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Aestabdella leiostomi sp. n. (Hirudinea: Piscicolidae) from the Gills of Spot, *Leiostomus xanthurus*, in the Lower Chesapeake Bay, Virginia¹

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ABSTRACT: *Aestabdella leiostomi* is characterized externally by a subcylindrical to flat, unpigmented body lacking tubercles, gills, and pulsatile vesicles; a large, very deeply cupped caudal sucker and 2 pairs of poorly formed eyes on the oral sucker are present. Internal anatomy is characterized by 2 pairs of esophageal diverticula, 5 pairs of testisacs, large bursa, paired conducting tissue strands connecting copulatory zone and ovisacs, expansive coelomic system lacking testicular sinuses, and 10 pairs of large nephridia. This leech is the first member of the genus from the Atlantic Ocean; it is known only from spot collected during summer from the lower Chesapeake Bay and from the Gulf of Mexico near Pascagoula, Mississippi.

KEY WORDS: *Aestabdella leiostomi* sp. n., Hirudinea, parasitology, taxonomy, *Leiostomus xanthurus*, Chesapeake Bay, Gulf of Mexico, western North Atlantic Ocean.

The marine leeches of the east coast of the United States have been relatively well studied (Sawyer et al., 1975; Appy and Dadswell, 1981; Burreson and Zwerner, 1982; Sawyer, 1986) and, thus, it was somewhat surprising to discover an undescribed species commonly infecting the abundant spot, *Leiostomus xanthurus* Lacépède (Sciaenidae), in the lower Chesapeake Bay. The leech has previously been reported as *Piscicola funduli* Pratt in a popular article on marine leeches from the Gulf of Mexico (Causey, 1954). Causey presented a photograph of a whole mount of a leech from the gill cavity of spot collected near Pascagoula, Mississippi. Sawyer et al. (1975) believed that the leech photographed by Causey was the same as their new species, *Malmiana philotherma*; however, none of the specimens of *M. philotherma* they collected was from spot. In addition, the very large, deeply cupped caudal sucker of the leech photographed by Causey (1954) resembles very closely the caudal sucker of specimens collected from spot in Chesapeake Bay. The type specimens of *M. philotherma*, on the other hand, all have smaller, much less deeply cupped caudal suckers. The similarities in morphology and host between the leeches collected by Causey and those collected by us lead us to conclude that they are the same species, described below.

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Materials and Methods

Leeches were collected during summer of 1987 from the gills of spot, *Leiostomus xanthurus*. Specimens were relaxed in weak alcohol and fixed in 10% neutral-buffered formalin; some were stained with Semichon's acetocarmine and mounted whole. Specimens to be sectioned were relaxed in weak alcohol and fixed in Bouin's fluid. Two complete series of frontal, sagittal, and transverse sections were cut at 6 μ m and stained with hematoxylin and eosin. Figures were drawn with the aid of a camera lucida. Measurements are in millimeters unless stated otherwise. Side by side comparisons of preserved specimens were made with the holotype (USNM 51481) and paratypes (USNM 51482, 51483, 51484) of *Malmiana philotherma* Sawyer, Lawler, and Overstreet.

Results

Leeches were most abundant near the mouth of the Chesapeake Bay below the Bay Bridge-Tunnel; however, specimens have been recovered from spot collected at the mouth of the York River. Spot were collected in most tows during a cruise in July 1987 that covered most of the Virginia portion of the Chesapeake Bay and the lower reaches of the major tributaries, but leeches were only recovered in the lower portion of the Bay near the Bay Bridge-Tunnel. During August 1987, 19 leeches were recovered from 10 of 73 spot examined, a prevalence of 13.7%. Intensity ranged from 1 to 4 leeches per host. All infested spot were collected in a single tow just inside the mouth of the Chesapeake Bay off Cape Charles, although many spot were collected at other locations in the lower Bay on the same day. Leeches were usually attached to the floor of the

opercular cavity under the filaments of the last gill arch, but were occasionally found attached to the gill filaments themselves.

Spot, *L. xanthurus*, and croaker, *Micropogonias undulatus* (Linnaeus), were collected in coastal waters between Cape Fear and Cape Hatteras, North Carolina, in September and March, and between Cape Hatteras and Delaware Bay in September during National Marine Fisheries service ground fish surveys aboard *R/V Albatross IV*. No leeches were recovered from the 141 spot and 156 croaker examined.

Family Piscicolidae

Subfamily Platybdellinae

Genus *Aestabdella* Burreson, 1976

Aestabdella leiostomi sp. n.

(Figs. 1–5)

DIAGNOSIS: Body elongate, subcylindrical to flat, up to 11 mm total length, lacking papillae, tubercles, gills and pulsatile vesicles. Midbody segments 6(12) annulate. Caudal sucker large and deeply cupped, wider than maximum body width; oral sucker well developed with 2 pairs of poorly formed eyes. Body and suckers usually unpigmented and appearing white to cream-colored. Occasionally very faint black segmental bands on urosome and 13 triangular pigment bands on the caudal sucker radiating from urosome/sucker junction toward outer edge of sucker. Coelomic system consisting of ventral and dorsal sinuses with expansive segmental connecting sinuses that ramify extensively dorsally and laterally to fill most spaces among clitellar gland cells. Testicular sinuses are absent. The presence of lateral sinuses could not be confirmed with certainty. Male reproductive system with 5 pairs of testisacs, accessory gland cells surrounding ejaculatory ducts and atrial cornua, and moderately large bursa. Paired strands of conducting tissue connect copulatory zone and ovisacs. Ten pairs of large nephridia open laterally from XIV through XXIII.

TYPE SPECIMENS: Holotype (USNM 132421)

and 10 paratypes (USNM 132422) deposited in Division of Worms, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560.

TYPE HOST: Spot, *Leiostomus xanthurus* Lacépède.

TYPE LOCALITY: Mouth of Chesapeake Bay, Virginia, U.S.A., 37°10'N, 76°00'W. Salinity: 30 ppt. Temperature: 28°C.

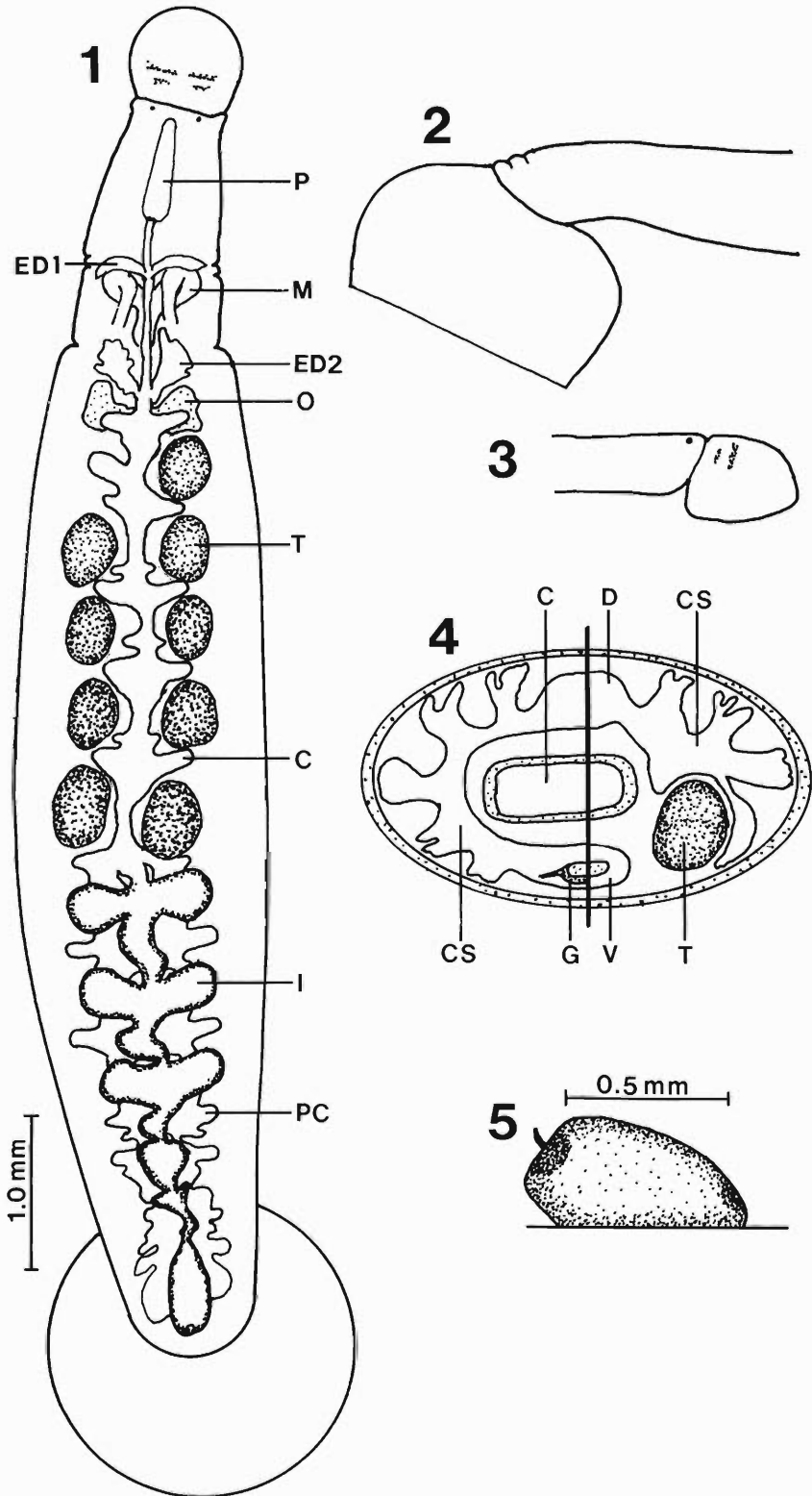
ETYMOLOGY: Named for the fish host.

EXTERNAL CHARACTERS (Figs. 1–3; measurements of holotype given first, with measurements of largest specimen in parentheses): Body elongate, subcylindrical to flat, especially after relaxation in weak alcohol; distinctly divided into trachelosome, clitellum, and urosome. Body surface smooth, lacking tubercles, papillae, gills, or pulsatile vesicles. Trachelosome usually devoid of pigment except for paired punctiform black spots dorsally and ventrally on the first and last segments. Clitellum narrower than posterior portion of trachelosome or anterior portion of urosome; first annulus of clitellum wide and distinctly demarcated. Urosome usually unpigmented except for 12 pairs of segmental punctiform black spots dorsally and ventrally. First segment of urosome often has no spots resulting in only 11 pairs in some individuals. Some individuals have faint black pigment in the form of segmental bands on the trachelosome and urosome. Total length, inclusive of suckers, 8.5 (11.0); maximum width 1.5 (2.0). Mouthpore centrally located in discoid oral sucker, 0.5 (0.8) in diameter, and eccentrically attached to trachelosome. Oral sucker unpigmented except for 2 pairs of poorly formed, diffuse eyes that appear as thin, straight, or slightly curved lines (Fig. 1). Eyes occasionally very close together, sometimes appearing as 1 pair. Caudal sucker large and very deeply cupped (Fig. 1, 2), 1.6 (2.2) in diameter, usually unpigmented, but occasionally with 13 faint black pigment bands radiating outward from urosome/sucker junction.

DIGESTIVE SYSTEM: Mouthpore centrally located in oral sucker. Proboscis extending to gan-

→

Figures 1–5. *Aestabdella leiostomi* sp. n. 1. Dorsal view of *A. leiostomi* drawn from a whole mount showing general body shape and portions of the reproductive and digestive systems, especially the position of the first (ED1) and second (ED2) pairs of esophageal diverticula. C, crop; I, intestine; M, terminal portion of male reproductive system; O, ovisac; P, proboscis; PC, postceca; T, testisac. 2. Caudal sucker, lateral view. Scale as for Figure 1. 3. Oral sucker, lateral view. Scale as for Figure 1. 4. Diagrammatic reconstruction of the coelomic system based on transverse sections. C, crop; CS, connecting sinus; D, dorsal sinus; G, ganglion; T, testisac; V, ventral sinus. 5. Cocoon, lateral view.



gion in IX; salivary glands located between ganglia in VII and IX. Typical esophageal diverticula emerge from crop in posterior portion of XI and project anteriorly to the level of the ganglion in XI, lying dorsal to ejaculatory bulbs. An unusual, second pair of esophageal diverticula emerge in the anterior portion of XIII and project anteriorly to the level of the ganglion in XII (Fig. 1). These large diverticula did not contain a portion of the blood meal, but were filled with a granular, basophilic material similar to that of the more typical esophageal diverticula located in segment XI. The crop lumen expands between the testisacs in the form of paired diverticula (Fig. 1). Usually there is a second, smaller pair of diverticula (Fig. 1), but these often appeared to be obliterated by the testisacs in large individuals. The intestine and postceca originate immediately posterior to the ganglion in XIX. The intestine has 3 pairs of bulbous diverticula and a series of smaller compartments prior to a tubular rectum (Fig. 1). The postceca are fused with fenestra at each ganglion.

REPRODUCTIVE SYSTEM: Five pairs of large testisacs located intersegmentally in XIV/XV. Vasa deferentia enlarge in XIII and enter loosely coiled epididymides in anterior portion of XIII, continue anteriorly, and become confluent with thick-walled ejaculatory bulbs. At ganglion in XI, ejaculatory bulbs bend ventrad and enter atrial cornua. Terminal portions of ejaculatory bulbs and dorsal portions of atrial cornua covered with accessory gland cells. Atrial cornua merge into common atrium that opens to moderately large bursa that terminates as the male gonopore in the anterior portion of XII.

Female reproductive system typical, with paired, convoluted ovisacs that merge into common oviduct and open through the female gonopore in the posterior portion of XII. Paired, narrow ducts of conducting tissue originate from the ventral body wall at the level of the ganglion in XIII and fuse with ventral portion of each ovisac in the posterior portion of XIV. Cocoons (Fig. 5) measure 0.7 long by 0.4 wide; 20 were deposited overnight around 1 isolated leech.

COELOMIC SYSTEM: Coelomic system consists of dorsal and ventral sinuses and, probably, lateral sinuses; presence of the latter could not be confirmed with certainty. No testicular sinuses. Connecting sinuses between the dorsal and ventral sinuses ramify extensively in the region of the ganglion in each segment to occupy most of the space among the clitellar gland cells (Fig. 4).

Ramifications of the connecting sinuses extend laterally to the position typically occupied by lateral sinuses, suggesting that lateral sinuses are present. Intersegmentally, the dorsal sinus ramifies ventrolaterally, but does not connect with the ventral sinus (Fig. 4).

EXCRETORY SYSTEM: Ten pairs of large nephridia occur intersegmentally in XIII/XIV through XXII/XXIII. The single large trunk of each nephridium originates near the ventral body wall in the posterior portion of the segment preceding the segment in which the nephridium opens through the body wall. Ciliated funnels were not observed. Each nephridium passes posteriorly near the ventral body wall until about the level of the ganglion in the next segment posteriorly where it bends dorsally and then laterally to occupy a mid-lateral position. The nephridium trunk enters a muscular bladder that opens through the body wall just posterior to the ganglion. Thus, the nephridium that originates in segment XIII opens to the outside just posterior to the ganglion in XIV.

Discussion

This leech fits readily in the genus *Aestabdella*, which is characterized by a smooth, subcylindrical to flat body with well-developed suckers, poorly formed eyes, 7(14)-annulate somites, extensive coelomic system, 5 pairs of testisacs, the presence of conducting tissue, and 10 pairs of large nephridia (Burreson, 1976). The new species described here differs in having 1) mid-body segments that are 6(12)-annulate; 2) a coelomic system with extensive ramifications of connecting sinuses; but 3) lacking testicular sinuses.

Aestabdella leiostomi is the first member of the genus known from the Atlantic Ocean. The other 2 members of the genus, *A. abditovesiculata* (Moore) and *A. platycephali* (Ingram), are known from the eastern North Pacific (Hawaii and the west coast of the United States) (Moore, 1952; Burreson, 1976) and from Tasmania (Ingram, 1957), respectively. In addition to host and geographical location, and characters listed above, *A. leiostomi* differs from the other members of the genus by the presence of a second pair of esophageal diverticula posterior to the bursa, a larger, much more deeply cupped caudal sucker, and greatly reduced pigmentation.

In the lower Chesapeake Bay, *A. leiostomi* has only been recovered from spot collected from June through September. Leeches were not recovered from spot collected offshore in the mid-

dle Atlantic region, or from croaker collected in the estuary or offshore. Causey (1954) collected specimens from spot collected "offshore" near Pascagoula, Mississippi. However, the number of hosts examined is small and the host and geographic range and temporal distribution of the leech have not been adequately determined.

Externally, *A. leiostomi* superficially resembles *M. philotherma*, which may be present in the same geographical area, although not reported from the same host. The 2 species can be easily distinguished on the basis of caudal sucker size and shape. The caudal sucker of *M. philotherma* is a shallow disc about the same diameter as the maximum body width. The caudal sucker of *A. leiostomi* is very deeply cupped and the diameter is greater than the maximum body width.

Burreson (1989), in a comparison of the new genus *Richardsonobdella* with *Aestabdella*, erroneously stated that *Aestabdella* lacked conductive tissue. Both *Richardsonobdella* and *Aestabdella* possess conductive tissue; thus, the 2 genera are more closely related than previously believed. However, *Richardsonobdella* is still justified because it lacks large, segmental nephridia.

Acknowledgment

We thank Beth McGovern for collecting leeches during a cruise of the NOAA ship *R/V Ferrel* in Chesapeake Bay.

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Accessorius peruensis gen. et sp. n. (Monogenea: Gyrodactylidea) from *Lebiasina bimaculata* (Characidae) in Peru

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ABSTRACT: *Accessorius peruensis* gen. et sp. n. (Gyrodactylidea: Polyclithrinae) is described from *Lebiasina bimaculata* (Characidae) in the Chicama River of Peru. *Accessorius* is identified by having 2 anterolateral, tubular-shaped accessory sclerites lying beside the hamulus root. The haptor is rectangular in shape, with marginal hooks I–III located in 2 anterolateral groups ahead of hooks IV–VIII. The penis is bulbous and armed with 1 large hooked spine and a single row of small spines. *Accessorius* is placed tentatively in the Polyclithrinae Rogers, 1967, because of its similarity to *Polyclithrum* Rogers, 1967, and *Swingleus* Rogers, 1969. *Accessorius* is the fifth gyrodactylid genus known to occur on South American freshwater fishes.

KEY WORDS: *Accessorius peruensis* gen. et sp. n., Monogenea, Gyrodactylidea, *Lebiasina bimaculata*, Peru.

During a study of gyrodactylid parasites of freshwater fishes of Peru, an undescribed species within a new genus was found. It is described herein as *Accessorius peruensis* gen. et sp. n.

distributed unevenly, with I–III located anterolaterally in 2 groups set away from IV–VIII. Parasites of freshwater teleosts.

TYPE SPECIES: *Accessorius peruensis*.

Materials and Methods

Host fishes were netted in the Chicama River, Ascope Province, Peru. The parasites were collected according to the method described by Mizelle and Kritsky (1967). Fixed worms were mounted unstained in glycerine jelly. Measurements, in micrometers, of the holotype are followed in parentheses by those of the paratypes. Descriptive terminology follows Jara and Cone (1989).

Results

Accessorius gen. n.

DIAGNOSIS: Gyrodactylidae Cobbold, 1864; Polyclithrinae Rogers, 1967. Body elongate with 2 cephalic lobes. Bulbous pharynx, short esophagus, intestinal crura ending blindly. Viviparous reproduction. Bulbous penis with large terminal hook and single row of small spines. Round testis immediately posterior to ovary. One pair of hamuli. Single superficial and deep bars. Pair of tubular accessory sclerites lateral to each hamulus root. Haptor with 16 marginal hooks (8 pairs)

Accessorius peruensis sp. n.

(Figs. 1–7)

DESCRIPTION (12 specimens measured): Flattened specimen 560 (472–640) long, 108 (80–144) wide at midbody. Pharynx 27 (26–41) long, 38 (33–40) wide. Penis 17 (16–23) in diameter with a large spine and a single row of 5 small spines. Hamuli 120 (109–121) long; root 35 (24–40), shaft 91 (86–97), point 31 (25–37). Tubular accessory sclerite 24 (20–25) in outer diameter and 50 (48–52) long. Ventral bar 13 (10–15) long, 35 (31–36) wide, with anterolateral processes 38 (30–51) long. Ventral bar membrane 71 (52–71) long. Dorsal bar 23 (18–26) long. Marginal hook 40 (32–41) long. Sickle 5 (4–6) long, 4 (3–4) wide proximally, 4 (4) wide distally. Handle 35 (32–36) long, with a terminal swelling and distinct ligament. Filament 17 (16–17)

HOST: *Lebiasina bimaculata* Cuvier and Valenciennes, 1846 (Characidae).

LOCALITY: Chicama River, Peru.

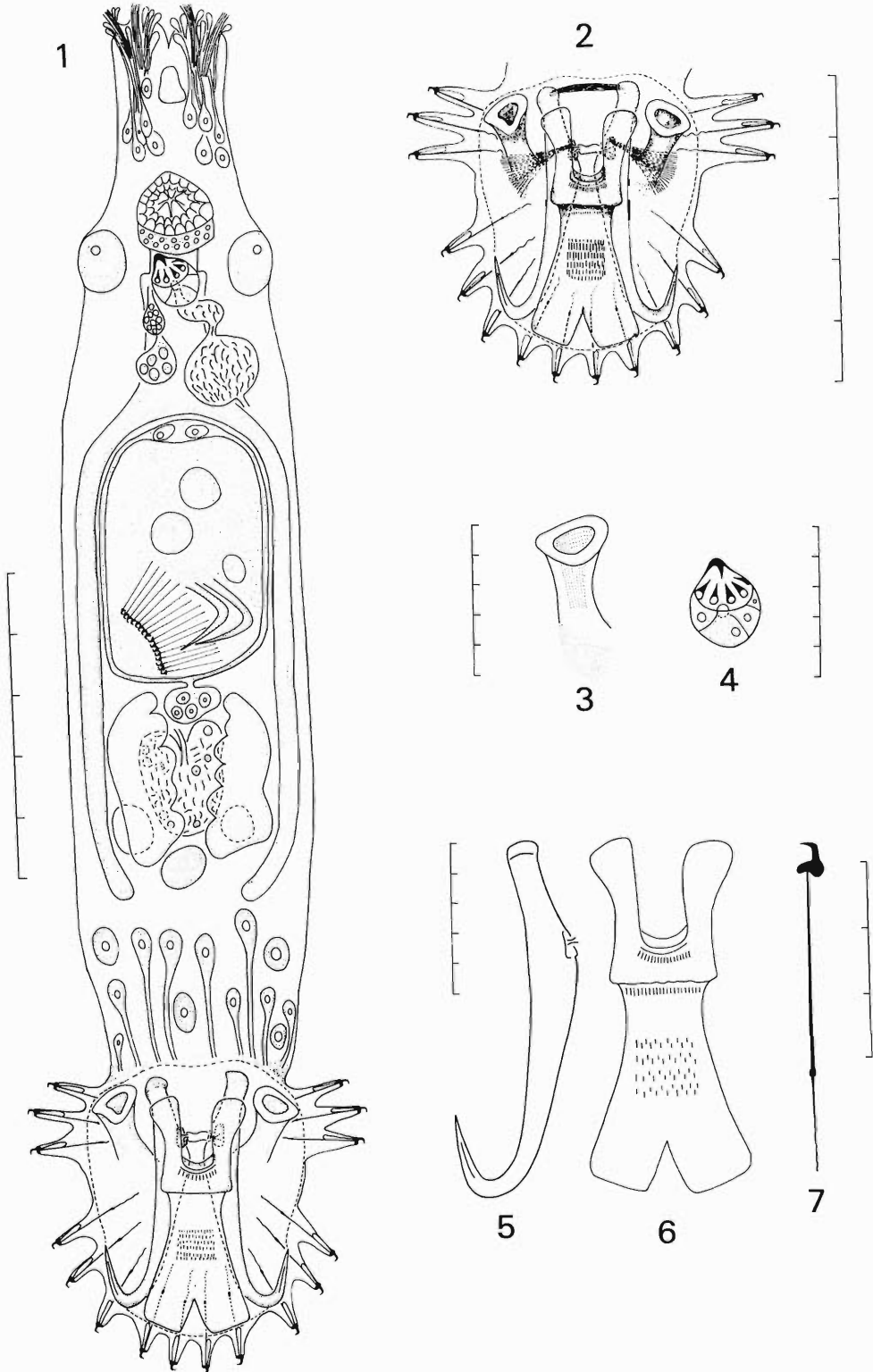
SITE OF INFECTION: Body washings.

HOLOTYPE: USNM Helm. Coll. Slide 81438.

PARATYPE: USNM Helm. Coll. Slide 81439.

⁴ Author to whom reprint requests should be addressed.

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Figures 1–7. *Accessorius peruensis*. 1. Whole mount in ventral view. Scale bar divisions 30 μ m. 2. Ventral view of the haptor. Scale bar divisions 30 μ m. 3. Accessory sclerite. Scale bar divisions 10 μ m. 4. Penis. Scale bar divisions 10 μ m. 5, 6. Ventral bar and hamulus. Scale bar divisions 10 μ m. 7. Marginal hook. Scale bar divisions 10 μ m.



Discussion

There are 19 genera and 375 species within the Gyrodactylidea recognized to date. They are found worldwide on freshwater and marine fishes. *Accessorius peruensis* is the tenth species to be described from South America. Previous reports include 5 species of *Gyrodactylus* Nordmann, 1832, and single species of *Scleroductus* Jara and Cone, 1989, *Oogyrodactylus* Harris, 1983, *Phanerothecium* Kritsky and Thatcher, 1977, and *Paragyrodactyloides* (Szidat, 1973), that parasitize as a group a characid, a poeciliid, and 4 siluriform fishes (Szidat, 1973; Kritsky and Thatcher, 1977; Harris, 1983; Jara and Cone, 1989; Ligou, Jara, and Cone, unpubl.). Given the apparently high host specificity of the gyrodactylids and the vast number of fishes in South America, we suspect a great number of gyrodactylideans from fishes of the continent is yet to be discovered.

Accessorius resembles *Polyclithrum* Rogers, 1967, and *Swingleus* Rogers, 1969, parasites of fundulid and mugilid fishes in the southeastern United States. *Polyclithrum* has 3 pairs of accessory bars, no peduncular bar, and marginal hooks I–IV grouped anteriorly. *Swingleus* has a peduncular bar, a pair of large winglike accessory sclerites, and hooks I–III grouped anteriorly. Rogers (1967) established the family Polyclithrinae on the basis of the species having the marginal hooks grouped anterolaterally on the margin of the haptor. However, Rogers (1969) then questioned the validity of the family after describing *Swingleus* with a peduncular bar similar to that present in *G. prolongis* Hargis, 1955. Kritsky and Thatcher (1977) removed *G. prolongis* and other gyrodactylids with peduncular bars from *Gyrodactylus* and placed the species into a new genus, *Fundulotrema* Kritsky and Thatcher, 1977. Cone and Odense (1988) recently redescribed *Fundulotrema* and revealed that, in all known species of the genus, marginal hooks I–III are grouped anteriorly on the haptor. We ten-

tatively place *Accessorius* in the family Polyclithrinae because of the anterolateral grouping of the marginal hooks. *Fundulotrema* likely belongs in the family as well, but a final decision on these rearrangements awaits a phylogenetic analysis of the group.

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Aspidogastrid (Trematoda) Parasites of Unionid (Bivalvia) Molluscs in Kentucky Lake¹

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ABSTRACT: A total of 219 bivalve molluscs (Unionidae) representing 7 genera (10 species) from 17 localities in Kentucky Lake was examined for aspidogastrid trematodes. Nine species were infected by *Aspidogaster conchicola* Baer: *Amblema plicata*, *Anodonta grandis*, *A. suborbiculata*, *Fusconaia ebena*, *F. undata*, *Megaloniaias gigantea*, *Plectomerus dombeyanus*, *Quadrula nodulata*, and *Q. quadrula*. One species, *A. suborbiculata*, served as a host for both *A. conchicola* and *Cotylaspis insignis* Leidy. A single specimen of *Arcidens confragosus* was not parasitized by either trematode species. Prevalence was moderate for *A. conchicola* infected bivalves with 63 (28.8%) individuals parasitized. Prevalence was much lower for *C. insignis* with only 2 (0.9%) host individuals parasitized. The highest parasite load occurred in *A. suborbiculata* (mean intensity = 6.3, maximum intensity = 20 *A. conchicola*).

KEY WORDS: *Aspidogaster conchicola*, *Cotylaspis insignis*, Trematoda, parasites, unionid, Unionidae, molluscs, Kentucky Lake.

Hendrix et al. (1985) stated that in North America aspidogastrid trematodes are common parasites of freshwater unionid mussels (Bivalvia), and there are large gaps in the known host range and geographic distribution of freshwater aspidogastrids. They also mentioned that distributional limits have not been established.

Although it is important to define the geographical boundaries of a parasite, it is also informative to map its distribution within those boundaries. Only 1 published account of the occurrence of an aspidogastrid exists for Kentucky, which reported *Cotylasteroides occidentalis* from a species of *Goniobasis* (Gastropoda) (Whittaker and Kozel, 1975). No published account exists for aspidogastrids from bivalves in Kentucky. This is the first report of the occurrence, prevalence, mean intensities, and maximum intensities of aspidogastrids from bivalves in Kentucky. Additionally, a new host record is established for *Aspidogaster conchicola* in *Anodonta suborbiculata*.

Materials and Methods

A total of 219 bivalve molluscs representing 7 genera and 10 species was collected from 17 localities in Kentucky Lake (12 in Kentucky, 5 in Tennessee) between 21 March and 27 October 1989 (Fig. 1). Bivalves were collected by diving (SCUBA), placed in containers filled with lake water, and transported to the laboratory with-

in 3 hr of collection. They were held in aerated tanks containing lake water for approximately 1-48 hr before necropsy. The valves were separated by severing the adductor muscles with an oyster knife. Subsequently, the gills, mantle, foot, visceral mass, kidney, and pericardial region were isolated and examined for aspidogastrid trematodes using a dissecting microscope. Trematodes were removed and fixed in warm alcohol-formalin-acetic acid (AFA) solution. Helminths were later rinsed in 70% ethanol, stained in dilute Semichon's acetocarmine, dehydrated in a graded ethanol series, cleared in xylene, and mounted in Permount®.

Ecological terms follow those proposed by Margolis et al. (1982). All bivalve names are based upon Burch (1975).

Voucher specimens have been deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705, as: *Aspidogaster conchicola* (Nos. 81277 and 81278), and *Cotylaspis insignis* (No. 81279).

Results

Aspidogaster conchicola Baer, 1827, parasitized 9 of the 10 bivalve species examined. *Cotylaspis insignis* Leidy, 1857, was isolated only from *Anodonta suborbiculata*, which also served as a host for *A. conchicola* (Table 1). *Aspidogaster conchicola* was found in the kidney and/or pericardial region of the hosts while *C. insignis* was removed from the surface of the kidney and/or suprabranchial cavity. Overall prevalence was moderate with 63 of 219 (28.8%) bivalves infected. Respectively, 62 (28.3%) host individuals were infected by *A. conchicola*, whereas only 2 *A. suborbiculata* (0.9%) harbored *C. insignis* (1 of which was also infected with *A. conchicola*). Sixty (27.4%) of the parasitized hosts were in-

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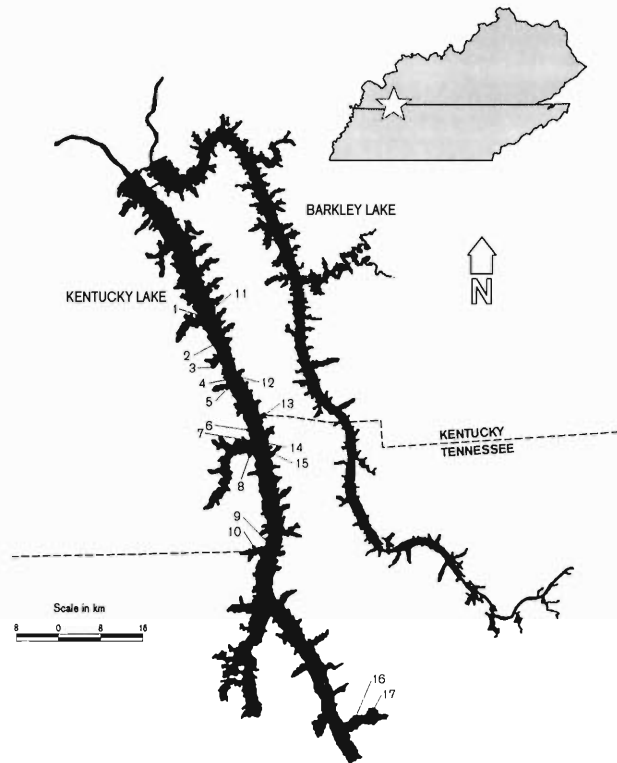


Figure 1. Map of collection localities on Kentucky Lake and its relationship with nearby Lake Barkley. The inset of Kentucky and Tennessee provides a relative geographic point of the area sampled.

ected with adult *A. conchicola*, while only 4 (1.8%) bivalve specimens harbored larval *A. conchicola*. These larvae were found in *A. suborbiculata*, *Megaloniais gigantea*, *Plectomerus dombeyanus*, and *Quadrula quadrula*. Larval forms of *A. conchicola* were only observed from May through July. No larval *C. insignis* were observed during the study.

A total of 158 adult *A. conchicola* was recovered from 63 parasitized bivalves for a mean intensity of 2.5. The heaviest adult *A. conchicola* infection was observed in *A. suborbiculata* with a mean intensity of 6.3 and a maximum intensity of 20 (Table 1). Only *A. suborbiculata* was parasitized by *C. insignis*. A total of 11 adult *C. insignis* was recovered from 2 *A. suborbiculata* for a mean intensity of 5.5 and a maximum intensity of 10.

Discussion

Aspidogaster conchicola was the most prevalent aspidogastrid found during this study. Similar results have been reported by other authors (Hendrix, 1968; Nelson et al., 1975; Danford and

Joy, 1984). Danford and Joy (1984) reported a lower overall prevalence of *A. conchicola* (9.4%) in West Virginia compared to the present study (28.3%). In contrast, Huehner and Etges (1981) found 42.8% of the hosts examined from Ohio parasitized by *A. conchicola*. Another study conducted by Stromberg (1970) in Ohio reported an overall prevalence of 23.8% for *A. conchicola*, which is similar to the prevalence rate in this study.

While *C. insignis* occurred only in *A. suborbiculata* and was less prevalent (0.9%) than *A. conchicola* in the present study, Hendrix and Short (1965) and Flook and Ubelaker (1972) reported higher prevalence rates for *C. insignis*. Based upon personal calculations from data presented by Najarian (1955), the overall prevalence of *C. insignis* from 29 bivalves (3 species) was 96.5%.

Mean intensities of aspidogastrids from bivalves in Kentucky Lake were low with a range of 1 to 6.3 for *A. conchicola* and a value of 5.5 for *C. insignis*. Higher mean intensities ranging from 14.4 to 20.7 have been reported for *A. con-*

Table 1. Summary data of *Aspidogaster conchicola* and *Cotylaspis insignis* in bivalve molluscs collected in Kentucky Lake between 21 March and 27 October 1989. Numbers in parentheses indicate values for *C. insignis*.

Bivalve species	Aspidogastrid parasites				
	No. examined	% prevalence/ no. of hosts infected	Mean intensity	Maximum intensity	Overall % prevalence N = 219
<i>Amblema plicata</i> Say, 1817	48	29.1/14	1.9	5	6.4
<i>Anodonta grandis</i> Say, 1829	5	40.0/2	3.0	5	0.9
<i>A. suborbiculata</i> Say, 1831*	14	28.6/4 (14.3/2)†	6.3 (5.5)	20 (10)	1.8 (0.9)
<i>Arcidens confragosus</i> (Say, 1829)	1	0/0	0	0	0
<i>Fusconaia ebena</i> (Lea, 1831)	1	100/1	1.0	1	0.5
<i>F. undata</i> (Barnes, 1823)	4	25.0/1	1.0	1	0.5
<i>Megaloniais gigantea</i> (Barnes, 1823)	68	7.4/5	5.2	17	2.3
<i>Plectomerus dombeyanus</i> (Valenciennes, 1827)	11	54.5/6	2.0	4	2.7
<i>Quadrula nodulata</i> Rafinesque, 1820	5	20.0/1	2.0	2	0.5
<i>Q. quadrula</i> (Rafinesque, 1820)	62	45.2/28	2.4	9	12.8
Total	219	-/63	-	-	28.8

* New host record.

† Dual infection in one host.

chicola by Stromberg (1970), Nelson et al. (1975), Huehner and Etges (1981), and Danford and Joy (1984). The mean intensity of *C. insignis* during this study is the same as that reported from West Virginia by Danford and Joy (1984) and similar to values of previous studies.

Higher maximum intensities of *A. conchicola* and *C. insignis* have been reported by some authors than were observed in Kentucky Lake. Nelson et al. (1975) reported >1,500 *A. conchicola* from a host and Najarian (1955) observed 212 *C. insignis* in a single bivalve. However, Danford and Joy (1984) stated that characteristically the number of aspidogastrids per infected bivalve is low.

Danford and Joy (1984) reported larval *A. conchicola* from hosts in May and July during a 14-month period in West Virginia. While the present study encompassed a shorter time period of 7 months, larval *A. conchicola* were only observed between May and July. This may indicate that *A. conchicola* reproduces during similar time periods in the 2 geographic areas sampled.

Acknowledgments

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MEETING SCHEDULE HELMINTHOLOGICAL SOCIETY OF WASHINGTON 1991–1992

- (Wed) 9 Oct 1991 “To Be Announced,” Uniformed Services University of the Health Sciences, Bethesda, MD
- (Wed) 13 Nov 1991 “Zoonoses,” Parasitology Unit, U.S. Department of Agriculture, Beltsville, MD
- (Wed) 11 Dec 1991 Tentative meeting at the Smithsonian Institution, Washington, DC
- (Wed) 8 Jan 1992 “To Be Announced,” Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD
- (Wed) 12 Feb 1992 “Recent Developments in Malaria Vaccine Research,” Department of Immunoparasitology, U.S. Naval Medical Research Institute, Bethesda, MD
- (Wed) 4 Mar 1992 “To Be Announced,” Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC
- (Wed) 8 Apr 1992 “To Be Announced,” Annual Joint Meeting with the Baltimore Tropical Medicine Dinner Club, Johns Hopkins University and University of Maryland
- (Sat) 2 May 1992 “To Be Announced,” Annual Joint Meeting with the New Jersey Society for Parasitology, to be held at the New Bolton Center, University of Pennsylvania, Kennett Square, PA

Helminth Parasites of Bowfin (*Amia calva*) from South Carolina

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ABSTRACT: Twelve bowfin (*Amia calva*) collected from 3 backwaters off the Savannah River in South Carolina were examined for helminth parasites. Thirteen species were found: 5 trematodes, 2 cestodes, 5 nematodes, and 1 acanthocephalan. Five species (2 trematodes, 2 cestodes, and 1 acanthocephalan) occur regularly and are considered core species. Species richness and community abundance were high compared to published studies on other species of freshwater fish. Although mean number of species and diversity were comparable to infracommunity patterns observed for many aquatic birds, the abundance of helminths was reduced. Host specificity and broad feeding preferences are suggested as important determinants of helminth community structure in bowfin.

KEY WORDS: bowfin, *Amia calva*, helminths, frequency, intensity, species richness, infracommunity composition.

Bowfin, *Amia calva* L., are the only extant species of the Amiidae. They are found throughout most of eastern North America in a diversity of freshwater habitats. Their large size and abundance makes them conspicuous components of the fish assemblage in swampy, vegetated bays of lakes and rivers (Scott and Crossman, 1973; Pfieger, 1975).

Compared to many North American freshwater fishes, comparatively little information is available on the natural history of *A. calva* (Reighard, 1904; Holland, 1964; Cartier and Magnin, 1967; Stacy et al., 1970). This also characterizes information on their helminth parasites with most studies providing either descriptions of new taxa or reports of species occurrences (e.g., Sogandares-Bernal, 1955; Sillman, 1962; Premvati, 1969). Several surveys indicate compositional variability between locations (Van Cleave and Mueller, 1934; Bangham and Hunter, 1939; Bangham, 1941; Bangham and Venard, 1942; Bangham, 1955; Robinson and Jahn, 1980); none provides analysis and few present data potentially useful to understanding the organization of parasite communities. Recent comparative treatments of helminth infracommunity structure have suggested that those in freshwater fish are species-poor (Kennedy et al., 1986). In the present study, we show that bowfin can have parasite communities that are surprisingly rich for freshwater fish, with high proportions of frequently co-occurring species.

Materials and Methods

Bowfin were collected during the second week of July 1988 by electrofishing 3 backwater locations off the Savannah River between river miles 136 and 158 in Aiken and Barnwell counties, South Carolina. Individuals were returned live to the laboratory where they were measured (total length) and sexed (6 male, 6 female) before necropsy. Age was determined from the gular plate (Holland, 1964). Alimentary tracts were immediately removed and quickly frozen in a dry ice-95% ethanol mixture as described by Bush and Holmes (1986). The remaining viscera and carcass were then individually frozen for later examination. Esophagus, stomach, and intestine were separated, measured, and then examined. All worms were counted and identified using either temporary wet mounts or specimens that were fixed and prepared as permanent mounts. Voucher specimens have been deposited in the United States National Museum Helminthological Collection (USNMH 81477-81483) and the University of Nebraska Parasitology Collection (HWML 31614-31621). Noncatalogued species (unidentified Spiruridae, *Capillaria* sp., *Thynascaris brachyurum*) were either uncommon or damaged, but specimens have been retained by 1 of the authors (J.M.A.).

Terminology follows definitions established by Margolis et al. (1982). Frequency of infection (N) is the number of infected hosts. Predictability of infracommunity composition was measured using Jaccard's coefficient (qualitative, based on species presence/absence) and percent similarity (quantitative, based on numerical proportions of each species). Brillouin's index, appropriate for fully censused communities (Pielou, 1975), provided a measure of infracommunity diversity. Values were calculated using common logarithms; for comparison with data of Kennedy et al. (1986), where Brillouin's index was calculated using natural logarithms, values can be multiplied by 2.303. Fisher exact tests (with an adjusted $P = 0.005$ to maintain an "experiment-wise" error rate of $P \leq 0.05$) were calculated for the common helminth species to test for differences in frequency of infection and host sex; differences in intensity of infection were examined using

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the Mann-Whitney *U*-test. Correlations between infection parameters and host factors were tested using Spearman rank correlation (r_s). Rejection of the null hypothesis was at $P \leq 0.05$. Values are expressed as mean \pm 1 SE.

Results

Twelve adult bowfin were examined for helminths. Fish ranged in size from 520 to 680 mm (589 ± 16 mm) and were 4–8 years old. The presence of recrudescing gonads indicated all individuals were sexually mature adults. The number of fish examined might be considered low relative to other studies on fish parasites but, based on cumulative species richness, >90% of the helminth species found were recovered from just 6 bowfin. Thus, the sample size should be sufficient to examine patterns of infracommunity structure.

Each bowfin was infected, and helminths were found only in the alimentary tract (Table 1). Individual fish harbored between 5 and 8 species of helminths and 18–269 individuals; mean species richness was 6.5 ± 0.3 and community abundance averaged 114 ± 27 individuals. Brillouin's index ranged from 0.32 to 0.80, with a mean value of 0.52 ± 0.04 . The intestinal helminth assemblage had the greatest number of species with 3 to 6 species (4.8 ± 0.3) per fish. Number of worms found in the intestine ranged from 7 to 230 individuals (63 ± 22). Community diversity of the intestinal helminth assemblage varied from 0.15 to 0.43 (0.30 ± 0.02). Mature or gravid individuals of all species were represented except for *Thynascaris brachyurum*. Frequency, prevalence, mean intensity, and site of infection are presented in Table 1.

Composition of the helminth assemblage was numerically dominated (>95% of individual worms) by trematodes (5 species) and cestodes (2 species). Two species of trematode, *Macroderoides typicus* and *M. trilobatus*, and the cestode, *Proteocephalus perplexus*, were commonly found and accounted for 85% of all individuals. *Azygia angusticauda*, *A. longa*, *Haplobothrium globuliforme*, and *Neoechinorhynchus cylindratus* also occurred frequently, but in moderate to low abundance. The remaining species, including the 5 nematode species, were infrequent and abundance was low.

There were no differences in the frequency or mean intensity of infection between host sexes for any helminth species. There were also no significant correlations between either the num-

ber of individuals of each species or the total number of helminth individuals and either host size or length of the intestine. These patterns suggest that the number of worms is not a function of the size of the host environment.

Qualitative faunal similarity among hosts was quite high (Jaccards coefficient = $60.0 \pm 1.9\%$). Quantitative similarity was comparatively lower (percent similarity = $45.3 \pm 2.8\%$) indicating some disparity in the abundance of species across infracommunities. Frequency of occurrence and infracommunity abundance were significantly correlated ($r_s = 0.67$, $P < 0.05$). The co-occurrence of *M. typicus* and *P. perplexus* in 11 hosts, which were often the 2 most abundant helminths, influenced this correlation. In pairwise comparisons between the intensities of all species pairs (using only species where $N \geq 4$), only *P. perplexus* and *M. typicus* had a significant association ($r_s = 0.67$, $P < 0.03$); there was an equivocal positive association between *Haplo-nema immutatum* and *N. cylindratus* ($r_s = 0.95$, $P = 0.05$). Frequency distribution of the prevalences of the helminth species distinguished 3 groups of species. Parasites whose prevalence was >80% were regarded as core species, and included *P. perplexus*, *H. globuliforme*, *A. angusticauda*, *N. cylindratus*, and *M. typicus*. Species with prevalence <20% are regarded as satellite species, and those intermediate in prevalence are regarded as secondary species (as in Bush and Holmes, 1986).

Discussion

The helminth fauna of bowfin from this area of the Savannah River is a representative subset of the species found in other parts of its geographical range (Van Cleave and Mueller, 1934; Bangham and Hunter, 1939; Bangham, 1941; Bangham and Venard, 1942; Bangham, 1955; Robinson and Jahn, 1980). With the exception of *Capillaria* sp., every species has previously been reported from bowfin. *Macroderoides trilobatus* had only been reported before from bowfin collected in the Ochlocknee River, Georgia (Taylor, 1978). In this study, it is the absence of monogeneans or larval trematodes, cestodes, or nematodes that is most unique.

The high species richness and diversity of the helminth assemblage from bowfin in this locality are unusual among freshwater fish. Most studies indicate helminth communities of a variety of freshwater fish are species-poor with low or variable abundance and low community diversity

Table 1. Frequency, prevalence, mean intensity, and site of infection for gastrointestinal helminths of bowfin (*Amia calva*).

Helminth species	Site*	N (%)†	Mean intensity (SE)
Trematoda			
<i>Azygia angusticauda</i> (Stafford, 1904) Manter, 1926	S, SI	12 (100)	6.8 (1.9)
<i>Azygia longa</i> (Leidy, 1851) Manter, 1926	S, SI	5 (42)	1.0 (0.0)
<i>Macroderoides typicus</i> (Winfield, 1929) Van Cleave and Mueller, 1932	LI	11 (92)	35.0 (13.2)
<i>Macroderoides trilobatus</i> Taylor, 1978	SI	6 (50)	44.7 (22.9)
<i>Microphallus opacus</i> (Ward, 1894) Ward, 1901	SI	2 (17)	8.5 (7.5)
Cestoda			
<i>Haplobothrium globuliforme</i> Cooper, 1914	SI	10 (83)	2.0 (0.5)
<i>Proteocephalus perplexus</i> LaRue, 1911	S, SI	12 (100)	44.9 (14.2)
Nematoda			
<i>Camallanus oxycephalus</i> Ward and Magath, 1917	LI	4 (33)	2.5 (1.5)
<i>Capillaria</i> sp.	E	1 (8)	1
<i>Haplonema immutatum</i> Ward and Magath, 1917	SI	4 (33)	3.0 (0.8)
Unidentified Spiruridae	SI	2 (17)	1.0 (0.0)
<i>Thynascaris brachyurum</i> (Ward and Magath, 1917 sensu Van Cleave and Mueller, 1934)	S	1 (8)	1
Acanthocephala			
<i>Neoechinorhynchus cylindratus</i> (Van Cleave, 1913) Van Cleave, 1919	SI	10 (83)	3.4 (0.8)

* E = esophagus; S = stomach; SI = small intestine; LI = large intestine.

