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Life Cycle Studies on Arcto-boreal Leeches (Hirudinea)

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ABSTRACT: This study provides further information on the life cycles of 6 piscicolid leeches inhabiting the arcto-boreal region of the northwestern Atlantic and northeastern Pacific oceans. Four species including *Platybdella olriki, Platybdella anarrhichae, Oceanobdella sexoculata,* and *Johanssonia arctica* inhabit the Atlantic primarily, but *Notostomum* (*Notostomobdella*) = *cyclostomum* and *Beringbdella rectangulata* have been recorded only from the Pacific. Some species (*P. olriki, J. arctica, N. cyclostomum,* and possibly *B. rectangulata*) deposit their cocoons on decapod crustaceans and a pycnogonid, whereas others (*O. sexoculata* and *P. anarrhichae*) utilize the eggs of host fish. Newly hatched leeches can readily locate their hosts that hatch simultaneously. It appears that 3 species, namely, *P. olriki, P. anarrhichae,* and *O. sexoculata,* have annual life cycles, whereas others such as *J. arctica, N. cyclostomum,* and *B. rectangulata* live more than 1 yr. The life cycle strategies, which include sites of cocoon deposition and host preferences, ensure that their progeny will successfully locate new hosts after emergence.

KEY WORDS: marine leeches, Hirudinea, *Platybdella, Oceanobdella, Johanssonia, Notostomum, Beringbdella,* northwestern Atlantic Ocean, Bering Sea, Gulf of Alaska.

There is limited information on the life cycles of marine leeches, especially species living in the arcto-boreal region. Some leeches are normally attached to their fish hosts in nature. Others are occasionally associated with decapod crustaceans. Increasing evidence indicates that this relationship is not parasitic but one in which the arthropod provides a hard substrate for cocoon deposition and dispersal (Moore and Meyer, 1951; Meyer and Barden, 1955). This relationship has been confirmed in studies on *Myzobdella lugubris* Leidy, 1851, on the blue crab, *Callinectes sapidus* Rathbun, 1896 (Daniels and Sawyer, 1975); *Johanssonia arctica* (Johansson, 1899) on the spider crab, *Chinonocetes opilio* (O. Fabricius, 1788) in the north Atlantic (Meyer and Khan, 1979; Khan, 1982a, b); and *Notostomum cyclostomum* (Johansson, 1898), which attaches to the red king crab, *Paralithodes camtschaticus* (Tilesius, 1815) in North Pacific waters (Moore and Meyer, 1951; Sloan et al., 1984). Some other reports of associations remain speculative, such as that of *Platybdella olriki* Malm, 1865, reported on *Hyas araneus* (Linnaeus, 1758) and on *Sclerocrangon* (=*Crangon*) *boreas* (Phipps, 1774; see Wesenberg-Lund, 1926). Additionally, little is known of the methods used by other leeches to ensure that their progeny will successfully locate new hosts after emergence. The present study provides further information on the life histories of some marine leeches inhabiting the arcto-boreal region and their strategies for locating their hosts.

Materials and Methods

Platybdella olriki were obtained from the toad crab, *Hyas araneus,* captured in baited conical traps set at 10–50 m deep in Conception Bay, Newfoundland (47°31′N, 53°05′W). After removal, leeches were held in ambient seawater and subsequently allowed to reattach to toad crabs held in a flow-through aquarium in the laboratory. Species of fish were introduced at 2–5-day intervals to ascertain host preferences. Additionally, a number of fish species inhabiting Logy Bay were examined by SCUBA divers at depths of 5–20 m for *P. olriki.*

Seaspiders, *Nymphon* sp. (Pycnogonidae), were collected by otter trawl off the northeast coast of Newfoundland and Ungava Bay during 1978 and 1982 and held in ambient seawater tanks until their return to the laboratory. Seaspiders harboring cocoons of undetermined leeches were retained until young emerged. Other pycnogonids, without leeches, were exposed to a number of species of leeches to determine which species would attach to deposit their cocoons.

Egg masses with adhering leech cocoons of an ocean-pout, *Macrozoarces americanus* (Schneider, 1801), and an unknown fish were held in aquaria (20 liters) through which ambient seawater (0–4°C) flowed after collection by SCUBA divers and otter trawl, respectively, off the northeastern coast of Newfoundland. After emergence, young leeches were removed and exposed to several fish species to ascertain their host preferences and growth rate.

Notostomum cyclostomum were obtained from red king crab and Tanner crab, *Chionoecetes bairdi* Rathbun, 1924, captured in baited traps set at 30–120 m

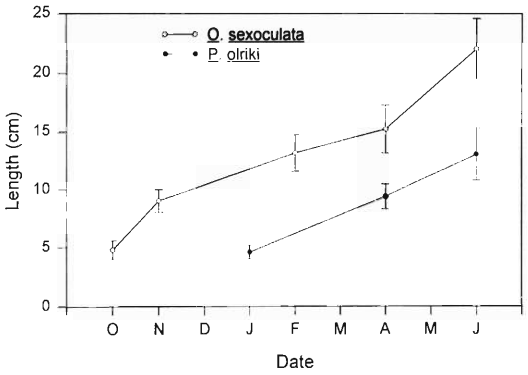


Figure 1. Growth rate of *Platybdella olriki* and *Oceanobdella sexoculata* at the ambient (0–14°C) seawater temperature. Date refers to months of the year.

in the Gulf of Alaska and the Bering Sea. Live specimens also were obtained from Pacific halibut, *Hippoglossus stenolepis* Schmidt, 1904, captured by hook and line in Resurrection Bay, Alaska (60°06′N, 149°28′W); after transportation to Newfoundland, they were held at 2–4°C in a recirculating water system. Leeches were exposed subsequently in an aquarium to a number of fish species to determine their host specificity.

Several species of fish caught by hook and line in Resurrection Bay, Alaska, were examined for the leech *Beringbdella rectangulata* (Levinsen, 1882). After removal, leeches were placed in 500-ml beakers with seawater at 4°C. The number of cocoons deposited and their dimensions were recorded as well as the period of incubation before young emerged.

Results and Discussion

Northwestern Atlantic leeches

PLATYBDELLA OLRIKI MALM, 1865: For species description and occurrence, see Meyer and Khan (1979).

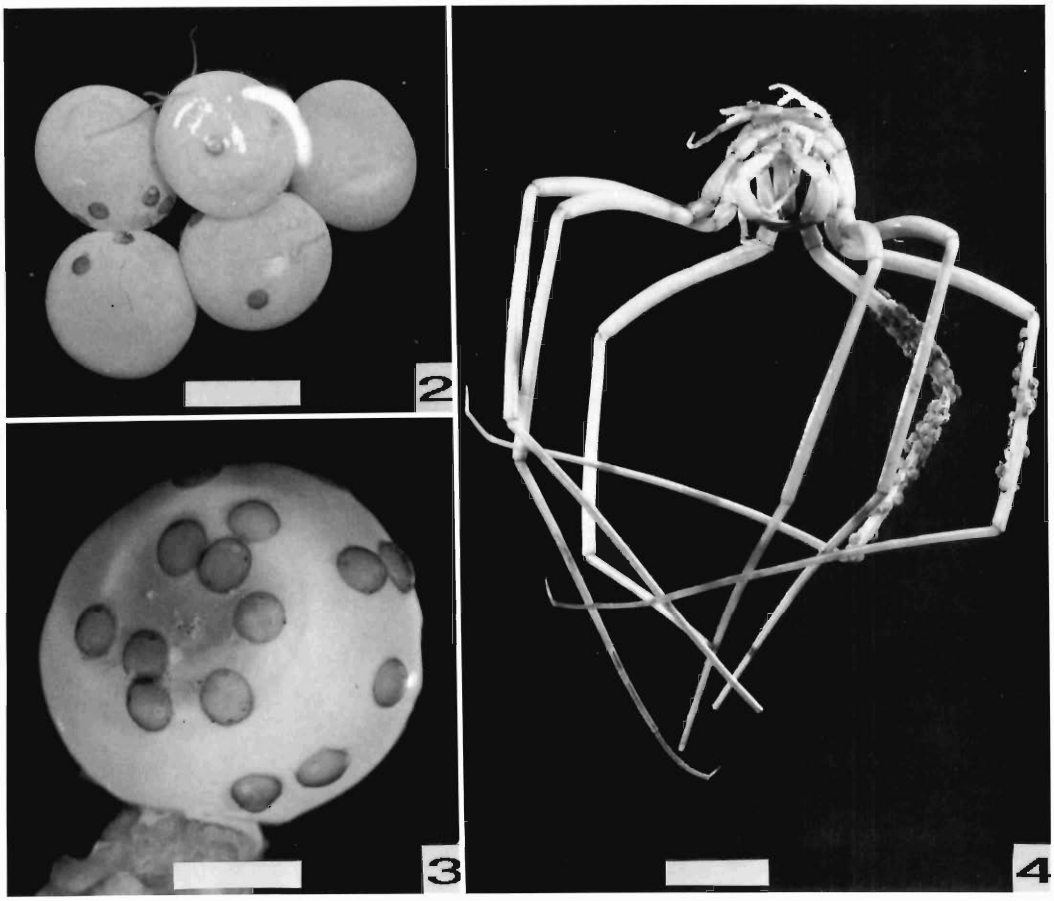
This leech was observed on the toad crab, *H. araneus,* taken at 10–50-m depth but not in deeper areas (100–190 m) or on other species of crabs (*Hyas coarctatus* Leach, 1815, and *C. opilio*). Specimens were obtained from January through July; none were observed between August and December. Its prevalence (2.4% of 250 toad crabs examined) and mean intensity (0.24 ± 0.002), although low, were greatest during the months of April (15%; $\bar{x}$, 0.14 ± 0.02) and June (25%; $\bar{x}$, 0.24 ± 0.3), especially on olive-brown–colored crabs. The leeches were irregularly distributed over the carapace and dorsal and ventral surfaces of the legs. Cocoons were deposited mainly on the ventral surfaces of the legs. Leeches of varying dimensions were collected during May to July mainly from winter flounder, *Pleuronectes* (=*Pseudopleuronectes*) *americanus* (Walbaum, 1792), and less often from sea raven, *Hemitrypterus americanus* (Gmelin, 1789), lumpfish, *Cyclopterus lumpus* Linnaeus, 1758, and longhorn sculpin, *Myoxocephalus octodecenspinosus* (Mitchell, 1815). Engorged adults, held in aquaria, each deposited 14 ± 4.1 cocoons between July and September (42 ± 7.2 days) and died subsequently. Cocoons measured 0.68 ± 0.14 × 0.58 ± 0.12 mm. Each produced 1 young that emerged from late December to January, about 92 ± 10.4 days later, coinciding with the time when they appear on toad crabs in nature. Young leeches measured 4.1 ± 0.6 mm in length. They attached and fed readily on winter flounder and ignored other species of fish such as longhorn sculpin and sea raven. Based on the red coloration of recently engorged leeches, about 5 blood meals were required before the leeches deposited cocoons in July (Fig. 1).

OCEANOBDELLA SEXOCULATA (Malm, 1863): For species description, see Khan and Meyer (1976).

Egg masses collected from oceanpout, *M. americanus,* in November were infested with cocoons of *O. sexoculata* (Fig. 2). Young leeches emerged during February and attached to the cephalic region of larval oceanpout that were hatching concurrently. Blood feeding occurred subsequently and many larvae died. Some leeches on surviving oceanpout larvae were removed at intervals, and their growth rate appeared to be similar in the laboratory (Fig. 1) to that in nature (see Khan and Meyer, 1978). Cocoon deposition, an indicator of maturity, occurred in July and August; by this time, most leeches had died. The natural life cycle appears, therefore, to be annual in nature but bi-annual in the laboratory. Leeches were distributed over the body of oceanpout, but many more occurred in the cephalic region. They appeared to feed continually when attached to their host.

PLATYBDELLA ANARRHICHAE (Diesing, 1859): For species description, see Meyer and Khan (1979).

An ovoid egg mass, ~20 cm in diameter, harbored 7 *P. anarrhichae* and numerous cocoons (Fig. 3) after collection in early December. Cocoons measured 1.51 ± 0.12 × 1.26 ± 0.15 mm. Larval wolffish, *Anarhichas* sp., and young leeches emerged simultaneously in mid-February, approximately 63 days after the egg mass was collected. The young attached immediately to larvae in the head region, including the eyes, and



Figures 2–4. 2. Cocoons and young leeches of *Oceanobdella sexoculata* attached to the eggs of an oceanpout. Scale bar = 4.5 mm. 3. Cocoons of *Platybdella anarrhichae* attached to eggs of an unknown species of wolffish. Scale bar = 3 mm. 4. Cocoons and adults of *Johanssonia arctica* attached to a pycnogonid. Scale bar = 1 cm.

subsequently fed. Young initially measured 4.5 ± 0.8 mm in length and grew rapidly over the following 6 wk (8.8 ± 1.3 mm in length). During this period, some (~300) were fasted for a 2-wk period and exposed to winter flounder, longhorn sculpin, oceanpout, Atlantic cod, spotted wolffish, *A. minor* Olafsen, 1774, and Atlantic wolffish, *A. lupus* Linnaeus, 1758. The leeches attached only to Atlantic wolffish from which blood was obtained. More than 50% of the leeches attached to the head region of the fish compared to other parts of the body. Most of the leeches left on the wolffish (measuring 10.8 ± 1.1 mm) had died by the end of June without deposition of cocoons. It is likely that growth rate and maturity of *P. anarrhichae* is similar to that of *P. olriki*. Both life cycles in nature appear to be annual. This leech is widely distributed in the northeast and northwest Atlantic (see Meyer and Khan, 1979).

JOHANSSONIA ARCTICA (Johansson, 1899): For species description, see Meyer and Khan (1979).

In the northwestern Atlantic, this leech is normally associated with the spider (queen) crab *C. opilio* and less often on *Hyas coarctatus* and *H. araneus,* on which cocoons also are deposited. Although rarely associated with fish, it can be found in an engorged state primarily on American plaice, *Hippoglossoides platessoides* (Fabricius, 1780), caught in gillnets off the northeastern coast of Newfoundland and Labrador, Ungava Bay, and the Davis Strait. It has been found attached to the pycnogonid, *Nymphon* sp. (Fig. 4), on which cocoons are deposited primarily on the upper 2 segments of the legs. When the seaspiders were held in ambient (0–4°C), running seawater,

Figure 5. Cocoons and an adult of *Notostomum cyclostomum* attached to the carapace of a Tanner crab.

small leeches eventually emerged after 201–262 days. These young fed more readily on American plaice than on longhorn sculpin, winter flounder, or Atlantic wolffish.

A group of 7 engorged adult *J. arctica* were allowed to attach and deposit cocoons on a sea-spider that had been collected alive from Ungava Bay. It was held initially at 0°C, and a total of 207 cocoons were deposited over a period of 26 days. Young leeches emerged 267 ± 27 days later, indicating that both crabs (*C. opilio*) and sea-spiders serve as sites for cocoon deposition. The leeches fed approximately 7 times over a 2-yr period before the terminal deposition of cocoons and death. Based on the present and a previous study (Khan, 1982b), the life cycle of *J. arctica* is completed in about 2 yr.

Northeastern Pacific leeches

NOTOSTOMUM CYCLOSTOMUM (=*Notostomobdella* cyclostoma) (Johansson, 1898): For species description and occurrence, see Moore and Meyer (1951).

In the Gulf of Alaska and the Bering Sea, leeches were collected more often from the red king crab than from the Tanner crab. The leech was more prevalent on the Tanner crab taken from the Bering Sea (7% of 101 crabs) than from the Gulf of Alaska (2% of 54). Cocoons also occurred more often on crabs from the Bering Sea (31.2%) than from the Gulf of Alaska (16.3%). Similarly, the mean intensity was greater on crabs taken from the Bering Sea (3.2 ± 1.2) than from the Gulf of Alaska (0.6 ± 0.3 per crab). Cocoons were deposited primarily on the main body carapace and less often on other parts of the body (Fig. 5). Large individuals were usually associated with crabs, whereas smaller forms were obtained mainly from species of flatfish. In the Gulf of Alaska, small leeches (29.2 ± 2.3 mm) were collected from yellowfin sole, *Pleuronectes* (=*Limanda*) *asper* (Pallas, 1814), rock sole, *P. bilineata* (Ayres, 1855), flathead sole, *Hippoglossoides elassodon* Jordan and Gilbert, 1880, and Pacific halibut from May to August. This leech is widely distributed in the northeast and northwest Pacific (Moore and Meyer, 1951; Epshtein, 1962; Sloan et al., 1984). According to Moore and Meyer (1951), there is no record of the leech's occurrence in the Arctic Ocean. The same authors (Moore and Meyer, 1951) also reported that most specimens (158 of 161) of *N. cyclos-*

tomum were dredged from soft substrate habitats free of their crab or fish hosts and concluded that it is ". . . a free-ranging predacious hunter which attaches to its prey to satisfy its sanguivorous requirements . . . p. 24." Epshtein (1961, 1962) concluded that *N. cyclostomum* was specific for *P. camtschatica* and *C. opilio.* However, Sloan et al. (1984) noted that although 3 species of crabs, primarily the golden king crab, *Lithodes aequispina* Benedict, 1895, was infested with cocoons of the leech collected from the deep fjords of British Columbia, Canada, gut contents revealed fish blood in various stages of digestion.

Examination of smears (10) of the gastrointestinal contents of leeches removed from crabs in the present study revealed the presence of erythrocytes of fish in all specimens. Five live leeches (72 ± 8.24 mm) obtained by one of us (A.J.P.) from Tanner crabs were maintained at ~4°C after transportation to Newfoundland. Four of these leeches deposited a total of 35 ($\bar{x}$, 8.75 ± 1.1) cocoons over a period of 84 days when held in a 1-liter plastic container. The leeches were subsequently placed in a flow-through seawater aquarium (100 liters), which included Atlantic cod, shorthorn sculpin, *Myoxocephalus scorpius* (Linnaeus, 1758), and winter flounder. They were observed at weekly intervals to ascertain host preferences. The leeches fed only on flounder. Shortly after feeding, 3 of these leeches attached to a toad crab, *H. araneus,* and were subsequently consumed by it. The remaining leech deposited 6 cocoons before it died.

Sixteen young leeches (20.0 ± 2.50 mm) emerged from cocoons 245–301 days later. Ten individuals fed readily on the blood of winter flounder and 6 on yellow tail, *Pleuronectes* (=*Limanda*) *ferrugineus* (Storer, 1839) but not on Atlantic cod or longhorn sculpin. They were maintained at ~4°C, but a sudden change of temperature (>10°C) in the incoming seawater over a period of a week resulted in total mortality. These observations support the view that flatfish species are primarily the source of blood meals of *N. cyclostomum* and the crab exoskeleton as a site for cocoon deposition.

BERINGBDELLA RECTANGULATA (Levinsen, 1882): For description of the species, see Moore and Meyer (1951).

Three of 12 Pacific cod, *Gadus macrocephalus* Tilesius, 1815, captured in Resurrection Bay at ~45 m were parasitized by a leech that occurred more often in the branchial chamber than on the body. As many as 37 leeches infested 1 fish. Preserved specimens of 25 leeches from Pacific cod measured 48.2 ± 9.4 × 4.8 ± 0.8 mm. Examination of preserved specimens revealed that the leech was *Beringbdella rectangulata* (Levinsen, 1882). The urosome was considerably wider and readily distinguished from the trachelosome. The cephalic sucker was smaller than the posterior sucker and eyes were lacking. This leech was originally described as *Piscicola rectangulata* by Levinsen in 1882, but Vasileyev (1939) transferred it to the newly created genus *Levinsenia.* Caballero (1970), however, pointed out that *Levinsenia* resulted in a homonym and proposed *Beringbdella rectangulata* (Levinsen, 1882) comb. n. Epshtein (1962) considered *Icthyobdella uobir* Oka, 1910, a synonym of *B. rectangulata.*

According to Moore and Meyer (1951), the leech was collected more often from the gills of Pacific cod than from free of its host in Alaskan waters of the eastern Pacific including the Pribilof and Aleutian islands. Levinsen's type material was obtained for the Amur region in the western Pacific. Vasileyev (1939) and Epshtein (1962) have recorded it from eastern Kamtchatka southward to the Sea of Japan, an area where Oka in 1910 made his collection. According to Burrreson (pers. comm.), the identification of *B. rectangulata* from the great sculpin, *M. polyacanthocephalus* (Pallas, 1814), is incorrect and was confused with *Heptacyclus virgatus* (Oka, 1910). The 2 leeches, namely, *B. rectangula* and *H. virgatus,* are superficially similar in appearance but can be distinguished because *B. rectangulata* has a much stockier body, a more muscular caudal sucker, a smaller oral sucker and lacks eyes (Burreson, pers. comm.) Measurements of 53 specimens of *H. virgatus* from *M. polyacanthocephalus* captured in the Bering Sea revealed that it is a smaller leech (43.7 ± 9.9 × 3.6 ± 0.8) than *B. rectangulata.* It appears, then, that *B. rectangulata* is restricted to gadid fish. Cocoons of this leech, initially opaque in color, become tanned and golden-brown within 48 hr. Three *B. rectangulata,* when held together at ~4°C in a flow-through, ambient seawater system, deposited 15 cocoons over a 26-day period. These, dome-shaped in appearance, measured 2.71 ± 0.28 × 1.79 ± 0.12 mm (n = 10). A sudden change in the temperature (~10°) of the incoming seawater caused mortality of the leeches, and the cocoons never hatched. Based on the leech's dimensions, it is likely that its life cycle exceeds 1 yr.

Marine leeches inhabiting the arcto-boreal re-

gion utilize different strategies to ensure that their offspring locate new hosts. Adult *J. arctica, M. lugubris, P. olriki,* and *N. cyclostoma* deposit their cocoons on the legs or carapace of decapod crustaceans and a pycnogonid for dispersal. More piscicolid species deposit cocoons on the legs than on the carapace. Presumably firm substrates are limited or absent in the area frequented by these leeches, but some might utilize rocks when available. Cocoons of *P. olriki,* for example, also were observed by SCUBA divers on rock outcrops frequently used by winter flounder in Logy Bay (Khan, unpubl. data). Other leeches such as *O. sexoculata* and *P. anarrhichae* deposit cocoons directly on the egg mass of host fish. Young leeches after emergence can locate their hosts that hatch simultaneously. A third group of leeches, such as *Malmiana scorpii, M. brunnea, O. microstoma* (Khan and Meyer, 1976), and *O. blenni* (Knight-Jones, 1940) (see Gibson and Tong, 1969) deposit their cocoons in spring on rocks where species of sculpins and blennies, *Blennius pholis* (Linnaeus, 1758), frequent so that the young subsequently can locate their hosts after emergence. The site(s) of cocoon deposition of *B. rectangulata* is unknown, but because the leech feeds on fish that inhabit areas where the substrate is soft, it is likely that a crustacean might also be used for cocoon deposition. Fish leeches that adhere to invertebrates for dispersal and cocoon deposition are less likely to be found in nature feeding on fish except during the period prior to the final blood meal and cocoon deposition. Generally, these leeches have a broader host range than those that attach permanently such as species of *Malmiana* and *Oceanobdella.* The various sites used by these fish-feeding leeches for cocoon deposition and their associated host preferences ensure that their offspring can locate new hosts successfully after emergence.

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Nippostrongylus marhaeniae sp. n. and Other Nematodes Collected from *Rattus* cf. *morotaiensis* in North Halmahera, Molucca Islands, Indonesia

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ABSTRACT: *Nippostrongylus marhaeniae* sp. n., 2 *Odilia* spp., *Orientostrongylus* sp., *Strongyloides ratti*, and *Mastophorus muris* were collected from *Rattus* cf. *morotaiensis* from Halmahera Island, North Moluccas, Indonesia. *Nippostrongylus marhaeniae* resembles *N. magnus* and *N. typicus* of Australian rats in the bursal structure but is readily distinguished by having only 12 ridges of synlophe in midbody of both sexes and in that the tips of spicules are not recurved strongly. Species of *Odilia* were first recorded outside of New Guinea–Australian region, and 1 of the present species closely resembles *O. mackerrasae* from the Australian rat by having intermittent ridges in the ventral side. Presence of the trichostrongyloids closely related to the Australian representatives suggests that these nematodes were introduced by some rats from the New Guinea–Australian region and have been maintained within the endemic rat community on Halmahera Island.

KEY WORDS: *Nippostrongylus marhaeniae* sp. n., nematodes, *Rattus* cf. *morotaiensis*, Halmahera Island, Indonesia, systematics, zoogeography.

Rattus morotaiensis Kellog, 1945, is distributed in North Moluccas, Indonesia (type locality: Morotai Island) (Musser and Carleton, 1993). Because only limited examples have been collected, the biology of this endemic rat has not been adequately elucidated. In 1993, we had a chance to collect murines for parasitological survey on Halmahera Island, located just south of Morotai Island. One individual of *R.* cf. *morotaiensis* was incidentally obtained, and its parasitological examination revealed 6 nematode species, of which 4 are trichostrongyloids of systematic interest. This paper deals with these nematodes with special reference to the zoogeography of the host and parasites.

Materials and Methods

The rat, captured by a domestic cat in the nearby forest of Kai village, Kao District, North Halmahera, Moluccas, Indonesia, was examined. Its viscera were fixed with 10% formalin solution on the same day of capture, and then parasites were collected under a stereomicroscope. Collected nematodes were rinsed in 70% ethanol solution, cleared in glycerol-alcohol solution, and mounted with 50% glycerol solution. Freehand cross-sections were made for observation of the synlophe of the trichostrongyloids. Figures were made with the aid of a drawing tube. Given measurements, in micrometers unless otherwise stated, are for the holotype male and the allotype female, followed in parentheses by the range of paratype males and females. The terminology of the synlophe follows Durette-Desset (1983).

Nematode specimens are deposited in the United States National Museum Helminthological Collection (USNM Helm. Coll.), Beltsville, Maryland, U.S.A. The stuffed skin and skull specimen of the host are deposited in the American Museum of Natural History, New York, U.S.A., AMNH267681.

Results

Four nematode species belonging to the subfamily Nippostrongylinae (Trichostrongyloidea: Heligmonellidae) were found in the small intestine as described later. *Strongyloides ratti* Sandground, 1925 (Rhabditoidea: Strongyloididae) (2 parasitic females: USNM Helm. Coll. No. 84315), and *Mastophorus muris* (Gmelin, 1790) (Spiruroidea: Spirocercidae) (3 males and 9 females: USNM Helm. Coll. No. 84316) were also collected from the small intestine and the stomach, respectively.

Nippostrongylus marhaeniae sp. n. (Trichostrongyloidea: Heligmonellidae: Nippostrongylinae) (Figs. 1–13)

GENERAL: Small red worms, forming sinistral tight or flat coils with ventral side located inside. Anterior end with cephalic vesicle (Figs. 1, 2). Mouth triangular (Fig. 1). Four large cephalic papillae, 6 small labial papillae and amphids present (Fig. 1). Cuticle finely striated. Synlophe well developed with pointed ridges, commencing immediately posterior to cephalic vesicle and ending slightly anterior to bursa in male,

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and at vulval level in female (Figs. 2, 4, 13). In midbody of both sexes 12 ridges present, carene of type A supported by hypertrophied left lateral ridge present; axis of orientation of ridges passing through ventral-right and dorsal-left sides, inclined about 45° from sagittal axis; 2 ridges in right to right-dorsal field and 3 ventral-left ridges well developed, 2 right-ventral ridges less developed; midventral to ventral-right portion devoid of ridges (Figs. 3, 12). Esophagus club-shaped (Fig. 2). Nerve ring posterior to midesophagus, excretory pore at midpoint between nerve ring and posterior end of esophagus, and deirids at same level or slightly posterior to excretory pore (Fig. 2).

MALE (holotype and 3 paratypes): Length 3.61 (3.20–3.88) mm, width at midbody 104 (94–112). Cephalic vesicle 66 (58–74) long by 36 (35–42) wide. Nerve ring 203 (154–193), excretory pore 268 (245–310), and deirids 288 (245–313) from cephalic end. Esophagus 320 (315–353) long and 26 (24–26) wide near posterior end. Bursa asymmetrical, right lobe larger than left lobe; bursal rays except posterolateral and externodorsal rays in right lobe thicker than in left lobe (Figs. 5, 8). Right lobe: ventral rays widely divergent; laterooventral ray slightly longer than ventroventral ray; externolateral and mediolateral rays thick, divergent distally; posterolateral ray short, small, arising from base of mediolateral ray, divergent widely from other laterals; externodorsal ray thin, arising from proximal half of trunk of dorsal ray (Figs. 5, 8). Left lobe: ventral rays moderately divergent, ventroventral ray slightly shorter than lateroventral ray; externolateral ray attached lateroventral ray along almost whole length, slightly shorter than lateroventral ray; mediolateral ray shortest among laterals, directed lateroventrally; posterolateral ray thickest among laterals, directed posterolaterally; externodorsal ray arising from distal half of trunk of dorsal ray, much thicker than right externodorsal ray (Figs. 5, 8). Dorsal ray with thick trunk, divided at distal ⅓ into 2 branches, each of which again divided into 2 offshoots. Outer offshoots longer than inner offshoots, directing posterolaterally; each inner offshoot provided with 2 papillae apically (Figs. 5, 7). Genital cone protruded prominently, with 1 pair of conical papillae apically; anterior lip of cloaca less protruded, provided with 1 papilla (Fig. 6). Spicules equal in length, alate, joined and slightly twisted distally (Fig. 9). Left spicule slightly thickened distally forming round tip (Fig. 10), and right spicule tapering distally forming pointed tip (Fig. 11). Spicule length 388 (335–385) (corresponding to 9.6–10.7% of worm length). Gubernaculum boat-shaped, 21 (21–22) long (Fig. 4).

FEMALE (allotype and 1 complete and 1 incomplete paratype): Length 4.13 (3.92) mm, width at midbody 109 (88). Cephalic vesicle 53 (56) long by 43 (53) wide. Nerve ring 144 (177), excretory pore 208 (270), and deirids 214 (273) from cephalic end. Esophagus 269 (310) long and 24 (32) wide near posterior end. Body narrowed at vulval level and postvulval body bent ventrally strongly (Fig. 13). Vulva 84 (68–96) and anus 33 (26–29) from caudal end (Fig. 13). Vagina vera 24 (32–50) long, forming diverticulum dorsally; vestibule narrowed distally, 80 (72–83) long; sphincter 31 (24–25) long; infundibulum 125 (64–128) long (Fig. 13). Cuticle between vulva and anus distended (Fig. 13). Tail conical (Fig. 13). Eggs ellipsoidal, thin-shelled, containing morula to tadpole-stage embryos, and 53–56 × 30–34.

TYPE HOST: *Rattus* cf. *morotaiensis* (Muridae: Murinae).

SITE: Small intestine.

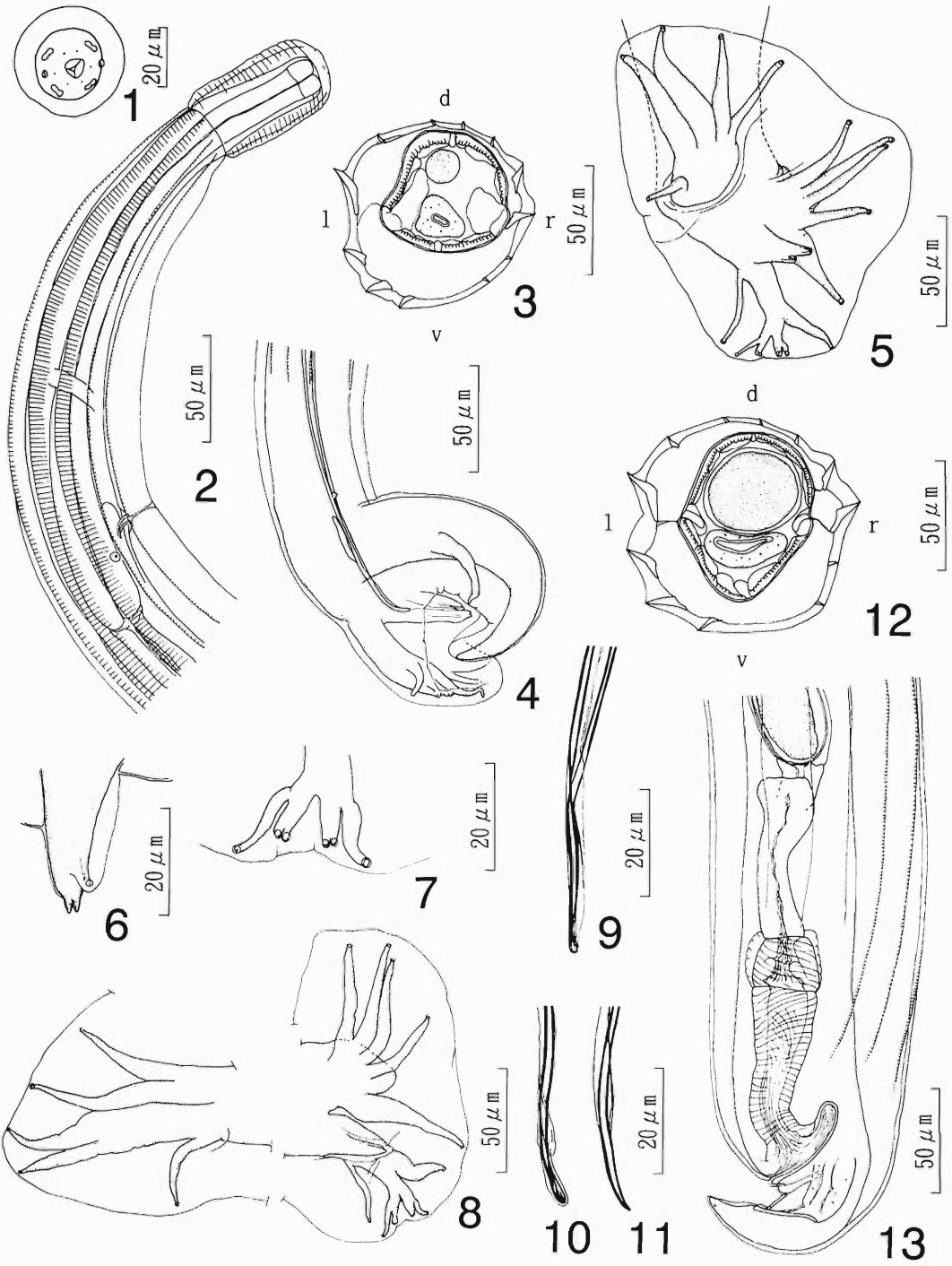
TYPE LOCALITY: Kai (1°32′N, 127°48′E; 100 m elevation), North Halmahera, Indonesia.

DATE OF COLLECTION: 11 July 1993.

ETYMOLOGY: Species name is dedicated to Dr. Marhaeni Hasan, director of the Kao Health Center, to whom we are greatly indebted for the survey.

TYPE SPECIMENS: USNM Helm. Coll. No. 84317 (holotype and allotype) and 84318 (3 male and 2 female paratypes).

REMARKS: The present species has every morphological characteristic of the genus *Nippostrongylus* Lane, 1923, although its synlophe consists of only 12 ridges (Durette-Desset, 1970a, 1983). *Nippostrongylus marhaeniae* resembles *Nippostrongylus typicus* (Mawson, 1961) and *Nippostrongylus magnus* (Mawson, 1961), both of which have been known from Australian murines, in that the left externodorsal ray is thicker and arising from a more distal level of the trunk of the dorsal ray than the right one (Mawson, 1961; Durette-Desset, 1969; Beveridge and Durette-Desset, 1992). Specimens of *N. marhaeniae* are easily distinguished by this character from *Nippostrongylus brasiliensis* (Travassos, 1914), *Nippostrongylus rysavyi* (Erhardová, 1959), *Nippostrongylus rauschi* Chabaud and Desset, 1966,



Figures 1–13. ***Nippostrongylus marhaeniae*** **sp. n. from** ***Rattus*** **cf.** ***morotaiensis*** **from Halmahera Island, North Moluccas, Indonesia. 1. Cephalic extremity of male, apical view. 2. Anterior part of holotype, right lateral view. 3. Cross-section of male through midbody. 4. Posterior part of holotype, right lateral view. 5. Bursa copulatrix of paratype, ventral oblique view. 6. Genital cone, subventral view. 7. Distal end of dorsal ray, ventral view. 8. Bursa copulatrix dissected, ventral view. 9. Distal ends of spicules. 10. Distal end of left spicule** ***dissected. 11.*** **Distal end of right spicule dissected. 12. Cross-section of female through midbody. 13. Posterior part of allotype, left lateral view. Abbreviations: d = dorsal, l = left, r = right, v = ventral.**

Nippostrongylus djumachani (Tenora, 1969), *Nippostrongylus witenbergi* Greenberg, 1972, and *Nippostrongylus* sp. of Hasegawa (1990) (Erhardová, 1959; Mawson, 1961; Chabaud and Desset, 1966; Durette-Desset, 1969, 1970a; Tenora, 1969; Greenberg, 1972; Hasegawa, 1990). *Nippostrongylus typicus* and *N. magnus* have a strongly recurved distal end of the spicule, being readily distinguished from the present species (Mawson, 1961; Beveridge and Durette-Desset, 1992).

Odilia sp. 1

(Trichostrongyloidea: Heligmonellidae: Nippostrongylinae)

HOST: *Rattus* cf. *morotaiensis* (Muridae: Murinae).

SITE: Small intestine.

LOCALITY: Kai (1°32′N, 127°48′E; 100 m elevation), North Halmahera, Indonesia.

DATE OF COLLECTION: 11 July 1993.

SPECIMENS: USNM Helm. Coll. No. 84319 (1 male and 3 females).

REMARKS: The present specimens belong to the genus *Odilia* Durette-Desset, 1973 (syn. *Austrostrongylus* sensu Durette-Desset, 1971, nec Chandler 1927), in that the left lateral ridge of the synlophe is hypertrophied with the adjacent dorsal one supporting the carene of type A, the bursa copulatrix is asymmetrical, the dorsal ray is divided in its basal half, and the externodorsal rays are of similar size (Durette-Desset, 1971, 1973, 1983). By having intermittent ridges in the ventral half of the body, this species is especially close to *Odilia mackerrasae* (Mawson, 1961) from *Melomys cervinipes, Melomys lutillus, Melomys* sp., and *Uromys caudimaculatus* of North Australia (Mawson, 1961; Durette-Desset, 1969) and from *Rattus fuscipes* of South Australia (Obendorf, 1979). It may be distinguished from *O. mackerrasae* by having shorter spicules and a longer esophagus and by lacking a gubernaculum (Mawson, 1961). However, proposal of a new species is withheld because only a small number of the worms was obtained.

Odilia sp. 2

(Trichostrongyloidea: Heligmonellidae: Nippostrongylinae)

HOST: *Rattus* cf. *morotaiensis* (Muridae: Murinae).

SITE: Small intestine.

LOCALITY: Kai (1°32′N, 127°48′E; 100 m elevation), North Halmahera, Indonesia.

DATE OF COLLECTION: 11 July 1993.

SPECIMEN: USNM Helm. Coll. No. 84320 (1 female).

REMARKS: Only 1 female was collected. Although a male was not collected, it is possible to classify this species in the genus *Odilia* by the typical arrangement of synlophe ridges (Durette-Desset, 1971, 1983). The present female was a coparasite of the former species but is easily distinguished by the fact that the synlophe ridges are all continuous and the right lateral ridge is quite small. The synlophe of the present female resembles that of *Odilia brachybursa* (Mawson, 1961) from *M. cervinipes* of Australia by having 15 ridges in midbody (Mawson, 1961; Durette-Desset, 1969).

Orientostrongylus sp.

(Trichostrongyloidea: Heligmonellidae: Nippostrongylinae)

HOST: *Rattus* cf. *morotaiensis* (Muridae: Murinae).

SITE: Small intestine.

LOCALITY: Kai (1°32′N, 127°48′E; 100 m elevation), North Halmahera, Indonesia.

DATE OF COLLECTION: 11 July 1993.

SPECIMENS: USNM Helm. Coll. No. 84321 (3 males and 1 female).

REMARKS: The present material resembles *Orientostrongylus tenorai* Durette-Desset, 1970, which has been known from various murines in the areas from Afghanistan to Taiwan (Durette-Desset, 1970b; Ohbayashi and Kamiya, 1980; Ow Yang et al., 1983; Hasegawa, 1990; Hasegawa et al., 1994), and also from *Rattus rattus* and *Rattus exulans* on Halmahera Island (Hasegawa and Syafruddin, 1995). It is distinguished from the examples of *O. tenorai* from these rats on Halmahera Island by having a much thicker body and longer spicules. However, more comparative study, especially on the host-dependent variations, is necessary to conclude whether or not it is conspecific with *O. tenorai*.

Discussion

The endemic murines of the Moluccas have been considered to be allied with those on New Guinea and its offshore islands, and *R. morotaiensis* is believed to be closely related to native *Rattus* of New Guinea (Musser, 1981; Musser and Carleton, 1993). The present nematode fau-

na also contains the species with close morphological resemblance to the New Guinea–Australian representatives. *Nippostrongylus marhaeniae* shares same bursal characteristics with *N. typicus* and *N. magnus* from Australian *Melomys*. The genus *Odilia* has been recorded only in Australia and New Guinea (Irian Jaya) (Durette-Desset, 1983; Hasegawa and Syafruddin, 1994). The presence of the *Odilia* species with intermittent synlophe ridges in the ventral cuticle on Halmahera Island is of special interest because its most allied species, *O. mackerrasae,* has been recorded from *Melomys,* which is distributed in Australia, New Guinea, and North Moluccas (Musser and Carleton, 1993). It is therefore probable that these nematodes were introduced by some endemic rats from New Guinea to Halmahera Island and have been maintained within the endemic murine populations on this island.

The trichostrongyloid fauna of *R.* cf. *morotaiensis* of Halmahera seems to be critically different from that of *R. rattus* and *R. exulans* on this island: only *N. brasiliensis* and *O. tenorai* were detected from the latter 2 species (Hasegawa and Syafruddin, 1995). The dispersal of these 2 murines in the Pacific islands is considered to have been facilitated by humans (cf. Musser and Carleton, 1993). *Nippostrongylus brasiliensis* is a cosmopolitan parasite of *R. rattus* and *Rattus norvegicus,* and *O. tenorai* is also a common nematode of the rats in Southeast and East Asia (cf. Ohbayashi and Kamiya, 1980; Ow Yang et al., 1983; Hasegawa et al., 1994). Thus, it is presumed that these 2 trichostrongyloids have been introduced to Halmahera by the commensal rats (Hasegawa and Syafruddin, 1995). The difference in trichostrongyloid fauna between the *R.* cf. *morotaiensis* and the commensal rats may be attributed to the host specificity of the parasites and/or the habitat segregation of the hosts.

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Digenetic Trematodes of Marine Fishes from Suva, Fiji: The Family Gyliauchenidae Ozaki, 1933

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ABSTRACT: Three new species of gyliauchenids are described from marine fishes taken at Suva, Fiji Islands: *Gyliauchen pomacentri* from *Pomacentrus philippinus*, *G. parapapillatus* from *Siganus virgatus*, and *G. zancli* from *Zanclus cornutus*. *Gyliauchen* sp. from *Siganus spinus* and *Apharyngogyliauchen* sp. from *Scarus ghobban* are described from immature specimens and classified to generic level. *Gyliauchen papillatus* of Durio and Manter (1969) nec Goto and Matsudaira (1918) and nec Goto (1919) is considered a synonym of *G. parapapillatus*. *Gyliauchen nahaensis* Ozaki, 1937, is reported from *Siganus punctatus* and *Zanclus cornutus*, both new locality records and the latter a new host record. A key to all 22 adult species of Gyliauchenidae and host-parasite and parasite-host lists are included as well as some observations on the zoogeography of the Gyliauchenidae.

KEY WORDS: digenetic trematodes, parasites, Gyliauchenidae, marine fishes, Fiji Islands.

Between 13 January and 7 February 1992, the senior author collected helminths of marine fishes at the Institute of Marine Resources, University of the South Pacific, Suva, Fiji Islands. Two previous collections of parasites of marine fishes from the Fiji Islands have been made: the first by Manter in 1951 (see Manter, 1953, 1961, 1963a, b, c; Manter and Prince, 1953), the second between 1979 and 1982 by the *Hatsutori Maru* and other fishing boats on charter to the government of New Zealand (see Lester et al., 1985). No gyliauchenids were reported in either study. The present paper deals with representatives of Gyliauchenidae Ozaki, 1933 (syn. Dissotrematidae Goto and Matsudaira, 1918).

To date, 19 species in 6 genera are known in the family Gyliauchenidae: *Gyliauchen* (8), *Paragyliauchen* (2), *Flagellotrema* (4), *Ichthyotrema* (1), *Leptobulbus* (1), and *Apharyngogyliauchen* (3). The description of 3 new species and 2 immature ones in this paper brings the total to 24.

Materials and Methods

A total of 236 fishes were obtained from several sources including traps, nets, spear fishing, and commercial fishermen. Except for a few fishes that were purchased, all were captured live on reefs and lagoons of Laucala Bay, Suva, a few miles from the Institute of Marine Resources. Fifty species representing 32 genera and 20 families were collected. Six species—*Pomacentrus philippinus* (family Pomacentridae), *Scarus ghobban* (family Scaridae), *Siganus punctatus*, *Siganus spinus*, *Siganus virgatus* (family Siganidae), and *Zanclus cornutus* (family Zanclidae)—harbored gyliauchenids. The fish were kept alive in tanks until shortly before examination. After removal from the host, the digeneans were washed in 0.7% saline, many studied alive before they were fixed in alcohol–formalin–acetic acid under slight coverslip pressure. The worms were then transferred to a dish, left in the fixative overnight, and stored in 70% ethanol. Most of the worms were stained with Semichon's acetocarmine, a few with aqueous Delafield hematoxylin, dehydrated in ascending series of isopropanol, cleared in methylsalicylate, rinsed in xylol, and mounted in Kleermount.

Measurements are expressed in millimeters except for eggs, which are in micrometers (μm). Sucker ratio was calculated from the mean of the length and the width and is expressed with the oral sucker taken as 1. Drawings of specimens obtained in this study were prepared by microprojection and details filled in through microscopic observations. Drawings of other species were made by tracing original figures. The number of specimens recovered from each infected fish and the number of fish examined are indicated next to each host species listed in the description.

Holotypes are deposited in the Parasite Collection of the United States National Museum (USNM), Beltsville, Maryland; vouchers of some species are in the Harold W. Manter Laboratory (HWML), University of Nebraska State Museum, Lincoln, and the British Museum of Natural History, BM(NH), London.

Fishes were identified by Johnson Seeto of the Institute of Marine Resources. References used included an unpublished manuscript on fishes of the Fiji Islands, Nelson (1984), Meyers (1989), and Randall et al. (1990).

Results

Gyliauchen pomacentri sp. n. (Fig. 1)

TYPE HOST: *Pomacentrus philippinus* Evermann and Seale (Pomacentridae) 1/1 of 1.

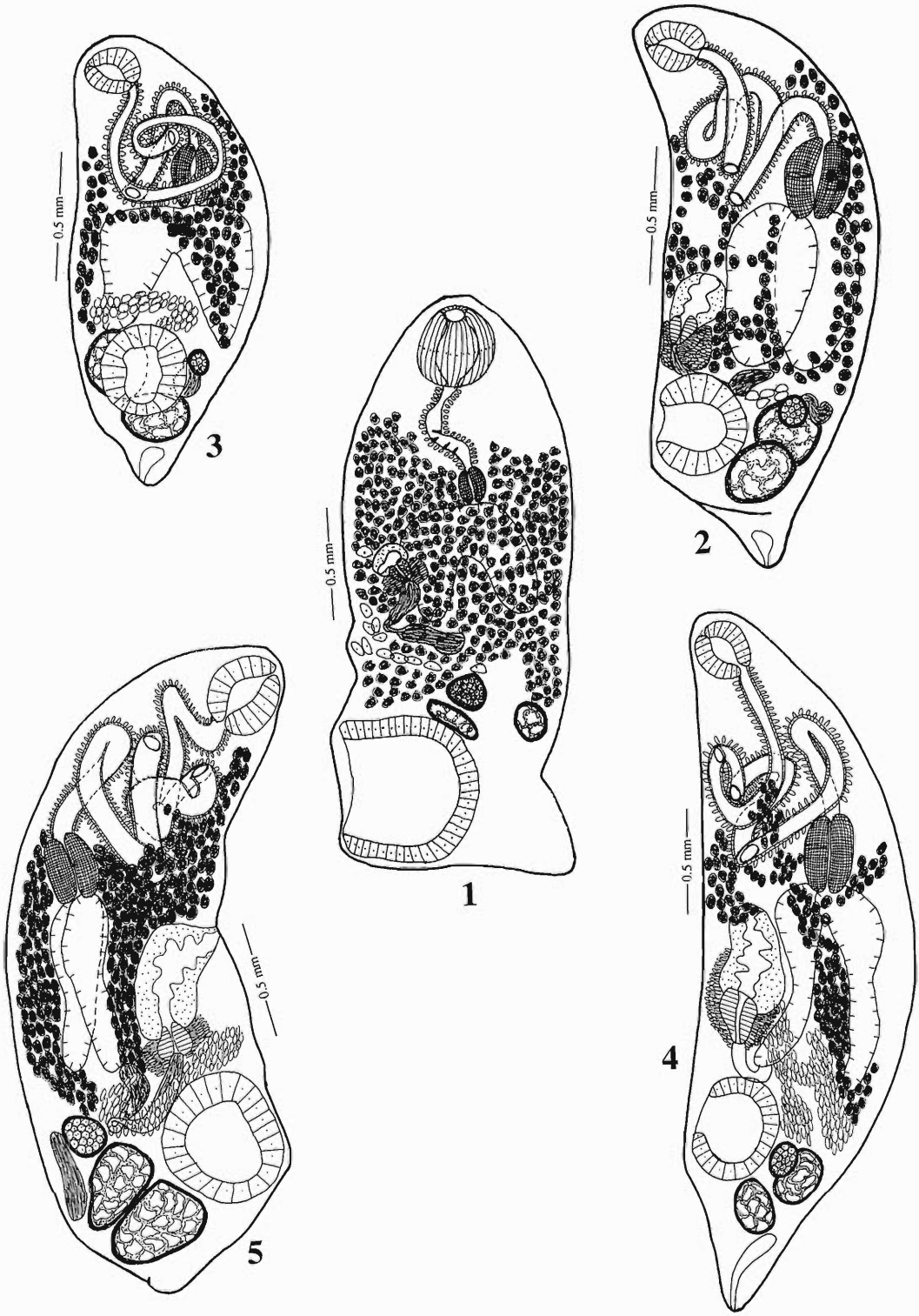
SITE IN HOST: Small intestine.

TYPE LOCALITY: Laucala Bay, Suva.

DATE OF COLLECTION: 3 February 1992.

HOLOTYPE: USNM Helm. Coll. No. 83915.

DESCRIPTION OF HOLOTYPE: Body broad, cylindrical, 2.50 long by 1.13 wide, rounded anteriorly, truncated posteriorly, with large excretory papilla projecting dorsally at level of ace-



Figures 1–5. 1. ***Gyliauchen pomacentri*** **sp. n. holotype from** ***Pomacentrus philippinus,*** **Suva, Fiji Islands. Ventral view. 2.** ***G. parapapillatus*** **sp. n. holotype from** ***Siganus virgatus,*** **Suva, Fiji Islands. Ventrolateral view. 3.** ***G. parapapillatus*** **sp. n. paratype from** ***Siganus virgatus,*** **Suva, Fiji Islands. Ventral view. 4.** ***G. parapapillatus*** **(Durio and Manter, 1969) from** ***Siganus lineatus,*** **Green Island, Queensland, Australia. Ventrolateral view. 5.** ***G. parapapillatus*** **(Durio and Manter, 1969) from** ***Siganus*** **sp., New Caledonia. Lateral view.**

tabulum. Cuticle thick and smooth. Oral sucker slightly subterminal, globular, 0.36 long by 0.37 wide. Ventral sucker cup-shaped, 0.69 long by 0.61 wide, at posterior end of body. Sucker ratio 1:1.78. Prepharynx 0.45 long by 0.10 wide or about one-fifth body length, sigmoid, surrounded by glands along entire length. Pharynx small, muscular, oblong, 0.21 long by 0.15 wide. Esophagus absent. Ceca 2, widely dilated, mostly in middle third of body.

Testes 2, symmetrical, anterior to acetabulum, right testis transversely elongate, 0.10 long by 0.23 wide, left testis subglobular, 0.14 long by 0.17 wide. Seminal vesicle bipartite, L-shaped, parts separated by narrow duct. Cirrus sac relatively small, well developed, muscular, containing ovoid pars prostatica and short cirrus. Prostatic cells well developed, surrounding junction of cirrus sac and anterior portion of seminal vesicle.

Ovary globular, pretesticular, 0.17 long by 0.20 wide. Seminal receptacle overlapping ovary, poorly stained, difficult to measure. Vitellaria follicular, extending from midprepharyngeal level, dorsally and ventrally, to near anterior level of gonads. Uterus short, preovarian, containing few eggs. Eggs 65–85 μm long by 38–45 μm wide.

Genital pore ventrolateral at level of cecal bifurcation. Excretory vesicle not observed, pore opening at tip of large posterodorsal excretory papilla. Lymphatic system present but details not determined.

REMARKS: *Gyliauchen pomacentri* may be distinguished from *G. caudatum* (syn. *Telotrema caudatum* Ozaki, 1933), the only other species in the genus with a relatively short prepharynx, by its body shape, greater sucker ratio, topography of gonads, and absence of a muscular sphincter near the genital opening.

When Ozaki (1933) described the genus *Telotrema* from the acanthurid *Xesurus scalprum*, he indicated that *Telotrema* can be differentiated from *Gyliauchen* by the configuration of the prepharynx, the assembly of the male parts, and the presence of a genital sphincter. Yamaguti (1934), however, stated that "*Telotrema caudatum*, Ozaki, 1933 is apparently congeneric with *Gyliauchen papillatus* (Goto and Matsudaira). It seems very probable that Ozaki misinterpreted the structure of the terminal genitalia, p. 529." Ozaki (1936a, b, 1937a, b) continued to refer to this species as *T. caudatum*. Winter (1960) agreed with Ozaki and reestablished the validity of *Telotrema*. The relatively short prepharynx compared to total body length in *G. pomacentri* may justify reestablishing *Telotrema* as a valid genus. However, a muscular genital sphincter is not evident, and in all other respects *T. caudatum* is typical of other species of *Gyliauchen*.

Gyliauchen parapapillatus sp. n. (Figs. 2–5)

G. papillatus of Durio and Manter (1969) nec *G. papillatus* (Goto and Matsudaira, 1918) Goto, 1919, new synonymy.

TYPE HOST: *Siganus virgatus* (Valenciennes) (Siganidae) 42/1 of 1.

SITE IN HOST: Small intestine.

TYPE LOCALITY: Laucala Bay, Suva.

DATE OF COLLECTION: 31 January 1992.

HOLOTYPE: USNM Helm. Coll. No. 83916.

PARATYPES: HWML 37619, BM(NH) 1994.6.14.3.

DESCRIPTION (based on 42 specimens and measurements on 17 mature ones; holotype measurements in parentheses): Body crescent-shaped in life and orange in color; fixed specimens somewhat convex dorsally, tapering gradually anteriorly, 1.43–2.18 (2.18) long by 0.40–0.83 (0.83) wide, with excretory papilla projecting posterodorsally. Cuticle thick and smooth. Oral sucker globular, slightly subterminal, 0.20–0.25 (0.24) long by 0.14–0.21 (0.21) wide. Ventral sucker globular, 0.28–0.36 (0.36) long by 0.22–0.31 (0.31) wide, near posterior end of body. Sucker ratio 1:1.28–1.59 (1.49). Prepharynx about 1.5 body length, convoluted, forming 3 or 4 coils, surrounded by glands along entire length. Pharynx oblong to cylindrical, muscular, 0.23–0.34 (0.31) long by 0.17–0.32 (0.24) wide. Esophagus absent. Ceca 2, mostly in midbody third, measuring about one-third to one-fourth body length.

Testes 2, globular, 0.14–0.30 (0.24–0.29) in diameter, oblique, dorsal to ventral sucker. Seminal vesicle bipartite, parts separated by narrow constriction. Cirrus sac well developed, containing ovoid prostatic vesicle and well-developed, muscular, eversible cirrus. Prostatic cells well developed, surrounding junction of cirrus sac and anterior portion of seminal vesicle.

Ovary globular, small compared to testes, dorsal to anterior testis or to junction of 2 testes, 0.04–0.19 (0.12) in diameter. Seminal receptacle globular to saccular, large, almost contiguous with ovary, 0.10–0.28 (0.17) long by 0.07–0.18 (0.11) wide. Vitellaria follicular, extending from midprepharyngeal region to near anterior level of

anterior testis. Uterus preovarian. Eggs yellow in life, 63–78 (73–78) μm long by 30–50 (38–40) μm wide in fixed specimens.

