) studies have been conducted.

The externolateral papillae and amphids of *P. equorum* as seen in Figure 7 have not been described in the literature in any detailed drawings or SEM micrographs. The role that the slits on the surface of the externolateral papillae might play is unknown. Detailed TEM work on the substructure of these papillae would undoubtedly shed light on their function. The amphids, with their centrally located pores have also been thought to function as chemoreceptors (McLaren, 1976). The externolateral papillae and amphids of *P. equorum* are similar to those of other ascaridoid nematodes (Uni and Takada, 1981; Kazacos and Turek, 1982). The inner circle of labial papillae as seen in *P. equorum* and other ascaridoid nematodes have also been thought to have a sensory function (Kazacos and Turek, 1982).

The shape and distribution of the denticles of *P. equorum* are similar to descriptions of other ascaridoid nematodes (Madden and Tromba, 1976; Snyder, 1983). Madden and Tromba (1976) found that the shape and size of denticles in *Ascaris suum* are related to the age of the worms and can be regarded as functional changes due to wear on the labia and denticles as these ascarids graze and feed on the intestinal mucosa and contents of the host. Due to this wear on the denticles with increasing age, the use of denticle shape and size is of questionable value in differentiating these ascaridoid nematodes at the generic and species level. Evidence that these denticles may be subject to continual wear are the small round holes seen in the labial cuticle directly above the pointed ends of individual denticles in some of the specimens (Fig. 5). However, the denticles as seen in Figure 5 appear to have experienced little wear.

The information obtained with the aid of the SEM in the present study of the posterior ends and associated structures of adult male and female *P. equorum* have not previously been reported in the literature. The general position and arrangement of pre- and postanal papillae in the male are similar to previous light microscope descriptions of *P. equorum* (Yorke and Maplestone, 1926; Lichtenfels, 1975) with the following exceptions. The double preanal central papilla as seen in Figures 9 and 10 is also depicted on a drawing (fig. 60) by Lichtenfels (1975), but was not described. Yorke and Maplestone (1926) did not show this structure on their drawing (fig. 179-D) of the ventrocaudal end of a male *P. equorum*. This double preanal central papilla has also been reported for other ascaridoid nematodes (Kikuchi et al., 1979; Uni and Takada, 1981).

Yorke and Maplestone (1926) and Lichtenfels (1975) reported about six pairs of postanal papillae on the ventrocaudal end of male *P. equorum*. In the present study, all the specimens ob-

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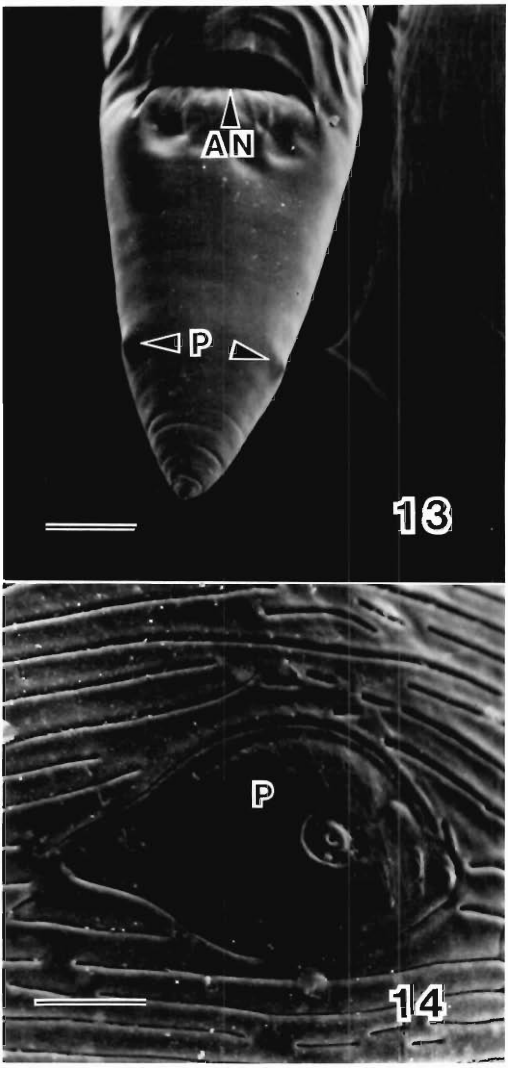
central papilla. ×950; bar = 10 μm. 11. Postanal papillae (labeled 1-5 on one side) of male showing phasmids (arrowheads) as the fourth pair of postanal papillae in relation to the third and fifth pairs of postanal papillae. ×175; bar = 100 μm. 12. Higher magnification of male phasmid (P). ×1,050; bar = 10 μm.



Figures 13, 14. Scanning electron microscopy of caudal end and associated structures of female *Parascaris equorum*. 13. Ventrocaudal end of female showing anus (AN) and phasmids (P). ×50; bar = 250 μm. 14. Higher magnification of female phasmid (P). ×1,570; bar = 10 μm.

served consistently had five pairs of postanal papillae, with the fourth pair of ventrocaudal postanal papillae being the phasmids. This general plan of five pairs of postanal papillae is the same as seen with several other ascaridoid nematodes (Kikuchi et al., 1979; Uni and Takada, 1981; Snyder, 1983). The position of the phasmids as the fourth pair of postanal papillae is similar to several *Baylisascaris* spp., but slightly different than *A. suum,* where the phasmids are the third pair of postanal papillae (Snyder, 1983). On what information is available, it appears that the phasmids may have both glandular and secretory functions (McLaren, 1976).

It is thought that the ventrocaudal papillae, which are localized around the anal region of the male, may assist in positioning the male anus with the female vulva prior to insertion of the male spicules, and ultimately copulation (McLaren, 1976). The reason for the different arrangements and numbers of ventrocaudal papillae in the various ascaridoid nematodes is not known. Additional information may be obtained when these ventrocaudal papillae are examined more closely with the aid of the TEM.

The caudal end of female *P. equorum* as described above is similar to the descriptions given by Yorke and Maplestone (1926) and Lichtenfels (1975); however, the presence of the phasmids were not described by these authors. A slit in the tail region of a female is depicted in a drawing (fig. 61) by Lichtenfels (1975), which would approximate the position of the phasmids as seen in Figure 13.

The microtopographic features as described above correspond to and provide new information on the adult structure of the horse ascarid, *Parascaris equorum.* This information may prove useful in comparisons and phylogenetic relationships between *P. equorum* and related ascaridoid nematodes.

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Response of Adult *Nippostrongylus brasiliensis* (Nematoda) to Fluid Velocity

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ABSTRACT: The retention of males and females of *Nippostrongylus brasiliensis* in an in vitro flow apparatus indicated that the helminths resisted a flow rate of 1 ml/min or less. Decreased temperature below 37°C or increased fluid viscosity greater than 0.025% agarose reduced the helminth's resistance to flow. The density of helminths from 10 to 75 worms, their sex ratio, or a pulsed flow had no effect on their retention in the flow. Rheotaxic orientation to flow was not observed, although locomotory activity was increased in both sexes of nematodes by fluid velocity.

Information about the response of parasitic nematodes to a flowing medium is limited. Wallace and Doncaster (1964) examined swimming by several species and found that larvae of zooparasitic nematodes moved moderately in nonflowing water. Weischer (1959) reported that larvae of the phytoparasite *Heterodera rostochiensis* were negatively rheotaxic; hence, the head of the larvae was oriented with, rather than against, the direction of flow. In contrast, *Ditylenchus dipsaci* showed no rheotaxis (Wallace, 1961). Croll (1970) discussed the disagreement regarding rheotaxes exhibited by *Ancylostoma caninum* and *Strongyloides stercoralis*.

The above investigations indicate that rheotaxic behavior by nematodes remains unclear, particularly for adult zooparasitic nematodes. However, several studies have suggested that intestinal propulsion by the host modulated helminth infection. Farmer (1981) proposed that changes in intestinal propulsion were related to establishment and subsequent expulsion of *Nippostrongylus brasiliensis* in rodents. Intestinal motility may also influence the distribution of *Trichinella spiralis* in the gut (Sukhdeo and Croll, 1981).

This study examined the response of the sexes of *N. brasiliensis* to fluid current. Additionally, selected biological and physical parameters that may interact with flow velocity were investigated.

Materials and Methods

Adults of *Nippostrongylus brasiliensis* were recovered from mice (ICR strain, Southern Animal Farm) at 7 days postinfection. Their responses to fluid current were tested in a lab-fabricated flow apparatus. Flow was provided by a peristaltic pump that was connected by Tygon tubing (3 mm ID) to the sidearm of a three-way valve (Millipore). The valve outlet was coupled by a rubber tubing sleeve to a 20-cm length of glass tubing (3 mm ID). The other inlet arm of the valve allowed injection of nematodes by syringe into the glass tube. Flow was started 1 min after injection of the worms. The distant end of the glass tube was tapered and was connected to a fraction collector (LKB) that allowed collection of the helminths in test tubes and determination of the fluid volumes that propelled the worms through the apparatus. The void volume of the apparatus was 2.94 ml. Flow rates were calibrated prior to and confirmed after experimentation. The fluid reservoir and valve-tubing apparatus were submerged in a water bath at 37°C, unless stated otherwise, above a white plastic background for observation.

Initially, the expulsion of the helminths by different flow rates was examined. Groups of 50 male or female worms were injected into the apparatus. Flow rates of 0.6–4.1 ml/min of Tyrode's solution were used to determine the mean retention time of the helminths by microscopic observation of recovered helminths in the test tubes. Based on these results, a 1 ml/min flow rate was used in subsequent studies unless stated otherwise.

The effect of temperature on worm expulsion was studied at 22, 27, 32, and 37°C. A pulsed flow was also tested by alternately turning the peristaltic pump on and off at 2-min intervals. Viscosity influences were examined with 0, 0.0025, 0.005, 0.025, 0.1, and 0.2% solutions of agarose (LKB) in Tyrode's solution. Groups of 50 male or female nematodes and a flow rate of 1 ml/min were used in these studies.

Selected biological parameters were studied. Groups of 10, 25, 35, 50, and 75 helminths of either sex were injected into the flow to determine if worm density altered their retention time. Sex ratios of 0:1, 3.5:1, 1:1, 1:3.5, and 1:0 female:male were used to determine if the presence of the opposite sex had any effect on retention time in the flow.

Behavioral analyses were done on the sexes of helminth in the flowing medium by microscopic observation of the worms as they passed through the optical plane of view. Percent activation of the nematodes was determined at flow rates of 0.6 and 1 ml/min. Activation was considered as the worm's thrashing appearance in which full body extension and rapid environmental sweeping by the anterior and posterior ends were evident. Body coiling was absent. Percent positive orientation to the flowing medium was determined by counting the number of nematodes whose

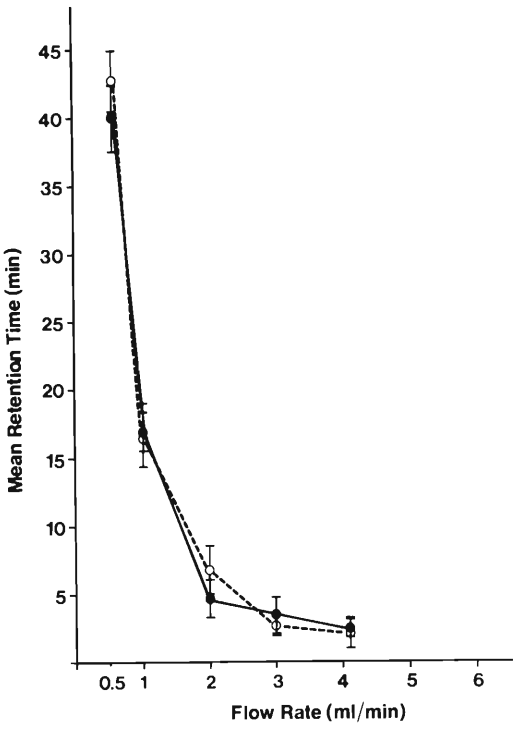

Figure 1. Mean retention time of males (●) and females (○) of *Nippostrongylus brasiliensis* in in vitro flow at the indicated rates.

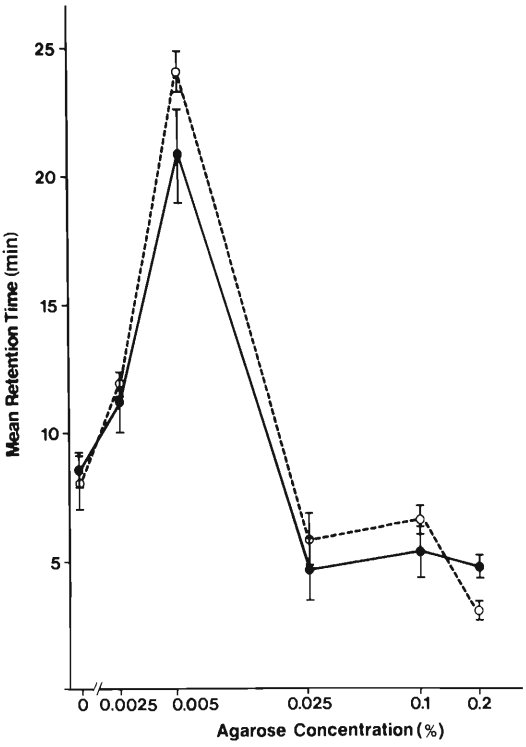

Figure 2. Mean retention time of males (●) and females (○) of *Nippostrongylus brasiliensis* in vitro in a 1 ml/min flow of agarose at the indicated concentrations.

anterior end was aligned against the direction of flow. Groups of 35 male or female *N. brasiliensis* were used in these studies.

Data were evaluated by linear regression or analysis of variance. The 0.05 probability level was considered statistically significant. Data are given as the mean (±SEM) of triplicate trials.

Results

The retention of males and females in various flow rates is shown in Figure 1. Both sexes were rapidly expelled by flow rates of 2 ml/min or greater. However, worms retained their position for 16 and 40–43 min in flow rates of 1 and 0.6 ml/min, respectively. Males and females were retained for 26.4 and 30.6 min, respectively, when a pulsed flow of 1 ml/min was given in alternate 2-min intervals.

Table 1. Effect of temperature on the propulsion of males and females of *N. brasiliensis* by a 1 ml/min flow rate in vitro.

Temperature (°C)	Mean retention time (SEM)	
	Male	Female
22	3.3 (0.54)	4.1 (0.81)
27	6.7 (0.67)	7.5 (0.59)
32	10.2 (1.4)	11.4 (1.73)
37	14.5 (1.12)	13.8 (2.09)

Table 1 gives the response of *N. brasiliensis* to flow at different temperatures. Decreased temperatures caused declines in the retention time of both sexes. Increases in the fluid's viscosity up to that produced by 0.005% agarose gave longer retention times of the helminths in the flowing medium (Fig. 2). However, the helminths were rapidly expelled in more viscous solutions of agarose.

No significant difference was found in the helminth's retention time as the density of either sex was increased from 10 to 75. Likewise, alterations in the sex ratios of the tested helminths had no apparent effect.

Any orientation of the helminths with or against the direction of flow was not evident. However, both sexes were activated by the velocity of flow. Females of *N. brasiliensis* were 29.33% (±3.06%)

and 64.03% (±9.03%) activated by flow rates of 0.6 and 1 ml/min, respectively, whereas the males showed 48.3% (±1.93%) and 82% (±1.24%) activation.

Discussion

The response of the sexes to fluid velocity seemingly is dependent on at least several factors. *N. brasiliensis* was retained for more than eight volumes of the flow apparatus at a 0.6 ml/min flow rate, rather than for three volumes at higher flow rates, which suggests that the worms' locomotor response enabled some resistance to flow in vitro. Surface features of the intestine may enhance the worm's ability to resist flow, and thus expulsion. Glassburg et al. (1981) suggested that male *N. brasiliensis* may move against peristalsis at a 1 cm/hr rate in the intestine when stimulated by the presence of females that were anterior in the intestine.

Increased temperatures may depress the general locomotor ability of the helminth and, thus, increase their rate of expulsion. Viscosity has an apparent bimodal effect since slightly viscous solutions increased the retention time in flow, whereas more viscous solutions caused a rapid elimination of the nematodes from the in vitro apparatus. Miller et al. (1981) reported that mucus trapping of *N. brasiliensis* was an early occurrence during immune exclusion of the parasite which suggests that viscosity has an in vivo effect.

Observations of *N. brasiliensis* in this study indicated that the nematode was not rheotaxic because no particular orientation to flow was evident. The response, however, may be kinetic because more worms were activated at a greater rate of flow. Mechanoreceptors of the helminth may be activated by the flowing medium with increased body movement as a response. Croll (1976) found that the impact of *Caenorhabditis elegans* with a glass bead initiated withdrawal and forward movement in another direction. However, any interactive effect of chemosensory information in a mobile phase should be considered for greater understanding of nematode responses to fluid velocity.

Acknowledgments

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New Species of Spiruridae (Nemata: Spirurida) from Endemic Hawaiian Honeycreepers (Passeriformes: Drepanididae), the Japanese White-eye (Passeriformes: Zosteropidae) and a New Species of Acuariidae (Nemata: Spirurida) from the Japanese White-eye Collected on the Island of Hawaii

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ABSTRACT: Three new species of Spirurida are described from the gizzards and proventriculi of three endemic birds and one introduced bird from the island of Hawaii. *Viguiera hawaiiensis* sp. n. is reported from *Himatione sanguinea* (Apane), *Hemignathus virens* (Amakihi), and *Vestiaria coccinea* (Iiwi); *Procyrnea longialatus* sp. n. was recovered from *H. sanguinea* and *Zosterops japonica* (Japanese white-eye); *Synhimantus* (*Dispharynx*) *zosteropsi* sp. n. was found only in the introduced Japanese white-eye. *Viguiera hawaiiensis* is characterized by the greater degree of development of the cephalic appendages, by the spicular ratio 20–32:1 and by the absence of inflation of the cervical region. *Procyrnea longialatus* is characterized by the lateral cuticular alae, which run along most of the length of the body, by the absence of unpaired papillae on the anterior lip of the cloaca of males, by the spicular ratio of 2.7:1, and by the presence of a small tubercle on the end of the female tail. *Synhimantus* (*D.*) *zosteropsi* is characterized by the lack of a ventral spine on the right spicule, by the bicuspid cervical papillae, and by the spicular ratio of 2.6:1.

During an ecological survey of birds from the southeastern slope of Mauna Loa, Volcano National Park, Hawaii (van Riper et al., 1985), 183 nematodes were collected from the tunic of the gizzard and the proventriculus of *Hemignathus virens* (Amakihi), *Vestiaria coccinea* (Iiwi), *Himatione sanguinea* (Apapane), and *Zosterops japonica* (Japanese white-eye).

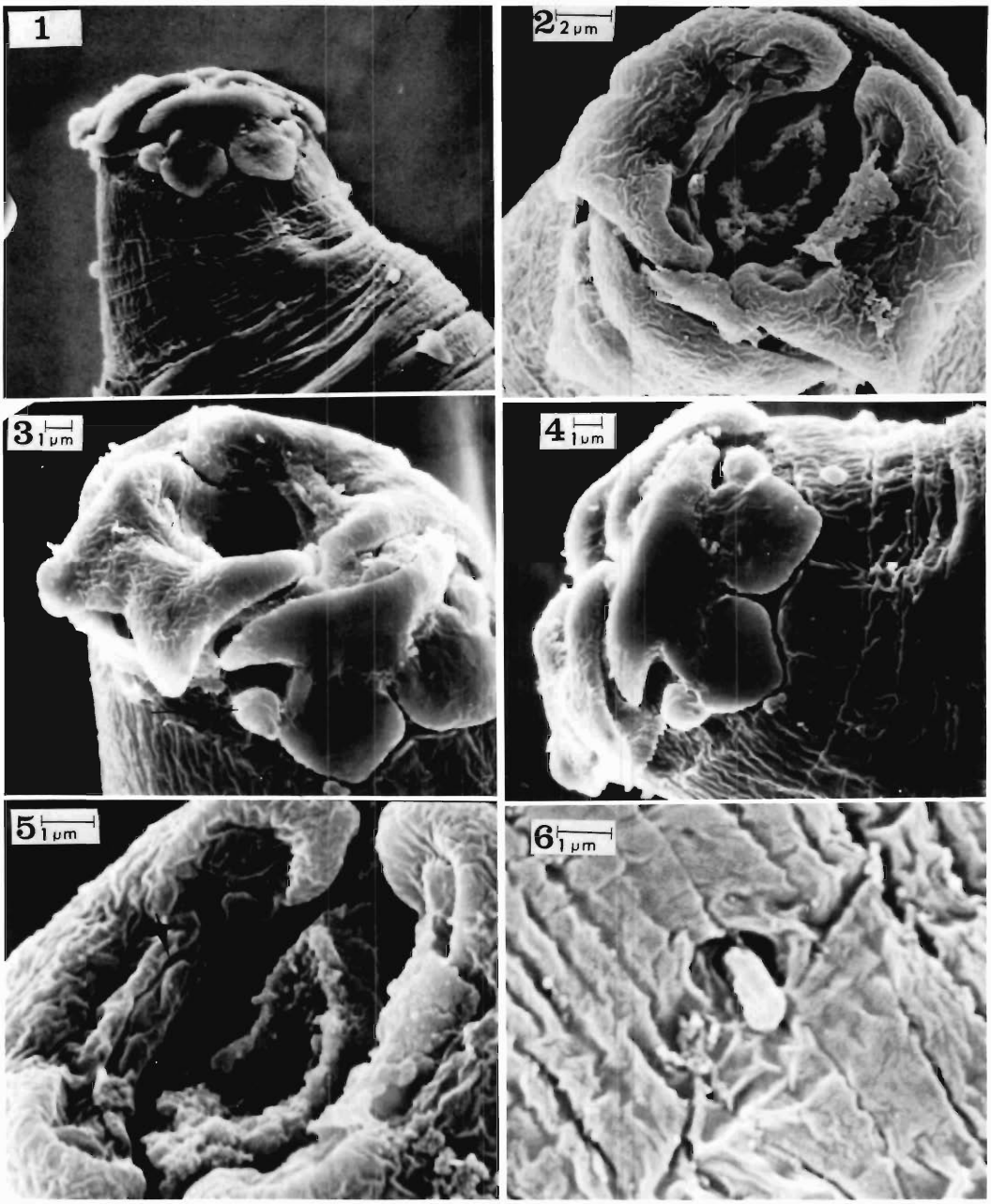
The spirurid nematodes collected from the 34 specimens of Hawaiian honeycreepers and six specimens of Japanese white-eye belong to two families of Spirurida: Spiruridae Oerley, 1885 and Acuariidae Seurat, 1913. The genus *Viguiera* Seurat, 1913 (Spiruridae) was distributed among all three species of Hawaiian honeycreepers (Drepanididae). *Procyrnea,* also a spirurid was found in both Hawaiian honeycreepers and the Japanese white-eye (Zosteropidae). The genus *Synhimantus* Railliet, Henry, and Sisoff, 1912 (Acuariidae) was recovered only from the Japanese white-eye. An unidentified *Capillaria* was found among the Hawaiian honeycreepers and an unknown nematode (broken and poorly fixed) was found in *Vestiaria coccinea.* The three nematode genera: *Procyrnea, Viguiera* and *Synhimantus* have not been previously reported from Hawaii, and the species described here are also new. All three genera are widely distributed and contain species from Asia, Europe, Australia, North America, and South America. *Synhimantus* has also been described from Cuba.

Materials and Methods

The nematodes were removed from the host, killed and fixed in 70% ethyl alcohol and glycerin following the procedure outlined by van Riper and van Riper (1980). At examination, some nematodes were dehydrated following Thorne's (1961) technique, and mounted in glycerin. Others, mainly the males, were cleared in lacto-phenol. Specimens selected for scanning electron microscopy (SEM) were dehydrated following the technique reported by Cid del Prado et al. (1983). They were mounted on specimen stubs, coated with gold 400–500 Å, and viewed with a ETEC electron microscope at 10 kV. Measurements are given in millimeters and micrometers with 95% confidence intervals ($\bar{x}$); figures were drawn with the aid of a camera lucida.

Viguiera hawaiiensis sp. n. (Figs. 1–11)

DESCRIPTION: Thirteen females, 11 males. Both sexes with long, slender body, tapering gradually at anterior and posterior extremities. Cuticle with fine annulations 3.2–4.8 μm wide. Cephalic region with dorsal and ventral expansions plus lateral bifurcate pseudolabia. Pseudolabia directed posteriorly (Fig. 2). Dorsal and ventral cuticular expansions with inverted triangular shape, with two small submedian lobes on each side of apex



Figures 1-6. Scanning electron micrographs of *Viguiera hawaiiensis* female. 1. Anterior end of body, dorsal view ×2,000. 2. Face view. 3. Labial region, latero-ventral view. 4. Labial region, ventral view. 5. Oral region. Arrow indicates denticle. 6. Cervical papilla of female.

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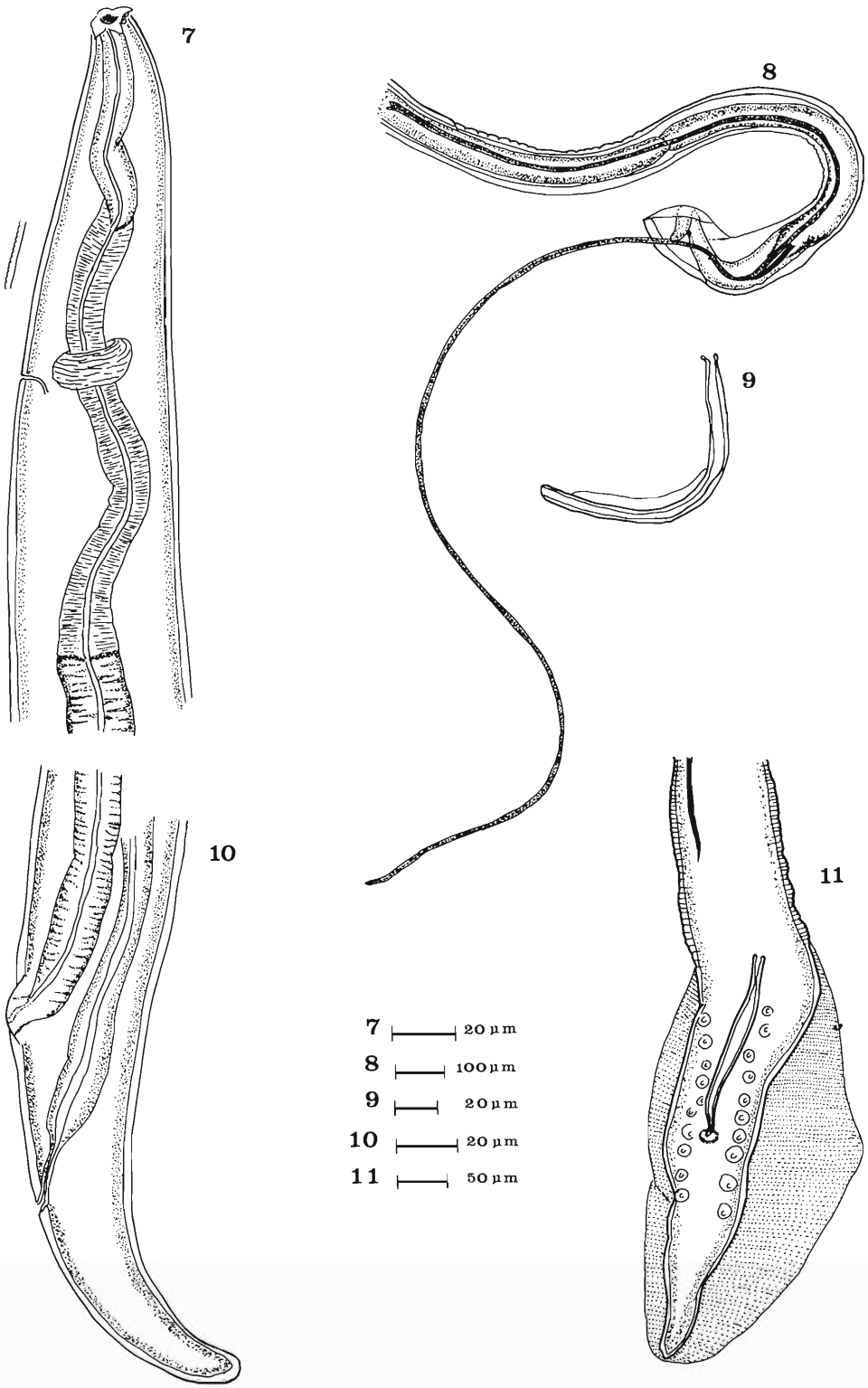
Figures 7-11. *Viguiera hawaiiensis.* 7. Anterior end of female body, lateral view. 8. Posterior end of male body. 9. Right spicule. 10. Posterior end of female body, lateral view. 11. Posterior end of male body, ventral view.



(Figs. 1, 4). One papilla located on exterior side of each small lobe, total four. Two prominent papillae on inner surface of each pseudolabium (Fig. 2). Four denticle-like structures on inner border of pseudolabia, interior to level of papillae (Figs. 2, 5). Simple cervical papillae (deirids) present at level of or slightly posterior to excretory pore (Fig. 6). Amphids located directly under midborder of pseudolabia (Fig. 3).

HOLOTYPE MALE: Body length 5.1 mm; body width, 0.10 mm; excretory pore, 0.18 mm; cervical papillae, 0.11 mm; anterior muscular esophagus, 0.16 mm; posterior glandular esophagus, 1.3 mm; right spicule, 0.17 mm; left spicule, 3.8 mm; left spicule 23.2 times as long right spicule.

MALES ($N = 11$): Length 5.1–7.6 mm ($\bar{x} = 6.1$ mm ± 0.5) and 0.07–0.10 mm ($\bar{x} = 0.1$ mm ± 0.01) wide. Stoma slender, 0.07–0.10 mm ($\bar{x} = 0.09$ mm ± 0.01) long. Anterior esophagus, 0.15–0.23 mm ($\bar{x} = 0.18$ mm ± 0.03); expanded glandular posterior esophagus, 1.01–1.49 mm ($\bar{x} = 1.20$ mm ± 0.09) long. Nerve ring, 0.12–0.18 mm ($\bar{x} = 0.15$ mm ± 0.01) from anterior end; excretory pore, 0.11–0.20 mm ($\bar{x} = 0.15$ mm ± 0.03) from anterior end. Cervical papillae, small lobes, slightly anterior to excretory pore, and 0.08–0.11 mm ($\bar{x} = 0.10$ mm ± 0.02) from anterior end. Tail spirally twisted, provided with asymmetrical caudal alae. Six pairs of pedunculate precloacal papillae, one pair adanal papillae, and three pairs of postcloacal papillae (Fig. 11). Spicules very unequal: right spicule short and rigid, 0.14–0.17 mm ($\bar{x} = 0.15$ mm ± 0.01) long (Fig. 9); left spicule large and flexible, 3.35–4.10 mm ($\bar{x} = 3.63$ mm ± 0.1) long, occupying 46–75% (60% ± 5.4) of body length and 20–32 times ($\bar{x} = 24$ times ± 2.5) longer than right spicule (Fig. 8).

FEMALES ($N = 13$): Body length 3.1–14.3 mm ($\bar{x} = 7.9$ mm ± 2.4) and 0.05–0.11 mm ($\bar{x} = 0.09$ mm ± 0.01) in width. Cuticle with fine transverse striae, more evident from midbody to posterior. Stoma cylindrical, 0.05–0.12 mm ($\bar{x} = 0.08$ mm ± 0.01) long (Fig. 7). Esophagus with narrow anterior muscular part, 0.13–0.21 mm ($\bar{x} = 0.17$ mm ± 0.02) long; expanded glandular posterior part, 0.80–1.72 mm ($\bar{x} = 1.16$ mm ± 0.21) long. Nerve ring 0.10–0.19 mm ($\bar{x} = 0.13$ mm ± 0.02) from anterior extremity; excretory pore 0.12–0.20 mm ($\bar{x} = 0.15$ mm ± 0.04) from anterior extremity (Fig. 7). Cervical papillae simple lobe (Fig. 6) approximately at level of excretory pore: 0.11–0.20 mm ($\bar{x} = 0.14$ mm ± 0.03) from anterior end. Anus 0.094–0.139 mm ($\bar{x} = 0.132$ mm ± 0.71) from posterior end. Vulva, two lips, anterior lip narrow 0.006 (0.004–0.008) mm wide; posterior lip large and protruding, 0.035 (0.028–0.044) mm wide. Vulva located far posterior at 95–99% of body length, opens anterior to anus 0.022–0.058 mm ($\bar{x} = 0.055$ mm ± 0.01), and 0.156–0.226 mm ($\bar{x} = 0.186$ ± 0.01) from posterior end (Fig. 10). Vagina with thick muscular layer communicating with muscular uteri. Eggs thick shelled, embryonated 29–37 μm ($\bar{x} = 34$ μm ± 0.9) long 15–23 μm ($\bar{x} = 19$ μm ± 1.2) wide. Tail conical and blunt or with a small, tuberculate end, 0.094–0.139 mm ($\bar{x} = 0.132$ mm ± 0.71).

SPECIMENS: Holotype (male): Deposited in University of California Davis Nematode Collection (UCDNC), Davis, California, USA, UCNC No. 2127. Paratypes deposited as follows: 5 females, UCNC No. 2128; 5 males, UCNC No. 2129; in the UCDNC, Davis, California, USA; 5 females, 1 male at Instituto de Biologia, Lab. Helmintologia, UNAM, Mexico; 3 females, 5 males at Laboratoire des Vers, Museum National d'Histoire naturelle, 61 rue de Buffon 75005, Paris, France.

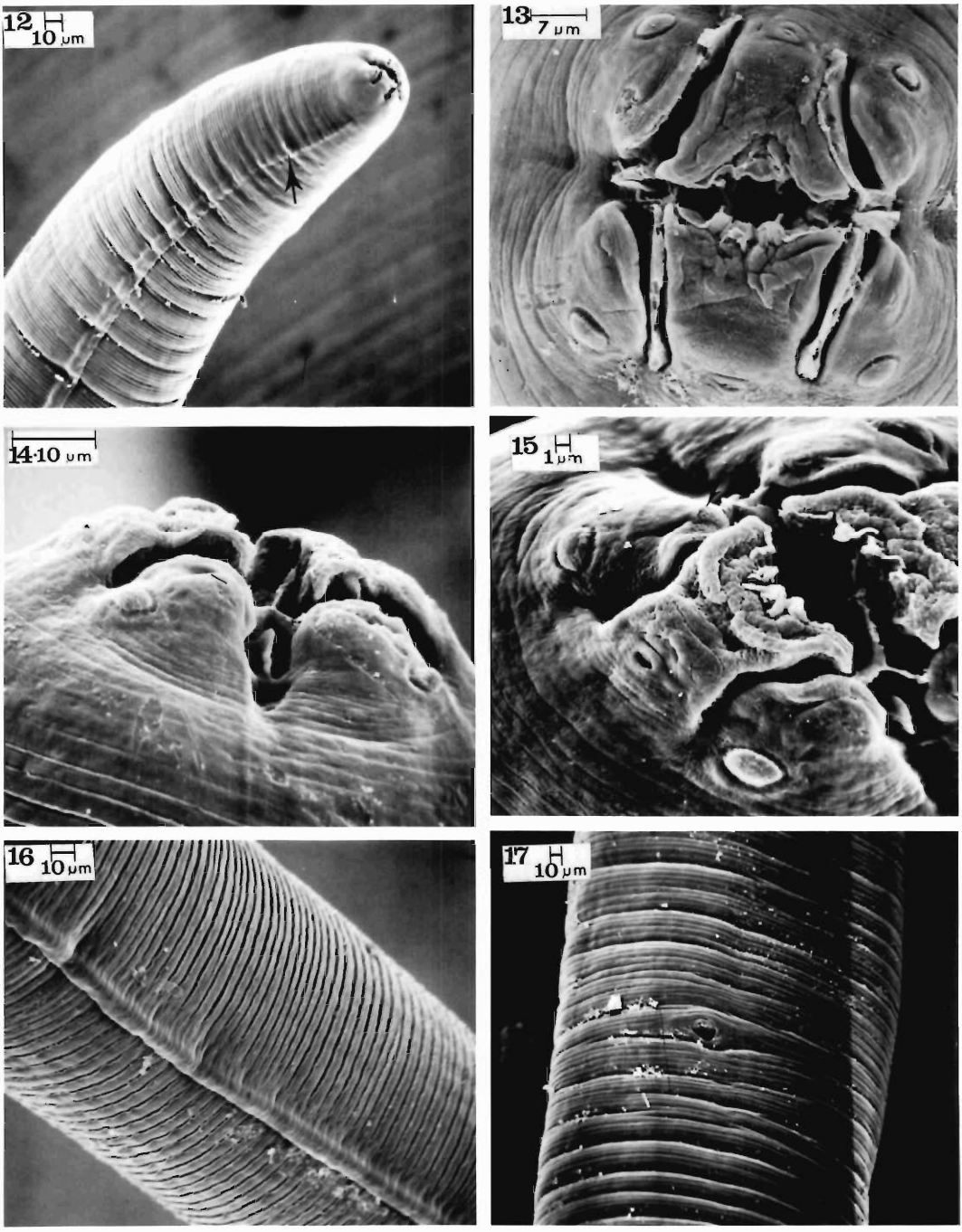
HOST: *Hemignathus virens* (Amakihi).

OTHER HOSTS: *Himatione sanguinea, Vestiaria coccinea.*

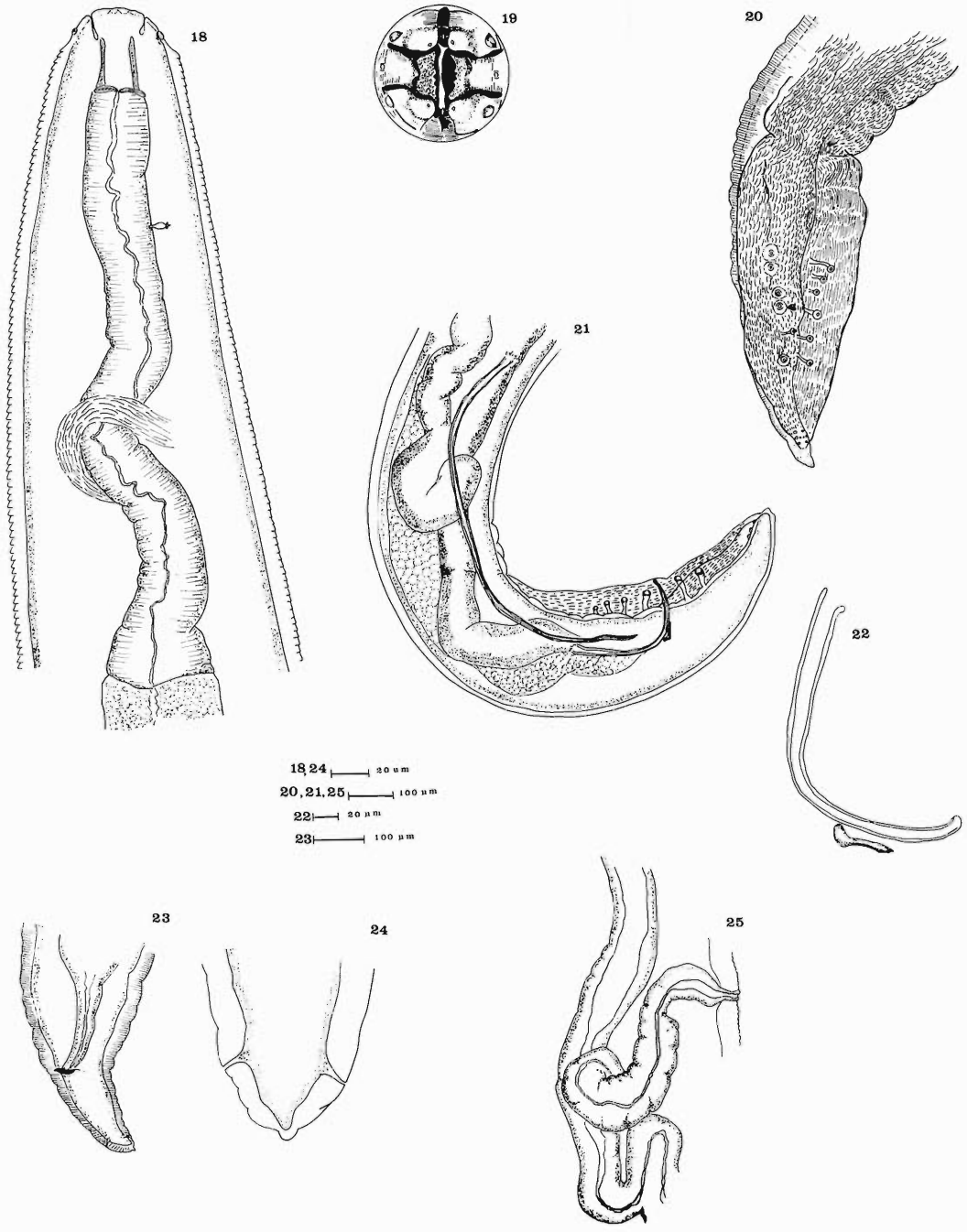
SITE OF INFECTION: Between the tunic layers of the gizzard.

