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Eimeria dixoni sp. n. (Apicomplexa: Eimeriidae) from an Introduced Population of Common House Geckos, *Hemidactylus frenatus* (Sauria: Gekkonidae), in Dallas County, Texas

CHRIS T. MCALLISTER,[1,2] STEVE J. UPTON,[3] AND DONAL M. BOYER[4]

[1] Renal-Metabolic Lab (151-G), Veterans Administration Medical Center,
4500 S. Lancaster Road, Dallas, Texas 75216,
[2] Department of Biological Sciences, University of North Texas, Denton, Texas 76203,
[3] Division of Biology, Ackert Hall, Kansas State University, Manhattan, Kansas 66506, and
[4] Reptile Department, Dallas Zoo, 621 East Clarendon Drive, Dallas, Texas 75203

ABSTRACT: *Eimeria dixoni* sp. n. is described from the feces of 11/16 (68%) common house geckos, *Hemidactylus frenatus,* in Texas. Sporulated oocysts of the new species are spherical or subspherical, 20.8 × 19.7 (17–22 × 17–21) μm, with a smooth, bilayered wall; shape index 1.06 (1.0–1.1). A micropyle, oocyst residuum, and polar granule are absent. Sporocysts are ovoid, 9.3 × 7.8 (8–11 × 7–8) μm; Stieda and substieda bodies are absent. A spherical or ovoid sporocyst residuum is present, composed of a compact mass of similar-sized membrane-bound granules. Sporozoites are vermiform, 9.8 × 2.8 (8–11 × 2.6–3.2) μm in situ, each containing spherical anterior and spherical or subspherical posterior refractile bodies. In addition to the new species, *Eimeria lineri* McAllister, Upton, and Freed, 1988, was found in 14/16 (88%) sympatric Mediterranean geckos, *Hemidactylus turcicus turcicus.* However, neither eimerian was found to be shared among congeneric geckos, suggesting strict host specificity.

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

The common house gecko, *Hemidactylus frenatus,* is a medium-sized Old World gekkonid lizard that ranges from southern Africa and Madagascar eastward to tropical southern Asia, the Malay Archipelago, and the western Pacific Islands of the Indian Ocean (Taylor, 1921; Smith, 1935). It has been widely introduced into northern Australia and Hawaii (McCoy and Busack, 1970) and also into the western hemisphere in Mexico (Edgren, 1956; Marcellini, 1971). Like the related Mediterranean gecko, *Hemidactylus turcicus turcicus* (Linnaeus), this gekkonid lizard occasionally enters the United States along shipping routes.

Much is known about the trematode (Killick and Beverley-Burton, 1982; Kennedy et al., 1987a, b), cestode (Kennedy et al., 1982; Jensen et al., 1983), and especially the nematode (Gupta, 1959; Caballero, 1968; Jehan, 1970; Oshmarin and Demshin, 1972; Schmidt and Kuntz, 1972; Jaing and Lin, 1980) parasites of *H. frenatus.* However, compared to other gekkonids, little is available on the coccidian parasites of the species (see Matuschka and Bannert, 1986a, b). Yamamoto (1933) reported 2 unnamed eimerians from *H. frenatus* in Taiwan, and Else and Colley (1975) described *Eimeria cicaki* in a single house gecko from Malaysia. Nothing is known about the coccidia of introduced North American populations of *H. frenatus.*

Between April and June 1989, we had the opportunity to examine select specimens of *H. frenatus* for intestinal protozoans. Fecal samples from some of these geckos contained coccidian oocysts of the genus *Eimeria,* which proved to represent a previously undescribed species. The following is a description of this new eimerian and presents preliminary evidence that supports the notion that this new species, as well as *Eimeria lineri* McAllister, Upton, and Freed, 1988, from *H. turcicus,* may have narrow host specificity.

Materials and Methods

Sixteen adult (7 male and 9 female) *H. frenatus* ($\bar{x}$ ± SEM snout–vent length [SVL] = 54.3 ± 1.5, range = 42–62 mm) were collected by one of us (D.M.B.) as they roamed free within the reptile facility of the Dallas Zoo, Dallas County, Texas. In addition, 16 wild adult (8 male and 8 female, 48.9 ± 1.3, 42–57 mm) *H. t. turcicus* were collected for comparative purposes from within the same building and examined for coccidial parasites. Individual geckos were assigned an accession number and placed in 3.8-L glass jars containing damp toweling and water. Freshly shed fecal pellets were obtained from captive geckos, placed in individual vials containing 2.5% (w/v) aqueous $K_2Cr_2O_7$, and stored at 4°C. Geckos were later toe-clipped following accepted

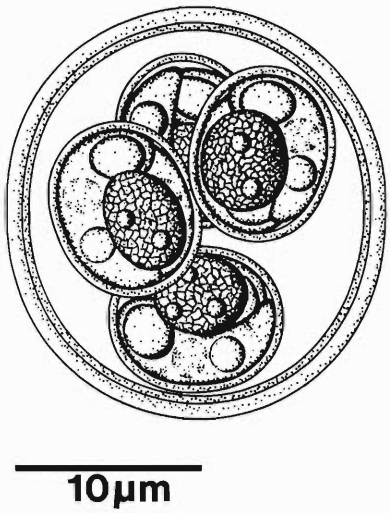


Figure 1. Line drawing of sporulated oocyst of *Eimeria dixoni* sp. n.

guidelines (ASIH–HL–SSAR, 1987) to avoid duplicate sampling and released unharmed where they were originally collected. Fecal samples were screened for coccidia by flotation in modified Sheather's sugar solution (sp. gr. 1.30) and positive samples containing unsporulated oocysts were allowed to sporulate at 22°C in petri dishes containing a thin layer of 2.5% potassium dichromate, then mailed to Kansas State University prior to further examination. Sporulated oocysts were concentrated by centrifugation–flotation (as above) and examined and photographed using Nomarski interference-contrast microscopy. Oocysts were measured using a calibrated ocular micrometer; measurements are reported in micrometers (µm) with the means followed by the ranges in parentheses. Oocysts of the new species were 15–22 days old when examined and photographed.

Results

Twenty-five of 32 (78%) geckos were infected with coccidia, including 11/16 (69%) *H. frenatus,* which harbored a previously undescribed species of *Eimeria,* and 14/16 (88%) *H. t. turcicus* infected with *Eimeria lineri* McAllister, Upton, and Freed, 1988. Neither of these 2 eimerians was found to be shared among geckos. Below is a description of the new species.

Eimeria dixoni sp. n. (Apicomplexa: Eimeriidae) (Figs. 1–4)

DESCRIPTION: Oocysts ($N = 30$) spherical or subspherical, 20.8 × 19.7 (17–22 × 17–21); shape index (length/width) 1.06 (1.0–1.1). Wall smooth and bilayered, ca. 1.2 thick, composed of a thick, colorless outer layer ca. 0.8 and thinner inner layer ca. 0.4. Micropyle, polar granule, and oocyst residuum absent. Sporocysts ($N = 30$) ovoid, 9.3 × 7.8 (8–11 × 7–8), with a smooth, thin wall ca. 0.4 thick; shape index 1.2 (1.1–1.4); Stieda and substieda bodies absent. Spherical or ovoid sporocyst residuum ($N = 9$) present, 5.8 × 4.5 (5–7 × 3–6), composed of a compact mass of similar-sized membrane-bound granules. Sporozoites ($N = 7$) vermiform, tapered anteriorly, 9.8 × 2.8 (8–11 × 2.6–3.2) in situ, and usually arranged head-to-tail within sporocyst. Each sporozoite contains a spherical anterior refractile body ($N = 6$), 1.9 (1.6–2.4), and a spherical or ovoid posterior refractile body ($N = 7$), 3.1 long × 2.5 wide (2.4–3.8 × 2.4–2.6). A nucleus lies between the refractile bodies.

TYPE SPECIMENS: Syntypes (oocysts in 10% formalin) are deposited in the U.S. National Museum, Beltsville, Maryland 20705, as USNM 80757.

TYPE HOST: *Hemidactylus frenatus* Schlegel *in* Duméril and Bibron, 1836 (Sauria: Gekkonidae), common house gecko, adult female, Arkansas State University Museum of Zoology, ASUMZ 13028, 9 May 1989.

TYPE LOCALITY: U.S.A., Texas, Dallas County, Dallas Zoo, 621 East Clarendon Drive.

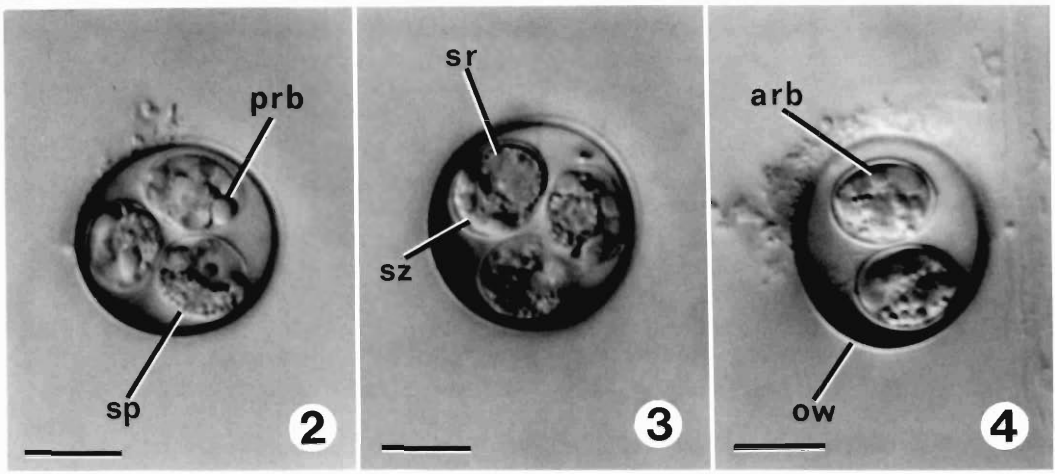
SITE OF INFECTION: Unknown. Oocysts removed from feces.

SPORULATION: Exogenous. Oocysts were passed unsporulated or partially sporulated and became fully sporulated within 48 hr at 22°C in 2.5% $K_2Cr_2O_7$.

PREVALENCE: 11/16 (69%) of *H. frenatus* examined.

ETYMOLOGY: Named in honor of James R. Dixon, Professor of Wildlife and Fisheries Sciences, Texas A&M University, for his contributions to gekkonid biology and neotropical herpetology.

REMARKS: *Eimeria dixoni* sp. n. resembles the following eimerians from gekkonid lizards: *Eimeria* sp. from *H. frenatus* in Taiwan (Yamamoto, 1933); *Eimeria boveroi* (Carini and Pinto, 1926) from the house gecko, *Hemidactylus mabouia* (Moreau de Jonnes, 1818) in Brazil and Mexico (Carini and Pinto, 1926; McAllister and Upton, 1989); *E. cicaki* Else and Colley, 1975, from the stump-toed gecko, *Gehyra mutilata* (Wiegmann, 1835) and *H. frenatus* in Malaysia (Else and Colley, 1975); and *Eimeria brygooi* Upton and Barnard, 1987, from the Madagascar day gecko, *Phelsuma madagascarensis grandis* (Gray, 1870), and golddust day



Figures 2–4. Nomarski interference-contrast photomicrographs of sporulated oocysts of *Eimeria dixoni* sp. n.: arb (anterior refractile body), ow (oocyst wall), prb (posterior refractile body), sp (sporocyst), sr (sporocyst residuum), sz (sporozoite). Scale bars = 10 μm.

gecko, *Phelsuma laticauda* (Boettger, 1880), in Madagascar (Upton and Barnard, 1987). The new species differs from Yamamoto's unnamed eimerian is being less elongate and not having a micropyle, from *E. boveroi* by possessing considerably larger sporocysts and by lacking a polar granule, and from *E. cicaki* and *E. brygooi* by having smaller oocysts and sporocysts and by lacking the 3–7 polar granules characteristic of *E. cicaki*.

Discussion

Hemidactylus frenatus and *H. turcicus* are native to the Old World. The population of *H. frenatus* at the Dallas Zoo was initially started during the 1970's for pest (i.e., insect) control. On the other hand, the *H. turcicus* were accidentally introduced into the zoo in the late 1970's, probably in hay bedding and shipping pallets transported from south Texas. Both geckos typically deposit fecal pellets on walls, sinks, floors, and even in other reptile cages within the reptile facility at the zoo. Because both geckos primarily utilize the same microhabitat within the building, they must come into contact with freshly deposited feces of either species, and the possibility for cross-transmission or autoinfection of coccidia exists. However, as stated previously, neither *E. dixoni* nor *E. lineri* was found to infect geckos other than the type host species.

In conclusion, although *E. turcicus* Upton, McAllister, and Freed, 1988, has been found commonly to infect *H. t. turcicus* at 2 sites in southern Texas and 1 locale in Louisiana (McAllister et al., 1988; Upton et al., 1988), it was not found in the population of Mediterranean geckos at the Dallas Zoo. This suggests that *H. t. turcicus* and *H. frenatus* may not share the same coccidian parasites and that at least some species of lizard coccidia may be species specific. However, caution must be exercised in the final interpretation of our preliminary data. It is known that even when 2 or more hosts have overlapping ranges in natural situations they may not share coccidians, even though both host species are equally susceptible and capable of being infected in the laboratory (see Doran, 1953).

Acknowledgments

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Larval Bucephalids (Trematoda: Digenea) Parasitizing Bivalve Molluscs in the Galveston Bay Area, Texas

WILLIAM J. WARDLE
Department of Marine Biology, Texas A&M University at Galveston,
P.O. Box 1675, Galveston, Texas 77553

ABSTRACT: Four species of larval bucephalids are reported from bivalve molluscs in the Galveston Bay area, Texas, and are designated as bucephalid cercaria A from *Anadara brasiliana,* B from *Ischadium recurvum,* C from *Rangia cuneata,* and D from *Periploma margaritaceum.* This report increases the number of bucephalid larvae reported from the Gulf of Mexico from 6 to 10. The metacercarial stage of B was obtained experimentally in fishes of the genus *Fundulus.*

KEY WORDS: Bucephalidae, Trematoda, Digenea, cercariae.

The family Bucephalidae is named for the distinctive "ox-head" appearance of the cercarial stage. It is basically furcocercous but with contractile furcae resembling horns attached to a wider-than-long modification of the tail stem. Bucephalid cercariae have long been known to develop in branching sporocysts parasitizing the visceral mass of both marine and freshwater bivalve molluscs. Reports of such infections from marine and estuarine waters of the Gulf of Mexico have been summarized recently (Wardle, 1988). Described below are 4 new cercariae from those waters in the Galveston Bay area, Texas. Some, or perhaps all 4, may be larvae of species whose adults have been named and described. For that reason, the 4 are designated as bucephalid cercaria A, bucephalid cercaria B, etc., instead of giving each a formal species name that would disappear as a junior synonym should life-history studies reveal the adult to be a previously named species.

Besides the distinctive tail, bucephalid cercariae have in common bodies with features whose presence need not be repeated in each description: a ventral mouth leading directly into the pharynx, followed by a rhabdocoele intestine; an anterior rhynchus independent of the digestive system; and a mesostomate excretory system, each side with a common tubule receiving a posterior and anterior collecting tubule, and entering a tubular to saccate bladder at a distance from its anterior end.

Drawings were made from living specimens stained with neutral red and observed under moderate pressure. All measurements, expressed in micrometers, were from 10 naturally emerged cercariae that were heat killed and mounted without coverslip pressure. For whole mounts, specimens were fixed in formalin–acetic acid–alcohol and stained with carmine.

Bucephalid Cercaria A
(Fig. 1)

DESCRIPTION: Body 120–155 long by 25–40 wide, minutely spinose anteriorly. Rhynchus 25–35 long by 15–23 wide, containing about 12 glands 10–12 in length, opening anteriorly through ducts arranged in a circle. Anterior end of body with 4 distinct lobes bearing coarse spines 1.0 in length. Pharynx spherical, 12–14 in diameter, in posterior half of body. Intestine rhabdocoele, 40–60 long, extending anteriad from pharynx, its contents staining red with neutral red. Excretory bladder I-shaped, 25–42 long, slightly displaced from midline anteriorly by pharynx and genital primordium. Flame cell formula 2([2 + 2 + 2 + 2] + [2 + 2 + 2]) = 28, anterior and posterior collecting tubules on each side unite anterior to pharynx, resulting common tubule enters anterior third of excretory bladder. Genital primordium situated posterior to pharynx, indistinct, staining red with neutral red. Nerve commissure posterior to rhynchus. Basal portion of tail 20–32 long and 35–60 wide, strongly indented posteriorly. Furcae 7–12 thick near base; very extensile, up to 10 times as long as the cercarial body. Sporocysts long and branching, 80–250 in width located in gonad of host, causing parasitic castration. Pigmentation in cuticula of sporocysts imparts orange color to gonadal area of infected clams.

Swimming was not observed but cercariae were seen crawling on the bottom of the glass container in which infected clams were kept.

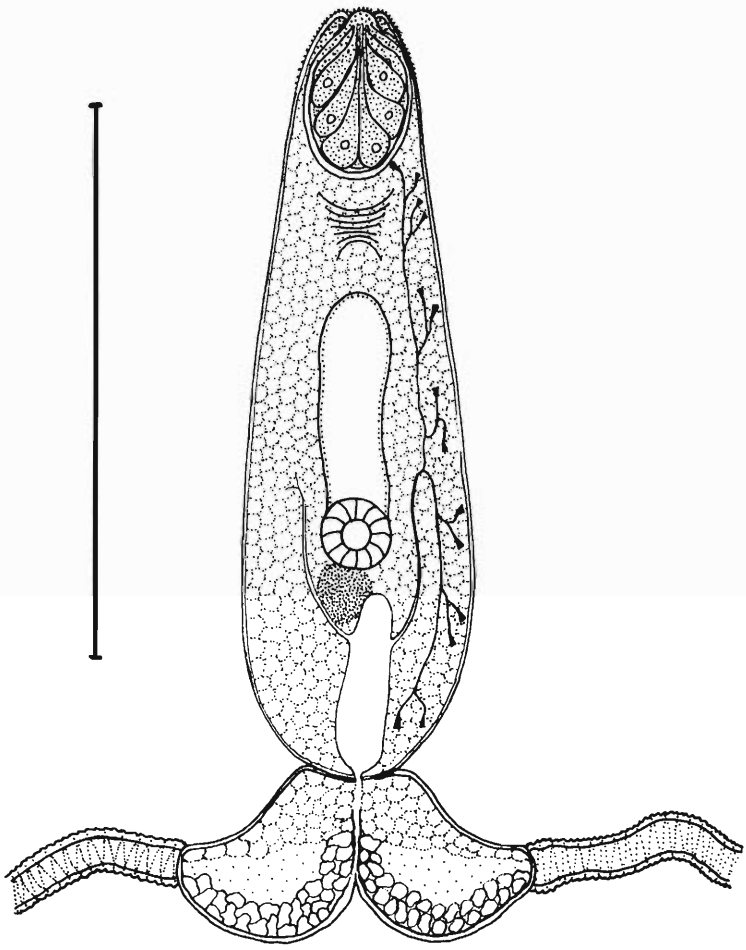


Figure 1. Bucephalid cercaria A from *Anadara brasiliana*. Ventral view of body and proximal portion of tail; details of excretory system shown only on left side of body. Scale bar = 100 μm.

HOST: *Anadara brasiliana* (Lamarck), incongruous ark.

LOCALITY: Gulf of Mexico at Galveston Beach, Galveston, Texas, U.S.A.

HABITAT: Subtidal, in sandy substrate just beyond surf zone, in waters of 25–32 ppt salinity.

PREVALENCE: One hundred seventy-two of 358 clams (48.0%).

VOUCHER SPECIMEN: U.S. National Parasite Collection, Beltsville, Maryland, Cat. No. 80839.

IDENTITY: In having a tail base with a distinct medial indentation posteriorly, this species resembles the cercaria of *Bucephalus loeschi* described by Hopkins (1958) from *Donax variabilis* from Mustang Island, Texas. However, the lateral cephalic glands and primordial cirrus sac reported for *B. loeschi* were not seen in the present species.

Bucephalid Cercaria B (Fig. 2)

DIAGNOSIS: Body 275–345 long, 55–75 wide, minutely spinose. Rhynchus oval to pyriform, 50–72 long, 40–55 wide, containing about 12 elongated glands 18–20 in length, opening anteriorly through ducts arranged in a circle. Rhynchus with 4 distinct lobes bearing coarse spines up to 2 in length. Pharynx oval, 30–40 wide by 25–35 long, located on ventral surface of posterior half of body. Intestine, 55–95 long by 22–30 wide, extending posteriorly from pharynx, contents staining with neutral red. Excretory bladder I-shaped, 50–70 long, its anterior extremity deflected to left of midline by intestine. Flame cell formula 2([2 + 2 + 2] + [2 + 2 + 2]) = 24. Anterior and posterior collecting tu-

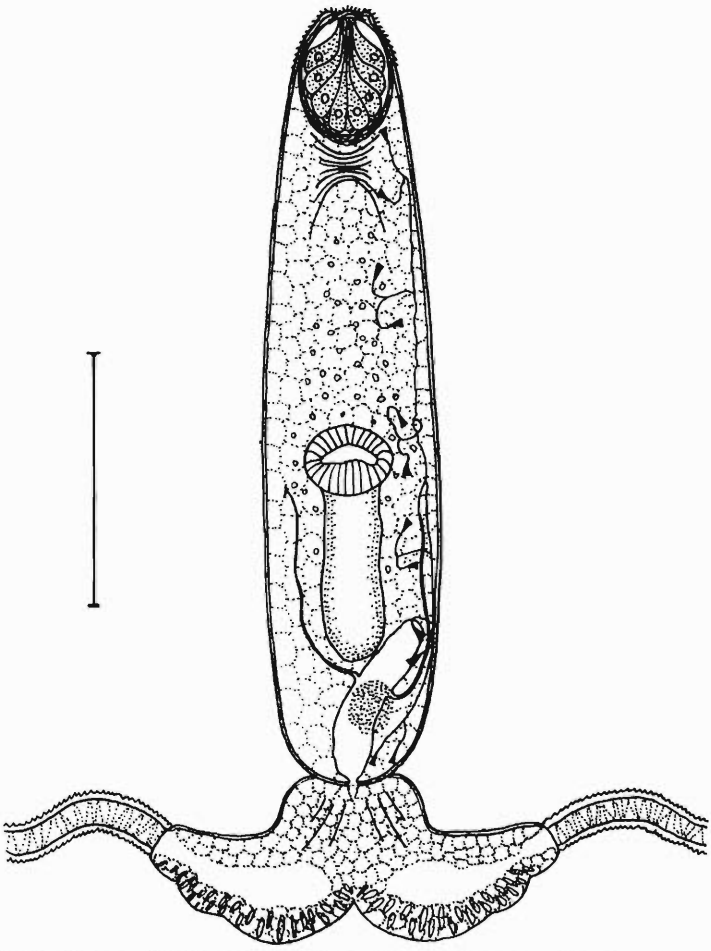


Figure 2. Bucephalid cercaria B from *Ischadium recurvum*. Ventral view of body and proximal tail region; details of excretory system shown only on left side. Scale bar = 100 μm.

bules uniting in posterior half of body to form common excretory tubules entering excretory bladder laterally at its midlevel. Genital primordium near the same level, staining pink with neutral red. Nerve commissure posterior to rhynchus. Basal portion of tail 70–150 wide by 40–50 in length, indented posteriorly. Furcae extensile, up to 10 times as long as body, 15–20 in thickness. Sporocysts as in cercaria A but branching, imparting pale yellow hue to gonadal area of infected mussels.

Swimming was not observed but cercariae were seen crawling on the bottom of containers with infected mussels.

HOST: *Ischadium recurvum* (Rafinesque), hooked mussel.

LOCALITIES AND PREVALENCES: Morgan's Point, Galveston Bay, Texas, U.S.A., 3 of 256 mussels (1.2%); Offat's Bayou, West Galveston Bay, Texas, U.S.A., 1 of 43 mussels (2.3%).

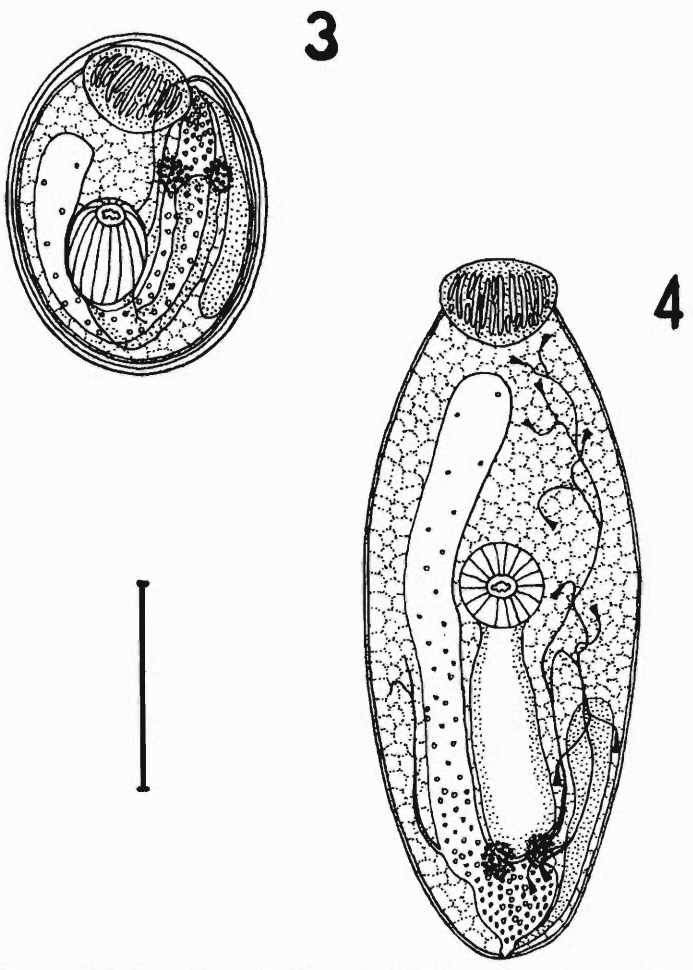
HABITAT: On coarse shell and rock substrates in intertidal and subtidal waters of up to 1 m in depth, 10–30 ppt salinity.

OVERALL PREVALENCE: Four of 305 mussels (1.3%).

VOUCHER SPECIMEN: U.S. National Parasite Collection, Beltsville, Maryland, Cat. No. 80840.

Metacercaria (Figs. 3, 4)

The metacercarial stage of bucephalid cercaria B was obtained experimentally by placing 2 species of killifishes with infected mussels in aquaria. Fishes were examined after 7 days of continuous exposure and all were found to contain numerous metacercariae encysted in the su-



Figures 3, 4. Metacercarial stage of bucephalid cercaria B. 3. Encysted metacercaria from body musculature of *Fundulus similis*. 4. Ventral view of excysted metacercaria; details of excretory system shown only for left side of body. Scale bar = 100 μm.

perficial musculature near the fin bases. Two control fishes of each species, which were not exposed to infected mussels, were found to be negative for bucephalid metacercariae. Drawings were prepared from mechanically excysted specimens under moderate coverslip pressure and stained with vital dyes. For measurements, 4 specimens were mechanically excysted, heat-killed, and measured under a floating coverslip.

DESCRIPTION: Cysts (Fig. 3) oval, 170–210 long by 120–150 wide, with body folded on itself inside. Cyst wall thin, transparent. Mechanically excysted specimens (Fig. 4) 310–390 long by 120–160 wide; tegument minutely spinose. Rhynchus 40–70 by 60–90, subtriangular in ventral view. Pharynx spherical, 45–55 in diameter, slightly anterior to midlevel of body. Intestine 120–170 long, extending posteriorly from pharynx. Excretory bladder 240–290 long, passing to right of intestine and pharynx to reach almost to rhynchus. Base of bladder with concretions that gradually disappear anteriorly. Flame cell formula $2([2 + 2 + 2] + [2 + 2 + 2]) = 24$. Three genital primordia stain pink with neutral red stain: that of the cirrus sac on left side of intestine, 90–140 in length; and 2 indistinct, roughly circular primordia 25–30 in diameter, posterior to intestine.

EXPERIMENTAL HOSTS: *Fundulus similis* (Baird and Girard), long-nosed killifish; *Fundulus grandis* (Baird and Girard), gulf killifish.

IDENTITY: The metacercarial stages of 3 species of *Rhipidocotyle* have been reported from the Gulf of Mexico. These include *R. transversale* described and reported by Chandler (1935); *R.*

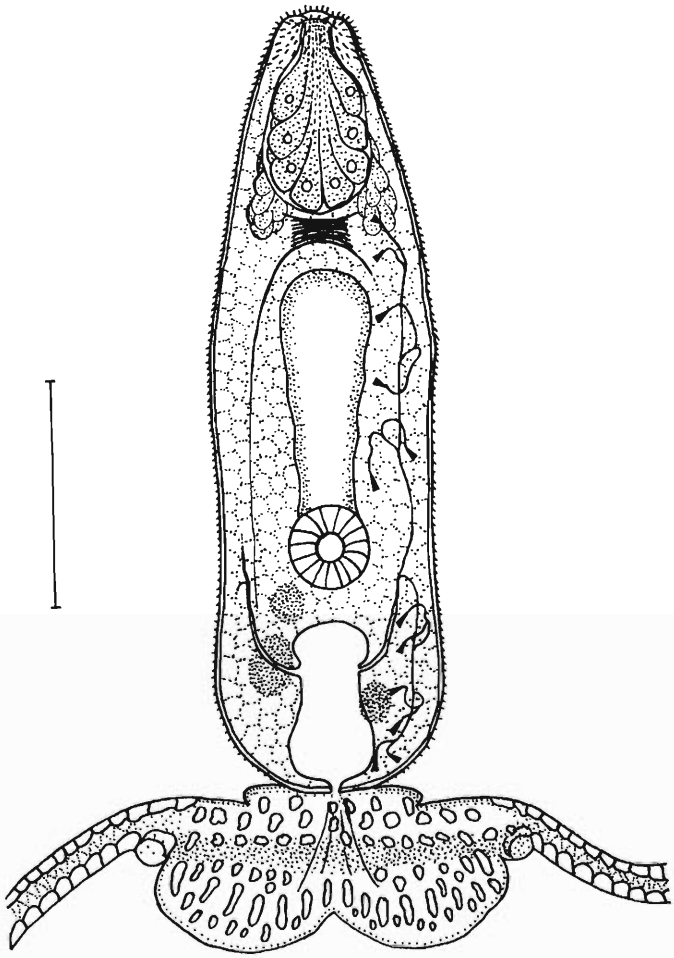


Figure 5. Bucephalid cercaria C from *Rangia cuneata*. Ventral view of body and proximal tail region; details of excretory system shown only for left side, posterior extension of nerve trunk for right side. Scale bar = 100 μm.

lintoni Hopkins, 1954 (reported by Sparks, 1957), and *R. lepisostei* Hopkins (1954). The life cycles of *R. transversale* and *R. lintoni* were resolved by Stunkard (1976). The metacercaria of bucephalid cercaria B reported here differs from the metacercaria of *R. transversale* and *R. lintoni* in having 24 (instead of 16) flame cells and in having a longer excretory bladder. The metacercaria of *R. lepisostei* differs from the present species in having 48 flame cells.

Bucephalid Cercaria C (Fig. 5)

DESCRIPTION: Body 300–418 long by 70–110; spinose with spines more prominent on anterior third. Relatively coarse spines up to 2 long on 4 distinct lobes at anterior end. Rhynchus 60–95 long by 50–65 wide, containing numerous indistinct glands with ducts opening anteriorly. Pharynx spherical, well posterior to midlevel of body, 28–42 in diameter. Intestine extending anteriad from pharynx, 80–140 in length, staining red with neutral red. Paired clusters of indistinct cephalic glands posterolateral to rhynchus; stained very light pink by neutral red. Excretory bladder I-shaped, 65–90 long. Flame cell formula 2([2 + 2 + 2] + [2 + 2 + 2]) = 24. Three genital primordia anterolateral to excretory bladder, each roughly circular in outline, 15–30 in diameter. Primordia staining pink with neutral red stain. Nerve commissure posterior to rhynchus, with trunks extending posteriad to level of excretory bladder. Basal portion of tail 115–160 wide by 45–70 in length, indented posteriorly. Caudal

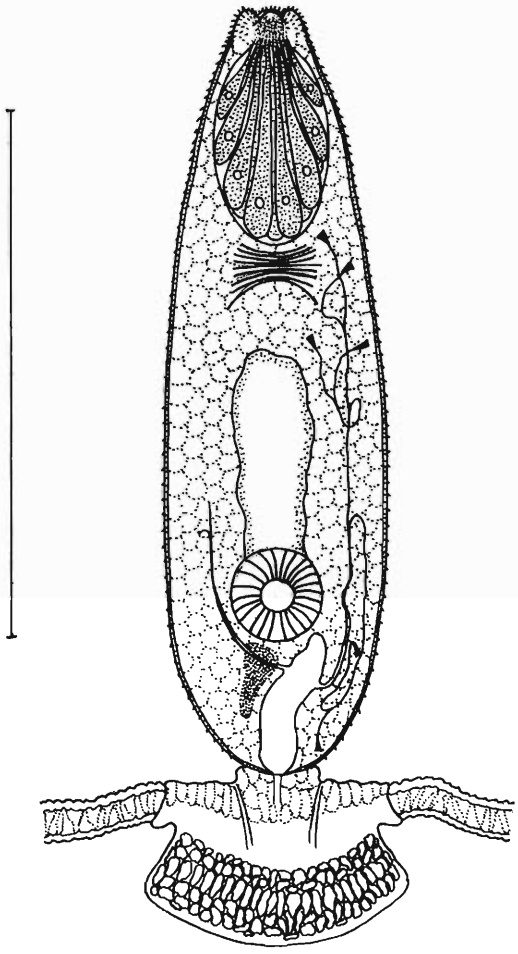


Figure 6. Bucephalid cercaria D from *Periploma margaritaceum*. Ventral view of body and proximal tail region of cercaria; details of excretory system shown only on left side. Scale bar = 100 μm.

furcae extremely protrusile, extending up to 10 times length of body, 17–22 in diameter. Sporocysts long and branching, 45–185 in width, walls containing orange pigmentation and imparting gross light orange color to gonadal area of infected clams. Cercariae not observed swimming but crawling on bottom of glass container.

HOST: *Rangia cuneata* Gray, common rangia.

LOCALITIES AND PREVALENCES: Lake Anahuac, Texas, U.S.A., 50 of 428 clams (11.6%); McCollum Park, Texas, U.S.A., 10 of 279 clams (3.6%).

HABITAT: In intertidal and subtidal mud–sand substrates up to 1 m in depth, in waters of 0.3–10.0 ppt salinity.

OVERALL PREVALENCE: Sixty of 707 clams (8.5%).

VOUCHER SPECIMEN: U.S. National Parasite Collection, Beltsville, Maryland, Cat. No. 80841.

IDENTITY: This cercaria resembles *Bucephalus cuculus* described by McCrady, 1874, from the oyster *Crassostrea virginica* (as figured by Hopkins, 1954) in being of similar size and in having the same flame cell formula. However, it differs from *B. cuculus* in lacking a constriction of the excretory bladder at its base and in having a distinct posterior cleft in the basal tail-segment.

Bucephalid Cercaria D (Fig. 6)

DESCRIPTION: Body 130–175 in length, 30–45 wide. Cuticula minutely spinose, spines coarser on anterior fifth of body; anterior end with 4 lobes bearing coarser spines up to 1.5 long. Rhynchus 30–45 long by 18–25 wide, containing about 12 glands with ducts opening in a circle at anterior end of body. Pharynx well posterior to midlevel of body, 18–25 in diameter. Intestine 25–45 in length; extending anteriad from pharynx, contents staining red with neutral red. Excretory bladder I-shaped, 25–32 long, its anterior portion deflected to left of midline by pharynx. Flame cell formula 2([2 + 2] + [2]) = 12. Genital primordium on right side of excretory bladder posterior to pharynx, 14–18 long by 6–12 wide, light pink with neutral red staining. Nerve commissure located posterior to rhynchus. Basal portion of tail 45–55 wide by 24–35 long, not indented posteriorly. Caudal furcae long and protrusile, up to 10 times length of body, 70–90 in diameter. Sporocysts branching, 20–65 in width, unpigmented. Gonadal area of infected clams pale white instead of the creamy white color of this area in uninfected clams. A few apparently normal host gametes (eggs) were observed in 1 infected clam, indicating that parasitic castration may not always be complete prior to cercarial production by the parasite.

Swimming of cercariae was not observed; specimens were found crawling on the bottom of the glass containers that held the host clams.

HOST: *Periploma margaritaceum* (Lamarck), spoon clam.

LOCALITY: Galveston Beach, Galveston, Texas, U.S.A.

HABITAT: Subtidal in sandy substrate just beyond surf zone in waters of 25–32 ppt salinity.

PREVALENCE: Four of 268 clams (1.5%).

VOUCHER SPECIMEN: U.S. National Parasite Collection, Beltsville, Maryland, Cat. No. 80842.

IDENTITY: This species is similar to *Cercaria apalachiensis* Holliman, 1961, from *Mulina lateralis* in Florida in that both have 12 flame cells, and about 12 glands in the rhynchus. However, *C. apalachiensis* is larger (150–200) and has a distinct cleft in the posterior margin of the tail base, which does not occur in the present species. Habitats of the molluscan hosts also differ markedly. Holliman (1961) found infected *M. lateralis* in a salt marsh environment, whereas spoon clams (*Periploma margaritaceum*) infected with cercaria D were from an exposed beach area.

Summary

This study increases the number of bucephalid cercariae reported from the Gulf of Mexico from 6 to 10, whereas about 30 species of adult bucephalids are known from those waters.

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Name Change for the *Proceedings*

At the 601st meeting of the Society on 15 February 1989, the members present voted on and passed a motion to amend the Constitution of the Society. Thus was the name of the *Proceedings of the Helminthological Society of Washington* changed to the *Journal of the Helminthological Society of Washington* effective with Volume 57. The principal reason for the change was to avoid the perception of some agencies and individuals that ours is not a peer-reviewed journal.

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Some Digeneans of the Neotropical Turtle Genus *Rhinoclemmys* in Mexico and South America

WILLIAM G. DYER[1] AND JOHN L. CARR[2]

[1] Department of Zoology, Southern Illinois University, Carbondale, Illinois 62901
[2] Conservation International, 1015 18th St. NW, Suite 1000, Washington, D.C. 20036

ABSTRACT: Four species of digeneans detected in freshwater turtles of Mexico and South America represent new host and/or locality records. *Rhinoclemmys areolata* of Mexico and *Rhinoclemmys punctularia* of French Guiana were infected with *Nematophila grandis. Rhinoclemmys nasuta* of Ecuador was infected with *Octangioides tlacotalpensis, Pseudocleptodiscus margaritae, Pseudallassostoma heteroxenus,* and *N. grandis. Rhinoclemmys pulcherrima pulcherrima* of Mexico, *Rhinoclemmys annulata, Rhinoclemmys melanosterna,* and *Kinosternon leucostomum* of Ecuador, and *Rhinoclemmys diademata* of Venezuela were negative for digeneans.

KEY WORDS: Digenea, *Octangioides tlacotalpensis, Nematophila grandis, Pseudocleptodiscus margaritae, Pseudallassostoma heteroxenus,* freshwater turtles, *Rhinoclemmys areolata, Rhinoclemmys nasuta, Rhinoclemmys punctularia,* new host records, new locality records, Mexico, Ecuador, French Guiana.