Genital pore ventral at level of intestinal bifurcation. Excretory bladder with short duct opening at tip of excretory papilla. Lymphatic system present, seen in sagittal sections as longitudinal canals extending from anterior to posterior end of body.

REMARKS: *Gyliauchen parapapillatus* (Figs. 2, 3) is most similar to *G. papillatus* (Goto and Matsudaira, 1918) Goto, 1919 (Figs. 18, 19), in the anterior extent of the vitellaria, which, in both, extend to at least the midlevel of the prepharynx. The Fijian specimens differ, however, in 2 major characters, a prepharynx that is longer than body length and the relatively larger size of the intestinal ceca compared to the body. We have examined and drawn 2 specimens of *G. papillatus* (Figs. 20, 21) (USNM 37889) deposited by Fischtal and Kuntz (1964) from *Anodontostoma chacunda* from Puerto Princesa, Palawan Island, Philippines. We have also examined and drawn 2 specimens reported as *G. papillatus* by Durio and Manter (1969) from *Siganus lineatus* (HWML "A274d"; Fig. 4) from Green Island, Queensland, Australia, and from *Siganus* sp. (HWML no 618; Fig. 5) from New Caledonia.

Based on the review of pertinent literature and the figures reproduced or drawn, it is evident that 2 groups exist: one group consisting of populations from Japanese and Palawan Island waters, the second of Fijian, New Caledonian, and Australian waters. The Australian (Fig. 4) and New Caledonian (Fig. 5) material share with the Fijian specimens the longer prepharynx and the relatively larger intestinal ceca. Fischtal and Kuntz's specimens (Figs. 20, 21) have a prepharynx shorter than body length and relatively smaller intestinal ceca. We consider *G. papillatus* of Durio and Manter a synonym of *G. parapapillatus* sp. n.

Ozaki (1937b) stated, "The degree of winding is variable according to species, and even in the same species it may vary over quite a wide range; so the topographical figure of the prepharynx if not presenting a major difference had better be neglected in identification, p. 175." Our observations do not support Ozaki's statement. In each of the 42 specimens of *G. parapapillatus,* which probably represent different infections, as evidenced by differences in size and maturity, the prepharynx is about 1.5 times that of body length.

One mature specimen from *Zanclus cornutus* is very similar in body shape to 2 others identified as *G. nahaensis* except for the absence of prepharyngeal glands, shorter prepharynx, and more anterior location of the ovary. The 3 specimens, recovered from the same host and processed at the same time, were not suspected to represent different species until the stained material was studied. The description of this worm as a new species follows.

Gyliauchen zancli sp. n. (Fig. 6)

TYPE HOST: *Zanclus cornutus* (Linnaeus) (Zanclidae) 1/1 of 2.

SITE IN HOST: Small intestine.

TYPE LOCALITY: Laucala Bay, Suva.

DATE OF COLLECTION: 6 February 1992.

HOLOTYPE: USNM Helm. Coll. No. 83917.

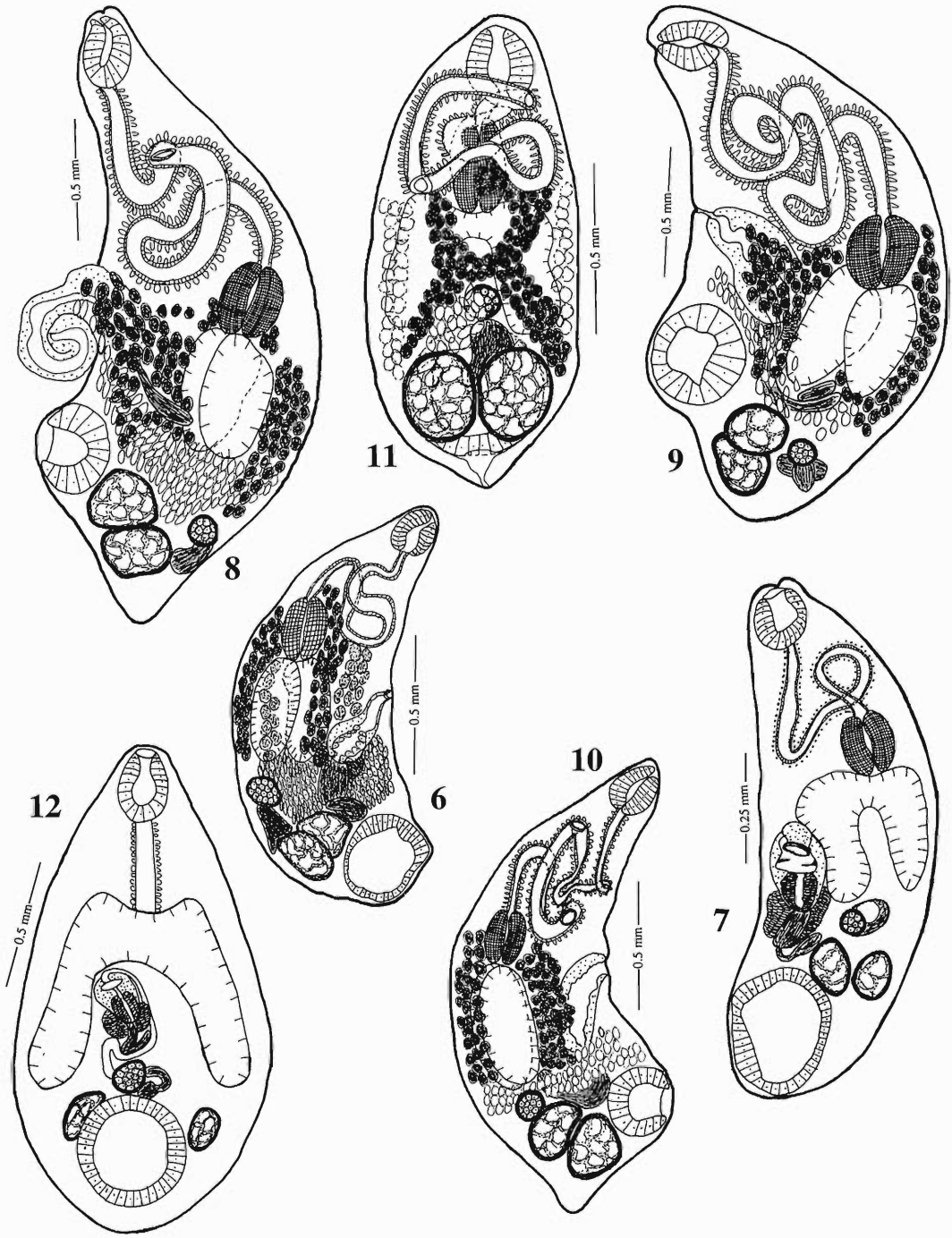
DESCRIPTION OF HOLOTYPE: Body crescent-shaped, 1.70 long by 0.65 deep. Cuticle thick and smooth. Oral sucker ovoid, slightly subterminal, 0.20 long by 0.16 wide. Ventral sucker globular, 0.33 long by 0.30 wide, at posterior end of body. Sucker ratio 1:1.76. Prepharynx thick-walled, convoluted, about three-quarters body length, not surrounded by glands. Pharynx oblong, muscular, 0.26 long by 0.15 wide. Esophagus absent. Ceca 2, about two-sevenths body length, occupying middle third of body.

Testes 2, slightly oblique; anterior testis subglobular, 0.26 long by 0.18 wide, left testis subglobular, 0.21 long by 0.18 wide. Seminal vesicle bipartite, saccular parts separated by constriction. Cirrus sac somewhat pyriform, well developed, containing ovoid pars prostatica and cirrus of equal length. Prostatic cells surrounding junction of cirrus sac and seminal vesicle.

Ovary globular, pretesticular, 0.14 long by 0.11 wide, between testes and intestinal ceca. Seminal receptacle not observed. Vitellaria follicular, extending just anterior to pharynx to posterior ends of ceca. Vitelline reservoir triangular, occupying space between testes and ovary. Uterus coiled, containing many eggs. Eggs yellow, ovoid, 55–85 μm long by 30–53 μm wide.

Genital pore ventral, near midbody level. Excretory papilla not evident. Lymphatic system not observed.

REMARKS: The only other species of *Gyliauchen* lacking prepharyngeal glands is *G. indicum* (Fig. 17). *Gyliauchen zancli* differs from *G. indicum* in its smaller size (1.70 by 0.65 com-



Figures 6–12. 6. ***Gyliauchen zancli*** **sp. n. holotype from** ***Zanclus cornutus,*** **Suva, Fiji Islands. Lateral view. 7.** ***Gyliauchen*** **sp. from** ***Siganus spinus,*** **Suva, Fiji Islands. Ventral view. 8.** ***G. nahaensis*** **Ozaki, 1937, from** ***Siganus punctatus,*** **Suva, Fiji Islands. Lateral view. 9.** ***G. nahaensis*** **Ozaki, 1937, from** ***Siganus punctatus,*** **Suva, Fiji Islands. Lateral view. 10.** ***G. nahaensis*** **Ozaki, 1937, from** ***Zanclus cornutus,*** **Suva, Fiji Islands. Lateral view. 11.** ***G. nahaensis*** **Ozaki, 1937, from** ***Siganus punctatus,*** **Suva, Fiji Islands. Dorsal view. 12.** ***Apharyngogyliauchen*** **sp. holotype from** ***Scarus ghobban,*** **Suva, Fiji Islands. Ventral view.**

pared to 2.11–2.40 by 0.72–0.88), relatively larger pharynx, and smaller testes. The testes in *G. zancli* are smaller than the ventral sucker; those of *G. indicum* are about the same size. The discovery of another species lacking prepharyngeal glands indicates that this feature is not necessarily a family characteristic even though the majority of species have them. There is no evidence in our specimen of any gland cells that have become exhausted and, therefore, would not stain. It should also be noted that in *G. oligoglandulosus,* Gu and Shen (1979) reported few gland cells surrounding the anterior portion of the prepharynx, but they are apparently absent around the more posterior part.

Gyliauchen sp. (Fig. 7)

HOST: *Siganus spinus* (Linnaeus) (Siganidae) 2/2 of 4.

SITE IN HOST: Small intestine.

LOCALITY: Laucala Bay, Suva.

DATE OF COLLECTION: 2 February 1992.

DEPOSITED SPECIMEN: USNM Helm. Coll. No. 83918.

DESCRIPTION (based on 2 specimens, 1 complete and 1 missing ventral sucker): Body convex dorsally, slightly concave ventrally, tapering anteriorly, rounded posteriorly, 1.33 long by 0.40–0.45 in greatest width. Cuticle smooth. Oral sucker globular, subterminal, 0.12–0.14 in diameter. Ventral sucker globular, 0.30 long by 0.25 wide, at posterior end of body. Sucker ratio 1:2.17. Prepharynx with single loop, about three-fourths body length, surrounded by diffuse glands along entire length. Pharynx muscular, ovoid, 0.16–0.19 long by 0.12–0.13 wide. Esophagus absent. Ceca 2, about two-ninths body length, occupying middle third of body.

Testes 2, symmetrical, anterodorsal to acetabulum, right testis globular, 0.13 long by 0.11 wide, left testis globular, 0.12 long by 0.10 wide. Seminal vesicle bipartite, larger anterior segment separated by narrow duct from posterior portion. Cirrus sac containing large, coiled cirrus and ovoid pars prostatica; prostatic cells surrounding junction of cirrus sac and anterior portion of seminal vesicle.

Ovary globular, pretesticular, 0.06 in diameter. Seminal receptacle ovoid, 0.15 long by 0.10 wide, overlapping ovary. Vitellaria not observed. Uterus preovarian. One collapsed egg 73 μm long by 30 μm wide.

Genital pore ventral to cecal bifurcation. Excretory system not observed. Excretory papilla and lymphatic system not evident.

REMARKS: *Gyliauchen* sp. from *Siganus spinus* agrees well with other species of *Gyliauchen* in general body shape and internal anatomy. However, it cannot be further classified because the vitellaria, which are an important specific character, are not evident.

Gyliauchen nahaensis Ozaki, 1937 (Figs. 8–11, 13, 14)

HOSTS: *Siganus punctatus* (Forster) (Siganidae) 189/1 of 2; *Zanclus cornutus* (Linnaeus) (Zanclidae) 2/1 of 2, new host record.

SITE IN HOSTS: Small intestine.

LOCALITY: Laucala Bay, Suva.

DATE OF COLLECTION: 27 January 1992; 6 February 1992.

DEPOSITED SPECIMENS: USNM Helm. Coll. No. 83920, HWML 37618, BM(NH) 1994.6.14.2.

DESCRIPTION (based on all mature and immature specimens from both host species; measurements on 33 mature specimens from *S.*

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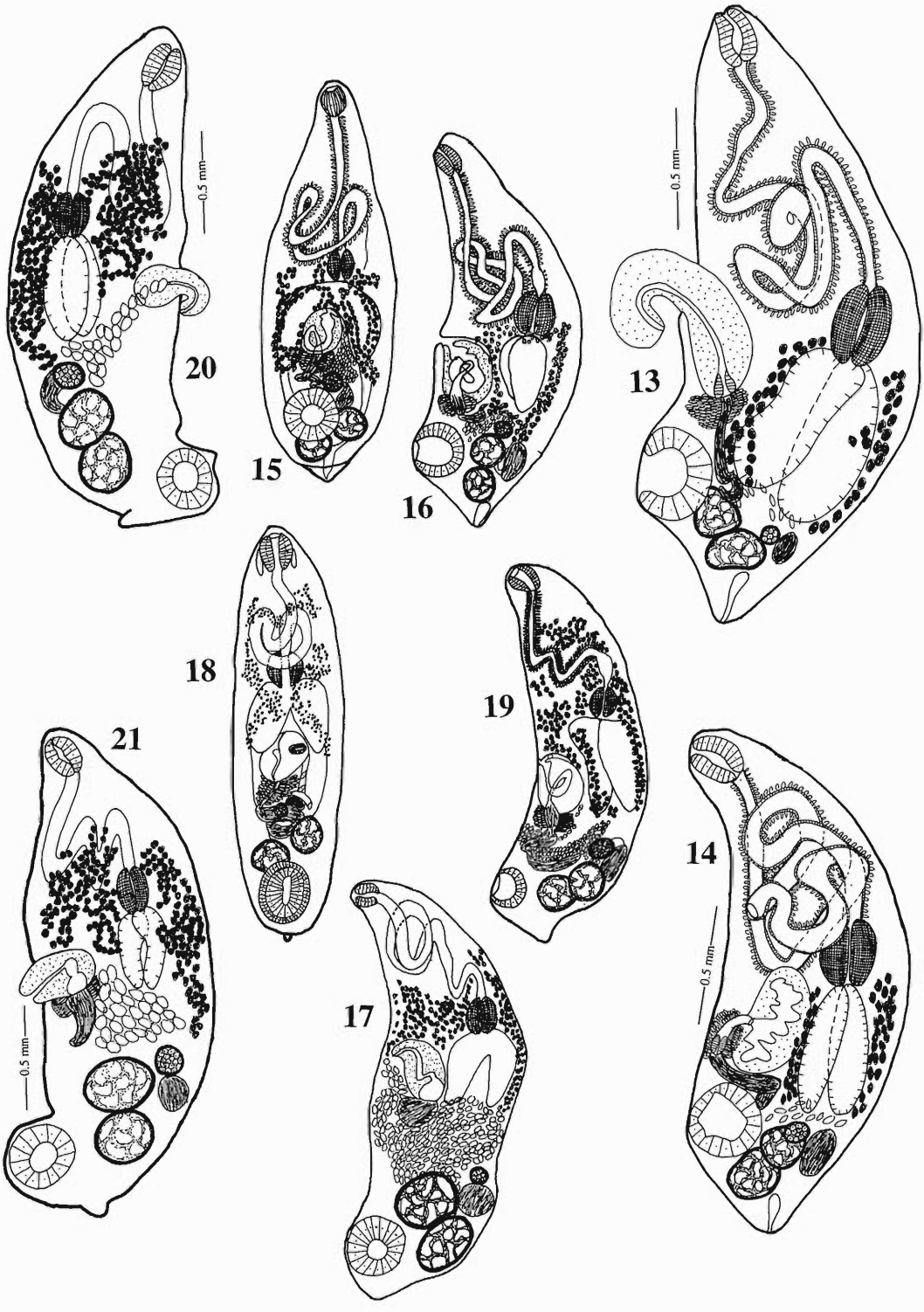
Figures 13–21. 13. *Gyliauchen nahaensis* Ozaki, 1937, from *Siganus chrysospilos* (=*S. punctatus*), locality unknown. Lateral view. 14. *G. nahaensis* Ozaki, 1937, from *Siganus chrysospilos* (=*S. punctatus*), locality unknown. Ventral view. 15. *G. nahaensis* Ozaki, 1937, from *Siganus punctatus,* Naha, Japan (after Ozaki, 1937b). Ventral view. 16. *G. nahaensis* Ozaki, 1937, from *Siganus punctatus,* Naha, Japan (after Ozaki, 1937). Lateral view. 17. *G. indicum* Gupta and Tandon, 1985, from *Engraulis hamiltoni,* Puri, Orissa, India (after Gupta and Tandon, 1985). Ventral view. 18. *G. papillatus* (Goto and Matsudaira, 1918) from *Siganus fuscescens,* Inland Sea and Pacific Coast of Mie and Wakayama prefectures, Japan (after Goto and Matsudaira, 1918). Ventral view; shows no gland cells surrounding prepharynx—as in original. 19. *G. papillatus* (Goto and Matsudaira, 1918) from *Siganus* sp., Pacific Coast and Inland Sea of Japan (after Ozaki, 1937b). Lateral view; shows gland cells surrounding prepharynx—as in original. 20. *G. papillatus* (Goto and Matsudaira, 1918) from *Anodontostoma chacunda,* Puerto Princesa, Palawan Island, Philippines. Lateral view; gland cells surrounding prepharynx not shown. 21. *G. papillatus* (Goto and Matsudaira, 1918) from *Anodontostoma chacunda,* Puerto Princesa, Palawan Island, Philippines. Lateral view; gland cells surrounding prepharynx not shown.



punctatus and 1 from *Z. cornutus*): In life, specimens were orange in color and crescent-shaped; fixed specimens convex dorsally, slightly concave ventrally. Body 1.30–2.45 long by 0.70–1.13 wide, greatest width at or near acetabular level; posterior end broadly pointed forming short, sometimes inconspicuous, excretory papilla. Cuticle thick and smooth. Oral sucker globular, slightly subterminal, 0.20–0.28 long by 0.17–0.24 wide. Ventral sucker globular, 0.27–0.43 long by 0.26–0.45 wide, near posterior end of body. Sucker ratio 1:1.45–1.95. Prepharynx long, convoluted, forming 3–4 coils, measuring about 1.5–2 times body length, surrounded by glands along entire length. Pharynx ovoid to cylindrical, muscular, 0.22–0.39 long by 0.18–0.28 wide. Esophagus absent. Ceca 2, occupying third quarter of body.

Testes 2, usually oblique to slightly tandem, rarely symmetrical, subglobular, dorsal or posterodorsal to ventral sucker, 0.18–0.33 long by 0.13–0.33 wide. Seminal vesicle large, bipartite, parts separated by constriction often concealed by uterus. Cirrus sac well developed, containing ovoid to cylindrical pars prostatica, and large, muscular, eversible cirrus. Numerous prostatic cells surrounding base of cirrus sac at junction with seminal vesicle.

Ovary globular, very small compared to testes, dorsal to anterior testis or junction of testes, 0.08–0.20 long by 0.07–0.15 wide. Seminal receptacle usually spherical, saccate, rarely coiled, larger than and posterodorsal to ovary, 0.10–0.52 long by 0.08–0.22 wide. Vitellaria follicular, extending from about midlevel of pharynx to midlevel of anterior testis, confluent dorsally just posterior to cecal bifurcation. Uterus preovarian. Eggs yellow in life, numerous, 63–85 μm long by 35–58 μm wide in fixed specimens.

Genital pore midventral near level of cecal bifurcation. Excretory pore opening at posterior end of body. Lymphatic system present, seen in sagittal sections as longitudinal canals extending from near posterior end of body to near oral sucker.

REMARKS: This is the fourth report of *Gyliauchen nahaensis* and the first outside of Japanese waters. In describing *G. nahaensis,* Ozaki (1937b) distinguished it from the other species by the conical shape of the body, absence of excretory papilla, and the postpharyngeal vitellaria; from both *G. papillatus* and *G. tarachodes* by the longer and more convoluted prepharynx, the subterminal acetabulum, and the testes lying on the posterodorsal side of the body. The Fijian specimens from *Siganus punctatus* (Figs. 8, 9, 11) and *Zanclus cornutus* (Fig. 10) are remarkably similar to 2 specimens (HWML 31261) (Figs. 13, 14) labeled *G. nahaensis* from *Siganus chrysospilos* (=*S. punctatus*) borrowed from the HWML, University of Nebraska. Unfortunately, these specimens, part of a gift to the Manter Laboratory, were labelled only with parasite and host; the geographic origin is unknown. They do, however, share a common host with the Fijian material and agree with the descriptions and measurements provided by Ozaki (1937b) and Yamaguti (1942, 1953).

The specific characters of this species are Vitellaria not extending into prepharyngeal region of body; testes usually oblique or tandem in lateral view, rarely symmetrical in ventral view; and a convoluted prepharynx, 1.5–2 times body length. An excretory papilla is present but poorly developed.

The specimen represented by Figure 11 is a dorsal view and in agreement with the general morphology and measurements of *G. nahaensis* except for the arrangement of the gonads; this specimen (1 out of 191 collected) was specifically manipulated and excessively flattened during live observation to determine the location of the genital pore and the relationship of the internal organs to each other.

The present finding represents a new locality record and includes a new host record.

Apharyngogyliauchen sp. (Fig. 12)

HOST: *Scarus ghobban* Forsskål (Scaridae) 1/1 of 2.

SITE IN HOST: Small intestine.

TYPE LOCALITY: Laucala Bay, Suva.

DATE OF COLLECTION: 27 January 1992.

DEPOSITED SPECIMEN: USNM Helm. Coll. No. 83919.

DESCRIPTION (based on a single immature specimen): Body pyriform, 2.03 long by 1.05 wide, greatest width just anterior to ventral sucker. Cuticle smooth. Oral sucker slightly subterminal, somewhat pear-shaped, 0.31 long by 0.24 wide. Ventral sucker spherical, 0.46 in diameter, near posterior end of body. Sucker ratio 1:1.67. Esophagus straight, 0.37 long by 0.10 wide or about one-fifth body length, surrounded by glands

Table 1. Host specificity of selected trematode families for species of marine fishes. Families 1-8 are from Curaçao and Jamaica; family 9 is from various parts of the world.

Families of trematodes	Number of species	Number of host species					
		1	2	3	4	5	6+
1. Acanthocolpidae	10	6 (60.0%)	1 (10.0%)	1 (10.0%)		1 (10.0%)	1 (10.0%)
2. Bucephalidae	15	11 (73.3%)	2 (13.3%)	1 (6.7%)			1 (6.7%)
3. Fellodistomatidae	12	8 (66.7%)	3 (25.0%)				1 (8.3%)
4. Hemiuridae	23	10 (43.5%)	2 (8.7%)	5 (21.7%)			6 (26.1%)
5. Haplosplanchnidae	11	5 (45.5%)	1 (9.1%)	1 (9.1%)		3 (27.3%)	1 (9.1%)
6. Lepocreadiidae	36	25 (69.4%)	7 (19.4%)	2 (5.6%)	1 (2.8%)	1 (2.8%)	
7. Monorchiidae	17	10 (58.8%)	3 (17.6%)	2 (11.8%)			2 (11.8%)
8. Opecoelidae	21	12 (57.1%)	5 (23.8%)	2 (9.5%)			2 (9.5%)
9. Gyliauchenidae	24	12 (50.0%)	4 (16.7%)	4 (16.7%)	3 (12.5%)	1 (4.2%)	

along entire length. Pharynx absent. Ceca 2, occupying middle third of body.

Testes 2, symmetrical, 1 on each side of anterior half of acetabulum; right testis elongate, 0.20 long by 0.10 wide; left testis ovoid, 0.13 long by 0.10 wide. Seminal vesicle tubular and curved. Cirrus sac tapering posteriorly, enclosing ovoid pars prostatica and cirrus; prostatic cells surrounding junction of cirrus sac with seminal vesicle.

Ovary ovoid, 0.16 long by 0.13 wide, just anterior to acetabulum. Seminal receptacle to left of ovary: anterior portion tubular, curved; posterior portion ovoid. Vitellaria undeveloped. Uterus extending from ovary laterally along left side of cirrus sac to genital atrium. Eggs not present.

Genital pore posterior to intestinal bifurcation, dextral to median line. Excretory system not observed. Excretory papilla absent. Wide canals, extending laterally along both sides from the posterior end to the anterior region of the body, are evident and probably represent a lymphatic system.

REMARKS: *Apharyngogyliauchen* sp. from *Scarus ghobban* agrees well with other species of *Apharyngogyliauchen* in general body shape, internal anatomy, and the absence of a pharynx. However, it cannot be further classified because it is immature, lacking both eggs and vitellaria.

Discussion

The present survey, part of a collection made during a 3-wk period from 13 January to 7 February 1992 by the senior author, is the third for the Fiji Islands and the second for Suva. In 1951, Manter examined 44 species of fish and recovered 35 species of digenetic trematodes (see Manter, 1953, 1961, 1963a, b, c; Manter and Prince, 1953); the second was reported by Lester et al. (1985) based on collections by the *Hatsutori Maru* and other fishing boats on charter to the government of New Zealand. This collection dealt with parasites of the skipjack, *Katsuwonus pelamis,* captured in various locations in the central and western Pacific including Fijian waters. No gyliauchenids were reported in either study. It should be noted, however, that none of the fish species harboring gyliauchenids in the Nahhas collection were examined by either Manter or Lester.

The present study adds 3 new species to the family Gyliauchenidae and describes, but does not name, 2 additional immature forms, for a total of 24; it also extends the geographic distribution of 1 known species, *G. nahaensis,* to Fijian waters.

Present knowledge indicates that gyliauchenids are widely scattered in the Indo-Pacific region, an area that stretches from the coast of East Africa to the easternmost islands of Oceania, as well as to Hawaii and along the Pacific coast of Mexico. Recently, Cribb et al. (1994) reported recovery of at least 9 species of gyliauchenids from Heron Island, Great Barrier Reef. There are no reports of any gyliauchenids from other parts of the world.

One principle of parasitism suggests that host specificity is related to zoogeography because, by definition, host specificity implies a restricted distribution of a parasite to certain particular host species (Manter, 1957, 1967). Another principle, at least as it applies to digenetic trematodes, is that this group of parasites tends to be more host-specific in their molluscan than in their vertebrate hosts. Consequently, even though a

Table 2. Host specificity of selected trematode families for genera of marine fishes. Families 1-8 are from Curaçao and Jamaica; family 9 is from various parts of the world.

Families of trematodes	Number of species	Number of host genera					
		1	2	3	4	5	6+
1. Acanthocolpidae	10	8 (80.0)	1 (10.0%)			1 (10.0%)	
2. Bucephalidae	15	14 (93.3%)		1 (6.7%)			
3. Fellodistomatidae	12	11 (91.7%)			1 (8.3%)		
4. Hemiuridae	23	14 (60.9%)	2 (8.7%)	1 (4.3%)		2 (8.7%)	4 (17.4%)
5. Haplosplanchnidae	11	7 (63.6%)	2 (18.2%)	1 (9.1%)	1 (9.1%)		
6. Lepocreadiidae	36	34 (94.4%)	1 (2.8%)	1 (2.8%)			
7. Monorchiidae	17	11 (64.7%)	5 (29.4%)			1 (5.9%)	
8. Opecoelidae	21	19 (90.5%)				1 (4.8%)	1 (4.8%)
9. Gyliauchenidae	24	14 (58.3%)	6 (25.0%)	4 (16.7%)			

species of fish may be widely distributed, its parasites are not expected to be similar except in the region where both the definitive and intermediate hosts occur together. It is not the intention of this paper to discuss zoogeography or host specificity in any detail, but a few observations on the family Gyliauchenidae are pertinent.

The 24 species of gyliauchenids, described or reported so far, are known from 42 species of fish representing 13 families (Tables 4, 5). Manter (1957) reviewed and summarized the extent to which digenetic trematodes as a group have been reported from 1 or more species of marine fishes in Tortugas, the Mediterranean, the British Isles, and Japan. Nahhas and Cable (1964) compared their data from Curaçao and Jamaica to that of Manter; more recently, Dyer et al. (1985, 1988, 1992), Barker et al. (1994), and Cribb et al. (1994) have made similar studies. All the preceding data suggest a certain degree of host specificity for digenetic trematodes of marine fishes but do not consider the differences among trematode families. Because the present paper deals only with the family Gyliauchenidae, it would be relevant to make such a comparison using 9 digenean families, each represented by 10 or more species from Curaçao and Jamaica. The data extracted from Nahhas and Cable (1964) along with the data on the family Gyliauchenidae are shown in Tables 1–3.

At the host species level (Table 1), 50% of the species of gyliauchenids show specificity to a single host species, 16.7% to 2, 16.7% to 3, 12.5% to 4, and 4.2% to 5. The data from Curaçao and Jamaica suggest that the greatest specificity to 1 host is seen in the bucephalids (73.3%), followed by lepocreadiids (69.4%), fellodistomatids (66.7%), and progressively less for the other trematodes, with least host specificity for the haplosplanchnids (45.5%) and the hemiurids (43.5%). Compared to these families, the gyliauchenids are among the least host-specific except for the haplosplanchnids and hemiurids.

When the data are considered at the level of the host genus (Table 2), the same families that show highest and lowest specificity at the host

Table 3. Host specificity of selected trematode families for families of marine fishes. Families 1-8 are from Curaçao and Jamaica; family 9 is from various parts of the world.

Families of trematodes	Number of species	Number of host families					
		1	2	3	4	5	6+
1. Acanthocolpidae	10	9 (90.0%)			1 (10.0%)		
2. Bucephalidae	15	15 (100%)					
3. Fellodistomatidae	12	11 (91.7%)	1 (8.3%)				
4. Hemiuridae	23	16 (69.6%)	1 (4.3%)	3 (13.0%)	1 (4.3%)		2 (8.6%)
5. Haplosplanchnidae	11	10 (90.9%)			1 (9.1%)		
6. Lepocreadiidae	36	34 (94.4%)	2 (5.6%)				
7. Monorchiidae	17	14 (82.4%)	2 (11.8%)	1 (5.9%)			
8. Opecoelidae	21	19 (90.5%)	1 (4.8%)				1 (4.8%)
9. Gyliauchenidae	24	16 (66.7%)	6 (25.0%)	2 (8.3%)			

Table 4. Host-parasite list.

Family Acanthuridae
Acanthurus sandvicensis Streets
1. *Flagellotrema potteri*
Acanthurus sp.
1. *Gyliauchen ozakii*
Xesurus punctatus Gill
1. *Ichthyotrema vogelsangi*
Xesurus scalprum (Cuvier and Valenciennes)
1. *Gyliauchen caudatus*
2. *Flagellotrema convolutum*
Family Blenniidae
Plagiotremus tapeinosoma (Bleeker)
1. *Paragyliauchen chaetodontis*
Family Chaetodontidae
Chaetodon corallicola Snyder
1. *Flagellotrema chaetodontis*
Chaetodon fremblii Bennet
1. *Flagellotrema chaetodontis*
Chaetodon miliaris Quoy and Gaimard
1. *Flagellotrema chaetodontis*
Chaetodon multicintus Garrett
1. *Flagellotrema chaetodontis*
Chaetodon sp.
1. *Paragyliauchen chaetodontis*
Family Dorosomidae
Anodontostoma (*Dorosoma*) *chacunda* (Fowler and Bean)
1. *Gyliauchen papillatus*
Family Engraulidae
Engraulis hamiltoni (Cuvier and Valenciennes)
1. *Gyliauchen indicum*
Family Harpodontidae
Harpodon nehereus Ham
1. *Gyliauchen ozakii*
Family Labridae
Anampses caeruleopunctatus Rüppell
1. *Apharyngogyliauchen callyodontis*
Cirrhilabrus sp.
1. *Apharyngogyliauchen opisthovarius*
Family Pomacanthidae
Arusetta sextriatus (Kuhl and VanHassett)
1. *Paragyliauchen arusettae*
Centropyge ferrugatus Randall and Burgess
1. *Flagellotrema convolutum*
Centropyge heraldi Woods and Schultz
1. *Paragyliauchen arusettae*
Centropyge potteri (Jordan and Metz)
1. *Flagellotrema potteri*
2. *Flagellotrema centropygis*
Holacanthus septentrionalis Temminck and Schlegel
1. *Paragyliauchen chaetodontis*
Family Pomacentridae
Pomacentrus philippinus Evermann and Seale
1. *Gyliauchen pomacentri* sp. n.
Family Scaridae
Calotomus sandvicensis (Valenciennes)
1. *L. magnacirratus*
Pseudoscarus harid Forsskål
1. *Apharyngogyliauchen callyodontis*
2. *Gyliauchen volubilis*
Scarus dubius Bennet
1. *Leptobulbus magnacirratus*

Table 4. Continued.

Scarus ghobban Forsskål
1. *Apharyngogyliauchen* sp.
Scarus sordidus Forsskl
1. *Leptobulbus magnacirratus*
2. *Apharyngogyliauchen scarustis*
Scarus (=*Callyodon*) sp.
1. *Apharyngogyliauchen callyodontis*
2. *Leptobulbus magnacirratus*
Family Siganidae
Amphacanthus sigan Rüppell
1. *Gyliauchen volubilis*
Siganus fuscescens (Houttuyn)
1. *Gyliauchen papillatus*
Siganus guttatus (Bloch)
1. *Gyliauchen oligoglandulosus*
Siganus lineatus (Valenciennes)
1. *Gyliauchen parapapillatus* sp. n.
Siganus (=*Teuthis*) *oramin* (Schneider)
1. *Gyliauchen ozakii*
Siganus punctatus (Forster)
1. *Gyliauchen nahaensis*
Siganus spinus (Linnaeus)
1. *Gyliauchen* sp.
Siganus (=*Lo*) *unimaculatus* (Evermann and Seale)
1. *Gyliauchen nahaensis*
Siganus vermiculatus (Valenciennes)
1. *Gyliauchen ozakii*
Siganus virgatus (Valenciennes)
1. *Gyliauchen parapapillatus* sp. n.
Siganus (=*Teuthis*) sp.
1. *Gyliauchen nahaensis*
Siganus sp.
1. *Gyliauchen papillatus*
Siganus sp.
1. *Gyliauchen parapapillatus* sp. n.
Family Tachysuridae
Tachysurus sp.
1. *Gyliauchen tarachodes*
Family Zanclidae
Zanclus cornutus (Linnaeus)
1. *Gyliauchen nahaensis*
2. *Gyliauchen zancli* sp. n.

species level show a similar trend at the host genus level; the lowest specificity is seen in the families Haplosplanchnidae and Hemiuridae. The gyliauchenids, with a specificity of 58.9%, are the least host-specific among the 9 families.

When the data are considered at the level of host family (Table 3), host specificity is greater than 90.0% for all the families except Monorchiidae (82.4%), Hemiuridae (69.4%), and Gyliauchenidae (66.7%). Thus, gyliauchenids are among the least host-specific at all 3 levels.

Based on a review of the literature, a key to all adult species and host-parasite and parasite-host lists are provided.

Table 5. Parasite-host list.

Subfamily Apharyngogyliaucheninae Yamaguti, 1942
Genus *Apharyngogyliauchen* Yamaguti, 1942
A. callyodontis Yamaguti, 1942
1. *Anampses caeruleopunctatus*
2. *Pseudoscaris harid*
3. *Scarus* (=*Callyodon*) sp.
A. opisthovarius Gu and Shen, 1983
1. *Cirrhilabrus* sp.
A. scarustis Gu and Shen, 1983
1. *Scarus sordidus*
Apharyngogyliauchen sp.
1. *Scarus ghobban*
Subfamily Gyliaucheninae Fukui, 1929
Genus *Flagellotrema* Oazki, 1936
F. centropygis Yamaguti, 1970
1. *Centropyge potteri*
F. chaetodontis (Manter and Pritchard, 1962) Yamaguti, 1970
1. *Chaetodon corallicola*
2. *Chaetodon fremblii*
3. *Chaetodon miliaris*
4. *Chaetodon multicintus*
F. convolutum Ozaki, 1936
1. *Xesurus scalprum*
2. *Centropyge ferrugatus*
F. potteri Yamaguti, 1970
1. *Centropyge potteri*
2. *Acanthurus sandvicensis*
Genus *Gyliauchen* Nicoll, 1915
G. caudatus (Ozaki, 1933)
1. *Xesurus scalprum*
G. indicum Gupta and Tandon, 1985
1. *Engraulis hamiltoni*
G. nahaensis Ozaki, 1937
1. *Siganus punctatus*
2. *Siganus* (=*Lo*) *unimaculatus*
3. *Siganus* (=*Teuthis*) sp.
4. *Zanclus cornutus*
G. oligoglandulosus Gu and Shen, 1979
1. *Siganus guttatus*
G. ozakii Srivastava, 1938
1. *Acanthurus* sp.
2. *Harpodon nehereus*
3. *Siganus* (=*Teuthis*) *oramin*
4. *Siganus vermiculatus*
G. papillatus (Goto and Matsudaira, 1918) Goto, 1919
1. *Anodontostoma chacunda*
2. *Siganus fuscescens*
3. *Siganus* sp.
G. parapapillatus sp. n.
1. *Siganus lineatus*
2. *Siganus virgatus*
3. *Siganus* sp.
Gyliauchen pomacentri sp. n.
1. *Pomacentrus philippines*
G. tarachodes Nicoll, 1915
1. *Tachysurus* sp.
G. volubilus Nagaty, 1956
1. *Amphacanthus sigan*
2. *Pseudoscarus harid*

Table 5. Continued.

Gyliauchen zancli sp. n.
1. *Zanclus cornutus*
Gyliauchen sp.
1. *Siganus spinus*
Genus *Ichthyotrema* Caballero and Bravo-Hollis, 1953
I. vogelsangi Caballero and Bravo-Hollis, 1953
1. *Xesurus punctatus*
Genus *Leptobulbus* Manter and Pritchard, 1962
L. magnacirratus Manter and Pritchard, 1962
1. *Calotomus sandvicensis*
2. *Scaridea zonarcha*
3. *Scarus dubius*
4. *Scarus sordidus*
5. *Scarus* (=*Callyodon*) sp.
Genus *Paragyliauchen* Yamaguti, 1934
P. arusettae Machida, 1984
1. *Arusetta sextriatus*
2. *Centropyge heraldi*
P. chaetodontis Yamaguti, 1934
1. *Chaetodon* sp.
2. *Holacanthus septentrionalis*
3. *Plagiotremus tapeinosoma*

Key to Species of the Family Gyliauchenidae

1a. Pharynx absent 2
1b. Pharynx present 4
2a. Testes larger than ventral sucker; ovary anterior to ventral sucker ..... *Apharyngogyliauchen callyodontis*
2b. Testes about same size or smaller than ventral sucker; ovary dorsal to ventral sucker 3
3a. Testes about same size as ventral sucker; ovary intertesticular ..... *Apharyngogyliauchen opisthovarius*
3b. Testes much smaller than ventral sucker; ovary pretesticular ..... *Apharyngogyliauchen scarustis*
4a. Pharynx poorly developed ..... *Leptobulbus magnacirratus*
4b. Pharynx well developed 5
5a. Testes symmetrical and posterior to ventral sucker 6
5b. Testes symmetrical, oblique, or tandem and anterodorsal to posterodorsal to ventral sucker 7
6a. Vitellaria follicular; genital pore anterior to cecal bifurcation ..... *Paragyliauchen chaetodontis*
6b. Vitellaria ramiform; genital pore posterior to cecal bifurcation .. *Paragyliauchen arusettae*
7a. Prepharynx straight; ovary greatly posttesticular ..... *Ichthyotrema vogelsangi*
7b. Prepharynx sigmoid, coiled, or convoluted; ovary pre-, inter-, or slightly posttesticular 8
8a. Ovary intertesticular or slightly posttesticular; testes anterior to ventral sucker 9

8b. Ovary pretesticular or dorsal to testes; testes anterior, at same level, or posterior to ventral sucker 12
9a. Pharynx at least as large as ventral sucker .. .. *Flagellotrema centropygis*
9b. Pharynx smaller than ventral sucker 10
10a. Genital pore at level of posterior end of ceca ..... *Flagellotrema convolutum*
10b. Genital pore at about level of cecal bifurcation 11
11a. Testes smaller than pharynx .... *Flagellotrema chaetodontis*
11b. Testes about same size or larger than pharynx ..... *Flagellotrema potteri*
12a. Prepharynx relatively short and slightly sinuous 13
12b. Prepharynx long and coiled 14
13a. Testes dorsal to ventral sucker with 1 testis located in the basal part of the excretory papilla; oral sucker slightly larger than pharynx; genital sphincter present .... *Gyliauchen caudatum*
13b. Testes anterodorsal to ventral sucker; oral sucker at least twice the diameter of the pharynx; genital sphincter absent .... *Gyliauchen pomacentri* sp. n.
14a. Prepharynx surrounded by glands 15
14b. Prepharynx not surrounded by glands 16
15a. Vitellaria usually not extending anteriorly beyond anterior level of the pharynx .. 17
15b. Vitellaria extending anteriorly to at least mid-prepharyngeal level 18
16a. Testes smaller than ventral sucker .... *Gyliauchen zancli* sp. n.
16b. Testes about same size or larger than ventral sucker ..... *Gyliauchen indicum*
17a. Testes dorsal or posterodorsal to ventral sucker ..... *Gyliauchen nahaensis*
17b. Testes anterior or anterodorsal to ventral sucker 19
18a. Vitellaria extensive, evenly distributed in prepharyngeal region, extending anteriorly to near oral sucker .... *Gyliauchen volubilis*
18b. Vitellaria less extensive than above, not evenly distributed in prepharyngeal region, not reaching anteriorly to oral sucker 20
19a. Seminal receptacle about same size or smaller than testes; seminal vesicle saccular and trilobed .. *Gyliauchen tarachodes*
19b. Seminal receptacle usually larger than testes; seminal vesicle tubular and convoluted ..... *Gyliauchen oligoglandulosus*
20a. Chitinous process in genital sinus present . ..... *Gyliauchen ozakii*
20b. Chitinous process in genital sinus absent . 21
21a. Prepharynx shorter than body length; ceca shorter than one-third body length .... *Gyliauchen papillatus*
21b. Prepharynx longer than body length; ceca about one-third body length .... *Gyliauchen parapapillatus* sp. n.

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Conodiplostomum asymmetricum sp. n. (Neodiplostomidae: Crassiphialinae), from *Niviventer cremoriventer* (Muridae) from Yunnan Province of the Peoples Republic of China

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ABSTRACT: One of 6 pencil-tailed rats, *Niviventer cremoriventer,* collected from Yunnan Province, Peoples Republic of China, in August 1987, was infected with 5 specimens of an undescribed species of *Conodiplostomum* (Neodiplostomidae). *Conodiplostomum asymmetricum* sp. n. differs from existing species of *Conodiplostomum* in having a larger body size (3,050–3,300 μm), smaller eggs (65–80 μm), the forebody shorter than the hindbody, the acetabulum located in the upper ⅓ of the forebody, and using a mammalian host.

KEY WORDS: *Conodiplostomum asymmetricum,* China, Crassiphialinae, Neodiplostomidae, *Niviventer cremoriventer,* Muridae.

Dubois (1937) divided the genus *Neodiplostomum* Railliet, 1919, into 2 subgenera, *Neodiplostomum* and *Conodiplostomum. Conodiplostomum* was elevated to generic status by Sudarikov (1962). *Neodiplostomum* is characterized by the absence of a genital cone and an asymmetrical arrangement of the testes, where the testes are of unequal size, whereas species of *Conodiplostomum* have a genital cone and testes of about the same size. Members of the genus *Fibricola* Dubois, 1932, bear a strong resemblance to species of both *Neodiplostomum* and *Conodiplostomum.* Dubois (1932) separated *Fibricola* from *Neodiplostomum* and *Conodiplostomum* based on the absence of vitelline follicles in the hindbody of species of *Fibricola* and on their specificity for mammals. Several authors have challenged the validity of this separation based primarily on the variability of the distribution of the vitellaria seen in species of *Fibricola* and on observations that suggest species of *Fibricola* are not exclusively mammal parasites (Chandler, 1942; Chandler and Rausch, 1946; Pearson, 1959; Shoop, 1989). Yamaguti (1971) recognized 12 species of the original subgenus *Conodiplostomum* from birds: *C. accipitris* Dubois and Rausch, 1948; *C. acutum* Dubois, 1937; *C. australiense* Dubois, 1937; *C. banghami* Penrod, 1947; *C. brachypteris* Chatterji, 1942; *C. brachyurum* (Nicoll, 1914) Dubois, 1937; *C. butasturinum* (Tubangui, 1932) Dubois, 1936; *C. krausei* Dubois, 1937; *C. palumbarii* Dubois, 1937; *C. perlatum* Ciurea, 1911; *C. sarcorhamphi* Dubois, 1937; and *C. spathula* (Creplin, 1829) La Rue, 1926. Betterton (1976) described *Neodiplostomum Conodiplostomum ramachandrani* from *Rattus muelleri* in Malaysia; however, Palmieri et al. (1979) examined additional specimens of this species and transferred it to the genus *Fibricola* Dubois, 1932. Dubois (1985) described *N. C. pitangi* from *Pitangus sulphuratus* in Paraguay. Shoop (1989) used systematic analysis of morphological characteristics and types of metacercariae to redefine the family Diplostomidae Poirier, 1886, and establish 2 new families, Neodiplostomidae and Bolbophoridae. Under this redefinition, members of the genus *Neodiplostomum,* which were previously assigned to the subgenus *Neodiplostomum,* were placed in the subfamily Neodiplostominae (Neodiplostomidae) and those previously assigned to the subgenus *Conodiplostomum* were placed in Crassiphialinae (Neodiplostomidae) under the genus *Conodiplostomum.* The vitelline follicles in Neodiplostominae are restricted to the forebody, a genital cone is lacking, there is a neodiplostomulum-type metacercariae in amphibians, and adults are found in both birds and mammals. The vitelline follicles in Crassiphialinae are distributed in the entire body, or exclusively in the hindbody, a genital cone is present, there is a neascus-type metacercariae in fish, and adults have been reported exclusively from birds.

During a survey of the helminths of mammals from Yunnan Province of the Peoples Republic of China, we found an undescribed species of

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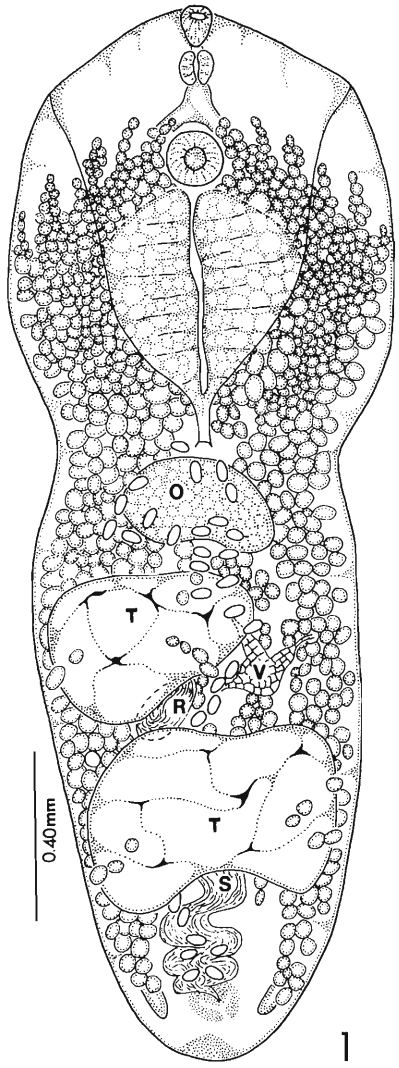

Figure 1. Camera lucida drawing of ventral view of adult *Conodiplostomum asymmetricum* sp. n. (Neodiplostomidae) from *Niviventer cremoriventer* showing the ovary (O), seminal receptacle (R), seminal vesicle (S), testes (T), and vitelline reservoir (V).

Conodiplostomum Sudarikov, 1962, in the pencil-tailed rat, *Niviventer cremoriventer* Miller, 1900.

Materials and Methods

Six specimens of the pencil-tailed rat, *N. cremoriventer,* were collected in August 1987, from Menglun, Yunnan Province, Peoples Republic of China, and examined for helminths. Trematodes were observed alive, fixed in hot alcohol–formalin–acetic acid under slight coverslip pressure, stained in Semichon's carmine, and mounted in Canada balsam. Measurements are in micrometers with the mean followed by the range in parentheses.

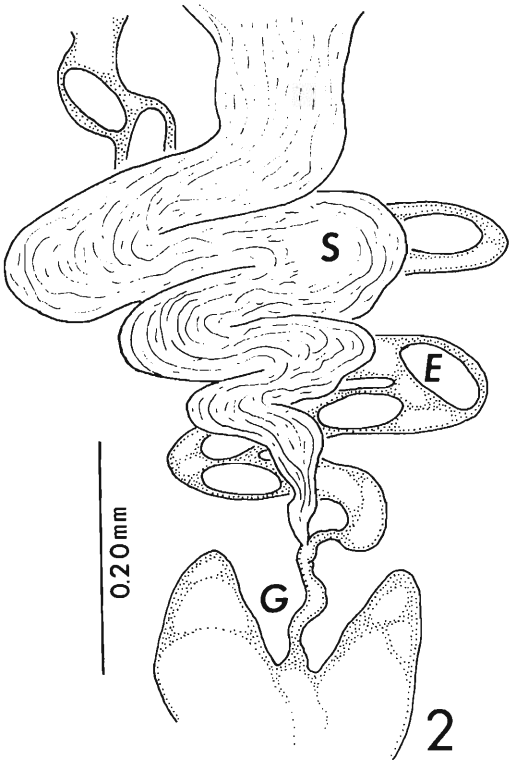

Figure 2. Enlarged view of genital cone region of *Conodiplostomum asymmetricum* sp. n. from *Niviventer cremoriventer* showing eggs in the uterus (E), the genital cone (C), and the seminal receptacle (S).

Results

One of 6 specimens of *N. cremoriventer* (Muridae) was infected with 5 specimens of *Conodiplostomum asymmetricum* sp. n.

Conodiplostomum asymmetricum sp. n. (Figs. 1, 2)

DESCRIPTION (based on 5 adult specimens): With characteristics of genus. Body 3,140 (3,050–3,300) long; distinctly divided into a short, finely spined forebody, 1,300 (1,275–1,400) long by 1,175 (1,150–1,200) wide, and a longer, more cylindrical hindbody, 1,840 (1,775–1,900) long by 975 (945–1,010) wide. Oral sucker subterminal, 118 (110–125) long by 100 (95–112) wide. Acetabulum, located ⅓ the distance down forebody, 145 (128–160) long by 165 (158–168) wide. Ratio of transverse diameter of oral sucker to acetabulum, 1:1.6. Tribocytic organ circular to elliptical, large, 750 (700–810) long by 610 (540–

680) wide, approximately ½ as long as forebody. Prepharynx 8 (2–18) long; pharynx 90 (85–96) long by 83 (80–85) wide; esophagus 20 (15–35) long, bifurcating midway between pharynx and acetabulum; ceca terminating near posterior extremity of hindbody. Testes tandem, in middle third of hindbody. Anterior testis asymmetrical, 560 (530–590) long by 635 (630–640) wide, smaller than posterior testis, 595 (550–640) long by 855 (750–960) wide. Seminal vesicle tubular, highly folded, extending anteriorly from near posterior extremity of hindbody to level of ovary. Copulatory bursa present, not evaginable; genital pore at tip of well-developed genital cone, opening dorsally near posterior end of body. Ovary median, immediately pretesticular, 250 (220–272) long by 470 (420–540) wide. Ootype located at level of anterior testis, near midline of body. Laurer's canal not observed. Vitelline follicles large, densely distributed in forebody and hindbody, extending from cecal bifurcation to near posterior extremity of hindbody. Uterus largely intercecal, confined to hindbody, occupying space between division of forebody and hindbody and genital cone. Eggs small, 74 (65–80) long by 48 (40–60) wide. Excretory pore slightly subterminal on ventral surface.

Taxonomic summary

SPECIMENS DEPOSITED: Holotype: USNM Helm. Coll. No. 84406. Paratypes: USNM Helm. Coll. No. 84407 (1 specimen), Texas Cooperative Wildlife Coll. No. CHI-87-1-3 (2 specimens), Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, Texas.

TYPE HOST: *Niviventer cremoriventer.*

SITE OF INFECTION: Small intestine.

TYPE LOCALITY: Yunnan Province, Peoples Republic of China, 21°55′N, 101°17′E.

ETYMOLOGY: The specific epithet refers to the asymmetrical shape of the anterior testis.

Discussion

Hong and Shoop (1994) emended Neodiplostominae to include species like *Neodiplostomum seoulensis,* which have nearly symmetrical testes, vitellaria distributed in both fore- and hindbodies, pseudosuckers absent, and a reduced genital cone. *Conodiplostomum asymmetricum* sp. n. was collected from a mammal; has an asymmetrical anterior testis, vitelline follicles that are heavily distributed in the fore- and hindbodies and a well-developed genital cone; and cannot be placed in *Fibricola* or *Neodiplostomum.* Based on these characteristics, we have placed the new species in *Conodiplostomum.* To facilitate placement of *C. asymmetricum* sp. n., the genus *Conodiplostomum* and the subfamily Crassiphialinae, as defined by Shoop (1989), should be emended to include mammalian hosts. *Conodiplostomum perlatum* Ciurea, 1929, is the only other species in the genus in which the anterior testis is smaller than the posterior testis; however, *C. asymmetricum* sp. n. can be distinguished from all species in the genus because it is larger (3,050–3,300), it has a smaller egg size (65–80), the acetabulum is in the upper ⅓ of the forebody, the forebody is shorter than the hindbody (approximately ¾ as long), and it is a parasite of mammals.

Acknowledgments

We thank the National Academy of Sciences and the government of the Peoples Republic of China, without whose cooperation and assistance this study would not have been possible. We especially thank Professor Wu Delin and Director Feng Yaozhong of the Kunming Institute of Ecology for their scientific advice and many courtesies and Dr. Wesley Shoop from the Merck Institute for Therapeutic Research, Rathway, New Jersey, for his suggestions in the preparation of this manuscript. We also thank Dr. Ralph Lichtenfels, National Parasite Collection, Beltsville, Maryland, and Dr. Rodney Bray, The Natural History Museum, London, for allowing us access to type materials.

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Report on the Brayton H. Ransom Memorial Trust Fund

The Brayton H. Ransom Memorial Trust Fund was established in 1936 to "Encourage and promote the study and advance of the Science of Parasitology and related sciences." Income from the Trust currently provides token support of the *Journal of the Helminthological Society of Washington* and limited support for publication of meritorious manuscripts by authors lacking institutional or other backing. Donations or memorial contributions may be directed to the Secretary-Treasurer. Information about the Trust may be found in the following articles: *Proceedings of the Helminthological Society of Washington* (1936) 3:48–87; (1983) 50:200–204; and (1993) 60:144–150.

Financial Report for 1994

Balance on hand, January 1, 1994		$14,773.12
Receipts:		
Interest received in 1994	$706.99	
Donations	$386.00	
Total		$ 1,092.99
Disbursements:		
Grant to the Helminthological Society of Washington for 1994	($ 50.00)	
Membership in the American Association for Zoological Nomenclature	($ 50.00)	
Total		($ 100.00)
On hand, December 31, 1994		$15,766.11

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Detection of Avian Malaria Infections in Wild and Captive Penguins

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ABSTRACT: Sera from wild African black-footed penguins (*Spheniscus demersus* L., 1758), Adelie penguins (*Pygoscelis adeliae* Houbron, 1841), Gentoo penguins (*Pygoscelis papua* Forster, 1781), king penguins (*Aptenodytes patagonicus* Miller, 1778), and little blue penguins (*Eudyptula minor* Forster, 1781) and from captive yellow-eyed penguins (*Megadyptes antipodes* Houbron, 1841) and Magellanic penguins (*Spheniscus magellanicus* Forster, 1781) were tested by enzyme-linked immunosorbent assays for the presence of avian malaria antibodies (Ab). *Plasmodium falciparum* sporozoite ($R32tet_{32}$) and gametocyte (P.F.R27) antigens were used. Specificity of anti–*S. demersus,* anti-duck, anti-chicken, and anti-turkey IgG labeled with alkaline phosphatase was determined for homologous and heterologous sera of 8 avian species (including 6 penguin species). The penguin conjugate was the most specific for the various penguin species immunoglobulins. It was possible to detect penguin immunoglobulins at a dilution of $10^{-4.11}$. The relative binding of anti–*S. demersus* IgG was equal to relative binding of commercial conjugates. Kinetic profiles and overall magnitudes of malarial Ab detected by the 2 antigens were not significantly different. Antarctic *P. adeliae* were negative for malarial Ab, all New Zealand *M. antipodes* were positive, and the positivity prevalence of the remaining penguins ranged from 33 to 92%. Antibody titers and the prevalence of infection of wild *S. demersus* were significantly lower than those reported for captive North American *S. demersus.*

KEY WORDS: avian malaria, penguins, *Plasmodium relictum, Plasmodium elongatum,* ELISA, New Zealand, Antarctic.