† N (%) = frequency, number of fish infected (prevalence, % of fish infected).

(Kennedy et al., 1986). Excluding this study, infracommunity richness for bowfin ranges from 2 to 4 species per location (Bangham and Hunter, 1939; Bangham, 1941; Bangham and Venard, 1942; Bangham, 1955), whereas most freshwater fish typically have fewer than 2 species per host individual (Kennedy et al., 1986). Patterns of species richness and diversity observed here for bowfin are comparable to helminth communities in many aquatic birds (Kennedy et al., 1986; Stock and Holmes, 1987; Holmes, 1990). Fundamental differences in helminth infracommunity structure between ectotherms and endotherms is suggested to be a function of physiological and behavioral processes affecting the acquisition of parasites by individual hosts. The energy demands of endothermy increase exposure to infected intermediate hosts, and contribute to the development of larger, more complex communities (Kennedy et al., 1986). Factors contributing to differences among fish species, and apparent parallels in community complexity with some aquatic birds are, however, still unclear.

Host specificity makes an important contribution to the helminth community structure for bowfin from this locality. Unlike the pattern of

low helminth assemblage similarity within and between locations for many species of freshwater fish (Esch et al., 1988; Kennedy 1990), the presence of a central block of frequent, regularly co-occurring core species results in considerable qualitative similarity among bowfin infracommunities. Based on patterns from the literature (see Hoffman, 1967; Margolis and Arthur, 1979 for summary checklists), 3 core species (*P. perplexus*, *H. globuliforme*, and *M. typicus*) are host specialists of *Amia* as are the 2 secondary species, *H. immutatum* and *M. trilobatus*. The remaining 2 core species (*N. cylindratus* and *A. angusticauda*), as well as all satellite species, are host generalists reported from a wide variety of freshwater fish.

Qualitative similarity for 6 surveys (Van Cleave and Mueller, 1934; Bangham and Hunter, 1939; Bangham, 1941; Bangham and Venard, 1942; Bangham, 1955; Robinson and Jahn, 1980) is low ($30.1 \pm 4.3\%$) suggesting considerable variability in the helminth assemblage among different bowfin populations. Compositional differences between populations are predominantly due to species replacements of host generalists; specialist species only account for ca. 25% of the helminth species reported from bowfin. This pat-

tern, therefore, suggests that processes determining the abundance of host generalists (e.g., availability of suitable intermediate hosts, geographic location, or composition of the host community) strongly influence the richness and diversity of the helminth assemblage. This view is contrary to suggestions by Toft (1986) that species richness in parasite communities should be enhanced by a high degree of host specificity.

A second factor that contributes to helminth community structure appears to be host feeding preferences. Examination of food habits of adult bowfin suggests that they are omnivorous, but forage extensively on a variety of fish and crustaceans such as grass shrimp and crayfish (Holland, 1964; Cartier and Magnin, 1967; Stacy et al., 1970). The proportions of parasites derived from different intermediate hosts support these patterns, and emphasize the importance of fish and crustaceans to helminth acquisition. Illustrated by the present study, helminths using fish intermediate hosts represent approximately 50% of the species and 80% of the community abundance while those using crustaceans account for 20–30% of the species. Life cycles for the remaining species are either direct (15% of the species) or are incompletely known (*H. immutatum*, *Capillaria*, unidentified Spiruridae).

Kennedy et al. (1986) suggest that broad diets should promote development of diverse helminth communities and that selective feeding can lead to large infrapopulations. Price and Clancy (1983) provide evidence that predaceous fishes have richer helminth communities than fishes lower in the food chain. The feeding preferences of bowfin are consistent with these predictions for the establishment of diverse communities and large infrapopulations ("large" relative to most other ectotherms). These features suggest the existence of predictable, strong trophic linkages among bowfin that encourage qualitative consistency in infracommunity composition within a location. Although other freshwater fish are opportunistic, generalist omnivores, depauperate helminth infracommunities in these species may reflect either greater temporal and spatial variation in patterns of prey utilization or intermediate hosts that do not have high prevalence or intensity of infection. Similar observations for the development of depauperate infracommunities in other ectotherms have been made by Goater et al. (1987) and Aho (1990).

The high helminth infracommunity richness and diversity of bowfin from this location, com-

pared to other studies on bowfin, are thought to be habitat related. Where information on collection site is provided, the helminth fauna of bowfin has been predominantly studied in lentic environments. Bowfin examined in this study were found within the vicinity of protected, vegetated backwaters off the main river channel. These are highly productive habitats compared to main channel reaches (Brinson et al., 1981); presence of woody debris and submerged aquatic vegetation associated with these areas serve as valuable cover for a diversity of fish species and substrate for invertebrate production. The preference of bowfin for low velocity, clear water habitats (Scott and Crossman, 1973; Pflieger, 1975) leads to high spatial and temporal overlap of host and parasite life histories. This can enhance helminth transmission and circulation dynamics. While their somewhat isolated nature makes them analogous to small lakes, these backwaters are also continually exposed to immigration by invertebrates and vertebrates dispersing helminth larval stages. As a result, helminth species present in a habitat may be reintroduced frequently (as in the rescue phenomenon of island biogeography; Schoener, 1983) or colonized by new helminth species. The dynamic processes operating within these backwaters provide, and maintain, conditions promoting development of complex local helminth communities. As a consequence of these processes, bowfin helminth infracommunity structure approaches helminth species richness and diversity features found in several species of aquatic birds. We would also expect other fish found in these environments to have richer, and more predictable helminth infracommunities than typically found in lentic habitats.

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Some Digeneans (Trematoda) of the Green Turtle, *Chelonia mydas* (Testudines: Cheloniidae) from Puerto Rico

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ABSTRACT: The Caribbean Aquatic Animal Health Project and the Caribbean Stranding Network attempted to rehabilitate a moribund green turtle, an endangered marine species, from Puerto Rico. The animal died and a necropsy was performed in an attempt to determine the cause of death. Several species of digeneans were found: a single spirorchid, *Learedius learedi*; 2 pronoccephalids, *Pyelosomum cochelear* and *Glyphicephalus lobatus*, recorded for the first time in green turtles of Puerto Rico; a single angiodictyid, *Deuterobaris proteus*, which represents a new locality record for the Caribbean; and 3 microscaphidiids, *Angiodictyum parallellum* and *Octangium sagitta*, which represent new locality records for the Caribbean and Atlantic Ocean, respectively, and *Polyangium linguatula*, a new locality record for Puerto Rico.

KEY WORDS: Digenea, *Learedius learedi*, *Pyelosomum cochelear*, *Glyphicephalus lobatus*, *Deuterobaris proteus*, *Angiodictyum parallellum*, *Octangium sagitta*, *Polyangium linguatula*, green turtle, *Chelonia mydas*, Puerto Rico.

The green turtle, *Chelonia mydas* (Linnaeus, 1758), is a marine species with a geographic distribution encompassing the Atlantic, Pacific, and Indian oceans (Ernst and Barbour, 1989). Although it usually prefers a tropical climate, it has been recorded as far north in the Pacific as Alaska (Hodge, 1981) and in the Atlantic to Great Britain (Brongersma, 1972). *Chelonia mydas* is a threatened species throughout its range and an endangered species in the breeding colony populations of Florida and the Pacific Coast of Mexico (Anonymous, 1979).

Few reports are available on the parasites of wild turtles of Puerto Rico. Fischthal and Acholonu (1976) reported 28 species of digeneans from 14 Atlantic hawksbill turtles, *Eretmochelys imbricata imbricata* (Linnaeus), from Cabo Rojo.

On 15 April 1990, a moribund green turtle was snagged in a fishing line off Ponce, Puerto Rico. The animal was emaciated and the plastron severely sunken. Rotation to the right of a normal horizontal position in the water suggested damage or failure of the left lung. The turtle was held for 2 days after rescue but its health deteriorated and it died on 17 April. A necropsy was performed in an attempt to determine the cause of death. The helminths collected during this investigation are reported herein. All species are recorded for the first time from *C. mydas* in Puerto Rican waters.

Materials and Methods

All helminths were recovered in situ at necropsy from the turtle shortly after death. The digestive tract,

lungs, circulatory system, and urinary bladder were examined for helminths. The digeneans were fixed in warm AFA with light coverglass pressure, stained in Harris' hematoxylin, dehydrated, cleared in beechwood creosote, and mounted in Canada balsam. All specimens were deposited in the United States National Museum Helminthological Collection (USNM Helm. Coll.) as noted.

Results and Discussion

Six species of digeneans, including 2 pronoccephalids, 1 angiodictyid, and 3 microscaphidiids, were recovered from the digestive tract and 1 spirorchid from the cardiac cavity. All other tissues examined were negative for digeneans.

Spirorchidae Stunkard, 1921

Spirorchinae Stunkard, 1921

Learedius learedi Price, 1934

Although 7 genera and 10 species of spirorchids have been recorded in *Chelonia mydas* from other parts of the world (Smith, 1972), none has been reported from this turtle in the West Indies.

Three specimens of a spirorchid identified as *Learedius learedi* were found in the cardiac cavity. The genus *Learedius* was proposed by Price (1934) for a single specimen of *L. learedi* found in the circulatory system of *Chelone mydas* (= *Chelonia mydas*), which died in the National Zoological Park, Washington, D.C. A more detailed account of *L. learedi* based on 45 specimens was given later by Caballero et al. (1955) from *C. mydas* of Panama. *Learedius learedi* has

also been reported in wild green turtles from Florida (Nigrelli, 1941) and Bermuda (Rand and Wiles, 1985) and mariculture-reared green turtles from the Cayman Islands (Greiner et al., 1980). It has also been reported in *Eretmochelys imbricata imbricata* from Puerto Rico (Fischthal and Acholonu, 1976).

Spirorchid eggs have been associated with histopathological changes occurring in wild *C. mydas*. Glazebrook et al. (1981) reported that eggs of *Haplotrema* sp. released into the circulation from the heart and other sites of a moribund green turtle captured near Townsville in North Queensland, Australia, elicited a generalized focal granulomatous response in the host. Rand and Wiles (1985) found that eggs of *Learedius learedi* evoked a granulomatous host reaction with multiple foci in all tissues examined from several moribund green turtles from inshore Bermuda waters after storms. Greiner et al. (1980) reported what appeared to be spirorchid eggs surrounded by discrete chronic granulomata consisting of epithelial cells, multinucleated giant cells, and mononuclear cells in tissues of several mariculture-reared green turtles from Grand Cayman. Jacobson et al. (1986) found few spirorchid eggs in lung tissue of green turtles with lung, eye, and trachea disease from Cayman Turtle Farm, Grand Cayman. We did not detect eggs of *L. learedi* in either the blood or other tissues examined.

This is the first report of the species in wild green turtles from the Caribbean. Voucher specimens of *L. learedi* have been deposited as USNM Helm. Coll. No. 81202.

Pronocephalidae Looss, 1902

Pronocephalinae Looss, 1899

***Pyelosomum cochelear* Looss, 1899**

Three digeneans from the cloaca were identified as *Pyelosomum cochelear*. Looss (1899) established the genus *Pyelosomum* with *P. cochelear* from the urinary bladder of *C. mydas* of Egypt as type species. This species has also been reported in *C. mydas* from Panama (Caballero, 1954) and *C. mydas* from Florida (Nigrelli, 1941). To our knowledge, the only other species of *Pyelosomum* reported in *C. mydas* is *Pyelosomum posterorhynchis* Oguro, 1936, which was originally described from the intestine of *Eretmochelys squamosa* (Linnaeus) of Palao Island and redescribed by Caballero et al. (1955) from *C. mydas*

of the Pacific coast of Panama. Fischthal and Acholonu (1976) reported *P. posterorhynchis* from the Atlantic hawksbill turtle, *Eretmochelys imbricata imbricata* of Cabo Rojo, Puerto Rico.

This represents the first report of *P. cochelear* in green turtles of Puerto Rico. Voucher specimens have been deposited as USNM Helm. Coll. No. 81203.

***Glyphicephalus lobatus* Looss, 1901**

Two immature and 1 mature specimen of a pronocephalid digenean from the large intestine were identified as *Glyphicephalus lobatus*. Looss (1901) erected the genus *Glyphicephalus* with the type species as *G. solidus* from *C. mydas* of Egypt. According to Yamaguti (1971), this species has also been reported in *Eretmochelys imbricata* from Cuba. *Glyphicephalus lobatus* was described from *C. mydas* in Egypt by Looss (1901). It has also been reported in *C. mydas* from Panama and in *Eretmochelys squamosa* from Palao Island. This species has been redescribed as *Pleurogonius lobatus* (Looss, 1901) by Ruiz (1946) and later redescribed as *P. lobatus* by Caballero et al. (1955) in *C. mydas*. It has also been reported from Puerto Rico in *E. imbricata imbricata* by Fischthal and Acholonu (1976).

This is the first report of this species in *C. mydas* of Puerto Rico. Specimens of *G. lobatus* have been deposited as USNM Helm. Coll. No. 81204.

Angiodictyidae Looss, 1902

Deuterobaridinae Looss, 1902

***Deuterobaris proteus* (Brandes, 1891) Looss, 1902**

Twenty-one angiodictyids from the small intestine were identified as *Deuterobaris proteus*. Two species of *Deuterobaris* have been described from marine turtles; *D. proteus* in *Chelone viridis* (= *Chelonia mydas*) from the Mediterranean, and *D. chelonei* Gupta, 1961, in *C. mydas* from Trinidad. Gupta (1961) did not mention the presence of ventral glands in the description. No type specimen was designated nor was any record of deposition of type material given. *Deuterobaris proteus* has been reported from green turtles of Florida (Nigrelli, 1941).

This is the first report of *D. proteus* from the Caribbean. Specimens of *D. proteus* have been deposited as USNM Helm. Coll. No. 81205.

Microscaphidiidae Travassos, 1922**Microscaphidiinae Looss, 1900*****Angiodictyum parallelum*****(Looss, 1901) Looss, 1902**

Sixty-five specimens of a microscaphidiid from the large intestine agree quite well with specimens of *Angiodictyum parallelum* described by Looss (1901) and reexamined later by Blair (1986). Four species of *Angiodictyum* are known from marine turtles: 1 from the hawksbill turtle, 2 from the green turtle, and 1 from both species. *Angiodictyum parallelum* has been reported in the large intestine of *C. mydas* from the Mediterranean coast of Egypt and from Florida (Nigrelli, 1941). *Angiodictyum glossoides* Blair, 1986, has been reported in the intestine of *E. imbricata* from Río Cañaveral, Caribbean coast of Panama. *Angiodictyum longum* Blair, 1986, has been reported in the pseudocecum of *C. mydas* from Queensland, Australia, and the large intestine of *C. mydas* from the Straits of Malacca, Malaysia and from Ceylon. *Angiodictyum posterovitellatum* Challopadyaya, 1972, has been recorded in the lower intestine of *E. imbricata* from the Gulf of Manar, India and the large intestine of *C. mydas* from Queensland, Australia. A key to the species of *Angiodictyum* was given by Blair (1986).

This is the first report of the digenean from the Caribbean. Specimens of *A. parallelum* have been deposited as USNM Helm. Coll. No. 81206.

Polyangium linguatula**(Looss, 1899) Looss, 1902**

Five specimens of a microscaphidiid from the large intestine were identified as *Polyangium linguatula*. Yamaguti (1971) lists *Monostomum reticulare* Walters, 1893, *Microscaphidium linguatula* Looss, 1899, and *Monostomum pseudamphistomum* Creplin, 1846, as synonyms of this species. Our specimens agree quite well with the specimens described by Looss (1902) except that our specimens are slightly shorter. Later descriptions of *P. linguatula* include those of Teixeira de Freitas and Lent (1938), Groschaft et al. (1977), and Blair (1986). Specimens examined by Groschaft et al. (1977), although mature, were smaller (maximum body length 2.96 mm) than any recorded including our specimens (maximum body length 3.20 mm). The tegument of our specimens is smooth and the esophageal bulb weakly developed as reported by Groschaft et al. (1977).

Polyangium linguatula has been reported in *C. mydas* from the Mediterranean coast of Egypt (Looss, 1899, 1902; Sey, 1977), Australia (Johnston, 1913), Singapore (Kobayashi, 1915), Brazil (Teixeira de Freitas and Lent, 1938), Florida (Nigrelli, 1941; Manter, 1954), India (Mehrotra, 1973; Blair, 1986), and Cuba (Groschaft et al., 1977). Other species of *Polyangium* reported from marine turtles include *P. miyajimai* Kobayashi, 1921, in *Chelonia mydas*, *P. longiseminale* Chattopadhyaya, 1972, in *Caretta caretta*, and *P. colymbi* (Poche, 1926) Price 1937. The latter species was described from the collection of the University of Granz as *Nephrobius colymbi* which was allegedly found in the kidney of *Colymbus arcticus*, a bird found in the digestive tract of a marine turtle. Price (1937), however, pointed out that the specimens are indistinguishable from *Polyangium* and suggested that they must have been mislabeled. We concur with Blair (1986) that *Polyangium* is represented by *P. linguatula* as the sole species and that specimens of *P. longiseminale*, *P. miyajimai*, and *P. colymbi* all lie within the range described for *P. linguatula*.

This is the first report of this digenean in a wild turtle from Puerto Rico. Specimens of *P. linguatula* have been deposited as USNM Helm. Coll. No. 81208.

Octangiinae Looss, 1902***Octangium sagitta* (Looss, 1899) Looss, 1902**

A single specimen of a microscaphidiid from the large intestine was identified as *Octangium sagitta* based on the description of this species as given by Blair (1987). Although several species of *Octangium* have been described, Blair (1987) recognized only 2, *O. sagitta* and *O. hypalum*. These may be differentiated by the latter species having 3 pairs of primary ducts in the excretory plexus, the more anterior placing of the testes, the shorter lateral vitelline field, and a relatively larger esophageal bulb than the former species. *Octangium sagitta*, of which *O. hasta* Looss, 1902, and *O. takanoi* Kobayashi, 1921, are considered synonyms according to Blair (1987), has been reported in the intestine of *C. mydas* from the Mediterranean coast of Egypt, Australia, India, Singapore, and Taiwan and also in the large intestine of *E. imbricata* from India. *Octangium hypalum*, of which *O. takonoi* Kobayashi sensu Mehrotra (1973) and Tandon and Gupta (1981) is considered a synonym according to Blair (1987), has been reported from the pseudocecum of *C. mydas* from Queensland, Australia.

This is the first report of *O. sagitta* from the Atlantic Ocean. A specimen has been deposited as USNM Helm. Coll. No. 81207.

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Report on the Brayton H. Ransom Memorial Trust Fund

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

Financial Report for 1990

Balance on hand, 1 January 1990	\$11,565.92
Receipts: Net interest received in 1990	990.92
	<u>\$12,556.84</u>
Disbursements:	
Grant to the Helminthological Society of Washington for 1990	(\$ 50.00)
Membership in the American Association for Zoological Nomenclature for 1990 ..	(\$ 50.00)
Page Charge Support	(\$ 400.00)
	<u>(\$ 500.00)</u>
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The Proliferative Tetrathyridium of *Mesocestoides vogae* sp. n. (Cestoda)

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ABSTRACT: The name *Mesocestoides vogae* sp. n. is proposed for the species of asexually proliferative tetrathyridium described by Specht and Voge (1965) and now in popular use in many laboratories in the world. A number of developmental differences are cited that make the tentative identification of this species as *Mesocestoides corti* Hoenpli, 1925, by Specht and Voge difficult to accept. Until further data become available which demonstrate its adult stage and definitive host, a different name should be used for this metacestode species.

KEY WORDS: *Mesocestoides corti*, *Mesocestoides lineatus*, *Mesocestoides vogae* sp. n., asexual proliferation, taxonomy, Cestoda.

Mesocestoides corti was first named and described in the adult form by Hoenpli (1925). Using a vial of about 100 preserved worms "collected by W. W. Cort in 1909 at Colorado Springs, Colorado" from "a specimen of the common house mouse, *Mus musculus*," Hoenpli described these adult worms and named them in honor of the collector. Specht and Voge (1965) described a form of asexually proliferative tetrathyridia in the fence lizard, *Sceloporus occidentalis biseriatus*, and demonstrated the remarkable proliferative ability of these worms in the coelom and liver of laboratory mice. They tentatively identified this tetrathyridium as *M. corti*, with the disclaimer that "this might suggest our species is not *M. corti*" because they were not able to obtain development to a definitive size in either mice or cats. Voge (1967) further expressed her doubt of the identity of these larvae, stating that "Specht and Voge (1965) described asexual multiplication of tetrathyridia apparently belonging to *Mesocestoides corti*" (emphasis added by the writer). Beaver (1989) seriously questioned that mice could be the type host of *Mesocestoides corti*, because *M. corti* adults have never been rediscovered in the house mouse in over 60 ensuing years, nor has any experimental study shown that the proliferative tetrathyridium isolated by Specht and Voge (1965) can develop into an adult worm comparable in size to Hoenpli's (1925) original description. Conn (1990) reviewed the literature concerning asexual reproduction in species of tetrathyridia and concluded that the only verified instance of this ability is seen in the strain isolated by Specht and Voge (1965). The present study is an attempt to resolve the question of the identity of this unique species of *Mesocestoides*.

Materials and Methods

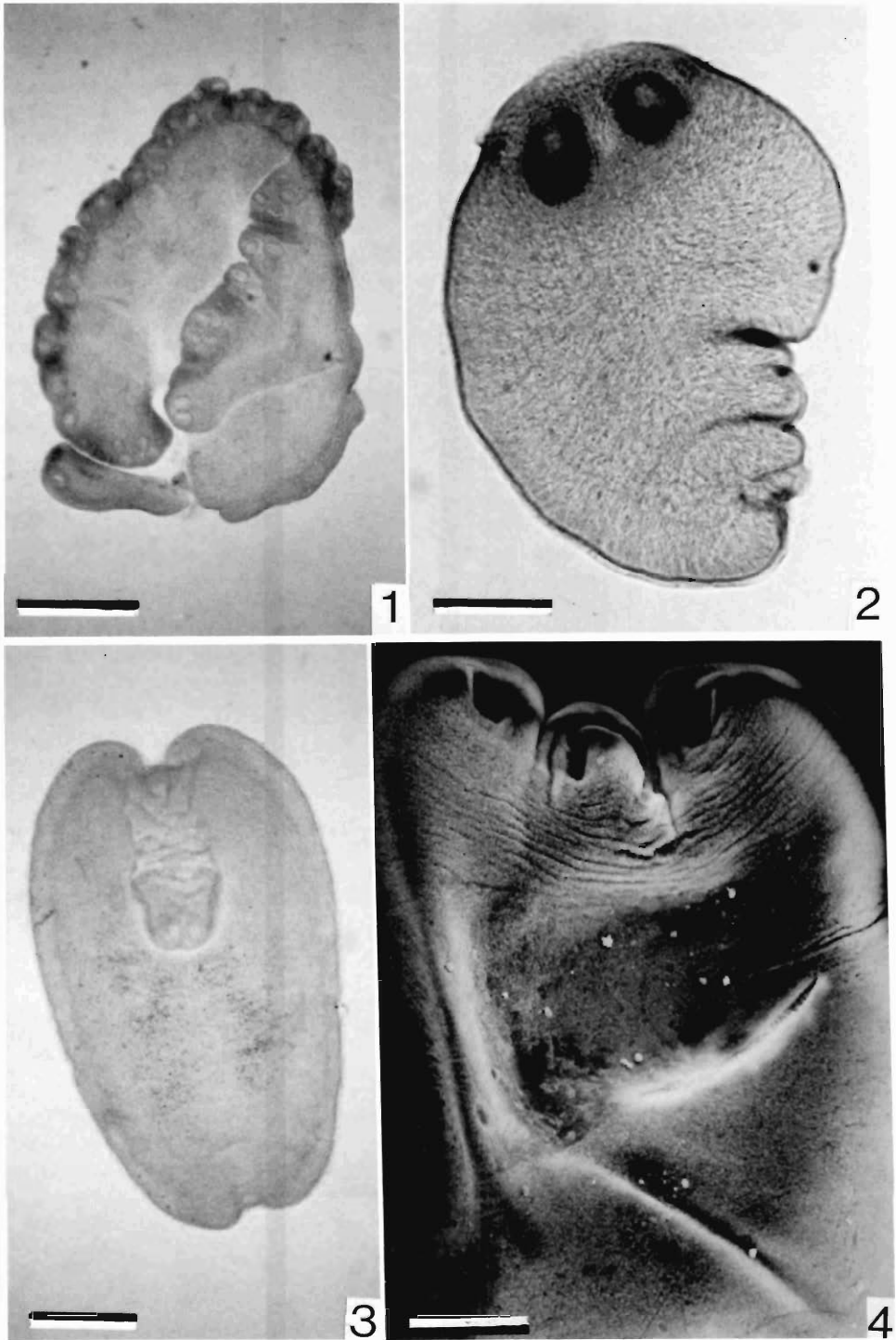
The asexually proliferative tetrathyridia used in this study were from a lineage originally obtained from the western fence swift by Specht and Voge (1965) and maintained in random bred BALB-C mice. About 150 worms obtained from the coelom of infected mice were introduced by stomach tube in all experimental infections. Non-proliferative tetrathyridia of *Mesocestoides lineatus* (Goeze, 1782) were obtained from *Anolis carolinensis* purchased from a commercial supplier as described by Conn and Etges (1984). Adults developed from these tetrathyridia in Syrian hamsters.

Microscope slide preparations were made of specimens fixed in neutral-buffered formalin and stained with Semichon's acetic acid carmine. When the non-specific esterase substrate bromindoxyl acetate was used, specimens were fixed after 1–3-hr periods in the substrate solution (Thompson, 1966). Cover glass pressure was used only when needed for large specimens.

Specimens for scanning electron microscopy were fixed in modified Karnovsky's fixative, soaked overnight in 0.1 M cacodylate buffer, dehydrated in ethanol, critical point dried with CO₂, mounted on aluminum stubs, coated with 30 nm of gold/palladium in a sputter coater, and observed with an ETEC Autoscan electron microscope at 20 kV.

Results

In a period of 6 to 12 mo, mice were infected by many thousands of tetrathyridia actively proliferating by both multiple fission (Fig. 1) and binary fission (fissiparity) (Fig. 4). All growing and reproductive stages showed typical everted scolices (Fig. 2). Most tetrathyridia were found free in the coelom, but a considerable number were encapsulated in liver tissue and (rarely) in other organs such as mammary glands. Proliferative tetrathyridia from mice never showed any significant increase in size or ability to strobilize in the hamster intestine, but they were able to survive for more than 2 mo in this location, while undergoing asexual proliferation.



Figures 1–4. Tetrathyridia of *Mesocestoides vogae* (1, 2, 4) and *M. lineatus* (3). 1. Holotype specimen showing multiple scolex formation (Semichon's acetocarmine); scale bar = 0.25 mm. 2. Paratype specimen showing typical everted scolex (Bromindoxyl acetate stain); scale bar = 0.12 mm. 3. *M. lineatus*, a typical nonproliferative tetrathyridium with inverted scolex (Semichon's acetocarmine); scale bar = 0.50 mm. 4. SEM image showing binary scolex formation (fissiparity); scale bar = 0.25 mm.

Mesocestoides lineatus tetrathyridia never underwent reproduction when transplanted into the coelom of mice or hamsters, but remained the same in size and form as when recovered from the coelom of their anole hosts (Fig. 3). In the small intestine of 1 hamster, 3 gravid adult worms were obtained experimentally after only 22 days; these strobilae were 35, 165, and 208 mm long and were composed of about 100–400 proglottids.

Proliferative tetrathyridia from mice are the basis for the description of a new species.

Description

Mesocestoides vogae sp. n. (Figs. 1, 2, 4)

DIAGNOSIS: Cestoda: Mesocestoidea. Metacestodes of the tetrathyridial type, ranging in size from 0.10 to 0.25 mm in length (Fig. 2); asexually proliferative by means of multiple (Fig. 1) and binary fission (fissiparity) (Fig. 4). Primarily situated in coelom and liver of reptiles (naturally) and small mammals (experimentally); occasionally able to invade other organs (of experimental hosts), such as mammary glands, mesenteries, testes, etc. Able to survive and replicate in small intestine of small mammals, elongate to 4–5 mm, but not strobilize. Occasionally able to strobilize slightly in experimental hosts such as cats and skunks, but rarely to fully gravid condition or length greater than 5 mm.

TYPE HOST: *Sceloporus occidentalis biseriatus* (Hallowell, 1854), the western fence lizard.

EXPERIMENTAL HOSTS: Mice, hamsters, cats, dogs, and other mammals.

TYPE LOCALITY: Riverside and Los Angeles counties, California.

ETYMOLOGY: The name is in honor of Marietta Vogé, late Professor of Microbiology at the University of California at Los Angeles, who discovered this strain of proliferative tetrathyridia, and drew attention to it as a useful experimental model system.

SPECIMENS DEPOSITED: Holotype: USNM Helm. Coll. No. 81558. Paratypes: USNM Helm. Coll. No. 81559.

Discussion

Since its erection by Vaillant (1863), the genus *Mesocestoides* has remained enigmatic. The complete life cycle has never been demonstrated experimentally (though generally believed to involve 3 obligate hosts), the systematic relationships of the genus are uncertain, and the validity

of many named species is questionable because controlled experimental infections are not possible without knowing the identity of the first intermediate host, presumably an arthropod (Webster, 1949). Judging from the recent comprehensive account of Schmidt (1986), adult *Mesocestoides* are almost always parasitic in the small intestines of various carnivores, except for 2 species found in birds and the original report of *M. corti* in the house mouse by Høeppli (1925). Mueller (1930) distinguished *M. corti* from *M. latus* Mueller, 1927, on the basis of the shorter length (4–8 cm), smaller number of proglottids (200–300), and smaller egg size (0.035 mm) in *M. corti*. Vogé (1955) revised the genus, placing *M. variabilis* Mueller, 1927, and *M. manteri* Chandler, 1942, into synonymy with *M. corti* Høeppli, 1925, based on her study of 3 preserved worms of the 100 used by Høeppli (1925). She concluded that "I am reasonably certain that *Mesocestoides corti* Høeppli and *Mesocestoides variabilis* Mueller are cospecific." Neither Høeppli, Mueller, nor Vogé commented on the presence of 100 adult worms in the intestine of a single house mouse.

Tetrathyridia have often been reported in host surveys, as has their development into various species of *Mesocestoides*. For example, Loos-Frank (1980) reported a typical case of adult development in cats experimentally exposed to tetrathyridia of *M. leptothylacus*, a natural parasite of foxes. She also showed that this species of tetrathyridium did not proliferate asexually in mice, birds, or voles. A similar result was obtained by the writer, using tetrathyridia from the common anole, *Anolis carolinensis*, reported by Conn and Etges (1984). In 1 Syrian hamster, 3 adult strobilae were obtained in only 22 days postinfection. These worms, tentatively identified as *M. lineatus* by Conn et al. (1984), were shown to survive indefinitely with no asexual proliferation as tetrathyridia (Fig. 3) in the coelom of mice and hamsters in several experiments, including the present report.

The first, and probably only, proven description of a proliferative tetrathyridium species is that of Specht and Vogé (1965), who recovered their material from the coelom of the fence lizard, *Sceloporus occidentalis biseriatus*, in southern California. They demonstrated the remarkable ability of these worms to proliferate asexually, by a process termed "fissiparity," in vitro maintenance of the tetrathyridia for 4.5 mo, and the ease with which this parasite can be

transferred from mouse to mouse by stomach intubation. They also stressed the possibility of exploiting this convenient experimental model, a suggestion that has led to hundreds of publications produced by laboratories worldwide. All such studies descend from this landmark report and the generosity of the discoverers who have provided this parasite for everyone's use. However, because Specht and Voge were unable to obtain adult worms in mice or cats, their identification of the species was very tentative; they suggested that "our species (might) not (be) *M. corti*. Voge (1967) expressed further uncertainty of its identity in her study, stating that Specht and Voge described asexual multiplication of tetrathyridia "apparently belonging to *Mesocestoides corti*." Uncertainty notwithstanding, the name *M. corti* is universally used to refer to this proliferative metacestode.

Several reports, mainly employing *in vitro* techniques (Voge, 1967; Eckert et al., 1969; Barrett et al., 1982; Kawamoto et al., 1986), have provided some evidence of growth (up to 1.84 cm), strobilization (up to 40 proglottids), and sexual maturation (a few embryonated eggs of about $\frac{2}{3}$ the diameter of those described for *M. corti* (0.035 mm) by Hoenpli [1925]). Because the first intermediate host of *Mesocestoides* is yet unknown, infectivity of eggs produced by the stunted adults derived from proliferative tetrathyridia cannot be tested. It would be of some interest if they could be shown to hatch and develop *in vitro* in the manner described by Voge (1967) for eggs taken from adults found in natural infections, but there has been no report of this to the writer's knowledge. In any case, identification of proliferative tetrathyridia will remain as tentative as Voge believed, until they can be shown to produce adult worms comparable to the 4–8 cm worms described by Hoenpli (1925).

The identity of the mouse as type host of *M. corti* was seriously questioned by Beaver (1989), because no confirmation of a natural infection in mice has been reported in over 60 yr. Furthermore, the presence of about 100 adult worms of 4–8-cm length in the intestine of so small a host is surprising, a point not commented upon by either Hoenpli (1925), Mueller (1930), or Voge (1955). One can only speculate on the reasons that might explain this peculiarity in the description of *M. corti* and its host.

Conn's (1990) comprehensive literature review led to his conclusion that asexual proliferation is a proven ability in only 1 species of

tetrathyridium, i.e., that described by Specht and Voge (1965), and the suggestion that it "may be no more than a rare anomaly that has been propagated because of its convenience as a laboratory model." The writer concurs in these views, but suggests that perhaps asexual proliferation in *Mesocestoides* tetrathyridia may be a valuable evolutionary adaptation to the scarcity of definitive hosts, allowing for it to be transmitted directly from one vertebrate to another by deleting intermediate and definitive hosts from the normal life cycle. Confirmation of this view will depend on fieldwork to confirm the presence of asexual proliferation in a larger number of cases than presently known (1 fence lizard and 1 horned toad; Voge, pers. comm.).

Because of the several questions raised by Conn (1990) and Beaver (1989), and the uncertain identity of the proliferative tetrathyridium species discovered by Specht and Voge (1965), the writer is convinced that another name should be coined for it, and that *M. corti* be restricted to the adult worms described by Hoenpli (1925) and any other morphologically similar adults found in any mammalian host. While Witenberg (1934) cautioned against the naming of species of tetrathyridia because "no specific morphological distinction may be recognized between them" and "only in limited instances is it possible to indicate to what species they should be attributed," the writer thinks that there are good and sufficient developmental and physiological grounds (cited above) for giving a separate name to the proliferative tetrathyridia isolated by Specht and Voge.

Acknowledgments

Sincere thanks are extended to Jerry Snider for photographic assistance, D. B. Conn for providing microscope slide mounts, and Ralph Thorson for providing the proliferative strain of tetrathyridia, which he had obtained from Marietta Voge.

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MEETING NOTICES

The Second International Symposium on Monogenea will be held in Montpellier, France, 5-8 July 1993. For information contact:

Dr. Alain Lambert
 Laboratoire de Parasitologie Comparée C. C. 105
 Université des Sciences et Techniques de Languedoc
 Place E. Bataillon - 34095
 Montpellier Cédex 05, FRANCE

The Twenty-first International Nematology Symposium will be held in Albufeira, Portugal, 12-17 April 1992. For information contact:

Secretary 21st International Nematology Symposium
 % Departamento de Zoologia
 Universidade de Coimbra
 3049 Coimbra Codex, PORTUGAL

Nematode Parasites from Marine Fishes of Okinawa, Japan

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ABSTRACT: *Paracapillaria sesokoensis* sp. n. is described from *Fistularia petimba*, *Cucullanus okinawanus* sp. n. from *Echidna delicatula*, and *Dichelyne (Neocucullanelus) laticeps* is redescribed from *Arothron mappa*. *Paracapillaria sesokoensis* is distinguished from other members in the genus by the shape and measurement of spicule, and *C. okinawanus* is readily distinguished from other representatives in the genus by its stout spicules and body dimensions. *Spirocamallanus istiblenni*, *Heliconema baylisi*, and *D. (N.) laticeps* are recorded from Okinawan fishes for the first time, and their presence suggests that the fish nematode fauna of this area has characteristics of the Indo-Polynesian Province of the Indo-West Pacific Region. Nematodes rarely occurred in the Okinawan shore fishes examined.

KEY WORDS: Nematoda, *Paracapillaria sesokoensis* sp. n., *Cucullanus okinawanus* sp. n., *Dichelyne (Neocucullanelus) laticeps* redescription, coral reefs, survey, fish, Okinawa, Japan, new hosts, new localities, habitat.

Few reports are available on the parasitic nematode fauna of marine fishes of Okinawa (cf. Yamaguti, 1941). Because of the need for additional information on the helminths of marine fishes of this area, the junior authors examined various fishes as listed by Dyer et al. (1988a, 1989) during their stay as invited researchers at the Sesoko Marine Science Center, University of the Ryukyus, from 27 May 1985 through 20 March 1986. This paper reports the nematodes collected from these fishes with descriptions of new species and new geographical and host records. Some other groups of parasites from these examinations that have been reported include: Digenea (Dyer et al., 1988a, b), Monogenea (Dyer et al., 1989), barnacles (Williams and Williams, 1986b), and nudibranchs (Williams and Williams, 1986a).

Materials and Methods

Fishes were collected with spearguns (Williams and Williams, 1986). They were either examined immediately or held alive and examined within 24 hr of capture. The alimentary system, coelomic cavity, gills, swim bladder, urinary bladder, and skin of all fishes were examined. Nematodes were fixed in AFA solution, prepared by the standard glycerin alcohol method, and mounted with 50% glycerin aqueous solution. Figures were made with the aid of a drawing tube. Measurements of holotypes and allotypes are followed by ranges for paratypes in parentheses. All measurements are in micrometers unless otherwise stated. Specimens of all nematode species are deposited in the United States National Museum Helminthological Collection (USNM). The following material was also examined: *Spirocamallanus istiblenni* Noble, 1966, syntypes (1 male and 1 female), USNM 72590 and 72591; *Spirocamallanus philippinensis* Machida and Taki, 1985 (3 males and 3 females), National Science Museum,

Tokyo, NSMT As-1802; *Dichelyne (Neocucullanelus) laticeps* Baylis, 1948, syntypes (2 males and 3 females), British Museum (Natural History), Reg. No. 1950.12.6. 167-178.

Results

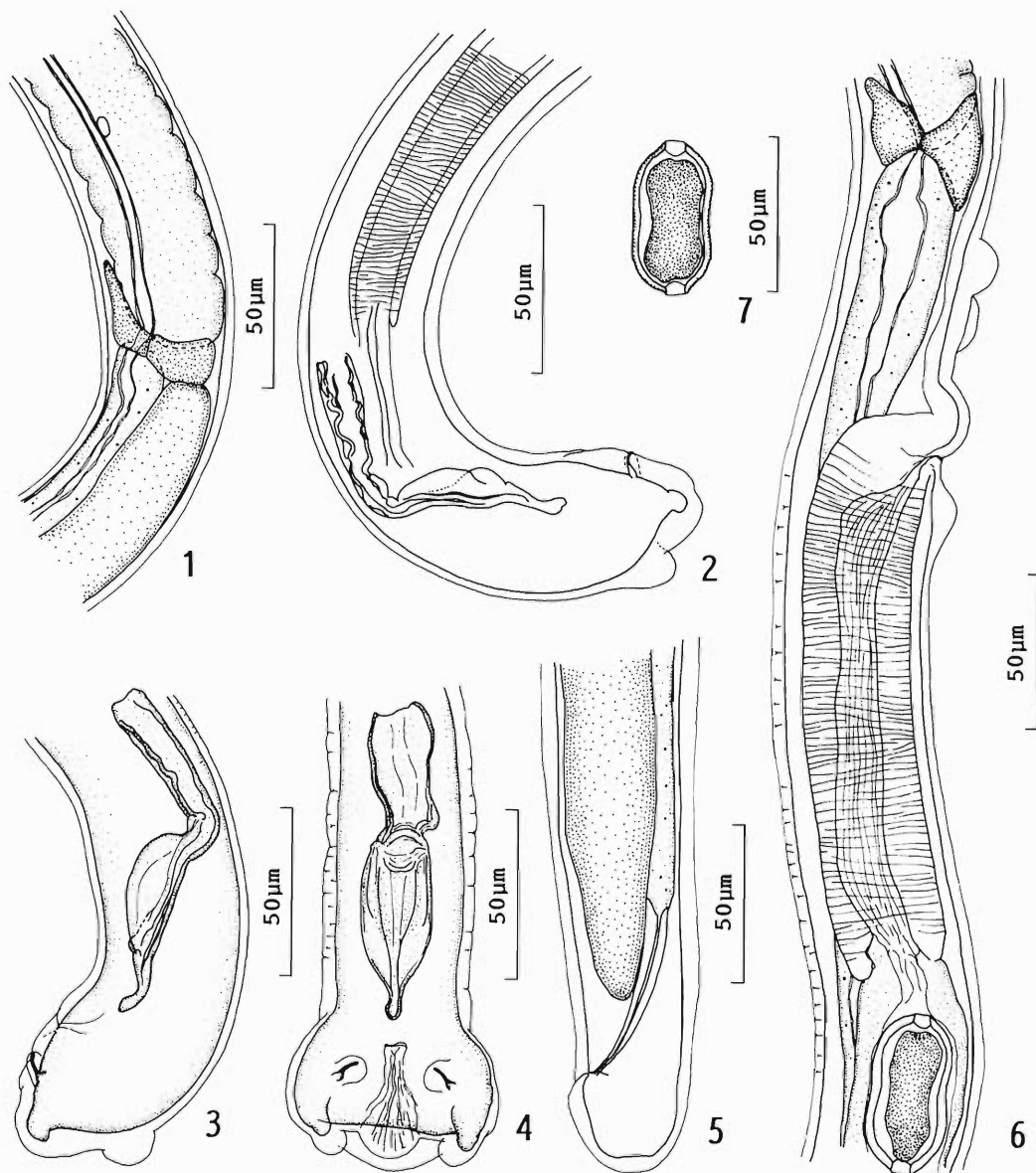
Nine species of nematodes were collected from 27 specimens of 24 species of fishes (Table 1). Nematodes occurred in 9.3% of the specimens and 12.9% of the species of fishes examined.

Paracapillaria sesokoensis sp. n. (Figs. 1-7)

Trichinelloidea, Trichuridae, Capillariinae.

Body slender, threadlike, forming complex tangles. Cuticle thin, with fine transverse striations. Anterior part extremely thin with pointed apex. Muscular esophagus short followed by stichocytes in single row. Stichocytes uniform in color, each with transverse annuli and small nucleus (Fig. 1). Esophago-intestinal junction with 2 glandular cells (Figs. 1, 6). Lateral bacillary bands present (Figs. 4, 6).

MALES (holotype and 1 paratype): Length 17 (18) mm and maximum width 48 (48) at about 4 mm anterior to posterior extremity. Nerve ring 106 from anterior extremity. Muscular esophagus 294 long. Esophago-intestinal junction 11.5 (10.7) mm from anterior extremity. Posterior extremity expanded laterally forming bursate structure; 1 pair of small papillae projecting ventrally and 1 pair of stout papillae present at posterior apex (Figs. 2-4). Spicule stout, short, bent ventrally, with lateral thickenings in distal half, with



Figures 1–7. *Paracapillaria sesokoensis* sp. n. from *Fistularia petimba*. 1. Esophago–intestinal junction of male (holotype), lateral view. 2. Posterior end of male (holotype), lateral view. 3. Posterior end of male (paratype), lateral view. 4. Posterior end of male (paratype), ventral view. 5. Posterior end of female (paratype), lateral view. 6. Vulval part of female (paratype), lateral view. 7. Egg.

rounded tip, 95 (100) long (Figs. 2–4). Ejaculatory duct with fine striations, 1.75 (1.69) mm long (Fig. 2).

FEMALE (1 paratype lacking anterior end): Length over 31 mm and maximum width 65 at about 10 mm anterior to posterior extremity. Esophago–intestinal junction 21.3 mm from posterior extremity. Vulva behind esophago–intes-

tinal junction (Fig. 6). Anterior lip of vulva protruded, 2 cuticular elevations in front of vulva and 1 posterior to vulva (Fig. 6). Vagina muscular, directed posteriorly, 167 long (Fig. 6). Anus subterminal, posterior extremity round (Fig. 5). Eggs elliptical, with polar plugs, slightly constricted at middle, surface with fine markings, 48–51 by 23–26 (at widest portion) (Fig. 7).

Table 1. Nematode parasites from marine fishes of Okinawa, Japan.

Host							
Species	Size of infected	No. examined/ no. infected	Collection		Parasite		
			Locality*	Date	Number	Location	Other parasites
<i>Paracapillaria sesokoensis</i>							
<i>Fistularia petimba</i>	37.0 cm SL	2/1	north reef	9 & 17 July 1985	8	intestine	10 immature cestodes 13 <i>Allolepidapedon fistulariae</i> †
<i>Spirocamallanus istiblenni</i>							
<i>Amphiprion clarkii</i>	10.5 cm SL	2/1	southeast	28 May 1985	1	intestine	4 <i>Bucephalopsis ozaki</i> †
<i>Bothus pantherinus</i>	13.2 cm SL	1/1	southeast	12 March 1986	5 ♀, 9 ♂	intestine	1 digenean
<i>Parapercis cylindrica</i>	7.7 cm SL	2/1	southeast	28 May 1985	1	intestine	
	6.7 cm SL	2/1	southeast	1 & 4 June 1985	1	intestine	10 <i>Sterrhurus magnacetabulum</i> †
<i>Parapercis polyphthalma</i>	11.9 cm SL	1/1	southeast	4 June 1985	2	intestine	
<i>Plectorhynchus pictus</i>	17.0 cm SL	1/1	north coast	12 July 1985	4	intestine	2 <i>Pseudopecoelus sesokoensis</i> ‡
<i>Scolopsis bilineatus</i>	15.0 cm SL	1/1	southeast	27 May 1985	1 ♀, 2 ♂	intestine	
<i>Soleichthys heterorhinos</i>	6.8 cm SL	1/1	southeast	12 October 1985	1	mid-intestine	
<i>Valencienna strigata</i>	13.0 & 15.0 cm SL	2/2	north reef	19 July 1985	3	intestine	
<i>Variola albimarginata</i>	32.0 cm SL	2/1	south reef	24 July 1985	5	intestine	37 <i>Proisorhynchus platycephali</i> †
<i>Variola louti</i>	20.5 cm SL	1/1	north coast	12 July 1985	1	intestine	20 <i>P. platycephali</i> †
<i>Heliconema baylisi</i>							
<i>Echidna delicatula</i>	49.8 cm TL	1/1	north reef	9 July 1985	50+	stomach	15 <i>Helicometrina quadrorchis</i> †
<i>Cucullanus okinawanus</i>							
<i>Echidna delicatula</i>	49.8 cm TL	1/1	north reef	9 July 1985	1 ♀, 1 ♂	intestine	15 <i>H. quadrorchis</i> †
<i>Dichelyne laticeps</i>							
<i>Arothron mappa</i>	26.5 cm SL	1/1	southeast	22 October 1985	5	intestine	
<i>Philometra</i> sp.							
<i>Epinephelus summana</i>	27.9 cm SL	1/1	north reef	18 & 19 July 1985	1	under skin	4 copepods
		1/0	southeast	27 June 1985			
		1/0	north coast	12 July 1985			
<i>Tylosurus crocodilus</i>	63.0 cm SL	1/1	southeast	26 June 1985	1	gular rays under skin	(encysted)
	76.0 cm SL	1/1	southeast	13 March 1986	1	anal fin rays under skin§	

Table 1. Continued.