Few references are available on angiodictyid and paramphistomid digeneans of freshwater turtles in Mexico and South America and especially so on those of Ecuador, Venezuela, and French Guiana. In conjunction with fieldwork by one of us (J.L.C.) on the ecologically diverse genus *Rhinoclemmys* (Emydidae) in Ecuador and Mexico during July–August 1986 and 1988, respectively, an opportunity became available to study the helminths of some of these turtles. Over the same time period, colleagues donated freshly collected *Rhinoclemmys* spp. from Venezuela and French Guiana. Included in this report are new host and/or geographic locality records for 4 species of digeneans found in *Rhinoclemmys areolata* (Dumeril and Bibron, 1851), *Rhinoclemmys nasuta* (Boulenger, 1902), and *Rhinoclemmys punctularia* (Daudin, 1801).

Materials and Methods

Helminths were recovered in situ by necropsy from the turtles shortly after death. Some turtles were transported to our laboratory in Carbondale, Illinois, and kept isolated from other specimens prior to necropsy. Helminths from *R. nasuta* were recovered from organs preserved in 10% formalin in the field. Only digestive tracts and lungs were examined for helminths. All other diageneans were fixed in cold AFA. Specimens were stained in Harris' hematoxylin, dehydrated, and cleared in beechwood creosote. Small and medium size digeneans were mounted in Canada balsam and large specimens were examined and stored in beechwood creosote. Trematode specimens are deposited in the United States National Museum Helminthological Collection (USNM Helm. Coll.). Representative specimens of the host species, *R. nasuta,* have been deposited in the United States National Museum Reptile Collection, Nos. 281887–281891. Specimens of *R. areolata* will be deposited in the USNM collection, and *R. punctularia* specimens are destined for the Texas Cooperative Wildlife Collection at Texas A&M University.

Results and Discussion

Of 37 freshwater, semiterrestrial, and terrestrial turtles examined from Mexico, western Ecuador, Venezuela, and French Guiana, 4 species of digeneans including 1 from Mexico, 1 from French Guiana, and 4 from Ecuador were recovered from digestive tracts. Lungs were negative for helminths. Nematodes recovered from digestive tracts are in the process of being studied and will be reported elsewhere.

Angiodictyidae Looss, 1902

Octangioidinae Yamaguti, 1958

Octangioides tlacotalpensis Caballero, 1942

Of 17 *R. nasuta* examined from various localities in Esmeraldas Province, Ecuador, 8 were infected with approximately 50–365 specimens per host of *Octangioides* in the large intestine. These included 2 of 7 turtles from Sarria, Río Bogotá (0.1°0.6′N, 78°48′W), and 6 of 8 from Estero El Ceibo (0.1°05′N, 78°48′W). Two turtles from Playa Grande (00°54′N, 78°58′W) were negative for *Octangioides.* Observation based on numerous specimens from each host with respect to the morphology of the excretory vesicle and canals, length of the intestinal ceca and their proximity to the excretory vesicles, body size, and organ configuration revealed that they fit the description of *Octangioides tlacotalpensis* as presented by Caballero (1942). Egg size was not given in the original description but was later reported by Thatcher (1963) as 90–100 μm long

by 50–65 μm wide. In the present study, eggs measured 93–110 μm long by 50–70 μm wide.

Two species of *Octangioides* have been described from turtles, namely *Octangioides skrjabini* Price, 1937, and *O. tlacotalpensis* Caballero, 1942. Both have been described from the same host, *Dermatemys mawii* Gray, and the same locality, Mexico. To our knowledge each species has subsequently been reported only once. Caballero Rodriquez (1960) redescribed and figured *O. skrjabini* from *D. mawii* of Tabasco, and Thatcher (1963) reported *O. tlacotalpensis* from 2 *D. mawii* of Veracruz and Tabasco, Mexico.

The finding of *O. tlacotalpensis* in *R. nasuta* from Ecuador establishes a new host record and extends the geographic distribution from Mexico to South America.

Voucher specimens of *O. tlacotalpensis* have been deposited in the USNM Helm. Coll. Nos. 80612–80613.

Paramphistomidae Fischoeder, 1901

Nematophilinae Skrjabin, 1949

Nematophila grandis (Diesing, 1839) Travassos, 1934

Six of 17 *R. nasuta* examined from various localities in Ecuador were infected with 2–6 *Nematophila grandis* (Diesing, 1839), Travassos, 1934 (=*Paramphistomum argentinum* Cordero and Vogelsang, 1940), in both the small and large intestines. These included 4 of 8 turtles from Estero El Ceibo and 2 of 7 from Sarria, Río Bogotá. In addition, 3 of 4 *R. punctularia* captured 1.6 km W and 1.8 km S of Iracoubo, French Guiana (0.5°28′N, 53°12′W), were each infected with a single *N. grandis*. Two of 7 *R. areolata*, one captured 24 km NE of Catazajá, Chiapas (17°52′N, 91°50′W) and the other in the vicinity of Emiliano Zapata, Tabasco, Mexico (17°45′N, 91°46′W), were also infected with 3 and 1 specimens of *N. grandis*, respectively.

Digeneans of the genus *Nematophila* have thus far been reported from freshwater turtles of Central and South America. *Nematophila grandis* occurs in several freshwater turtles of Panama including *Rhinoclemmys melanosterna* (Caballero et al., 1958), and Argentina. Alho (1964) reported *N. grandis* from the large intestines of *Kinosternon scorpioides scorpioides* (Linnaeus) and *Geoemyda punctularia punctularia* (Daudin) (=*Rhinoclemmys p. punctularia*) in Brazil. *Nematophila venezuelensis* (Cordero and Vogelsang, 1940) Yamaguti, 1958 (=*Allassostoma venezuelensis* Cordero and Vogelsang, 1940), and *Nematophila ovalis* Cordero and Vogelsang, 1940, have both been reported from *Podocnemis* sp. from Venezuela.

Rhinoclemmys areolata and *R. nasuta* are new host records for *N. grandis*. The finding of *N. grandis* in *R. nasuta* from Ecuador, *R. punctularia* from French Guiana, and *R. areolata* from Mexico constitute new locality records.

Voucher specimens of *N. grandis* have been deposited in the USNM Helm. Coll. Nos. 80614–80617.

Dadaytrematinae Yamaguti, 1958

Pseudocleptodiscus margaritae Caballero, 1961

Seven of 17 *R. nasuta* from Ecuador were infected with *Pseudocleptodiscus margaritae* in either the terminus of the small intestine or the upper part of the large intestine; the range of infection being 1–20 specimens per host. These included 5 of 8 turtles from Sarria, Río Bogotá, and 2 of 7 from Estero El Ceibo. Two turtles from Playa Grande were negative for *P. margaritae*.

Except for those species of *Pseudocleptodiscus* that occur in turtles, dadaytrematinid digeneans are parasites of fish. The types species, *P. margaritae*, was described by Caballero (1961) from the large intestine of a freshwater turtle, *D. mawii*, from tributaries of the Río Grijalva near Villahermosa, Estado de Tabasco, Mexico. Later, *Pseudocleptodiscus sphaerorchidum* (Thatcher, 1963) Yamaguti, 1971 (=*Dadaytrema sphaerorchidum* Thatcher, 1963), was described from the same host species captured 15 mi south of Villahermosa, Estado de Tabasco. On examination of the holotype (USNM Helm. Coll. No. 60309), Yamaguti (1971) concluded that it appears conspecific with *P. margaritae*.

The finding of *P. margaritae* in *R. nasuta* of Ecuador constitutes a new host record and a southern extension of the geographic range of this parasite.

Specimens of *P. margaritae* have been deposited in the USNM Helm. Coll. No. 80618.

Schizamphistominae Looss, 1912

Pseudallassostoma heteroxenus (Cordero and Vogelsang, 1940) Yamaguti, 1958

One of 8 *R. nasuta* from Estero El Ceibo, Ecuador was found infected with 50 *Pseudallassostoma heteroxenus* (=*Cladorchis heteroxenus*

Cordero and Vogelsang, 1940). This species was originally described from the stomach of *Podocnemis* sp. from Estado de Guarica, Venezuela. The finding of *P. heteroxenus* in *R. nasuta* from Ecuador constitutes a new host record and extends the geographic distribution from Venezuela to Ecuador.

Specimens of *P. heteroxenus* have been deposited in the USNM Helm. Coll. No. 80619.

Specimens of *Kinosternon leucostomum* (Dumeril and Bibron, 1851), *Rhinoclemmys annulata* (Gray, 1860), *R. melanosterna* (Gray, 1861) of Ecuador, *Rhinoclemmys diademata* (Mertens, 1954) of Venezuela, and *Rhinoclemmys pulcherrima pulcherrima* (Gray, 1855) of Mexico were negative for digeneans.

Acknowledgments

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Collecting in Ecuador was conducted with permits from the Ministerio de Agricultura y Ganaderia, and accomplished with the support of Professor G. Orces, V., L. Albuja, R. Barriga, and A. Almendáriz. J. E. Simmons and M. T. Nielsen also assisted in the fieldwork. Collecting in Mexico was conducted with permit no. 461 from the Instituto Nacional de la Pesca, with the logistical help of V. Gonzalez and R. C. Vogt.

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Grillotia similis (Linton, 1908) comb. n. (Cestoda: Trypanorhyncha) from Nurse Sharks in the Florida Keys

J. N. Caira and M. M. Gavarrino
Ecology and Evolutionary Biology, University of Connecticut, U-43, Rm. 312,
75 N. Eagleville Rd., Storrs, Connecticut 06268

ABSTRACT: Based on the examination of type material as well as light and scanning electron microscopy of voucher specimens collected from the nurse shark (*Ginglymostoma cirratum* (Bonnaterre, 1788)) in the Florida Keys, it was determined that *Grillotia simmonsi* Dollfus, 1969, is a junior synonym of *Rhynchobothrium simile* Linton, 1908. As the generic name *Rhynchobothrium* is no longer available, and the species is consistent with the diagnosis of *Grillotia* Guiart, 1927, the new combination *Grillotia similis* (Linton, 1908) is proposed and the description of this species is emended. Segment morphology is described and figured for the first time. The description of the vitellaria in the generic diagnosis of *Grillotia* is expanded to accommodate all of the species currently recognized in the genus. *Grillotia similis* was present in 81.8% of the 22 nurse sharks necropsied, with a mean intensity of 15.05 (±19.69) worms per host. The worms were found in the anteriormost 7 of the 16 spiral valve chambers and most abundantly in the anteriormost 3.

KEY WORDS: trypanorhynch, *Grillotia,* Lacistorhynchidae, nurse shark, Florida Keys.

Linton (1908) described *Rhynchobothrium simile* Linton, 1908, a species of relatively large trypanorhynch, from the nurse shark (*Ginglymostoma cirratum* (Bonnaterre, 1788)) off the Dry Tortuga Islands, west of the Florida Keys. Based on 3 immature specimens collected from the same host species off Sarasota, Florida by J. E. Simmons, Dollfus (1969) described *Grillotia simmonsi* Dollfus, 1969. Neither author was able to describe the segment morphology in any detail; however, Dollfus presented a very detailed account of the scolex morphology. Comparison of Linton's description and specimens with Dollfus' description and specimens leads us to believe that these species are synonymous. We recently collected numerous specimens of this species from nurse sharks in the Florida Keys. This additional material allows us to describe the segment morphology for the first time, to emend slightly the description of the tentacle armature, and to comment on the vitellaria in the genus *Grillotia* Guiart, 1927, in general. In addition, the exact location of this species within the spiral valve of the nurse shark was investigated.

Materials and Methods

During the summers of 1986 through 1988, 22 nurse sharks were examined for the presence and location of specimens of this large trypanorhynch species. Nurse sharks were caught on hook and line in the vicinity of Lower Matecumbe Key, in the Florida Keys, temporarily held in a floating wire pen, and pithed immediately prior to necropsy. The entire spiral valve was removed and cut along the dorsomedian line to expose the internal chambers. Each of the 16 chambers was examined separately for tapeworms. The tapeworms from each chamber were placed in a vial of AFA, such that there was 1 vial for each of the 16 chambers for each of the 22 nurse sharks.

Specimens for whole mounts were stained with Harris' hematoxylin or Semichon's acetocarmine, dehydrated in ethanol, cleared in xylene, and mounted in Canada balsam. The tentacles of 2 specimens were dissected away from the scolex musculature and stained and mounted as above. To circumvent the problem of viewing segment morphology through the dense circumcortical longitudinal musculature of this species, segments from 3 worms were cut longitudinally into dorsal and ventral halves using a razor blade. Each half was then stained and mounted as above. For cross sections, specimens were embedded in paraplast, sectioned at 10-μm intervals with an American Optics rotary microtome, stained in Gill's hematoxylin and eosin, dehydrated in ethanol, cleared in xylene, and mounted in Canada balsam. Specimens for scanning electron microscopy were hydrated, placed in 1% osmium tetroxide overnight, dehydrated in ethanol, critical point-dried in liquid CO_2, and mounted on stubs with double-sided adhesive tape and carbon paint, sputter-coated with gold, and examined with a Coates and Welter field emission scanning electron microscope.

The scolex terminology and hook numbering systems used follow those of Dollfus (1942, 1969, respectively). The genital terminology used follows that of Beveridge and Sakanari (1987). Measurements are in micrometers unless otherwise stated. The range is given for each numerical character, followed in parentheses by the mean, the standard deviation, the number of worms examined, and the total number of observations (when more than 1 structure per worm was examined). Illustrations were drawn with the aid of a drawing tube. Whole mounts of 10 voucher specimens were deposited in the U.S. National Museum Helminthological Collection (No. 80896) in Beltsville, Maryland. The sections from which Figures 1 and 2 were

drawn were deposited at the H. W. Manter Laboratory in Lincoln, Nebraska (No. 31166) as were the slides of tentacles isolated from their respective scolices (No. 31167), and whole mounts of 8 voucher specimens including the scolices from which the tentacle hooks in Figures 3 and 4 were drawn (No. 31165). Linton's specimens of *R. simile* (No. 8993) were borrowed from the U.S. National Museum Helminthological Collection. Two of Dollfus' syntype specimens of *G. simmonsi* were borrowed from the Laboratoire de Zoologie (Vers) at the Muséum National d'Histoire Naturelle in Paris.

Grillotia similis (Linton, 1908) comb. n. (Figs. 1–9)

The following information should emend Dollfus' (1969) description of the scolex of this species: Scolex unspined. Basal hooks on tentacles irregular in position; external surface of tentacle with group of 4 rose-thorn-shaped hooks of differing sizes arranged in a diamond pattern (Figs. 3, 4, 9).

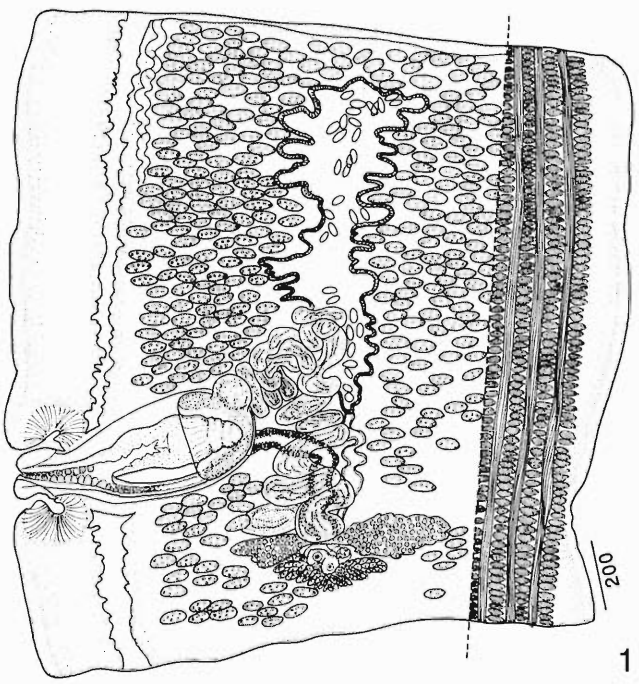
The following information should emend the descriptions of Linton (1908) and Dollfus (1969) for the strobila of this species: Worms 14.2–41.5 mm (28.1, 8.8, 22) long, maximum width 1,075–1,450 (1,240, 121, 22) occurring at bulbs. Strobila unspined. Neck lacking. Segments with numerous conspicuous bands of longitudinal muscle fibers. Immature segments wider than long; mature segments longer than wide, 550–1,725 (1,120.9, 297.4, 20) long by 375–1,500 (833.3, 285.7, 20) wide, ratio of length to width 0.52–2.95 (0.97, 0.44, 15, 31) acraspedote, apolytic. Genital atrium lateral, in posterior one-third of segment, 27.6–48.1% (34.8, 5.24, 16, 30) from posterior end, surrounded by sphincter-like muscle fibers. Hermaphroditic sac pyriform, thin-walled, 180–280 (221.5, 28.2, 5, 13) long by 490–670 (574, 47.7, 5, 13) wide; common genital duct of variable length, divides within hermaphroditic sac into vagina and cirrus. Cirrus unarmed, enters saccate internal seminal vesicle at proximal end. Small external seminal vesicle present; vas deferens greatly coiled, extending anteriorly then posteriorly along midline to ovarian isthmus. Testes numerous, 20–32.5 (24.8, 5.1, 20) long by 42.5–90 (62.1, 14.5, 20) wide, occupying medulla of entire segment median to excretory ducts, interrupted by hermaphroditic sac, 2–3 layers deep in cross section. Vagina piercing posterolateral wall of hermaphroditic sac, extending to midline of segment and then posterior to ovarian bridge. Ovary bilobed in dorsoventral view, 40–135 (101, 27.8, 8, 18) long by 320–650 (301.7, 216.3, 8, 18) wide, tetralobed in cross section. Mehlis' gland postovarian, between ovarian lobes, 55–130 (78.3, 21.7, 6, 10) long by 130–302.5 (220.2, 57.9, 6, 10) wide. Uterus medial, extending almost to anterior end of segment, with lateral branches; no uterine pore seen. Vitellaria follicular, circumcortical, arranged in longitudinal columns that alternate with bands of longitudinal muscle fibers. Eggs elipsoidal, 27.5–50 (40.9, 6.6, 20, 30) long by 17.5–23.7 (21.3, 1.9, 20, 30) wide, nonoperculated.

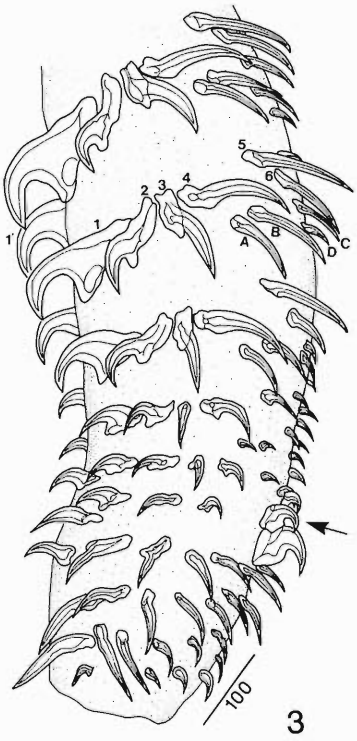
Remarks

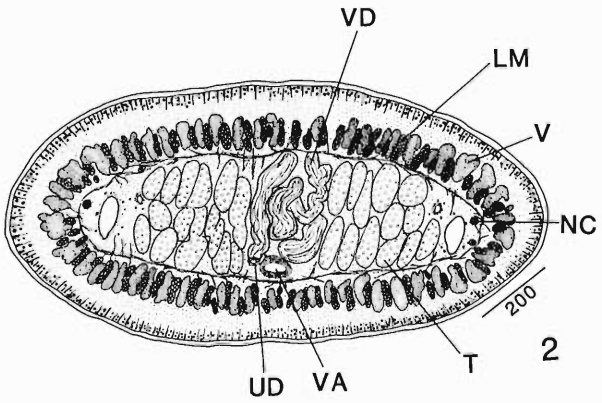
Comparison of Linton's specimens of *R. simile* (USNM No. 8993) and Dollfus' specimens of *G. simmonsi* confirms that they represent the same species. The distinctive basal group of 4 hooks (Figs. 3, 4, 9) was clearly visible on the tentacles of these specimens although they were not described by either Linton or Dollfus. As we first observed the presence of these hooks with scanning electron microscopy and then confirmed their presence with light microscopy, it is not surprising that these hooks were overlooked by these authors.

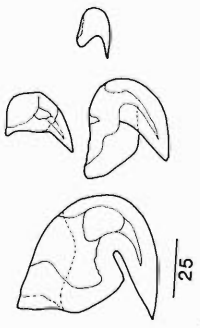
As the name *R. simile* predates the name *G. simmonsi,* and these 2 species appear to be synonymous, the correct specific epithet for this species should be "*simil.*" The name *Rynchobothrium,* however, was rejected by Dollfus (1929) and therefore is no longer available. Based on characteristics of the tentacle armature, Dollfus (1969) placed his material into the genus *Grillotia,* in the monogeneric subfamily Grillotiinae, of the family Lacistorhynchidae. With the exception of the vitellaria, this species conforms to the diagnoses of *Grillotia* presented by Dollfus (1942), Yamaguti (1959), and more recently by Schmidt (1986), including the presence of a special basal group of small hooks on the external surface of the tentacles. With alteration of the gender of the specific epithet to match that of "*Grillotia,*" we propose the new combination *G. similis* (Linton, 1908) for this species.

At present there appears to be some confusion as to the configuration of the vitellaria in *Grillotia.* Dollfus (1942, p. 345) described the vitellaria of the genus as "á la fois en dedans et en dehors de la musculature longitudinal interne," and somewhat consistent with this characterization, Yamaguti (1959, p. 126) described the vitellaria as "medullary, intruding into cortex." Schmidt (1986, p. 64), however, described the vitellaria in this genus as "lateral, mostly med-



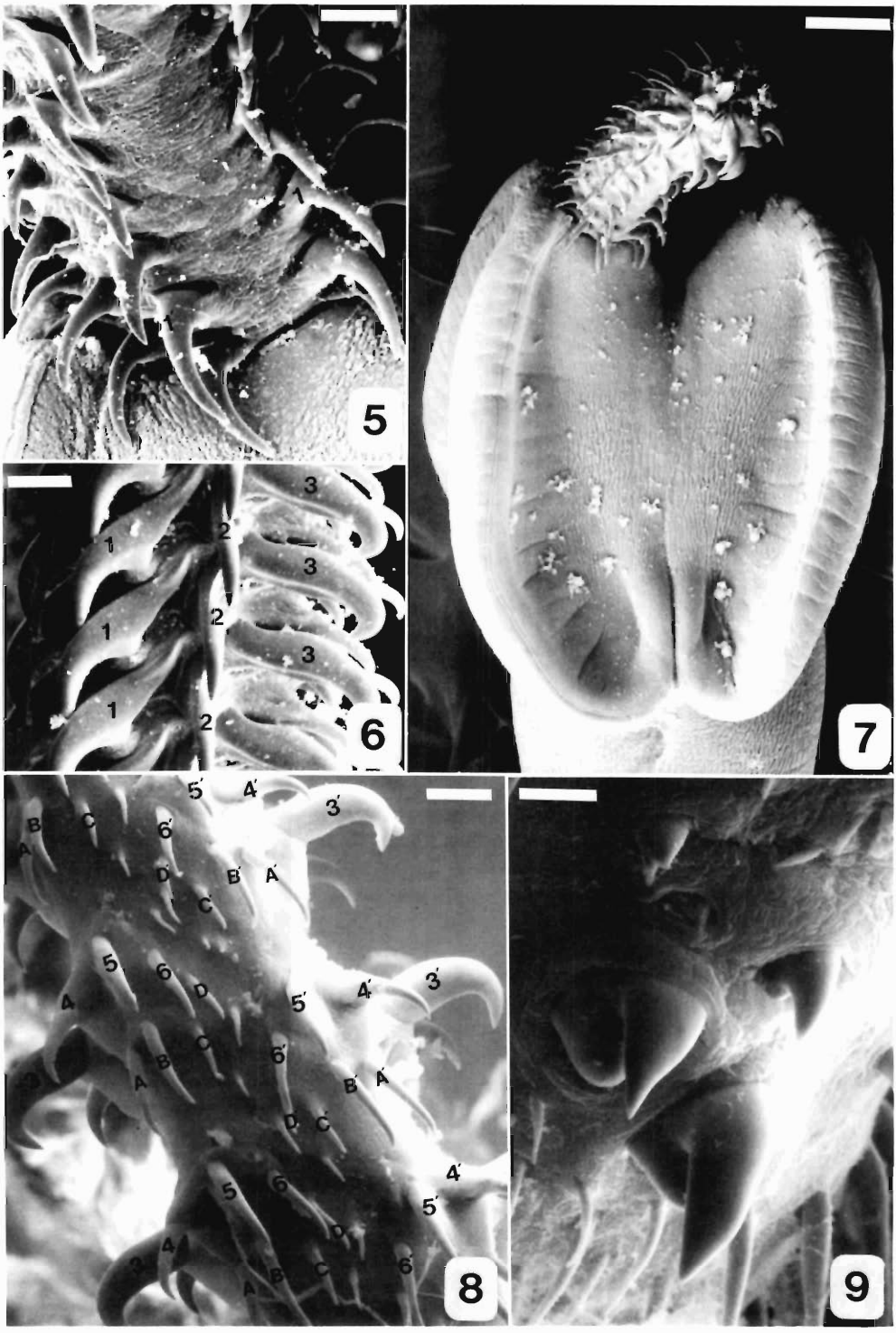






Figures 1, 2. ***Grillotia similis.*** **1. Mature proglottid from razor-blade section. Vitellaria and longitudinal muscle bundles are circumcortical but are drawn only to the reader's right of the dashed line. 2. Cross section through mature proglottid. Figure 3. Bothridial surface of tentacle showing location of distinctive diamond pattern of basal hooks on external surface (arrow). Figure 4. Enlarged view of distinctive basal hooks arranged as found on tentacle. VD—vas deferens, LM—longitudinal muscle bundle, V—vitellaria, NC—nerve cord, T—testis, VA—vagina, UD—uterine duct.**

ullary." Contrary to this latter characterization, cross sections of our specimens reveal vitellaria that encircle the segment, alternating with the longitudinal muscle bundles that divide the medullary region from the cortex (Fig. 2). As most of the 22 species of *Grillotia* (see Schmidt, 1986) are known only from larvae or immature adults, data on the vitellaria in other species of this genus are limited. Hart (1936) described vitellaria similar to that of *G. similis,* in *Grillotia musculara* (Hart, 1936) Dollfus, 1942, and Yamaguti (1959) published a cross section of *Grillotia erinaceus* (van Beneden, 1858) Guiart, 1927, illustrating this same configuration. But Hart (1936, p. 375)



Figures 5–9. Scanning electron micrographs of scolex of *Grillotia similis*. **5.** Internal surface of tentacle. Scale bar = 50 μm. Note hook-free region between hooks 1 and 1′. **6.** Metabasal hooks. Scale bar = 50 μm. Internal surface is to the left. **7.** Entire scolex. Scale bar = 200 μm. Note large posterior bothridial notch. **8.** Metabasal hooks on external surface of tentacle. Scale bar = 50 μm. Note the sinuous band of unlabeled small hooks

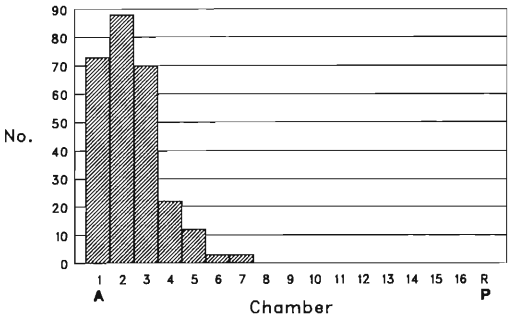


Figure 10. Distribution of 271 specimens of *G. similis* throughout the spiral valve chambers of 22 nurse sharks. Spiral valve chambers are numbered 1–16. R—rectum, A—anterior, P—posterior.

Table 1. Prevalence and intensity of *Grillotia similis* infections based on position in the gut in 22 nurse sharks.

Position (numbers indicate chambers of spiral valve)	Prevalence (%)	Intensity (mean no. worms/infected shark ± SD; range in parentheses)
1	59.1	5.6 ± 5.02 (1–17)
2	77.3	5.2 ± 5.21 (1–19)
3	54.5	5.8 ± 7.13 (1–24)
4	36.4	2.8 ± 2.9 (1–9)
5	27.3	2.0 ± 2.0 (1–6)
6	4.5	3.0 ± 0 (3)
7	13.4	1.0 ± 0 (1)
8–16	0.0	0.0
Rectum	0.0	0.0

also described vitellaria in *Grillotia megabothridia* (Hart, 1936) Dollfus, 1942, that "encircle the proglottids internal to the longitudinal muscle bundles" and are therefore medullary. In order to accommodate this variation we suggest that it would be best to describe the vitellaria of this genus as "encircling the segment, either internal to, or intermingled with the longitudinal muscle bundles dividing the cortex from the medulla."

The presence of a hermaphroditic sac in *G. similis* is worthy of some comment. A sac containing both the cirrus and the vagina, or a hermaphroditic sac, has been reported previously in *Lacistorhynchus* Pintner, 1913, *Mustelicolla* Dollfus, 1969, and *Callitetrarhynchus* Pintner, 1931 (see Beveridge and Sakanari, 1987; Campbell and Beveridge, 1988). Campbell and Beveridge consider this character to be of some taxonomic significance at the generic level. The segment morphologies of too few species of *Grillotia* have been examined in sufficient detail for the generality of this feature within the genus to be discussed; however, Dollfus (1942) described an organ that would appear to be a hermaphroditic sac (although he did not use that term) in *G. erinaceus* (van Beneden, 1858) Guiart, 1927, the type species of the genus.

Site specificity

The prevalence of *G. similis* in 22 nurse sharks was 81.8%. The overall mean intensity was 15.05 (±19.69) worms per infected shark with a range of 1–79. With respect to the 16 chambers of the nurse shark spiral valve, *G. similis* was restricted to the anterior 7, with a distinct preference for chambers 1–3. The spiral valve locations for the 271 specimens of *G. similis* recovered from 22 nurse sharks are given in Figure 10. Prevalence and mean intensity values, by spiral valve chamber, are given in Table 1. These results are consistent with the report of Linton (1908, p. 178) that, on 6 July 1906, 59 specimens of *R. simile* were found in "the upper part" of the spiral valve of a nurse shark.

Acknowledgments

We are grateful to W. and M. Servatt for collecting the nurse sharks used in this study, as well as for allowing us to use their backyard as a necropsy facility. In addition, we thank N. M. Caira, M. B. Caira, C. M. Tarca, and J. Ward for their assistance with collection of the tapeworms; J. R. Lichtenfels of the USNM and A. Petter of the MNHN for lending specimens; and G. W. Benz for his comments on an earlier version of this manuscript. Special thanks are extended to 2 anonymous reviewers for their helpful comments, as well as for indicating the location of Dollfus' type material of *G. simmonsi*. This work was supported by a grant from the University of Connecticut Research Foundation and grant no. BSR-8722468 from the National Science Foundation to J.N.C.

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occupying the center of the external face of the tentacle. Compare to figure 19 in Dollfus (1969). 9. Distinctive diamond pattern of 4 hooks at base of external surface of tentacle. Scale bar = 20 μm. Hooks in Figures 5, 6, and 8 are numbered according to the system of Dollfus (1969).

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J. Helminthol. Soc. Wash.
57(1), 1990, pp. 21–25

Pinworms from Water Scavenger Beetles (Coleoptera: Hydrophilidae) with a Description of a New Species, *Zonothrix columbianus* sp. n. (Oxyurida: Pseudonymidae), from Western Canada

MARTIN L. ADAMSON AND ADRIENNE BUCK
Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada V6T 2A9

ABSTRACT: *Zonothrix columbianus* sp. n. (Oxyurida: Pseudonymidae) is described from hydrophilid beetles in the lower mainland of British Columbia, Canada. The new species most closely resembles *Zonothrix adversa* in body shape, in having irregularly inflated annules in the cervical region, and by the shape of the female tail. It is distinguished by its shorter tail, the greater distance between the vulva and anus (20–28% of body length in the new species compared with 12–15% in *Z. adversa*), and by its longer spicule. The new species occurred in 172 of 211 *Tropisternus columbianus,* 3 of 5 *Tropisternus lateralis marginatus,* and 2 of 8 *Hydrobius fuscipes.*
KEY WORDS: pinworms, Oxyurida, entomophilic nematodes, *Zonothrix,* new species.

The Oxyurida is a major order of zooparasitic nematodes richly represented in arthropods and vertebrates. The broad host distribution of the group belies a rather narrow ecological specificity; they are found only in hosts in which the posterior gut is modified to form a fermentation chamber. Among arthropods, oxyuridans are most common in Diplopoda, Blattoida, and Gryllotalpoidea. The vast majority of species have been reported from terrestrial hosts in tropical and subtropical regions. The present communication describes a new species of Oxyurida from hydrophilid beetles in the lower mainland of British Columbia and represents the first report of an oxyuridan from Canadian insects.

Materials and Methods

Beetles were collected from drainage ditches and ponds in the lower mainland of British Columbia using long-handled nets and transported to the laboratory in polyethylene bags. The following hosts were examined: *Tropisternus columbianus, Tropisternus lateralis marginatus, Hydrobius fuscipes,* and *Enochrus lacustris.* Dr. Ales Smetana (Biosystematic Research Centre, Agriculture Canada, Ottawa, Ontario, Canada K1A 0C6) confirmed identifications of beetles.

Beetles were placed ventral side up in stacking dishes containing a small volume of 0.60% sodium chloride solution. The abdomen was separated from the thorax using fine forceps, and the intestine removed and opened using fine dissecting needles. Nematodes were fixed in hot glycerin–ethanol and cleared in lactophenol. Drawings were made with the aid of a drawing tube.

Description

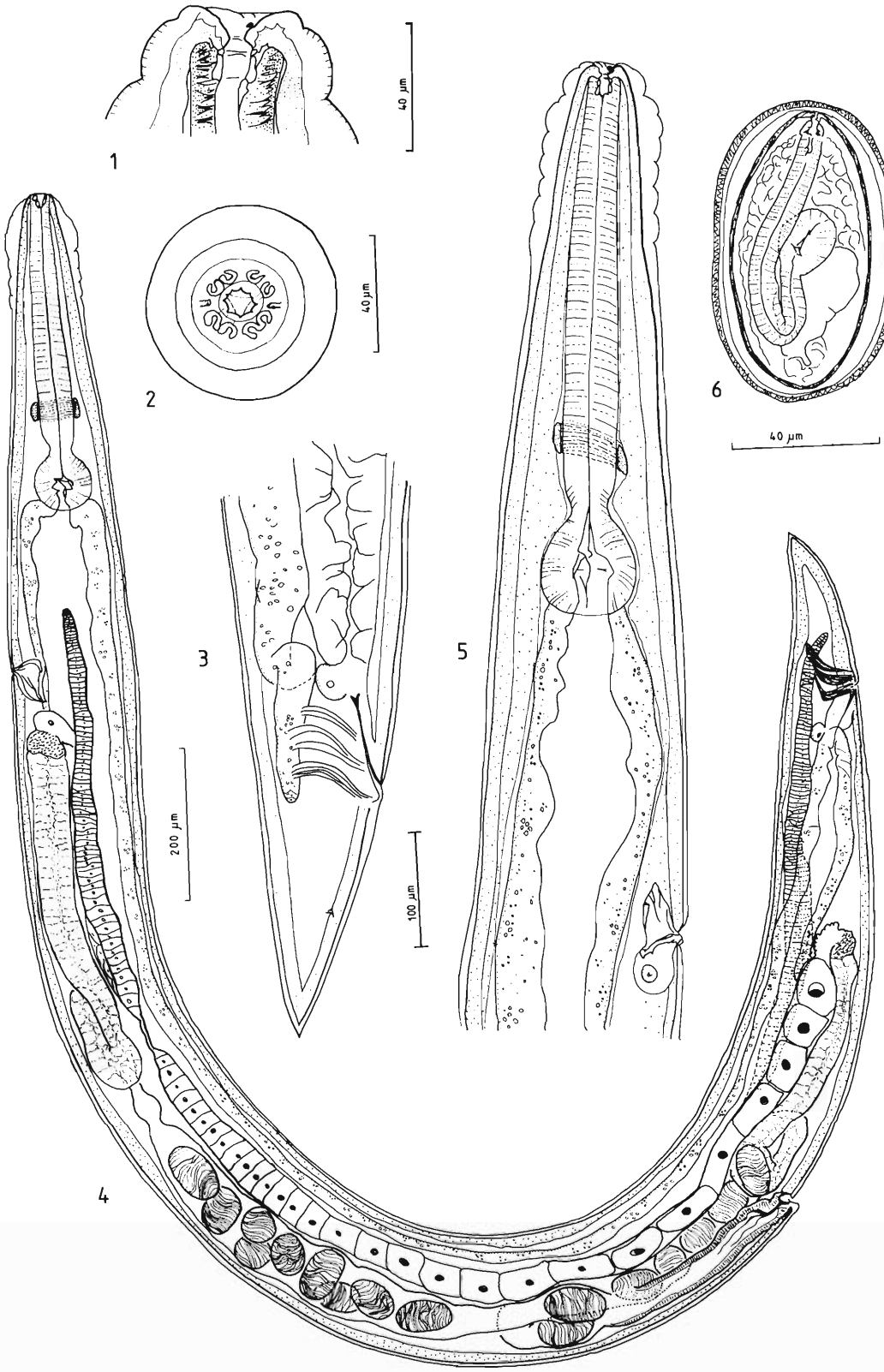
Zonothrix columbianus sp. n. (Figs. 1–12)

GENERAL (all measurements in μm unless otherwise stated): Oral opening polygonal with 8 submedian papillae and 2 lateral amphids. Inner papilla not observed.

MALE (range of 15 specimens, holotype in parentheses): Length 0.791–1.665 (1.463) mm. Maximum width 37–91 (67). Buccal cavity 8–14 (12) long, its posterior 4–7 (5) surrounded by esophageal tissue. Esophagus 163–255 (255) long including corpus 116–209 (197) and isthmus 16–20 (18) long, and valved bulb 27–42 (36) long and 25–42 (33) wide. Nerve ring 109–183 (183), excretory pore 200–385 (320), and anterior tip of testis 424–847 (806) from cephalic extremity.

Seven pairs of caudal papillae: 2 pairs preanal subventral; 1 pair subventral, 1 pair sublateral and 1 median duplex papillae immediately posterior to anus; 1 pair subventral and 1 pair sublateral, 7–11 (8) from caudal extremity. Spicule 23–27 (26) long. Tail roughly conical, 35–44 (35) long. Phasmids 12–21 posterior to anus.

FEMALE (range of 13 specimens, allotype in parentheses): Habitus C-shaped. Length 2.215–4.343 (4.343) mm. Maximum width 152–225 (225) near midbody. Buccal cavity 13–20 (20) long, its posterior 6–10 (10) surrounded by esophageal tissue. Body cuticle bearing faint transverse striations about 4 apart. Cuticle in cervical region inflated, forming 8–11 irregular annule-like swellings. Esophagus 365–471 (471) long including corpus 277–397 (397), and bulb 77–84 (84) long and 66–88 (88) wide. Nerve ring 250–349 (349), excretory pore 532–852 (852), anterior spermatheca 670–899 (899), vulva 2.097–3.033 (3.033) mm, and posterior spermatheca 2.457–3.794 (3.794) mm from cephalic extremity. Amphidelphic. Ovary associated with anterior uterus beginning just posterior to anus, extending anteriorly before flexing posteriorly at



spermatheca just behind excretory pore. Ovary associated with posterior uterus beginning just anterior to excretory pore, extending posteriorly and flexing anteriorly at spermatheca just anterior to anus. Oviducts coiling once before emptying into uteri. Anterior lip of vulva forming prominent projection. Tail subconical 180–303 (303) long. Phasmids 94–169 posterior to anus.