LOCALITY: Mauna Loa, Volcano National Park, Hawaii.

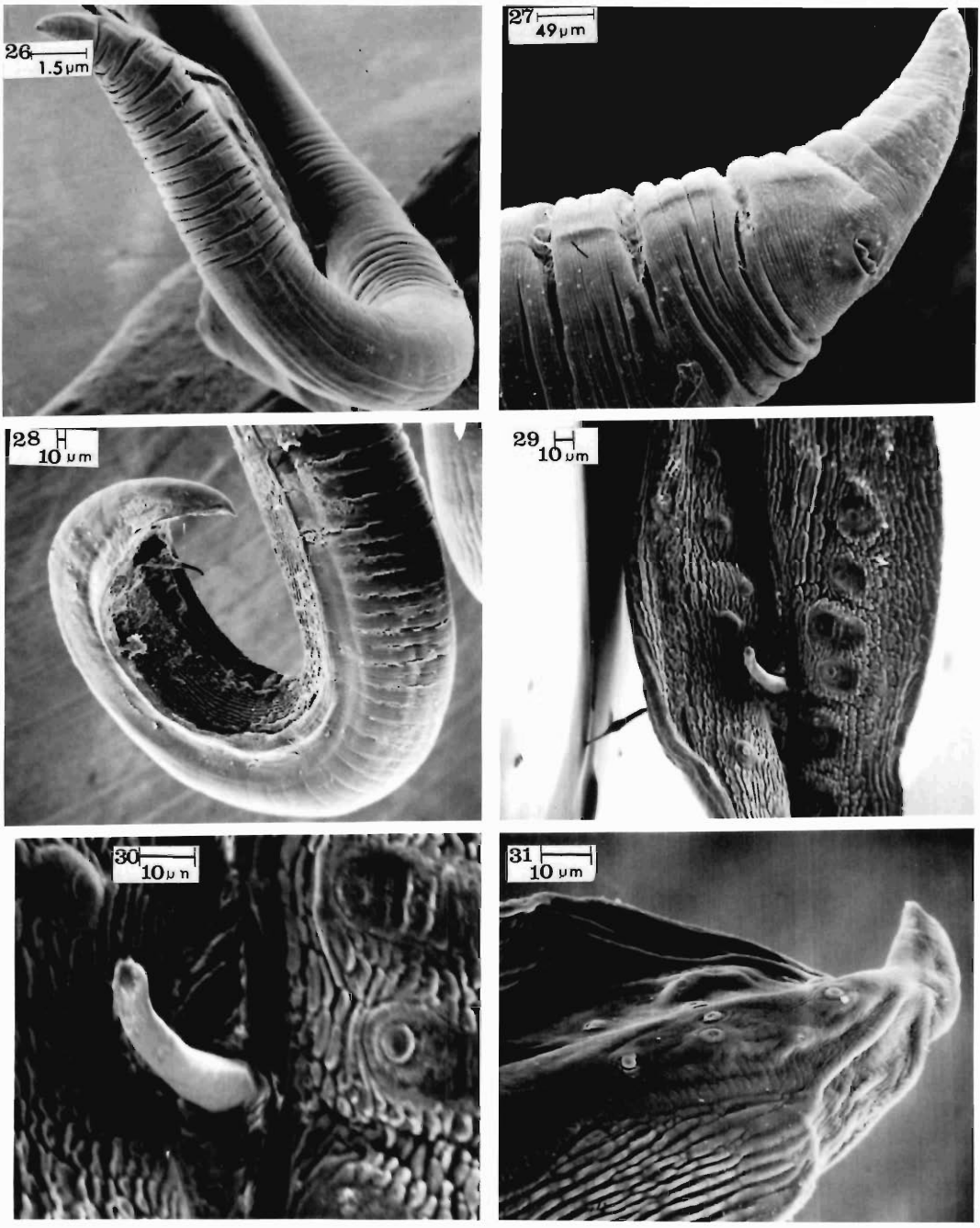
DIAGNOSIS: *Viguiera hawaiiensis* is very close to *V. osmanhilli* (Yeh, 1954) Chabaud, 1957 from Brazil in having a spicular ratio 20:1. Only one male of *V. osmanhilli* was originally studied. Our 11 specimens have a range of 20–32:1. However, our specimens differ in that the head is continuous with the body and the cervical area is not slightly inflated as in *V. osmanhilli*; also our specimens differ in the position of cervical papillae 0.11–0.20 mm in females and 0.08–0.19 mm in males of *V. hawaiiensis* n. sp. versus 0.22–0.24 mm in females and 0.21 mm in males of *V. osmanhilli.* Finally, the males of *V. hawaiiensis* have 6 pairs of precloacal papillae, 1 pair adanal and 3 pairs postcloacal; whereas in *V. osmanhilli* there are 8 pairs precloacal and 2 pairs postcloacal. Also our specimens are close to *V. terpsiphonei* Jairajpuri and Siddiqi, 1971 by the spicular ratio, but differ by the shape of the cuticular expansion of the head and in the number and arrangement of cloacal papillae, in *V. terp-*



Figures 12–17. Scanning electron micrographs of *Procyrnea longialatus* female. 12. Anterior end of body. Arrow indicates cervical papilla. 13. Face view. 14. Anterior end, dorsal view. 15. Anterior end, lateral view. 16. Left cuticular ala. 17. Middle part of the body (vulval area), ventral view.



Figures 18–25. ***Procyrnea longialatus.*** **18. Anterior end of female body, lateral view. 19. Face view, female. 20. Posterior end of male body, ventral view. 21. Posterior end of male body, lateral view. 22. Right spicule and gubernaculum. 23. Posterior end of female body, latero-ventral view. 24. Female tail, ventral view. 25. End of reproductive tract female, lateral view.**



Figures 26-31. Scanning electron micrographs of *Procyrnea longialatus*. 26, 27. Posterior regions of the female body, lateral and latero-ventral view. 28. Posterior end of the male body, lateral view. 29. Caudal alae, ventral view. 30. Cloacal region and end right spicule. 31. Male tail, ventral view.

siphonei, there are 10 pairs precloacal and 1 pair postcloacal.

Procyrnea longialatus sp. n. (Figs. 12–31)

DESCRIPTION: Seven females, 3 males. Body with distinct annuli, anterior annuli projecting posteriorly; lip region continuous with body contour, no constriction present. Labial region in females and males consists of two wide lateral pseudolabia, four narrow dorsal and ventral submedian lobes with interlabia at base of each (Figs. 13 and 19). Pore-shaped amphids located on pseudolabia (Fig. 15). Pseudolabia wider at mouth than at base, three small teeth on interior border of pseudolabia, papillae not observed. Ventral and dorsal submedian lobes separated from pseudolabia by deep groove (Figs. 14, 15). Prominent papillae at base of each submedian lobe in lateroventral and laterodorsal positions; each submedian lobe with a small papilla at apex, papillae located on either side of interlabia (Figs. 13, 15). A solid plate or tooth located dorsally and ventrally at base of submedian lobes (Fig. 14). Lateral unequal alae present along sides of body, extending from lip region to a point between anus and tail tip. Left ala conspicuous (Figs. 16, 26), right ala less developed and difficult to see on posterior extreme of body; in SEM it reached same level posteriorly as left ala. Cervical papillae, simple protuberance, located anterior to nerve ring (Fig. 18); excretory pore slightly posterior to nerve ring.

HOLOTYPE MALE: Body length, 7.8 mm; body width, 0.29 mm; cervical papillae, 0.12 mm; anterior muscular esophagus 0.36 mm; posterior glandular esophagus, 2.2 mm; right spicule, 0.37 mm; left spicule, 0.87 mm; ratio of the spicules, 2.4:1.

MALES: ($N = 3$): Body smaller than females, 5.8–7.8 mm ($\bar{x} = 6.7$ mm $\pm$ 3.0) long, 0.29–0.39 mm ($\bar{x} = 0.34$ mm $\pm$ 0.13) wide. Stoma strongly cuticularized 49–57 μm ($\bar{x} = 52$ μm $\pm$ 9.0) long (includes lips), 14–23 μm ($\bar{x} = 16$ μm $\pm$ 14.0) wide. Anterior muscular esophagus short, 0.35–0.36 mm ($\bar{x} = 0.35$ mm $\pm$ 0.08) long; posterior glandular esophagus one-third of total body length, 2.16–2.20 mm ($\bar{x} = 2.18$ mm $\pm$ 0.23) long, connected to intestine by conspicuous esophago-intestinal valve. Cervical papillae anterior to nerve ring, 0.12–0.16 mm ($\bar{x} = 0.14$ mm $\pm$ 0.08) from anterior extremity; nerve ring, 0.25–0.27 mm ($\bar{x} = 0.26$ mm $\pm$ 0.09) from anterior end, excretory pore, 0.28 mm from anterior extremity. Tail curved ventrally (Figs. 21, 28), bursa asymmetrical; two lateral alae, one 0.73 mm long, one 0.58 mm long; meet anterior to tail tip (Fig. 20). Cuticular platelets variable in size, located ventrally, from just anterior to bursa to end of caudal alae (Figs. 20, 29). Four pairs of precloacal pedunculate papillae (Fig. 29). No sessile papillae present around cloacal pore (Figs. 29, 30). Two pairs of symmetrical pedunculate papillae located posterior to cloacal pore (Fig. 20). Eight small sessile papillae located near tail tip (Fig. 31). Spicules unequal; left spicule long and flexible with acute tip, 0.87–1.0 mm ($\bar{x} = 0.96$ mm $\pm$ 0.06) long (Fig. 21) and right spicule slightly C-shaped, stout, distal tip rounded, 0.32–0.37 mm ($\bar{x} = 0.3$ mm $\pm$ 0.06) long. Gubernaculum present, bar-shaped, 0.06 mm long (Figs. 22, 30).

FEMALES: ($N = 7$): Body, 13.3–16.5 mm ($\bar{x} = 14.3$ mm $\pm$ 1.1) long, 0.41–0.49 mm wide. Annuli project posteriorly. Stoma with strong cuticular walls, 40–70 μm ($\bar{x} = 52$ μm $\pm$ 10.4) (including the lips) long, 17–30 μm ($\bar{x} = 23$ μm $\pm$ 4.6) wide. Esophagus divided into two parts, muscular anterior part, 0.43–0.49 mm ($\bar{x} = 0.45$ mm $\pm$ 0.02) long and posterior glandular part 2.2–3.1 mm ($\bar{x} = 2.8$ mm $\pm$ 0.25) long, connected to intestine by conspicuous esophago-intestinal valve. Cervical papillae found anterior to nerve ring and approximately 40 μm from beginning of cuticular alae, 0.12–0.23 mm ($\bar{x} = 0.17$ mm $\pm$ 0.04) from anterior end (Fig. 18); nerve ring located 0.21–0.28 mm ($\bar{x} = 0.25$ mm $\pm$ 0.03) from anterior end; excretory pore located 0.33–0.38 mm ($\bar{x} = 0.36$ mm $\pm$ 0.03) from anterior end. Vulva located near midbody 42–44% and 5.84–7.12 mm ($\bar{x} = 6.4$ mm $\pm$ 0.95) from anterior extremity. Vulva circular pore approximately 40 μm in diameter (Fig. 17); vulval lips protrude slightly, poorly developed in lateral view (Fig. 25). Vagina approximately 0.64 mm in length to point of bifurcation (Fig. 25). Eggs

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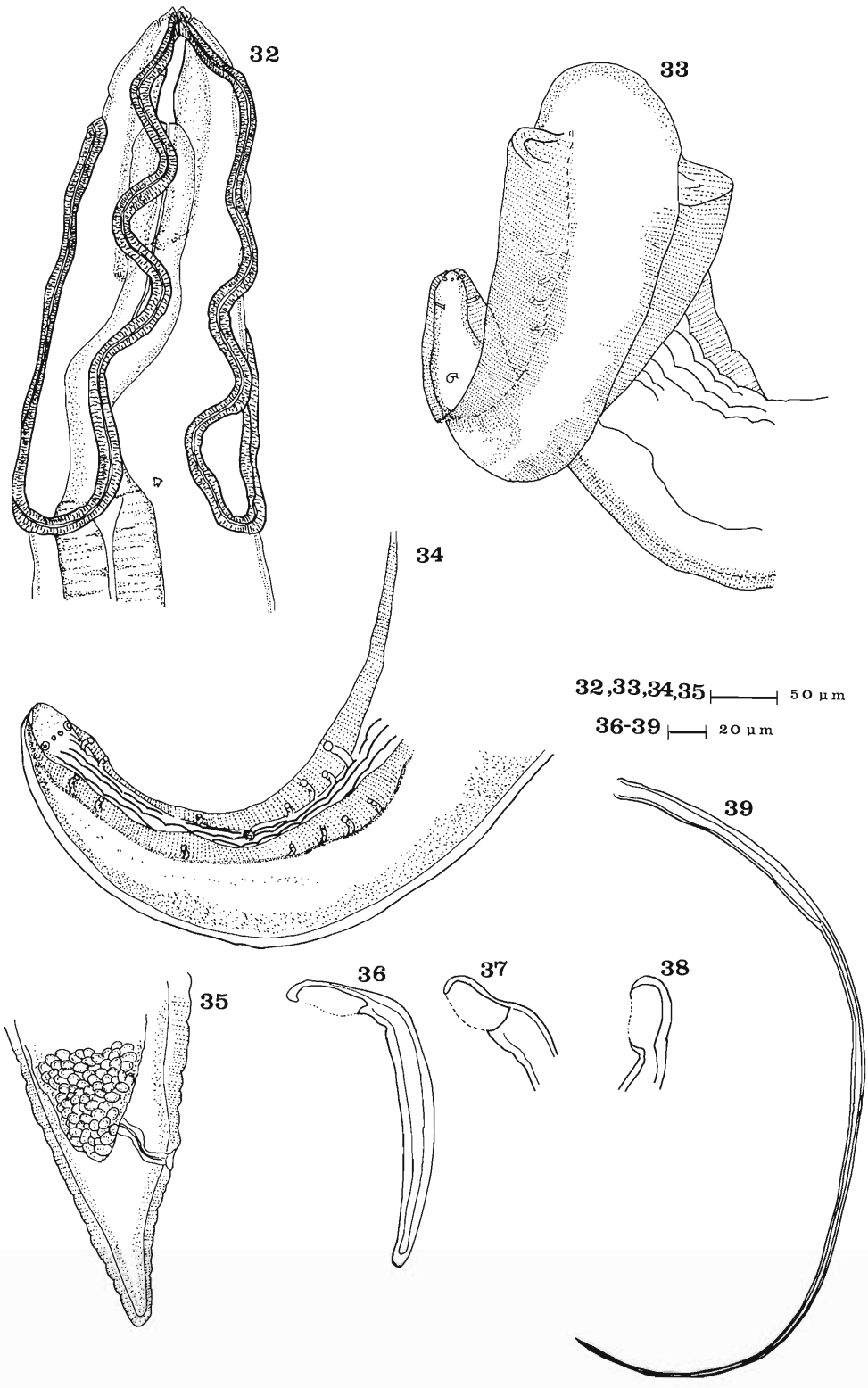
Figures 32–39. ***Synhimantus* (*Dispharynx*) *zosteropsi*. 32. Anterior end of female body, lateral view. 33. Posterior end of male body. 34. Posterior end of male body, lateral view. 35. Posterior end of female body, lateral view. 36–38. Right spicule. 39. Left spicule.**



oval with smooth surface, 30.7–38.4 μm ($\bar{x}$ = 34.9 μm ± 1.7) long, 19.2–21.5 μm ($\bar{x}$ = 20.4 μm ± 0.25) wide. Tail conical, rounded, oriented dorsad with small tubercle on end and 0.21–0.37 mm ($\bar{x}$ = 0.28 mm ± 0.05) long, paired papillae, 50–73 μm from tail tip (Figs. 23, 24). Annuli of body much reduced on ventral region of tail, fine annulation continues to tail tip (Fig. 27).

HOLOTYPE (male): Deposited in University of California Davis Nematode Collection (UCDNC), Davis, California, USA, UCNC No. 2133. Paratypes deposited as follows: 5 females, UCNC No. 2134, in the UCDNC, Davis, California, USA; 1 female, 1 male at Instituto de Biologia, Lab. Helmintologia, UNAM, Mexico; 1 female at Laboratoire des Vers, Museum National d'Histoire naturelle, Paris, France.

HOST: *Zosterops japonica* (Japanese white-eye).

OTHER HOST: *Himatione sanguinea* (Apapane).

SITE OF INFECTION: Proventriculus.

LOCALITY: Mauna Loa, Volcano National Park, Hawaii.

DIAGNOSIS: Most species of the genus *Procyrnea* (Chabaud, 1958) Chabaud, 1975, have cuticular alae that start anterior to the cervical papillae and extend only to the first half of the body. Alae are not seen on the posterior extreme of the body. *Procyrnea longialatus* is distinguished by the lateral cuticular alae starting anterior to the cervical papillae and ending in the area of the anus on females and at the point of the beginning of the bursa on males.

No papillae were observed on the lateral pseudolabia. *Procyrnea longialatus* is closely related to *P. mansioni* (Seurat, 1914; Chabaud, 1958, 1975) but differs in the length and prominence of lateral cuticular alae, and absence of unpaired papilla on the anterior lip of the cloaca of the male. When the spicules are withdrawn, the cloacal opening has a papilla-like appearance. Seurat (1914) described an unpaired precloacal papilla that was visible when the spicule was extended. Also, our material differs in the size of the spicules: in *P. longialatus* the left spicule is 0.866–1.003 mm long and the right spicule 0.318–0.366 mm long versus *P. mansioni* where the left spicule is 0.680 mm long and the right spicule 0.315 mm long. Also, the ratio of the spicules differs 2.1:1 in *P. mansioni* versus 2.7:1 in *P. longialatus*.

Synhimantus (*Dispharynx*) *zosteropsi* sp. n. (Figs. 32–45)

DESCRIPTION: Six females, 8 males. Both sexes: body stout, tapering gradually at both extremes. Cuticle with fine annuli, about 3.4 μm apart at midbody. Four distinct convoluted recurrent nonanastomosing cordons, extending from pseudolabia to approximately one-half of anterior esophagus. Cervical papilla bicuspid (Fig. 42), located laterally between cordons and near most posterior part of cordons (Fig. 32). Two simple pseudolabia present (Figs. 41, 43). Amphids slitlike located laterally, just posterior to lips. Two cephalic sensilla (papillae) located posterior to amphids (Figs. 41, 43).

HOLOTYPE MALE: Body length, 6.5 mm; body width, 0.24 mm; excretory pore, 0.40 mm; anterior muscular esophagus, 0.56 mm; posterior glandular esophagus, 2.0 mm; right spicule, 0.20 mm; left spicule, 0.44 mm; left spicule 2.2 times as long right spicule.

MALES (N = 8): Body length 5.5–7.0 mm ($\bar{x}$ = 6.1 mm ± 0.46), width 0.11–0.29 mm (0.24 mm ± 0.1). Stoma, 0.11–0.14 mm ($\bar{x}$ = 0.11 mm ± 0.02) long. Anterior esophagus slightly convoluted, 0.39–0.71 mm ($\bar{x}$ = 0.52 mm ± 0.08) long, posterior esophagus occupies one-third of body length, 1.6–2.3 mm ($\bar{x}$ = 1.9 mm ± 0.19) long; combined length of esophagus, 1.9–2.9 mm ($\bar{x}$ = 2.5 mm ± 0.25). Nerve ring surrounds anterior esophagus, 263–284 μm ($\bar{x}$ = 270 μm ± 0.059) from anterior end. Excretory pore immediately posterior to posterior limit of cordons. Cordons, 0.47–0.69 mm ($\bar{x}$ = 0.64 mm ± 0.07) in total length; descending branch, 0.31–0.47 mm ($\bar{x}$ = 0.41 mm ± 0.05) long, recurrent branch, 0.17–0.28 mm ($\bar{x}$ = 0.23 mm ± 0.04) long. Tail coiled, caudal alae long, narrow, asymmetrical, 0.50–0.58 mm long and 0.25 mm wide (Figs. 33, 44). Pedunculate caudal papillae present, four pairs preanal, four pairs postanal; one pair of sessile papillae near tail tip (Fig. 45). Left spicule slender, 439–529 μm ($\bar{x}$ = 478 μm ± 0.04) long

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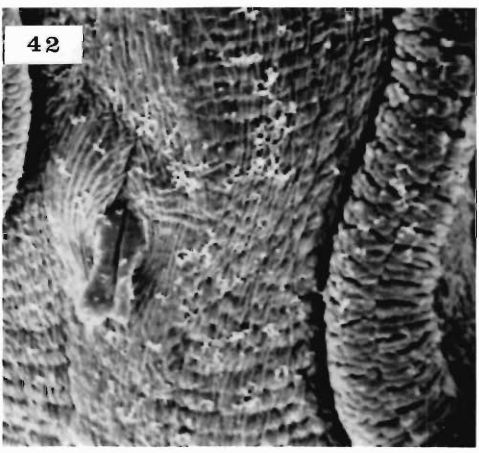
Figures 40–45. Scanning electron micrographs of *Synhimantus* (*Dispharynx*) *zosteropsi*. 40. Anterior end of female body, lateral view. 41. Anterior end of female body, dorso-lateral view. 42. Cervical papilla of female. 43. Face view of female. 44. Posterior end of male. 45. Male tail tip, ventral view. Arrow indicates sessile papilla.



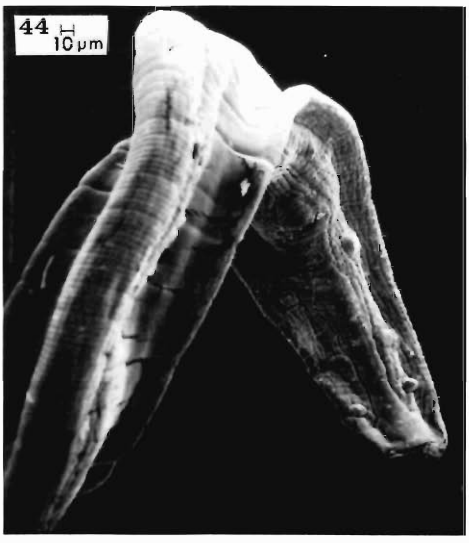


Table 1. Nematode parasite numbers and the infestation rate among Hawaiian birds.

Host species	Host no.	Parasite species	Infestation per bird: Total ♀	Total ♂	Lowest ♀	Lowest ♂	Highest ♀	Highest ♂	Loci
Himatione sanguinea (Apanane)	21	*Viguiera hawaiiensis*	52	36	1	—	9	1	Gizzard
		Procyrnea longialatus	6	—	1	—	3	—	Proventriculus
		Capillaria sp.	14	—	—	1	13	—	Small intestine
Hemignathus virens (Amakihi)	9	*V. hawaiiensis*	30	14	1	2	17	12	Proventriculus Gizzard
Vestiaria coccinea (Iiwi)	4	*V. hawaiiensis*	2	7	—	4	2	3	Gizzard
		Capillaria sp.	1	1	—	—	1	1	Small intestine
Zosterops japonica (Japanese white-eye)	6	*P. longialatus*	45	15	3	—	20	15	Gizzard Proventriculus
	1	*Synhimantus* (*Dispharynx*) *zosteropsi*	20	15	—	—	—	—	Gizzard

(Fig. 39); right spicule with well-developed manubrium (appears hook-like) (Figs. 36–38), tip blunt, length 132–201 μm ($\bar{x}$ = 180 μm ± 0.03). Length ratio of spicules, 2.6:1. Ventral surface of caudal part of body with prominent longitudinal ridges, most evident in precloacal region.

FEMALE (N = 6): Females 5.6–7.0 mm ($\bar{x}$ = 6.0 mm ± 0.49) long, 0.12–0.47 mm ($\bar{x}$ = 0.38 mm ± 0.12) wide. Stoma, 0.10–0.14 mm ($\bar{x}$ = 0.12 mm ± 0.02) long. Anterior esophagus slightly convoluted, 0.31–0.57 mm ($\bar{x}$ = 0.43 mm ± 0.26) long, ends far posterior to cordons; posterior esophagus, 1.69–2.13 mm ($\bar{x}$ = 1.96 mm ± 0.48) long. Nerve ring obscure. Excretory pore not observed. Cordons, 1.38–2.28 mm ($\bar{x}$ = 1.74 mm ± 0.34) long; descending branch 0.86–1.47 mm ($\bar{x}$ = 1.13 mm ± 0.20) long; recurrent branch, 0.37–0.82 mm ($\bar{x}$ = 0.61 mm ± 0.17) long, non-anastomosing (Figs. 32, 40). Vulva, 75–84% (78% ± 3.2), 1.08–1.48 mm ($\bar{x}$ = 1.31 mm ± 0.15) from tail tip. Tail blunt 126–200 μm ($\bar{x}$ = 163 μm ± 0.03) long, no papillae present (Fig. 35). Eggs, 34–40 μm ($\bar{x}$ = 38 μm ± 1.2) long, 18–23 μm ($\bar{x}$ = 22 μm ± 1.0) wide.

HOLOTYPE (male): Deposited in University of California Davis Nematode Collection (UCDNC), Davis, California, USA, UCNC No. 2130. Paratypes deposited as follows: 9 females, UCNC No. 2131; 4 males, UCNC No. 2132; in the UCDNC, Davis, California, USA; 3 females, 4 males at Instituto de Biologia, Lab. Helmintologia, UNAM, Mexico; 2 females, 4 males at Laboratoire des Vers, Museum National d'Histoire naturelle, Paris, France.

HOST: *Zosterops japonica* (Japanese white-eye).

SITE OF INFECTION: Between the tunic layers of the gizzard.

LOCALITY: Mauna Loa, Volcano National Park, Hawaii.

DIAGNOSIS: *Synhimantus* (*D.*) *zosteropsi* is closely related to *S.* (*D.*) *pipilonis* (Olsen, 1939). *S.* (*D.*) *zosteropsi* differs from this species, by the absence of the ventral spine at the point where the calomus merges with the lamina of the right spicule, by the bicuspid cervical papillae in *S.* (*D.*) *zosteropsi* and simple papillae in *S.* (*D.*) *pipilonis*. The length of the descending branch of the cordon in females also differs from those of *S.* (*D.*) *pipilonis*, 433–616 μm versus 0.860–1.473 mm in *S.* (*D.*) *zosteropsi*. Finally, the spicule ratio (left:right) in our specimens was 2.6:1 versus 3.4:1 in *S.* (*D.*) *pipilonis*.

S. (*D.*) *zosteropsi* bears a certain resemblance to *S.* (*D.*) *emberizae* (Yamaguti, 1935) by the presence of a pair of papillae immediately below the two simple lips, by the size of the anterior and posterior esophagus, and by the type and size of the cordons. *S.* (*D.*) *zosteropsi* differs from *S.* (*D.*) *emberizae* by the bicuspid type of cervical papillae in males and females, by the presence of 4 small sessile papillae in front of the male tail end in *S.* (*D.*) *zosteropsi* and only 2 in *S.* (*D.*) *emberizae*, and by the size and shape of the right spicule, 0.15–0.16 mm in *S.* (*D.*) *emberizae*, and in *S.* (*D.*) *zosteropsi* 0.13–0.20 mm.

Discussion

Viguiera hawaiiensis was the most commonly found species and had the widest host range. It was found in 30 of the 40 birds examined in this

study (Table 1), parasitizing the gizzard and proventriculus of *Himatione sanguinea* and *Hemignathus virens,* but occurred only in the gizzard of *Vestiaria coccinea.* The number of nematodes found in each host was variable from 1 female to 17 females and 12 males. *Procyrnea longialatus* was the second most abundant species. It was found in 8 birds, parasitizing the gizzard and proventriculus of *H. sanguinea*; the number of nematodes found per bird was from 1 female to 8 females and 8 males. In three cases, we found mixed infections with the two above-mentioned nematode species parasitizing the gizzard of *Hematione sanguinea,* with a total of 21 nematodes. In one specimen, 9 females and 1 male of *V. hawaiiensis* were found versus 1 female of *Procyrnea longialatus.* In another specimen of *H. sanguinea, Viguiera hawaiiensis* was parasitizing the gizzard and *Capillaria* sp. the intestine.

Synhimantus (*Dispharynx*) *zosteropsi* was found parasitizing the gizzard only in the introduced Japanese white-eye, *Zosterops japonica*; this could indicate that *S. zosteropsi* was introduced to Hawaii via the Japanese white-eye. *P. longialatus* was also collected from the Japanese white-eye. This could mean that it too was introduced and became adapted to *H. sanguinea* or the reverse, i.e., *P. longialatus* was endemic and became adapted to the Japanese white-eye.

Acknowledgments

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Species of Small Strongyles and Other Internal Parasites Recovered from Donkeys at Necropsy in Kentucky[1]

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ABSTRACT: Donkeys ($N = 8$) were examined at necropsy in Kentucky for infections of parasites in the eyes, lungs, abdominal cavity, cranial mesenteric artery, stomach, and small intestine. The cecum, ventral colon, and dorsal colon were examined for large strongyles, tapeworms, and adult pinworms (*Oxyuris equi*) in six of these donkeys, for small strongyles and immature *O. equi* in four, and for the minute pinworm (*Probstmayria vivipara*) in one.

Parasites recovered from the donkeys were: the lungworm (*Dictyocaulus arnfieldi*), the filariid (*Setaria* spp.), bots (*Gasterophilus intestinalis*—2nd and 3rd instars and *Gasterophilus nasalis*—2nd and 3rd instars), stomach worms (*Trichostrongylus axei*—mature, *Habronema muscae*—immature and mature, and *Draschia megastoma*—mature), the ascarid (*Parascaris equorum*—mature), large strongyles (*Strongylus vulgaris* and *Strongylus edentatus*), the minute pinworm (*Probstmayria vivipara*), and small strongyles (eight genera and 26 species). Three species of small strongyles (*Cyathostomum alveatum, Cyathostomum tetracanthum,* and *Cylicocyclus auriculatus*) had not been found previously in equids in Kentucky by the present authors. The finding of *C. alveatum* may be the first confirmed identification of this species in equids in North America.

Publications on internal parasites found at necropsy in donkeys in this country appear to be sparse except for reports dealing with individual species, e.g., lungworms (Lyons et al., 1985b). The purpose of the present investigation was to determine the species and numbers of endoparasites in donkeys purchased recently in Kentucky; with special interest on identification to genus and species of the heterogeneous group commonly referred to as small strongyles.

Materials and Methods

Eight donkeys, including a horse/donkey cross (No. 2077), purchased from a local livestock dealer who procured them mainly from sale yards in Kentucky, were killed and examined for internal parasites. Background history on the donkeys prior to purchase was not possible except for two that had spent some time in Ohio. Five (Nos. 2070—♀, 2072—gelding, 2073—♂, 2076—♀, and 2077—♂) of the donkeys were selected by fecal examination for lungworm (*Dictyocaulus arnfieldi*) infections prior to purchase in June 1983; they were immediately used in a controlled test evaluation of activity of ivermectin against lungworms (Lyons et al., 1985a). The other three donkeys (Nos. 2081—♂, 2082—♂, and 2083—gelding) were purchased in May 1984 for immediate examination for internal parasite infections, with particular interest in diagnosis of the species of small strongyles. Exact ages of the donkeys were unknown but all were mature and most were deemed to be aged.

Sites examined for parasites included the eyes, lungs, abdominal cavity, cranial mesenteric artery, and gastrointestinal tract. Techniques for examinations of these organs were published previously (Drudge et al., 1963; Lyons and Drudge, 1975; Lyons et al., 1976, 1981, 1983, 1985a). The cecum, ventral colon, and dorsal colon were examined from only six donkeys for large parasites (large strongyles, tapeworms, and adult pinworms—*Oxyuris equi*); and from four for small strongyles including species enumeration and immature pinworms (*O. equi*). The minute pinworm, *Probstmayria vivipara,* was enumerated in one donkey (No. 2083) after specimens were found in samples of contents from the dorsal colon during the search for small strongyles.

Scientific names of helminths found in this investigation are as listed in Lichtenfels (1975). Representative specimens of all endoparaites found have been deposited in the USDA Parasite Collection, Beltsville, Maryland 20705 (Nos. 78676–78711; and *Cyathostomum alveatum*—No. 69736, *Cyathostomum tetracanthum*—No. 69976, *Cylicocyclus auriculatus*—No. 69977, *Triodontophorus tenuicollis*—No. 69978, and *Poteriostomum ratzii*—No. 78721).

Results and Discussion

Data on most of the internal parasites recovered from the donkeys are summarized (Table 1). Bots were recovered from stomachs of all donkeys; *Gasterophilus intestinalis* (DeGeer, 1776) Leach, 1817 2nd instars from 1 donkey and 3rd instars from all 8 donkeys. Only 1 donkey was infected with *Gasterophilus nasalis* (Clark, 1797) Leach, 1817 and both instars were present. The numbers of bots were relatively low; whether this was due to host difference or some other factor is unknown. *Trichostrongylus axei* was found in all 8 donkeys and data on 5 of these

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were published previously (Lyons et al., 1985a). Six of the 8 donkeys harbored *Habronema muscae* and 2 were infected with *Draschia megastoma.*

Parascaris equorum was present in only 1 of the 8 donkeys. The low prevalence was probably because most of the donkeys were too old to harbor this parasite. Two species of large strongyles were found—*Strongylus vulgaris* was recovered from all 6 donkeys examined and *Strongylus edentatus* from 3 of the 6 examined. Numbers of small strongyles from the 4 examined for these parasites ranged from 104,300 to 261,250. Six of the 8 donkeys harbored migratory stages of *S. vulgaris* in the cranial mesenteric artery.

Eyeworms (*Thelazia* spp.) were not found in any of the 8 donkeys. The lungworm (*Dictyocaulus arnfieldi*) was found (3–315 specimens each) in the lungs of 5 donkeys selected because of presence of lungworm larvae in feces (Lyons et al., 1985a); one of the other 3 donkeys was infected (258 specimens) with *D. arnfieldi. Setaria* spp. was recovered from 3 of the 8 donkeys (1–4 specimens each). Of 4 donkeys examined for immature *O. equi,* only 1 animal was infected (100 specimens). Enumeration of *Probst. vivipara* in 1 donkey resulted in an estimated total of 50,155,000 present.

Tapeworms were not found in any of the 8 donkeys.

Comparison of the number of donkeys infected with the aforementioned parasites in relation to recent examination for internal parasites in horses is difficult, mainly because of the small number of donkeys examined. It is of particular interest that a much higher proportion of donkeys (100%) were infected with *T. axei* than have been found recently in dead thoroughbreds (4%) in Kentucky (Lyons et al., 1983). Although donkeys traditionally have a high prevalence of *D. arnfieldi,* the selection of five of the lungworm-positive donkeys for a controlled test prejudices the prevalence of this species.

The species of small strongyles from four donkeys are summarized (Table 2)—eight genera and 26 species were found. Three of the species (*Cyathostomum alveatum, Cyathostomum tetracanthum,* and *Cylicocyclus auriculatus*) had not been found in equids in Kentucky previously. *Cyathostomum alveatum* has not previously been confirmed as being present in North America by Lichtenfels (1975). *Cyathostomum tetracanthum* and *C. auriculatus* are reported as being

Table 1. Prevalence of parasites recovered from donkeys at necropsy.

Donkey no.	Stomach							Small intestine	Large intestine*			Cranial mesenteric artery	
	Gasterophilus intestinalis		*Gasterophilus nasalis*		*Trichostrongylus axei*	*Habronema muscae*		*Draschia megastoma*	*Parascaris equorum*	*Strongylus vulgaris*	*Strongylus edentatus*	Small strongyles	*Strongylus vulgaris*
	2nd instar	3rd instar	2nd instar	3rd instar	mature	Immature	Mature	mature	mature				4th	5th
2070	0	2	0	0	1,600	0	20	24	0	3	0	104,300	0	0
2072	0	6	0	0	18,400	0	0	0	0	16	13	ND	2	0
2073	0	44	0	0	34,300	0	0	0	0	ND	ND	ND	11	0
2076	0	5	6	1	1,550	0	9	167	0	ND	ND	ND	17	6
2077	2	1	0	0	3,280	60	63	0	0	57	25	ND	0	0
2081	0	19	0	0	500	0	109	0	0	2	5	111,600	8	10
2082	0	1	0	0	200	0	3	0	0	54	0	261,250	27	34
2083	0	6	0	0	3,930	0	79	0	1	12	0	146,400	0	3

ND = Not determined.
* = Cecum, ventral colon, dorsal colon.

Table 2. Genus and species of small strongyles recovered from four donkeys at necropsy.

Genus and species of small strongyles	Counts for individual donkeys				Total for all donkeys	Average for all donkeys	Average no. for infected donkeys	% of infections†
	No. 2070	No. 2081*	No. 2082	No. 2083				
Craterostomum								
acuticaudatum	0	300	800	0	1,100	275	550 (2)‡	<1
Cyathostomum								
alveatum	600	0	0	0	600	150	600 (1)	<1
catinatum	23,600	7,050	0	1,900	32,550	8,138	10,850 (3)	8
coronatum	0	0	1,400	50	1,450	363	725 (2)	<1
labiatum	2,000	5,600	7,350	18,650	33,600	8,400	8,400 (4)	9
labratum	3,600	13,100	9,450	600	26,750	6,688	6,688 (4)	7
pateratum	0	0	0	1,150	1,150	288	1,150 (1)	<1
tetracanthum	500	0	0	1,650	2,150	538	1,075 (2)	1
Cylicocyclus								
auriculatus	0	0	1,800	2,400	4,200	1,050	2,100 (2)	1
elongatus	0	0	150	0	150	38	150 (1)	<1
insigne	0	150	0	Pos§	150	38	150 (2)‖	<1
leptostomus	3,200	1,450	150	12,200	17,000	4,250	4,250 (4)	4
nassatus	21,450	6,850	6,150	24,400	58,850	14,713	14,713 (4)	15
radiatus	500	1,900	0	200	2,600	650	867 (3)	1
ultrajectinus	0	0	0	350	350	88	350 (1)	<1
Cylicodontophorus								
bicoronatus	0	200	750	200	1,150	288	383 (3)	<1
Cylicostephanus								
calicatus	0	0	250	650	900	225	450 (2)	<1
goldi	9,400	1,500	2,000	0	12,900	3,225	4,300 (3)	3
longibursatus	28,300	15,700	57,100	2,500	103,600	25,900	25,900 (4)	27
minutus	2,550	13,300	9,750	53,350	78,950	19,738	19,738 (4)	20
poculatus	0	0	50	0	50	13	50 (1)	<1
Gyalocephalus								
capitatus	0	200	150	200	550	138	183 (3)	<1
Poteriostomum								
imparidentatum	0	100	0	0	100	25	100 (1)	<1
Triodontophorus								
serratus	0	400	150	0	550	138	275 (2)	<1
tenuicollis	200	1,500	2,800	0	4,500	1,125	1,500 (3)	1
Total mature small strongyles	95,900	69,300	100,250	120,450	385,900	96,475	96,475 (4)	62¶
Total unidentified small strongyles#	50	0	0	0	50	13	50 (1)	<1¶
Total immature small strongyles	8,350	42,300	161,000	25,950	237,600	59,400	59,400 (4)	38¶
Total small strongyles	104,300	111,600	261,250	146,400	623,550			

* *Poteriostomum ratzii* was found in donkey No. 2081, but in too low numbers for aliquot count.
† % for each species is based on the total no. of mature small strongyles.
‡ No. in parentheses is no. of infected donkeys.
§ Present but too few specimens for a factored count.
‖ Two infected but no factored count on one.
No head or tail.
¶ % is based on combined total no. of mature, unidentified, and immature small strongyles.
Pos = positive.