Host		No. examined/ no. infected	Collection		Parasite		
Species	Size of infected		Locality*	Date	Number	Location	Other parasites
<i>Hysterothylacium</i> sp.							
<i>Epinephelus hoedtii</i>	27.2 cm SL	1/1	west coast	11 September 1985	1	stomach	1 digenea immature cestodes
<i>Heniochus chrysostomus</i>	12.5 cm SL	2/1	north reef	11 July 1985	1	intestine	7 <i>Hysterolecitha</i> sp.†
		1/0	north reef	30 May 1985			
<i>Raphidascaris</i> sp. A							
<i>Amblyglyphidodon leucogaster</i>	11.0 cm SL	1/1	north reef	11 July 1985	1	intestine	
<i>Lutjanus fulviflamma</i>	24.4 cm SL	1/1	west coast	11 September 1985	1	ceca	5 <i>Hamacreadium</i> <i>lethrini</i> †
		1/0	north coast	12 July 1985			
<i>Saurida gracilis</i>	15.6 cm SL	1/1	southeast	3 June 1985	1	intestine	1 digenea
<i>Synodus variegatus</i>	20.9 cm SL	1/1	southeast	4 June 1985	1	intestine	2 <i>Sterrhurus</i> sp.† immature cestodes
<i>Raphidascaris</i> sp. B							
<i>Gymnothorax flavimarginatus</i>	78.0 cm TL	1/1	north coast	4 July 1985	1 ♀, 2 ♂	intestine	1 <i>Aponurus</i> <i>acropomati</i> ‡
Unknown nematodes							
<i>Diplogrammus xenicus</i>	4.0 cm SL	3/1	southeast	3, 12 & 13 June 1985	1	intestine	(immature worm, lost)
<i>Bathygobius fuscus</i>	4.9 cm SL	3/1	southeast	3 July 1985	1	intestine	(<i>Spirocamallanus</i> sp.?)

* Localities around Sesoko Island follow Williams and Williams (1986). Southeast = off Sesoko Marine Science Center; north coast = north coast of Sesoko Island; north reef = reefs north of Sesoko Island; south reef = reef extending off south coast of Sesoko Island; west coast = west coast of Sesoko Island.

† Dyer et al., 1988a.

‡ Dyer et al., 1988b.

§ Not removed from host. Fish preserved intact and deposited in USNM Ichthyological Collection by B. B. Collette.

HOST: *Fistularia petimba* Lacépède (Fistulariidae).

SITE IN HOST: Intestine.

SPECIMENS DEPOSITED: Holotype, USNM 81813; paratypes, USNM 81814.

REMARKS. The new species belongs to the genus *Paracapillaria* Mendonça, 1963, because the male lacks a spiny spicular sheath and lateral caudal alae, but has a membranous bursa supported by 2 lateral rays (cf. Moravec, 1986, 1987). Fourteen species have been recognized in the genus and 6 of them are parasitic in fishes (Moravec, 1986, 1987, 1990). *Paracapillaria sesokoensis* differs from all other members of the genus by having a much shorter spicule of characteristic shape (over 0.2 mm in length in other species).

The specimen of *F. petimba* without nematodes and the 1 with *P. sesokoensis* were also infected with 2 different digeneans, 15 *Opecoelus sphaericus*, and 13 *Allolepidapedon fistulariae*, respectively, also both were infected with immature cestodes (3 and 10, respectively), all in the intestine. The *O. sphaericus* occurred in the posterior intestine, while *P. sesokoensis* and *A. fistulariae* occurred throughout.

Spirocamallanus istiblenni Noble, 1966

Camallanoidea, Camallanidae.

MALES (19 specimens): Length 7.6–15.4 mm and maximum width 120–240. Buccal capsule 68–86 long and 52–87 wide. Nerve ring 190–260 and excretory pore 370–590 from anterior extremity. Muscular esophagus 310–420 long and glandular esophagus 380–590 long. Right spicule 220–290 long, left spicule 150–200 long. Tail 120–230 long.

FEMALES (10 specimens): Length 9.4–25.7 mm and maximum width 150–420 in midbody. Buccal capsule 75–96 long and 67–75 wide. Nerve ring 240–310, excretory pore 400–660 and vulva 4.77–11.7 mm from anterior extremity. Muscular esophagus 390–470 long and glandular esophagus 400–680 long. Tail 150–190 long. Eggs 40–42 by 27–31.

HOSTS: *Amphiprion clarkii* (Bennett) (Pomacentridae), *Bothus pantherinus* (Ruppell) (Bothidae), *Parapercis cylindrica* (Bloch) (Mugiloididae), *Parapercis polyophtalma* (Cuvier) (Mugiloididae), *Plectorhynchus pictus* (Thunberg) (Pomadasyidae), *Scolopsis bilineatus* (Bloch) (Nemipteridae), *Soleichthys heterorhinos* Bleeker (Soleidae), *Valenciennesa strigata* (Broussonet)

(Gobiidae), *Variola albimarginata* Baissac (Serranidae), *Variola louti* (Forsskål) (Serranidae).

SITE IN HOST: Intestine.

SPECIMENS DEPOSITED: USNM 81815–81824.

REMARKS: Although the present worms have a somewhat longer esophagus, morphological characteristics are otherwise identical to those in the original description and of type specimens of *S. istiblenni* from *Istiblennius zebrae* (Blennidae) in Hawaii (Noble, 1966). The fishes listed above are recorded for the first time as hosts of *S. istiblenni*. *Spirocamallanus istiblenni* resembles *Spirocamallanus philippinensis* Machida and Taki, 1985, from *Siganus guttatus* (Siganidae) of the Philippines in general morphology, but is distinguished in that the mature adults of the latter species have much larger body (males 17.6–19.8 mm long and females 32.7–40.1 mm long; Machida and Taki, 1985).

Spirocamallanus istiblenni seems to have little host specificity, occurring in 8 families of fishes in our study. It also seemed to be widespread in habitat preferences, occurring in all localities around Sesoko Island (Table 1). The food habits of these hosts, which would presumably be important in transmission to the final host, varied from large predators and minipredators, through generalists and herbivores (Williams and Williams, 1986).

The 5 specimens of *S. istiblenni* in *V. albimarginata* occurred in the posterior intestine while 22 specimens of *Proisorhynchus platycephali* (Digenea) (Dyer et al., 1988a) occupied the mid-intestine and 17 in the anterior intestine. This sort of habitat differentiation was not noted in other digeneans associated with this nematode. Two *Sterrhurus* sp. occurred in the stomach of a second *V. albimarginata* (Dyer et al., 1988a).

Heliconema baylisi Ogdén, 1969

Physalopteroidea, Physalopteridae, Proleptinae.

MALES (10 specimens): Length 21–37 mm and maximum width 300–530. Diameter of head 104–127. Buccal cavity 30–44 long. Nerve ring 260–330, excretory pore 450–600 and deirids 250–350 from anterior extremity. Muscular esophagus 440–570 long and glandular esophagus 2.42–3.62 mm long. Right spicule 350–410 long, left spicule 380–500 long, and spicule ratio 1:1.05–1.22. Tail 380–550 long.

FEMALES (8 specimens): Length 15–40 mm and maximum width 280–620. Diameter of head

99–147. Buccal cavity 31–50 long. Nerve ring 220–350, excretory pore 350–550 and deirids 240–400 from anterior extremity. Muscular esophagus 370–570 long and glandular esophagus 2.50–4.42 mm long. Vulva 9.2–21.5 mm from anterior extremity. Tail 200–280 long. Eggs 40–42 by 27–31.

HOST: *Echidna delicatula* (Kaup) (Muraeniidae).

SITE IN HOST: Intestine and stomach.

SPECIMENS DEPOSITED: USNM 81825.

REMARKS: *Heliconema baylisi* was originally described from *Echidna nebulosa* caught at Cocos Keeling Island, Indian Ocean (Ogden, 1969). Fusco and Palmieri (1980) stated that the average spicule ratio of *H. baylisi* is 1:1.5. However, this value may be incorrect because the spicule ratio calculated from the original data by Ogden (1969) is 1:1.11–1.36. The present specimens are morphologically identical with the original description of *H. baylisi*, but some worms are much larger (cf. Ogden, 1969). *Echidna delicatula* represents a new host.

More than 50 *H. baylisi* were attached in the anterior stomach of *E. delicatula*. Fifteen specimens of *Helicometrina quadrorchis* (Dyer et al., 1988a) occurred in the anterior intestine and 2 specimens of *Cucullanus okinawanus* were found in the posterior intestine of the same host.

***Cucullanus okinawanus* sp. n.**
(Figs. 8–15)

Seuratoidea, Chitwoodchabaudiidae, Cucullininae.

Body stout with maximum width at midbody. Cuticle thin, with fine transverse striations. Lateral alae present. Pseudobuccal cavity relatively developed; inner surface with numerous minute tubercles; Y-shaped suture present; transverse ventral plate small; reniform structures and dorsal arrow-shaped structures well developed (Figs. 8–10). Four submedian papillae and amphids present (Figs. 9, 10), 6 inner labial papillae present. Mouth dorsoventrally slitlike, bordered by collarette bearing many teeth on inner surface (Figs. 9, 10). Esophagus club-shaped; posterior width larger than anterior width in lateral view (Fig. 8). Nerve ring at anterior 1/3 of esophagus (Fig. 8). Deirids prominent, at posterior 1/3 of esophagus (Fig. 8). Excretory pore near esophago-intestinal junction (Fig. 8); excretory system with relatively long terminal duct connecting posteriorly small sinus from which 2 anteriorly

directed and 2 posteriorly directed lateral canals arise (Fig. 11). Intestinal cecum absent.

MALE (holotype): Length 7.6 mm and maximum width 220. Esophagus 750 long, anterior width 140 and posterior width 180. Nerve ring 260, excretory pore 720 and deirids 590 from anterior extremity. Ventral sucker without cuticular rim present preanally (Fig. 12). Tail conical, narrowed abruptly at a short distance behind anus, 210 long (Fig. 12). Caudal alae absent. Ten pairs of sessile papillae present in caudal region: anterior 4 pairs well spaced; 1st pair anterior to sucker; 2nd pair posterior to sucker; 3rd pair midway between sucker and anus; 4th to 6th pairs grouped adanally; 7th and 8th pairs positioned laterally; 9th and 10th pairs near tail tip (Fig. 12). Spicules subequal, stout, well chitinized, widest at midportion, tapering to both ends, and pointed distally; right spicule 390 long and left spicule 400 long (Fig. 12). Gubernaculum well chitinized, Y-shaped in ventral view, 127 long.

FEMALE (allotype): Length 9.9 mm, maximum width 300. Esophagus 890 long, anterior width 150 and posterior width 210. Nerve ring 300, excretory pore 790 and deirids 580 from anterior extremity. Vulva 5.95 mm from anterior extremity. Amphidelphic; vagina directed anteriorly, then flexed posteriorly (Fig. 13). Tail conical, abruptly narrowed behind anus, 234 long (Fig. 14). Eggs elliptical, thin-shelled, containing 1- to 2-cell stage embryos at deposition, 65–71 by 35–42 (Fig. 15).

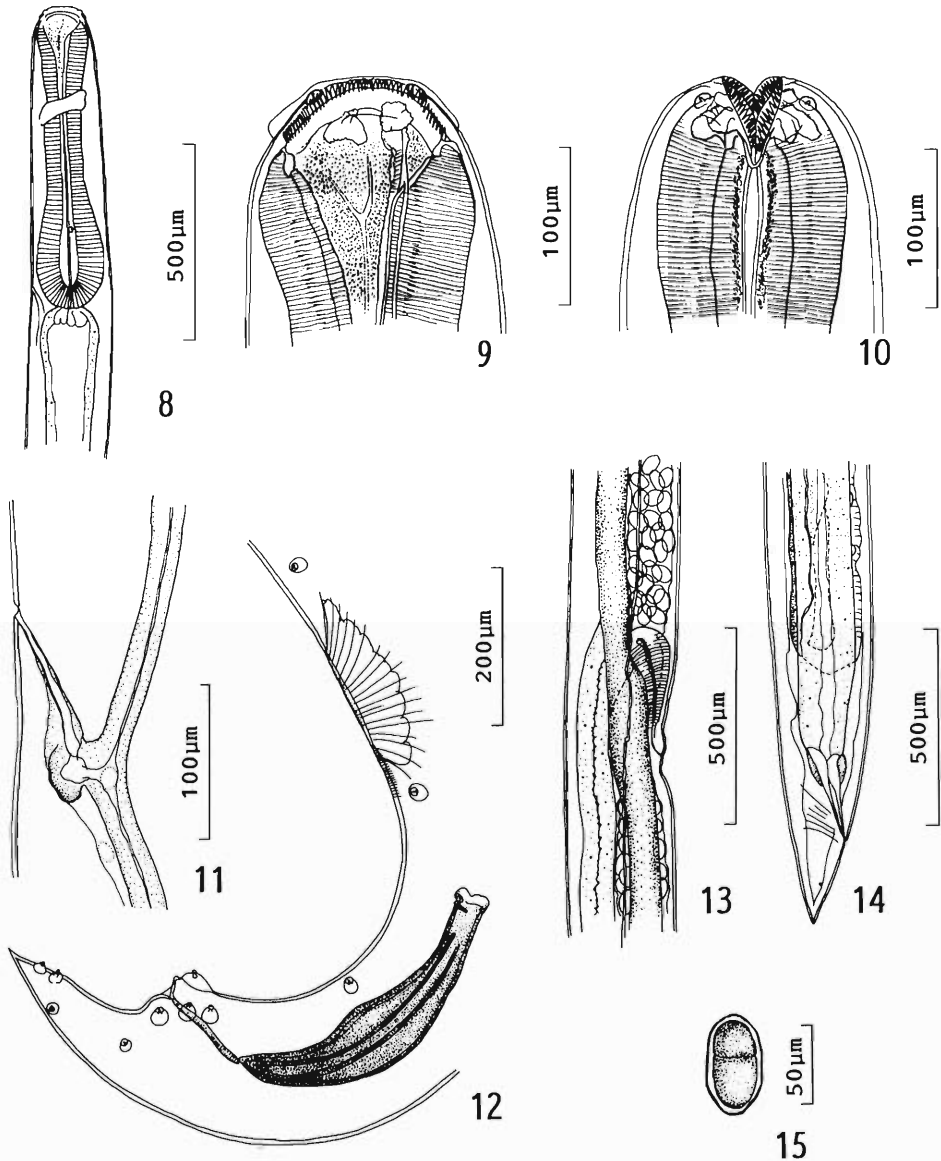
HOST: *Echidna delicatula* (Kaup) (Muraeniidae).

SITE IN HOST: Intestine.

SPECIMENS DEPOSITED: Holotype and allotype, USNM 81826.

REMARKS: The new species belongs to the genus *Cucullanus* Mueller, 1777, in having a developed pseudobuccal cavity with Y-shaped suture and dorsoventrally elongated mouth, and in lacking intestinal cecum and alae in male tail (cf. Chabaud, 1978). Petter (1974b) revised the family Cucullanidae Cobbold, 1864, and recognized 68 species in the genus *Cucullanus*. Subsequently, 31 species have been added in the genus. From Okinawan waters, *Cucullanus himezi* Yamaguti, 1941, has been described from *Upeneu bensasi* (Mullidae) (Yamaguti, 1941).

Cucullanus antipodeus Baylis, 1932, *C. bagre* Petter, 1974, *C. caballeroi* Petter, 1977, *C. chrysochrydis* Gendreau, 1927, *C. micropapillatus*



Figures 8–15. *Cucullanus okinawanus* sp. n. from *Echidna delicatula*. 8. Anterior part of female (allotype), lateral view. 9. Anterior extremity of female (allotype), lateral view. 10. Anterior extremity of female (allotype), ventral view. 11. Excretory system, lateral view. 12. Posterior end of male (holotype), lateral view. 13. Vulval part of allotype, lateral view. 14. Posterior end of female (allotype). 15. Egg.

Törnquist, 1931, *C. muraenesocis* Yamaguti, 1961, *C. murenophidis* Campana-Rouget, 1957, *C. pseudeutrophi* Agrawal, 1967, *C. quadrii* Bliques and Fatima, 1980, and *C. vachai* Gupta and Bakshi, 1983, resemble *C. okinawanus* by having males with a preanal sucker and spicules shorter than 0.5 mm. However, all except *C. murenophidis* are readily distinguished from *C.*

okinawanus by having spicules with parallel sides, lacking markedly expanded middle portion (cf. Yamaguti, 1961; Petter, 1974a, 1977; Ivashkin and Khromova, 1976; Bilques and Fatima, 1980; Gupta and Bakshi, 1983). Although the shape of spicules of *C. murenophidis* has not been described or figured, it differs from *C. okinawanus* in having an excretory pore that is situated pos-

terior to the esophago-intestinal junction, an esophagus which is more inflated anteriorly than posteriorly, and 2 pairs of large papillae on the mid-ventral portion of the tail (Campana-Rouget, 1957).

Cucullanus okinawanus can also be distinguished from the *Cucullanus* species for which males have not been described: *C. callichroi* (Stewart, 1914) has a shorter but thicker body (6.63–7.225 mm long by 0.39–0.43 mm wide) and larger eggs (85 by 56); *C. carangis* (MacCallum, 1921) has an esophagus, the anterior portion of which is much wider than the posterior; *C. girellae* Yamaguti, 1941, is a much larger worm (19–20 mm long) with larger eggs (75–81 by 41–45); *C. bilqeesi* Petter, 1974, has 5 finger-like projections in the pseudobuccal cavity (cf. MacCallum, 1921; Yamaguti, 1941; Bilqees et al., 1971; Ivashkin and Khromova, 1976).

***Dichelyne (Neocucullanellus) laticeps*
Baylis, 1948
(Figs. 16–29)**

Seuratoidea, Chitwoodchabaudiidae, Cucullaninae.

Body relatively stout, widest at postesophageal portion and tapering to both extremities. Cuticle thick, with fine transverse striations; lateral alae absent. Prominent somatic papillae with rounded tips (Fig. 20) and intracuticular papilla-like structures (Fig. 21) scattered on body. Cephalic extremity slightly inclined ventrally (Fig. 16). Pseudobuccal cavity relatively developed; inner surface with numerous minute tubercles; Y-shaped suture present; transverse ventral plate small; reniform structures and dorsal arrow-shaped structures small (Figs. 17–19). Four submedian papillae and amphids present, 6 small inner labial papillae present (Figs. 17–19). Mouth dorsoventrally slitlike, bordered by collarete bearing many minute teeth on inner surface (Fig. 17). Esophagus club-shaped, anterior width larger than posterior width (Fig. 16). Intestinal cecum present on dorsal side of esophagus (Fig. 16). One pair of well-developed cervical glands present laterally extending to mid-esophagus (Fig. 16). Nerve ring immediately posterior to pseudobuccal cavity (Fig. 16). Deirids sharply pointed, posterior to cervical glands (Figs. 16, 22). Excretory pore anterior to posterior end of esophagus (Fig. 16); excretory system with extremely short terminal duct attached to posteriorly directed sinus from which 2 anteriorly di-

rected and 2 posteriorly directed lateral canals arise (Figs. 23, 24).

MALE (1 specimen): Length 11.7 mm and maximum width 380. Esophagus 880 long, anterior width 210 and posterior width 120. Intestinal cecum 630 long. Nerve ring 330, excretory pore 980, deirids 860 from anterior extremity. Oblique muscular bands present anterior to anus. Tail conical, with pointed tip, 170 long (Figs. 25, 26). Eleven irregular pairs of caudal papillae present: 8 pairs preanal and 3 pairs postanal; 1st to 6th pairs well spaced; 6th, 8th and 9th pairs set closely; 7th pair situated laterally; 10th and 11th pairs near posterior end (Figs. 25, 26). In addition, 1 pair of small papillae and 1 unpaired median papilla present on anterior anal lip (Fig. 26). Phasmids at middle of tail (Fig. 26). Spicules weakly chitinized, simple, slender, tapering distally; each distal end forming a small hook (Fig. 25). Right spicule 1.59 mm long, left spicule 1.52 mm long. Gubernaculum rodlike in lateral view, 82 long (Fig. 25).

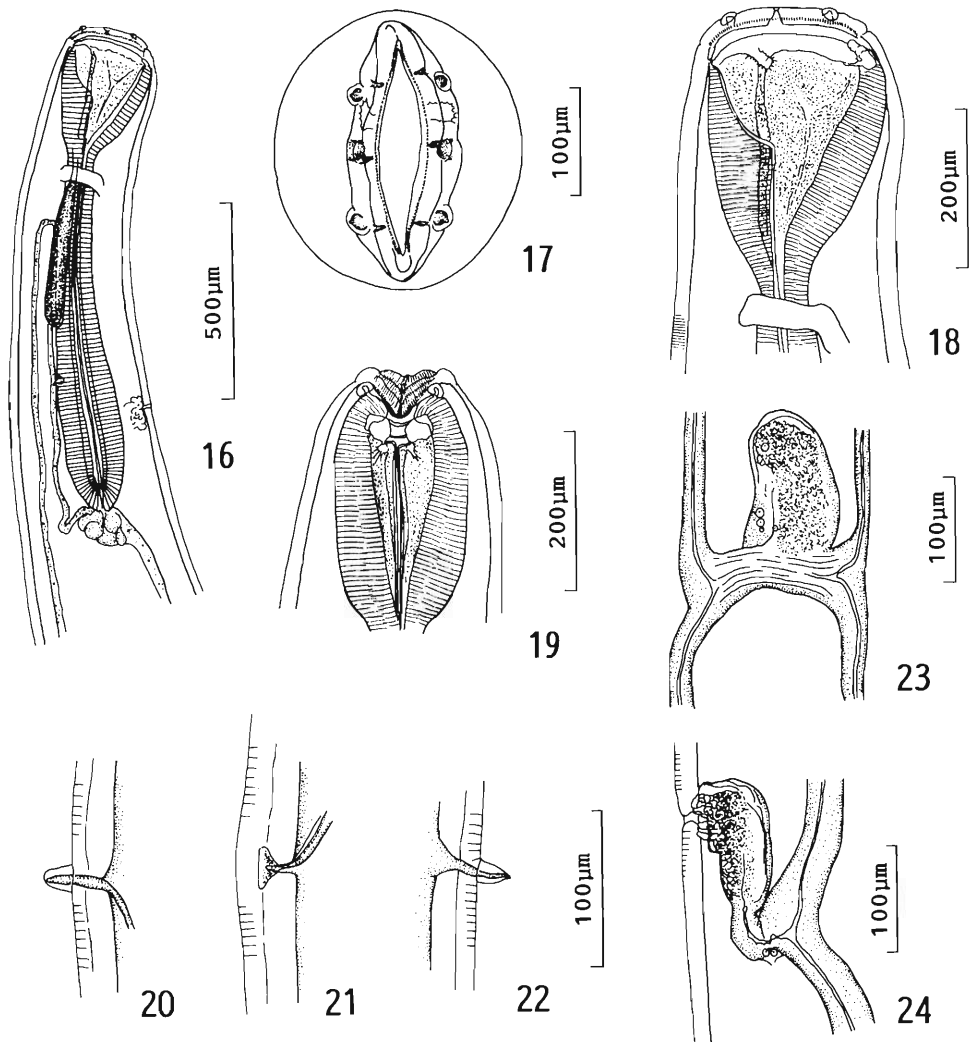
FEMALES (4 specimens): Length 9.8–16.8 mm and maximum width 360–670. Esophagus 1.00–1.30 mm long, anterior width 190–260 and posterior width 120–170. Intestinal cecum 660–880 long. Nerve ring 310–370, excretory pore 0.87–1.03 mm, deirids 780–960 from anterior extremity. Vulva 5.28–8.93 mm from anterior extremity (Fig. 27). Amphidelphic, vagina muscular, 370–760 long, directed anteriorly and joining to uteri (Fig. 27). Tail conical, with prominent phasmids at middle, tip hornlike, 200–250 long (Fig. 28). Eggs elliptical, thin-shelled, containing 1- to 2-cell stage embryo at deposition, 55–60 by 40–45 (Fig. 29).

HOST: *Arothron mappa* (Lesson) (Tetraodontidae).

SITE IN HOST: Intestine.

SPECIMENS DEPOSITED: USNM 81827.

REMARKS: *Dichelyne (N.) laticeps* is the only representative of the subgenus *Neocucullanellus* parasitic in Tetraodontiformes (Petter, 1974b). This species was first described from *Arothron hispidus* (syn. *Tetraodon hispidum*) from Cleveland Bay, North Queensland, Australia (Baylis, 1948). This species was in need of redescription because the original type materials were shrunken and distorted by being mounted in Canada balsam (Baylis, 1948). The present specimens are somewhat larger and have longer spicules but otherwise agree with the type specimens and the original description. The spicules are about 1 mm



Figures 16–24. *Dichelyne (Neocucullanellus) laticeps* Baylis, 1948, from *Arothron mappa*. 16. Anterior part of female, lateral view. 17. Anterior extremity of female, apical view. 18. Anterior extremity of female, lateral view. 19. Anterior extremity of female, ventral view. 20. Somatic papilla. 21. Intracuticular papilla-like structure. 22. Deirid. 23. Excretory system, ventral view. 24. Excretory system, lateral view.

in the original description (Baylis, 1948). In the type specimens, the spicules have numerous wrinkles, which may have resulted from distortion of these weakly chitinized structures.

From Okinawan waters *Dichelyne (Neocucullanellus) apharesi* Yamaguti, 1941, has been described from *Aphareus furcatus* (Lutjanidae) (Yamaguti, 1941). *Dichelyne (N.) laticeps* is readily distinguished from *D. (N.) apharesi* by the dorsal position of the intestinal cecum (ventral side in *D. (N.) apharesi*) and larger body (males 3.5–4.9

mm long and females 4.0–6.43 mm long in *D. (N.) apharesi*) (Yamaguti, 1941).

***Philometra* sp. (mature female)**

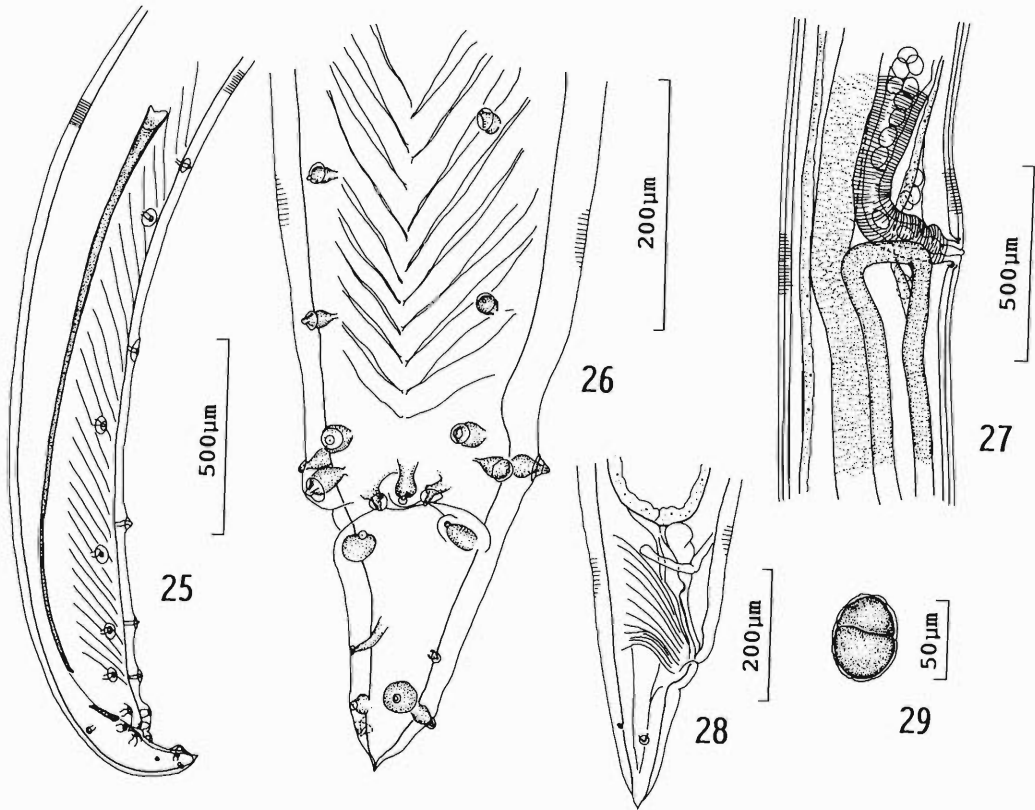
Dracunculoidea, Philometridae.

HOSTS: *Epinephelus summana* (Forsskål) (Serranidae), *Tylosurus crocodilus* (Lesueur) (Belontiidae).

SITE IN HOST: Under skin.

SPECIMENS DEPOSITED: USNM 81828, 81829.

REMARKS: This red-colored nematode can be



Figures 25–29. *Dichelyne (Neocuccullanelus) laticeps* Baylis, 1948 from *Arothron mappa*. 25. Posterior part of male, lateral view. 26. Posterior extremity of male, ventral view. 27. Vulval part of female, lateral view. 28. Posterior end of female, lateral view. 29. Egg.

observed easily through the skin of the host in the gular areas or fin rays (Table 1).

***Hysterothylacium* sp.**
(larva or immature adult)

Ascaridoidea: Anisakidae.

HOSTS: *Epinephelus hoedtii* (Bleeker) (Serranidae), *Heniochus chrysostomus* Cuvier (Chaetodontidae).

SITE IN HOST: Intestine and stomach.

SPECIMENS DEPOSITED: USNM 81830, 81831.

***Raphidascaris* sp. A (adult)**

Ascaridoidea: Anisakidae.

HOSTS: *Amblyglyphidodon leucogaster* (Bleeker) (Pomacentridae), *Lutjanus fulviflamma* (Forsskål) (Lutjanidae), *Saurida gracilis* (Quoy and Gaimard) (Synodontidae), *Synodus variegatus* (Lacépède) (Synodontidae).

SITE IN HOST: Intestine and ceca.

SPECIMENS DEPOSITED: USNM 81832–81835.

***Raphidascaris* sp. B (larva)**

Ascaridoidea: Anisakidae.

HOST: *Gymnothorax flavimarginatus* (Ruppell) (Muraenidae).

SITE IN HOST: Intestine.

SPECIMENS DEPOSITED: USNM 81836.

Discussion

Okinawa is located in the middle of the Ryukyu Archipelago which lies between Taiwan and the mainland of Japan. Marine zoogeographically, Okinawa belongs to the Indo-Polynesian Province of Indo-West Pacific Region, and most marine fishes of Okinawa are shared with the subtropical and tropical West Pacific and Indian oceans (cf. Briggs, 1974). It is thus natural that some of the nematode parasites recorded in this study, namely *S. istiblenni*, *H. baylisi*, and *D. (N.) laticeps*, are shared with Okinawan and Tropical Pacific or Indian Ocean fishes. A similar

pattern was noted in the trematode fauna of Okinawa (Dyer et al., 1988a, 1989): among 12 species of monogeneans recorded 5 had been described from Sulawesi waters, and among 34 digenean species collected, 7 had been reported from Sulawesi, Philippines, and Tropical Pacific waters (cf. Yamaguti, 1963, 1970).

The nematodes in subtropical Okinawan shore fishes seemed to occur rather rarely compared to those the junior authors have examined in similar fishes in the temperate northern Gulf of Mexico (Williams, 1983) and tropical Puerto Rico and other Caribbean locations (Bashirullah and Williams, 1980; Williams, 1983; Dyer et al., 1985). Similar results were obtained by Myers and Kuntz (1967) in Taiwan waters, which are south of Okinawa. They examined many marine fishes including shore fishes, but most nematodes collected were larval or immature forms, and few species of mature nematodes, i.e., *Procamallanus sigani* Yamaguti, 1935, *Rhabdochona* sp., and *Spinitectus* sp., occurred. The marine shore fishes of the Ryukyu Archipelago-Taiwan areas seem to have a depauperate nematode fauna.

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New Book Available

WHO MODEL PRESCRIBING INFORMATION, DRUGS USED IN PARASITIC DISEASES ISBN 92 4 140102 8, 1990, 126 pp. (in English) is available from: WHO Publications Center USA, 49 Sheridan Avenue, Albany, New York 12210. US\$18.90.

Abomasal Nematodes from White-tailed Deer (*Odocoileus virginianus*) in Maine¹

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ABSTRACT: Abomasa of 35 Maine white-tailed deer, collected from January to August during 1988, 1989, and 1990, were examined for nematode parasites. Six species of nematodes, *Mazamastrongylus odocoilei*, *Ostertagia mossi*, *Ostertagia dikmansi*, *Ostertagia ostertagi*, *Trichostrongylus askivali*, and *Trichostrongylus axei*, were recovered with prevalences of 100%, 94%, 29%, 2%, 14%, and 3%, respectively. Mean intensities of infection with adult worms were not high (max. 779) and highest levels were seen during the period April–July. Numerous, seasonally inhibited ostertagine larvae were also found. Prior to February, inhibition rates were very high (around 80%), but subsequently began to decrease. By May they were low (17%) and negligible in July and August, indicating a gradual resumption of development of inhibited larvae during the spring months.

KEY WORDS: nematodes, Ostertagiinae, *Odocoileus virginianus*, inhibited development, prevalence, Maine, *Mazamastrongylus odocoilei*, *Ostertagia mossi*, *Ostertagia dikmansi*, *Ostertagia ostertagi*, *Trichostrongylus askivali*, *Trichostrongylus axei*.

Species of the subfamily Ostertagiinae have been found to be widely distributed in white-tailed deer (*Odocoileus virginianus* (Zimmerman, 1780)) in the eastern parts of North America, from southern Florida (27°N lat.) to Barrie, Ontario (44°N lat.) (Becklund and Walker, 1968; Walker and Becklund, 1970; Prestwood et al., 1973; Baker and Anderson, 1975; Prestwood and Pursglove, 1981). Since ostertagiasis in domestic animals can present a serious problem, investigation of its occurrence in deer could be important to game management. Information on the distribution of ostertagine nematodes in white-tailed deer has begun to accumulate and some research (Baker and Anderson, 1975) has been done on the significance of inhibited development to their seasonal prevalence. The present research was aimed at studying the prevalence of Ostertagiinae species and their inhibited development in Maine in white-tailed deer.

Materials and Methods

Animals

Thirty-five abomasa from road-killed, adult white-tailed deer were collected over the months of January to August during 1988, 1989, and 1990 and were kept frozen until examined (usually within 2 wk). The animals were from 20 townships in central Maine.

Worm recovery

Worm recovery followed a modification of the method of Powers et al. (1982). After the abomasum was

opened, its content was screened through 60- and 200-mesh sieves. All the screened material was collected and made up to a volume of 4,000 ml. A 10% sample was taken from each screening. Five percent formalin to a final concentration was added to preserve the sample for later worm counting. The abomasal mucosa was scraped and the scraping was digested in a jar for 4 hr at 40°C. For 100 g of mucosa, a digestion mixture of 5 g pepsin, 15 ml hydrochloric acid, and 500 ml water was used. After screening through a 200-mesh screen, 10% of the digesta was formalized for later worm counting.

Worm counting and identification

All counting was performed using a dissecting stereoscope. Parasites were then collected and preserved in 70% ethanol. Adult worms were identified using a compound microscope with the key of Becklund and Walker (1968) and species descriptions by Davidson and Prestwood (1979), Rickard and Zimmerman (1986), and Dunn (1965). Inhibited larvae were identified by the descriptions of Baker and Anderson (1975). Representative specimens have been deposited in the United States National Museum Helminthological Collections, Beltsville, Maryland and assigned accession numbers 81805 through 81808.

Results

Mean monthly worm counts for the 35 deer are shown in Table 1. Six species of worms were found, 4 of them belonging to the subfamily Ostertagiinae. All of the deer had worms in their abomasa, although the total worm counts were generally not very high (maximum 2,419). Highest numbers of worms were found during the winter and spring months (January–April). Of the deer, 97% were infected by more than 1 species, 40% were infected by 3 species, and 3% by 4 species of parasites. The 6 species and their

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prevalences were: *Mazamastrongylus odocoilei* (Dikmans, 1931) (100%), *Ostertagia mossi* (Dikmans, 1931) (94%), *O. dikmansii* (Becklund and Walker, 1968) (29%), *O. ostertagi* (Stiles, 1892) (3%), *Trichostrongylus askivali* (Dunn, 1964) (14%), and *T. axei* (Cobbold, 1879) (3%). The most prevalent and dominant species were *M. odocoilei* and *O. mossi*.

Seasonal inhibition of the ostertagine worms was also observed. For most deer, during the winter months (January–March), numbers of inhibited larvae were higher than those of adults. As shown in Table 1, before February the inhibition rates were high (around 80%). After this, the inhibition rates began to decrease and by May the inhibition rate was low (17%) and negligible during July and August. As the proportion of inhibited larvae decreased, numbers of adult and developing ostertagines increased. Highest numbers of adult worms were seen during the period of April–June.

Discussion

The most prevalent of the 6 species of nematodes found in Maine white-tailed deer were *M. odocoilei*, *O. mossi*, and *O. dikmansii*. *Ostertagia ostertagi* and *T. axei* are mainly parasites of cattle and each was found in only 1 deer; therefore, they represent accidental parasites of the deer. Their presence in white-tailed deer has previously been reported (Walker and Becklund, 1970; Prestwood et al., 1973). *Ostertagia ostertagi* has been reported as causing clinical disease in 1 white-tailed deer (Conti and Howerth, 1987). *Trichostrongylus askivali* was originally reported as a parasite of red deer in Scotland (Dunn, 1965). Its presence in white-tailed deer was reported by Prestwood et al. (1973) in the southeastern United States, but it has not been reported in northern parts of North America before. Its distribution may thus be wider than previously considered. Another common species in the southeast, *Mazamastrongylus pурсglovei* (syn. *Apteragia pурсglovei*) (Davidson and Prestwood, 1979), was not found in Maine.

The northernmost point where species in the subfamily Ostertagiinae have previously been reported in white-tailed deer was Barrie, Ontario (44°N lat.) (Baker and Anderson, 1975). The present report thus confirms that members of the Ostertagiinae are present in colder areas; in this instance the northernmost location was Greenville, Maine (45.5°N lat.). Ostertagines in white-

Table 1. Mean monthly worm recovery from deer abomasa.

Month	No. of deer	Ostertagiinae						EL4†	Inhibition (%)	Trichostrongylus		Total worms
		<i>M. odocoilei</i>	<i>O. mossi</i>	<i>O. dikmansii</i>	<i>O. ostertagi</i>	Adults	LL4*			<i>T. askivali</i>	<i>T. axei</i>	
Jan.	3	175	52	0	0	279	0	919	80.8	0	0	1,146
Feb.	4	467	93	0	0	560	43	1,811	76.5	5	0	2,419
March	7	157	41	12	0	210	11	545	67.3	4	0	770
April	8	409	268	62	14	753	199	960	46.9	1	0	1,913
May	3	642	121	13	0	776	70	153	17.2	3	0	1,002
June	5	624	79	20	0	723	47	68	10.1	8	38	884
July	3	592	84	0	0	676	33	0	0.0	0	0	709
Aug.	2	289	16	0	0	305	35	0	0.0	0	0	340

* Late fourth-stage larvae.

† Early fourth-stage larvae.

tailed deer appear to be, therefore, widely distributed in North America. Unlike reports from other regions, *O. mossi* was much more common in Maine, with a prevalence of 94%. In the Southeast, Prestwood et al. (1973) reported a prevalence of 20%, while in Ontario, Canada, Baker and Anderson (1975) reported a prevalence of 66%. Interestingly, *O. dikmansii* was found only between March and June, together with *O. mossi*, in Maine.

Baker and Anderson (1975) reported that there was a seasonal prevalence of inhibited larvae of ostertagine species in white-tailed deer in Long Point, Ontario. They recovered numerous inhibited, early fourth-stage larvae during winter and early spring, and a few in July and August. The present research is in agreement and showed that inhibited development of Ostertagine nematodes also occurs in Maine white-tailed deer. Inhibition rates were very high before February, then decreased gradually. Resumption of development of inhibited larvae in bovine ostertagiasis in different geographical locations is either spontaneous and synchronous or unsynchronous and unsynchronous (Armour and Ogbourne, 1982). This may be the case in deer ostertagiasis. At Long Point, Ontario, Baker and Anderson (1975) found that except in May, most worms recovered from deer were either early fourth-stage larvae or fully developed adults, a sign of synchronous resumption of development. However, since developing larvae were found frequently in Maine deer throughout the spring, this implied a gradual or unsynchronous resumption of inhibited larval development in the worms in this region. In Maine cattle, it was found that inhibited larvae of *O. ostertagi* resume their development gradually (Gibbs, 1988).

The highest inhibition rate we observed (81%) was higher than that (65%) obtained by Baker and Anderson (1975). Inhibited development may thus be a widespread event in deer ostertagine nematodes. The studies in Ontario and Maine are in agreement with the results obtained from bovine ostertagiasis of north temperate regions of North America (Gibbs, 1988), with occurrence of inhibited development in winter. In south temperate regions, studies of seasonal prevalence of ostertagine worms in adult deer showed high adult worm burdens in summer and fall (Eve and Kellogg, 1977), suggesting that the situation in deer from these areas is similar to that in bovine ostertagiasis (Williams and Knox,

1988) from that region, with the probable occurrence of inhibited development in summer.

Acknowledgment

We thank the personnel of the Maine Department of Inland Fisheries and Wildlife, who submitted the deer abomasa.

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Student Presentation Competition Results

The Second Student Presentation Competition, sponsored by The Helminthological Society of Washington, was held at the 618th Meeting of the Society at the Walter Reed Army Institute of Research on 13 March 1991.

John Hawdon, a graduate student in the Department of Parasitology, School of Veterinary Medicine, University of Pennsylvania, captured the First Place award for his paper, "Reduced glutathione induces feeding by *Ancylostoma caninum* infective larvae." Mr. Hawdon received \$300 from the Society and a 1-yr subscription to the *Journal of Parasitology* from the American Society of Parasitologists. If his paper is accepted for publication in the *Journal of the Helminthological Society of Washington*, page charges will be waived.

James Higgins, a graduate student in the Department of Immunology, School of Hygiene and Public Health, Johns Hopkins University, received the Second Place award of \$200 for his presentation, "Development of *Brugia malayi* in resistant strains of *Aedes aegypti*."

Victor Apanius, a graduate student in the Department of Biology, University of Pennsylvania, captured the Third Place award of \$100 for his presentation, "Chronic blood parasitism, immunity and reproduction in wild birds."

Nancy Briscoe, an undergraduate student in the Department of Biological Sciences, Marshall University in West Virginia, presented an interesting study on "Population dynamics of *Orchopeas leucopus* (Siphonaptera: Ceratophyllidae) and *Epitedia wenmanni* (Siphonaptera: Hystrichopsyllidae) from the white-footed mouse, *Peromyscus leucopus*, in Mason Co., West Virginia."

Robert Maxe, a graduate student in the Department of Parasitology, School of Veterinary Medicine, University of Pennsylvania, gave his presentation on "The application of the factorial experimental design in the investigation of the impact the nematode *Parelaphostrongylus tenuis* has on the fecundity of the intermediate host *Triodopsis albolabris*."

Ultrastructure of the Intestinal Epithelium, Lumen, and Associated Bacteria in *Heterorhabditis bacteriophora*¹

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ABSTRACT: *Heterorhabditis bacteriophora* is an obligate parasite of insects that contains the bacterial symbiont, *Xenorhabdus luminescens*, in its intestinal lumen. Ultrastructural observations were made of the intestinal epithelium, lumen, and associated bacteria. The oligocytous intestine of the infective juvenile of *H. bacteriophora* has junctional complexes that delineate the boundaries of cells forming the intestinal lumen. Fine structure of the intestinal cells includes microvilli on the apical membranes bordered by a dense matrix of vesicular endoplasmic reticulum and accumulations of variably stained electron-dense granules within each cell. Rod-shaped bacterial cells occur singly or en masse in the lumen of the intestine. Some bacteria appear to undergo autolysis or show interaction with the lumen contents.

KEY WORDS: ultrastructure, *Heterorhabditis bacteriophora*, Nematoda.

Current interest in developing alternatives to the use of chemicals for control of agricultural pests focuses attention on nematode species that can be used for biological control (Gaugler, 1987; Ghally, 1987; Gaugler et al., 1989; Kondo and Ishibashi, 1989; Figueroa and Roman, 1990; Glazer and Wysoki, 1990; Nickle and Cantelo, 1991). Among such species are nematodes, harboring bacteria within their intestinal lumen, that serve as vectors of potential toxicants to insects after host tissue penetration (Dutky and Hough, 1955; Dutky, 1959; Poinar, 1979). Bacteria have been observed in the lumen of the pharynx and intestines of nematodes, and in a certain species, in the epithelial cells forming the lumen (Poinar, 1966; Poinar and Leutenegger, 1968; Poinar et al., 1977). Microvilli, which are a consistent feature of intestinal epithelia of these nematodes, occur on the apical membrane of intestinal cells. Among the animal parasitic nematodes, the brush border of the infective larvae of *Trichinella spiralis* was shown to consist of microvilli (Bruce, 1966). Similarly, an ultrastructural study of the intestine of *Capillaria hepatica* showed microvilli occurring on the epithelial surfaces (Wright, 1963). Microvilli have been found and described in a wide range of nematode species that include animal, insect, and plant-parasitic species

(Wright, 1963; Sheffield, 1964; Shepherd and Clark, 1976; Munn and Greenwood, 1984; Endo, 1988).

With a goal to establish criteria for evaluating the effectiveness of nematodes as biocontrol agents of insects such as the wax moth, *Galleria mellonella* (L.), observations were made on the fine structure of the intestinal epithelia of *Heterorhabditis bacteriophora* Poinar (1975). Emphasis was on: (1) cell morphology and its components, (2) surface membrane modifications related to microvilli formation, and (3) distribution of associated bacteria, *Xenorhabdus luminescens* Thomas and Poinar, 1979, in the intestinal lumen.