The African black-footed penguin, *Spheniscus demersus,* is an endangered species. Populations have been drastically decreasing along the southern coast of the Republic of South Africa (RSA) (Crawford et al., 1990) due to oil contamination, injuries, and diseases (Brossy, 1992). The first avian malaria case (*Plasmodium relictum*) in a penguin was discovered in *S. demersus* in 1927 (Fantham and Porter, 1944) from Saldanha Bay (32°26′S, 17°455′E) in the RSA. Later, the parasite was found in captive *S. demersus* in Europe, and the disease was associated with infected *Culex pipiens* mosquitoes (Rodhain, 1937). Over 60 yr later, Brossy (1992) reported a 0.7% prevalence of *P. relictum* infection in *S. demersus* from Saldanha Bay (RSA) and a markedly higher prevalence (22%) (with fatal outcome) in injured or oiled penguins along the southern coast of the RSA.

The malaria-related mortality of *S. demersus* in a North American Zoo (Baltimore, Maryland, U.S.A.) fluctuated between 75% (Stoskopf and Beier, 1979) and 50% (Cranfield et al., 1990). Recently, Cranfield et al. (1994) demonstrated that avian malaria infections, once acquired, last for the duration of the penguin's lifetime. In the wild, or during transport from the natural habitat to captivity (i.e., from the Southern to Northern Hemisphere), such birds may die. This raised the question of whether mortality was caused by a newly acquired infection or recrudescence of a preexisting one. A high, posttransport, malaria-related mortality of nonparasitemic wild-caught penguins was reported by Fix et al. (1988). This problem has remained controversial because the results were based on the examination of blood smears. This technique determines only the prevalence of parasitemia, not the actual prevalence of infection.

The enzyme-linked immunosorbent assay (ELISA) developed for the diagnosis of avian

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malaria in captive *S. demersus* facilitates the evaluation of exposure of individual birds to parasites (Graczyk et al., 1994c). The assay utilizes anti–*S. demersus* IgG labeled with alkaline phosphatase. The purpose of the present study was to determine the applicability of this conjugate for the detection of immunoglobulins directed against avian malarial parasites in various species of penguins.

Materials and Methods

Blood samples were collected from 44 wild African black-footed penguins (*S. demersus*) at Boulders, Simon's Town Colony (33°26′S, 17°45′E), RSA; from 12 Gentoo penguins (*Pygoscelis papua*) and 12 king penguins (*Aptenodytes patagonicus*) from Kerguelen (49°15′S, 70°10′E) and Crozet islands (46°21′S, 57°32′E), French Subantarctic Territories; from 5 yellow-eyed penguins (*Megadyptes antipodes*) from Dudenin (46°05′S, 171°23′E), New Zealand (NZ); and from 5 Adelie penguins (*Pygoscelis adeliae*) at Cape Birds, Ross Island (77°13′S, 166°29′E), Antarctica. Captive bird collection included samples from 12 little blue penguins (*Eudyptula minor*) from Napier Zoo (39°30′S, 176°40′E), Napier, NZ; 7 Magellanic penguins (*Spheniscus magellanicus*) from Sea World of California (32°40′N, 117°12′W), San Diego, U.S.A.; and 9 *S. demersus* from the Baltimore Zoo (39°21′N, 76°34′W), Baltimore, Maryland, U.S.A. All birds were adult. The blood was collected by heparinized syringe venipuncture from the jugular vein or brachial vein, centrifuged (1,200 × *g*, 10 min), and the plasma stored, air-dried, on filter paper as described in Graczyk et al. (1993).

To determine the specificity of anti–*S. demersus* IgG labeled with alkaline phosphatase to homologous and heterologous sera (6 penguin species, duck, chicken, turkey), a direct ELISA was performed according to the protocol of Graczyk et al. (1994c). The air-dried samples were eluted into buffer (Graczyk et al., 1994c), and a pooled sample for each penguin species was prepared with 200 μl of the eluate from individual specimens. The 6 penguin serum pools were used at 1/100, 1/200, 1/400, 1/800, 1/1,600, 1/3,200, 1/6,400, and 1/12,800 dilutions in triplicate to coat the ELISA plate. The relative binding of anti–*S. demersus* conjugate was compared to anti-chicken IgG (Sigma Chemical Co., St. Louis, Missouri, U.S.A.), anti-duck IgG, and anti-turkey IgG (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Maryland, U.S.A.); all ligands were labeled with alkaline phosphatase. Human serum was used as a negative control (NC). The remaining eluate from an individual penguin sample (800 μl) was used for the indirect ELISA (Graczyk et al., 1994c) to determine the presence of anti–*Plasmodium* spp. immunoglobulins. Two antigens of *Plasmodium falciparum* were used: $R32tet_{32}$ and P.F.R27. Immunoglobulins directed against *P. relictum* and *P. elongatum* recognized these antigens (Graczyk et al., 1993). These immunoglobulins did not cross-react with antigens from another avian hemosporidian blood parasite, *Haemoproteus columbae* (Graczyk et al., 1994b), and anti–*H. columbae* immunoglobulins did not cross-react with $R32tet_{32}$ or P.F.R27 antigens (Graczyk et al., 1994b). Additionally, sera of *S. demersus* infected with *Babesia* sp., as determined by Giemsa-stained thin blood smear, gave an ELISA-negative reaction with $R32tet_{32}$ or P.F.R27 antigens (Graczyk et al., unpubl.). Pooled serum from 4 3-mo-old *S. demersus* chicks housed in indoors under mosquito-free conditions was used as a NC. At this age, maternally transmitted anti–*Plasmodium* spp. immunoglobulins were not detectable by ELISA (Graczyk et al., 1994a). The positive cutoff level was an absorbance greater than the mean ± 3 SD of 8 NC wells. A pool of serum from 2 captive, 2-yr-old *S. demersus* with clinical *P. relictum* and 3 2-yr-old penguins with clinical *P. elongatum* infections were used as a positive control (PC). The method of Schwartz et al. (1991) was used to compare the absorbance values from indirect ELISA trials.

Statistical analysis was performed with Analytical Software Statistix 3.5 (Analytical Software, St. Paul, Minnesota, U.S.A.). Analysis of variance (ANOVA) was performed to determine the significance of among-species effect. A 2-sample *t*-test was used to compare the mean absorbance values from different ELISA plates, paired *t*-test for the means derived from the same ELISA plate, and *G*-test to compare the prevalence of ELISA positivity among the penguin species. The degree of linear association among variables was compared using Pearson's correlation coefficient (*r*). Statistical significance was considered to be $P < 0.05$. Other statistical treatment followed the procedures of Sokal and Rohlf (1981).

Results

The most specific conjugate for the detection of immunoglobulins in the 6 penguin species sera was anti–*S. demersus* IgG (Table 1). The mean absorbance value obtained by this conjugate for the penguins (0.945) was significantly higher (paired *t*-test; $t = 12.24$, $P < 0.01$) than the mean absorbance obtained by anti-duck IgG (0.296), anti-chicken IgG (0.594), or anti-turkey (0.414) IgG. Additionally, the mean absorbance for an individual penguin species was significantly elevated when compared to those obtained by the 3 other ligands (ANOVA test; $F = 64.81$, $P < 0.01$) (Table 1). The mean absorbance (±SD) of the penguin serum pool was 0.987 ± 0.086. When this pool was tested for relative binding of the 4 ligands, the mean absorbance was 0.809 for anti-chicken IgG, 0.699 for anti-turkey IgG, 0.607 for anti-penguin IgG, and 0.473 for anti-duck IgG. ANOVA showed that absorbances obtained with anti-penguin IgG were not significantly ($F = 1.36$, $P = 0.31$) lower than those obtained by the commercially available conjugates. The specificity of immunoglobulin detection in the penguin sera increased with incremental penguin serum dilution (up to 1/400). At a dilution of 1/400, the absorbances obtained by duck, chicken, and turkey conjugates did not reach the threshold ELISA

Table 1. Specificity of anti-*Spheniscus demersus* IgG to the immunoglobulins in homologous and heterologous sera expressed by the mean absorbance values obtained at 405 nm. Sera diluted 1/100 with phosphate-buffered saline.

Avian sera	Alkaline phosphatase–labeled conjugates			
	*Spheniscus demersus**	Duck†	Chicken*	Turkey†
Spheniscus demersus	1.033‡	0.340	0.643	0.577
Pygoscelis adeliae	0.883‡	0.300	0.630	0.393
Pygoscelis papua	0.980‡	0.213	0.513	0.403
Aptenodytes patagonicus	0.895‡	0.317	0.550	0.327
Megadyptes antipodes	0.950‡	0.263	0.547	0.377
Spheniscus magellanicus	0.997‡	0.327	0.667	0.420
Eudyptula minor	0.879‡	0.310	0.610	0.401
Duck	0.387	0.727	0.713	0.426
Chicken	0.577	0.403	1.112	0.803
Turkey	0.478	0.467	0.817	0.920

* Developed in rabbit.
† Developed in goat.
‡ ANOVA test; $F = 64.81$, $P < 0.01$.

cutoff level (0.134). However, a 1/400 dilution of penguin serum significantly diminished (2-sample t-test; $t = 3.45$, $P < 0.05$) the detection of avian malaria antibodies (Ab) from an absorbance of 1.033 (dilution 1/100) (Table 1) to 0.565 (Fig. 1). Because the 3 other conjugates were not used for detection of avian malaria Ab, the dilution of 1/100 of penguin serum was selected for the ELISA. Using anti–*S. demersus* alkaline phosphatase–labeled IgG it was possible to detect penguin immunoglobulins up to a dilution of 1/12,800 ($10^{-4.11}$) (Fig. 1). The decreasing pattern of absorbance associated with the incremental serum dilutions was not significant (ANOVA; $F = 1.25$, $P = 0.345$) among the 7 penguin species.

In the indirect ELISA, the range of PC serum absorbances was 1.010–1.15 ($\bar{x} = 1.077 \pm 0.043$) for $R32tet_{32}$ and 1.09–0.899 ($\bar{x} = 0.971 \pm 0.060$) for P.F.R27. The range of NC absorbances was 0.082–0.120 ($\bar{x} = 0.095 \pm 0.013$), and the cutoff level was 0.134. All the Antarctic *P. adeliae* sera were negative for anti–*P. relictum* or anti–*P. elongatum* immunoglobulins, and all *M. antipodes* were positive (Table 2). The prevalence of ELISA positivity ranges between 33 and 92% among the remaining 5 penguin species (Table 2). The mean absorbance of positive penguin sera was significantly elevated (2-sample t-test; $t = 4.21$, $P < 0.05$) when compared to the mean NC absorbance. The mean absorbance of wild ELISA-positive *S. demersus* (0.356) was significantly lower (2-sample t-test; $t = 3.04$, $P < 0.05$) than the mean absorbance (1.024) of captive *S. demersus* with clinical *P. relictum* and *P. elongatum* infections. Kinetic profiles of Ab detected by $R32tet_{32}$ were similar to those detected by P.F.R27 for all penguin species ($0.826 < r < 0.965$, $P < 0.05$). The overall magnitudes of Ab titers detected by these antigens were not significantly different from each other (2-sample t-test; $t = 1.31$, $P = 0.13$).

The prevalence of ELISA positivity among species of wild penguins was significantly different (G-test; $G = 7.14$, $P < 0.05$). The same effect was seen between species of captive penguins (G-test; $G = 22.60$, $P < 0.05$).

Discussion

The positivity for malarial Ab of wild penguins is generated by a single contact with the parasites, because the infection is acquired for a lifetime

Table 2. The mean absorbance values (±SE) obtained at 405 nm in the indirect ELISA for detection of immunoglobulins against *Plasmodium relictum* or *P. elongatum* in the penguin sera diluted 1/100 with phosphate-buffered saline.

Penguin species	*Plasmodium falciparum* antigen		
	Sporozoite ($R32tet_{32}$) $\bar{x} \pm$ SE	Gametocyte (P.F.R27) $\bar{x} \pm$ SE	Positive* (%)
Spheniscus demersus ($n = 44$)	0.402 ± 0.034	0.313 ± 0.027	52
Pygoscelis papua ($n = 12$)	0.544 ± 0.053	0.391 ± 0.021	33
Aptenodytes patagonicus ($n = 12$)	0.488 ± 0.018	0.437 ± 0.021	58
Megadyptes antipodes ($n = 5$)	0.782 ± 0.022	0.661 ± 0.038	100
Spheniscus magellanicus ($n = 7$)	0.301 ± 0.031	0.241 ± 0.012	43
Eudyptula minor ($n = 12$)	0.402 ± 0.019	0.444 ± 0.021	92

* Above the cutoff level of 0.134.

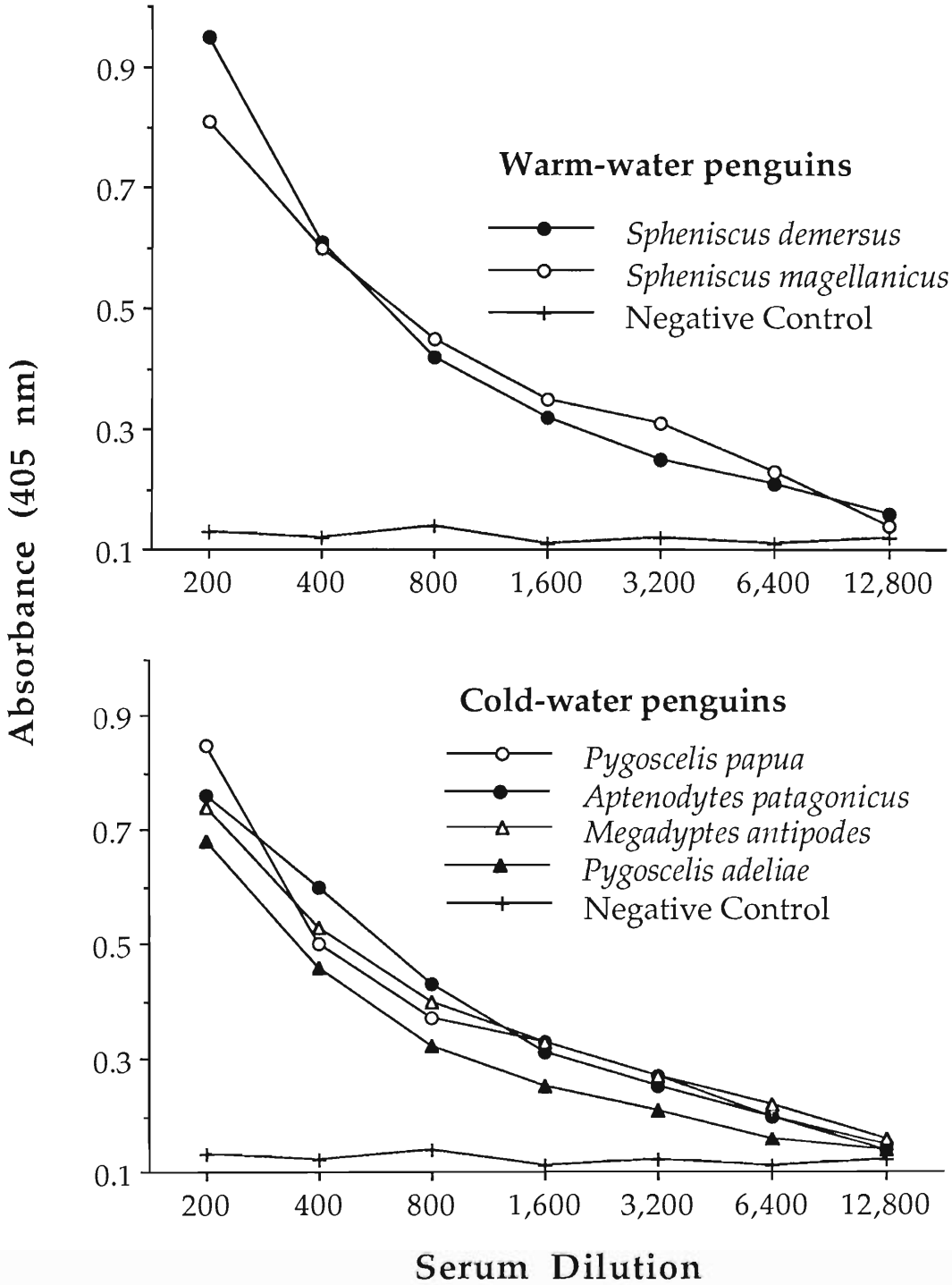

Figure 1. Mean absorbance values in a direct ELISA for detection of immunoglobulins in homologous and heterologous penguin sera by anti-*Spheniscus demersus* IgG labeled with alkaline phosphatase. Human serum was used as a NC.

(Cranfield et al., 1994). The detection of maternally transmitted Ab (Graczyk et al., 1994a) is excluded in the present study because all the birds were adults. The global distribution of warm-water penguins (Davis and Darby, 1990) is overlapped by the occurrence of *Culex* spp. mosquitoes (Knight and Stone, 1977). The contact with vectors is less likely for the cold-water penguins whose distribution is only partially covered by the occurrence of *Culex* spp. ELISA positivity of penguins in the areas with documented mosquito absence may be explained by exposure of birds during migration.

The prevalences of parasitemias of wild *S. demersus* at the southern coast of RSA were 7%, 5% (Fantham and Porter, 1944), and 0.7% (Brossy, 1992); however, the parasitemia prevalence of injured or oiled penguins increased to 22% (Brossy, 1992). The occurrence of *Culex* spp. had been reported in this region (34°41′S) by Edwards (1941). The Ab titers of wild *S. demersus* were significantly lower (2-sample t-test; $t = 2.22$, $P < 0.05$) than those reported by Graczyk et al. (1994c) in subclinically infected, captive *S. demersus* and were markedly lower than Ab titers of parasitemic penguins in the present study. The prevalence of ELISA-positive wild African penguins (52%) was significantly lower (G-test; $G = 49.86$, $P < 0.05$) when compared to 100% positivity among captive birds (Graczyk et al., 1994c). These facts may indicate that the malarial infections of wild birds were subclinical. Additionally, 22% of parasitemia prevalence (Brossy, 1992) among oiled and injured wild penguins is still significantly lower (G-test; $G = 51.41$, $P < 0.05$) than the >50% reported by Cranfield et al. (1990) and 62% (Graczyk et al., 1994d).

In addition to avian malaria, *Babesia* sp. (Brossy, 1992) and *Babesia percei* (Earle et al., 1993) were reported from wild *S. demersus* from the RSA. Sera of *S. demersus* infected with *Babesia* sp., as determined by Giemsa-stained thin blood smear, gave ELISA-negative reaction with *P. falciparum* $R32tet_{32}$ or P.F.R27 antigens (Graczyk et al., unpubl.). The potential cross-reactivity between these antigens and the closely related avian hemosporidian blood parasite (*Haemoproteus columbae*) was excluded by Graczyk et al. (1994b).

No *Culex* spp. mosquitoes were found at Crozet (46°21′S) and Kerguelen (49°15′S) islands (Crafford et al., 1986). Chastel et al. (1993) reported a tick species (*Ixodes uriae*) parasitizing the penguins. However, based on blood smear examination, Fantham and Porter (1944) reported *P. relictum spheniscide* from *A. patagonicus* from the higher latitudes, South Georgia Island (54°15′S), and *Culex* spp. mosquitoes have been reported from southern coastal points of Argentina (Knight and Stone, 1977). Twenty percent of rock-hopper penguins (*Eudyptes crestatus*) at Gough Island (41°31′S) were parasitemic with *P. r. spheniscide* (Fantham and Porter, 1944). The ELISA positivity of *P. papua* and *A. patagonicus* from Crozet and Kerguelen islands may reflect exposure to the parasites during migration. Bost and Jouventin (1990) reported that banded Gentoo penguin females were not seen on the Crozet Islands for up to 5 mo.

The lack of anti–*P. relictum* or anti–*P. elongatum* immunoglobulins in malaria-susceptible Antarctic *P. adeliae* is a consequence of lack of exposure due to the absence of the vectors. Antarctic *P. adeliae* that breed on the shores migrate northward in winter but remain in the southern oceans around the continent (Cockrem, 1990). Therefore, it is not likely that ELISA-negative penguins may have been infected with other than *P. relictum* or *P. elongatum* parasites. However, they may develop disease in areas of vector presence. Sladen et al. (1979) reported 46% malaria-induced mortality of Antarctic *E. crestatus* in North America.

The prevalence of *P. r. spheniscide* parasitemia in NZ yellow-eyed penguins (*E. antipodes*) from Stewart Island (47°12′S) was 10% (Fantham and Porter, 1944). Garnham (1966) reported *P. relictum* in NZ penguins but never in birds that remained in their Antarctic haunts. Plasmodial parasites from the NZ penguins were also reported by Laird (1950). Two indigenous (*C. pervigilans* and *C. asteliae*) and 1 exotic (*C. quinquefasciatus*) avian malaria vector in NZ had been reported frequently (see Laird, 1990) from the beginning of this century (Miller, 1920). *Culex pervigilans* and *C. quinquefasciatus* have a remarkable wide range of tolerance in NZ; however, the latter is primarily confined to the coastal area (Laird, 1990). The 100% ELISA positivity of *M. antipodes* in the present study, and the highest absorbance compared to other penguin species, indicate intense exposure to the malarial parasites. The mainland *M. antipodes* has been reported to have unidentified disease problems (Gill and Darby, 1993) not observed in Antarctic *P. adeliae*.

The results of the present study showed that the ELISA developed for captive *S. demersus* can be utilized for diagnostic surveys of exposure to *P. relictum* or *P. elongatum* in wild warm- and cold-water penguins. The overall magnitudes of ELISA-detected Ab were not significantly different for the 2 antigens used. Consequently, the test can be simplified by the elimination of 1 antigen. The ELISA wells that gave absorbance of 0.120 or higher (the cutoff level was 0.136) at 405 nm wavelength can be clearly visually distinguished (particularly on a white background) from the negative wells. Thus, the need for an automated ELISA reader is eliminated, making this method suitable for field surveys.

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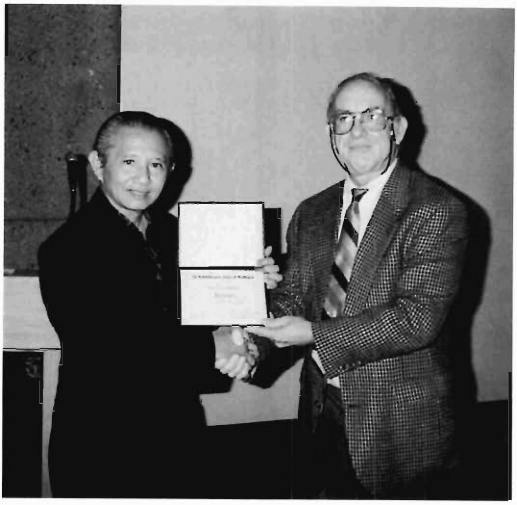
Second Seminar on Food-Borne Parasitic Zoonoses: Current Problems, Epidemiology, Food Safety, and Control

Because the first seminar was a success, the SEAMEO-TROPMED PROJECT is organizing a Second Seminar on Food-borne Parasitic Zoonoses to be held in Khon Kaen, Thailand, 6–9 December 1995. In addition to scientific sessions, a 1-day trip will be made into Laos. Additional information can be obtained from the SEAMEO-TROPMED PROJECT, Faculty of Tropical Medicine, Mahidol University, 420/6 Rajvithi Road, Bangkok 10400, Thailand, or from Dr. John H. Cross, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814; phone (301) 295-3139; fax (301) 295-1971.



Purnomo receiving the Honorary Membership Certificate from Willis A. Reid, Jr., November 9, 1994.

Louis S. Diamond receiving the Life Membership Certificate from Willis A. Reid, Jr., November 9, 1994.

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Parasites of Wood Frogs, *Rana sylvatica* (Ranidae), from Arkansas, with a Description of a New Species of *Eimeria* (Apicomplexa: Eimeriidae)

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ABSTRACT: Thirteen wood frogs, *Rana sylvatica* LeConte, 1825, were collected in February 1994 from Izard County, Arkansas, and examined for parasites. Twelve (92%) were infected with 1 or more parasites, including 8 (62%) with *Opalina* sp., 3 (23%) with *Myxidium serotinum* Kudo and Sprague, 1940, 5 (38%) with unidentified trematode metacercariae, 4 (31%) with *Brachycoelium salamandrae* (Frölich, 1789) Dujardin, 1845, 2 (15%) with *Mesocestoides* sp. tetrathyridia, 1 (8%) with *Abbreviata* sp., 1 (8%) with *Oswaldocruzia pipiens* Walton, 1929, and 1 (8%) with *Desserobdella picta* (Verrill, 1872). In addition, 11 (85%) were found to harbor a previously unreported eimerian. Oocysts of *Eimeria fitchi* sp. n. were ovoidal, 21.9 × 14.3 (20.0–24.0 × 13.2–15.2) μm, with a smooth, thin, single-layered wall; shape index (length/width) 1.5 (1.3–1.7). A micropyle, oocyst residuum, and polar granule were absent. The sporocysts were ovoidal, 10.9 × 7.4 (9.8–11.2 × 7.0–8.0) μm; shape index 1.5 (1.3–1.6). One end of the sporocyst was thickened slightly to form an indistinct Stieda body, and a substieda body was absent. A sporocyst residuum was present, 3.6 × 1.6, consisting of large, coarse granules often scattered free among sporozoites. Sporozoites were elongate, 11.1 × 1.7 (10.4–12.0 × 1.6–1.8) in situ, each with 2 refractile bodies. Three new host records are reported for parasites of *R. sylvatica*.

KEY WORDS: *Rana sylvatica*, wood frog, Anura, Ranidae, *Opalina* sp., *Myxidium serotinum*, metacercariae, *Brachycoelium salamandrae*, *Mesocestoides* sp. tetrathyridia, *Abbreviata* sp., *Oswaldocruzia pipiens*, *Desserobdella picta*, *Eimeria fitchi* sp. n.

The wood frog, *Rana sylvatica* LeConte, 1825, is a medium-sized anuran that ranges throughout much of northern North America, from Labrador to Alaska, south and eastward to the southern Appalachians; disjunct populations occur in Newfoundland, Alabama, Arkansas, Colorado, Missouri, North Dakota, and Wyoming (Conant and Collins, 1991). The wood frog is an explosive late winter–early spring breeder in small, fishless, mesic woodland ponds and pools (Johnson, 1987). For most of the year, *R. sylvatica* is secretive and solitary and often difficult to observe among shady ravines, forests near clear streams, leafy pools, cave entrances, and damp wooded hillsides (Johnson, 1987).

Martof (1970) provided a summary of the biology of *R. palustris* in a species account. Walton (1964) provided a summary of the protozoans known to infect *R. sylvatica*, and additional information regarding the parasites of wood frogs is available for individuals from Canada (Stafford, 1905; Fantham et al., 1942; Pearson, 1956; Baker, 1978a, b, 1979a, b; Adamson, 1980; Barta and Desser, 1984; Jones, 1987; Chen and Desser, 1989), Alaska (Metcalf, 1923), Maine (Bouchard, 1951), Maryland (Walton, 1931), Massachusetts (Rankin, 1945), Michigan (Najarian, 1955; Muzzall and Peebles, 1991), New York (Harwood, 1930, 1932), North Carolina (Metcalf, 1923), Ohio (Metcalf, 1923; Odlaug, 1954), and Wisconsin (Williams and Taft, 1980). However, nothing has been published on disjunct populations from the southwesternmost extent of its range in Arkansas. Herein, we provide information on parasites of a small sample of *R. sylvatica* from northern Arkansas, including a description of a new species of *Eimeria*.

Materials and Methods

Thirteen juvenile and adult male *R. sylvatica* ($\bar{x}$ ± SEM snout–vent length [SVL] = 58.5 ± 1.7, range 49–69 mm) were collected by hand during breeding activ-

Table 1. Parasites of *Rana sylvatica* from Izard County, Arkansas.

Parasite	Location in host	Prevalence*
Protozoa		
Eimeria fitchi sp. n.	Intestinal contents, feces	11/13 (85%)
Opalina sp.	Rectum	8/13 (62%)
Myxidium serotinum†	Gall bladder	3/13 (23%)
Trematoda		
Unidentified metacercariae	Mesenteries	5/13 (38%)
Brachycoelium salamandrae	Small intestine	4/13 (31%)
Cestoidea		
Mesocestoides sp.†	Liver, mesenteries	2/13 (15%)
Nematoda		
Abbreviata sp.	Stomach	1/13 (8%)
Oswaldocruzia pipiens	Small intestine	1/13 (8%)
Hirudinea		
Desserobdella picta†	Skin	1/13 (8%)

* Number infected/number examined (percent).
† New host record.

ities in February 1994 from a pond in Izard County, Arkansas (36°03'N, 91°54'W, elev. 195 m), and examined for parasites. Specimens were placed in plastic bags on ice and returned to the laboratory within 24 hr for processing. Frogs were sacrificed by sodium pentobarbital (Nembutal®) overdose. Methods for necropsy and preparation and staining of parasites follow McAllister et al. (1989). For coccidial isolation, individual samples of rectal contents and feces in Hank's balanced salt solution (HBSS) were initially examined for coccidia using Brightfield microscopy following flotation in Sheather's sucrose solution (sp. gr. 1.30). Positive samples containing unsporulated oocysts were placed in individual Petri dishes containing a thin layer of HBSS supplemented with 100 IU penicillin G/ml and 100 μg streptomycin/ml. Following a sporulation period of 5 days at room temperature (ca. 23°C), oocysts were mailed to Kansas State University. Oocysts were concentrated by flotation, measured using a calibrated ocular micrometer, and examined and photographed using Nomarski interference-contrast optics. Measurements are reported in micrometers (μm) with means followed by the ranges in parentheses. Oocysts were 1.5 wk old when measured and photographed.

Symbiotypes of *R. sylvatica* from which parasites were collected are deposited in the Arkansas State University Museum of Zoology (ASUMZ 19434–19446). Voucher specimens of parasites are deposited in the U.S. National Parasite Collection, Beltsville, Maryland 20705, as follows: *Opalina* sp. (USNM 83928), *Myxidium serotinum* (USNM 83927), *Brachycoelium salamandrae* (USNM 83925), unidentifiable trematode metacercariae (USNM 83926), *Mesocestoides* sp. tetrathyridia (USNM 83929), *Abbreviata* sp. (USNM 83931), and *Oswaldocruzia pipiens* (USNM 83930).

Results and Discussion

Three protozoan and 6 metazoan parasites infected *R. sylvatica* (Table 1). Of the 13 wood frogs examined, 1 (8%) was uninfected and 12 (92%) harbored multiple infections. None of the frogs were infected with apicomplexan or trypanosomal parasites in the blood.

Endocommensal *Opalina* sp. Purkinje and Valentin, 1840, not identifiable to species, were found in the rectum of 8 *R. sylvatica* (56.5 ± 1.9, 49–66 mm SVL). *Opalina virguloidea* Metcalf, 1923, has been reported from *R. sylvatica* in Ohio and North Carolina (Metcalf, 1923) and *O. obtrigonoidea* Metcalf, 1923, was reported from wood frogs in Ohio (Odlaug, 1954). In addition, Metcalf (1923) reported *Cepedea cantabrigensis* from *R. cantabrigensis* (=*R. sylvatica*) from Manitoba, Canada, Alaska, and Michigan. A similar opalinid was recently reported by McAllister et al. (1995b) in the pickerel frog, *Rana palustris*, from Independence County, Arkansas.

Trophozoites and spores of the myxosporean, *Myxidium serotinum* Kudo and Sprague, 1940, were found in 3 frogs (57.7 ± 5.5, 49–68 mm SVL). This represents a new host record and the second time *M. serotinum* has been reported from Arkansas, as McAllister et al. (1995b) recently recovered the parasite from *R. palustris*. *Myxidium serotinum* also infects other members of the Ranidae, including *Rana* sp. and *R. clamitans* from Louisiana, *R. pipiens* from unspecified locales in the United States, and *R. utricularia* from Florida (Kudo and Sprague, 1940; Kudo, 1943).

Specimens of the plagiorchid trematode, *Brachycoelium salamandrae* (Frölich, 1789) Du-

jardin, 1845, were found in 4 frogs (64.0 ± 3.7, 53–69 mm SVL) with a mean intensity of 2.5 ± 0.9 (range 1–4) worms per host. This parasite has been reported previously in wood frogs from Michigan (Najarian, 1955) and Ohio (Odlaug, 1954). McAllister et al. (1995a, b) reported *B. salamandrae* from Arkansas in graybelly salamanders, *Eurycea multiplicata griseogaster* and *R. palustris* (respectively).

Unidentified trematode metacercariae were found encapsulated in tissues of 5 frogs (62.0 ± 3.5, 52–69 mm SVL). Various unidentified, echinostome, and gorgoderid metacercariae have been reported in *R. sylvatica* from Massachusetts and Michigan (Rankin, 1945; Najarian, 1955; Muzzall and Peebles, 1991).

Numerous tetrathyridia of the cyclophyllidean tapeworm, *Mesocestoides* sp. Vaillant, 1863, were encapsulated in tissues within the body cavity of 2 *R. sylvatica* (53 and 58 mm SVL). This represents a new host record for *Mesocestoides* sp. McAllister et al. (1995b) recently reported *Mesocestoides* sp. tetrathyridia in *R. palustris* from Arkansas. In addition, other ranid hosts include *R. berlandieri* from Texas, *R. clamitans* from Wisconsin, and *R. pipiens* from Iowa, Minnesota, New York, South Dakota, and Wisconsin (see McAllister and Conn, 1990).

A single third-stage larval spiruroid nematode, *Abbreviata* sp. Travassos, 1920, was found in a 56-mm SVL wood frog. Walton (1931) provided a description of *Physaloptera* (=*Abbreviata*) *ranae* from *R. sylvatica* based on larval specimens. However, Baker (1987) designated *A. ranae* a species inquirenda. McAllister et al. (1993, 1995b) also reported *Hyla avivoca* and *R. palustris* from Arkansas as hosts of *Abbreviata* sp. Other ranids infected with *Abbreviata* sp. include *R. catesbeiana, R. clamitans, R. pipiens,* and *R. sphenocephala* (Walton, 1931; Morgan, 1945; Baker, 1987).

A single male strongylid nematode *Oswaldocruzia pipiens* Walton, 1929, was found in a 55-mm SVL *R. sylvatica.* This nematode has been reported previously in *R. sylvatica* from Canada (Baker, 1978a), Massachusetts (Rankin, 1945), Michigan (Muzzall and Peebles, 1991), and New York (Harwood, 1930, 1932). McAllister et al. (1993, 1995b) reported the species in *H. avivoca* and *R. palustris* from Arkansas. There is little host specificity in this parasite, as other frogs (ranids and hylids), toads, salamanders, turtles, and lizards have been reported as hosts of *O. pipiens* (see Baker, 1987).

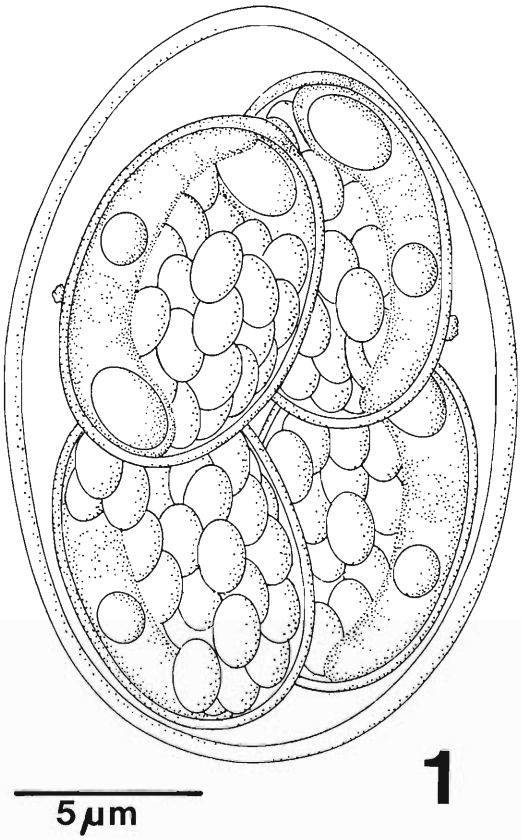
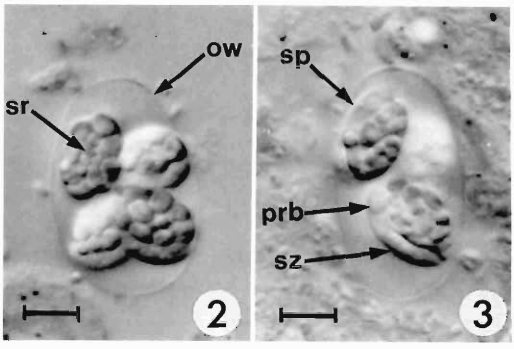


Figure 1. Composite line drawing of sporulated oocyst of *Eimeria fitchi* sp. n. from *Rana sylvatica*.

A glossiphoniid leech, *Desserobdella* (syn. *Batrachobdella*) *picta* (Verrill, 1872) was found firmly attached to the dorsal skin of a male *R. sylvatica* (56 mm SVL). This leech is widely dis-



Figures 2, 3. Nomarski interference-contrast photomicrographs of sporulated oocysts of *Eimeria fitchi* sp. n. Abbreviations: ow = oocyst wall, prb = posterior refractile body, sp = sporocyst, sr = sporocyst residuum, sz = sporozoite. Scale bars = 5.0 µm.

Table 2. Helminths reported from *Rana sylvatica* from various North American localities.

Helminth	Locality	Reference
Trematoda		
*Alaria arisaemoides**	Canada	Pearson, 1956
Brachycoelium salamandrae	Arkansas	This report
	Michigan	Najarian, 1955
	Ohio	Odlaug, 1954
Echinostome cysts	Michigan	Najarian, 1955
Glypthelmins quieta	Michigan	Muzzall and Peebles, 1991
Gorgoderid cysts	Massachusetts	Rankin, 1945
	Michigan	Najarian, 1955
Gorgoderina attenuata	Massachusetts	Rankin, 1945
G. translucida	Idaho	Waitz, 1961†
	Maine	Bouchard, 1951
Haematoloechus complexus	Ohio	Catalano and White, 1977
H. medioplexus	Wisconsin	Williams and Taft, 1980
H. parviplexus	Michigan	Muzzall and Peebles, 1991
H. varioplexus	Idaho	Waitz, 1961†
	Michigan	Najarian, 1955
Megalodiscus temperatus	Canada	Stafford, 1905
Unidentified metacercariae	Arkansas	This study
	Michigan	Muzzall and Peebles, 1991
Cestoidea		
Cylindrotaenia americana	Canada	Jones, 1987
Mesocestoides sp.	Arkansas	This report
Plerocercoid larva (cysts)	Massachusetts	Rankin, 1945
Nematoda		
Abbreviata sp.	Arkansas	This report
	Maryland	Walton, 1931
Cosmocercoides dukae	Michigan	Muzzall and Peebles, 1991
or *C. variabilis*	New York	Harwood, 1930, 1932
	Ohio	Odlaug, 1954
Gyrinicola batrachiensis‡	Canada	Adamson, 1980
Megalobatrachonema gigantica	Idaho	Waitz, 1961†
Microfilaria ranae-sylvaticae§	Canada	Fantham et al., 1942
Oswaldocruzia pipiens	Arkansas	This report
	Massachusetts	Rankin, 1945
	Michigan	Muzzall and Peebles, 1991
	New York	Harwood, 1932
	Canada	Baker, 1978a
Rhabdias ranae	Canada	Baker, 1978b, 1979a, b
	Massachusetts	Rankin, 1945
	Michigan	Muzzall and Peebles, 1991
	Wisconsin	Williams and Taft, 1980
Spiroxys sp.	Michigan	Muzzall and Peebles, 1991
Acanthocephala		
Unidentified cystacanth	Ohio	Odlaug, 1954

* Experimental infection in tadpoles.
† Reported from *Rana pretiosa* (spotted frog) × *R. sylvatica* hybrids.
‡ Only tadpoles are reported to be infected.
§ Considered a species inquirenda by Baker (1987).

tributed in the United States and was reported previously from southeastern Arkansas (see Klemm, 1982). It is found in small woodland ponds and typically arrives shortly before breeding aggregations of amphibians. There is little host specificity in *D. picta,* as other hosts include *Ambystoma maculatum, A. talpoideum, A. tigrinum, Bufo americanus, Hyla versicolor, Pseudacris crucifer, R. clamitans, R. catesbeiana,* and *R. septentrionalis* (Sawyer, 1972; Sawyer and

Shelly, 1976; Klemm, 1982; Barta and Desser, 1984).

In addition to the parasites already noted, numerous eimerian oocysts were found in the feces of *R. sylvatica* (58.4 ± 2.0, 49–69 mm), which proved to be the most commonly observed parasite in this host sample. On further examination, these oocysts were found to represent a previously undescribed species. Here we present a description of this new coccidian.

Eimeria fitchi sp. n. (Figs. 1–3).

DESCRIPTION OF OOCYSTS: Oocysts ovoidal, contents with light greenish tint, 21.9 × 14.3 (20.0–24.0 × 13.2–15.2) ($n = 25$), with smooth, thin, single-layered wall ca. 0.5 thick; shape index (length/width) 1.5 (1.3–1.7). Micropyle, oocyst residuum, and intact polar granule absent, although 1–3 fragments are sometimes seen attached to outer walls of sporocysts. Sporocysts ovoidal, 10.9 × 7.4 (9.8–11.2 × 7.0–8.0), with smooth, thin, single-layered wall; shape index 1.5 (1.3–1.6). One end of sporocyst thickened slightly to form what appears to be indistinct Stieda body; substieda body absent. Sporocyst residuum present, 3.6 × 1.6, consisting of about 25 large, coarse granules often scattered free among sporozoites. Sporozoites elongate, 11.1 × 1.7 (10.4–12.0 × 1.6–1.8) in situ, each with 2 refractile bodies. Spherical anterio-central refractile body, 1.1 (0.8–1.4) in diameter; posterior refractile body subspherical to ovoidal, 2.9 long × 1.6 wide (2.2–3.4 × 1.4–1.6). An indistinct nucleus located between refractile bodies.

TYPE HOST: *Rana sylvatica* LeConte, 1825, "wood frog" (Anura: Ranidae), adult male, 69 mm SVL, collected 20 February 1994 by S. E. Trauth. Symbiotype deposited as ASUMZ 19434.

TYPE SPECIMENS: Phototype (see Bandoni and Duszynski, 1988) of sporulated oocysts in the U.S. National Parasite Collection, Beltsville, Maryland, as USNMPC No. 84163.

TYPE LOCALITY: 6.0 km SW Melbourne, off State Hwy 9, Izard County, Arkansas.

PREVALENCE: Found in 11 (85%) of the 13 frogs examined.

SITE OF INFECTION: Unknown. Oocysts recovered from rectal contents and feces.

SPORULATION: Exogenous. All oocysts were passed unsporulated or partially sporulated and became fully sporulated within 5 days at ca. 23°C.

ETYMOLOGY: The specific epithet is given in honor of Henry S. Fitch, Professor Emeritus, University of Kansas, in recognition of his numerous contributions to our understanding of the natural history and ecology of North American amphibians and reptiles.

REMARKS: Oocysts of *E. fitchi* sp. n. can be distinguished from *E. kermiti* Chen and Desser, 1989, and *E. algonquini* Chen and Desser, 1989, from *R. sylvatica* in Ontario, Canada, as follows: oocysts of *E. kermiti* are larger and possess an oocyst residuum and polar granule, and sporocysts have a distinct Stieda body; oocysts of *E. algonquini* are spherical, and sporocysts are distinctly different (see Chen and Desser, 1989). Further, oocysts of *E. fitchi* sp. n. are unlike those found in other anurans, including *Rana* spp. (see Upton and McAllister, 1988). *Rana sylvatica* represents the first ranid frog from the United States known to harbor coccidia.

In summary, parasites of our sample of Arkansas *R. sylvatica* were similar to those reported from other surveys on *R. sylvatica* from various parts of its range (Table 2). We also noted that several parasites of *R. sylvatica* are shared with *R. palustris* and *H. avivoca* in Arkansas. This was not surprising given that many anuran parasites have a wide geographic range and exhibit little host specificity. Admittedly, our sample size was small, and the survey lacked data for female *R. sylvatica.* In the most exhaustive helminth survey on *R. sylvatica* to date, Muzzall and Peebles (1991) reported 3 trematode and 4 nematode parasites in 100 wood frogs (combined prevalence = 77%) from Michigan. However, all frogs were reported to be under 47 mm SVL and, as such, represent juveniles. Therefore, additional surveys on protozoan and metazoan parasites of *R. sylvatica* should include examination of all age and size classes of male and female wood frogs collected throughout the year.

Acknowledgment

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Meeting Schedule

1995–1996

11 October 1995	National Institutes of Health (NIH), Bethesda, MD. Contact: Louis Miller (301) 496-2183.
8 November 1995	Anniversary Dinner, Uniformed Services University of Health Sciences (USUHS), Bethesda, MD. Contact: John Cross (301) 295-3139.
14 February 1996	Nematology Laboratory, United States Department of Agriculture, Beltsville, MD. Contact: David Chitwood (301) 504-5660.
20 March 1996	Johns Hopkins Montgomery County Center, Rockville, MD. Contacts: Thomas Simpson (410) 366-8814 and Alan Scott (410) 955-3442.
4 May 1996	New Bolton Center, University of Pennsylvania, Kennett Square, PA. Contact: Gerhard Schad (215) 898-6680.

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Parasites of *Desmognathus brimleyorum* (Caudata: Plethodontidae) from the Ouachita Mountains of Arkansas and Oklahoma

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ABSTRACT: Forty-one juvenile and adult Ouachita dusky salamanders, *Desmognathus brimleyorum,* were collected from Arkansas and Oklahoma and examined for parasites. Thirty-two (78%) were infected with 1 or more parasites, including 25 (61%) with *Chloromyxum salamandrae,* 1 (2%) with *Brachycoelium salamandrae,* 2 (5%) with *Cylindrotaenia americana,* 8 (20%) with *Mesocestoides* sp. tetrathyridia, 9 (22%) with *Batracholandros magnavulvaris,* 3 (7%) with *Desmognathinema nantahalaensis,* 4 (10%) with *Hedruris pendula,* 6 (15%) with *Omeia papillocauda,* 1 (2%) with unidentified Ascaridoidea larvae, 1 (2%) with an acanthocephalan cystacanth, and 28 (68%) with larval *Hannemania* sp. In addition, 10 (24%) salamanders harbored an intraerythrocytic inclusion, thought to represent a rickettsia or virus of undetermined taxonomic status. Several new host and distributional records are documented for parasites of *D. brimleyorum,* including the first report of *Mesocestoides* sp. in a caudate amphibian worldwide.

KEY WORDS: *Cylindrotaenia americana, Mesocestoides* sp., *Desmognathus brimleyorum, Hannemania* sp., *Chloromyxum salamandrae, Batracholandros magnavulvaris, Omeia papillocauda, Brachycoelium salamandrae,* acanthocephalan cystacanth, *Desmognathinema nantahalaensis, Hedruris pendula.*

The Ouachita dusky salamander, *Desmognathus brimleyorum* Stejneger, 1894, is a large, robust amphibian that is restricted in range to the Ouachita uplift of central Arkansas and southeastern Oklahoma (Conant and Collins, 1991). This semi-aquatic salamander is found in seepages around rocky and gravelly streams where it hides under rubble and leaf litter. To our knowledge, there is only 1 previous published report on helminths of *D. brimleyorum* (Winter et al., 1986). We report new host and distributional records on several parasites from *D. brimleyorum* from Arkansas and Oklahoma, including the first record of *Mesocestoides* sp. from a caudate amphibian.

Materials and Methods

During March and May 1994, 41 (29 male, 12 female) juvenile and adult ($\bar{x}$ ± SE snout–vent length [SVL] = 58.0 ± 2.8, range 19–93 mm) *D. brimleyorum* were collected by handraking streamlets in Polk County, Arkansas (N = 37), and LeFlore County, Oklahoma (*N* = 4), and examined for parasites. Of the 41 *D. brimleyorum,* 22 were considered juveniles with SVL's of ≤62 mm (Trauth et al., 1990). Salamanders were placed in bags containing stream water and transported on ice to the laboratory within 48 hr. Specimens were sacrificed by prolonged immersion in a dilute chlorotone solution. Methods for salamander necropsy and preparation and staining of blood smears, helminths, myxozoans, and coccidial isolation follow McAllister and Upton (1987) and Upton et al. (1995). Mites were gently teased from capsules, fixed in 70% ethanol, dehydrated, heated to 60°C for 5–10 min in lactophenol, and mounted in Hoyer's medium. Voucher specimens of hosts are deposited in the Arkansas State University Museum of Zoology (ASUMZ 19494–19520, 19522–19524, 19769–19780). Specimens of parasites are deposited in the U.S. National Parasite Collection, Beltsville, Maryland 20705, as follows: *Chloromyxum salamandrae* (USNM 83947), intraerythrocytic inclusion (USNM 83944), *Brachycoelium salamandrae* (83945), *Cylindrotaenia americana* (USNM 84048), *Mesocestoides* sp. tetrathyridia (USNM 83943), *Batracholandros magnavulvaris* (83948), *Desmognathinema nantahalaensis* (USNM 83950, 84047), *Hedruris pendula* (USNM 83951, 84046), *Omeia papillocauda* (USNM 83949), Ascaridoidea larvae (USNM 83952), acanthocephalan cystacanth (USNM 83946), and *Hannemania* sp. (USNM 83953).

Table 1. Parasites of *Desmognathus brimleyorum* from Arkansas and Oklahoma.

Parasite	Prevalence*	Mean intensity ± 1 SE (range)	Locality†
Intraerythrocytic inclusion‡§	10/41 (24)	—	1, 2
Protozoa			
Chloromyxum salamandrae§	25/41 (61)	—	1, 2
Trematoda			
Brachycoelium salamandrae§	1/41 (2)	2.0 ± — (—)	1
Cestoidea			
Cylindrotaenia americana	2/41 (5)	1.0 ± 1.0 (1)	1
Mesocestoides sp.§	8/41 (20)	—	1
Nematoda			
Ascaridoidea (larvae)§	1/41 (2)	1.0 ± — (—)	1
Batracholandros magnavulvaris	12/41 (27)	2.6 ± 0.7 (1–8)	1
Desmognathinema nantahalaensis§	3/41 (7)	3.0 ± 2.0 (1–7)	1
Hedruris pendula§‖	4/41 (10)	6.3 ± 2.3 (2–12)	1
Omeia papillocauda§‖	6/41 (15)	2.6 ± 0.7 (1–6)	1
Acanthocephala			
Unidentified cystacanth	1/41 (2)	1.0 ± — (—)	1
Acari			
Hannemania sp.	28/41 (68)	—	1, 2

* Number infected/number examined (percent).

† Localities: 1 = 1.0 km S of Rich Mountain, off State Hwy 272, Polk County, Arkansas; 2 = N face of Kiamichi Mountain, off State Hwy 259, LeFlore County, Oklahoma.

‡ An intracellular parasite of unknown classification thought to be rickettsial in nature and may represent *Aegyptianella bactifera* (Labbé, 1894) Barta, Boulard, and Desser, 1989.

§ New host record.

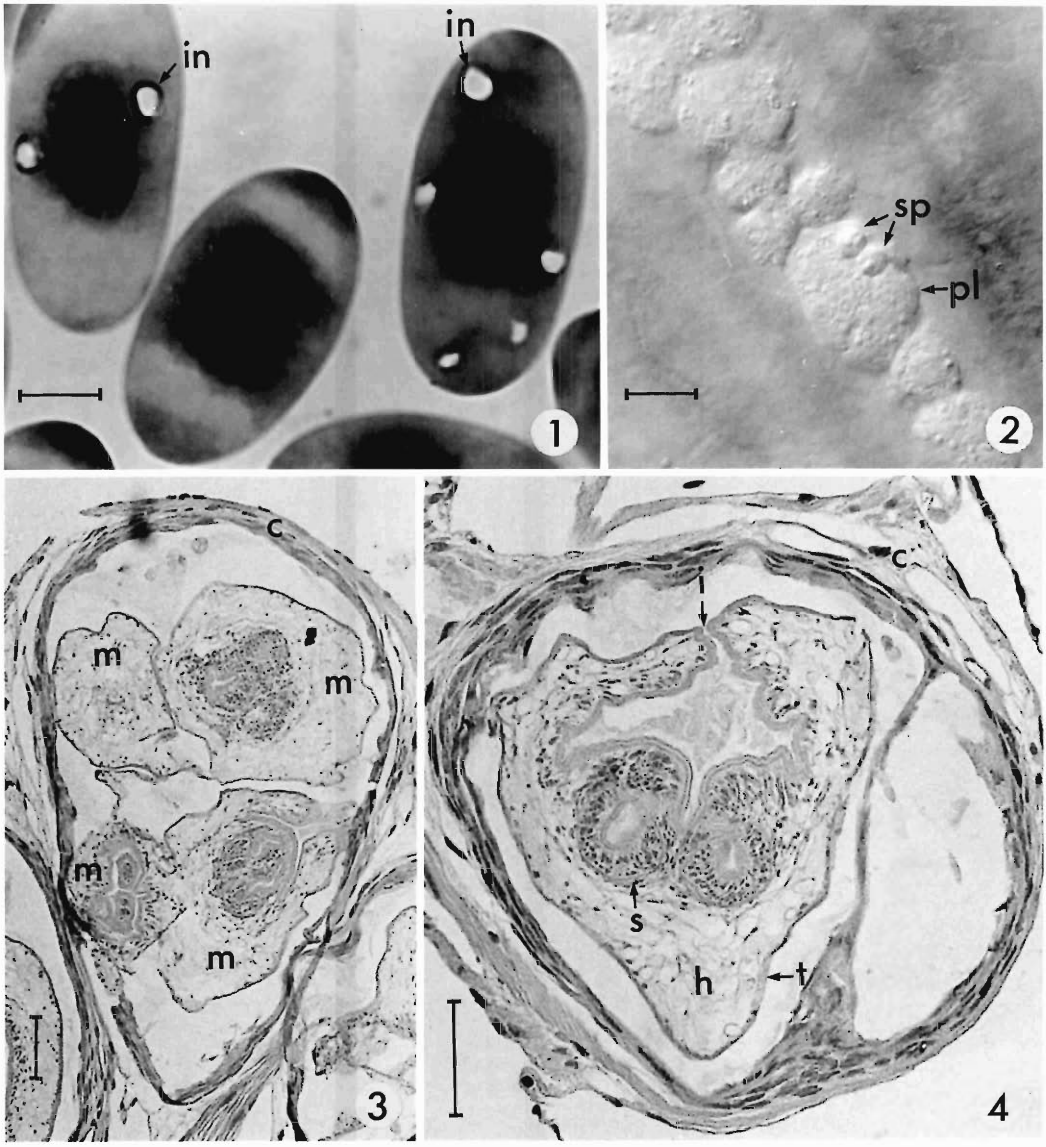
‖ New distributional record.

Results and Discussion

Thirty-two of 41 (78%) *D. brimleyorum* were infected with 1 or more parasites (Table 1). No coccidia were found in the feces or intestinal contents of salamanders. All helminth parasites were found exclusively in *D. brimleyorum* from the Rich Mountain (Arkansas) site, whereas the modest sample from Kiamichi Mountain (Oklahoma) harbored only the intraerythrocytic inclusion, *C. salamandrae,* and *Hannemania* sp. (Table 1).