Table 3. Checklist of genus and species of small strongyles recorded from equids in Kentucky.*

Genus and species of small strongyles in equids in Kentucky†	First report	Host§		
		Horse	Pony	Donkey
Craterostomum				
acuticaudatum	Drudge and Lyons, 1972	X	–	X
Cyathostomum				
alveatum	Present paper, 1985	–	–	X
catinatum	Drudge et al., 1955	X	X	X
coronatum	Drudge et al., 1955	X	X	X
labiatum	Drudge et al., 1955	X	X	X
labratum	Drudge et al., 1955	X	X	X
pateratum	Drudge et al., 1974a	X	X	X
tetracanthum	Present paper, 1985	–	–	X
Cylicocyclus				
auriculatus	Present paper, 1985	–	–	X
brevicapsulatus	Drudge et al., 1979	X	X	–
elongatus	Drudge et al., 1963	X	X	X
insigne	Drudge et al., 1955	X	X	X
leptostomus	Drudge et al., 1955	X	X	X
nassatus	Drudge et al., 1955	X	X	X
radiatus	Drudge et al., 1972	X	X	X
ultrajectinus	Drudge et al., 1974b	X	–	X
Cylicodontophorus				
bicoronatus	Drudge et al., 1955	X	X	X
euproctus	Drudge et al., 1974a	X	–	–
mettami	Lyons et al., 1974	X	–	–
Cylicostephanus				
asymetricus	Drudge et al., 1974a	X	X	–
calicatus	Drudge et al., 1955	X	X	X
goldi	Drudge et al., 1963	X	X	X
longibursatus	Drudge et al., 1955	X	X	X
minutus	Drudge et al., 1955	X	X	X
poculatus	Drudge et al., 1955	X	X	X
Gyalocephalus				
capitatus	Drudge et al., 1955	X	X	X
Oesophagodontus				
robustus	Drudge et al., 1974a	X	–	–
Poteriostomum				
imparidentatum	Drudge et al., 1963	X	X	X
ratzii	Drudge et al., 1963	X	–	X
Triodontophorus				
brevicauda	Drudge et al., 1963	X	X	–
nipponicus‡	Drudge et al., 1979	X	–	–
serratus	Drudge et al., 1963	X	X	X
tenuicollis	Drudge et al., 1963	X	X	X

* Summarized from publications by one or more authors of the present paper.

† Lichtenfels (1975) previously verified occurrence in North America of all the same species listed except for *C. alveatum.*

‡ Although *T. nipponicus* was first reported in Kentucky in 1979, it was mistakenly identified and reported by us previously as *T. minor* (Drudge et al., 1974a).

§ Composite from all of our publications.

rare in North America (Lichtenfels, 1975). Becklund (1963) listed these two species and also *C. alveatum* in a checklist of parasites in horses, mules, and asses in the U.S., its possessions, and Canada. Two recent publications report finding *C. tetracanthum* from horses (Wescott et al., 1982) and ponies (Klei and Torbert, 1980) and *C. auriculatus* from ponies (Klei and Torbert, 1980). However, the report of *C. tetracanthum* from ponies by Klei and Torbert (1980) was a misidentification (Klei, pers. comm.). Upon review of several publications on species of small strongyles found in equids outside North America, there seems to be some indication that the donkey, and possibly zebras, are more common hosts than the horse for *C. alveatum, C. tetracanthum,* and *C. auriculatus* (Boulenger, 1920; Theiler, 1923; LeRoux, 1924; Round, 1962; Soulsby, 1965; Scialdo-Krecek et al., 1983).

A checklist, summarizing all of the species of small strongyles that have been found in equids at necropsy in Kentucky to date is presented (Table 3). This list was compiled from publications by one or more of the present authors and includes nine genera and 33 species. Lichtenfels (1975) has confirmed the presence of nine genera and 35 species of small strongyles in equids in North America. Only three of these species (*Cylicocyclus triramosus, Cylicostephanus bidentatus,* and *Cylicostephanus hybridus*) have not been found by the present authors in equids. With the identification of *C. alveatum* from the present investigation, at least nine genera and 36 species of small strongyles are now known to be present in equids in North America.

Although we reported that *Triodontophorus minor* (Drudge et al., 1974a) was found in Kentucky, this species was actually *Triodontophorus nipponicus* (Drudge et al., 1979). *Triodontophorus minor* has been reported by others in North America but Lichtenfels (1975) was not able to confirm its presence because the available specimens that had been diagnosed as *T. minor* were deemed to be *T. nipponicus.*

The present authors could find only one publication on species of small strongyles found in equids in Kentucky by others. Olsen et al. (1949) reported finding several species of small strongyles in equids in Kentucky (apparently during the period 1947–1949): *Cyathostomum catinatum, Cyathostomum labiatum, Cyathostomum labratum, Cylicocyclus insigne, Cylicocyclus nassatus, Cylicodontophorus bicoronatus, Cylicostephanus hybridus, Cylicostephanus poculatus, Cylicostephanus* spp. (apparently three species), *Gyalocephalus capitatus, Triodontophorus brevicauda,* and *Triodontophorus tenuicollis.* Specimens from the collection by these authors were not available but it is of interest to note the foregoing includes one species (*C. hybridus*) which has not been found subsequently by the present authors.

In a recent survey of species of small strongyles in horses in Ohio, six genera and 21 species were found by Reinemeyer et al. (1984). All of these species were reported previously from equids in Kentucky.

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The Pentastomid *Sebekia mississippiensis* sp. n. in the American Alligator and Other Hosts

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ABSTRACT: *Sebekia mississippiensis* sp. n. is described from *Alligator mississippiensis* in Louisiana, Mississippi, and Florida. Closely related to *S. oxycephala* in South American crocodilians, it differs by having a smaller and less spinous hook shield, a broader base for the posterior extensions of the oral cadre, and a thinner and more delicate tegument. The male reproductive system differs somewhat from that described for other pentastomids. Nymphs parasitize several fishes as well as turtles, snakes, and mammals.

The American alligator, *Alligator mississippiensis* Daudin, over most of its range in the southeastern United States, hosts a pentastomid infection in its lungs and viscera. We consider the parasite, commonly referred to as, but distinct from, *Sebekia oxycephala* (Diesing, 1835) of South American crocodilians, to represent a new species. This study describes the new species, expands the ecological data reported by Overstreet (1978), and lists several hosts for the nymphal stage.

Materials and Methods

Alligator hosts for this study came from several sources. Three were collected alive in Rockefeller Refuge, Cameron Parish, Louisiana, in 1970–1971. During experimental alligator harvest programs (Palmisano et al., 1974) in that same general area conducted by the Louisiana Department of Wildlife and Fisheries in 1972, 1973, and 1975, we examined viscera from 10, 7, and 8 recently killed alligators. All but one were males. Two alligators from Ocean Springs and one from Cat Island, Mississippi, were examined soon after they drowned by entangling in lines or fishermen's nets. Three were examined fresh from near St. Augustine, Florida. Nymphs from hosts other than the alligator came from routine parasitological examinations not specific for pentastomids.

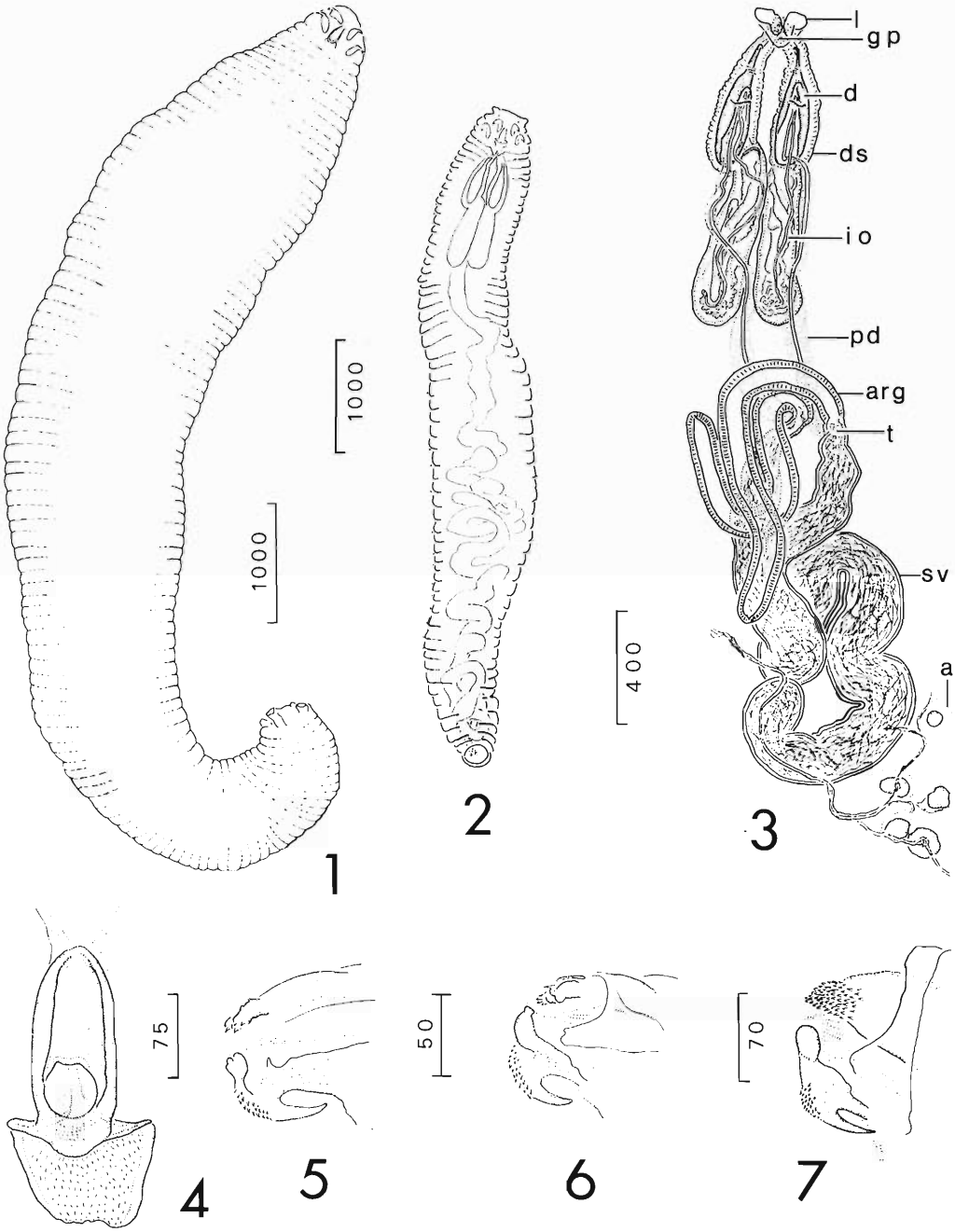
Pentastomid specimens mounted directly from 70% ethanol in Hoyer's medium were studied progressively as they cleared. This method provided distinct annuli, because specimens in ethanol were too opaque and fully cleared ones were too transparent. The transparent preparations allowed the best view of sclerotized hooks, oral cadre, and male genitalia. Three male specimens were serially sectioned at 6 μm and stained using Harris' hematoxylin and eosin. Hooks were measured using the method of Fain (1961). Illustrations were made from projected images. All measurements are in millimeters.

Results

Sebekia mississippiensis sp. n. (Figs. 1–6, 9–27)

MALE: Body 5.6 long; width 0.05 at cephalothorax, 0.9 at abdomen; cephalothorax triangular in some individuals. Annuli numbering 69–70, with spinelike cuticular projections prominent in some individuals. Sensory papillae 2 pairs, with each group forward of anterior hook and parallel to annuli; cephalic lobe paired, at anterior of body forward of sensory papillae. Hooks claw-shaped, having broad base with dimensions measuring AB = 0.04, AC = 0.05, and BC = 0.03; talon relatively short; outer convex surface forming hump with minute spines (Figs. 5, 6, 9). Fulcrum 0.13 long (straight line between ends); outer surface rugose, without spinules; anterior outer portion projecting against base of hook as relatively small spinose shield; shield not touching hook when hook bent forward. Oral cadre oblong, 0.17 long by 0.07 wide, with opening an oblong ring; distal portion of mouthring lightly sclerotized dorsally and open ventrally (Fig. 4). Intestine sinuous especially in posterior half (Fig. 2), with hemosiderin usually abundant in gastrodermis (Figs. 22, 26). Rectum length about equal to midbody diameter (Fig. 15). Anus terminal.

Testis bound by membrane (Figs. 23, 24), sinuous and surrounding portions of sinuous posterior half of intestine (Figs. 26, 27), with all or most spent in some individuals, succeeded by nearly spherical spermatic aggregates (Figs. 23–25). Seminal vesicle Y-shaped, with short arms anteriorly, sinuous, bound by conspicuous muscular sheath, located near midbody, with mus-



Figures 1-6. ***Sebekia mississippiensis*****, from whole mounts. 1. Female, ventral view. All examined adult females were gravid, but annuli in central abdominal region were seldom as visible as shown. 2. Male, ventral view. Note that anterior end is more blunt than for female. 3. Male terminal genitalia, ventral view. Note genital pore (gp), cuticular lobes (l) above pore, dilator (d), dilator sac (ds), multifold intromittent organ (io), prostatic duct (pd), accessory reproductive gland (arg), triad (t), seminal vesicle (sv), and spermatic aggregate (a). 4. Oral cadre, male, ventral view. 5. Posterior right hook bent forward away from the fulcrum's shield. 6. Anterior right hook showing slightly more spinous shield than on posterior hook.**

Figure 7. ***Sebekia oxycephala*** **from** ***Caiman sclerops*** **in Brazil (Instituto Oswaldo Cruz Coll. No. 32.104b), anterior right hook showing more spinous and larger shield of the fulcrum than on comparable structure of** ***S. mississippiensis*** **in Figure 6. Scale measurements for Figures 1-7 are micrometers.**

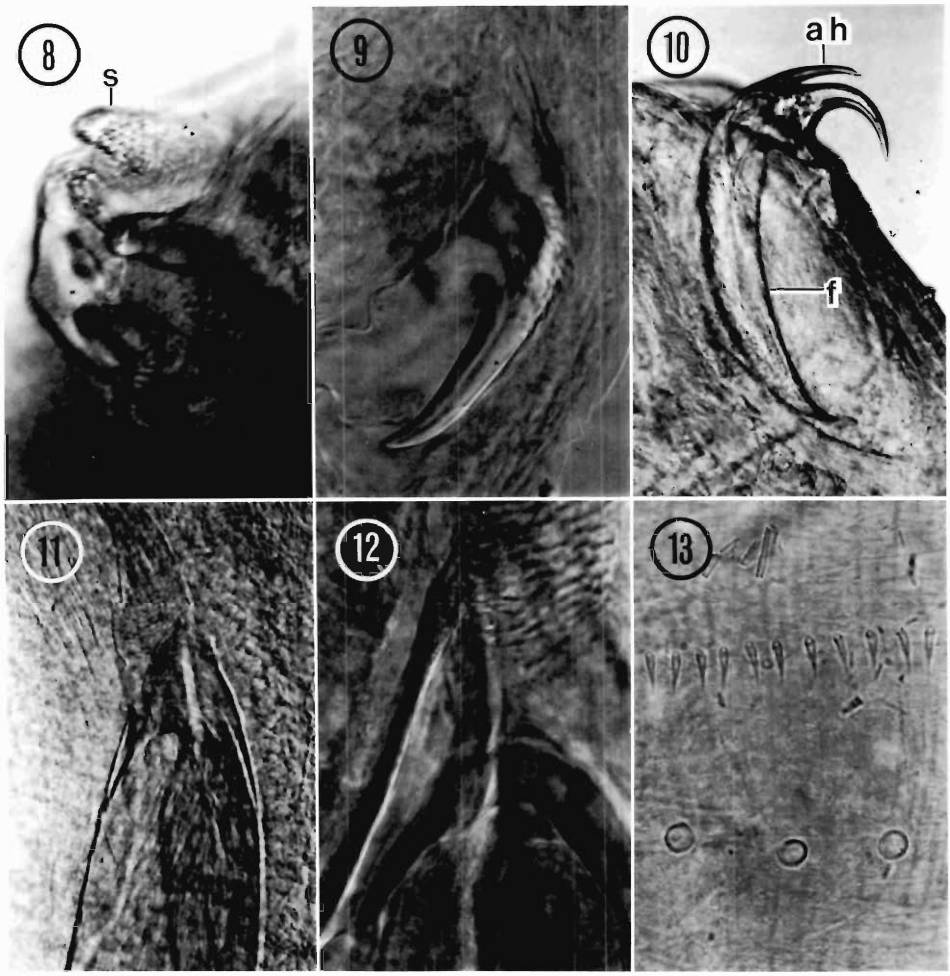


Figure 8. ***Sebekia oxycephala,*** **same hook as shown in Figure 7. Note projecting shield (s).**

Figures 9–13. ***Sebekia mississippiensis.*** **9. Anterior right hook of male protruding through cuticle. 10. Posterior left hook of nymph showing thin diagnostic accessory hook (ah) and long narrow fulcrum (f). 11. Mid-portion of sclerotized dilator showing rugose area of surrounding genital duct. 12. Close-up of rugose area in Figure 11 into which serrated portion of dilator's narrow neck fits. 13. Cuticular spines and pores (three shown) of male.**

cular vas deferens at proximal end (Figs. 24, 25). Vas efferens ramose, connecting aggregates and portions of testis with glandular-encased vas deferens. Terminal genitalia paired, similar, with each member of pair consisting of muscular sac-complex, sperm duct, and accessory reproductive gland (ARG), with each member of pair joining arm of seminal vesicle as triad with ARG and glandular portion of sperm duct (Fig. 21); ARG relatively long, sinuous in some specimens, highly glandular with basal nuclei in tall epithelial secretory cells; epithelial layer separated from thick longitudinal muscular layer by conspicuous eosinophilic hyaline basement membrane; muscle layer bound by layer of cells usually appearing with relatively clear cytoplasm (Fig. 22). Sac-complex a thick longitudinal muscular ensheathment of two connected cavities (Fig. 18); anterior sac (dilator sac; Figs. 17–19) a continuation of highly muscular duct, with basal swollen portion containing complex sclerotized dilator; dilator somewhat ladle-shaped, opened posteriorly and ventrally in posterior half, attached to sac near midlevel, with head portion not attached to sac

and narrowing anteriorly, with fine serrations on ventral portion of neck base fitting into villous and rugose area of sclerotized lining of genital duct, with short blunt hook at distal tip; posterior sac containing multifold sperm duct; sperm duct sclerotized, with presumed distal free end heavily reinforced on one side as thick minutely corrugated intromittent organ (Fig. 20), becoming relatively thinner and narrower as progressing proximally (anteriorly and looping), traversing from near rear of posterior sac to near midlevel of dilator sac as narrow duct within flat pliable muscular conduit connecting both sacs, seldom twisted and nearly uniform in width when within conduit, with proximal (located anteriormost) portion looped through ventral chock near middle of dilator (Fig. 19) and then becoming encased by glandular sheath (Fig. 18) and continuing to seminal vesicle as prostatic duct (Figs. 20, 21); prostatic duct passing through either posterior end or level of posterior fourth of dilator sac depending on individual and state of contraction of sac, sclerotized moderately within dilator sac where covered by sheath thickness of one to few small cells having small nuclei, becoming lined internally by thinner sclerotized layer coated with layer 1 small cell thick overlayed by another layer of 1–3 relatively large cells thick as leaving sac, with outer prostatic cells having relatively large nuclei, extending to triad with ARG and seminal vesicle. Common male duct muscular (Fig. 16), with pore opening at ventral surface at first abdominal annulus, longer than ½ body width, formed by union of muscular secondary genital duct extensions of dilator sacs; pore with 2 overhanging cuticular cephalic lobes anteriorly.

FEMALE: Body 10 long maximum, with width of cephalothorax 0.6; cephalothorax roughly triangular, not demarcated sharply from abdomen; abdomen 1.6 at widest level. Annuli numbering approximately 70, difficult to distinguish in midregion. Anus terminal. Uterovaginal pore anterior to but not contiguous with anus on same annulus. Tegument relatively thin, delicate. Hooks similar to those of male, with dimensions AB = 0.05, AC = 0.07, and BC = 0.04; fulcrum similar to that in male, 0.19 long. Oral cadre similar to that of male, 0.21 long by 0.09 wide.

TYPE HOST: *Alligator mississippiensis* Daudin.

SITES FOR ADULTS: Lungs, occasionally along lining of pleuroperitoneal cavity possibly resulting from postmortem migration.

HOSTS FOR NYMPHS: *Alligator mississippiensis* plus fishes, turtles, snakes, and mammals as indicated in text.

LOCALITIES: Numerous freshwater, brackish, and saline habitats in Cameron and Vermilion parishes, Louisiana (type locality); brackish bayous in Ocean Springs, Mississippi; near St. Augustine, Florida; additional localities for specimens, not all of which have been examined, occur in text.

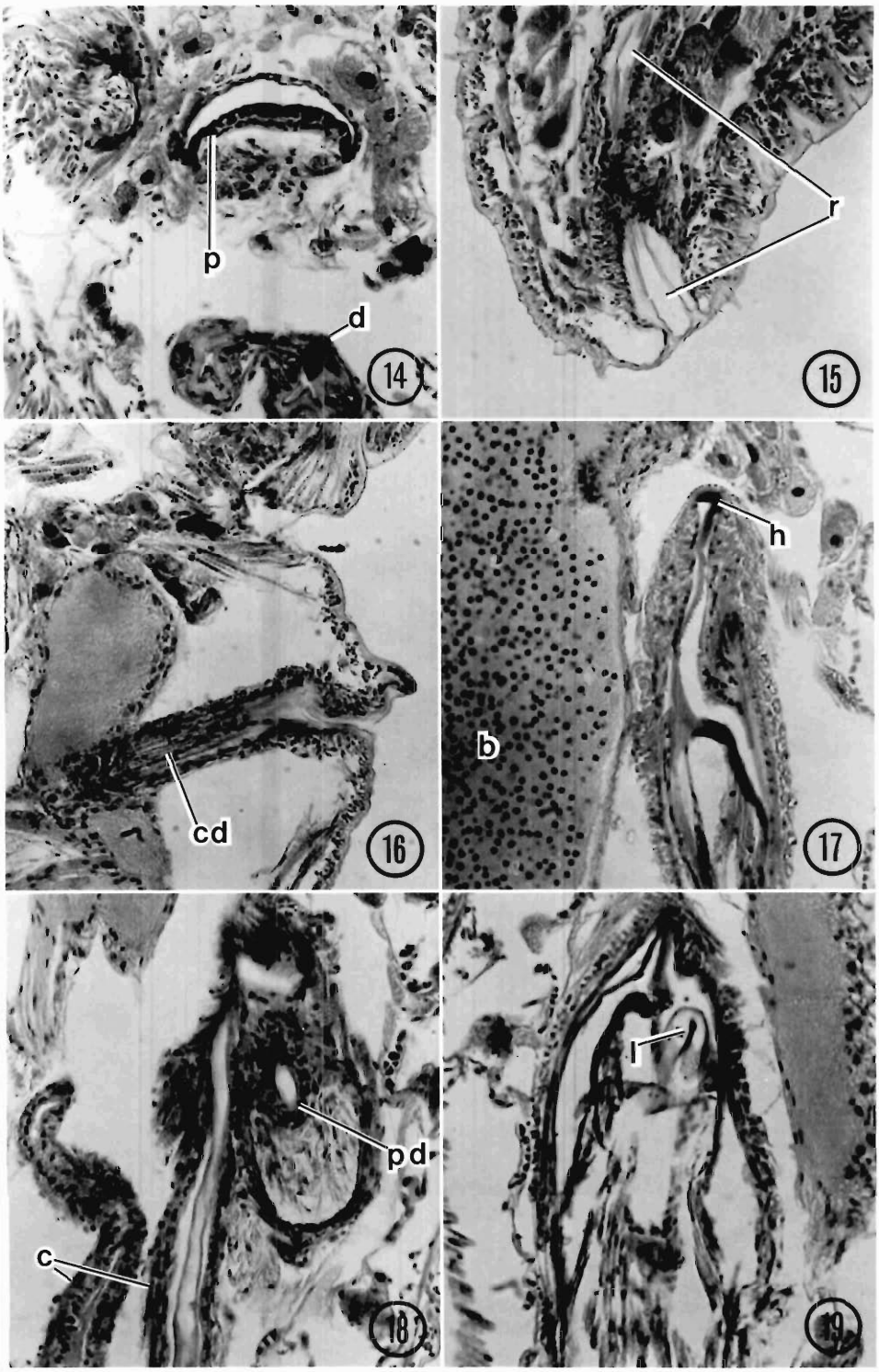
SPECIMENS DEPOSITED: Holotype, male: Pentastomida Collection No. 529A, American Museum of Natural History; allotype, female; paratype crushed for hook and oral cadre measurements: AMNH Pentastomida Coll. No. 529; additional paratypes: AMNH Pentastomida Coll. Nos. 575–576.

ETYMOLOGY: The adjective *mississippiensis* refers both to the parasite's alligator host and the general region in which the parasite commonly occurs.

Remarks

Of 31 alligators 147–311 cm long examined from Louisiana and Mississippi, 20 harbored infections of *Sebekia mississippiensis,* and 17 of those contained adults in their lungs or on the pleuroperitoneal lining. Adults occurred during all months sampled: March, May, July, and September. Three infected alligators harbored no adults but had nymphs in their lungs or liver. Most of the examined nymphs occurred in the liver, presumably before migrating to the lungs, but some also occurred within or under connective tissues lining the spleen, stomach, and mesentery. Relatively high numbers of approximately 14 adults were obtained from the lungs of each of three alligators, but most had 1–6. Three alligators contained over 15 nymphs in their liver. Those hosts from brackish marshes had as many or more pentastomids as those from freshwater habitats.

Confirmed secondary hosts with nymphs include the fishes *Lepomis macrochirus* Rafinesque (bluegill) and *Fundulus grandis* Baird and Girard (gulf killifish) from brackish marsh areas of Jackson County, Mississippi; *Micropogonias undulatus* (Linnaeus) (Atlantic croaker) from 64 m of water in the Gulf of Mexico (29°15′N, 88°38′W); and *Xiphophorus helleri* (Heckel) (swordtail) from a tropical fish supplier rearing fish in outdoor ponds in the southeastern U.S. These nymphs occurred under the connective tissues lining



Figures 14-19. Histological sections of male *Sebekia mississippiensis*. 14. Cross section of crescent-shaped pharyngeal segment (p) of oral cadre showing thick ventral portion; the two genital ducts before uniting, one of which contains the dilator's tip (d); and muscular sheath and associated musculature for proximal portion of

muscle, kidney, liver, and swim bladder. A specimen donated by K. C. Corkum occurred in skeletal muscle of *Micropterus salmoides* (Lacépède) (largemouth bass) from Lake St. John, Louisiana, near Natchez, Mississippi. A specimen of the Virginia opossum, *Didelphis virginiana* Kerr, collected in the "Carolinas" and shipped to Tulane Medical School for parasitological examination by us had a nymph beneath the hepatic capsule. Nymphs collected by others from several hosts indicated below are also *S. mississippiensis* as determined from representative specimens. Francis C. Rabalais collected them in Louisiana from the snakes *Nerodia rhombifera* (Hallowell), *N. sipedon* (Linnaeus), *N. cyclopion cyclopion* (Dumeril and Bibron), and *N. fasciata confluens* Blanchard; John E. Ubelaker collected them from *N. erythrogaster* Forster; A. D. W. Acholunu collected them in Louisiana from the turtles *Chrysemys scripta elegans* (Weid), *Sternotherus odoratus* (Latreille), and *Trionyx spiniferus* Lesueur; and Donald J. Forrester collected them from the nearctic river otter, *Lutra canadensis lataxina* (Cuvier).

Discussion

The male reproductive system of *Sebekia mississippiensis* presents problems dealing with accepted terminology for pentastomids, uncertainties about some functions, and a potentially valuable set of taxonomic characters for both specific and higher levels. Problems with this system concern both the paired terminal genitalia and testis.

A few terms for structures of the terminal genitalia used in the above description differ from those used in and repeated from classic works and reviews (e.g., data from Leuckart and Spencer in Heymons [1935], Fain [1961], and Self [1983]). In most papers, the intromittent organ has been referred to as a cirrus within a cirrus sac. By definition, a cirrus, according to most parasitological literature, is the evaginable terminal portion of the male ejaculatory duct, but in *S. mississippiensis, S. oxycephala, Linguatula serrata* Frölich, 1789 (see Heymons, 1935), and other species, the organ probably protrudes without evagination. Because it is not clearly an extension of the mouth of the ejaculatory duct, we prefer intromittent organ or the general term "copulatory apparatus" to the term "penis." We could not confirm the presence of a distal free end reported by others and assumed by us. The basal, or proximal, continuation of the sperm duct leading to the seminal vesicle and ensheathed by glandular cells has been referred to as the "vas deferens." Because that duct does not deliver spermatozoa from testis to seminal vesicle, it is not the vas deferens, but it probably serves as a true prostatic duct. Also involved in secretion is the structure we consider the accessory reproductive gland (ARG), a structure probably homologous to the one or more structures referred to as ARG's in insects (e.g., Odhiambo, 1969). Whereas members of different groups of insects have different numbers of ARG's, some have a single pair of tubular glands similar to that in *S. mississippiensis* except that the encapsulating muscular sheath in those is thin and sometimes hardly detectable and secretions from different segments of the gland may differ (e.g., Musgrave, 1937). The ARG in pentastomids other than *S. mississippiensis* has been referred to routinely as an ejaculatory sac or duct. An ejaculatory duct transmits spermatozoa, whereas an ARG produces one or more seminal fluid products to mix with spermatozoa from a seminal vesicle or testis. Other terms defining components of the terminal genitalia are not so well established. For example, the sclerotized structure in the muscular dilator sac has been referred to as the "Kopulationszapfen" (abbreviated as "pen" and translated as copulatory cone, or spigot), spicule, and other terms including dilator, which we prefer here.

Functions of the male structures have not all been demonstrated, but we assume that the longitudinal musculature covering the dilator sac

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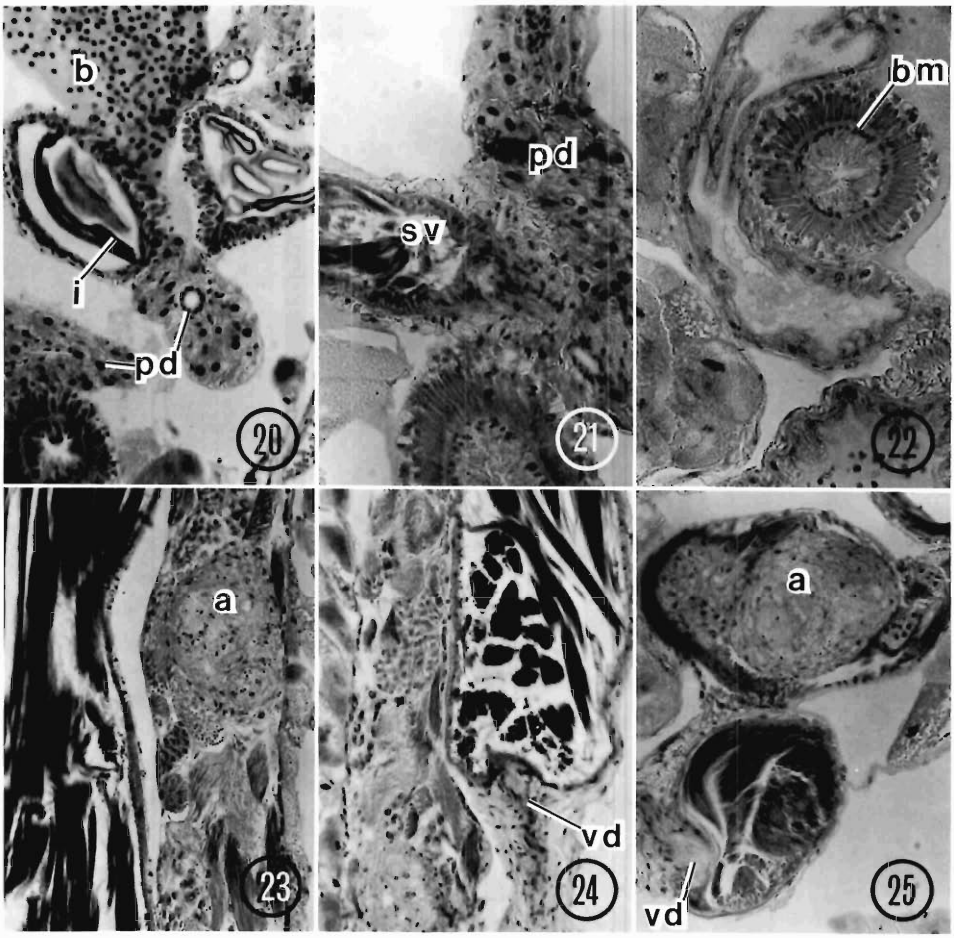
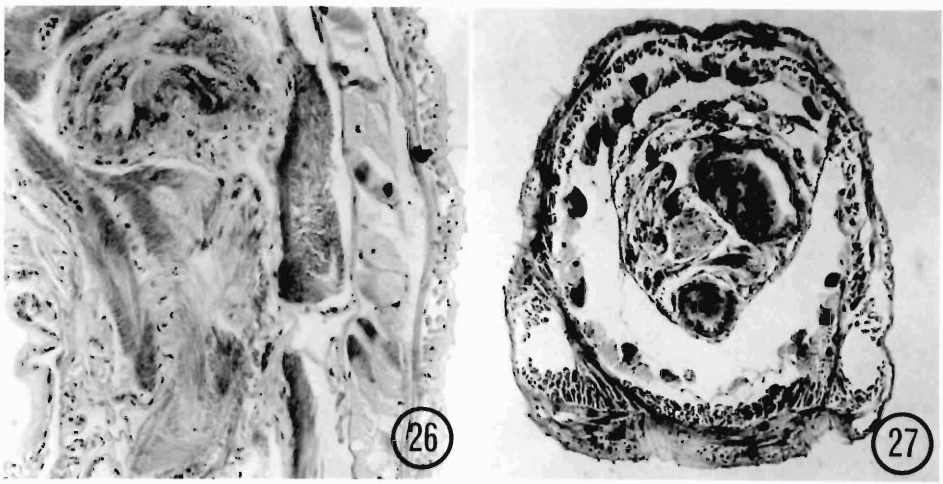
fulcrum in upper left corner of micrograph. 15. Near-frontal section of rectum (r) and associated glands. 16. Sagittal section of common genital duct (cd) showing pore and overhanging lip. Duct deflects anterior of intestine near left of micrograph. 17. Sagittal section of dilator sac with enclosed dilator showing serrated portion of neck and blunt distal hook (h). Note that muscular genital duct surrounding neck is an anterior continuation of dilator sac. Intestine at left is filled with alligator blood (b). 18. Frontal section of dilator sac showing origin of prostatic duct (pd) portion of sperm duct before exiting sac. Associated with the anterior portion of sac is the flat conduit (c) that joins dilator and posterior sacs and carries the sperm duct; adjacent to it is the conduit for other member of the pair. 19. Frontal section of dilator sac at level showing anteriormost loop (l) of sperm duct in chock of dilator; this portion of duct may serve as base of protruded intromittent organ.



Figures 20–25. Histological sections of male *Sebekia mississippiensis*. 20. Sagittal section through two artifactually divided portions of an arcuate posterior sac showing the dark bluish-stained, reinforced side portion of intromittent organ (i) at left and several sections through multifold completely reddish-stained intromittent organ at right. Sac is divided anteriorly by alligator blood-filled intestine (b) and posteriorly by prostatic duct (pd) leading toward accessory reproductive gland (ARG) at lower left. 21. Sagittal section showing plane through triad of prostatic duct (pd) at top, arm of seminal vesicle (sv) at left, and ARG at bottom. 22. Cross section of ARG showing hyaline basement membrane (bm) lined internally by basally nucleated glandular epithelium and externally by layer of longitudinal muscle overlaid by boundary cells. Epithelium of intestine curving around ARG has an abundance of hemosiderin particles. 23. Sagittal section of testis adjacent to seminal vesicle filled with mature spermatozoa. Note testis contains germinal cysts at various spermatogenic stages and a presumed recently formed spermatic aggregate (a). 24. Sagittal section of ensheathed vas deferens (vd) joining spermatozoan-filled seminal vesicle adjacent to testis showing different spermatogenic stages. 25. Sagittal section of different specimen showing similar union of vas deferens. Note spermatic aggregate (a) above with surrounding mature spermatozoa. Such aggregates, many without surrounding spermatogenic stages, are all that remain from the gonad in some specimens.

and genital duct constricts, allowing protrusion of the distal one-third of the dilator. The dilator probably inserts into the female's genital pore, aligning the mating pair with aid from the cuticular papillae to allow penetration by the intromittent organ. Sperm stored in the seminal vesicle can be expelled by the outer muscular layer of that vesicle. The exceptionally muscular layer of the ARG extrudes secretions from the glandular epithelium. Its constriction may also help force spermatozoa from the seminal vesicle to mix with products from its epithelium and from the prostatic duct, which would then advance into and finally protrude the long, relatively thin copu-



Figures 26, 27. Histological sections of male *Sebekia mississippiensis*. 26. Sagittal section through heavily hemosiderin-laden intestine surrounded by testis abundant with nearly developed spermatozoa. 27. Cross section of posterior end showing two sections through sinuous intestine at a level close to rectum. Note testicular tissue between sections at this level.

latory apparatus as well as cause ejaculation of the semen. The base of the copulatory apparatus is probably the portion of the sperm duct looped in the dilator's chock.

Unlike the cephalobaenids in which males often produce one group of spermatozoa that all mature and fill the seminal vesicle at essentially the same time, the porocephalids supposedly can mate repeatedly over long periods (e.g., Self, 1983). At least the porocephalid *S. mississippiensis* may be exceptional. An apparent depletion of spermatogenic stages other than some spermatic aggregates seems to accompany the filling of the seminal vesicle with spermatozoa. Moreover, the long sclerotized copulatory apparatus has no obvious means to retract into its sac. On the other hand, a portion of the copulatory apparatus may form spermatophores and the spermatic aggregates may contain either a second type of sperm or resting immature forms to be utilized later. The aggregates do not contain mature spermatozoa as found in the seminal vesicle and adjacent germinal cysts, but the tailed structures are not similar to the degenerating stages in the testis of *Raillietiella* sp. (see fig. 6 from Self, 1983). Even though the contents may undergo resorption, we observed no pyknosis, not necessarily an associated process. These different structures and possibilities need investigation.

Features of the complex male genitalia certainly have taxonomic and probably phylogenetic significance. Until such structures in other pentastomids can be adequately described and compared, the level of that significance cannot be judged. Still, dilators have specific differences in *Raillietiella* Sambon, 1910 (see Ali et al., 1984) and probably do for members of *Sebekia.* Rather than being a specific characteristic, the Y-shaped seminal vesicle may be diagnostic at a higher level. The testis of some pentastomids has a more defined shape and does not surround the midgut.