EGGS: Eggs 70–80 long and 40–45 wide, surrounded by 2 filaments originating at spherical structure on surface of eggshell. Eggs nearest vagina containing larva flexed once at midbody and once just behind tail. Larva contracting to form ovoid resting stage with thickened cuticle in eggs dissected from female and incubated for 3 days at room temperature.

Taxonomic Summary

Diagnosis

Aside from the present species, 8 species of *Zonothrix* have been described: *Z. tropisterna* Todd, 1942, from *Tropisternus nimbatus* in Nebraska, *Z. hydroi* (Galeb, 1878) Todd, 1942, from *Hydrous caraboides* in Europe, and *Z. adversa* Kloss, 1958, from *Tropisternus collaris, Z. galebi* Kloss, 1959, from *Neohydrophilus medius, Z. gladius* Kloss, 1959, from *Coleostoma luederwaldi, Z. helocharesae* Kloss, 1959, from *Helochares pallipes, Z. izecksohni* from *Tropisternus lateralis,* and *Z. paraense* Kloss, 1959, from *Tropisternus chalybeus* from Brazil. The present species most closely resembles *Z. adversa* in body shape, in having irregularly inflated annules in the cervical region, and by the shape of the female tail. The new species is distinguished by its shorter tail and by the greater distance between the vulva and anus (20–28% of body length in the new species compared with 12–15% in *Z. adversa*). The new species is further distinguished by its longer spicule.

HOLOTYPE MALE AND ALLOTYPE FEMALE: Collected from same host individual and stored in the National Museums of Canada Parasite Collection, Ottawa (NMCP 1989-0233, holotype; NMCP 1989-0234, allotype).

OTHER SPECIMENS: The following material was collected from the type host and locality. National Museums of Canada Parasite Collection, Ottawa (NMCP 1989-0235, 0236, 0238, 0240, males; NMCP 1989-0237, 0239, 0241, females). United States National Museum Parasite Collection, 4 vials, each with 1 male and 1 female (USNM 80860). Laboratoire de Zoologie (Vers), Museum national d'Histoire naturelle, Paris (Bocal N568: 63 HF-C 307, 64 HF-C 285, 65 HF-C 286).

HOSTS: *Tropisternus columbianus* (type host); *T. lateralis marginatus, H. fuscipes* (Hydrophilidae; Coleoptera) (Table 1).

LOCATION IN HOST: Worms were located just behind the level at which the Malpighian tubules enter the posterior gut and were oriented with their anterior extremities facing toward the midgut.

LOCALITY: Drainage ditches surrounding the Southlands Riding Stables, Vancouver, British Columbia, Canada (type locality); retention ponds of the Little Campbell River System, Whiterock, British Columbia, Canada.

Remarks

The Oxyurida parasites of Hydrophilidae are a well-defined clade characterized by having 7 pairs of caudal papillae in the male and spiral filaments on the eggshell. The first species described was *Pseudonymus spirotheca* (Gyory, 1856) Diesing, 1857 (=*Oxyuris spirotheca* Gyory, 1856, and *Ptychocephalus spirotheca* (Gyory, 1856) Diesing, 1860, from *Hydrous piceus* in Austria. Galeb's (1878) *Oxyuris* (*Helicothrix*) *spirotheca,* also from *H. piceus* in Europe, is not conspecific with Gyory's material. Kloss (1959) renamed it *Gyoryia europea,* type species of *Gyoryia* Kloss, 1959, but Leibersperger (1960) and Jarry (1964) consider it a synonym of *Pseudonymus islamabadi* (Basir, 1941) Basir, 1956. The first suprageneric taxon proposed for the group was Kloss' (1959) subfamily Gyoryiinae. However, because there is disagreement as to the validity of *Gyoryia,* Adamson (1980, 1989) proposed the Pseudonyminae and Pseudonymidae for these worms. The following genera are referable to the family: *Pseudonymus* Diesing, 1857, *Zonothrix* Todd, 1942, *Stegonema* Travassos,

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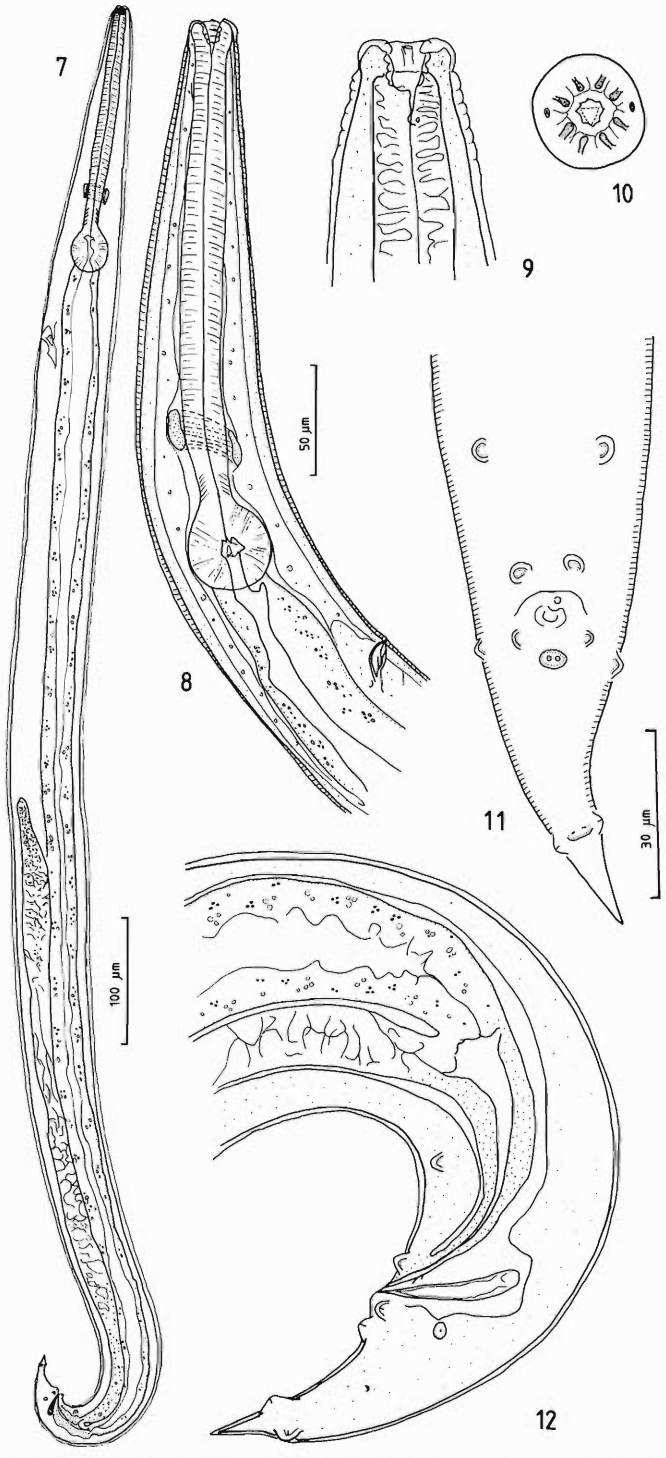
Figures 1–6. ***Zonothrix columbianus*** **sp. n., female. 1, 2. Cephalic extremity in lateral and apical views (scale = 40 μm). 3. Caudal extremity (scale = 100 μm). 4. Entire worm, lateral view (scale = 200 μm). 5. Esophageal region, lateral view (scale = 100 μm). 6. Egg containing infective larva after 4 days of incubation at 22°C; filaments on the egg shell are not depicted (scale = 40 μm).**



Figures 7–12. ***Zonothrix columbianus*** **sp. n., male. 7. Entire worm, lateral view (scale = 100 µm). 8. Esophageal region, lateral view (scale = 50 µm). 9, 10. Cephalic extremity in lateral and apical views (scale = 30 µm). 11, 12. Caudal extremity in ventral and lateral views (scale = 30 µm).**

Table 1. Prevalence (% of hosts infected) and intensity (number of worms per infected host) of *Zonothrix columbianus* in 4 species of Hydrophilidae (Coleoptera) from the lower mainland of British Columbia, Canada.

Host	Number examined	Number infected (%)	Intensity (range)
Tropisternus columbianus	211	172 (82)	1–7
T. lateralis	5	3 (60)	2–3
Hydrobius fuscipes	8	2 (25)	1–2
Enochrus lacustris	14	0 (0)	N/A

1954, *Gyoryia* Kloss, 1957, *Itaguaina* Kloss, 1959, and *Jarryella* Van Waerebeke and Remillet, 1973. Genera are distinguished on the basis of vulvar position and the form of cuticular annulations in the cervical region of the female.

Zonothrix Todd, 1942, was proposed to accommodate *Z. tropisterna* and was distinguished from its most closely related genus *Pseudonymus* in lacking inflated cervical annules and by the posterior position of the vulva. In fact, some *Zonothrix* spp. do have inflated annules but they are discontinuous and irregular in number and size; those of *Pseudonymus* spp. form a complete ring around the cervical region although they appear to vary in size and number (Jarry, 1964).

Only 1 of the 9 species of *Zonothrix* has been described outside of the New World, namely *Z. hydroi,* described by Galeb (1878). This species has not been redescribed and is considered a species inquirenda by Jarry (1964). Of the remaining species, 6 occur in Brazil and 5 are parasites of members of the genus *Tropisternus.*

In the following key to the species, zoogeographic localities and hosts are included as ancillary information; they are not considered key characters;

1(16). Body of female C-shaped after fixation. Maximum width greater than 5% total length.
2(3). Posterior end of corpus in female swollen, almost as broad as esophageal bulb. Ex: *Hydrous caraboides* in France ..... *Z. hydroi*
3(2). Posterior end of corpus in female much narrower than bulb.
4(5). Mature female less than 2.0 mm long. Ex: *Helochares pallipes* in Brazil ..... *Z. helocharesae*
5(4). Mature female 3.0 mm or larger.
6(15). Swollen annulations present in cephalic extremity of female.
7(8). Tail of female narrowing abruptly behind anus and continuing as spinelike caudal extension. Ex: *Coleostoma luederwaldi* in Brazil ..... *Z. gladius*
8(7). Tail of female not as above.
9(12). Caudal extremity of female terminating in short spinelike structure.
10(11). Caudal spine of female 26–35 μm long. Ex: *Neohydrophilus medius* in Brazil ..... *Z. galebi*
11(10). Caudal spine of female less than 10 μm long. Ex: *Tropisternus chalybeus* in Brazil ..... *Z. paraense*
12(9). Caudal extremity of female without spinelike appendage.
13(14). Distance between vulva and anus 20–28% total body length. Tail of female 5–8% body length. Ex: *Tropisternus columbianus, T. lateralis marginatus,* and *Hydrobius fuscipes* in Canada ..... *Z. columbianus*
14(13). Distance between vulva and anus less than 15% total body length. Tail of female about 10% body length. Ex: *Tropisternus collaris* in Brazil ..... *Z. adversa*
15(6). Swollen cuticular annulations absent on cervical region of female. Ex: *Tropisternus nimbatus* in the United States ..... *Z. tropisterna*
16(1). Body of female coiled one and a half times after fixation. Maximum width about 3.5% of total length. Ex: *Tropisternus lateralis* in Brazil ..... *Z. izecksohni*

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Redescription of *Chroniodiplogaster aerivora* (Cobb) gen. n., comb. n. (Rhabditida: Diplogasteridae) from Termites

GEORGE O. POINAR, JR.
Department of Entomology, University of California, Berkeley, California 94720

ABSTRACT: The nematode originally described as *Diplogaster aerivora* by Cobb is redescribed in the new genus *Chroniodiplogaster*. Diagnostic characters of the new genus include (1) the presence of 9 genital papillae, 3 of which are closely associated in a triplet set at the base of the tail, (2) 2 separate bursae, 1 which extends ventrolaterally along the tail, and the second, which is associated with the triplet papillae, and (3) a dorsal metarhabdion with a large mobile tooth and subventral metarhabdions with variably sized teeth. Dauer stages of *C. aerivora* occurred in the heads of worker termites of *Reticulitermes tibialis*. On occasion, the nematodes would enter the body cavity and kill the termites.

KEY WORDS: *Chroniodiplogaster aerivora* gen. n., comb. n., Diplogasteridae, Rhabditida, Nematoda, termite, parasite.

In 1916, Cobb described *Diplogaster labiata* and *Diplogaster aerivora* in a paper by Merrill and Ford (1916) who described the associations of these 2 nematodes with insects. In this study, the latter species was reported by Merrill and Ford (1916) to occur in the heads of the termite, *Leucotermes lucifugus,* with insect death resulting from high numbers of nematodes. Along with his description, Cobb also reported that *D. aerivora* fed on grasshopper eggs. Banks and Snyder (1920) reported juveniles of *D. aerivora* in the heads of active, normal-appearing *Reticulitermes flavipes* and adult nematodes in sick and dead insects.

Davis (1919) found *Mesodiplogaster* (=*Diplogaster*) *aerivora* in dead and dying larvae of the beetle, *Phyllophaga* sp., and suggested that the nematodes were the cause of death. Cobb identified *Mesodiplogaster aerivora* as the nematode responsible for natural mortality of corn earworm larvae, *Heliothis obsoleta* (Winburn and Painter, 1932). Lim et al. (1981) isolated *M. aerivora* from both living and dead grubs of the June beetle, *Phyllophaga anxia.* Recently, the present author received a culture of *M. aerivora* that was isolated from *Reticulitermes tibialis* in Colorado.

Recognition of *M. aerivora* is made difficult by the absence of detailed illustrations and quantitative data in the original description. Neither Weingärtner (1955) nor Goodey (1963) treated *M. aerivora* in their taxonomic presentation of the family Diplogasteridae and the species *M. aerivora* has been treated under the genera *Diplogaster, Micoletzkya,* and *Pristionchus.*

After a detailed morphological study of *M. aerivora,* the present author redescribes the species and places it in a new genus, *Chroniodiplogaster.*

Materials and Methods

Populations of *M. aerivora* studied here were isolated from the heads of worker termites (*R. tibialis*) (Banks) in Colorado in 1987 by John L. Capinera. They were maintained on nutrient agar plates and taxonomic studies were made on adults and dauer juveniles removed from these cultures. All nematodes were killed in hot (55°C) water, fixed in TAF, and processed to glycerin for measurements and drawings. Four of Cobb's original slides (nos. 1–4) containing adult male and female *M. aerivora* were received from A. Morgan Golden, Nematology Laboratory, U.S. Dept. of Agriculture, Beltsville, Maryland, for comparative purposes.

Results

Populations of nematodes removed from the heads of *R. tibialis* in Colorado agreed with the description of *M. aerivora* by Cobb (*in* Merrill and Ford, 1916) and were similar to Cobb's original specimens of *M. aerivora.* Therefore the Colorado population was considered to be *M. aerivora* Cobb.

This reexamination also revealed certain characters that made it difficult to place *M. aerivora* in any of the existing genera of Diplogasteridae. These characters consist of 9 pairs of genital papillae including a posterior set of 3, of which 2 are always reduced, a gubernaculum with ventral processes, which encloses the distal portion of the spicule shafts, a long narrow bursa associated with the 3 ventrolateral papillae, and a separate short bursa associated with the posterior set of

3 unequal papillae. The genus *Diplogaster* Schultze lacks a bursa, the gubernaculum does not encircle the spicule shafts, and there are 8 pairs of genital papillae without a posterior set of 3 (Goodey, 1963). The genus *Micoletzkya* (Weingärtner) contains 10 pairs of genital papillae with the first 2 pairs adjacent (Goodey, 1963). Members of the genus *Mesodiplogaster* Goodey (nec. Weingärtner; see Loof [1976]) possess 2 different stomal forms (Goodey, 1963). The genus *Pristionchus* Kreis, which was not mentioned by Goodey (1963), was originally described as having a small postanal bursa supported by 4 papillae (Kreis, 1933). However, Fedorko and Stanuszek (1971) show a long narrow bursa running posteriorly from the anus to the tail spike in *Pristionchus uniformis*. They illustrate 10 genital papillae pairs but state that only 9 pairs exist.

The generic status of the Diplogasteridae is in a state of confusion and the family needs to be revised. The present author prefers to redescribe the species *M. aerivora* Cobb in a new genus in the family Diplogasteridae, based on the discovery of new morphological characters not reported or seriously considered previously.

In the quantitative portion of the description, all figures are given in micrometers unless otherwise specified. The first number following the character is the mean and numbers in parentheses indicate the range.

Chroniodiplogaster gen. n. (Diplogasteridae (Micoletzky) Steiner)

DESCRIPTION: Medium-sized nematodes with smooth cuticle; 6 lips united at base but distinct at tip. Stoma massive, slightly longer than wide, variable in shape but with only a single basic form, dorsal metarhabdion with large mobile tooth, subventral metarhabdions variable; right subventral bearing tooth usually wide and pointed or notched at tip; left subventral bearing smaller pyramidal or lacinate tooth; median bulb of pharynx distinct, with strong crescent-shaped valves; female didelphic; female and male tails variable, short and acute or long and tapering or long and offset leading to filamentous tip; spicules large (length greater than body width at cloaca), slender, with small but distinct capitulum; gubernaculum with proximal (terminal) portion bent up and enclosing spicule tip region; with 9 pairs of genital papillae including 3 pairs located together in an off-ventral position near base of tail constriction; 2 bursae normally present; 1 extending ventrolaterally along tail region and second smaller ventral 1 associated with the triplet papillae at base of tail.

The genus *Chroniodiplogaster* can be separated from previously described genera in the Diplogasteridae having a similarly shaped stomal opening with the dorsal metarhabdion containing a distinct movable tooth by the normal presence of 2 separate bursae, a gubernaculum that surrounds the terminal spicular tips, and the presence of 9 basic genital papillae always including a postanal set of 3 (triplet).

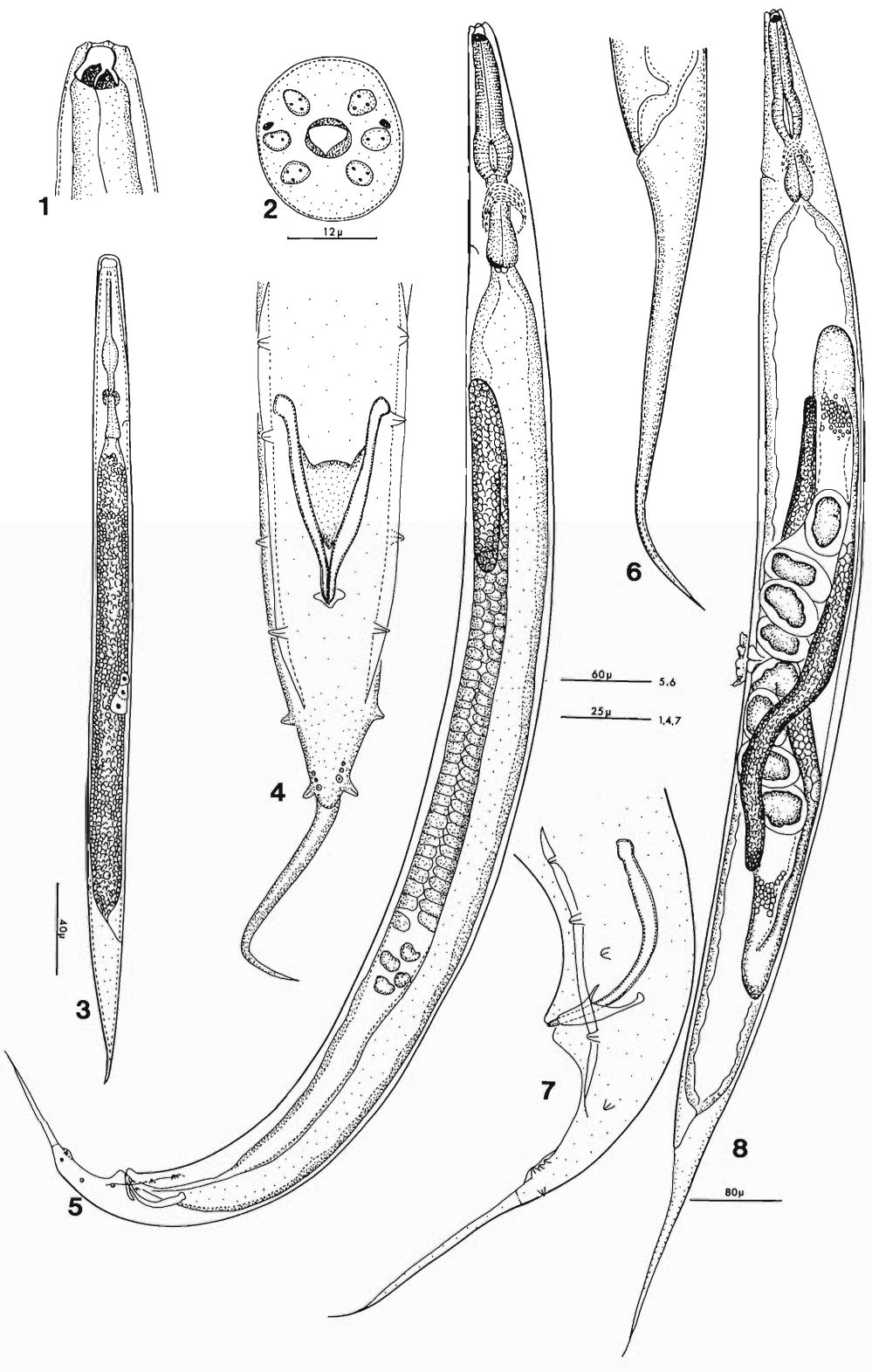
Chroniodiplogaster aerivora (Cobb) 1916 comb. n.

The following description also incorporates features cited by Cobb (*in* Merrill and Ford, 1916) in his original description.

DESCRIPTION: Adults: Cuticle transparent, with faint transverse striae and 24 longitudinal striae; lateral lines present; anterior end truncated; not offset, lips 6, fused at base, each bearing single inner labial papilla, submedial lips also bearing 2 additional papillae, lateral lips with single additional papilla, amphids located at base of lateral lips (on dorsal side), stoma walls highly cuticularized; dorsal metarhabdion with large protruding movable tooth, the inner contour of which fits into the contour of the protruding tooth on the right subventral metarhabdion, which may be notched at apex; left subventral metarhabdion bearing a smaller, conoid-lacinate tooth, which is closely associated with that of the right subventral metarhabdion: pharynx extending from base of stoma, gradually widening, until reaching swollen metacorpus containing elongated valvular apparatus, followed by isthmus surrounded by nerve ring; pharyngeal base expanding into pyriform bulb lacking distinct valve; excretory pore opening in vicinity of basal bulb; small, 2-celled pharyngeal–intestinal valve present; intestine forming narrow rectum before leading to anus or cloacal vent.

FEMALE (Figs. 1, 2, 6, 8): Vulva located in midbody region, roughly spherical in shape in ventral view; gonads paired and opposite with ovaries reflexed past vulvar opening; tail tapering gradually to fine hairline terminus; eggs ellipsoidal with smooth and relatively thick shell; embryonation occurs within female uterus.

MALE (Figs. 4, 5, 7): Similar to female in respect to cuticle, stoma, pharynx and intestine; tail tapering abruptly posterior to cloaca; tail tapering uniformly to small hairline terminus pos-



terior to triplet group of ventrolateral genital papillae, faint constriction present just posterior to triplet group of ventrolateral papillae; spicules slender, light brown, and curved almost to 90° angle, with distinct, but variably shaped capitulum, slender shaft and sharply pointed tips; gubernaculum with lateral crurae enclosing tips of spicules; extended proximal portion of corpus frequently, but not always, hooked; pair of ventral processes extending anteriorly from surrounding corpus-crurae portion; genital papillae consisting of 3 preanal pairs, 2 ventrolateral (submedian), 1 lateral (or sublateral), and 6 postanal pairs, 1 of which is ventrolateral just posterior to anus, another lateral and another dorsolateral just anterior to constricted tail area; another 3 pairs (triplet) are in a ventrolateral group just anterior to tail constriction; these latter 3 pairs of papillae not uniform in structure, the anterior 2 reduced and show little cuticular support with posterior pair larger; this set of 3 papillae is placed more anteriorly on left side than on right side of tail; single testis reflexed; 2 bursae present, ventrolateral bursa extends preanally and postanally in association with 3 ventrolateral papillae; another smaller bursa associated with posterior set of 3 (triplet) papillae.

Measurements

FEMALES (N = 10) (from nutrient agar cultures): Length, 1.39 (1.22–1.60) mm; greatest width, 82 (69–95); length stoma, 9 (6–11); width stoma, 7 (5–8); distance from anterior end to base of metacorpus, 116 (98–133); distance from anterior end to excretory pore, 159 (139–184); distance from anterior end to nerve ring, 144 (133–152); distance from anterior end to base of pharynx, 177 (152–200); length tail, 215 (162–273); width at anus, 36 (32–41); percent vulva, 49 (47–52); length of eggs in utero, 57 (48–64); width of eggs in utero, 32 (28–35).

MALES (N = 10) (from nutrient agar cultures): Length, 960 (832–1,120); greatest width, 62 (57–70); length stoma, 9 (8–10); width stoma, 7 (5–8); distance from anterior end to base of metacorpus, 104 (88–120); distance from anterior end to excretory pore, 152 (147–160); distance from anterior end to nerve ring, 119 (109–136); distance from anterior end to base of pharynx, 162 (142–181); reflexion of testis, 155 (120–192); length tail, 112 (88–138); width at cloaca, 36 (30–40); length tail posterior to constriction, 63 (35–90); length spicules, 56 (48–69); width spicules, 4 (2–4); length gubernaculum, 18 (14–22); width gubernaculum, 3 (2–4).

DAUER JUVENILES (Fig. 3) (N = 15): Length, 337 (284–397), greatest width, 15 (12–19); mouth and pharynx collapsed, head slightly offset, third-stage juvenile surrounded by second-stage cuticle.

Discussion

The population of *C. aerivora* redescribed here originated from colonies of *R. tibialis.* Dauer stages occurred in the heads of workers and when the termites were stressed or weakened, the nematodes entered the insect's body cavity and initiated development (Capinera, pers. comm.). The original population of *C. aerivora* was recovered from the heads of *L. lucifugus* where they occurred in the immediate region of the mouth parts and in the upper part of the head cavity (Merrill and Ford, 1916). The latter authors mentioned that the nematodes were usually found in masses, feeding upon the bodies of dead termites or other available decaying matter. In laboratory infection experiments where *L. lucifugus* workers were placed in moist soil containing cultures of the nematodes, the average number of nematodes in each termite head increased from 3 (natural infection) to 46 over a period of 4 days. After 12 days, all the termites had died and their bodies were being consumed by nematodes. Nutrients were probably obtained from bacteria and insect breakdown products.

In studies on *C. aerivora* in the scarabaeid beetle, *Phyllophaga anxia,* by Lim (1979) and Lim et al. (1981), nematodes were recovered from moribund field-collected second- and third-instar grubs. Measurements provided by Lim (1979) of the white grub population of *C. aerivora* agree in general with the measurements cited in the present study. However, Lim (1979) recorded a female tail length of 169 (128–176) (N = 10), which is somewhat shorter than that of the termite population. Also the distance from the anterior end to the base of the pharynx was given as 185 (176–191) (N = 4) for the male and 194

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Figures 1–8. ***Chroniodiplogaster aerivora*** **(Cobb): 1. Lateral view of female head. 2. En face view of female. 3. Dauer juvenile. 4. Ventral view of male tail. 5. Male. 6. Lateral view of female tail. 7. Lateral view of male tail. 8. Female.**

(178–223) (N = 10) for the female of the white grub population (Lim, 1979), which are larger values than noted in the present study. These quantitative differences could be attributed to strain differences or availability of nutrients, and both nematodes are considered the same species by the present author.

Detailed studies on events leading to the actual infection and death of any insect with *C. aerivora* are lacking. Earlier studies with *Mesodiplogaster lheritieri* (Maupas) and *Pristionchus uniformis* Fedorko and Stanuzek indicated that mortality of *Galleria mellonella* larvae resulted from reproducing nematodes breaking through the intestinal wall and entering the hemocoel (Poinar, 1969). Thus, these latter nematodes as well as *C. aerivora* are facultative parasites (Poinar, 1972) whose dauer stages enter the natural openings of insects and remain quiescent or begin to develop in the alimentary tract, head glands, or other body areas. This behavior causes the rupture of protective membranes, allowing the nematodes, together with their microbial associates, to enter the insect's hemocoel, resulting in immediate or eventual death. Insects undergoing stress from starvation, physical factors, or other disease-producing agents are probably more susceptible to infection by *C. aerivora* and related species. In the case of *C. aerivora* and termites, the nematodes may remain for relatively long periods of time in the head glands of the insects before initiating development and invading the hemocoel. Thus the insect may also serve as a refuge against adverse environmental conditions as well as being a potential food source and/or culture medium for edible bacteria.

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Errata

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

July 1989, 56(2):201–203, in the article by Canals and Gasbarre:

In Table 1, all the protein concentrations of the ES given as mg/ml should be μg/ml.

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Parasites of Summer Flounder, *Paralichthys dentatus*, in the Chesapeake Bay

MAURA E. JANSEN AND EUGENE M. BURRESON[1]
School of Marine Science, College of William and Mary, Gloucester Point, Virginia 23062

ABSTRACT: A total of 38 species of parasites was collected from 341 summer flounder, *Paralichthys dentatus*, in the Chesapeake Bay. The parasites included 8 species of protozoans, 1 monogenean, 11 digeneans, 2 acanthocephalans, 1 copepod, 1 branchiuran, 1 leech, 10 cestodes, and 4 nematodes. Of the 38 parasites, only 18 species were found in more than 10% of the hosts. Protozoans included the flagellates *Cryptobia* sp. from the gills and *Trypanoplasma bullocki* from the blood, the myxozoan *Davisia branchiophora* from the gall bladder and the ciliate *Trichodina* sp. from the gills. The monogenean *Neoheterobothrium affine* occurred on the gills, and digeneans included *Stephanostomum dentatum* and *Opecoeloides vitellosus* from the intestine and *Stephanostomum tenue* encysted in the gills. The acanthocephalan *Serrasentis sagittifer* was encapsulated in the mesentery. Metacestodes included *Nybelinia bisulcata* encapsulated in the intestinal wall, *Grillotia smarisgora* encapsulated in the mesentary, *Rhinobothrium* sp. in the intestine, and 3 different forms of the group *Scolex pleuronectis*, 1 in the intestine, 1 in the gall bladder, and 1 encapsulated in the gills. Other abundant parasites included the nematodes *Dichelyne cylindricus* in the intestine and a juvenile *Hysterothylacium* sp. encapsulated in the mesentery, and the branchiuran *Argulus chesapeakensis* on the skin. Parasite abundance was analyzed with respect to host size, season, and host migration into and out of Chesapeake Bay. Some parasites, such as *Bucephalopsis paralichthydis* and *A. chesapeakensis*, were clearly acquired in the Bay during summer, whereas *Bothriocephalus scorpii*, *Hysterothylacium habena*, *Acanthochondria galerita*, and *Rhinobothrium* sp. appeared to be acquired offshore. *Cryptobia* sp. and *T. bullocki* were more prevalent in fish less than 300 mm total length (TL), whereas *S. dentatum*, the various *S. pleuronectis* types, *Rhinobothrium* sp., *D. cylindricus*, and the encysted species were more prevalent in fish greater than 300 mm TL.

KEY WORDS: *Paralichthys dentatus*, parasites, Chesapeake Bay, seasonality, Protozoa, Monogenea, Digenea, Cestoda, Nematoda, Acanthocephala, Branchiura, Copepoda, Hirudinea.

The summer flounder, *Paralichthys dentatus* (L.), is an important commercial and recreational species along the middle Atlantic coast of the United States. The species ranges from Nova Scotia to Florida (Guntherz, 1967) but it is most abundant between Cape Cod, Massachusetts, and Cape Fear, North Carolina. Adult summer flounder normally inhabit coastal and estuarine waters during the warmer months and remain offshore in 36–182 m of water during the fall and winter (Bigelow and Schroeder, 1953; Rogers and Van Den Avyle, 1983). Mature individuals migrate out of Chesapeake Bay in October and spawning takes place on the bottom as fish migrate to their overwintering grounds on the continental shelf (Morse, 1981). Eggs and larvae rise and drift inshore to coastal and estuarine nursery areas (Smith, 1973). Juvenile flounder remain in nursery areas until October of their second year of life (Powell and Schwartz, 1977). At that time, at least in Chesapeake Bay, a portion of these juveniles migrates out of the estuary and overwinters in nearshore waters along the coasts of Virginia and North Carolina. In April each year fish return to shallow coastal waters and estuaries (Hildebrand and Schroeder, 1928).

The food habits of summer flounder have not been extensively studied. The investigations that have been conducted suggest that fish less than 300 mm total length feed predominantly on crustacea and small fish, whereas larger flounder consume higher percentages of large fish and squid (Smith and Daiber, 1977; Powell and Schwartz, 1979; Langton and Bowman, 1981).

The parasite fauna of summer flounder is not well known. There has been no thorough survey of summer flounder parasites, but summer flounder were included in fish parasite surveys conducted by Linton (1889, 1897, 1898, 1900, 1901, 1905, 1940) from fish collected at Woods Hole, Massachusetts, and Beaufort, North Carolina, by Davis (1917) and Manter (1931) at Beaufort, and by Meyers (1978) from frozen samples originally collected in Raritan Bay, New Jersey. It is not clear if the flounder studied by Linton (1905) from North Carolina were summer flounder or the closely related southern flounder, *Paralich-*

[1] Author to whom reprint requests should be addressed.

thys lethostigma Jordan and Gilbert. Various parasites of summer flounder have been reported in other studies, including myxozoa by Walford (1958), hematozoa by Laird and Bullock (1969), Burreson and Zwerner (1982), and Khan and Newman (1982), gill flagellates by Burreson and Sypek (1981), nematodes by Cheng (1976), branchiurans by Cressey (1978), leeches by Burreson and Zwerner (1982), and a digenean by Hunninen and Cable (1941).

The purpose of this study was to survey the parasites of summer flounder collected from the Chesapeake Bay region and to attempt to relate parasite fauna to host size and migratory patterns.

Materials and Methods

Summer flounder were collected monthly from the lower York River and lower Chesapeake Bay, Virginia, by otter trawl between April 1980 and October 1982. Overwintering juveniles were also collected 1.5 km off Virginia Beach, Virginia, from October through March. Samples of migrating flounder returning to the Bay were collected near the mouth of Chesapeake Bay during April and May each year, and samples of flounder leaving the Bay were collected there during September and October. Based upon published feeding and migratory habits, hosts were divided into 2 groups designated juveniles (<300 mm) and adults (>300 mm).

Live flounder were brought to the laboratory, held in flowing seawater tanks, and examined for parasites within 5 days of capture. One drop of blood from the caudal vessels was mixed with a drop of 0.6% saline and examined at 100× for the presence of flagellates. Permanent blood smears were stained in Giemsa and scanned for protozoa at 400×. The external surfaces were examined for parasites with the unaided eye. Individual gill arches were placed in sea water and examined with a dissecting microscope. Filaments from 1 arch were scraped onto a glass slide and examined at 400× for protozoa. Permanent smears were treated as above for blood smears. The liver, spleen, gonads, musculature, and internal and external surfaces of the gastrointestinal tract were examined with a dissecting microscope. Bile and urine were examined at 400×; permanent smears were treated as blood smears. Protozoa were enumerated on a subjective scale from 1 to 4 representing light to heavy infections. Helminths, excluding nematodes, were relaxed in distilled H_2O and fixed in AFA; crustaceans were fixed unrelaxed in AFA; and nematodes were fixed unrelaxed in glacial acetic acid. Specimens were stored in 70% ethanol. Most helminths were stained in Semichon's acetocarmine, cleared in methyl salicylate, and mounted in permount. Crustaceans were stained with picric acid fuchsin and mounted in Hoyer's medium ringed with Zut. The terms prevalence, mean intensity, and abundance follow the definitions of Margolis et al. (1982).

Gill filaments with encysted parasites and portions of operculum with embedded monogeneans were fixed in 10% buffered formalin, decalcified in HCl, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin.

Differences in parasite prevalence and mean intensity among season, sex, size, and migrating fish were tested using 2 × 2 contingency tables and Chi-square analyses. The Yates correction for continuity was applied when expected frequencies were small. Values were calculated only for those parasites that occurred in at least 10% of the hosts. All statistical tests of significance were made with alpha = 0.05.

Results and Discussion

A total of 341 summer flounder was examined for parasites; 260 juveniles (<300 mm) and 81 adults (>300 mm) were collected. Twenty-three adults were collected as they returned to the Bay in the spring, 37 adults were collected during summer in the Bay, and 21 adults were collected as they migrated out of the Bay in the fall.

A total of 38 species of parasites was collected (Table 1); 20 of these, indicated by asterisks, are new host records. Twenty of the species were found in fewer than 10% of the hosts and most of these species will not be considered further in this report. There was no difference in prevalence and intensity between male and female fish for any parasite.

Morphology of *Davisia brachiophora* differed from that of the original description (Davis, 1917). Spore appendages varied greatly in length from 10 to 100 μm as opposed to 18–22 μm in the original description. In addition, many spores had long threadlike filaments, up to 100 μm long, projecting from the ends of the appendages. These filaments were not mentioned in the original description, but figure 113 (Davis, 1917) shows a short projection from one of the appendages that may be a broken filament. *Davisia longibrachia* Kabata also has threadlike lateral appendages, but this species has gradually tapering appendages (Kabata, 1962), whereas *D. brachiophora* has blunt appendages.

Stephanostomum dentatum was the most abundant digenean and more data were collected for this species (Table 2) than for others. Preserved individuals ranged in length from 0.3 to 4.1 mm. The smallest worm observed with eggs was 0.9 mm long. During spring, most individuals were small and immature; mean size and egg production increased through summer and fall, although new infections continued to be acquired. Egg production and acquisition of worms ceased during winter, and most, if not all, of the worms were lost.

All cestodes collected were plerocercoids ex-

Table 1. Parasites of summer flounder ($N = 341$) in the Chesapeake Bay.

Parasite	Location	Peak abundance	Prevalence %	USNM No.
Sarcomastigophora				
Amyloodinium sp.*	G	S	2.9	
Cryptobia sp.	G	S	38.7	
Trypanoplasma bullocki (Strout, 1965)	B	W	26.7	80707
Ichthyobodo sp.*	G	S	0.9	
Hexamita sp.*	GB	S	1.2	
Apicomplexa				
Haemogregarina platessae Lebailly, 1904	B	YR	5.9	80708
Myxozoa				
Davisia branchiophora (Davis, 1917)*	UB	YR	17.6	
Ciliophora				
Trichodina sp.*	G	S	17.0	
Monogenea				
Neoheterobothrium affine (Linton, 1898)	G, M	S	11.4	80709
Digenea				
Bucephalopsis paralichthydis (Corkum, 1961)*	RM	S	6.7	80710
Stephanostomum dentatum (Linton, 1900)	I	S	37.5	80711
Stephanostomum tenue (Linton, 1898)*	G (E)	S	10.0	80712
Lepocreadium setiferoides (Miller and Northup, 1926)	I	R	0.3	80713
Lepocreadium areolatum (Linton, 1900)*	I	R	0.6	80714
Opecoeloides fimbriatus (Linton, 1934)*	I	R	0.6	80715
Opecoeloides vitellosus (Linton, 1900)	I	S	13.5	80716
Parahemiurus merus (Linton, 1910)*	ST	R	1.5	80717
Lecithochirium synodi Manter, 1931	ST	R	1.2	80718
Hirudinella ventricosa (Pallas, 1774)*	ST	R	0.3	80719
Microphallus turgidus (Leigh, 1958)*	ST	R	0.3	80720
Acanthocephala				
Serrasentis sagittifer (Linton, 1889)	I, MS (E)	YR	10.0	80721
Dollfusentis chandleri Golvan, 1969*	I	R	0.9	80722
Cestoda				
Nybelinia bisulcata (Linton, 1889)	I (E)	S	10.9	80723
Grillotia smarisgora (Wagener, 1854)*	MS (E)	S	19.6	80724
Bothriocephalus scorpii (Mueller, 1776)	I	S	4.4	80725
Scolex pleuronectis type A	I	S	50.7	80726
Scolex pleuronectis type B	I	W	6.5	80727
Scolex pleuronectis type C	GB	S	34.6	80728
Scolex pleuronectis type D*	G (E)	S	12.0	
Ceratobothrium xanthocephalum Monticelli, 1892*	I	YR	7.9	80729
Rhinobothrium sp.*	I	S	10.0	80730
Nematoda				
Dichelyne cylindricus Chandler, 1935	I	S	13.5	80731
Capillaria sp.*	I	S	4.7	80732
Hysterothylacium habena (Linton, 1900)*	I	S	5.0	80733
Hysterothylacium type A*	MS (E)	YR	27.6	80734
Hirudinea				
Calliobdella vivida (Verrill, 1872)	SK	W	1.2	80735
Branchiura				
Argulus chesapeakensis Cressey, 1971	SK	S	12.9	80736
Copepoda				
Acanthochondria galerita (Rathbun, 1886)	MS	S	4.7	

* = New host record, B = blood, (E) = encysted, G = gills, GB = gall bladder, I = intestine, M = mouth, MS = mesentery, R = rare, RM = rectum, S = summer, SK = skin, ST = stomach, UB = urinary bladder, W = winter, YR = year-round.