Intraerythrocytic inclusions of an unknown classification (Fig. 1) were found in nearly one-fourth of all salamanders examined (10 adults [4 male, 6 female]; 75.3 ± 2.5, 67–92 mm). No infections were observed in juvenile salamanders or any salamanders collected in May. These organisms resembled a frog intraerythrocytic virus or rickettsia reported from *Rana catesbeiana, R. clamitans,* and *R. septentrionalis* in Canada (Barta and Desser, 1984; Barta et al., 1989; Gruia-Gray and Desser, 1992). McAllister et al. (1993) reported similar intraerythrocytic inclusions thought to be *Aegyptianella* (syn. *Cytamoeba*) *bactifera* (Labbé, 1894) Barta, Boulard, and Desser, 1989, in *Plethodon albagula* from Arkansas. In addition, Rankin (1937a) reported *A. bactifera* in *Desmognathus fuscus fuscus, D. ochrophaeus, D. imitator, D. monticola,* and *D. quadramaculatus* from North Carolina. Ultrastructural examination will be necessary to determine the identity of this enigmatic organism in *D. brimleyorum.*

Adhering to the gall bladder epithelium in 14 (64%) of the juvenile (10 male, 4 female; 52.1 ± 1.7, 40–62 mm) and 11 (58%) of the adult (7 male, 4 female; 72.9 ± 2.7, 65–83 mm) *D. brimleyorum* were myxozoan plasmodia of *Chloromyxum salamandrae* Upton, McAllister, and Trauth, 1995 (Fig. 2). Two distinct types of plasmodia were observed in *D. brimleyorum,* including a dendritic sheet-like form of *C. salamandrae* in 13 (52%), a compact form in 7 (28%), and a mixture of both forms in 5 (20%) salamanders. The Ouachita dusky salamander is a new host of *C. salamandrae.* Other hosts of this



Figures 1–4. Parasites of *Desmognathus brimleyorum* from Arkansas and Oklahoma. 1. Intraerythrocytic inclusion (in); scale bar = 50 μm. 2. *Chloromyxum salamandrae* plasmodia (pl) and spores (sp) adhering to gall bladder epithelium; scale bar = 20 μm. 3. *Mesocestoides* sp. tetrathyridia encapsulated in mesenteries showing 4 metacestodes (m) in single host-derived fibrous capsule (c); scale bar = 200 μm. 4. Single tetrathyridium of *Mesocestoides* sp. showing structure of parasite within capsule (c). Note the solid cellular hindbody (h), deep invagination canal (i), well-developed tetracetabulate scolex (s), distinct tegument (t), and absence of an apical organ; scale bar = 200 μm.

myxozoan include *Eurycea multiplicata griseogaster* and *E. multiplicata multiplicata* from Arkansas and *E. neotenes* from Texas (Upton et al., 1995).

Two specimens of the plagiorchid trematode, *Brachycoelium salamandrae* (Frölich, 1789) Dujardin, 1845, were found in the small intestine of a single adult male *D. brimleyorum* (92 mm SVL). *Brachycoelium elongatum* Cheng, 1958, was reported previously in *Desmognathus fuscus conanti* from Arkansas by Rosen and Manis (1976). Therefore, *D. brimleyorum* represents a

new host record for *B. salamandrae*. Other dusky salamanders have been reported to harbor *Brachycoelium* spp., including *D. ochrophaeus, D. monticola,* and *D. quadramaculatus* from North Carolina (Rankin, 1937a; Goater et al., 1987) and Tennessee (Dunbar and Moore, 1979) and *D. fuscus* from Georgia (Byrd, 1937; Parker, 1941), Illinois (Dyer et al., 1980), New York (Fischthal, 1955a), North Carolina (Rankin, 1937a), Pennsylvania (Fischthal, 1955b), and Tennessee (Dunbar and Moore, 1979). McAllister et al. (1995a, b, c) previously reported *B. salamandrae* from Arkansas in *E. multiplicata griseogaster, Rana palustris,* and *Rana sylvatica* (respectively).

Rankin's (1938) review of the genus *Brachycoelium* reduced all known species to synonymy with *B. salamandrae,* a view not universally accepted (see Dyer and Brandon, 1973). McAllister et al. (1995a) suggested adopting a conservative approach until an exhaustive revision has been completed of this morphologically variable genus.

Two immature cyclophyllidean cestodes, most closely matching the description of *Cylindrotaenia americana* (Jewell, 1916) were found in the small intestine of 2 juvenile *D. brimleyorum* (male and female, 45 and 40 mm SVL) collected in Polk County. In an unpublished thesis, Bouchard (1953) reported 1/11 (9%) *D. brimleyorum* from Oklahoma to harbor *C. americana.* Other species of *Desmognathus* have been reported previously as hosts of *C. americana,* including *D. fuscus fuscus* from New York and North Carolina, *D. ochrophaeus* and *D. monticola* from North Carolina and Tennessee, and *D. quadramaculatus* from North Carolina (see McAllister, 1991). *Cylindrotaenia americana* has been reported previously from Arkansas in *P. albagula* (McAllister et al., 1993). However, Jones (1987) considers *C. americana* to be an anuran parasite, whereas *Cylindrotaenia idahoensis* (Waitz and Mehra, 1961) Jones, 1987, has been reported only in plethodontid salamanders. Jones (1987) further suggests a reexamination of material from salamander hosts to determine whether or not the material is indeed *C. americana.*

Winter et al. (1986) reported immature nematotaeniid cestodes in *D. brimleyorum* from Arkansas. However, because these cestodes were considered to contain a single parauterine organ, the authors tentatively placed them in the family Nematotaeniidae, without generic designation. Currently, no known genera within the Nematotaeniidae have fewer than 2 uterine capsules per segment (Jones, 1987); therefore, Winter et al. (1986) may have observed a single parauterine complex per segment, as by definition in species of *Cylindrotaenia,* consists of 2 parauterine organs joined basally and sharing a common uterine mass (Jones, 1987).

Numerous tetrathyridia of *Mesocestoides* sp. were found in 5 (23%) juvenile (4 male, 1 female; 58.2 ± 2.0, 53–62 mm) and 3 (16%) adult (3 male; 68.7 ± 0.9, 67–70 mm) salamanders. These parasites were encapsulated in the mesenteries of their hosts, either in groups (Fig. 3) or as solitary worms (Fig. 4). All the tetrathyridia appeared healthy, as did the surrounding tissue outside the host-derived capsule. Each tetrathyridium possessed a single tetracetabulate scolex, which lacked hooks, and a rostellum, or apical organ, which was invaginated into a solid hindbody (i.e., lacking a primary lacuna). The tetrathyridia showed no evidence of asexual proliferation, thus conforming to the usual pattern for the genus (Conn, 1990). Furthermore, the presence of groups of tetrathyridia occurring in single host capsules (Fig. 3), but lacking morphological evidence of proliferation, supports the interpretation of Conn (1990), McAllister and Conn (1990), and McAllister et al. (1992) that such groups result from multiple encapsulation rather than asexual activity within a capsule.

This is the first definitive report of *Mesocestoides* from any salamander species; however, proteocephalan metacestodes have been reported from salamanders, including several species of *Desmognathus.* Rankin (1937a) reported "proteocephalid cysts" from *D. fuscus* in North Carolina; Dunbar and Moore (1979) reported "plerocercoids . . . probably of the order Proteocephalidea" from *D. monticola* in Tennessee; Goater et al. (1987) reported "proteocephalan plerocercoids" from *D. quadramaculatus, D. monticola,* and *D. ochrophaeus* in North Carolina. It is possible that some or all of these were actually *Mesocestoides*; because proteocephalideans have a tetracetabulate acetabulum and solid hindbody, some potential for misdiagnosis exists. The distinguishing characteristic is the presence of an apical organ only in proteocephalideans. However, Rankin (1937a) and Dunbar and Moore (1979) identified their specimens only on the basis of tetracetabulate scoleces. Tetrathyridia have been reported from 10 anuran species in North America, including 4 bufonids, 5 ranids, and 1 hylid (McAllister and Conn, 1990; McAllister et al., 1995b, c). Tetrathyridia have

also been reported from lizards in Arkansas (McAllister et al., 1991, 1992).

Two larval and 7 adult (3 male, 4 female) seuratoid nematodes, *Desmognathinema nantahalaensis* Baker, Goater, and Esch, 1987, were found in the small intestine of 3 juvenile male *D. brimleyorum* (56.0 ± 2.6, 51–60 mm). This nematode was originally described from desmognathine salamanders in North Carolina (Baker et al., 1987) and reported recently from *E. multiplicata griseogaster* and *Eurycea lucifuga* in Arkansas (McAllister et al., 1995a). *Desmognathus brimleyorum* is a new host and fifth species of plethodontid salamander known to harbor this worm. The Polk County site is approximately 160 km SE of the nearest previously recorded locale for *D. nantahalaensis* in Arkansas.

An unknown species of Ascaridoidea larvae was found encapsulated in the dorsal body wall musculature of a juvenile male (56 mm SVL) *D. brimleyorum*. Goater et al. (1987) reported similar larvae in *D. monticola, D. ochrophaeus,* and *D. quadramaculatus* from North Carolina. The present finding represents a new host record for *D. brimleyorum*.

Twenty-five specimens (10 male, 15 female) of the habronematoid nematode, *Hedruris pendula* (Leidy, 1851) Chandler, 1919, were found in the stomach of 1 juvenile male (62 mm) and 3 adult male (69.7 ± 0.3, 69–70 mm) salamanders. Specimens of *H. pendula* from *D. brimleyorum* were only about one-half the size reported for the species (Baker, 1986); however, they matched the description in every other detail, including possessing mature ova without lateral projections and the appropriate ratio for measurements of the distance from end of body to anus. *Desmognathus brimleyorum* is a new host and Arkansas a new locality for *H. pendula*. The species has been reported previously in other North American vertebrates (see Baker, 1987). A similar species, *H. siredonis* Baird, 1858, has been reported from various salamanders, including *D. fuscus* from Georgia (Baker, 1986, 1987).

A total of 16 (7 male, 9 female) seuratoid nematodes, *Omeia papillocauda* Rankin, 1937, were found in the stomach of 2 juvenile (male and female, 57 mm SVL) and 4 adult (2 male, 2 female, 76.8 ± 5.5, 67–92 mm) *D. brimleyorum*. A new host and locality record is documented for *O. papillocauda*. This nematode was described from *Desmognathus* spp. and *Gyrinophilus porphyriticus danielsi* in North Carolina (Rankin, 1937b). It exhibits little host specificity and is a common parasite of numerous plethodontid salamanders from North America (see Baker, 1987). In addition, survey data indicate that prevalence of infection with *O. papillocauda* can vary widely depending on the host species and geographic locality and has been reported to range from 4 to 8% in *D. ochrophaeus,* 13 to 30% in *D. quadramaculatus,* and 20 to 40% in *D. monticola* (Dunbar and Moore, 1979; Baker et al., 1987; Goater et al., 1987; Joy et al., 1993).

A single male and 22 female oxyurid nematodes, *Batracholandros magnavulvaris* (Rankin, 1937) Petter and Quentin, 1976, were found in the rectum of 11 male and 1 female (64.5 ± 4.0, 51–92 mm) *D. brimleyorum*. Of the infected salamanders, 6 (50%) were juveniles (54.8 ± 1.1, 51–58 mm) and 6 (42%) were adults (75.2 ± 4.2, 65–92 mm). In addition, the smallest infected salamander (51 mm SVL) had 4 worms, whereas the largest (92 mm SVL) had a single *B. magnavulvaris*. Mean intensity of *B. magnavulvaris* was slightly higher in smaller salamanders, as juveniles had 2.5 ± 0.7 (range 1–4) worms per host whereas adults had 1.9 ± 0.8 (1–6) worms per host. Also, there was a 2-fold difference in prevalence of infection depending on the month of collection, as 34% of *D. brimleyorum* collected in mid-March (N = 29 examined, 57.2 ± 3.6 mm) versus only 17% collected in late May (N = 12 examined, 59.8 ± 4.0 mm) harbored *B. magnavulvaris*. This is probably the result of salamanders congregating for courtship and breeding in March–April. Prevalence data can vary greatly, as Winter et al. (1986) reported 77% of the *D. brimleyorum* they examined had *B. magnavulvaris* with a mean intensity of 4.6 (range 1–19). Similarly, prevalence in other desmognathine hosts and locales can be variable and has been reported to range from 6 to 27% in *D. fuscus,* 38 to 50% in *D. monticola,* 14 to 25% in *D. ochrophaeus,* and 7 to 85% in *D. quadramaculatus* (Fischthal, 1955a; Dunbar and Moore, 1979; Dyer et al., 1980; Goater et al., 1987; Joy et al., 1993). This nematode exhibits little host specificity and infects other plethodontids (Muzzall, 1990) as well as salamandrids (Rankin, 1937b; Baker, 1987).

A single acanthocephalan cystacanth was recovered from the body musculature of a juvenile (53 mm SVL) male *D. brimleyorum*. Winter et al. (1986) and McAllister et al. (1993) previously have reported cystacanths in *D. brimleyorum* and *P. albagula* from Arkansas, respectively. Cysts

of *Acanthocephalus acutulus* Van Cleave, 1931, have been reported from various salamanders, including *D. fuscus* and *D. quadramaculatus* from North Carolina (Rankin, 1937a). In addition, larval *Centrorynchus conspectus* Van Cleave and Pratt, 1940, has been reported from the colon of *D. quadramaculatus* and *D. monticola* in North Carolina (Goater et al., 1987).

The most common parasite of *D. brimleyorum* were larval intradermal mites, *Hannemania* sp. Twenty-eight infected salamanders measured 62.3 ± 2.4 (range 40–92 mm SVL), whereas the 13 uninfected salamanders were 48.7 ± 6.6 (19–93 mm). Unengorged and partially engorged larvae were encapsulated primarily on the appendages and digits by host dermal connective tissue causing nodular projections of the digital skin. Specific identity of *Hannemania* sp. was not possible because only larvae were found. However, *H. dunni* Sambon, 1928, was reported previously by Winter et al. (1986) on *D. brimleyorum* from Polk County, Arkansas. Prevalence was reported to be 77% and intensity averaged 21 chiggers/host (Winter et al., 1986). *Hannemania dunni* was described from *D. fuscus fuscus* from an unnamed locality in the southeastern United States (Sambon, 1928) and has been reported on *Desmognathus auriculatus* from Cass, Lee, and McLennon counties, Texas, and *D. brimleyorum* from Montgomery and Polk counties, Arkansas, and LeFlore and Woods counties, Oklahoma (Loomis, 1956). *Hannemania* sp. has also been reported in Arkansas on *E. multiplicata griseogaster* (McAllister et al., 1995a) and *R. palustris* (McAllister et al., 1995b).

In addition to the parasites of *D. brimleyorum* already mentioned, Winter et al. (1986) reported *Oswaldocruzia pipiens* (syn. *O. euryceae* Reiber, Byrd, and Parker, 1940) and an *Oxysomatium* sp. Railliet and Henry, 1916, from this host species. However, the latter genus is known only from Old World amphibians and reptiles (Baker, 1987), and other reports from North America (Walton, 1927; Fischthal, 1955a; Landewe, 1963) are doubtful. Most likely the parasite is *Cosmocercoides* sp.

In summary, 8 new host and 2 new distributional records are reported for parasites of *D. brimleyorum* from Arkansas. The parasite community of our sample of *D. brimleyorum* is variable yet somewhat similar when compared to other desmognathine salamanders. Goater et al. (1987) surveyed 4 species of desmognathine salamanders and reported isolationist parasite infracommunities that they correlated with host diet, size, and habitat preferences. Host range, diet, and parasite life cycles are important in determining what species are present and how intense the infection may be in a given host. Our survey tends to support Aho's (1990) contention of a depauperate noninteractive community structure observed in helminth communities of most amphibians and reptiles.

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Scutogyrus gen. n. (Monogenea: Ancyrocephalidae) for *Cichlidogyrus longicornis minus* Dossou, 1982, *C. l. longicornis*, and *C. l. gravivaginus* Paperna and Thurston, 1969, with Description of Three New Species Parasitic on African Cichlids

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ABSTRACT: *Scutogyrus* gen. n. (Monogenae: Ancyrocephalidae) is defined for *Cichlidogyrus longicornis minus* Dossou, 1982, on *Sarotherodon melanotheron* (Cichlidae). This new genus is characterized by a dorsal transversal bar enlarged laterally with, in its median portion, 2 very long auricles hollow at their base and by the ventral transversal bar arched, rigid, and supporting 1 large, thin, oval plate. In agreement with Douëllou (1993), *C. longicornis* Paperna and Thurston, 1969, on *Oreochromis niloticus* and *C. gravivaginus* Paperna and Thurston, 1969, on *O. leucostictus* are considered valid; the new combinations *Scutogyrus longicornis* (Paperna and Thurston, 1969) and *S. gravivaginus* (Paperna and Thurston, 1969) are proposed for them. Three new species are also described: *Scutogyrus bailloni* sp. n. on *Sarotherodon galilaeus, S. ecoutini* sp. n. on *S. occidentalis,* and *S. chikhii* sp. n. on *O. mossambicus.* A key to the species of *Scutogyrus* is given.

RESUME: Un nouveau genre *Scutogyrus* gen. n. (Monogenea: Ancyrocephalidae) est défini pour *Cichlidogyrus longicornis minus* Dossou, 1982 parasite de *Sarotherodon melanotheron.* Le nouveau genre est caractérisé par la morphologie de la barre tranversale dorsale élargie latéralement et munie de 2 très longs auricules et de la barre transversale ventrale arquée, rigide, supportant 1 mince plaque ovoide. En accord avec Douëllou (1993) *Cichlidogyrus longicornis* Paperna et Thurston, 1969 de *Oreochromis niloticus* et *C. gravivaginus* Paperna et Thurston, 1969 de *O. leucostictus* sont considerés comme de bonnes espèces, nous proposons les nouvelles combinaisons *Scutogyrus longicornis* (Paperna et Thurston, 1969) et *S. gravivaginus* (Paperna et Thurston, 1969). Trois nouvelles espèces sont décrites: *S. bailloni* chez *Sarotherodon galilaeus, S. ecoutini* chez *S. occidentalis* et *S. chikhii* chez *Oreochomis mossambicus.* On propose une clé de détermination des *Scutogyrus.*

KEY WORDS: *Scutogyrus* gen. n., Monogenea, gills parasite, Cichlidae, freshwater, Africa.

This article addresses the finding, in West and Central Africa, on *Oreochromis niloticus* (L., 1758), on *O. mossambicus* (Peters, 1852), and on 3 species of *Sarotherodon* (*S. galilaeus* (L., 1758), *S. melanotheron* Rüppel, 1852, and *S. occidentalis* (Daget, 1962)) of monogeneans that, by the structure of their haptor, clearly belong to *Cichlidogyrus longicornis.* After careful examination of these parasites, it is believed that they represent, in this area, some specificity toward the hosts.

Materials and Methods

Fish were captured in various rivers and lagoons of Senegal, Gambia, Guinea, the Ivory Coast, and the Congo using gill nets or cast nets or after poisoning with Rotenone (Predatox®). Fish were either dissected on site immediately after capture or kept fresh and dissected later in the laboratory. In both cases, the left gill arches, separated by dorsal and ventral sections, were frozen at −20°C or in liquid nitrogen until examination. To verify the specific identity of host fishes, the carcasses were numbered, fixed, and preserved in formalin. After thawing, the parasites were detached from the gill using a strong water current and transferred individually with a mounted needle directly into a drop of ammonium picrate–glycerine mixture, according to Malmberg (1957). The preparation was then covered with a round coverslip, and after several hours (necessary for proper impregnation by the mounting medium) the coverslip was sealed with Glyceel (GURR-BDH Chemicals Ltd.). From these preparations, drawings were made of the sclerotized pieces of the haptor and of the copulatory complex using a camera lucida. All measurements were made with a digitizer. Measurements, given in micrometers as the range mean ± standard deviation (minimum–maximum), are those proposed by Gussev (1962) (Fig. 1).

The method of lettering and numbering the haptoral pieces is that adopted at ICOPA IV (Euzet and Prost, 1981), whereas the method of naming is that proposed by Pariselle and Euzet (in press a): *uncinulus* for the

little marginal hooklets, and *gripus* for the large median hooks.

Results

The discovery of Monogenea whose haptor presents a morphology similar to that of *Cichlidogyrus longicornis,* but processing a penis and vagina with different morphologies, implying reproductive isolation, leads to the description of 3 new species. This characteristic of the haptor has profoundly influenced taxonomic studies. Until now, and despite the differences in the size and shape of the copulatory complex, authors have only distinguished subspecies; therefore, it has been necessary to reexamine the taxonomic status of the 3 subspecies already described.

After careful examination, it is believed that these species, possessing the very particular haptor characteristics of *C. longicornis* and specificity toward the genera *Oreochromis* and *Sarotherodon,* lead to the proposal of a new genus. The name *Scutogyrus* gen. n. is proposed to point out the shield-like shape (*scutus* in Latin) of the ventral transverse bar.

It is certain that *Scutogyrus* gen. n. is very close to *Cichlidogyrus,* particularly in the presence of auricles on the dorsal transverse bar. The 2 genera are both gill parasites of African cichlids, but a detailed examination of the haptor shows some significant differences between the two genera: very long auricles and lateral outgrowths of the dorsal transverse bar and rigidity of the ventral transverse bar supporting 1 large sclerotized plate on *Scutogyrus.* The anatomical differences of the haptor translate into functional peculiarities of this organ and therefore indicate an original attachment of *Scutogyrus* on the gill of the host fish.

Scutogyrus gen. n.

Ancyrocephalidae. Three pairs of cephalic glands. Two posterior ocelli with crystalline lenses. Two small anterior ocelli, not always present. Simple intestinal branches joined posteriorly. Two pairs of gripi, 1 dorsal and 1 ventral. Dorsal transverse bar highly arched, enlarged laterally, winged, having in its median portion 2 very long auricles hollow at their bases. Ventral transverse bar arched, rigid, supporting 1 large, thin, oval plate marked by fan-shaped median thickenings. Fourteen uncinuli. Testis median, posterior. Vas deferens dextral, not encircling intestinal branch. Seminal vesicle present. One prostatic reservoir. Male copulatory complex with penis and accessory piece. Ovary median pretesticular. Vaginal opening sublateral dextral. Vagina sclerified. Seminal receptacle present. Parasites of African Cichlidae.

TYPE SPECIES: *Scutogyrus minus* (Dossou, 1982) comb. n. for *Cichlidogyrus longicornis minus* Dossou, 1982.

TYPE HOST: *Sarotherodon melanotheron* Rüppel, 1852.

REMARKS: The choice of the type species of this new genus was complex because, when establishing *Cichlidogyrus longicornis,* Paperna and Thurston (1969) distinguished two subspecies. For the first cited *C. longicornis longicornis* (only 3 specimens from *Oreochromis niloticus,* which are probably lost), there is no type material. Paperna's (1979) designation of a parasite of *Sarotherodon galilaeus,* collected from Volta Lake in Ghana, as holotype for *C. l. longicornis* is erroneous because it contradicts the rules of the International Code of Zoological Nomenclature. This specimen cannot represent a lectotype, because it does not belong to the type series of *C. l. longicornis.* For the second subspecies (*C. longicornis gravivaginus* from *Tilapia leucosticta*), there exists, in the collection of the Musée Royal de l'Afrique Centrale at Tervuren, 1 preparation (MT 35 932) that was designated by Paperna (1979) as the "type" of this subspecies. According to D. C. Kritsky (pers. comm.), the type for *C. longicornis* cannot be chosen from the subset collected from *Tilapia leucosticta* because these specimens are not part of the series used to establish the nominotypical subspecies. The type for the new genus *Scutogyrus* can be any of the valid species we have. Because *Cichlidogyrus minus* Dossou, 1982, is a well-described species, without any doubt concerning the morphology of its haptor and its host (see later), it was selected to be the type species of *Scutogyrus.*

Scutogyrus minus (Dossou, 1982) comb. n. (Figs. 2, 3)

Cichlidogyrus longicornis minus Dossou, 1982.

HOST: *Sarotherodon melanotheron* Rüppel, 1852.

SITE: Gills.

TYPE LOCALITY: Ouémé River, Bénin.

MATERIAL STUDIED: Thirty individuals coming from the Ivory Coast, stained and mounted according to Malmberg (1957).

Material deposited at the Muséum National d'Histoire Naturelle, Paris: 460 H.F. Tg. 55 (1 specimen), Tg. 56 (1 specimen), Tg. 57 (1 spec-

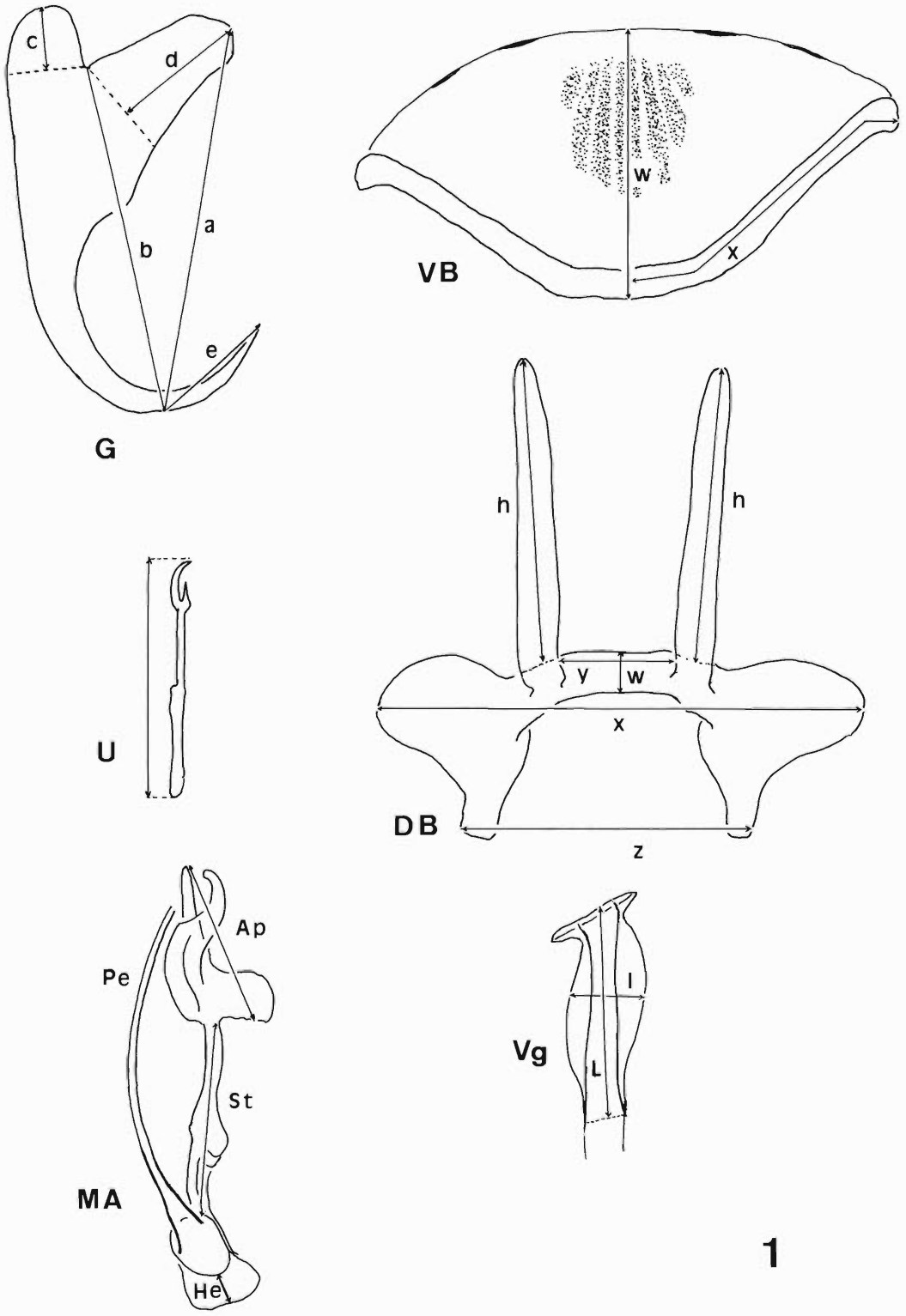

Figure 1. Measurements used in this study. Ap = accessory piece, DB = dorsal transverse bar, G = gripus, He = heel, MA = male apparatus, Pe = penis, St = stalk, U = uncinuli, VB = ventral transverse bar, Vg = vagina.

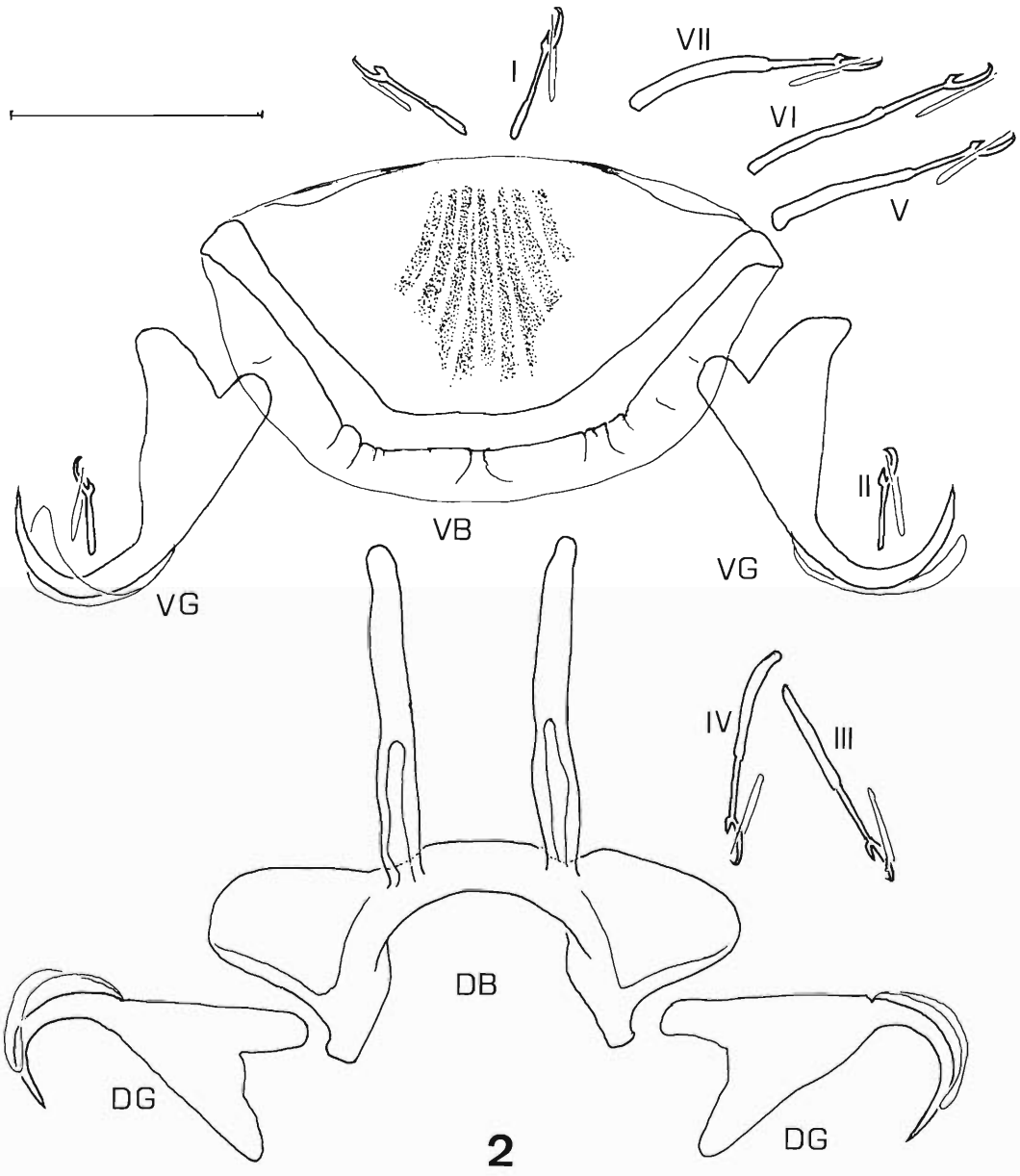

Figure 2. ***Scutogyrus minus*** **(Dossou, 1982). Haptoral sclerites. DG = dorsal gripus, VG = ventral gripus, I-VII = uncinulus. Scale bar = 30 μm.**

imen); at The Natural History Museum, London: Reg. No. 1994.4.7.1 (1 specimen); at the Musée Royal d'Afrique Centrale, Tervuren: M.R.A.C. 37.357 (2 specimens).

S. minus was also found (nobis) on the same host in the Ivory Coast: at the Layo research station, Ebrié Lagoon (4 January 1991); in Bakré Lake, Abidjan offshore bar (3 March 1992); in Ayamé Lake, Bia River (4 November 1991); and in the Comoé River at Abengourou (29 January 1991). In Guinea in the Konkouré River at Wassou bridge (17 April 1992); and in the Bourouma River 10 km SW from La Ramié (19 April 1992).

DESCRIPTION: Adults 665 ± 85.5 (509–884) long, 106 ± 18.1 (72–139) wide at level of vagina.

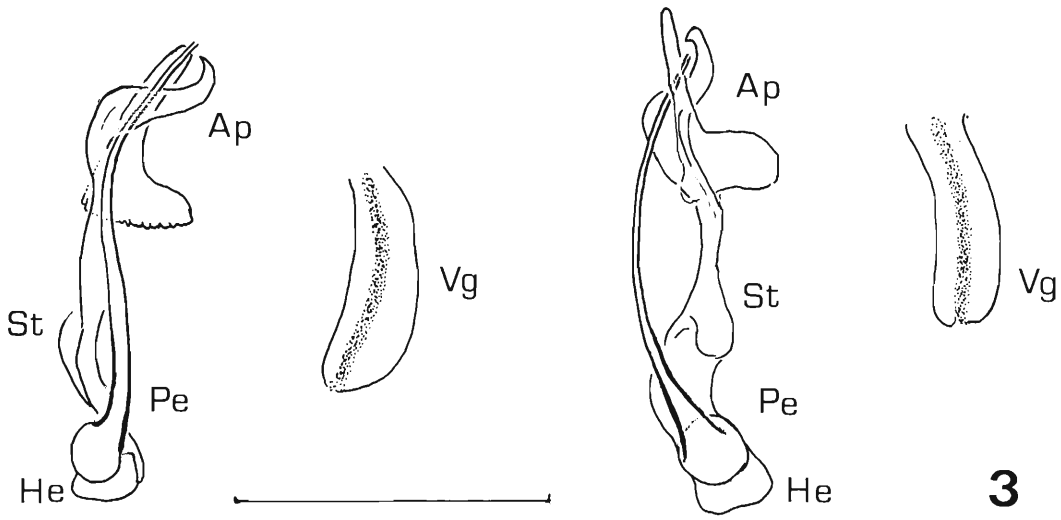

Figure 3. ***Scutogyrus minus*** **(Dossou, 1982). Two genital apparatus. Scale bar = 30 μm.**

Pharynx 56 ± 7.9 (38–70) at its widest point. Dorsal gripus with root fused to shaft, blade arched: a = 30 ± 1.1 (24–33), b = 24 ± 1.4 (19–27), c = 8 ± 1.2 (5–11), d = 11 ± 1.2 (8–14), e = 9 ± 0.7 (8–11). Dorsal transverse bar: x = 62 ± 2.6 (55–67), w = 6 ± 0.7 (5–8), y = 14 ± 0.8 (13–16), z = 31 ± 3.4 (25–38), h = 39 ± 1.6 (36–43). Ventral gripus comparable to dorsal, with root more fused to shaft: a = 29 ± 0.9 (27–31), b = 29 ± 1.4 (26–32), c = 4 ± 1.1 (1–7), d = 8 ± 1.2 (6–11), e = 12 ± 0.8 (10–14). Ventral transverse bar arched and rigid: x = 39 ± 1.8 (34–43), w = 31 ± 3.4 (18–37). Uncinulus: I = 16 ± 0.6 (15–17), II = 12 ± 0.4 (11–14), III = 28 ± 1.1 (24–30), IV = 29 ± 1.2 (24–31), V = 29 ± 0.9 (26–30), VI = 25 ± 1.1 (22–28), VII = 25 ± 0.9 (23–28).

Penis slightly arched, tubular: Pe = 43 ± 1.5 (39–45), He = 3 ± 0.5 (3–4). Accessory piece with large widening at the base, terminates in 2 unequal opposed outgrowths, largest hook-shaped: Ap = 17 ± 0.9 (15–19), St = 26 ± 1.8 (21–30). Vagina forms a moderately wide tube: L = 20 ± 1.5 (16–23), l = 6 ± 0.5 (5–7).

REMARKS: For Douëllou (1993), the creation by Dossou (1982), on the basis of measurements (inferior) and morphology of haptoral and sclerotized genitalia pieces, the subspecies *Cichlidogyrus longicornis minus* is not justified. We observed that the difference related by Dossou (1982) to the haptor was not confirmed; however, sufficient differences were noted in the morphology of the accessory piece and the vagina (see comparisons with the following species). Thus, we propose to elevate to species status the subspecies *C. longicornis minus* described by Dossou (1982).

Scutogyrus longicornis (Paperna and Thurston, 1969) comb. n. (Figs. 4, 5)

Cichlidogyrus longicornis longicornis Paperna and Thurston, 1969.

Cichlidogyrus longicornis Paperna and Thurston, 1969, of Douëllou, 1993.

HOST: *Oreochromis niloticus* (L., 1758).

SITE: Gills.

TYPE LOCALITY: Lakes George and Albert, Uganda.

MATERIAL STUDIED: Thirty individuals from Senegal and the Ivory Coast, stained and mounted according to Malmberg (1957).

Material deposited at the Muséum National d'Histoire Naturelle, Paris: 461 H.F. Tg. 58 (1 specimen), Tg. 59 (4 specimens); at The Natural History Museum, London: Reg. No. 1994.4.7.2 (1 specimen); at the Musée Royal d'Afrique Centrale, Tervuren: M.R.A.C. 37.358 (3 specimens).

This species was found on *O. niloticus* in Ghana (Paperna, 1969), Egypt (Ergens, 1981), the Philippines (Natividad et al., 1986; Bondad-Reantaso and Arthur, 1990), the Ivory Coast (*nobis*) in Ayamé Lake, Bia River (4 November 1991), the Comoé River at Abengourou (29 January 1991), and the IDESSA Research Station at

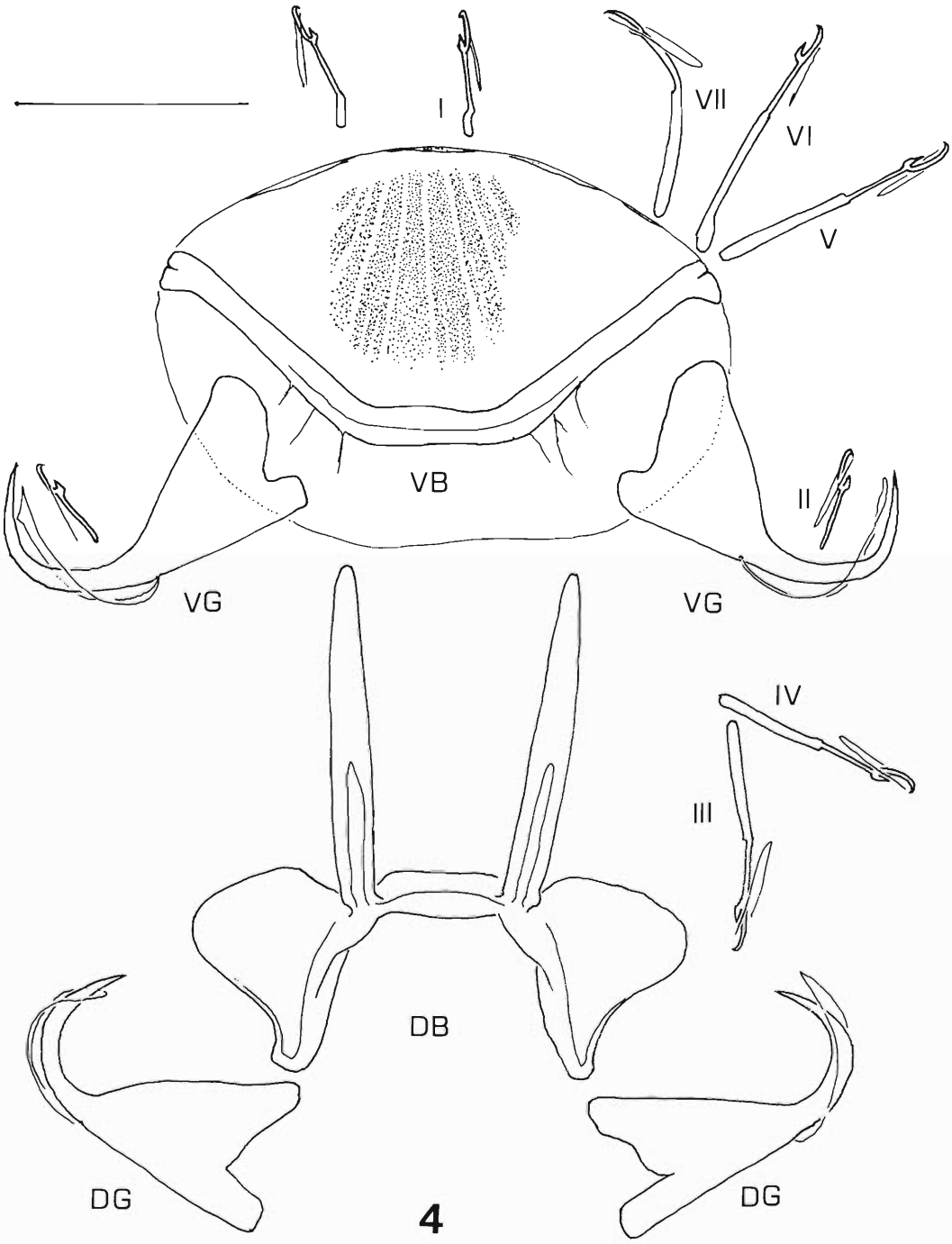

Figure 4. ***Scutogyrus longicornis*** **(Paperna and Thurston, 1969). Haptoral sclerites. DG = dorsal gripus, VG = ventral gripus, I-VII = uncinuli. Scale bar = 30 μm.**

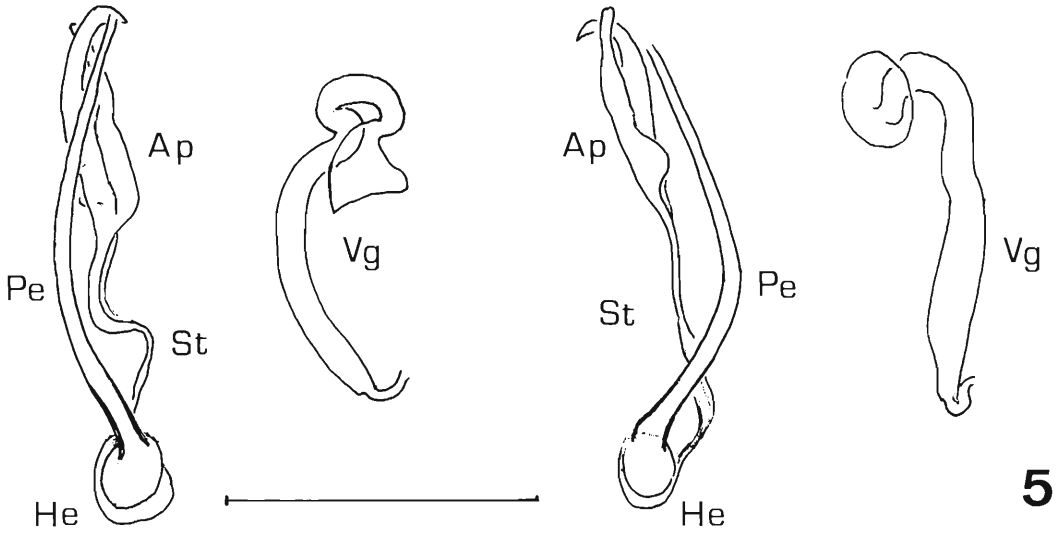

Figure 5. ***Scutogyrus longicornis*** **(Paperna and Thurston, 1969). Two genital apparatus. Scale bar = 30 μm.**

Bouaké; also on *Sarotherodon galilaeus*(?) and *Tilapia zillii*(?) in Ghana (Paperna, 1969), on *Oreochromis mortimeri* in Lake Kariba Zimbabwe (Douëllou, 1993), and on *O. aureus* (nobis) in the Senegal River at Djouj National Park (13 April 1991).

DESCRIPTION: Adults 786 ± 99.5 (541–971) long, 157 ± 15.8 (127–193) wide at vagina. Pharynx 82 ± 3.8 (72–90) at its widest point. Dorsal gripus with root fused to shaft, blade regularly arched: a = 33 ± 1.4 (30–39), b = 28 ± 2 (21–32), c = 8 ± 2 (4–16), d = 11 ± 1.4 (8–16), e = 10 ± 0.8 (8–12). Dorsal transverse bar: x = 62 ± 2.6 (56–67), y = 15 ± 1.1 (13–18), z = 30 ± 2.5 (25–36), w = 5 ± 0.6 (4–6), h = 41 ± 1.8 (37–45). Ventral gripus: a = 34 ± 1.4 (30–37), b = 33 ± 1.4 (30–37), c = 4 ± 0.7 (3–6), d = 10 ± 1 (8–13), e = 13 ± 1 (10–15). Ventral transverse bar arched and rigid: x = 41 ± 2.2 (37–47), w = 34 ± 3.1 (27–41). Uncinulus: I = 17 ± 0.8 (16–19), II = 13 ± 0.4 (12–14), III = 31 ± 1.5 (28–34), IV = 33 ± 1.1 (30–36), V = 33 ± 1.2 (28–36), VI = 29 ± 1.6 (26–35), VII = 28 ± 1.1 (26–32).

Penis slightly arched, tubular: Pe = 48 ± 3.1 (40–56); with a poorly developed heel: He = 2 ± 0.5 (1–3). Accessory piece with small enlargement at base, terminates in 2 opposing unequal and straight outgrowths: Ap = 21 ± 1.2 (19–24), St = 20 ± 2.8 (15–25). Sinuous vagina a narrow tube: L = 41 ± 4.4 (34–55), l = 4 ± 0.9 (3–7).

REMARKS: This species can be distinguished from *Scutogyrus minus* by the dimension of the penis (48 vs. 43), the heel (2 vs. 3), and the accessory piece (length: 21 vs. 17, enlargement small vs. large widening), by the shape of the largest terminal outgrowth of this piece (straight vs. hook-shaped), and by the morphology and the length of the sclerified portion of the vagina (narrow tube vs. wider, length 41 vs. 20). This parasite corresponds to *Cichlidogyrus longicornis longicornis* described also on *O. niloticus* by Paperna and Thurston (1969) and to *C. longicornis* described by Douëllou (1993) on *O. mortimeri,* and according to this author *Cichlidogyrus longicornis longicornis* needs to be elevated to specific status.

Scutogyrus gravivaginus (Paperna and Thurston, 1969) comb. n. (Figs. 6, 7)

Cichlidogyrus longicornis gravivaginus Paperna and Thurston, 1969.

Cichlidogyrus gravivaginus Paperna and Thurston, 1969, of Douëllou, 1993.

HOST: *Tilapia leucosticta* = *Oreochromis leucostictus* (Trewavas, 1933).

SITE: Gills.

TYPE LOCALITY: Jinja, Lake Victoria, Uganda.

MATERIAL STUDIED (holotype): MT 35 932 of the Musée Royal d'Afrique Centrale, Tervuren.

This species was also found by Paperna (1979)

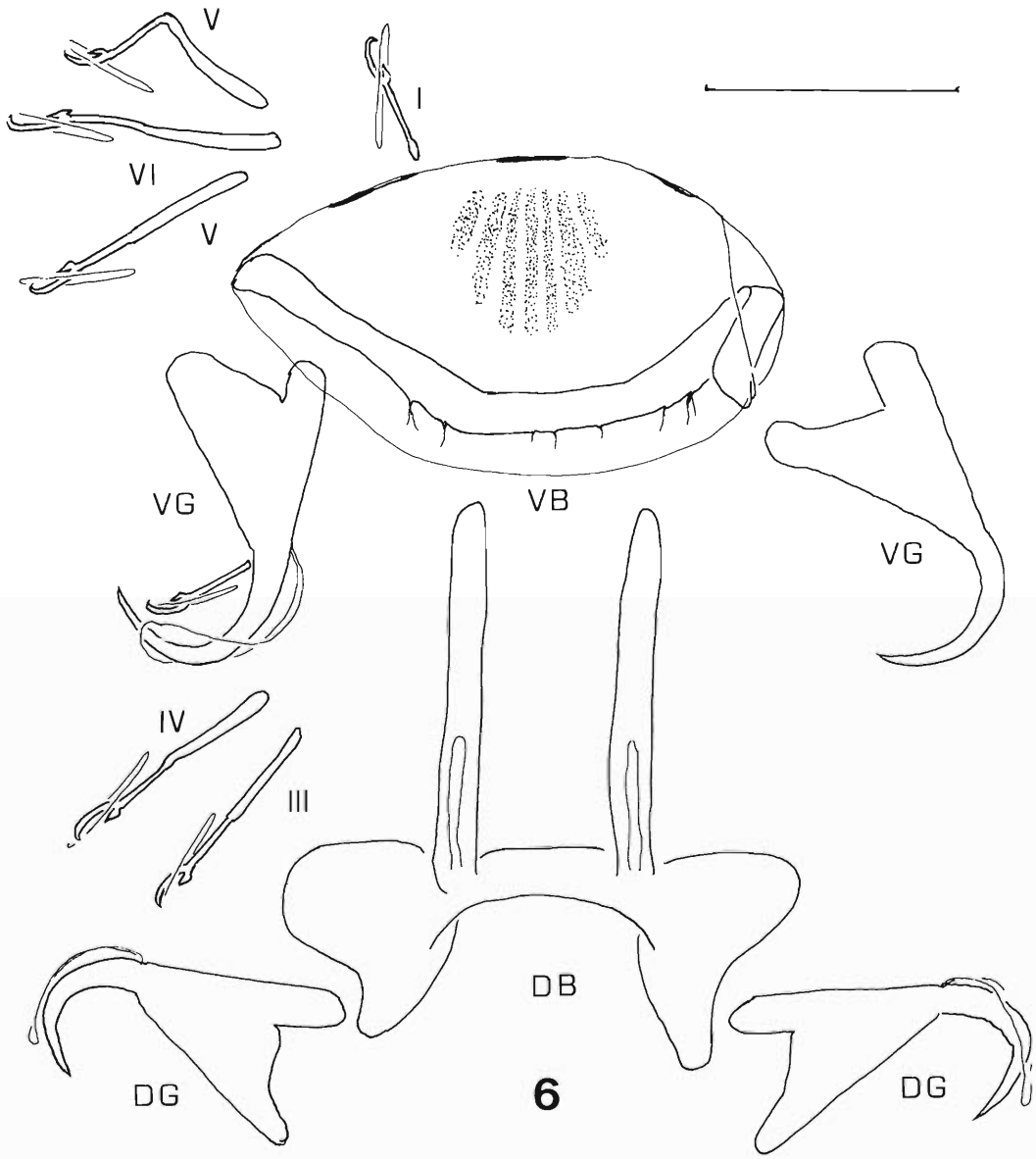

Figure 6. ***Scutogyrus gravivaginus*** **(Paperna and Thurston, 1969). Haptoral sclerites. DG = dorsal gripus, VG = ventral gripus, I-VII = uncinuli. Scale bar = 30 μm.**

on *Oreochromis variabilis* in Lake Victoria, Uganda, and by Douëllou (1993) on *O. mortimeri* in Lake Kariba, Zimbabwe.

DESCRIPTION: The condition of the holotype did not permit detailed study of the anatomy, as only a few sclerified pieces of the haptor and of the genital system could be observed.

Dorsal gripus (anchors X of original description): a = 35, b = 28, c = 8, d = 12, e = 10. Dorsal transverse bar (bar X), enlarged into wings at each side, has 2 very long median appendices: x = 58, y = 16, z = 37, w = 5, h = 41. Ventral gripus (anchor V of original description): a = 35, b = 30, c = 8, d = 12, e = 11. Ventral transverse bar (bar V) has 1 thin, oval plate transversally arranged: x = 77, w = 32. Uncinulus: U = 25–30, except I and II U = 15. Male copulatory complex composed of a thin tubular penis (Pe = 77), whose basal bulb is marked by 1 trapezoidal heel (He = 16) and 1 accessory piece enlarged to

form a triangle, finished in 2 opposed spikes (Ap = 26). The accessory piece is linked to base of penis by a sinuous stalk: St = 35. S-shaped tubular vagina: L = 55; diameter varies from 15 (at opening) to 6.

REMARKS: The examination of the type specimen showed that the measurements of the haptoral pieces correspond, very nearly, to those of the original description, while those for the copulatory complex differ (77 vs. 53–57). However, the morphology of this complex, which consists of a penis with a developed heel and an accessory piece with a triangular enlargement, leads us (according to Douëllou, 1993) to believe that an inversion was introduced in the publication of Paperna and Thurston (1969, p. 21) between the legends of Figure 3c and 3d. Therefore, Figure 3d given as *Cichlidogyrus longicornis longicornis* would be that of *Cichlidogyrus longicornis gravivaginus* and conversely for Figure 3c. This inversion would help to explain the difference noted in the size of the penis between the type material and the original description.

This specimen, regarding the drawings and measurements, shows no significant differences with the subspecies *C. longicornis gravivaginus* as described by Paperna and Thurston (1969) or *C. gravivaginus* as described by Douëllou (1993). The great differences in the measurements of the penis (77 vs. 43 or 48), the heel (16 vs. 3 or 2), and the vagina (55 vs. 20 or 41 in length, 15 vs. 6 or 4 maximum diameter) between this species and the one previously cited are sufficient to consider it a valid species.

Scutogyrus bailloni sp. n. (Figs. 8, 9)

HOST: *Sarotherodon galilaeus* (L., 1758).

SITE: Gills.

TYPE LOCALITY: Mékrou River at "W" National Park, Niger (18 February 1993).

MATERIAL STUDIED: Twenty-four specimens stained and mounted according to Malmberg (1957).

Holotype and 1 paratype deposited at the Muséum National d'Histoire Naturelle, Paris: 462 H.F. Tg. 60.

Paratypes deposited at the Muséum National d'Histoire Naturelle, Paris: 462 H.F. Tg. 61 (2 specimens); at The Natural History Museum, London: Reg. No. 1994.4.7.3 (2 specimens); at the Musée Royal d'Afrique Centrale, Tervuren: M.R.A.C. 37.359 (2 specimens).

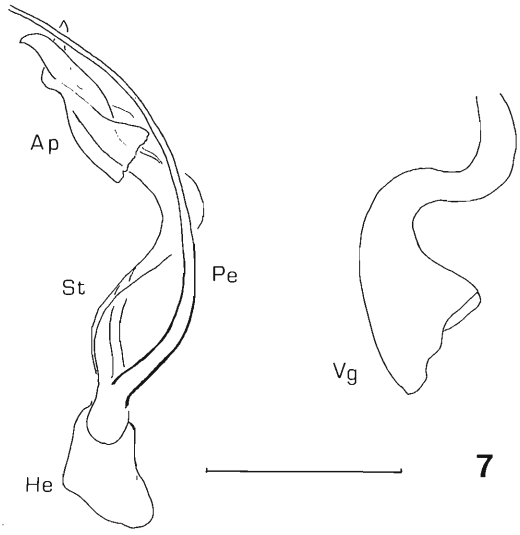

Figure 7. ***Scutogyrus gravivaginus*** **(Paperna and Thurston, 1969). Genital apparatus. Scale bar = 30 μm.**

This species was also found on the same host in the Kou River (Volta Noire River tributary) at Bama near Bobodioulasso, Burkina Fasso (12 August 1991).

DESCRIPTION: Adult 816 ± 160 (502–1114) long; 159 ± 26.5 (103–212) wide at level of vagina. Pharynx 95 ± 16 (66–128) at widest point. Dorsal gripus with root fused to shaft, blade arched: a = 31 ± 1.1 (28–33), b = 25 ± 1.1 (23–27), c = 8 ± 1 (6–10), d = 11 ± 1.2 (9–13), e = 9 ± 0.6 (7–11). Dorsal transverse bar: x = 60 ± 2.6 (55–64), w = 6 ± 0.9 (4–7), y = 14 ± 1 (13–17), z = 30 ± 2.1 (27–34), h = 37 ± 1.9 (34–42). Ventral gripus comparable to dorsal: a = 31 ± 0.8 (29–32), b = 29 ± 1.2 (26–31), c = 5 ± 1.2 (2–8), d = 9 ± 1.1 (7–11), e = 13 ± 1 (10–15). Ventral transverse bar arched and rigid: x = 40 ± 1.8 (36–43), w = 34 ± 2.9 (28–39). Uncinulus: I = 17.4 ± 0.8 (16–19), II = 13 ± 0.4 (12–14), III = 27 ± 1.3 (24–30), IV = 29 ± 1.7 (24–32), V = 29 ± 1.1 (27–32), VI = 25 ± 1.4 (24–30), VII = 25 ± 1.5 (22–29).