Nymphs of *S. mississippiensis* and presumably most members of *Sebekia* Sambon, 1922 morphologically resemble adults except for the absence of genitalia and the presence of accessory hooks on each of the four principal hooks. Nymphs lose their accessory hooks during the molting process. Whereas the function of these hooks has not been established, we believe that they, along with the spinose cuticle, aid the nymph to move within the intermediate or secondary host as well as to migrate within the viscera of the final host and to transit into its lungs. We have counted as few as 61 annuli in nymphs, suggesting that either more variation exists in the number of annuli in adults or the number may increase by adulthood. Venard and Bangham (1941) reported 64–68 segments for the nymph.

Sebekia mississippiensis has close affinities with *S. oxycephala,* common in some South American crocodilians. It has previously been considered that species in North America (e.g., Venard and Bangham, 1941) but differs by having a

smaller, less spinous shield of the fulcrum overlying the base of the hook (compare Figs. 5, 6 with Figs. 7, 8 of *S. oxycephala*), a broader base for the sclerotized pharyngeal extensions of the oral cadre, and a thinner and more delicate tegument. Without sectioning and critically comparing with our findings those of the tegument of comparative-sized specimens of *S. oxycephala,* we present a subjective interpretation of delicateness. As determined for the study by Self and Rego (1985), the females could not be readily flattened, stain did not penetrate their tegument, and they did not clear readily in solvent. Examination of internal structures and mounts of the hooks and oral cadre necessitated either dissecting specimens or shaving off their surface layer. In contrast, the delicate gravid female *S. mississippiensis* often swells, making annuli difficult to distinguish. The spinose annular rings in males were absent in examined *S. oxycephala,* but also in some individuals of *S. mississippiensis.* The two species also apparently differ in average size. Self and Rego (1985) reported the length of *S. oxycephala* as 15–24 mm compared to 10 mm maximum for *S. mississippiensis.* Heymons (1935) reported a range of 15–19 mm for the former, but he may have had a mixture of species because he cited North America as a locality for *S. oxycephala.* He (1935, fig. 123) illustrated a specimen with narrow-based posterior extensions on the oral cadre, characteristic of *S. oxycephala,* which showed the intromittent organs in the posterior sacs (sacs not illustrated) to be in a spherical rather than an elongated mass and the dilators to have shorter necks than those of *S. mississippiensis,* suggesting diagnostic differences in terminal genitalia of the two species.

The status of several members of *Sebekia* and closely related genera has been reviewed recently by Self and Rego (1985), who provided means of differentiating species. Briefly, *Sebekia* includes in addition to *S. mississippiensis, S. oxycephala* (synonyms *S. wedli* Giglioli, 1922 and *S. divestei* Giglioli, 1922), *S. megastoma* (Diesing, 1835) (synonym *Diesingia m.*), and *S. microhamus* Self and Rego, 1985. *Sebekia acuminata* Travassos, 1924, *S. cesarisi* Giglioli, 1922, *S. jubini* (Vaney and Sambon, 1910), and *S. samboni* Travassos, 1924 all need to be redescribed. With the exception of *S. megastoma,* which has a chelonian definitive host, all species infect crocodilians. *Alofia platycephala* (Lohrmann, 1889) (synonym *A. merki* Giglioli, 1922) may in the future be placed in *Sebekia. Diesingia kachugensis* (Shipley, 1910) is a nymph of uncertain taxonomic position. Also, the sebekid nymph *Pentastomum gracile* Diesing, 1835 may be conspecific with *Leiperia cincinnalis* Sambon, 1922. Nymphs of most or all species exhibit less specificity to secondary hosts than the adults. Adults usually infect the lungs of one or few host species.

Species of *Sebekia* have apparently coevolved as a faunal unit with their host group similarly to species of other pentastomids, *Porocephalus* Humboldt, 1811, *Kiricephalus* Sambon, 1922, and *Raillietiella* Sambon, 1910, as well as to several crocodilian digenean groups as hypothesized and discussed by Brooks (1979) and to crocodilian ascaridoid nematodes (e.g., Sprent, 1977, 1979).

Values for prevalence and intensity of infection present some problems. Our samples are heterogeneous because hosts come from different habitats, from different years, and from a predominately male population of alligators. Most material represents coastal southwestern Louisiana samples in September, and postmortem migration and loss of worms probably occurred in some cases. The alligator occurs in large numbers in southwestern Louisiana where most of the material for this study was collected. Based on nest counts in the collection-area, McNease and Joanen (1979b) estimated an increase in alligators from 1970 to 1977 with most, 1 per 3.2 ha, occurring in marshes intermediate between fresh and brackish. Habitats for immature, adult male, and adult female alligators differ (e.g., McNease and Joanen, 1975), as does an individual's range. An immature male has had a minimum documented range as great as 605 ha, averaging 229 ha (McNease and Joanen, 1975), and adult males ranged within 183–5,083 ha in 11 months or less, with a migration of 8,458 m recorded for one day (Joanen and McNease, 1973). Some do not move from one type of habitat. Brooks et al. (1977) noted that from a larger sample of the same collection, the presumed freshwater proterodiplostome digeneans, unlike the pentastomid, occurred in individuals from exclusively freshwater areas. Most of our sample consisted of males because in September during the harvests, males occupied readily accessible canals, bayous, and lakes (Palmisano et al., 1974).

Based on vague reports, some alligators along the Atlantic coast may be more heavily infected

than typically encountered in the northern Gulf of Mexico where the alligator is probably more prevalent. However, as also pointed out by Deakins (1971), adults easily penetrate lung serosa immediately following evisceration. Consequently, some of our values are probably low because we examined most alligators a few hours after their death. Twelve alligators from three locations in South Carolina had infections ranging from 1 to 44 individuals, not differentiating lung from liver infections (Hazen et al., 1978). Seven alligators from McIntosh County, Georgia, each had about 30 or 40 adults in their lungs (Deakins, 1971). We have confirmed representative specimens from those studies as *S. mississippiensis.* Cherry and Ager (1982) examined 30 alligators from seven counties in southern Florida, and from 1 to 77 (average 10.6) pentastomids infected each of 93% of the sample. They determined no difference in infections in male versus female hosts. Shotts et al. (1972) reported 43 individuals in the lungs of a large male from Lake Beaudair, Florida. From near an alligator farm in St. Augustine, Florida, one host examined in September had two adults and another had 12; two hosts in January each had one specimen, one a female and one a nymph. Twelve alligators examined by Penn (1942) from Sabine Refuge near our collecting sites in Cameron Parish, Louisiana, had no infections.

Whereas the alligator may acquire most of its pentastomids from fishes, it can also obtain them from turtles, snakes, and mammals. All these animals constitute prey for this opportunistic feeder, greatly augmenting the alligator's potential to acquire infections. The alligator's diet varies some from saltwater to freshwater habitats and from year to year (McNease and Joanen, 1979a). Our data on stomach contents from alligators examined for pentastomids in 1972 and 1973 were included in the evaluation by McNease and Joanen (1979a). In 1975 we examined about 25 stomachs for food, even though the lungs and liver of only eight were examined for pentastomids. They contained less food than in 1972 and 1973, and the ambient temperature in 1975 was lower than in those years. Fewer nutria bodies occurred in the stomachs than in earlier samples, but nutria hair was present in nearly all individuals. As in other years, other mammals also made up much of the diet: two with white-tailed deer, one with calf remains, one with skunk remains, and one with a dog skull and hair. Turtle fragments occurred in three and gar in two. Invertebrates such as crayfish and clams were in three and one, respectively, being less frequent than in previous years. We found redwing blackbirds and an occasional grackle in nearly all stomachs, but these species had been used as bait. Nevertheless, most alligators eat many fish (McNease and Joanen, 1979a), and large numbers can be eaten over a period of time.

In Rockefeller Refuge, Albert P. Gaudé, III (pers. comm.) examined fishes from two locations specifically for pentastomid nymphs and found infections in 8 of 8 specimens of the spotted gar, *Lepisosteus oculatus* (Winchell), 3 of 29 specimens of *Lepomis macrochirus,* and 1 of 8 specimens of the blue catfish, *Ictalurus furcatus* (Lesueur), all of which are reported prey of alligators from that area (McNease and Joanen, 1979a). Gaudé found no nymphs in 84 individuals of 12 other species of fish.

In Ocean Springs, we saw nymphs in fish from bayous where alligators occurred. Nymphs not available for study but probably *S. mississippiensis* infected the ladyfish, *Elops saurus* Linnaeus, and the longnose killifish, *Fundulus similis* (Baird and Girard), in high salinity water near Horn Island, Mississippi, where alligators occur. We did not examine any alligators from that island, and one from nearby Cat Island had no infection. The offshore infected fish (207 mm SL gravid *Micropogonias undulatus,* 32 km off Pass a Loutre, Louisiana, on October 31, 1975) probably had acquired its infection months earlier in a bayou nursery ground in Louisiana or Mississippi.

Numerous other fishes have been reported as hosts, some harboring many specimens. Dukes et al. (1971) found 2–23 nymphs located in musculature near the ribs and backbone of 23 of 34 individuals of *Micropterus salmoides.* Those were from Lake St. John, Louisiana, from where we examined a specimen. Specimens assumed to be *S. mississippiensis* have been reported by Holl (1928) from the liver, mesentery, and swimbladder of *Ictalurus natalis* (Lesueur) (as *Ameiurus n.*) and *Lepomis gibbous* (Linnaeus) (as *Eupomotis g.*) from a lake near Gibsonville, North Carolina. Bangham reported others from Florida in three publications (e.g., Venard and Bangham, 1941) as obtained from cysts in the mesentery of *Amia calva* Linnaeus, *Gambusia affinis* (Baird and Girard) (as *G. a. holbrookii*), *Lepomis punctatus* (Valenciennes) (as *Sclerotis p. punctatus*),

L. marginatus (Holbrook) (as *Xenotis megalotis marginatus*), *L. microlophus* (Günther) (as *Eupomotis m.*), *L. gulosus* (Cuvier) (as *Chaenobrytus g.*), and *Pomoxis nigromaculatus* (Lesueur) (as *P. sparoides*). Boyce et al. (1984) also reported them from *G. affinis* in Florida.

Life cycle studies of *S. mississippiensis* need to be conducted. They might show that only specific fishes serve as true intermediate hosts, and that others plus turtles, snakes, and mammals act as paratenic hosts. Dukes et al. (1971) recovered nymphs of *S. mississippiensis* from lungs of the snapping turtle, *Chelydra serpentina* (Linnaeus), but not from four species of snakes (three of which we report as hosts of nymphs), one month after feeding them encapsulated nymphs from the largemouth bass. We examined nymphs occurring naturally in turtles from Louisiana, and, according to Holl (1928), Stunkard has observed pentastomids in lungs of turtles. Whether these nymphs can mature in those hosts and whether they and those in mammals were acquired from nymphs in infected fish or from eggs need to be established.

Sebekia mississippiensis can cause necrosis and hemorrhaging in the lungs and liver of the alligator (e.g., Hazen et al., 1978), it apparently can kill hatchlings (Boyce et al., 1984), its infections have been suggested as producing or accentuating fatal pneumonic aeromonad bacterial infections in that host (Shotts et al., 1972; Hazen et al., 1978), and its nymphs can be relatively large or numerous in some fish, threatening their health. The fact that nymphs infect mammalian hosts poses the possibility of human infections, either from consuming nymphs with fish or other vertebrates or maybe even from swallowing eggs.

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The Prevalence of *Sebekia mississippiensis* (Pentastomida) in American Alligators (*Alligator mississippiensis*) in North Florida and Experimental Infection of Paratenic Hosts

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ABSTRACT: The pentastomid *Sebekia mississippiensis* was found in 29 of 31 alligators examined from two lakes in Alachua County, Florida. The majority of parasites found in the lungs were nymphs and no eggs were found on fecal examination.

Nymphs obtained from naturally infected mosquitofish, *Gambusia affinis,* survived in two species of laboratory rodents, two species of turtles, and one frog species when administered by oral gavage. Live nymphs were recovered 28 and 120 days after infection from the rodents and turtles, respectively, and 24 hr after administration in the frog. Hemorrhage due to nymphal migration killed the single infected frog within 24 hr and resulted in significant intra-abdominal adhesions in other experimentally infected animals. There was no apparent development beyond the nymphal stage in any of the experimentally infected animals.

Studies on the prevalence and intensity of parasites infecting the American Alligator (*Alligator mississippiensis* Daudin) have reported infection rates by the pentastomid *Sebekia oxycephala* (Desing, 1835) to exceed 93% (Hazen et al., 1978; Cherry and Ager, 1982). This pentastomid occurring in American alligators has recently been described as *Sebekia mississippiensis* Overstreet, Self, and Vliet, 1985, to distinguish it from *Sebekia oxycephala,* which occurs in South American crocodilians (Overstreet et al., 1985). Adults of *S. mississippiensis* occur in the respiratory passages of crocodilians and nymphal stages are found in crocodilians, turtles, mammals, and several species of fish (Vernard and Bangham, 1941; Dukes et al., 1971; Overstreet et al., 1985). The entire life cycle is not known; however, adults of *S. mississippiensis* mature in the lungs of alligators and shed eggs that are passed with the feces (Deakins, 1971) into the aquatic environment and serve as the source of infection for fish. Alligators acquire infections by ingesting infected fish (Boyce et al., 1984).

The high incidence of infection of *S. mississippiensis* in alligators, coupled with the diversity of species harboring nymphs, suggests that numerous opportunities may exist for species associated with aquatic environments to serve as paratenic hosts of *S. mississippiensis.* This paper reports the prevalence of *S. mississippiensis* in alligators from north Florida, and the results of experimental studies designed to determine whether or not warm-blooded vertebrates (rodents), turtles, and frogs could serve as paratenic hosts when infected with nymphs obtained from naturally infected fish.

Materials and Methods

Alligators were collected from Orange Lake ($N = 21$) and Newnan's Lake ($N = 10$) in Alachua County, Florida, as part of an experimental alligator hunt sponsored by the Florida Game and Freshwater Fish Commission during September 1983. Most of the alligators were males ($N = 21$) and weights ranged from 18 to 234 kg at Newnan's Lake and from 6 to 170 kg at Orange Lake. Alligators were killed at night and stored under refrigeration until the following morning when the lungs were removed and frozen. Fecal samples were obtained per rectum and examined by both flotation and sedimentation techniques. Later, the lungs were thawed, teased apart under a magnifying lamp, and pentastomes recovered and preserved in 70% glycerin alcohol. Specimens were cleared in lactophenol and nymphs and adults differentiated by the presence or absence of accessory hooks on each of the four principal hooks.

Prevalence and intensity of infection of *S. mississippiensis* were determined in mosquitofish (*Gambusia affinis* (Baird and Girard)) from a manmade lake heavily populated by adult alligators in Hillsborough County, Florida. Mosquitofish were collected and examined in October 1983 ($N = 30$); March 1984 ($N = 127$); and July 1984 ($N = 28$). The nymphs used in the experimental infections were obtained from mosquitofish from this manmade lake in October 1984. The mosquitofish were maintained in aquaria for up to 1 wk prior to dissection and recovery of viable nymphs. Upon dissection nymphs were placed in tap water and administered via tube directly into the stomach of the experimental host.

Six female golden hamsters (*Mesocricetus auratus* (Waterhouse)) were each given 30 nymphs of *S. mississippiensis* and two other rats served as uninfected controls. All hamsters were full siblings and 1 mo of

Table 1. Prevalence and intensity of *Sebekia mississippiensis* in alligators from Alachua County, Florida.

Lake	*N*	Prevalence	Intensity		Total number	
			Mean (SD)	Range	Adults	Nymphs
Orange	21	90.4	14.0 (14.0)	1–53	9	258
Newnan's	10	100	25.6 (30.4)	4–107	10	252

age at time of infection. One infected hamster was necropsied at 1 day and 7 days post-infection, and two infected hamsters were necropsied 14 and 28 days after infection. One control hamster was necropsied at 14 and 28 days post-infection.

Six male Sprague Dawley rats (*Rattus norvegicus* (Berkenhaut)) were each given 30 nymphs and two other rats served as uninfected controls. All rats were 6 wk old at the time of infection. Two infected rats were necropsied at 7, 14, and 28 days post-infection. One control rat was necropsied at 14 and 28 days post-infection.

Ten Florida cooters (*Pseudemys nelsoni* Carr) and one Florida softshell (*Trionyx ferox* Schneider) were each given 10 nymphs; four additional cooters and one additional softshell served as uninfected controls. All turtles were hatched in captivity from eggs obtained from the wild and were 1 mo of age at time of infection. Both species of turtles were maintained in aquaria and fed a commercial diet (Tetra ReptoMin, TetraWerke, West Germany). Three infected cooters and one uninfected control were necropsied at 7 days post-infection, two infected cooters and an accompanying uninfected control were necropsied at both 14 and 28 days post-infection, and three infected cooters and one uninfected control were necropsied 120 days post-infection. The infected and uninfected softshell turtles were necropsied 14 days after infection.

A single wild caught pig frog (*Rana grylio* Stejneger) was dosed with 20 nymphs and maintained in an aquarium for 24 hr at which time it died and was necropsied.

All experimental animals, except for the pig frog, were killed with an overdose of methoxyflurane (Metofane, Pittman-Moore Inc., Washington Crossing, New Jersey) prior to necropsy. At necropsy, tissues were examined for gross lesions and then teased apart to recover intact nymphs. Viability of recovered nymphs was assessed under a dissecting microscope at 10× by the presence or absence of movement. Histopathologic examination was not attempted due to the destructive nature of the nymphal recovery procedure.

Results

The prevalence and intensity of *S. mississippiensis* in alligators from Alachua County, Florida, are presented in Table 1. Pentastome eggs were not recovered on fecal examination from any of the 31 alligators examined; however, eggs were present within the uteri of five adult females of *S. mississippiensis* recovered from the lungs. Representative specimens have been deposited in the U.S. National Parasite Collection in Beltsville, Maryland, and given the accession number 78415. The prevalence and intensity of nymphs of *S. mississippiensis* in mosquitofish from Hillsborough County, Florida, are presented in Table 2. Nymphs were encapsulated and located primarily in the body cavity.

Experimental infections

All nymphs of *S. mississippiensis* recovered at necropsy were viable and at the same developmental stage as at the time of administration, i.e., all nymphs still possessed accessory hooks on the principal hooks.

HAMSTERS AND RATS: Nymphs of *S. mississippiensis* were recovered from all experimentally infected rats and hamsters and similar numbers were recovered at each time interval (Table 3). No nymphs were recovered and no pathologic lesions were noted in the non-infected controls of each species necropsied 14 and 28 days after infection.

Clinical signs of infection were not detected in the infected rats whereas the infected hamsters became markedly lethargic and depressed in the 24-hr period immediately following infection. One hamster became moribund during this period and was euthanized. On necropsy adhesions were found between the stomach, liver, and mesentery, and three nymphs were found penetrating the stomach wall in the region of the adhesions. Marked hemorrhage surrounded these lesions and there was a small amount of blood free in the peritoneal cavity.

Lesions were remarkably consistent among the

Table 2. Prevalence and intensity of *Sebekia mississippiensis* in mosquitofish from Hillsborough County, Florida.

Date	*N*	Prevalence	Intensity	
			Mean (SD)	Range
October 1983	30	60.0	9.06 (7.47)	1–28
March 1984	127	71.6	4.48 (7.11)	1–51
July 1984	28	85.7	9.86 (6.26)	1–79

Table 3. Intensity of nymphs of *Sebekia mississippiensis* recovered from individual experimentally infected hosts.

Host species*	Intensity				
	1 day	7 days	14 days	28 days	120 days
M. auratus (30)	9	12	16	9	—
hamster	—	—	7	9	—
R. norvegicus (30)	—	12	9	6	—
rat	—	10	9	10	—
P. nelsoni (10)	—	8	6	5	4
Florida cooter	—	5	4	4	6
	—	4	—	—	1
T. ferox (10)	—	—	7	—	—
Florida softshell					
R. grylio (20)	14	—	—	—	—
pig frog					

* Size of infective dose of *S. mississippiensis* nymphs.

remaining infected rats and hamsters necropsied at later time intervals. Adhesions between the stomach and surrounding tissues were a universal finding and were usually associated with the presence of nymphs. Nymphs were recovered mainly from the stomach wall and adjacent organs and tissues (liver and mesentery); however, some nymphs were found within skeletal muscle and subcutaneous tissues in both hamsters and rats. A single nymph was recovered from the lungs of an infected rat necropsied 28 days after infection, whereas nymphs were not found in the lungs of any of the hamsters. Nymphs were usually folded within a fibrous capsule in host tissues from which they began to actively exit upon death of the host. Therefore, it was not possible to determine if all nymphs were encapsulated prior to euthanasia.

PIG FROG AND TURTLES: The single infected pig frog became markedly lethargic soon after infection and died within 24 hr. At necropsy the oral cavity contained a bloody froth and nymphs were found free in the body cavity along with a substantial volume of bloody fluid. Nymphs were also found penetrating the stomach wall and within muscle tissues.

Nymphs were recovered from all experimentally infected turtles, whereas no nymphs or lesions were found in the noninfected controls. Marked adhesions were noted among visceral organs usually associated with the presence of nymphs. The infected Florida softshell displayed a unilateral swelling on its neck that was found to contain a single nymph located just under the skin at necropsy 14 days post-infection. Nymphs were found in the stomach walls, body cavities, and lungs of both the Florida softshell and Florida cooters. Nymphs were also present in the musculature of all infected Florida cooters.

Discussion

The high prevalence of infection of *S. mississippiensis* seen in alligators from Alachua County, Florida (Table 1) is consistent with infection rates reported from South Carolina (Hazen et al., 1978) and south Florida (Cherry and Ager, 1982). Although adults were recovered from 6 of 10 alligators from Newnan's Lake and 5 of 21 alligators from Orange Lake, the vast majority of parasites found in the lungs were nymphs. Overstreet et al. (1985) reported finding small numbers of adults (1–14) of *S. mississippiensis* in alligators collected during the months of March, May, July, and September from Mississippi and Louisiana, and Deakins (1971) found large numbers (30–40) of adults in the lungs and eggs in the feces of seven alligators from McIntosh County, Georgia. Pentastome eggs were not found on fecal examination of the 31 alligators in the present study, and eggs were found in the uteri of only 5 of 529 parasites found in the lungs. However, nymphs were found in mosquitofish in October, March, and July in Hillsborough County, Florida (Table 2), and infected mosquitofish and turtles (unpubl. data) have been maintained in laboratory aquaria for over 6 mo demonstrating that infective nymphs probably occur in natural environments year round.

These results suggest that there may be a seasonal cycle in the occurrence of nymphs and adults in the lungs and in the shedding of eggs in the feces, possibly related to the feeding habits of the alligator. Food consumption decreases with decreasing ambient temperature (Overstreet et al., 1985), and although nymphs are present in fish throughout the year they are less likely to be ingested during colder months. Therefore, in the southeastern United States, one might expect to find higher numbers of nymphs in alligators during and after the warm summer months when alligators are feeding most actively. Further studies are needed to determine the longevity and maturation of nymphs and adults of *S. mississippiensis* to clarify the epidemiological picture.

Nymphs of *S. mississippiensis* were recovered from a variety of locations from all experimental

hosts and resulted in a larval migrans syndrome similar to that described by Sprent (1963). In alligators, a natural host of *S. mississippiensis*, nymphs are found predominantly in the liver and lungs in both natural and experimental infections soon after infection (Boyce et al., 1984). This suggests that the parasite utilizes some type of cue in alligators to orient its migratory route anteriorly towards the lungs where it will eventually reside as an adult. The lack of such a cueing system in these experimentally infected hosts was suggested from the location of recovered nymphs.

The distribution of nymphs in the rodent hosts was fairly uniform with the majority of nymphs occurring in the stomach wall or other tissues in the abdomen. Significant numbers of nymphs were also found in liver, muscle, and subcutaneous tissues; however, only a single nymph was found in the lungs. Apparently, nymphs penetrated through the stomach wall soon after ingestion and migrated randomly throughout the tissues. Most nymphs ceased migration near the region of the stomach whereas others continued to migrate into the muscles or through the body wall into the subcutaneous tissues. Distribution of nymphs of *S. mississippiensis* in the hamster and rat was similar to the distribution of nymphs of *Porocephalus crotali* (Humboldt, 1808) reported by Layne (1967) in the Florida mouse (*Peromyscus floridanus* (Chapman)), the cotton mouse (*Peromyscus gossypinus* (Le Conte)), and the cotton rat (*Sigmodon hispidus* Say and Ord). The pentastomid *P. crotali* occurs as an adult in crotaline snakes and utilizes a wide variety of mammals as intermediate hosts. The behavior of *S. mississippiensis* in these experimentally infected rodents suggests that it also is capable of utilizing mammals as transport hosts.

The distribution of nymphs in the frog and turtles was similar to that seen in the rats and hamsters, with the majority of nymphs located in the stomach wall or nearby in the body cavity. Although nymphs were recovered from the lungs in both species of turtles it is not clear whether this represents a predilection for this site. Dukes et al. (1971) were able to successfully infect a snapping turtle (*Chelydra serpentina* (Linnaeus)) by force feeding largemouth bass (*Micropterus salmoides* (Lacépède)) tissues containing viable nymphs of *S. mississippiensis* and they recovered healthy nymphs, at the same stage of development, 1 mo later from the lungs. In the present study, viable nymphs were recovered 4 mo after infection, and further studies are underway to determine if nymphs of *S. mississippiensis* will develop into adults in turtles over a longer period of time. Whether or not they serve as definitive hosts it appears likely that fish-eating turtles could become infected with nymphs of *S. mississippiensis* and serve as a source of infection for alligators, especially large adults, which commonly feed on turtles.

Moderate numbers of nymphs were recovered from the musculature of the frog and turtles in this study and they have been reported from several important game fish (Vernard and Bangham, 1941; Dukes et al., 1971). This is significant in that this study demonstates that warm-blooded vertebrates are susceptible to infection, and fish, frogs, turtles, and alligators are an important diet item for many species including man. Pentastomid infections, sometimes fatal, have been reported in a variety of species including man, and further work is needed to determine the role this particular parasite plays in human and animal health (Cosgrove et al., 1970; Self et al., 1972; Boyce et al., 1984).

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In Vitro and In Vivo Development of the Tetracotyles of *Cotylurus flabelliformis* (Trematoda: Strigeidae)

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ABSTRACT: Tetracotyles (metacercariae) of the strigeoid trematode *Cotylurus flabelliformis* readily activated in frog Ringer's solution at 41°C. In vitro growth rates of the tetracotyles were best in the defined medium NCTC 135 supplemented with either 50% inactivated chicken serum or 50% inactivated duck serum. By 72 hr ovigerous adults were present, but only non-shelled or thin-shelled eggs incapable of embryonation were produced. Ovigerous adults were not observed until at least day 5 in NCTC 135 supplemented with either 40% inactivated chicken serum plus 20% chicken upper intestine mucosal extract or with 40% inactivated duck serum plus 20% duck upper intestine mucosal extract; all eggs were abnormal and inviable. Ovigerous adults did not develop in NCTC 135 with 50% inactivated duck serum plus 20% duck upper intestine mucosal extract. Adult worms containing shelled eggs were first recovered 3 days post-infection from experimentally infected Muscovy ducklings.

Several in vitro culture studies have been made on strigeoid trematodes beginning with metacercariae (Ferguson, 1940; Bell and Smyth, 1958; Wyllie et al., 1960; Williams et al., 1961; Voge and Jeong, 1971; Basch et al., 1973; Kannangara and Smyth, 1974; Fried et al., 1978; Mitchell et al., 1978). Ovigerous adult worms developed in vitro in all of these studies but only in one species, *Cotylurus lutzi* Basch, 1969, were normal eggs capable of embryonation produced (Basch et al., 1973). However, this only occurred when a mucosal extract from the upper intestine of chickens was added to the culture medium.

In vitro studies have been made on two other *Cotylurus* species: *C. strigeoides* Dubois, 1958 by Fried et al. (1978) and *C. erraticus* Dubois, 1938 by Mitchell et al. (1978). In both studies all eggs formed in vitro were abnormal, even though in the case of *C. strigeoides* an upper intestine mucosal extract of chickens was added to the culture medium.

Kannangara and Smyth (1974) have emphasized that those metacercariae almost developed to the adult stage are more easily cultured to ovigerous adults in vitro. In fact, in all cases where adult worms have produced eggs capable of hatching, the metacercariae possess preformed genital primordia (Berntzen and Macy, 1969; Basch et al., 1973; Yasuraoka et al., 1974).

The subject of the present in vitro culture study is the strigeoid trematode *Cotylurus flabelliformis* (Faust, 1917). Identification was based on measurements and morphological features of metacercariae from naturally infected snails and adults from an experimentally infected duckling (Hughes, 1929; van Haitsman, 1931; Dubois, 1968; Campbell, 1973). The metacercariae (tetracotyles) of this species are well developed, with preformed genital primordia. No previous attempts have been made to culture the tetracotyles of this species.

Materials and Methods

Source of inoculum

Lymnaeid snails, *Stagnicola elodes* (Say), infected with the tetracotyles of *Cotylurus flabelliformis,* were collected from a game production pond north of Vermillion, South Dakota (SW corner, Section 4, Township 93N, Range 51W). They were maintained in aerated aquaria in the laboratory and fed Purina catfish chow. Tetracotyles were teased from the tissues of infected snails in frog Ringer's solution (MacInnis and Voge, 1970) containing 100 units of penicillin/ml and 100 μg of streptomycin/ml at 41°C. These larvae were contracted and surrounded by a thin cyst wall. Larval activation readily occurred in frog Ringer's at 41°C, with the worms rapidly elongating and contracting (Fig. 1).

The tetracotyles of *C. flabelliformis* were adequately described by Hughes (1929). These metacercariae possess a well-developed hindbody (Fig. 1). In stained specimens the genital primordium is seen as a distinct bilobed mass of cells.

Preparation of inoculum

Tetracotyles were rinsed four times in frog Ringer's solution and transferred to 16 × 125-ml screw cap tubes (12–20/tube) containing 4 ml of medium with an initial pH ranging from 7 to 7.4 and with no special gas phase. Cultures were maintained in a stationary water bath at 40 ± 1°C. Half of the culture medium was replaced with fresh fluid every 48 hr to prevent the possible accumulation of toxic wastes.

The cultures were examined daily and worms removed at different periods. The criteria used to assess

development were increase in worm size and time of development of vitellaria, gonads, and uterine eggs. Eggs, when present, were removed from culture tubes with a sterile Pasteur pipette. They were sedimented several times in distilled water and then cultured in distilled water. Measurements and morphology of in vitro worms were compared those of in vivo worms. The latter worms were obtained from five 2-day-old unfed Muscovy ducklings experimentally infected by force-feeding each three visceral masses of infected snails. The ducklings were killed 1, 2, and 3 days post-infection and the entire intestine examined for worms. Worms used for measurements were fixed without pressure in hot 10% formalin, cleared in cedarwood oil, and mounted in Permount. Measurement of total body length was made with the aid of a camera lucida.

Preparation of culture media

The defined medium NCTC 135 was purchased from Grand Island Biological Company (Grand Island, New York). The following culture media were used: (1) NCTC 135 + chicken serum (1:1) (NCTC-CS); (2) NCTC 135 + duck serum (1:1) (NCTC-DS); (3) NCTC 135 + chicken serum + chicken upper intestine mucosal extract (2:2:1) (NCTC-CS-CEX); (4) NCTC 135 + duck serum + duck upper intestine mucosal extract (2:2:1) (NCTC-DS-DEX1); and (5) NCTC 135 + duck serum + duck upper intestine mucosal extract (3:5:2) (NCTC-DS-DEX2). All culture media contained 100 units of penicillin/ml and 100 μg of streptomycin/ml. The chicken serum was purchased for Grand Island Biological Company, and the duck serum was prepared from domestic mallard duck blood obtained by cardiac puncture. The blood was allowed to clot overnight at 4°C and then centrifuged at about 2,000 rpm for 15 min. The serum was drawn off and stored frozen at −20°C. The chicken and duck sera were inactivated at 56°C for 30 min prior to use. A modification of the method given by Basch et al. (1973) was used to prepare the upper intestine mucosal extract of both chickens and ducks. The first 50 cm of the intestine was cut open longitudinally and washed in five changes of cold (4°C) normal saline. The mucosal layer was scraped off with a glass slide in a small amount of cold normal saline. This material was sonicated until it was evident by microscopic examination that the cells were broken up. The resulting liquid mixture was made up to 20 ml with normal saline and centrifuged for 1 hr at 3,000 rpm at 5°C. The supernatant was filtered twice through filter paper and then through a Seitz filter (pore size 0.45 μm) for sterilization. The filtrate was stored frozen at −20°C until used.

Results

Growth in vivo (Tables 1, 2)

Worms recovered from the five experimentally infected ducklings were found primarily in the posterior half of the intestine firmly attached to the intestinal wall. None of the ducklings was heavily infected (3–25 worms) even though each was fed an estimated 300 tetracotyles. The increase in length after 48 hr in vivo ($\bar{x}$ = 698 ± 47.02) was similar to that of worms in vitro in NCTC-CS ($\bar{x}$ = 679 ± 50.89) and in NCTC-DS ($\bar{x}$ = 685 ± 82.83). The time in appearance of the vitellaria, gonads, and uterine eggs was similar in the in vitro and in vivo worms, but the latter were more robust and the gonads better

Table 1. Measurements of *Cotylurus flabelliformis* in micrometers.*

Treatment	Region measured	Activated larvae	Days in culture			
			Day 1	Day 2	Day 3	Day 4
In vitro						
NCTC-CS†	FB‡	257 ± 34.76	268 ± 13.23	316 ± 42.65	305 ± 31.16	326 ± 21.33
	HB	263 ± 39.70	262 ± 39.70	363 ± 23.44	453 ± 80.26	464 ± 49.06
	TL	393 ± 55.31	531 ± 45.85	679 ± 50.89	758 ± 91.03	786 ± 55.18
NCTC-DS	FB		276 ± 28.40	327 ± 31.18	341 ± 33.37	355 ± 17.20
	HB		223 ± 22.81	357 ± 68.53	376 ± 57.94	476 ± 74.93
	TL		500 ± 38.60	685 ± 82.83	717 ± 73.53	741 ± 93.22
NCTC-CS-CEX	FB		255 ± 11.48	254 ± 22.40	259 ± 44.87	272 ± 28.64
	HB		195 ± 17.83	219 ± 57.72	337 ± 117.56	350 ± 39.32
	TL		450 ± 22.81	472 ± 74.26	596 ± 155.32	621 ± 61.22
In vivo						
Muscovy ducklings	FB		306 ± 33.92	300 ± 25.25	367 ± 2.12	
	HB		270 ± 30.95	399 ± 29.68	542 ± 52.33	
	TL		575 ± 55.66	698 ± 47.02	909 ± 54.45	

* All measurements from 10 worms, except NCTC-CS-CEX, day 3 from seven worms and in vivo, day 3 from two worms.
† NCTC-CS = NCTC 135 + chicken serum (1:1), NCTC-DS = NCTC 135 ± duck serum (1:1), NCTC-CS-CEX = NCTC 135 + chicken serum + upper intestine chicken mucosal extract (2:2:1).
‡ FB = forebody, HB = hindbody, TL = total length.

developed (Fig. 5). The in vivo worms by 72 hr were longer ($\bar{x}$ = 909 ± 54.45) than those in vitro in NCTC-CS ($\bar{x}$ = 758 ± 91.03) and in NCTC-DS ($\bar{x}$ = 717 ± 73.53) and contained a number of shelled eggs (Fig. 5).

Growth in vitro (Tables 1, 2)

Tetracotyle growth rates and morphological changes up to 96 hr in vitro were similar in NCTC-CS and NCTC-DS and the best growth rates occurred in these two media. The fore- and hindbodies of the worms gradually increased in length, with the latter showing the greatest increase (Figs. 2, 3). The forebody had assumed after 1 day in culture the cup-shaped structure typical of adults of *C. flabelliformis.* Some worms even up to 72 hr in culture were observed in a membranous case (Fig. 2). The source of this material is not known but it is possibly, at least in part, an expanded cyst wall, as even activated larvae retained the cyst wall (Fig. 1). The worms would eventually escape from this case. Reproductive system development and egg appearance were as follows: 24 hr, the two testes were readily evident and in some worms the ovary was seen; 48 hr, the testes and ovary were well developed and the vitellaria present; 72 hr, eggs present in the uterus of some worms (Fig. 3) and the vitellaria more extensively developed; and 96 hr, eggs present in many worms and many eggs in the culture medium. Worms were observed cross-copulating in the cultures, but only non-shelled or thin-shelled eggs were produced (Figs. 6, 7) and no development occurred in eggs cultured at room temperature (21°C) in distilled water. Although bursting of eggs was not observed, naked clusters of yolk cells on slide preparations indicated that this had apparently occurred (Fig. 6).

The development of worms in NCTC-DS-DEX1 and NCTC-CS-CEX was similar, although measurements are only from worms cultured in the latter medium (Fig. 4). The total length of the worms in this medium after 96 hr ($\bar{x}$ = 621 ± 61.22) was less than that of worms in NCTC-CS ($\bar{x}$ = 786 ± 55.18) and NCTC-DS ($\bar{x}$ = 741 ± 93.22). Eggs were first observed in worms in vitro in NCTC-CS-CEX and NCTC-DS-DEX1 on days 5 and 6, respectively. Only a small number of eggs was produced and all were abnormal and incapable of embryonation. The poorest development was in NCTC-DS-DEX2; ovigerous adults did not develop in this medium.

Table 2. Egg production of adults of *Cotylurus flabelliformis* in vitro and in vivo.

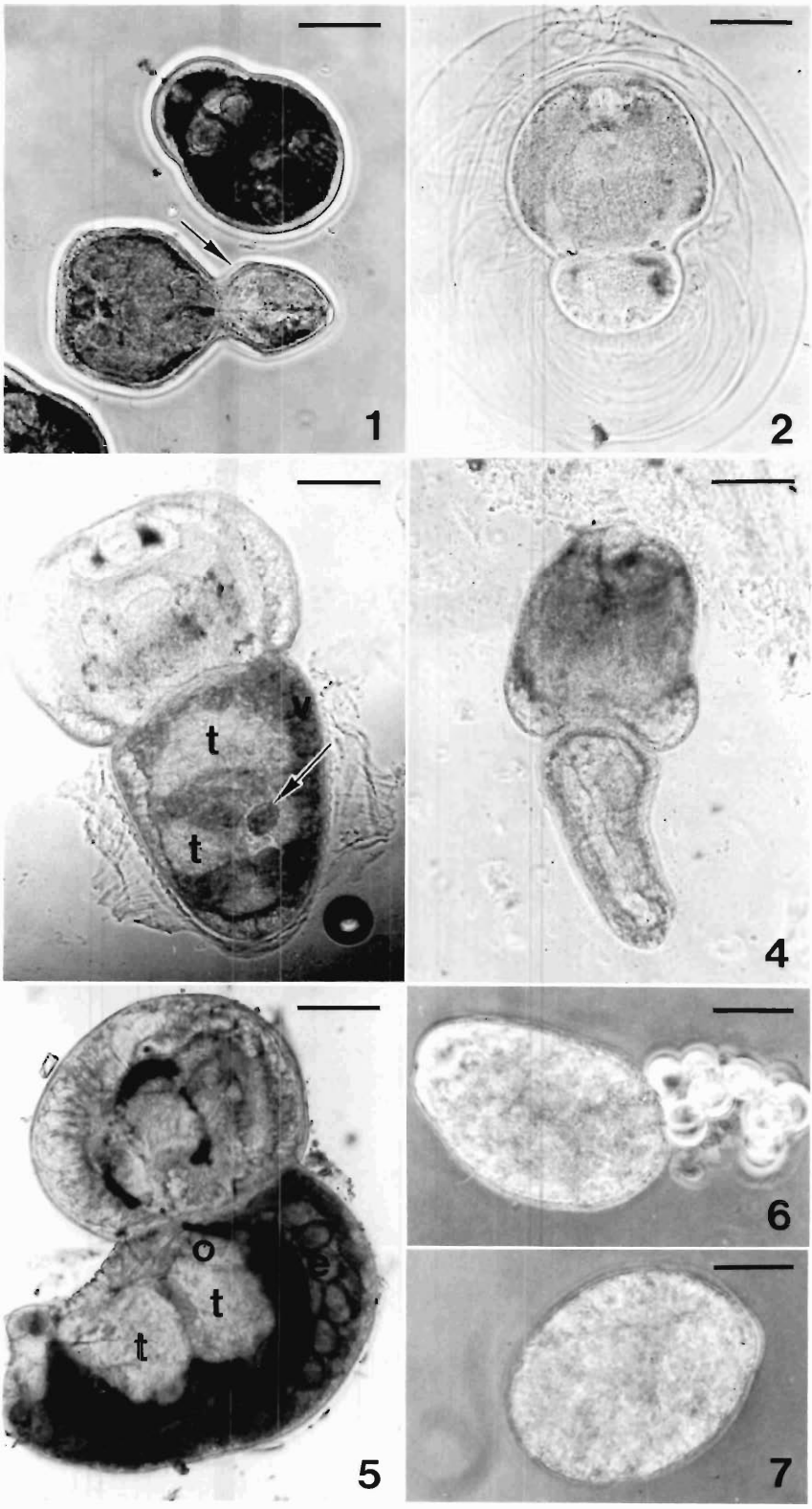
Treatment	In vitro/ in vivo (days)	First egg production (days)	Normal eggs
In vitro			
NCTC-CS*	10	3	No
NCTC-DS	6	3	No
NCTC-CS-CEX	5	5	No
NCTC-DS-DEX1	8	6	No
NCTC-DS-DEX2	8	—	—
In vivo			
Muscovy ducklings	3	3	Yes

* NCTC-CS = NCTC 135 + chicken serum (1:1); NCTC-DS = NCTC 135 + duck serum (1:1); NCTC-CS-CEX = NCTC 135 + chicken serum + chicken upper intestine mucosal extract (2:2:1); NCTC-DS-DEX1 = NCTC 135 + duck serum + duck upper intestine mucosal extract (2:2:1); NCTC-DS-DEX2 = NCTC 135 + duck serum + duck upper intestine mucosal (3:5:2).