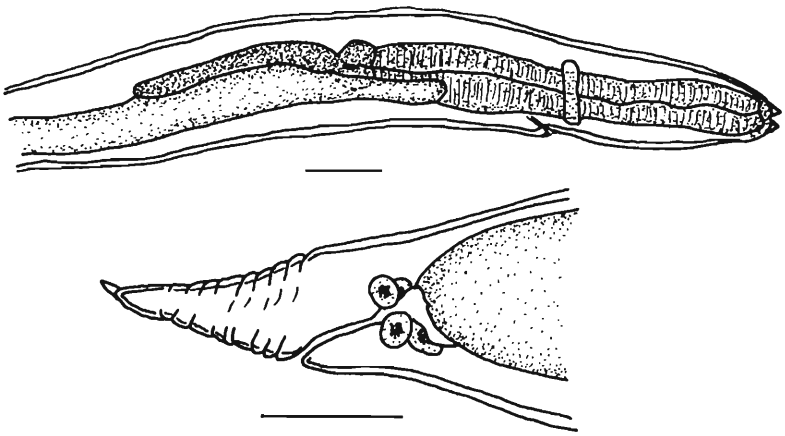

Figure 1. Anterior and posterior extremities of *Hysterothylacium* type A. Scale bars = 1.0 mm.

cept *Bothriocephalus scorpii*. *Scolex pleuronectis* encompasses a wide variety of metacestodes having 4 bothridia and an apical sucker. Four different types were determined based on morphology and location in the host (Table 1). Types A, B, and C appear identical to *Echeneibothria* sp. from summer flounder illustrated by Linton (1897) in plate LXI, figures 4, 10, and 12, respectively. It is not known if these types represent separate species or only different developmental stages of the same species. Type D was distinguished by its location embedded in host gills.

The juvenile nematode *Hysterothylacium* type A has a body 11–22 mm long by 0.2–0.4 wide; cuticular alae lacking; cuticle with inconspicuous annules becoming prominent on tail; single boring tooth; esophagus 2.3–4.3 mm long, 19.5–21.9% of body length; ventriculus 0.40–0.43 mm long; ventricular appendage 5.4–8.0 mm long; intestinal cecum 2.8–3.5 mm long; ratio of cecal to ventricular appendage 1:1.9–2.3; nerve ring in anterior 28–39% of esophagus, 0.15–0.22 mm in breadth; tail with single spine at tip (Fig. 1). This nematode is similar to *Hysterothylacium* type HA of Deardorff et al. (1982), except that it has a longer esophagus and cecal appendage and the tail has marked caudal annulations. *Hysterothylacium* type A also resembles *Hysterothylacium* type MD of Deardorff and Overstreet (1981) except that type A is longer, the nerve ring is more anterior, and the excretory port opens caudad to the nerve ring. Type A also has a smaller cecal to ventricular appendage length ratio and has marked cuticular annulations on the tail.

Seasonality

The seasonal variation in parasitism in summer flounder is shown in Table 3 and, for young-of-the-year fish, in Table 4. The data from Table 4 are also included in Table 3. Seasonality was similar in the York River and at the lower Bay station, reflecting the widespread distribution of the host as well as similar environmental conditions between the 2 areas. Only *Cryptobia* sp. was more prevalent in the York River. Most parasites, even the encapsulated cestodes, were more

Table 2. Seasonal changes in egg production, maturity, size, prevalence, and intensity of *Stephanostomum dentatum* in summer flounder from the Chesapeake Bay.

	Sample month			
	Apr–Jun	Jul–Sep	Oct–Nov	Dec–Mar
No. worms sampled	123	151	101	25
Percent mature	10.6	54.4	91.1	100.0
Mean number of eggs	5.1	13.0	26.6	0.0
Mean worm length (mm) ± SD	0.75 ± 0.25	1.00 ± 0.65	2.08 ± 2.00	1.11 ± 0.15
Prevalence (%)	35.6	56.3	46.3	9.8
Mean intensity	11.6	14.4	14.7	2.6
No. hosts sampled	105	119	41	76

Table 3. Seasonal prevalence (P%) and mean intensity (I) of parasites of summer flounder in the Chesapeake Bay.

Parasite	Apr–Jun				Jul–Oct				Nov–Mar			
	P		I		P		I		P		I	
	J (70)*	A (35)	J	A	J (100)	A (46)	J	A	J (90)	A (0)	J	A
Cryptobia sp.	58.6	25.7	2.8	2.6	60.0	19.6	2.0	1.4	21.1	—	2.4	—
Trypanoplasma bullocki	18.6	0.0	2.1	0.0	2.0	0.0	1.0	0.0	80.0	—	2.5	—
Trichodina sp.	14.3	28.6	1.9	1.9	23.0	19.6	1.5	1.6	6.7	—	2.0	—
Neoheterobothrium affine	20.0	14.3	1.4	1.6	3.0	4.3	1.0	1.0	16.7	—	1.2	—
Opecoeloides vitellosus	14.3	25.7	6.0	2.1	24.0	4.3	1.0	1.0	8.9	—	2.9	—
Stephanostomum dentatum	21.4	62.9	15.3	7.7	46.0	76.0	7.0	25.1	10.0	—	2.6	—
Stephanostomum tenue	12.9	37.1	18.3	17.3	3.0	15.2	5.0	132.0	0.0	—	0.0	—
Serrasentis sagittifer	1.4	40.0	4.0	2.7	1.0	34.8	3.0	4.1	2.2	—	1.0	—
Nybelinia bisulcata	15.7	28.6	7.5	10.3	6.0	13.0	10.0	12.7	4.4	—	5.3	—
Grillotia smarisgora	10.0	57.1	8.1	5.2	13.0	2.2	9.2	9.6	3.3	—	3.0	—
Scolex pleuronectis type A	54.3	94.3	27.0	55.1	43.0	43.5	27.5	40.4	43.3	—	9.0	—
Scolex pleuronectis type C	15.7	60.0	23.6	49.9	44.0	82.6	27.5	24.8	4.4	—	11.5	—
Scolex pleuronectis type D	4.3	37.1	44.7	78.9	1.0	47.8	1.0	262.1	0.0	—	0.0	—
Ceratobothrium xanthocephalum	5.7	17.1	9.0	33.3	5.0	6.5	23.8	14.7	0.0	—	0.0	—
Rhinobothrium sp.	15.7	57.1	7.1	6.9	2.0	0.0	1.8	0.0	2.2	—	1.5	—
Dichelyne cylindricus	4.3	20.0	2.0	5.9	11.0	52.2	1.8	2.2	2.2	—	1.5	—
Hysterothylacium sp. type A	12.9	37.1	9.8	5.5	32.0	28.3	3.8	9.2	28.9	—	4.5	—
Argulus chesapeakensis	2.9	0.0	1.0	0.0	30.0	26.1	3.3	3.8	0.0	—	0.0	—

* J = fish <300 mm (juveniles), A = fish >300 mm (adults); sample size shown in parentheses. No adult flounder occur in the Bay during winter.

prevalent during spring and summer; however, there was no seasonality in the abundance of the encapsulated acanthocephalan *Serrasentis sagittifer,* and the hemoflagellate *Trypanoplasma bullocki* was most abundant during winter. *Trypanoplasma bullocki* is known to be transmitted by the leech *Calliobdella vivida,* which is only present during winter (Burreson and Zwerner, 1982). This accounts for the peak prevalence of *T. bullocki* in juveniles during winter. Adult flounder are not present in the estuary during winter and, thus, are never exposed to *T. bullocki.* In addition, as the water temperature increases in late spring, flounder are able to eliminate the parasite (Burreson and Frizzell, 1986). *Neoheterobothrium affine* was not recovered from any flounder from September through November. Immature worms with developing opisthaptor clamps were found on gills from December through March. After all 4 pairs of clamps had developed, worms migrated to the oral cavity and matured while partially embedded in the oral mucosa and musculature. No *Rhinobothrium* sp. plerocercoids were found after June in fish from the Bay. The infected juvenile fish (Table 3) were all collected in nearshore waters off Virginia Beach from October through March. Seasonality was not as pronounced in the parasite fauna of young-of-the-year fish (Table 4) except for the blood protozoans *T. bullocki* and *Haemogregarina platessae.* Lack of seasonality in the other parasites may be because young-of-the-year fish have lived only in the estuarine system.

Effect of host size

The size (age) of a host can influence the parasite fauna through changes in diet, habitat, or immune competency. Parasites of 2 size groups of summer flounder are listed in Table 3; in addition, parasites of young-of-the-year fish are listed in Table 4. *Cryptobia* sp. and *T. bullocki* were more prevalent in smaller fish, reflecting habitat and, at least for *T. bullocki,* immune competence of the host. *Stephanostomum dentatum, Stephanostomum tenue, Nybelinia bisulcata, S. pleuronectis* types A, C, and D, *Rhinobothrium* sp., *Dichelyne cylindricus,* and *S. sagittifer* were more prevalent in large fish. Higher prevalence in large fish may result from an increasing occurrence of fish in the diet of larger hosts (Powell and Schwartz, 1979) and, for the larval forms, increased time for accumulation of worms. For example, known second intermediate hosts (Stunkard, 1961) for *S. dentatum* are

Table 4. Seasonal prevalence and mean intensity of parasites from young-of-the-year (<180 mm) summer flounder in the Chesapeake Bay.

Parasite	Oct–Mar $N = 62$		Apr–Sep $N = 52$	
	Prevalence	Intensity	Prevalence	Intensity
Cryptobia sp.	21.0	2.8	61.5	2.5
Trypanoplasma bullocki	66.1	2.4	1.9	1.0
Haemogregarina platessae	11.3	1.2	0.0	0.0
Davisia branchiophora	12.9	2.4	13.5	1.8
Trichodina sp.	9.7	2.0	15.4	1.7
Neoheterobothrium affine	22.6	1.1	7.7	1.0
Bucephalopsis paralichthydis	6.5	76.5	5.8	9.0
Opecoeloides vitellosus	6.5	3.5	11.5	3.0
Stephanostomum dentatum	11.3	4.0	13.5	3.6
Scolex pleuronectis type C	35.5	5.7	11.5	3.0
Hysterothylacium type A	27.4	4.5	9.6	2.6

the teleosts *Fundulus heteroclitus* and *Menidia menidia,* both common inhabitants of Chesapeake Bay. Larger flounder may feed more readily on these fishes and, thus, have a higher prevalence and intensity of *S. dentatum* (Table 3). The abundance of *Opecoeloides vitellosus,* on the other hand, appeared to be independent of host size. Metacercariae of this digenean encyst in the hemocoels of some marine amphipods. Relatively high prevalence of this worm in large flounder during spring indicates that these fish are feeding on amphipods.

Table 5. Prevalence (%) of parasites in adult summer flounder (>300 mm) migrating into Chesapeake Bay from offshore (Apr–May), within Chesapeake Bay (Jun–Aug), and migrating out of Chesapeake Bay (Sep–Oct).

Parasite	Apr–May $N = 23$	Jun–Aug $N = 37$	Sep–Oct $N = 21$
Cryptobia sp.	30.4	21.6	14.3
Trichodina sp.	39.1	16.2	19.0
Neoheterobothrium affine	13.0	10.8	0.0
Bucephalopsis paralichthydis	0.0	10.8	14.3
Opecoeloides vitellosus	30.4	5.4	9.5
Stephanostomum dentatum	73.9	62.2	81.0
Stephanostomum tenue	39.8	21.6	19.0
Serrasentis sagittifer	56.5	8.1	66.7
Nybelinia bisulcata	39.1	13.5	9.5
Grillotia smarisgora	73.9	40.5	57.1
Bothriocephalus scorpii	26.1	0.0	0.0
Scolex pleuronectis type A	47.8	83.8	81.0
S. pleuronectis type B	8.7	2.7	0.0
S. pleuronectis type C	91.3	59.5	47.6
S. pleuronectis type D	30.4	37.8	66.7
Rhinobothrium sp.	60.9	51.4	0.0
Ceratobothrium xanthocephalum	17.4	10.8	4.8
Dichelyne cylindricus	17.4	51.4	38.1
Hysterothylacium habena	30.4	0.0	0.0
Hysterothylacium type A	47.8	29.7	19.0
Argulus chesapeakensis	0.0	16.2	28.6
Acanthochondria galerita	17.4	16.2	0.0

Effect of host migration

The prevalence of parasites in adult flounder migrating into and out of Chesapeake Bay, as well as of those residing in the Bay during summer, is shown in Table 5. No definite conclusions can be reached about offshore or estuarine origin of parasites whose prevalence was relatively constant in all 3 groups of fish, e.g., *S. dentatum* or *S. pleuronectis* type A. Parasites with such even distributions may be acquired both offshore and in the Bay, or they may be long-lived parasites acquired in only one, or both, areas. Two parasites, *Bucephalopsis paralichthydis* and *Argulus chesapeakensis,* were clearly acquired in the Bay based upon their absence in April and May samples (incoming hosts) and their presence in summer (Bay) and fall (departing hosts) samples. Those parasites present in April/May and summer samples, but absent in fall samples, appear to be acquired offshore. Older flounder migrate into the Bay already parasitized with these species and gradually lose the parasites during summer without reacquisition. Thus, they are no longer parasitized by these species when they migrate out of the Bay during fall. Parasites such as *Rhinobothrium* sp., *B. scorpii,* and *H. habena* are lost quickly, whereas others, such as *Acanthochondria galerita,* are lost more slowly (Table 5). However, absence in fall samples does not necessarily mean the parasite is acquired only offshore. For example, *N. affine* is absent in fall samples but is present in young-of-the-year fish,

which have never migrated out of the Bay. This parasite appears to have an annual cycle with death of all worms by August. Thus, absence of this parasite in the fall is a function of the timing of its life cycle and not its area of acquisition.

Host specificity

Four groups of parasites can be identified based on their affinity for summer flounder. The first group consists of parasites found only in summer flounder; the nematode *D. cylindricus* is the only parasite in this group. The second group is found in association with summer flounder or closely related pleuronectiform fishes. Parasites include *D. branchiophora, B. paralichthydis, A. galerita,* and *N. affine.* The third group consists of parasites with little host specificity that can be recovered from a wide variety of unrelated fish species. Included are *Cryptobia* sp., *Trichodina* sp., *T. bullocki, S. dentatum, S. tenue, O. vitellosus, Ceratobothrium xanthocephalum, Rhinobothrium* sp., *S. pleuronectis, N. bisulcata, Grillotia smarisgora, C. vivida, A. chesapeakensis, S. sagittifer,* and *H. habena.* These parasites infect a large number of hosts that reflect functional similarities such as diet or habitat. The fourth group of parasites consists of the rare species with prevalences below 10% in Table 1. *Hirudinella ventricosa* and *Microphallus turgidus* are accidental parasites incapable of maturing in summer flounder. Oceanic fishes (Gibson and Bray, 1977) and aquatic birds (Heard and Overstreet, 1983), respectively, are the definitive hosts for these parasites. This pattern is similar to that of other northern fishes that are often infected with a large component of nonspecific enteric metazoan parasites and a smaller component of parasites that are more or less restricted to 1 host or host family (Appy and Burt, 1982; Bray, 1987).

Latitudinal gradients

It is difficult to draw conclusions on latitudinal gradients of summer flounder parasites because this study is the only thorough analysis involving a large number of parasites and hosts. For example, protozoa were not examined by Linton (1940) at Woods Hole or by Meyers (1978) in New Jersey, and, except for the blood protozoa (Laird and Bullock, 1969; Khan and Newman, (1982), this component of the fauna is unknown in the northern portion of the host range. However, even with limited collections at the extremes of the host range, it appears that most parasites prevalent in summer flounder in Virginia are widespread. The blood protozoans *T. bullocki* and *H. platessae* and the digenean *S. dentatum* are known from Massachusetts to North Carolina; the monogenean *N. affine,* the nematode *D. cylindricus,* and the copepod *A. galerita* are known from Massachusetts to Virginia, but probably occur in North Carolina. The digenean *O. vitellosus* is known from Massachusetts and Virginia, but was not reported by Meyers from New Jersey. However, other than the original description from *P. lethostigma* in Louisiana, the digenean *B. paralichthydis* is known only from Virginia. Many parasites of summer flounder reported by others, mainly from Woods Hole, New Jersey, and North Carolina, were not found in Virginia. In particular, fish from the northern portion of their range seemed to have a greater number and variety of juvenile nematodes.

Pathogenicity

Of the parasites listed in Table 1, only *T. bullocki* has been associated with mortality of feral summer flounder (Burreson and Zwerner, 1984). However, other listed parasites or related species are known pathogens in other fishes. These include *Amyloodinium* sp. (Paperna, 1980), *Ichthyobodo* sp. (Robertson et al., 1981; Cone and Wiles, 1984), *Haemogregarina sachai* (Kirmse, 1980), *Trichodina* sp. (Pearse, 1972), and *Argulus* sp. (Kolipinski, 1969; Kroger and Guthrie, 1972).

Although not implicated in mortality, some other parasites were associated with varying degrees of pathology. *Neoheterobothrium affine* elicited a chronic granulomatous inflammatory host response around the opisthaptor, which was embedded in the oral mucosa and musculature of the host. The response resulted in a fibrous tissue collar around the isthmus of the parasite. An inflammatory response was also elicited by *S. tenue* and *S. pleuronectis* type D embedded in the gills. Heavy infections of these 2 parasites were observed during this study and could interfere with gill function.

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Obituary Notice

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Gastrointestinal Parasites of the Blue Catfish (*Ictalurus furcatus*) in Kentucky Lake, Tennessee

TIMOTHY L. HOFFNAGLE,[1] R. A. COLE,[2] AND WESLEY L. SHOOP[3]
Hancock Biological Station, Murray State University, Murray, Kentucky 42071

ABSTRACT: Gastrointestinal tracts were collected from 56 blue catfish (*Ictalurus furcatus*) caught on commercial baitlines in Kentucky Lake, Tennessee. Fish ages ranged from 2 to 13 yr, lengths from 280 to 931 mm, and weights from 190 to 10,800 g. Eight species comprised the helminth community and included the following: 3 trematodes, *Allacanthochasmus varius, Crepidostomum cooperi,* and *Megalogonia ictaluri,* in 3.6% (2), 28.6% (16), and 89.3% (50) of the catfish, respectively; 3 cestodes, *Corallobothrium fimbriatum, Corallobothrium giganteum,* and *Proteocephalus fragile,* in 57.1% (32), 14.3% (8), and 7.1% (4) of the catfish, respectively; and 2 nematodes, *Dichelyne robusta* and *Spinitectus gracilis,* in 46.4% (26) and 92.8% (52) of the catfish, respectively. Certain helminth species showed increasing prevalence with greater age and/or size of the host (*A. varius, C. giganteum,* and *P. fragile*) and others showed the converse (*C. cooperi* and *M. ictaluri*). Each of these 2 opposing trends appeared to be correlated with food items of the catfish, i.e., infective stages of helminths prevalent in the younger/smaller catfish have been reported from mayflies, whereas infective stages of species prevalent in the older/larger catfish have been reported from small fish. Thus, as the catfish changes feeding habits from predominantly invertebrates to one including fish, it also changes the composition of its helminth community.

KEY WORDS: blue catfish, helminths, *Ictalurus furcatus,* survey, Tennessee.

Although numerous studies of the parasites of various catfish species have been conducted, virtually nothing is known about the helminths of the blue catfish, *Ictalurus furcatus.* The blue catfish is easily capable of living in excess of a decade, reaching over a meter in length, and weighing in excess of 50 kg. We had the opportunity to examine the helminths from the gastrointestinal tract of blue catfish from Kentucky Lake, Tennessee. Herein, we report 8 species previously unknown from this host.

Methods

All blue catfish were caught on commercial baitlines in Kentucky Lake, Tennessee, during July and August 1986. A maximum of 10 fish was sampled in sequential 100-mm length classes in an effort to obtain as wide a sampling of various age and size classes as possible. Because of the commercial baitlines, only length classes 200 mm and above were available for sampling, and because of the rarity of very old specimens, only length classes up to and including the 600-mm class reached their maximum. Blue catfish in the 600-mm length class were estimated to be in the 9-yr age class based on age–length relationships established for that species in that area of Kentucky Lake by Timmons et al. (1986). Although no length classes above 600 mm reached their maximum, blue catfish up to 931 mm were included in this study.

All catfish were measured, weighed, and their left pectoral spine removed. The latter was done for precise age determination upon return to the laboratory. Precise ageing was accomplished by counting annual rings on cross sections of pectoral spines. Also, the gastrointestinal tracts were removed, placed in separate plastic bags, and refrigerated at 4°C until necropsy. Necropsy procedures were standard and helminths were prepared using routine histological techniques. Voucher specimens have been deposited in the USNM Helminthological Collection.

Results

Fifty-six gastrointestinal tracts were collected from blue catfish ranging in age from 2 to 13 yr, in length from 280 to 931 mm, and in weight from 190 to 10,800 g. Eight species of helminths, apparently none of which has been reported previously from blue catfish, were found in their gastrointestinal tracts (Table 1). Each fish harbored a mean of 3.4 (range 0–6) helminth species with a distribution as follows: 1 fish had none, 1 had 1 species of helminth, 11 had 2, 13 had 3, 24 had 4, 5 had 5, 1 had 6, and no fish harbored either 7 or all 8 species.

As was expected, age, length, and weight of the hosts were highly correlated (Table 2), and thus, in Table 3 only the age of the hosts relative to the individual parasite species is given to illus-

[1] Present address: Department of Biology, University of North Dakota, Grand Forks, North Dakota 58202.

[2] Present address: Department of Pathobiology, School of Veterinary Medicine, Auburn University, Auburn, Alabama 36849.

[3] Present address: Merck, Sharp & Dohme Research Laboratories, P.O. Box 2000, Rahway, New Jersey 07065-0900.

Table 1. Prevalence and site of infection of gastrointestinal tract parasites of blue catfish from Kentucky Lake, Tennessee.

Species	Site*	No. infected	Prevalence (%)
Trematoda			
Allacanthochasmus varius (USNM #80737)	SI	2	3.6
Crepidostomum cooperi (USNM #80738)	SI	16	28.6
Megalogonia ictaluri (USNM #80739)	SI	50	89.3
Cestoda			
Corallobothrium fimbriatum (USNM #80740)	SI	32	57.1
C. giganteum (USNM #80741)	SI	8	14.3
Proteocephalus fragile (USNM #80742)	SI	4	7.1
Nematoda			
Dichelyne robusta (USNM #80743)	SI	26	46.4
Spinitectus gracilis (USNM #80744)	SI, S	52	92.8

* SI = small intestine; S = stomach.

trate our sample. The number of helminth species the blue catfish harbored increased with greater age until the 9-yr class, whereafter the numbers decreased. There were individual helminth species that varied with age of the host as well as others that did not. Among those that did were 2 groups: those that showed an increase in prevalence over age and size (*Allacanthochasmus varius, Corallobothrium giganteum, Proteocephalus fragile*) and those that showed a decrease (*Crepidostomum cooperi, Megalogonia ictaluri*). It should be noted that even though certain species decreased in prevalence over age classes they were still present in low numbers throughout. In contrast, those species that increased in prevalence were completely absent in the early age classes. Thus, when the mean numbers of species per host were plotted against the various age classes, the resulting curve resembled a sine wave (Fig. 1).

Discussion

The blue catfish is a known opportunistic omnivore feeding on whatever is available from an early age (Brown and Dendy, 1961). In Kentucky Lake, the 2 main food sources for blue catfish are mayflies and fish (Cannamela et al., 1978; Davis, 1979). Not surprisingly, most of the gastrointestinal parasites identified in this study can be classified by their intermediate hosts into those 2 major groups. For example, metacercariae of *M. ictaluri* encyst in the gills of mayfly nymphs that are ingested by catfish (Hopkins, 1934). Metacercariae of *C. cooperi* encyst in the gills of mayfly nymphs and may establish as adults in a variety of fish species (Hopkins, 1934). The infective stages of *Spinitectus gracilis* can also occur in mayflies (Hoffman, 1967).

On the other hand, the metacercariae of *A. varius* encyst in minnow and other fishes (Hoffman, 1967). The life cycle of *P. fragile* is unknown but may be similar to that of other members of its genus in that small fish harbor the infective plerocercoid stage. *Corallobothrium fimbriatum* also has a plerocercoid that has been found in small fish (Allison, 1957), and presumably such is the case with *C. giganteum*. Unfortunately, *Dichelyne robusta* is the only species that cannot be categorized because, at least to our knowledge, nothing is known of its life cycle nor for any other member of its genus.

It may be inferred from the discussion above and Figure 1 that the high level of infection observed in the 2-yr age class of catfish was predominantly the result of ingestion of infective stages in mayflies. The 3 helminth species, 2 trematode and 1 nematode, that occur in mayflies can be thought of as "guild" (sensu Holmes, 1987) or, in other words, an association of parasites within a community that utilizes a similar

Table 2. Correlation coefficients for age, length, and weight of blue catfish collected in Kentucky Lake, Tennessee.

	Pearson correlation coefficients ($P > /R/$ under HO:RHO = 0)			Spearman correlation coefficients ($P > /R/$ under HO:RHO = 0)		
	Length	Weight	Age	Length	Weight	Age
Length	1.00000	0.87943	0.97816	1.00000	0.98486	0.98493
Weight	—	1.00000	0.89452	—	1.00000	0.97577
Age	—	—	1.00000	—	—	1.00000

Table 3. Prevalence and number of helminth species in age classes of blue catfish from Kentucky Lake, Tennessee.

Age (yr)	No. fish	Prevalence (%)*								No. helminth species
		AV	CC	MI	CF	CG	PF	DR	SG	
2	9	0	78	100	89	0	0	44	100	5
3	5	0	20	100	80	0	0	40	100	5
4	6	0	17	100	33	0	0	17	83	5
5	8	0	25	100	50	0	0	38	88	5
6	9	0	22	89	56	44	0	33	89	6
7	5	20	20	100	100	20	0	80	80	7
8	3	0	33	100	33	33	0	67	100	6
9	5	20	20	80	60	40	20	80	100	8
10+	6	0	0	33	0	0	50	50	100	4

* Abbreviations are: AV, *Allacanthochasmus varius*; CC, *Crepidostomum cooperi*; MI, *Megalogonia ictaluri*; CF, *Corallobothrium fimbriatum*; CG, *C. giganteum*; PF, *Proteocephalus fragile*; DR, *Dichelyne robusta*; SG, *Spinitectus gracilis*.

resource (e.g., mayflies) in a similar manner. The subsequent decrease in parasitism observed in 3- and 4-yr-old catfish resulted from the decline of the mayfly guild and likely reflects the migration of catfish from shallow to deeper water that occurs at about 2 yr of age. For instance, with respect to the 2 trematodes that comprise the mayfly guild, *C. cooperi* and *M. ictaluri,* the shallow water is where the molluscs, mayflies, trematodes, and catfish overlap. As long as the catfish remain in the shallow water, the mayfly guild dominates. In deeper water, there is a decrease in number of both molluscs (first intermediate host) and mayflies (second intermediate host). Thus, in the deeper water 2 major factors for transmission are diminished. As a result, the total overall number of helminth species to which these age groups of catfish are exposed does not change, but the prevalence does.

After the fourth year of life it appears that the catfish diet begins to include a greater proportion of fish as evidenced by the acquisition of helminths comprising the fish "guild"; e.g., *A. varius, P. fragile,* and *C. giganteum* are known or suspected to utilize fish as second intermediate hosts. As a consequence of the overlap between the mayfly and fish guilds, both the total and mean number of helminth species per host increased until the ninth year class.

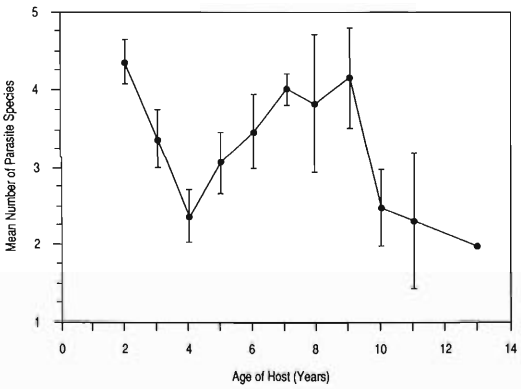

Figure 1. The mean number of helminth species per blue catfish plotted against the age of the host (in years).

The decreased parasitism in the very old catfish (>9 yr of age) is less readily explicable. Interestingly, Dogiel (1961, and references contained therein) noted this phenomenon, but could offer no explanation. In the present study, it may have resulted from 3 possibilities. First, it may be an artifact due to sampling error, because fish greater than 9 yr of age are so rare we were able to examine only 6. Second, it may be real and have resulted from the natural dearth of very old catfish coupled with a continuation of the ontogenetic change in feeding habit in which progressively larger fish prey (up to 400 mm in length) comprise the bulk of the diet. Conceivably, the small numbers of very old catfish and the parasite progeny generated therefrom may be below the threshold levels needed to propagate infection in a new group of larger prey. Third, rather than an ontogenetic explanation, it could be that these rare old catfish are sole survivors because they represented a subpopulation that was genetically less susceptible from the beginning of life. Thus, our results may be due to natural sampling error.

Acknowledgments

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International Symposium on Emerging Problems in Food-borne Parasitic Zoonoses

Chiang Mai, Thailand

14–17 November 1990

It is becoming more evident that the problem of animal product-borne diseases, such as toxoplasmosis, cysticercosis, trichinosis, capillariasis, angiostrongyliasis, gnathostomiasis, sarcosporidiosis, and trematodiases, is increasing. The economic consequences of these zoonoses for agriculture, and their associated public health impact, are quite severe in some regions of Southeast Asia and elsewhere in the world.

The development of animal production systems is handicapped by these problems, and a better understanding of the problems is needed before effective control strategies can be developed. Therefore, there is a need to bring capable scientists with the relevant experience from various parts of the world into contact with Southeast Asian agricultural and public health scientists to provide guidance on how to deal with the problems. We welcome participation by all interested individuals and groups.

For further information, contact Professor Chamlong Harinasuta, SEAMEO TROPMED Project, 420/6 Rajvithi Road, Bangkok 10400, Thailand; or Dr. John H. Cross, Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, Maryland 20814, telephone (202) 295-3139.

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Some Helminth Parasites of Dunlin (*Calidris alpina*) and Western Willet (*Catoptrophorus semipalmatus inornatus*) from California

HILDA LEI CHING
Hydra Enterprises Ltd., P.O. Box 2184, Vancouver, British Columbia, Canada V6B 3V7

ABSTRACT: Dunlins and western willets were collected on their winter feeding grounds at Bodega Bay and Bolinas Lagoon, California, during 1986. Six digeneans, 8 cestodes, 2 acanthocephalans, and a new nematode were found in dunlins. Seven digeneans, 2 cestodes, 1 acanthocephalan, and the new nematode were found in western willets; 4 helminths including the new roundworm were common to dunlins. *Thominx cecumitis* sp. n. differs from other capillarid nematodes with a spinous spicule-sheath in having a short, globular vulvar appendage, peanut-shaped eggs, a cuticular ball at the posterior tip of the body, narrow lateral alae, and a cuticular genital bursa.

KEY WORDS: helminths, *Calidris alpina, Catoptrophorus semipalmatus inornatus,* California, *Thominx cecumitis* sp. n. (Nematoda), new host records.

Dunlin, *Calidris alpina* (L.), and western willet, *Catoptrophorus semipalmatus inornatus* (Brewster), were sampled on their winter feeding grounds in California during 1986. The purpose of this study was to identify the helminth parasites that were mainly from the digestive system, to redescribe *Levinseniella gymnopocha,* and to report on a new species of nematode.

Materials and Methods

Nine dunlins were sampled in February and in March 1986 at Bodega Bay, California, and in March 1986 in Bolinas Lagoon, California for a total of 27 birds. Nine western willets were sampled in January 1986 at Bodega Bay. Dunlins collected at Bodega Bay represented distinct populations; those in February remained to feed in the area, whereas those sampled in March left the area each day (Ruiz, 1986, pers. comm.). Birds were collected by nets and killed in the field by Drs. Greg M. Ruiz and Wayne P. Sousa. Intestinal tracts were removed and immediately frozen using the technique described by Bush and Threlfall (1984). For necropsies, the samples were thawed. The portion posterior to the gizzard and anterior to the ceca was divided into 4 sections, and the remainder of the posterior intestine was separated into the ceca, large intestine, and cloaca. Mesenteric veins were also examined. Parasites were preserved in 5% buffered formalin and studied using standard permanent mounts. Scolices of some tapeworms were mounted in Berlese's fluid and lightly flattened to study the rostellar hooks. Drawings were made with the aid of a camera lucida. Lengths of nematodes were taken using a map measurer after magnified drawings were made with a camera lucida. Measurements are in micrometers unless stated otherwise with ranges presented first followed by the means in parentheses. Voucher specimens and types have been deposited in the Helminthological Collections of the U.S. National Museum and the accession numbers are listed in Tables 1 and 2.

Results and Discussion

Parasites of dunlin

Seventeen parasite taxa were found from the intestine and mesenteric veins from a total of 27 dunlins (Table 1). The taxa consisted of 6 digeneans, 8 cestodes, 2 acanthocephalans, and 1 nematode.

Digenea

Ascorhytis charadriformis (Microphallidae) was found in the large intestine of dunlins and was originally described from charadriiform birds in California (Young, 1949). Gulls are other avian hosts in the life cycle, which involves littorine snail and grapsid crab intermediate hosts (Ching, 1963a). Five specimens of *Austrobilharzia penneri* (Schistosomatidae) were found in the mesenteric veins. They were in the minimum size range but had similar anterior locations of the spiral ovary and spatial relationships of the suckers as described by Short and Holliman (1961). Martin (1972) suggested that the schistosomatid cercaria, which he found in *Cerithidea californica,* was *A. penneri* although experimental infections of chicks and pigeons were not successful. Dunlins are the first natural host reported for this blood fluke. *Cloacitrema michiganense* (Philophthalmidae) and *Parorchis acanthus* (Philophthalmidae) were found in the cloaca; of 4 coinfections, 2 involved immature worms. Both digeneans utilize *C. californica* as the first intermediate host (Martin, 1972; LeFlore et al., 1985) and infect their next hosts passively through ingested matter containing metacercarial cysts. *Hi-*

Table 1. Parasites of dunlin, *Calidris alpina,* from Bodega Bay and Bolinas Lagoon, California, 1986.

Parasite	Location, month*			USNM coll. no.
	Bodega Bay, February	Bodega Bay, March	Bolinas Lagoon	
Digenea				
Ascorhytis charadriformis (Young, 1949)	0	3, 13	0	80771
Austrobilharzia penneri Short and Holliman, 1961	0	0	3, 5	80772
Cloacitrema michiganense McIntosh, 1938	0	0	6, 13	80773
Himasthla leptosoma (Creplin, 1829)	5, 86	7, 31	7, 26	80774
Levinseniella gymnopocha Coil, 1956	0	7, 29	1, 1	80775
Parorchis acanthus (Nicoll, 1906)	0	0	6, 9	80776
Cestoda				
Aploparaksis brachyphallos (Krabbe, 1869)	6, 13	4, 30	0	80777
A. crassirostris (Krabbe, 1869)	7, 121	7, 104	7, 26	80778
A. retroversa Spasskii and Gubanov, 1961	6, 26	6, 40	4, 9	80779
Dicranotaenia amphitricha Lopez-Neyra, 1942	3, 19	5, 20	4, 13	80780
Echinocotyle nitida (Krabbe, 1869)	7, 21	5, 19	6, 12	80781
Nadejdolepis paranitidulans (Golikowa, 1959)	9, 1,928	9, 2,431	9, 2,760	80782
Retinometra deblocki (Schmidt and Neiland, 1968)	4, 8	3, 7	2, 5	80783
Trichocephaloides megalocephala (Krabbe, 1869)	6, 13	4, 16	1, 2	80784
Acanthocephala				
Arhythmorhynchus comptus Van Cleave and Rausch, 1950	0	0	2, 4	80788
A. eroliae (Yamaguti, 1939)	1, 1	0	5, 9	80789
Nematoda				
Thominx cecumitis sp. n.	1, 1	6, 9	2, 3	80785, 80786

* At all locations 9 birds were sampled. Measurements indicate: number of infected birds, number of parasites.

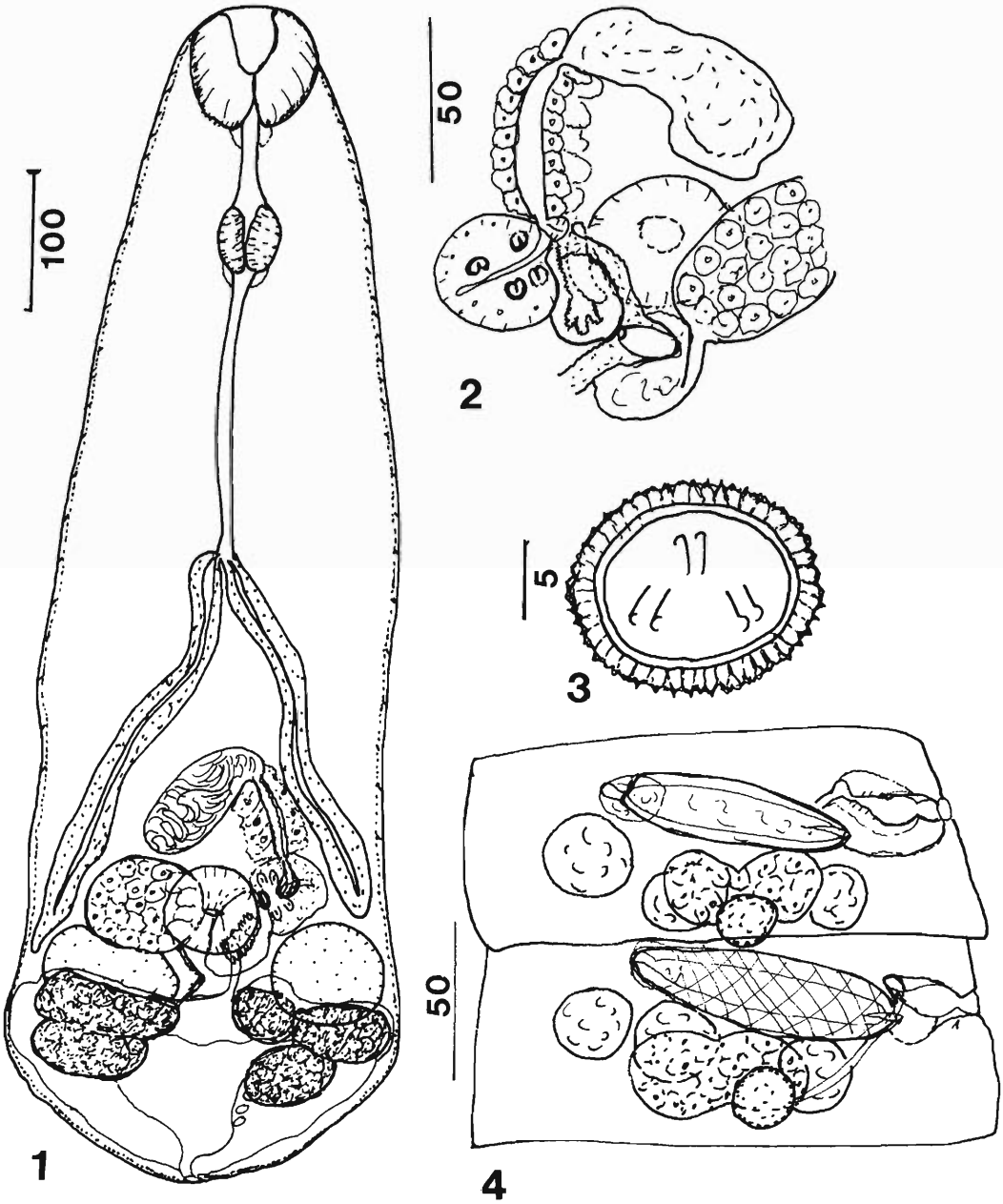
masthla leptosoma (Echinostomatidae) was found in the posterior half of the intestine of dunlins from all collection sites with immature worms comprising 13% of 86 specimens in February, 23% of 31 in March (Bodega Bay) and 89% of 26 specimens in March (Bolinas Lagoon). Loos-Frank (1967) has reported this species in dunlins in Europe.