Penis long, with basal globular bulb and large trapezoidal heel: Pe = 84 ± 3.4 (76–90), He = 17 ± 1.9 (14–23). Accessory piece terminates in a single hook: Ap = 31 ± 3 (26–39). Stalk thick: St = 39 ± 6.3 (23–47). Vagina long, with crenelated lining, terminates in a thin-walled folded pocket: L = 69 ± 5.5 (56–83), l = 11 ± 4.2 (5–19).

REMARKS: This species is easily distinguishable from the preceding species by the size of the

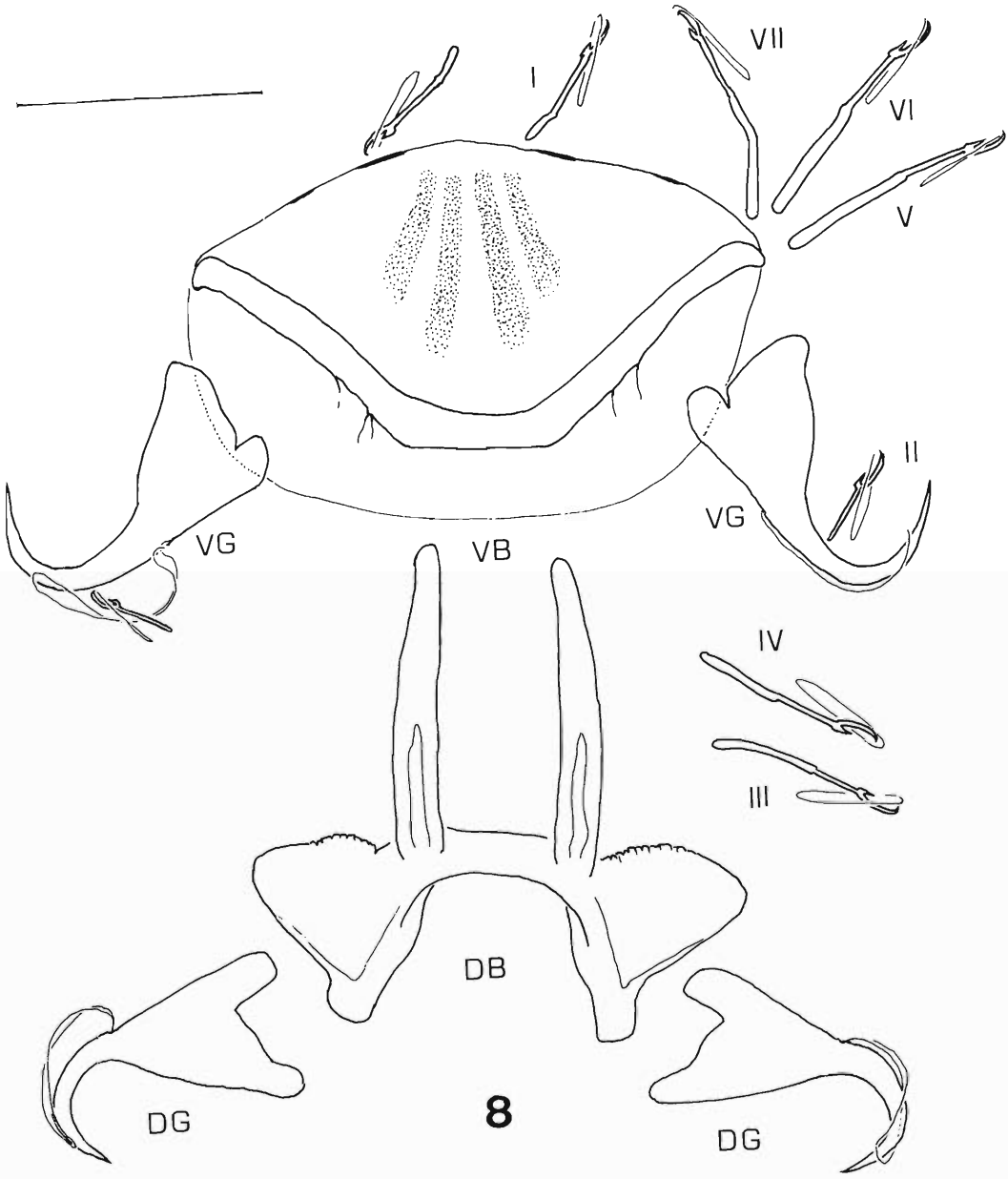

Figure 8. ***Scutogyrus bailloni*** **sp. n. Haptoral sclerites. DG = dorsal gripus, VG = ventral gripus, I-VII = uncinuli. Scale bar = 30 μm.**

male copulatory organ (84 vs. 77 at the most) and the vagina (69 vs. 55 at the most), and by the shape of the extremity of the accessory piece (single vs. double for all previous species) and of the stalk (thick vs. thin). Therefore, we consider it a new species and propose the name *Scutogyrus bailloni* in honor of F. Baillon, who kindly assisted in the acquisition of material.

The parasite of *S. galilaeus*, deposited under the name *Cichlidogyrus longicornis longicornis* (Paperna and Thurston, 1969) at the Music Royal d'Afrique Centrale, Tervuren, with the number MT 35 931, was also examined. Despite the state of the material, the length of the male copulatory complex (48) shows that it is not the species from *S. galilaeus* as described herein. The

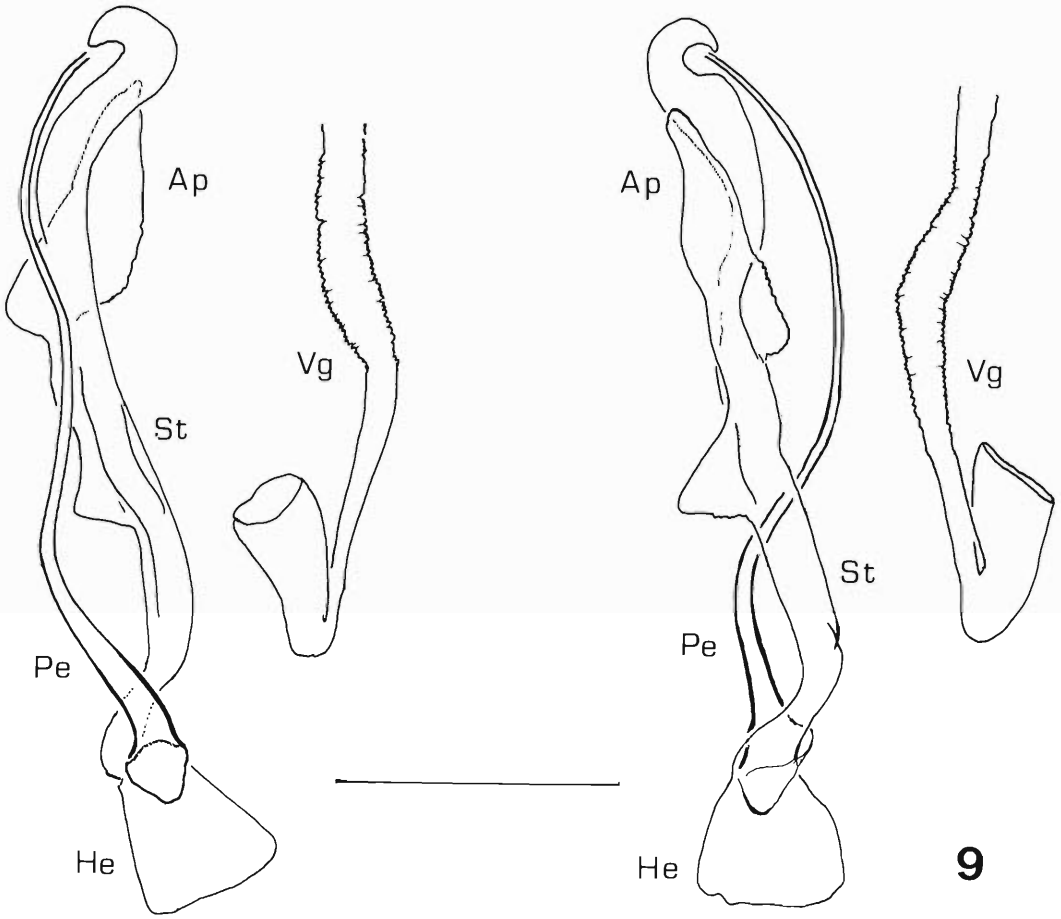

Figure 9. ***Scutogyrus bailloni*** **sp. n. Two genital apparatus. Scale bar = 30 μm.**

morphology of the accessory piece is close to that of *S. minus*, but because the vagina could not be observed no conclusions could be drawn.

Scutogyrus ecoutini sp. n.
(Figs. 10, 11)

HOST: *Sarotherodon occidentalis* (Daget, 1962).

SITE: Gills.

TYPE LOCALITY: Bourouma River 10 km SW from La Ramié, Guinea (19 April 1992).

MATERIAL STUDIED: Twenty-six specimens stained and mounted according to Malmberg (1957).

Holotype deposited at the Muséum National d'Histoire Naturelle, Paris: 463 H.F. Tg. 62.

Paratypes deposited at the Muséum National d'Histoire Naturelle, Paris: 463 H.F. Tg. 63 (2 specimens); at The Natural History Museum, London: Reg. No. 1994.4.7.5 (1 specimen); at the Musée Royal d'Afrique Centrale, Tervuren: M.R.A.C. 37.360 (2 specimens).

DESCRIPTION: Adult 652 ± 88.2 (533–833) long, 118 ± 13.6 (91–143) wide at level of vagina. Pharynx 69 ± 10.3 (51–104) wide at its widest point. Dorsal gripus with root fused to shaft, blade arched: a = 32 ± 1.1 (27–34), b = 27 ± 1.3 (24–29), c = 8 ± 1.2 (5–11), d = 10 ± 1.4 (7–13), e = 10 ± 0.8 (8–12). Dorsal transverse bar: x = 65 ± 3.1 (58–70), w = 6 ± 0.7 (5–7), y = 15 ± 1 (13–17), z = 30 ± 2.4 (27–36), h = 37 ± 1.8 (31–40). Ventral gripus comparable to dorsal, with shorter root and shaft: a = 32 ± 1.3 (25–34), b = 30 ± 1.3 (27–34), c = 4 ± 0.8 (2–7), d = 8 ± 1 (7–12), e = 13 ± 1 (11–15). Ventral transverse bar arched and rigid: x = 37 ± 1.8 (31–40), w = 31 ± 2.5 (28–37). Uncinulus: I = 16 ± 0.5 (15–18), II = 12 ± 0.5 (10–13), III =

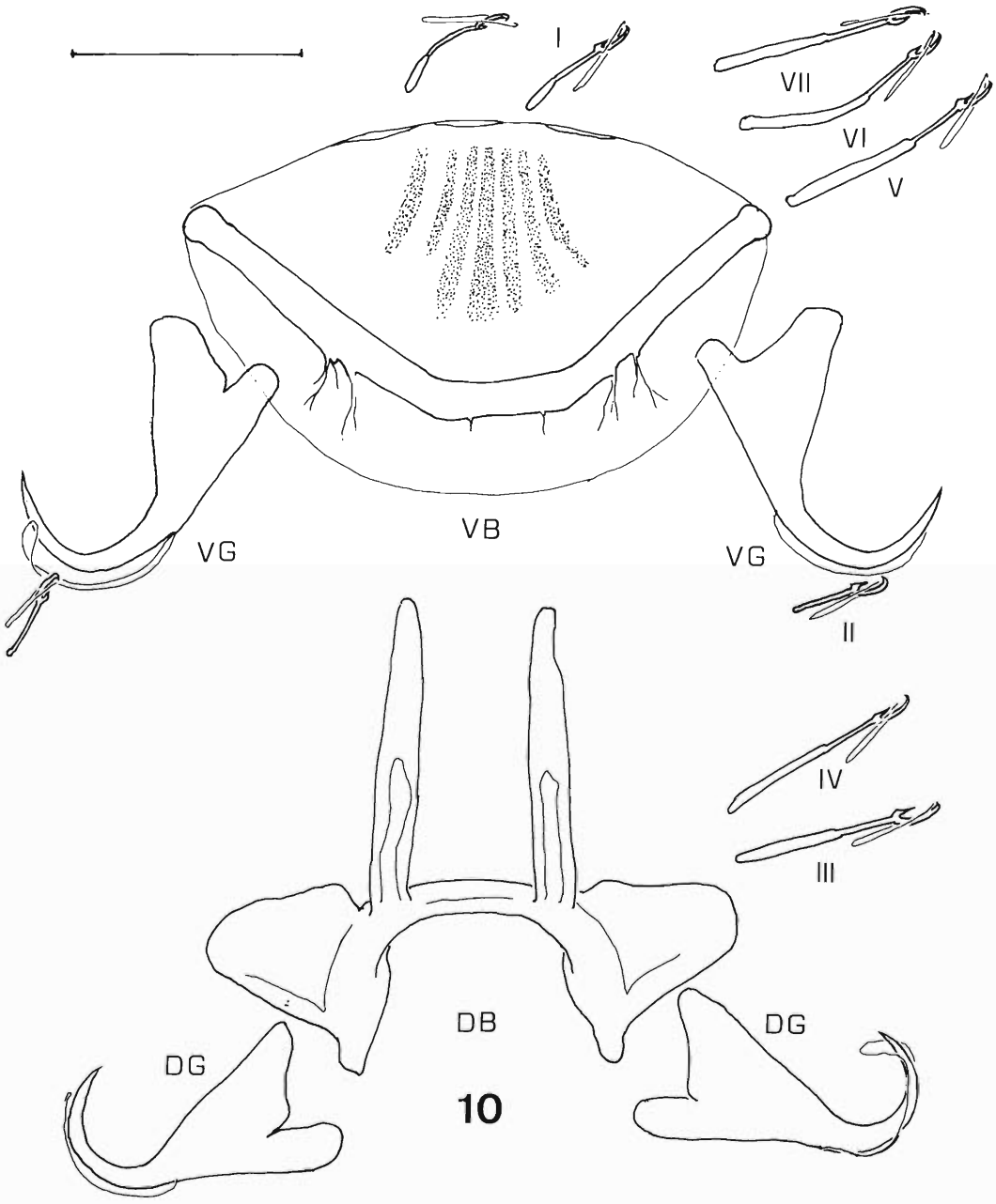

Figure 10. ***Scutogyrus ecoutini*** **sp. n. Haptoral sclerites. DG = dorsal gripus, VG = ventral gripus, I-VII = uncinuli. Scale bar = 30 μm.**

27 ± 1.4 (25–32), IV = 29 ± 1.2 (25–31), V = 29 ± 1.1 (26–32), VI = 26 ± 1.4 (20–28), VII = 25 ± 1 (23–28).

Penis very long, sinuous, filiform, (Fig. 11) with small globular bulb and thin irregular heel: Pe = 411 ± 22.7 (376–455), He = 5 ± 0.8 (3–6). Accessory piece terminates in a large hook: Ap = 40 ± 1.5 (37–43). Stalk very fine: St = 14 ± 3.2 (6–19). Vagina tubular, thin, forming 1 spiral (18 ± 1.6 (13–21) in diameter) linked by a straight portion (28 ± 5.2 (20–41) long) to the genital opening.

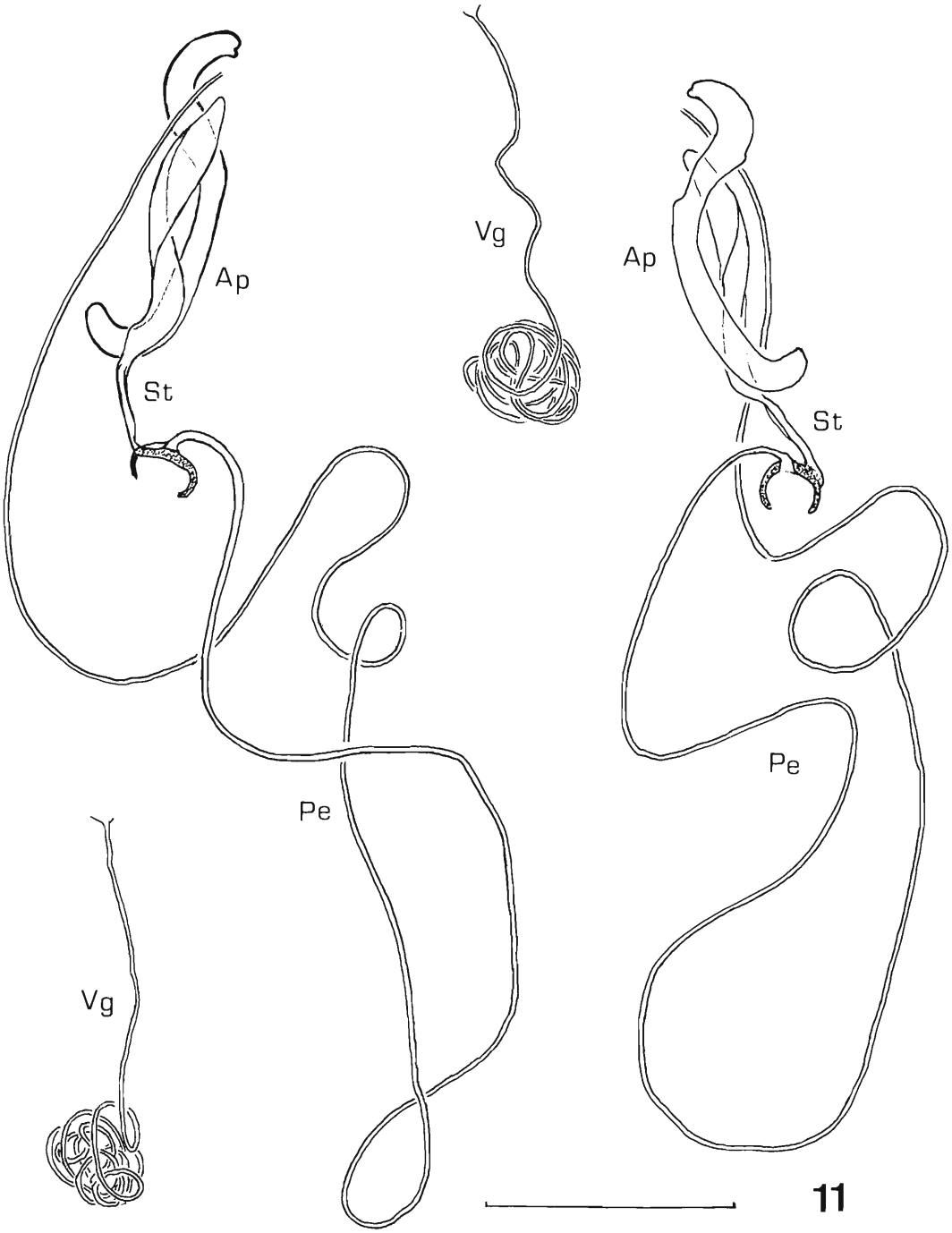

Figure 11. ***Scutogyrus ecoutini*** **sp. n. Two genital apparatus. Scale bar = 30 μm.**

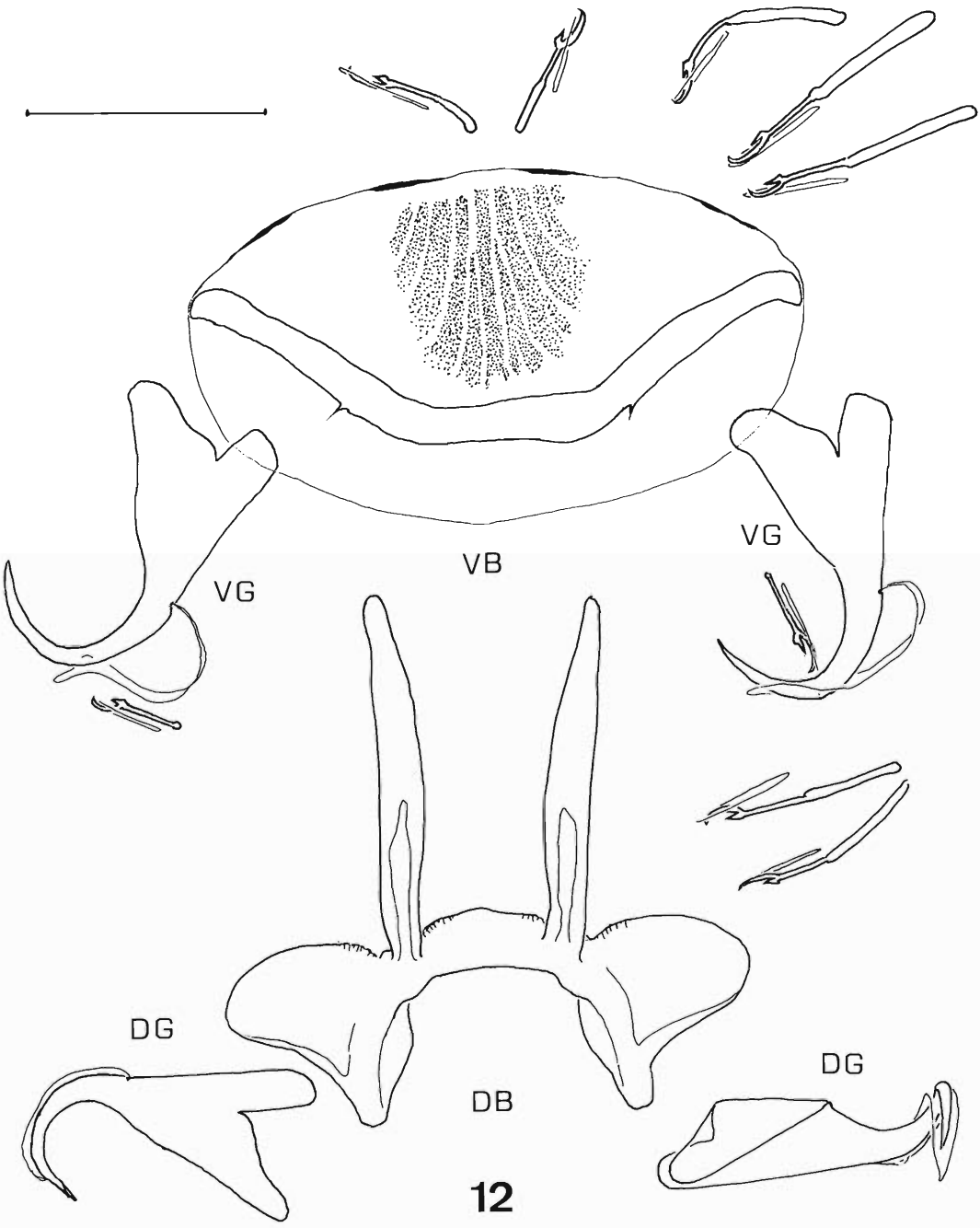

Figure 12. ***Scutogyrus chikhii*** **sp. n. Haptoral sclerites. DG = dorsal gripus, VG = ventral gripus, I-VII = uncinuli. Scale bar = 30 μm.**

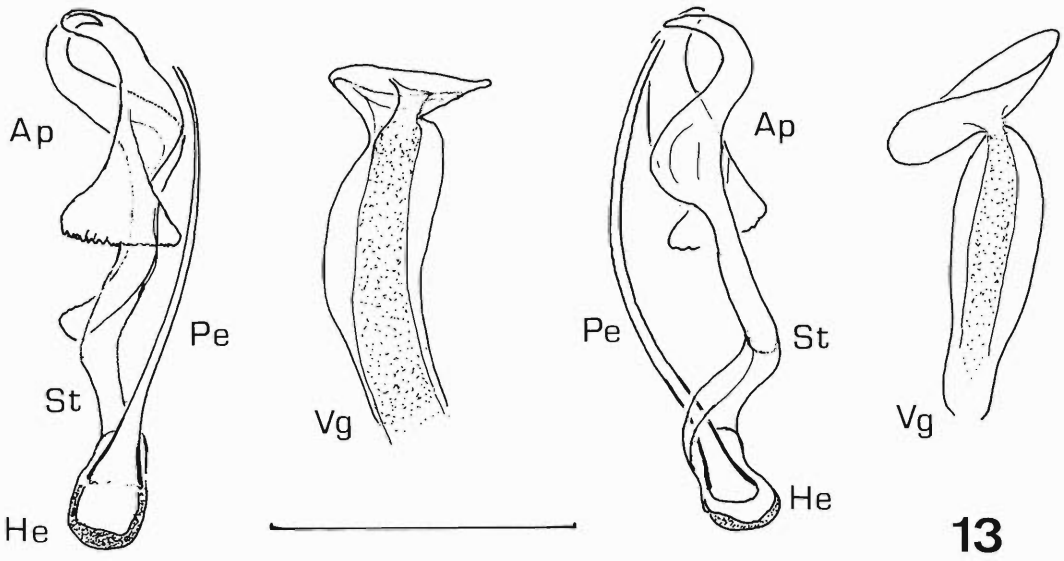

Figure 13. ***Scutogyrus chikhii*** **sp. n. Two genital apparatus. Scale bar = 30 μm.**

REMARKS: The morphology and size of the penis (sinuous and filiform vs. thin tubular and slightly arched, 411 vs. 89 at the most in length) and the vagina (spiraled vs. sinuous at the most) separate this parasite of *Sarotherodon occidentalis* from all the precedent species. We propose the name *Scutogyrus ecoutini* sp. n. in honor of Dr. Jean-Marc Ecoutin, who assisted in the collection of material.

Scutogyrus chikhii sp. n. (Figs. 12, 13)

HOST: *Oreochromis mossambicus* (Peters, 1852).

SITE: Gills.

TYPE LOCALITY: Cayo Lake, near Pointe Noire, Congo (25 May 1993).

MATERIAL STUDIED: Thirteen specimens stained and mounted according to Malmberg (1957).

Holotype deposited at the Muséum National d'Histoire Naturelle, Paris: 464 H.F. Tg. 64.

Paratypes deposited at The Natural History Museum, London: Reg. No. 1994.4.7.5 (1 specimen); at the Musée Royal d'Afrique Centrale, Tervuren: M.R.A.C. 37.361 (1 specimen).

DESCRIPTION: Adults 796 ± 89.2 (671–1,000) long, 145 ± 23.9 (111–185) wide at level of vagina. Pharynx 85 ± 11.6 (73–113) at widest point. Dorsal gripus with root fused to shaft, blade arched: a = 32 ± 1.4 (29–35), b = 27 ± 1.3 (24–29), c = 8 ± 1.6 (3–11), d = 11 ± 1.5 (9–15), e = 9 ± 1.2 (8–11). Dorsal transverse bar: x = 64 ± 3.9 (55–68), w = 7 ± 1 (6–10), y = 15 ± 1.1 (13–17), z = 33 ± 2 (29–36), h = 42 ± 2 (38–46). Ventral gripus: a = 32 ± 1.4 (29–35), b = 27 ± 1.3 (24–29), c = 5 ± 1.3 (3–8), d = 8 ± 1.3 (5–11), e = 13 ± 1 (11–15). Ventral transverse bar arched and rigid: x = 42 ± 1.7 (38–45), w = 34 ± 2.9 (27–38). Uncinulus: I = 17 ± 0.6 (17–19), II = 13 ± 0.3 (12–13), III = 29 ± 1.2 (27–32), IV = 31 ± 1 (29–34), V = 31 ± 1.2 (29–34), VI = 26 ± 0.8 (24–27), VII = 26 ± 1.1 (24–29).

Penis short, slightly arched: Pe = 49 ± 1.8 (45–52), He = 7 ± 2.7 (2–10). Accessory piece, marked by a lateral subtriangular widening and a posterior club-like expansion, terminates in 2 pincer-like hooks: Ap = 23 ± 1.4 (20–26), St = 23 ± 3.6 (17–29). Vagina with sclerified portion pocket-like: L = 26 ± 3.8 (19–32), l = 9 ± 0.9 (8–11).

REMARKS: This species differs from previous species by the morphology of the accessory piece (2 opposite hook-shaped outgrowths vs. only 1 [*S. bailloni* or *S. ecoutini*] or 2 with only 1 hook-shaped [*S. minus*] or 2 straight [*S. longicornis* or *S. gravivaginus*] and of the vagina (pocket-like vs. tubular for all the previous species [except *S. ecoutini* filiform]). These characteristics are sufficient to consider the parasite of *O. mossambicus* from the Congo as a new species. We propose

the name *Scutogyrus chikhii* in honor of Lounès Chikhi, who provided the first material.

Discussion

The new genus described in the preceding is found only on hosts from *Oreochromis* and *Sarotherodon,** and all the hosts from these 2 genera, sampled in this study's area (or elsewhere; see e.g., Ergens, 1981; Douëllou, 1993), present at least 1 *Scutogyrus*—this is why we can say that it is a good biological tag for these 2 Tilapiine genera and probably a good example of coevolution between host and parasite.

If 4 species within *Scutogyrus* are host-specific, 2 have been found on several species of fishes: *S. longicornis* on *Oreochromis niloticus* (type host), *O. aureus,* and *O. mortimeri,* and *Sarotherodon galilaeus* (and *T. zillii*; see preceding footnote) and *S. gravivaginus* on *Oreochromis leucostictus* (type host), *O. variabilis,* and *O. mortimeri.* However, we noticed that, in the case of *S. longicornis,* 3 hosts listed have a very similar parasitic fauna: all the Monogenea that we have found (nobis) on *O. aureus* have been described on *O. niloticus* (*Cichlidogyrus halli, C. thurstonae, C. tilapiae,* and *S. longicornis*). In the same way, *O. mortimeri* possesses, in addition to *Scutogyrus longicornis* and *S. gravivaginus, Cichlidogyrus halli, C. sclerosus, C. tilapiae,* and *S. longicornis,* which are known from *O. niloticus,* and occasionally 3 other species (*C. dossoui, C. karibae,* and *C. zambezensis*) described by Douëllou (1993). This fact (different species of hosts possessing the same parasitic fauna) must be compared to the study on the subgenus Coptodon (Cichlidae) (Pariselle and Euzet, in press b), where the parasitic specificity is related to the genetic proximity of host. This represents a great danger for native fishes because the parasites followed their hosts when they were introduced (see Natividad et al., 1986; Bondad-Reantaso and Arthur, 1990).

Key for *Scutogyrus* Species

1a. Penis and vagina filiform, very long ... *S. ecoutini*
1b. Penis and vagina nonfiliform 2
2a. Penis more than 70 μm long 3
2b. Penis less than 70 μm long 4
3a. Vagina tubular and narrow ..... *S. bailloni*
3b. Vagina wide ..... *S. gravivaginus*
4a. Accessory piece terminates in equal pincer-like hooks ..... *S. chikhii*
4b. Accessory piece terminates in unequal hooks 5
5a. Lateral outgrowth of accessory piece well marked ..... *S. minus*
5b. Lateral outgrowth of accessory piece poorly marked ..... *S. longicornis*

* Paperna (1979) indicated *Cichlidogyrus longicornis longicornis* as host for *Tilapia zillii,* insofar as we have never found again this parasite on this host, despite numerous samples studied from Senegal, Guinea, Mali, Benin, Niger, the Ivory Coast, Egypt, and so forth. It seems to be an accidental catching or an erroneous determination of the host species.

Acknowledgments

We are grateful to Dr. D. C. Kritsky of Idaho State University at Pocatello for remarks on taxonomic problems, to Dr. Puyleart of the Musée Royal de l'Afrique Centrale, Tervuren, for lending type material, to Dr. L. Douëllou for advice and the loan of specimens, and to Dr. X. Rognon for providing host individuals.

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Obituary Notice

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Armatae Xiphidiocercariae of North Carolina, with a Description of Five New Cercarial Species

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ABSTRACT: During a survey of larval trematodes from the piedmont region of the Neuse River in eastern North Carolina, 6 armatae xiphidiocercariae were reported from gastropods. New locality records are reported for the cercaria of *Dasymetra conferta* Nicoll, 1911, and the 5 remaining cercariae are reported as new species.

KEY WORDS: *Cercaria peribolentera* sp. n., *Cercaria acanthicocystis* sp. n., *Cercaria elachiocotyle* sp. n., *Cercaria acanthocotyleda* sp. n., *Cercaria pleophysinx* sp. n., *Dasymetra conferta*, Plagiorchiida, Digenea, Trematoda, North Carolina.

Armatae xiphidiocercariae possess a stylet, a midventral sucker, and a tail that tapers to a single point (leptocercous). These cercariae generally develop in sporocysts in aquatic snails. Adults of these cercariae are from the families Plagiorchiidae, Telorchiidae, Auridistomidae, Ochetosomatidae, and Cephalogonimidae. Adult plagiorchiids parasitize the intestine, gall bladder, bile ducts, and cloaca of vertebrates, whereas telorchiids parasitize the intestines of amphibians and reptiles. The definitive hosts of the family Auridistomidae are turtles. Adult ochetosomatids parasitize the mouth, esophagus, and respiratory tract of snakes, whereas cephalogonimids parasitize the intestines of fishes, amphibians, and reptiles (Schell, 1985).

While surveying for larval trematodes in the upper Neuse River basin in eastern North Carolina, 28 cercarial species were found, 6 of which were of the armatae xiphidiocercaria type. The following is the report of these 6 cercarial species.

Materials and Methods

Mollusks were collected from 50 stations in the upper Neuse River basin of North Carolina from October 1989 to October 1990.

Snails were tentatively identified with the aid of taxonomic keys (Burch, 1982). Mr. William F. Adams of the Army Corps of Engineers, Wilmington, North Carolina, verified these identifications.

In the laboratory, mollusks were separated by species and stored in glass dishes with distilled water for 48 hr. Dishes were examined after the first 24 hr. After the initial examination, the mollusks releasing cercariae were isolated and the water in the remaining dishes was changed. If no cercariae emerged after the second 24 hr, the mollusks were crushed and examined for immature infections.

Naturally emerging cercariae were first examined for swimming habits and tropisms. For microscopic study, cercariae were transferred in a drop of water to a slide, whereupon a coverslip was applied. Coverslip pressure was controlled by adding a drop of water to the edge of the coverslip or by removing excess water by absorbtion with a paper towel. Numerous preparations of each species were examined during the process of making preliminary drawings.

After preliminary drawings were made, cercariae were transferred to steaming 10% formalin in order to obtain relaxed and extended specimens for measurement.

After study of naturally emerged cercariae, the host was crushed and the precercarial stages were examined, drawn, and measured.

Measurements were taken from 10 fixed cercariae and 10 live sporocysts. Maximum, minimum, and average measurements for each feature are reported in the descriptions, with the average measurements in parentheses. All text measurements are in micrometers.

Preliminary drawings were made freehand from living material. Final drawings were drawn to scale using preliminary drawings and average measurements. All scale bars are in millimeters.

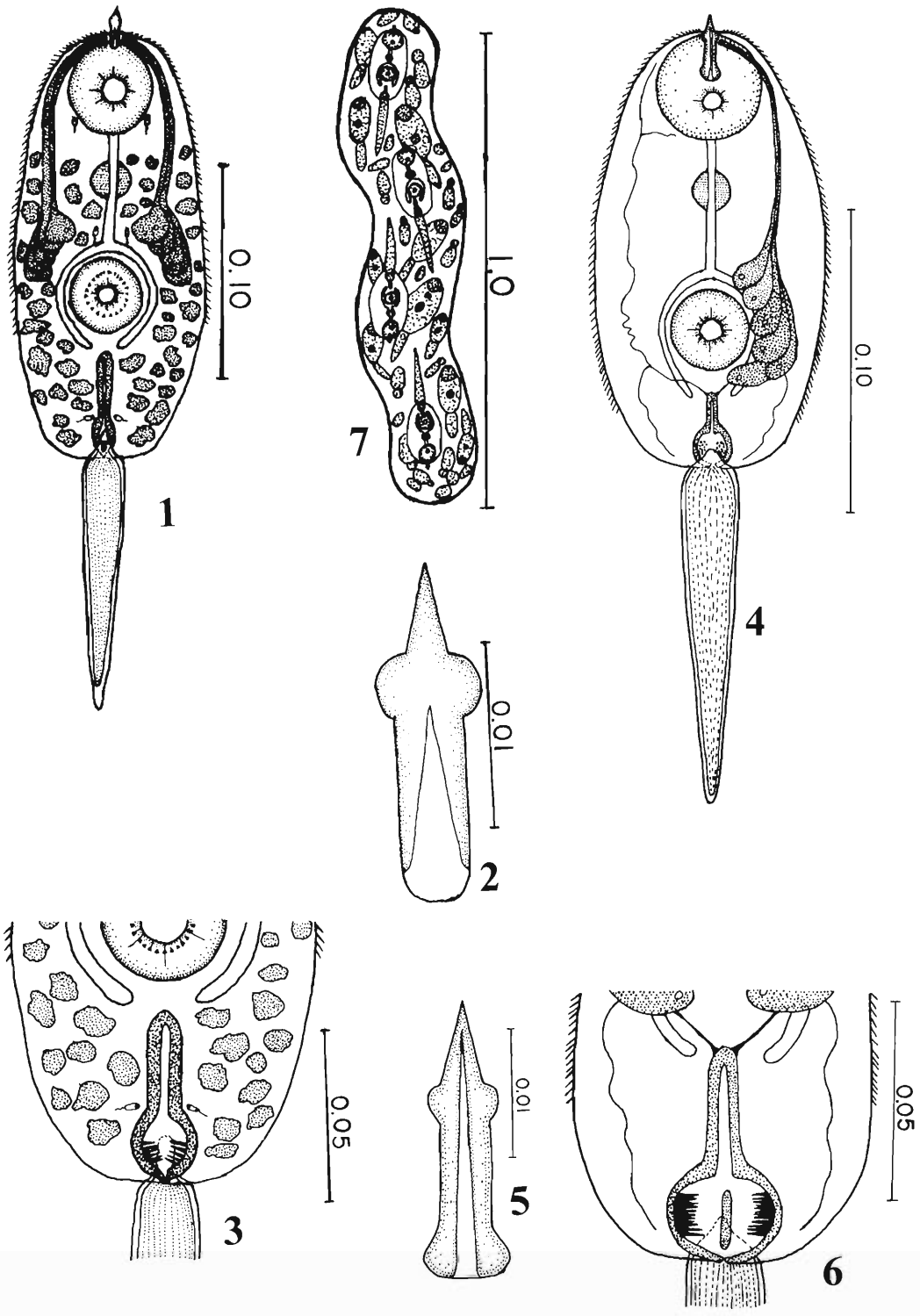
Specimens to be deposited in the USNM Helminth collection were stained with Mayers acid carmine and mounted on slides with xylene-based mounting medium. Snails infected with *Cercaria peribolentera* and *Cercaria pleophysinx* died before permanent slides were made of these cercariae.

Results and Discussion

Cercaria peribolentera sp. n. (Figs. 1–3)

Description

Body 154.4–196.0 (179.6) long, 73.5–105.4 (89.9) wide at level of ventral sucker. Tail 85.8–134.8 (118.3) long, 19.6–22.1 (20.1) wide at base. Base of tail inserted into a caudal pocket, which possesses spines in its lateral walls (Fig. 3). Oral sucker 37.0–48.0 (43.7) long, 37.0–45.0 (41.6) wide at greatest width, surrounding a subterminal ventral mouth and possessing a well-developed stylet. Stylet (Fig. 2): shaft 10.0 long, 3.7 wide; shoulders 3.3 long, 5.7 wide; tip 5.0 long, 2.3 wide at base. Ventral sucker 34.0–37.0 (35.3)



Figures 1–7. 1–3. *Cercaria peribolentera* sp. n. 1. Cercaria. 2. Stylet. 3. Excretory bladder. 4–7. *Cercaria acanthicocystis* sp. n. 4. Cercaria. 5. Stylet. 6. Excretory bladder. 7. Sporocyst.

long, 32.0–40.0 (36.5) wide; approximately 60.0 μm from posterior margin of body. Prepharynx extending from mouth to muscular pharynx, 14.0–21.0 (17.4) long, 18.0–26.0 (21.2) wide. Pharynx approximately 60.3 μm from anterior extremity. Long esophagus, extending posteriorly from pharynx, bifurcates just prior ventral sucker. Ceca narrow, encircling ventral sucker, ending just posterior to ventral sucker. Five granular, nucleated penetration glands occur lateral to esophagus. Duct from each gland passes anteriorly and through oral sucker's lateral wall. Excretory bladder is I-shaped, extending posteriorly into bulbous caudal pocket. Only 3 pairs of flame cells are distinguishable. Irregular-shaped, granular cystogenous glands occurring throughout cercarial body obstruct view of other flame cells. Small spines occur from the anterior margin to just posterior of ventral sucker. Small spines also located around ventral sucker's opening.

Cercaria swims tail-first through the water, ventral side up, its tail whipping in a figure-8 motion. The cercaria swims to the surface of the water column, then sinks motionless with its tail pointed toward the surface of the water. Once the cercaria touches the bottom substrate, it swims back to the surface. No periodicity in the release of cercariae was observed. No tropisms were noted.

Cercaria peribolentera develops in a sac-like sporocyst that occurs in the digestive gland of its gastropod host. The sporocyst is 1,125.0–1,400.0 (1,212.5) long, 200.0–250.0 (217.9) wide, containing an average of 5 mature cercariae. The rest of the sporocyst is filled with immature or embryonic cercariae.

TYPE HOST: *Helisoma anceps* (Menke, 1830).

TYPE LOCALITY: North Fork of the Little River in Durham County, North Carolina, access SR 1461 (36°09′50″, 78°56′57″).

PREVALENCE OF INFECTION: One of 233 *Helisoma anceps.*

Discussion

Cercaria peribolentera sp. n. shares the characteristic of having an I-shaped excretory bladder with 8 other U.S. armatae xiphidiocercariae—those being *Cercaria acanthicocystis* n. sp. of this study, *Cercaria cystonchoides* Miller, 1935, described by Miller (1935, 1936), *Cercaria welleri* McMullen, 1938, by McMullen (1938), *Cercaria candelabra* Faust, 1919, by Faust (1919), *Cercaria leptacantha* Cort, 1914, by Cort (1914), cercaria of *Alloglossidium macrobdellensis* Beckerdite and Corkum, 1974, described by Corkum and Beckerdite (1975), *Alloglossidium corti* (Lamont, 1921) Van Cleave and Mueller, 1934, by McCoy (1928), McMullen (1935), and Crawford (1937), and cercaria of *Macroderoides spinifer* Pearse, 1924, by Leigh (1958). *Cercaria cystonchoides* and *C. leptacantha* develop in prosobranch gastropods and thus are not considered to be related to *C. peribolentera.* Including *C. peribolentera,* all other armate cercaria that have I-shaped bladders develop in planorbid snails. Except for *C. welleri,* the remaining cercariae possess spines in their caudal pockets or sphincters of the excretory bladder. Of the remaining 5 cercariae in question, *C. acanthicocystis* sp. n. of this study most resembles *C. peribolentera.* However, *C. peribolentera* is distinct from that cercaria due to its number of penetration glands, distinct cystogenous glands, spinous ventral sucker, and the cecal length. Also, this is the only cercaria to have the ceca completely encircling the ventral sucker, thus the name *Cercaria peribolentera,* the cercaria with the encircling intestines.

Cercaria acanthicocystis sp. n. (Figs. 4–7)

Description

Body 125.0–191.1 (143.8) long, 66.2–88.2 (77.3) wide at ventral sucker. Tail 80.9–132.3 (110.8) long, 17.2–22.1 (19.1) wide at base. Base of tail situated in a caudal pocket that possesses several spines (Fig. 6). Oral sucker 30.0–40.0 (35.1) long, 34.0–39.0 (35.8) wide at greatest width, surrounding subterminal ventral mouth, and armed with a stylet. Stylet (Fig. 5): base 4.0 long, 6.7 wide; shaft 8.0 long, 4.0 wide; shoulders 3.0 long, 5.7 wide; tip 6.3 long, 3.3 wide at its base. Ventral sucker 22.0–31.0 (27.0) long, 23.0–32.0 (27.0) wide; approximately 55.1 μm from cercaria's posterior margin. Prepharynx extending from mouth to muscular pharynx. Pharynx: 13.0–15.0 (14.1) long, 13.0–15.0 (13.6) wide; approximately 44.8 μm from cercaria's anterior extremity. Esophagus extending posteriorly from pharynx, bifurcates into ceca at ventral sucker. Narrow ceca extend to excretory bladder. Six pairs of granular and nucleated penetration glands lateral to ventral sucker. First 2 glands on each side are round and finely granulated, while last 4 glands on each side are irregularly shaped and coarsely granulated. Duct from each gland passes

anteriorly and through lateral wall of oral sucker. Excretory bladder is I-shaped with thick, granulated walls; extends posteriorly into the spinous caudal pocket. Pair of excretory ducts passes laterally from anterior of bladder and bifurcates into 2 tubules. Small tegumental spines occur from the anterior margin to excretory bladder.

Cercaria swims randomly, tail-first through the water, ventral surface up. Eventually, it sinks motionless with its tail pointed toward the surface of the water. Once the cercaria touches the bottom substrate it swims through the water column again. No periodicity of the release of cercariae was noted. No tropisms were noted.

Cercaria acanthicocystis sp. n. develops in a sac-like sporocyst (Fig. 7) that is entwined in the tissues of the gastropod's ovotestis and digestive gland. The sporocyst is 750.0–1,300.0 (1,085.0) long, 150–225 (192.5) wide, and is filled with cercariae at various levels of development.

TYPE HOST: *Helisoma anceps* (Menke, 1830).

TYPE LOCALITY: Crabtree Creek in Wake County, North Carolina, access SR 1664 (35°50′41″, 78°43′43″).

PREVALENCE OF INFECTION: Three of 233 *Helisoma anceps.*

SPECIMENS DEPOSITED: USNM Helminthological Collection Accession No. 84557.

Discussion

Similar to *C. peribolentera, Cercaria acanthicocystis* sp. n. possesses an I-shaped excretory bladder, spines in the caudal pocket, and develops in a planorbid snail. Also like *C. peribolentera, C. acanthicocystis* does not resemble any of the previously described armatae cercaria. However, unlike *C. peribolentera, C. acanthicocystis* possesses 6 pairs of penetration glands compared to *C. peribolentera*'s 5. *Cercaria acanthicocystis* lacks distinct cystogenus glands prevalent in *C. peribolentera.* The name, *Cercaria acanthicocystis,* refers to the spiny bladder.

Cercaria elachiocotyle sp. n. (Figs. 8–11)

Description

Body 306.3–375.4 (340.9) long, 108.7–153.1 (133.4) wide at ventral sucker. Tail 193.6–291.6 (238.0) long, 27.0–29.4 (28.6) wide at base. Oral sucker 22.0–26.0 (23.7) long, 18.0–22.0 (20.6) wide at its greatest width; surrounding a subterminal ventral mouth; armed with a stylet. Stylet (Fig. 9): shaft 14.2 long, 5.5 wide; tip 5.7 long, 3.0 wide at its base. Ventral sucker 18.5–23.0 (20.8) long, 21.0–27.0 (23.4) wide; approximately 116.2 μm from posterior extremity. Prepharynx extends from mouth to muscular pharynx. Pharynx 8.0–9.0 (8.5) long, 7.0–8.5 (7.9) wide; approximately 93.8 μm from anterior margin. Esophagus extends posteriorly from pharynx; bifurcates approximately 8.0 μm anterior to ventral sucker. Narrow ceca extend to excretory bladder. Five nucleated penetration glands occur on each side of the ventral sucker. First 3 are anterior to the ventral sucker while the last 2 are lateral to the ventral sucker. All penetration glands are finely granulated. Duct, passing anteriorly from each gland, enters oral sucker through sucker's lateral wall. Excretory bladder Y-shaped, with bulbous base and very short branches; thick, muscular, and granulated walls. Bulbous part of bladder is a muscular sphincter that contains several spines on its lateral walls. This spinous excretory sphincter is closely associated with the base of the tail. Small tegumental spines occur from cercaria's anterior margin to ventral sucker. Refractile bodies, ranging from 2.0 to 6.0 μm in diameter, occur throughout the body parenchyma, except in suckers. Refractile bodies occur most abundantly posterior to pharynx, with only a few anterior to pharynx.

This cercaria swims tail-first through the water, ventral side up, with its tail whipping in a figure-8 motion. The cercaria swims to the surface of the water column, then sinks motionless with its tail pointed anteriorly toward its oral sucker (Fig. 11). Once the cercaria touches the bottom substrate, it swims back to the surface. No periodicity of release of cercariae was noted. No tropisms were noted.

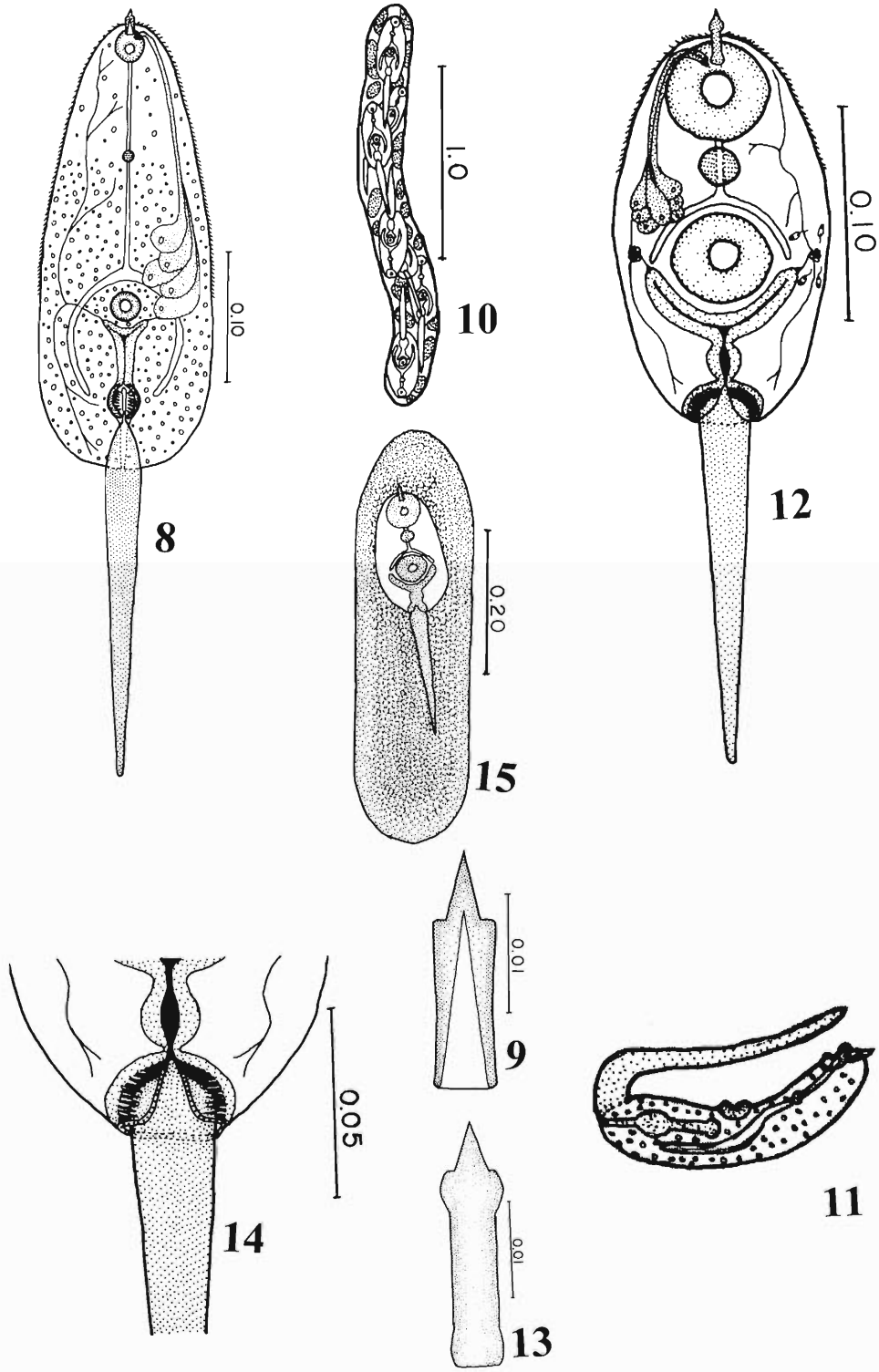
Cercaria elachiocotyle sp. n. develops in a sac-like sporocyst (Fig. 10) that infects the snail's digestive gland. The sporocyst is 1,500.0–2,750.0 (2,102.5) long, 175.0–250.0 (222.5) wide, with an average of 7 mature cercariae within. The rest of the sporocyst is filled with immature and embryonic cercariae. The thickness of the sporocyst's tegument is notable, being approximately 36.8 μm.

TYPE HOST: *Helisoma anceps* (Menke, 1830).

TYPE LOCALITY: Crabtree Creek in Wake County, North Carolina, access CSX Railroad (35°48′48″, 78°37′13″).

PREVALENCE OF INFECTION: One of 233 *Helisoma anceps.*

SPECIMENS DEPOSITED: USNM Helminthological Collection Accession No. 84559.



Figures 8–15. Scale bars in millimeters. 8–11. *Cercaria elachiocotyle* sp. n. 8. Cercaria. 9. Stylet. 10. Sporocyst. 11. Position in water column. 12–15. *Cercaria acanthocotyleda* sp. n. 12. Cercaria. 13. Stylet. 14. Excretory bladder. 15. Sporocyst.

Discussion

The following are a few distinct characteristics of *Cercaria elachiocotyle* sp. n.: short branches of the Y-shaped excretory bladder; ceca extend to the excretory bladder, spines within the muscular sphincter of the excretory bladder, and refractile bodies scattered throughout the parenchyma. Five, previously described, armatae cercariae were found to possess these same characteristics; these species are *Cercaria 3B* Kreitzer, 1966, and *Cercaria 5B* Kreitzer, 1966, described by Kreitzer (1966), *Stylet Cercaria four* Erlandson, 1972, and *Stylet Cercaria three* Erlandson, 1972, by Erlandson (1972), and *Cercaria nolfi* Brooks, 1943, by Brooks (1943). Of these, only *Cercaria 3B* shares the ontogenic characteristic of developing in a planorbid snail. However, *Cercaria elachiocotyle* is distinct from that cercaria by the shape of the stylet, the number of penetration glands, and size differences. Finally, the most definitive characteristic is the minute size of its suckers and pharynx relative to its body size, hence the name *Cercaria elachiocotyle,* the cercaria with small suckers.

Cercaria acanthocotyleda sp. n. (Figs. 12–15)

Description

Body 142.1–196.0 (172.0) long, 83.3–107.8 (101.7) wide at ventral sucker. Tail 132.3–203.4 (167.4) long, 19.6–31.9 (24.4) wide at base. Base of tail situated within a caudal pocket that possesses several spines (Fig. 14). Oral sucker 31.0–56.0 (47.5) long, 46.0–55.0 (50.1) wide at greatest width; surrounds subterminal ventral mouth; armed with stylet. Stylet (Fig. 13): base 4.0 long, 5.5 wide; shaft 12.0 long, 4.8 wide; shoulders 4.5 long, 6.5 wide; tip 5.0 long, 3.5 wide at base. Ventral sucker 30.0–51.0 (40.9) long, 44.0–52.0 (47.9) wide; approximately 48.3 μm from cercaria's posterior margin. Prepharynx extends from mouth to muscular pharynx. Pharynx 14.0–18.0 (16.2) long, 18.0–21.0 (19.5) wide; approximately 50.8 μm from cercaria's anterior margin. Esophagus extends posteriorly from pharynx; bifurcates anterior to ventral sucker. Narrow ceca extend to midline of ventral sucker. Approximately 7 granular, nucleated penetration glands occur on each side of esophagus. Four ducts from each set of glands pass anteriorly through lateral wall of oral sucker; empty through pores posterior and lateral to stylet. Excretory bladder Y-shaped, thick-walled, ungranulated. Small tegumental spines occur over the cercaria's anterior to the pharynx.

This cercaria swims tail-first through the water, ventral side up. No periodicity of release of cercariae nor tropisms were noted.

Cercaria acanthocotyleda sp. n. develops in sac-like sporocyst (Fig. 15), which infects the digestive gland of its gastropod host. Sporocyst 444.6–686.7 (575.2) long, 128.4–207.5 (162.3) wide, with an average of 1 mature cercaria. The tegument of the sporocyst is relatively transparent without noticeable pigmentation.

HOSTS: *Menetus dilatatus* (Gould, 1841) (type host), and *Planorbella* (*P.*) *trivolvis* (Say, 1817).

LOCALITIES: Little River in Franklin County, North Carolina, access SR 1106 (35°58′41″, 78°25′17″) (type locality), and US 401 (35°57′37″, 78°24′31″).

PREVALENCE OF INFECTION: One of 494 *Menetus dilatatus,* 3 of 14 *Planorbella* (*P.*) *trivolvis.*

SPECIMENS DEPOSITED: USNM Helminthological Collection Accession No. 84558.