Discussion

The tetracotyles of *C. flabelliformis* are similar to those of *C. lutzi* in that activation occurred at 41°C in the absence of digestive enzymes and bile salts (Voge and Jeong, 1971; Basch et al., 1973). However, Fried et al. (1978) with *C. strigeoides* and Mitchell et al. (1978) with *C. erraticus* found that pretreatment was necessary for activation and excystation to occur. Apparently, the activated larvae of *C. lutzi* easily ruptured the delicate cyst wall (Voge and Jeong, 1971). In the case of *C. flabelliformis* the cyst wall is apparently retained and becomes an expanded membranous case from which most larvae had escaped by 72 hr in culture (Fig. 2).

Development of metacercariae to adult worms in vitro is in general slower than that in vivo. This was the case even in two digenetic trematodes where viable eggs were produced (Berntzen and Macy, 1969; Yasuraoka et al., 1974). The one exception is *C. lutzi* as Voge and Jeong (1971) and Basch et al. (1973) reported that development to ovigerous adults in vitro was the same as that in experimentally infected finches. In our study of *C. flabelliformis,* the time for development (3 days) to ovigerous adults in vitro and in Muscovy ducklings was the same when the defined medium NCTC 135 was supplemented with either 50% chicken serum or 50% duck serum. However, Campbell (1973) reported ovigerous adults present after 2 days in the mallard duck.



In the present study only non-shelled or thin-shelled eggs were formed by *C. flabelliformis* adults in vitro. The eggs were often mis-shaped and lacked the rigid outer shell seen in adults in vivo. Basch et al. (1973) reported 10–20% normal eggs produced by adults of *C. lutzi* when an extract of the upper intestine mucosa of chickens was added to the culture medium (40% NCTC 135 + 40% chicken serum). In our investigation, only inviable eggs were present when an extract of the upper intestine mucosa of either chickens or ducks was added to similar basic media (40% NCTC 135 + 40% chicken serum or 40% NCTC 135 + 40% duck serum). In fact, worm development was slower in these media than in those without the extract and adult worms were smaller (Table 1).

Fried et al. (1978) noted that adults of *C. strigeoides* did not produce eggs unless an upper intestine mucosal extract of chickens was added to the culture medium but all eggs were abnormal. In two other species of digenetic trematodes, the addition of an intestinal mucosal extract was harmful. Davies and Smyth (1979) reported that in *Microphallus similis* all worms died after only 3 days in vitro, whereas without the extract, abnormal eggs were produced by day 4 and in some media worms survived up to 30 days. Halton and Johnston (1983) found with *Bucephaloides gracilescens* that all worms died within 7 days in vitro when an extract was added to the medium, whereas without it, eggs were produced by day 14.

The reason for the formation of abnormal eggs in vitro is not known. It has been suggested that the nutrients and/or growth factors needed for normal development and egg-shell formation are lacking (Halton and Johnston, 1983). It may be that egg-shell abnormality is caused by a premature tanning of the shell-protein constituents in cultured worms as pretanning has been observed in certain trematodes (Davies and Smyth, 1979; Halton and Johnston, 1983).

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Figures 1-5. Living *Cotylurus flabelliformis*. Figures 6, 7. *C. flabelliformis* in vitro eggs. Phase contrast microscopy. 1. Tetracotyles in frog Ringer's solution at 41°C. Note cyst wall retained by an activated larva (arrow). 2. Tetracotyle 24 hr in vitro, NCTC 135 with 50% duck serum. Note membranous case surrounding the worm. 3. Ovigerous adult, 72 hr in vitro, NCTC 135 with 50% duck serum. Note egg (arrow), anterior and posterior testes (t), and vitellaria (v). 4. Worm, 72 hr in vitro, NCTC 135 with 40% chicken serum and 20% chicken upper intestine mucosal extract. 5. Ovigerous adult, 72 hr in vivo, Muscovy duckling. Note eggs (e), ovary (o), and anterior and posterior testes (t). 6. Egg from 5-day-old culture, NCTC 135 with 50% duck serum. Note abnormal shape and thin shell of the egg, and cluster of yolk cells adjacent to the egg. 7. Same as Figure 6. Note thin shell of the egg. Scale bar equals approximately 150 μm in Figures 1-5; 25 μm in Figures 6, 7.

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A List of Records of Freshwater Aspidogastrids (Trematoda) and Their Hosts in North America

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ABSTRACT: Published records for the six species of North American freshwater aspidogastrid trematodes from molluscs and vertebrates have been compiled, listing both hosts and localities for state or province. Seventeen new unionid mussel hosts (Bivalvia) are reported for *Aspidogaster conchicola* along with new state records for Arkansas, Connecticut, Delaware, Maryland, Mississippi, North Carolina, New York, Virginia, and San Luis Potosi (Mexico); 12 new unionid host species for *Cotylaspis insignis,* with new state records for Arkansas, Delaware, Massachusetts, Mississippi, New Jersey, Rhode Island, and Wisconsin; five new unionid hosts for *Cotylogaster occidentalis,* with new state records for Connecticut, Delaware, Florida, and Texas; and a new unionid host record for *Lophotaspis interiora.* No new records are given for *Cotylaspis cokeri* nor for *C. stunkardi* from turtles.

In North America, aspidogastrid trematodes are common parasites of freshwater unionid mussels (Bivalvia). They occur less often in gastropods, fishes, and turtles (Dollfus, 1958; Rohde, 1972). *Aspidogaster conchicola* von Baer, 1826, *Cotylaspis insignis* Leidy, 1857 (=*Platyaspis anodontae* Osborn, 1898 and *C. reelfootensis* Najarian, 1961), and *Cotylogaster occidentalis* Nickerson, 1902 (=*C. barrowi* Huehner and Etges, 1972) are widely distributed, but distributional limits have not been established, particularly at the northern and western boundaries of their ranges. Information on *Cotylaspis cokeri* Barker and Parsons, 1914, *C. stunkardi* Rumbold, 1928, and *Lophotaspis interiora* Ward and Hopkins, 1931 are based on from one to five reports each, and the distributions are poorly known.

Distribution and host records are scattered in the literature; the nomenclature of unionid mussels has been unstable and changing; and recent surveys have almost consistently recorded declines in mussel species, diversity, and ranges as habitats are modified by human activities (Suloway, 1981; Havlik, 1983). To create a checklist of locality records for North American aspidogastrids, we reviewed and numbered 74 publications (Appendix 1) (note that the numbers are also cited in the checklist).

The checklist (Appendix 2) includes previously unpublished host and locality data from the authors' collections of *A. conchicola, C. insignis, C. occidentalis,* and *L. interiora.* To conserve space in the checklist, we are listing the stations as given in Appendix B of Vidrine (1980), in parentheses. Photocopies of this Appendix have been deposited at the U.S. National Parasite Colection, USDA, ARS, BARC-East No. 1180, Beltsville, Maryland 20705; the Harold W. Manter Laboratory, University of Nebraska State Museum, Lincoln, Nebraska 68588-0514; and the Biology Department of Gettysburg College. Localities for our new state records are listed in Table 1. Two additional localities from Pennsylvania (§7 and §8, are included in this table but are not new state records. Mussel names are based on the works of many authors, especially Burch (1975), but the higher taxa are based on Davis and Fuller (1981); gastropod names are based on Burch and Tottenham (1980) and Burch (1982); turtle names are based on Ernst and Ernst (1977). Junior synonyms of host names are included when those names have been used by authors reporting aspidogastrids.

Mussels were collected by hand, with a modified quahog clamming rake, by snorkeling, or with a crowfoot apparatus. The soft parts were dissected in a manner similar to that of Hendrix and Short (1965, 1972), but the visceral mass was also cut open to locate any *C. occidentalis* that might be in the intestine. The worms were collected, fixed, and identified using standard parasitological techniques. Voucher specimens have been deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705, Nos. 78804–78810.

Table 1. Localities of new state records for aspidogastrids.

*Locality no.	Species	Location
279	†A.c./C.i.	Lake Chicot, junct. of US 82 and US 65, ca. 5 mi east of Lake Village, Chicot Co., AR
391	C.i.	Saline River at AR 160, east of Johnsville, Ashley and Bradley cos., AR
396	A.c./C.i.	North Cadron Cr., US 65, north of Greenbriar, Faulkner Co., AR
398	C.i.	Saline River at US 167, Grant and Dallas cos., AR
400	C.i.	Ouachita River at AR 270, Rocky Shoals Park, Montgomery Co., AR
404	A.c./C.i.	Ouachita River, 6 mi southeast of Ink, Polk Co., AR
406	C.i.	Strawberry River at US 167, 2 mi north of Evening Shade, Sharp Co., AR
§1	A.c.	Carlson's Pond, junct. of CT 207 and Pond Rd., North Franklin, New London Co., CT
§2	A.c./C.o.	Williams Pond, off CT 207, Amston, New London Co., CT
3	A.c./C.i./C.o.	Deep Cr. at Nanticoke Acres, DE 20, Seaford, Sussex Co., DE
§3	A.c./C.i.	Mashpee Pond, Mashpee Twp., Barnstable Co., MA
§4	A.c./C.i.	Sargo Lake, Town landing, Dennis, Barnstable Co., MA
§5	A.c./C.i.	Upper Mill Pond, Brewster, Barnstable Co., MA
74	C.o.	Little Withlacooche River, at US 301, south of Bushnell, Sumter and Hernando cos., FL
9	A.c.	Chester River, east of junct. of MD 297 and MD 313, Millington, Kent Co., MD
110	A.c./C.i.	Tombigbee River at US 82, Columbus, Lowndes Co., MS
111	A.c.	Tombigbee River at MS 50, ca. 6 mi northwest of Columbus, Lowndes and Clay cos., MS
133	C.i.	Tickfaw River at MS 584, east of Gilsburg, Amite Co., MS
134	C.i.	East Fork of Amite River, ca. 4 mi north of LA state line, Amite Co., MS
135	C.i.	East Fork of Amite River at MS 584, Amite Co., MS
138	C.i.	Leaf River at US 98, Greene Co., MS
139	C.i.	Yokanookany River at MS 429, Leake Co., MS
140	C.i.	Bogue Chitto River at US 84, Bogue Chitto, Lincoln Co., MS
142	A.c.	Pearl River at US 98, Marion Co., MS
143	A.c./C.i.	Hobolochitto Cr. at MS 11, Pearl River Co., MS
144	C.i.	Wolf River at MS 26, Pearl River Co., MS
145	C.i.	Bogue Chitto River at US 98, Pike Co., MS
146	C.i.	Tangipahoa River at US 51, Pike Co., MS
386	A.c.	Big Black River at MS 12, Holmes and Attala cos., MS
388	C.i.	Sunflower River at MS 14, ca. 3 mi east of Anguilla, Sharkey Co., MS
45	A.c.	Chowan River at Raye's Beach Fishing Club, Gates Co., NC
14	A.c./C.i.	Delaware River at Kinkora Island, Roebling, Burlington Co., NJ
§6	A.c.	Susquehanna River at Recreation Park, off NY 7, Conklin, NY
§7	C.i.	Schuylkill River at Hawes Ave. Park, Norristown, Montgomery Co., PA
§8	A.c./C.i.	Susquehanna River at Selinsgrove, Snyder Co., PA
§9	C.i.	30 Acre Pond, off RI 138 and RI 110, Univ. of Rhode Island, Kingston, Washington Co., RI
174	C.o.	Village Cr. at US 96, south of Silsbee, Hardin Co., TX
446	A.c.	Possum Cr., near Gate City, Scott Co., VA
231	C.i.	Mississippi River at DeSoto, above Indian Camp Light, Vernon Co., WI
462	A.c.	Valles River below RR station in Micos, San Luis Potosi, MEX

* Localities of Vidrine (1980) unless marked § (see checklist).
† A.c. = *A. conchicola*, C.i. = *C. insignis*, C.o. = *C. occidentalis*.

Discussion

Although the checklist includes numerous unionid mussel and other hosts, there are large gaps in the known host range and geographic distribution of these freshwater aspidogastrids. Burch (1975) lists 227 species of unionacean mussels north of Mexico, only a fraction of which are reported to have aspidogastrids. For example, there may be no North American records of aspidogastrids from the more primitive subfamily Margaritiferinae because few specimens and localities have been examined. Aspidogastrids are presently reported from only 30 states, one Canadian province, and one Mexican state. *A. conchicola* is the only aspidogastrid reported from

the western third of the continent (Pauley and Becker, 1968); the remaining records come from the midwestern, southern, and eastern regions of North America, primarily the United States. The northern boundary of the aspidogastrid range is uncertain because, although unionid mussels have migrated into previously glaciated areas of North America, it appears that the aspidogastrids have not necessarily accompanied them. Several mussel collections in Washington County, Maine yielded no aspidogastrids, yet we report them in Connecticut, Rhode Island, and Massachusetts. Further, Dr. M. D. B. Burt (pers. comm.) has examined numerous unionids in New Brunswick, Canada without finding these helminths. No aspidogastrids were found upon examination of numerous mussels in the Canadian National Museum collection by M.F.V. (*C. occidentalis* was not sought). Gaps in known host and geographic distribution probably reflect more the interests of workers in various laboratories and the availability of host material than true gaps. In drainages where these helminths have been reported, usually not all of the potential host species from those drainages have been examined.

Both *A. conchicola* and *C. insignis* have rather low unionid host specificity and a large distributional range which suggests an ancient coevolutionary relationship between mussels and these two aspidogastrids. This specificity may however, be limited primarily by habitat preferences of the hosts rather than physiological preferences of the parasites. As yet, too little is known about the ecology, life histories and host–parasite relationships of these species in mussels to make generalizations.

The host and geographic ranges of *C. occidentalis* and *L. interiora* also are known incompletely, perhaps because they utilize vertebrate hosts as well as molluscs. *Lophotaspis interiora* appears to have a two-host life cycle; adults are known only from a single turtle originally from Arkansas (Ward and Hopkins, 1931), whereas juveniles are known only from Florida mussels (Hendrix and Short, 1972). The availability of a fish host, *A. grunniens* Raf., may partially explain the fairly wide geographic range of *C. occidentalis*. The fact that the mussel visceral mass must be dissected in order to locate these worms in the intestine may contribute to the relatively few reports of this species.

We report in the checklist an additional 17 new mussel hosts for *A. conchicola,* 12 for *C. insignis,* 5 for *C. occidentalis,* and 1 for *L. interiora.* New state records (Table 1) are given for all of these except *L. interiora* which has yet to be reported from mussels outside of Florida. The range of *A. conchicola* is extended to Arkansas, Connecticut, Delaware, Maryland, Mississippi, New York, North Carolina, Virginia, and San Luis Potosi (Mexico); that of *C. insignis* to Arkansas, Delaware, Massachusetts, Mississippi, New Jersey, Rhode Island, and Wisconsin; that of *C. occidentalis* to Connecticut, Delaware, Florida, and Texas.

Both *A. conchicola* and *C. insignis* are found in Mexico. *A. conchicola* is found in a number of the Atlantic drainages in the northern portion of the country whereas *C. insignis* occurs in the more southern ones (Vidrine et al., 1983). The taxonomy of Mexican mussels is being revised, and the best availabile names are in the checklist.

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Appendix 1

Publications Containing Locality Information on North American Aspidogastrids

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Appendix 2

Checklist of State and Province Records of Known Molluscan and Vertebrate Hosts of Freshwater Aspidogastrids in North America

CLASS GASTROPODA, Subclass Prosobranchia, Family Pleuroceridae: ***Elimia livescens*** (Menke) as *Goniobiasis livescens, A. conchicola* †OH 28, 31; ***Elimia virginica*** (Say) as *Goniobiasis virginica, A. conchicola* NJ 33; ***Elimia*** sp. as *Goniobiasis* sp., *C. occidentalis* KY 69, OH 12; ***Pleurocera acuta*** Rafinesque, *C. occidentalis* IN 8, *C. cokeri* IN 8. Family Viviparidae: ***Campeloma decisum*** (Say) as *Paludina decisa, A. conchicola* PA? 43; ***Cipangopaludina japonica*** (Martens) as *Viviparous japonicus, A. conchicola* MA 47; ***Cipangopaludina chinensis malleata*** (Reeve) as *Viviparous malleatus, A. conchicola* MA 47, OH 28, 29, 31.

CLASS BIVALVIA, Superfamily Unionacea, Family Unionidae: ***Actinonaias carinata*** (Barnes) as *A. ligamentina carinata, A. l. ligamentina, Lampsilis ligamentinus,* and *Unio ligamentinus, A. conchicola* MO 27, OH 32, *C. insignis* IA 37, IL 37, 39, MO 27, *C. occidentalis* MO 27; ***Actinonaias ellipsiformis*** (Conrad) as *Venustaconchia ellipsiformis ellipsiformis* and *V. e. pleasii, A. conchicola* MO 27, *C. insignis* ‡AR(406), MO 27; ***Alasmidonta marginata*** Say, *A. conchicola* OH 60, PA 37; ***Amblema dombeyana*** (Val.) as *Plectomerus dombeyana, A. conchicola* LA(289, 294, 302, 305, 320, 335, 341, 346, 347, 363, 369) 66, 67, *C. insignis* LA(304), ‡MS(138); ***Amblema gigantea*** (Barnes) as *Megalonaias gigantea, Magnoniais nervosa,* and *Quadrula undulata, A. conchicola* IN 61,

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* = New host record.

‡( ) = New state and locality record (see Table 1).

( ) = Vidrine (1980) locality.

(§) = Locality listed in Table 1, not in Vidrine (1980).

† State codes: AL—Alabama, AR—Arkansas, CT—Connecticut, DE—Delaware, FL—Florida, GA—Georgia, IA—Iowa, IL—Illinois, IN—Indiana, KY—Kentucky, LA—Louisiana, MA—Massachusetts, MD—Maryland, MI—Michigan, MN—Minnesota, MO—Missouri, MS—Mississippi, NC—North Carolina, NJ—New Jersey, NY—New York, OH—Ohio, OK—Oklahoma, ONT—Ontario (Canada), PA—Pennsylvania, RI—Rhode Island, SLP—San Luis Potosi (Mexico), TN—Tennessee, TX—Texas, VA—Virginia, WA—Washington, WI—Wisconsin, WV—West Virginia.

LA(302, 368), 67, MO 27, *C. insignis* IN 73; ***Amblema neisleri*** Lea as *Carunculina neisleri, A. conchicola* FL 25; ***Amblema plicata*** (Say) as *A. costata, A. perplicata, A. peruviana, Crenodonta rariplicata,* and *Quadrula plicata, A. conchicola* ‡AR(404), IA 37, IL 37, 65, LA(151, 168, 293, 294, 302, 303, 304, 341, 345, 349, 363, 369, 370) 67, MO 27, OH 6, 60, OK 50, TN 24, TX(175, 183) 13, 18, 19, WI 71, WV 11, *C. insignis* MO 27, OH 60, TX 18; ***Anodonta californiensis*** Lea, *A. conchicola* WA 55; ***Anodonta cataracta*** Say as *Anodonta fluviatilus, A. lacustris,* and *A. marginata, A. conchicola* ‡CT (§1, §2), ‡DE(3), MA(§3, §4, §5), NJ(14), OH 32, PA? 40, 41, 44, *C. insignis* ‡DE(3), ‡MA(§3, §4), ‡NJ(14), PA(§7) 41, 42, ‡RI(§9), **C. occidentalis* ‡CT(§2); ***Anodonta cowperiana*** Lea, *C. insignis* FL 25; ***Anodonta gibbosa*** Say, *A. conchicola* FL 25, *C. insignis* FL 25; ***Anodonta grandis*** Say as *A. g. corpulenta, A. corpulenta, A. ovata,* and *A. plana, A. conchicola* ‡AR(404), IA 37, IL 22, 23, 37, 61, IN 72, 73, LA(154, 288, 289, 294, 304, 305, 318, 335, 341, 345, 361, 363, 366, 367, 370, 373) 66, 67, MO 27, OH 6, 32, 60, OK(389) 3, 50, TX(176) 2, 13, 18, 19, WI 70, WV 11, *C. insignis* ‡AR(404), IA 37, IL 37, 39, 61, IN 72, 73, LA(288, 289, 303, 304, 305, 316, 317, 318, 319, 334, 335, 361, 366, 367, 370, 376) 10, 66, 67, MN 45, MO 27, 64, NY 53, 54, OH(207, 201) 60, OK(389) 50, TN 48, 49, TX 1, 13, 18, 19, WV 11, *C. occidentalis* MI 15, 74; ***Anodonta hallenbecki*** Lea, *A. conchicola* AL 25, FL(96), *C. insignis* AL 25, FL(96); ***Anodonta imbecillus*** Say, *A. conchicola* AL 25, ‡AR(404), FL 25, IL 22, 23, LA(287, 322, 323, 363) 67, ‡NC(45), OH 60, OK 50, PA(22), WI 71, *C. insignis* AL 25, ‡AR(404), FL(95) 25, IL 37, 61, LA(113, 314, 322, 380, 381) 67, OH 60, OK 50, ‡PA(§7); ***Anodonta implicata*** Say, **A. conchicola* MA(§3, §5), ‡MD(9), PA(22), **C. insignis* ‡MA(§3, §5), ‡RI(§9); ***Anodonta oregonensis*** Lea, *A. conchicola* WA 55; ***Anodonta peggyae*** Johnson, **A. conchicola* FL(98), **C. insignis* FL(98); ***Anodonta suborbiculata*** Say, *C. insignis* IL 37; ***Anodonta* sp.,** *A. conchicola* OK 62, 63; ***Anodontoides ferussacianus*** (Lea) as *Anodonta ferrus, C. insignis* IL 61; ***Arcidens confragosus*** (Say) as *Alasmidonta confragosus* and *Unio confragosus, C. insignis* IL 37, 39; ***Carunculina parva*** (Barnes) as *C. minor,* and *Lampsilis parvus, A. conchicola* LA(289, 322, 335, 341, 363) 67, TX(178), *C. insignis* FL 25, IL 37, LA(289, 297, 312, 317, 322, 326, 333, 338, 364, 367, 376, 385) 67, ‡MS(388), OH 60; ***Cyclonaias tuberculata*** (Raf.), *A. conchicola* MO 27, OH 60, TN(437) 24, *C. insignis* MO 27; ***Cyrtonaias tampicoensis*** (Lea) as *Lampsilis tampicoensis berlandierii, *A. conchicola* TX(185), *C. insignis* TX(185) 19; ***Disconaias fimbriata*** (Frierson), **A. conchicola* ‡SLP(462); ***Ellipsaria lineolata*** (Raf.) as *Plagiola lineolata, A. conchicola* TN 24; ***Elliptio arctata*** (Conrad), **C. insignis* FL(96); ***Elliptio buckleyi*** (Lea), **C. insignis* FL(69), **C. occidentalis* ‡FL(74); ***Elliptio complanata*** (Lightfoot) as *Unio purpureus, A. conchicola* ‡CT(§1, §2), ‡DE(3), NJ(16), MA(§3, §4), PA(21) 37, 40, 44, *C. insignis* NC 46, PA(§8), *C. occidentalis* ONT 34, 35, 36; ***Elliptio crassidens*** (Lam.), *A. conchicola* FL 25, TN 24, *C. insignis* FL 25, ‡MS(134, 146), *L. interiora* FL 26; ***Elliptio dilatata*** (Raf.), *A. conchicola* IL 37, MO 27, OH 60, WI 71; ***Elliptio folliculatus*** (Lea), *C. insignis* GA 25; ***Elliptio icterina*** (Conrad) as *E. toumeyi, A. conchicola* AL 25; ***Elliptio jayensis*** (Lea), **C. insignis* FL(68); ***Elliptio lanceolata*** (Lea) as *E. gibbosus, C. insignis* NC 46; ***Elliptio strigosus*** (Lea), *A. conchicola* FL 25, *C. insignis* FL 25, GA 25, *L. interiora* FL 26; ***Elliptoideus sloatianus*** (Lea) as *Elliptio sloatianus, A. conchicola* FL 25; ***Friersonia iridella*** (Pilsbry and Frierson), **A. conchicola* ‡SLP(462); ***Fusconaia ebena*** (Lea), *A. conchicola* TN 24, WI 71; ***Fusconaia escambia*** Clench and Turner, *A. conchicola* FL 25, *C. insignis* FL 25; ***Fusconaia flava*** (Raf.) as *Quadrula rubiginosa, A. conchicola* IN 72, LA(349), MO 27, ‡MS(143), OH(207) 60, OK 50, *C. insignis* IN 72, LA(341, 377, 384), MO 27, ‡MS(146), OK 50; ***Fusconaia subrotunda*** (Lea), *A. conchicola* OH 6; ***Fusconaia succissa*** (Lea), *A. conchicola* FL 25, *L. interiora* FL 26; ***Fusconaia undata*** (Barnes) as *Quadrula trigona, A. conchicola* WI 71, *C. insignis* IL 37; ***Glebula rotundata*** (Lam.), *A. conchicola* LA(291, 313, 323, 361, 370, 373, 375) 66, 67, ‡MS(138, 142), *C. insignis* LA(120, 310, 313, 316, 317, 320, 361, 365, 370, 373, 375) 66, 67; ***Gonidea angulata*** (Lea), *A. conchicola* WA 55; ***Lampsilis cariosa*** (Say), **A. conchicola* ‡NY(§6), **C. insignis* PA(§8); ***Lampsilis claibornensis*** (Lea), *C. insignis* FL(97), 25, LA(123), ‡MS(135, 139, 146); ***Lampsilis fasciola*** Raf., *A. conchicola* OH 60; ***Lampsilis higginsi*** (Lea), *C. insignis* IL 37; ***Lampsilis hydiana*** (Lea) as *L. radiata hydiana, *A. conchicola* LA(157, 168, 337, 358), *C. insignis* LA(151, 154, 160, 165, 168, 171, 289, 304, 305, 311, 337, 338, 341, 349, 377, 383, 384) 67, TX(181) 13, *L. interiora* FL 26; ***Lampsilis ochracea*** (Say), *C. insignis* NC 46, **C. occidentalis* ‡DE(3); ***Lampsilis ovata ovata*** (Say), *A. conchicola* LA(151, 154, 157) 67, OK 50, TX(174, 181), *C. insignis* ‡AR(400, 406), LA(151, 152, 154, 157, 171, 349) 67, OK 50, TX(174, 181), **C. occidentalis* ‡TX(174); ***Lampsilis ovata ventricosa*** (Barnes) as *Lampsilis ventricosa, A. conchicola* IA 37, IL 37, MO 27, OH 32, *C. insignis* IA 37, IL 37, 65, IN 73, MO 27, OH 60, WV 11, *C. occidentalis* OH 30, MO 27; ***Lampsilis radiata radiata*** (Gmelin), **A. conchicola* ‡CT(§1), ‡DE(3), MA(§3), **C. occidentalis* ‡DE(3); ***Lampsilis radiata siliquodea*** (Barnes) as *L. radiata luteola, L. siliquoidea* and *L. luteolus, A. conchicola* IL 37, IN 72, LA 67, OH 32, OK 50, WI 71, WV 11, *C. insignis* IL 37, IN 72, 73, LA 67, NY 53, 54, OH(206) 60, OK 50, WV 11, *C. occidentalis* IA 14, 15, 38, OH 30; ***Lampsilis reeviana brevicula*** (Call), *C. insignis* MO 27; ***Lampsilis subangulata*** (Lea) as *Ligumia subangulata, A. conchicola* FL 25, *C. insignis* FL 25, *L. interiora* FL 26; ***Lampsilis teres*** (Raf.) as *L. anodontoides* and *L. anodontoides floridana, A. conchicola* FL 25, IA 37, IL(227) 37, LA(151, 154, 157, 168, 171, 287, 303, 304, 305, 309, 312, 322, 323, 324, 334, 335, 349, 363, 373, 384) 66, 67, MO 27, OK(414) 50, TX(174, 181), *C. insignis* ‡AR(391), FL(96) 25, GA 25, IA 37, IL 37, 39, LA(151, 160, 165, 168, 171, 292, 304, 305, 309, 311, 312, 319, 322, 324, 341, 349, 363, 364, 370, 373, 377, 384) 10, 67, MO 27, ‡MS(139), OK 50, TX(181) 13, 19; ***Lasmigona complanata*** (Barnes) as *Alasmodonta complanata, Symphynota complanata* and *Unio katharinae, A. conchicola* IA 37, IL 37, IN 72, MO 27, OH 6, 32, OK 50, *C. insignis* IL 37, 39, OK 50; ***Lasmigona costata*** (Raf.), *A. conchicola* OH 6, 60; ***Leptodea fragilis*** (Raf.) as *Lampsilis fragilis, L. gracilis,* and *Unio gracilis, A. conchicola* IA 37, IL 37, 65, LA(151, 157, 287, 304, 320, 324, 363,

365, 367, 370) 67, MO 27, 64, ‡MS(110, 111), OH 6, 60, OK 50, TX(175, 176), WI(231), *C. insignis* ‡AR(279), IA 37, IL 37, 39, 61, LA(151, 157, 303, 320, 334, 367, 368, 370) 67, ‡MS(139), OK 50, TX(175, 181), ‡WI(231); ***Leptodea leptodon*** (Raf.) as *Lampsilis tenuissimus, C. insignis* IL 37; ***Ligumia nasuta*** (Say) as *Lampsilis nasutus* and *Unio nasutus, A. conchicola* ‡CT(§2), ‡DE(3), NJ(14), OH 60, PA 40, 44, *C. insignis* ‡NJ(14), OH 60, PA 37, ‡RI(§9), *C. occidentalis* ‡CT(§2), MI 15, 16, 74; ***Ligumia recta*** (Lam.) as *Lampsilis rectus* and *Unio rectus, A. conchicola* IA 37, IL 37, OH 60, *C. insignis* IA 37, IL 37, 39, IN 72, 73, LA(349), MO 27, OH 72, *C. occidentalis* MI 45; ***Ligumia subrostrata*** (Say) as *Lampsilis subrostratus, A. conchicola* LA(314), MO 27, *C. insignis* LA(113, 297, 338) 67, MO 27, TN 48, 49; ***Medionidus conradicus*** (Lea), **A. conchicola* ‡VA(446): ***Obliquaria reflexa*** Raf., *A. conchicola* MO 27, ‡MS(110), OH 60, OK 50, TN 24; ***Obovaria castenea*** (Lea), **A. conchicola* LA(157, 168); ***Obovaria olivaria*** (Raf.) as *Obliquaria olivaria* and *Lampsilis ellipsis, A. conchicola* IA 37, IL 37, 65, TN 24, *C. insignis* IA 37, IL 37; ***Obovaria retusa*** (Lam.) as *Obliquaria retusa, A. conchicola* TN 24; ***Obovaria subrotunda*** (Raf.), *A. conchicola* OH 6; ***Orthonymus cylindrica*** (Say) as *Quadrula cylindrica, *A. conchicola* TN (437); ***Orthonymus metanevra*** (Raf.) as *Quadrula metanevra, A. conchicola* MO 27, TN 24, *C. insignis* IL 37; ***Plagiola triquetra*** (Raf.) as *Dysonomia triquetra, A. conchicola* OH 60; ***Plethobasis cyphyus*** (Raf.), *C. insignis* TN 24; ***Pleurobema cordatum*** (Conrad) as *P. coccineum, A. conchicola* IL 65, OH 6, 60, TN 24, WV 11; ***Pleurobema sintoxia*** (Raf.), *A. conchicola* MO 27; ***Pleurobema strodeanum*** (B. H. Wright), *A. conchicola* FL 25, *L. interiora* FL 26; ***Popenaias* sp.**, **A. conchicola* ‡SLP(462); ***Proptera alata*** (Say) as *Lampsilis alatus* and *Potamilus alatus, A. conchicola* IA 37, IL 37, MO 27, OH 6, 60, OK 50, TN 24, WI 71, WV 11, *C. insignis* IA 37, IL 37, 39, MO 27, OK 50; ***Proptera amphichaena*** (Frierson), **A. conchicola* TX(176), **C. insignis* TX(176); ***Proptera laevissima*** (Lea) as *Leptodea laevissima* and *Potamilus ohioensis, A. conchicola* ‡AR(279), MO 27, WI 71, **C. insignis* ‡AR(279); ***Proptera purpurata*** (Lam.) as *Potamilus purpuratus, A. conchicola* LA(154, 168, 287, 304, 305, 311, 312, 320, 323, 334, 337, 345, 347, 349, 363, 369, 370, 377) 66, 67, ‡MS(110), OK(389) 2, 50, TX(175, 181) 1, *C. insignis* ‡AR(398, 404), LA(154, 168, 305, 311, 334, 341, 345, 347, 349, 363, 368, 369, 377) 67, ‡MS(139), OK(389) 50, TX(175, 176) 1, 13; ***Ptychobranchus fasciolare*** (Raf.), *A. conchicola* OH 6, 60, *C. insignis* MO 27, OH (207); ***Ptychobranchus subtentum*** (Say), **A. conchicola* TN(437); ***Quadrula apiculata*** (Say), *A. conchicola* LA(287, 293, 294, 323, 324, 375, 377) 66, *C. insignis* LA(375) 67; ***Quadrula nodulata*** (Say) as *Q. pustulata, A. conchicola* LA(312, 326) 67 *C. insignis,* IL 37, LA(326, 368); ***Quadrula pustulosa*** (Lea) as *Q. houstonensis* and *Unio pustulosus, A. conchicola* IA 37, IL 37, 65, LA(151, 168, 171, 294, 312, 324, 326, 349, 378) 67, MO 27, ‡MS(386), OH 6, 60, OK 50, TN 24, TX 19, WI 71, WV 11, *C. insignis* IL 37, 61, LA(363, 368) 67, MO 27, ‡MS(110, 139), TX 19, WV 11, *C. occidentalis* MO 27; ***Quadrula quadrula*** (Raf.) as *Q. forsheyi, A. conchicola* LA(171), MO 27, OH(206, 208) 6, 32, 60, OK 2, 50, TN 24, TX 19, *C. insignis* LA(303, 345, 347, 368), TX 19, WV 11; ***Quincuncina burkei*** (Walker), **A. conchicola* FL(100); ***Quincuncina infucata*** (Conrad), *A. conchicola* FL 25, *C. insignis* FL(97) 25, **L. interiora* FL(97); ***Strophitus subvexus*** (Conrad), **A. conchicola* LA(157), **C. insignis* ‡MS(143); ***Strophitus undulatus*** (Say) as *S. edentulus, S. rugosus* and *Unio edentulus, A. conchicola* IA 37, IL 37, LA(337) 3, MO 27, OH 6, *C. insignis* IA 37, IL 37, 39, LA(337), OH 60, WV 11; ***Tritogonia verrucosa*** (Raf.) as *Quadrula tuberculata* and *Unio tuberculata, A. conchicola* ‡AR(396), IL 37, LA(162, 168, 171, 303, 323, 347, 369, 370) 66, 67, MO 27, ‡MS(111), OH 6, 60, OK 2, 50, TN 24, WI 71, WV 11, *C. insignis* ‡AR(396), IL 37, 39, LA(154, 171, 305, 341, 349, 370) 66, 67, MO 27, ‡MS(139, 140, 146) OK 50; ***Truncilla donaciformis*** (Lea) as *Plagiola donaciformis, A. conchicola* IA 37, IL 37, OH 60, OK 50, **C. insignis* LA(363); ***Truncilla truncata*** Raf. as *Plagiola elegans* and *Unio elegans, A. conchicola* IL 37, OK 50, WI 71, *C. insignis* IL 39, OK 50; ***Uniomerus tetralasmus*** (Say) as *U. obesus, A. conchicola* LA(324) 67, MO 27, *C. insignis* FL 25, GA 25, LA(310, 324, 338, 364) 67, MO 27, TN 48, 49, TX(390); ***Villosa delumbis*** (Conrad), **C. insignis* NC(40); ***Villosa iris*** (Lea), **A. conchicola* ‡AR(404), **C. insignis* ‡AR(404), OH (207); ***Villosoa lienosa*** (Conrad), *A. conchicola* FL(100) 25, LA(168, 377), ‡MS(143), *C. insignis* FL(97) 25, GA 25, LA(123, 124, 168, 322, 338, 377), ‡MS(133, 134, 140, 143, 144, 145), OK(414); ***Villosa vibex*** (Conrad), *A. conchicola* LA 67, ‡MS(143, 145), *C. insignis* FL 25, LA(123), ‡MS(143, 146); ***Villosa villosa*** (B. H. Wright) as *Carunculina villosa, C. insignis* FL(95) 25; **Unionidae or Mussels,** *A. conchicola* IL 70, PA 20, 21.

SUBPHYLUM VERTEBRATA, CLASS OSTEICHTHYES, Order Acipenseriformes, Family Polyodontidae: ***Polyodon spathula*** (Walbaum), *C. cokeri* MS 58. Order Perciformes, Family Sciaenidae: ***Aplodinotus grunniens*** Raf., *C. occidentalis* IA 38, LA 59, MN 14, 15, 51?, 52, MS 58, OH 12, TN 4.

CLASS REPTILIA, Order Testudines, Family Chelydridae: ***Chelydra serpentina*** (L.), *C. stunkardi* NC 57; ***Macroclemys temminckii*** (Troost), *L. interiora* AR 68. Family Emydidae: ***Chrysemys scripta*** (Schoepff), *Cotylaspis* sp. LA 7; ***Graptemys geographica*** (LeSueur) as *Malacoclemmys lesueuri, C. cokeri* IN 8, OH 56, TX 61; ***Graptemys pseudogeographica*** (Gray) as *Malacoclemmys lesueurii* and Lesseur's terrapin, *C. cokeri* IA 5. Family Trionychidae: ***Trionyx ferox*** (Schneider), *C. insignis* OK 17 (incidental host).