Levinseniella gymnopocha (Microphallidae) was found exclusively in the ceca and was originally described from 4 specimens taken from 1 dunlin in Ohio (Coil, 1956). This microphallid is characterized by uneven ceca, a female pouch with fine internal folds, a male pouch with 4 simple pockets, a large seminal receptacle, and massive vitellaria (Figs. 1, 2). Because the specimens were proportionately larger in size than originally reported, 10 were measured: body length 736–1,122 (912), body width at the level of the ventral sucker 164–265 (237). Transverse width of oral sucker 54–100 (80), ventral sucker diameter, 43–64 (55) with sucker ratio, 1:0.5–1:0.8. Prepharynx length 49–98 (67); pharynx 33–51 by 28–91 (40 × 35); esophagus length 151–286 (221). Ceca long, reaching to level of ovary and testes, right longer than left. Gonads spherical, ovary 54–92 (72); left testis 61–110 (84), right testis 51–94 (77). Seminal vesicle elongate, 54–89 (70), male pouch 33–56 (46) in diameter, female pouch slightly smaller. Vitellaria massed laterally in 2 and 3 clumps. Uterus on left with older eggs. Eggs 18–20 by 10–11. Flame cells visible in preserved specimens, with typical microphallid pattern of 2[(2 + 2) + (2 + 2)]. Excretory bladder V-shaped.

The temporal and spatial distributions of digeneans show some patterns of presence and absence. *Ascorhytis* and *Levinseniella* were absent in February but present in March in dunlins at Bodega Bay, but were rarely present in Bolinas Lagoon. *Himasthla* were present in all dunlin populations and were most mature in February but were immature in March at Bolinas Lagoon. *Austrobilharzia, Cloacitrema,* and *Parorchis* were not found in Bodega Bay but were present in Bolinas Lagoon, where a high level of infected snails was found (Sousa, 1983).

Cestoda

Three species of *Aploparaksis* (Hymenolepidae) were identified using the descriptions by Spasskii (1963) and Deblock and Rausch (1968).



Figures 1–4. 1. Whole mount of *Levinseniella gymnopocha,* ventral view. 2. Terminal genitalia of *L. gymnopocha,* dorsal view. 3. Egg of *Aploparaxis crassirostris.* 4. Segments of *Nadjedolepis paranitidulans.* Bar numbers are in micrometers.

Webster (1955) described *A. schilleri* and *A. rauschi* from dunlins in Alaska; these are recognized as synonyms respectively of *A. brachyphallos* and *A. crassirostris* (Deblock and Rausch, 1968). They were found together in the posterior half of the intestine but with more of *A. crassirostris.* Six hosts had infections in which the scolices of *A. crassirostris* were embedded in red intestinal mucosa surrounded by fibrous tissue. The eggs of *A. crassirostris* averaged 32 by 26 with a finely notched surface not previously described (Fig. 3). The third species, *A. retroversa,* was ex-

Table 2. Parasites of 9 western willet, *Catoptrophorus semipalmatus inornatus*, Bodega Bay, California, 1986.

Parasite	No. birds infected, no. parasites	USNM coll. no.
Digenea		
Ascorhytis charadriformis (Young, 1949)	9, 1,936	
Cloacitrema michiganense McIntosh, 1938	1, 1	80790
Levinseniella gymnopocha Coil, 1956	8, 344	80791
Maritrema laricola Ching, 1963	9, 3,098	80792
Odhneria odhneri Travassos, 1921	1, 8	80793
Parorchis acanthus (Nicoll, 1906)	1, 1	80794
Parvatrema borealis Stunkard and Uzmann, 1958	1, 100	
Cestoda		
Cysticercoid stage	8, 54	
Hymenolepidae	1, 1	
Acanthocephala		
Polymorphidae	1, 1	
Nematoda		
Thominx cecumitis sp. n.	7, 20	80787

clusively found in the ceca and has been reported from the short-billed dowitcher (*Limnodromus griseus*) in Alaska by Schmidt and Neiland (1968) and shorebirds in the Yakutskaya region of the Soviet Union by Spasskii (1963).

Dicranotaenia amphitricha (Hymenolepidae) was identified from the description by Deblock and Rose (1962) and, probably because of the varied appearance of the strobila (Belopolskaia, 1970), has had a complex taxonomic history (Schmidt, 1986).

Two small cestodes were found in coinfections in the anterior fourth part of the intestine. *Echinocotyle nitida* has been described from sandpipers from the Barents Sea and France by Deblock and Rose (1962). This species has fine spines on its suckers and should not be confused with *Echinocotyle dubininae* (syn. of *Hym. (Ech.) nitida* of Clerc, 1902) which has tiny aploparaxoid-shaped hooks on its suckers. The size range of *E. nitida* was 5–10 mm, whereas *Nadjedolepis paranitidulans* at the same site was smaller, 3–7 mm, and more numerous. Four species of *Nadejdolepis* have been reported from dunlins, and the pointed rostellum and rostellar hook shape of *N. paranitidulans*, as in the figure of the scolex by Spasskii and Bobova (1962), were most similar to my specimens. The rostellar hook measurements given were 40–42; my measurements of 28 were slightly larger, 45–50 (48), handle 19–23 (22), blade 22–31 (27). Segments of *N. paranitidulans* are shown for reference purposes (Fig. 4).

Trichocephaloides macrocephala and *Retinometra deblock* (Hymenolepidae) were found in low intensity in the anterior half of the intestine. The former has been reported from sandpipers in North America, Europe, and the Soviet Union (Schmidt, 1986), and the latter was described from *Limnodromus griseus* in Alaska by Schmidt and Neiland (1968). Schmidt (1986) transferred *Hymenolepis (H.) deblocki* to *Retinometra* even though this species has an accessory sac and other members of the genus do not. Use of Schmidt's key could also result in allocation of this species to *Sobolevicanthus*, but its species have skrjabinoid-shaped rostellar hooks. The rostellar hooks of *R. deblocki* are nitidoid-shaped and large; 25 were 102–115 (108 ± 4.6). Specimens were shorter than the type specimen, <22 mm in length with ½ the numbers of proglottids. The spined, copulatory portion of the vagina was enlarged, 32–42 (38) anteroventral to the cirrus sac. Eggs were not mature but 36–70 were counted per proglottid, with maximum size of 31 × 26. Dr. Eric Hoberg identified this tapeworm for me.

The cestode populations were evenly distributed in numbers in both localities. There appeared to be fewer tapeworms and no *A. brachyphallos* at Bolinas Lagoon.

Acanthocephala and Nematoda

The acanthocephalans, *Arhythmorhynchus comptus* and *A. eroliae* were found in the third quarter of the intestine; the former was originally described from dunlin in Alaska by Van Cleave and Rausch (1950) and the latter from the same host in Japan by Yamaguti (1939). Both species

occurred at Bolinas Lagoon; *A. eroliae* consisted of 2 immature, 1 male, and 1 nongravid female specimens. The new species of nematode occurred in the ceca of 6 dunlins sampled in March at Bodega Bay. Its description will follow.

Parasites of western willet

Eleven parasite taxa were found in the intestine of western willets (Table 2). The taxa consisted of 8 digeneans, 2 cestodes including a cysticercoid stage, 1 acanthocephalan, and 1 nematode. Four helminths were common to dunlin. Microphallid trematodes (*Ascorhytis, Maritrema, Levinseniella*) occurred in high prevalence and intensity; the first 2 species use the same intermediate hosts, which are shorecrabs in muddy bays and rocky shores (Ching, 1963a, b). Other parasites were comparatively rare including *Parvatrema borealis* (Gymnophallidae), with 100 minute specimens estimated to be present in 1 western willet. A dunlin was experimentally infected after feedings with the clam intermediate host, *Nuticola tantilla* (Gould). Shorebirds are new hosts on the Pacific coast of North America. The cysticercoid stage, partial specimen of hymenolepid and single sample of polymorphid acanthocephalan could not be further identified. According to Bush (1989), western willets can have varied and abundant helminth populations; those from California can be considered depauperate.

Thominx cecumitis sp. n. (Figs. 5–9)

Description

GENERAL: Body hairlike, long, extremely slender. Bacillary bands extend length of body. Anterior end pointed with no cuticular swelling, posterior end bluntly rounded. Esophagus with short, weakly muscular, long glandular portions. Eggs operculate with cuticular plug at each end. Males with single spicule at posterior end, spicule enclosed in spinous sheath.

MALE (N = 5): Body length 5–12 (8) mm, anterior width 8–10, width at midbody and posterior end, 33–43 (Fig. 5). Esophagus with maximum of 47 stichocytes. Spicule 777–979 (878) in length, composed of 4 cuticular rods somewhat cuboidal in cross section, uniformly wide 6–13 (8.7), terminating at posterior end with bluntly rounded bladelike tip. Spicule protrudes more than ½ its length from posterior end, when fully withdrawn, 44–132 from posterior end (Fig. 6). Sheath transversely wrinkled, finely spinous. Cuticular, bursa-like extensions at posterior end; median ball at tip. Pair of postanal papillae present. Lateral alae narrow, somewhat asymmetrical extending anteriorly to level of withdrawn spicule (Fig. 7).

FEMALE (N = 15): Body length 12.8–29.5 mm, immature worms 11–13.8 mm. Anterior end narrow, width 8–13, width at vulva 51–64, posterior end blunt, width 33–51, with median cuticular ball. Glandular esophagus with maximum of 64 stichocytes. Anus subterminal, caudal papillae present. Vulva 25–39% from cephalic end, 43–47% from cephalic end in immature specimens. Vulva with cuticular appendage shorter than width of body; when expanded, goblet-shaped with cuticular supports 32–59 by 20–38 (Fig. 8). Vagina muscular, uterus with 27–86 (51) eggs. Eggs with cuticular plugs, slightly flexed at middle like peanut shells, with homogeneous granular surface (Fig. 9), 100 measured 49–69 × 23–33 (62 × 30).

TYPE HOSTS: *Calidris alpina* (L.), dunlin; *Catoptrophorus semipalmatus inornatus* (Brewster), western willet.

SITE OF INFECTION: Ceca.

TYPE LOCALITY: Bodega Bay, California.

SPECIMENS DEPOSITED: Holotype male, USNM Helm. Coll. No. 80785; allotype female, USNM Coll. No. 80786, paratypes USNM Coll. No. 80787.

Remarks

Capillarid species with a spinous spicule sheath were placed in the genus *Thominx* Dujardin, 1845, by Skrjabin et al. (1957). Of 51 species listed, 6 were reported from avian hosts. The new species differs from *T. anatis* (Schrank, 1790), *T. contorta* (Creplin, 1839), *T. raillieti* (Lopez-Neyra, 1946), and *T. spinulosum* (Linstow, 1890) in having a vulvar appendage, peanut shell shape of the eggs, and presence, in both males and females, of a median cuticular ball at the posterior end. It is similar to *T. ellisi* (Johnston and Mawson, 1945) and *T. nyrococinarum* (Madsen, 1945), which have a vulvar appendage. However, it dif-

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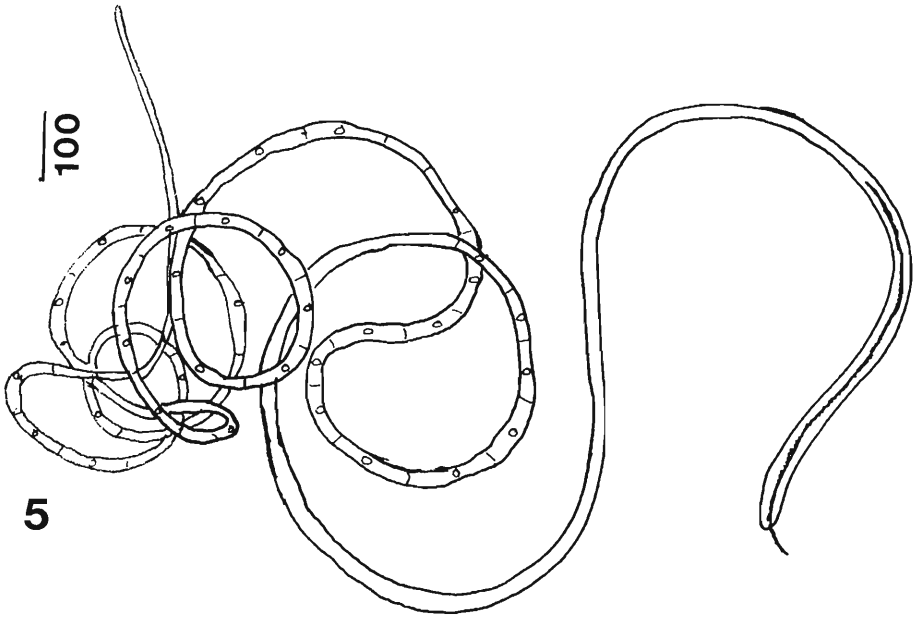
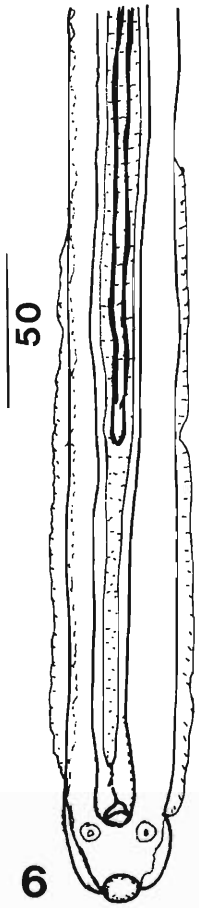
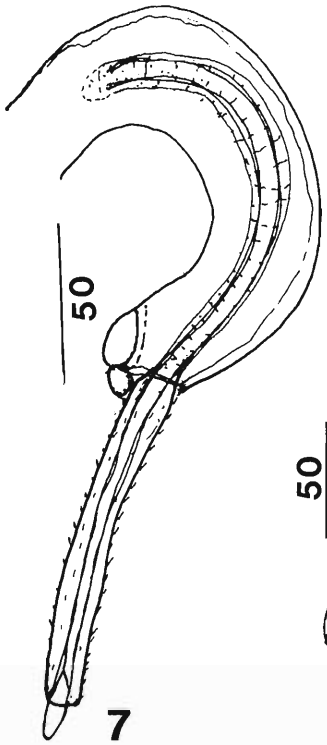
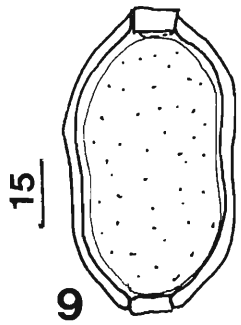
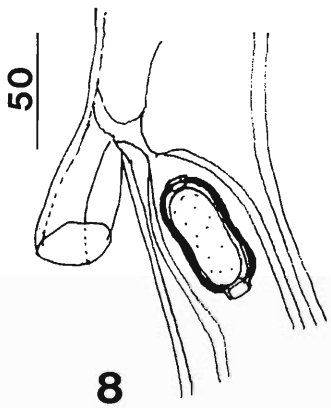
Figures 5–9. ***Thominx cecumitis*** **sp. n. 5. Whole mount of male holotype. 6. Posterior portion of male. 7. Everted spicule of male. 8. Vulvar appendage of female. 9. Egg. Bar numbers are in micrometers.**











fers from *T. ellisi* in the shorter proportions of the vulvar appendage and shorter length of the spicule (1.4 mm in *T. ellisi* vs. <1 mm in *T. cecumitis*). It differs from *T. nyrococinarum* in the symmetry of the eggs and presence of lateral alae in males. According to McDonald's (1974) key to species of *Capillaria,* 4 capillarids from the ceca and small intestine of waterfowl are similar to the *T. cecumitis* in having a short vulvar appendage. *Capillaria pudendotecta* Liubimova, 1947, has a globular-shaped vulvar appendage but its eggs are asymmetrical, and the female has a terminal anus. The male of *C. pudendotecta* has not been described. *Capillaria mergi* Madsen, 1945, has a small vulvar appendage, and eggs with the inner shell reflexed at the ends. The male of *C. mergi* has a nonspinous, transversely striated spicule sheath and no genital bursa. *Capillaria bursata* Freitas and Almeida, 1934, has eggs with fine longitudinal ridges and shell layers with terminal curves. The male has a nonspinous, transversely striated spicule-sheath, genital bursa, and lateral alae. *Capillaria exilis* (Dujardin, 1845) has eggs with a lattice pattern and prominent striae at the ends. The male has a nonspinous spicule-sheath, sharply pointed spicule, and small genital bursa and caudal alae.

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Effects of Aeration and Temperature in In Vitro and In Vivo Studies on Developing and Infective Eggs of *Ascaris suum*

R. J. BOISVENUE
Animal Science Division, Lilly Research Laboratories,
A Division of Eli Lilly and Company, Greenfield, Indiana 46140

ABSTRACT: Studies were conducted on the in vitro development of eggs of *Ascaris suum* using temperatures between 20 and 32°C and continuous aeration for 46 days. The optimum temperature, defined as the temperature at which 50% of the eggs develop to the motile first larval (L1) stage in the shortest period of time was 30°C. Times for 50% development of tadpole, L1, and second larval (L2) stages were shortest at 30°C. Development appeared suboptimal at 32°C, as greater than 50% of the infective eggs showed a gradual decline in L2 motility and healthy protoplasmic appearance from 30 to 46 days in aerated cultures. Due to the inability to relate in vitro hatchability with infectivity of eggs, an in vivo system of index infectivity was established in the lungs of mice inoculated orally with 10,000 infective eggs. Eggs conditioned to growth at 30°C exhibited the greatest index of infectivity in mice at the earliest time of 21 days. The most severe index of infectivity (mortality) was obtained in mice inoculated with eggs cultured for 39 days at 26°C. There were no mortalities in mice given eggs cultured at 20 and 23°C. The rate of aeration did appear to be a differential factor in enhancing infectivity of eggs during the first 25 days of culture.

KEY WORDS: *Ascaris suum*, eggs, cultivation, temperature, aeration, mice, lung lesions.

Several authors reported that the infective larvae of *Ascaris suum* develop in decoated eggs between the temperatures of 16 and 34°C with the maximum rate of development at 31°C (Martin, 1913; Ransom and Foster, 1920; Seamster, 1950; Jaskoski, 1952; Arene, 1986). Jaskoski (1954) presented minimum and average developmental periods of 9 and 10 days at 31°C with temperatures of 31–33°C appearing to be near optimum. Timoshin (1967) reported optimal temperatures for egg development between 17 and 30°C and that the rate of development was directly proportional to temperature. Velocity curves of swine ascarid egg development at various temperatures were constructed by Seamster (1950). Cleeland and Laurence (1962) reported that decoated swine ascarid eggs were maintained at 22–26°C with frequent manual agitation until a majority of eggs had reached the infective state, usually within 30 days. Fairbairn (1961) decoated eggs and embryonated them in 0.1 N sulfuric acid at 30°C for about 20 days when they were fully infective. Rogers (1958) reported that in vitro hatching of L2 eggs could be induced following 20 days of embryonation at 30°C. Fairbairn (1961) supported this observation and stated that hatching increased linearly to near the maximum of 80% after 19 days. Arene (1986) stated that eggs embryonated at $\geqq 28 + 1$°C had less ability to hatch and to penetrate tissue membranes in vitro than larvae from eggs embryonated at lower temperatures.

The purpose of this paper is to determine the optimum temperature for the development of eggs of *A. suum* using continuous agitation provided by slow and rapid aeration rates and various temperatures. Optimum temperatures, defined as the temperature at which 50% of the eggs would develop to the motile first larval (L1) stage in the shortest period of time, will be determined. Also, this work is intended to relate hatchability and infectivity of eggs embryonated at various conditions by experimentally infecting mice with embryonated eggs and subsequently determining mortality or lung lesion scores in mice.

Materials and Methods

Adult female *Ascaris suum* were collected from the intestines of market pigs slaughtered at a local abbatoir. The last centimeter of uteri was removed and placed in a 0.5 N NaOH solution in a Thomas tissue grinder with a grooved teflon pestle. Uteri were broken up and the eggs freed by grinding. The decoated eggs were then sterilized with a 5% peracetic acid solution for approximately 20 min. After exposure to NaOH and peracetic acid, the egg material was washed several times in sterile distilled water, each time with the aid of gentle centrifugation at 700 rpm for 5 min. The eggs were placed in 300 ml of sterile distilled water at the rate of 42,000 fertilized eggs/ml in a 500-ml Bellco dispensing funnel flask (no. 5608) tooled for rubber tubing connection at the bottom of the flask. Estimated number of eggs per flask was 12.6 million. Attached to the bottom of the funnel flask was a sterilized latex tube that was connected to an aquarium air pump. A sterile cotton filter was inserted into the air feeder tube system at the connection. Each flask had a sterilized liquid

Table 1. Time for development of eggs of *Ascaris suum* at various aeration rates and temperatures.

Temperature	BPM*	% L1 at 10 days	Mean number of days to reach stage†			
			50% tadpole	50% L1	50% L1/L2	90% L2
20	75–85	0	19.50[a]	24.00[a]	35.10[d]	38.80[f]
20	175–185	0	17.50[b]	23.00[b]	33.10[b]	40.00[d]
23	75–85	0	11.60[c]	15.00[d]	20.40[i]	33.00[i]
23	175–185	0	11.30[d]	15.00[d]	21.00[h]	40.00[d]
26	75–85	83	7.80[f]	9.30[f]	25.00[c]	38.30[h]
26	175–185	90	7.50[g]	9.00[g]	24.00[e]	37.00[j]
28	75–85	81	6.20[h]	8.80[j]	24.00[e]	41.00[b]
28	175–185	76	8.20[e]	9.80[e]	22.70[f]	41.00[b]
30	75–85	93	5.60[k]	8.25[l]	15.50[l]	24.00[k]
30	175–185	96	5.00[l]	8.50[k]	15.70[k]	20.50[l]
32	75–85	82	5.70[j]	8.80[j]	21.30[g]	39.00[c]
32	175–185	86	5.80[i]	8.80[j]	19.80[j]	36.50[g]

* BPM = number of bubbles of air per minute.

† Student–Newman–Keuls analysis ($P < 0.05$); 1-way analysis of variance. Means followed by the same letter are not significantly different at the 0.05 level.

probe thermistor connected to a telethermometer. This instrument was coupled with an automatic temperature recorder. A sterile cotton plug was then inserted in the mouth of the funnel. The flask with tubing was placed in a similar area of each environmental chamber. The aquarium air pump and telethermometer were situated outside of the chamber. A series of experiments was designed in which thermostats of 6 chambers were regulated to within ±1°C for the following temperatures: 20, 23, 26, 28, 30, and 32°C. These temperatures were selected because they were within the minimum and maximum range of 16 and 34°C reported by several authors for the development of embryonated eggs. Aeration ranges were determined by auscultation of air tubes, calculating the approximate number of sounds per minute. Ranges varied from 75 to 85 (slow aeration) and 175 to 185 (fast aeration) bubbles of air per minute. The percentage of stage of development was calculated by pipetting approximately 200 eggs from each flask in 2 equal samples taken every 2 days onto a clean microscope slide and counting the number of eggs in each stage of growth. Air was constantly introduced in the bottom of the flask during these sampling periods.

Though there were several stages in early development, i.e., single-celled, 2-celled, 4-celled, 8-celled, morula, and tadpole, the tadpole stage was chosen for the first measurement of growth. The next measurement was the percent of L1 in eggs cultured for 10 days. This time period was arbitrarily selected from growth data presented by Arene (1986), Maung (1978), and Seamster (1950). The time for these eggs of *A. suum* to reach about 50% population of L1 was the third growth assessment. To obtain the next 2 measurements, approximately 50% L1/L2 and ≧90% L2, an aliquot sample of approximately 200 eggs was taken per measurement from each culture, placed on a glass slide, and a coverslip superimposed on the egg sample. Pressure was applied to the coverslip, which liberated larvae from the eggs. According to Maung (1978), L1 larvae are so delicate that they could not withstand being pressed. After the first molt, the L2 larvae were able to withstand pressure. In addition, the L1 cuticle separated from the L2 until a loose sheath was formed around the L2. Eventually the L1 cuticle was lost inside the egg, so that when pressure was applied a naked L2 appeared. L1 and L2 larvae were differentiated and counted using the guidelines of Maung (1978).

To conduct infectivity studies, Cox White Swiss mice, weighing approximately 20 g each, were allotted to groups of 6 mice each. The number of animal groups per experiment was 4. An oral dose of approximately 10,000 embryonated eggs of *A. suum* per mouse was given by intubation (Boisvenue et al., 1968) at periodic times during development. Ten days after infection, all mice were killed in a carbon dioxide gas chamber, and the excised lungs were immediately examined and scored according to Brown and Chan (1955). The degree of lung damage caused by migrating L2 was classified based on the extent of hemorrhage and consolidation and scored from 0 to 5. Lungs apparently normal were scored 0; complete hemorrhage of both lungs was scored 5. Mean lung lesion scores were determined for each group. Infectivity of eggs was measured by the number of rodent deaths, the range of individual scores, and the group mean score of 6 mice. Statistical analysis was conducted using Student–Newman–Keuls method ($P < 0.05$), 1-way analysis of variance.

Results

The greatest percentage of L1 stages observed at 10 days was in cultures at 30°C, followed closely by those eggs cultivated at 26°C (Table 1). There were no L1 stages seen at this time in cultures set at 20 and 23°C. The temperature at which swine ascarid eggs developed to a 50% motile L1 population in the shortest period, i.e., optimum

Table 2. Mean lung lesion scores of groups of mice experimentally infected with 10,000 eggs of *Ascaris suum* cultured at 20 and 26°C.

Age of cultures (days)	Group no.	Temp. °C	Aeration (BPM)*	Mean group score†	Range of scores	Mortalities
25	1	20	75–85	0.00[d]	(0)	0
	2	26	75–85	2.00[a]	(0)	0
	3	20	175–185	0.71[c]	(0–1)	0
	4	26	175–185	1.14[b]	(1–2)	0
31	1	20	75–85	1.00[c]	(0)	0
	2	26	75–85	2.71[b]	(1–4)	1
	3	20	175–185	1.00[c]	(0)	0
	4	26	175–185	4.14[a]	(2–5)	3
39	1	20	75–85	1.00[c]	(0)	0
	2	26	75–85	2.00[b]	(1–4)	2
	3	20	175–185	0.00[d]	(0)	0
	4	26	175–185	4.16[a]	(3–5)	4
46	1	20	75–85	0.33[c]	(0–1)	0
	2	26	75–85	1.50[a]	(1–4)	1
	3	20	175–185	1.00[b]	(0)	0
	4	26	175–185	1.33[a]	(1–3)	1

* BPM = bubbles per minute.
† Student–Newman–Keuls analysis ($P < 0.05$).

time, was 30°C. In addition, this temperature promoted the greatest development in tadpoles, 50% L1/L2, and 90% L2 stages (Table 1). In general, it appeared that a faster aeration rate improved the development of tadpoles and 50% L1 at temperatures from 20 to 26°C.

Mean lung lesion scores determined from groups of mice experimentally infected with embryonated eggs cultured at various temperatures, time, and aeration are presented in Tables 2–4. Scores were very low in mice infected with eggs cultured for 25 days at 20°C (Table 2). Infectivity of these eggs was generally poor following 31, 39, and 46 days cultivation. Eggs developed at 26°C were determined to be more infective in mice inoculated at these times. Twenty-five to 58% mortalities were observed in mice given eggs cultured for 31 and 39 days. Rapid aeration of cultures appeared to enhance infectivity of eggs grown at 26°C during this high mortality period, but not at 46 days. Mice in this group were not infected with eggs cultured for 21 days.

In the second experimental group (Table 3), eggs cultured at 23°C for 21 and 25 days were noninfective. However, as culturing continued, rapid aeration generally improved infectivity of these eggs at this temperature. Eggs cultured at 30°C were more infective in mice in the initial growth period, i.e., 21, 25, and 32 days. There appears to be some difference in parasitic infectivity in mice due to a difference in aeration of eggs set at 30°C. With aging to 39 and 46 days, eggs exposed to 23°C had similar infectivity (lesion scores) in mice as those conditioned at 30°C.

Infectivity was more pronounced at 21 days in mice infected with eggs cultured at 28 and 32°C (Table 4). However, at 31 days of continuous growth, the infectivity of eggs set at 32°C diminished. This condition was corroborated by microscopic observation of embryos that were gradually deteriorating to a poor protoplasmic appearance with little L2 motility. Eggs maintained in the 28°C environment retained a good level of infectivity throughout. The rate of aeration did appear to be a differential factor in enhancing infectivity of these eggs during the first 25 days of culture.

Discussion

The lower percentage of L1 found in eggs after 10 days in culture (DIC) at 28°C, especially at the higher aeration rate, cannot be explained by a difference in pooled egg material (Table 1). However, it was observed microscopically at 12 days that the L1 percentages in both aerated cultures were at least equal to those percentages in eggs set at 26°C. In general, the mean number of days at the temperature required to produce 50% L1 was significantly less in the present study compared to Seamster's (1950) study (Table 5).

Table 3. Mean lung lesion scores of groups of mice experimentally infected with 10,000 eggs of *Ascaris suum* cultured at 23 and 30°C.

Age of cultures (days)	Group no.	Temp. °C	Aeration (BPM)*	Mean group score†	Range of scores	Mortalities
21	1	23	75–85	0.0[d]	(0)	0
	2	30	75–85	2.8[b]	(2–5)	2
	3	23	175–185	0.0[d]	(0)	0
	4	30	175–185	2.8[b]	(2–4)	1
25	1	23	75–85	0.0[d]	(0)	0
	2	30	75–85	2.66[a]	(2–3)	0
	3	23	175–185	0.0[d]	(0)	0
	4	30	175–185	2.50[a]	(2–3)	0
31	1	23	75–85	1.50[b]	(1–2)	0
	2	30	75–85	2.50[a]	(2–4)	1
	3	23	175–185	1.00[c]	(0–2)	0
	4	30	175–185	2.11[a]	(2–3)	1
39	1	23	75–85	1.66[b]	(1–3)	0
	2	30	75–85	1.83[b]	(1–2)	0
	3	23	175–185	2.00[b]	(0)	0
	4	30	175–185	2.66[a]	(2–3)	0
46	1	23	75–85	1.83[d]	(1–2)	0
	2	30	75–85	2.16[b]	(2–3)	0
	3	23	175–185	2.50[a]	(2–3)	0
	4	30	175–185	1.83[d]	(0–3)	1

* BPM = bubbles per minute.

† Student–Newman–Keuls analysis ($P < 0.05$).

Table 4. Mean lung lesion scores of groups of mice experimentally infected with 10,000 eggs of *Ascaris suum* cultured at 28 and 32°C.

Age of cultures (days)	Group no.	Temp. °C	Aeration (BPM)*	Mean group score†	Range of scores	Mortalities
21	1	28	75–85	2.60[b]	(2–3)	1
	2	32	75–85	2.16[d]	(2–3)	0
	3	28	175–185	2.20[c]	(1–3)	1
	4	32	175–185	2.75[a]	(1–5)	2
25	1	28	75–85	2.16[b]	(1–4)	2
	2	32	75–85	1.33[d]	(1–2)	0
	3	28	175–185	1.83[c]	(1–2)	0
	4	32	175–185	2.33[a]	(1–3)	0
31	1	28	75–85	2.50[a]	(2–3)	0
	2	32	75–85	1.00[c]	(0)	0
	3	28	175–185	2.33[a]	(2–3)	0
	4	32	175–185	1.16[b]	(1–2)	0
39	1	28	75–85	1.66[a]	(1–3)	0
	2	32	75–85	0.83[c]	(0–1)	0
	3	28	175–185	1.83[a]	(1–3)	0
	4	32	175–185	1.00[b]	(0–2)	0
46	1	28	75–85	2.33[a]	(2–3)	0
	2	32	75–85	1.33[c]	(1–2)	0
	3	28	175–185	2.00[b]	(1–3)	0
	4	32	175–185	1.20[d]	(1–2)	0

* BPM = bubbles per minutes.

† Student–Newman–Keuls analysis ($P < 0.05$).

Table 5. Time required for development of eggs of *Ascaris suum* to reach 50% motile L1 at various temperatures.

	Mean number of days at temperature (°C)					
	20	23	26	28	30	32
Seamster (1950)	19.17[a]*	12.96[c]	12.95[d]	9.71[f]	8.88[g]	8.75[h]
This study†	18.50[b]	11.45[e]	7.65[i]	7.20[j]	5.30[l]	5.75[k]

* Student–Newman–Keuls analysis ($P < 0.05$).
† Mean of slow and fast aeration rates

This difference was greatly significant at ≧26°C and is attributed to the continuous agitation of eggs by means of aeration, which was not conducted in the 1950 experiment. The earlier study involved humidity requirements for culturing that were not necessary in the present study. Again, eggs cultured at 30°C required the least amount of time for development.

There appear to be conflicting reports about the sequence of events occurring during the second molt in the development of *A. suum* larvae. Roberts (1934) stated that the second molt was in the lungs, and Douvres et al. (1969) reported that this molt occurred in the liver. Araujo (1972) and Maung (1978) related that the early stages of development in the egg comprised 2 molts, each occurring soon after the other. However, the time of completion of the second molt varied considerably, which sometimes was not completed until the larvae reached the liver (Maung, 1978). The author of this report presents the case that the larval stage identified in the infective egg (Table 1) is the L2 stage and makes no attempt to present larval types A–I as did Maung (1978). L1 and L2 stages were identified by their ability to withstand hatching by the coverslip pressure method and by the presence of the L1 cuticle loose at the anterior and posterior ends, around the body of the liberated L2.

Due to the inability to relate in vitro hatchability with infectivity of eggs, an in vivo system in mice was established. On the basis of lung lesion scores in mice, it was possible to identify egg samples cultured at lower temperatures of 20 and 23°C. Eggs conditioned to growth at 30°C exhibited the greatest index of infectivity at the earliest time of 21 days. The most severe index of infectivity (i.e., mortality) was attained in mice inoculated with eggs cultured for 31 and 39 days at 26°C (Table 6). Arene (1986) stated that eggs embryonated at ≧28°C had less ability to hatch and to penetrate tissue membranes in vitro than larvae from eggs embryonated at lower temperatures. In the present study, there were no mortalities in mice infected with eggs cultured at 20 and 23°C regardless of time. However, the infectivity rates indicated by mortalities were higher in mice inoculated with eggs in the early days of culture at ≧28°C. Cultivation temperatures of 28 and 32°C were correlated with points of infectivity. The latter temperature is interpreted as approaching the maximum tolerated temperature, as L2 larvae in the eggs were observed microscopically to show a gradual decline in larval motility and in healthy protoplasmic appearance at 30–46 days of cultivation. This interpretation approximates that given by Seamster (1950) and was verified by lower lung lesion scores obtained

Table 6. Mean lung lesion scores of groups of mice inoculated orally with 10,000 eggs of *Ascaris suum* cultured at different temperatures.

	Temperature (°C)											
	20†		23		26		28		30		32	
DIC*	S	F	S	F	S	F	S	F	S	F	S	F
21	N/D	N/D	0.00[h]*	0.00[h]	N/D	N/D	2.60[d]	2.20[e]	2.80[b]	2.80[b]	2.16[f]	2.75[c]
25	0.00[l]	0.71[i]	0.00[l]	0.00[l]	2.00[d]	1.14[h]	1.75[f]	1.83[e]	2.66[a]	2.50[b]	1.33[g]	2.33[c]
31	1.00[k]	1.00[j]	1.50[g]	1.00[j]	2.71[b]	4.14[a]	2.50[d]	2.33[e]	2.50[d]	2.11[f]	1.00[j]	1.16[h]
39	1.00[j]	0.00[l]	1.66[g]	2.00[c]	1.00[j]	4.16[a]	1.66[g]	1.83[e]	1.83[e]	2.66[b]	0.83[k]	1.00[j]
46	0.33[l]	1.00[k]	1.83[f]	2.50[a]	1.00[k]	1.00[k]	2.33[b]	2.00[d]	2.16[c]	1.83[f]	1.33[g]	1.20[h]

* DIC = days in culture.
† S/F = slow and fast aeration; N/D = not done; Student–Newman–Keuls analysis ($P < 0.05$).

in mice inoculated with eggs set at 32°C for 31, 39, and 46 days.

The present study suggests that the aeration rate and temperature at which eggs of *A. suum* are embryonated have an effect on the viability, hatchability, and subsequent infectivity of L2 larvae in mice.

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1990 Student Presentation Competition

The October 1990 meeting of the Society will be devoted to the second student research presentation competition, which will be held at the Uniformed Services University of the Health Sciences. Cash awards will again be made for the best paper(s). A call for papers will appear in the July issue of the *Journal.* For more information, contact Dr. Patrick Carney, Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, Maryland 20814.

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Freezing Resistance of a *Trichinella spiralis nativa* Isolate from a Gray Wolf, *Canis lupus,* in Montana, with Observations on Genetic and Biological Characteristics of the Biotype

DAVID E. WORLEY,[1] DANTE S. ZARLENGA,[2] AND FLOYD M. SEESEE[1]

[1] Veterinary Research Laboratory, Department of Veterinary Science, Montana State University, Bozeman, Montana 59717 and

[2] U.S. Department of Agriculture, Agricultural Research Service, Livestock and Poultry Sciences Institute, Beltsville, Maryland 20705

ABSTRACT: A *Trichinella spiralis* isolate obtained from a gray wolf (*Canis lupus*) in northwestern Montana in 1987 was evaluated for infectivity and resistance to freezing and its DNA restriction fragment length polymorphisms (RFLP's) compared to that from other sylvatic and domestic isolates. Infectivity indices in albino mice were low (≤2.6) and correlated well with mouse infectivity data from other sylvatic isolates. Larvae did not survive freezing after storage at −20 to −30°C in wolf musculature for 1- and 2-mo periods. Analysis of DNA extracted from the wolf isolate by RFLP's and DNA dot blots using a *Trichinella spiralis spiralis*-specific probe indicated that this isolate belongs to the *Trichinella spiralis nativa* group. Inability of the Montana wolf isolate to survive subfreezing temperatures contrasts with published data indicating long-term survival (>18 mo at −10°C) of a Canadian wolf isolate. This disparity in cold-hardiness of *T. spiralis* isolates from wolves in neighboring zoogeographic regions as well as variations in RFLP banding patterns between the Montana wolf isolate and other freeze-resistant isolates (e.g., polar bear) indicates the coexistence of dissimilar sylvatic strains at higher latitudes where Arctic or freeze-resistant biotypes predominate.