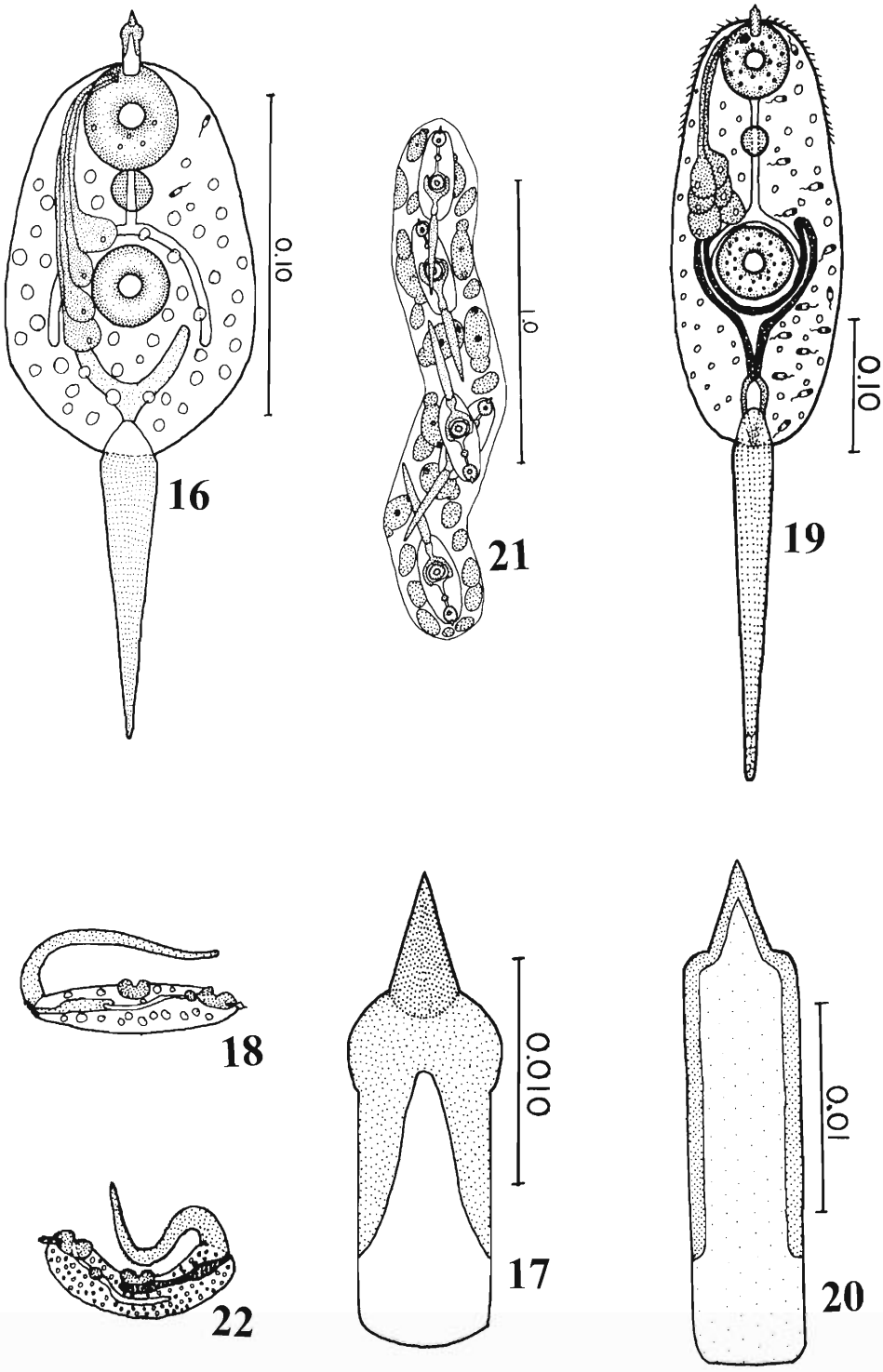
Discussion

Cercaria acanthocotyleda sp. n. is 1 of 9 armatae cercaria to have the branches of the Y-shaped excretory bladder extend anteriorly to a level lateral to the ventral sucker. Of the other 8 cercariae, *Cercaria amherstensis* Rankin, 1939, most closely resembles *C. acanthocotyleda.* These 2 cercariae share other characteristics, such as ceca that extend posteriorly to a level lateral to the ventral sucker and spines within the caudal pocket. Characteristics that preclude referring *C. acanthocotyleda* to *Cercaria amherstensis* are the shape of the stylet, the number of penetration glands, and general size differences. Also, Rankin (1939) reported that the tip of *C. amherstensis*' tail is concave, "as if enclosing an opening," whereas *C. acanthocotyleda*'s tail is pointed. Finally, *C. acanthocotyleda* develops in planorbid snails, whereas *C. amherstensis* develops in *Pseudosuccinea columella,* a lymnaeid. This cercaria is named for the spines within the caudal pocket; *Cercaria acanthocotyleda* means the cercaria with a thorny socket.

Cercaria pleophysinx sp. n. (Figs. 16–18)

Description

Body 100.5–144.6 (123.4) long, 68.6–95.6 (78.7) wide at ventral sucker. Tail 71.1–102.9



Figures 16–22. Scale bars in millimeters. **16–18.** ***Cercaria pleophysinx*** **sp. n. 16. Cercaria. 17. Stylet. 18. Position in water column. 19–22. Cercaria of** ***Dasymetra conferta.*** **19. Cercaria. 20. Stylet. 21. Sporocyst. 22. Position in water column.**

(89.4) long, 14.7–24.5 (18.2) wide at base. Oral sucker 30.0–36.0 (32.9) long, 26.0–33.0 (28.7) wide at greatest width; surrounds subterminal ventral mouth; armed with stylet. Stylet (Fig. 17): shaft 11.3 long, 6.0 wide; shoulders 4.8 long, 6.8 wide; tip 5.0 long, 3.0 wide at its base. Ventral sucker 20.0–30.0 (25.1) long, 21.0–32.0 (25.0) wide; approximately 40.7 μm from cercaria's posterior extremity. Short prepharynx extends from mouth to muscular pharynx. Pharynx 12.0–13.0 (12.4) long, 9.0–15.0 (11.4) wide; approximately 33.0 μm from anterior extremity. Esophagus extends posteriorly from pharynx; bifurcates approximately 20.0 μm anterior to ventral sucker. Narrow ceca extend to a paraacetabular level. Four pairs of nucleated, finely granulated penetration glands occur adjacent to ventral sucker. First pair is preacetabular, second pair is paraacetabular, and last 2 pairs are postacetabular. Duct passes anteriorly from each gland; enters lateral wall of oral sucker; empties lateral to stylet. Excretory bladder Y-shaped; extends anteriorly to ventral sucker. Excretory bladder walls very thin. Large refractile bodies occurring throughout the parenchyma make flame cell pattern indistinguishable. Refractile bodies range from 3.0 to 12.0 μm in diameter; occur throughout body, except in the musculature of the ventral sucker. Interestingly, 4–6 small refractile bodies are located within the tissue of the oral sucker.

Inverted and slightly flexed, this cercaria is pulled through the water column by its whipping tail. The cercaria swims, almost constantly, near the surface of the water, only stopping periodically. The cercaria "rests" inverted in the water column with its body extended and its tail held above the body pointing anteriorly (Fig. 18). Cercariae are released from the gastropod host when the host is subjected to lighted conditions.

Cercaria pleophysinx sp. n. develops in a branched sporocyst, infecting the digestive gland of the snail host. A branch of the sporocyst is approximately 513.8 long, 217.4 wide. The branches of the sporocyst contain cercariae at various stages of development, while the central part of the sporocyst contains embryonic cells and large clusters of refractile bodies.

TYPE HOST: *Campeloma decisum* (Say, 1817).

TYPE LOCALITY: Moccasin Creek in Franklin County, North Carolina, access NC 97 (35°50′03″, 78°15′39″).

PREVALENCE OF INFECTION: One of 288 *Campeloma decisum.*

Discussion

Cercaria pleophysinx sp. n. is morphologically similar to *Cercaria leptacantha* Cort, 1914, a cercaria that Cort (1914) placed in the xiphidiocercariae subgroup, Microcotylae Cercariae. The cercariae of this group are described as developing in small round or oval sporocysts, being under 0.2 mm in length, possessing an acetabulum smaller than the oral sucker and behind the midline of the body, having 4 or less penetration glands arranged in a row, lateral to the acetabulum, and possessing an undeveloped digestive system with only a short prepharynx and small pharynx present. Except for developing in a branched sporocyst and having a fully developed digestive system, *Cercaria pleophysinx* appears to be a microcotyle-type xiphidiocercaria and possibly synonymous with *C. leptacantha.* Not only is this cercaria morphologically similar to *C. leptacantha,* but both cercariae use viviparid snails of the genus *Campeloma* for intermediate hosts. However, *C. pleophysinx*'s development in a branched sporocyst, fully developed digestive system, and size differences prevent this synonymy. It is recognized that Cort's description of *C. leptacantha* was based on immature material, and further investigations may show the actual relationship of these 2 cercariae.

The most striking characteristic of *C. pleophysinx* is the large number of refractile bodies throughout the cercaria's body, giving the appearance that the cercaria is full of bubbles. Thus, this cercaria is named *Cercaria pleophysinx,* the cercaria full of bubbles.

Cercaria of *Dasymetra conferta* Nicoll, 1911 (Figs. 19–22)

HOST: *Physella* sp. Haldeman, 1843.

LOCALITIES: Richland Creek in Franklin County, North Carolina, access SR 1147 (36°01′25″, 78°29′53″); tributary of Little River in Franklin County, North Carolina, access SR 1101 (35°57′26″, 78°23′52°); and Crabtree Creek in Wake County, North Carolina, access SR 1664 (35°50′41″, 78°43′43″).

PREVALENCE OF INFECTION: Four of 5,505 *Physella* sp.

Discussion

The only armatae xiphidiocercaria to be identified as a previously described species is that of *Dasymetra conferta* Nicoll, 1911. This cercaria was first described by McCoy (1928). More re-

cently, Viyanant (1973) briefly described this cercaria from Rutherford County, Tennessee. A comparison of the cercaria found during this study and the description given for the cercaria of *D. conferta* by McCoy (1928) reveals that only the number of flame cells is contradictory. McCoy (1928) reported the cercaria to possess 22 flame cells, whereas this study found 28. The altered view of flame cells due to the large numbers of refractile bodies may account for this difference. However, the similarity of other morphologic characteristics support the supposition that the cercaria of the present study is the cercaria of *D. conferta*. Two ontogenic factors also support this supposition. First, the definitive host of *Dasymetra conferta,* the northern water snake (*Nerodia sipedon*), is abundant in North Carolina, and, second, McCoy (1928) and Viyanant (1973) have reported this cercaria from *Physa* (=*Physella*) *integra* (Haldeman, 1841), and this study confirms the utilization of a physid snail by this parasite.

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Spauligodon caymanensis sp. n. (Nematoda: Pharyngodonidae) from *Anolis conspersus* (Sauria: Polychridae) from Grand Cayman Island, British West Indies

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ABSTRACT: *Spauligodon caymanensis* sp. n. (Nematoda: Pharyngodonidae), a new oxyurid nematode, discovered in the large intestine of *Anolis conspersus* is described and illustrated. Six of 24 adult specimens of *A. conspersus* collected from Grand Cayman Island harbored a total of 67 specimens of *S. caymanensis* sp. n.; prevalence of infection was 25% (mean intensity 11.2, range 1–29). *Spauligodon caymanensis* sp. n. is distinguished from all other Neotropical species by the possession of oval eggs.

KEY WORDS: *Spauligodon caymanensis* sp. n., nematode, *Anolis conspersus*, lizard.

In a recent helminthological survey of Caribbean anoles, 6 specimens of *Anolis conspersus* Garman, 1887, were found to harbor a previously undescribed species of *Spauligodon*. *Anolis conspersus* is known only from the Cayman Islands where it occurs on Grand Cayman Island and Booby Cay (Schwartz and Henderson, 1991). It is probably derived from ancestors that invaded the western Antilles from Central America (Williams, 1969) and is sympatric with the amphibians *Eleutherodactylus planirostris* Cope, 1863, and *Osteopilus septentrionalis* Duméril and Bibron, 1841; the lizards *Anolis sagrei* Duméril and Bibron, 1837, *Aristelliger praesignis* Hallowell, 1857, *Cyclura nubila* Gray, 1831, *Gonatodes albogularis* Duméril and Bibron, 1836, *Leiocephalus carinatus* Gray, 1827, and *Sphaerodactylus argivus* Garman, 1888; and the snakes *Alsophis cantherigerus* Bibron, 1840, *Tretanorhinus variabilis* Duméril and Bibron, 1854, *Tropidophis caymanensis* Battersby, 1938, and *Typhlops caymanensis* Sackett, 1940.

Materials and Methods

Ten specimens of *Anolis conspersus conspersus* (snout vent length [SVL] = 50.8 ± 8.6 mm, range 30–60 mm) and 14 of *A. c. lewisi* Grant, 1940 (SVL = 54.7 ± 9.5 mm, range 43–66 mm), were collected by hand-held noose on Grand Cayman Island August 1993 and fixed in neutral-buffered 10% formalin. The body cavity was opened by a longitudinal incision from vent to throat, and the gastrointestinal tract was removed by cutting across the anterior esophagus and rectum. The esophagus, stomach, small intestine, and large intestine of each lizard were examined separately. Two specimens of *A. c. conspersus* were found to harbor a total of 40 oxyurid nematodes (prevalence 20%, mean intensity 20, range 11–29) and 4 of *A. c. lewisi* harbored a total of 27 oxyurid nematodes (prevalence 29%, mean intensity 6.8, range 1–22). These nematodes were placed in undiluted glycerol, allowed to clear, examined under a light microscope, and determined to represent a new species, *Spauligdon caymanensis*. Measurements in the text are given in millimeters, unless otherwise noted. All anoles were deposited in the herpetology collection of the Natural History Museum of Los Angeles County: *A. c. conspersus*, LACM 140959–140968; *A. c. lewisi*, LACM 140945–140958.

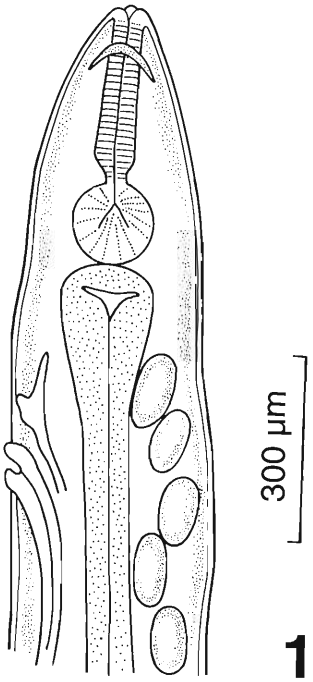
Results and Discussion

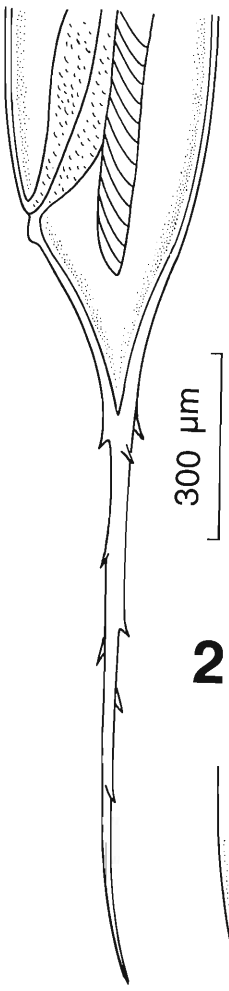
Spauligodon caymanensis sp. n. (Figs. 1–6)

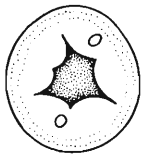
Description

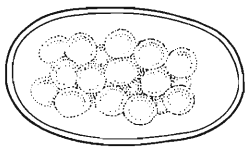
With characters of the genus: specifically, males having caudal alae that do not envelop posterior postcloacal pair of pedunculate papillae; females having vulva in anterior half of body. Nematodes of small size with cylindrical body tapering both anteriorly and posteriorly. Body ending in long, thin tail that supports several cuticular spines. Cuticle transversely striated. Lateral alae present in males and females. Mouth opening is triangular, bounded by 3 lips, each with shallow midline indentation. Esophagus ends in valvulate, subspherical bulb that is separated from esophageal body by small constriction. Excretory pore behind esophageal bulb in males and females.

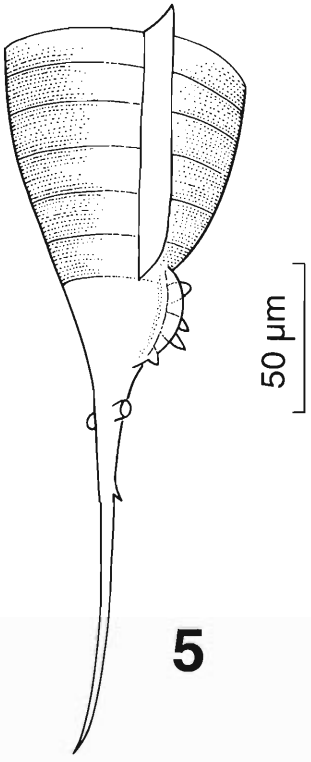
MALE (based on 10 specimens): Small, white, fusiform nematodes tapering both anteriorly and posteriorly; length, 1.36 (1.25–1.43); maximum width, 0.20 (0.18–0.23). Lateral alae, 0.17 (0.014–0.021) wide extending from halfway between nerve ring and lips to anterior border of caudal

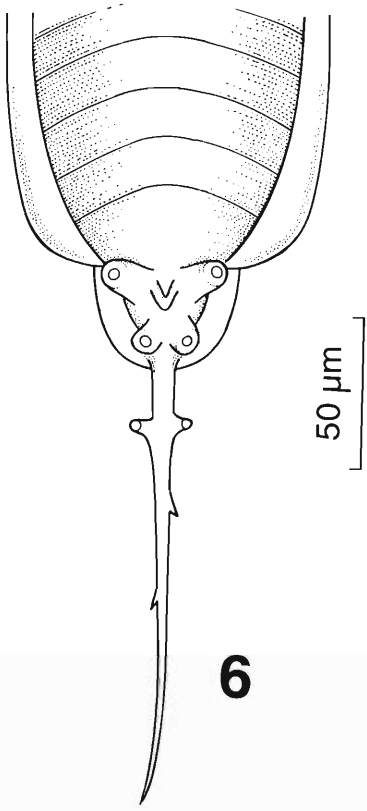












alae. Cuticle with striations of approximately 1 μm width; every eighth to tenth striation deepened as an annulus. Mouth bounded by 3 lips, each with shallow midline indentation to produce bilobed appearance. Esophagus (including bulb), 0.224 (0.200–0.228); bulb length, 0.062 (0.057–0.066); bulb width, 0.059 (0.054–0.063). Nerve ring, 0.090 (0.080–0.097); excretory pore, 0.360 (0.332–0.408) from anterior end. Narrow caudal alae present, 0.005 (0.005–0.006) wide by 0.042 (0.040–0.045) long. Three pairs of caudal papillae present; precloacal pair situated on slightly inflated ventral surface of caudal end, first postcloacal pair posteriolaterally directed; second postcloacal pair not enclosed by caudal alae, 0.035 (0.030–0.040) behind first postcloacal pair. Prominent genital cone in midventral line consisting of small, pointed anterior cloacal lip and larger, pointed posterior cloacal lip; spicule absent. Cloacal opening 0.266 (0.242–0.281) from posterior extremity. Filiform tail extends 0.235 (0.204–0.255) beyond second postcloacal papillae; 3 (1–5) cuticular spines.

FEMALE (based on 10 gravid specimens): Small, white, nematodes tapering anteriorly and posteriorly; length, 4.30 (3.50–5.10); maximum width, 0.30 (0.27–0.32). Lateral alae, 0.035 (0.030–0.040) wide, extending from level of nerve ring to base of filiform portion of tail. Cuticle with striations of approximately 1–1.5 μm width; every eighth to tenth striation deepened as an annulus. Esophagus (including bulb), 0.330 (0.320–0.348); bulb length, 0.88 (0.086–0.091); bulb width, 0.90 (0.088–0.097). Nerve ring, 0.080 (0.074–0.086); excretory pore, 0.510 (0.460–0.536); vulva, 0.540 (0.536–0.612), from anterior end. Thick-walled muscular ovijector extends posteriorly 0.300 continuing as thin-walled vagina 0.300 joining 2 uteri, one directed anteriorly and the other posteriorly. Ovarian and uterine coils do not extend anteriorly as far as the esophageal bulb. Anus 1.20 (1.05–1.44) from posterior end of body. Filamentous portion of tail 0.92 (0.80–1.05) in length and with 9 (8–11) cuticular spines. Eggs oval, 0.105 (0.099–0.111) by 0.55 (0.048–0.057), no polar adornment; development to morula stage at deposition.

TYPE SPECIMENS: Holotype. Male (U.S. National Museum Helminthological Collection, Beltsville, Maryland, accession No. 83748. Allotype: Female (83749). Paratypes (9 males, 9 females, 83750).

TYPE HOST: *Anolis conspersus lewisi* (LACM 140954). Other host, *A. c. conspersus.*

TYPE LOCALITY: Grand Cayman Island (19°20′N, 81°15′W)

ETYMOLOGY: The specific epithet is derived from the name of the island of occurrence.

Discussion

The general morphology of *Spauligodon caymanensis* sp. n. allows its assignment to the superfamily Oxyuroidea Railliet, 1916, family Pharyngodonidae Travassos, 1919, which currently contains 21 genera (see Petter and Quentin, 1976). Of these, 3 genera characteristic of reptiles exhibit a vulvar opening in the anterior part of the body just behind the postbulbar excretory pore: *Pharyngodon* Diesing, 1861, *Spauligodon,* Skrjabin, Schikhobalova, and Lagodovskaja, 1960, and *Skrjabinodon,* Inglis, 1968. These genera are separated by the relationship of the caudal alae to the genital papillae: males of the genus *Pharyngodon* have well-developed caudal alae that envelop all genital papillae; in males of the genus *Spauligodon,* the posterior pair of papillae are excluded from envelopment by the caudal alae, and males of the genus *Skrjabinodon* lack caudal alae. The inclusion of the described specimens in the genus *Spauligodon* is based on the position of the vulva and the configuration of the caudal alae.

The genus *Spauligodon* contains 26 species that are separated on the basis of the egg shape, presence or absence of spines on tail filament, and geographical distribution (Table 1). Only 2 other species have been reported to have eggs with rounded ends: *S. tarentolae* Spaul, 1926, and *S. cabrerae* Castaño-Fernández, Zapatero-Ramos, and Solera Ruertas, 1988. These species are geographically isolated from *S. caymanensis* sp. n. Chabaud and Brygoo (1962) suggested that geographical distribution is the most important factor in the speciation of reptilian oxyurids. Tail spines provide a second criterion in separating these 3 species: *S. cabrerae,* male smooth, female spiny; *S. tarentolae,* male smooth, female smooth;

←

Figures 1–6. ***Spauligodon caymanensis*** **sp. n. 1. Anterior end of female, lateral view. 2. Posterior end of female, lateral view. 3.** ***En face*** **view. 4. Egg. 5. Posterior end of male, lateral view. 6. Posterior end of male, ventral view.**

Table 1. Geographical distribution and selected characters of species of *Spauligodon*.

Biogeographic Realm *Spauligodon* species	Male characters		Female characters		Reference
	Spicule	Tail	Tail	Egg ends	
Palaearctic Realm					
S. auziensis (Seurat, 1917)	49 μm	Smooth	Smooth	Pointed, no knobs	Skrjabin et al., 1960
S. azerbajdzanicus Sharpilo, 1974	49 μm	Smooth	Spiny	Truncated	Sharpilo, 1974
S. carbonelli Roca and Garcia-Adell, 1988	15–35 μm	1–5 spines	6–11 spines	Truncated	Roca and Garcia-Adell, 1988
S. cabrerae Castaño-Fernández, Zapatero-Ramos, and Solera Puertas, 1988	Absent	Smooth	Spiny	Rounded, no knobs	Castaño-Fernández et al., 1988
S. eremiasi Markov and Bogdanov, 1961	Absent	Smooth	Smooth	Truncated	Markov and Bogdanov, 1961
S. extenuatus (Rudolphi, 1819)	70 μm	Smooth	Spiny	Truncated	Skrjabin et al., 1960
S. lacertae Sharpilo, 1966	Absent	Smooth	Smooth	Truncated	Sharpilo, 1966
S. laevicauda (Seurat, 1914)	70 μm	Smooth	Smooth	Truncated	Skrjabin et al., 1960
S. parasskiffi Markov and Bogdanov, 1961	Absent	Smooth	Smooth	Truncated	Markov and Bogdanov, 1961
S. paratectipenis (Chabaud and Golvan, 1957)	Absent	Smooth	Smooth	Truncated	Chabaud and Golvan, 1957
S. phrynocephali Sharpilo, 1976	Absent	Smooth	Smooth	Truncated	Sharpilo, 1976
S. pseudoeremiasi Sharpiilo, 1976	Absent	Smooth	Somoth	Truncated	Sharpilo, 1976
S. saxicolae Sharpilo, 1961	Absent	Smooth	Smooth	Truncated	Sharpilo, 1961
S. tarentolae (Spaul, 1926)	Absent	Smooth	Smooth	Rounded, no knobs	Spaul, 1926
S. tectipenis (Gedoelst, 1919)	Absent	Spiny	Smooth	Truncated	Skrjabin et al., 1960
Ethiopian Realm					
S. dimorpha (Chabaud and Brygoo, 1962)	Absent	Smooth	Smooth	Truncated	Chabaud and Brygoo, 1962
S. morgani (Fitzsimmons, 1961)	Absent	3–6 spines	9–11 spiones	Pointed, each knobed	Fitzsimmons, 1961
Nearctic Realm					
S. californiensis (Read and Amrein, 1953)	Absent	Smooth	9–12 spines	1 truncated, 1 rounded	Read and Amrein, 1953
S. giganticus (Read and Amrein, 1953)	Absent	0–2 spines	10–11 spines	Pointed, 1 with knob	Read and Amrein, 1953
S. mearnsi (Edgerly, 1952)	75–80 μm	Smooth	Spiny	Truncated	Edgerly, 1952
Neotropical Realm					
S. antillarum Barus and Coy Otero, 1974	Absent	3 spines	8–15 spines	1 truncated, 1 pointed with knob	Barus and Coy Otero, 1974
S. caymanensis sp. n.	Absent	3–5 spines	9–11 spines	Rounded, no knobs	Present study
S. cuensis (Read and Amrein, 1953)	Absent	Smooth	Smooth	Pointed, each knobed	Read and Amrein, 1953
S. maytacapaci (Vicente and Ibáñez, 1968)	Absent	Smooth	2 spines	Pointed, each knobed	Vicente and Ibáñez, 1968
S. oxkutzcabiensis (Chitwood, 1938)	Absent	Smooth	13–15 spines	Pointed, each knobed	Chitwood, 1938
S. viracochai (Freitas, Vicente, and Ibáñez, 1986)	Absent	Smooth	Smooth	Pointed, no knobs	Freitas, et al., 1968

and *S. caymanensis* n. sp., male spiny, female spiny.

Five previously described species are found in the Neotropical Realm: *S. antillarum* Barus and Coy Otero, 1974, *S. cubensis* (Read and Amrein, 1953) Skrjabin, Schikhobalova, and Lagodovskaja, 1960, *S. maytacapaci* (Vicente and Ibáñez, 1968) Barus and Coy Otero, 1974, *S. oxkutzcabiensis* (Chitwood, 1938) Skrjabin, Schikhobalova, and Lagodovskaja, 1960, and *S. viracochai* (Freitas, Vicente, and Ibanez, 1968) Barus and Coy Otero, 1974. *S. caymanensis* sp. n. differs from these 5 species in the possession of oval eggs, i.e., eggs with rounded ends without polar adornments. The other Neotropical species have eggs with pointed or flat ends, and all but *S. viracochai* have polar adornments. Additionally, all males of previously described Neotropical species, with the exception of *S. antillarum,* have smooth tails. These comparisons were based on published descriptions; no type specimens were examined.

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Gastrointestinal Helminths of Nine Species of *Sceloporus* Lizards (Phrynosomatidae) from Texas

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ABSTRACT: A total of 276 individuals of 9 species of sceloporine lizards from Texas were examined for gastrointestinal helminths. New host records include *Oochoristica* sp. in *Sceloporus merriami merriami* and *Sceloporus variabilis*; *Oochoristica scelopori* in *Sceloporus olivaceus*; *Atractis penneri* in *Sceloporus merriami merriami, Sceloporus olivaceus,* and *Sceloporus variabilis*; *Cosmocercoides variabilis* in *Sceloporus undulatus hyacinthinus*; *Physaloptera retusa* in *Sceloporus merriami merriami, Sceloporus olivaceus, Sceloporus serrifer,* and *Sceloporus variabilis*; *Physocephalus* sp. (larvae) in *Sceloporus magister bimaculosis*; *Spauligodon giganticus* in *Sceloporus merriami merriami* and *Sceloporus serrifer*; *Strongyluris similis* in *Sceloporus grammicus microlepidotus, Sceloporus merriami merriami, Sceloporus olivaceus, Sceloporus serrifer, Sceloporus undulatus hyacinthinus,* and *Sceloporus variabilis*; *Thubunaea iguanae* in *Sceloporus merriami longipunctatus*; and an acanthocephalan in *Sceloporus magister bimaculosis* and *Sceloporus merriami longipunctatus.* The highest prevalence in the study was recorded for *Spauligodon giganticus* in *Sceloporus poinsettii* (92%). The greatest mean intensity was recorded for *Atractis penneri* in *Sceloporus olivaceus* (206). Helminth species diversity varied from a high of 8 in *Sceloporus merriami* to 0 in *Sceloporus graciosus.*

KEY WORDS: Cestoda, *Mesocestoides* sp., *Oochoristica* sp., *Oochoristica scelopori,* Nematoda, *Atractis penneri, Cosmocercoides variablis, Oswaldocruzia pipiens, Parathelandros texanus, Physaloptera retusa, Physocephalus* sp., *Skrjabinoptera phrynosoma, Spauligodon giganticus, Strongyluris similis, Thubunaea iguanae,* Acanthocephala, prevalence, intensity.

Nine species of lizards in the genus *Sceloporus* occur in Texas (Garrett and Barker, 1987). These include *Sceloporus graciosus arenicolous* Degenhardt and Jones, 1960; *Sceloporus grammicus microlepidoptus* Wiegmann, 1834; *Sceloporus magister bimaculosis* Phelan and Brattstrom, 1955; *Sceloporus merriami* Stejneger, 1904, represented by 3 subspecies, *S. m. annulatus* Smith, 1937, *S. m. longipunctatus* Olson, 1973, and *S. m. merriami* Stejneger, 1904; *Sceloporus olivaceus* Smith, 1934; *Sceloporus poinsettii* Baird and Girard, 1852; *Sceloporus serrifer* Cope, 1866; *Sceloporus undulatus* (Bosc and Daudin, 1801), represented by 3 subspecies, *S. u. consobrinus* Baird and Girard, 1853, *S. u. garmani* Boulenger, 1882, and *S. u. hyacinthinus* (Green, 1818); and *Sceloporus variabilis marmoratus* Hallowell, 1853. Geographic ranges are given in Garrett and Barker (1987).

There are only a few reports of helminths from sceloporine lizards in Texas: Harwood (1932) for *S. undulatus*; Specian and Ubelaker (1974a) for *S. merriami* and *S. undulatus*; McAllister (1988) for *S. olivaceus*; Goldberg et al. (1993) for *S. poinsettii*; and Goldberg et al. (1994a) for *S. serrifer.* In addition, there are reports of helminths from populations of some of these lizards in other states and Mexico: *S. graciosus* in California (Stebbins and Robinson, 1946; Goldberg and Bursey, 1989a, b) and Utah (Woodbury, 1934; Pearce and Tanner, 1973); *S. magister* in Arizona (Walker and Matthias, 1973; Benes, 1985; Goldberg et al., 1994b); *S. undulatus* in Arizona (Goldberg et al., 1994b), New Mexico (Gambino and Heyneman, 1960), and Utah (Pearce and Tanner, 1973); and *S. grammicus* in Mexico (Prado Vera, 1971). There are apparently no published accounts of helminths from *S. variabilis.* The purpose of this report is to present data on helminths from 9 species of sceloporine lizards from Texas and to compare helminth infections among the various species of Texas lizards.

Materials and Methods

All specimens utilized in this study ($N = 276$) were borrowed from museums or collected and deposited in museums: *Sceloporus graciosus,* Department of Biology, Appalachian State University (APPSU), Texas Cooperative Wildlife Collection, Texas A&M University (TCWC), and the Museum, Texas Tech University (TTU); *S. grammicus,* Herpetology Collection, Texas A&I University (TAIC); *S. magister,* Herpetology Col-

Table 1. Helminths found in Texas lizards.

Host Helminth	Prevalence	$\bar{x}$ intensity (range)	Site of infection	County	Reference
Anolis carolinensis					
Oochoristica anolis	3% (1/30)	1	Small intestine	Harris	Harwood, 1932
Proteocephalus sp. (immature)	3% (1/30)	1	Small intestine	Harris	Harwood, 1932
Cnemidophorus dixoni					
Mesocestoides sp. (tetrathyridia)	5% (3/58)	Massive	Body cavity/viscera	Presidio	McAllister et al., 1991a
Oochoristica bivitellobata	16% (9/58)	5 (1–13)	Small intestine	Presidio	McAllister et al., 1991b
Oochoristica sp.	5% (3/58)	2 (1–5)	Small intestine	Presidio	McAllister et al., 1991b
Parathelandros texanus	5% (3/58)	1	Large intestine	Presidio	McAllister et al., 1991b
Physaloptera sp. (larvae)	19% (11/58)	3 (1–11)	Stomach	Presidio	McAllister et al., 1991b
Acanthocephala (juvenile)	21% (12/58)	2 (1–11)	Mesentery/muscle	Presidio	McAllister et al., 1991b
*Cnemidophorus exsanguis**					
Oochoristica bivitellobata	11% (4/37)	5 (1–9)	Small intestine	Brewster, Culberson, Hudspeth	McAllister, 1990c
Pharyngodon warneri	16% (6/37)	8 (1–15)	Large intestine	Culberson, El Paso, Hudspeth, Pecos, Presidio	McAllister, 1990c
Physaloptera sp. (larvae)	22% (8/37)	10 (1–45)	Stomach	Brewster, Jeff Davis, Presidio	McAllister, 1990c
*Cnemidophorus gularis**					
Oochoristica bivitelobata	1% (1/118)	4	Small intestine	Brewster	McAllister, 1990d
Oochoristica sp.	6% (7/118)	4 (1–7)	Small intestine	Irion, Pecos, Taylor	McAllister, 1990d
Parathelandros texanus	1% (1/118)	1	Large intestine	Jeff Davis	McAllister, 1990d
Pharyngodon kirbii	2% (2/118)	6 (1–10)	Large intestine	Andrews, Brewster	McAllister, 1990d
Pharyngodon warneri	37% (44/118)	Massive	Large intestine	Not given	McAllister, 1990d
Physaloptera sp. (larvae)	19% (23/118)	3 (1–13)	Stomach	Hidalgo, Jeff Davis, Pecos Starr, Tarrant	McAllister, 1990d
Acanthocephala (juvenile)	1% (1/118)	1	Body cavity/muscle	Johnson	McAllister, 1990d
Cnemidophorus inornatus heptagrammus					
Parathelandros texanus	Not given	Not given	Large intestine	Brewster	Specian and Ubelaker, 1974a
Pharyngodon warneri	Not given	Not given	Not given	Brewster	Specian and Ubelaker, 1974b
Cnemidophorus laredoensis					
Pharyngodon warneri	15% (5/34)	Massive	Large intestine	Webb	McAllister et al., 1986
Physaloptera sp. (larva)	3% (1/34)	1	Stomach	Webb	McAllister et al., 1986
Cnemidophorus marmoratus					
Mesocestoides sp. (tetrathyridia)	3% (1/35)	>200	Body cavity/liver	Presidio	McAllister et al., 1991a

Table 1. Continued.

Host Helminth	Prevalence	$\bar{x}$ intensity (range)	Site of infection	County	Reference
*Cnemidophorus neomexicanus**					
Oochoristica bivitellobata	2% (1/44)	1	Small intestine	El Paso	McAllister, 1990b
Pharyngodon warneri	5% (2/44)	7 (3–10)	Large intestine	El Paso	McAllister, 1990b
Physaloptera sp. (larvae)	5% (2/44)	6 (1–10)	Stomach	El Paso	McAllister, 1990b
Acanthocephala (juvenile)	2% (1/44)	1	Muscle fascia	El Paso	McAllister, 1990b
*Cnemidophorus septemvittatus**					
Mesocestoides sp.	8% (7/83)	Massive	Coelom/viscera	Presidio	McAllister et al., 1995
Oochoristica bivitellobata	1% (1/83)	2	Small intestine	Presidio	McAllister et al., 1995
Parathelandros texanus	Not given	Not given	Large intestine	Brewster	Specian and Ubelaker, 1974a
	5% (4/83)	2 (1–6)	Large intestine	Presidio	McAllister et al., 1995
Pharyngodon kirbii	Not given	Not given	Large intestine	Brewster	Specian and Ubelaker, 1974b
Pharyngodon warneri	2% (2/83)	10 (10)	Large intestine	Presidio	McAllister et al., 1995
Physaloptera sp. (larvae)	1% (1/83)	3	Stomach	Presidio	McAllister et al., 1995
Acanthocephala (juvenile)	6% (5/83)	2 (2–5)	Muscle fascia	Presidio	McAllister et al., 1995
Cnemidophorus sexlineatus					
Pharyngodon warneri	50% (2/4)	Not given	Large intestine	Walker	Harwood, 1932
Cnemidophorus tesselatus					
Oochoristica bivitellobata	11% (3/27)	2 (1–3)	Small intestine	Brewster, Presidio	McAllister, 1990a
Parathelandros texanus	11% (3/27)	7 (1–15)	Large intestine	Presidio	McAllister, 1990a
Parapharyngodon warneri	15% (4/27)	65 (1–>200)	Large intestine	Brewster, Culberson, Presidio	McAllister, 1990a
Physaloptera sp. (larvae)	19% (5/27)	7 (1–25)	Stomach	Brewster, Presidio	McAllister, 1990a
Cnemidophorus tigris					
Parathelandros texanus	Not given	Not given	Large intestine	Brewster	Specian and Ubelaker, 1974a
Pharyngodon cnemidophori	Not given	Not given	Not given	Brewster	Specian and Ubelaker, 1974b
Coleonyx brevis					
Pharyngodon mudgi	Not given	Not given	Large intestine	Brewster	Specian and Ubelaker, 1974b
Cophosaurus texanus scitulus					
Parathelandros texanus	Not given	Not given	Large intestine	Brewster	Specian and Ubelaker, 1974a
Cophosaurus texanus texanus					
Mesocestoides sp. (tetrathyridia)	5% (1/21)	90	Body cavity	Johnson	McAllister, 1988
Crotaphytus collaris					
Atractis penneri	25% (1/4)	Not given	Large intestine	Terrell	Gambino and Heyneman, 1960

Table 1. Continued.

Host Helminth	Prevalence	$\bar{x}$ intensity (range)	Site of infection	County	Reference
Eumeces fasciatus					
Mesocoelium monas	11% (1/9)	1	Small intestine	Harris	Harwood, 1932
Mesocestoides sp. (tetrathyridia)†	11% (1/9)	1	Body cavity/mesentery	Harris	Harwood, 1932
Oochoristica eumecis	11% (1/9)	1	Small intestine	Harris	Harwood, 1932
Cosmocercoides dukae	44% (4/9)	Not given	Large intestine	not given	Harwood, 1932
Oswaldocruzia pipiens	22% (2/9)	Not given	Digestive tract	Walker	Harwood, 1932
Hemidactylus turcicus					
Ascarops sp. (larvae)	9% (9/98)	Not determined	Viscera	Harris	McAllister et al., 1993
Raillietiella frenatus	44% (210/480)	14 (1–72)	Lungs	Hidalgo	Pence and Selcer, 1988
Raillietiella teagueselfi	20% (17/86)	Not given	Lungs	Harris	Riley et al., 1988
Ophisaurus ventralis					
Cosmocercoides variabilis	25% (1/4)	Not given	Large intestine	Harris	Harwood, 1930
Phrynosoma cornutum					
Diochetos phrynosomatis	57% (4/7)	Not given	Small intestine	Grimes, Harris	Harwood, 1932
Skrjabinoptera phrynosoma	43% (3/7)	Not given	Stomach	Grimes, Harris	Harwood, 1932
Sceloporus grammicus microlepidotus					
Strongyluris similis‡	9% (1/11)	1	Large intestine	Refugio	This study
Sceloporus magister bimaculosis					
Oochoristica scelopori	6% (1/17)	1	Small intestine	Brewster	This study
Atractis penneri	6% (1/17)	709	Large intestine	El Paso	This study
Physaloptera retusa	6% (1/17)	1	Stomach	El Paso	This study
Physocephalus sp. (larvae)‡	12% (2/17)	>100	Viscera	Brewster, Presidio	This study
Thubunaea iguanae	12% (2/17)	2 (1–2)	Stomach	Brewster, Presidio	This study
Acanthocephala (juvenile)‡	6% (1/17)	5	Muscle	Presidio	This study
Sceloporus merriami annulatus					
Parathelandros texanus	Not given	Not given	Large intestine	Brewster	Specian and Ubelaker, 1974a
Sceloporus meriami longipunctatus					
Parathelandros texanus	33% (13/39)	17 (1–42)	Large intestine	Presidio	This study
Thubunaea iguanae‡	8% (3/39)	3 (1–7)	Stomach	Presidio	This study
Acanthocephala (juvenile)‡	3% (1/39)	1	Small intestine	Presidio	This study
Sceloporus merriami merriami					
Oochoristica sp.‡	9% (2/23)	2 (1–2)	Small intestine	Brewster, Val Verde	This study
Atractis penneri‡	17% (4/23)	174 (47–329)	Large intestine	Val Verde	This study
Parathelandros texanus	13% (3/23)	7 (4–12)	Large intestine	Val Verde	This study

Table 1. Continued.

Host Helminth	Prevalence	$\bar{x}$ intensity (range)	Site of infection	County	Reference
Physaloptera retusa‡	4% (1/23)	1	Stomach	Val Verde	This study
Spauligodon giganticus‡	4% (1/23)	1	Large intestine	Val Verde	This study
Strongyluris similis‡	9% (2/23)	6 (5–7)	Intestine	Val Verde	This study
Sceloporus olivaceus					
Mesocestoides sp. (tetrathyridia)	14% (1/7)	>200	Body cavity	Johnson	McAllister, 1988
Oochoristica scelopori‡	3% (2/61)	1	Small intestine	Johnson	This study
Atractis penneri‡	2% (1/61)	206	Large intestine	Hidalgo	This study
Physaloptera retusa‡	48% (29/61)	20 (1–78)	Stomach	Johnson, Tom Green, Travis	This study
Strongyluris similis‡	20% (12/61)	11 (1–31)	Large intestine	Blanco, Hood, Johnson, Travis	This study
Sceloporus poinsettii					
Oochoristica scelopori	30% (3/10)	7 (3–15)	Small intestine	El Paso	Goldberg et al., 1993
Physaloptera retusa	54% (7/13)	66 (1–229)	Stomach	Blanco, Jeff Davis, Llano, Pecos	This study
Spaligodon giganticus	92% (12/13)	30 (2–103)	Large intestine	Blanco, Jeff Davis, Llano, Pecos, Sutton	This study
Skrjabinoptera phrynosoma	80% (8/10)	27 (4–68)	Stomach	El Paso	Goldberg et al., 1993
Thubunaea iguanae	20% (2/10)	1	Stoamch	El Paso	Goldberg et al., 1993
Sceloporus serrifer					
Physaloptera retusa‡	60% (15/25)	5 (1–36)	Stomach	Starr, Webb, Zapata	This study
Physocephalus sp. (larvae)	29% (7/24)	>50	Stomach wall	Webb, Zapata	Goldberg et al., 1994a
Spauligodon giganticus‡	88% (22/25)	11 (1–76)	Large intestine	McMullen, Starr, Webb, Zapata	This study
Strongyluris similis‡	36% (9/25)	7 (1–25)	Digestive tract	McMullen, Starr, Webb	This study
Scleoporus undulatus consobrinus					
Physaloptera retusa	20% (2/10)	54 (3–104)	Stomach	Hudspeth, Val Verde	This study

Table 1. Continued.

Host Helminth	Prevalence	$\bar{x}$ intensity (range)	Site of infection	County	Reference
Scleoporus undulatus hyacinthinus					
Cosmocercoides variabilis‡	4% (1/23)	2	Large intestine	San Jacinto	This study
Oswaldocruzia pipiens	33% (1/3)	Not given	Digestive tract	Walker	Harwood, 1932
	13% (3/23)	1	Digestive tract	Nacogdoches, San Jacinto	This study
Parathelandros texanus	Not given	Not given	Large intestine	Brewster	Specian and Ubelaker, 1974a
Physaloptera retusa	17% (4/23)	3 (1–6)	Stomach	Colorado, Harris, Travis	This study
Strongyluris similis‡	4% (1/23)	1	Large intestine	Colorado	This study
Sceloporus variabilis					
Oochoristica sp.‡	2% (1/42)	1	Small intestine	Nueces	This study
Atractis penneri‡	10% (4/42)	96 (1–230)	Large intestine	Cameron	This study
Physaloptera retusa‡	26% (11/42)	29 (1–249)	Stomach	Live Oak, Nueces, Uvalde	This study
Strongyluris similis‡	2% (1/42)	1	Large intestine	Uvalde	This study
Scincella lateralis					
Brachycoelium daviesi	23% (26/111)	Not given	Small intestine	Walker, Harris	Harwood, 1932
Mesocoelium monas	5% (5/111)	Not given	Small intestine	Harris	Harwood, 1932
Cylindrotaenia americana	37% (41/111)	Not given	Digestive tract	Not given	Harwood, 1932
Mesocestoides sp. (tetrathyridia)†	2% (2/111)	1	Body cavity/mesentery	Not given	Harwood, 1932
Cosmocercoides variabilis	4% (4/111)	Not given	Large intestine	Harris	Harwood, 1930
Oswaldocruzia pipiens	5% (6/111)	Not given	Digestive tract	Walker	Harwood, 1932
Physaloptera squamatae	4% (4/111)	Not given	Stomach	Harris	Harwood, 1932
Thubunaea leiolopismae	20% (22/111)	Not given	Stomach	Harris	Harwood, 1932
Urosaurus ornatus					
Parathelandros texanus	Not given	Not given	Large intestine	Brewster	Specian and Ubelaker, 1974a

* Mean intensity data for *Cnemidophorus* was recalculated from McAllister (1990b, c, d) and McAllister et al. (1994).
† Possibly *Mesocestoides* sp; originally given as *Cysticercus* (larva).
‡ New host record.

lection at Dallas Museum of Natural History (DMNH), Natural History Museum of Los Angeles County (LACM), TCWC, and Department of Zoology, University of Arkansas (UADZ); *S. merriami,* APPSU, Herpetology Collection, Sul Ross State University (SRSU), and Herpetology Collection, University of Texas at Austin (TNHC); *S. olivaceus,* Arkansas State University Museum of Zoology (ASUMZ), LACM, TNHC, and DMNH; *S. poinsettii,* LACM and ASUMZ; *S. serrifer,* Museum of Natural Science, Louisiana State University (LSUMZ), SRSU, and TNHC; *S. undulatus,* LACM and ASUMZ; and *S. variabilis,* TAIC and TCWC.

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 rectum. The esophagus, stomach, and small and large intestines were slit longitudinally and examined under a dissecting microscope. Each helminth was examined and identified using the standard glycerol wet mount. Cestodes were stained with Semichon's acetocarmine or hematoxylin and mounted in balsam. Representative specimens were deposited in the U.S. National Parasite Collection, Beltsville, Maryland 20705. Accession numbers are given in the Appendix.

Results and Discussion

The known helminth fauna for Texas lizards including prevalences and mean intensities (sensu Margolis et al., 1982), infection sites, and localities (county) are presented in Table 1. For sceloporine lizards, known helminths consist of 3 cestode species, *Mesocestoides* sp. represented as tetrathyridia, *Oochoristica scelopori* Voge and Fox, 1950, and an unidentified (perhaps undescribed) species of *Oochoristica*; 10 nematode species, *Atractis penneri* (Gambino, 1957) Baker, 1987, *Cosmocercoides variabilis* (Harwood, 1930) Travassos, 1931, *Oswaldocruzia pipiens* Walton, 1929, *Parathelandros texanus* Specian and Ubelaker, 1974, *Physaloptera retusa* Rudolphi, 1819, *Physocephalus* sp. (encysted larvae), *Skrjabinoptera phrynosoma* (Ortlepp, 1922) Schulz, 1927, *Spauligodon giganticus* (Read and Amrein, 1953) Skrjabin, Schikhobalova, and Lagodovskaja, 1960, *Strongyluris similis* Caballero, 1938, and *Thubunaea iguanae* Telford, 1965; and 1 species from the phylum Acanthocephala, an unidentified cystacanth.

Helminth diversity ranged from 0 helminth species in *Sceloporus graciosus arenicolous* to 8 in *Sceloporus merriami.* Of the 51 species of lizards in Texas (Garrett and Barker, 1987), 30 (59%) are now reported to harbor helminths. *Parathelandros texanus* infects the greatest number of lizard species (10) but is apparently limited in range to west Texas. *Physaloptera retusa* has been recorded in more Texas counties (18) than any other lizard helminth.

None of the 14 species of helminths infecting sceloporine lizards from Texas are unique to the genus *Sceloporus*; all are shared with other amphibian or reptilian host species. Eight are heteroxenous helminths requiring an arthropod intermediate host: *Mesocestoides* sp., *Oochoristica* sp., *Oochoristica scelopori, Physaloptera retusa, Physocephalus* sp., *Skrjabinoptera phrynosoma, Thubunaea iguanae,* and an acanthocephalan. Six are monoxenous with skin penetration, egg ingestion, or autoinfective routes of infection: *Atractis penneri, Cosmocercoides variabilis, Oswaldocruzia pipiens, Parathelandros texanus, Spauligodon giganticus,* and *Strongyluris similis.*

In Texas, *Sceloporus merriami* harbored 8 species of helminths, *S. magister* 6, *S. olivaceus, S. poinsettii,* and *S. undulatus* 5 each, *S. serrifer* and *S. variabilis* 4, *S. grammicus* 1, and *S. graciosus* 0. The failure to find any helminths in *S. graciosus* may be due to the small sample size ($N = 12$); however, Burkholder and Tanner (1974) reported very low helminth prevalences in large sample sizes (>300) of *S. graciosus* from Salt Lake and Wasatch counties, Utah.

In conclusion, our investigations along with previous studies have indicated 14 species of helminths in sceloporine lizards from Texas. Six are monoxenous species; lizard density may be most important in determining the intensity of infection by these helminth species. The remaining 8 species are heteroxenous; intermediate host distribution and lizard diet may be most important in determining infection intensities for these species.

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Appendix: USNM Helminthological Collection Numbers

S. grammicus: *Strongyluris similis*, 84169.

S. magister: *Oochoristica scelopori*, 84170; *Atractis penneri*, 84171; *Physaloptera retusa*, 84172; *Physocephalus* sp. 83420; *Thubunaea iguanae*, 84173; Acanthocephala 83419.

S. merriami: *Oochoristica* sp., 84233; *Atractis penneri*, 84174; *Parathelandros texanus*, 84175; *Physaloptera retusa*, 84232; *Spauligodon giganticus*, 84176; *Strongyluris similis*, 84177; *Thubunaea iguanae*, 84178; Acanthocephala, 84179.

S. olivaceus: *Oochoristica scelopori*, 84234; *Atractis penneri*, 84180; *Physaloptera retusa*, 84181; *Strongyluris similis*, 84182.

S. poinsettii: *Physaloptera retusa*, 84183; *Spauligodon giganticus* (female and alate male) 84184, (analate male) 84185.

S. serrifer: *Physaloptera retusa*, 84186; *Spauligodon giganticus* (female and alate male) 84187, (analate male) 84188; *Strongyluris similis*, 84189.

S. undulatus: *Cosmocercoides variabilis*, 84190; *Oswaldocruzia pipiens*, 84191; *Physaloptera retusa*, 84192; *Strongyluris similis*, 84193.

S. variabilis: *Oochoristica* sp., 84235; *Atractis penneri*, 84194; *Physaloptera retusa*, 84195; *Strongyluris similis*, 84196.

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Helminths of the Opossum, *Didelphis virginiana,* in Southern Illinois, with a Compilation of All Helminths Reported from This Host in North America

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ABSTRACT: Twelve species of helminths were recovered from 46 opossums, *Didelphis virginiana,* in southern Illinois. These species and prevalence of infection are as follows: *Brachylaima virginiana* (32.6%), *Capillaria didelphis* (17.4%), *Capillaria longicauda* (52.2%), *Cruzia americana* (78.3%), *Didelphodiplostomum variabile* (21.7%), *Echinostoma trivolvis* (4.30%), *Longistriata didelphis* (63.0%), *Mesocestoides latus* (15.2%), *Oligacanthorhynchus tortuosa* (17.4%), *Paragonimus westermani* (6.52%), *Physaloptera turgida* (100%), and *Rhopalias macracanthus* (15.2%). Of these helminthic infections, the mean intensity was greatest in *Didelphodiplostomum variabile* (66.9 specimens per infected host) and *Cruzia americana* (50.0 specimens per infected host). In addition, a report of all the helminths known to infect this host is included.

KEY WORDS: opossum, *Didelphis virginiana,* helminths, survey.

The opossum, *Didelphis virginiana* Kerr, 1792, the only member of the family Didelphidae found north of Mexico, occurs from southern Canada through much of the contiguous United States, into Mexico and Costa Rica (Gardner, 1982). At one time, the Virginia opossum was considered to be a subspecies of *D. marsupialis*; however, since revision of the genus by Gardner (1973), the Virginia opossum has been considered distinct. The two species are sympatric from northeastern Mexico to northwestern Costa Rica (Gardner, 1982), but only the helminths of *D. virginiana* are considered here.

One can infer that, due to the apparent success as a species, *D. virginiana* has expanded both its population and range. This expansion is primarily due to the wide array of acceptable habitats, its high reproductive potential, and omnivorous diet (Stieglitz and Klimstra, 1962). In addition, the opossum is a hardy creature, and it seems to adapt to heavy parasitic infections quite well. Remarkably few species of helminths observed in this study caused any overt tissue damage. However, the opossum is a short-lived animal; few live longer than 2 yr (Hamilton, 1963). The question of whether or not helminths affect the short life span of the opossum has not been fully investigated. Therefore, in light of this information, the aim of this study was twofold: (a) to examine both the prevalence and intensity of the parasites that infect this host in southern Illinois, and (b) to provide an annotated list of the helminths previously reported in this host.

Materials and Methods

Forty-six opossums, *Didelphis virginiana,* were collected between September 1992 and January 1993 in the following Illinois counties, with quantities in parentheses: Jackson (14), Saline (12), Union (2), and Washington (18). Opossums were gathered by means of road kills and live trapping and through local hunters during the trapping season.

After euthanasia, the hosts were eviscerated, and the organs were separated and placed into containers filled with normal saline. The esophagus, stomach, small intestine, large intestine, body cavity, and lungs were then examined with a dissecting microscope. All parasites were prepared for study utilizing standard parasitological procedures as outlined by Schmidt (1988). Trematodes and cestodes were fixed in alcohol–formalin–acetic acid, stained in Harris' hematoxylin, dehydrated, cleared in beechwood creosote, and mounted in Canada balsam. Nematodes were fixed in hot 70% ethanol and cleared in a 5% glycerine/95% ethanol solution. The ethanol was allowed to evaporate, and they were studied as temporary mounts in 100% glycerine. Mature acanthocephalans were chilled in physiologic saline in order to evert the proboscis, fixed in formalin, and studied without the aid of a permanent mount.

The terms used in this study, including prevalence, intensity, and range of intensity, follow the definitions outlined by Margolis et al. (1982). Specimens have also been deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705.

Results and Discussion

Five nematode, 5 trematode, 1 acanthocephalan, and 1 cestode species were recovered from 46 hosts. The species, respective location within the hosts, prevalence, mean intensity, and range of infection are listed in Table 1. Every host had

Table 1. Helminths recovered from 46 opossums, *Didelphis virginiana*, in southern Illinois.