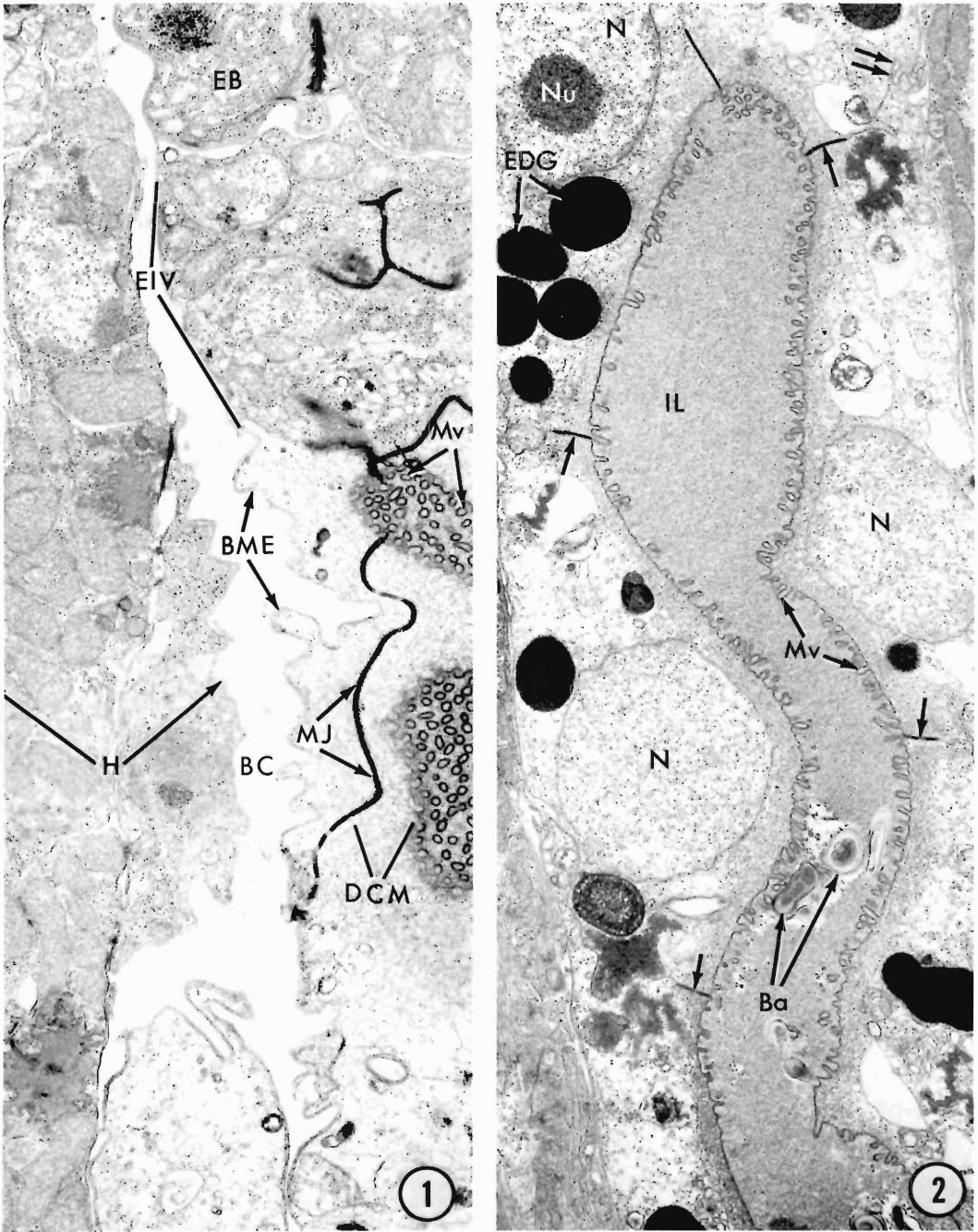
Materials and Methods

Dauer larvae of *Heterorhabditis bacteriophora* from a culture maintained by Biosys, Inc. in an alginate gel were released in water. The infective juveniles (J3) were then concentrated and suspended in water agar prior to fixation and embedded according to previously published procedures (Endo and Wergin, 1973; Endo, 1984, 1987). Briefly, nematodes in a suspension of water were mixed with warm liquefied 2% water-agar and the mixture was poured into small grooves in agar-filled petri dishes. The solidified agar, containing the nematodes, was diced into 2-3-mm blocks that were transferred to glass vials containing 3% glutaraldehyde in 0.05 M phosphate buffer (pH 6.8) at 22°C for chemical fixation of the larvae. Subsequent rinsing and postfixation in osmium tetroxide were also carried out in 0.05 M phosphate buffer (pH 6.8). The glutaraldehyde fixation (for 1.5 hr) was followed by washing in 6 changes of buffer over a period of 1 hr. The agar blocks were then postfixated in 2% osmium tetroxide for 2 hr at 22°C, dehydrated in an acetone series and infiltrated with a low viscosity embedding medium (Spurr, 1969). Silver-gray sections of selected nematodes were cut with a dia-

¹ Mention of a trade name, warranty, proprietary product, or vendor does not constitute a guarantee of a product and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

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³ Research Zoologist.



Figures 1, 2. Tangential and mid-longitudinal sections through base of esophagus and intestine of a J3 juvenile of *Heterorhabditis bacteriophora*. 1. Longitudinal-tangential section shows basal boundary of the esophageal bulb (EB) and adjacent 3-tiered cells of the esophago-intestinal valve (EIV). The body cavity (BC) occurs between convoluted basal epithelial membranes (BME) and hypodermis (H). Tangential section through intestine shows base of microvilli (Mv), dense cytoplasmic matrix (DCM), and membrane junctions (MJ). $\times 11,700$. 2. Mid-longitudinal section of mid-region of intestine shows alternate cell arrangements of intestinal epithelium as delineated by nuclei (N), membrane junctions (arrows), and cell membrane appositions (double arrows). The intestinal lumen lined with microvilli (Mv) and containing bacteria (Ba). EDG, electron-dense granules; IL, intestinal lumen; Nu, nucleolus. $\times 7,190$.

mond knife and mounted on uncoated 75 × 300-mesh copper grids. The sections were stained with uranyl acetate and lead citrate and viewed in a Philips 400 electron microscope that was operated at 60 kV with a 20- μ m aperture.

Results

Immediately anterior to the intestinal epithelium of *Heterorhabditis bacteriophora* is a tier of cells that forms the esophago-intestinal valve (Fig. 1). These cells are linked to adjacent intestinal cells by clearly defined membrane junctions (Figs. 1, 4). Beyond the membrane junctions, membranes of adjacent cells are in close apposition and follow convoluted pathways to the surface of the epithelium (Fig. 2). Intercellular spaces near the basal surface of the epithelium merge with the body cavity (Figs. 1, 2) which is extensive in the region of the anterior intestinal epithelium and hypodermis. The apical-lateral borders of the epithelium are defined by membrane junctions (Figs. 2, 5, 11). Longitudinal or cross sections of a nematode showed that the cylindrical central lumen of the intestine was lined and supported by a series of paired cells (Figs. 2, 5). These same junctions at the terminus of the apical-lateral positions of paired epithelial cells appeared as elongated membrane junctions when the cells were sectioned tangentially (Fig. 1). Mid-longitudinal sections of the same or similar cells showed a staggered arrangement of epithelial cells that were well defined by the alternate positions of membrane junctions (Figs. 2, 6). The basal portion of the epithelial cells were joined by a labyrinth of interfolded membranes. As the basal-lateral membranes separated, the widened spaces merged with the body cavity (Fig. 9).

Apical membranes of intestinal epithelia have irregular arrangements of microvilli as they evaginate from the anterior surface membranes of the epithelial cells. The microvilli of the anterior to mid-intestinal region were blunt or elongate as the lumen was distended (Figs. 2, 3, 7, 9). In the nondistended sector of the anterior (Fig. 3) and

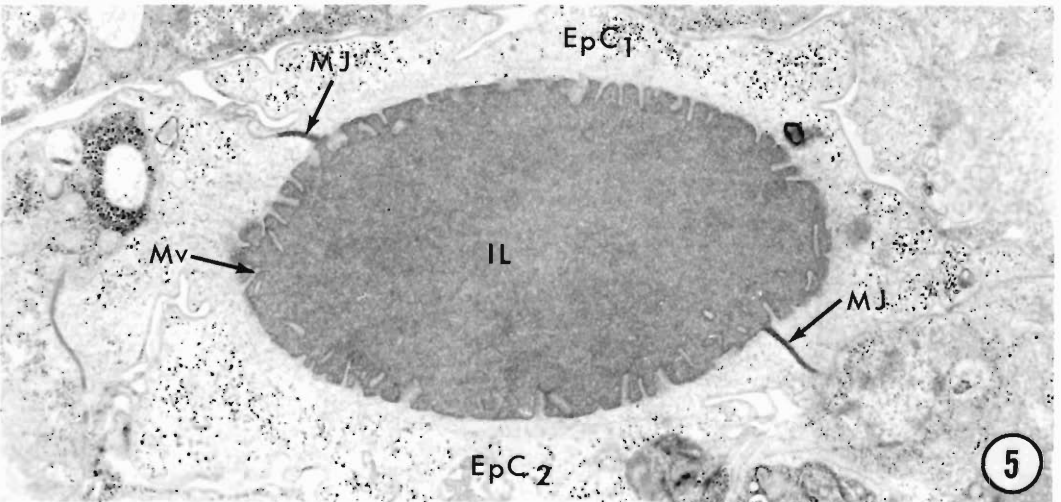
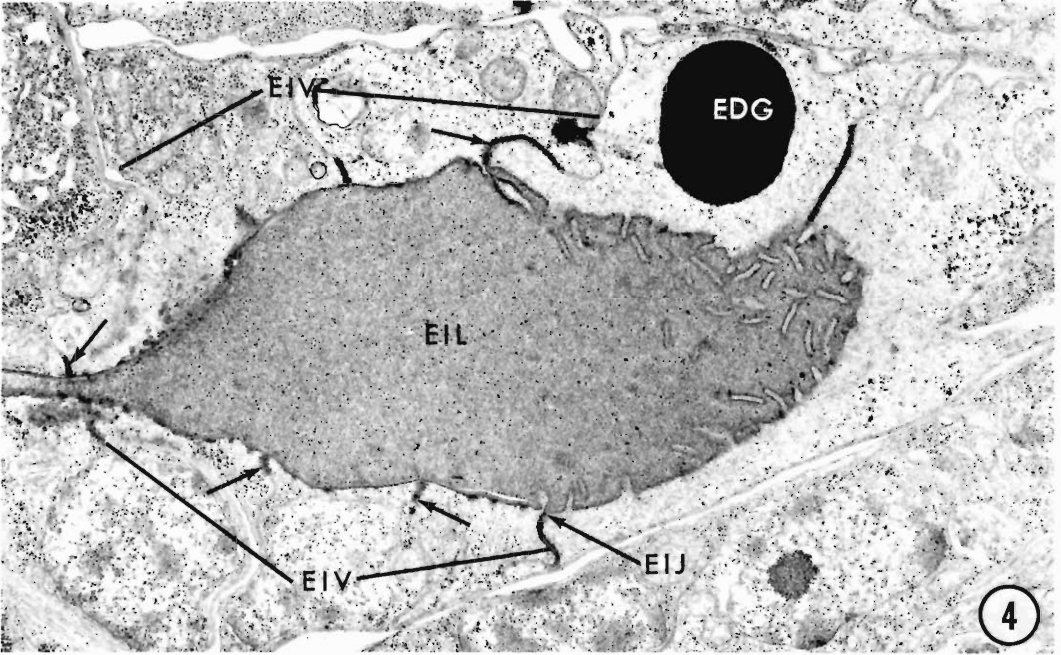
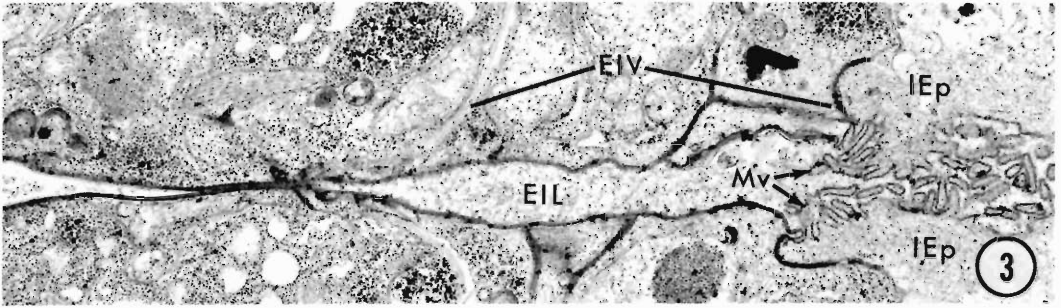
posterior regions of the intestinal epithelium, the microvilli are elongate (Figs. 14, 15). Each microvillus has a few irregularly spaced filaments that extend from its apex to the base. An enteric fibrous coating (EFC) occurs on the surface of microvilli (Fig. 10) and the inter-microvillar surfaces of the apical membranes of the intestinal epithelium (Fig. 9). This coating occurs along the entire length of the intestine, but is variable in density. Integration of microvilli with cellular contents occurs by contact of microvillar filaments with the cellular matrix (Figs. 7, 9, 10). The dense cytoplasmic matrix (DCM) (Figs. 7, 9) of smooth and rough vesicular endoplasmic reticulum occurs adjacent to the intestinal lumen. Each of the intestinal cells appears to have a central prominent nucleus with various concentrations of cytoplasmic inclusions including glycogen, lipid droplets, and granules (Fig. 2). Certain epithelial cells have moderate to dense accumulations of granules with characteristic morphology (Figs. 2, 4, 9, 12). The electron density of these granules varies from uniformly dense to alternating levels of light to dark bands that appear as concentric rings in cross sections (Fig. 12). The intestinal lumen is terminated with a closed cuticularly lined rectum (Fig. 16).

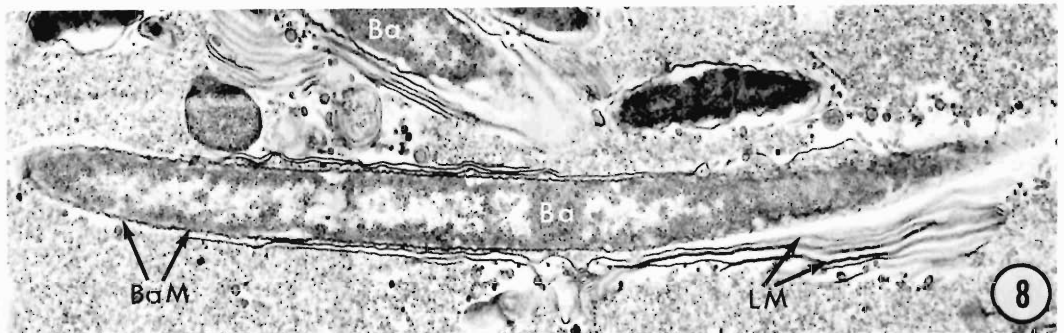
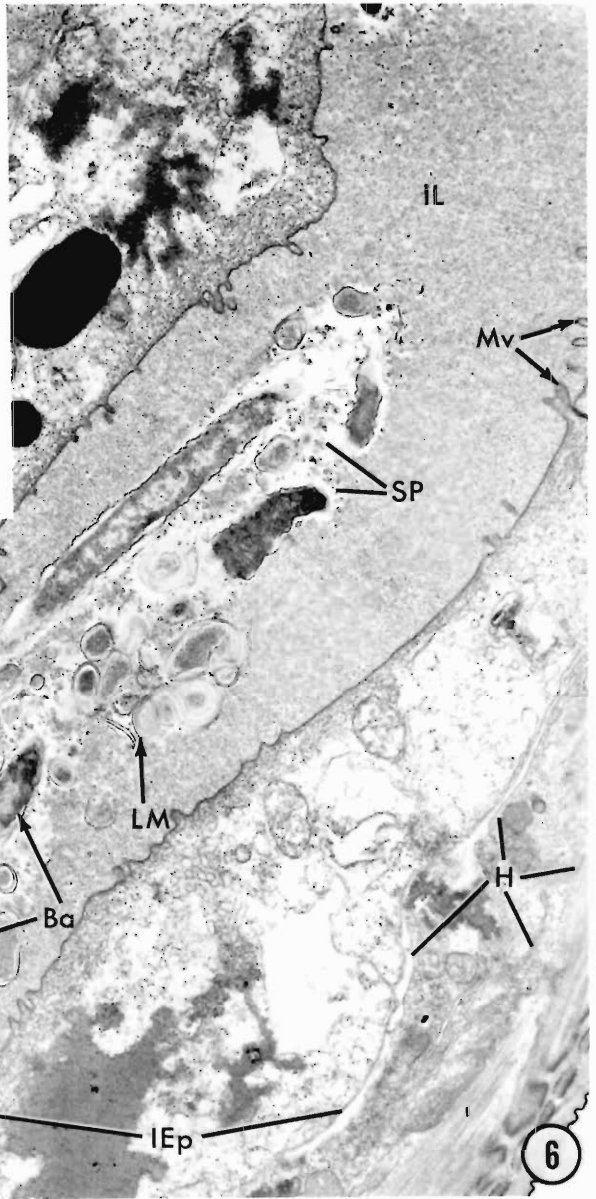
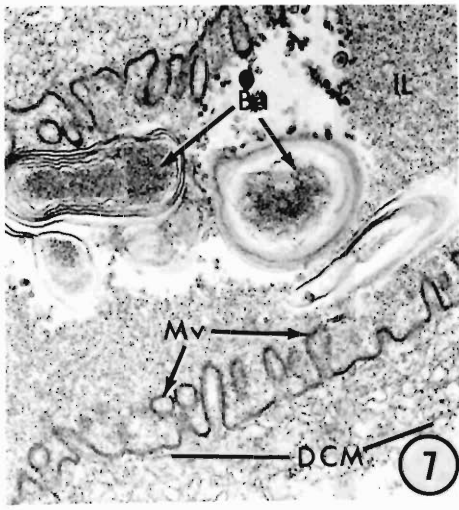
The lumen of the intestine contained populations of bacteria that occurred singly or in large masses within the lumen of the intestine (Figs. 2, 6–8, 13). The rod-shaped bacterial cells were often enclosed or surrounded by membrane-like images (Fig. 13). In certain regions, the lumen appeared clear and the once uniform granular matrix was absent or replaced with spherical to elongate particles (Figs. 6, 13).

Discussion

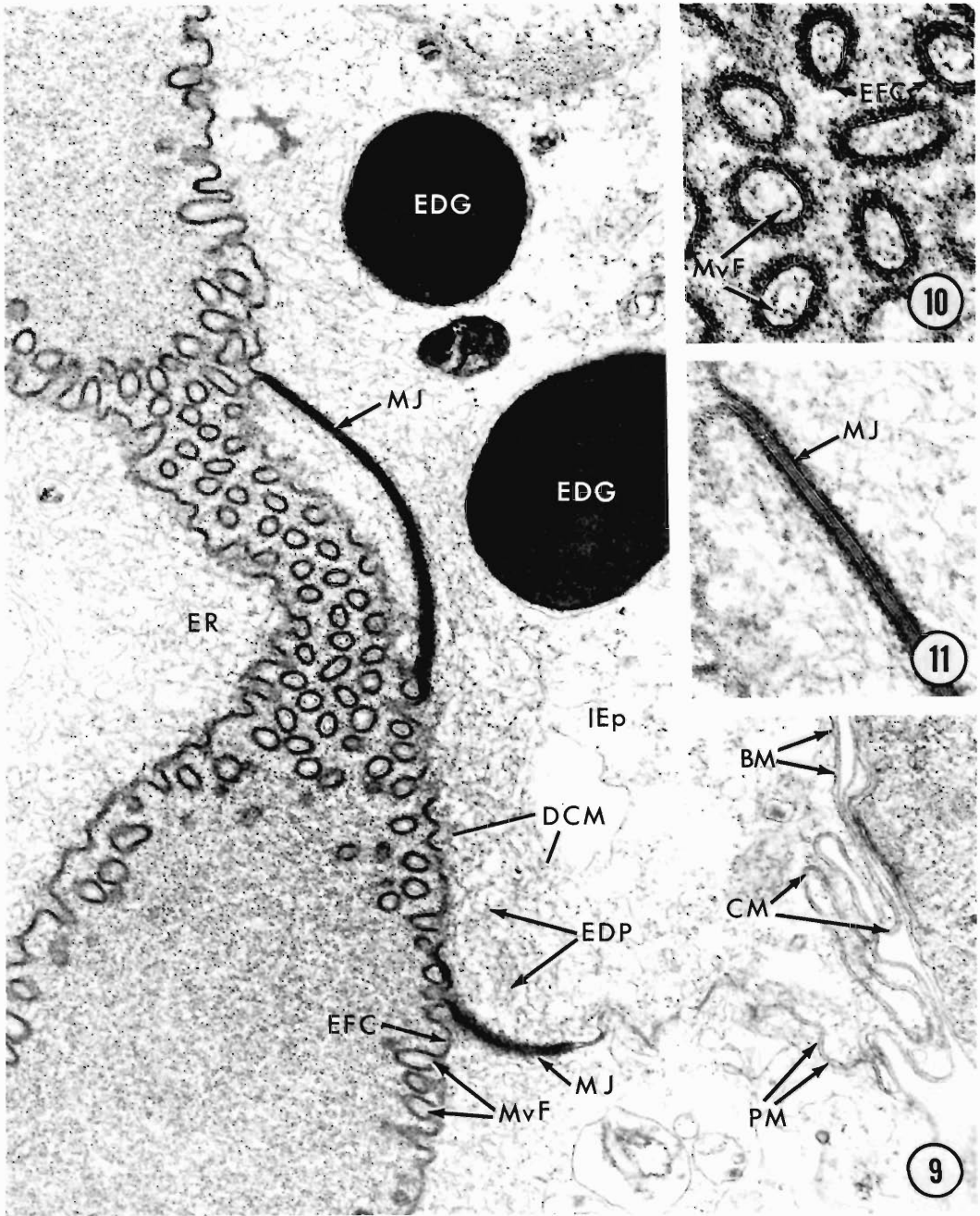
The anterior limits of the intestine are delineated by the presence of microvilli in the apical membrane of the intestinal epithelium. Closely allied anteriorly and adjacent to the intestine was a 3-tiered group of cells. This 3-tiered organ functions as an esophago-intestinal valve because its

Figures 3–5. Longitudinal-tangential section through esophago-intestinal region and cross section of mid-intestine of *Heterorhabditis bacteriophora*. 3. Longitudinal section showing an esophago-intestinal valve (EIV) in partially closed position with transition to intestinal lumen (EIL) lined by elongated microvilli (Mv) of intestinal epithelium (IEp). ×14,840. 4. Midsection of esophago-intestinal valve (EIV) formed by 3 tiers of cells defined by membrane junctions (arrows) near cuticular wall of lumen. Noncuticularized lumen wall and cell junction delineate beginning of intestinal epithelium with microvilli on apical surface of cells (EIJ). EDG, electron-dense granule; EIL, esophago-intestinal lumen. ×12,460. 5. Cross section of mid-intestine showing paired arrangement of epithelial cells (EpC₁ and EpC₂). IL, intestinal lumen; MJ, membrane junction; Mv, microvilli. ×10,350.





Figures 6–8. Longitudinal sections of mid-region of intestine of infective third stage of *Heterorhabditis bacteriophora*. 6. Broad region of intestinal lumen partially occupied by rod-shaped bacteria (Ba) and associated lamellar membranes (LM). Spherical (SP) and linear particles (LP) occur within clear regions of the lumen. Microvillar (Mv) surfaces are irregular and sparse with portions of the apical membranes devoid of surface evaginations. H, hypodermis; IEp, intestinal epithelium; IL, intestinal lumen. $\times 10,680$. 7. Anterior region of



Figures 9–11. Longitudinal mid- to tangential sections of intestine of *Heterorhabditis bacteriophora*. 9. A portion of epithelial cell (IEp) with electron-dense granules (EDG) with apical membrane surfaces lined with endoplasmic reticulum (ER) and electron-dense particles (EDP). Microvilli contain longitudinally oriented filaments (MvF). Adjacent epithelial cells are joined near lumen with membrane junctions (MJ). Paired membrane (PM) closely apposed laterally and convoluted (CM) especially near the basal membrane (BM). DCM, dense cytoplasmic matrix; EFC, enteric fibrous coat. $\times 27,150$. 10. Enlargement of portion of microvilli showing irregular arrangements of filaments (MvF) and trilaminar membranes of microvilli. EFC, enteric fibrous coat. $\times 107,360$. 11. Enlargement of membrane junction (MJ) of Figure 9. Note zone of cell membrane apposition in which an intercellular space is evident, uniform in width, and filled with electron-dense material. $\times 70,960$.

← intestine of Figure 6 shows a narrowing of the lumen, bordered by vesicular endoplasmic reticulum as part of dense cytoplasmic matrix (DCM), high number of microvilli (Mv), and apical cell membranes. Ba, bacterial cells; IL, intestinal lumen. $\times 24,000$. 8. Section through intestinal lumen showing a bacterium (Ba) with a distinct cell wall membrane. BaM, bacterial membrane; LM, lamellar membrane. $\times 18,240$.

cuticular lumen is continuous with the broad intestinal lumen.

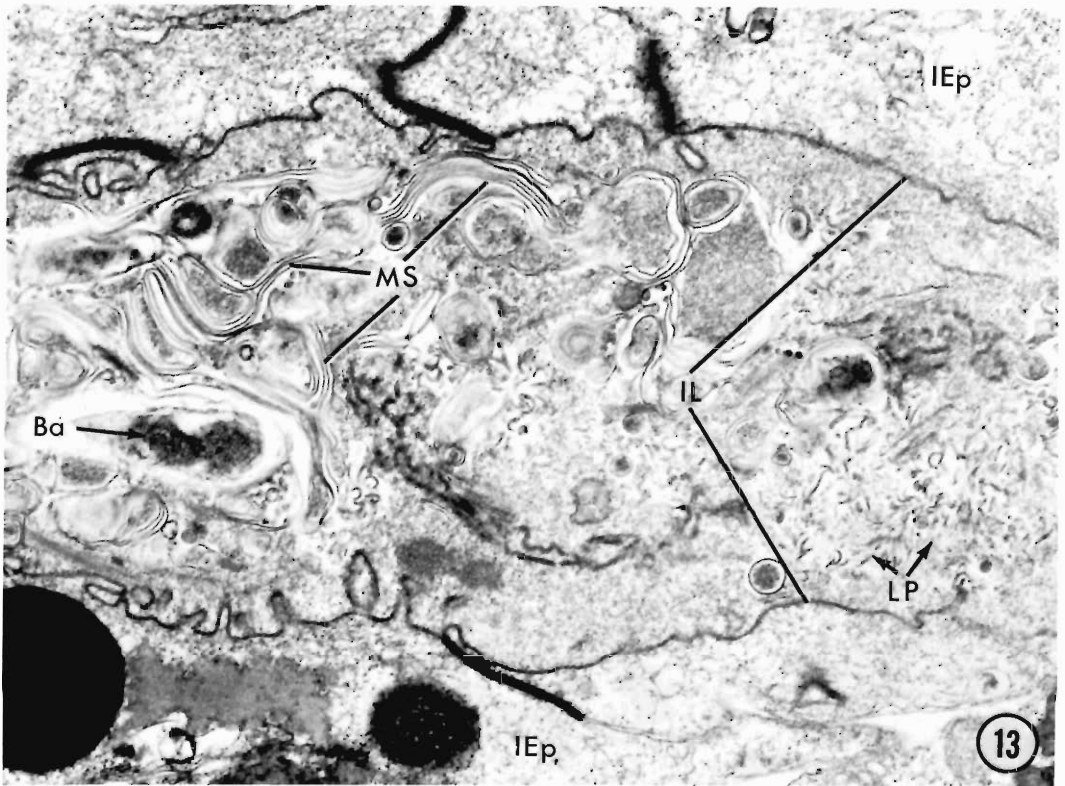
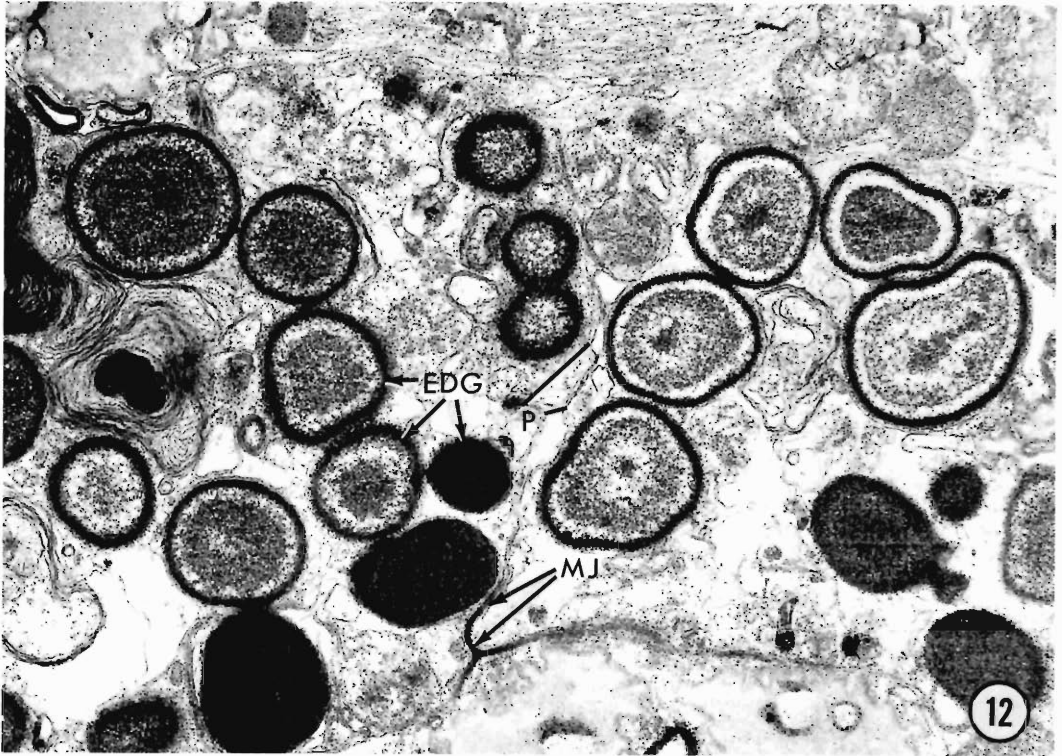
Longitudinal sections through the intestine of *H. bacteriophora* show the moderate number of nucleated cells that comprise the intestinal epithelium. This species follows the oligocytous arrangement of intestinal epithelial cells consisting of fewer than 128 cells per organ (Chitwood and Chitwood, 1950; Bird, 1971). In contrast, *Ascaris suum* has a myriocytous arrangement that exceeds 8,000 cells per specimen. Although the numbers of cells comprising intestinal epithelia vary widely among nematodes, the presence of microvilli and the terminal web are common features. The inner core of the microvilli of *A. suum* has elongated fibers that extend for short distances into the epithelial cell and terminate in a subacillary layer. This layer, with adjacent fibrous network and small granules, forms the terminal web (Sheffield, 1964). The filaments in the central core of the microvilli in *H. bacteriophora* were similar but less numerous than those described for *A. suum*. The dense cellular matrix at the bases of microvilli appear not to be a terminal web in the form discussed by Munn and Greenwood (1984) but may be a region of intestinal cell secretion and absorption related to the digestive functions of the nematode. The membrane junctions of adjacent intestinal epithelial cells near the lumen appeared to form a continuous belt-like junction with a mechanism to retain intestinal cell and lumen continuity. Similar cell junctions occur between epithelial cells of higher animals (Fawcett, 1966).

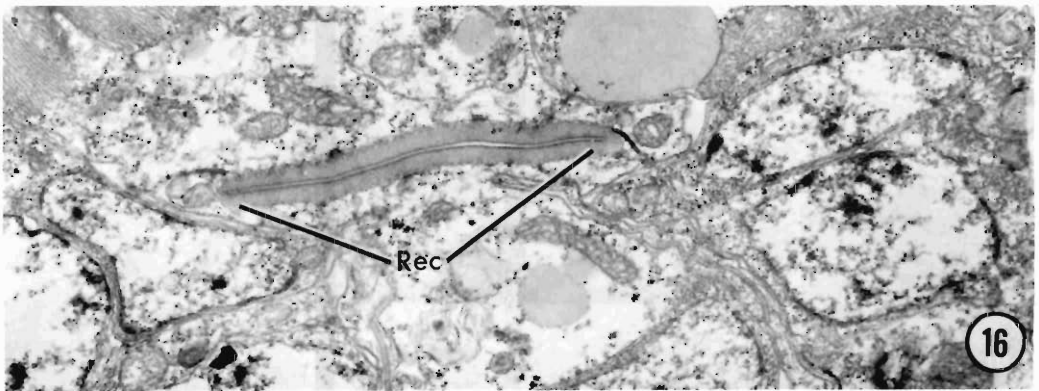
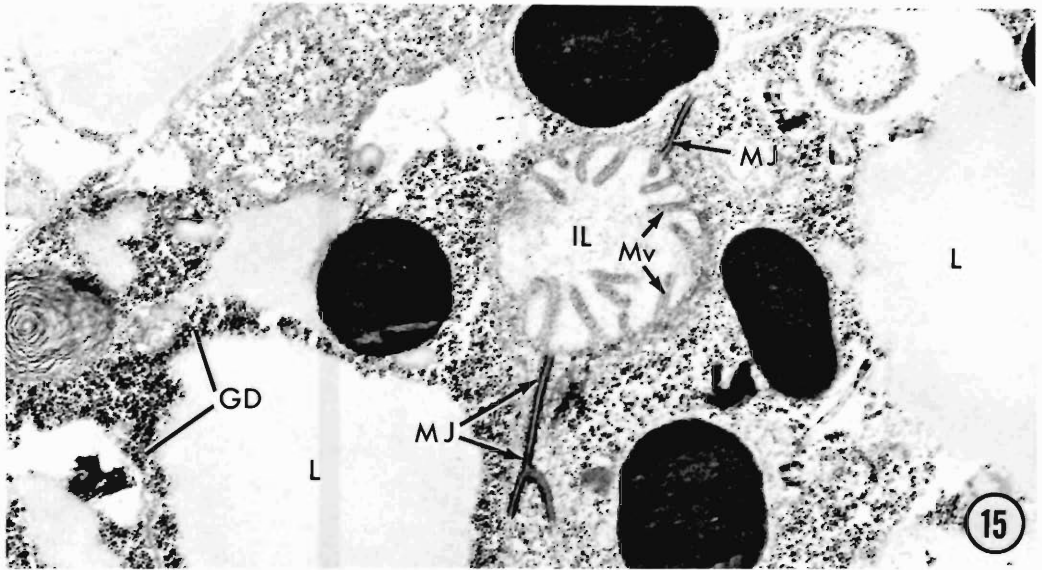
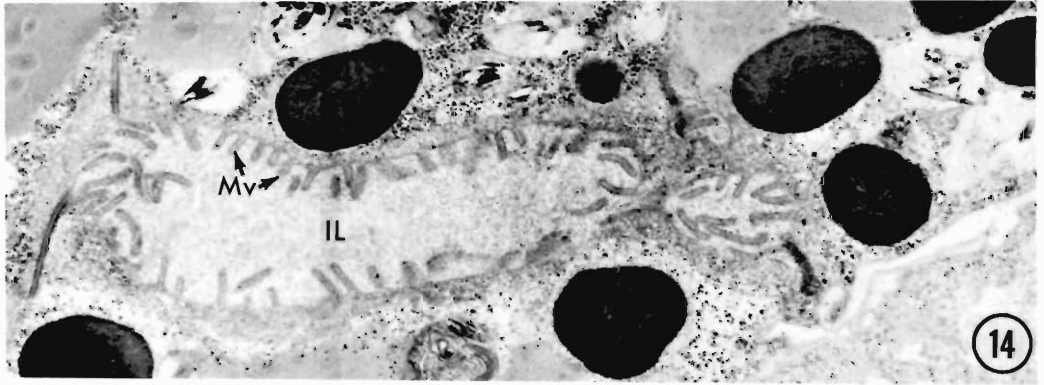
The enteric coating on the microvilli and intermicrovillar surface of the apical membranes of the intestinal epithelium is similar to the coating that occurs on the apical membranes of intestines of *Aphelenchoides blastophthorus* (Shepherd et al., 1980) and of *Heterodera glycines* (Endo, 1988) but lack the highly sculptured appearance of the latter species. Additional research is needed to determine the nature of the enteric material and its possible role in food uptake and digestion among plant and insect-parasitic species.

The granules with concentric rings of electron density present in the intestinal epithelial cells of *H. bacteriophora* were quite distinct from liquid droplets. The granules also occurred in insects and appeared like glycolipids or lipoproteins that had been partially degraded and gave a differential stain reaction. However, cytochemical analyses will be necessary to elucidate the true nature of these particles. In terms of the fine structure, abundance, and general presence of granules in the intestinal epithelia, the granules may be similar to the particles described in rhabditoids using the light microscope. They may constitute the birefringent-spherocrystals that were observed in rhabditoid species (Cobb, 1914, Jacobs and Chitwood, 1937). The spherocrystals of the intestines were reported as gray in color, bright spots in dark field illumination, and bright spots with a central cross when observed with polarized light. Similar dense bodies were observed within the midgut of the housefly, *Musca domestica*. It is suggested that mineralized dense bodies are sites of intercellular sequestration of minerals and may play a vital functional role in the excretory system (Sohal et al., 1977). Among lepidopterous larvae, dense bodies within the midgut take on a laminated appearance and are termed spherites. They appear first before ecdysis and disappear during differentiation of regenerative cells to columnar and goblet cells (Turbeck, 1974).

Physiological studies were not used to identify the bacteria observed in the lumen of *H. bacteriophora* of this study. However, the nematodes were samples taken from cultures previously used for infectivity studies that were consistent with observations of Poinar et al. (1977) in which the bacteria were identified as *X. luminescens* (Thomas and Poinar, 1979). They were able to liberate bacteria from the pharynges and intestines of surface sterilized juveniles of infective J3 of *H. bacteriophora* by placing the nematodes in a drop of insect blood. It is evident that when the J3 juveniles enter an insect host, and penetrate the body cavity, bacteria are released into the insect hemolymph. The bacteria multiply

→
Figures 12, 13. Longitudinal sections through intestinal epithelial cell and lumen of intestine showing granules and bacteria, respectively. 12. Median section through portions of 2 epithelial cells with granules (EDG) of varying degrees of electron density and size; appearance of concentric rings of stain apparently dependent on sectioning levels through each spheroid granule. P, plasmalemmae; MJ, membrane junctions. $\times 14,180$. 13. Mid-longitudinal section through lumen of intestine (IL) with bacterium (Ba) and associated membrane swirls (MS) and linear particles (LP). Number of microvilli evaginated from the apical cell surface are variable; microvilli may be absent along portions of lumen surface. IEP, intestinal epithelium. $\times 21,600$.





Figures 14–16. Longitudinal and cross sections through posterior region of intestinal epithelium and cuticularized rectum. 14. Longitudinal–tangential section of posterior intestinal lumen (IL) lined with microvilli (Mv). Epithelial cells contain prominent lipid droplets and granules. $\times 11,590$. 15. Cross section of a different nematode showing a circular form of intestinal lumen (IL) lined with microvilli (Mv). Epithelial and hypodermal sections have large lipid droplets (L), some surrounded by dense glycogen deposits (GD). MJ, membrane junctions. $\times 18,240$. 16. Cross section of tail region of *Heterorhabditis* shows cuticle lined rectum (Rec) and supporting cells. $\times 14,350$.

rapidly, and their large populations become toxic to the insect and cause septicemia. In a related study, Poinar (1966) showed that *Achromobacter nematophilus* was contained in the ventricular region of the intestinal lumen of *Steinernema carpocapsae*, strain DD 136. In addition, bacteria were found in the epithelial cells of the intestine. In our study, bacteria were not observed in epithelial cells of *H. bacteriophora*.

Future work should focus on changes in bacterial morphology and multiplication in the intestinal lumen during the transition of the infective J3 nematode from the nonfeeding stage to the feeding stage within the insect host. Observations should be made to determine the nature and accumulation of bacteria-associated membrane-like particles that may be products of autolysis or lytic action of bacteria on the lumen contents. Ultimately, it may be possible to correlate the fine structure observations of the bacteria in nematodes with the effectiveness of species or strains of rhabditoid nematodes as biological control agents against important agricultural insect pests.

Acknowledgments

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ANNOUNCEMENT

Ostertagia Workshop 1-4 March 1992

A 2½-day workshop on *Ostertagia* will be held 1-4 March 1992 at the University of Maryland, University College Center of Adult Education, College Park, Maryland. The scientific program is intended to be comprehensive on all aspects of research related to *Ostertagia ostertagi* and diseases associated with this parasite. The workshop will include invited speakers and roundtable discussions on topics to promote research and control. These include systematics, epidemiology, immunity, modeling, genetics, chemotherapy, diagnosis, and effects on host physiology. A poster session will be included in the program as an evening session to allow interested participants an opportunity to display current research from their laboratories related to ostertagiosis. Participation will be limited. Prompt registration is suggested.

For further information about the workshop, agenda, and scientific sessions, please call or write: Dr. Daniel E. Snyder, USDA, ARS, Animal Parasite Research Laboratory, P.O. Box 952, Auburn, Alabama 36831; Telephone (205) 887-3741; FAX (205) 821-1732.

Transmission of Some Internal Parasites in Horses Born in 1989 on a Farm in Central Kentucky¹

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ABSTRACT: The dynamics of acquisition of infections of internal parasites of equids in central Kentucky were observed. Aspects studied were the relationship of the life cycle of the parasites, seasonal occurrence, and age of the horses. Ten horses born in 1989 and kept on a pasture on a farm in central Kentucky were naturally infected with internal parasites and examined (1 a month) at necropsy at 64-222 days of age between 20 June 1989 and 14 March 1990. Antiparasitic compounds were never given to these horses and were not generally used in the breeding band for over 10 years.

Parasites found and the months of their recovery were: bots, *Gasterophilus intestinalis*, in the mouth from September to December and in the stomach September-March; stomach worms, *Trichostrongylus axei* in June-September, November, and March and *Habronema* sp. in August and January; ascarids, *Parascaris equorum*, in all months; intestinal threadworms, *Strongyloides westeri*, in all months but January and February; large strongyles, *Strongylus vulgaris* in the cranial mesenteric artery in all months and in the large intestine from September to March, and *Strongylus edentatus* in the ventral abdominal wall from August to March; small strongyles in all months; pinworms, *Oxyuris equi*, in all months; and eyeworms, *Thelazia lacrymalis*, in August, October, and November. Identification of small strongyles from 5 of the horses revealed 7 genera and 22 species present.

This research provided insight into the transmission pattern of several species of internal parasites of equids and should be useful in establishing more definite control measures.

KEY WORDS: horses, internal parasites, seasonal transmission, Kentucky.

Knowledge on transmission of internal parasites of equids is important; understanding this aspect of the biology of parasites is of special interest in providing a basis for control recommendations for these organisms. Specific research on ecology of internal parasites of equids is not extensive. Particularly lacking are studies on equids themselves regarding age when infections are acquired and the influence of life cycle of the parasites and season of transmission. In studying transmission of internal parasites, availability of adequate numbers of equids, born and raised under similar conditions, is usually a limitation.

Previous studies on parasite transmission in equids in Kentucky have generally included composite data. For example, investigations have been on horses born in 1982 on several farms (Lyons et al., 1985) and born over a 19-yr period on 1 farm (Lyons et al., 1990). While valuable information on seasonal transmission of internal

parasites was found in these studies, it did not include monthly serial examination of horses born on the same pasture in the same year.

The purpose of the present research was to follow monthly the progressive acquisition of infections of endoparasites over a 10-mo period in horses born in 1989 on the same pasture (Field No. 10) in central Kentucky.

Materials and Methods

Ten foals were born on a pasture (Field No. 10) in central Kentucky between 17 April 1989 and 4 August 1989. Details on the birth date, month and age at necropsy, and sex of the horses are recorded (Table 1). History on size of the pasture, treatment of the breeding band, and other aspects of horses kept in Field No. 10 has been published recently (Lyons et al., 1990). Parasiticides have not been used in the breeding band in Field No. 10 since 1979, except for treatment of occasional replacement animals. However, beginning in 1987, parasite control was initiated in horses in surrounding fields and several antiparasitic compounds were routinely given.

None of the present foals examined was ever treated with an anthelmintic. Each foal was with its dam until necropsy.

Organs, tissues, or parts of the horses examined at necropsy were: eyes, mouth (tongue and gums), pharynx, brain, heart, mesenteric lymph nodes, ligamentum

¹ The investigation reported in this paper (No. 90-4-124) was conducted in connection with a project of the Kentucky Agricultural Experiment Station and is published with the approval of the director.

Table 1. Internal parasites recovered from 10 horses born on a farm in central Kentucky in 1989.

ID no.	Sex	Horse		Parasites									
		Birth date	Necropsy		Teeth and/or tongue		Stomach			Small intestine			
			Mo.	Age (days)	<i>G. intest.</i>		<i>G. intest.</i>		<i>T. axei</i>	<i>S. westeri</i>	<i>P. equor.</i>		
					1st	2nd	2nd	3rd			Imm.	Mat.	
		(1989)	(1989)										
1	♀	4/17	June	64	0	0	0	0	30	130	640	0	
2	♀	5/2	July	78	0	0	0	0	30	1,760	1,770	0	
3	♀	5/2	Aug.	107	0	0	0	0	10	1,850	390	17	
4	♂	5/10	Sept.	125	4	2	4	0	180	2,270	110	168	
5	♂	6/1	Oct.	139	27	21	70	9	0	4,420	230	223	
6	♀	6/4	Nov.	164	2	1	21	21	30	2,610	510	68	
7	♀	6/22	Dec.	174	3	3	25	73	0	6,850	1,890	5	
			(1990)										
8	♀	6/25	Jan.	205	0	0	1	120	0	0	0	8	
9	♀	7/25	Feb.	204	0	0	0	97	0	0	340	51	
10	♂	8/4	Mar.	222	0	0	0	147	10	90	530	6	

G. intest. = *Gasterophilus intestinalis*; *T. axei* = *Trichostrongylus axei*; *S. westeri* = *Strongyloides westeri*; *P. equor.* = *Parascaris equorum*; *S. vulg.* = *Strongylus vulgaris*; *O. equi* = *Oxyuris equi*; SS or ss = small strongyles; *S. edent.* = *Strongylus edentatus*. Mo. = month; Imm. = immature; Mat. = mature; VAW = ventral abdominal wall; CMA = cranial mesenteric artery. ND = not determined.

nuchae, the cranial mesenteric artery, the ventral abdominal walls, lungs, esophagus (wash and mucosa), stomach (contents and mucosa), small intestine (contents and mucosa), ileocecal valve, contents and mucosa of the cecum, ventral colon, dorsal colon, small colon, and rectum.

Examination of the contents of the various portions of the gastrointestinal tract included inspections of aliquot samples to estimate numbers of smaller parasites and visual search of the remainder of the contents for the larger parasites.

Organs, tissues, and other areas were examined grossly, after which all (except for material artificially digested) were suspended in containers of water for about 16 hr. The material remaining after decanting or reducing the volume of liquid by pouring it into a 200-mesh sieve was fixed with 5% formalin. Later the preserved residue was washed over a 200-mesh sieve and examined under a stereoscopic microscope at about 10×.

Small strongyles, found in the contents of the large intestine, were identified to the species level for 5 of the horses. This was begun with the first horse (No. 1) examined, and done on every other horse thereafter.

Artificial digestive juice (1% pepsin and 1% HCl) was used for recovery of parasites from the esophageal mucosa, small intestine (anterior half), stomach (glandular mucosa), and cranial mesenteric artery.

The method for enumerating encysted small strongyles in the mucosa of the large intestines is given as follows: Separation of the large intestine was made into 3 portions—cecum, ventral colon, and dorsal colon. Following the removal of the contents, each portion was weighed and 10% was excised randomly with scissors. Thickened areas were trimmed from the serosal surface (e.g., lymph nodes and arteries) of these tissue

samples, which were cut into smaller pieces, pressed between 2 petri dishes, and examined under a stereoscopic microscope at about 10×. The top, or smaller dish, turned upside down inside the bottom one, had a grid that aided in counting the larvae. High intensity light was used to illuminate the tissue (Reinemeyer and Herd, 1986a). Encysted small strongyles were enumerated for estimations of the total numbers present.

Feces were used for determining worm eggs per gram (epg) and larvae per gram (lpg) of feces. For the first 3 months of the study, lpg were not determined. More detailed descriptions on techniques for recovery of internal parasites and on methods for epg and lpg have been published (Drudge et al., 1963, 1975; Lyons and Drudge, 1975; Lyons et al., 1976b, 1981a, 1983).

Data on bots found in the mouth, eyeworms, and *Habronema* sp. from the stomach are not included in the tables. Comparisons are made of data for some of the internal parasites recovered from horses in Field No. 10 in the present study and from horses from this field and an adjacent field in a previous study (Lyons et al., 1990). Some parasites found in the present investigation were not sought in the earlier study. A major difference between the 2 studies is that the current investigation is on a group of horses born the same year and sampled monthly over a 10-mo period, whereas the earlier study was a random sampling of parasitic infections by month in foals born over a 19-yr period (Lyons et al., 1990).

Results

One species of bot and several species of nematodes were recovered at necropsy of the horses (Table 1).

In the mouth, *Gasterophilus intestinalis* first

Table 1. Continued.

Parasites									
Lungs	Large intestine					VAW		CMA	
<i>P. equor.</i> Imm.	<i>S. vulg.</i>	<i>O. equi</i>		SS	Encysted ss	<i>S. edent.</i>		<i>S. vulg.</i>	
		Imm.	Mat.			4th	5th	4th	5th
0	0	20	1	20,970	8,610	0	0	55	0
25	0	30	74	15,260	3,030	ND	ND	126	2
4	0	750	64	34,650	2,750	77	1	236	43
1	5	50	0	42,575	2,050	261	122	206	111
1	62	2,200	275	30,700	4,240	41	89	147	95
2	146	1,650	87	82,550	9,270	47	161	284	82
11	66	1,100	465	105,050	16,450	98	28	250	46
0	248	3,050	85	102,200	1,420	32	90	273	108
0	129	7,050	162	49,250	3,590	59	87	110	49
2	173	8,050	485	3,770	1,360	39	155	23	12

instars (2–27/horse) and second instars (1–21/horse) were found from September through December. *Gasterophilus intestinalis* second instars were found in the stomach from September through January. Third instars of this species were present from October through March.