KEY WORDS: *Trichinella spiralis,* wolf strain freeze resistance, infectivity in deer mice, DNA polymorphisms.

Selected biological features such as host affinity, infectivity, and fecundity in laboratory rodents, and resistance to freezing have been proposed as working criteria for differentiating *Trichinella spiralis* subspecies (Dick and Chadee, 1981; Leiby et al., 1985; Worley et al., 1985). Recently, isozyme analysis (Pozio et al., 1989), DNA probes (Klassen et al., 1986b; Dame et al., 1987), and characteristic banding patterns of enzyme-digested parasite DNA separated on agarose gels (Curran et al., 1985; Chambers et al., 1986; Klassen et al., 1986a; Zarlenga and Murrell, 1989) have also been used to distinguish *T. spiralis* isolates. Furthermore, some physiological and ecological characteristics appear to be relatively constant within subspecies and hence may have value for differentiating *Trichinella spiralis spiralis* and *Trichinella spiralis nativa* (Belosevic and Dick, 1980; Smith, 1983). Experimental data derived from a wolf isolate collected in northwestern Montana demonstrated that isolates of *T. s. nativa* from the same host species and geographic region may have contrasting biological characteristics such as resistance to freezing.

Materials and Methods

Trichinella larvae obtained by peptic digestion of skeletal musculature from an adult male gray wolf (*Canis lupus*) killed on the Blackfeet Indian Reservation adjoining Glacier National Park at approximately 48°30′N latitude, 113°W longitude in 1987 were evaluated for resistance to freezing as described previously (Worley et al., 1986). Parasite infectivity was verified by passages in both deer mice (*Peromyscus maniculatus*) and CD-1 strain albino mice (*Mus musculus*). Genomic DNA extracted from the Montana wolf isolate by proteinase-K SDS digestion (Dame and McCutchan, 1983) was compared by dot blot and RFLP analysis to DNA isolated from several pig and *T. s. nativa* isolates (see Dame et al., 1987, for complete listing and source of parasite isolates).

Dot blots were prepared by diluting 0.5 μg of each genomic DNA to 400 μl in 10 mM Tris, pH 7.6, 1 mM EDTA, and heating the solution in a boiling waterbath for 10 min. The solutions were adjusted to 4× SSC (1× SSC is 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0) and vacuum-filtered through a nitrocellulose membrane, which was subsequently baked at 80°C for 2 hr. The filters were prehybridized at 65°C for 6 hr in hybridization buffer. Dot blots were then screened overnight with a ^{32}P-labeled *T. s. spiralis*-specific probe (pBP-2) (Dame et al., 1987) prepared by nick translation (Rigby et al., 1977) to determine the relationship of the Montana wolf isolate to the pig isolate. Filters were washed in 0.2× SSC, 0.1% SDS at 50°C, air dried, and then autoradiographed.

Southern blot analysis was used to compare restriction enzyme-digested *T. spiralis* DNA from Montana wolf to DNA isolated from *T. s. spiralis* collected from pig and various sylvatic hosts. DNA samples (2 μg) were digested to completion for 2 hr with Dra I (10 units of enzyme/μg DNA), electrophoresed through a 0.8% agarose gel, and then blotted to a Nytran mem-

Table 1. Infectivity in deer mice (*Peromyscus maniculatus*) of a wolf isolate of *Trichinella spiralis nativa* before and after freezing in wolf muscle.

Time frozen (days)	No. mice inoculated	Inoculum* (larvae/mouse)	Mice infected/total no. inoculated	Larvae/g bodyweight
0	3	100	3/3	83, 89, 283
30	3	100	0/3	—
60	3	100	0/3	—

* Given orally in saline suspension.

Table 2. Comparative infectivity of *Trichinella spiralis* isolates from domestic pig and gray wolf in albino mice.

Host	No. mice inoculated	Infectivity index*
Pig	7	24.25
Wolf	10	2.53

* Index: ratio of larvae inoculated to total muscle larvae recovered 44 days postinoculation.

brane according to Southern (1975). Membrane filters were baked and prehybridized as described above. Blots were screened with [γ^{32}P]ATP-kinased total RNA (Zarlenga and Gamble, 1987) then hybridized and washed as indicated for dot blot analysis.

Results and Discussion

Digestion of a 25-g sample of wolf biceps brachii muscle revealed a larval concentration of 26.3 larvae/g of tissue. Parasite infectivity was verified by successful passages in deer mice (Table 1); however, infectivities in albino mice were significantly lower than *T. s. spiralis,* suggesting that the isolates from the wolf and pig were not the same (Table 2).

Because it had been demonstrated elsewhere (Dame et al., 1987; Murrell et al., 1987) that *T. s. spiralis* is capable of infecting sylvatic hosts, it was necessary to determine whether the gray wolf isolate was of the pig type (*T. s. spiralis*) or more closely related to other sylvatic isolates (*T. s. nativa*). Dot blots screened with a ^{32}P-labeled probe (pBP-2), specific for *T. s. spiralis,* verified that the Montana wolf isolate was not of the pig biotype by its failure to hybridize to the pBP-2 probe (Fig. 1). Furthermore, Southern blot analysis comparing restriction enzyme-digested DNA from pig and sylvatic *T. spiralis* isolates indicated similarities between the wolf isolate and *T. s. nativa* (Fig. 2, lanes 2–5) and significant differences in the major rDNA bands from the pig type (Fig. 2, lane 1).

No viable *Trichinella* larvae were retrieved from wolf leg musculature stored at −20 to −30°C for 1- and 2-mo periods as indicated by failure to induce infections in laboratory-reared deer

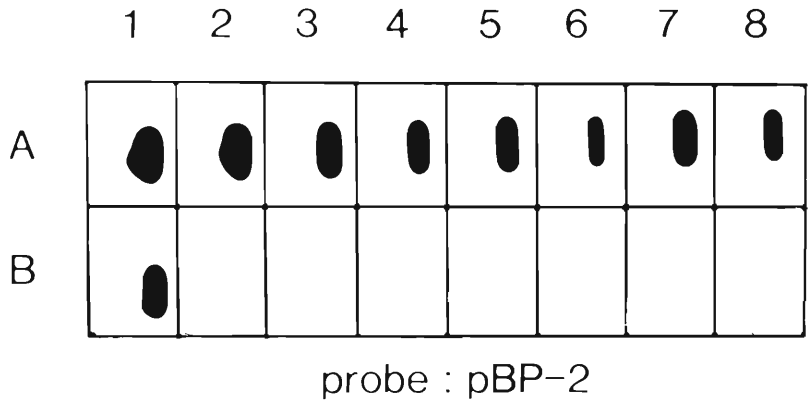

Figure 1. Dot blot analysis of *T. spiralis* isolates using pBP-2 probe. DNA samples (0.5 μg) were denatured by boiling and then vacuum-filtered through a nitrocellulose membrane. Blots were baked at 80°C for 2 hr and then hybridized to the ^{32}P-labeled pBP-2 DNA probe. A: 1, Beltsville pig (*Sus scrofa*); 2, Maine pig (*S. scrofa*); 3, Scott pig (*S. scrofa*); 4, Thai pig (*S. scrofa*); 5, bobcat (*Felis rufus*); 6, polar bear—4 (*Ursus maritimus*); 7, raccoon—1 (*Procyon lotor*); 8, UPB-6 (*Ursus americanus*). B: 1, UPB-8 (*U. americanus*); 2, Montana wolf (*Canis lupus*); 3, grizzly—1 (*Ursus arctos*); 4, polar bear—1 (*U. maritimus*); 5, Pennsylvania fox (*Urocyon cinereoargenteus*); 6, UPB-3 (*U. americanus*); 7, UPB-11 (*U. americanus*).

mice. The absence of freezing resistance in muscle larvae from the Montana wolf, which was a member of a pack believed to have originated farther north along the Canadian Rockies in Alberta (Ream and Harris, 1986), contrasts with long-term survival (18 mo at −10°C) reported by Dies (1980) for *Trichinella* larvae in skeletal muscle of a wolf collected near Fort McMurray in northwestern Alberta. Larval viability of the Canadian isolate was also confirmed by mouse inoculation. Because both isolates were derived from wolves inhabiting adjoining biotic provinces differing more in physiographic features than climate (Dice, 1943), and because each had been shown to be true sylvatic isolates by DNA hybridization studies and freeze resistance, an obvious explanation for the disparity in cold hardiness of the 2 isolates is lacking. RFLP comparison of the Montana wolf isolate (Fig. 2, lane 2) with a previously determined freeze-resistant isolate, PB-1 (Fig. 2, lane 4), reveals some variation in banding patterns, i.e., absence of the 1.8-kb, 2.6-kb, and 4.1-kb bands in the freeze-resistant strain. Whether these band differences are indicative of the cold-hardy biotype or are attributable to normal variation within the various sylvatic isolates is not yet known.

Previous studies have clearly demonstrated the existence of freeze-resistant isolates of *T. spiralis* in certain host species (e.g., grizzly bear and wolverine) at latitudes comparable to that where the Montana wolf isolate originated (Worley et al., 1986). As neither host muscle type nor relative depth of larvae within infected tissue appear to alter the ability of trichinae to survive subfreezing temperatures (Chadee and Dick, 1982), factors other than host range, geographic origin, and tissue distribution of larvae must be considered in evaluating the existence of so-called Arctic or cold-hardy *T. spiralis* isolates occurring at higher latitudes in North America. The regional variability in parasite subpopulations from the same host inhabiting different sylvatic ecosystems may better be studied by developing DNA probes for the freeze-resistant genotype.

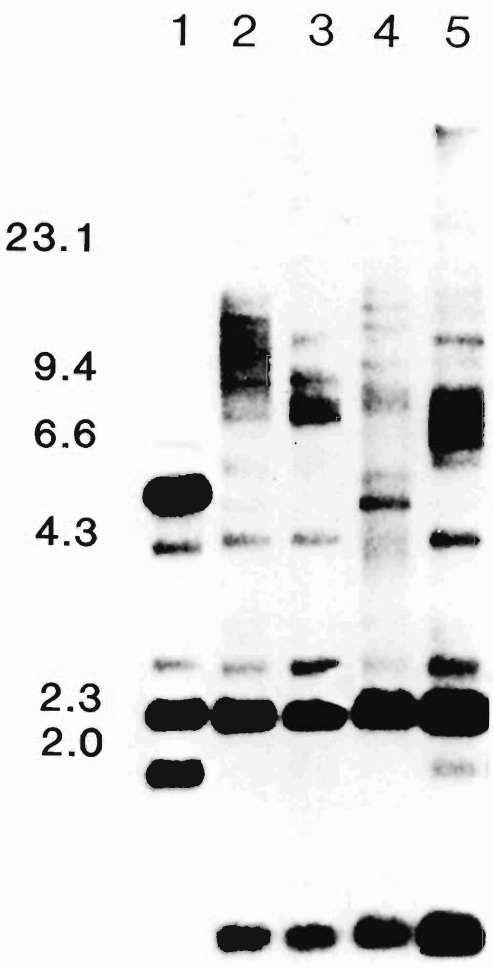

Figure 2. Southern blots of *T. spiralis* DNA screened with ^{32}P-labeled total RNA. DNA samples were digested to completion with Dra I, electrophoresed through a 0.8% agarose gel, and then blotted to a Nytran membrane filter. Blots were probed with [^{32}P]ATP-kinased *T. spiralis* RNA. Lane 1, Beltsville pig (*Sus scrofa*); 2, Montana wolf (*Canis lupus*); 3, grizzly bear (*Ursus arctos*); 4, polar bear (*Ursus maritimus*); 5, Pennsylvania fox (*Urocyon cinereoargenteus*).

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Cuticular Ridge Pattern in *Ostertagia gruehneri* and *Ostertagia arctica* (Nematoda: Trichostrongyloidea) from Caribou, *Rangifer tarandus*

J. R. LICHTENFELS,[1] P. A. PILITT,[1] AND M. FRUETEL[2]

[1] Biosystematic Parasitology Laboratory, Livestock and Poultry Sciences Institute, Agricultural Research Service, USDA, Beltsville, Maryland 20705 and

[2] Department of Biology, Lakehead University, Thunder Bay, Ontario, Canada P7B 5E1

ABSTRACT: Two species of medium stomach worms are common parasites of the caribou, *Rangifer tarandus*. The 2 species, *Ostertagia gruehneri* Skrjabin, 1929, and *O. arctica* Mitzkewitzsch, 1929, differ so markedly in morphology of the spicules and genital cone that many nematode systematists place them in different genera. Recent studies of similar pairs of species parasitic in other ruminants have provided evidence that such pairs of species may be morphotypes of 1 species. The 2 species from caribou are redescribed with emphasis on the pattern of surface cuticular ridges and the structure of the esophagus, characters considered useful for distinguishing species of trichostrongyloid nematodes. *Ostertagia gruehneri* and *O. arctica* were found to have identical ridge patterns and esophageal characteristics. Both species had 5 lateral ridges, a long eosphageal valve, and ducts for the subventral esophageal glands that opened internally posterior to the level of the cervical papillae.

KEY WORDS: *Ostertagia gruehneri, Ostertagia arctica,* Nematoda, Trichostrongyloidea, Ostertagiinae, synlophe, nematode morphology, cuticle, ruminants.

Two species of "medium stomach worms" (Ostertagiinae: Trichostrongylidae) are commonly found as coparasites of the caribou, *Rangifer tarandus*. *Ostertagia gruehneri* Skrjabin, 1929 was found in virtually 100% of abomasa of Norwegian *Rangifer tarandus* by Bye (1987) and it comprised 85–99% of the nematode population. Another species frequently found with *O. gruehneri* is *O. arctica* Mitzkewitsch, 1929. Recently, Lancaster et al. (1983) proposed that polymorphism is common in the Ostertagiinae and that species with morphological characteristics of the genus *Skrjabinagia* may be morphotypes of associate dominant species such as *O. gruehneri*. We have studied several suspected pairs of species or associates from other ruminants using newly employed characters to supplement characters commonly used to identify trichostrongyloid species such as the shape of the spicules, genital cone, and copulatory bursa (Lichtenfels et al., 1988a, b; Lichtenfels and Pilitt, 1989). These newly employed characters include the pattern of surface cuticular ridges (synlophe) and the structure of the esophagus.

The objective of the present study was to describe the synlophe and esophageal characteristics of *O. gruehneri* and *O. arctica*. Previous studies have shown the synlophe to be one of the most useful morphological characters for separating species of the Trichostrongyloidea (Lichtenfels, 1977; Lichtenfels and Pilitt, 1983a, b; Measures and Anderson, 1983; Fukumoto, 1986; Lichtenfels et al., 1986; Hoberg and Rickard, 1988). Our hypothesis was that if *O. gruehneri* and *O. arctica* are different species, they would have different synlophes.

Materials and Methods

Nematodes

All specimens were obtained from the USDA Parasite Collection maintained in the Beltsville laboratory. Host and locality data (Table 1) were obtained from the records of the collection. Common and scientific names of hosts and synonymies of nematodes are provided (Table 1). The species identities of male nematodes were confirmed on the basis of spicule and genital cone morphology (Drozdz, 1965). Females were identified by matching synlophes to that of the males.

Hosts

Both woodland caribou, *Rangifer tarandus caribou,* and barren-ground caribou, *R. tarandus groenlandicus,* were included in the collections from Canada (Fruetel and Lankester, 1989). The subspecies in Norway was the Svalbard reindeer, *Rangifer tarandus platyrhynchus,* and the host subspecies for the collection from Russia was *Rangifer tarandus sibiricus*. Common names frequently used for these ruminants are caribou in North America and reindeer in Europe and Asia. For simplicity herein we will refer to all as *Rangifer tarandus* or caribou.

Microscopy

Specimens were studied either as (1) temporary whole mounts cleared in phenol–alcohol (80 parts melted phenol crystals and 20 parts absolute ethanol) and examined with ordinary light microscopy or interference-contrast light microscopy; or, (2) critical point-dried, coated with gold palladium, and viewed at 5–20 kV with scanning electron microscopy (SEM) (Madden and

Table 1. Specimens of *Ostertagia gruehneri* and *Ostertagia arctica* studied by host and locality.

Species and synonyms	Host, locality (number of lots/number of specimens by host, locality, and sex)
Ostertagia gruehneri Skrjabin, 1929 Syn. *Grühneria grühneri* Sarwar, 1956	*Rangifer tarandus,* Canada 2/9 ♂, 4 ♀ Norway 1/10 ♂, 4 ♀ USSR 1/1 ♂ Alaska 2/4 ♂
Ostertagia arctica Mitzkewitsch, 1929 Syn. *Sjobergia arctica* (Mitzkewitsch, 1929) Sarwar, 1956; *Ostertagiella arctica* (Mitzkewitsch, 1929) Andreeva, 1957; *Skrjabinagia arctica* (Mitzkewitsch, 1929) Drozdz, 1965	*Rangifer tarandus,* Canada 2/7 ♂ Norway 1/15 ♂ USSR 1/2 ♂ Alaska 1/1 ♂

Tromba, 1976). Measurements are in millimeters unless indicated otherwise.

Characters studied

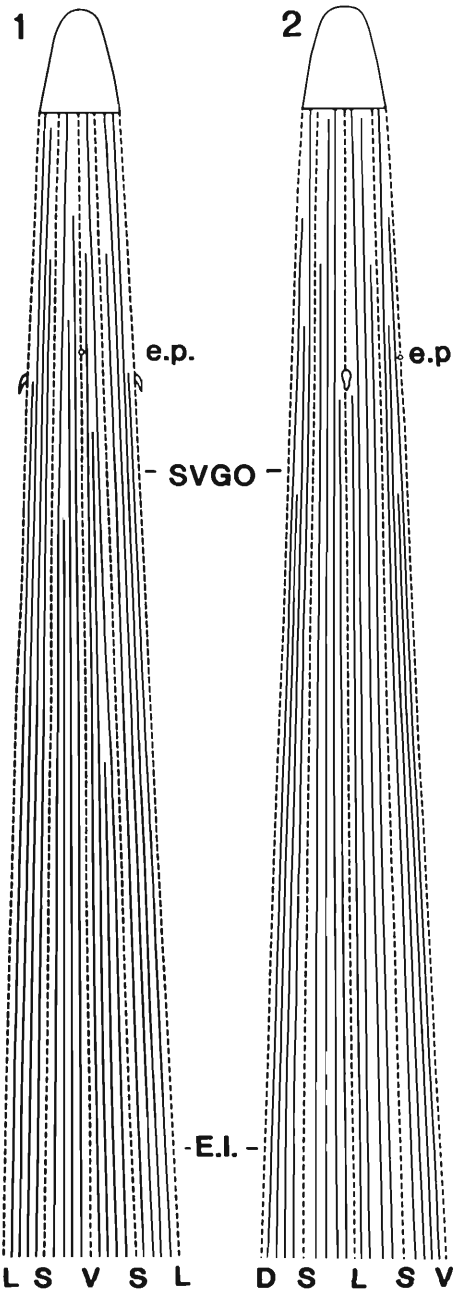
In addition to the synlophes of the nematodes, several morphometric characteristics were studied (Table 2). Student's *t*-test was employed to test apparent difference among mean measurements.

Results

Synlophe

The synlophes of *O. gruehneri* and *O. arctica* were found to be identical. Because no differences between the 2 species were found they are described together below and in the drawings (Figs. 1, 2). The most distinctive and easily recognizable feature of the synlophe of these 2 species is the group of 5 closely spaced ridges in each lateral field (Figs. 1–5). The 5 lateral ridges can be recognized because they are closer together than other ridges. There is a gradient toward less space between ridges in lateral areas than in dorsal and ventral areas (Figs. 3–5). The pattern of ridges in the region of the esophagus is illustrated in lateral and ventral views (Figs. 1, 2). Like other members of the Ostertagiinae the synlophe of these 2 species consisted, in the region of the esophagus, of 40 ridges. For convenience in understanding the pattern the ridges can be grouped into 4 symmetrical and relatively equal fields. The ventral and dorsal fields include 9 ridges each and the lateral fields include 11 ridges each.

The lateral ridge (dashed line L in Fig. 1) is ventral to the cervical papilla. The 5 closely spaced lateral ridges include the lateral, a pair of



Figures 1, 2. Diagrammatic drawings of synlophe present in both *Ostertagia gruehneri* and *Ostertagia arctica,* with lateral (L), ventral (V), and subventral and subdorsal (S) ridges indicated by dashed lines. Other abbreviations: ep = excretory pore; E-I = esophageal-intestinal junction; SVGO = subventral esophageal gland duct opening. 1. Ventral view. 2. Lateral view. Note: dashed lines are for emphasis only; the ridges are not interrupted.

Table 2. Morphometrics (in micrometers; range with mean in parentheses) of males* of *Ostertagia gruehneri* and *Ostertagia arctica* in *Rangifer tarandus*.

Character	Species	
	Ostertagia gruehneri ($N = 23$)	*Ostertagia arctica* ($N = 20$)
Body length	6,600–9,600 (7,950)	5,280–10,500 (7,250)
Cephalic inflation length†	85–120 (100)	82–130 (097)
Nerve ring†	221–288 (259)	223–296 (256)
Execretory pore†	269–332 (305)	257–344 (300)
Cephalic papillae†	284–446 (322)	280–371 (315)
Subventral gland orifices†	296–395 (348)	296–387 (353)
Esophagus length†	806–999 (905)	802–1,149 (948)
Esophageal–intestinal valve length	96–143 (120)‡	110–171 (131)
Spicule length	182–226 (204)	181–263 (206)
Soberg's organ	Absent	Present
Bursal ray pattern§	2-1-2	2-1-2
Length of dorsal ray of bursa	68–96 (81)‖	75–143 (111)‖
Length of bursa#	211–304 (251)	190–365 (272)

* Females not measured.
† Distance measured from anterior end.
‡ $N = 22$.
§ Pattern following system of Durette-Desset (1983).
‖ Significant differences between means with Student's t-test; probability of greater t value less than 0.001.
Measured from prebursal papillae; $N = 20$.

adjacent ridges (1 ventral and 1 dorsal) anterior to the cervical papilla that like the lateral ridge extend from the cephalic expansion to the posterior end of the nematode, and a second pair of ridges that flank the lateral ridge between it and the first pair. The second pair usually begin just posterior to the cervical papilla but may begin slightly anterior to the cervical papilla (Fig. 3) or as much as 200–300 μm posterior to the papilla. Each lateral field also includes 2 additional pairs of ridges for a total of 9 ridges each.

The ventral ridge (dashed line "V" in Figs. 1, 2) intersects with the excretory pore and extends anteriorly to the cephalic inflation. The pair of ridges that flank the ventral ridge extend anteriorly about half of the distance from the excretory pore to the cephalic expansion. The next 2 pairs of ridges flank the 3 ventralmost ridges and are variable in length, extending anteriorly to the middle of the esophagus or in some specimens to a point slightly anterior to the excretory pore. The lateralmost 2 pairs of ridges in the ventral field both extend anteriorly to the cephalic expansion.

The ridges in the dorsal field are a mirror image of those in the ventral field. Each dorsal and ventral field includes 11 ridges. The lateralmost ridge of the ventral field is drawn with a dashed line and labeled "S" in Figures 1 and 2.

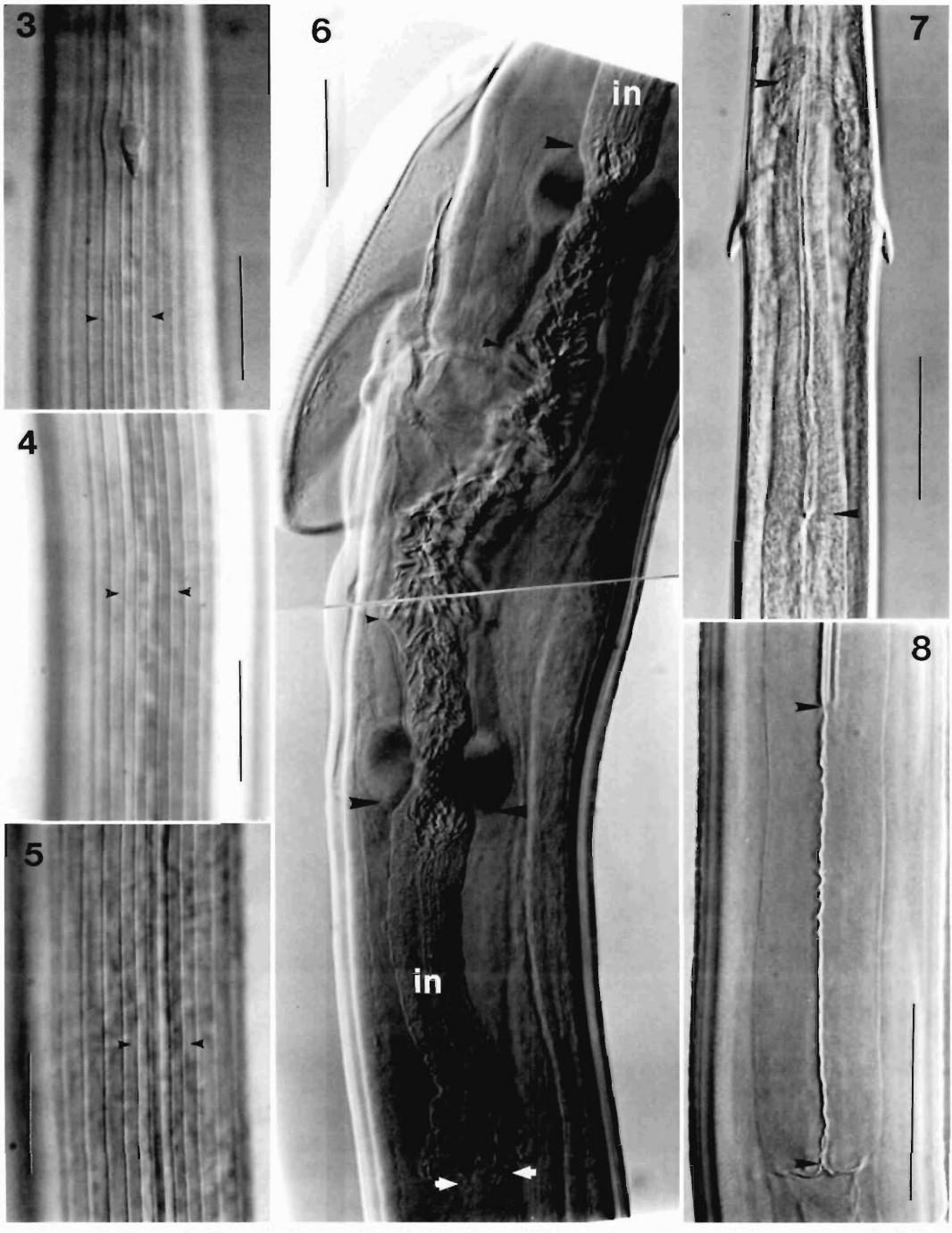
The total number of ridges at the level of the esophageal valve and for most of the rest of the nematode is 40. The ridges are exceptionally straight and continuous with few crossovers, interruptions, or additions. In the posterior half of the lateral fields, 1 or 2 of a group of 5 closely spaced ridges may branch to form a group of 6 or 7 closely spaced ridges (Fig. 5). Near the copulatory bursa in the males the lateral ridges extend almost to the level of the prebursal papillae, but ventral and dorsal ridges end 400–500 μm anterior to the bursa. In the female the ridges are interrupted at the vulva and the vulval flap (if the flap is present), but most of the ridges extend almost to the tip of the tail.

Esophagus

The esophageal valves of *O. gruehneri* and *O. arctica* were found to be similar in length (Table 2). The valves were more than 3 times as long as thick (Fig. 8). The position of the openings of the subventral esophageal gland ducts (SVGO) in relation to the position of the cervical papillae was variable, but was usually posterior to the papillae (Table 2; Fig. 7) in both species.

Bursal rays

The bursal ray formula (2-1-2) described by Durette-Desset (1983) for the genus *Ostertagia* was present in both species studied herein. The only difference noted in the copulatory bursae



Figures 3–8. Synlophe and other morphological features of *Ostertagia gruehneri* and *Ostertagia arctica*. Light micrographs with the aid of interference microscopy. All scale bars, 50 μm. 3. Lateral synlophe of male *O. gruehneri* in region of left cervical papilla showing 5 closely spaced lateral ridges (between arrows). 4. Lateral synlophe of male *O. arctica* in region of esophageal-intestinal junction showing 5 closely spaced lateral ridges (between arrows). 5. Lateral synlophe of male *O. gruehneri* near midbody showing 5 closely spaced lateral ridges (between arrows). 6. Female reproductive system of *O. gruehneri* showing vulva with vulval flap, vestibule (between upper and lower small arrows), sphincters (between small and large arrows), and infundibula (in). The white arrows indicate the end of the posterior infundibulum where it joins the uterus. The anterior infundibulum (only

between the 2 species was in the lengths of the dorsal ray. In *O. gruehneri* the dorsal ray was significantly ($P < 0.001$) shorter than that of *O. arctica* (Table 2).

Genital cones

The genital cones of the 2 species were quite different. Ventrally, *O. gruehneri* had a prominent proconus (Fig. 10), but this structure was completely lacking in *O. arctica* (Fig. 12). Dorsally, in *O. arctica* the accessory bursal membrane was enlarged and sclerotized (Figs. 11, 12), but this structure was small and unsclerotized in *O. gruehneri* (Figs. 9, 10).

The spicules of the 2 species were similar in length (Table 2) but differed markedly in shape. The spicules of *O. gruehneri* were slender and divided into 3 dissimilar branches in their distal third (Figs. 13, 14). The spicules of *O. arctica* are relatively thicker and divided into 3 dissimilar branches in their distal half (Figs. 15, 16). A gubernaculum is present in both species (Figs. 10, 14, 15).

Females

We found no differences among the females so they were all regarded to be *O. gruehneri.*

Discussion

The original spelling, *Ostertagia grühneri,* is not acceptable because of the umlaut sign. The International Code of Zoological Nomenclature (Third Edition, 1985), Article 32(d)(i), clearly requires the deletion of the umlaut from a vowel and the insertion of the letter "e" after the vowel. Accordingly, we have spelled the species name as *O. gruehneri* herein, although most earlier workers either followed the original spelling with the umlaut or dropped the umlaut but did not add the required "e" after the "u."

Females of the 2 species have never been clearly distinguished; being separated by some workers (see Skrjabin et al., 1954) as without a vulval flap (*O. gruehneri*) or with a vulval flap (*O. arctica*). We do not regard presence or absence of a vulval flap to be a useful character for identifying species (Hong and Timms, 1989), and, as no differences were found among the females, they were all regarded to be 1 species.

In recent studies of other species of the Ostertagiinae, Lichtenfels et al. (1988a, b) and Lichtenfels and Pilitt (1989) found that characteristics of the synlophe and the esophagus were useful for identifying species. In the present study however, no differences in these characters were found between *O. gruehneri* and *O. arctica.* Thus, the results are consistent with the hypothesis proposed by Lancaster and Hong (1981) that species such as *O. arctica,* with the characteristics of the genus *Skrjabinagia* as described by Drozdz (1965), may be morphotypes of associate dominant species such as *O. gruehneri.*

The unique synlophe described herein for *O. gruehneri* and *O. arctica* provides a new useful character for identifying both males and females of this species. The distinctive 5 lateral ridges in the region of the esophagus can be observed with light microscopy (at 400× or greater magnification) in whole mounts of living, frozen, or cleared and fixed specimens. Cross sections were not made because an insufficient number of specimens of *O. arctica* were available. Therefore, in order to have comparative data for both species, studies were made on whole specimens.

The newly described characters of the cuticle and esophagus provide new insight into the evolutionary relationships of the Ostertagiinae. The 5 lateral ridge synlophe appears to be very similar to the 3 lateral ridge synlophe present in *O. leptospicularis* (=*O. kolchida*) and *Marshallagia marshalli* (=*O. occidentalis*). Those species also share with *O. gruehneri* (=*O. arctica*) a long esophageal valve and a bursal ray pattern of 2-1-2 (Lichtenfels et al., 1988b). In addition, *O. gruehneri* shared with *O. leptospicularis* the characteristics of 3 ventral ridges, a relatively posterior SVGO, and cervid hosts. It appears that the species from cervids may be more closely related to each other than they are to the Ostertagiinae parasitic in bovids. Lichtenfels and Pilitt (1983a, b) and Hoberg et al. (1989) came to similar conclusions for *Nematodirella* and *Nematodirus,* respectively. However, *O. odocoilei* and *O. mossi* have yet to be redescribed, and an hypothesis on the evolution of the Ostertagiinae is beyond the scope of this work.

The percentage of the population consisting of the minor species, *O. arctica,* in the present study

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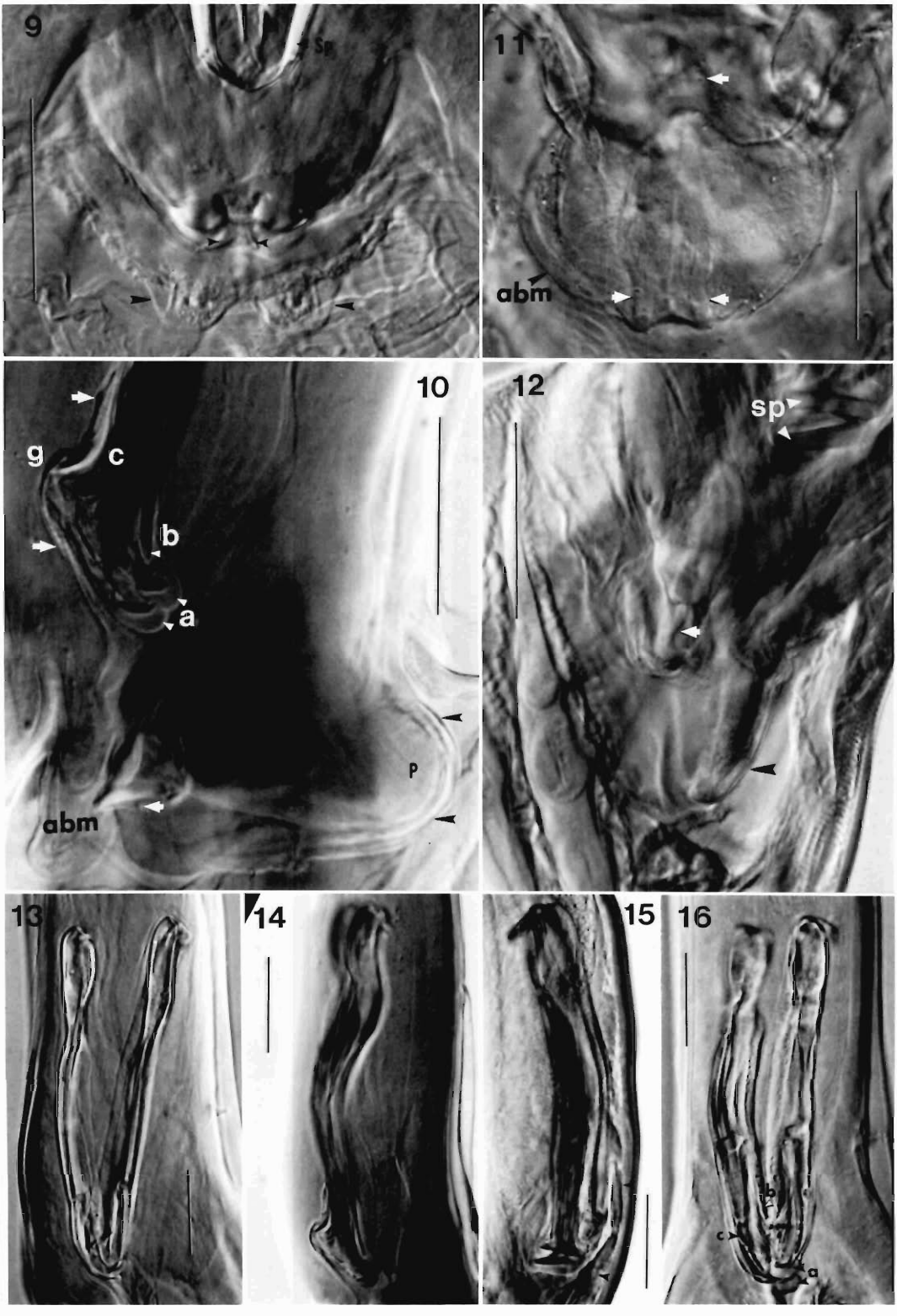
partially shown) is the same size as the posterior one. 7. Male *O. gruehneri,* dorsoventral view, showing the position of the nerve ring (upper arrow) and the anterior margin of the subventral esophageal gland (lower arrow) in relation to the prominent cervical papillae. 8. Esophageal valve of male *O. gruehneri* showing thick cuticular lining of esophagus anterior to the valve (above upper arrow) and the posterior end of the valve (lower arrow).



(0–11%, mean 2%) was similar to that reported for other minor species. In all pairs of species examined previously (Lichtenfels et al., 1988a, b; Lichtenfels and Pilitt, 1989) and suspected to be examples of polymorphism (Lancaster and Hong, 1981), the member of the pair with the relatively slender spicules and unsclerotized accessory bursal membrane has comprised the major proportion of the nematode population (85–99%); and the species with relatively stout spicules and an enlarged and sclerotized dorsal part of the genital cone has comprised a minor proportion of the population (1–15%). This consistent pattern of differences has been described previously by Lancaster and Hong (1981). Others (Fruetel and Lankester, 1989) have published excellent drawings of the spicules and genital cones of the males of these 2 species. However, photomicrographs of these structures as presented herein have not been published previously. The photomicrographs of the spicules and genital cones do not provide new information on their structure, but do provide an example of these structures as seen with interference light microscopy.

The distribution of *O. gruehneri* and *O. arctica* extends throughout the range of *Rangifer tarandus*. We were able to study specimens from that host from North America, Europe, and Asia. Both nematode species have been reported also from many other cervids and bovids. We have determined however, that errors in identifying these species are common because of the difficulty earlier workers had in distinguishing *O. gruehneri* and *O. arctica* from *O. ostertagi* and *O. lyrata*. Therefore, caution is urged in accepting reports in the literature. The new characters described herein and by Lichtenfels et al. (1988a, b) make the identification of these 2 pairs of species exceptionally simple. Although the spicules and genital cones of the 2 pairs of species are quite similar, characteristics of the synlophe and esophagus indicate that *O. gruehneri* and *O. arctica* probably are not closely related to *O. ostertagi* and *O. lyrata*. With the aid of the newly described characteristics, an accurate host range for the species of *Ostertagia* in caribou can be developed.

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Figures 9–16. Genital cones and spicules of *Ostertagia gruehneri* and *O. arctica*. All scale bars, 50 μm. 9. Genital cone, ventral view, of *O. gruehneri* showing dorsal bilobed accessory bursal membrane (large arrows), ventral paired papillae (small arrows), and distal end of spicules (sp). 10. Genital cone, lateral view, of *O. gruehneri* showing accessory bursal membrane (abm), 1 of paired ventral papillae (lowest white arrow), proconus (p, between black arrows), and distal ends of spicules (a—main branch of each spicule with cuticular pad; b—bladelike ventral branch of 1 spicule; c—dorsal curved branch of 1 spicule; g—gubernaculum, upper white arrows). 11. Genital cone, ventral view, of *O. arctica* showing enlarged, oval, sclerotized accessory bursal membrane (abm) with elongate papillae (lower arrows) and paired ventral papillae out of focus (upper arrow). 12. Genital cone, lateral view, of *O. arctica* showing enlarged, sclerotized accessory bursal membrane (large arrow), 1 of the ventral paired papillae (small arrow), and the broad, flat, distal ends of the spicules (sp). 13, 14. Spicules of *O. gruehneri* showing shape and 3 distal branches separated in distal quarter. 13. Subventral view. 14. Lateral view showing gubernaculum. 15, 16. Spicules of *O. arctica* showing shape and 3 distal branches (a, b, c) separated in distal half. 15. Lateral view showing gubernaculum (arrows). 16. Ventral view.