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Ecology of Parasitic Helminths of Wood Ducks, *Aix sponsa,* in the Atlantic Flyway[1]

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ABSTRACT: Two hundred and fifteen wood ducks from 24 counties in 12 states of the Atlantic Flyway were infected with 15 species of trematodes, 6 cestodes, 20 nematodes, and 1 acanthocephalan. Twenty-two of these are believed to represent new host records and three are believed to be undescribed species. Although some differences were observed across host's age and sex, the helminth fauna generally showed a high degree of consistency across these variables. Geographic distribution was responsible for most of the association between common species of helminths. Due largely to high abundances throughout the flyway of three dominant helminths (*Trichobilharzia* sp., *Amidostomum acutum,* and *Tetrameres sponsae*), helminth communities were similar across geographic regions. However, 20 of the 24 common species differed significantly in prevalences or abundances between northern and southern regions. This indicated that, in spite of the high similarities, differently structured helminth communities were characteristic of wood ducks from these regions. These regional differences are possibly related to migration and other zoogeographic factors.

The wood duck is a common species of waterfowl in North America. It is, however, unusual among Nearctic anatids because it has a non-migratory population, the members of which breed across the southeastern United States, as well as a more typical migratory northern population, the members of which breed as far north as southern Canada (Bowers and Martin, 1975; Bellrose, 1976; Schorger, 1976). Small numbers of wood ducks have been examined from scattered localities in North America and from zoological parks around the world, but an analysis of their parasites throughout a flyway has not been reported (Lapage, 1961; McDonald, 1969; Thul, 1979). In 1976, a study was initiated to determine the parasitic fauna of the wood duck throughout most of its range in the Atlantic Flyway. The survey was conducted during the host's post-breeding and premigratory period to compare the parasite faunas of migratory northern and non-migratory southern wood ducks prior to the movement of northern migrants into the southern wood duck's range. Reports on the blood parasites (Thul et al., 1980) and the parasitic arthropods (Thul, 1985) have been presented. This paper examines the ecological interrelationships of the helminths, host and environment.

Materials and Methods

Two hundred and fifteen wood ducks from 24 counties in 12 states in the Atlantic Flyway were collected by live-trapping and shooting from May 18 to October 8, 1976, and from August 23 to October 4, 1977. The sample comprised 67 adult males, 48 adult females, 50 immature males and 50 immature females. Ages and sexes were determined by plumage and cloacal examination (Kortright, 1942; Carney, 1964). Immature birds were those that hatched within the calender year, and adults were birds in at least their second calendar year.

Techniques for recovering, killing, fixing, and examining helminths followed Kinsella and Forrester (1972) including the use of fine mesh screens (100-mesh or 150-μm opening). Representative specimens have been deposited in the U.S. National Parasite Collection, Beltsville, Maryland (USNM Nos. 75328–75371).

To investigate the possibility of overdispersion, Chi-square tests were conducted on frequency distributions. These tests determined that non-parametric statistical techniques would be appropriate for many subsequent analyses because most populations were not randomly distributed. Statistical analyses to measure 1) the effects of host age and sex on helminths and 2) helminth associations were conducted to satisfy the prerequisites (Pence et al., 1983) for measuring faunal similarity across a host's range. Chi-square analysis and Kruskal-Wallis tests (or Mann-Whitney *U*-statistics for two sample comparisons) were used to deter-

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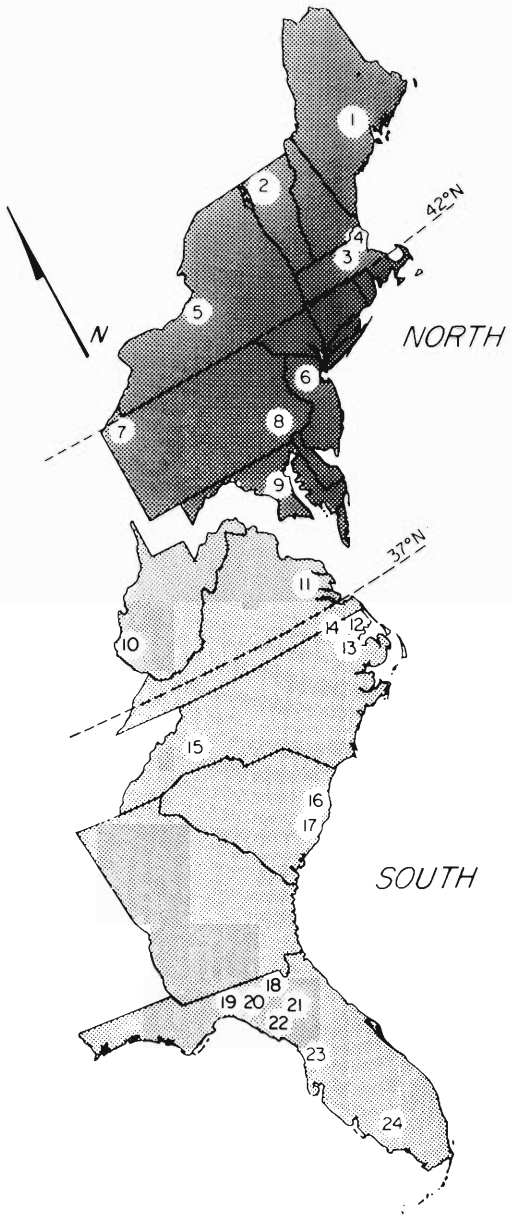

Figure 1. Collection sites of 215 wood ducks collected in Atlantic Flyway in 1976 and 1977 (N = number of ducks examined). 1. Penobscot County, Maine (N = 24); 2. Franklin County, Vermont (N = 10); 3. Worcester County, Massachusetts (N = 9); 4. Essex County, Massachusetts (N = 3); 5. Cayuga County, New York (N = 12); 6. Morris County, New Jersey (N = 12); 7. Erie County, Pennsylvania (N = 8); 8. Lancaster County, Pennsylvania (N = 3); 9. Montgomery County, Maryland (N = 11); 10. Mason County, West Virginia (N = 5); 11. Charles City County, Virginia (N = 12); 12. Chowan County, North Carolina (N = 2); 13. Tyrrell County, North Carolina (N = 4); 14. Washington County, North Carolina (N = 8); 15. Burke County, North Carolina (N = 12); 16. Georgetown County, South Carolina (N = 2); 17. Berkeley County, South Carolina (N = 15); 18. Hamilton County, Florida (N = 4); 19. Gadsden County, Florida (N = 10); 20. Leon County, Florida (N = 35); 21. Alachua County, Florida (N = 3); 22. Levy County, Florida (N = 4); 23. Hernando County, Florida (N = 6); 24. Glades County, Florida (N = 1).

mine significant differences in prevalence and abundance, respectively, across variables of age, sex, and geographic locality. In addition, prevalence data were analyzed as a linear model using FUNCAT from the Statistical Analysis System (SAS) (Helwig and Council, 1979). This was a multiway contingency analysis incorporating tests of age, sex, and locality simultaneously. Square root transformations were performed on intensity data and analyzed by ANOVA and Duncan's Multiple Range tests. Affinities among nine common helminth species were examined by Chi-square tests that compared expected versus actual pairwise occurrences. Nine species with prevalence $\geq 10\%$ and abundance ≥ 100 were selected. Associations based on numbers of individuals shared among pairwise combinations of helminth species were determined with Kendall Tau B correlation coefficients. Paired rank correlations with associated statistical tests were computed for each combination of nine common helminth species. Throughout this paper, statements of statistical significance refer to $P \leq 0.05$.

Indices of similarity were prepared according to Holmes and Podesta (1968). The reciprocal of Simpson's index (1/SI) was used as a measure of diversity (Simpson, 1949). This was calculated as $1/SI = 1/\sum_{i=1}^{n}(p_i)^2$, where p_i is the prevalence of the i^{th} species in the sample divided by the sum of all n prevalences (Leong and Holmes, 1981). Helminth fauna similarity across geographical regions was also examined using cluster analysis, a data sorting technique, on helminth abundance data (Custer and Pence, 1981). The CLUSTER procedure from the Statistical Analysis System (SAS; Sarle, 1982) was used to perform hierarchical clustering of abundance data from 14 areas (combined from 24 collection sites) using Ward's method. In this procedure, the distance between pairs of clusters is defined as the sum of squares between two clusters totaled over all variables. At each clustering step, two clusters are combined so as to minimize the within-cluster sum of squares over all possible partitions (Sarle, 1982). The data set was transformed according to Conover and Iman (1981) prior to cluster analysis (though results for non-transformed data were essentially similar). The SAS TREE procedure (Howell and Sarle, 1982) was used to construct dendrograms. Vertical axes on these dendrograms are in values of R^2, the sum of squares between clusters divided by the total sum of squares. Because there is no standard objective method for determining the appropriate number of clusters (Everitt, 1979; Ray, 1982; Afifi and Clark, 1984), meaningful clusters were selected largely on biological and geographical grounds; single member clusters were

avoided. Of course, R^2 values and Cubic Clustering Criterion (Sarle, 1982) were also considered. Stepwise multiple discriminant analyses were used to determine which helminth species were the best discriminators between clusters. Chi-square and Kruskal-Wallis tests respectively were used to determine significant differences in prevalences and abundances of helminth species across the regions delineated by important clusters.

Helminth community structures during the host's post-breeding, premigratory period were examined according to the methodology of Bush (1973). Based on importance values, helminth species were classified into the following four categories:

DOMINANT: Species strongly characteristic of the community ($I \geq 1.0$).

CODOMINANT: Species contributing significantly to the community, though to a lesser degree than dominant species ($0.01 \leq I < 1.0$).

SUBORDINATE: Species occurring infrequently; although they may develop and reproduce, they do not contribute significantly to the community ($0 < I < 0.01$).

UNSUCCESSFUL PIONEER: Species that gain access to the host but do not mature and reproduce; they contribute little to the community and are characteristic of another host ($I = 0$).

These definitions are broader than Bush's to compensate for the temporal changes exhibited by most species of helminths in migratory waterfowl.

The importance value, I, was calculated for each of the helminth species as follows:

$$I_j = (M_j)\frac{A_jB_j}{\sum_{i=1}^{42} A_iB_i} \times 100$$

where A_j = number of individual parasites in species j, B_j = number of hosts infected with parasite species j, and M_j is a maturity factor equal to 1.0 if at least one mature specimen of species j is found and equal to 0 otherwise.

The geographic regions employed are as follows (numerals refer to site numbers in Fig. 1):

MID-ATLANTIC: New York (5), New Jersey (6), and Lancaster County, Pennsylvania (8).

NORTHEAST: Maine (1), Vermont (2), Massachusetts (3, 4), Erie County, Pennsylvania (7), and Maryland (9).

NORTH: Mid-Atlantic and Northeast combined.

SOUTH: West Virginia (10), Virginia (11), North Carolina (12–15), South Carolina (16, 17), and Florida (18–24).

These definitions were based upon the results of cluster analysis and our knowledge of wood duck migration patterns in the Atlantic Flyway. They are introduced here for clarity of presentation. The rationale for these groupings will be covered in the results.

For purposes of discussion, the helminth fauna of Florida ducks (*Anas fulvigula*) was compared to that of wood ducks collected within the latter's home range, peninsular Florida. Data from a study by Kinsella and Forrester (1972) were provided; community structures and indices of similarity and diversity were determined as described above.

Results

Forty-two helminth species (15 trematodes, 6 cestodes, 20 nematodes and 1 acanthocephalan) were found. The site, prevalence, intensity (mean, standard error and range), and geographic distribution of each species are presented in Table 1. Of the 33 species of helminths reported previously from wood ducks (reviewed by Thul, 1979), 20 were collected in the present study. This difference may be related to confusion in taxonomy and to the large number of reports from zoos outside North America. In addition, 22 helminth species not previously reported in wood ducks were identified in the present study.

Every bird was infected with at least one species of helminth ($\bar{x}$ = 5.1; 1–14 species). The mean intensity was 47.9 (range 1–542). A total of 10,365 individual helminths was recovered. An overdispersed distribution of individuals was evident as 20.5% of the ducks harbored 61.5% of the total helminth population. In fact, every species, with the exception of four rarely encountered species, *Psilochasmus oxyurus, Hystrichis tricolor, Streptocara crassicauda,* and *Streptocara* sp., exhibited highly significantly overdispersed populations.

Only three hosts (1.4%) were infected with a single species of helminth. In the 215-bird sample, multiple infections of 2–14 helminth species were, respectively, 23 (10.7%), 27 (12.6%), 46 (21.4%), 40 (18.6%), 24 (11.2%), 29 (13.5%), 7 (3.3%), 3 (1.4%), 6 (2.8%), 4 (1.9%), 1 (0.5%), 1 (0.5%), 1 (0.5%), and 1 (0.5%).

Eighty-eight percent of the wood ducks were infected with trematodes (mean 2.3 and range 1–7 species). Trematodes comprised 57% of all helminth individuals. The mean intensity of trematode infections was 31.2 worms (range 1–519). Individuals of *Trichobilharzia* sp. and *Notocotylus urbanensis* comprised over half the trematode sample (33% and 25%, respectively).

Cestode infections occurred in 46% of the wood ducks with a mean of 1.3 species per infected bird (range 1–4). Cestodes comprised only 9% of the total helminth sample, with a mean intensity of 9.2 (range 1–206). *Sobolevicanthus* sp. was the most abundant cestode, comprising 61% of all cestode individuals recovered.

Nematode infections occurred in 96% of the wood ducks, with a mean of 2.6 species per infected bird (range 1–7). Mean intensity was 16.9 (range 1–128). Nematodes comprised 34% of the total helminth sample. *Amidostomum acutum*

Table 1. Helminths of 215 wood ducks from the Atlantic Flyway.

Helminth	% prev.	No. worms/infected duck				Distribution†
		Mean	SE	Median	Range	
Trematoda						
Trichobilharzia sp. (12)*‡	48	19.5	4.2	6	1–292	1, 3–11, 13–15, 16, 17, 19–23
Notocotylus urbanensis (6)	33	22.8	8.7	4	1–519	1, 3, 5, 8–11, 13–23
Apatemon gracilis (4, 5)	27	8.2	1.6	4	1–72	1, 3, 6, 8, 9, 13–15, 17–24
Zygocotyle lunata (6)	24	2.7	0.3	2	1–8	1–3, 5–10, 11, 14, 20
Prosthogonimus ovatus (8, 15)‡	15	6.0	1.1	4	1–23	1–3, 5, 7, 9, 13, 14, 16, 17, 20, 22
Psilostomum sp. (4, 5)‡§	13	11.4	3.7	5	1–93	1, 2, 5–7, 11, 13, 17, 19, 20
Echinostoma revolutum (5)	13	5.1	1.8	1	1–44	1, 2, 4–6, 8, 9, 11, 14, 15, 17, 19, 20, 23
Ribeiroia ondantrae (2)‡	10	26.3	7.8	13	1–133	1–3, 5–7, 9, 15, 20
Echinoparyphium recurvatum (4, 5)	9	12.2	5.3	2	1–92	1, 4–7, 9, 15
Dendritobilharzia pulverulenta (12)	5	1.8	0.2	2	1–3	5, 6, 8, 9, 20
Sphaeridiotrema globulus (4, 5)‡	2	54.2	35.4	29	1–194	1, 2
Maritrema sp. (5)‡	2	3.4	1.4	2	1–9	1, 3, 7
Eucotyle warreni (13)‡	1	3.0	1.0	3	2–4	13, 14
Typhlocoelum cucumerinum (11)‡	1	5.7	4.7	1	1–15	1, 3, 24
Psilochasmus oxyurus (5)‡	<1	1.0	0.0	1	—	17
Cestoda						
Sobolevicanthus sp. (5)§‡	17	16.4	6.4	2	1–206	1–3, 5–7, 11, 13, 14, 17, 19–23
Diorchis bulbodes (5)	15	6.0	2.0	1	1–53	1, 2, 5, 6, 8, 13–15, 17–20, 23
Hymenolepis sp. (6)	12	1.9	0.4	1	1–10	2, 5, 6, 12, 15, 17–22
Microsomacanthus sp. (4, 5)	7	3.8	1.4	2	1–18	1, 3, 5, 6, 8, 9, 13, 17
Cloacotaenia megalops (8)‡	2	1.2	0.2	1	1–2	1, 6, 8
Fimbriaria fasciolaris (5, 6)	1	2.7	1.7	1	1–6	5
Nematoda						
Amidostomum acutum (3)	79	8.7	0.7	5	1–56	1–24
Tetrameres sponsae (2)	63	8.5	1.0	4	1–66	1–23
Tetrameres galericulata (2)	20	10.3	3.5	2	1–128	1–4, 7–11, 13–17, 19–22
Capillaria contorta (1)	14	2.9	1.1	1	1–34	1, 2, 5–7, 9–15, 17, 19, 20
Tetrameres fissispina (2)	8	2.7	0.5	2	1–8	1, 2, 4, 5, 8, 9, 13, 14
Hadjelia neglecta (2, 3)‡	7	1.8	0.3	1	1–6	1, 15, 17, 19, 20, 22, 23
Strongyloides sp. (6)‡	7	6.7	1.4	6	1–21	1, 17, 19, 20, 23, 24
Capillaria spinulosa (6)‡	7	2.4	0.5	1	1–6	1–6, 9, 14
Tetrameres ryjikovi (2)	6	2.1	0.6	1	1–8	1, 2, 5, 8, 9, 11, 13, 20, 24
Capillaria anatis (6)	5	1.8	0.3	1	1–4	1, 3, 4, 8, 14
Aproctella stoddardi (11)‡	4	2.0	0.6	1	1–6	11, 13, 14, 17, 20
Contracaecum spiculigerum (5)‡	2	1.2	0.2	1	1–2	6, 20, 22
Echinuria uncinata (2)	2	6.5	1.3	7	3–9	4, 9
Alifilaria pseudolabiata (14)‡	2	1.2	0.2	1	1–2	6, 7, 13, 17
Streptocara sp. (3)‡§	1	1.0	0.0	1	—	10, 11
Cyathostoma bronchialis (9)‡	1	2.0	1.0	2	1–3	8, 15
Epomidiostomum uncinatum (3)‡	1	1.5	0.5	1	1–2	5, 6
Tetrameres striata (2)‡	<1	36.0	0.0	36	—	8
Hystrichis tricolor (3)‡	<1	1.0	0.0	1	—	23
Streptocara crassicauda (3)	<1	1.0	0.0	1	—	8
Acanthocephala						
Polymorphus minutus (4, 5)	1	3.7	2.7	1	1–9	6

was the most abundant nematode comprising 42% of all nematode individuals.

One species of acanthocephalan, *Polymorphus minutus,* occurred in three (1.4%) wood ducks. A total of 11 worms was recovered; this was 0.1% of all helminth individuals recovered.

Effects of host age and sex

Immature wood ducks harbored from 1 to 12 species of helminths ($\bar{x} = 5.0$). One to 14 species occurred in adult birds ($\bar{x} = 5.1$). A mean of 5.0 species (range 1–12) was recovered from male wood ducks and a mean of 5.1 species (range 1–14) was recovered from female birds.

Effects of age and sex on helminth prevalence and intensity were relatively few. Only 5 of 42 species exhibited significant effects for age, sex, or age and sex combined. The prevalences of *Trichobilharzia* sp. and *Tetrameres sponsae* were significantly higher in adult than immature wood ducks (54–42% and 73–52%, respectively). The intensity of *Apatemon gracilis* infections was significantly higher in male than female ducks ($\bar{x} =$ 12.5 and $\bar{x} = 4.5$, respectively). Significant interactions of age and sex were evident in the prevalence of *Amidostomum acutum* in adult males and immature males (91% and 68%, respectively), in the intensity of *Prosthogonimus ovatus* in immature males and immature females ($\bar{x} = 8.1$ and 2.8, respectively) and in the intensity of *Tetrameres sponsae* in adult females and adult males ($\bar{x} = 12.7$ and $\bar{x} = 5.8$, respectively).

Helminth species associations

The nine most abundant species were selected to examine associations among wood duck helminths that might result from interference competition, competitive exclusion, mutualism, or other factors. Chi-square tests on co-occurrence data demonstrated significant affinities, all positive, among 4/36 species pairings (Table 2). Correlation coefficients (Kendall's Tau B) were computed for all 36 species pairings to determine whether abundances varied in concert, oppositely, or independently. Overall, 21 pairings were positive and 15 were negative. Statistically significant positive correlations were observed in four pairings, significant negative correlations in one (Table 2).

Table 2. Significant affinities of nine common helminth species* based upon Chi-square tests (χ^2) on frequency and Kendall Tau B Correlation Coefficients (T) and associated level of significance (P) on intensity data.

Helminth pair	χ^2†	T	P
Apatemon–Notocotylus	0.0449	0.11	0.0595
Psiolostomum–T. sponsae	0.5440	−0.12	0.0450
Ribeiroia–Trichobilharzia	0.0637	0.14	0.0188
Ribeiroia–Sobolevicanthus	0.0058	0.19	0.0038
Trichobilharzia–Amidostomum	0.0033	0.22	<0.0001
T. galericulata–T. sponsae	0.0005	0.29	<0.0001

* Species included: *Apatemon gracilis, Notocotylus urbanensis, Psilostomum* sp., *Ribeiroia ondatrae, Trichobilharzia* sp., *Sobolevicanthus* sp., *Amidostomum acutum, Tetrameres galericulata, T. sponsae.*

† Probabilities associated with χ^2 values.

Faunal similarity across the host's range

Similarities among helminth faunas across geography were examined by cluster analysis using abundance data (Custer and Pence, 1981). The most prevalent 24 helminth species (≥5% prevalence) were considered, eliminating rare species. Most sites strongly tended to cluster with their geographical neighbors. Exceptions that could be explained on biogeographical grounds were sites in Pennsylvania (sites number 7 and 8) and North Carolina (sites number 12–14 and 15). To simplify presentation, a final analysis was performed to cluster 14 localities (the 12 states plus sites number 7 and 15) hierarchically. In addition, to indicate the diversity of helminth species in each area, the reciprocal of Simpson's index of diversity (Simpson, 1949) was calculated for each of the 14 localities (Fig. 2). The values ranged from a high of 14.3 in Lancaster County, Pennsylvania (EPA, site number 8) to a low of 7.3 in Maryland, with a value of 14.1 for the Atlantic Flyway.

Evaluated from a biological standpoint and also by somewhat subjective consideration of the

←

* Numbers in parentheses indicate location in host: (1) esophagus, (2) proventriculus, (3) gizzard lining, (4) duodenum, (5) small intestine, (6) cecum, (7) large intestine, (8) cloaca, (9) trachea, (10) lungs, (11) body cavity, (12) blood vessels, (13) kidneys, (14) subcutaneous, (15) bursa of Fabricius.

† Numbers refer to collection localities given in Figure 1.

‡ New host record.

§ Undescribed species.

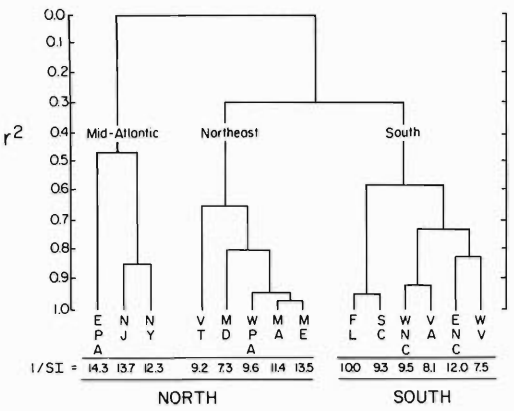

Figure 2. Dendrogram derived from cluster analysis based on mean abundances of 24 common helminth species of 215 wood ducks from 14 localities in the Atlantic Flyway* and 1/Simpson's index of diversity values for all helminth species for each locality. * EPA = eastern Pennsylvania, NJ = New Jersey, NY = New York, VT = Vermont, MD = Maryland, WPA = western Pennsylvania, MA = Massachusetts, ME = Maine, FL = Florida, SC = South Carolina, WNC = western North Carolina, VA = Virginia, ENC = eastern North Carolina, WV = West Virginia.

Cubic Clustering Criterion (Ray, 1982), cluster analysis was judged to yield three important clusters (Fig. 2), labelled Mid-Atlantic, Northeast, and South. The Mid-Atlantic cluster contained 32 helminth species with 1/SI = 14.9, the highest diversity value among the three clusters. This Mid-Atlantic cluster shared 25 helminth species with the Northeast and 26 species with the South. Stepwise discriminant analysis was used to identify which parasite species were particularly important in differentiating among the three clusters. Significant discriminating helminth species (in order of decreasing variance accounted for by the species) in the Mid-Atlantic cluster were *Zygocotyle lunata, Dendritobilharzia pulverulenta, Diorchis bulbodes, Echinoparyphium recurvatum,* and *Microsomacanthus* sp. Species unique to the cluster were *Fimbriaria fasciolaris, Epomidiostomum unicatum, Tetrameres striata,* and *Polymorphus minutus.*

The Northeast contained 30 species of helminths with 1/SI = 12.6. Twenty-six species were shared with the South. Significant discriminating species (in order of decreasing variance accounted for by the species) in this region were *Ribeiroia ondatrae, Zygocotyle lunata, Capillaria spinulosa, Prosthogonimus ovatus, Echinoparyphium recurvatum,* and *Psilostomum* sp. Species unique to this region were *Maritrema* sp., *Sphaeridiotrema globulus,* and *Echinuria uncinata.*

Wood ducks from the South contained 33 species of helminths with 1/SI = 11.4. This region had the most species, but the lowest diversity. Significant discriminating species (in order of decreasing variance accounted for by the species) were *Apatemon gracilis, Notocotylus urbanensis,* and *Tetrameres sponsae.* Species unique to the South were *Eucotyle warreni, Psilochasmus oxyurus, Aproctella stoddardi, Hystrichis tricolor,* and *Streptocara* sp.

The classification criterion developed by discriminant analysis was then applied to the same 215-bird sample to determine the percentage of cases correctly classified into each respective cluster. Overall, 85% (183/215) of the observations were correctly classified. Of those wood ducks from the Northeast cluster 74% (48/65) were correctly classified with 3% (2/65) and 23% (15/65) classified into the Mid-Atlantic and Southeast, respectively. Eighty-nine percent (24/27) of the Mid-Atlantic wood ducks were correctly classified with 4% (1/27) and 7% (2/27) classified into the Northeast and Southeast, respectively. Of the Southeast wood ducks, 90% (111/121) were classified into the Southeast, 10% (12/123) into the Northeast, and zero into the Mid-Atlantic.

To compare the helminth fauna of northern, migratory wood ducks versus the fauna of southern, predominantly resident birds, a new regional breakdown was established. This scheme formed a single "North" region by combining "Northeast" and "Mid-Atlantic" sites. The prevalence and intensity (mean, standard error, range) of the helminths by these regions are presented in Table 3. Of the 42 species of helminths found in the Atlantic Flyway, 37 species were collected from northern birds, and 33 species were collected from southern birds. Twenty-eight species were shared, including 13 species of nematodes, 11 species of trematodes, and 4 species of cestodes. There were no significant regional effects for total number of helminths. Of the 24 common helminths, 20 species exhibited significant differences in prevalence between regions and 19 exhibited significant differences in abundance. These species are denoted in Table 3. Values of 1/SI were calculated for each region to indicate the diversity of species present in each area. The values were 14.2 in the North and 11.4 in the South. A similarity index value of 61.0% was calculated for the two regions.

Table 3. Helminths of northern and southern wood ducks in the Atlantic Flyway.

	North (N = 92)				South (N = 123)			
		Intensity				Intensity		
	% prev.	Mean	SE	Range	% prev.	Mean	SE	Range
*Apatemon gracilis**†	9.8	1.8	0.36	1–4	39.8	9.4	1.90	1–72
*Dendritobilharzia pulverulenta**	9.8	1.9	0.26	1–3	0.8	1.0	0.00	1–1
*Echinoparyphium recurvatum**†	17.4	12.9	6.21	1–92	2.4	1.0	0.00	1–1
*Echinostoma revolutum**†	20.7	5.4	2.41	1–44	6.5	4.4	1.72	1–13
Eucotyle warreni	0.0	—	—	—	2.4	2.3	0.88	1–4
Maritrema sp.	6.5	3.4	1.44	1–9	0.0	—	—	—
*Notocotylus urbanensis**†	14.1	4.1	1.27	1–16	46.3	26.6	10.63	1–519
*Prosthogonimus ovatus**†	22.8	5.9	1.32	1–23	9.8	5.7	1.86	1–22
Psilochasmus oxyurus	0.0	—	—	—	0.8	1.0	0.0	1–1
Psilostomum sp.†	18.5	5.9	1.61	1–28	8.9	19.9	8.57	1–93
*Ribeiroia ondatrae**†	20.7	28.6	8.44	1–133	1.6	4.0	1.00	3–5
Sphaeridiotrema globulus	5.4	48.7	29.46	1–194	0.0	—	—	—
Trichobilharzia sp.*†	59.8	19.7	5.68	1–292	38.2	14.3	6.42	1–272
Typhlocoelum cucumerinum	2.2	1.0	0.00	1–1	0.8	15.0	0.00	15–15
*Zygocotyle lunata**†	45.7	2.9	0.34	1–9	7.3	1.4	0.34	1–4
Cloacotaenia megalops	4.3	1.2	0.18	1–2	0.0	—	—	—
Diorchis bulbodes	14.1	8.7	3.95	1–53	14.6	4.1	2.06	1–38
Fimbriaria fasciolaris	3.3	2.7	1.67	1–6	0.0	—	—	—
Hymenolepis sp.*†	3.3	3.3	1.20	1–5	17.9	1.7	0.42	1–10
Microsomacanthus sp.*†	74.1	4.2	1.66	1–18	2.4	12.8	6.83	1–84
Sobolevicanthus sp.*†	23.9	18.7	9.65	1–206	11.4	2.0	0.58	1–3
Alifilaria pseudolabiata	2.2	1.0	0.00	1–1	1.6	1.5	0.50	1–2
*Amidostomum acutum**†	94.6	10.2	1.09	1–56	66.7	7.1	0.89	1–44
Aproctella stoddardi	0.0	—	—	—	7.3	2.0	0.60	1–6
*Capillaria anatis**†	9.8	1.9	0.39	1–4	1.6	1.5	0.50	1–2
Capillaria contorta	13.0	1.5	0.19	1–3	14.6	3.9	1.89	1–34
*Capillaria spinulosa**†	13.0	2.6	0.58	1–6	1.6	1.0	0.00	1–1
Contracaecum spiculigerum	2.2	1.5	0.50	1–2	1.6	1.0	0.00	1–1
Cyathostoma bronchialis	1.1	3.0	0.00	3–3	0.8	1.0	0.00	1–1
Echinuria uncinata	4.3	6.2	1.23	3–9	0.0	—	—	—
Epomidiostomum uncinatum	2.2	1.5	0.50	1–2	0.0	—	—	—
*Hadjelia neglecta**†	2.2	1.0	0.00	1–1	11.4	1.9	0.37	1–6
Hystrichis tricolor	0.0	—	—	—	0.8	1.0	0.00	1–1
Streptocara crassicauda	1.1	1.0	0.00	1–1	0.0	—	—	—
Streptocara sp.	0.0	—	—	—	2.4	1.0	0.00	1–1
Strongyloides sp.*†	1.1	2.0	0.00	2–2	12.2	7.1	1.43	1–21
*Tetrameres fissispina**	17.4	2.9	0.56	1–8	1.6	1.0	0.00	1–1
*Tetrameres galericulata**†	12.0	6.8	3.50	1–37	25.2	11.6	0.49	1–128
Tetrameres ryjikovi	8.7	2.7	0.88	1–8	4.1	1.0	0.00	1–1
*Tetrameres sponsae**†	53.3	4.4	0.52	1–18	69.1	10.8	1.48	1–66
Tetrameres striata	1.1	36.0	0.00	36–36	0.0	—	—	—
Polymorphus minutus	3.3	3.7	2.67	1–9	0.0	—	—	—

* Denotes significant difference in prevalence at $P \leq 0.05$ level between North and South all birds; only species ≥5% overall prevalence considered.

† Denotes significant differences in abundance at $P \leq 0.05$ level between North and South all birds; only species ≥5% overall prevalence considered.

Helminth species from the North and South were classified according to importance values as dominant, codominant, and subordinate (no species were classified as unsuccessful immigrant) (Table 4). There were eight dominant species in the North and six in the South with only three species shared. The dominant species accounted for 75.3% of the helminths recovered in the North and 85.4% in the South. *Amidostomum acutum, Trichobilharzia* sp., and *Tetrameres sponsae* were dominant in both regions. Other dominant species were relegated to codominant status in the opposite regions, except for *Ribeiroia ondatrae,* a northern dominant species, which was only subordinate in the South. Twenty-one species were classified as codomi-

Table 4. Classification and importance values (I) of helminth species of wood ducks in the Atlantic Flyway.

North	I	South	I
		Dominant species	
Amidostomum acutum	41.02	*Notocotylus urbanensis*	28.96
Trichobilharzia sp.	31.69	*Tetrameres sponsae*	26.15
Tetrameres sponsae	5.62	*Amidostomum acutum*	15.99
Ribeiroia ondatrae	5.48	*Trichobilharzia* sp.	14.29
Sobolevicanthus sp.	4.81	*Apatemon gracilis*	7.57
Zygocotyle lunata	2.72	*Tetrameres galericulata*	3.74
Prosthogonimus ovatus	1.38		
Echinostoma revolutum	1.04		
		Codominant species	
Echinoparyphium recurvatum	0.99	*Psilostomum* sp.	0.81
Psilostomum sp.	0.90	*Strongyloides* sp.	0.54
Diorchis bulbodes	0.78	*Diorchis bulbodes*	0.45
Sphaeridiotrema globulus	0.65	*Capillaria contorta*	0.42
Echinuria uncinata	0.53	*Prosthogonimus ovatus*	0.27
Tetrameres galericulata	0.44	*Hymenolepis* sp.	0.27
Tetrameres fissispina	0.39	*Sobolevicanthus* sp.	0.13
Microsomacanthus sp.	0.38	*Hadjelia neglecta*	0.13
Notocotylus urbanensis	0.37	*Echinostoma revolutum*	0.09
Capillaria spinulosa	0.20	*Aproctella stoddardi*	0.05
Capillaria contorta	0.11	*Zygocotyle lunata*	0.04
Tetrameres ryjikovi	0.09	*Microsomacanthus* sp.	0.04
Dendritobilharzia pulverulenta	0.08		
Capillaria anatis	0.08		
Apatemon gracilis	0.08		
Maritrema sp.	0.06		
Hymenolepis sp.	0.02		
Tetrameres striata	0.02		
Polymorphus minutus	0.02		
Fimbriaria fasciolaris	0.01		
Cloacotaenia megalops	0.01		
		Subordinate species	
Contracaecum spiculigerum	0.003	*Tetrameres ryjikovi*	0.008
Epomidiostomum uncinatum	0.003	*Eucotyle warreni*	0.007
Alifilaria pseudolabiata	0.002	*Ribeiroia ondatrae*	0.005
Typhlocoelum cucumerinum	0.002	*Typhlocoelum cucumerinum*	0.005
Cyathostoma bronchialis	0.002	*Echinoparyphium recurvatum*	0.003
Hadjelia neglecta	0.002	*Streptocara* sp.	0.003
Streptocara crassicauda	0.0004	*Alifilaria pseudolabiata*	0.002
Strongyloides sp.	0.0004	*Capillaria anatis*	0.002
		Capillaria spinulosa	0.001
		Contracaecum spiculigerum	0.001
		Tetrameres fissispina	0.001
		Dendritobilharzia pulverulenta	0.0004
		Psilochasmus oxyurus	0.0004
		Cyathostoma bronchialis	0.0004
		Hystrichis tricolor	0.0004

nant in the North; only 12 were classified codominant in the South. Five species were shared in this classification. Subordinate species included 8 species from the North and 15 species from the South with 4 species shared in this category.

Discussion

The endoparasite fauna of wood ducks in the Atlantic Flyway was found to consist of at least 42 species of helminths. Not all species would be expected to occur in every geographical region, much less in any individual duck. The host population averaged only five parasite species per bird with the transmission of many species apparently restricted to parts of the host's range or varying in abundance from one part to another. Nearly all the helminths exhibited overdispersed (or clumped) distributions within the host pop-

ulation. A complex relationship exists between the host, the parasite community, geographic conditions, and other zoogeographic factors. Several complicating factors encountered in our investigation of the parasites of wood ducks were considered worthy of further comment.

Many recent ecological studies of helminth infracommunities across a host's range have concentrated on large mammalian predators such as wolves, coyotes, bobcats, and bears (Holmes and Podesta, 1968; Stone and Pence, 1978; Pence and Meinzer, 1979; Custer and Pence, 1981; Pence and Eason, 1981; Pence et al., 1983). Because the territorial nature and limited mobility of these animals reduces potential ecological interactions, this group lends itself well to such analyses. On the other hand, the wood duck's host/parasite/environment interrelationships are more complicated. The birds can move quickly over great distances, they are not territorial, and they are highly gregarious. In addition, northern wood ducks migrate into the range of non-migratory southern wood ducks during the fall and winter. Thus, there are actually two host populations with sympatric winter ranges. This complex situation provides obvious mechanisms for distributing parasites among individual hosts throughout the Flyway, yet ecological barriers have undoubtedly limited the range and extent of transmission for many parasite species.

Seasonal effects on wood duck helminths are substantial (Ogburn-Cahoon, 1979; Drobney et al., 1983); therefore, to minimize this source of unwanted variation, hosts were collected over as short a time span as possible. Also, the necessarily brief collecting period was scheduled to take birds near their breeding or natal areas and thereby avoid confusing migratory and resident birds—which are not morphologically separable. This timing of collection further served to avoid variation due to helminth infracommunity changes that may result from the stress of host migration (Dogiel, 1962; Cornwell and Cowan, 1963; Buscher, 1965; Drobney et al., 1983). Overall, we believed that studying the helminth infracommunities prior to the host's migration was the least complicated approach and that this approach would lead to a better understanding of the ecological aspects of the helminth communities of each host population (migratory and non-migratory) in the Flyway.

Pence et al. (1983) stated that the following prerequisites are assumed when measuring faunal similarity across a host's range: 1) stability of the helminth fauna across intrinsic host variables (age and sex), and 2) established interrelationships of species within the helminth community (associations). Accordingly, prior to comparing wood duck helminth faunas across geography, we examined our data to determine whether the above prerequisites were satisfied.

Effects of host age and sex

In general, the helminth faunas were stable across host-intrinsic variables of age and sex. Indeed, only five of 42 species of helminths exhibited significant differences in prevalence or abundance, and these are briefly discussed below.

The higher prevalence of *Trichobilharzia* sp. in adult wood ducks was attributed to a greater chance of exposure in older birds or to a peak transmission period prior to the hatching of young of the year. This fluke actively infects the host by penetrating the skin of the feet (McDonald, 1969), so cercariae would have opportunity to infect the adults during the spring and summer before ducklings hatched and became infected.

The reason for a higher mean intensity of infection of *Apatemon gracilis* in male wood ducks was unclear, especially because adult females would be expected to ingest more infective invertebrate intermediate hosts during the laying period in the spring (Drobney et al., 1983). Turner and Threlfall (1975) reported no age or sex effect for *A. gracilis* infections in blue-winged and green-winged teal (*Anas discors, A. crecca*) during the late summer in Canada.

Intensity of *Prosthogonimus ovatus* infections was significantly greater in immature females than immature males. This could be attributed to anatomical or immunological differences between the sexes. *Prosthogonimus ovatus* also was more prevalent in immature birds than in adults (27% and 5%, respectively). A higher abundance would be expected in immature birds because their diet includes a higher percentage of dragonfly nymph intermediate hosts (McDonald, 1969; Hocutt and Dimmick, 1971), and the preferred infection site for this fluke is the bursa of Fabricius, which atrophies with maturity.

The higher intensity of *T. sponsae* in adult females than in adult males may have been due to higher invertebrate consumption by adult females (Drobney et al., 1983). These nematodes inhabit protected sites in the mucosa and glands of the proventriculus and may be relatively persistent over the stressful incubation and molt period into late summer and fall.