The stomach collections also included species of 2 genera of nematodes. *Trichostrongylus axei* were present in low numbers from June to September and in November and March. A second species of nematode in the stomach was *Habronema* sp. (immature) in 2 horses (August [$N = 36$] and January [$N = 20$]).

In the small intestine 2 species of nematodes were found. *Strongyloides westeri* were recovered in all months except January and February. Immature *Parascaris equorum* were represented in all months but January, and mature specimens in every month except the initial months of June and July when the foals were only 64 and 78 days old, respectively. From July to December, and in March, immature *P. equorum* were also found in the lungs.

Examination of the large intestine revealed 1 species of large strongyle, 22 species of small strongyles, and 1 species of pinworm. *Strongylus vulgaris* were first seen in September and observed each month through March. Small strongyles were found in the mucosa and contents for all months. The month with the highest number

of small strongyles was December in both the mucosa and contents. Regarding the mucosa of the dorsal colon, encysted small strongyle larvae were found in much lower numbers than from the mucosae of the cecum and ventral colon; there was none present in the dorsal colon mucosa in November, December, February, and March.

A total of 7 genera and 22 species of small strongyles was present in the contents of the large intestine of 5 horses (Table 2). The most prevalent were *Cyathostomum catinatum* and *Cylicostephanus longibursatus*. Those 2 species, plus *Cyathostomum coronatum* and *Cylicostephanus goldi*, were the only small strongyles found in horse No. 1, examined in June at 64 days of age. Also, about 80% of the small strongyles in horse No. 1 were immature; whereas in the subsequent horses, the majority were mature.

Oxyuris equi immatures were found in all months and matures in all months except September.

Strongylus vulgaris were found in the cranial mesenteric arteries for all months and *Strongylus edentatus* were found in the ventral abdominal walls from August (no examination in July) to March.

The eyeworm, *Thelazia lacrymalis* (1–2/horse) was found in only 3 horses (33%) in August, October, and November.

Table 2. Numbers of small strongyles recovered from 5 horses born on a farm in central Kentucky in 1989.

Genus and species	Counts for individual horses*					All horses		
	1	3	5	7	9	Counts		% of total†
						Total	Aggregate average	
<i>Cyathostomum</i>								
<i>C. catinatum</i>	50	6,500	3,700	28,100	14,650	53,000	10,600	26
<i>C. coronatum</i>	20	200	750	1,100	250	2,320	464	1
<i>C. labiatum</i>	0	200	100	600	200	1,100	220	1
<i>C. labratum</i>	0	800	300	650	700	2,450	490	1
<i>Cylicocyclus</i>								
<i>C. elongatus</i>	0	100	0	0	0	100	20	<1
<i>C. insigne</i>	0	100	600	750	1,350	2,800	560	1
<i>C. leptostomus</i>	0	900	600	2,300	1,350	5,150	1,030	2
<i>C. nassatus</i>	0	2,500	900	8,750	4,900	17,050	3,410	8
<i>C. radiatus</i>	0	0	0	150	200	350	70	<1
<i>Cylicodontophorus</i>								
<i>C. bicoronatus</i>	0	300	100	300	200	900	180	<1
<i>C. mettami</i>	0	0	200	0	0	200	40	<1
<i>Cylicostephanus</i>								
<i>C. asymmetricus</i>	0	100	200	0	0	300	60	<1
<i>C. calicatus</i>	0	500	750	1,450	650	3,350	670	2
<i>C. goldi</i>	950	3,300	3,300	8,400	1,700	17,650	3,530	9
<i>C. longibursatus</i>	2,870	12,000	12,500	36,400	17,300	81,070	16,214	39
<i>C. minutus</i>	0	2,500	2,300	6,100	3,050	13,950	2,790	7
<i>C. poculatus</i> §	0	0	100	100	0	200	40	<1
<i>Poteriostomum</i>								
<i>P. imparidentatum</i>	0	100	0	450	500	1,050	210	1
<i>Craterostomum</i>								
<i>C. acuticaudatum</i>	0	0	400	150	0	550	110	<1
<i>Triodontophorus</i>								
<i>T. brevicauda</i>	0	200	200	1,050	200	1,650	330	1
<i>T. serratus</i>	0	100	300	650	400	1,450	290	1
<i>T. tenuicollis</i>	0	0	200	150	0	350	70	<1
Total mature	3,890	30,400	27,500	97,600	47,600	206,990	41,398	86‡
Total immature	17,080	4,250	3,200	7,450	1,650	33,630	6,726	14‡
Total small strongyles	20,970	34,650	30,700	105,050	49,250	240,620	48,124	100

* Month of necropsy was June (No. 1), August (No. 3), October (No. 5), December (No. 7), and February (No. 9).

† % for each species is based on the total no. of mature small strongyles.

‡ % based on combined total No. of mature and immature small strongyles.

§ Lichtenfels and Klei (1988) cite and accept the placing of *C. poculatus* in the genus *Petrovinema* by Hartwich.

Egg and lpg data are recorded (Table 3). Ascarid eggs were found first in August and also in all succeeding months except December and March. Eggs of strongyles were present for all months and of *Strongyloides* for all but the last 3 months of the study. Larvae of *S. vulgaris* appeared first in December, but also in the months of January, February, and March. *Strongyloides westeri* larvae were found in the same months as were worm specimens. *Eimeria leuckarti*-type oocysts were found in July.

Discussion

Finding that *G. intestinalis* were not present until September was of interest because, in the earlier observations in the 19-yr study on horses in Field No. 10 (Lyons et al., 1990), second instars were found in all months of the year except June and third instars in all months. The numbers of both instars were much fewer in the present than previous study. Possibly, this is related to the recent use of boticides in horses in sur-

Table 3. Worm eggs per gram (epg) and larvae per gram (lpg) of feces of 10 horses born on a farm in central Kentucky in 1989.

ID No.	Egg			Lpg		
	Ascarids	Strongyles	Strongyloides	<i>Strongylus vulgaris</i>	Small strongyles	<i>Strongyloides westeri</i>
1	0	20	10	ND	ND	ND
2*	Neg	Pos	Pos	ND	ND	ND
3	10	1,690	2,190	ND	ND	ND
4	180	590	170	0	770	Pos
5	150	830	180	0	135	Pos
6	100	290	130	0	310	Pos
7	0	650	250	10	860	Pos
8	20	600	0	10	440	Neg
9	70	750	0	30	950	Neg
10	0	780	0	15	355	Pos

* No epg, but float was positive for strongyles, *Strongyloides*, and *Eimeria* oocysts.
 ND = no data; Pos = positive; Neg = negative.

rounding fields and the consequent reduction of the pool of botflies.

Comparison of data on stomach worms reveals that in the present study only *T. axei* and immature *Habronema* sp. were found, whereas in the previous study *T. axei* were not found, but prevalence of *Habronema muscae* was 79% and *Draschia megastoma*, 37% (Lyons et al., 1990).

The high rate of occurrence of *Strongyloides westeri* in the horses born in 1989 was not unexpected because usually there is a high prevalence in young horses. But, infections are self-limiting and tend to disappear after foals are a few months of age (Drudge and Lyons, 1986). Examination was not made in the previous study for this parasite.

Regarding *Parascaris equorum*, results were similar to the previous observation (Lyons et al., 1990) where immatures were found in all months but June and August; matures were present in all months. Recovery of immature *P. equorum* in the lungs in the present study from July to December, and in March, provided an index of the ongoing acquisition of ascarid infections, because invasive ascarid larvae reach the lungs by about 14 days after ingestion (Lyons et al., 1976a).

While intestinal stages of *Strongylus vulgaris* were first found in September, patency was not indicated until December when larvae were first present in fecal cultures. In the 19-yr study, *S. vulgaris* were present in all months except August.

A recent publication listing 9 genera and 33 species of small strongyles found in equids in

Kentucky by the present authors over about a 30-yr period (Tolliver et al., 1985) includes the 7 genera and 22 species found in the present study. Another investigation in this geographical area (Ohio) revealed 6 genera and 21 species of small strongyles present in horses (Reinemeyer et al., 1984). The finding of very few encysted small strongyles in the mucosa of the dorsal colon has been reported previously (Reinemeyer and Herd, 1986b).

A comparison of findings on parenteral stages of large strongyles reveals that, in the other study (Lyons et al., 1990), *S. vulgaris* were found in the cranial mesenteric artery in every month but July and *S. edentatus* were found in the ventral abdominal wall in all months except February, July, and August.

Presence of mature *Oxyuris equi* was similar to the previous study in which they were found in all months except February, July, August, and November (Lyons et al., 1990).

For the previous data on prevalence, *Thelazia lacrymalis* were found from January through May and in September and December (Lyons et al., 1990). Also in that survey, the prevalence (75%) and the mean number of eyeworms (6) were much higher than in the present study. A possible reason for the differences is that the availability of the intermediate host, the face fly (*Musca autumnalis*) (Lyons et al., 1980), was probably less in 1989 because of recent removal of grazing cattle from a farm in close proximity to Field No. 10. Therefore, suitable breeding material, mainly cattle feces (Drudge and Lyons, 1986), for the face flies was less available. Also, possible

effect of 1 compound (ivermectin), used several times in horses in surrounding fields, on reducing *T. lacrymalis* infections available for face fly transmission to the present horses is unknown. Single treatments with ivermectin appear to be ineffective on *T. lacrymalis* (Lyons et al., 1981b; Drudge et al., 1984), but data are not available for multi-treatments.

A relationship between the prepatent periods (Drudge and Lyons, 1986) of some of the parasites and ages of the horses when infected was evident. That is, there was evidence that some foals became infected soon after birth. For *P. equorum*, the prepatent period is approximately 10–12 wk. Eggs of this parasite were not found in the 78-day-old foal, but were present in the 107-day-old. Larvae of *S. vulgaris* were first observed in a fecal culture from the foal 174 days old, although adult specimens were present in 3 younger foals (125, 139, and 164 days old). For this large strongyle, the prepatent period is approximately 6 mo. The foals were not examined at a young enough age to record first occurrence of patent infections for some parasites, e.g., *S. westeri* and small strongyles. The prepatent period for *S. westeri* is about 2 wk and small strongyles approximately 6–10 wk; the youngest foal examined was 64 days old. Classic field research by Russell (1948) and Todd et al. (1949) has established the typical acquisition and development profile of several species of internal parasites in young equids.

Several species of parasites, previously found in horses born in Field No. 10, were not recovered from horses born there in 1989. They were *Gasterophilus nasalis*, *Anoplocephala perfoliata*, *Habronema muscae* (adult), *Draschia megastoma*, and *Strongylus edentatus* (in the large intestine). The reason for absence of *G. nasalis*, *H. muscae* (a few immature specimens of *Habronema* sp. were found), and *D. megastoma* is probably because horses in surrounding fields had been treated with parasiticides, therefore reducing the pool of these parasites. The absence of *A. perfoliata* is not readily explainable, but it is cyclic (Drudge and Lyons, 1986). This species of tapeworm was found in 33% of of horses born in Field No. 10 over a 19-yr period (Lyons et al., 1990). Absence of *S. edentatus* in the large intestine is accounted for by the young age of the horses. The prepatent period for *S. edentatus* is about 11 mo and the oldest horse was less than 8 mo old.

In the present study, the serial examination at monthly intervals of horses born the same year in the same field provided insights on the transmission dynamics of several species of endoparasites. Although not investigated previously in this manner in central Kentucky, several essentially predictable findings were verified. These included seasonal presence of some parasites and their stage of development according to established life cycle features. Several other observations on seasonal transmission of parasites were not anticipated because previous data were limited or lacking, e.g., numbers of encysted small strongyles, pinworm infectivity and development, and actual numerical values on migrating large strongyles. Probable effects of factors outside the pasture under research were found, causing lessening of available dipterid parasites and dipterid intermediate hosts themselves, or parasites they transmit. This research, besides filling at least some of the void in the biology of these parasites under natural conditions, may be a contributing factor in improving control recommendations.

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Transmission of Gastrointestinal Parasites in Dairy Calves on Pasture in Central Kentucky from 1987 through 1989¹

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ABSTRACT: Parasite-naïve tester dairy calves ($N = 50$) were placed on helminth-infested pasture once a month (1–3 calves) for 1 mo during the period July 1987–November 1989 (except for January 1989). At necropsy, the predominant mature parasites and mean numbers recovered from the tester calves were *Ostertagia ostertagi*—703, *Nematodirus helvetianus*—1,626, *Cooperia oncophora*—3,401, and *Trichuris* spp. (includes immatures)—138. The average total number of these parasites per calf was 5,885, with the highest monthly values found in July for 1987, April for 1988, and June for 1989. For each of these parasites, the month (each year) with the maximal numbers of mature specimens was: *O. ostertagi* in July 1987, May 1988, and June 1989; *N. helvetianus* in October 1987, May 1988, and June 1989; *C. oncophora* in July 1987, April 1988, and June 1989; *Trichuris* spp. (includes immatures) in August 1987, May 1988, and August 1989.

Ostertagia ostertagi L₄ were found only in November 1987, April and May 1988, and June, September, and October 1989; the average numbers were low, varying from 20 to 170. Low numbers of L₄ *N. helvetianus* and *C. oncophora* were present also in calves in several months. Other parasites present were *Moniezia* spp. (1–31 specimens each) in 17 calves and *Bunostomum phlebotomum* (3 specimens) in 1 calf. The eyeworm, *Thelazia gulosa*, was found (1–7 specimens per calf) in 6 calves.

KEY WORDS: internal parasites, natural infections, transmission, dairy calves, Kentucky.

Transmission of internal parasites of cattle has been studied extensively in many parts of the world. A review of epizootiology of nematodes of cattle in North America has been published recently (Williams, 1986). Studies on transmission of helminths of cattle in central Kentucky have been minimal; mainly they have been on overwintering of internal parasites (Drudge et al., 1958; Lyons et al., 1983).

The purpose of the present research was to obtain information on the seasonal transmission pattern of internal parasites of dairy calves on a farm in central Kentucky, with special interest in observing hypobiosis of *Ostertagia*.

Materials and Methods

A total of 50 calves (28 Jerseys and 22 Holsteins) was obtained from a local dairy farm. Sexes of the calves were 46 males (intact) and 4 females. They were raised parasite-free after being removed from their dams at about 2–4 days of age. Until weaning, they were fed a commercial milk replacer and a supplemental grain ration. They eventually accepted a complete pelleted ration which was fed during the remaining periods of confinement. The calves were kept individually in wire-bottom cages, situated in separate rooms, during pre- and postpasture exposure periods. Cages, food and

water containers, and areas surrounding the cages, including the floor and walls, were washed twice daily.

From July 1987 through November 1989 (except for January 1989), 1–3 calves (designated as “testers”) per month, at about 60–180 days of age, were placed on pasture. Data for the months of May–November 1989 are evaluated cautiously because only 1 tester calf was examined for each of these months.

The tester calves were located on pastures grazed concurrently by older dairy cattle naturally infected with internal parasites. These nontester cattle on pasture were mostly yearlings, but some were younger; numbers varied from about 6–35. Anthelmintic therapy for them consisted of 2 treatments (spring and fall) each year with levamisole.

Tester calves were kept on pasture for a 1-mo period, and then returned to confinement in individual cages. They were held in these cages for an additional month to allow time for advanced development or maturation of the parasites to occur. The testers were then killed and the gastrointestinal tracts were examined for internal parasites. Also, the eyes were examined for eyeworms except for July 1987 and December 1987–April 1988. Fecal samples were collected from the rectum of each calf at necropsy for epg (worm eggs per gram of feces).

Processing of the gastrointestinal tract included 2 ligatures: at the junctions between the abomasum and duodenum and between the ileum and cecum. Contents of the 3 portions—the abomasum, the small intestine, and the combination of cecum and large intestine—were expressed into separate containers. Then, the various segments of the tract were flushed 3 times with water to rinse out remaining particulate material and parasites. Rinses were added to the appropriate containers of contents. The 3 portions (abomasum,

¹ The investigation reported in this paper (No. 90-4-131) was made in connection with a project of the Kentucky Agricultural Experiment Station and is published with the approval of the director.

Table 1. Worm data for tester calves ($N = 12$) at necropsy in 1987.

Tester calves		No. of parasites for individual calves							Total
		<i>Ostertagia ostertagi</i>		<i>Nematodirus helvetianus</i>		<i>Cooperia oncophora</i>		<i>Trichuris</i> * spp.	
Month on pasture	No.	Imm	Mat	Imm	Mat	Imm	Mat		
July	2	0	480	20	2,640	20	9,320	269	12,749
		0	840	20	1,180	20	14,300	88	16,448
August	2	0	220	0	1,780	0	4,460	176	6,636
		0	300	80	2,300	20	8,080	286	11,066
September	2	0	40	60	3,660	0	780	245	4,785
		0	100	120	5,020	0	1,400	136	6,776
October	2	0	80	60	4,720	0	660	14	5,534
		0	20	20	5,980	0	640	42	6,702
November	2	80	60	40	680	0	300	40	1,200
		100	40	60	1,980	0	360	0	2,540
December	2	0	40	0	220	20	100	109	489
		0	60	0	100	20	420	3	603
Aggregate average		15	190	40	2,522	8	3,402	118	6,294

* Stages not determined.

Imm = immature; Mat = mature.

small intestine, and cecum-large intestine) of the gastrointestinal tract were cut open and any observed parasites were removed and saved. Each section of the tract was then suspended, with large paper clips, in containers of water (1-gallon each for the abomasum and for the cecum-large intestine and 2.5 gallons for the small intestine) for about 6 hr to permit migration of parasitic stages from the mucosa (Williams et al., 1979). Afterwards, the walls of the tract were rubbed (Williams et al., 1979) by hand under tap water into a 200-mesh sieve. The residue, along with the water and materials in the containers in which the tissues had been suspended, were washed on the 200-mesh sieve; the residue was saved and preserved with 5% formalin. Artificial digestive juice was not used for parasite recovery from any portions of the gastrointestinal tract. Further processing of all of the material for recovery of parasites was similar to that published previously (Drudge et al., 1963). Basically, it included washing aliquot samples (50 ml/liter) of fixed material from the abomasum and intestine onto 100-mesh (contents and washes) or 200-mesh ("soaks" of tissue) sieves. The residue from the samples was examined for worms through a stereoscopic microscope at about $10\times$. After sampling, excess material was washed onto a 40-mesh sieve and the remains were examined grossly for larger parasites. Procedures for recovery of eyeworms (Lyons and Drudge, 1975) and for determining epg were likewise as described previously (Drudge et al., 1963).

Results and Discussion

Four main genera (*Ostertagia*, *Nematodirus*, *Cooperia*, and *Trichuris*) of internal parasites were recovered at necropsy from the tester calves (Tables 1-3). Species of these genera include *Ostertagia ostertagi* from the abomasum, *Nematodi-*

rus helvetianus and *Cooperia oncophora* from the small intestine, and *Trichuris* spp. from the cecum-large intestine. They are the same species predominating in the same dairy herd in past research (Lyons et al., 1983).

For each year the highest monthly mean number of mature *O. ostertagi* was in July (660) for 1987, May (5,400) for 1988, and June (2,620) for 1989. The total numbers ranged from 0 in February and November 1988, and November 1989, to 6,320 in May 1988. The numbers generally declined in the months after the highest values, except for moderate increases in September and October for both 1988 and 1989.

For mature *N. helvetianus*, the highest average numbers were in October 1987 (5,350), May 1988 (3,390), and June 1989 (9,440). The total numbers of this parasite varied from a low of 0 for 5 months (February and December 1988 and February, March, and November 1989) to a high of 9,440 for 1 month (June 1989). Numbers were generally much lower by 1-2 mo after the highest values. The decline was over a longer period though for 1989.

Mature *C. oncophora* were present in highest average numbers in July 1987 (11,810), April 1988 (30,440), and June 1989 (7,780). The total numbers varied from a low of 0 (February 1988 and November 1989) to a high of 37,100 (April 1988). After the month with the highest numbers, a gradual decrease was evident in the succeeding months in 1987. This same pattern oc-

Table 2. Worm data for tester calves ($N = 25$) at necropsy in 1988.

Tester calves		No. of parasites for individual calves							Total
		<i>Ostertagia ostertagi</i>		<i>Nematodirus helvetianus</i>		<i>Cooperia oncophora</i>		<i>Trichuris</i> * spp.	
Month on pasture	No.	Imm	Mat	Imm	Mat	Imm	Mat		
January	3	0	220	0	120	0	360	2	702
		0	200	20	300	0	920	3	1,443
		0	340	105	585	0	1,185	40	2,255
February	2	0	0	0	0	0	0	2	2
		0	0	0	20	0	0	8	28
March	2	0	120	0	100	0	60	20	300
		0	210	0	220	0	750	19	1,199
April	2	60	2,440	0	700	20	23,780	16	27,016
		80	4,320	220	100	640	37,100	20	42,480
May	2	0	6,320	0	1,600	0	10,020	219	18,159
		340	4,480	200	5,180	0	16,020	106	26,326
June	2	0	220	20	2,320	0	300	112	2,972
		0	200	80	4,013	0	560	135	4,988
July	2	0	180	20	500	0	300	27	1,027
		0	140	40	1,860	0	700	113	2,853
August	2	0	280	100	100	0	640	27	1,147
		0	260	80	1,060	0	700	79	2,179
September	2	0	380	0	160	0	1,720	10	2,270
		0	540	0	340	0	2,440	77	3,397
October	2	0	240	0	60	0	1,460	59	1,819
		0	320	0	60	20	2,100	132	2,632
November	2	0	0	0	20	0	140	20	180
		0	40	0	40	0	280	40	400
December	2	0	115	0	15	0	655	32	817
		0	280	0	0	0	750	16	1,046
Aggregate average		19	874	35	779	27	4,118	53	5,905

* Stages not determined.

Imm = immature; Mat = mature.

curred in 1988, except for a rebound during September and October. For 1989, the postpeak decline was relatively less than observed for the other years, but there was a repetition of 1988 in the increase in numbers in September and October.

For *Trichuris* spp. (mature and immature), the months with the highest average numbers per calf were August 1987 (231), May 1988 (163), and August 1989 (1,479). The total numbers varied from a low of 0 (November 1987 and February and March 1989) to a high of 1,479 (August 1989). Numbers of specimens were quite variable for most of the months following the peak periods.

Immature forms of *O. ostertagi* (L₄) were found in low numbers (20–340 per calf) in only 8 calves during 6 mo (November 1987, April and May 1988, and June, September, and October 1989). Measurements of L₄ specimens indicated all were

≤ 2.0 mm long except for 2 (2.5 mm and 3.8 mm long).

For L₄ *N. helvetianus*, low numbers (20–260) were recovered from about one-half (26) of the calves. The highest average numbers of L₄ *N. helvetianus* (1.3–7.0 mm long) per calf were in September 1987 (90), April 1988 (110), and June 1989 (260). *Cooperia oncophora* L₄ (20–640) were present in only 12 calves; the highest numbers (640) were in April 1988 (all were ≤ 2.5 mm long, except 1 which measured 6.0 mm long).

For all stages of the 4 predominant genera (*Ostertagia*, *Nematodirus*, *Cooperia*, and *Trichuris*) in the tester calves, the month with the highest average number was July 1987 (14,599), April 1988 (34,748), and June 1989 (20,520). For the entire study, the aggregate mean number of these 4 genera per calf was 6,294 in 1987, 5,905 in 1988, and 5,465 in 1989. This value for all 50 calves was 5,885.

Table 3. Worm data for tester calves (N = 13) at necropsy in 1989.

Tester calves		No. of parasites for individual calves							Total
		<i>Ostertagia ostertagi</i>		<i>Nematodirus helvetianus</i>		<i>Cooperia oncophora</i>		<i>Trichostrongylus</i> * spp.	
Month on pasture	No.	Imm	Mat	Imm	Mat	Imm	Mat		
February	2	0	140	0	0	0	60	21	221
		0	380	0	0	0	120	0	500
March	2	0	440	0	0	20	340	0	800
		0	440	0	20	0	360	61	881
April	2	0	1,500	20	620	0	1,060	750	3,950
		0	1,200	20	2,680	0	1,160	45	5,105
May	1	0	360	0	860	0	380	865	2,465
June	1	20	2,620	260	9,440	0	7,780	400	20,520
July	1	0	920	100	6,760	20	3,800	206	11,806
August	1	0	660	40	1,960	20	1,440	1,479	5,599
September	1	20	1,180	40	3,000	0	4,460	186	8,886
October	1	20	1,160	160	3,520	20	5,280	150	10,310
November	1	0	0	0	0	0	0	2	2
Aggregate average		5	846	49	2,220	6	2,018	320	5,465

* Stages not determined.

Imm = immature; Mat = mature.

Other helminths were also recovered from the tester calves, but the data are not presented in tabular form. *Moniezia* spp. were found in 17 calves (1–31 specimens each) in July through October 1987, in April through October 1988, and in April 1989. *Bunostomum phlebotomum* were found in 1 calf (3 specimens) in September 1988. The eyeworm, *Thelazia gulosa*, was recovered from 6 calves (1–7 specimens each) in August and September 1987 and in June, August, and September 1988.

Trichostrongyle egg data are recorded (Table 4). The egg values generally had similar patterns observed for the worm counts.

All of the parasites except *B. phlebotomum* had been found previously in the dairy herd used for the present study (Lyons et al., 1981a, b, 1983). However, only in 1 earlier study had the small and large intestine been examined for parasites (Lyons et al., 1983). Gastrointestinal parasites found in the herd in the past but not present research were *Trichostrongylus axei* and *Haemonchus* spp. (Lyons et al., 1981a). Other internal parasites recovered from calves in additional herds in this geographical area are *Cooperia punctata*, *Oesophagostomum radiatum*, and *Strongyloides papillosus* (Lyons et al., 1972, 1982).

Establishment of a seasonal inhibition pattern or hypobiosis of *L*₄ *O. ostertagi*, *N. helvetianus*, and *C. oncophora* in this geographical area was

not revealed in the present observations because of generally low numbers recovered. It is unfortunate that the numbers of *L*₄ were so low, because the present study does not clarify the inhibition pattern of *O. ostertagi*, in particular, in cattle in central Kentucky. Also, the numbers of mature *O. ostertagi* were not high. This indicates that nontester cattle were not shedding many *O. ostertagi* eggs on pasture. Therefore, few larvae of the parasites were available for ingestion by tester calves. Possibly, the numbers of *L*₄ trichostrongyles might have been higher if recovery methods from the gastrointestinal tract had been for a longer time, e.g., 24 hr (Williams et al., 1981; Gasbarre, 1987) instead of 6 hr (Williams et al., 1979). Also, the size of the sieve openings (200 mesh) may have been too large for maximum retention of *L*₄ during washing of material.

The nonimmune state of the tester calves, because of a lack of previous exposure to *Ostertagia* and other parasites, may have been a factor in low numbers of *L*₄. Supporting this view is research by Michel (1970), showing that the numbers of inhibited *Ostertagia* were higher, in previously infected, than naive calves. However, Anderson (1988) states that seasonal inhibition of *Ostertagia* occurred in both naive and previously infected calves. Another view on hypobiosis is that it is due to environmental factors (Williams, 1986).

It seems clear that if much higher numbers of

Table 4. Worm eggs per gram of feces (epg) for tester calves ($N = 50$) at necropsy in 1987 ($N = 12$), 1988 ($N = 25$), and 1989 ($N = 13$).

Month on pasture	Total no.*	Epg for individual calves								
		Trichostrongyle†			<i>Nematodirus</i>			Total		
		1987	1988	1989	1987	1988	1989	1987	1988	1989
January	3	—	60	—	—	0	—	—	60	—
		—	20	—	—	0	—	—	20	—
		—	30	—	—	10	—	—	40	—
February	4	—	0	60	—	0	0	—	0	60
		—	0	10	—	0	0	—	0	10
March	4	—	20	20	—	0	0	—	20	20
		—	60	40	—	0	0	—	60	40
April	4	—	90	180	—	0	0	—	90	180
		—	1,350	160	—	0	0	—	1,350	160
May	3	—	1,040	50	—	40	0	—	1,080	50
		—	1,110	—	—	10	—	—	1,120	—
June	3	—	60	150	—	20	30	—	80	180
		—	0	—	—	10	—	—	10	—
July	5	810	70	590	30	0	120	840	70	710
		1,010	30	—	90	10	—	1,100	40	—
August	5	150	20	30	60	0	20	210	20	50
		310	110	—	10	0	—	320	110	—
September	5	70	230	450	70	0	40	140	230	490
		90	60	—	60	0	—	150	60	—
October	5	0	180	70	30	0	0	30	180	70
		20	110	—	40	0	—	60	110	—
November	5	20	10	0	40	0	10	60	10	10
		30	10	—	30	0	—	60	10	—
December	4	0	90	—	0	0	—	0	90	—
		0	30	—	0	0	—	0	30	—
Aggregate average		209	192	139	38	4	17	248	196	156

* Total number of calves examined for each month for all 3 years; the number of calves examined for each month for individual years can be obtained by counting the total number of epg values for each time period.

† Excluding *Nematodirus*.

L_4 nematodes had been on pasture, the tester calves would have had greater levels of parasites, allowing more meaningful interpretation of seasonal transmission, including inhibition of these parasites. Further research is necessary to establish the inhibition phenomenon of larval nematodes in central Kentucky.

Regarding mature specimens of *Ostertagia*, *Nematodirus*, and *Cooperia*, the time of the year (April, May, and/or June in 1988 and 1989), with the highest numbers present, were similar. *Trichostrongylus* spp., although found in all months of the study, were most numerous in 1989. The total numbers of all stages of the 4 main genera of gastrointestinal parasites followed a similar pattern (1988 and 1989) of being most prevalent in the spring, summer, and fall months (April–October). There were fewer total parasites recov-

ered from the calves in June and July 1988 than for a comparable time in 1989 and July in 1987. Possibly, this was because of a drought in 1988, particularly in June and early July (Table 5). However, in 1987 a drought occurred in August, September, and October (Table 5) and values for total parasites were higher than in 1988, but similar to those in 1989.

It is of interest that transmission of the parasites from pasture to calves occurred in varying degrees year-round. Lower numbers of parasites in calves in the coldest months may have been partially the result of these animals preferring to eat hay, rather than graze the poor quality vegetation on pasture at that time of the year. Previously it was determined in tester calves that several species of helminths (*O. ostertagi*, *T. axei*, *N. helvetianus*, *Nematodirus* spp., *C. oncophora*,

Table 5. Climatological data* for 1987, 1988, and 1989 for the vicinity of pasture used for the parasite transmission study.

Date	Temperature (F°)			Precipitation (inches)
	Minimum	Maximum	(Av)	
1987				
January	7	59	(34)	1.20
February	14	58	(40)	4.15
March	23	76	(49)	3.43
April	30	84	(55)	2.40
May	40	91	(71)	1.70
June	53	92	(76)	5.96
July	56	95	(78)	2.95
August	53	97	(77)	0.77
September	42	89	(70)	0.92
October	25	80	(53)	0.52
November	15	83	(51)	3.32
December	16	66	(39)	6.28
Total	—	—	—	33.60
1988				
January	-3	64	(31)	3.68
February	-1	67	(34)	3.37
March	18	78	(45)	2.12
April	28	88	(54)	3.78
May	36	90	(63)	2.55
June	39	100	(72)	0.55
July	46	103	(78)	3.87
August	47	100	(78)	3.41
September	42	87	(68)	4.94
October	22	76	(50)	1.81
November	23	72	(47)	6.08
December	6	66	(37)	3.76
Total	—	—	—	39.92
1989				
January	16	69	(40)	3.71
February	3	65	(33)	9.85
March	18	80	(48)	7.09
April	20	85	(54)	3.19
May	31	88	(61)	4.97
June	48	92	(72)	5.68
July	53	93	(76)	3.85
August	46	91	(74)	3.89
September	33	89	(67)	4.12
October	28	84	(56)	2.90
November	17	73	(45)	2.89
December	-20	59	(23)	1.80
Total	—	—	—	53.94

* Data were obtained from the University of Kentucky weather station, located on Spindletop Farm about 1 mile from the parasite study area. They were supplied by K. T. Priddy, Department of Agricultural Engineering, University of Kentucky.

Trichuris spp., *Moniezia* spp., and *Dictyocaulus viviparus*) overwinter on pasture in central Kentucky (Drudge et al., 1958; Lyons et al., 1981a, 1983).

From the present investigation, a seasonal pat-

tern of greatest transmission of the 3 most prevalent genera of mature gastrointestinal parasites, found in cattle during this study in central Kentucky, was defined as during the spring months.

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Molineus barbatus (Trichostrongylidae) and other Helminthic Infections of the Cat in Arkansas

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ABSTRACT: During anthelmintic studies on naturally infected cats in Arkansas, we encountered a species never before reported from cats among a 10-species helminthic assemblage in 13 untreated, control animals. The helminthic assemblage included the following: 1 trematode, *Eurytrema procyonis* in 8% of the cats; 3 cestodes, *Dipylidium caninum*, *Mesocestoides variabilis*, and *Taenia* sp. in 8%, 15%, and 8% of the cats, respectively; and 6 nematodes, *Ancylostoma tubaeforme*, *Capillaria* sp., *Molineus barbatus*, *Physaloptera rara*, *Toxascaris leonina*, and *Toxocara cati* in 77%, 62%, 54%, 38%, 8%, and 92% of the cats, respectively. To our knowledge, this is the first report of the trichostrongylid, *M. barbatus*, from domestic cats. Adult worms were found in 7 of 13 animals with a maximum intensity of 21 worms. It is significant to note that fecal analysis made prior to necropsy failed to reveal trichostrongyle eggs in any of the cats. Given the similarity of *M. barbatus* eggs to hookworm eggs in shape (both are thin-shelled and elliptical) and size (both are 60 × 40 μm), it is possible that we mistook *M. barbatus* eggs for those of hookworms. It is also possible that practitioners could make the same mistake resulting in animals treated for hookworm infections that they may not have.

KEY WORDS: *Ancylostoma tubaeforme*, *Capillaria* sp., cats, *Dipylidium caninum*, *Eurytrema procyonis*, helminths, *Mesocestoides variabilis*, *Molineus barbatus*, *Physaloptera rara*, *Taenia* sp., *Toxascaris leonina*, *Toxocara cati*.

Recently we conducted anthelmintic trials on naturally infected cats in Arkansas. Of the 50 cats involved in the studies, 13 remained as untreated controls. Within those untreated control animals we encountered a helminthic assemblage that comprised 10 species; 1 that appeared commonly had never been reported from the cat.

Materials and Methods

In preparation for anthelmintic trials, 50, mixed-breed, sexually mature cats from the Conway, Arkansas area underwent fecal examination during 1989 and 1990 for the presence of natural infections of intestinal helminths. Fecal exams consisted of coverslip flotations using magnesium-sulfate. Eggs of hookworms, ascarids, capillarids, physalopterans, and tapeworms were observed (Table 1). Based on the presence and number of eggs, the 50 cats were allocated to treatment groups such that each group had an equal representation of parasite species and number of worms (or as close to equality as possible for quantitative fecal analysis). Of the 50 cats, 13 were allocated to controls and remained untreated. Table 1 demonstrates that the 13 cats left untreated were a representative subsample of the 50-cat population. The data from animals allocated to the treatment groups were excluded from this report. At necropsy, the entire gastrointestinal tract from each control cat was removed, slit longitudinally, and the mucosa washed over a 200-mesh screen (pore size 75 μm). The contents collected from that screen were placed in formalin. The gastrointestinal tracts were then incubated individually overnight in saline and Combiotic (Pfizer) at room temperature. The next day they again were washed and the sievings fixed in the same manner as previously described. The formalin-pre-

served contents from the 2 washings were examined microscopically and total residual worm counts were made.

Results

Ten species of helminths were found in the 13 control animals at necropsy and are presented in Table 2. Adults of *Molineus barbatus* Chandler, 1942 (Figs. 1-4), were found in 7 of the 13 cats. It is important to note that eggs recognizable as belonging to a trichostrongylid were not distinguished at any time during the fecal analysis.

Table 1. Prevalence of helminths in Arkansas cats as evidenced from fecal exams.

Species	Prevalence	
	Entire 50 animal group	Control group aliquot
<i>Ancylostoma tubaeforme</i>	72% (36/50)	77% (10/13)
<i>Capillaria</i> sp.	22% (11/50)	23% (3/13)
<i>Dipylidium caninum</i>	0% (0/50)	0% (0/13)
<i>Eurytrema procyonis</i>	0% (0/50)	0% (0/13)
<i>Mesocestoides variabilis</i>	0% (0/50)	0% (0/13)
<i>Molineus barbatus</i>	0% (0/50)	0% (0/13)
<i>Physaloptera rara</i>	2% (1/50)	0% (0/13)
<i>Taenia</i> sp.	8% (4/50)	8% (1/13)
<i>Toxascaris leonina</i>	0% (0/50)	0% (0/13)
<i>Toxocara cati</i>	48% (24/50)	54% (7/13)

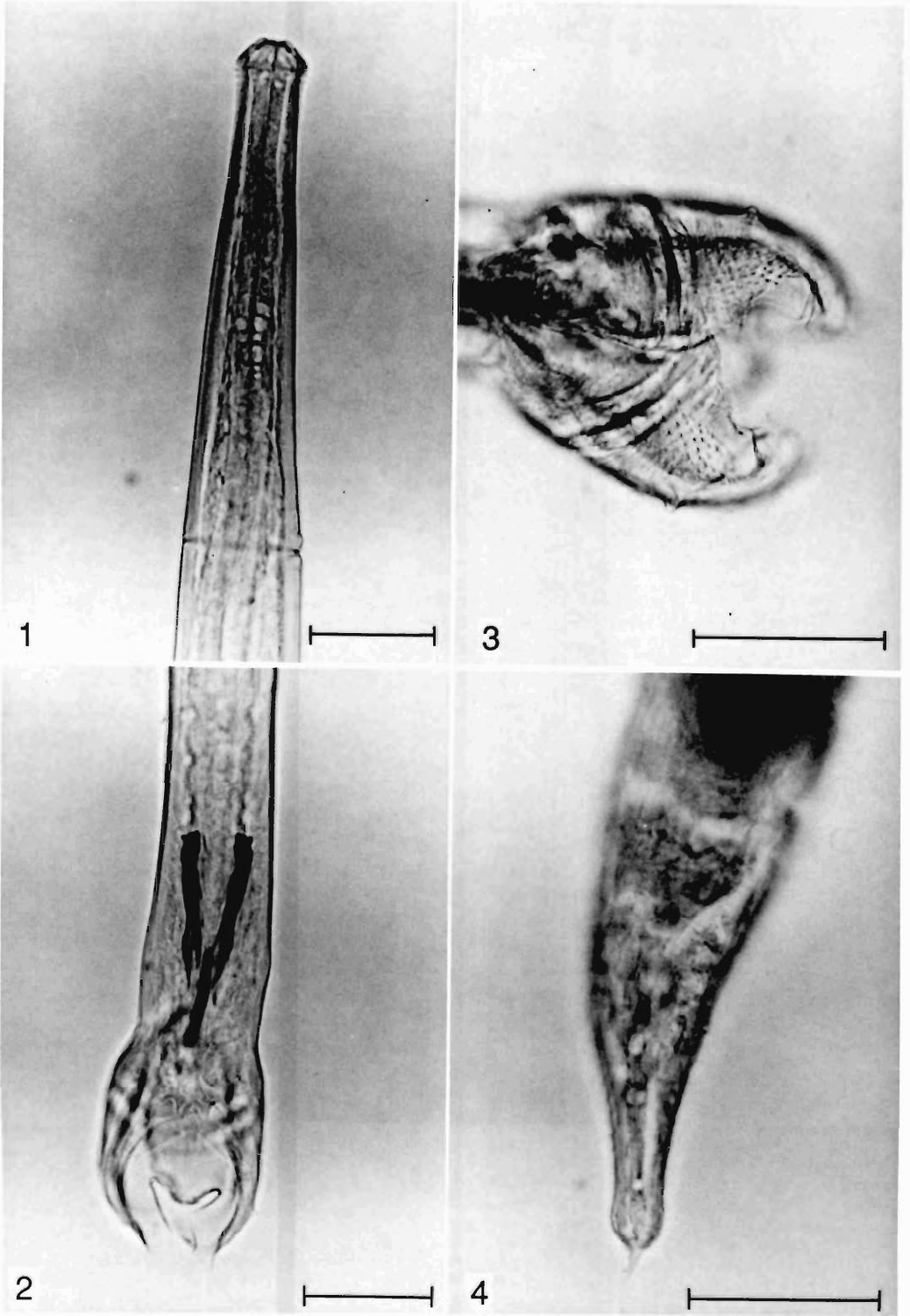


Table 2. Prevalence and intensity of helminths in Arkansas cats after necropsy.

Species	USNM Helminth Collection Number	Prevalence	Intensity
<i>Ancylostoma tubaeforme</i>	81875	77% (10/13)	17 (0-100)
<i>Capillaria</i> sp.	81876	62% (8/13)	6 (0-27)
<i>Dipylidium caninum</i>	81877	8% (1/13)	<1 (0-1)
<i>Eurytrema procyonis</i>	81878	8% (1/13)	<1 (0-1)
<i>Mesocestoides variabilis</i>	81879	15% (2/13)	<1 (0-3)
<i>Molineus barbatus</i>	81880	54% (7/13)	4 (0-21)
<i>Physaloptera rara</i>	81881	38% (5/13)	4 (0-27)
<i>P. rara</i> L ₄	81881	38% (5/13)	8 (0-75)
<i>Taenia</i> sp.	81882	8% (1/13)	<1 (0-3)
<i>Toxascaris leonina</i> L ₄	81883	8% (1/13)	<1 (0-1)
<i>Toxocara cati</i>	81884	92% (12/13)	13 (0-46)

Discussion

The results from the 13 untreated control cats reveal 10 species of helminths, including 1 *Molineus barbatus*, which has not been reported previously from this host. Of the 13 control animals, 7 had natural infections of adult *M. barbatus*. *Molineus barbatus* is a trichostrongylid parasite commonly found in the small intestine of raccoons (Chandler, 1942; Harkema and Miller, 1964) and bobcats (Miller and Harkema, 1968; Watson et al., 1981). In addition, it has also been observed once from the cougar (Forrester et al., 1985) and skunk (Babero, 1960). To our knowledge, it has never been reported from domestic cats. Significantly, this species was fourth in prevalence after *Toxocara cati*, *Ancylostoma tubaeforme*, and *Capillaria* sp. based on necropsy results.

The finding of these trichostrongylid worms in the intestines of cats is more than just of zoological interest. The eggs of *M. barbatus* are similar to those of cat hookworms such as *Ancylostoma tubaeforme* in shape (both are thin-shelled and elliptical) and size (both are 60 × 40 μm). In fact, we are not at all confident that we did not observe trichostrongyle eggs in the cat feces, but because they look so similar to hookworm eggs they may have been mistaken as such. More important than confusing the outcome of an anthelmintic trial is the possibility that cats

observed passing these eggs may be considered hookworm positive by practitioners and thus made to undergo expensive and sometimes dangerous treatment for worms they might not have.

These studies indicate that *M. barbatus* may be a common parasite of cats in Arkansas. This trichostrongylid is more generally known as a parasite of raccoons, but it was reported to be a common component of the helminth fauna of bobcats in the southeastern United States (Watson et al., 1981). Thus, it is no surprise that domestic cats may also become infected. Although the infective stages of trichostrongyles are usually ingested, Gupta's (1961) study of the life cycle of *M. barbatus* showed that the infective stage of this species is also capable of skin penetration. This may be a more likely explanation of how carnivores become infected.

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We would like to thank our collaborators in Arkansas, Jerry Cunningham and Ron Everett, for their help in conducting this study.

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Figures 1-4. Adult male and female *Molineus barbatus* from naturally infected domestic cats. 1. Anterior end showing the transversely striated anterior swelling and the cervical groove with the excretory pore surrounded by liplike swellings. 2. Posterior end of male showing the spicules and gubernaculum. 3. Copulatory bursa showing the characteristic spines. 4. Posterior end of the female with characteristic spine. All scale bars = 75 μm.

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A Redescription of *Ostertagia bisonis* (Nematoda: Trichostrongyloidea) and a Key to Species of Ostertagiinae with a Tapering Lateral Synlophe from Domestic Ruminants in North America

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ABSTRACT: *Ostertagia bisonis* Chapin, 1925, is an abomasal worm of the American buffalo, *Bison bison*, and other ruminants including cattle in which it can cause clinical nematodiasis. This report describes characteristics of *O. bisonis*, especially details of the synlophe and esophagus, that are necessary for constructing a key to the species of medium stomach worms (Ostertagiinae) parasitic in domestic ruminants in North America. The synlophe of *O. bisonis* is most similar to the single ridge tapering lateral synlophe of *Ostertagia ostertagi*. Ducts of the subventral glands of the esophagus empty anterior to the cervical papillae and the esophageal–intestinal valve is more than twice as long as wide. We follow earlier workers in considering *Ostertagia orloffii* Sankin, 1930, a synonym of *O. bisonis*. *Ostertagia bisonis* may have more generalized characters than any other species with a tapering lateral synlophe that are parasites of domestic ruminants in North America, but polarization of some characters cannot be considered reliable until additional outgroups are studied.

KEY WORDS: *Ostertagia bisonis* (= *O. orloffii*), synlophe, redescription, nematode morphology, Nematoda, *Bison bison*, Trichostrongyloidea, Ostertagiinae, *Ostertagia ostertagi*, *Ostertagia lyrata*, *Teladorsagia circumcincta* (= *Teladorsagia davtiani*), *Teladorsagia trifurcata*, cuticle, ruminants, SEM.

Ostertagia bisonis Chapin, 1925, is one of a group of medium stomach worms that is among the most serious nematode pathogens of ruminants (Anonymous, 1983). It was described from the American buffalo, *Bison bison*, and has been reported from a wide range of ruminants including Bovidae, Cervidae, and Antilocapridae. It is of interest to veterinary parasitologists because of the clinical nematodiasis it can cause in cattle (Worley and Sharman, 1966).

The present study is part of an effort to prepare an identification key to the species of medium stomach worms of domestic ruminants of North America. Several characters of primary importance in the identification keys, including the synlophe (longitudinal surface cuticular ridges) and structure of the esophagus, have not been described for *O. bisonis*, and are described herein. An earlier redescription of *O. bisonis* by Becklund and Walker (1967) included excellent drawings of spicules and the copulatory bursa. The structure of the genital cone was described in detail by Stringfellow (1971). In order to separate female *O. bisonis* from other species with a similar synlophe, a detailed study of females of 3 species was necessary including *O. bisonis*, *O. ostertagi*, and *Teladorsagia circumcincta*. A key to males of 5 species and females of 3 species is presented at the end of the Discussion section. This group includes all species with a tapering lateral synlophe.

Materials and Methods

Nematodes

Specimens were obtained from the National Parasite Collection maintained in this laboratory and others were provided by Drs. David E. Worley and Newton Kingston (Table 1). Among specimens studied were paratypes of *Ostertagia bisonis* (USNM Helm. Coll. Nos. 25960 and 26103).

Microscopy

Specimens were studied as: (1) temporary whole mounts cleared in phenol–alcohol (80 parts melted phenol crystals and 20 parts absolute ethanol) and examined with regular light microscopy or interference-contrast microscopy at a magnification of 400 to 1,000; (2) cross sections in free-hand cuts made with a cataract knife and mounted in glycerine jelly; (3) critical point dried, coated with gold palladium, and viewed at 5–20 kV with scanning electron microscopy (SEM) (Madden and Tromba, 1976).

Characters studied

Male specimens were identified to species on the basis of the morphology of the spicules and genital cones (Andreeva, 1958; Drózdź, 1965; Becklund and Walker, 1967, 1971; Stringfellow, 1971) prior to study of the synlophe and esophagus. Bursal ray patterns were determined and described using the system of Durette-Desset and Chabaud (1981). Papillae of the genital cone and rays of the copulatory bursa followed the numbering system of Chabaud et al. (1970). The lengths of the esophageal–intestinal (E-I) valves, determined to extend from the posterior end of the cuticular lining of the triradiate lumen of the esophagus to the posterior end of the esophagus (Figs. 14–16), were measured (Table 2). For measurements of the

Table 1. Specimens of *Ostertagia bisonis* and related species* studied by host, locality, and sex.