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Research Note

Metacercaria of *Clinostomum complanatum* (Rudolphi, 1814) (Trematoda: Digenea) in a Texas Salamander, *Eurycea neotenes* (Amphibia: Caudata), with Comments on *C. marginatum* (Rudolphi, 1819)

CHRIS T. MCALLISTER

Renal-Metabolic Laboratory (151-G), Veterans Administration Medical Center,
4500 S. Lancaster Road, Dallas, Texas 75216 and
Department of Biological Sciences, University of North Texas, Denton, Texas 76203

ABSTRACT: *Clinostomum complanatum* (Rudolphi, 1814) (Digenea: Clinostomoidea) metacercariae were recovered from 1/86 (1.2%) Texas salamanders, *Eurycea neotenes*, in south-central Texas. Three encysted flukes were found to be developing in host connective tissue and muscle adjacent to heart and liver. This finding represents the first report of *C. complanatum* from a plethodontid salamander and the fourth time the genus has been reported from a caudate amphibian. Based on comparative morphological data and opinions proposed by earlier workers, the synonymy of *Clinostomum marginatum* (Rudolphi, 1819) with *Clinostomum complanatum* (Rudolphi, 1814) is provisionally supported, and *Clinostomum attenuatum* Cort, 1913, is recognized as a distinct species.

KEY WORDS: Clinostomidae, *Clinostomum attenuatum, C. complanatum, C. marginatum, Eurycea neotenes*, metacercariae, prevalence, synonymy, Texas salamander, Trematoda.

Trematodes of the genus *Clinostomum* Leidy, 1856, as adults, are normally endoparasites of the oral cavity and anterior esophagus of piscivorous birds, including those within the orders Charadriiformes (alcids, gulls, and shorebirds) and Ciconiiformes (herons, ibises, and storks). Occasional human cases of *Clinostomum* infection have been reported following ingestion of raw fishes (Hirai et al., 1987). Snails serve as first intermediate hosts (Yamaguti, 1971), and metacercariae have been reported previously to infect second intermediate hosts such as fishes (Hoffman, 1967), ranid frogs (Osborn, 1911; Cort, 1913; Fortner, 1923; Manter, 1938), a siren (Manter, 1938), a 3-toed amphiuma (Bennett and Humes, 1938), a spotted salamander (Fowler, 1947), and a plains garter snake (Hopkins, 1933). The purpose of this note is to report, for the first time, the occurrence of metacercariae of *Clinostomum complanatum* (Rudolphi, 1814) in a plethodontid salamander. Also, information is provided on the synonym *C. marginatum* (Rudolphi, 1819).

Between March 1982 and September 1985, 86 juvenile and adult (wet mass range, 0.2–0.9 g) Texas salamanders, *Eurycea neotenes* Bishop and Wright, 1937, were collected with dipnets from permanent springs located at 29°45′N, 99°33′W and 29°51′N, 99°40′W in Bandera and Real counties of south-central Texas. Following metabolic experiments (see McAllister and Fitzpatrick, 1989), salamanders were anesthetized with 0.1% TMS-222 (tricaine methanesulfonate), examined with a dissecting microscope for metacercarial cysts, and later preserved in 10% formalin. When cysts were observed, metacercariae were gently teased from membranes and killed in hot distilled water. For permanent slides, worms were flattened slightly under a cover glass, fixed in AFA (alcohol–formalin–acetic acid), stained with Mayer's hematoxylin and eosin counterstain, and mounted in Permount®. Representative specimens have been deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705, as USNM Helm. Coll. No. 80631. Voucher specimens of salamanders have been deposited in the Arkansas State University Museum of Zoology (ASUMZ 8421–8426).

Only 1 (1.4%) of the 70 *E. neotenes* from the Real County site (off E. Frio River) was found to be harboring metacercariae; all of the 16 salamanders from the Bandera County site (Mill Creek) were negative. Three ovoid to spherical yellow metacercarial cysts (2.3–2.5 mm in diameter) were visible to the unaided eye through the venter of the infected host and situated in connective tissue and muscles adjacent to heart and liver. The 3 mechanically excysted metacercariae had the following characteristics and measurements (mean followed by the range in micrometers in parentheses, except where noted): body length 3.5 (2.9–4.3) mm; width 1.0 (0.8–1.2) mm; fine cuticular spines protruding from tegumental surface; oral sucker length and

width 272 × 218 (184–321 × 173–258); ventral sucker length and width 538 × 614 (449–639 × 534–727); vitellaria not completely formed; immature ovary between testes, 139 × 188 (119–160 × 140–223); immature testes 122 × 227 (85–261 × 58–242). Based on the descriptions provided in Osborn (1912), Cort (1913), Ukoli (1966), Kagei et al. (1984), and Feizullaev and Mirzoeva (1986), specimens were identified as *C. complanatum* (Rudolphi, 1814).

Larson et al. (1988) recently noted that "outside of North America, *C. marginatum* is referred to as *C. complanatum,*" a view that earlier was also noted by Manter (1938). However, Ukoli (1966) revised the genus and following Baer's (1933) and Yamaguti's (1933) independent suggestions, proposed that *C. marginatum* Rudolphi, 1819, is 1 of at least 20 synonyms of *C. complanatum.* Ukoli (1966) further recognized the similar *C. attenuatum* Cort, 1913, as a distinct species occurring primarily in frogs. Other investigators have agreed that *C. marginatum* is a junior synonym of *C. complanatum* (see Dowsett and Lubinsky, 1980; Feizullaev and Mirzoeva, 1983). Indeed, the slight morphological differences between *complanatum* and *marginatum* that Braun (1901) regarded as convincing evidence for recognizing distinct species is probably due to individual variability (see Baer, 1933); thus, the synonymy originally proposed by Baer (1933) and reiterated by Ukoli (1966) and Dowsett and Lubinsky (1980) is provisionally supported. In spite of this revised taxonomic information and the difficulty differentiating these 2 species (see Baer, 1933), the name *marginatum* continues to be perpetuated in the North American literature (Fried and Foley, 1970; Fried et al., 1970; Taber, 1972; Hazen and Esch, 1978; Ingham and Dronen, 1980; Uglem and Larson, 1987; Larson et al., 1988; and others), and it may persist as a viable subjective synonym.

Lastly, as there are at least 2 valid species of *Clinostomum* (i.e., *attenuatum* and *complanatum*) found as metacercariae in various North American fishes, amphibians, and at least 1 reptile, caution should be exercised before arriving at an identification based solely on the traditional view of host specificity. Cort (1913) suggested that the metacercarial forms found in frogs represent *C. attenuatum* and those in fishes were *C. marginatum* (=*complanatum*). However, the latter species is not restricted to fishes because it has been reported in a pig frog, *Rana grylio,* and siren, *Siren* sp., from Florida (Manter, 1938), a 3-toed amphiuma, *Amphiuma tridactylum,* from Louisiana (Bennett and Humes, 1938), leopard frogs, *Rana pipiens,* from New Jersey (Fried and Foley, 1970), a plains garter snake, *Thamnophis radix,* from an unknown locality in the United States (Hopkins, 1933), and a plethodontid salamander from Texas (McAllister, this study).

I thank Dr. J. E. Ubelaker for examining the metacercariae, members of the H. E. Butt Foundation Camp for allowing me access to springs, and the Texas Parks and Wildlife Department for Scientific Collecting Permit No. SPO44-1. I also thank Dr. J. R. Lichtenfels for helpful comments and curatorial assistance and 2 anonymous reviewers for critically reviewing the manuscript.

Addendum

While this manuscript was in press, I was informed (R. P. Eckerlin, pers. comm.) of another salamander that now can be included as a new host for *C. complanatum.* The host, an adult male red-spotted newt, *Notophthalmus viridescens viridescens* (Rafinesque), was collected in early 1968 from an unknown locale in Massachusetts. Five metacercariae were encysted near the eyes and forelimbs of the newt. After removal, 4 of the metacercariae were fed to a pigeon in an unsuccessful attempt to obtain mature specimens; 1 was retained as a voucher and later deposited as USNM Helm. Coll. No. 80823. The single *C. complanatum* metacercaria measured 5.0 long × 1.5 mm wide and possessed fine cuticular spines, immature testes, and ovary. The family Salamandridae, to which *N. v. viridescens* belongs, further extends the host range for the genus *Clinostomum* to include 4 families of salamanders.

I thank Dr. R. P. Eckerlin for bringing this information to my attention and allowing the record to be included in the present report.

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Research Note

Intra- and Interspecific Chemoattraction in *Echinostoma caproni* and *E. trivolvis* Adults In Vitro

BERNARD FRIED AND M. A. HASEEB

Department of Biology, Lafayette College, Easton, Pennsylvania 18042 and
Department of Microbiology and Immunology, State University of New York Health Science Center, Brooklyn, New York 11203

ABSTRACT: Intra- and interspecific chemoattraction was studied in 14-day-old *Echinostoma caproni* and *E. trivolvis* from domestic chicks. Experiments were carried out at 38.5 ± 1°C in petri dishes with an agar substratum overlaid with Locke's solution. *Echinostoma trivolvis* exhibited significantly greater intraspecific attraction than *E. caproni*; this attraction was significantly greater than the interspecific attraction.

KEY WORDS: *Echinostoma trivolvis, Echinostoma caproni,* attraction, pairing, intraspecific, interspecific, in vitro.

In accord with the recent review of Christensen et al. (1988), this study uses the names *Echinostoma caproni* and *Echinostoma trivolvis* for 2 related species of 37-collar-spined echinostomes, previously referred to as *E. liei* and *E. revolutum,* respectively.

Larval and adult stages of closely related 37-collar-spined echinostomes are morphologically quite similar, yet differ physiologically. Thus, Fried and Emili (1988) noted only subtle morphologic differences between the metacercarial cysts of *E. trivolvis* and *E. caproni,* but more obvious specific differences in both the percent and rate of excystation of cysts in vitro.

To document further physiologic differences between these 2 closely related species, we examined behavior and pairing patterns of the echinostomes in vitro. Although numerous studies on the in vitro pairing tendency of *E. trivolvis* are available (Fried, 1986; Haseeb and Fried, 1988), similar studies on *E. caproni* are not; interspecific pairing in echinostomes has not been demonstrated. The present study compares intra- and interspecific pairing between *E. caproni* and *E. trivolvis* adults in vitro.

Echinostoma trivolvis and *E. caproni* were grown for 14 days in domestic chicks (Fried and Weaver, 1969; Fried et al., 1988). Worms were quickly removed from the ileum at necropsy, washed in several changes of Locke's solution, and maintained individually in petri dishes containing 10 ml Locke's solution for about 0.5 hr prior to use.

The bioassay consisted of a petri dish (6 cm diameter) with a nutrient agar substratum and a 10-ml Locke's overlay (Fried and Roberts, 1972). Two worms were placed 20 mm apart in each dish and incubated at 38.5 ± 1°C under subdued light. The distances between worm centers were measured in millimeters at 15-min intervals for 90 min without removing the worms from the incubator. All worms were alive at the end of the experiments. Observations were based on 16 different pairs of worms for each combination (Fig. 1). The unmodified data were compared using a 2-tailed Kolmogorov–Smirnov test (TRUE EPISTAT®, Epistat Services, Richardson, Texas). The percent attraction was determined by the

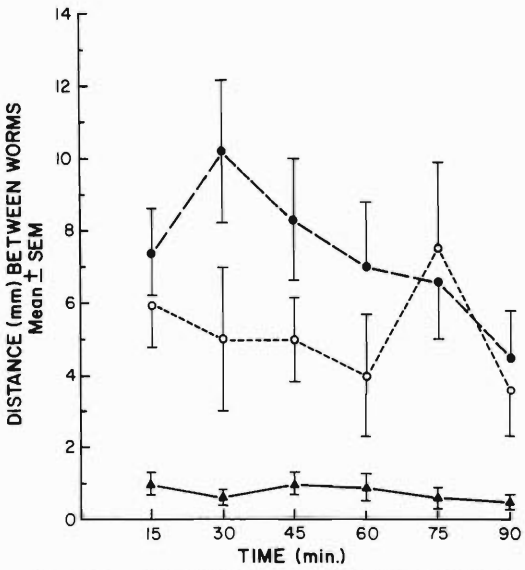

Figure 1. The distance (mm) between worm pairs is shown as mean ± SEM. Open circles, *Echinostoma caproni* vs. *E. caproni*; triangles, *E. trivolvis* vs. *E. trivolvis*; closed circles, *E. caproni* vs. *E. trivolvis*.

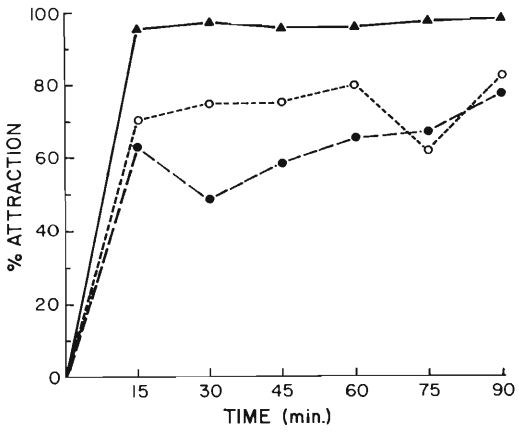

Figure 2. Intra- and interspecific attraction in *Echinostoma caproni* and *E. trivolvis* is shown as percent attraction, which was calculated by the formula: ([20 − D]/20) × 100, where 20 is the starting distance and D is the distance between a worm pair at an observation time. Open circles, *E. caproni* vs. *E. caproni*; triangles, *E. trivolvis* vs. *E. trivolvis*; closed circles, *E. caproni* vs. *E. trivolvis*.

formula: ([20 − D]/20) × 100, where 20 represents the initial distance between worms and D is the actual distance between a worm pair at a particular time point. Thus, by this formula 0% attraction would indicate that the worms remained at their original distance (20 mm) or moved further apart and 100% attraction would indicate that a worm pair was in contact.

The distance between worms averaged about 1 mm for *E. trivolvis*, 5 mm for *E. caproni*, and 8 mm for *E. trivolvis* vs. *E. caproni*. The mean distances at each time between worms are shown in Figure 1. Contact pairing (worms in physical contact along any 2 surfaces) was observed 60% of the time in *E. trivolvis*, 25% of the time in *E. caproni*, and only 9% of the time in the interspecific trials.

Intraspecific attraction of *E. trivolvis* was significantly greater ($P < 0.05$) than that of *E. caproni* at all time points except 30 and 60 min. The intraspecific attraction of *E. trivolvis* was significantly greater ($P < 0.05$) than interspecific attraction at all time points except at 90 min. The intraspecific attraction of *E. caproni* was significantly greater ($P < 0.05$) than interspecific attraction at 30 and 60 min. The percent attraction in the intraspecific and interspecific studies is shown in Figure 2.

The results of these experiments clearly show that *E. trivolvis* and *E. caproni* each has its own in vitro pairing pattern and that the interspecific pattern is yet different from either intraspecific pattern. In a previous study, interspecific pairing between *Zygocotyle lunata* and *E. trivolvis* had a pattern identical to the intraspecific pattern of *Z. lunata* (Fried and Wilson, 1981). Differences in the results between these 2 studies are not clear at this time. Moreover, the significance of in vitro pairing is not well understood; protrusion of cirri was never seen in the present study, suggesting that this pairing is not related to cross-copulation.

Lipophilic factors, particularly sterols, are presumably responsible for mediating intraspecific pairing of *E. trivolvis* (Fried et al., 1980; Fried, 1986). It is not known if a similar mechanism exists for intraspecific pairing in *E. caproni* or for interspecific pairing between *E. trivolvis* and *E. caproni*.

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Research Note

Occurrence of *Trichospirura teixeirai* (Spirurida: Rhabdochonidae) in *Hemidactylus brookii haitianus* (Sauria: Gekkonidae) from Hispaniola

ROBERT POWELL,[1] PATRICIA J. HALL,[2] JOHN H. GREVE,[3] AND DONALD D. SMITH[4]

[1] Department of Natural Sciences, Avila College, Kansas City, Missouri 64145,
[2] Pharmacia Deltech Inc., St. Paul, Minnesota 55112,
[3] Department of Veterinary Pathology, Iowa State University, Ames, Iowa 50011, and
[4] Department of Medicine, University of Kansas, Kansas City, Kansas 66103

ABSTRACT: Two female *Trichospirura teixeirai* were found free in the lumina of gall bladders of *Hemidactylus brookii haitianus* from Hispaniola (Dominican Republic). These represent new site, host, and locality records, respectively.

KEY WORDS: parasite, rhabdochonid nematode, *Trichospirura teixeirai,* lizard host, *Hemidactylus brookii haitianus,* Hispaniola, Dominican Republic.

Trichospirura teixeirai (Barus and Coy Otero, 1968) Moravec, 1975, have been previously reported only from iguanid lizards in Cuba (Barus and Coy Otero, 1969; Coy Otero, 1970; Coy Otero and Barus, 1979). Here we report its occurrence in a new host from Hispaniola. A total of 41 *Hemidactylus brookii haitianus* Meerwarth, 1901, was collected in human habitations near Juan Dolio, San Pedro de Macoris Province, in March 1988, and from Barahona, Barahona Province, in January and March 1986 and August 1987. This subspecies, endemic to the Greater Antilles, is common on Cuba, Puerto Rico, and Hispaniola (Schwartz and Henderson, 1988). Specimens were collected at night on walls and ceilings of buildings as lizards emerged from refugia occupied during the day. Animals were killed, preserved in formalin, and, after return to the laboratory, transferred to 75% ethanol. Stomachs, livers, gall bladders, small intestines, bile and pancreatic ducts, and lungs were excised and examined. Lizards were deposited in the Bobby Witcher Memorial Collection at Avila College, Kansas City, Missouri, U.S.A. (BWMC 02464, 02476, 02742–02746, 03123–03135, 03303–03317); at Appalachian State University, Boone, North Carolina, U.S.A. (ASU 13017–13018); at the Museum of Natural History, University of Kansas, Lawrence, Kansas, U.S.A. (KUMNH 206693–206694); and the Parque Zoológico Nacional, Santo Domingo, República Dominicana ($N = 2$, no numbers). The last 2 specimens were not available for examination of lungs, small intestines, and bile and pancreatic ducts.

Single nonoviferous female *Trichospirura teixeirai* were found in gall bladders of 2 hosts collected during March 1986 and August 1987 in Barahona. Both were free in the lumen. The slender nematodes are about 12 mm long, 45 μm at the junction of esophagus and intestine, and 300 μm wide at the widest point near the start of the posterior quarter of body length. The pharynx is remarkable by being long (0.95–1.00 mm) and slender (10 μm throughout its entire length, except for a slight dilation as it enters the esophagus). The muscular esophagus is about 300 μm long, the glandular esophagus about 900 μm long. The vulva is about 600 μm from the posterior tip, located in a dishlike depression, surrounded by an array of glandular tissue. Didelphic uteri are devoid of eggs. The tail is an elongated cone, the anus 300 μm from the posterior tip. As *Trichospirura teixeirai* in previously published reports were in the small intestine, this constitutes a new site record, although fixation-induced migration cannot be ruled out. Examination of excised organs revealed no other nematodes, although lungs in 23 of 39 animals were infected with pentastomes, genus *Raillietiella.* Organs of lizards in the genera *Anolis* ($N = 57$), *Leiocephalus* ($N = 17$) (Iguanidae), and *Ameiva* ($N = 19$) (Teiidae), taken from the same localities, were also examined, but no additional rhabdochonid parasites were observed. Nematodes were deposited in the National Parasite Collection, Beltsville, Maryland 20705 (USNM Helminthological Collection Nos. 80797–80798).

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Research Note

Occurrence of *Skrjabinoptera leiocephalorum* (Spirurida: Physalopteridae) in *Leiocephalus* spp. (Sauria: Iguanidae) from Hispaniola

ROBERT POWELL,[1] PATRICIA J. HALL,[2] AND JOHN H. GREVE[3]

[1] Department of Natural Sciences, Avila College, Kansas City, Missouri 64145,
[2] Pharmacia Deltech Inc., St. Paul, Minnesota 55112, and
[3] Department of Veterinary Pathology, Iowa State University, Ames, Iowa 50011

ABSTRACT: *Skrjabinoptera leiocephalorum* were found free and attached in stomach lumina of *Leiocephalus schreibersi* and *L. barahonensis* from Hispaniola (Dominican Republic). Prevalence was 0.40 and 0.56 and intensities ranged from 1 to 250 and 1 to 45 in the 2 host species, respectively. There was no obvious relationship between prey selection and the presence of parasites. There was a positive correlation between larger size of hosts and prevalence, but none between size and intensities. Both males and females were infected, and reproductive condition was insignificant. Habitat was not related to the presence of parasites.

KEY WORDS: parasite, physalopterid nematode, *Skrjabinoptera leiocephalorum*, lizard hosts, *Leiocephalus schreibersi*, *Leiocephalus barahonensis*, Hispaniola, Dominican Republic.

Skrjabinoptera spp. occur in a number of reptilian hosts (Baker, 1987). Here we describe the occurrence of *Skrjabinoptera leiocephalorum* Greve and Powell, 1989, in 2 hosts from Hispaniola. Stomachs of 60 *Leiocephalus schreibersi* Gravenhorst, 1837, and 54 *Leiocephalus barahonensis* Schmidt, 1921, were examined for the presence of nematodes. Both host species are endemic to Hispaniola (Schwartz and Henderson, 1988).

Leiocephalus schreibersi were sampled from 6 and *L. barahonensis* from 5 localities in the Dominican Republic (Fig. 1). The 2 species are sympatric in Barahona (site 4). Localities, dates of collection, numbers sampled, and infected are summarized in Tables 1 and 2. Terminology follows Margolis et al. (1982). All collection sites were in acacia or agave scrub, except site 7, which was a coastal coconut palm stand near the mouth of a small stream. Sites 1, 5, 6, and 7 were near permanent water, and site 4 provided access to water via numerous leaks in the public water system. Sites 2, 3, 8, 9, and 10 were arid with no apparent access to surface water. Specimens were collected during the day, kept on ice until evening, killed, and preserved in formalin. After return from the field, animals were transferred to 75% ethanol, stomachs were excised, and contents analyzed. Lizards were deposited in the Bobby Witcher Memorial Collection at Avila College, Kansas City, Missouri (*L. schreibersi*:

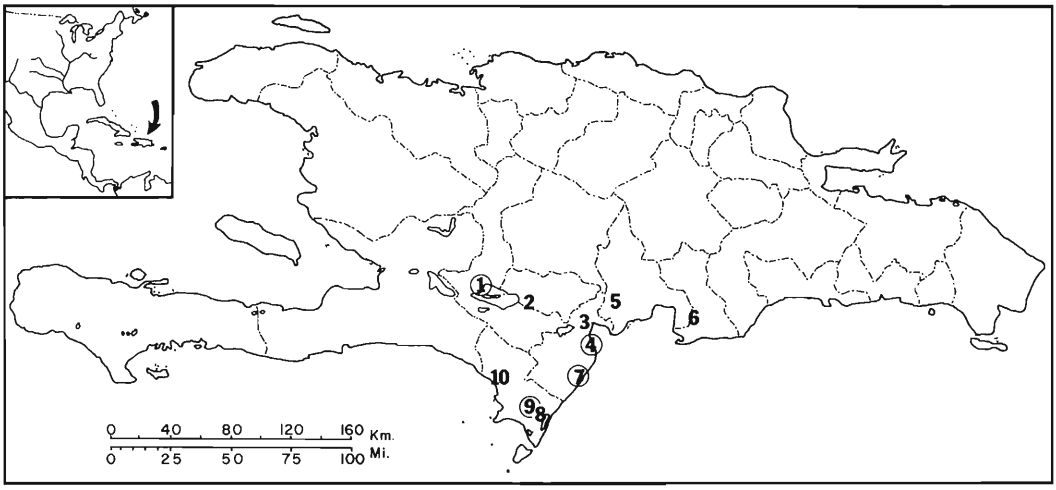

Figure 1. Hispaniola; sites from which *Leiocephalus schreibersi* and *Leiocephalus barahonensis* were collected. See Tables 1 and 2 for specific localities. Circled numbers represent sites from which animals infected with *Skrjabinoptera leiocephalorum* were taken.

BWMC 03010–03030, 03177–03201, 03289–03302; *L. barahonensis*: BWMC 03002–03009, 03202–03235, 03378–03389).

Adult and juvenile parasites of both sexes were found in the stomachs of hosts. Most were free in the lumen, admixed with ingesta, although individuals in 2 hosts from site 1 were attached to the stomach wall. Free (especially smaller) nematodes in *L. barahonensis* were concentrated in the pylorus.

There was no obvious relationship between prey selection and the presence of nematodes. Sixteen arthropod orders were identified among the ingesta. No orders were represented exclusively in infected populations. There was a positive correlation between larger size and prevalence, but none between size and intensities ($P < 0.05$) (Sokal and Rohlf, 1987). Snout–vent lengths ranged from 31 to 87 mm ($\bar{x} = 60.2$) in

Table 1. *Skrjabinoptera leiocephalorum* in *Leiocephalus schreibersi* from the Dominican Republic.

Site*	Date	*N*	Prev-alence	Inten-sity	Mean	Me-dian
1	Mar 86	10	0.90	2–250	39	12
	Aug 87	11	0.36	2–9	4	2
2	Mar 86	3	0			
	Aug 87	2	0			
3	Mar 88	2	0			
4	Mar 86	1	0			
	Aug 87	6	0.50	7–22	14	13
	Mar 88	8	1.00	1–51	18	17
5	Aug 87	5	0			
	Mar 88	4	0			
6	Mar 86	7	0			
	Aug 87	1	0			
Total		60	0.40	1–250	23	10

* Collection sites (elevations to the nearest 10 m): 1, Provincia de Independencia, Boca de Cachon, elev. −20 m; 2, Provincia de Independencia, 10 km S Neiba, elev. −20 m; 3, Provincia de Barahona, 26 km N Barahona, elev. 200 m; 4, Provincia de Barahona, Barahona, elev. 0 m; 5, Provincia de Azua, Río Tábara at Hwy. 44, elev. 130 m; 6, Provincia de Peravia, Río Ocoa at Hwy. 2, elev. 170 m.

Table 2. *Skrjabinoptera leiocephalorum* in *Leiocephalus barahonensis* from the Dominican Republic.

Site*	Date	*N*	Prev-alence	Inten-sity	Mean	Me-dian
4	Mar 86	1	0			
	Mar 88	1	1.00	29	—	—
7	Aug 87	26	0.73	1–45	10	7
	Mar 88	11	0.73	1–44	14	2
8	Mar 86	3	0			
	Aug 87	4	0			
9	Mar 86	3	0			
	Aug 87	4	0.50	10–14	12	—
10	Mar 86	1	0			
Total		54	0.56	1–45	12	7

* Collection sites (elevations to the nearest 10 m): 4, Provincia de Barahona, Barahona, elev. 0 m; 7, Provincia de Barahona, Paraiso, elev. 0 m; 8, Provincia de Pedernales, 5 km NW Oviedo, elev. 40 m; 9, Provincia de Pedernales, 16.4 km NW Oviedo, elev. 50 m; 10, Provincia de Pedernales, 10 km N Cabo Rojo, elev. 20 m.

L. schreibersi and from 26 to 72 mm ($\bar{x}$ = 49.6) in *L. barahonensis*. Both males and females were infected, and reproductive condition was insignificant (Williams' corrected *G*-test, $P < 0.05$). Juveniles of both sexes, reproductively active males, and females with unyolked and yolked ovarian follicles, oviducal eggs, and corpora lutea were infected. Habitat (i.e., access to water) did not appear important (Williams' corrected *G*-test, $P < 0.05$).

Stomachs of *Leiocephalus semilineatus* Dunn, 1920 (N = 35), and lizards in the genera *Anolis* (N = 146) (Iguanidae), *Ameiva* (N = 46) (Teiidae), *Hemidactylus* (N = 26) (Gekkonidae), and *Celestus* (N = 9) (Anguidae), taken from the same localities as infected specimens, were also examined. No physalopterid nematodes were found. Voucher specimens of *S. leiocephalorum* were deposited in the USNM Helminthological Collection (80581–80584) and the parasitological collection at Avila College, Kansas City, Missouri, U.S.A. (no numbers assigned).

We wish to thank Donald D. Smith, John S. Parmerlee, Jr., Scott A. Maxey, Mark A. Rice, S. Scott Duer, Sascha Oerter, and members of the Avila Field Biology classes of 1986 and 1988 for their assistance in the field and laboratory. Sixto and Ivonne Incháustequi provided assistance in the Dominican Republic. José A. Ottenwalder of the Parque Zoológico Nacional facilitated opportunities for field work. Permits wre graciously provided by Emilio A. Bautista M., Director, Departamento Vida Silvestre, República Dominicana.

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Research Note

Reduction of the *Syphacia* sp. Infection in the Laboratory Rat by Viprostol Treatment

MICHAEL R. IRWIN, REYNALDO J. ARCEO, AND THOMAS DAVIS

Experimental Pathology Department, Medical Research Division, American Cyanamid Company, Pearl River, New York 10965

ABSTRACT: During the conduct of routine chronic preclinical safety evaluation studies it was found that orally administered viprostol, a prostaglandin E_2 analogue, reduced or removed the *Syphacia* sp. infection in laboratory rats. This apparent anti-nematodal activity tended to correlate with the presence of gastrointestinal trophic changes, suggesting that the activity may be due to an altered environment of the parasite.

KEY WORDS: *Syphacia* sp., laboratory rat, prostaglandin E_2, viprostol.

We report an interesting observation of apparent anti-pinworm (*Syphacia* sp.) activity of a prostaglandin E_2 (PGE_2) analogue, viprostol, which was observed in 2 chronic preclinical toxicology studies conducted in rats (COBS CD.®, Charles River Breeding Laboratories Inc.). In the studies, groups of rats, unrestricted but individually housed, were treated with 0, 0.5, 2, or 10 mg/kg/day given orally in one study and applied topically in petrolatum in the other for 1 yr, followed by 1, 2, or 3 mo of posttreatment observation. Surviving rats were killed at the end of each period. A single stained tissue section of the colon of all rats was examined microscopically

Table 1. The prevalence of *Syphacia* sp. observed in rats after a year of viprostol treatment with or without a subsequent observation period.

Route	Dose mg/kg/day	Males				Females			
		After treatment period		After observation period		After treatment period		After observation period	
		N	% infected	*N*	% infected	*N*	% infected	*N*	% infected
Topical	0.00	25	20	30	43	25	16	29	17
	0.50	25	28	30	23	27	15	28	21
	2.00	27	0	28	14	27	0	28	11
	10.00	25	0	30	13	26	0	29	7
Oral	0.00	26	31	34	18	33	6	27	15
	0.50	28	0*	32	31	27	4	33	9
	2.00	27	0*	33	15	26	0	34	3
	10.00	33	0*	27	22	29	0	31	6

* $P < 0.05$.

as part of the routine histopathologic evaluation. The *Syphacia* sp., most probably *S. muris,* was identified (we thank Dr. Jack D. Tiner) using specimens dissected from formalin-fixed tissue. The criterion for establishing the infection in the various treatment groups was the presence of pinworm sections in stained (hematoxylin and eosin) colon tissue slides from individual rats.

Results of the evaluation are presented in Table 1. In both studies, no pinworms were observed in the colons of rats of both sexes treated orally or topically with either 2 or 10 mg/kg/day, nor in the males treated with 0.5 mg/kg/day orally. Using Fisher's exact test, the males treated orally with viprostol were the only ones to show statistical significance ($P < 0.05$). The female rats treated with 0.5 mg/kg/day had a prevalence of pinworms similar to the vehicle-treated controls. The rats treated with 2 or 10 mg/kg/day of viprostol apparently lost the pinworm infection and remained free of it while treatment was being administered. During the recovery period following treatment for 1 yr, the previously treated rats became rapidly reinfected within a month following the cessation of treatment.

Investigators using the *Nippostrongylus brasiliensis* rat model have suggested that the mechanism of PGE worm expulsion from the small intestine may either be by the direct effect on worm metabolism or a change in the gastrointestinal environment (Richards et al., 1977). We believe that the latter mechanism may be involved with *Syphacia* sp., as the prevention of epithelial gastrointestinal cell exfoliation, thought to be responsible for the PGE_2 cytoprotection (Reinhart et al., 1983), was observed in both orally and topically PGE-treated rats in these studies. These trophic gastrointestinal epithelial changes were not found in rats after a treatment-free period of 1 mo, during which the infection reappeared. No anthelmintic activity was observed in a standard diet test using 200 ppm of viprostol on 7–11-day-old *Trichostrongylus colubriformis* in gerbils. Viprostol may therefore be added to the list of compounds, like cimetidine (Rew and Fetterer, 1986), having an indirect antinematodal activity by altering the environment of the parasite.

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Research Note

Scanning and Transmission Electron Microscopic Observations on Metacercariae of *Echinostoma trivolvis* and *Echinostoma caproni* During In Vitro Excystation

S. W. B. IRWIN[1] AND B. FRIED[2]

[1] Department of Biology, University of Ulster at Jordanstown, Shore Road, Newtownabbey, Co. Antrim, Northern Ireland, BT37 0QB and
[2] Department of Biology, Lafayette College, Easton, Pennsylvania 18042

ABSTRACT: Electron microscopy was used to study the metacercariae of *Echinostoma trivolvis* and *Echinostoma caproni* during in vitro excystation. Untreated cysts of both species possessed a particulate outer layer, an amorphous middle layer, and a lamellated inner layer. Larvae of cysts treated in an alkaline bile salts–trypsin medium first emerged into a space between the outer and inner layers. Breaching of the inner cyst layer by the larva involved fraying at 1 point so that the lamellated layer spread to become more diffuse. Globules found in close proximity to this region may be secretions implicated in the disruption of the lamellated layer. Prior to final emergence, the outer layer was breached. The only differences in the 2 species were that *E. trivolvis* had a thicker middle layer and the surface of its inner layer was rougher than that of *E. caproni.*

KEY WORDS: Trematoda, Digenea, *Echinostoma trivolvis, Echinostoma caproni,* in vitro excystation, scanning electron microscopy, transmission electron microscopy.

According to Kanev (1985), for 37-collar-spined echinostomes, *Echinostoma caproni* is the correct name for a species described by Jeyarasasingam et al. (1972) as *Echinostoma liei,* and *Echinostoma trivolvis* is the correct name for a North American form worked with by Beaver (1937) as *Echinostoma revolutum.* Our study uses the Egyptian and North American species and refers to them as *E. caproni* and *E. trivolvis,* respectively. A recent review by Christensen et al. (1988) has discussed fundamental differences in the biology of these 2 related species. However, there is sparse ultrastructural information on larval or adult stages of these 2 echinostomes.

Fried and Emili (1988) examined chemical excystation of the 2 related 37-collar-spined echinostomes by light microscopy. Only subtle differences between the 2 species were observed in the morphology of metacercariae, i.e., width of the outer cyst wall, diameter of the excretory concretions, and length of the excysted larvae. The present study uses scanning and transmission electron microscopy to show similarities and differences in the ultrastructure of the metacercariae of *E. trivolvis* and *E. caproni* during in vitro excystation.

Metacercarial cysts of both echinostome species were maintained in laboratory-infected snails, *Biomphalaria glabrata,* as described by Jeyara-

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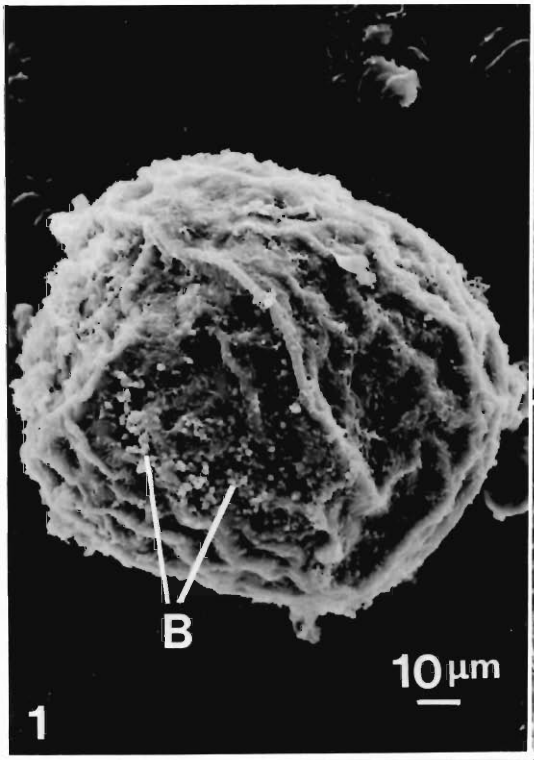
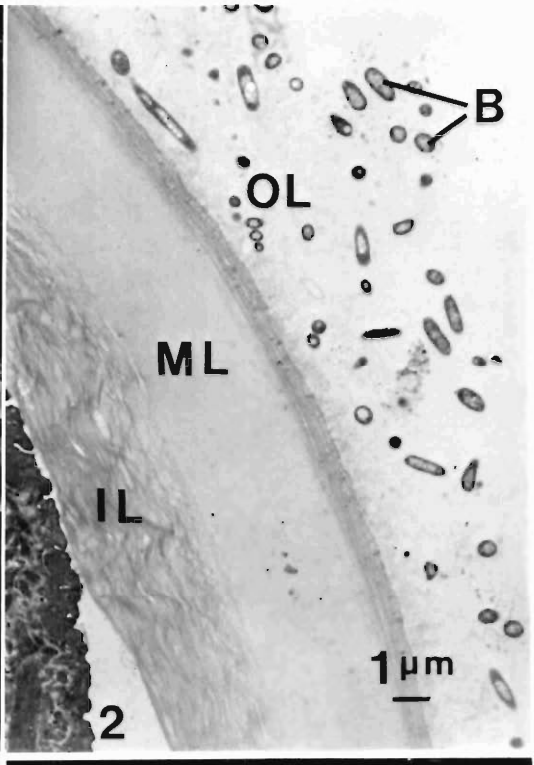
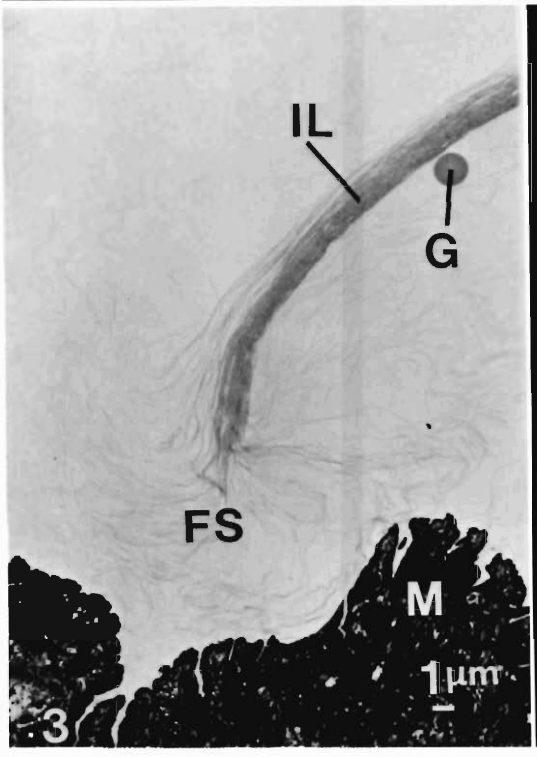
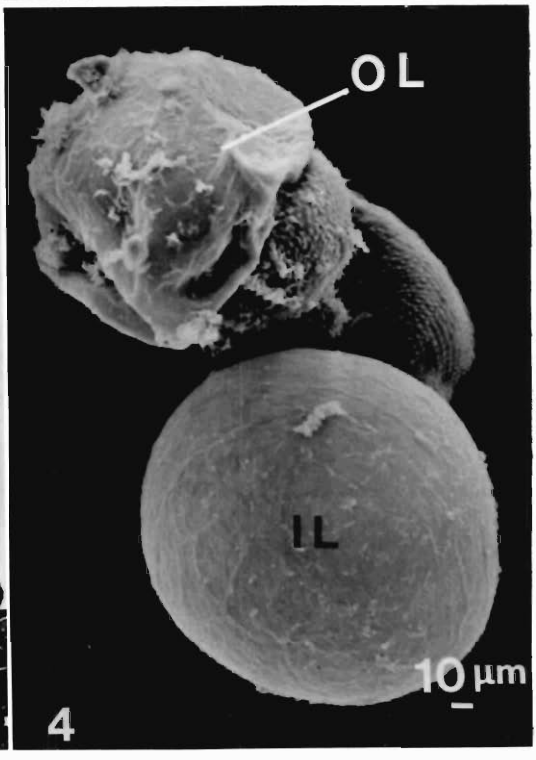
Figures 1–4. Electron micrographs of *Echinostoma trivolvis*. 1. Scanning electron micrograph of a whole cyst. Bacteria can be seen adhering to the outer surface. 2. Transmission electron micrograph of the untreated cyst wall showing the inner lamellated layer, the amorphous middle layer, and the outer layer, which has collagen-like material containing bacteria on the outside and is lamellated on the inner aspect. 3. The frayed structure of a breached inner layer. Part of a metacercaria can be seen passing through the breach. Note the presence of an electron-dense globule in the area. 4. This specimen is emerging from its inner cyst layer. The outer layer is still encapsulating the anterior end of the fluke. Abbreviations: B, bacteria; FR, frayed region; FS, frayed structure; G, globule; IL, inner layer; M, metacercaria; ML, middle layer; OL, outer layer.