The higher prevalence of *Amidostomum acutum* in adult males than in adult females was attributed to behavioral differences between the sexes leading to differences in host density. Because this nematode possesses a direct life cycle (McDonald, 1969), the probability of ingesting infective larvae would increase in areas of higher host density. Shortly after incubation is initiated, the male birds typically abandon the hens and congregate in bachelor groups. Hens spend less time in the company of other adult birds as they attend their brood (Bellrose, 1976; Schorger, 1976). This increases the effective host density for the drakes and may increase the frequency that infective larvae are ingested, especially in the North where the breeding season is shorter and host densities are higher.

Overall, these results indicate a high degree of similarity in the helminth faunal composition across age and sex variables during the late summer and early fall and generally agree with the observations of Drobney et al. (1983) in wood ducks at that time of year in Missouri. The relatively few differences across intrinsic variables justified the combining of the helminth data for host age and sex.

Helminth species associations

Patterned interspecific interaction could be caused by such phenomena as competition for space or nutrients (negative association), or by commensalism or sharing an intermediate host (positive association). A relatively simple approach was used to investigate these relationships.

Prevalence was examined to determine when frequency of pairwise species co-occurrence deviated significantly from expectations under a null hypothesis of statistical independence. Abundance data were examined by correlation analysis to determine whether helminth numbers varied in concert, oppositely, or independently. Five of 36 species pairs demonstrated significant relationships in co-occurrence and/or abundance. Four relationships were positive and all but one of these were attributed to high prevalences and abundances of the paired species in a common geographic area. Similarly, the only case of negative association was accounted for by higher prevalences and abundances of the relevant species in opposite geographic areas. Only the relationship between *Tetrameres sponsae* and *T. galericulata* could not be explained by geographical factors. The significant co-occurrence of these two species was attributed to their utilizing a common or similar intermediate host. Generally, we concluded that most significant associations were not caused by interspecific interactions, but were predominantly geographic in origin.

Faunal similarity across the host's range

Because the above results indicated that the prerequisites stated in Pence et al. (1983) were satisfied, we proceeded to analyze helminth faunal composition across geographic regions.

Cluster analysis using abundance data suggested three major clusters (Fig. 2), two northern clusters (Mid-Atlantic and Northeast) and one southern cluster (South). The Mid-Atlantic possessed the most diverse and distinct fauna (1/SI = 14.9). This could have been related to the particular collection areas in this region, where intense management for wood ducks resulted in high host densities and possibly, therefore, richer parasite communities of similar composition.

Because the wood ducks of both the Mid-Atlantic and Northeast regions are migratory, these regions were combined to compare the helminth fauna of migratory, northern wood ducks with that of southern resident wood ducks. The "North" region was consistent in that wood ducks from the region are migratory and very few wood ducks overwinter in the region. Although wood ducks from Virginia and West Virginia are considered to be migratory, these states, particularly Virginia, represent areas with poorly defined migratory trends among their native wood ducks (Bowers and Martin, 1975). In fact, many birds breeding in West Virginia probably migrate down the Mississippi Flyway; this might explain the dissimilarity of the helminth faunas between the wood ducks from West Virginia and the northern Atlantic Flyway states. In the present analysis, helminth faunas of birds from these two intermediate states clearly clustered with those of the other southern states.

Overall, the helminth community was found to have only 7 of 42 (16%) helminth species with $\geq$20% prevalence and only 12 of 42 (28%) with $\geq$10% prevalence. This indicated that a very large secondary community of helminth species existed which accounted for much of the diversity across geographic regions and a small primary community of a few species existed which accounted for the majority of similarity across geographic regions. Index of similarity values between the South and Mid-Atlantic (57.1) and

South and Northeast (62.2) indicated a moderately strong relationship of similarity between helminth faunas of northern and southern wood ducks. Much of this similarity was accounted for by three dominant species, *Amidostomum acutum, Trichobilharzia* sp., and *Tetrameres sponsae,* which were common in both the North and South. These species accounted for 75.3% of the helminths recovered in the North and 85.4% recovered in the South. Even so, significant differences in both prevalence and abundance between regions were evident in all three species. Indeed, 20 of 24 common helminth species demonstrated significant differences in prevalence and/or abundance between North and South (Table 3). Therefore, although similarities in helminth composition are evident (27 species shared and 61% index of similarity), important differences existed among both the dominant and the less abundant species of the helminth communities. In fact, these differences were dramatic considering that birds of each host population are apparently similar morphologically (Thul, 1979) and share the same wintering ground for a minimum of several months each year (Bellrose, 1976).

The community classification methodology was useful in quantitatively assessing the relative importance of helminth species within an infracommunity. The helminth communities profiled herein should be considered representative of only the late summer and early fall, because helminth abundances can vary with the seasons and parasite species can change classifications from one season to another.

Proportionally, each region had a similar number of dominant helminths. The North, however, possessed more codominant species (57%) and appreciably fewer subordinate species (21%), whereas the South maintained a moderate proportion of codominant species (36%) and more subordinate species (46%). Of the 27 species shared by wood ducks from the North and South, only 12 were categorized the same way in both regions. This indicated regional differences in relative importance for many species.

The pattern of high proportions of codominant species in the North and subordinate species in the South was attributed to the abundance of other waterfowl species in the northern Atlantic Flyway. This situation probably contributed to the higher diversity of helminths in the North.

The species of the subfamily Anatinae are very similar phylogenetically and consequently many parasites infecting ducks have broad host specificities. Other species of ducks frequently found within the migratory wood duck's range in the Atlantic Flyway include black ducks (*Anas rubripes*), mallards (*Anas platyrhynchos*), blue-winged teal (*A. discors*), common goldeneyes (*Bucephala clangula*), and hooded mergansers (*Mergus cucullatus*) (Bellrose, 1976). Of the 42 helminth species reported from wood ducks in the present study, at least 17 have been reported in black ducks, 28 in mallards, 14 in blue-winged teal, 21 in common goldeneyes, and 2 in hooded mergansers (McDonald, 1969). It is apparent that the presence of other species of ducks can effectively increase host density, thus also increasing the probability of infection.

Helminth community structure in southern resident wood ducks may be influenced by the same density factors, but in the South this would occur only during the fall and winter (as migrants move southward) rather than during the spring and summer as in the North. It would therefore be interesting to study acquisition and population dynamics of helminths in resident wood ducks during the fall and winter to examine the influence of migrants on the helminth community. Unfortunately, the arrival of morphologically indistinguishable northern wood ducks confounds any attempt to do this directly.

On the other hand, another resident waterfowl species might experience seasonal changes in its helminth fauna similar to that in wood ducks. The mottled, or Florida, duck (*Anas fulvigula*), a non-migratory relative of the mallard and black duck, shares at least 21 species with the wood duck (Kinsella and Forrester, 1972), and an analysis of its helminth community yielded important general insights into the population dynamics of helminths in southern resident waterfowl. Helminth community structure during summer and early fall (May–September) in Florida ducks was 16% dominant, 71% codominant, and 13% subordinate. The winter (October–February) structure was 55% dominant, 29% codominant, and 16% subordinate. Apparently, an important portion of the Florida duck's relatively diverse helminth fauna was acquired from late fall to early spring, coinciding with the presence of other migratory waterfowl species in the region. Similar mechanisms could function in the wood duck population, enabling wintering wood ducks to acquire substantial helminth infections during the late fall, winter and early spring in the southern Atlantic Flyway. Many of these winter-ac-

quired infections might be reduced in migratory birds as they fly north in the spring, but in resident birds similar infections might persist over the summer with little or no transmission because the helminths of residents do not experience the same stresses associated with long migration. These remnant helminth infections may be responsible for a substantial portion of the subordinate species in southern wood ducks in late summer.

A comparison of the helminth community of Florida ducks with that of wood ducks also proved useful. Of the 21 helminth species common to Florida ducks and wood ducks, only eight were shared by ducks during the summer in Florida. The index of similarity was only 28%. Interestingly, a comparison of the helminths of northern wood ducks (collected during the summer) and wintering Florida ducks yielded results indicating a higher degree of similarity. In this case, 19 species of helminths were shared with an index of similarity value of 38%. It is apparent that an important relationship exists between northern migratory waterfowl and southern resident waterfowl.

Changes in prevalences of shared species were studied from summer to winter to provide insights into helminth acquisition. Ten species of helminths in Florida ducks increased in prevalence during the summer and early fall; they included the following species found in wood ducks: *Apatemon gracilis, Strongyloides* sp., *Echinoparyphium recurvatum,* and *Prosthogonimus ovatus.* In immature wood ducks, the first two species demonstrated significantly higher abundances in the South, the other two in the North. *Echinoparyphium recurvatum,* and *P. ovatus* have broad host specificities (McDonald, 1969) and, according to our distribution data from immature birds, are transmitted in wood ducks throughout the Flyway. The higher prevalence of infection of these two species in northern wood ducks may be influenced in part by higher waterfowl densities in the North. *Apatemon gracilis,* a common fluke of ducks in North America, is also transmitted throughout the Flyway, but it is significantly more abundant in southern wood ducks, especially adults. This same observation was evident in another common trematode, *Notocotylus urbanensis.* These predominantly southern parasites acquired primarily during the summer should be considered important wood duck parasites because, in the virtual absence of other waterfowl across that region, the wood duck is probably the primary host within the supracommunity of these helminth species. All of the above species, except *Strongyloides* sp. are trematodes that require invertebrate intermediate hosts whose activity could be related to warm summer temperatures. *Strongyloides* sp. is an alternately parasitic and free-living nematode that infects a broad range of avian hosts (McDonald, 1969). It was transmitted only in southern ducks and is probably temperature dependent, limited by low temperatures and humidity during the winter in the North.

Thirteen of 34 helminth species in the Florida duck increased in prevalence during the late fall and winter. Among them were the following species also found in wood ducks: *Amidostomum acutum, Trichobilharzia* sp., *Zygocotyle lunata, Dendritobilharzia pulverulenta, Capillaria contorta, Cloacotaenia megalops,* and *Echinuria uncinata.* The first three species were common in wood ducks and exhibited significantly higher abundances among immature wood ducks in the North, indicating a higher infection rate among northern wood ducks during the summer. However, the prevalence and abundance of infections of *Amidostomum acutum* were high in adult wood ducks in both regions. This indicated that the *A. acutum* infection rate is highest when host densities are at their maximum: summers in the North and winters in the South. The protected site under the gizzard lining may make this helminth persistent, allowing accumulation with age and explaining its higher abundance in older southern birds compared to the immature southern birds.

Primary acquisition of helminth infections in wood ducks, then, could be expected to occur during fall and winter for some helminths and during spring and summer for others. Of the species of parasites increasing in abundance during the winter, most do not require ingestion of an intermediate host. On the other hand, most species increasing in prevalence over the summer do require ingestion of an active intermediate host. Of course this pattern is not absolute. The relatively temperate climate of the South could allow winter acquisition of infections, even by species requiring an intermediate host. That is, the warmer weather could permit some invertebrate intermediate host activity to continue and could thus allow adequate survival of infective stages throughout the winter.

The probability of acquiring any helminth infection during the winter undoubtedly declines

with increasing latitude and the accompanying increase in severity of winter conditions. This could contribute to the lower diversity values in Maryland, West Virginia, and Virginia. In these middle Flyway states, temperatures are too severe to allow acquisition of the helminth infections during winter that occurs in states further south. Furthermore, most migratory wood ducks do not overwinter in these states but migrate through to points further south in the fall and further north in the spring. These factors may limit introduction and establishment of helminth supracommunities in the middle Flyway states relative to areas at either end of the Flyway.

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Research Note

Haemoproteus antigonis from the Sandhill Crane in Western North America

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The three subspecies of sandhill cranes (lesser sandhill crane, *Grus canadensis canadensis*; Canadian sandhill crane, *G. canadensis rowani*; greater sandhill crane, *G. canadensis tabida*) range over much of western North America and their breeding grounds extend from eastern Siberia and Alaska, through Canada, eastward to Michigan (Lewis, 1977, pp. 5–43 *in* G. C. Sanderson, ed. Management of Migratory Shore and Upland Game Birds in North America. Int. Assoc. Fish and Wildl. Agencies, Washington, D.C.). These cranes largely overwinter on the High Plains of western Texas, eastern New Mexico, and western Oklahoma and in the coastal prairies of southern Texas. Overwintering sandhill cranes have been regulated migratory game birds in Texas since 1961 when a hunting season was first authorized.

As part of a study on the genetics, morphology, and parasites of sandhill crane populations from western North America (Gaines and Warren, 1984, J. Wildl. Manage. 48:1387–1393), blood films were collected from 99 sandhill cranes from Alaska (seven hosts), Canada (14), the Texas Panhandle (50), and the Gulf coastal prairies of southern Texas (28). A description of these localities is found in Gaines et al. (1984, op. cit.). This report presents the results of a survey for blood protozoa from these populations of sandhill cranes in western North America.

Sandhill cranes were collected by shooting. Two thin blood films were prepared from heart blood of each bird as quickly as possible following death. Blood films were air dried, stored in wooden boxes, and fixed in 100% methanol prior to staining with Wright-Giemsa's stain. Representative specimens are deposited in the International Reference Centre for Avian Hematozoa, Memorial University, St. John's, Newfoundland A1B 3X9, Canada (nos. 95038–95039).

A single species, *Haemoproteus antigonis* de Mello, 1935 was observed on blood films from 10 of 99 (10%) sandhill cranes examined. The prevalence of *H. antigonis* was much higher in sandhill cranes collected in summer (June–July) on the northern breeding grounds than in cranes on the Texas wintering grounds (collected November–March). Two of seven (29%) and five of 14 (36%) sandhill cranes from Alaska and Canada, respectively, were infected (33% across both regions), whereas only three of 78 (4%) sandhill cranes from the wintering grounds in Texas (3 of 50 [6%] from Texas Panhandle; 0 of 28 from southern Texas) were infected. *Haemoproteus antigonis* was reported previously from greater sandhill cranes in Florida (Forrester et al., 1976, Proc. Int. Crane Workshop 1:284–290) and Wisconsin and Indiana (Windingstad, 1978, Disease and Parasites of the Greater Sandhill Crane, M.S. Thesis, Univ. Wisconsin, Madison, 66 pp.) and from the Florida sandhill crane (*Grus canadensis pratensis*) in Florida (Forrester et al., 1976, op. cit.). It was also reported from several species of the Old World Gruidae (Bennett et al., 1975, Can. J. Zool. 53:72–81). A redescription of this species was provided by Bennett et al. (1975, op. cit.).

The much higher prevalence of *H. antigonis* in sandhill cranes from Canada and Alaska versus Texas indicates that transmission probably occurs on the breeding grounds rather than on the wintering grounds. Similarly, Windingstad (1978, op. cit.) found *H. antigonis* in 19% and 45% ($\bar{x}$ = 34%) of the greater sandhill cranes on the breeding grounds in Indiana and Wisconsin, respectively. Forrester et al. (1976, op. cit.) reported an 8% prevalence of *H. antigonis* in greater sandhill cranes overwintering in Florida whereas the resident population of Florida sandhill cranes had a 14% prevalence of this species.

The vector of *H. antigonis* is unknown although a number of species of the Hippoboscidae and Ceratopogonidae are implicated in the trans-

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mission of related species of the genus *Haemoproteus* (Levine, 1973, Protozoan Parasites of Domestic Animals and Man, Burgess Publ. Co., Minneapolis, Minnesota, 406 pp.). Hippoboscids were not recovered from any of the cranes examined in this study.

We appreciate the assistance of Dr. Donald J. Forrester who confirmed our identification of *Hemoproteus antigonis* and the U.S. Fish and Wildlife Service, Canadian Wildlife Service, Alaska Fish and Game Department, and the Texas Parks and Wildlife Department for assistance in field collections of sandhill cranes. This project was funded in part by the Caesar Kleberg Foundation for Wildlife Conservation and in part by the Institute for Museum Research, The Museum of Texas Tech University.

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Research Note

Tapeworms from Turkey and Syria in the Collection of the Late George G. Witenberg

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In 1930 Professor George G. Witenberg participated in two faunistic expeditions organized by the Department of Zoology of the Hebrew University, Jerusalem. Collection sites, at that time under a French Mandatory Government, included the surroundings of Palmyra and Dayr-az-Zawr, presently in Syria, and Kirikhan now in southern Turkey (see map Fig. 1). One hundred seventy-two birds of 20 species were examined of which 48 were infected with cestodes (28% prevalence); one rodent was examined. The worms were relaxed in water, fixed in warm 70% alcohol, stained with alum-carmine and mounted in Canada balsam. The parasites apparently were studied by Witenberg but were not identified.

In the present study, 23 species were identified. Seven samples were identified to six genera for lack of scolices and/or gravid proglottids (Table 1). The cestode classification is based on Schmidt (1985, Handbook of Tapeworm Identification, CRC Publishers, Boca Raton).

Although no new species were found, all parasites represent new locality records, and there are three new host records. The findings thus further our knowledge of the distribution of cestodes.

This paper is published in memory of the late Professor G. Witenberg (died January 30, 1979).

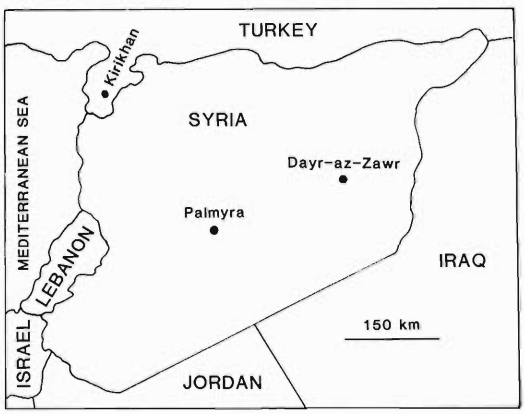

Figure 1. Map of the collection area. Spelling of place names according to Rand McNally, The New International Atlas, 1979.

[4] G.W., on sabbatical leave from the Hebrew University, wishes to express her appreciation to the Department of Biological Sciences, University of Northern Colorado, for its cooperation and hospitality during the period of this research.

Table 1. Cestodes of vertebrates from Turkey and Syria.

Host	Cestode	Locality	Voucher number HUJP*
Birds			
Anas platyrhynchos	*Aploparaksis* sp.	Kirikhan	C-178
Anas penelope	*Diploposthe laevis* (Bloch, 1782) Jacobi, 1896	Kirikhan	C-228
	Fimbriarioides intermedia (Fuhrmann, 1913) Fuhrmann, 1932	Kirikhan	C-195
	Microsomacanthus sp.	Kirikhan	C-227
Ardea cinerea	*Neogryporhynchus cheilancristatus* Baer and Bona, 1960	Palmyra	C-304
Asio brachiatus†	*Paruterina rauschi* Freeman, 1957	Dayr-az-Zawr	C-90
Aythya fuligula	*Hispaniolepis arcuata* (Kowalewskii, 1904) Lopez-Neyra, 1942	Kirikhan	C-194
	Schillerius ransomi (Schultz, 1940) Schmidt, 1985	Kirikhan	C-189
	Confluaria sp.	Kirikhan	C-194A
Aythya marila	*Diploposthe laevis*	Kirikhan	C-222
	Fimbriarioides intermedia	Kirikhan	C-223
	Microsomacanthus sp. (juv.)	Kirikhan	C-231
	Schillerius ransomi	Kirikhan	C-219
Aythya nyroca	*Diploposthe laevis*	Kirikhan	C-340
	Fimbriarioides intermedia	Kirikhan	C-197
Ciconia ciconia	*Anomotaenia discoidea* (Beneden, 1868) Fuhrmann, 1908	Kirikhan	C-179
Circaetus gallicus	*Mesocestoides perlatus* (Goeze, 1782) Mühling, 1898	Kirikhan	C-183
Fulica atra	*Diorchis inflata* (Rudolphi, 1819) Clerc, 1903	Kirikhan	C-167
	Diorchis sp.	Kirikhan	C-168
Gallinago gallinago	*Aploparaksis crassirostris* (Krabbe, 1866) Clerc, 1903	Kirikhan	C-190
	Dicranotaenia calumnacantha (Schmidt, 1963) Schmidt, 1985	Kirikhan	C-191
Himantopus himantopus	*Acoleus vaginatus* (Rudolphi, 1819) Fuhrmann, 1899	Kirikhan	C-204
	Dicranotaenia tzengi (Joyeux and Baer, 1940) Yamaguti, 1959	Kirikhan	C-205
	Diplophallus polymorphus (Rudolphi, 1819) Fuhrmann, 1900	Kirikhan	C-182
Hoplopterus spinosus	*Amoebotaenia brevicollis* Fuhrmann, 1907	Kirikhan	C-200
Nycticorax nycticorax	*Choanotaenia mutabilis* (Linton, 1927) Meggitt, 1933	Kirikhan	C-199
Pelecanus onocrotalus	*Armadoskrjabinia medici* (Stossich, 1890) Spasskii and Spasskaja, 1954	Kirikhan	C-214
Pica pica	*Passerilepis stylosa* (Rudolphi, 1809) Spasskii and Spasskaja, 1954	Dayr-az-Zawr	C-93
Plegadis falcinellus†	*Cloacotaenia megalops* (Nitzsch in Creplin, 1829) Wolfhügel, 1938	Kirikhan	C-188
	Paradilepis urceus (Wedl, 1855) Hsü, 1935	Kirikhan	C-342
	Paradilepis sp.	Kirikhan	C-186
	Retinometra sp.	Kirikhan	C-342A
Sturnus vulgaris	*Variolepis farciminosa* (Goeze, 1782) Spasskii and Spasskaja, 1954	Dayr-az-Zawr	C-87
Turdus merula	*Fernandezia spinosissima* (Linstow, 1894) Lopez-Neyra, 1936	Dayr-az-Zawr	C-88
Rodents			
Psammomys obesus†	*Pseudocatenotaenia matovi* (Genov, 1971) Tenora, Mas-Coma, Murai, and Feliu, 1980	Dayr-az-Zawr	C-159

* HUJP = Hebrew University Jerusalem, Parasitological Collection.
† New host record.

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Research Note

Ectoparasites of the Blackstripe Topminnow, *Fundulus notatus*, from Harrods Creek, Oldham County, Kentucky

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The blackstripe topminnow *Fundulus notatus* (Rafinesque, 1820) is a surface-feeding killifish most often found in rivers and streams of low gradient, with clay or mud bottoms, in the middle and lower Mississippi River drainage system (Braasch and Smith, 1965, Copeia 1965:46–53). In parts of north-central Kentucky, however, it can be collected along the vegetated margins of streams with less turbidity, greater current, and a hard substrate such as Harrods Creek, Oldham County. Fifty-two *F. notatus,* which represented excess capture from another project, were selected at random and examined to determine the ectoparasite fauna of the species near the eastern edge of its range, in what may be less than optimum habitat.

Fish were captured by dipnetting from three collection stations near highway bridges (A—KY 329, B—KY 1694, C—KY 393) between October 17, 1973 and October 5, 1974. Host transport, examination and parasite processing methods were identical to those reported by Kozel and Whittaker (1982, Proc. Helminthol. Soc. Wash. 49:138–139).

Ectoparasites were observed on 12 of 52 (23.1%) of the hosts examined. Two species of ectoparasite were found on 3 (5.8%) fish and 9 (17.3%) fish had one species. Table 1 shows the percent prevalence, intensity, and location of the parasites on *F. notatus*.

Thirty percent (3 of 10) of the hosts from station A, 15.6% (5 of 32) from station B and 40% (4 of 10) from station C harbored ectoparasites. The mean intensity of *Trichodina* sp. infestation on hosts from stations A (5.5) and C (4.0) was elevated compared with station B (1.3). Limnologic characteristics of all three stations were apparently similar with the exception of a small slough-like cul-de-sac located at both stations A and C. Although connected directly with the main channel, the current velocity in the sloughs was reduced (0.01–0.1 m/sec) compared with that in the main channel (0.1–0.5 m/sec). Hosts were seen to be somewhat more concentrated in the slower water and it is likely that opportunities for host–parasite contact and attachment were increased in these areas. Interestingly, the cul-de-sacs at stations A and C more closely resembled optimum habitat for *F. notatus* than did main channel habitat.

No relationship between *F. notatus* standard length ($\bar{x}$ = 28.6 mm, range 22–50 mm) and parasite prevalence or intensity was noticed. Eight of 24 (33.3%) males and 4 of 28 (14.3%) females were parasitized. A slight increase in prevalence and intensity of *Trichodina* sp. was seen in March and April, but no other seasonal variations were observed.

The *Trichodina* sp. recovered was not identified to species, but it most closely resembles *T. funduli* Wellborn, 1967, and represents a new record of the parasite for the host. *Fundulotrema megacanthus* (Wellborn and Rogers, 1967) has previously been seen on *F. notatus* from the Embarrass and Sangamon rivers, Champaign Co., Illinois (Kritsky and Thatcher, 1977, Publicaciones Espec. (4), Inst. Biol., Univ. Nac. Autonom. Mexico, pp. 53–60) and from the starhead topminnow, *F. notti* (Agassiz, 1854) on fins and body in Perry Co., Alabama. *Fundulotrema megacanthus* has also been reported from the blackspotted topminnow, *F. olivaceus* (Storer, 1845) on fins and body in Harrison Co., Mississippi (Wellborn, 1967, Proc. Helminthol. Soc. Wash. 34:55–59). Kritsky and Leiby (1973, Can. J. Zool. 51:1057–1063) reported the presence of *Urocleidus umbraensis* Mizelle, 1938 from *F. notatus* in the Embarrass River, Champaign Co., Illinois. Mizelle (1938, Ill. Biol. Monogr. 17:1–81) had earlier reported the monogenean on *F. notatus* from the same area and also from the Kaskaskia River near Bondville, Illinois (Miz-

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Table 1. Percent prevalence, intensity, and location of ectoparasites recovered from 52 blackstripe topminnows, *Fundulus notatus* (Rafinesque), from Oldham County, Kentucky.

Parasite	Percent prevalence	Intensity (mean and range)	Location
Protozoa			
Trichodina sp.	17.3	3.7 (1–8)	Anal, caudal, dorsal fins
Trematoda			
Fundulotrema megacanthus	5.8	2.3 (1–5)	Dorsal, caudal fins, upper body surface
Urocleidus umbraensis	3.8	1.5 (1–2)	Gills
Crustacea			
Lernea cyprinacea	1.9	1	Upper body surface

elle, 1938, Am. Midl. Nat. 19:465–470). *Urocleidus fundulus* Mizelle, 1940 has been observed on the gills of *F. notatus* from eastern Texas (Meade and Bedinger, 1972, Southwest Nat. 16: 281–295). *Lernaea cyprinacea* Linnaeus, 1761 is known from *F. notatus* in the White River near Petersburg, Indiana (Whitaker and Schlueter, 1975, Am. Midl. Nat. 93:446–450). McKee and Parker (1982, Can. J. Zool. 60:1347–1358) reported 2 of 16 *F. notatus* studied from the North Sydenham River system in extreme southern Ontario were parasitized by *Lernea* sp. Lastly, Shira (1913, U.S. Bur. Fish. Econ. Circ. No. 6, pp. 1–10) mentioned that 1 of 4 *F. notatus* from Caddo Lake, Caddo Co., Louisiana harbored "well-encysted, developed" glochidia approximately 50 × 110 μm on its gills.

During the second week of September 1982 an opportunity to collect *F. notatus* from stations A and B occurred. Five females (S.L. $\bar{x}$ = 32.8 mm, range 28–42 mm) from station A, and three males and two females (S.L. $\bar{x}$ = 30.8 mm, range 25–39 mm) from station B were examined as above. One 34-mm specimen from station A harbored a single *F. megacanthus* at the base of its caudal fin and one *L. cyprinacea* was seen on the anterior ventral body surface of a 32-mm male from station B. The overall percent prevalence drops slightly to 22.6 when the most recent collection data are included.

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Research Note

Scanning Electron Microscopy of *Ollulanus tricuspis* (Nematoda)

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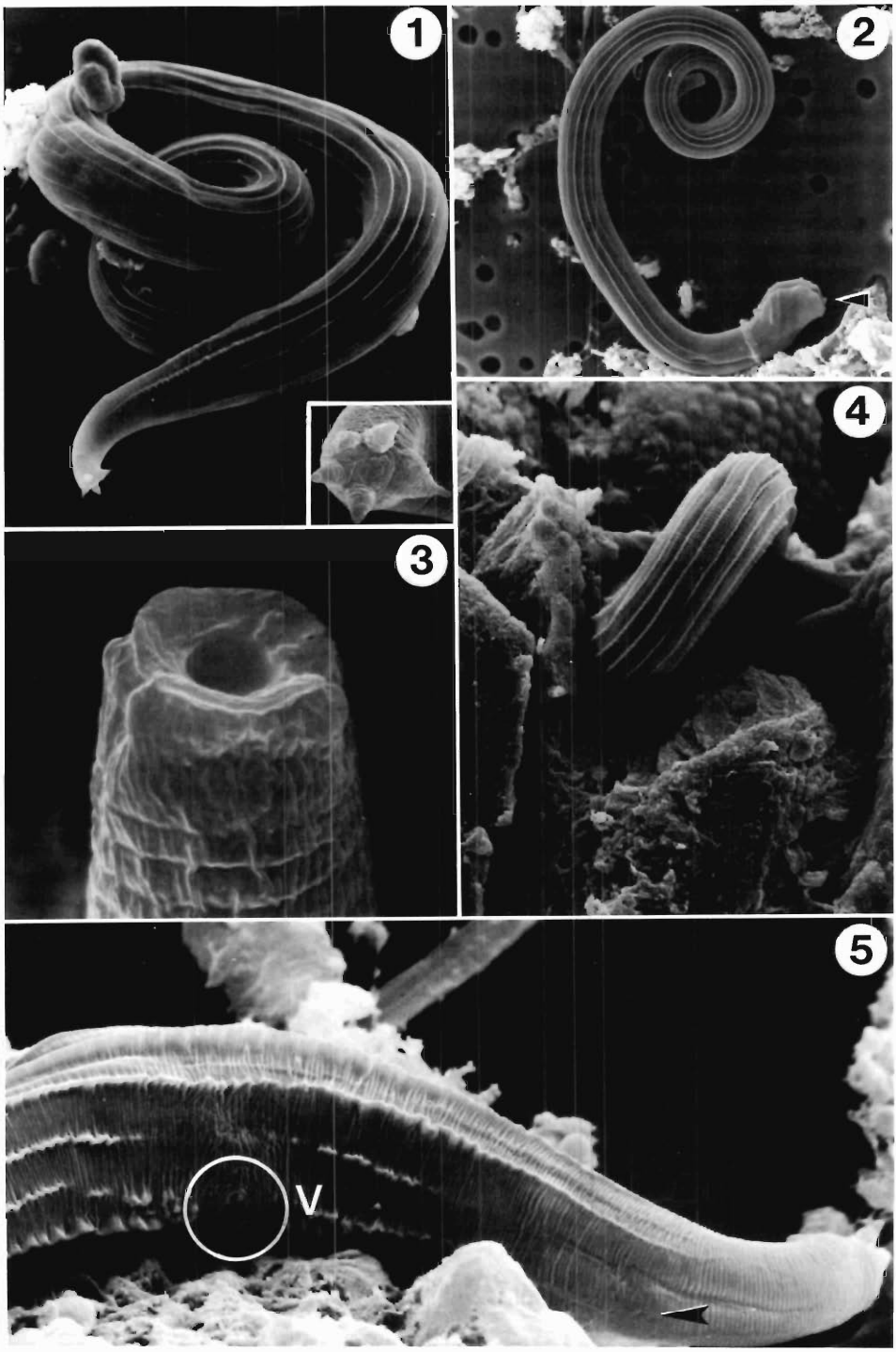
Ollulanus tricuspis is a small gastric nematode (about 1 mm in length) found in domestic cats. Infections have been reported from Australia, New Zealand, England, Germany, and Chile (Bearup, 1960, Austral. Vet. J. 36:352–354; Torres et al., 1972, Arch. Med. Vet. 4:20–29; Collins, 1973, N.Z. Vet. J. 21:175–176; Lewis, 1975, J. Helminthol. 5:171–182; Hasslinger, 1979, Berlmunch. Tierarztl. Wochenschr. 92:316–318), and more recently in the United States (Hargis et al., 1981, J. Am. Vet. Med. Assoc. 178:475–478). The prevalence of infection can be as high as 27% within breeding colonies (Hargis et al., 1983a, Vet. Path. 20:71–79).

According to recent taxonomic reviews (Commonwealth Institute of Helminthology, 1983, Key to Nematode Parasites of Vertebrates), *O. tricuspis* is placed in the superfamily Trichostron-



Figures 1–5. Scanning electron micrographs of *Ollulanus tricuspis*. **1.** Adult female has longitudinal ridges and tail cusps (inset). Many females have more than three tail cusps, ×650. **2.** Adult male has well-developed bursa (arrow). Longitudinal ridges are also present, ×250. **3.** Anterior end of parasite has a small buccal cavity, ×6,500. **4.** Parasite is coiled in crypt of stomach mucosa, ×750. **5.** Longitudinal ridges are interrupted in region of vulva (label). Anus is depicted by arrow, ×2,500.

gyloidea, family Molineidae, and subfamily Ollulaninae. *Ollulanus* is the only genus and *O. tricuspis* is the only species in this subfamily, and is one of the few members of the Trichostrongyloidea that is viviparous. Other differentiating characteristics include its small size and the absence of caudal or cervical spines.

Ollulanus tricuspis has caused gastric lesions ranging from mild catarrhal gastritis to severe fibrosis, with an increase in gastric lymphoid follicles and mucosal globule leukocytes (Hargis et al., 1983a, loc. cit.; Hargis et al., 1982b, Vet. Path. 19:320–323). The absence of eggs or larvae in feces and the small size of adult worms undoubtedly have caused many infections to be overlooked during routine parasitologic examination. Because of the small size of the adult worm, this scanning electron microscope (SEM) study was performed to better identify external morphologic features and appearance of the worm in situ.

Adult *O. tricuspis* obtained from the stomachs of three naturally infected cats and samples of stomach from one severely infected cat were fixed in neutral-buffered formalin. Samples of stomach and isolated worms were processed according to methods described by Seesee et al. (1977, J. Parasitol. 63:1135–1137) for observation using an ISI 60 SEM.

Living and fixed adult *O. tricuspis* were usually coiled (Fig. 1). Male adult *O. tricuspis* had a well-developed bursa (Fig. 2). Many female *O. tricuspis* had between three and six tail cusps. Generally three cusps were prominent projections and one or two were smaller cusps (Fig. 1, inset). Both male and female adult worms had prominent longitudinal ridges (Figs. 1, 2). The anterior end had no cervical spines and the buccal cavity was small (Fig. 3), typical of most Trichostrongyloidea. Examination of the stomach of an infected cat indicated that many of the worms were located in recessed areas of the rugae, closely associated with the gastric mucosa (Fig. 4); often with the anterior end buried deeply into gastric crypts. In the female, the longitudinal ridges were interrupted in the region of the vulva (Fig. 5) and near the anus (arrow).

The observations made in this scanning electron microscope investigation of *O. tricuspis* confirm many of the observations made by Cameron (1927, J. Helminthol. 5:67–80) concerning external morphologic features. These features include the presence of three or more tail cusps on the female, a well-developed bursa in the male, presence of longitudinal cuticular ridges, a small buccal cavity, and the location and appearance of the vulva and anus.

In addition, observations made of parasites in situ provide information on the position of the worm in the stomach that would be difficult to obtain by any other means. The increased mucus production seen in catarrhal gastritis and the fibrosis seen in severe infections with *O. tricuspis* may be a result of mechanical damage caused by the close association of the worm with the gastric mucosa (Hargis et al., 1982b, loc. cit.).

The authors wish to thank Ron Davis of the University of Idaho Electron Microscope Laboratory for his generous help in the preparation of micrographs for this manuscript. Supported in part by Grant RR00515 from the National Institutes of Health.

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Research Note

Cuticular Lesions on Adult *Baylisascaris procyonis* (Nematoda) Passed Naturally from a Raccoon

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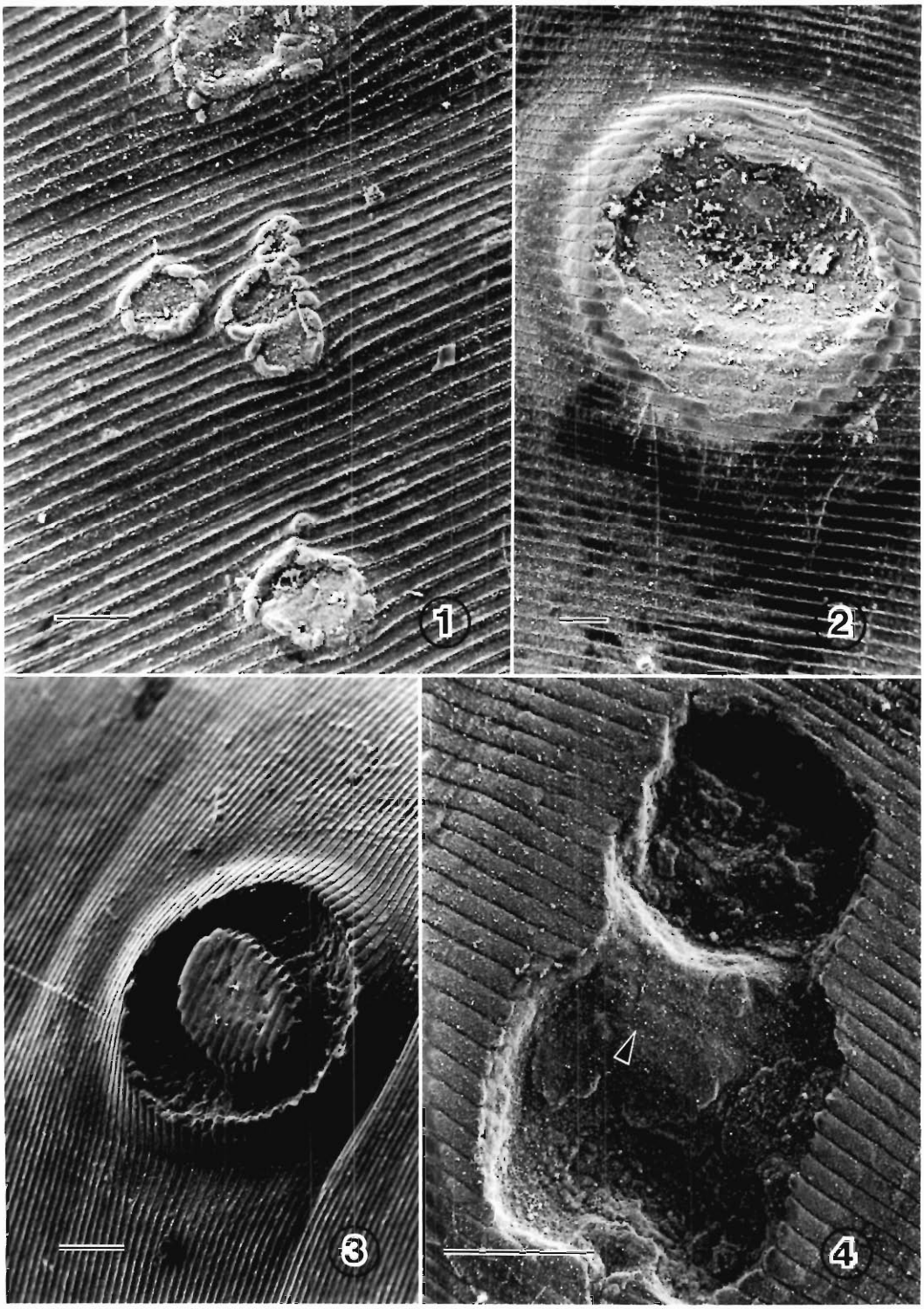
Several *Baylisascaris procyonis* adults, passed naturally from a captive raccoon kept as a source of these ascarid eggs, had numerous grossly visible blackened areas of variable shape and size scattered over the surface of the cuticle. Under a dissecting microscope (×50), these blackened areas on *B. procyonis* appeared as lesions of the cuticle and were of two sizes: smaller and more



Figures 1–4. Cuticular lesions on adult *Baylisascaris procyonis*. Line = 50 μm. Figures 1–3. Cuticular lesions with raised circumscribed peripheries and varying degrees of degeneration in their centers. Figure 4. More extensive cuticular lesion in terms of size, shape, and depth of certain portions.

numerous lesions approximately 0.5–1.0 mm in diameter and larger lesions 1.0–2.0 mm in diameter. In order to better visualize the microtopographic features of the lesions, segments with the blackened areas were processed for examination with the aid of the scanning electron microscope (SEM). The method of preparation was described by Snyder (1983, Ph.D. Dissertation, University of Illinois, Urbana, Illinois. 233 pp.).