Species and synonyms	Number of lots/number of specimens by host, locality and sex	
<i>Ostertagia bisonis</i> Chapin, 1925 = <i>O. orloffii</i> Sankin, 1930 = <i>O. bellae</i> , nomum nudum	<i>Ammotragus lervia</i> , Barbary sheep	
	New Mexico	1/2 males
	<i>Antilocapra americana</i> , pronghorn	
	South Dakota	4/15 males, 1 female
	Wyoming	1/5 males
	<i>Bison bison</i> , buffalo	
	Alberta, Canada	2/6 males, 6 females
	South Dakota	1/6 males, 7 females
	<i>Bos taurus</i> , cattle	
	Colorado	1/1 male
	Montana	5/25 males, 16 females
	Wyoming	6/20 males, 1 female
	<i>Odocoileus hemionus</i> , mule deer	
	Montana	2/7 males, 6 females
	South Dakota	2/4 males
<i>Ovis aries</i> , sheep		
USSR, Leningrad	1/1 male, 1 female	
<i>Ostertagia ostertagi</i> *	<i>Antilocapra americana</i> , pronghorn	
	South Dakota	1/1 female
	<i>Bos taurus</i> , cattle	
	England, Weybridge	1/2 males, 1 female
	Georgia	2/3 females
	Louisiana	1/7 males
	Maryland	5/11 males, † 14 females
	Montana	3/13 males, 15 females
	New York	1/2 males
	Wyoming	1/7 males, 1 female
<i>Odocoileus hemionus</i> , mule deer		
Montana	1/3 females	
<i>Teladorsagia circumcincta</i> *	<i>Capra hircus</i> , goat	
	England, Weybridge	3/4 males, 8 females
	<i>Oreamnos americanus</i> , mountain goat	
	South Dakota	1/1 female
	<i>Ovis aries</i> , sheep	
	England, Weybridge	1/1 male‡
	Georgia	1/2 males, 5 females
	Indiana	1/1 male
	Maryland	1/5 females
	Virginia	1/1 female

* For additional specimens studied and synonymies see Lichtenfels et al. (1988a).

† Includes 1 male *Ostertagia lyrata*.

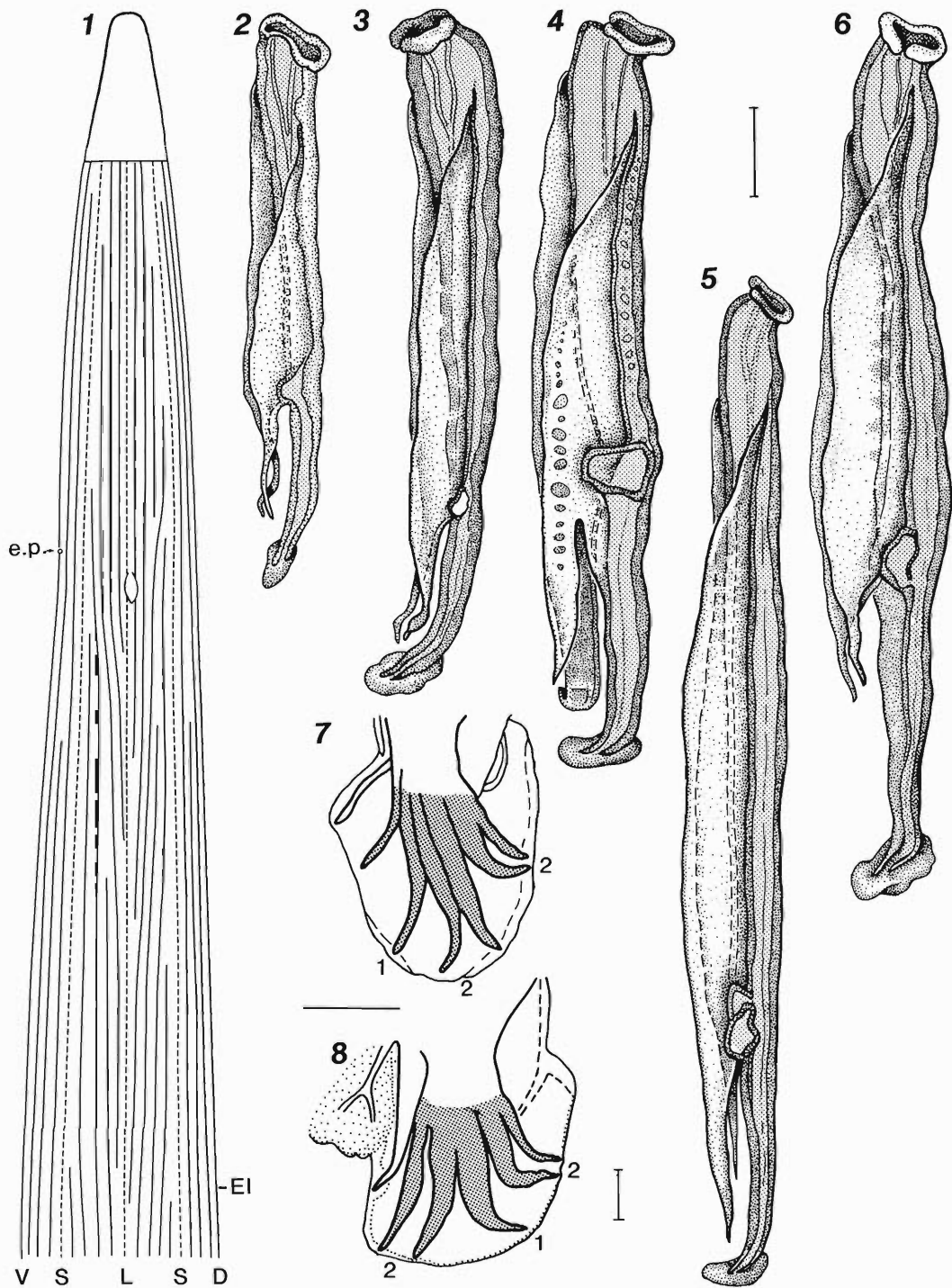
‡ *Teladorsagia trifurcata*.

infundibula and sphincters of the ovejectors (Table 3), the edge of the muscular portion of the sphincter was used as a dividing line between them, and the fluffy coat around the sphincter and the portion of the infundibulum overlapped by the muscles of the sphincters were ignored (Figs. 36–38). Because the separation of the vestibule from the sphincter was difficult or impossible to determine, the vestibule was counted as part of the sphincter. The measurement for length of the sphincter includes the distance from the distal end of the sphincter to the vulva. Measurements are in micrometers unless indicated otherwise. Synonymies in Table 1 follow Drózdź (1965) and Skrjabin et al. (1954).

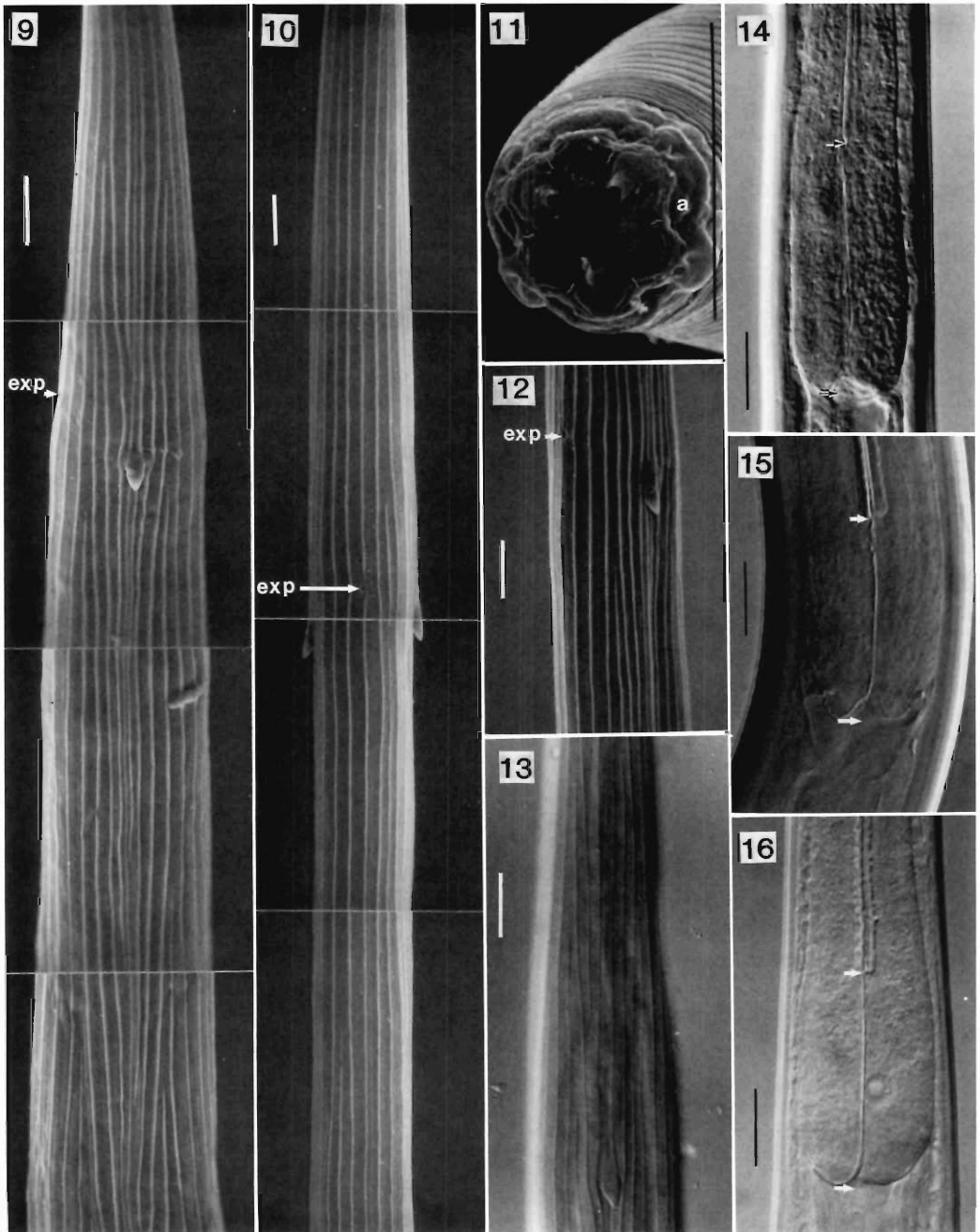
Results

Synopse of *Ostertagia bisonis*

There were about 20–38 ridges in the region of the esophagus (Fig. 1) with the smaller number anteriorly. The pattern observed was the Type I lateral synopse (Lichtenfels et al., 1988a) in which there is 1 continuous lateral ridge (L in Fig. 1) running just ventral to the cervical papilla and other adjacent ridges in the lateral field angle posteriorly toward the lateral ridge and end adjacent to it (Figs. 1, 9, 12, 13). This pattern was



Figures 1–8. Drawings of 5 species of Ostertagiinae. 1. Synlophe of *Ostertagia bisonis*, diagram of left lateral view. Note: Dashed lines for emphasis only; ridges are not interrupted (see Figs. 9, 10, 12, 13). 2. Spicule of *Ostertagia bisonis*. 3. Spicule of *Ostertagia ostertagi*. 4. Spicule of *Ostertagia lyrata*. 5. Spicule of *Teladorsagia circumcincta*. 6. Spicule of *Teladorsagia trifurcata*. 7. Bursal ray pattern of the *Teladorsagia* species. 8. Bursal ray pattern of *Ostertagia bisonis* (from Becklund and Walker, 1967). e.p., excretory pore; EI, esophageal–intestinal junction; L, lateral ridge; S, sublateral ridges; V, ventral ridge; D, dorsal ridge. All scale bars = 25 μ m.



Figures 9–16. Lateral tapering synlophe of *Ostertagia bisonis* and esophageal–intestinal (E–I) valves of 3 species of Ostertagiinae. 9. Synlophe, left lateral view, SEM. 10. Synlophe, ventral view, SEM. 11. En face view, SEM, showing 6 labial papillae (arrows), 1 of the 2 lateral amphids (a). Outer circle of 8 cephalic papillae not discernable. 12. Synlophe showing number of ridges between excretory pore and cervical papilla, SEM. 13. Synlophe, left lateral view, light microscopy. 14. E–I valve of *Ostertagia bisonis* showing anterior and posterior ends of valve between arrows. 15. E–I valve of *Ostertagia ostertagi* showing anterior and posterior ends of valve between arrows. 16. E–I valve of *Teladorsagia circumcincta* showing anterior and posterior ends of valve between arrows. exp, excretory pore. All scale bars = 25 μ m.

Table 2. Morphometrics (in micrometers; range with mean in parentheses) of male *Ostertagia bisonis*, *Ostertagia ostertagi*, *Ostertagia lyrata*, *Teladorsagia circumcincta*, and *Teladorsagia trifurcata*.

Character	<i>Ostertagia bisonis</i> (N = 40)	<i>Ostertagia ostertagi</i> * (N = 18)	<i>Ostertagia lyrata</i> * (N = 16)	<i>Teladorsagia circumcincta</i> * (N = 35)	<i>Teladorsagia trifurcata</i> * (N = 24)
Body length (mm)	5.64–8.64 (7.23)	5.40–7.44 (6.12)	5.04–7.32 (6.23)	5.40–11.4 (8.13)	6.24–10.7 (8.44)
Subventral esophageal gland orifices†	225–350 (285)	204–280 (248)‡	224–293 (260)	188–256 (219)	188–280 (236)
Cervical papillae†	225–388 (312)	296–372 (339)	278–392 (344)	264–408 (332)	296–396 (352)§
Esophagus length†	604–869 (741)	556–764 (672)	525–740 (665)	502–892 (620)	528–684 (618)
Esophageal-intestinal valve length	82–141 (108)	51–89 (76)	50–77 (64)	53–97 (79)	51–99 (79)
Esophagus as percent of body length	8.4–12.3 (10.3)	7.6–13.1 (11.1)	7.9–13.9 (10.8)	5.6–10.3 (7.7)	6.3–8.8 (7.4)
Spicule length	129–188 (162)	212–264 (238)	229–268 (238)	242–408 (317)	188–319 (252)
Genital cone shape	Normal	Proconus	Sjöberg's organ	Normal	Sjöberg's organ
Bursal ray formula	2-1-2	2-1-2	2-1-2	2-2-1	2-2-1

* Emended measurements published by Lichtenfels et al. (1988a).

† From anterior end.

‡ N = 16.

§ N = 23.

|| Pattern of rays in lateral lobes of bursa following system of Durette-Desset and Chabaud (1981).

repeated through the anterior half of the specimen. In the posterior half of the nematode the lateral ridges became more parallel to each other. In the region of the esophagus posterior to the cervical papillae, 1–4 pairs of lateral ridges ended adjacent to the lateral ridge. Most specimens had 3 parallel ventral ridges with the ventralmost ridge being interrupted by the excretory pore (Fig. 10). However, 3 or 4 of the 130 specimens studied had only 1 ventral ridge and 1 or more ridges beginning near the excretory pore next to the ventral ridge rather than running parallel to the ventral ridge anteriorly to the cephalic region. At the level of the excretory pore *O. bisonis* had 28 ridges (Fig. 12). More posteriorly in the body the number of ridges increased to 38–40 at the E-I junction (Figs. 1, 9). The number of ridges remained relatively constant through the middle three-fourths of the nematode (Fig. 39). In the prebursal or postvulvar region only the lateral ridges remained (Table 4).

Esophagus

The most useful characteristic of the esophagus, as in other related species, was the length of the E-I valve (Tables 2, 3). The valve was found to be longer than that of other species (Figs. 14–16) with a similar tapering lateral synlophe, but shorter than related *Ostertagiinae* with a parallel lateral synlophe. In addition other characteristics

of the esophagus were studied (Tables 2, 3). The length of the esophagus as a percentage of the total body length was found to differ among similar species and was useful in identifying females (Table 3). The subventral esophageal gland orifices (SVGO) were anterior to the excretory pore (Table 3).

Male characters

We confirmed the presence of a gubernaculum (Figs. 18, 32) and found it to have an expanded proximal portion. The paired spicules are mirror images of each other, of medium to slender thickness, split into 3 branches in the distal third, with a central shaft that ends distally in a medially curved point covered with a fleshy pad, and a pair of thin branches supported by membranous alae that extend from the proximal shaft and end in slightly sinuous tips of nearly equal length (Figs. 2, 18, 19). The ventral surface of the genital cone does not have a proconus (Fig. 18). In lateral view the genital cone is bilobed with ventral and dorsal lobes, and with the saclike dorsal ray of the bursa the appearance is trilobed (Fig. 18). In ventral view the ventral lobe (Fig. 17) of the genital cone appears to be a bipartite velum that encloses the paired number 0 papillae. The dorsal part of the genital cone bears an oval accessory bursal membrane (Figs. 17, 18) that encloses the paired papillae number 7. The dorsal lobe is

Table 3. Morphometrics (in micrometers; range with mean in parentheses) of female *Ostertagia bisonis*, *Ostertagia ostertagi*, and *Teladorsagia circumcincta*.

Character	<i>Ostertagia bisonis</i> * (N = 18)	<i>Ostertagia ostertagi</i> (N = 16)	<i>Teladorsagia circumcincta</i> (N = 15)
Body length (mm)	6.38–8.90 (7.95)	8.90–10.5 (9.07)	9.10–12.4 (10.7)
Nerve ring†	199–280 (249)	229–293 (267)	235–304 (266)
Subventral esophageal gland orifices†	252–328 (290)	194–296 (262)§	209–310 (246)
Excretory pore†	269–328 (300)	259–343 (309)	308–386 (349)
Cervical papillae†	280–353 (322)	289–369 (341)	323–418 (372)
Esophagus length†	643–849 (743)	651–825 (724)	548–688 (615)
Esophageal–intestinal valve length	89–124 (107)	56–86 (76)	63–82 (74)
Esophagus as percent of body length	8.6–10.3 (9.4)	7.1–8.9 (8.0)	5.1–6.8 (5.8)
Vulva percent†	79–83 (81)	84–87 (85)	80–85 (83)
Anterior infundibulum	124–199 (163)	131–189 (162)	136–196 (168)
Anterior sphincter to vulva length‡	168–332 (257)	114–196 (140)	159–253 (203)
Posterior infundibulum	112–178 (143)	128–180 (151)	129–175 (150)
Posterior sphincter to vulva length‡	145–295 (224)	103–159 (123)	147–211 (171)
Eggs (l × w)	70–84 (78) × 30–43 (39)	70–86 (77) × 39–49 (41)	84–101 (91) × 40–56 (49)
Tail length	126–164 (148)	105–168 (136)	133–164 (154)

* Includes 5 paratypes.

† From anterior end.

‡ Sphincter to vulva includes vestibule and sphincter length.

§ N = 14.

|| N = 15.

¶ N = 13.

about half to two-thirds as long as the lateral lobes of the bursa, saclike in lateral view, and usually has a granular appearance (Fig. 18). The fleshy rays of the lateral lobes of the copulatory bursa are oriented in a 2-1-2 pattern with the ventroventral (No. 2) and the lateroventral (No. 3) rays close together, the anterolateral (No. 4) ray by itself and the mediolateral (No. 5) and posterolateral (No. 6) rays close together (Fig. 8).

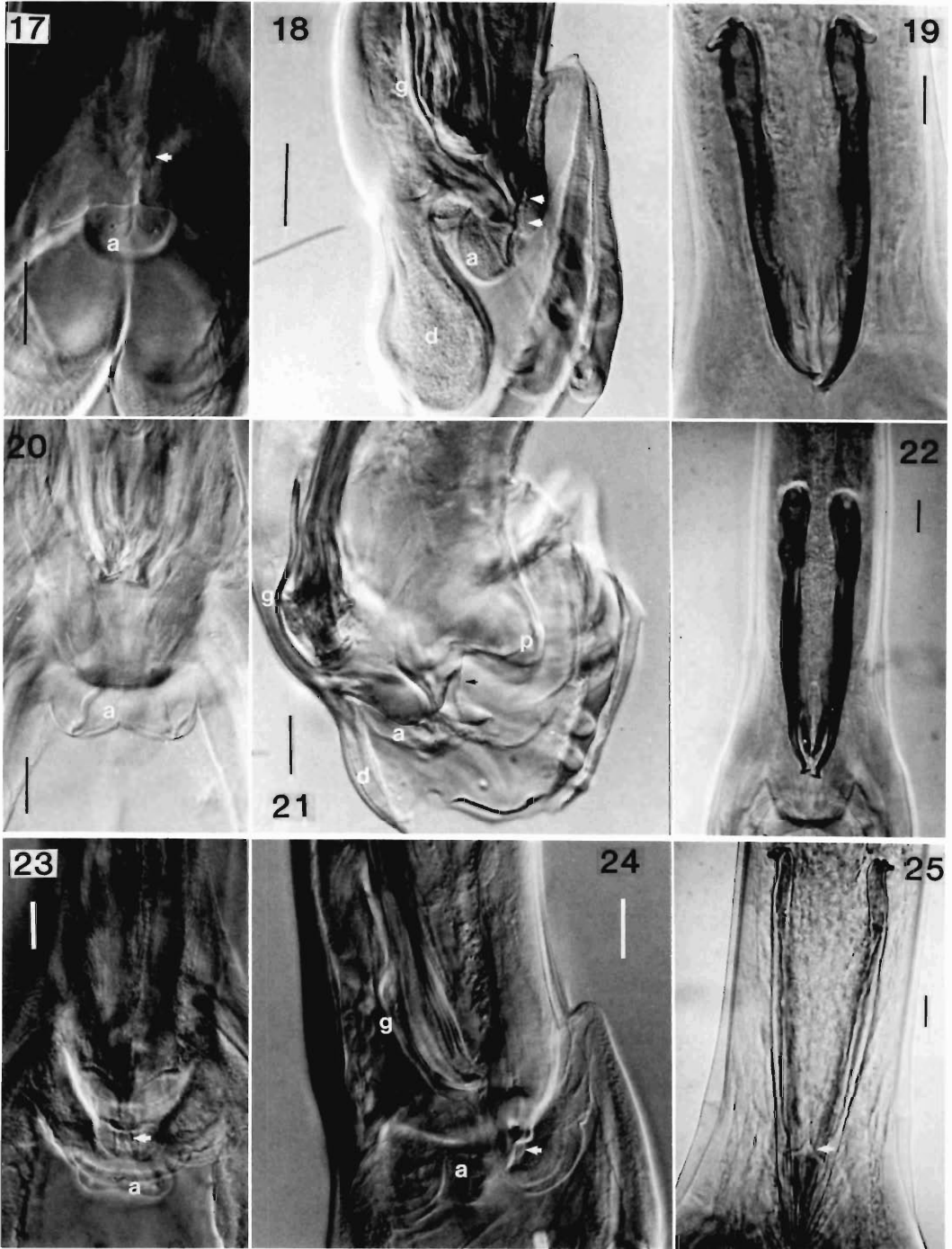
Female characters

The vulva is located about one-fifth of the body length from the posterior end. The vulva is a ventral transverse slit, usually without a vulval

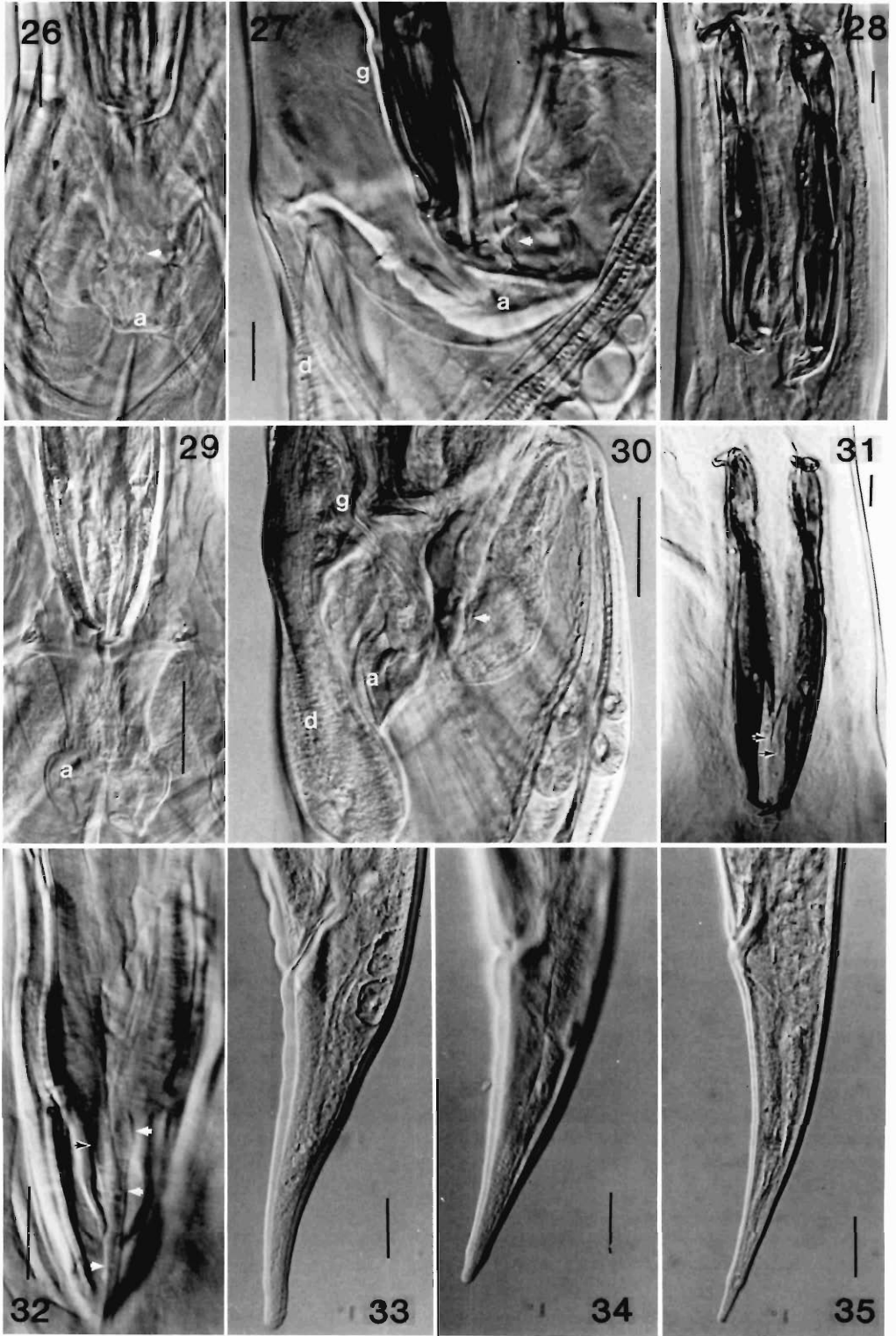
flap and with a thickened cuticle around the opening (Fig. 36). In 2 of 20 specimens studied, a small cuticular lobe was present on 1 side, slightly anterior to the vulva. The ovejectors fill most of the diameter of the body cavity (Fig. 36). The infundibula are slightly shorter than the distance from the vulva to the junction of the sphincter and infundibulum. The anterior ovejector is longer than the posterior ovejector in all specimens. The tip of the tail is completely covered by annulated cuticle and is usually slightly swollen or digitiform (Fig. 33). Eggs are cylindrical with rounded ends 70–84 (78) long by 30–43 (39) wide.

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Figures 17–25. Genital cones, spicules and gubernacula of some male *Ostertagiinae*. 17–19. *Ostertagia bisonis*. 17. Genital cone, ventral view, showing paired papillae number 0 (arrow) within the ventral velumlike expansion, and paired papillae number 7 within accessory bursal membrane (a). 18. Genital cone and dorsal bursal lobe (d), lateral view, showing ventral lobe of genital cone enclosing 1 of paired papillae number 0 (arrows), and dorsal lobe of genital cone bearing accessory bursal membrane (a) enclosing 1 of paired papillae number 7. 19. Spicules, ventral view, showing 3 distal branches on each spicule. 20–22. *Ostertagia ostertagi*. 20. Genital cone, ventral view, showing accessory bursal membrane (a) enclosing paired papillae number 7 and distal ends of central branches of spicules. 21. Posterior extremity of male, lateral view, showing dorsal bursal lobe (d), gubernaculum (g), distal end of spicules and genital cone with accessory bursal membrane (a), and 1 of paired papillae number



0 (arrow), and proconus (p). 22. Spicules and gubernaculum, ventral view. 23–25. *Teladorsagia circumcincta*. 23. Genital cone, ventral view, showing ventral velumlike expansion of genital cone enclosing papillae number 0 (arrow), and accessory bursal membrane (a) enclosing papillae number 7. 24. Genital cone, lateral view, showing distal end of spicules, gubernaculum (g), ventral expansion of cone enclosing 1 of paired papillae number 0 (arrow) and accessory bursal membrane (a) enclosing 1 of paired papillae number 7. 25. Spicules, ventral view and gubernaculum (arrow). All scale bars = 25 μ m.



Discussion

New information on *Ostertagia bisonis* presented herein includes the description of the synlophe and the esophagus. This new information was required in order to develop an identification key to both males and females of *O. bisonis* and related species. The tapering lateral synlophe described herein for *O. bisonis* is very similar to that described earlier (Lichtenfels et al., 1988a) for *O. ostertagi* and *Teladorsagia circumcincta*. Lichtenfels et al. (1988a) reported that the tapering pattern was more marked in *T. circumcincta* than in *O. ostertagi* with 2 or 3 pairs of ridges tapering toward and ending near the lateral ridge in the region of the esophagus in *T. circumcincta*, but only 1 pair ending in that region in *O. ostertagi*. However, in *O. bisonis* there was a greater range of variation in this character with 1–4 pairs of ridges ending near the lateral ridge in the region of the esophagus (Figs. 1, 9). The variability of the ventral synlophe pattern in *O. bisonis* was similar to that reported earlier (Lichtenfels et al., 1988a) for *O. ostertagi*. Most specimens had 3 parallel ventral ridges (Fig. 10), but fewer than 5% of *O. bisonis* specimens were similar to the single ventral ridge pattern described by Lichtenfels et al. (1988a) for *T. circumcincta*. In number of ridges in 1 circumference at various points along the body, *O. bisonis* is very similar to *O. ostertagi* except for its smaller number of postvulvar and prebursal ridges (Table 4). The cross sections also show, in addition to the number and distribution of ridges, that *O. bisonis* is only about half as thick as the 2 related species (Figs. 39–41).

Two characteristics of the esophagus were found to be consistent within species, variable among species, and easily observable making them useful key characters for identifying females of *O. bisonis*, *O. ostertagi*, and *T. circum-*

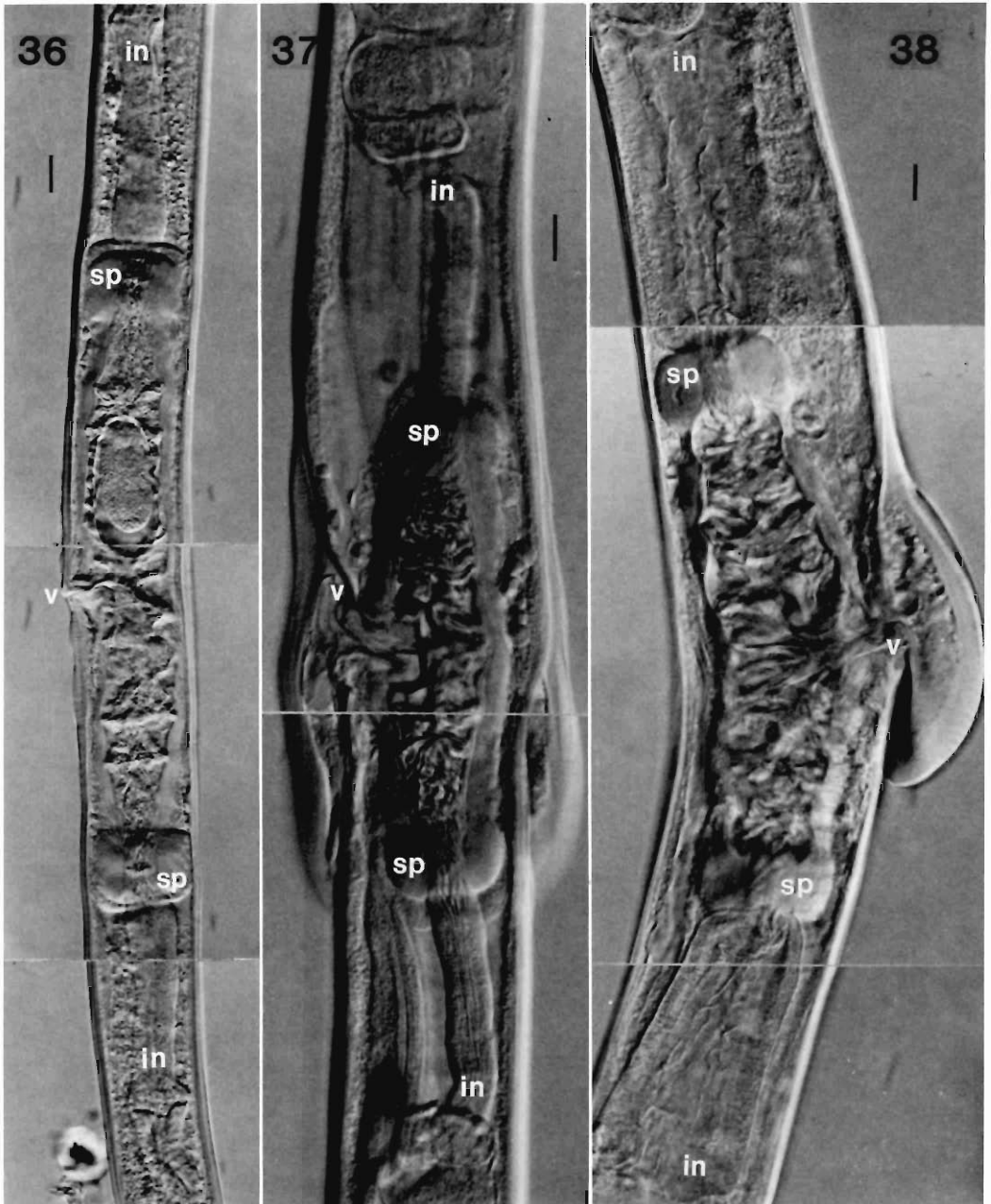
cincta. The length of the E-I valve of *O. bisonis* (Fig. 14) is longer than that of *O. ostertagi* (Fig. 15) or *T. circumcincta* (Fig. 16) (Tables 2, 3). The length of the esophagus as a percentage of total body length is smaller in *T. circumcincta* than in the other 2 species, and is especially useful in separating females of *O. bisonis* and *T. circumcincta* (Tables 2, 3) as a supplementary character to the length of the E-I valve.

The ducts of the subventral glands of the esophagus empty anterior to the cervical papillae (Tables 2, 3). As reported earlier (Lichtenfels et al., 1988a), the position of these ducts relative to the nerve ring, cervical papillae, and excretory pore has a wide variability because of the degree of shrinkage or stretching of the esophagus. In addition, the position of the ducts is difficult to determine compared to the length of the E-I valve which we recommend, along with the length of the esophagus as a percentage of total body length, as key characters for this group of species.

The most useful characteristics for identifying males to species, in addition to the length of the E-I valve, are the morphology of the spicules and genital cone, lateral bursal ray pattern, and presence or absence of a proconus. The spicules, gubernaculum, genital cone, and copulatory bursa of *O. bisonis* were illustrated in detailed drawings by Becklund and Walker (1967). Herein we provide, for comparison with related species, drawings of the left spicule (Figs. 2–6) and photomicrographs of the genital cone, gubernaculum, and spicules (Figs. 17–32) of 5 species with a tapering lateral synlophe. The spicules of *O. bisonis* (Figs. 2, 18, 19) appear to be typical for the Ostertagiinae with a central main stem and 2 medial branches (Figs. 3–6). In characteristics of the genital cone, especially in lacking a proconus, *O. bisonis* (Fig. 18) is more similar to *T. circumcincta* (Fig. 24) than to *O. ostertagi* (Fig. 21). The

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Figures 26–35. Genital cones, spicules and gubernacula (26–32), and female tails (33–35) of some Ostertagiinae. 26–28. *Ostertagia lyrata*. 26. Genital cone and spicule tips, ventral view, showing paired papillae number 0 (arrow) and accessory bursal membrane (a). 27. Genital cone and spicule tips, lateral view, showing 1 of paired papillae number 0 (arrow), sclerotized accessory bursal membrane (a), gubernaculum (g), and proximal portion of dorsal bursal lobe (d). 28. Spicules, ventral view, showing 3 distal branches on each spicule. 29–31. *Teladorsagia trifurcata*. 29. Genital cone and spicule tips, ventral view, showing accessory bursal membrane (a) enclosing paired papillae number 7. 30. Genital cone and spicule tips, lateral view, showing ventral lobe of cone enclosing 1 of paired papillae number 0 (arrow), accessory bursal membrane (a), dorsal bursal lobe (d), and gubernaculum (g). 31. Spicules, ventral view, showing 3 distal branches with arrows indicating 2 medial branches of left spicule. 32. *Ostertagia bisonis* gubernaculum (between arrows), dorsal view. 33–35. Female tails, left lateral views. 33. *Ostertagia bisonis*. 34. *Ostertagia ostertagi*. 35. *Teladorsagia circumcincta*. All scale bars = 25 μ m.



Figures 36–38. Ovejectors of some female Ostertagiinae. 36. *Ostertagia bisonis*. 37. *Ostertagia ostertagi*. 38. *Teladorsagia circumcincta*. V, vulva; sp, sphincter; in, infundibula (lateral view). All scale bars = 25 μ m.

2-1-2 pattern of rays in the copulatory bursa of *O. bisonis* is like that of *O. ostertagi*.

Additional characters for separating females of *O. ostertagi* and *T. circumcincta* include the synlophe, vulval morphology, and egg size. Differences in the synlophes of *O. ostertagi* and *T.*

circumcincta were described by Lichtenfels et al. (1988a). The lateral synlophe between the cervical papillae and the posterior end of the esophagus of *O. ostertagi* has a single pair of ridges ending adjacent to the lateral ridge, but *T. circumcincta* has 2 or 3 pairs of ridges ending next

Table 4. Number of longitudinal cuticular ridges in cross sections at various body regions of 3 species of Ostertagiinae.

Body region	<i>Ostertagia bisonis</i>		<i>Ostertagia ostertagi</i> *		<i>Teladorsagia circumcincta</i> †	
	Male (N = 2)	Female (N = 5)	Male (N = 4)	Female (N = 4)	Male (N = 4)	Female (N = 5)
Junction of E-I	29-38	36-40	39-40	33-39	31-36	30-43
End of first quarter	36-38	38-40	38-41	33-40	29-36	33-43
Midbody	38-41	36-38	39-41	32-36	25-39	29-42
End of third quarter	29-37	38-40	37-41	36-44	25-34	28-36
Prebursal or postvulvar‡	18-30§	16-32§	30-36§	39-49	14-28§	36-54§

* Includes 1 male *Ostertagia lyrata*.

† Includes 1 male *Teladorsagia trifurcata*.

‡ Sectioned 200-300 µm anterior to prebursal papillae or midway between vulva and tail tip.

§ Total number distributed in 2 equal lateral fields, no ridges dorsally or ventrally.

to the lateral ridge in this region. Ventrally ridges beginning between the area of the excretory pore and the posterior end of the esophagus are lateral to a single ventral ridge in *T. circumcincta*, but usually lateral to 3 parallel ventral ridges in *O. ostertagi*. The vulval flap of *O. ostertagi* is usually present and it usually wraps around the body almost 360° (Fig. 37). However, Michel et al. (1972a, b) described considerable variation in the degree of development of the vulval flap in *O. ostertagi*. Specimens may be found that vary from smooth to a fully developed flap that wraps around the body almost 360°. In *T. circumcincta* if a vulval flap is present it wraps 180° or less of the body circumference (Fig. 38). This character has limited use in a key, however, since flapless or specimens with incompletely developed flaps would not be identified. The egg size of *O. bisonis* is almost identical to that of *O. ostertagi*, but differs from the larger eggs of *T. circumcincta* (Table 3).

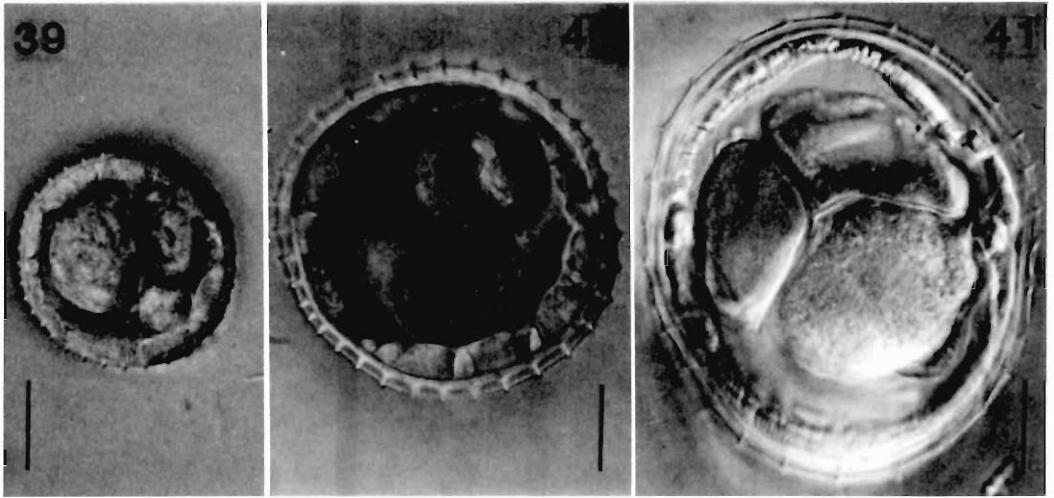
Two additional characteristics of females, morphometrics of the ovejectors and shape of the tail, were useful for identifying some specimens but were variable enough to disqualify them as key characters. The anterior ovejectors were longer than the posterior ovejectors in all 3 species, *O. bisonis*, *O. ostertagi*, and *T. circumcincta* (Figs. 36-38). It will be interesting to determine how widespread this asymmetry in ovejectors is within the Ostertagiinae. Morphometrics of the ovejectors were found to be useful in separating most specimens of *O. ostertagi* from *O. bisonis* and *T. circumcincta* (Table 3). In most specimens of *O. ostertagi* the infundibula were longer than the combined length of the sphincter and vestibule but the reverse was true in the other 2 species (Figs. 36-38). This was true for both anterior

and posterior ovejectors. In characteristics of the ovejectors and vulvar morphology, *O. bisonis* is more similar to *T. circumcincta* than to *O. ostertagi*. The shape of the tip of the female tail of most specimens of *O. bisonis* is slightly swollen or digitiform (Fig. 33) while the tail tips of *O. ostertagi* and *T. circumcincta* females usually are sharply tapered (Figs. 34, 35, respectively).

Recently, characteristics useful for identifying 4 species of female Ostertagiinae were described by Lancaster and Hong (1990). They did include *O. ostertagi* and *T. circumcincta*, but not *O. bisonis*, and they used different characters than used here.

The possible synonymy of *O. orloffii* with *O. bisonis* was discussed by Becklund and Walker (1967) and they preferred to recognize both species because of possible minor differences in shape of the gubernaculum and medial branches of the spicules. The original description of *O. bisonis* indicated a gubernaculum was not present. The presence of a gubernaculum in *Ostertagia orloffii* Sankin, 1930, was the primary difference between the 2 species until a gubernaculum was described in *O. bisonis* by Olsen (1949). After studying the synlophe, spicules, gubernaculum, genital cone, esophagus, ovejectors, and other characteristics of 1 male and 1 female *O. orloffii* collected from *Ovis aries* in Leningrad in 1936, we regard *O. orloffii* Sankin, 1930, to be a junior synonym of *O. bisonis* following Karamendin (1967). However, Drózdź (1979) and Petrov (1983) continued to use the name *O. orloffii* for the Palaearctic populations.

Since the review of *O. bisonis* literature in North America by Becklund and Walker (1967), this species has been reported in *Antilocapra americana*, *Bison bison*, and *Odocoileus hemionus* in



Figures 39–41. Mid-body cross sections of some female Ostertagiinae. 39. *Ostertagia bisonis* showing 39 ridges. 40. *Ostertagia ostertagi* showing 36 ridges. 41. *Teladorsagia circumcincta* showing 30 ridges. All scale bars = 25 μ m.

South Dakota (Boddicker and Hughhins, 1969), in *O. hemionus* in Montana (Worley and Eustace, 1972), and in cattle in Wyoming (Hones and Bergstrom, 1971). A review of the literature on *O. orloffii* in Asia and North America supports the conclusions of Worley and Sharman (1966) that *O. bisonis* (= *O. orloffii*) normally occurs in wild ruminants and appears in cattle and sheep in specialized conditions when grazing land is shared. It is clear, however, that *O. bisonis* can be an important pathogen of cattle under those conditions (Worley and Sharman, 1966).

The Ostertagiinae of domestic ruminants of North America is a group of 10 species with unsettled generic level taxonomy (Lichtenfels et al., 1988b). Recently, Durette-Desset (1989) placed *O. bisonis* in the genus *Camelostrongylus* Orloff, 1933, based on characteristics of the copulatory bursa and synlophe. The species she transferred to *Camelostrongylus* included *O. lyrata*, but not *O. ostertagi*. We prefer to retain *O. bisonis* and *O. lyrata* in the genus *Ostertagia* with *O. ostertagi*. Realignment of species at the generic level will be required in the Ostertagiinae, but must await clarification of relationships in future studies.

Several synlophe patterns have been identified in the Ostertagiinae from domestic ruminants (Lichtenfels et al., 1988a, b). The following key includes only species with a tapering lateral synlophe. Data on all key characters for 5 species of

males are included in Table 2. Comparative data on the main characters of females include only 3 species in both Table 3 and the key, because females of *O. lyrata* and *T. trifurcata* have never been identified. Drózdź (1974) reported that species pairs were common within the Ostertagiinae. Lancaster and Hong (1981) and Lancaster et al. (1983) have proposed that *O. lyrata* (Figs. 26–28) and *T. trifurcata* (Figs. 29–31) (= *T. davtiani* Andreeva and Satubaldin, 1954) are morphotypes of *O. ostertagi* and *T. circumcincta*, respectively. Included among 14 species pairs proposed by Drózdź (1974) were *O. orloffii* (which we consider to be a synonym of *O. bisonis*) and *Teladorsagia kazakhstanica* Dikov and Neki-pelova, 1963. In North America no associated minor species has been identified for *O. bisonis*. The associated minor species in Asia (*T. kazakhstanica*) was described (Dikov and Neki-pelova, 1963) as similar to *T. trifurcata* (= *T. davtiani*) but with longer branches on the spicules, longer ventral papillae on the genital cone (pair number 0), a dorsal ray that bifurcates in its proximal third, and lateral rays in a 2-1-2 pattern rather than 2-2-1 as in *T. trifurcata*. In addition, we can predict that *T. kazakhstanica* will share characteristics of the synlophe and esophagus as described herein with *O. bisonis* (= *O. orloffii*).

The comparisons with other species have been limited to those species with a tapering lateral

synlophe that are parasitic in domestic ruminants in North America. Two species, *Ostertagia leptospicularis* Assadov, 1953, and *O. mossi* Dikmans, 1931, without a tapering lateral synlophe are sufficiently similar in other characteristics, especially in size and spicule morphology, that distinguishing characteristics for separating them from *O. bisonis* are given here. Both *O. leptospicularis* and *O. mossi* have 3 parallel lateral ridges, and both sexes of both species can be distinguished from *O. bisonis* on that character alone. In addition, males of *O. leptospicularis* and *O. mossi* have a proconus, but *O. bisonis* males do not.