Species	Anatomical location	Prevalence	Mean intensity	Range of infection	USNM Helm. Coll. No.
Acanthocephala					
Oligacanthorhynchus tortuosa	Small intestine	17.4%	8.5	1–33	83346
Cestoda					
Mesocestoides latus	Small intestine	15.2%	5.4	1–10	83340
Nematoda					
Capillaria didelphis	Lungs	17.4%	2.8	1–5	83351
Capillaria longicauda	Esophagus	52.5%	1.7	1–4	83350
Cruzia americana	Large intestine	78.3%	50.0	1–200	83349
Longistriata didelphis	Small intestine	63.0%	17.0	1–52	83348
Physaloptera turgida	Stomach	100.0%	18.1	4–60	83347
Larval nematode (unidentified)	Coelomic adipose tissue	8.7%	1.6	1–2	83352
Trematoda					
Brachylaima virginiana	Small intestine	32.6%	15.6	1–32	83341
Didelphodiplostomum variabile	Small intestine	21.7%	66.9	1–500	83342
Echinostoma trivolvis	Small intestine	4.3%	2.0	1–3	83343
Paragonimus westermani	Lungs	15.2%	13.8	2–12	83345
Rhodpalias macracanthus	Small intestine	15.2%	13.8	1–36	83344

at least 1 infection, but the highest prevalence resulted from nematode parasites (100%), followed by trematodes (63%), acanthocephalans (17%), and finally cestodes (15%). In addition, as shown in Table 2, it can be demonstrated from the literature that nematodes are more prevalent than trematodes, and cestodes are roughly equivalent to acanthocephalans in prevalence. The latter 2 groups are considerably less prevalent than the former groups. The present study reflected a similar trend.

Larval nematodes, most likely third-stage larvae, were recovered from the adipose tissue surrounding the right kidney in 4 hosts. These larvae were undergoing a molt in this region when they were discovered; however, the exact identification was impossible to determine. Thus, 11 genera comprising 12 species of helminths were recovered from the 46 opossums examined from southern Illinois. A brief discussion of each of these species is presented.

Acanthocephala

Oligacanthorhynchus tortuosa (Leidy, 1850) Schmidt, 1972

Oligacanthorhynchus tortuosa caused the most overt harm of all the helminthic infections observed in this survey. The particular opossum with 33 worms had almost complete mechanical obstruction of the small intestine and seemed to have smaller fat reserves than most of the hosts examined. Opossums are known to accumulate large quantities of fat, and this host in comparison to the others appeared to be malnourished.

Oligacanthorhynchus tortuosa attachment to the intestinal mucosa produces a small nodule that was demonstrated in many hosts. Babero (1957) observed that this parasite caused destruction of the mucosal and submucosal layers of the intestinal tract, and the penetration of the proboscis into the intestinal lining is the main cause for this necrosis.

Oligacanthorhynchus tortuosa was originally reported from the opossum by Leidy in 1850 (Van Cleave, 1953). Other investigators have recovered this helminth from Illinois, Georgia, Colorado, Arkansas, and Washington. Despite these scattered and infrequent reports, this author believes that *O. tortuosa* is a rather common parasite of the opossum, because it has been reported from widely distributed localities throughout this animal's range.

Cestoda

Mesocestoides latus Mueller, 1927

The presence of *M. latus* caused little gross tissue destruction, for there was no visible host reaction at the attachment sites. There have been numerous reports of 2 species in this genus within the Virginia opossum: *M. latus* and *M. var-*

Table 2. Helminths recorded from *Didelphis virginiana* in North America.

Species	Anatomical location	Geographic locality	Reference
Acanthocephala			
Centrorynchus sp. Luhe, 1911	Small intestine	North Carolina	Miller and Harkema, 1970
Centrorhynchus wardae Holloway, 1958	Small intestine	Arkansas	Richardson, 1993
Macracanthorhynchus ingens (Linstow, 1879) Meyer, 1932	Small intestine	North Carolina	Sherwood et al., 1969
		New Jersey	Fahnestock, 1985
Oligacanthorhynchus tortuosa (Leidy, 1850) Schmidt, 1972	Small intestine	Illinois	Babero, 1957
		Georgia	Babero, 1960
		Colorado	Krupp and Quillin, 1964
		Georgia	Stewart and Dean, 1971
		Illinois	Wong et al., 1979
		Arkansas	Richardson, 1993
		Washington	Richardson, 1993
		Illinois	Present study
Oligacanthorhynchus tumida (Van Cleave, 1947) Schmidt, 1972	Small intestine	Oklahoma	Van Cleave, 1947
		Pennsylvania	Blumenthal and Kirkland, 1976
Cestoda			
Anoplocephala sp. Blanchard, 1848	Small intestine	Colorado	Krupp and Quillin, 1964
Hymenolepis sp. Weinland, 1858	Small intestine	Illinois	Leigh, 1940
		Colorado	Krupp and Quillin, 1964
Mesocestoides sp. Vaillant, 1863	Small intestine	Louisiana	Dikmans, 1931
Mesocestoides latus Mueller, 1927	Small intestine	Illinois	Mueller, 1930
		Wisconsin	Rausch and Tiner, 1949
		California	Voge, 1953
		Pennsylvania	Blumenthal and Kirkland, 1976
		Illinois	Present study
Mesocestoides variabilis Mueller, 1927	Small intestine	Mississippi	Byrd and Ward, 1942
		Mississippi	Byrd and Ward, 1943
		Illinois	Babero, 1957
		Georgia	Babero, 1960
		North Carolina	Miller and Harkema, 1970
		Georgia	Stewart and Dean, 1971
		North Carolina	Feldman et al., 1972
Oochoristica sp. Luhe, 1898	Small intestine	Illinois	Leigh, 1940
Spirometra mansonoides Mueller, 1935	Small intestine	Louisiana	Corkum, 1966
Nematoda			
Anatrichosoma buccalis Pence and Little, 1972	Gums and buccal muscosa	Louisiana	Pence and Little, 1972
		Costa Rica	Pence and Little, 1972
		Florida	Kinsell and Winegarner, 1975
Aspidodera harwoodi Chandler, 1932	Cecum	Texas	Chandler, 1932
Capillaria sp. Zeder, 1800	Lungs	North Carolina	Sherwood et al., 1969
		Georgia	Prestwood et al., 1977
		Louisiana	Brow, 1988
Capillaria didelphis Butterworth and Beverley-Burton, 1977	Lungs	North Carolina	Miller and Harkema, 1970
		North Carolina	Feldman et al., 1972
		Norht Carolina	Feldman and Self, 1973
		Georgia	Nettles et al., 1975
		Georgia	Butterworth and Beverley-Burton, 1977
		Virginia	Snyder et al., 1991
		Illinois	Present study
Capillaria longicauda Freitas and Lent, 1935	Esophagus	Georgia	Babero, 1960
		N. Carolina	Feldman et al., 1972
		Illinois	Present study
Cruzia americana Maplestone, 1930	Large intestine	Texas	Chandler, 1932
		Illinois	Leigh, 1940
		Ohio	Crites, 1956
		Illinois	Babero, 1957

Table 2. Continued.

Species	Anatomical location	Geographic locality	Reference
		Georgia	Babero, 1960
		Virginia	Holloway and Dowler, 1963
		Virginia	Holloway, 1966
		North Carolina	Miller and Harkema, 1970
		Georgia	Stewart and Dean, 1971
		North Carolina	Feldman et al., 1972
		North Carolina	Feldman and Self, 1973
		Georgia	Nettles et al., 1975
		Pennsylvania	Blumenthal and Kirkland, 1976
		Georgia	Prestwood et al., 1977
		Virginia	Snyder et al., 1991
		Illinois	Present study
Cruzia tentaculata Rudolphi, 1819	Large intestine	Pennsylvania	Canavan, 1929
		Louisiana	Dikmans, 1931
		Pennsylvania	Canavan, 1931
		Texas	Chandler, 1932
		Tennessee	Reiber and Byrd, 1942
		Wisconsin	Rausch and Tiner, 1949
		North Carolina	Sherwood et al., 1969
		Mexico	Lamothe et al., 1981
Didelphonema longispiculata (Hill, 1939) Wolfgang, 1953	Stomach	Oklahoma	Hill, 1939b
		Georgia	Stewart and Dean, 1971
Didelphostrongylus hayesi Prestwood, 1976	Lung pleura	Georgia	Prestwood, 1976
		Georgia	Prestwood et al., 1977
		Georgia	Anderson et al., 1980
		Louisiana	Brown, 1988
		Tennessee	Duncan et al., 1989
Dipetalonema didelphis Esslinger and Smith, 1979	Esophageal connective tissue	Georgia	Babero, 1960
		North Carolina	Feldman et al., 1972
		Louisiana	Esslinger and Smith, 1979
Dipetalonema pricei Vaz and Pereira, 1934	Connective tissue	Pennyslvania	Blumenthal and Kirkland, 1976
Dirofilaria sp. Railliet and Henry, 1911	Heart	Georgia	Babero, 1960
		North Carolina	Feldman et al., 1972
Dracunculus sp. Reichard, 1759	Connective tissue	Canada	Crichton and Beverley-Burton, 1973
Gnathostoma sp. Owen, 1836	Stomach	Louisiana	Dikmans, 1931
		Texas	Chandler, 1932
		Georgia	Babero, 1960
		Georgia	Stewart and Dean, 1971
Gnathostoma didelphis Chandler, 1932	Liver	Pennsylvania	Canavan, 1929
		Pennsylvania	Canavan, 1931
		Georgia	Babero, 1960
		Georgia	Flores-Barroeta et al., 1961
		Louisiana	Flores-Barroeta et al., 1961
Gnathostoma spinigerum Owen, 1836	Stomach	Georgia	Babero, 1960
		Pennsylvania	Blumenthal and Kirkland, 1976
Gongylonema longispiculum Schults, 1927	Esophagus	Georgia	Babero, 1960
Lagochilascaris sprenti Bowman, 1983	Stomach	Louisiana	Bowman et al., 1983
Lagochilascaris turgida (Stossich 1902) Travassos, 1924	Stomach	Pennyslvania	Canavan, 1931
Longistriata didelphis (Travassos, 1914) Travassos and Darriba, 1929	Small intestine	Louisiana	Dikmans, 1931
		Illinois	Leigh, 1940
		Tennessee	Reiber and Byrd, 1942
		Maryland	Dikmans, 1943
		Illinois	Babero, 1957
		Georgia	Babero, 1960
		North Carolina	Miller and Harkema, 1970

Table 2. Continued.

Species	Anatomical location	Geographic locality	Reference
		Georgia	Stewart and Dean, 1971
		North Carolina	Feldman et al., 1972
		North Carolina	Feldman and Self, 1973
		Illinois	Present study
Oesophagostomum sp. Molin, 1861	Lungs	Louisiana	Dikmans, 1931
Physaloptera turgida Rudolphi, 1819	Stomach	Pennsylvania	Canavan, 1929
		Louisiana	Dikmans, 1931
		Pennsylvania	Canavan, 1931
		Texas	Chandler, 1932
		Kansas	Haley, 1938
		Oklahoma	Hill, 1939a
		Illinois	Leigh, 1940
		Tennessee	Reiber and Byrd, 1942
		New York	Stoner, 1945
		Wisconsin	Rausch and Tiner, 1949
		New York	Hamilton, 1951
		Illinois	Babero, 1957
		New York	Babero, 1960
		Georgia	Krupp, 1962
		Texas	Hamilton, 1963
		Virginia	Holloway and Dowler, 1963
		Colorado	Krupp and Quillin, 1964
		Virginia	Holloway, 1966
		North Carolina	Sherwood et al., 1969
		North Carolina	Miller and Harkema, 1970
		Georgia	Stewart and Dean, 1971
		North Carolina	Feldman et al., 1972
		Georgia	Nettles et al., 1975
		Pennsylvania	Blumenthal and Kirkland, 1976
		Georgia	Prestwood et al., 1977
		Louisiana	Green, 1980
		Mexico	Lamothe et al,. 1981
		Florida	Gray and Anderson, 1982
		Tennessee	Duncan et al., 1989
		Virginia	Snyder et al., 1991
		Illinois	Present study
Strongyloides sp. Grassi, 1870	Small intestine	Louisiana	Contacos, 1954
		Louisiana	Little, 1966
Toxocara canis Werner, 1782	Stomach decomposed	Pennsylvania	Blumenthal and Kirkland, 1976
Trichinella spiralis Owen, 1835	Diaphragm tongue	Iowa	Zimmerman et al., 1956
		Iowa	Zimmerman et al., 1959
		Virginia	Solomon and Warner, 1969
		Florida	Scholtens and Norman, 1971
		Pennsylvania	Schad et al., 1984
		New Jersey	Leiby et al., 1988
Trichostrongylus sp. Loos, 1905	Lungs	Louisiana	Dikmans, 1931
Trichuris sp. Roederer, 1761	Cecum	Louisiana	Dikmans, 1931
		North Carolina	Miller and Harkema, 1970
		North Carolina	Feldman et al., 1972
		North Carolina	Feldman and Self, 1973
Trichuris didelphis Babero, 1960	Cecum	Georgia	Babero, 1960
Trichuris marsupialis Foster, 1939	Cecum	Georgia	Stewart and Dean, 1971
Trichuris minuta Rudolphi, 1819	Cecum	Georgia	Babero, 1960
		Colorado	Krupp and Quilin, 1964
Viannaia hamata Travassos, 1914	Small intestine	North Carolina	Miller and Harkema, 1970
		North Carolina	Feldman et al., 1972
		North Carolina	Feldman and Self, 1973
Viannaia viannai Travassos, 1914	Small intestine	Maryland	Dikmans, 1943

Table 2. Continued.

Species	Anatomical location	Geographic locality	Reference
Trematoda			
Alaria marcianiae (La Rue, 1917) Walton, 1949	subcutaneous fat and lungs	Louisiana	Shoop and Corkum, 1981a (mesocercarial stage)
Amphimerus pseudofelineus Ward, 1901	Ducts of liver and gall bladder	Illinois	Leigh, 1940
Brachylaima didelphus Premvati and Bair, 1979	Small intestine	Florida	Premvati and Bair, 1979
Brachylaima virginiana Dickerson, 1930	Small intestine	Virginia	Dickerson, 1930
		Louisiana	Dikmans, 1931
		Texas	Chandler, 1932
		Maryland	Krull, 1935
		Illinois	Leigh, 1940
		Tennessee	Byrd et al., 1942a
		Wisconsin	Rausch and Tiner, 1949
		Illinois	Babero, 1957
		Georgia	Babero, 1960
		Virginia	Holloway and Dowler, 1963
		Louisiana	Kaplan, 1964
		Virginia	Holloway, 1966
		North Carolina	Miller and Harkema, 1970
		North Carolina	Feldman et al., 1972
		North Carolina	Feldman and Self, 1973
		Georgia	Nettles et al., 1975
		Pennsylvania	Blumenthal and Kirkland, 1976
		Georgia	Prestwood et al., 1977
		Louisiana	Shoop and Corkum, 1981b
		Louisiana	Shoop and Corkum, 1982
		Illinois	Present study
Didelphodiplostomum variabile (Chandler, 1932) Dubois, 1945	Small intestine	Texas	Chandler, 1932
		Illinois	Leigh, 1940
		Tennessee	Byrd et al., 1942a
		Illinois	Babero, 1957
		Georgia	Babero, 1960
		North Carolina	Miller and Harkema, 1970
		North Carolina	Feldman et al., 1972
		Florida	Premvati and Bair, 1979
		Illinois	Present study
Echinostoma trivolvis Cort, 1914	Small intestine	Louisiana	Dikmans, 1931
		Oklahoma	Park, 1936
		Illinois	Leigh, 1940
		Tennessee	Byrd et al., 1942a
		Wisconsin	Rausch and Tiner, 1949
		North Carolina	Feldman et al., 1972
		Pennsylvania	Blumenthal and Kirkland, 1976
		Illinois	Present study
Fibricola cratera (Barker and Noll, 1915) Dubois, 1932	Small intestine	Tennessee	Byrd et al., 1942a
		Michigan	Chandler and Rausch, 1946
		Wisconsin	Rausch and Tiner, 1949
		Florida	Premvati and Bair, 1979
		Louisiana	Shoop and Corkum, 1981b
		Louisiana	Shoop and Corkum, 1982
Fibricola lucida (LaRue and Bosma, 1927) Dubois and Rausch, 1950	Small intestine	Texas	LaRue and Bosma, 1927
		Louisiana	Dikmans, 1931
		Oklahoma	Park, 1936
		Tennessee	Byrd et al., 1942a
		Illinois	Babero, 1957
		Louisiana	Lumsden and Zischke, 1961
		Louisiana	Kaplan, 1964
		Florida	Premvati and Bair, 1979
		Louisiana	Shoop and Corkum, 1982

Table 2. Continued.

Species	Anatomical location	Geographic locality	Reference
Heterobilharzia americana Price, 1929	Mesenteric venules	Louisiana	Kaplan, 1964
		Louisiana	Shoop and Corkum, 1981b
Linstowiella szidati Anderson, 1944	Small intestine	Louisiana	Lumsden and Winkler, 1962
		Louisiana	Shoop and Corkum, 1982
Maritreminoides nettae (Gower, 1938) Rankin, 1939	Small intestine	North Carolina	Miller and Harkema, 1970
Paragonimus kellicotti Ward, 1908	Lungs	Georgia	McKeever, 1958
		North Carolina	Sherwood et al., 1969
		North Carolina	Feldman et al., 1972
		Louisiana	Shoop and Corkum, 1982
Paragonimus rudis (Diesing, 1850) Stiles and Hassall, 1900	Lungs	Mexico	Lamothe et al., 1981
		Mexico	Lamothe et al., 1986
Paragonimus westermani (Kerbert, 1878) Braun, 1899	Lungs	Tennessee	Byrd, 1941
		Tenneessee	Byrd et al., 1941
		Tennessee	Byrd et al., 1942b
		Illinois	Present study
Phagicola lageniformis (Chandler, 1941) Morozov, 1952	Lungs	Florida	Premvati and Bair, 1979
Rhopalias macracanthus Chandler, 1932	Small intestine	Louisiana	Dikmans, 1931
		Texas	Chandler, 1932
		Illinois	Leigh, 1940
		Tennessee	Byrd et al., 1942a
		Oklahoma	Self and McKnight, 1950
		Illinois	Babero, 1957
		Georgia	Babero, 1960
		Louisiana	Lumsden and Zischke, 1961
		North Carolina	Miller and Harkema, 1970
		Georgia	Stewart and Dean, 1971
		North Carolina	Feldman et al., 1972
		North Carolina	Feldman and Self, 1973
		Florida	Premvati and Bair, 1979
		Louisiana	Shoop and Corkum, 1981b
		Louisiana	Shoope and Corkum, 1982
		Illinois	Present study
Strictodora cursitans Holliman, 1961	Small intestine	Florida	Kinsella and Heard, 1974
Zonorchis allentoshi (Foster, 1939)	Gallbladder	Texas	Denton, 1944

iabilis (Table 2). At this time, the specific rank of these tapeworms has been questioned, and morphological differences between the 2 are indistinct. In fact, there is a great deal of variability in both the hosts and the morphology, causing even further confusion.

Nematoda

Capillaria didelphis Butterworth and Beverley-Burton, 1977

Adult *C. didelphis* were found encysted in lung tissue such that yellow patches appear just beneath the surface. The finding of this species in the Illinois opossum constitutes a new locality record. The genus *Capillaria* Zeder, 1800, contains numerous species that parasitize virtually all classes of vertebrates. Representatives of this genus have been reported as parasites of the digestive tract, respiratory system, genitourinary tract, and subcutaneous tissues of various North American mammals (Read, 1949).

Capillaria longicauda Freitas and Lent, 1935

In a typical infection, there was only one *C. longicauda* worm present per animal. The finding of this species in Illinois represents a new locality record for this host. Previous to this survey, this parasite has only been reported from the opossum in Georgia (Babero, 1960) and North Carolina (Feldman et al., 1972).

Because over 50% of the hosts examined in this survey were infected with this parasite, one can conclude that it is a rather common helminth in opossums. The paucity of reports may be due to the small size and often obscure location of

infection. These nematodes are long and slender and burrow into the mucosa of the esophagus, forming several intertwining loops and making removal difficult.

Cruzia americana Maplestone, 1930

Normally, *C. americana* resides in the cecum; however, upon the death of the host, they usually migrate to other regions of the intestinal tract. This species is one of the most common helminths in the opossum, with reported findings from numerous states (Table 2). In addition to *C. americana,* there have been numerous reports of *C. tentaculata* Rudolphi, 1819, in the Virginia opossum from several states and *C. cameroni* in opossums from Trinidad (Wolfgang, 1951).

Nettles et al. (1975) examined a debilitated opossum from Georgia and reported a large number of *C. americana.* They asserted that despite this seemingly innocuous appearance, *C. americana* in sufficient numbers could interfere with host nutrition. In conjunction with other helminths, this species may produce some degree of debilitation.

Longistriata didelphis (Travassos, 1914) Travassos and Darriba, 1929

Longistriata didelphis are red-colored in vivo because they feed on the blood of the host. They are rather small, tightly coiled worms that possess a moderately expanded cuticle with very fine transverse striations. Reports of *L. didelphis* are common in the opossum, as demonstrated by the plethora of published accounts in numerous localities throughout North America (Table 2).

Despite their prevalence in this survey, there was no sign of inflammation or other gross tissue destruction. Feldman et al. (1972) reported that there seemed to be little host response to this parasite. The results of this survey suggest that the opossum can adapt to its presence rather easily.

Physaloptera turgida Rudolphi, 1819

There have been more than 30 reports of *P. turgida* in the opossum, and nearly every publication surveying helminths of this host has mentioned its presence. This species seems to be present throughout the range of the Virginia opossum. Adult worms were always concentrated in a large group along the greater curvature of the stomach near the fundus, producing a large fibrous ulceration at the point of attachment. It has additionally been surmised that the ulcerations produced in the gastric epithelium may open up avenues for infection by bacteria (Sherwood et al., 1969). Larvae of the nematode parasite *Lagochilascaris* sp. may use these openings as a migration route as well (Smith et al., 1983). Adults of *L. sprenti* can be found encysted in the lungs, brain, mesentery, and muscle tissue.

Food studies on the opossum (Hamilton [1951] in New York and Stieglitz and Klimstra [1962] in Illinois) note the importance of grasshoppers and beetles as food items. These insects are a likely intermediate host for this helminth.

Trematoda

Brachylaima virginiana Dickerson, 1930

Brachylaima virginiana was the most prevalent trematode found in this survey, a trend reflected in the literature with more than 20 reports of its presence from approximately 10 states. In addition to the opossum, there have been reports of *B. virginiana* in the mink, *Mustela vison,* and the skunk, *Mephitis mephitis* (Yamaguti, 1958).

Didelphodiplostomum variabile (Chandler, 1932) Dubois, 1945

One opossum from a marshy area had an intense infection, suggesting that this particular host fed primarily on snails and amphibians and consequently harbored a very large number of adult parasites. *Didelphodiplostomum variabile* is one of several common trematode parasites in the opossum. Reports of *D. variabile* have been cited in most surveys. Several authors disagree about the generic placement of this species; even the establishment of this genus was questioned for some time. Adults within the subfamily Diplostominae Monticelli, 1888, are usually found in fish-eating birds (Shoop, 1989); however, adults in several genera are known to occur in mammals. The genus *Didelphodiplostomum* was erected to account for their presence in mammals rather than birds. Chandler and Rausch (1946) disagreed because substantial morphological differences were absent, and the debate has continued since. Harris et al. (1967) called for the suppression of *Didelphodiplostomum,* arguing that host specificity cannot be relied upon.

Shoop (1989) presented a systematic analysis of the strigeoid trematodes and asserted that the considerable adult similarities are typical of this group. The phylogeny suggests that this group originally infected reptiles and then radiated to

birds. The final step in their evolution resulted in the infection of mammals, which was accomplished by shifting the second intermediate host from fish to amphibians. Shoop (1989) concluded that these genera are valid, based primarily on body shape, citing the degree of separation of the anterior and posterior body regions as the major criterion.

Echinostoma trivolvis Cort, 1914

Echinostoma trivolvis is a rather uncommon helminth of the opossum, having been reported only from a few states. This species is a cosmopolitan parasite and shows little host specificity, as it is known to occur in waterfowl, muskrats, terrestrial birds, and beavers. Because it is associated with aquatic and semiaquatic vertebrates, its low prevalence (in only 2 animals) is reflected by the fact that most of the opossums in this study were collected from wooded habitats.

Due to variability in its life cycle, *E. trivolvis* can mature in numerous vertebrate hosts, and as a result of the distinct physiology of a given definitive host, considerable morphological variation exists in the adult form. This has given rise to a number of descriptions of new species within this genus. Beaver (1937) was able to discount several of these species and synonymized close to 15 forms under the name *E. revolutum.* More recently, this has been determined to be incorrect, and the current name, *E. trivolvis,* is now in use (Huffman and Fried, 1990).

Paragonimus westermani (Kerbert, 1878) Braun, 1899

Previous to the present survey, *P. westermani* had only been reported in the opossum from Tennessee. The finding of this species constitutes a new locality report for this host.

There has been a great deal of taxonomic difficulty surrounding this genus. In the opossum, there have been reports of *P. kellicotti* in Georgia, North Carolina, and Louisiana (Table 2). Additionally, *P. rudis* (Lamothe et al., 1981) is known to occur in the opossum in Mexico. *Paragonimus westermani* infects a number of vertebrate hosts including the mink (Olsen, 1974), its normal definitive host, as well as dogs, cats, and humans. Because the mink is considered to be the normal host for this helminth, its presence in the opossum demonstrates that the opossum feeds on crayfish, the second intermediate host.

Ameel (1934) originally described the life cycle and discussed the taxonomy of this genus. To differentiate these species, Ishii (1966) placed great importance on the nature of the tegumental spines, egg morphology, and construction of the testes. The most conclusive way to differentiate the adults of *P. westermani* and *P. kellicotti* is through the examination of the ovary. Ishii (1966) observed that the branching of the ovary in *P. kellicotti* is more distinct and extensive than the ovary of *P. westermani,* which is less branched.

In the present study, the specimens reflect this simpler branching and are consistent with the description given by Byrd et al. (1942b). Although some authors believe these forms to be conspecific (Olsen, 1974), these specimens will be assigned to *P. westermani* until the taxonomic debate is resolved or until more substantial criteria for differentiation are established.

Rhopalias macracanthus Chandler, 1932

Rhopalias macracanthus is considered to be one of the few ubiquitous trematode parasites in the opossum in North America, having been reported from numerous localities. Characteristic of this genus are 2 retractable proboscises resting on either side of the oral sucker. These structures can protrude from their receptacles, allowing *R. macracanthus* to attach to the intestinal mucosa by means of 10 well-developed spines on each proboscis.

As observed in this study, the opossum harbors a diverse and sometimes intense helminth population. How these animals seem to thrive with the enormous burdens associated with heavy helminthic infections is unknown. This apparent adaptability to the presence of these parasites may give these animals an enhanced capacity to act as a reservoir for several species of helminths. The prevalence of these species in other mammals as well as the effects on the life expectancy and overall health of the hosts are not presently understood. Further research is needed to test for the presence of these helminths in other mammals in order to elucidate the role of the opossum in spreading disease.

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Helminth Parasites of the Alimentary Tract of the Harbor Porpoise, *Phocoena phocoena* (L.), from Newfoundland and Labrador

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ABSTRACT: Stomachs ($N = 80$) and intestines ($N = 29$) of the harbor porpoise, *Phocoena phocoena,* caught as a by-catch in fishing gear off southeastern Newfoundland and adjacent areas during summer and fall 1987–1991 were examined for helminth parasites. Three species of ascaridoid nematode (*Anisakis simplex, Contracaecum osculatum,* and *Phocascaris* sp.), 3 cestodes (*Diphyllobothrium* sp. plerocercoids, *D. stemmacephalum,* and *Tetrabothrius* sp.), 1 acanthocephalan (*Bolbosoma* sp.), and 1 digenean (*Campula oblongata*) were found. All age groups except calves (<1 yr old) were infected with helminths, but there were no significant differences in prevalence or abundance of parasite species among the remaining host age groups or between sexes. Porpoises acquired many larvae of the phocid parasites *C. osculatum* and *Phocascaris* sp., apparently from feeding on capelin, *Mallotus villosus,* but these parasites did not develop to maturity. Small numbers (1–38) of adult *A. simplex* were found in the forestomach of 5% of the porpoises; other helminths were rare. Data on numbers of adult *A. simplex* in other local species of cetaceans are limited, but the numbers of adult *A. simplex* found in *P. phocoena* are consistently lower, suggesting that the harbor porpoise occupying inshore waters during the summer months is not a major source of larval *A. simplex* for local fish stocks.

KEY WORDS: harbor porpoise, helminths, *Anisakis simplex, Contracaecum osculatum, Phocascaris* sp., *Campula oblonga, Tetrabothrius* sp., *Diphyllobothrium stemmacephalum, Bolbosoma* sp.

The harbor porpoise, *Phocoena phocoena* (L.), is one of the most abundant small cetaceans in temperate waters of the Northern Hemisphere. There are numerous records of gastrointestinal helminths in this marine mammal from the Atlantic (Scott and Fisher, 1958a; Vik, 1963; van Thiel, 1966; Young, 1972; F. R. Smith and Threlfall, 1973; Margolis and Arai, 1989 and references therein; J. W. Smith, 1989; Baker and Martin, 1992), but most parasitological studies are based on examination of small numbers (<10) of animals. In this study, a large number of porpoise stomachs ($N = 80$) and intestines ($N = 29$), collected during a program to estimate the total by-catch of these marine mammals in fishing gear, were subjected to parasitological examination.

The main objectives of this study were to determine the numbers of adult *Anisakis simplex* (Nematoda: Ascaridoidea) in harbor porpoises and thereby obtain information on the role of these cetaceans in the transmission of this parasite to local fish stocks. The third-stage larvae (L3's) of *A. simplex* B (=*A. simplex* sensu stricto; for taxonomy, see Nascetti et al., 1986) are common in the flesh of marine fishes off Atlantic Canada (McClelland et al., 1985, 1990; McGladdery, 1986; Brattey and Bishop, 1992) and are potential human pathogens when consumed in raw, marinated, or lightly cooked seafood (Oshima, 1987; McKerrow et al., 1988). Cetaceans normally serve as definitive hosts for species of *Anisakis* (van Thiel, 1966; Davey, 1971; J. W. Smith and Wootten, 1978; Margolis and Arai, 1989), but it is not clear which of the many species of cetaceans occurring off eastern Canada are important definitive hosts for this nematode. Information is presented on the levels of infection in the harbor porpoise of *A. simplex* and other helminths, together with data on the stages of maturity of the parasites and their distribution along the alimentary tract.

Materials and Methods

Harbor porpoises were caught by fishermen as an incidental by-catch in fishing gear set at 40–90 m deep in St. Mary's Bay and Placentia Bay and around the Avalon Peninsula and adjacent areas (Table 1, Fig. 1) during June–August 1990–1991. Two porpoises were obtained from these areas during the summer of 1987. Whole porpoises were placed on ice immediately upon arrival at the wharf and transported within 2 hr to the laboratory, where they were frozen (−30°C) whole for storage. One additional animal caught in October 1991 off southern Labrador during a Department of Fisheries and Oceans survey was dissected upon capture and the gastrointestinal tract frozen immediately. Porpoises were thawed in the laboratory and the sex, length (nearest centimeter), and weight (nearest kilogram) of each animal were recorded. The lower jaw was also removed and a tooth extracted from the middle of the lower mandible; the age of all except 5 animals was determined by examining growth layer groups in stained sections of the tooth using methods described by Rich-

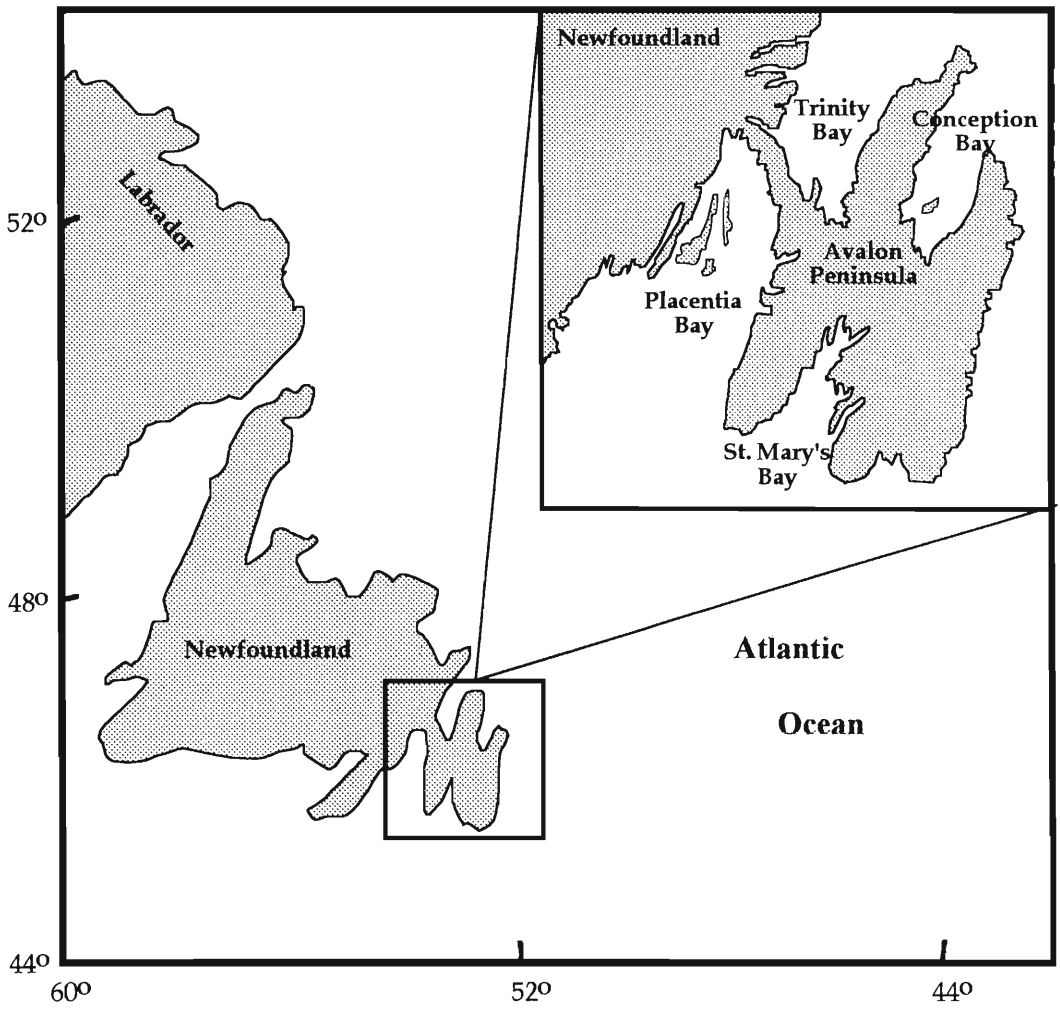

Figure 1. Sampling area for the harbor porpoise, *Phocoena phocoena*.

ardson (1992). Thirty-three female and 49 male porpoises of a wide range of ages were examined (maximum 9 yr old for females, 13 for males); they were classified into 4 age groups for analysis: calves (<1 yr old), immatures (1–3), young adults (4–6), and old adults (≥7 yr) (Table 1). Stomachs and intestines were removed, usually while the animal was still partially frozen, and parasites were extracted from food items and mucosa. Digestive tracts were in excellent condition upon examination. Gut contents together with scrapings of mucosa were washed through 4 fine-meshed sieves (850, 500, 355, and 53 μm), and the washings were collected and searched with a binocular dissecting microscope (×40 magnification). Major prey items were identified. The intestine was divided into 10 sections of approximately equal length, and separate counts of helminths were kept for each of the 3 stomach compartments (G. J. D. Smith, 1972) and the 10 intestinal sections. Ascaridoid nematodes were fixed in glacial acetic acid, preserved in glycerin–alcohol, and cleared in glycerin or lactic acid; they were identified and categorized as L3's or L4's, adult males, immature adult females (with no eggs in the uterus), or mature adult females (with fully developed eggs in the uterus). Other helminths (cestodes, digeneans, and acanthocephalans) were fixed in alcohol–formalin–acetic acid, stained in borax carmine or Ehrlich's hematoxylin, and mounted in Canada balsam. Parasite occurrence was expressed in terms of prevalence (% infected) and abundance (mean number of parasites per host including uninfected hosts ± standard error) following Margolis et al. (1982). Representative specimens have been deposited at the Atlantic Reference Centre, Huntsman Marine Laboratory, St. Andrews, New Brunswick, Canada E0G 2X0.

Results

A total of 8 helminth taxa, comprising 3 ascaridoid nematodes (*Anisakis simplex* Rudolphi, 1809, *Contracaecum osculatum* Rudolphi, 1802,

Table 1. Sampling details for the harbor porpoise, *Phocoena phocoena*, collected off Newfoundland and Labrador and examined for gastrointestinal helminths.

		Age group (yr)									
		Female					Male				
Location	Year	<1	1–3	4–6	≥7	Unknown	<1	1–3	4–6	≥7	Unknown
Southern Labrador	1991	—	—	—	—	1	—	—	—	—	—
Placentia Bay	1991	1	10	5	2	1	2	13	13	11	—
St. Mary's Bay	1987	—	—	—	—	1	—	—	—	—	1
	1990	1	—	1	1	—	—	1	—	1	1
	1991	—	1	2	—	—	—	—	1	—	—
Eastern Avalon	1990	—	—	2	—	—	1	—	—	—	—
	1991	—	1	—	—	—	—	—	1	—	—
Conception Bay	1990	—	1	—	—	—	—	—	—	—	—
	1991	—	2	—	—	—	—	1	1	1	—

and *Phocascaris* sp.), 3 cestodes (*Diphyllobothrium* sp. plerocercoids, *D. stemmacephalum* Cobbold, 1858, and *Tetrabothrius* sp.), 1 acanthocephalan (*Bolbosoma* sp.), and 1 digenean (*Campula oblongata* Cobbold, 1858) were found in the alimentary tracts examined (Table 2). The acanthocephalan and the cestode *D. stemmacephalum* were found only in the intestine, whereas the single species of digenean and the cestode *Tetrabothrius* sp. were found in the third stomach; nematodes were found throughout the alimentary tract (see later). Gastric ulcers, associated with the presence of larval and adult *A. simplex,* were observed in 1 porpoise; no other pathology was observed.

Ascaridoid nematodes, particularly *C. osculatum* (=*C. osculatum* B; for taxonomy, see Nascetti et al., 1993) were the most prevalent and abundant helminths in harbor porpoise stomachs and intestines (Table 2); other species were

Table 2. Helminth parasites of the alimentary tract of the harbor porpoise, *Phocoena phocoena*, from Newfoundland and Labrador.

Parasite	Prevalence	Abundance (±SE)	Maximum
A. Stomach (N = 80)			
Nematoda			
Anisakis simplex	47.5	3.18 ± 1.47	100
Contracaecum osculatum	83.8	25.31 ± 4.94	307
Phocascaris sp.*	30.0	0.89 ± 0.23	11
Cestoda			
Diphyllobothrium sp. (plerocercoids)	3.8	0.05 ± 0.05	2
Tetrabothrius sp.*	1.3	0.01 ± 0.01	1
Digenea			
Campula oblonga	7.5	0.15 ± 1.88	6
B. Intestines (N = 29)			
Nematoda			
A. simplex	13.8	0.17 ± 0.09	2
C. osculatum	75.9	14.76 ± 2.73	51
Phocascaris sp.	13.8	0.21 ± 0.10	2
Acanthocephala			
Bolbosoma sp.	6.9	0.07 ± 0.09	1
Cestoda			
D. stemmacephalum	6.9	0.07 ± 0.09	1

* Denotes new host record.

Table 3. Numbers and developmental stages of ascaridoid nematodes recovered from various regions of the alimentary tract of the harbor porpoise, *Phocoena phocoena*, from Newfoundland and Labrador.

Nematode	Developmental stage†	Stomach compartment*			Intestinal section*									
		1	2	3	1	2	3	4	5	6	7	8	9	10
Anisakis simplex	L3	46	12	21	0	2	1	1	1	0	0	0	0	0
	L4	129	0	0	0	0	0	0	0	0	0	0	0	0
	Adult male	13	0	0	0	0	0	0	0	0	0	0	0	0
	Adult female (imm.)	24	0	0	0	0	0	0	0	0	0	0	0	0
	Adult female (mat.)	8	0	0	0	0	0	0	0	0	0	0	0	0
Contracaecum osculatum	L3	348	283	1,383	130	103	46	64	44	21	10	3	3	1
	L4	2	2	7	0	2	1	0	0	0	0	0	0	0
Phocascaris sp.	L3	35	12	24	1	0	1	0	4	0	0	0	0	0

* Each stomach (N = 80) consisted of 3 compartments, which were examined separately, 1 = keratinized forestomach, 2 = main stomach, 3 = pyloric stomach (after Smith, 1972). Intestines (N = 29) were divided into 10 sections of approximately equal length, numbered from anterior to posterior.

† Imm. and mat. = adult females without and with fully developed eggs in the uterus, respectively.

uncommon (prevalences <10%, abundances <1 per host). Mature specimens (i.e., with fully developed reproductive organs) were observed among only 3 of the 8 taxa found; these were *A. simplex* (3.0% of 267), *C. oblonga* (100% of 18), and *D. stemmacephalum* (both specimens with gravid proglottids). Both acanthocephalan specimens were immature females whose proboscides were not fully extended; the single specimen of *Tetrabothrius* sp. was approximately 6 cm long and immature.

The numbers of each developmental stage in various regions of the alimentary tract were determined for the 3 species of ascaridoid nematodes (Table 3). Adults and L4's of *A. simplex* were restricted to the keratinized forestomach (Table 3); L3's were also common in this region, but small numbers were found in other stomach compartments and also in intestinal sections 2–5. Specimens of *C. osculatum* were much more abundant and widely distributed throughout the alimentary tract, particularly in the third (pyloric) stomach and the anterior sections of the intestines. All *C. osculatum* recovered were larvae (L3's and L4's). *Phocascaris* sp., all L3's, were much rarer than other nematodes but were found in all stomach compartments and occasionally in the anterior sections of the intestine.

Calves (<1 yr old) were the only age group not infected with gastrointestinal helminths. Among the remaining age groups, there were no significant differences in prevalence ($P > 0.05$, multiway contingency analysis or Fishers exact test) or abundance ($P > 0.05$, Kruskal-Wallis and Wilcoxon tests) among host age groups and sexes for any of the species of helminths. Adults of *A. simplex* occurred in porpoises of a wide range of ages (2–9 yr) and in both males and females.

Examination of stomach contents indicated that most porpoises had recently fed; 93.4% of the stomachs contained recognizable food items. There was no evidence (from examination of the gullet during extraction of teeth) that food items had been regurgitated during capture. In terms of the percentage of stomachs containing a particular prey species, the dominant food item was capelin, *Mallotus villosus* (Muller) (88.5%), which also comprised the bulk of the stomach contents in most animals. Other dietary items included gadoids (19.2%), sand lance (*Ammodytes* sp., 16.7%), herring (*Clupea harengus harengus* L., 10.3%), and squid (Teuthoidea, 1.3%). Amphipods were also common (41.0%), whereas pandalid shrimps (2.6%) were rare.

Discussion

Adults of *A. simplex* are known from several species of marine mammals on the Pacific and Atlantic coasts of North America (see summaries by Margolis and Dailey, 1972; Margolis and Arai, 1989). Although adults of *A. simplex* have been observed in the stomach of a phocid, *Halichoerus grypus* Fabricius (McClelland, 1980; Brattey and Stenson, 1993), cetaceans are the principle definitive hosts. There have been few large-scale studies on the abundance of *A. simplex* in Cetacea from the Northwest Atlantic, and these data combined with those from numerous smaller-scale studies and anecdotal reports permit only tentative conclusions about the importance of

the harbor porpoise relative to other Cetacea as definitive hosts of *A. simplex.*

Cowan (1967) reported variable numbers of *Anisakis* sp. in clusters of up to 100 worms in the stomach of 55 pilot whales, *Globicephala melaena* Traill, collected off Newfoundland, suggesting much higher abundances of *A. simplex* than those reported here. Scott and Fisher (1958a) found only 3 adults of *Anisakis* in the stomach of 150 harbor porpoises collected in the lower Bay of Fundy during May–November 1952–1956 but found 427 *Anisakis* in the stomach of a single beluga whale, *Delphinapterus leucas* Pallas. Vladykov (1944) reported that belugas from the Gulf of St. Lawrence were heavily infected with *Anisakis* spp., and Sergeant and Fisher (1957) observed nematodes, presumed to be *Anisakis,* in the stomach of each of 5 white-beaked dolphins, *Lagenorhynchus albirostris* Gray, from Conception Bay, Newfoundland. We observed 130 adults of *A. simplex* in the stomach of a *L. albirostris* from the same locality in March 1988 and several thousand adult *A. simplex* in the stomach of a humpback whale (*Megaptera novaeangliae* Borowski) caught in similar fashion in August 1992 in Lord's Cove, Burin Peninsula, Newfoundland (unpubl. obs.). Other records of *Anisakis* spp. in Cetacea are given in Margolis and Arai (1989).

There are undoubtedly some biases in the literature on parasites of Cetacea because animals with no worms are seldom reported; also, stranded animals are often the only source of samples, and their parasite faunas may not be representative. Nonetheless, our results generally agree with the extensive survey by Scott and Fisher (1958a) and suggests that the harbor porpoise in the inshore waters off Eastern Canada during the summer carry relatively few adults of *Anisakis.* The preceding summary suggests that cetaceans such as the *G. melaena, M. novaeangliae,* and *L. albirostris,* which are common around Newfoundland during summer (Sergeant and Fisher, 1957; Hay, 1982), carry on a per capita basis much heavier burdens of *A. simplex* and may therefore be more important in the transmission of the parasite to local fish stocks. The role of other common species, such as the minke (*Balaenoptera acutorostrata* Lacepede) and finback (*Balaenoptera physalus* (L.)), remains unknown because few specimens have been examined.

The presence of *A. simplex* in less than half of the harbor porpoises and the general rarity of adults of *A. simplex* in Northwest Atlantic harbor porpoises contrasts with the much heavier *Anisakis* burdens reported by J. W. Smith (1989) for *P. phocoena* from U.K. waters. However, differences in findings may partly be a seasonal effect because our samples were collected in summer (June–August) whereas Smith's samples were collected during winter (November–January). Surveys have shown that in the general area where our porpoises were collected 3 of the taxa we observed had in their stomachs larvae of *A. simplex* (e.g., capelin [prevalence 29%, abundance 0.37; Pálsson, 1986], adult herring [prevalence 33%, abundance 0.91; Parsons and Hodder, 1971], Atlantic cod, *Gadus morhua* L. (prevalence 37%, abundance 0.67; Brattey and Bishop, 1992]). These species of fish are therefore probably important dietary sources of larval *A. simplex* for harbor porpoise in our study area.

The most prevalent and abundant parasites were larvae of the ascaridoid nematode *Contracaecum osculatum.* The broad distribution of these larvae throughout the alimentary tract and the absence of adults suggest that they are unable to complete their development in this host and were being expelled. This species of nematode, along with *Phocascaris* sp., is common in the gastrointestinal tract of various phocids, particularly grey (*Halichoerus grypus*), harp (*Phoca groenlandica* Erxleben), and hooded (*Cystophora cristata* Erxleben) seals off Newfoundland and Labrador (Scott and Fisher, 1958b; Brattey and Ni, 1992; Brattey and Stenson, 1993). Harbor porpoises probably acquired the larvae of these nematodes by preying heavily on capelin. Data given by Pálsson (1986) indicate that larvae of *C. osculatum* (reported as *Contracaecum* sp.) were much more common (prevalence 63%, abundance 1.23) than larvae of *A. simplex* (prevalence 29%, abundance 0.37) in capelin from St. Mary's Bay, which broadly agrees with our findings on the relative abundance of these nematodes in harbor porpoise from the same general area.

Although water temperatures in the sampling area (at 40–90 m deep) are generally below 4°C during June–August (Colbourne and Fitzpatrick, 1994) and animals were kept cold and frozen as soon as possible after capture, postmortem migrations may have influenced the distribution of some of the parasites. In particular, *C. oblonga* and *Tetrabothrius* sp. possibly migrated as they normally occur in the bile ducts and anterior intestine, respectively, rather than the third stomach. Although adults of *A. simplex* were firmly anchored to the gastric mucosa, larvae were usually unattached, and the extent to which

postmortem migrations influenced the distribution of larval nematodes remains unknown. However, the intestines of porpoises were more than 20 m long and, because chilling dramatically reduces the mobility of larval ascaridoids, it seems unlikely they would have migrated along a significant proportion of the intestines during the interval between capture and freezing.

This study provides the first record of the acanthocephalan *Bolbosoma* sp. from the harbor porpoise in the Atlantic, although Dailey and Stroud (1978) recorded *Bolbosoma* sp. from 1 of 4 *P. phocoena* from the Pacific coast of North America. Records of *Bolbosoma* spp. from other cetaceans are numerous, but individual hosts usually carry few specimens that are often recovered from hosts in poor condition, making the identification of species difficult (see Measures, 1992; Hoberg et al., 1993). Measures (1992) summarized records of *Bolbosoma* from North America and described *B. turbinella* (Deising, 1851) from blue whales, *Balaenoptera musculus* (L.) from the Gulf of St. Lawrence. *Bolbosoma capitatum* (Linstow, 1880) Porta, 1808, has been found in pilot whales, *G. melaena* (Cowan, 1967), and sperm whales, *Physeter macrocephalus* L. (Hoberg et al., 1993), in Canadian Atlantic waters. *Bolbosoma* sp. has also been found in Atlantic white-sided dolphins, *Lagenorynchus acutus* Gray (Beverley-Burton, 1978), off Maine. Balbuena and Raga (1993) found that *B. capitatum* was common (prevalence 46.5%, abundance 6.3) in the intestine of long-finned pilot whales, *Globicephala melas* Traill, at the Faroe Islands in the Northeast Atlantic. However, the general rarity of *Bolbosoma* in the harbor porpoise and other marine mammals in Canadian waters suggests that this parasite is generally rare in the Northwest Atlantic. The absence of mature specimens further suggests that the harbor porpoise is an atypical host.

Our findings together with those of Scott and Fisher (1958a) and Baker and Martin (1992) suggests that the helminth community in the alimentary tract of harbor porpoises is species-poor and is therefore consistent with that of other toothed whales (Cowan, 1967; Wazura et al., 1986; Balbuena and Raga, 1993; Aznar et al., 1994). Other notable characteristics of the helminth fauna are that none of the species recovered are specific to the harbor porpoise; a few occur in several species of cetacean (e.g., *A. simplex, C. oblonga*), but most appear to be either rare species (e.g., *D. stemmacephalum*) or "accidental infections" (e.g., *C. osculatum* and *Phocascaris, Bolbosoma, Diphyllobothrium* plerocercoids, and possibly *Tetrabothrius*). Samples from a broader range of localities and seasons that include collection of organs other than the alimentary tract are required to further characterize the helminth parasite fauna of the harbor porpoise. More detailed parasitological information could also help elucidate the stock structure of the Northwest Atlantic harbor porpoise, which at the moment is largely a matter of conjecture (see Gaskin 1984, 1992).

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Filaricidal Activity of CGP 20376 against *Brugia malayi* Microfilariae, Larvae, and Adults

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ABSTRACT: The macrofilaricidal drug CGP 20376 was evaluated for its capacity to kill all stages of subperiodic *Brugia malayi* at various doses in inbred *Meriones unguiculatus* (jirds). Killing of microfilariae (MF) and infective stage larvae (third-stage larvae [L3's]) was also studied at various drug concentrations in vitro. Studies in vitro were performed in 24-well culture plates to evaluate drug concentrations ranging from 1,000 to 0.01 μg/ml. Culture wells containing 500 MF each or 20 L3's each were dosed with 10-fold dilutions of CGP 20376 suspended in dimethyl sulfoxide (DMSO) and serum-free medium. Three replicates of each experiment were performed. MF were killed within 2 hr at drug concentrations of 1,000 and 100 μg/ml. Killing reached 100% by 24 hr with 0.1 μg/ml of the drug, whereas at the lowest concentration, 0.01 μg/ml, complete killing required 35 hr. MF in medium only or in medium with DMSO remained viable after 35 hr in culture. For L3's, drug concentrations of 1000 and 100 μg/ml killed 100% of the larvae by 2.5 hr and by 15 hr with 10 μg/ml. In 1 μg/ml, 50% were dead by 20 hr and 90% by 25 hr. However, at this concentration, a few L3's remained alive and sluggishly motile for 165 hr. The effects of CGP 20376 on MF, adults, and developing larvae were evaluated in groups of age-matched inbred male jirds. A single dose of 25 mg/kg of CGP 20376 was more than 99% effective against fourth-stage larvae in vivo. Higher doses were required to kill adult worms within lymphatics.

KEY WORDS: CGP 20376, filaricidal drug, *Brugia malayi*, jirds, filariasis.

Lymphatic filariasis caused by *Wuchereria bancrofti* and *Brugia* species remains a serious health problem affecting more than 78 million people in tropical and subtropical regions of the world (World Health Organization, 1992). The lack of safe and reliable chemotherapeutic agents against larvae and adult worms has been a major hindrance to successful filarial control efforts. Diethylcarbamazine (DEC) has been the drug of choice for treatment of *Wuchereria* and *Brugia* infections for several decades; however, DEC is primarily a microfilaricide that is administered in large doses over several days, and its use is often plagued by serious side effects. The efficacy of single doses of DEC has recently been evaluated (Kimera et al., 1985; Paniker et al., 1991; Cartel et al., 1992; Kazura et al., 1993; Mataika et al., 1993; Shenoy et al., 1993; Dreyer et al., 1994). Ivermectin, a newer antifilarial drug that kills microfilariae and suppresses microfilaremias is currently undergoing field trials (reviewed in Ottesen and Campbell, 1994; Chodakewitz, 1995).

Several compounds that are chemically classed as isothiocyanates and their derivatives have antifilarial activity (Subrahmanyam, 1987; Townson et al., 1990). One such compound, CGP 20376, has been evaluated in vitro against *Onchocerca volvulus* (Strote, 1989) and *Litomoside carinii* (Davies et al., 1989) and in several animal models, including *O. volvulus*–infected rats (Strote, 1989), *Dipetalonema*-infected chimpanzees (Moysan et al., 1988), and *B. malayi* in monkeys (Mak et al., 1990), rats (Zahner et al., 1990), and jirds (Chandrashekar et al., 1990, 1991). Here we report on the efficacy of CGP 20376 against *B. malayi* microfilariae (MF) and infective stage larvae (third-stage larvae [L3's]) in vitro as well as larval and adult stages in jirds.

Materials and Methods

Subperiodic *B. malayi* was used throughout these studies. Infected jirds as well as stock *Aedes aegypti* eggs for maintenance of the entire life cycle were obtained from the U.S.–Japan Cooperative Filariasis Program repository (University of Georgia, Athens, Georgia). Inbred *Meriones unguiculatus* were obtained from Tumblebrook Farms (West Brookfield, Massachusetts). L3's were harvested from *Ae. aegypti* 14 days after the infecting blood meal as previously described (Yates et al., 1994), except that mosquitos were surface-sterilized in 95% ethanol before L3's were collected. MF were collected by syringe and needle from jirds with intraperitoneal infections (McCall et al., 1973).

In vitro studies were performed in triplicate in 24-well culture plates to evaluate the effects of drug concentrations ranging from 1,000 to 0.01 μg/ml on L3's and MF. Cultures were incubated at 37°C with 95% relative humidity and 5% CO_2 in air. For the experi-

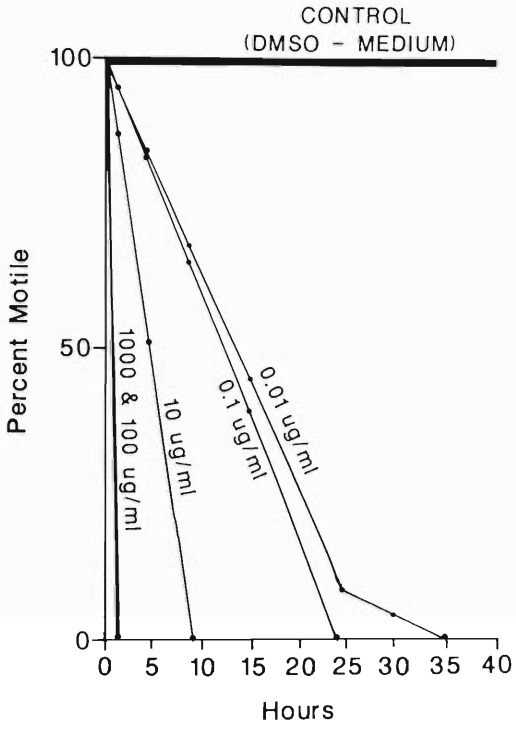

Figure 1. In vitro effect of CGP 20376 on *Brugia malayi* MF.

ments with MF, culture wells each containing medium F12 and 500 MF were dosed with 10-fold dilutions of CGP 20376 suspended in dimethyl sulfoxide (DMSO) and medium. Control culture wells contained medium with the maximum DMSO concentration used or medium only. All wells contained a final volume of 1 ml and a pH of 7.2. For the in vitro experiments with L3's, culture conditions were similar except that 20 larvae were placed in each well and doses of 0.5 and 0.05 μg/ml were also evaluated. Mortality was determined on the basis of absent motility and the apparent loss of structural integrity. Larvae considered dead by these criteria did not regain motility after washing and reincubation in fresh medium.