The normal epicuticle of *B. procyonis,* as well as several other related ascaridoid nematodes, consists of transverse grooves running parallel between epicuticular ridges (striae). An example of normal epicuticle can best be seen surrounding the cuticular lesion presented in Figure 4. The cuticular lesions, as seen in Figures 1–3, have raised circumscribed peripheries with depressed central cavities in which most or all of the transverse cuticular grooves and striations are no longer evident. In Figure 1, several epicuticular grooves and striations are barely discernable in the centers of these lesions, suggesting that they have not yet extended into the deeper portions of the cuticle. In Figure 2, the single lesion still has grooves and striations along its periphery whereas the center has none of the characteristics of a normal epicuticle, suggesting that this lesion is more advanced than the lesions in Figure 1. The cuticular lesion in Figure 3 has a raised circumscribed periphery with a central island of relatively normal epicuticle.

The cuticular lesion seen in Figure 4 was different from those in Figures 1–3 in that it was deeper, had a different shape and was larger. There are two confluent, almost circular portions to the lesion with the upper portion appearing to be deeper and not containing any remnants of the epicuticle. The lower portion of the lesion appeared to involve both deep and superficial parts of the epicuticle. A portion of a cuticular stria (arrowhead) is partially visible in this part of the lesion.

These cuticular lesions are similar to others reported from several genera of nematodes. Weinberg and Keilin (1912, C. R. Soc. Biol. 73: 260–262) described similar cuticular lesions on *Parascaris equorum,* attributing their formation to "large cocci." Manter (1929, J. Parasitol. 16: 101) described cuticular lesions in *Ascaris suum,* with bacteria resembling *Clostridium welchii* (=*C. perfringens*) as the possible etiologic agent. Lubinsky (1931, Z. Parasitenkd. 3:755–779) reported similar cuticular lesions in eight species of nematodes: *Parascaris equorum, Ascaris suum, A. lumbricoides, Toxascaris limbata, Heterakis perspicillum, Oxyuris curvula, Strongylus equinus,* and *S. vulgaris.* McKinnon and Lubinsky (1966, Can. J. Zool. 44:1090–1091) found cuticular lesions in approximately 2% of *A. suum* examined from swine in Canada. Stewart and Goodwin (1963, J. Parasitol. 49:231–234) isolated the bacteria *Escherichia coli* and *Pseudomonas* sp. and a yeast *Candida* sp. from cuticular lesions of *A. suum* and concluded that only the pseudomonad was capable of causing lesions on the cuticles of healthy worms in vitro. Anderson et al. (1971, J. Parasitol. 57:1010–1014) found two morphologically distinct types of cuticular lesions on *A. suum*; however, they found similar bacterial species associated with both types of lesions and also healthy cuticle. Cuticular lesions on nematodes other than ascarids have also been reported for *Stephanurus dentatus* (Anderson et al., 1973, J. Parasitol. 59:765–769) and *Strongylus edentatus* (Anderson et al., 1978, Proc. Helminthol. Soc. Wash. 45:219–225). Poinar (1973, Proc. Helminthol. Soc. Wash. 40:37–42) described a cuticular infection on *Thelastoma pterygoton,* a nematode parasitic in beetles. Transmission electron micrographs by Poinar showed numerous unidentified microorganisms in close association with the infected cuticle. Due to the close association between the microorganisms and the infected cuticle, the author considered that the microorganisms were capable of dissolving at least part of the nematode's cuticle and establishing colonies on the surface of the nematode.

It is clear that the cuticle of various nematode species may be inhabited by several types of bacteria and/or yeast. In certain instances these microorganisms may be pathogenic to the nematode's cuticle. At the magnifications used in Figures 1–4 neither bacteria nor yeast were evident on either the normal or diseased cuticle. The methods used to process these worms for viewing with the SEM may have precluded one from seeing any bacteria or yeast. The mechanisms that initiate these cuticular lesions are not clearly understood; however, it is likely that host-produced substances, along with mechanical, chemical, and microbial agents, or a combination of these, may play an important role. Anderson et al. (1971, loc. cit.) suggested that if the cuticular integrity is compromised to the extent that the lesions debilitate the worm, then the

outcome may ultimately be fatal. More work is needed to understand the mechanisms that initiate these cuticular lesions of nematode parasites and the role they might play, if any, in causing the expulsion of these parasites from their respective hosts.

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Research Note

Eustrongylides sp. (Nematoda: Dioctophymatoidea): First Report of an Invertebrate Host (Oligochaeta: Tubificidae) in North America

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As adults, nematodes of the genus *Eustrongylides* parasitize piscivorous birds. Infection results in mortality of the avian host (Locke, 1961, Avian Dis. 5:135–138; Locke et al., 1964, Avian Dis. 8:420–427). Third- and especially fourth-stage larvae of *Eustrongylides* spp. are known to parasitize a wide variety of vertebrate intermediate hosts. Freshwater fish are the most common vertebrate intermediate hosts, but fourth-stage larvae from fish will invade the tissues of various reptilian and mammalian hosts (including man) if infected fish are ingested uncooked. An invertebrate as the first intermediate host has been suspected, but has only been confirmed for *Eustrongylides excisus* Jägerskiöld, 1909 in freshwater oligochaetes in the delta of the Volga River (Karmanova, 1965, Trudy Gel'mint. Lab. Akad. Nauk SSSR 15:86–87).

In the Chesapeake Bay area of the United States a frequent vertebrate intermediate host for *Eustrongylides* is the benthic mummichog, *Fundulus heteroclitus* (L.). A recent study of the prevalence of larval *Eustrongylides* in the mummichog (Hirshfield et al., 1983, J. Fish Biol. 23: 135–142) led those authors to hypothesize that an increased prevalence of the nematode in mummichogs in the warmer waters of a power plant might be related to an increased abundance of oligochaetes, the suspected first intermediate hosts. The present report is part of a study of oligochaetes undertaken in areas where mummichogs were infected to determine whether the oligochaetes were infected with larval *Eustrongylides*. This report describes the single third-

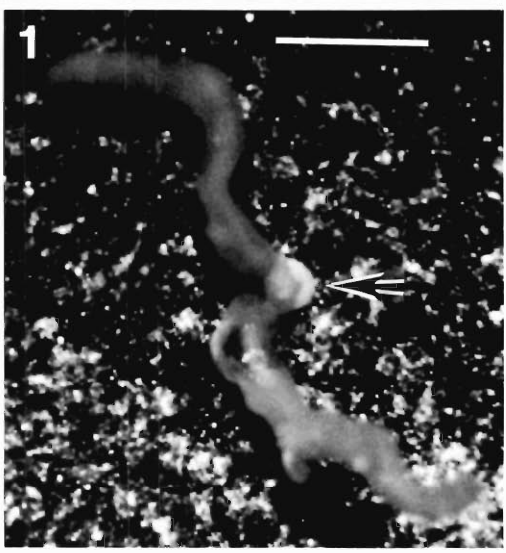
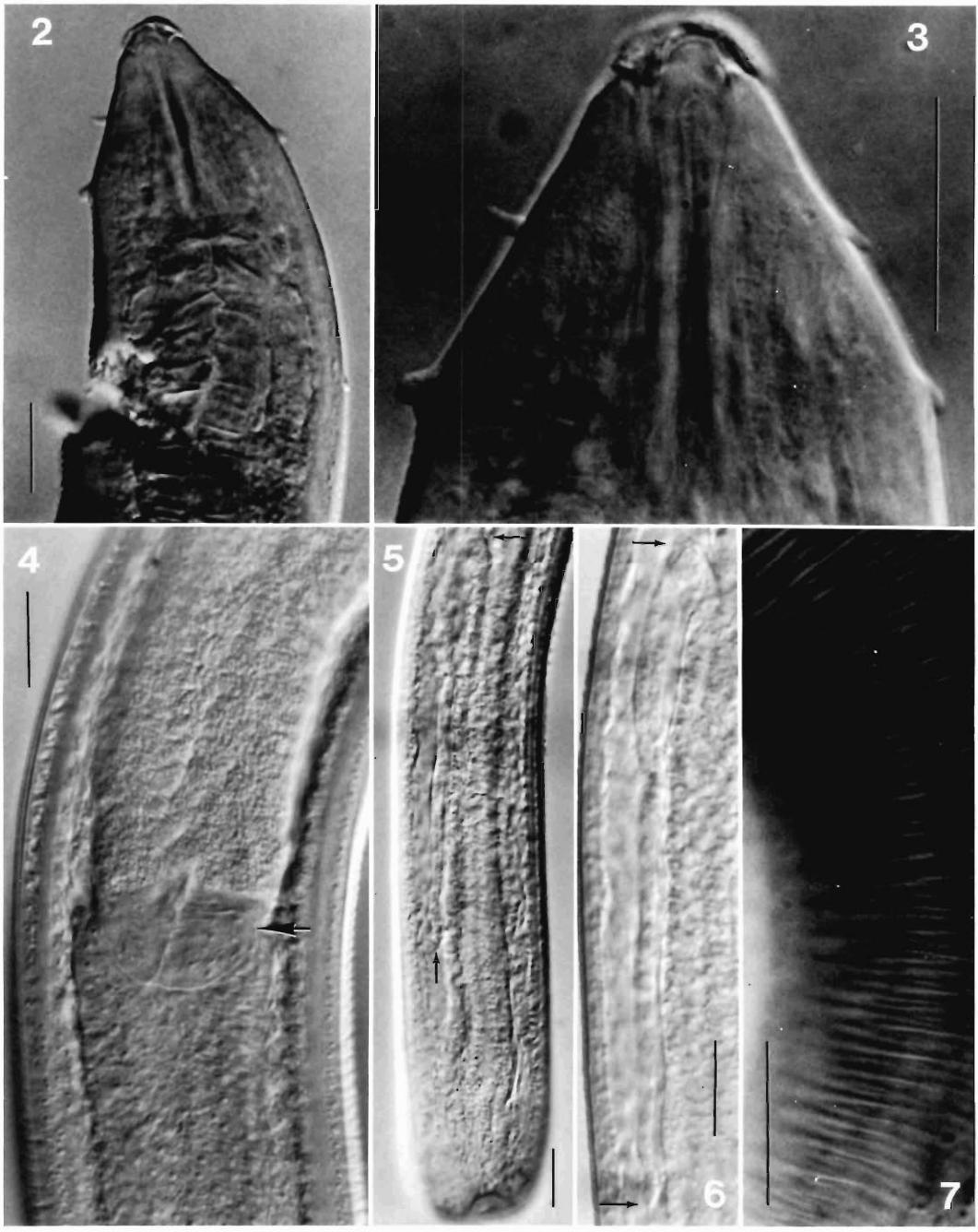


Figure 1. Oligochaete (Tubificidae) ruptured by parasitic nematode, *Eustrogylides* sp. (third-stage larva) (arrow); scale bar, 1 mm.



Figures 2–7. ***Eustrongylides*** **sp. third-stage larva from tubificid oligochaete. Scale bars, 25 μm. 2. Anterior end showing conical head and tubular buccal capsule. 3. Anterior extremity showing spine-like papilla of internal circle and lobe-like papilla of external circle. 4. Esophageal-intestinal valve (arrow) separating esophagus (with granules) from intestine. 5, 6. Posterior end; arrows at anterior flexure and blind terminus of immature reproductive tract (6, higher magnification). 7. Cuticle showing irregular annulation.**

stage larval *Eustrongylides* sp. recovered in the study. This is the first report of a natural infection of an oligochaete by *Eustrongylides* in North America.

The oligochaete host was collected August 16, 1983 from a subtidal fringe marsh in Quantico Creek, near the Possum Point Power Station. Quantico Creek is a small shallow tributary of the Potomac River between Occaquan Bay and Aquia Creek on the Virginia shore. This area is transitional between fresh and oligohaline water (salinity varies annually between 0.0 and 0.5 ppt). The sediment was 95% sand, 5% silt/clay and contained a large amount of organic detritus. A total of 1,767 oligochaetes was collected from seven locations throughout the Maryland portion of the Chesapeake Bay of which 422 were collected at Quantico Creek. Only one of 1,767 oligochaetes examined was infected, and it contained a single *Eustrongylides* third-stage larva (Fig. 1). Because the infected oligochaete was immature and damaged by the nematode, it could not be identified past the family level. The oligochaete was classified as a Tubificidae without capilliform chaetae. All of the mature tubificids collected in the sample were *Limnodrilus* spp., with approximately half identified as *Limnodrilus hoffmeisteri* Claparede and half as *Limnodrilus cervix* Brinkhurst (both typical and variant forms). Therefore, the infected oligochaete was probably a *Limnodrilus*. The remains of the oligochaete and the nematode have been deposited in the U.S. National Parasite Collection, USDA, Beltsville, Maryland as USNM Helm. Coll. No. 78225.

The identification of *Eustrongylides* to species is based on characteristics of adults so we must refer to our larval specimens as *Eustrongylides* sp. However, von Brand and Simpson (1944, J. Parasitol. 30:121–129) obtained an adult male *Eustrongylides ignotus* Jägerskiöld, 1909 from an in vitro culture of larval specimens from *Fundulus heteroclitus* collected in the Chesapeake Bay area. This appears to be the only species of *Eustrongylides* that has been identified from this area.

Fundulus heteroclitus is a non-selective benthic feeder, gaining its energy from animal matter (Prinslow et al., 1974, J. Exp. Mar. Biol. Ecol. 16:1–10), and it is likely that *F. heteroclitus* consumes oligochaetes proportional to their abundance in the study area. However, the feeding habits of *F. heteroclitus* have not been studied in areas of low salinity such as the study area. The low incidence of infection in oligochaetes in the area sampled should not rule them out as normal intermediate hosts for *Eustrongylides*. If large numbers of the oligochaetes are eaten by the fish, a low incidence of infection in the oligochaetes may still be effective in establishing a high incidence of infection in the fish. Such a relationship was demonstrated for a tapeworm, *Echinococcus multilocularis* Leuckart, 1863, with a low incidence of infection in rodents and a high incidence in foxes (Rausch and Schiller, 1951, Science 113:57–58).

The third-stage nematode recovered from the oligochaete is 3.89 mm long with a conical anterior extremity and a bluntly rounded posterior extremity. The mouth is dorsoventrally elongated with one large, broad, lateral lip on each side, and with single, shorter and narrower dorsal and ventral lips. The buccal capsule is thin and 65 μm long (Fig. 2). The conical anterior extremity bears two circles of cephalic papillae. The lateral papillae of both internal and external circles are located more anteriorly than the subventrals and subdorsals. The subdorsal and subventral pairs of the internal circle of six spine-like papillae are located slightly more than half way from the dorsoventrally elongated mouth to the external circle of papillae, which is located 47 μm posterior to the anterior extremity (Figs. 2, 3). The six papillae of the external circle are lobe-like, broader, and protrude less than those of the internal circle (Figs. 2, 3). The esophagus is 1.17 mm long, uniformly thick, occupies two-thirds of the body diameter, and has a prominent cuticular lining and an esophageal–intestinal valve (Fig. 4). The posterior half of the esophagus is full of granules (Fig. 4). The nerve ring is near the anterior end of the esophagus, 100 μm from the anterior extremity. The lumen of the intestine is open and the anus is terminal and recessed (Fig. 5). A reproductive system extends anteriorly 300 μm before reflexing to a narrow blind terminus (Figs. 5, 6). The cuticle is annulated irregularly (Fig. 7). Somatic papillae are not visible on the surface of the cuticle with oil immersion, perhaps due to the annulation, but their presence is indicated by lateral rows of subsurface nuclei in optical section.

Descriptions of third-stage larvae of *Eustrongylides* spp. are extremely rare. To our knowledge

the only previous descriptions are: 1) third-stage *E. excisus* by Karmanova (1965, loc. cit.); 2) third-stage *E. tubifex* by Sprinkle (1973, Thesis, Ohio State Univ. 60 pp.) and by Crites (1982, Clear Technical Report No. 258, Ohio State Univ., Ctr. for Lake Erie Area Res., Columbus, Ohio, July 1982, 83 pp.); and 3) third-stage *E. mergorum* (Rudolphi, 1809) by Fagerholm (1982, Acta Acad. Aboensis, Ser. B 40:11–19). Recently, two third-stage larval *Eustrongylides* sp. from *Fundulus* sp. collected in the Chesapeake Bay area became available for study. They will be described elsewhere (Lichtenfels and Pilitt, 1986, Proc. Helminthol. Soc. Wash. 53. In press) along with a discussion of characters useful for separating third- and fourth-stage *Eustrongylides* sp.

Portions of this work were supported by the State of Maryland Power Plant Siting Program under the technical advisory of the Environmental Research Guidance Committee. Peer review of this report was handled by Dr. David R. Lincicome, Editor, International Goat and Sheep Research, as a courtesy, to protect the confidentiality of the review process and to avoid any appearance of a conflict of interest because the first author is Editor of the Proceedings.

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Research Note

Methods for Long-Term Collection of Fish Feces

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Center for Marine Studies, University of California, Santa Cruz, California 95064

The collection of helminth eggs and protozoan spores from the feces of fish over an extended period is often required in parasite life history studies. For fish several centimeters in length, a separation funnel apparatus can be modified to accommodate a fish (Fig. 1). If a benthic or benthopelagic host is used, a plastic screen can be placed on the bottom of the funnel. The grid must be large enough to allow fecal material to pass through to the collection tube. This method, however, may not be suitable for larger fish or fish that would not readily adapt to the above apparatus. For benthic and benthopelagic hosts, a rubber sheath can be used to collect fecal material. Fish approximately 15 cm in length can be anesthetized and placed in a nonlubricated condom (Fig. 2). Smaller fish can be placed in a physician's finger cot. A small split-shot fishing weight should be placed in the end of the sheath to prevent the tip from floating. The method of securing the sheath depends upon the species of host. Usually the sheath's collar can be secured between the pectoral fin and the opercular cover by a rubber band. Care should be taken that the band not be so tight as to injure the host. The pectoral fin can be placed through a small cut in the sheath. If necessary, the sheath can be further attached by spot glueing (Histoacryl, Tri-Hawk, Los Angeles, California) the collar to the fish. To guard against possible bacterial infection or accelerated fecal decomposition, antibiotics can be added to the sheath without affecting the viability of helminth eggs or protozoan spores. The caudal fin should be checked for pathology due to compression. In a helminth life cycle experiment using *Gillichthys mirabilis* the sheaths were removed and the contents collected weekly for 3 wk with no apparent pathology to the host. The helminth eggs were separated from the feces and mucus using standard concentration techniques.

We thank the staff at Long Marine Lab for their help in this study.

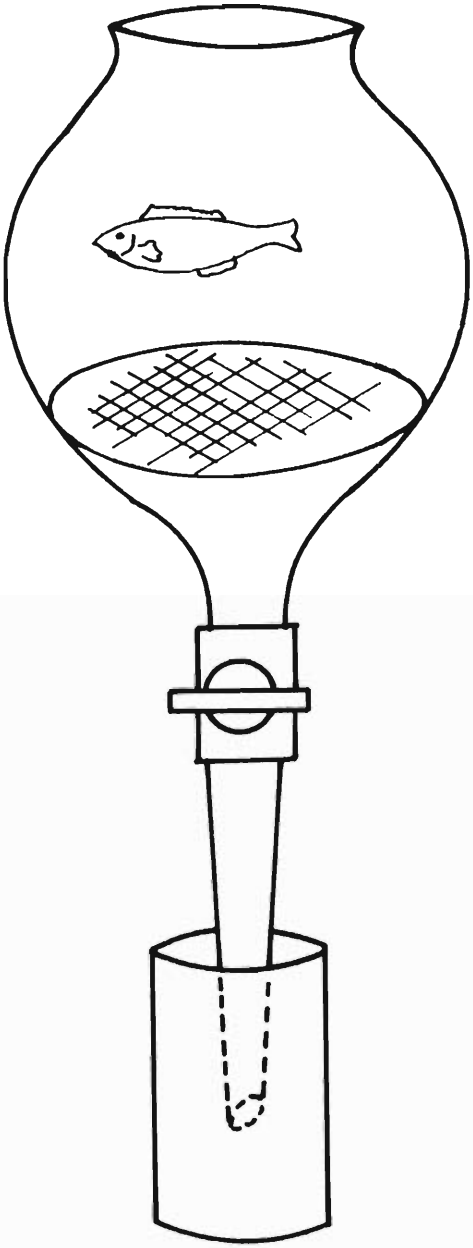

Figure 1. A separation funnel modified by the addition of a plastic grid to hold a small fish.

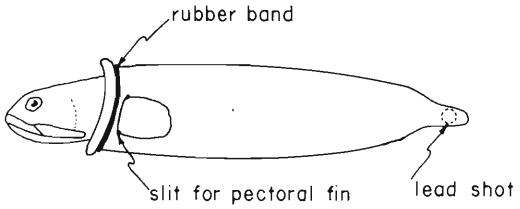

Figure 2. Fish placed in rubber sheath to collect fecal material.

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President's Note on Constitution

In the 1982 volume of these Proceedings (49: 55), the Immediate Past President, Nancy D. Pacheco, announced the incorporation of the Helminthological Society of Washington in the State of Maryland. Inherent in these legal proceedings was the ratification of the Constitution and By-Laws of the Society consistent with the Articles of Incorporation; this was duly accomplished by the membership present at the 541st meeting held on October 16, 1981. Since that time, one constitutional amendment creating the emeritus membership classification has been implemented and was published in the Proceedings in 1984 (51:371). It has been seven years since the Constitution and By-Laws were last published in their entirety (45:144–148 of the Proceedings), and the time is appropriate to remedy that omission. This and the Articles of Incorporation of the Society are presented below. I wish to take this opportunity to express my and the Society's gratitude for the dedication of the numerous members who contributed to the completion of this project. Through their efforts, and the support of the membership in general, our Society continues to provide a model forum of scientific excellence and communication.

WILLIS A. REID, JR.
President

THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON, INC.
CONSTITUTION

The name of the Society shall be the Helminthological Society of Washington.

The object of the Society shall be to provide for the association of persons interested in parasitology and related sciences for the presentation and discussion of items of interest pertaining to those sciences.

BY-LAWS

ARTICLE 1

Membership

Section 1. There shall be four classes of members, namely, regular, life, honorary, and emeritus.

Section 2. Any person interested in parasitology or related sciences may be elected to regular membership in the Society. The privileges and responsibilities of regular members include eligibility to hold office, to vote, and to receive Society publications. Spouses of regular members may apply for election to regular membership with all the privileges and responsibilities except that they will not receive the Society publications and will pay annual dues at a reduced rate.

Section 3. Any person who has rendered conspicuous and continuous service as a member of the Society for a period of not less than 15 years, and has reached the age of retirement, may be elected to life membership. Life members shall have all the privileges of regular members, but shall be exempted from payment of dues. The number of life members shall not exceed five percent of the membership at the time of election.

Section 4. Any person who has attained eminent distinction in parasitology or related sciences may be elected to honorary membership. An honorary member shall have all the privileges of membership except voting, holding office, or having any interest in the real or personal property of the Society, and shall be exempted from the payment of dues. The number of honorary members shall not exceed ten at any one time and not more than one honorary member shall be elected in any one year.

Section 5. Any person who has been a member in good standing for not less than ten years, and who has retired from active professional life, may upon application in writing to the Corresponding Secretary-Treasurer have his membership status changed to emeritus. An emeritus member shall be exempt from payment of dues, and with the exception of receiving the Proceedings, shall enjoy all privileges of membership. An emeritus member, upon payment of 75 per-

cent of the current dues, may elect to receive the Proceedings.

Section 6. Candidates for election to regular membership may be sponsored and proposed only by members in good standing. The candidate shall submit a duly executed and signed application to the Recording Secretary, who in turn shall submit the application to the Executive Committee. The Committee shall review the application and submit its findings to the Society. Voting may be either by voice or by ballot. The Corresponding Secretary-Treasurer shall inform the candidate of the action of the Society.

Section 7. Payment of dues shall be considered as evidence of acceptance of membership in the Society. Election to membership shall be void if the person elected does not pay dues within 3 months after the date of notification of election.

Section 8. Nominations for Honorary and Life Membership, approved by the Executive Committee, shall be submitted to the membership for election at a regular meeting.

ARTICLE 2

Officers

Section 1. The officers of the Society shall be a President, a Vice-President, a Recording Secretary, a Corresponding Secretary-Treasurer, and such other officers as the Society may deem necessary. The four named officers shall also be the Directors of the Society. Only members in good standing and whose dues are not in arrears shall be eligible for election to office. Terms of office shall be one year.

Section 2. The President shall preside over all meetings, appoint all committees except the Executive Committee, and perform such other duties as may properly devolve upon a presiding officer. The President may appoint an Archivist, a Librarian, a Custodian of Back Issues, and an Assistant Corresponding Secretary-Treasurer, as needed.

Section 3. The Vice-President shall preside in the absence of the President, and when so acting shall perform such duties as would otherwise devolve upon the President. The Vice-President shall serve as Program Officer.

Section 4. In the absence of both President and Vice-President, the member, among those present, who last held the office of President shall be the presiding officer. Under other circumstances, members may elect a presiding officer but business action taken shall be reviewed by the Executive Committee.

Section 5. The Recording Secretary shall record the proceedings of all meetings and shall present at each meeting a written report of the transactions of the preceding meeting, shall keep an accurate and complete record of the business transacted by the Society in its meetings, and shall notify the Corresponding Secretary-Treasurer of the election of new members. He shall prepare for publication in the Proceedings an annual digest of scientific meetings and business transacted, including elections of officers and new members.

Section 6. The Corresponding Secretary-Treasurer shall be responsible for all funds, collections, payment of bills, and maintenance of financial records. At the beginning of each year, he shall present to the Society an itemized statement of the receipts and expenditures of the previous year; this statement shall be audited by at least two members of the Society.

ARTICLE 3

Executive Committee

Section 1. There shall be an Executive Committee which shall be the administrative body of the Society.

Section 2. The Executive Committee shall consist of ten members in good standing as follows: The President, Vice-President, Recording Secretary, Corresponding Secretary-Treasurer, Editor, Immediate Past-President, and four Members-at-Large. The Committee shall represent to the fullest practicable degree the varied scientific interests of the Society's membership and the local distribution of its members.

Section 3. The President shall serve as chairman of the Executive Committee.

Section 4. Members-at-large shall serve for a term of two years. Two members-at-large shall be appointed each year in November by the President-elect for the prescribed term of two years.

Section 5. Vacancies occurring on the Executive Committee for any reason shall be filled by appointment by the President, except as otherwise provided, the appointee to serve for the remainder of the unexpired term.

Section 6. The Executive Committee shall carry out the provisions of the Constitution and By-Laws and shall make decisions on all matters of general and financial policy not otherwise set forth in the Constitution and By-Laws and shall report its actions to the Society annually at the last regular meeting.

Section 7. The Executive Committee shall approve the selection of a depository for the current funds, direct the investment of the permanent funds, and act as the administrative body of the Society on all matters involving finance. It shall prepare and present to the Society at the beginning of each calendar year a budget based on the estimated receipts and expenditures of the coming year with such recommendations as may seem desirable.

Section 8. With the presentation of the annual budget the Executive Committee shall present to the Society, if feasible, the estimated cost for publication to be charged to contributors to the Society's publication for that year.

Section 9. Costs of publication, in excess of amounts borne by the Society, shall be borne by authors in accordance with guidelines established by the Executive Committee.

Section 10. The Executive Committee shall pass on all nominations for membership and on the reinstatement of delinquent members, except as otherwise provided, and shall make its recommendations to the Society.

ARTICLE 4

Nomination and Election of Officers

Section 1. The Executive Committee, acting as the Nominating Committee of the Society, shall prepare a slate of officers and present this to the Society at the October meeting of each year. Independent nominations may be made in writing by any five members. In order to receive consideration, such nominations must be in the hands of the Recording Secretary at the time of the election at the November meeting.

Section 2. The election of officers shall be held prior to the presentation of notes and papers at the November meeting. Voting may be either by voice or by ballot.

Section 3. The last order of business at the December meeting shall be the installation of officers, and the naming of officers, and the naming of necessary appointees.

ARTICLE 5

Awards Committee

Section 1. There shall be an Awards Committee to select individuals for special commendation. The Committee shall consist of three members.

Section 2. Members shall serve for a term of 3 years with appointments staggered so that one new member is added each year. The senior member of the Committee shall serve as Chairman.

Section 3. The Awards Committee shall be charged with the duty of recommending candidates for the Anniversary Award which may be given annually or less frequently at the discretion of the Committee.

Section 4. The recipient of the Anniversary Award shall be, or have been, a Society member who is honored for one or more achievements of the following nature: (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.

Section 5. The individual recommended shall be subject to approval by the Executive Committee.

ARTICLE 6

Editorial Board

Section 1. There shall be an Editorial Board for the Society's publications, which shall include *The Proceedings of the Helminthological Society of Washington.*

Section 2. The Editorial Board shall consist of an Editor and other members in good standing, representing to the fullest practicable degree the varied scientific interests and the employment-group affiliations of the Society's membership.

Section 3. The Editor shall be elected by the Society for a term of five years on nomination by the Executive Commmittee.

Section 4. Other members of the Editorial Board shall be appointed for terms of three years.

Section 5. The Editor, after consultation with the Editorial Board, shall appoint new members,

formulate publication policies, and make all decisions with respect to format and content of the Society's publications. The Editor shall operate within financial limitations determined by the Executive Committee.

ARTICLE 7

Publication

The publications of the Society shall be issued at such times and in such form as the Society through its Editorial Board may determine.

ARTICLE 8

Meetings

Section 1. Meetings of the Society shall be held monthly during January, February, March, April, May, October, November, and December, the time and place to be determined by the officers of the Society.

Section 2. The October meeting shall be known as the Anniversary Meeting and the Anniversary Award, when made, ordinarily shall be presented at this meeting.

Section 3. Notice of the time and place of meetings shall be given by the Recording Secretary at least ten days before the date of the meeting.

ARTICLE 9

Procedure

The rules contained in Robert's *Rules of Order,* Revised, shall govern the Society in all cases to which they are applicable, and in which they are not inconsistent with the By-Laws or the special rules of order of this Society.

ARTICLE 10

Order of Business

Call to order.
Reading of minutes of previous meeting.
Election of new members.
Reports of committees.
Unfinished business.
New business.
Presentation of notes and papers.

ARTICLE 11

Quorum

The members in attendance at any regular meeting shall constitute a quorum.

ARTICLE 12

Dues and Debts Owed to the Society

Section 1. Annual dues for regular and spouse members shall be fixed by the Executive Committee, subject to ratification by the Society. Spouse members will pay dues at a reduced rate.

Section 2. The fiscal year for payment of dues and for all other business purposes shall be the same as the calendar year, that is, from 1 January to 31 December, and dues shall be payable on or before 1 January. The dues of a newly elected member paid prior to 1 July of the year of his election shall be credited to that year; if paid after 1 July, they shall be credited either to the current fiscal year or to the following one, at the option of the new member. The dues shall include subscription to the Society's publication; only those members whose dues are paid shall receive the publication.

Section 3. All other obligations owed to the Society by members or nonmembers shall be due and payable 30 days after bills are rendered; the further extension of credit to those whose obligations are in arrears shall be a matter for decision by the Executive Committee.

ARTICLE 13

Suspension and Reinstatement

Any member whose dues are in arrears for two years shall be dropped from membership. Members who have been dropped for nonpayment of dues may be reinstated automatically upon payment of the dues in arrears and the dues for the current year, or may be otherwise reinstated by action of the Executive Committee.

ARTICLE 14

Provision for Dissolution of Funds

In the event the Society is disbanded, all monies shall be presented to the Trustees of the Brayton Howard Ransom Memorial Trust Fund for such purposes as that continuing body may deem advisable.

ARTICLE 15

Amendments to the By-Laws

Any amendment to these By-Laws shall be presented in writing at a regular meeting. It shall not be acted upon until the following meeting. A two-thirds vote of the members in attendance shall be required for adoption.

ARTICLES OF INCORPORATION
OF
THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON, INC.
(A Non-stock Corporation)

FIRST, I, the undersigned, Charles A. Dukes, Jr., whose post office address is 300 Landover Mall West, Landover, Maryland 20785, being at least twenty-one years of age, do hereby form a corporation under and by virtue of the General Laws of the State of Maryland.

SECOND: The name of the corporation (which is hereinafter called the Corporation) is The Helminthological Society of Washington, Inc.

THIRD: The purposes for which the Corporation is formed are as follows:

(a) To provide for the association of persons interested in parasitology and related sciences for the presentation and discussion of items of interest pertaining to those sciences.

(b) To advance the science of parasitology, in both its fundamental and its economic aspects; to act as an agency for the exchange of information; to hold regular meetings and to promote and extend knowledge in all phases of parasitology.

(c) And generally to carry on any other business in connection therewith not contrary to the laws of the State of Maryland, and with all the powers conferred upon non-profit corporations which are contained in the General Laws of the State of Maryland.

FOURTH: The post office address of the principal office of the Corporation in this state is 9110 Drake Place, College Park, Maryland 20740. The name and post office address of the resident agent of the Corporation in this state is A. Morgan Golden, 9110 Drake Place, College Park, Maryland 20740. Said resident agent is a citizen of this State and actually resides herein.

FIFTH: The Corporation is not authorized to issue capital stock.

SIXTH: The number of directors of the Corporation shall be four which number may be increased or decreased pursuant to the By-Laws of the Corporation, but shall never be less than three; and the names of the directors who shall act until their successors are duly chosen and qualified are Nancy D. Pacheco, Louis S. Diamond, Sherman S. Hendrix and Milford N. Lunde.

SEVENTH: The duration of the Corporation shall be perpetual.

IN WITNESS WHEREOF, I have signed these Articles of Incorporation on the 3rd day of November, 1981. I acknowledge these articles and this signature to be my act.

WITNESS:

[signed]	[signed]
Gary Greenwald	Charles A. Dukes, Jr.

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MINUTES

Five Hundred Sixty-Fifth Through Five Hundred Seventy-Second Meetings

565th Meeting: Naval Medical Research Institute, Bethesda, MD, 10 October 1984. A moment of silence was observed in honor of deceased former members John Bucheit and Marietta Voge. Richard Beaudoin presented the Anniversary Award to Harley G. Sheffield. The following slate of officers was presented to the society: President, Willis A. Reid; Vice-President, Ralph P. Eckerlin; Recording Secretary, Jeffrey W. Bier; and Corresponding Secretary-Treasurer, Michael D. Ruff. LTC Bryce C. Redington presided over the following papers: "Characterization of *Schistosoma mansoni* surface antigens by lectin affinity chromatography," Eugene Hayunga; "Knobs and sequestration in vivo in *Plasmodium falciparum,*" Elizabeth Peterson; and "Studies on the Brazilian snail host of *Schistosoma mansoni,*" Edward Michelson.

566th Meeting: Animal Parasitology Institute, USDA, Beltsville, MD, 14 November 1984. J. R. Lichtenfels reported that the January 1985 issue was sent to Allen Press. The slate of officers presented at the 565th meeting was elected. Dr. Joan K. Lunney presided over the following papers: "The status of trichinosis research in the United States: areas of study and goals," Ray Gamble; "Multi-disciplinary research on the biology of coccidia: the discipline and the findings," M. D. Ruff; "Morphogenesis of developmental stages of *Dirofilaria immitis* in the dog," J. R. Lichtenfels, P. A. Pilitt, K. G. Powers, and T. Kotani; and "Characterization of bovine interleukin II," L. Gasbarre.

567th Meeting: Plant Protection Institute, USDA, Beltsville, MD, 12 December 1984. Cosponsor, Oxford Biological Laboratory, Oxford, MD. Dr. Raymond V. Rebois presided over the following papers: "*Caenorhabditis elegans,* sperm development," S. G. Ward; "Movement and dispersal of disease agents in mariculture systems," Aaron Rosenfield; "Disease and parasitism in gills of the winter flounder *Pseudopleuronectes americanus,*" T. K. Sawyer and E. J. Lewis; "Ultrastructure of regeneration of stylets of infective juveniles of *Heterodera glycines,*" B. Y. Endo; and "Spaceflight of nematodes," Christine Choate.

568th Meeting: The National Institutes of Health, Bethesda, MD, 14 January 1985. M. D. Ruff presented the proposed 1985 budget to the membership. Dr. Allen Cheever presided over the following papers: "Evidence of antigenic diversity of *P. falciparum* isolates from The Gambia as assayed by serum induced agglutination of parasitized RBC's," J. Sherwood, M. Marsh, and R. J. Howard; "Tricyclic antidepressants and antipsychotic drugs cause lethal disruption of membrane function in *Leishmania,*" Dan Zilberstein; "Cultured schistosomulae undergo an intrinsic loss in susceptibility to immune killing while retaining their surface antigenicity," Edward Pearce; and "Cloning by expression-screening of 2 genes encoding major schistosome antigens," David Lanar.

569th Meeting: Naval Medical Research Institute, Bethesda, MD, 13 February 1985. Cosponsor, Food and Drug Administration. The report of the audit committee on the 1984 balance sheet was presented to the membership. R. Beaudoin presided over the following papers: "Serodiagnosis of anisakiasis," Richard Raybourne; "Micro ELISA for detection of malarial sporozoites: a novel 2 site assay," L. V. Peters and F. W. Carson; and "Cerebral malaria in Jayapura, Irian Jaya, Indonesia," Stephen L. Hoffman.

570th Meeting: Walter Reed Army Institute of Research, Washington, DC, 13 March 1985. Cosponsor, Armed Forces Institute of Pathology. The business advisory committee's recommendation to not solicit advertising for the Proceedings was accepted. J. R. Lichtenfels announced the January issue of the Proceedings was mailed 7 March 1985. James R. Palmieri presided over the following papers: "Lymph node changes in silvered leaf monkeys infected with *Wuchereria bancrofti* (experimental) and *Wuchereria kalimantani* (natural)," J. R. Palmieri and D. H. Connor; "In vitro antimalarial activity of Quinghaosu," U. K. Milhous and D. L. Clayman;

"Massive infection in human ascariasis," J. K. Baird and D. H. Connor; "Isolation and characterization of an extracellular acid phosphatase in *Leishmania donovani,*" J. K. Lovelace and M. Gottleib; and "*Plasmodium falciparum*: continuous culture in a plasma free modified 1640 culture medium," G. P. Millet, J. Canfield, and W. A. Reid.

571st Meeting: The Johns Hopkins University, Baltimore, MD, 17 April 1985. Howard Goodman presided over the following presentations: "Processing of the 'C' terminus of trypanosome variant surface glycoprotein," James Bangs; "Limiting effects of O_2 on *Schistosoma mansoni* egg survival in vitro and in vivo," Gerald Feldman; and "Antigenic peptides on the surface of *Dirofilaria immitis* microfilariae," Wesley Tamashiro.

572nd Meeting: University of Pennsylvania New Bolton Center, Kennett Square, PA, Joint Meeting with the New Jersey Society for Parasitology, 11 May 1985. Ralph Eckerlin announced the tentative meeting schedule for next year. G. A. Schad presided over a symposium, "Host reactions to parasitism: recent observations and some underlying mechanisms," which consisted of the following presentations: "*Trichinella* and structural change in mammalian muscle fibers: observations on a non-malignant transformation process," Dickson D. Despommier; "Parasites, macrophages and cachectin, a monokine that induces a catabolic state," Anthony Cerami; and "Parasitism and haematopoiesis," Leon Weiss.

Respectfully submitted,
JEFFREY W. BIER

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