Ostertagia bisonis includes some characteristics of both genera *Ostertagia* and *Teladorsagia*. Like *Ostertagia* it has a 2-1-2 arrangement of lateral bursal rays. Like *Teladorsagia* it lacks a proconus. In characteristics of the ovejectors and vulval morphology, *O. bisonis* is more similar to *T. circumcincta* than to *O. ostertagi*. In egg size *O. bisonis* is almost identical to *O. ostertagi*, and both differ from *T. circumcincta*. The synlophe pattern and other characteristics shared by this group of species may indicate that they are each other's closest relatives, but this cannot be determined until sufficient information is available for these key characters to be polarized. Additional characters, including DNA comparisons, should be completed prior to making generic level changes.

Key to Male Ostertagiinae with a Tapering Lateral Synlophe from Domestic Ruminants in North America

- 1a. Esophageal-intestinal valve length more than twice its width (Fig. 14); spicules 129-188 μm long, dorsal and ventral medial branches longer than half of distance from branching point to distal end of central branch (Figs. 2, 19); proconus absent (Fig. 18) *O. bisonis*
- 1b. Esophageal-intestinal valve length less than twice its width (Figs. 15, 16); spicules usually longer than 200 μm 2
- 2a. Lateral rays of copulatory bursa with fleshy rays in 2-1-2 pattern (Fig. 8); proconus present (Fig. 21) or absent (Fig. 27) 3
- 2b. Lateral rays of copulatory bursa with fleshy rays in 2-2-1 pattern (Fig. 7); proconus absent (Figs. 24, 30) 4
- 3a. Proconus present, remainder of genital cone not prominent (Figs. 20, 21); spicules as in Figures 3, 21, 22 *O. ostertagi*
- 3b. Proconus absent, dorsal part of genital cone enlarged and sclerotized (Figs. 26, 27); spicules as in Figures 4, 27, 28 *O. lyrata*
- 4a. Spicules divided in distal fourth, dorsal and

- ventral branches more than half as long as main branch (Figs. 5, 25); dorsal genital cone unsclerotized (Figs. 23, 24) *T. circumcincta*
- 4b. Spicules divided at beginning of distal third, dorsal and ventral medial branches about half as long as main branch (Figs. 6, 31); dorsal genital cone enlarged and sclerotized (Figs. 29, 30) *T. trifurcata*

Key to Female Ostertagiinae with a Tapering Lateral Synlophe from Domestic Ruminants of North America

- 1a. Esophageal-intestinal valve length more than twice its width (Fig. 14); esophageal length 643-849 (743) μm long and is 8.6-10.3 (9.4) percent of body length (Table 3) *O. bisonis*
- 1b. Esophageal-intestinal valve length less than twice its width (Figs. 15, 16) 2
- 2a. Lateral synlophe with 1 pair of ridges ending next to lateral ridge between cervical papilla and posterior end of esophagus; in region of esophagus shorter ventral ridges usually begin lateral to 3 parallel ventral ridges; esophagus length 651-825 (724) μm long and is 7.1-8.9 (8.0) percent of body length (Table 3); egg length 70-86 (77) μm; egg width 39-49 (41) μm *O. ostertagi*
- 2b. Lateral synlophe with 2 or 3 pairs of ridges ending next to lateral ridge between cervical papilla and posterior end of esophagus; in region of esophagus shorter ventral ridges begin next to single ventral ridge; esophagus length 548-688 (615) μm long and is 5.1-6.8 (5.8) percent of body length; egg length 84-101 (91) μm; egg width 40-56 (49) μm *T. circumcincta*

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Research Note

New Locality for *Leptorhynchoides aphredoderi*
(Acanthocephala: Rhadinorhynchidae)

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ABSTRACT: *Leptorhynchoides aphredoderi* Buckner and Buckner, 1976, was obtained from 1 of 12 *Lepomis gulosus* collected during 1988 in Greene County, Alabama, and 5 of 7 *Aphredoderus sayanus* collected during 1990 in Sumter County, Alabama. *Leptorhynchoides aphredoderi* is previously reported only from *Aphredoderus sayanus*, *Lepomis auritus*, and *Lepomis punctatus* collected in several localities of southeastern Louisiana. No pronounced geographic variation was observed when specimens from Alabama were compared with specimens from Louisiana.

KEY WORDS: Acanthocephala, *Leptorhynchoides aphredoderi*, *Aphredoderus sayanus*, pirate perch, new host record, new locality record, survey.

Two species of *Leptorhynchoides* are known from North America: *Leptorhynchoides thecatus* (Linton, 1891) Kostylev, 1924, occurring in a variety of fish hosts, primarily centrarchids, from numerous localities throughout the eastern half of North America, and *Leptorhynchoides aphredoderus sayanus*, of a few localities in southeastern Louisiana (Buckner and Buckner, 1976). Collection of *L. aphredoderi* from fish obtained

in west-central Alabama extends the known distribution of this parasite.

Leptorhynchoides aphredoderi was obtained from fish collected at 2 localities in west-central Alabama. At the first locality (Parker Creek, southwest of Livingston, Sumter County, Alabama, R3W T18N, Sec. 12), a male *L. aphredoderi* was obtained from a warmouth, *Lepomis gulosus*, collected during May 1988. The worm was found attached in the midsection of the intestine. This is the first report of *L. aphredoderi* from this host species. The specimen was short (trunk length = 2.1 mm) and showed no evidence of sperm production or cement gland activity. Buckner and Buckner (1976) collected specimens of *L. aphredoderi* from the midintestinal region of 2 species of centrarchids: redbreast sunfish, *Lepomis auritus* and spotted sunfish, *Lepomis punctatus*. They reported the specimens as being unattached, immature, and shrouded in mucus. The uncommon occurrence of *Leptorhynchoides aphredoderi* in species of *Lepomis* and its loca-

Table 1. Measurements, in micrometers, of selected features of *Leptorhynchoides aphredoderi* as originally described from Louisiana and for specimens from *Aphredoderus sayanus* of Alabama.

	Males		Females	
	LA (N = 21)	AL (N = 5)	LA (N = 22)	AL (N = 3)
Trunk length	2,600–4,400	3,500–5,100	2,600–6,400	3,600–4,700
Proboscis length	510–690	513–667	600–690	609–629
Receptacle length	650–900	890–1,200	750–1,150	1,025–1,073
Number of hook rows	15–18	15–18	16–18	16–17
Number of hooks per row	11–16	12–15	11–15	13–15
Dorsal hook length				
Anterior	30–48	40–47	40–48	45–52
Middle	33–45	36–45	40–48	45–47
Basal	28–45	31–40	30–43	40–43
Ventral hook length				
Anterior	30–45	40–47	45–48	47–50
Middle	38–45	40–45	40–50	45–52
Basal	30–40	36–40	33–45	38–43
Egg length	—	—	145–150	135

tion in the intestine of these fish rather than in the usual site, the pyloric ceca, suggest that *Lepomis* spp. may not be its preferred host.

Other fish from this locality, number examined in parentheses, were not infected with *L. aphredoderi*: *Centrarchus macropterus* (5), *Ictalurus melas* (9), *Ictalurus natalis* (4), *Lepomis cyanellus* (4), *Lepomis gulosus* (12), *Lepomis macrochirus* (17), *Lepomis megalotis* (2), *Notemigonus crysoleucas* (12), *Notropis chrysocephalus* (5), *Pomoxis annularis* (2), and *Semotilus atromaculatus* (2).

At the second locality (McConnico Creek), southwest of Forkland, Greene County, Alabama, R2E T19N, Sec. 7), 4 male and 4 female (2 with eggs) *Leptorhynchoides aphredoderi*, ranging from 1 to 4 worms per infected fish, were obtained from 5 of 7 *Aphredoderus sayanus* collected during September 1990. Other fish from

this locality, number examined in parentheses, were not infected: *Esox americanus* (1), *Lepomis cyanellus* (1), and *Lepomis gulosus* (3).

No pronounced geographic variation was noted among the acanthocephalans when measurements of specimens from *Aphredoderus sayanus* of Alabama were compared with the original description from *A. sayanus* of Louisiana (Table 1).

Specimens have been deposited in the Manter Laboratory of the University of Nebraska State Museum, HWML No. 31745.

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Research Note

Gastrointestinal Helminths of the Northwestern Alligator Lizard, *Gerrhonotus coeruleus principis* (Anguillidae)

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ABSTRACT: One hundred four specimens of *Gerrhonotus coeruleus principis* from Whatcom County, Washington, were examined for helminths. Three lizards (3% prevalence) were infected. Findings consisted of 1 *Oswaldocruzia* sp. from 1 specimen and 1 *Cosmocercoides* sp. from each of 2 specimens. These represent the first nematodes reported from *G. coeruleus principis*. Our findings are in agreement with previous studies which have indicated species-poor helminth communities for members of the genus *Gerrhonotus*.

KEY WORDS: Nematoda, *Oswaldocruzia* sp., *Cosmocercoides* sp., prevalence, helminth community.

The northern alligator lizard, *Gerrhonotus coeruleus*, ranges from British Columbia to the central coast and Sierra Nevada of California; it is also found in the Rocky Mountains of western Montana and northern Idaho (Stebbins, 1985). To our knowledge there have been no helminth

surveys of *G. coeruleus*, although Fitch (1935) reported nematodes to be abundant sometimes in the stomachs and intestines of *Gerrhonotus coeruleus coeruleus*. Voge (1953) found metacercariae, *Mesocercoides* sp., in *G. coeruleus*. The purpose of this note is to report the results of a helminth survey for *Gerrhonotus coeruleus principis* Baird and Girard, 1852, from western Washington.

We examined 104 museum specimens of *G. coeruleus principis* (mean snout-vent length, SVL = 79 mm ± 14 mm; range 33-100 SVL). The specimens had been collected by hand along Chuckanut Drive, 1.6 km south of Bellingham, Whatcom County, Washington (48°42'N, 122°30'W), elevation ca. 30 m, during 1966, 1969, and 1970. The specimens were originally utilized

Table 1. Prevalence of gastrointestinal helminths in *Gerrhonotus* sp. of North America.

<i>Gerrhonotus</i> sp. Parasite	Locality	Prevalence (%)	Reference
<i>Gerrhonotus coeruleus</i>			
Cestode			
<i>Mesocestoides</i> sp. (metacestodes)	Contra Costa Co., CA	Not given	Voge, 1953
<i>Gerrhonotus coeruleus principis</i>			
Nematode			
<i>Cosmocercoides</i> sp.	Whatcom Co., WA	2 (2/104)	This paper
<i>Oswaldocruzia</i> sp.	Whatcom Co., WA	1 (1/104)	This paper
<i>Gerrhonotus multicarinatus webbi</i>			
Nematode			
<i>Oswaldocruzia pipiens</i>	Los Angeles Co., CA	2 (2/96)	Goldberg and Bursley, 1990
<i>Physaloptera retusa</i>	Riverside Co., CA	13 (4/30)	Telford, 1970
<i>Physaloptera</i> sp. (3rd stage)	Los Angeles Co., CA	1 (1/96)	Goldberg and Bursley, 1990
Cestode			
<i>Baerietta gerrhonoti</i>	Los Angeles Co., CA	64 (16/25)	Telford, 1965
<i>Mesocestoides</i> sp. (metacestodes)	Riverside Co., CA	7 (2/30)	Telford, 1970
<i>Oochoristica</i> sp.	Los Angeles Co., CA	1 (1/96)	Goldberg and Bursley, 1990

in a reproductive study (Vitt, 1973). They had been preserved in 10% buffered formalin. The body cavity was opened by a longitudinal incision from vent to throat. The esophagus, stomach, small intestine, and large intestine were slit longitudinally and examined under a dissecting microscope. The liver and body cavity were examined for the presence of *Mesocestoides* sp. Each helminth was identified using a glycerol wet mount.

Only 3 of the 104 specimens (3%) were infected with helminths. One female *Oswaldocruzia* sp. was found (Western Washington State College #5082, female, SVL 78 mm, large intestine). Two female *Cosmocercoides* sp. were recovered (CPS [=College of Puget Sound] #6838, male, SVL 83 mm, large intestine; CPS #8650, male, SVL 75 mm, small intestine). Since no male nematodes were recovered, we did not attempt specific identification. Prevalence for *Oswaldocruzia* sp. was 1% (1/104); prevalence for *Cosmocercoides* sp. was 2% (2/104). These nematodes represent new host records and they are the first identified nematodes from *G. coeruleus*. The nematodes were deposited in the U.S. National Parasite Collection (Beltsville, Maryland 20705, U.S.A.) as USNMHC Nos. 81215 and 81216 for *Oswaldocruzia* sp. and *Cosmocercoides* sp., respectively.

Investigations of *Gerrhonotus* sp. helminths are

summarized in Table 1. These data indicate a rather limited helminth fauna and infection rate when compared to lizards from other families (see Baker, 1987). Anguid lizards have a non-selective diet including small mammals, reptiles, insects, arachnids, millipedes, and snails (Stebbins, 1985). Pence (1989) suggested that a non-selective diet was 1 of several host criteria that could be used to predict a low-density, species-poor helminth community in mammals. The data of Table 1 would appear to support that claim for this species of reptile.

The observation of Fitch (1935) that abundant nematodes are sometimes seen in the stomach and intestines does not alter the suggestion of a species-poor helminth community for these lizards, but only suggests that there may be variability in helminth densities in various populations of *Gerrhonotus*. Fitch (1935) also mentioned finding flukes in the body cavity which we believe might have been *Mesocestoides* sp. metacestodes. The presence of *Mesocestoides* sp. has been reported for *Gerrhonotus* by Voge (1953) and Telford (1970). Whether limited helminth faunas are characteristic of *Gerrhonotus* must await further investigation of other members of this genus.

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Research Note

Biology of Cave Crickets, *Hadenoeus subterraneus*, and Camel Crickets, *Ceuthophilus stygius* (Insecta: Orthoptera): Parasitism by Hairworms (Nematomorpha)

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ABSTRACT: Gordiid hairworms identified as *Chordodes morgani* were collected from a rivulet in Floyd Collins' Crystal Cave, Kentucky, and the hemocoel of camel crickets, *Ceuthophilus stygius*, and cave crickets, *Hadenoeus subterraneus*. These collections extend the range for *C. morgani* to include Kentucky and add 2 new host species for this parasite. Infection prevalences for adult camel crickets were 16.9% for females and 2.9% for males. Adult cave crickets showed low infection rates of 0.8% and 0.9% for males and females, respectively. Based on average hairworm biomass, growth was slow during the summer while hosts were sexually immature and then became very rapid as host crickets matured. Repression of ova development was seen in parasitized female camel crickets (34.0 ova/female vs. 2.2 ova/parasitized female).

KEY WORDS: *Chordodes morgani*, *Hadenoeus subterraneus*, *Ceuthophilus stygius*, parasite load, hairworm growth rate, crickets, Nematomorpha.

The occurrence of internal helminths (unidentified gordiid hairworms) in the camel cricket, *Ceuthophilus stygius*, and cave cricket *Hadenoe-*

cus subterraneus, was very briefly mentioned in Hubbell (1936) and in Hubbell and Norton (1978), respectively. Hubbell (1936) also indicated fly larvae of *Oedematocera flaveola* Coquillet as frequent parasites of camel crickets.

From March 1986 through July 1987, nearly monthly collections of cave and camel crickets were made in several caves in or near Mammoth Cave National Park, Kentucky (Walnut Hill, Great Onyx, White, and Floyd Collins' Crystal Caves as well as the Frozen Niagara and Austin Entrances to and Sophys and Marion Avenues of Mammoth Cave). In association with ongoing studies of the biology of these crickets (Studier et al., 1986, 1987a), collected individuals were dissected for several purposes including examination for macroscopic internal parasites.

Juvenile horsehair worms were found in some crickets of both species in the May through December samples. Additionally, 2 adult hair-

worms were collected on the floor of the entrance to Floyd Collins' Crystal Cave from a temporary rivulet created by heavy epigeal rain in late July 1987. These adult hairworms and 1 juvenile from the hemocoel of an adult female *C. stygius* were identified as *Chordodes morgani*. A second juvenile worm from an adult female *C. stygius* hemocoel was tentatively identified as *C. morgani*. These collections extend the range for *C. morgani* (Chandler, 1985) to include Kentucky and add 2 new host species for this parasite. The voucher specimens are deposited in the U.S. National Parasite Collection, accession numbers USNM Helm. Coll. Nos. 81287–81289.

Although details of reproductive and population biology will be presented elsewhere, cave crickets (*Hadenoeus subterraneus*) reproduce throughout the year and adult life span exceeds 1 year. Individuals of all age classes are, therefore, present in all seasons. Only 2 hairworm juveniles were found in adult cave crickets: 1 in a male (of 106 examined = 0.9%) and 1 in a female (of 130 = 0.8%) both collected on 9 December 1986 from Floyd Collins' Crystal Cave. Of 153 juvenile cave crickets examined (49 in May, 50 in August, and 54 in November), none was parasitized by hairworms.

Camel crickets (*Ceuthophilus stygius*) complete their life cycle in 1 year and reproduce only in the fall, so adults were found only in the July through October collections. Of 70 males examined, 2 (2.9%) harbored hairworms while 11 of 65 (16.9%) females were parasitized; thus, females are hosts more frequently than males. Parasitized individuals came from several caves, representing where collection efforts were made in any given month. The 3 May and 4 October crickets came from Great Onyx Cave; the 24 October crickets, 1 each from Frozen Niagara and Austin Entrances, and 4 from Great Onyx Cave.

O'Brien and Etges (1981) reported that camel crickets collected about 100 mi northeast of Mammoth Cave National Park function as common intermediate hosts for the roundworm, *Pterygodermatites coloradensis*, but they make no mention of the occurrence of hairworms in the animals they examined. In addition to the horse-hair worms, we collected 1 unidentified roundworm from a *Hadenoeus* and 1 unidentified fly larva from a *Ceuthophilus*. No voucher specimens of either parasite are available.

Including all age hosts, camel crickets (9.6%)

are much more heavily parasitized than cave crickets (0.5%). The higher prevalence of hairworms in camel crickets may relate to their need to drink water to maintain water balance whereas cave crickets do not (Studier et al., 1987b). Of parasitized male camel crickets, 1 contained 1 hairworm while the other harbored 2. Of 11 parasitized females, 7 contained 1, 3 contained 2, and 1 contained 3 juvenile hairworms. Hairworm parasite load in individual camel crickets (1.46) is somewhat higher than in cave crickets (1.00).

Some indication of growth rate of the hairworms can be determined by following changes in average worm biomass with time. Among camel crickets, average hairworm biomass was 46.3 mg in 2 parasitized individuals on 3 May 1986, 67.4 mg in 5 individuals on 4 October 1986, and 168.8 mg in 6 individuals on 24 October 1986. Based on these limited data, hairworms grow very slowly during the summer, while the host camel crickets are subadults and young adults and grow very rapidly when host crickets rapidly develop gonads and become sexually active.

Energy which would be devoted to ova growth appears to be diverted to parasite nutrition in adult camel crickets. Seven nonparasitized female camel crickets collected in October 1986 contained an average of 34.0 ova, whereas 9 parasitized females collected at the same time contained an average of 2.2 ova. In fact, 7 parasitized females contained no ova at all. A similar phenomenon has been reported for Mormon crickets parasitized by hairworms (Thorne, 1940).

We thank the many students and colleagues who participated in the fieldwork and the Cave Research Foundation for the use of their field facilities. This work was done under MACA-N-103 with the cooperation of National Park Service personnel at Mammoth Cave National Park. Funding was provided by a University of Michigan–Flint Faculty Development Grant to E.H.S.

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Research Note

Development of a *Sarcocystis*-like Apicomplexan Protozoan in the Brain of a Raccoon (*Procyon lotor*)

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ABSTRACT: Schizonts of a *Sarcocystis*-like protozoan were found in the brain of a raccoon (*Procyon lotor*). The parasites, located directly in the cytoplasm of macrophages, neurons, and multinucleated giant cells, were not surrounded by a parasitophorous vacuole. The parasite divided by endopolygony, leaving a residual body. Schizonts were 5-35 × 5-20 μm and contained up to 35 merozoites. The merozoites had no rhoptries. The parasite was antigenically and structurally similar to *Sarcocystis neurona*, the organism of equine protozoal myeloencephalitis.

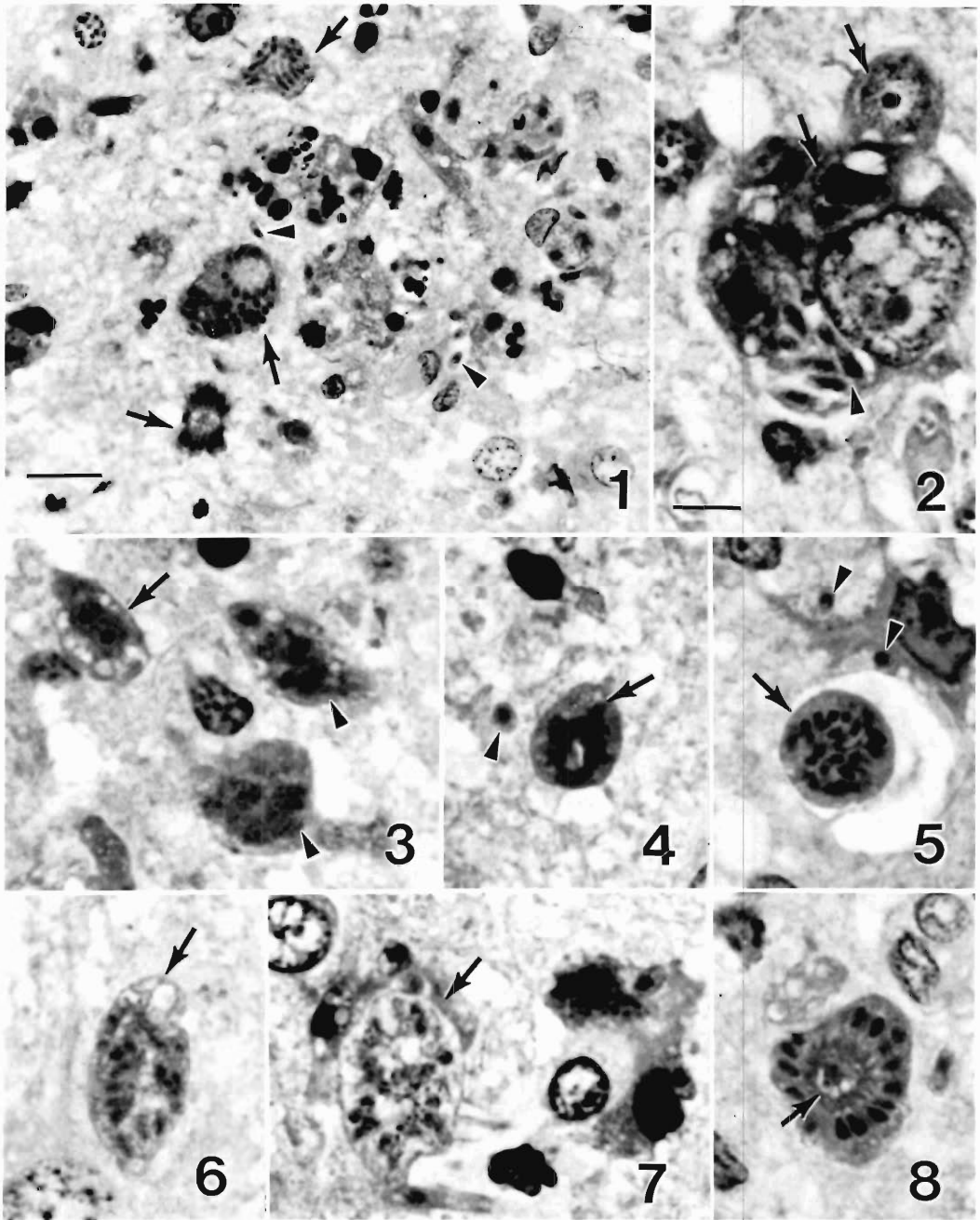
KEY WORDS: Protozoa, Apicomplexa, coccidia, *Sarcocystis*, encephalitis, schizonts, merozoites.

Toxoplasma gondii and *Neospora caninum* are the only known apicomplexan coccidians to cause fatal encephalomyelitis in carnivores (Dubey and Beattie, 1988). Recently Dubey et al. (1990) reported encephalitis in a raccoon associated with a *Sarcocystis*-like protozoan distinct from *T. gondii* and *N. caninum*. In this paper we report

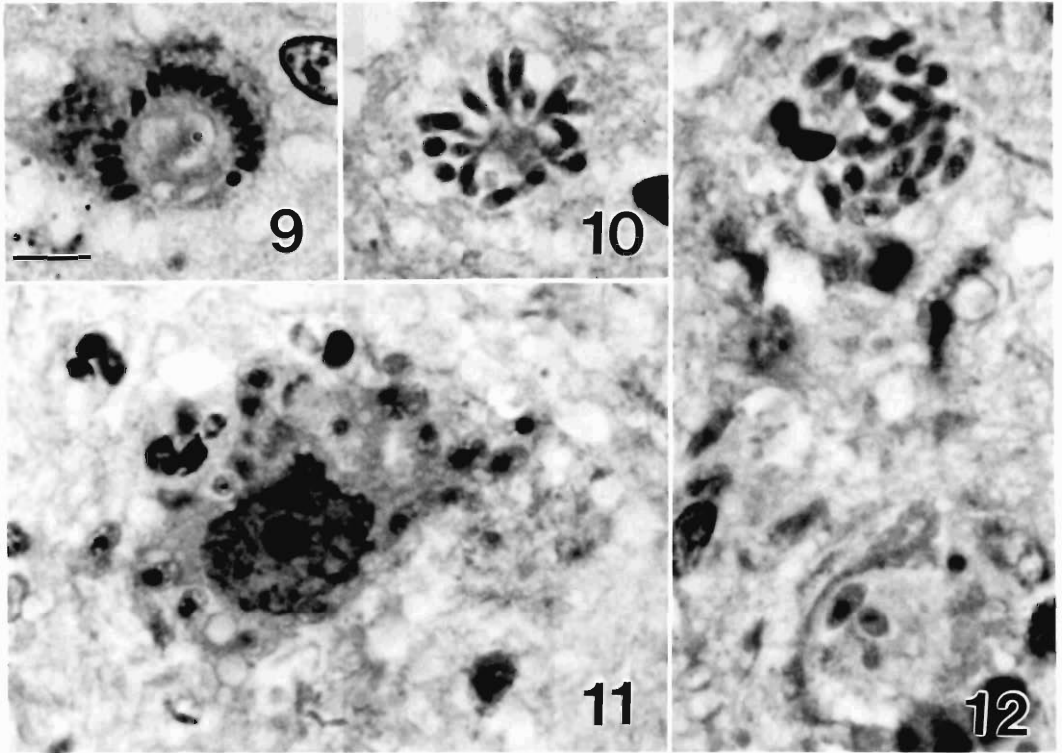
the development of the protozoan from the raccoon, *Procyon lotor* (L.), from Ohio.

Specimens of cerebrum were fixed in 10% buffered neutral formalin. Paraffin-embedded sections were cut at 3-6 μm, stained with hematoxylin and eosin (H&E), and examined microscopically. Selected specimens were embedded in glycol methacrylate and 2-3-μm sections were stained with H&E or periodic acid-Schiff hematoxylin (PASH). Formalin-fixed tissue was also processed for transmission electron microscopy. All measurements are given in micrometers.

Only asexual stages were seen (Figs. 1-12). Schizonts were located in neurons and macrophages. Individual merozoites were seen in neutrophils and mononuclear cells in lesions and in mononuclear cells in meningeal blood vessels. Most organisms seen were in macrophages. The



Figures 1-8. Stages of a *Sarcocystis* sp. in plastic-embedded sections of cerebrum of a naturally infected raccoon. 1. Individual merozoites (arrowheads) and developing schizonts (arrows) in a focus of necrosis. 2. Infected neuron with several schizonts. Arrows point to early schizonts with prominent nucleoli. Arrowhead points to mature schizont with merozoites. 3. Schizonts with undifferentiated nucleus or nuclei. Arrow points to a bilobed nucleus and arrowheads point to dividing nucleus. 4. Lobes of irregularly shaped nucleus. Arrowhead points to an extracellular merozoite. 5. Multinucleated schizont. 6. Schizont with a thin limiting membrane (arrow) and forming merozoites. 7. Schizont (arrow) with irregularly arranged developing merozoites. 8. Merozoites budding around a residual body (arrow). All figures H&E. Figure 1, scale bar = 13.3 μm , $\times 750$; Figures 2-8, scale bar = 6.6 μm , $\times 1,500$.



Figures 9–12. Schizonts and merozoites of a *Sarcocystis* sp. in plastic-embedded sections of cerebrum from a naturally infected raccoon. 9. Rosette of 19 merozoites. 10. Ruptured schizont with merozoites still attached to the residual body. 11. Numerous individual merozoites dispersed in the cytoplasm of a neuron. 12. Schizont with haphazardly arranged merozoites (above) and 2 merozoites in a cell (below). Merozoites in Figures 10–12 appear morphologically dissimilar, probably due to plane of sectioning and the stage of fixation. Figure 9, PASH; Figures 10–12, H&E. Scale bar = 6.6 μm . All figures $\times 1,500$.

structure of the parasites was difficult to discern in thick (5–6 μm) H&E stained sections and because of multiple stages being present in a single cell (Fig. 2). More details were visible in 2–3 μm plastic embedded sections (Figs. 2–12).

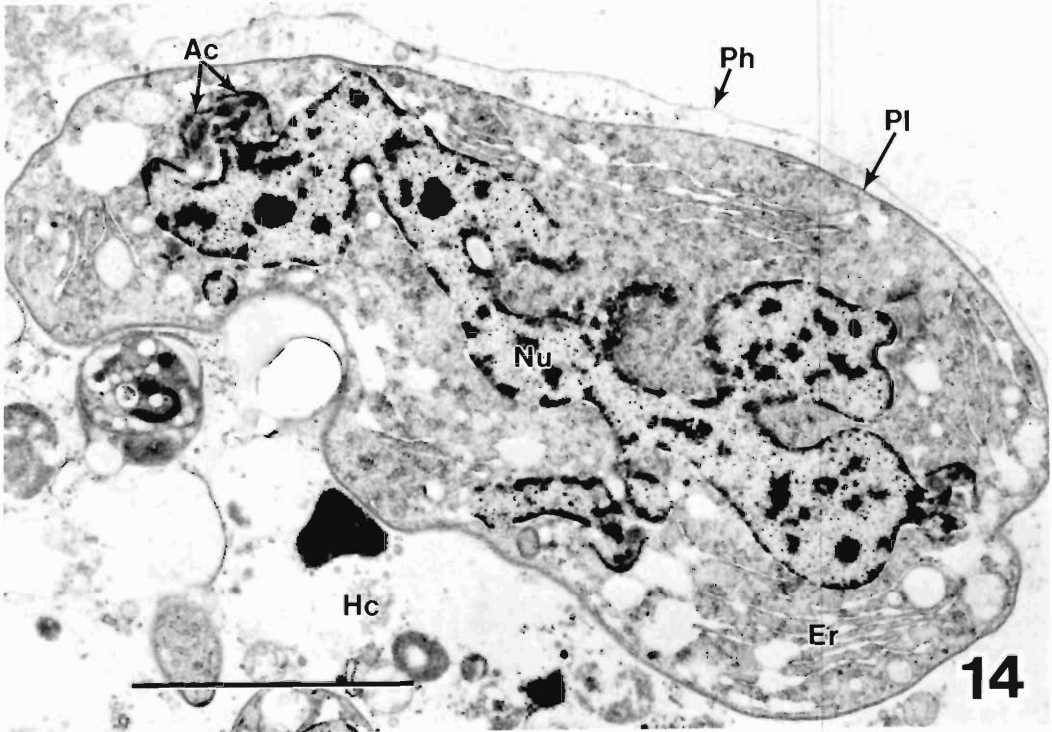
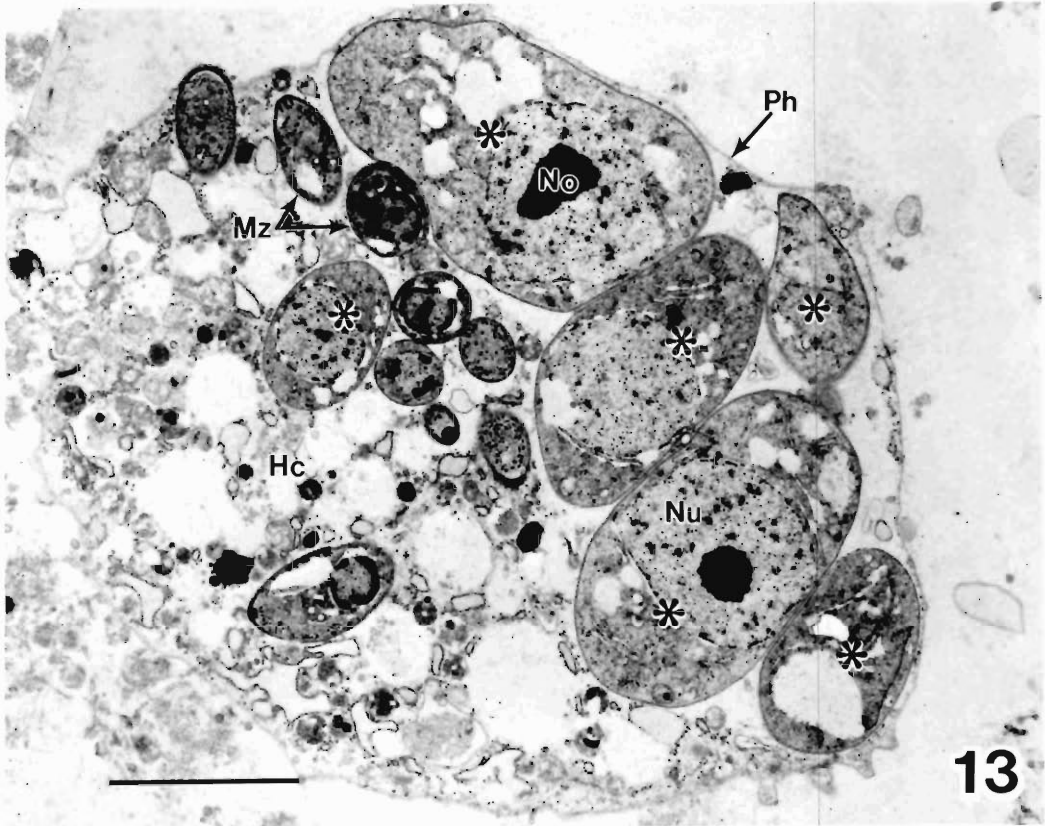
Schizonts divided by endopolygeny, a divisional process similar to that in *Sarcocystis* schizonts (Dubey et al., 1989). The early schizont (7 long \times 5 wide) contained a large nucleus with a prominent nucleolus. The nucleus became multilobed as the schizonts matured (Figs. 3, 4). Up to 35 nuclei or nuclear lobes were seen (Figs. 5,

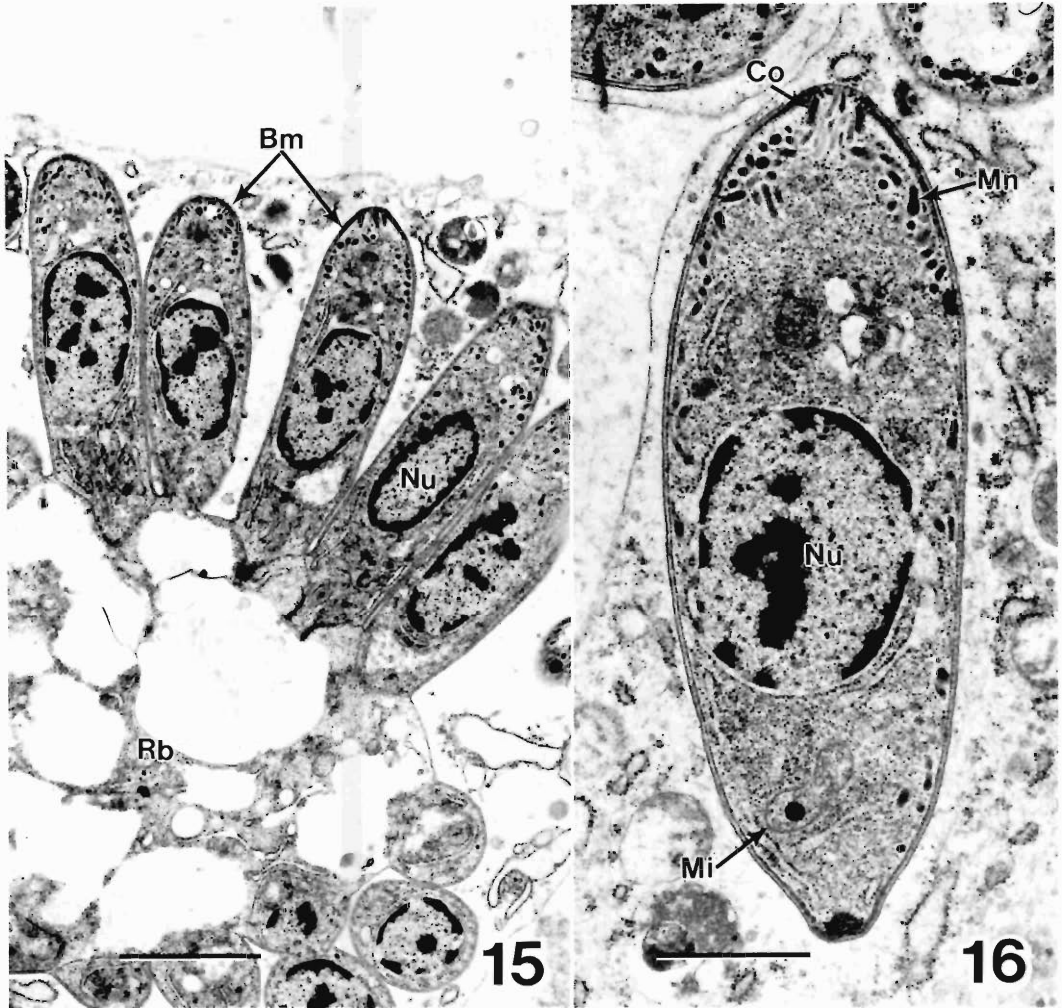
6) and merozoites were formed internally as well as peripherally (Figs. 6–8). Sometimes merozoites were arranged in a rosette around an eosinophilic residual body (Figs. 8–10). However, not all merozoites were arranged peripherally (Figs. 11, 12). Individual merozoites were pear-shaped with a central nucleus (Fig. 12).

Ultrastructurally, all parasite stages were located in the host cell cytoplasm without a parasitophorous vacuole (Fig. 13–16). Host cells appeared to be fibroblasts and macrophages and some harbored multiple parasites in various

→

Figures 13, 14. Transmission electron micrographs of *Sarcocystis* sp. in cells of a raccoon. 13. Degenerate host cell infected with merozoites (Mz) and early schizonts (*). Hc, host cell cytoplasm; Ph, plasmalemma of host cell. Bar = 5 μm . $\times 5,000$. 14. Intermediate schizont in early stage of merozoite formation. Ac, developing apical complex of merozoite; Er, parasite endoplasmic reticulum; Hc, host cell cytoplasm; Nu, nucleus of schizont; Ph, plasmalemma of host cell; Pl, plasmalemma of schizont. Bar = 5 μm . $\times 7,500$.





Figures 15, 16. Transmission electron micrographs of *Sarcocystis* sp. in cells of a raccoon. 15. Nearly mature schizont with merozoites (Bm) budding from a large centrally located residual body (Rb). Nu, merozoite nucleus. Bar = 2 μm . $\times 9,000$. 16. Merozoite showing conoid (Co), mitochondrion (Mi), micronemes (Mn) and nucleus (Nu). Bar = 1 μm . $\times 20,000$.

stages of multiplication (Fig. 13). The parasite multiplied exclusively by endopolygony in which numerous merozoites began development internally and later budded simultaneously at the surface of the schizont. Young ovoid schizonts (14 long \times 9 wide) contained a large nucleus with a single nucleolus plus other organelles characteristic of *Sarcocystis* spp. (Fig. 13). In more advanced schizonts, the nucleus became irregularly shaped with several nucleoli. Intermediate schizonts contained anlagen of developing merozoites and a highly lobulated nucleus (Fig. 14). A spindle apparatus consisting of several microtubules

appeared in association with each lobe of the nucleus. Merozoite anlagen formed immediately above each spindle apparatus (Fig. 14). Each merozoite anlage elongated by posterior extension of its inner membrane complex and subpellicular microtubules and eventually incorporated within it a part of the nucleus and cytoplasm. The pellicular membrane folded in around the developing merozoites until they appeared to bud at the surface of the schizont (Fig. 15). Schizonts contained approximately 50–60 merozoites. The merozoites measured from TEM photographs were 5.8 \times 1.8 (5.4–6.0 long \times 1.6–2.0 wide; *N*

= 12) and contained all of the organelles and inclusion bodies characteristic of a species of *Sarcocystis* (Dubey et al., 1989). Rhoptries were absent (Fig. 16).

The parasite in the raccoon was not *T. gondii* or *N. caninum* because it divided by endopolygony, was not located in a parasitophorous vacuole, and did not react with antisera against *T. gondii* and *N. caninum* in an immunohistochemical test (Dubey et al., 1990). Structurally, the raccoon parasite appears identical with the newly named organism, *Sarcocystis neurona* Dubey, Davis, Speer, Bowman, de Lahunta, Granstrom, Topper, Hamir, Cummings, and Suter, 1991, that causes fatal neurological disease in horses (Fayer and Dubey, 1987; Dubey et al., 1989, 1991).

The life cycle of *S. neurona* is not known. Only schizonts in the central nervous system of horses have been found. *Sarcocystis neurona* was recently grown in bovine monocytes in culture (Dubey et al., 1991). Further studies in the raccoon and with cultured organisms might help elucidate the life cycle of these parasites that cause encephalomyelitis in animals.

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Research Note

Helminth Parasites from Some Tichigan Lake Fishes in Southeast Wisconsin

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ABSTRACT: A total of 2,525 fishes of 30 species from Tichigan Lake and associated waters (Racine County) was examined for parasites between 1977 and 1979. *Corallobothrium fimbriatum*, *C. giganteum*, *Ophiotaenia fragilis*, *Proteocephalus* sp. (Cestoda), and *Polylekithum ictaluri* (Trematoda) are reported from *Ictalurus punctatus*, and *Triaenophorus nodulosus* pleroceocoids from *Catostomus commersoni*. All records are new to southeastern Wisconsin. Channel catfish appeared to be the major host of *C. giganteum*. *Corallobothrium* recruitment occurred during all seasons, but was maximal during autumn. Development, maturation, prevalence, and intensity of infection increased during spring and summer. Infection with *Corallobothrium* was not associated with host sex or size; no posterior migration was observed. The white sucker

appears to be the more common host of *T. nodulosus* in North America. The seasonality of *Acanthocephalus dirus* (Acanthocephala) in 9 common fish hosts was similar to that of *Corallobothrium*. Infections in 1977–1979 were, however, very light and fecundity unusually low. The spring population was compared with those from other years, e.g., 1984, and from a riverine habitat, the Pike River.

KEY WORDS: Wisconsin, catfish, *Corallobothrium*, *Ophiotaenia*, *Proteocephalus*, *Polylekithum*, *Triaenophorus*, *Acanthocephalus*, seasonality, host size and sex, site selection.

The seasonal ecology of cestode parasites of catfish, and of *Acanthocephalus dirus* from 9 fish

species in Tichigan Lake, Racine County, Wisconsin, are reported here for the first time. The seasonality of *A. dirus* has not been previously reported in a lake situation. Cestodes and trematodes were commonly reported from catfish in Wisconsin (Fischthal, 1947; Anthony, 1963) and elsewhere (Bangham and Hunter, 1939; Bangham, 1940, 1972; Bangham and Venard, 1942), but are recorded in southeastern Wisconsin for the first time.

Fishes were collected from Tichigan Lake (Racine County), a 458-ha lake in an advanced state of eutrophication on the Fox River (tributary of the Mississippi River). Seasonal biweekly collections were made during spring (April), summer (June, July, and early August), and autumn (late October and November) between 1977 and 1979. A total of 982 fishes representing 29 species and 10 families was captured by electroshocking. An additional 1,543 fishes representing 27 species and 11 families were collected in a channel draining the swampy western area of Tichigan Lake using seines or minnow traps (see Amin, 1990, for more details).

Fish were systematically dissected shortly after capture. Cestodes were fixed, stained, and mounted as in Amin (1990) and categorized as juveniles (small, proglottids immature or not yet formed, scolex not fully developed), adults (posterior proglottids sexually mature), and gravid (at least some proglottids with eggs). The trematode and acanthocephalan parasites were processed as in Amin, 1982 and 1987, respectively. Means refer to number of worms recovered/number of fish examined. Representative specimens were deposited in the U.S. National Museum Helminthological Collection (USNM Helm. Coll.) and in the University of Nebraska State Museum Harold W. Manter Laboratory Collection (HWML Coll.) as follows:

Corallobothrium giganteum Essex, 1927: USNM Helm. Coll. Nos. 81487–81491; HWML Coll. Nos. 31624–31627. *Corallobothrium fimbriatum* Essex, 1927: USNM Helm. Coll. No. 81486; HWML Coll. No. 31623. *Trianophorus nodulosus* Pallas, 1760: USNM Helm. Coll. No. 81485; HWML Coll. No. 31622. *Acanthocephalus dirus* (Van Cleave, 1931) Van Cleave and Townsend, 1936: USNM Helm. Coll. Nos. 79791–79798.

A total of 209 *Corallobothrium giganteum* and 44 *C. fimbriatum* was recovered from 20 and 7 channel catfish, *Ictalurus punctatus*, respectively, from Tichigan Lake proper. Fishes were more

frequently and heavily infected with *C. giganteum* (prevalence, 57%; mean intensity, 6.0) than with *C. fimbriatum* (20%; 1.3) (Table 1). This suggests that channel catfish is the more optimum definitive host of the first rather than the latter cestode species. Most previous reports indicate *C. giganteum* infections in *I. punctatus* and *C. fimbriatum* in other species of *Ictalurus* (Bangham and Hunter, 1939; Bangham, 1940, 1972; Bangham and Venard, 1942; Anthony, 1963). Recruitment of *C. giganteum* occurred during all seasons but was maximal (highest prevalence of juveniles) during autumn. Development, maturation, prevalence, and intensity of infection generally increased during spring and summer. The seasonality of *C. fimbriatum* was somewhat similar to that of *C. giganteum* except for its decreased maturation in *I. punctatus*; no gravid worms were recovered (Table 1). This observed seasonality agrees with the patterns reported for both cestode species in *I. punctatus* from the Rock, Mississippi, and Illinois rivers by Essex (1928), from Lake Carl Blackwell, Oklahoma, by Spall and Summerfelt (1969), and from the Kentucky and Ohio rivers by Edwards et al. (1977). Haderlie (1953) also observed similar seasonality of *C. fimbriatum* in *Ictalurus nebulosus* and *C. giganteum* in *Ictalurus catus* from Clear Lake, California. Gruninger et al. (1977), however, observed no seasonality in numbers of *C. fimbriatum* from *I. punctatus* in Eagle Mountain Lake, Texas. No relationship with host size or sex was observed. This is contrasted with the observations of Haderlie (1953) in California and Hoffnagle et al. (1990) in Tennessee. The differences in these findings may be related to the considerable diversity in the omnivorous channel catfish diet which was noted by Cannamella et al. (1978) to vary with forage availability, geographic location, and relative abundance and age of catfish. There was no evidence of seasonal posterior migration of *Corallobothrium*; the proportion of both species was highest in the small intestine directly behind the stomach during autumn (61%), spring (54%), and summer (72%). The remainder of the worms were located in decreasing frequencies in more posterior intestinal regions. The 7 *I. punctatus* infected with *C. fimbriatum* were also concurrently infected with *C. giganteum*; they were not spatially segregated. Haderlie (1953) reported anterior intestinal localization of *C. fimbriatum* in *I. nebulosus* but middle and posterior gut sites for *C. giganteum* in *I. catus*.

Table 1. Prevalence, mean intensity, and seasonal development of *Corallobothrium fimbriatum* and *C. giganteum* in *Ictalurus punctatus* from Tichigan Lake, 1977-1979.