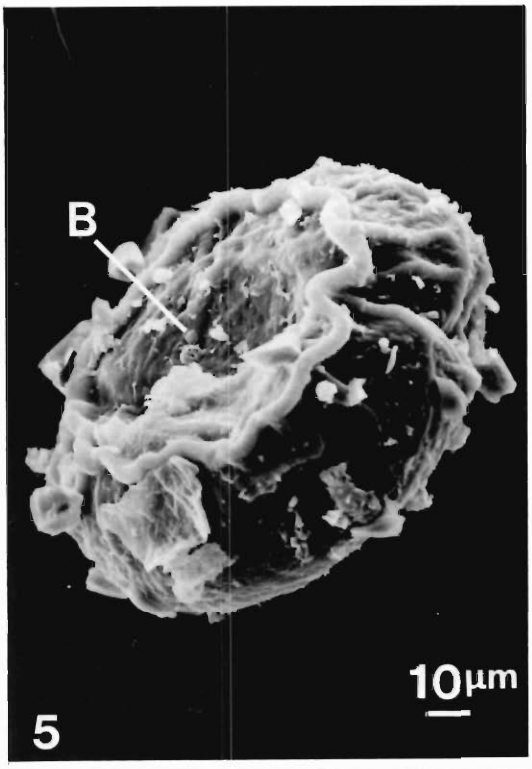
Figures 5–8. Electron micrographs of *Echinostoma caproni*. 5. A scanning electron micrograph of a whole cyst. A few bacteria are present on its surface. 6. The inner lamellated layer, middle amorphous layer, and outer layer, which is lamellated at its base and has collagen-like particles containing a bacterium on its outer aspect. 7. The frayed region of the inner layer of a breached cyst. A number of electron-dense globules are present close to the frayed region. 8. A metacercaria having emerged from the very smooth inner layer. The edge of the escape aperture (arrows) is thickened and frayed. A discarded outer layer remains nearby. Abbreviations: B, bacteria; FR, frayed region; FS, frayed structure; G, globule; IL, inner layer; M, metacercaria; ML, middle layer; OL, outer layer.

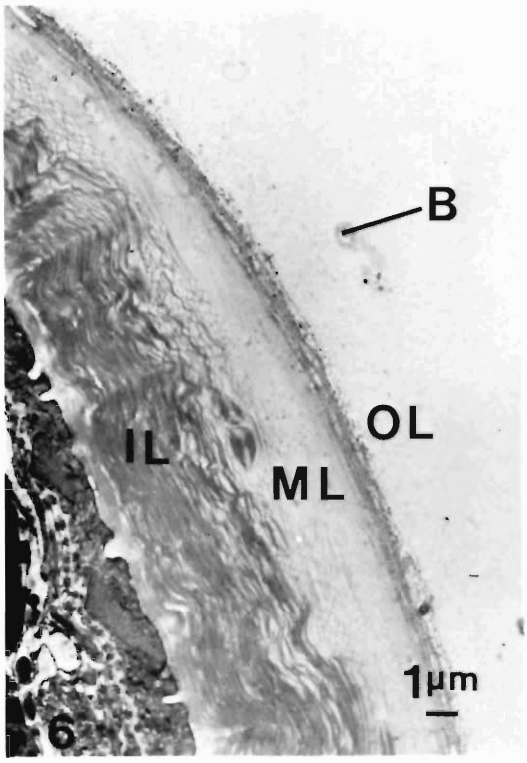


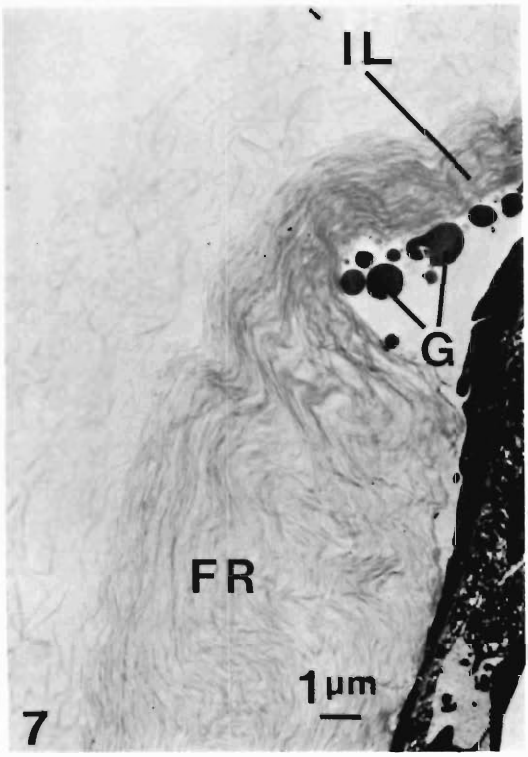


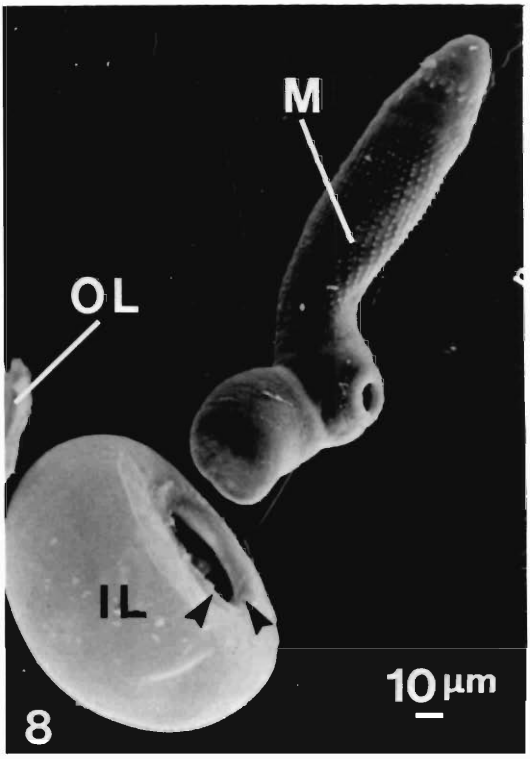












sasingam et al. (1972) and Anderson and Fried (1987) and were dissected from the saccular kidney 1–10 days postencystment. Both species of metacercariae were excysted following the method of Fried and Emili (1988) using an alkaline medium containing trypsin and bile salts at 41°C, and organisms at various stages of excystment were fixed along with untreated metacercarial cysts for examination by electron microscopy. Preparation of material followed procedures adopted by Irwin et al. (1984) and observations were made using JEOL 100S and JEOL JSM-840 electron microscopes.

Electron micrographs of *E. trivolvis* are shown in Figures 1–4 and of *E. caproni* in Figures 5–8. Scanning electron microscopy demonstrated that untreated cysts of *E. trivolvis* and *E. caproni* had a wrinkled or folded appearance; bacteria were observed on the cyst surfaces (Figs. 1, 5). Transmission electron microscopy of both species showed that untreated cysts had an inner lamellated layer merging into a middle layer of uniformly dense material that was thicker in *E. trivolvis* (Fig. 2) than in *E. caproni* (Fig. 6). The peripheral aspect of the outer layer had a particulate appearance that resembled collagen fibers and was somewhat lamellated toward its base where it merged with the amorphous middle layer. Sections through walls of metacercarial cysts of both species following exposure to the excystation medium demonstrated that much of the collagen-like material on the outside of the cyst had gone. The lamellated base of the outer layer and the inner lamellated layer were relatively unchanged, whereas the intervening amorphous layer (middle layer) was either diminished or not present. Breaching of the inner cyst layer by the larva involved a fraying and fragmenting at 1 point on the cyst wall so that the lamellated inner layer widened and became more diffuse (Figs. 3, 7). The organism passed through the frayed area, and in both species small electron-dense globules were often found in close proximity to the breached area (Figs. 3, 7). As the larva entered the cavity between the outer and inner cyst layers, fragments of the inner lamellated layer were carried into that space. The larvae at this stage were still constrained by the outer cyst layer. Excystation was completed by the disruption of the outer cyst layer. In some cases it was breached and the organism passed directly through the ruptured zone. In other cases the outer layer was separated from the inner layer, and the larvae could be observed becoming free as they passed through the now well-dispersed fragments of the inner lamellated layer. The outer layer could be retained for a short time as a cap over the anterior end of the escaping larva (Fig. 4), and eventually these outer layers were left scattered among the vacated and semivacated inner layers (Fig. 8). Those inner layers, which were now devoid of their outer covering, had markedly smooth surfaces although those of *E. caproni* were somewhat smoother than those of *E. trivolvis*. Scanning electron microscopy also demonstrated that vacated inner layers were somewhat flattened on the side from which the larvae escaped (Fig. 4). In each case the edge of the aperture was everted and had a ragged appearance consistent with the frayed and fragmented lamellar configuration demonstrated by transmission electron microscopy (Figs. 3, 7).

Breaching of the inner layer and the presence of electron-dense globules only at the ruptured site of the cysts in both species suggest that the organisms play an important role in excystment. The fraying and widening of the inner layer probably represented weakening that allowed the larva to breach this area. The globules may be glandular secretions involved in the disruption of the lamellated layer. The only other ultrastructural study on excystation of an echinostome was by Irwin et al. (1984) on *Himasthla leptosoma*. It showed that *H. leptosoma* metacercariae escape through regions of the inner cyst wall without lamellae. Unlike *E. trivolvis* and *E. caproni*, which break through the lamellated layer, no fraying or fragmenting of lamellae was observed in that species.

Although a previous study (Fried and Emili, 1988) demonstrated physiological differences in excystation and subtle morphologic differences in encysted and excysted metacercariae of *E. trivolvis* and *E. caproni*, the present ultrastructural study failed to demonstrate any differences in the excystation process. Indeed the only differences revealed by electron microscopy were the relative thickness of the middle cyst layer of *E. trivolvis* compared to *E. caproni* and the relative coarseness of the inner layer surface of *E. trivolvis* compared to that of *E. caproni*. Although these 2 echinostomes are distinct species, differences in larval morphology at the electron microscope level are not readily apparent in intact cysts or those undergoing excystation. It is apparent that EM studies alone on species of closely related

echinostome metacercariae may not be sufficient for precise identification solely on the basis of morphology.

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Research Note

Helminths of the Arizona Little Striped Whiptail, *Cnemidophorus inornatus arizonae*, and the Desert Grassland Whiptail, *Cnemidophorus uniparens* (Sauria: Teiidae), from Southeastern Arizona

STEPHEN R. GOLDBERG[1] AND CHARLES R. BURSEY[2]

[1] Department of Biology, Whittier College, Whittier, California 90608 and

[2] Department of Biology, Pennsylvania State University, Shenango Valley Campus, Sharon, Pennsylvania 16146

ABSTRACT: Examination of the gastrointestinal tract of 78 *Cnemidophorus inornatus arizonae* Van Denburgh, 1896, revealed the presence of the nematodes, *Pharyngodon warneri* Harwood, 1932, and *Physaloptera* sp. Rudolphi, 1819, and a cestode, *Oochoristica bivitellobata* Loewen, 1940. Overall prevalence of infection was 33%. The highest prevalence and mean intensity was for *P. warneri,* 23% and 15.4, respectively. Examination of the gastrointestinal tract of 31 *Cnemidophorus uniparens* Wright and Lowe, 1965, revealed only the cestode *O. bivitellobata*; prevalence was 26% and mean intensity was 2.1. One juvenile acanthocephalan, *Acanthocephalus* sp. Koelreuther, 1771, was also found. Presence of *Physaloptera* sp., O. *bivitellobata,* and *Acanthocephalus* sp. are new host records.

KEY WORDS: Nematoda, Cestoda, Acanthocephala, prevalence, intensity, survey, Teiidae, *Cnemidophorus inornatus arizonae, Cnemidophorus uniparens.*

The Arizona little striped whiptail, *Cnemidophorus inornatus arizonae* Van Denburgh, 1896, occurs in arid and semiarid grasslands of western New Mexico and southeastern Arizona (Behler and King, 1979). The desert grassland whiptail, *Cnemidophorus uniparens* Wright and Lowe, 1965, occurs in desert scrub from central Arizona through southern New Mexico to El Paso, Texas, and south into Chihuahua, Mexico (Steb-

Table 1. Prevalence, location, and intensity of gastrointestinal helminths in 78 *Cnemidophorus inornatus* and 31 *C. uniparens*.

Parasite	*C. inornatus*		*C. uniparens*	
	Prevalence (%)	Mean intensity (range)	Prevalence (%)	Mean intensity (range)
Nematoda				
*Pharyngodon warneri**†	23	15.4 (1–73)	—	—
Physaloptera sp.‡	1	—	—	—
Cestoda				
*Oochoristica bivitellobata**	13	1.7 (1–4)	26	2.1 (1–8)
Acanthocephala				
Acanthocephalus sp.‡	—	—	3	—

* Small intestine.
† Large intestine.
‡ Stomach.

bins, 1985); only females are known (Wright and Lowe, 1965). The purpose of this note is to describe the prevalence and intensity of helminth infections in *C. i. arizonae* and *C. uniparens* from the Willcox Playa of southeastern Arizona. Specian and Ubelaker (1974a, b) have previously reported nematodes from the Trans-Pecos little striped whiptail, *Cnemidophorus inornatus heptagrammus* Axtell, 1961, from west Texas. To our knowledge, there are no reports on the endoparasites of *C. uniparens*.

A total of 109 adult lizards collected by the senior author was examined. Seventy-eight *C. i. arizonae* (37 male, 41 female) were collected in May–August 1966 and May 1967, from the southern edge of Willcox (32°14′N, 109°50′W; elevation 1,269 m), Cochise County, Arizona. Thirty-one female *C. uniparens* were collected in June–August 1966, 0.8 km from the junction of Arizona Highway 186 and Kansas Settlement Road on Arizona Highway 186 (32°11′N, 109°45′W; elevation 1,280 m), Cochise County, Arizona. Lizards were shot with 22-caliber dust shot and preserved in Bouin's fixative. They were later transferred to neutral buffered 10% formalin. This collection was recently rediscovered. The body cavity was opened by a longitudinal incision from vent to throat and the gastrointestinal tract was excised by cutting across the anterior esophagus and the rectum. Esophagus, stomach, small intestine, and large intestine were examined separately. Each organ was slit longitudinally and examined under a dissecting microscope. Each helminth was examined and identified utilizing a glycerol wet mount. For detailed microscopy, selected nematodes were stained with iodine and selected cestodes were stained with hematoxylin.

Of the 78 *C. i. arizonae* examined, 26 contained helminths, a prevalence of 33%. Prevalence, location, and mean intensity by species are presented in Table 1. *Pharyngodon warneri* Harwood, 1932, was the major intestinal parasite and was found in the posterior segment of the small intestine as well as the large intestine (18 infected lizards harbored 277 nematodes). *Oochoristica bivitellobata* Loewen, 1940, was recovered from the small intestine (10 infected lizards; 17 cestodes) and third-stage *Physaloptera* sp. Rudolphi, 1819 (1 lizard; 2 nematodes), was found in the stomach. Eight of the 31 *C. uniparens* (Table 1) contained a total of 17 *O. bivitellobata*, all from the small intestine. A juvenile acanthocephalan was found among the stomach contents of 1 *C. uniparens*. The juvenile acanthocephalan had 16 rows of hooks with 5–6 hooks per row typical of the genus *Acanthocephalus* Koelreuther, 1771 (see Petrochenko, 1971). The *C. uniparens* sample had a helminth prevalence of 29%. Representative specimens were deposited in the USNM Helminthological Collection,

Table 2. Monthly prevalences of *Pharyngodon warneri* in *Cnemidophorus inornatus arizonae*.

Month	Male		Female	
	N	% infected	*N*	% infected
1966 May	5	0	9	0
June	3	33	1	0
July	15	40	18	28
August	8	25	9	33
1967 May	6	0	4	25

Table 3. Monthly prevalences of *Oochoristica bivitellobata* in *Cnemidophorus inornatus arizonae* and *C. uniparens*.

	C. i. arizonae				*C. uniparens*	
	Male		Female			
Month	*N*	% infected	*N*	% infected	*N*	% infected
1966 May	5	0	9	11	—	—
June	3	0	1	0	8	0
July	15	13	18	22	20	35
August	8	0	9	22	3	33
1967 May	6	0	4	25	—	—

USDA, Beltsville, Maryland 20705: for *C. i. arizonae, Oochoristica bivitellobata* (80770); *Pharyngodon warneri* (80769); *Physaloptera* sp. (80768); *Acanthocephalus* sp. (80767); for *C. uniparens, Oochoristica bivitellobata* (80766).

Infection prevalence between male and female *C. i. arizonae* (Table 2) was evaluated by the Kruskal–Wallis test, a rank-order analysis (Eckblad, 1984). The infection prevalence between males and females was not significantly different (for *P. warneri*: $j = 0.01$, 1 df, $P > 0.05$; for *O. bivitellobata*: $j = 3.15$, 1 df, $P > 0.05$). Prevalence between *C. i. arizonae* and *C. uniparens* for *O. bivitellobata* (Table 3) was also evaluated by the Kruskal–Wallis test ($j = 0.8$, 1 df, $P > 0.05$); again, there was no significant difference.

Pharyngodon warneri has been previously reported from *C. inornatus* (Specian and Ubelaker, 1974a) as well as from *Cnemidophorus laredoensis* (McAllister et al., 1986), *Cnemidophorus sexlineatus* (Dyer, 1971), *Cnemidophorus tigris* (Grundmann, 1959; Babero and Matthias, 1967), and *Urosaurus ornatus* (Walker and Matthias, 1973). *Oochoristica bivitellobata* was originally described from *C. sexlineatus* (Loewen, 1940) and has been recovered from *C. tigris* (Grundmann, 1959; Babero and Matthias, 1967; Telford, 1970; Benes, 1985; Lyon, 1986), *Cnemidophorus hyperythrus* (Bostic, 1965), and *Cnemidophorus burti stictogrammus* (Goldberg and Bursey, 1989). See Baker (1987) for a list of *Physaloptera* species recorded from lizards.

Mitchell (1979) found a large overlap in the insectivorous diets of *C. i. arizonae* and *C. uniparens* from the Willcox Playa. Thus, it is not suprising that both species are infected by *O. bivitellobata* which, if it is like *Oochoristica anolis* Harwood, 1932, requires an insect intermediate host (Conn, 1985). Oxyurid nematode infections are acquired directly by egg ingestion (Olsen, 1974). Thus, some aspect of life history other than diet apparently controls *P. warneri* infection; its absence in *C. uniparens* deserves further study.

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Research Note

Serological Prevalence of *Neospora caninum* and *Toxoplasma gondii* in Dogs from Kansas

D. S. LINDSAY,[1,4] J. P. DUBEY,[1] S. J. UPTON,[2] AND R. K. RIDLEY[3]

[1] U.S. Department of Agriculture, Livestock and Poultry Sciences Institute, Zoonotic Diseases Laboratory, BARC-East, Building 1040, Beltsville, Maryland 20705,
[2] Division of Biology, Kansas State University, Manhattan, Kansas 66506, and
[3] Department of Laboratory Medicine, Kansas State University, Manhattan, Kansas 66506

ABSTRACT: Sera from 229 dogs were examined for antibodies to *Neospora caninum* using an indirect immunofluorescence assay and for antibodies to *Toxoplasma gondii* using a direct agglutination test. Five of the dogs (2%) were positive for antibodies to *N. caninum* and 57 (25%) were positive for antibodies to *T. gondii*. Three (1%) of the dogs had antibodies to both protozoans. Results indicate that *N. caninum* is less prevalent in the canine population than *T. gondii*.

KEY WORDS: *Neospora caninum, Toxoplasma gondii,* dog, prevalence.

Neospora caninum Dubey, Carpenter, Speer, Topper, and Uggla, 1988, is a recently described protozoan parasite of dogs (Dubey et al., 1988a, b). It is similar to *Toxoplasma gondii* Nicolle and Manceaux, 1909, with light microscopy but can be differentiated by using transmission electron microscopy (Dubey et al., 1988a; Speer and Dubey, 1989) or serological and immunohistochemical testing (Bjerkås and Presthus, 1988; Lindsay and Dubey, 1989b, c). Clinical neosporosis in dogs manifests itself as polymyositis, encephalitis, polyradiculoneuritis, and ascending paralysis (Cummings et al., 1988; Dubey et al., 1988a, b). The disease can be fatal in young or old dogs but is more serious in transplacentally infected puppies. Clinical toxoplasmosis in dogs is usually seen in young animals and is associated with concurrent distemper virus infection (reviewed by Dubey, 1985).

Nothing is known about the prevalence of *N. caninum* infection in the canine population, whereas *T. gondii* infection is common (Dubey, 1985). In the present study we examined sera from 229 dogs for antibodies to *N. caninum* and *T. gondii*.

All dogs were patients at the Veterinary Med-

[4] Present address: Department of Pathobiology, Auburn University, Alabama 36849.

ical Teaching Hospital, Kansas State University, from 1988 to 1989. Samples were shipped frozen to the Zoonotic Diseases Laboratory, Beltsville, Maryland, and were stored at −20°C until examined. Samples were given a coded number and results of *N. caninum* and *T. gondii* testing were kept separate until all samples had been tested. Case histories were obtained for dogs serologically positive for *N. caninum*.

The indirect immunofluorescent assay (IFA) for detection of IgG antibodies to *N. caninum* was done as described (Dubey et al., 1988b) using tachyzoites of *N. caninum* that were grown in cell cultures (Lindsay and Dubey, 1989a) as antigen. Serum was screened at 1:50 dilution in phosphate-buffered saline (PBS), and positive samples were then titrated to an end-point using doubling dilutions of serum in PBS. Serum from 2 dogs with known *N. caninum* infections and serum from 3 dogs negative for *N. caninum* antibodies were used as positive and negative controls at dilutions of 1:50 in PBS. The direct agglutination test for *T. gondii* was performed as described by Dubey and Desmonts (1987). Serum from each dog was tested at dilutions of 1:25, 1:100, and 1:400. We arbitrarily screened canine sera at 1:50 in the IFA and at 1:25 in the agglutination test but have no evidence that titers less than these are not specific.

Antibodies to *N. caninum* tachyzoites were found in 5 of 229 (2%) serum samples. Antibody titers were 1:100 in 1 dog, 1:200 in 2 dogs, and 1:400 in 2 dogs. None of the dogs had clinical signs that could conclusively be associated with active *N. caninum* infection.

Antibodies to *T. gondii* were found in 57 (25%) of the 229 dogs. Forty (17%) had titers of 1:25, 11 (5%) had titers of 1:100, and 6 (3%) had titers of 1:400.

Three of the 5 dogs positive for *N. caninum* were also positive for *T. gondii*. One dog had titers of 1:200 for *N. caninum* and 1:25 for *T. gondii*, 1 had titers of 1:100 for *N. caninum* and 1:25 for *T. gondii*, and 1 had titers of 1:200 for *N. caninum* and 1:100 for *T. gondii*.

Bitches may have antibodies to *N. caninum*, but show no clinical signs of diseases (Dubey et al., 1988b; Hay et al., 1990). These bitches may give birth to transplacentally infected puppies not all of which show signs of disease (Dubey and Lindsay, 1989b; Hay et al., 1990).

Toxoplasma gondii infection was more common in the dogs examined in this study than was *N. caninum*. Oocysts, infected meat, and transplacental modes of transmission are ways in which animals become infected with *T. gondii* (see Dubey and Beattie, 1988). Presently, all that is known about the transmission of *N. caninum* is that parenteral inoculation of cell culture grown tachyzoites produces infections in some animals (Dubey et al., 1988b; Dubey and Lindsay, 1989a; Lindsay and Dubey, 1989c), that transplacental transmission occurs in dogs and cats (Dubey and Lindsay, 1989b, c), and that infected brain containing both tachyzoites and bradyzoites produced infection in a cat (Dubey and Lindsay, 1989c). Immunocompetent animals are more resistant to inoculation with *N. caninum* than are immunocompromised (Lindsay and Dubey, 1989c) or very young animals (Dubey and Lindsay, 1989a). Other modes of transmission, such as oocysts, probably exist but have not been found. It is possible that *N. caninum* may be less prevalent in the canine population because it is not as readily transmitted as is *T. gondii*.

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Research Note

Potentially Pathogenic Species of *Acanthamoeba* and *Hartmannella* (Protozoa: Amoebida) in Sediment of the Potomac River Near Washington, D.C.

SUAD MOHAMMED BIN AMER ASIRI, R. J. CHINNIS, AND W. C. BANTA[1]
Department of Biology, The American University, Washington, D.C. 20016

ABSTRACT: Sediments from 6 stations sampled from the shores of the Potomac River were taken from above the Monocacy River in Maryland to below the Blue Plains Sewage Treatment Plant in Washington, D.C. Sediments were cultured on agar plates streaked with *Escherichia coli* at 20, 37, and 40°C. *Hartmannella vermiformis,* not known to cause human disease, was found at all stations. *Acanthamoeba* species occurred downstream from Great Falls. There was an abrupt increase in the number of species of *Acanthamoeba* at about the limnological fall line, near Chain Bridge. As some of the 7 species of *Acanthamoeba* encountered are serious pathogens, the tidal part of the Potomac below Chain Bridge should be regarded as a potential source of *Acanthamoeba* infections.

KEY WORDS: *Acanthamoeba, Hartmannella,* Potomac, fall line, pathogen, Amoebida, ameba.

Acanthamoeba Volkonsky, 1931, and *Hartmannella* Alexeieff, 1912, may inhabit natural areas such as the warm moist banks of any natural body of water where there is an abundance of organic matter and bacteria (Sawyer et al., 1977). They also occur in freshwater and saltwater sediments and soils throughout the world. Studies of ocean sediments show a positive correlation between the presence of sewage-associated bacteria and the presence of both *Acanthamoeba* and *Hartmannella* (Griffin, 1972; Sawyer et al., 1977; Daggett et al., 1982, 1985).

Free-living amebae of genus *Acanthamoeba* are frequent contaminants of animal tissue culture cells and are potentially pathogenic to man and animals (Sawyer et al., 1977; Daggett et al., 1982). Human infections by *Acanthamoeba* have been recorded throughout the world, and probably occur more commonly than presently recognized (Daggett et al., 1982). Some cases of amebic meningoencephalitis and corneal ulcers have been traced to *Acanthamoeba. Acanthamoeba* may contaminate contact lenses or lens-cleaning/soaking fluids (Centers for Disease Control, 1986). Chronic granulomatous infections of the skin have been reported (Brown and Neva, 1983). Patients with Acquired Immune Deficiency Syndrome (AIDS) are susceptible to infections by many uncommon pathogens, including *Acanthamoeba*; this is true of experimental infections in immunocompromised mice (Wiley, 1987).

Human infections typically occur after contact with banks or bodies of water where *Acanthamoeba* occurs (Daggett et al., 1982; Callicott et al., 1988). No human or animal diseases have been demonstrated to have been caused by any species of *Hartmannella* (Wang and Feldman,

[1] To whom reprint requests should be addressed.

1967; Sawyer et al., 1977; Brown and Neva, 1983).

Sediment samples were taken at the following sites on the Potomac River: 1: North of the mouth of the Monocacy River, off Chick Road, Tuscarora, Frederick County, Maryland: 2: Monocacy River at the Maryland Rt. 28 Bridge; 3: Great Falls, Maryland, in front of the Visitor's Center; 4: Fletcher's Boat House, Canal Road and Reservoir Road, Washington, D.C.; 5: the Virginia bank of the Potomac at the bridge, Theodore Roosevelt Island; 6: the Virginia bank about 0.8 km below the Blue Plains Sewage Treatment Plant.

Five sediment samples at each station were obtained about 10 cm horizontally above the water line. Samples were collected with sterile wooden splints, placed in sterile 1.5-ml plastic centrifuge tubes, capped tightly, and refrigerated at 4°C for 1 wk.

A bacterial culture medium was made with 1.0% agar, 0.01% malt extract, and yeast extract dissolved in distilled water and autoclaved 15 min at 150°C. The medium was poured into sterile 50-mm-diameter snap-top plastic culture dishes. Replicate dishes were inoculated with *Escherichia coli* and sediment and incubated 3 days at 20, 37, and 40°C. The bacteria are food for amebae and other organisms in the sediment cultures (Sawyer et al., 1977). Five sediment samples from each station were cultured in triplicate for each of the 3 experimental incubation temperatures (9 dishes per sample). After 3 days, the incubated cultures were examined at 40× under an inverted microscope for the presence or absence of *Acanthamoeba* and *Hartmannella* cysts; each cyst encountered was identified to species.

Cultures at room temperature contained numerous invertebrates, including ciliates, nematodes, annelids, and trophozoites of *Hartmannella* and *Acanthamoeba*. Cultures incubated at 37°C and 40°C contained only species of *Hartmannella* and *Acanthamoeba*. These "high-temperature species" are generally considered to be potentially pathogenic (Page, 1976; Sawyer et al., 1977; Daggett et al., 1982).

Hartmannella vermiformis cysts occurred at all 6 stations. In culture plates where *Hartmannella* dominated, *Acanthamoeba* cysts were found in a small region of the dish; if there was a small number of *Hartmannella* cysts, *Acanthamoeba* cysts were distributed more or less evenly all over the dish.

Seven species of *Acanthamoeba* were encountered at stations 3, 4, 5, and 6; cysts of *A. astronyxis* and *A. comandoni* were found in stations 4 and 6; cysts of *A castellanii, A. culbertsoni,* and *A. polyphaga* were recovered from stations 4, 5, and 6; *A. hatchetti* cysts were found in stations 3, 4, 5, and 6; *A. rhysodes* was found at stations 3, 5, and 6.

The fall line of the Potomac River occurs at about Chain Bridge, below which the river becomes tidal. Water from the Washington, D.C. metropolitan area is retained for some days in the tidal part of the river. As in most eastern American cities, the human population increases greatly near the fall line; the Potomac may therefore be divided into 2 parts: the relatively clean, nontidal water above the fall line; and tidal, more lentic water influenced by human activities below the fall line.

Acanthamoeba species number increased abruptly between stations 3 and 4. As these *Acanthamoeba* species are known to be associated with sewage, it is likely that contamination of the tidal Potomac with raw sewage probably occurs at least occasionally. The lower Potomac River below Little Falls should be regarded as a potential source of *Acanthamoeba* infections.

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WORKSHOP ANNOUNCEMENT

A Workshop on Artificial Intelligence, Expert Systems, and Modern Computer Methods in Systematic Biology, will be held September 9 to 14, 1990, at the University of California, Davis. There will be about 45 participants representing an even mixture of biologists and computer scientists. Hotel expenses and per diem will be paid and travel will be paid to a maximum of US $500. Attendance by invitation only. These are the workshop subject areas:

1. Scientific workstations for systematics;
2. Expert systems, expert workstations and other tools for identification;
3. Phylogenetic inference and mapping characters onto tree topologies;
4. Literature data extraction and geographical data;
5. Machine vision and feature extraction applied to systematics.

To apply, please send the following information 1) name, address, and phone number; 2) whether you apply as a computer scientist or as a biologist; 3) a short resume; 4) a description of your previous work related to the workshop topic; 5) a description of your planned research and how it relates to the workshop; 6) whether you, as a biologist (or as a computer scientist), have taken or would like to take steps to establish permanent collaboration with computer scientists (or biologists). Mail to:

Renaud Fortuner, ARTISYST Workshop Chairman,
California Department of Food and Agriculture
Analysis & Identification, room 340
P.O. Box 942871
Sacramento, CA 94271-0001
Phone: (916) 445-4521; Fax: (916) 322-5913; E-mail: rfortuner@ucdavis.edu

APPLICATIONS RECEIVED AFTER APRIL 15, 1990 WILL NOT BE ACCEPTED.

J. Helminthol. Soc. Wash.
57(1), 1990, 91–92

PRESENTATION OF THE 1989 ANNIVERSARY AWARD TO J. RALPH LICHTENFELS

Dr. J. Ralph Lichtenfels (left) receiving the 1989 Anniversary Award from Dr. Sherman S. Hendrix.

Mr. President, members of The Helminthological Society of Washington, and guests. It is fitting that the 1989 Anniversary Award be presented here at the Animal Parasitology Institute, where this year's recipient, Dr. J. Ralph Lichtenfels, has, to date, spent his entire scientific career.

The Awards Committee unanimously recommended Ralph to the Executive Committee, which, in turn, supported this recommendation. In looking over Ralph's career and achievements as a parasitologist and member of The Helminthological Society of Washington, it was clear to us that he met all, not just one, award criteria as specified in the Bylaws. As chairman of the Awards Committee, it is a distinct pleasure for me to be here making this presentation.

Dr. Lichtenfels was born on 14 February 1939, in the village of Robinson, Pennsylvania. He received the B.S. degree in 1962 from Indiana University of Pennsylvania, where his interest in parasitology was fostered by Dr. Walter Gallati, who had Ralph running traplines, picking up road kills, and hunting for specimens, in a classical helminthology course. After this experience, he moved to the Washington area to pursue graduate studies in parasitology at the University of Maryland, earning an M.S. in 1966 under Dr. A. James Haley and a Ph.D. in 1968 under Dr. Leo A. Jachowski, working on the biology of the nematode *Nippostrongylus brasiliensis.*

In 1967 he joined the Agricultural Research service at Beltsville, where he became curator of the National Parasite Collection. Under his leadership, the Parasite Classification and Distribution Unit, with emphasis on classical morphological studies, evolved into a Biosystematic Parasitology Laboratory that combines an experimental approach of the organismal, morphological systematics with molecular genetics. As curator of the largest parasite collection in the world, Ralph corresponds with literally hundreds of scientists around the globe regarding the accession or loan of specimens, as well as answering questions related to systematics of parasites.

As a leading authority on the phylogenetic classification of nematode parasites of vertebrates, particularly the superfamilies Strongyloidea and Ancylostomatoidea, he has been able to

correlate their coevolution with that of the mammalian hosts. His work on cuticular ridge patterns in trichostrongyloid nematodes of ruminants has made it possible to make accurate identifications to species of these economically important helminths. Among his more than 100 publications over 20 years, several are considered classics, one of which deserves special mention here. A paper coauthored with another Anniversary Award recipient, Maybelle Chitwood, was the first publication describing characters to be used in identifying helminth parasites in host tissue sections, and has become an essential tool for medical and veterinary pathologists and diagnosticians. Other workers have recognized his preeminence in the field of systematic parasitology by naming species after him. To date, there are two nematodes, a trematode, and monogenean with the specific epithet *lichtenfelsi.*

In addition to his duties as curator and his research program, Dr. Lichtenfels is active in several professional societies, including the American Society of Parasitologists, Wildlife Disease Association, American Microscopical Society, Association of Systematics Collections, and the American Association for Zoological Nomenclature, serving on committees as well as an officer in several of the above. I would like to mention especially his service to The Helminthological Society of Washington. Dr. Lichtenfels joined the Society at the 405th meeting in 1964, and has since served with enthusiasm on numerous committees as well as the Editorial Board, and was the Corresponding Secretary-Treasurer, Recording Secretary, President, and, most recently, Editor of the *Proceedings,* ably assisted by Patricia Pilitt.

As you can see from this brief summary of his outstanding accomplishments in parasitology and service to this Society, Dr. Lichtenfels is richly deserving of this award. On behalf of The Helminthological Society of Washington and the Awards Committee (Ralph Eckerlin and Suzanne Giannini), I am pleased and honored to present the 1989 Anniversary Award to Dr. J. Ralph Lichtenfels.

SHERMAN S. HENDRIX
Chair, Awards Committee

ANNIVERSARY AWARD RECIPIENTS

* Edna M. Buhrer	1960	Margaret A. Stirewalt	1975
Mildred A. Doss	1961	* Leo A. Jachowski, Jr.	1976
* Allen McIntosh	1962	* Horace W. Stunkard	1977
* Jesse R. Christie	1964	Kenneth C. Kates	1978
Gilbert F. Otto	1965	* Everett E. Wehr	1979
* George R. LaRue	1966	O. Wilford Olsen	1980
* William W. Cort	1966	Frank D. Enzie	1981
* Gerard Dikmans	1967	Lloyd E. Rozeboom	1982
* Benjamin Schwartz	1969	Leon Jacobs	1983
* Willard H. Wright	1969	Harley G. Sheffield	1984
Aurel O. Foster	1970	A. Morgan Golden	1985
Carlton M. Herman	1971	Louis S. Diamond	1986
May Belle Chitwood	1972	Everett L. Schiller	1987
* Elvio H. Sadun	1973	Milford N. Lunde	1988
E. J. Lawson Soulsby	1974	J. Ralph Lichtenfels	1989
David R. Lincicome	1975		

HONORARY MEMBERS

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* Norman R. Stoll	1976	Bernard Bezubik	1980
* Horace W. Stunkard	1977	Hugh M. Gordon	1981

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* Nathan A. Cobb	* Joseph Goldberger	* Albert Hassall	* Brayton H. Ransom
* Howard Crawley	* Henry W. Graybill	* George F. Leonard	* Charles W. Stiles
* Winthrop D. Foster			

LIFE MEMBERS

* Maurice C. Hall	1931	David R. Lincicome	1976
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* Charles W. Stiles	1931	* Willard H. Wright	1976
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* William W. Cort	1952	* Everett E. Wehr	1977
* Gerard Dikmans	1953	Marion M. Farr	1979
* Jesse R. Christie	1956	John T. Lucker, Jr.	1979
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* Gerald Thorne	1961	Kenneth C. Kates	1981
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Gilbert F. Otto	1972	Harry Herlich	1987
* Theodor von Brand	1975	Glenn L. Hoffman	1988
May Belle Chitwood	1975	Robert E. Kuntz	1988
Carlton M. Herman	1975	Raymond V. Rebois	1988
Lloyd E. Rozeboom	1975	Frank W. Douvres	1989
* Albert L. Taylor	1975	Thomas K. Sawyer	1989

* Deceased.

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