	Autumn (late Oct., Nov.)	Spring (April)	Summer (June-early Aug.)	Total
<i>Corallobothrium fimbriatum</i>				
No. of cestodes (mean/fish) max.	0	16 (1.0) 14	28 (2.3) 22	44 (1.3) 22
Fish infected/examined (%)	0/6	2/17 (12)	5/12 (42)	7/35 (20)
% juvenile, mature, gravid cestodes	—	94, 6, 0	82, 18, 0	86, 14, 0
<i>Corallobothrium giganteum</i>				
No. of cestodes (mean/fish) max.	18 (3.0) 8	64 (3.8) 26	127 (10.6) 46	209 (6.0) 46
Fish infected/examined (%)	4/6 (67)	7/17 (41)	9/12 (75)	20/35 (57)
% juvenile, mature, gravid cestodes	89, 0, 11	58, 42, 0	22, 20, 58	39, 25, 36

One of 3 channel catfish examined from Tichigan Lake Canal was also infected with 14 gravid *C. giganteum* and 3 mature *C. fimbriatum* in May 1979. One of 5 yellow bullhead, *Ictalurus natalis*, examined also from Tichigan Lake Canal was infected with 1 mature *C. fimbriatum* in June 1978. An additional *C. fimbriatum* adult was recovered from 1 of 4 *I. natalis* in Silver Lake, a 188-ha land-locked eutrophic lake in adjacent Kenosha County. The absence of *C. giganteum* from Silver Lake is attributed to the absence of its major host, channel catfish, from that lake.

Three juvenile *Ophiotaenia fragilis* Essex, 1929, were recovered from a 50-cm long female *I. punctatus* in Tichigan Lake proper during spring 1977. The fish was also infected with 26 *C. giganteum* and 2 *C. fimbriatum*.

Six *Triaenophorus nodulosus* plerocercoids were coiled in 5 intestinal nodules recovered from 3 white suckers, *Catostomus commersoni*, in Tichigan Lake proper (3 worms from 2 suckers in July and October 1978), and Tichigan Lake Canal (3 worms from 1 sucker in May 1979). A total of 54 and 11 white suckers were examined from each location, respectively. Of the 85 species of fish intermediate hosts of *T. nodulosus* (see Valtonen et al., 1989), 24 species are reported from North America (Kuperman, 1973). In Europe, the preferred host appears to be perch (Andrews, 1979; Scholz, 1987; Ieshko et al., 1989). None of 77 yellow perch, *Perca flavescens*, examined from Tichigan Lake was infected. The white sucker appears to be the common host in North America and has often been infected when *P. flavescens* sampled from the same waters were free from infection (Fischthal, 1950, 1952).

One cestode of an undetermined species of *Proteocephalus* was found in the intestine of a

channel catfish from Tichigan Lake Canal in July 1979.

One gravid trematode, *Polylekithum ictaluri* (Pearse, 1924) Arnold, 1934 (= *Allocreadium ictaluri* Pearse, 1924; *A. halli* Mueller and Van Cleave, 1932), was found in the intestine of a 46-cm-long male channel catfish from Tichigan Lake proper during autumn 1979. The specimen fit the descriptions given by Pearse (1924), Mueller and Van Cleave (1932), and Arnold (1934). The vitellaria in my specimen and Pearse's (1924) material, also from Wisconsin, were discontinuous at the level of the acetabulum. Those in Mueller and Van Cleave's (1932) and Arnold's (1934) material from New York were, however, continuous.

Records of *Acanthocephalus dirus* in 16 fish species and 7 families from Tichigan Lake were listed in Amin (1985). The seasonal distribution of adult *A. dirus* from 9 common fish species collected in 1977-1978 is indicated in Table 2. The spring collection was compared with another from the same host species during the same season in 1984. The size of fishes of the same species from both spring collections was similar. The seasonal pattern of *A. dirus* during 1977-1978 was characterized by low infection intensities, slow development, low fecundity, and early elimination (none was recovered in the summer) compared to the 1984 spring pattern or the pattern in a river setting (Amin, 1975). There was no unusual weather condition noted for 1977-1978. Seasonal collections of other helminths from the same lake during the same period were not unusual. The 1977-1978 pattern was not evident in subsequent collections. For example, the 1984 spring distribution of *A. dirus* (the usual peak breeding season with maximum intensity)

Table 2. Seasonality of adult *Acanthocephalus dirus* from common fish hosts in Tichigan Lake during 1977 and 1978 compared with spring distribution in 1984.

Fish species	1977, 1978						1984			
	Autumn (Oct., Nov.)			Spring (April)			Summer (July, Aug.)	Spring (April)		
	Fish	<i>Acanthocephalus dirus</i>		Fish	<i>Acanthocephalus dirus</i>		Fish	Fish	<i>Acanthocephalus dirus</i>	
	Inf./exam. (%)	Total (mean/fish*)	% ♀ with eggs (with plugs)	Inf./exam. (%)	Total (mean/fish)	% ♀ with eggs (with plugs)	Inf./exam.	Inf./exam. (%)	Total (mean/fish*)	% ♀ with eggs (with plugs)
Catostomidae										
<i>Catostomus commersoni</i>	4/25 (16)	25 (1.00)	0 (0)	0/4 (0)	—	—	0/25	14/14 (100)	727 (51.93)	47 (13)
Centrarchidae										
<i>Lepomis cyanellus</i>	1/6 (17)	1 (0.17)	—	5/7 (71)	48 (6.86)	4 (48)	0/5	0/0 (0)	—	—
<i>Lepomis gibbosus</i>	0/13 (0)	—	—	9/15 (60)	67 (4.47)	3 (35)	0/32	1/1 (100)	4 (4.00)	0 (1)
<i>Lepomis macrochirus</i>	1/87 (1)	2 (0.02)	0 (0)	12/51 (23)	66 (1.29)	0 (5)	0/74	15/23 (65)	82 (3.56)	10 (29)
<i>Micropterus salmoides</i>	0/23 (0)	—	—	0/2 (0)	—	—	0/19	11/12 (92)	50 (4.17)	4 (22)
<i>Pomoxis nigromaculatus</i>	1/59 (2)	1 (0.02)	—	2/70 (3)	3 (0.04)	0 (0)	0/33	3/13 (23)	6 (0.46)	0 (0)
Cyprinidae										
<i>Cyprinus carpio</i>	0/30 (0)	—	—	0/14 (0)	6 (0.43)	0 (0)	0/22	4/5 (80)	78 (15.60)	10 (38)
Ictaluridae										
<i>Ictalurus natalis</i>	0/0 (0)	—	—	6/7 (86)	209 (29.86)	0 (29)	0/1	8/8 (100)	171 (21.37)	2 (55)
Percidae										
<i>Perca flavescens</i>	0/17 (0)	—	—	15/57 (26)	20 (0.35)	0 (0)	0/3	5/68 (7)	17 (0.25)	14 (43)
Total	7/260 (3)	29 (0.11)	0 (0)	49/227 (22)	419 (1.84)	1 (20)	0/214 (0)	61/144 (42)	1,135 (7.88)	34 (22)

* Mean = number of worms recovered/number of fishes examined.

shows considerably higher prevalence (42%), mean intensity (7.88), and fecundity (34% of females with eggs) compared to the spring of 1977–1978 in the same fish species (22%, 1.84, 1%) (Table 2). Whether *A. dirus* undergoes annual cycles in fecundity and abundance is unknown. Changes in *A. dirus* populations at the intermediate host level could be involved, but these could hardly explain differences in seasonal maturity and fecundity. Intestinal distribution of *A. dirus* in fish hosts during the spring of 1977–1978 was more anterior than what is normally found in fish infected with breeding worms during the spring (Amin, 1975). This suggests that posterior migration of acanthocephalans is more closely associated with worm maturation than with season.

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Research Note

First Report of *Ostertagia leptospicularis* (Nematoda: Trichostrongyloidea) in Calves (*Bos taurus*) from North America¹

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ABSTRACT: Specimens of *Ostertagia leptospicularis* were recovered from abomasa of 17 of 23 naturally infected calves in Oregon. Also present were: *Ostertagia kolchida*, *Ostertagia lyrata*, and *Ostertagia ostertagi*. The co-occurrence of specific pairs of species (*O. leptospicularis*: *O. kolchida*, and *O. ostertagi*: *O. lyrata*) supports the hypothesis of polymorphic species pairs within the Ostertagiinae. This is the first report of *O. leptospicularis* and the second of *O. kolchida* in cattle from North America.

KEY WORDS: Oregon, *Ostertagia leptospicularis*, Nematoda

Ostertagia leptospicularis Asadov, 1953, is a common abomasal parasite of members of the Cervidae and has been found in the abomasum of other sylvatic and domestic ruminants. The known geographic range of *O. leptospicularis* has until recently been confined to the Palearctic region and New Zealand where it is considered to be fairly common in cervids and less common in cattle. Common hosts include elk, *Cervus elaphus* (Jansen, 1960; Drozd, 1966; Kutzer and Hinaidy, 1969; Dunn, 1983), moose, *Alces alces* (Drozd, 1966; Nilsson, 1971), sika deer, *Cervus nippon* (Drozd, 1966), fallow deer, *Cervus dama* (Swierstra et al., 1959; Drozd, 1966), roe deer, *Capreolus capreolus* (Swierstra et al., 1959; Dunn, 1965; Drozd, 1966; Kutzer and Hinaidy, 1969; Nilsson, 1971; Andrews et al., 1974; Drozd et al., 1987), chamois, *Rupicapra rupicapra*

(Kutzer and Hinaidy, 1969), caribou, *Rangifer tarandus* (Freutel and Lankester, 1989), cattle, *Bos taurus* (Rose, 1963, 1968; Hinaidy et al., 1972), and sheep, *Ovis aries* (Swierstra et al., 1959; Nilsson, 1971).

The data presented in this report support the hypothesis of polymorphism suggested by Lancaster and Hong (1981). A report by Lichtenfels et al. (1988) also supports this hypothesis and provides a redescription of 7 species of the Ostertagiinae that are considered to be polymorphs of only 3 species, with each species pair being morphological variants of a single species. Between each polymorphic species pair, the major and minor species are usually found together, with 1 partner always dominant. An exception to this was reported by Rickard and Zimmerman (1986) when *O. kolchida* was discovered in the absence of its major species, *O. leptospicularis*.

The recovery of *O. leptospicularis* and *O. kolchida* during the present study represents the first report of *O. leptospicularis*, and the second of *O. kolchida*, from cattle in North America. The first report of *O. kolchida* from North America was by Rickard and Zimmerman (1986) in cattle from Oregon. Freutel and Lankester (1989) reported the recovery of *O. leptospicularis* from captive caribou at the Kakabeka Falls Game Farm, Canada, representing the first report from North America. Lichtenfels et al. (1988) listed *O. leptospicularis* from California cattle in a table of specimens studied, but we have learned (Lichtenfels, pers. comm.) that the item was a typo-

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Table 1. Intensity and prevalence of species of *Ostertagia* nematodes* recovered from Oregon calves (*Bos taurus*).

Species	Mean number per infected host		Prevalence (%)	
	Control	Treated	Control	Treated
<i>O. ostertagi</i>	5,620	4,187	100	100
<i>O. lyrata</i>	16	22	55	50
<i>O. leptospicularis</i>	98	88	73	75
<i>O. kolchida</i>	4	2	18	8

* Male specimens only.

graphical error, and no specimens of *O. leptospicularis* from North American cattle were included in that study. Since these reports, *O. leptospicularis* has again been collected from cattle in Oregon (Rickard, pers. comm.) and most recently from Montana (Decker and Mulrooney, unpubl. data).

During a routine anthelmintic efficacy trial, several specimens of *Ostertagia leptospicularis* were recovered. The trial was conducted early in the summer of 1988 at Oregon State University. Calves (*Bos taurus*) of mixed breed (less than 12 months of age), harboring naturally acquired gastrointestinal nematodes, were transported from the ranch of origin in Molalla, Oregon, to pastures located at the Berry Creek Beef Ranch of the Oregon State University Department of Animal Science, Corvallis, Oregon. A total of 23 animals was used for the study and was divided into 2 groups (11 in the nontreated, control group and 12 in the treated group). The treated animals were provided with free-choice medicated mineral mix (morantel tartrate), while nonmedicated mineral mix was available free-choice for the control animals. The quantity of mineral mix consumed by each individual animal is unknown. The 2 groups of animals were kept on separate pastures for a total of 63 days, then necropsied for recovery and identification of gastrointestinal nematodes present.

Based on the identification of male nematode specimens, 4 species of *Ostertagia* were identified: *O. ostertagi*, *O. lyrata*, *O. kolchida*, and *O. leptospicularis*. *Ostertagia leptospicularis* was recovered from 17 of the 23 cattle (8 nontreated, control animals and 9 treated animals). Intensity of *Ostertagia* species for each group of calves is presented in Table 1.

The mean values for the ratios of polymorphic species pairs were 99.7% *O. ostertagi*:0.3% *O. lyrata* and 96.1% *O. leptospicularis*:3.9% *O. kol-*

Table 2. Morphometrics of male specimens of *Ostertagia leptospicularis* recovered from Oregon calves (*Bos taurus*).

Character	Number of specimens measured	Ranges (mean) in μm	
		Control	Treated
Body length	82	4,750-6,833 (5,817)	
Esophagus length	82	617-864 (779)	
Esophagus width at base	78	27-59 (43)	
Esophageal-intestinal valve length	82	74-167 (118)	
Cervical papillae*	82	179-387 (298)	
Excretory pore*	82	154-370 (283)	
Width of body†	79	67-163 (96)	
Sub-ventral gland orifices*	77	189-380 (296)	
Spicule length	79	137-195 (159)	
Trifurcation of spicule tips‡	77	97-147 (118)	
Length of gubernaculum	70	13-48 (29)	
Width of gubernaculum	71	4-13 (7)	
Sjoberg's organ	82	absent	
Bursal ray pattern§	82	2-1-2	

* Distance measured from anterior end.

† Distance measured at pre-bursal papillae.

‡ Distance measured from anterior end of spicules.

§ Pattern following system of Durette-Desset (1983).

chida for the nontreated group of animals. From the group that received the medicated mineral mix, the mean values were 99.5% *O. ostertagi*:0.5% *O. lyrata* and 97.8% *O. leptospicularis*:2.2% *O. kolchida*. Of the polymorphic species pairs, *O. leptospicularis* and *O. ostertagi* are the dominant species, whereas *O. kolchida* and *O. lyrata* comprise the minor species, respectively. Morphological measurements of the specimens identified as *O. leptospicularis* were taken from approximately 80 male specimens (Table 2) and compared very well to those reported by Lichtenfels et al. (1988). By ANOVA, no statistical differences ($P > 0.05$) were observed in measurements between the treated and control groups of animals. Several specimens of *O. leptospicularis* have been deposited with the United States National Museum (USNM), Helminth Collection Nos. 81033 and 81034.

The mode of introduction and geographic distribution of *O. leptospicularis* in domestic ruminants in North America has yet to be determined. This species may have been: (1) present in North America but not previously recognized; (2) brought into the United States recently with animals imported from an area where *O. leptospicularis* is endemic; or (3) introduced by direct

interchange of parasites between sylvatic and domestic hosts, being cervids and cattle in this case. The range of origin in the present study is populated by black-tailed deer (*Odocoileus hemionis*) and occasionally utilized by elk (*Cervus elaphus*), which could account for direct interchange of parasites between these sylvatic cervids and cattle. The exchange of parasites between sylvatic and domestic hosts has been previously suggested by reports of the common deer parasites *Ostertagia kolchida* (Rickard and Zimmerman, 1986) and *Oesophagostomum venulosum* (Hoberg et al., 1988) in cattle from Oregon.

Ostertagia leptospicularis is considered to be highly pathogenic to cattle (Al Saqur et al., 1980, 1982a, b, 1984; Bisset et al., 1984; Sulger Buel et al., 1984) and could be considered as a potential threat to the livestock industry.

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Research Note

Helminths of the Wood Frog, *Rana sylvatica*, and Spring Peeper, *Pseudacris c. crucifer*, from Southern Michigan

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ABSTRACT: Eight helminth species (3 Trematoda, 5 Nematoda) were found in 100 wood frogs, *Rana sylvatica* LeConte, and 5 helminth species (1 Trematoda, 4 Nematoda) were found in 88 northern spring peepers, *Pseudacris c. crucifer* (Wied-Neuwied), collected from southern lower Michigan in spring 1989 and 1990. Of the species identified, *Oswaldocruzia pipiens* and *Haematoloechus parvplexus* had the highest prevalence and mean intensity in wood frogs, respectively. *Glypthelmins pennsylvaniensis* had the highest prevalence and mean intensity in spring peepers. Michigan is a new locality record for *G. pennsylvaniensis*, *Cosmocercoides dukae*, *O. pipiens*, and *Rhabdias ranae*.

KEY WORDS: *Rana sylvatica*, wood frog, *Pseudacris c. crucifer*, spring peeper, helminths, survey, Trematoda, Nematoda, Michigan.

Parasites of wood frogs, *Rana sylvatica* (family Ranidae), and (or) spring peepers, *Pseudacris* (syn. *Hyla*) *crucifer* (family Hylidae), have been surveyed by Brandt (1936), Rankin (1945), Odlaug (1954), Ashton and Rabalais (1978), Baker (1979), Williams and Taft (1980), and Coggins and Sajdak (1982). However, little information on helminths of these frog species from Michigan is available. Najarian (1955) found 4 helminth species each in wood frogs and spring peepers collected near Ann Arbor, Michigan. This note presents new information on the helminths of wood frogs and spring peepers from the Great Lakes area.

One hundred ($\bar{x} \pm$ SD snout–vent length = 40 \pm 3.8, range 27–47 mm) wood frogs, *R. sylvatica*, 88 (24 \pm 1.6, 20–28 mm) northern spring peepers, *P. c. crucifer*, and 1 (29 mm) western chorus frog, *Pseudacris triseriata* (Wied-Neufeld), were collected in April–May 1990 by dip net from a marsh in the Rose Lake Wildlife Area, Shiawassee and Clinton counties, southcentral Michigan. Sixty-six spring peepers (23 \pm 1.6, 21–31 mm) were also collected from a marsh southwest of Otis Lake in the Barry Game Area, Barry County, southwestern lower Michigan in March–May 1989. Frogs were pithed and all visceral organs, musculature and skin, were examined within 24 hours of collection. Helminths were processed

using conventional techniques. Prevalence is the percentage of infected frogs in a sample; mean intensity is the mean number of worms per infected frog, and values are expressed as a mean \pm 1 SD. Values for Brillouin's index for use in diversity and evenness (Pielou, 1975) were calculated using common logarithms for all helminths irrespective of their site of infection. Representative specimens of helminths have been deposited in the U.S. National Parasite Collection (USNM), Beltsville, Maryland (accession nos. 81867–81872).

Eight helminth species infected wood frogs and 5 species infected spring peepers. This number of helminth species found in wood frogs is the largest reported to date. Of the species identified from wood frogs, *Oswaldocruzia pipiens* and *Haematoloechus parvplexus* had the highest prevalence and mean intensity, respectively (Table 1). Fifty-three wood frogs harbored 1 helminth species, 16 harbored 2 species, and 8 harbored 3 species; overall prevalence of infection was 77%. The mean number of helminth species, helminth abundance, Brillouin's diversity and evenness for helminth infracommunities in wood frogs were 1.1 \pm 0.8 (0–3), 3.5 \pm 4.7 (0–24), 0.0434 \pm 0.0846 (0.0266–0.0602), and 0.1195 \pm 0.2232 (0.0752–0.1638), respectively. *Glypthelmins pennsylvaniensis* had the highest prevalence and mean intensity in spring peepers. Of the 21 (24%) spring peepers infected, none harbored more than 1 species. In contrast, Brandt (1936) in North Carolina found 13 helminth species in 60 spring peepers. In our study, there were no significant differences in prevalence (chi-square analysis, $P > 0.05$) and intensity (Student's *t*-test, $P > 0.05$) of parasitism between females and males of each frog species. There were also no distinct increases in infection for each helminth species or in helminth infracommunity descriptors with frog length.

Twenty-three (38%) spring peepers from the Barry Game Area were infected with *G. penn-*

Table 1. Prevalence and mean intensity of helminths in 100 wood frogs and 88 spring peepers from the Rose Lake area.

	Wood frog		Spring peeper		Site of infection
	Prevalence	Mean intensity ± 1 SD (range)	Prevalence	Mean intensity ± 1 SD (range)	
Digenea					
<i>Glythelmins pennsylvaniensis</i> Cheng, 1961*	—	—	10	9.0 ± 6.1 (1–20)	small intestine
<i>Glythelmins quieta</i> (Stafford, 1900) Stafford, 1905*†	2	1.0	—	—	small intestine
<i>Haematoloechus parviplexus</i> (Irwin, 1929) Harwood, 1932*†	9	3.7 ± 5.2 (1–17)	—	—	lung
Unidentified metacercariae	7	10.3 ± 8.6 (1–23)	—	—	mesenteries
Nematoda					
<i>Cosmocercoides dukae</i> (Holl, 1928) Travassos, 1931*	12	2.3 ± 1.7 (1–10)	—	—	rectum
<i>Cosmocercoides</i> sp.	—	—	1	1.0	rectum
<i>Oswaldocruzia pipiens</i> Walton, 1929*	34	2.0 ± 1.4 (1–7)	5	2.5 ± 3.0 (1–7)	stomach, small intestine, rectum
<i>Rhabdias ranae</i> Walton, 1929*	23	2.6 ± 3.6 (1–15)	2	1.0	lung, body cavity
<i>Spiroxys</i> sp.	15	3.2 ± 2.9 (1–10)	6	1.8 ± 0.8 (1–3)	mesenteries, stomach wall

* Gravid.

† New host record.

sylvaniensis with a mean intensity (range) of 10 ± 26 (1–126); no other helminth was found. The single western chorus frog from the Rose Lake Area was infected with 16 *G. pennsylvaniensis* and 4 *Cosmocercoides dukae*.

Most helminths were identified except for 1 male *Cosmocercoides* sp. from a spring peeper. It was similar to *C. dukae*, having 15 rosette papillae in its subventral rows, but total body length, spicule and gubernaculum measurements were within the ranges for *C. variabilis*. Therefore, we cannot determine its specific identity. Measurements of specimens from wood frogs and the western chorus frog fall within the ranges of *C. dukae* given by Vanderburgh and Anderson (1987). *Cosmocercoides dukae* is known to mature in molluscs, but frogs serve as incidental hosts. Anderson (1960) showed that *C. dukae* infections in frogs were of short duration. *Spiroxys* sp. is reported for the first time from wood frog and spring peeper and the recovery of it from tissues may indicate these frog species serve as second intermediate hosts (Hedrick, 1935) or paratenic hosts. *Glythelmins pennsylvaniensis*

was described by Cheng (1961) from spring peepers in Pennsylvania. Since then it has been found in *Pseudacris* spp. from Georgia by Sullivan and Byrd (1970) and Wisconsin by Coggins and Sajdak (1982). The occurrence of *G. pennsylvaniensis* in Michigan is a new locality record and provides additional evidence for host specificity in *Pseudacris*. In the present study, *G. quieta* was found in only 2 wood frogs; Rankin (1945) found *G. quieta* in a spring peeper. The wood frog is a new host record for *G. quieta* and *H. parviplexus* and the western chorus frog for *G. pennsylvaniensis*. Michigan is a new locality record for *C. dukae*, *O. pipiens*, and *Rhabdias ranae*.

Frog collections were made under a permit from the Michigan Department of Natural Resources (MDNR). We thank John Lerg and Mark Bishop, Barry Game Area, and Glenn Belyea, Rose Lake Wildlife Research Center, MDNR, for their cooperation; David Schinderle, Maxine Lipon, and Stephanie Simstad for their assistance in the field and laboratory. Funding for this study was provided by the College of Natural Science, Michigan State University.

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Research Note

***Parapharyngodon kartana* in Two Skinks, *Emoia nigra* and *Emoia samoense* (Sauria: Scincidae), from Samoa**

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ABSTRACT: Examination of 9 *Emoia nigra* revealed the presence of a nematode, *Parapharyngodon kartana* (prevalence 44%, mean intensity 3), in the large intestine and third-stage spirurid larvae in the small intestine. A single specimen of *Emoia samoense* also harbored *P. kartana* in the large intestine. These are new host records.

KEY WORDS: Nematoda, *Parapharyngodon kartana*, spirurid larvae, *Emoia nigra*, *Emoia samoense*, Scincidae.

The black skink, *Emoia nigra* (Hombron and Guichenot, 1853) Sternfeld, 1920, occurs in the South Pacific on the Caroline Islands, Bismarck Archipelago, Solomon Islands, New Hebrides, Fiji, Samoa, and Tonga (McCoy, 1980). The Sa-

moan skink, *Emoia samoense* (Duméril, 1851) Schmidt, 1923, is known from Fiji, Loyalty Islands, Samoa, and Tonga (Burt and Burt, 1932). The purpose of this note is to report the presence of the nematode, *Parapharyngodon kartana* (Johnston and Mawson, 1941) Mawson, 1971, and spirurid larvae in *E. nigra* and *P. kartana* in *E. samoense*. These findings represent new host records.

Nine *E. nigra*, mean snout–vent length (SVL) 89 mm ± 7 mm SD, were examined. Eight were from Tutuila Island, American Samoa (14°17'S, 170°41'W); 1 was from Upolu Island, Western Samoa (13°50'S, 171°45'W). A single *E. sa-*

moense (SVL 100 mm), also from Tutuila Island, was examined. Specimens were collected January 1990 with the exception of 1 *E. nigra* collected May 1989. Specimens were deposited in the herpetology collection of the Los Angeles County Museum of Natural History (LACM): *E. nigra* (138546–138554) and *E. samoense* (138545). The body cavity was opened ventrally and the esophagus, stomach, small intestine, and large intestine were slit longitudinally and examined under a dissecting microscope. The liver and body cavity were also examined for helminths. Each helminth was identified utilizing a glycerol wet mount.

Five of 9 (56%) *E. nigra* were infected with helminths; 4 contained the nematode *P. kartana* (1 male, 12 females) (44% prevalence; mean intensity 3) in the large intestine and 1 had spirurid larvae in the small intestine (11% prevalence; mean intensity 2). Seven *P. kartana* were recovered from the large intestine of the single specimen of *E. samoense*. Representative nematodes from *E. nigra* were deposited in the U.S. National Parasite Collection (Beltsville, Maryland 20705): *Parapharyngodon kartana* (81410) and spirurid larva (81411). We believe this to be the first report of nematodes recovered from the genus *Emoia*.

Parapharyngodon kartana was originally described from the scincid lizard, *Hemiergis peronii*, as *Thelandros kartana* by Johnston and Mawson (1941), but was moved to the genus *Parapharyngodon* by Mawson (1971). It has previously been reported only from South Australian lizards: Agamidae, *Amphibolurus fionni* by Mawson (1971); Gekkonidae, *Phyllodactylus marmoratus* by Angel and Mawson (1968); and Scincidae, *Lerista* sp. and *Rhodona* sp. by Mawson (1971). The measurements of the specimens from *E. nigra* are within the range of those from *H. peronii* as amended by Angel and Mawson (1968). The oral opening was surrounded by 6 lips each with a small papilla. Alae were present in the male for about two-thirds the body length, but were absent in the female. The male was 1.8 mm long with the caudal appendage inserted terminally. There was 1 pair of adanal papillae, 1 median and 2 lateral postanal papillae, and 1 pair

of papillae about midway along the caudal appendage. The spicule was 50 μ m long and very slightly chitinized. Females were 3.8–6.0 mm in length. There were distinct transverse annulations on the body approximately 11 μ m apart. Coils of the ovary wound around the corpus of the esophagus. The vulva was located in the third quarter of the body or about 1.5 mm from the posterior end in a 5-mm specimen. The caudal extremity tapered to a conical point. Eggs were asymmetrical with a pitted shell and subpolar operculum, 35 \times 85 μ m.

One species of amphibian and 15 species of reptiles occur in American Samoa (Amerson et al., 1982). The amphibian is the introduced giant toad, *Bufo marinus*; the reptiles include 2 marine turtles, 7 skinks, 5 geckos, and 1 snake. The degree to which *P. kartana* parasitizes other Samoan herpetofauna must await the examination of additional species.

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Research Note

Helminths of the Red-spotted Toad, *Bufo punctatus* (Anura: Bufonidae), from Southern Arizona

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ABSTRACT: The gastrointestinal tracts and lungs of 21 *Bufo punctatus* were examined for helminths. The cestode *Distoichometra bufonis* Dickey, 1921, and the nematodes *Aplectana itzocanensis* Bravo H., 1943, and *Oswaldocruzia pipiens* Walton, 1929, were present. *Aplectana itzocanensis* had the greatest prevalence (29%) and highest mean intensity (12). Our findings represent new host records.

KEY WORDS: Cestoda, *Distoichometra bufonis*, Nematoda, *Aplectana itzocanensis*, *Oswaldocruzia pipiens*, Bufonidae, *Bufo punctatus*, prevalence, intensity, survey.

The red-spotted toad, *Bufo punctatus* Baird and Girard, 1852, a toad of desert streams as well as canyonlands, ranges from southeastern California, southern Nevada, Utah, Colorado, southwestern Kansas, western Oklahoma, and central Texas to Hidalgo, Mexico and the tip of Baja California; it is found from below sea level (Death Valley, California) to 1,980 m (Stebbins, 1985). To our knowledge, there are no reports on the helminth fauna of *B. punctatus*. The purpose of this note is to describe the prevalence and intensity of the helminth fauna of a population of *B. punctatus* from southern Arizona.

Twenty-one *B. punctatus* (mean snout–vent length, SVL = 50.1 mm ± 10.0 SD (range 36–68 mm SVL) were hand collected and fixed in 10% formalin. The abdominal wall was slit to allow rapid penetration of fixative into the internal organs. Specimens were deposited in the herpetology collection of the Los Angeles County Natural History Museum (LACM). Six were collected July 1989 (LACM 138664–138669) from Gates Pass, Tucson Mountains, Pima County, Arizona (32°13'N, 111°06'W, 966 m elevation), 1 was collected August 1990 (LACM 138670) from Tucson, Pima County, Arizona (32°20'N, 111°02'W, 688 m elevation), and 14 were collected September 1990 (LACM 138671–138684) from Lukeville, Pima County, Arizona (31°53'N, 112°48'W, 424 m elevation). The body cavity was opened by a longitudinal incision from throat

to vent and the gastrointestinal tract was excised by cutting across the anterior esophagus and the rectum. The lungs, but not the urinary bladder, were also removed for examination. The esophagus, stomach, small intestine, large intestine, and lungs were examined separately. Each helminth was removed and identified utilizing a glycerol wet mount. Representative cestodes were stained with hematoxylin and mounted in balsam. Selected intact specimens were placed in vials of alcohol and deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705: *Distoichometra bufonis* (80802), *Aplectana itzocanensis* (80803), and *Oswaldocruzia pipiens* (80804).

No helminths were recovered from the esophagus or lungs. Prevalence, location, and mean intensity of recovered helminths are given in Table 1. There were 3 (23%, 3/13) infected female toads and 6 (75%, 6/8) infected male toads in the survey sample. Five of 6 toads infected with *A. itzocanensis* were males, all toads infected with *D. bufonis* were females, and a single female toad harbored *O. pipiens*. Only 1 toad had a mixed helminth infection.

The prevalence and mean intensity for *D. bufonis* reported here (14, 2, respectively) are lower than previously reported. Hardin and Janovy (1988) found prevalences of 70–100% and mean intensities of 2.7–14.8 in populations of *B. woodhousii* from Nebraska. Goldberg and Bursey (1991) reported prevalences of 19% (mean intensity 4) and 20% (mean intensity 3), respectively, from populations of *Bufo cognatus* and *Scaphiopus couchii*. It should be noted that the population of *B. punctatus* examined in this study is sympatric with the populations of *B. cognatus* and *S. couchii* examined by Goldberg and Bursey (1991). *Distoichometra bufonis* has also been reported from *Bufo terrestris*, *Bufo woodhousii fowleri*, and *Scaphiopus* sp. by Douglas (1958) and from *Bufo debilis debilis* and *Bufo woodhousii*

Table 1. Prevalence (%), mean intensity (range), and location of helminths from 21 *Bufo punctatus*.

Parasite	Prevalence	Mean intensity (range)	Location*
Cestoidea			
<i>Distoichometra bufonis</i>	14	2 (1-4)	b
Nematoda			
<i>Aplectana itzacanensis</i>	29	12 (4-33)	b, c
<i>Oswaldocruzia pipiens</i>	5	2 (2)	a

* a = stomach, b = small intestine, c = large intestine.

woodhousii by McAllister et al. (1989). It was originally described from *Bufo terrestris* (= *lentiginosus*) by Dickey (1921). Cyclophyllidean cestodes are acquired through infected invertebrate intermediate hosts (Schmidt, 1986).

Of the parasites recovered in this study, *Aplectana itzacanensis* had the greatest prevalence (29%) and highest mean intensity (12). It was first described by Bravo H. (1943) in *Scaphiopus multiplicatus* from Puebla, Mexico, and was subsequently redescribed from *Bufo woodhousii* by Baker (1985). *Aplectana itzacanensis* has been found in *Bufo marinus* from Costa Rica (Brenes and Bravo Hollis, 1959) and Veracruz, Mexico (Caballero Deloya, 1974), and in *Bufo alvarius* and *Bufo cognatus* from southern Arizona (Goldberg and Bursey, 1991). Although the life history of *A. itzacanensis* apparently has not been studied, Chabaud and Brygoo (1958) studied a species of *Aplectana* and reported that the life cycle has 2 phases: a preinfection, free-living phase (hatching stage to third-stage larvae) and a parasitic stage (infective third-stage larvae that become parasitic in the intestine of adult amphibians). Thus, infection in adult toads is acquired when larvae are swallowed by tadpoles and retained through metamorphosis or when larvae are accidentally swallowed by adult toads.

Oswaldocruzia pipiens has been found frequently in North American amphibians (see Baker, 1987). Among the toads, it has been reported in *B. woodhousii fowleri* by Brandt (1936), Rankin (1945), and Campbell (1968); in *Bufo americanus* by Ashton and Rabalais (1978); in *Bufo terrestris*, *Bufo valliceps*, and *Bufo houstonensis* by Thomas et al. (1984); in *B. alvarius* and *B. cognatus* by Goldberg and Bursey (1991); in *Schaphiopus holbrooki* by Brandt (1936); and in *S. couchii* by Goldberg and Bursey (1991). The life cycle of *O. pipiens* has been studied by Baker

(1978) who reported that the life cycle has 2 phases: a preinfection, free-living phase and a parasitic stage. Infection of adult toads is gained by penetration through the skin by 3rd stage larvae.

None of the parasites found in this study is unique to *Bufo punctatus*, but each represents a new host record. Based upon data from Baker (1987), McAllister et al. (1989), and Goldberg and Bursey (1991), we calculated an average of 4.1 (± 5.2 SD) species of nematodes (range 1-22) from 56 species of the family Bufonidae. Thus, the number of nematode species recovered in this study falls within the range that might be expected. Whether the differential gender infection rates reported here are an artifact of sampling or a reflection of microhabitat differences remains to be determined.

The population of *B. punctatus* examined in this study is sympatric with the populations of *B. alvarius*, *B. cognatus*, and *S. couchii* studied by Goldberg and Bursey (1991). Thus, it is not unexpected that these populations should share some of the same helminth species. However, we have no explanation for the apparent cestode substitution that occurs (*Nematotaenia dispar* in *B. alvarius* and *Distoichometra bufonis* in *B. punctatus*, *B. cognatus*, and *S. couchii*), nor can we explain the occurrence of *Aplectana itzacanensis* in *B. punctatus*, *B. alvarius*, and *B. cognatus*, and its replacement by *A. incerta* in *S. couchii*.

We thank C. H. Lowe and T. W. Yang for supplying us with our sample of *B. punctatus* and Rana Tawil for assistance in recovery of helminths.

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PRESENTATION OF THE 1990 ANNIVERSARY AWARD TO A. JAMES HALEY



Dr. A. James Haley (left) receiving the 1990 Anniversary Award from Dr. Ralph P. Eckerlin.

The recipient of the Anniversary Award for 1990 is A. James Haley. Dr. Haley wanted to receive the award at this meeting at Johns Hopkins University because it was here that he gave his first scientific paper presentation to the Helminthological Society of Washington while a graduate student at Hopkins.

Jim is a New Englander. He was born in New Hampshire and received his early education there. He received the B.S. degree in Biology from the University of New Hampshire in 1949 and stayed on there to get an M.S. in Zoology working under Dr. Wilbur Bullock. For 2 more years he was an Instructor of Zoology at the University of New Hampshire. Jim came to Baltimore in 1952 and was awarded the Sc.D. degree in Parasitology from Johns Hopkins University in 1955.

The criteria for selecting an Anniversary Award recipient are listed in the Society bylaws and generally state that the recipient must have done 1 or more of the following: made outstanding contributions to parasitology, outstanding service to

the Society, or made some other contribution warranting special recognition. The Awards Committee felt that Jim Haley qualified in all areas.

Jim wrote that there were several things of which he was particularly proud and the first of these was the teaching awards he received from students. In fact, teaching is Jim Haley's major contribution which warrants special recognition. Jim began teaching as Instructor of Zoology at the University of New Hampshire from 1950 to 1952. He was Instructor of Parasitology at Johns Hopkins University from 1955 to 1956, the year following the receipt of his doctorate. In the autumn of 1956, he moved to College Park, Maryland, where he rose in just 8 years from Assistant Professor to Professor of Zoology at the University of Maryland. Twenty years later, a total of 28 years at the University of Maryland, Jim retired as Professor Emeritus, a rank he still holds. However, even in retirement he is still teaching! In 1985 he was Visiting Professor at the Uni-

versity of Florida, and since 1986 has been adjunct Professor at the University of New England, School of Osteopathic Medicine.

During those years Haley had graduate students; 14 Master's students, 9 Ph.D. students, and a Postdoctoral Fellow. He has touched the lives of countless undergraduate students. His teaching excellence was recognized and rewarded by students at all levels. He won both the Student's Award for Excellence in Teaching and later the Mortarboard Honor Society Teaching Award while he was at the University of Maryland. In 1989 he was voted the Student's Outstanding Visiting Instructor Award by students at the University of New England.

There are some aspects of teaching excellence that cannot be counted or quantitated. One of Jim's former students told me Haley was the best and most organized lecturer he had ever had. Another told me he picked parasitology as his graduate major because Haley was the best teacher.

A second criterion for the Anniversary Award is outstanding contribution to parasitology. Perhaps the most important research contribution from Dr. Haley's laboratory was a series of papers on the adaptation of a parasite, *Nippostrongylus brasiliensis*, to its rat host over many generations. These represent now classical studies in intraspecific variation in a parasitic nematode. The *Nippostrongylus* model is still used today in numerous laboratories.

Over the years Jim published on microsporidians, acanthocephalans, nematodes, monogeneans, and tapeworms. I think it is safe to say he has a curious bent to him. In later years, most of Jim's publications were co-authored with students. In all he published 24 full papers, 6 research notes, 2 WHO "working papers," an undergraduate laboratory manual, 13 book reviews, and an important monograph on the immunodiagnosis of helminth infections.

But, Jim remembers most fondly 2 years of parasitology research he did in the villages of the

Punjab region of Pakistan during 1966–1968. Four publications on human protozoan and helminth infections resulted. Most importantly, in Jim's concern for people, perhaps some were benefitted as a result of his having been there and reporting his findings.

Finally, in the category of service to the Helminthological Society of Washington, Jim Haley qualifies for recognition. Jim attended his first meeting 39 years ago and he writes "and I have had a love affair with the Society ever since." He is most proud of his 8 years as Editor of the *Proceedings* of the Society. Only 2 Editors have served longer. During his tenure as Editor, the *Proceedings* improved and increased in stature as an important journal. Jim greatly enjoyed interacting with all the parasitologists who wrote and called him.

Prior to the editorship, Haley was on the Editorial Board of the *Proceedings* for 5 years and held just about every office. He was Recording Secretary in 1960, Vice President in 1969, and President the next year. He served on committees too numerous to list. In 1984 he was elected Life Member of the Society.

However, lest you think our honored guest is perfect, let me advise you that there is a flaw in his character. Perhaps he does not always tell the truth. When Ralph Lichtenfels was going to assume the Editorship following Haley, Ralph tried to pin Haley down on the approximate number of hours a week he devoted to the journal. Haley told Lichtenfels he did it all on Friday afternoons! Ralph finds this difficult to believe.

I think you will agree that A. James Haley qualifies for the Anniversary Award. On behalf of the Helminthological Society of Washington and the members of the Awards Committee (Suzanne H. Giannini and Edward H. Michelson), I am happy to present the 1990 Anniversary Award to Dr. A. James Haley.

RALPH P. ECKERLIN, Chair
Awards Committee

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MINUTES

Six Hundred Thirteenth Through Six Hundred Twentieth Meetings

613th Meeting: Uniformed Services University of Health Sciences, Bethesda, MD, 10 October 1990. John Cross presided over the business meeting and a slate of nominees for officers was announced and further recommendations were to be sent to the Recording Secretary. Bryce Reddington presided over the scientific program. Rodger Martin spoke on multiple drug resistance in parasitic protozoa. Peter J. Weina discussed a clinically based model for Katayama Fever. Phillip Lawyer presented work on the development of *Leishmania major* in *Phlebotomus duboscqi* and *Sergentomyia schwetzi*. Daniel Gordon discussed progress and plans on the DOD malaria vaccine program.

614th Meeting: Animal Parasitology Unit, ARS, USDA, Beltsville, MD, 20 November 1990. Hyun Lillehoj presided over the business meeting and the election of the new officers for 1990: Nancy Pacheco, President; Ruth Kulstad, Vice-President; David Chitwood, Secretary-Treasurer; Mark Jenkins, Recording Secretary. The deaths of the following members were announced with a moment of silence: Gerald D. Schmidt and Louis J. Olivier. Dr. Ralph Bram presided over the scientific portion of the meeting. Hyun S. Lillehoj spoke on immunological studies of host-parasite interactions in avian coccidiosis. Patricia Augustine discussed studies on cellular invasion and host cell-parasite interactions in avian coccidia. Mark Jenkins discussed approaches to identifying and cloning genes for protective coccidial antigens. Harry Danforth presented work on protective antigens of coccidia. Patricia Allen discussed pathophysiology of carotenoid malabsorption with emphasis on non-coccidial causative agents.

615th Meeting: Plant Protection Institute, USDA, ARS, Beltsville, MD, 5 December 1990. John Cross presided over the business meeting and the new officers were installed. Robin Huettel presided over the scientific meeting. Susan Meyer discussed recent work on mutant fungi and fungus/bioregulator combinations as biocontrol agents for plant parasitic nematodes. Sarwar

Hashmi presented studies on the influence of temperature and other factors on development of corn cyst nematodes. Ghazala Hashmi spoke on determination of optimum inoculation levels of *Meloidogyne incognita*. Sanaa Haroon discussed plant-parasitic nematode problems and control programs in Egypt.

616th Meeting: Laboratory of Parasitic Diseases, NIH, Bethesda, MD, 9 January 1991. Nancy Pacheco presided over the business meeting. The deaths of the following members were noted with a moment of silence: George W. Hunter III and Donal Meyer. Franklin Neva presided over the scientific meeting. Yuhui Xu presented studies on kinetic analysis of TH-1 and TH-2 cell responses in *Schistosoma japonicum* infected mice. Annie Walker-Jonah discussed identification of the chloroquine-resistance locus in *Plasmodium falciparum* cross. Amy Klion presented recent work on Loiasis in endemic vs. non-endemic populations.

617th Meeting: Naval Medical Research Institute, Bethesda, MD, 13 February 1991. Nancy Pacheco presided over the business meeting and Stephen Hoffman presided over the scientific meeting. Anita Malik discussed studies on human cytotoxic T lymphocytes against the *Plasmodium falciparum* circumsporozoite protein. Srisin Khusmith spoke on immunization with radiation-attenuated *Plasmodium yoelii* sporozoites induces cytotoxic T cells specific for the 140 kD sporozoite protein 2. Martha Sedegah presented recent work on immunization of Balb/C mice with *Plasmodium berghei* and *P. yoelii* irradiated sporozoites protects via different immune mechanisms. Mucide Ak discussed epitope mapping and antibody affinity determination of protective synthetic peptide- and irradiated sporozoite-induced monoclonal antibodies against the *Plasmodium yoelii* circumsporozoite protein.

618th Meeting: Walter Reed Army Institute of Research, Washington, DC, 13 March 1991. Nancy Pacheco presided over the business meet-

ing. Pat Carney presided over the Student Competition consisting of the following presentations: Victor Apanius showed his work on chronic blood parasitism, immunity, and reproduction in wild birds; Nancy J. Briscoe discussed her studies on population dynamics of *Orchopeas leucopus* and *Epitedia wenmanni* in Mason Co., West Virginia; and John M. Hawdon presented his findings on reduced glutathione (GHS) induces feeding by *Ancylostoma caninum* infective larvae. James Higgins discussed recent studies on development of *Brugia malayi* in resistant strains of *Aedes aegypti*. Robert J. Maze showed his data on the application of the factorial experimental design in an investigation of the impact the nematode *Parelaphostrongylus tenuis* has on the fecundity of the intermediate host *Triodontopsis albolabris*. The winners of the competition were as follows: 1st Place, John M. Hawdon; 2nd Place, James Higgins; 3rd Place, Victor Apanius.

619th Meeting: School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, MD, 10 April 1991, cosponsored by the Tropical Medicine Dinner Club. Nancy Pacheco presided over the business meeting. Ralph Eckertlin presented the Anniversary Award to A. James Haley. Milan Trpis presided over the scientific session. Jefferson A. Vaughan spoke on *Plasmodium falciparum* sporogonic development in vectors. Benjamin Wizel discussed recent work on the identification of a cross-reacting continuous epitope by *Plasmodium falciparum* transmission-blocking monoclonal antibodies. Michael Cranfield presented recent studies on protection of black-footed penguins against malaria infection at the Baltimore Zoo. M. Sofi Ibrahim showed data on protein composition and synthesis in early developing larvae of *Brugia malayi*.

620th Meeting: University of Pennsylvania, New Bolton Center, Kennett Square, PA, 11 May 1991;

joint meeting with the New Jersey Society for Parasitology and cosponsored by SmithKline Beecham Animal Health. Nancy Pacheco presided over the business meeting and Gerald A. Schad presided over the scientific meeting. Sandra Evans gave an introduction on Lyme disease. Cynthia Lord presented data on the population biology of Lyme disease. Durland Fish discussed current results from Lyme disease surveillance.

The Helminthological Society welcomed 51 new members to the Society during the meetings indicated: *613th:* I. Armendariz, Ciancio Aurelio, Antonio Gomez Barcina, Niels O. Christensen, James J. Daly, W. Decraemer, M. Hernandez, F. D. McElroy, J. F. Misonne, Nelson Simoes, M. E. Sweelam, Stephen H. Thomas, and Gary L. Windham; *614th:* Robert A. Clare, Richard D. Conklin, Vina R. Diderrich, Leon F. Dubinis-Gray, Sharon File, Martin E. Gordon, Andrea M. Gorman-Gelder, David E. Granstrom, Mary E. Gray, Randal Lampley, Harold E. Laubach, Linda Pote, C. D. Mackenzie, Luis G. L. Reis, George Riekirk, Richard G. Robbins, Thomas Simpson, Anthony O. W. Stretton, Herman Zaiman, and Anne Zajac; *615th:* Takashi Isobe and Gerardo Perez Ponce de Leon; *616th:* Barry G. Campbell, James R. Flowers, Timothy Foard, Javier Franco, H. D. Alan Lindquist, Richard O. McCracken, Charles Overstreet and Michael Sukhdeo; *617th:* Mucide C. Ak, Ann R. Donoghue, Sylvie Mellouk, and Griselda G. Zaraspe; *618th:* Robert J. Maze, Elizabeth Pierce, and Ignacio Cid del Prado Vera; *619th:* Gayle Pittman Noblet; *620th:* Ingo H. Gaida.

Respectfully submitted,

MARK C. JENKINS
Recording Secretary

The Helminthological Society of Washington

Application for Membership

Any person interested in parasitology or related fields is eligible for membership. Subscription to the Society's Proceedings is included in the dues. Members are privileged to publish therein at reduced rates. The annual dues are payable on notification of election. Send this completed form to:

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* Deceased.

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