The in vivo effects of CGP 20376 on developing larvae, adult worms, and MF in *M. unguiculatus* were evaluated in groups of age-matched inbred male jirds. Three types of experiments were designed to evaluate the following: (a) the effect of a single dose (25 mg/kg, by stomach tube) on fourth-stage larvae (L4's) and the level of residual killing 3 wk after such a single dose; (b) the effect on development of microfilaremia after 1 or 2 doses (25 mg/kg) given at various intervals after infection; and (c) the effect of 2-×-25-mg/kg doses given to jirds with stable, patent infections. The jirds were 8 wk old at the beginning of each study. To facilitate drug delivery by stomach tube, CGP 20376 was suspended in DMSO and diluted in RPMI-1640. Jirds receiving 2 doses were given 6 hr rest between doses. In each experiment, the drug was suspended immediately prior to use. Sham-treated control jirds received doses of the vehicle (RPMI-1640 with 1.8% DMSO) alone. Blood for MF counts and serological testing was collected from the retroorbital venous plexus. Serum from selected jirds was assayed for anti-*Brugia* immunoglobulins by enzyme-linked immunosorbent assay. Jirds were necropsied and adult worms were enumerated as described previously (Yates and Higashi, 1985).

Results

Brugia malayi MF and L3's were highly sensitive to the filaricidal activity of CGP 20376 in vitro without complement or added serum. MF were killed within 2 hr at drug concentrations of 1,000 and 100 μg/ml (Fig. 1). Killing reached 100% by 24 hr with 0.1 μg/ml of the drug, whereas complete killing required 35 hr at the lowest concentration, 0.01 μg/ml. MF in medium only or in medium with DMSO were more than 99% viable after 35 hr in culture. For L3's, drug concentrations of 1,000 and 100 μg/ml killed 100% of the larvae by 2.5 hr in culture and by 15 hr all were killed with 10 μg/ml (Fig. 2). In 1 μg/ml, 50% were dead by 20 hr increasing to 90% by 25 hr. Interestingly, 6 L3's in 3 different culture wells remained alive and sluggishly motile for 165 hr in the 1-μg/ml concentration. Drug concentrations of 0.5 and 0.05 μg/ml had no apparent effect. Larvae in medium only and medium containing DMSO remained active and normal in appearance up to the end of the 170-hr culture period. The maximum serum concentration in healthy human volunteers given single doses of 8 mg/kg of CGP 20376 during toxicity testing by Ciba-Geigy was in the range of 1 μg/ml (Dr. H. P. Streibel, Ciba-Geigy Limited, Basel, Switzerland, pers. comm.).

The effects of CGP 20376 on developing L4's and potential residual activity of the drug 3 wk after treatment were evaluated in 3 groups of age-matched inbred male jirds (14 animals per group). At the outset, 1 group of jirds was given 25 mg/kg of CGP 20376 each by stomach tube. Three weeks later, all 3 groups were infected with *B. malayi* by subcutaneous injection of 75 infective stage larvae per jird. After an additional 3 wk, 1 of the previously untreated groups of jirds was treated with 25 mg/kg each of the compound. The jirds were then kept for another 15 wk before infections were evaluated in terms of microfilaremias and recovery of adult worms from the lymphatics and viscera. CGP 20376 was very effective against L4's of *B. malayi,* treated after infection (Table 1). None of the 14 jirds in this group were microfilaremic after 18 wk of poten-

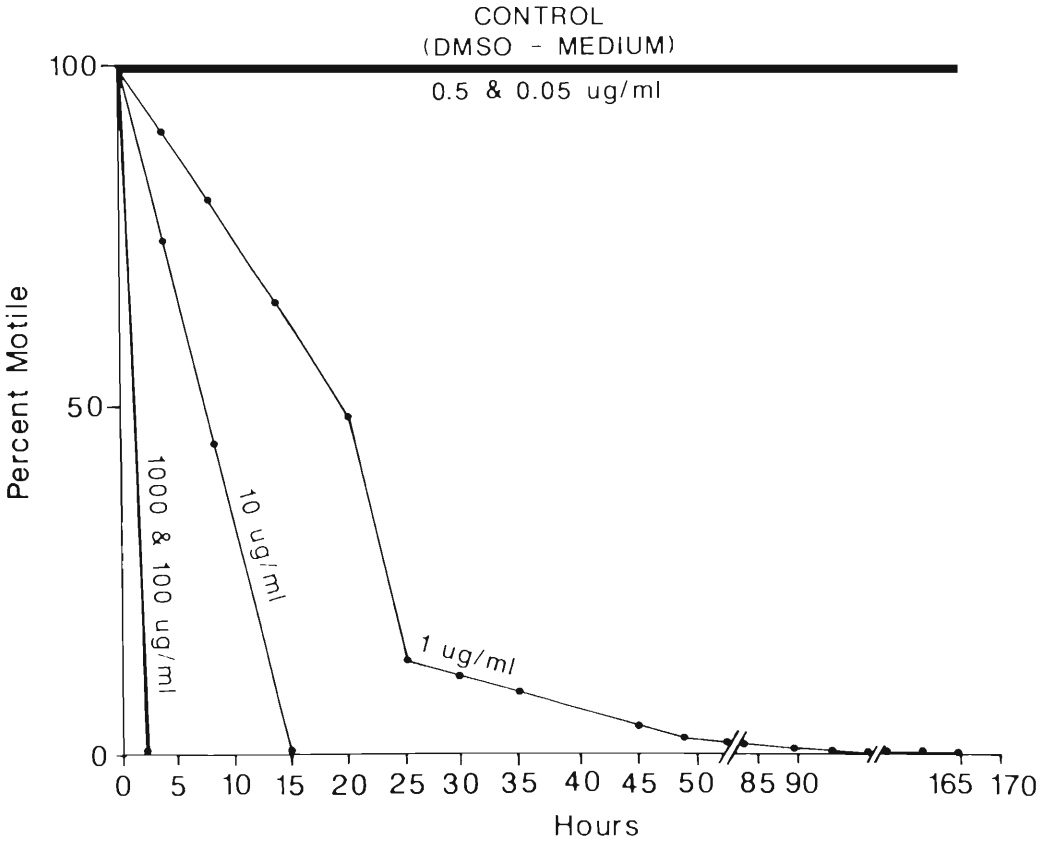

Figure 2. In vitro effect of CGP 20376 on *Brugia malayi* L3's.

tial development and at necropsy only 3 worms were found in the lymphatics or viscera of these jirds. In contrast, the other groups of jirds were heavily and similarly infected (Table 1). Normal development of *B. malayi* in jirds that were infected 3 wk after treatment with the drug was apparent. Microfilaremias were detected in 10 of these animals, and adult worms were found in the lymphatics of every jird in this group. Indeed, these findings were not significantly different from the sham-treated control group. The results of this experiment indicated that a single dose of 25 mg/kg of CGP 20376 was highly effective against the L4 stage, although a few worms survived this treatment. Filaricidal levels of the compound did not persist in the jirds 3 wk post-treatment.

From our preliminary experiments, it was apparent that a single dose protocol using 25 mg/kg as suggested by the drug manufacturer provided less than total clearance of worms from some jirds but seemed to produce amicrofilaremic infections in those jirds that harbored residual worms. In our early studies, treatments had always been given when the parasites were at the L3 or L4 stage and substantial but incomplete killing was noted (e.g., Table 1). Therefore, it was of interest to evaluate the effect on development of microfilaremia after 1 or 2 doses (25 mg/kg) given at different times in the course of infection. To that end, 144 jirds (12 groups of

Table 1. Effect of CGP 20376 (1 dose, 25 mg/kg) given to jirds 3 wk before or 3 wk after *Brugia malayi* infection.

Treatment	No. of jirds	No. of jirds with microfilaremia*	Mean No. of worms recovered
3 Wk before	14	10/14	7.4 (SD 4.11)
3 Wk after	14	0/14	0.2
Sham treatment	14	8/14	10.1 (SD 5.72)

* MF counts and necropsy 18 wk after infection.

Table 2. Effect on microfilaremia of CGP 20376 treatment (group A, 1 dose, 25 mg/kg; group B, 2 doses, 25 mg/kg) given to jirds at various intervals after *Brugia malayi* infection.

Treatment	No. of jirds		No. of jirds with microfilaremia			
			At 5 mo		At 10 mo	
	A	B	A	B	A	B
Sham treatment	12	12	12/12	12/12	11/12	12/12
After 7 days	12	12	1/12	0/12	1/12	0/12
After 20 days	12	12	3/12	0/12	3/12	0/12
After 32 days	12	12	2/12	0/12	3/12	0/12
After 42 days	12	12	3/12	0/12	5/12	0/12
After 100 days	12	12	5/12	0/12	9/12	0/12

12 jirds each) were infected with 100 *B. malayi* L3's each by the subcutaneous route. Ten groups were treated at 5 different times postinfection (PI); half the groups with the single-dose protocol (25 mg/kg) and half with 2 doses given 6 hr apart for a total of 50 mg/kg. As a control, 2 groups were sham-treated, one with a single dose of the vehicle only and the other with 2 doses of the vehicle. The sham treatments were given 6 days after infection. Because of the large number of L3's required for this study, it was necessary to infect the groups of jirds on 2 occasions. It was convenient to divide the experiment in half so that all jirds receiving the same treatment dose were infected on the same day. The 5 treatment times were chosen to correlate with various stages in the course of worm development. Treatments at 7 days of development were directed against the L3 stage a few days before the molt to L4, which occurs at about day 9 or 10. Treatments at days 20 and 32 PI were directed at the mid- and late L4, respectively, with the molt to immature adults (L5's) occurring between days 35 and 40. L5's were the target of treatments on day 42 PI, whereas day 100 treatments were directed at mature adults. Microfilaremias were evaluated at 5 and 10 mo PI. Two doses of 25 mg/kg given 6 hr apart prevented microfilaremia regardless of the developmental stage of the worms at the time of treatment (Table 2). However, with the single-dose protocol, there were patent infections in every treated group. All of the sham-treated jirds developed patent infections.

With an adequate treatment dose for preventing microfilaremia established at 50 mg/kg given in 2 equal doses, a third study was conducted to determine the effectiveness of this treatment in jirds with stable, patent infections. Sixteen jirds were infected with 100 L3's each. These jirds developed microfilaremias by 4 mo PI and maintained moderate MF levels (13–366 MF/20 μl of venous blood) until 10 mo PI. At that time, the jirds were randomly divided into 2 equal groups and either treated or given a sham treatment. MF were cleared from the blood of the treated jirds by 1 wk posttreatment and did not return for 6 mo (Table 3), at which time the jirds were necropsied. Microfilaremias persisted in the sham-treated group over the same time period. At necropsy, the CGP 20376–treated jirds were free of detectable worms whereas the sham-treated jirds were heavily infected with adult worms (Table 3). Correspondingly, serum antibody levels decreased slightly after treatment but persisted at high levels 6 mo after treatment.

Table 3. Effect of CGP 20 376 (2 doses, 25 mg/kg, each) given to jirds with stable, patent *Brugia malayi* infections.

Treatment	No. of jirds	Microfilaremia posttreatment			Mean adults recovered
		1 wk	3 mo	6 mo	
Sham treatment after 10 mo	8	8/8	8/8 (SD 16.3)	7/8	21.2
After 10 mo	8	0/8	0/8	0/8	0

Discussion

We have shown that CGP 20376 kills *B. malayi* MF and L3's in vitro without the aid of complement, cells, or other host factors at various drug concentrations. Furthermore, killing occurred at drug concentrations that were similar to serum levels seen in healthy human volunteers who had taken a single dose of the compound during toxicity testing conducted by the drug's manufacturer (Dr. H. P. Streibel, Ciba-Geigy Limited, Basel, Switzerland, pers. comm.). These

results are consistent with in vitro studies of CGP 20376 against *O. volvulus* L3's and L4's, *L. carinii* MF and adults, and *B. malayi* MF (Davies et al., 1989; Strote, 1989; Zahner et al., 1991). We have also confirmed the utility of DMSO as a carrier in place of ethanol (Davies et al., 1989).

Studies in jirds clearly showed the efficacy of this drug against MF, all larval stages, and adult worms established in the lymphatics. Treatment with 25 or 50 mg/kg was well tolerated by jirds and no untoward side effects were noted. However, hepatotoxicity has been observed in humans over the course of drug trials with this compound (Mak et al., 1991; Kohler et al., 1992). A single dose of 25 mg/kg of body weight was suggested to us as a starting point for treatment of *B. malayi* in jirds by the compound's manufacturer. This treatment was effective against the L4's but did not provide a complete clearance of worms. Treatment with a total dose of 50 mg/kg of body weight given in 2 oral doses 6 hr apart, however, provided apparently total killing of worms regardless of the developmental stage of the parasites. Previous studies showed similar results in *B. malayi*–infected jirds, however, were not as extensive and typically utilized carboxymethyl cellulose to solubilize the drug (Chandrashekar et al., 1991). Multiple doses of the drug have also been effective against *B. malayi* in *Mastomys natalensis* (Zahner et al., 1988). The killing of adult worms in jirds with stable, patent infections was substantiated by the loss of microfilaremia and its continued absence for 6 mo, as well as the absence of worms at necropsy.

Other filaricidal compounds have been shown to produce a residual prophylactic effect after treatment that may persist for months (Chusattayanond and Denham, 1984), and in some cases experimental treatments with antifilarial drugs apparently may lead to enhanced immunoresponsiveness and resistance to future infections (McCall et al., 1978; Blair and Cambell, 1981; Grieve et al., 1988). From our studies, it is clear that CGP 20376 produced insignificant residual chemoprophylaxis against *B. malayi* in jirds 3 wk posttreatment. Enhanced posttreatment immunoresponsiveness was not evaluated.

Acknowledgments

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Research Note

Effects of Reduced Oxygen Atmosphere on Motility, Penetration of Host Cells, and Intracellular Survival of *Eimeria nieschulzi* Sporozoites in Vitro

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ABSTRACT: We compared the ability of sporozoites of the rat coccidian, *Eimeria nieschulzi,* to become motile, penetrate Madin-Darby bovine kidney cells, and remain viable within host cells for up to 15 hr both in candle jars and in a 5% CO_2/95% air incubator. Results showed both motility and invasion of host cells to be unaffected by atmosphere whereas longer term survival was enhanced in the presence of a reduced oxygen atmosphere.

KEY WORDS: *Eimeria nieschulzi,* Apicomplexa, coccidia, blind well chamber, in vitro, oxygen.

Tilley and Upton (1988) reported development of the rat coccidian, *Eimeria nieschulzi* Dieben, 1924, to be enhanced when cultures were grown under reduced oxygen concentrations. Fukata et al. (1992) provided one explanation for this phenomenon by showing that reducing the oxygen concentration results in greater numbers of intracellular sporozoites of the chicken coccidian, *E. tenella.* Both Ricketts (1992) and Wrede et al. (1993) have found reduced O_2 to have no effect on growth of *E. tenella* in vitro, and Wrede et al. (1993) concluded that O_2 concentration affects only host cell invasion and not asexual development. Our recent studies, however, have indicated that this is not so for *E. nieschulzi,* the results of which are presented here.

Oocysts of *E. nieschulzi* were obtained from experimentally infected rats, strained through a graded series of sieves, sporulated, and stored in an aqueous 2.5% (w/v) $K_2Cr_2O_7$ solution as described by Bristol et al. (1983). Prior to use, oocysts were concentrated by sucrose flotation (Barnard and Upton, 1994) and incubated for 15 hr at 4°C in 100% Clorox® bleach to weaken oocyst walls (Hosek et al., 1988). Oocysts were then washed 3× in distilled water and 2× in phosphate-buffered saline (PBS) by centrifugation. Sporocysts were liberated from oocyst walls by grinding oocysts in a hand-held, Ten-Broeck glass-ground tissue grinder (Fisher Scientific, Pittsburgh, Pennsylvania). Free sporozoites were obtained by incubating sporocysts for 45 min in an excystation solution consisting of 0.25% (w/v) trypsin–0.75% (w/v) sodium taurocholate in PBS at 37°C. For migration assays (later), sufficient units of soybean trypsin inhibitor (Sigma Inc., St. Louis) dissolved in PBS were added to the mixture to neutralize 100% of the trypsin. Sporozoites were then purified from debris by nylon wool column filtration (Tilahun and Stockdale, 1982), washed by centrifugation at 800 *g* 1× in PBS and 2× in either Dulbecco's modified Eagle's medium (DMEM) for migration assays or RPMI 1640 for cell penetration assays. Mean numbers of sporozoites were calculated in each experiment by hemacytometer. All parasites were <2 mo old when utilized.

To assess whether or not motility of sporozoites was affected by atmosphere, sporozoite migration assays were performed as described by Upton and Tilley (1992). Briefly, after sporozoites were purified by filtration through a nylon wool column, 200 μl of DMEM containing 5.0 × 10^5 sporozoites was added to the top chamber of each blind well chamber (NeuroProbe Inc., Cabin John, Maryland). Sporozoites were separated from chemoattractant in the bottom chambers by a cellulose/acetate millipore filter with a pore size of 8.0 μm (Millipore Inc., Bedford, Massachusetts). Chambers were then divided into 2 equal groups and incubated at 37°C in either a 5% CO_2/95% air incubator or in candle jars (Tilley and Upton, 1988; Upton et al., 1991, 1994; Upton and Tilley, 1992). The O_2 concentration in candle jars and the 5% CO_2/95% air incubator was measured at 16.3–16.9 and 19.6%, respectively (Upton et al., 1994). Candle jars were preheated to 37°C prior to use to more carefully mimic the situation within the 5% CO_2/95% air

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incubator where cultures rise to incubator temperature rapidly. Positive chemoattractant consisted of 10% fetal bovine serum (FBS) in DMEM, and negative controls employed 100% DMEM only. The number of parasites that migrated through the filters and collected in the bottom chambers after 3 hr was assessed by counting sporozoites by hemacytometer (Upton and Tilley, 1992). Each test solution was examined at least in triplicate and the contents from each test well counted 10× (i.e., 10 counts/well × ≥3 wells = ≥30 counts per test solution).

Although long-term development of *E. nieschulzi* in Madin-Darby bovine kidney (MDBK) cells has been shown to be enhanced by reduced oxygen concentrations (Tilley and Upton, 1988), the effects of reduced O_2 on parasite survival in culture has been unknown. Therefore, we assessed parasite survival following penetration of host cells at 3 and 15 hr postinoculation using 2 different O_2 concentrations. MDBK cells (MDBK [NBL-1]; ATCC CCL 22) were grown to confluency on 22-mm² coverslips (VWR Scientific, San Francisco) in 6 well cluster plates (Costar Inc., Cambridge, Massachusetts). Media consisted of RPMI 1640 supplemented with 10% FBS, 10 mM HEPES, Na-bicarbonate to pH 7.4, 100 IU penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml fungizone. Prior to inoculation into cultures, each well was washed 2× with PBS and then inoculated either with 1.0×10^6 sporozoites for 3-hr incubations or 1.0×10^5 sporozoites for 15-hr incubations. These intervals were chosen because by 3 hr large numbers of sporozoites had penetrated cells but nonviable parasites had not yet degenerated; at 15 hr, sporozoites had not yet differentiated into first generation meronts, but nonviable sporozoites in cells were degenerating and could be distinguished from viable intracellular forms. Prior to inoculation, sporozoites were suspended evenly in the cell culture medium for uniform distribution over cells. Plates were incubated either in candle jars or in a 5% CO_2/95% air incubator. Coverslips were removed from wells using forceps, washed vigorously in a beaker of PBS to remove extracellular sporozoites, and examined by Nomarski interference contrast microscopy. The number of intact, intracellular sporozoites was then counted in 25, × 40 fields for each coverslip. Each experiment was replicated 4–8×. Total number of intracellular sporozoites in cells within each entire well was then calculated based on the assumption that monolayers were confluent. Results of all experiments were compared using the Wilcoxen Mann-Whitney *U*-test. Results are expressed as means followed by ± standard deviations.

Table 1. Effect of atmosphere on migration of sporozoites of *Eimeria nieschulzi* through a porous filter.

Attractant*	Replicates (*N*)	Number of migrating sporozoites ($\bar{x}$ ± SD)	% migration
5% CO_2/95% air			
100% DMEM	6	67 (163)	0.013
10% FBS in DMEM	6	123,733 (9,981)	24.7
Candle jars			
100% DMEM	6	400 (438)	0.08
10% FBS in DMEM	5	115,760 (12,217)	23.2

* $P > 0.05$ between same groups.

A reduced oxygen atmosphere in candle jars did not significantly affect motility of sporozoites in the blind well chambers (Table 1) ($P > 0.05$). Nearly 25% of the sporozoites migrated through the filters in the reduced oxygen atmosphere, whereas over 23% of the sporozoites did so in the 5% CO_2/95% air atmosphere. Likewise, atmosphere had no effect on penetration of sporozoites into host cells (Table 2) ($P > 0.05$). Over 22% of the sporozoites penetrated cells in both atmospheres. In contrast to the short-term studies, however, survival of sporozoites was affected by atmosphere after 15 hr (Table 2). Less than 39% of the sporozoites appeared intracellular and viable (nondegenerate) in the 5% CO_2/95% air atmosphere; many others were intracellular but appeared in various phases of degeneration. Approximately twice as many were observed to be viable in cells incubated in candle jars. This difference was significant ($P < 0.05$).

These results demonstrate that the effects of low O_2 on sporozoites of *E. nieschulzi* are different than for *E. tenella*. Sporozoite motility and host cell penetration of the former do not appear to be atmosphere-dependent, at least under the conditions of this study. However, survival within host cells, at least in the short term, are affected by atmospheric O_2. These differences may be due to a variety of factors, including differences in the class of vertebrate infected, site of invasion, and types of host cells targeted. The ability of many *E. nieschulzi* sporozoites to actively invade cells only to die later may be explained in

Table 2. Effect of atmosphere on penetration and survival of sporozoites of *Eimeria nieschulzi* in MDBK cells in vitro.*

3 hr postinoculation		15 hr postinoculation	
Candle jars ($N = 6$)	5% CO_2/95% air ($N = 8$)	Candle jars ($N = 6$)	5% CO_2/95% air ($N = 8$)
222,608 (40,128)	223,011 (39,069)	76,662† (4,726)	38,560† (2,260)

* 1.0×10^6 (3-hr study) or 1.0×10^5 (15-hr study) sporozoites inoculated/well onto monolayers of MDBK cells on 22-mm^2 coverslips. Coverslips were examined 3 or 15 hr postinoculation and the mean number of sporozoites/25 ×40 objective fields/coverslip counted. Results are expressed as the projected mean number of sporozoites/well (±SD).

† $P < 0.05$ between groups.

several ways. For instance, prolonged exposure of *E. nieschulzi* sporozoites to high oxygen concentrations may result in eventual, rather than immediate, sporozoite death. Eimerian sporozoites are known to have low levels of oxygen scavenging enzymes (Hughes et al., 1989; Michalski and Prowse, 1991), which may be exhausted relatively quickly and in an oxygen-dependent manner during the penetration process. Alternatively, lower oxygen concentrations may enhance the ability of sporozoites to successfully establish a functional parasitophorous vacuole by affecting extrusion of rhoptry contents. Tachyzoites of *Toxoplasma gondii* are known to be capable of invading host cells with or without extruding rhoptry contents, but these latter organisms are thought to eventually die and degenerate (Silva et al., 1982). Finally, Upton et al. (1994) recently showed that host cell type plays an important role in survival of *Cryptosporidium parvum* under different atmospheres in vitro. In these studies, survival of *C. parvum* in MDBK cell was enhanced in a reduced O_2 atmosphere, whereas higher numbers of parasites were found in a 5% CO_2/95% atmosphere when human HCT-8 cells were employed. These results suggest that the observed effects may be due to lower O_2 on host cells rather than parasites, which would in turn affect survival of intracellular parasites.

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Research Note

Parasites of the Round Goby, *Neogobius melanostomus,* and Tubenose Goby, *Proterorhinus marmoratus* (Perciformes: Gobiidae), from the St. Clair River and Lake St. Clair, Michigan

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ABSTRACT: Totals of 144 round gobies, *Neogobius melanostomus* (Pallas), and 48 tubenose gobies, *Proterorhinus marmoratus* (Pallas), were collected in June through September 1994 from the St. Clair River and Lake St. Clair, Michigan, and examined for parasites. Seven species (*Diplostomum* sp., *Eustrongylides tubifex, Rhabdochona decaturensis, Spinitectus* sp., *Spiroxys* sp., *Leptorhynchoides thecatus,* and glochidia) infected round gobies. More parasite species infected gobies from Lake St. Clair than from the St. Clair River, with *Diplostomum* sp. being most common at both locations. Four species (*Trichodina* sp., *Contracaecum* sp., *Spiroxys* sp., and *Neoechinorhynchus* sp.) infrequently infected tubenose gobies. All species infecting gobies have been reported from other fish species in Lake Huron and Lake Erie. Apparently, no parasites from the Black Sea have become established in this system with the original goby colonizers.

KEY WORDS: gobies, *Neogobius melanostomus, Proterorhinus marmoratus,* exotic fish, parasites, Michigan, Great Lakes.

Mills et al. (1993) discussed the animal species that have made their way to the Great Lakes of North America. The parasites of some of these exotic species have been studied. Toews et al. (1993) reported on the parasites of zebra mussels, *Dreissena polymorpha,* from Lake St. Clair and Lake Erie. Cone et al. (1994) found *Dactylogyrus amphibothrium* on the Eurasian ruffe, *Gymnocephalus cernuus,* in western Lake Superior and suggested that this monogenean arrived in North America with the original ruffe colonizers. Crossman et al. (1991) and Jude et al. (1992) have reported on the occurrence of the round goby, *Neogobius melanostomus,* and tubenose goby, *Proterorhinus marmoratus,* in the St. Clair River. Both species of gobies probably were transported from the Black Sea in Europe to the St. Clair River system in ballast water by freighter between 1986 and 1988. Jude et al. (1992) discussed the biology and potential impact of gobies on fishes in these waters. The present study reports on parasites that these goby species acquired in the St. Clair River and Lake St. Clair and whether or not Eurasian parasites were introduced into this system with the original goby colonizers.

Gobies were collected by angling, trawling, and electrofishing from the St. Clair River and Lake St. Clair, Michigan. The St. Clair River is a 63-km-long strait connecting Lake Huron to Lake St. Clair; midchannel depths range from 8.2 to 21.5 m and current velocity can approach 1.8 m/sec (Derecki, 1984). Lake St. Clair is a small, shallow body of water connecting the St. Clair and Detroit rivers, with a surface area of 1,114 km^2, a mean depth of 3 m, and a maximum depth of 8 m along a dredged shipping channel. The following fish data include information on location, month and year of collection, number of fish examined, and total length with range in millimeters (followed by mean ± SD):

1. Round gobies, St. Clair River (Marine City, Richardson Island), July and August 1994, n = 82, 62–142 (96 ± 18.5); Lake St. Clair (Anchor Bay, Huron Point, Middle Channel), August and September 1994; n = 62, 60–117 (86 ± 14.6).
2. Tubenose gobies, Lake St. Clair (Anchor Bay, Goosebay, Huron Point); June and August 1994; n = 48; 35–87 (56 ± 15.3).

Gobies were frozen in the field, measured (in millimeters), and sexed at necropsy. The entire fish was examined. Parasites were collected and processed using routine procedures. Prevalence is the percentage of fish infected, and mean intensity is the mean number of worms of a species per infected fish. Voucher specimens have been deposited in the U.S. National Parasite Collection, Beltsville, Maryland 20705: *Diplostomum* sp. (84550), *Contracaecum* sp. (84552), *Eustrongylides tubifex* (84545), *Rhabdochona decaturensis* (84546), *Spinitectus* sp. (84547), *Spiroxys* sp. (84548, 84553), *Leptorhynchoides thecatus*

Table 1. Prevalence (P), mean intensity (MI), and maximum number of parasites (max.) found in *Neogobius melanostomus* from the St. Clair River and Lake St. Clair, 1994.

Parasite	St. Clair River (n = 82) P	St. Clair River (n = 82) MI ± 1SD (max.)	Lake St. Clair (n = 62) P	Lake St. Clair (n = 62) MI ± 1SD (max.)	Site
Digenea					
Diplostomum sp.*	11	1.6 ± 0.7 (3)	89	9.8 ± 16.1 (82)	Lens
Nematoda					
*Eustrongylides tubifex**	—	—	2	1	Encysted in mesenteries
Rhabdochona decaturensis†	—	—	21	4.0 ± 5.0 (16)	Intestine
Spinitectus sp.‡	—	—	2	2	Intestine
Spiroxys sp.*	—	—	5	1	Encysted in mesenteries
Acanthocephala					
*Leptorhynchoides thecatus**	—	—	2	3	Encysted in mesenteries
Mollusca					
Glochidia*	1	—	—	—	Gills

* Larval or immature stages.
† Gravid females.
‡ Immature females.

(84551), *Neoechinorhynchus* sp. (84554), and glochidia (84549).

The present study is the first report of parasites from naturalized gobies in the Great Lakes area. Ten (12%) of 82 round gobies from the St. Clair River and 55 (89%) of 62 round gobies from Lake St. Clair harbored 1 or more metazoan parasite species. A total of 7 species (2 from the St. Clair River and 6 from Lake St. Clair) infected round gobies (Table 1). Most helminth species were represented as larval or encysted stages. *Diplostomum* sp. was the most common parasite at each location. *Rhabdochona decaturensis* Gustafson, 1949, also commonly infected gobies from Lake St. Clair. The other parasite species were infrequent. There were no significant differences in prevalence (chi-square analysis, $P > 0.05$) and intensity (Student's t-test, $P > 0.05$) of parasitism for *Diplostomum* sp. and *R. decaturensis* between female and male gobies. The round goby is a new host record for *R. decaturensis* and *Leptorhynchoides thecatus* (Linton, 1891) Kostylew, 1924.

Diplostomum sp. was the only species from round gobies shared between locations. The correlation coefficients for *Diplostomum* sp. intensity and host length were significant at the St. Clair River ($r = 0.579$, $P < 0.05$) and Lake St. Clair ($r = 0.537$, $P < 0.01$), indicating that *Diplostomum* sp. intensity increased with host length. *Diplostomum* sp. had a higher mean intensity and a significantly higher prevalence (chi-square, $\chi^2 = 35.9$, $P < 0.005$) in round gobies from Lake St. Clair than from the St. Clair River. However, infected gobies from the St. Clair River had a significantly larger mean length ± SD (112 ± 18.1) than their counterparts (88 ± 13.7) from Lake St. Clair (Student's t-test, $t = 21.0$, $P < 0.001$). Therefore, fish length does not play a major role in this difference. *Diplostomum* sp. was more common in gobies from Lake St. Clair because the snail intermediate host probably was more common there.

Only 5 (10%) of the 48 tubenose gobies from Lake St. Clair were infected with 1 or more parasites. The protozoan, *Trichodina* sp., occurred on the gills of 1 goby from Anchor Bay. Two larval nematodes, *Spiroxys* sp., were encysted in the mesentery of another goby from Anchor Bay. Two other larval nematodes, *Contracaecum* sp., and 1 acanthocephalan, *Neoechinorhynchus* sp., were encysted in the livers of 3 tubenose gobies from Goosebay.

Gobies from each location had a varied diet. Amphipods, isopods, and ostracods often were found in tubenose gobies. Zebra mussels, fingernail clams, snails, amphipods, chironomids, and caddisfly larvae were present in round gobies.

Hexagenia sp., mayfly naiads that are intermediate hosts for *R. decaturensis* and *Spinitectus* sp., were found in round gobies from Lake St. Clair but not from the St. Clair River. This probably explains why these nematodes were present in the lake but not the river.

The low intensities for most helminth species in round and tubenose gobies may be due to the limited time they have been in this system. However, native fish species (sculpins, *Cottus* spp., and johnny darter, *Etheostoma nigrum*) also may have low intensities in similar niches and merit examination for comparative purposes. Each goby species harbors helminths as larvae (*Diplostomum* sp., *E. tubifex, Spiroxys* sp., and *Contracaecum* sp.), which occur as larval stages in other Great Lakes fishes and mature in vertebrates common to the region. Endemic parasites known from other Great Lakes fishes (Dechtiar et al., 1988; Dechtiar and Nepszy, 1988) were acquired by both goby species. Apparently, none of the 10 parasites species found in gobies in the present study arrived with the original goby colonizers. In contrast, at least 1 Eurasian helminth species has entered the Great Lakes through the naturalization of an exotic fish species (Cone et al., 1994).

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Research Note

Ultrastructural Observations on *Myxidium serotinum* (Protozoa: Myxosporea) from *Bufo speciosus* (Anura: Bufonidae), in Texas

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ABSTRACT: An ultrastructural study employing scanning electron microscopy (SEM) was conducted on the myxosporean, *Myxidium serotinum* Kudo and Sprague, 1940. Trophozoites of *M. serotinum* were recovered from the gall bladder of 1 of 3 Texas toads, *Bufo speciosus* Girard, 1854, from Llano County, Texas. SEM observations on the spore of *M. serotinum* are reported for the first time. Comparisons are made with other *Myxidium* spp. from Old and New World amphibians.

KEY WORDS: Amphibia, *Bufo speciosus, Myxidium serotinum,* Myxosporea, Protozoa, toad, ultrastructure.

Four species of myxozoans of the genus *Myxidium* have thus far been reported in the gall bladder of amphibians worldwide (Delvinquier et al., 1992). *Myxidium immersum* (Lutz, 1889) was originally described and reported commonly from South American anurans (Lutz, 1889; Cordero, 1919, 1928; Carini, 1932) and has since been recovered from Australian frogs and the introduced giant toad, *Bufo marinus* (Delvinquier, 1986). *Myxidium haldari* Sarkar, 1982, is known only from the common treefrog, *Hyla arborea,* in west Bengal, India (Sarkar, 1982). *Myxidium lesminteri* Delvinquier, Markus, and Passmore, 1992, has been reported from 3 species of southern African anurans (Delvinquier et al., 1992). The North American species *Myxidium serotinum* was first described by Kudo and Sprague (1940). It has been subsequently reported in the gall bladder of various amphibians, including frogs and toads (Kudo, 1943; McAllister, 1987, 1991; McAllister et al., 1989, 1995a, b) and salamanders (Clark and Shoemaker, 1973; Clark, 1982; McAllister and Upton, 1987).

Ultrastructural observations have been published on spores of *M. immersum* (Delvinquier, 1986) and *M. lesminteri* (Delvinquier et al., 1992). Although Clark (1982), in an unpublished thesis, presented ultrastructural data on *M. serotinum,* nothing has been published previously on the ultrastructure of this myxozoan. Herein, we present a description of *M. serotinum* based mainly on ultrastructural studies (scanning electron microscopy [SEM]) carried out on the spores and compare the information with the ultrastructure of *M. immersum* and *M. lesminteri.*

Three (1 male, 2 females, $\bar{x} \pm$ SE snout–vent length [SVL] = 49.3 ± 0.67, range 48–50 mm) Texas toads, *Bufo speciosus* Girard, 1854, were collected in May 1993 by hand from Llano County, Texas, and examined for myxozoans. Toads were placed in individual bags on ice and returned to the laboratory for processing within 48 hr. They were sacrificed by pithing, and whole gall bladders were removed and rinsed in Hank's balanced salt solution, and their contents emptied and smeared onto microscopic slides. Gall bladder contents were searched by light microscopy for trophozoites, and measurements were taken of fresh material. For SEM, trophozoites were fixed in 2% glutaraldehyde containing 0.1 M sodium cacodylic acid buffer (pH = 7.4) for 2 hr, washed 3 times (10 min each) in 0.1 M

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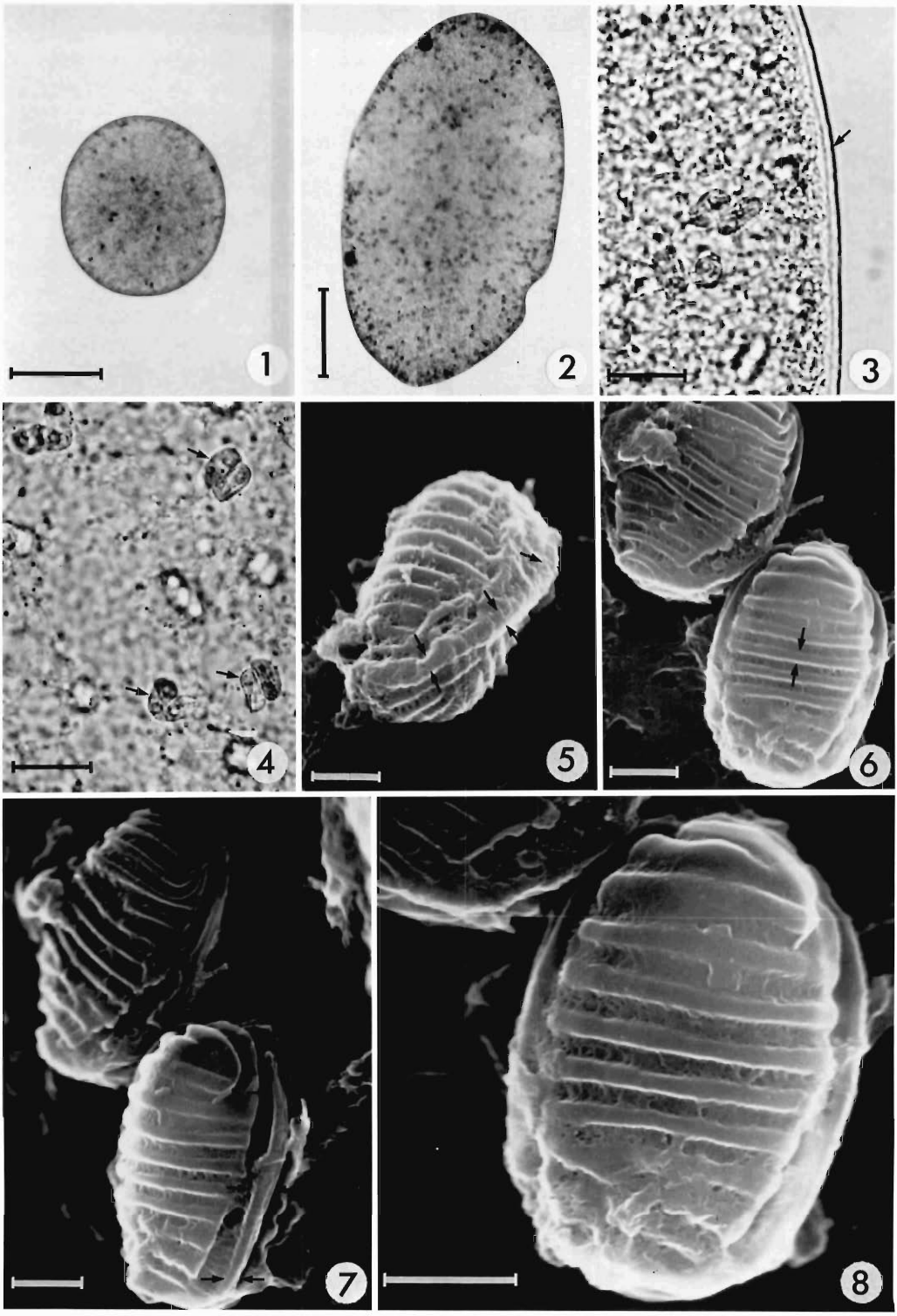
Figures 1–8. Trophozoites and spores of *Myxidium serotinum* Kudo and Sprague, 1940, from *Bufo speciosus*. 1. Spherical trophozoite. Scale bar = 250 μm. 2. Elongate or ellipsoidal trophozoite. Scale bar = 250 μm. 3. Trophozoite showing well-defined ectoplasmic layer (arrow). Scale bar = 20 μm. 4. Closer view of spores (arrows) inside medullary zone (endoplasm) of trophozoite. Scale bar = 20 μm. 5. Scanning electron micrograph (SEM) of spore showing valve suture (arrows). Scale bar = 2 μm. 6. SEM of spores showing individual transverse ridge



(arrows). Scale bar = 2 μm. 7. SEM of spores showing sutural ridge (arrows) paralleled by depression. Scale bar = 2 μm. 8. SEM of spore showing at least 12 transverse ridges. Scale bar = 2.5 μm.

cacodylic buffer, and transferred to 70% ethanol. Trophozoites were gently removed and placed onto strips of No. 2 coverslips that had been subbed in 0.1% poly-L-lysine (MW 164,000). Following maceration using teasing needles to release spores, both trophozoites and spores were allowed to settle onto coated coverslips for 1 min. Coverslips were then transferred temporarily to vials of 70% ethanol. Dehydration of samples was accomplished by dipping coverslips through a graded ethanol series followed by 2 changes in amyl acetate. Coverslips were critical point–dried using liquid carbon dioxide. For SEM, coverslips were attached to copper specimen strips by double adhesive tape and plasma-coated with 90% gold/10% palladium. SEM images were recorded on Polaroid type 55 positive–negative film at 40 kV (70 μA) with a JEOL 100CXII TEMSCAN electron microscope. Measurements were made with a calibrated ocular micrometer and are reported as means in micrometers followed by the ranges in parentheses.

Voucher specimens of *B. speciosus* were deposited in the Arkansas State University Museum of Zoology as ASUMZ 19083–19085. Specimens of *M. serotinum* examined by SEM (on grid) were deposited in the U.S. National Parasite Collection, Beltsville, Maryland 20705, as USNM 84236.

One of 3 of the *B. speciosus* (adult female, 50 mm SVL, ASUMZ 19083) was found to be infected with a myxozoan matching the description of *M. serotinum*. Spherical or ovoidal to elongate trophozoites (Figs. 1, 2) were flattened and floating freely in bile contents and measured (length × width): 800 (500–1,100) × 540 (450–630) μm (n = 10). Spores could be seen inside the trophozoites in the medullary zone or endoplasm. A well-defined ectoplasmic layer surrounded the internal spore-containing portion of the trophozoite (Fig. 3). Ovoidal spores containing 2 polar capsules (Fig. 4) were observed within trophozoites and measured: 8.0 (8.5–9.5) × 6.2 (5.8–6.5) μm (n = 10). Spheroid polar capsules were seen at each pole, but the number of polar filament turns within the capsule was not observed.

On SEM examination, spores appeared plump, ovoid, and bivalved and clearly showed a valve suture running obliquely along the spore (Fig. 5). The spore shell had numerous transverse striations (Figs. 6, 7) and was bissected by a sutural line bordered by parallel ridges. The suture was marked along its length in each valve by a pronounced ridge. The ridges were closely appressed along the suture except for a small zone subterminal to each pole of the spore where the ridges broadened around the polar filament eversion pole (Figs. 7, 8). The valves were marked by a pattern of transverse ridges (Fig. 8), which were remarkably similar from spore to spore. The sutural ridge was paralleled in each valve by a depression and a second oblique ridge continuous with the alternating transverse ridges and depressions. Transverse ridges numbered 10–13 (Fig. 8), confirming the light microscopic studies of Kudo and Sprague (1940).

When compared to other *Myxidium* spp. from Old and New World amphibians, spores of *M. serotinum* are most similar ultrastructurally to spores of *M. immersum* (see Delvinquier, 1986, figs. 4–7), as both possess shells with transverse striations. However, the subterminal valve region of *M. immersum* is devoid of transverse striations, whereas transverse striations completely cover both valves to the sutural lines at both ends of *M. serotinum*. In contrast, *M. haldari* has a shell with longitudinal striations (Sarkar, 1983), whereas the shell of *M. lesminteri* is smooth, which differentiates it from all 3 species (Delvinquier et al., 1992).

The ultrastructural configuration of spores of *M. serotinum* from *B. speciosus* were similar to those reported by Clark (1982) from two-lined salamanders, *Eurycea bislineata,* from West Virginia. However, differences in measurements of trophozoites and spores of *M. serotinum* reported by Kudo and Sprague (1940) and Clark and Shoemaker (1973) from those presented herein are probably the result of differences in amphibian hosts.

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Research Note

A Comparison of the Helminth Fauna of Two *Plethodon cinereus* Populations

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ABSTRACT: Two populations of *Plethodon cinereus* were examined for gastrointestinal helminths. The Pennsylvania population harbored 2 species, the cestode *Cylindrotaenia idahoensis* and the nematode *Batracholandros magnavulvaris,* and the Virginia population harbored 2 species, the trematode *Brachycoelium obesum* and the nematode *Cosmocercoides variabilis.* The presence of *Cylindrotaenia idahoensis* and *Cosmocercoides variabilis* in *Plethodon cinereus* establishes new host records. Pennsylvania is a new locality record for *Batracholandros magnavulvaris* and *Cylindrotaenia idahoensis.*

KEY WORDS: Caudata, *Plethodon cinereus,* Trematoda, *Brachycoelium obesum,* Cestoda, *Cylindrotaenia idahoensis,* Nematoda, *Batracholandros magnavulvaris, Cosmocercoides variabilis.*

The red-backed and lead-backed salamander, *Plethodon cinereus* (Green, 1818) Baird, 1850, is a small terrestrial salamander inhabiting forest-floor litter in cool, mesic coniferous and hardwood forests; it is found from southern Labrador and the Maritime Provinces of Canada to North Carolina, Indiana, and Minnesota (Smith, 1963). Some natural history of this salamander was pre-

Table 1. Helminths reported from *Plethodon cinereus* from various North American localities.

Helminth	Prevalence	Abundance	Mean intensity (range)	Locality	Reference
Trematoda					
Brachycoelium salamandrae	—*	50	—	Giles Co., VA	Cheng, 1958
	3.4	—	—	Durham Co., NC	Rankin, 1937a
	47.9	—	2.14	NC	Rankin, 1937a
	—	—	—	Avery Co., NC	Rankin, 1938
	25% (9/35)	—	—	Western MA	Rankin, 1945
	3% (1/36)	—	3.0	South-central NY	Fischthal, 1955a
	21% (5/24)	16	3.2	Pike Co., PA	Fischthal, 1955b
	57% (8/14)	8	1.0	WI	Coggins and Sajdak, 1982
	15% (26/171)	—	2.9 (1–16)	Barry Co., MI	Muzzall, 1990
Brachycoelium louisianai	—	21	—	VA	Cheng, 1960
Brachycoelium obesum	—	—	—	Giles Co., VA	Cheng, 1958
	—	>350	—	Giles and Albmarle cos., VA; Chester Co., PA	Cheng, 1960
	22% (13/60)	62	4.8 (1–38)	Accomack Co., VA	This study
Brachycoelium storeriae	—	4	—	Giles Co., VA	Cheng, 1958
	100% (4/4)	4	1.0	Bucks Co., PA	Cheng and Chase, 1960
Cestoda					
Plerocercoids	8.3	—	—	Durham Co., NC	Rankin, 1937a
Cylindrotaenia americana	12% (2/17)	—	—	Washington Co., TN	Dunbar and Moore, 1979
Cylindrotaenia idahoensis	7% (3/45)	17	5.6 (1–10)	Mercer Co., PA	This study
Nematoda					
Angiostoma plethodontis	—	—	—	VA	Chitwood, 1933
Batracholandros magnavulvaris	2.08	—	0.02	Buncombe Co., NC	Rankin, 1937b
	50% (6/12)	—	1.5 (1–12)	Fairfax Co., VA	Ernst, 1974
	28% (48/171)	—	1.9 (1–7)	Barry Co., MI	Muzzall, 1990
	9% (4/45)	7	1.8 (1–3)	Mercer Co., PA	This study
Cosmocercoides dukae	3.4	—	—	Durham Co., NC	Rankin, 1937a
	8% (3/35)	—	—	Western MA	Rankin, 1945
	7% (1/14)	1	1.0	WI	Coggins and Sajdak, 1982
Cosmocercoides variabilis	22% (13/60)	27	2.0 (1–4)	Accomack Co., VA	This study
Oswaldocruzia pipiens	3% (1/35)	—	—	Western MA	Rankin, 1945
Rhabdias ranae	7% (1/14)	—	—	WI	Coggins and Sajdak, 1982
Unidentified	25% (3/12)	—	1.3 (1–2)	Fairfax Co., VA	Ernst, 1974

* Not given.

sented by Dunn (1926); information on its parasites has been published by Chitwood (1933), Rankin (1937a, b, 1945), Fischthal (1955a, b), Ernst (1974), Dunbar and Moore (1979), Coggins and Sajdak (1982), and Muzzall (1990) (Table 1). This report compares the helminth fauna of 2 populations of *P. cinereus*.

Sixty *Plethodon cinereus* were collected by hand at Wallops Station, Accomack County, Virginia (30°57′N, 75°24′W), May 1992, from under logs in a pine-oak forest; 45 were collected from Buhl Ravine, Mercer County, Pennsylvania (41°12′N, 80°30′W), September 1991, from under rocks in an oak-maple forest. Both color phases (red-backed and lead-backed) were present in each sample. Salamanders were sacrificed by intraperitoneal injection of 70% ethanol and fixed in 5% formalin, washed in water, then transferred to 70% ethanol for storage. The body cavity was opened by a longitudinal incision from vent to throat, and the gastrointestinal tract was excised by cutting across the esophagus and rectum. The stomach and small and large intestines were slit longitudinally and examined under a dissecting

microscope. Each helminth was examined and identified using a glycerol wet mount. Selected cestodes were stained with hematoxylin and mounted in Canada balsam. Representative specimens were placed in vials of ethanol and deposited in the U.S. National Parasite Collection, Beltsville, Maryland 20705 (*Brachycoelium obesum,* 84389; *Cosmoceroides variabilis,* 84390; *Cylindrotaenia idahoensis,* 84391; *Batracholandros magnavulvaris,* 84392). Terminology use is in accordance with Margolis et al. (1982).

Six of 45 (13%) salamanders from Pennsylvania harbored helminths: 3 (7%) had a total of 17 individuals of the cestode species *Cylindrotaenia idahoensis* (Waitz and Mehra, 1961) Jones, 1987; 4 (9%) had a total of 7 individuals, 3 male, 4 female, of the nematode species *Batracholandros magnavulvaris* (Rankin, 1937) Petter and Quentin, 1976; and 1 salamander (2%) had a dual infection. Twenty-four of 60 (40%) salamanders from Virginia harbored helminths: 13 (22%) had a total of 62 individuals of the trematode species *Brachycoelium obesum* Cheng, 1958; 13 (22%) had a total of 27 individuals, 3 male, 24 female, of the nematode species *Cosmocercoides variabilis* (Harwood, 1930) Travassos, 1931; and 2 salamanders (3%) had dual infections.

The only trematode species found in this study was *Brachycoelium obesum,* which occurred in the small intestine of salamanders from Virginia. Although this trematode ranged from 1 to 38 individuals per infected host, only 5 hosts had 2 or more parasites. There has been controversy surrounding the assignment of species to the genus *Brachycoelium.* Rankin (1938) reduced all the American species to synonymy with *Brachycoelium salamandrae,* a European species and the type species; however, Parker (1941) and Cheng (1958) did not accept the synonymy and recognized 7 and 10 species, respectively. Later, Cheng and Chase (1960) and Couch (1966) described additional species to bring to 13 the number of species assigned to the genus. The specimens collected in this study most closely resemble *B. obesum* as described by Cheng (1958); they are oval distomes, rounded anteriorly, and less than 1.10 mm long with a large cirrus sac extending across the acetabulum. *Brachycoelium obesum* has also been found in *Ambystoma opacum* from West Virginia (Joy and Mills, 1975); *Desmognathus fuscus* from Illinois (Dyer et al., 1980) and Georgia (Byrd, 1937; Parker, 1941); *Eurycea bislineata* and *Plethodon glutinosus* from Georgia (Parker, 1941); and *P. glutinosus* from eastern Pennsylvania (Cheng, 1960), South Carolina (Byrd, 1937), and Virginia (Cheng, 1958, 1960). Infection requires ingestion of appropriate intermediate hosts. Both the common land snail *Zonatoides ligerus* and the common slug *Agriolimax agrestris* are suspected to be intermediate hosts (Cheng, 1960).

The only cestode found in this study was *Cylindrotaenia idahoensis,* which was collected from the small intestine of salamanders from Pennsylvania. This finding represents new locality and host records. Jones (1987) introduced some uncertainty in the host lists for species of *Cylindrotaenia* with his revision of the genus: *C. americana,* frequently reported in species of *Desmognathus* and *Plethodon* (Mann, 1932; Dunbar and Moore, 1979; Goater et al., 1987; McAllister et al., 1993), is considered to be a parasite of anurans, whereas *C. idahoensis,* originally described from *Plethodon idahoensis* by Waitz and Mehra (1961) and also known from *P. vehiculum* in Oregon (Panitz, 1969), is suggested to be the representative species in caudata. Dyer (1983) recorded *C. americana* from *Plethodon jordani* in North Carolina; but when Jones (1987) reexamined the material from *P. jordani,* it was determined to belong to *C. idahoensis.* Specimens collected in this study had paruterine complexes in a longitudinal orientation, a characteristic of *C. idahoensis*; in *C. americana*, the paruterine complexes have a transverse or diagonal orientation.

Two nematode species were found, 1 in salamanders from Virginia and 1 in salamanders from Pennsylvania. *Batracholandros magnavulvaris* was originally described as *Oxyuris magnavulvaris* by Rankin (1937b), who had a large number of female oxyurids from salamanders collected in North Carolina. Schad (1960) assigned *Oxyuris magnavulvaris* to the genus *Thelandros* and later described the male (Schad, 1963). Petter and Quentin (1976) expanded the genus *Batracholandros* to include species parasitic in American amphibians previously attributed to the genus *Thelandros. Batracholandros magnavulvaris* has been reported from a variety of salamanders (see Muzzall, 1990; Muzzall and Schinderle, 1992; Joy et al., 1993). *Batracholandros magnavulvaris* was found only in the Pennsylvania population, which represents a new locality record.

As in the cases of identity of species of *Brachyocoelium* and *Clylindrotaenia,* some uncertainty exists for species of *Cosmocercoides. Cosmocercoides variabilis* was first described as *Ox-*

ysomatium variabilis by Harwood (1930) from *Bufo valliceps* from Houston, Texas, as well as a number of other species of amphibians and reptiles. *Cosmocercoides dukae* was first described as *Cosmocerca dukae* by Holl (1928) from *Trituris virdisens* from North Carolina. Wilkie (1930) established the genus *Cosmocercoides,* and Travassos (1931) included both *C. dukae* and *C. variabilis* in his monograph on the Cosmocercidae. Harwood (1932) synomyzied *O. variabilis* with *Cosmocercoides dukae* and expressed some dismay that Travassos (1931) had the impression that Harwood (1930) had included a number of species in his original discussion of *Oxysomatium variabilis.* The major difference in the 2 species was the number of rosette papillae: *C. dukae* with 12 pairs of rosette papillae, and *C. variabilis* with 14–20 pairs. *Cosmocercoides dukae* is considered to be a parasite of terrestrial molluscs with inadvertent occurrence in animals feeding upon terrestrial molluscs; *C. variabilis* is considered to be a parasite of amphibians (see Vanderburgh and Anderson, 1987). Each male specimen in this study possessed 19 pairs of rosette papillae, thus the designation *Cosmocercoides variabilis.* This species was found only in the Virginia population and represents a new host record.

This is the first study of red-backed salamanders from western Pennsylvania: Mercer County is in the Ohio River drainage system. The 2 populations studied here harbor different helminth species. The results of this study are similar to those in other parasitologic surveys of *Plethodon cinereus* in that the number of parasite species found is low and the number of salamanders infected is also low. Not enough information is yet available to make any generalizations about the distribution patterns of the helminths of *P. cinereus.*

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Research Note

Gastrointestinal Nematodes of Two Australian Skinks, *Ctenotus regius* and *Ctenotus schomburgkii* (Sauria: Scincidae)

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ABSTRACT: Two species of Australian skinks, *Ctenotus regius* and *Ctenotus schomburgkii,* were examined for gastrointestinal helminths. *Abbreviata* sp. were found in connective tissue cysts on the outer surface of the stomach and small intestines of both species (73% prevalence in *C. regius*; 87% prevalence in *C. schomburgkii*). *Maxvachonia chabaudi* was found in *C. regius*; *Skrjabinelazia* sp. was found in *C. schomburgkii.* All findings represent new host records.

KEY WORDS: Nematoda, *Abbreviata* sp., *Maxvachonia chabaudi, Skrjabinelazia* sp., Sauria, Scincidae, *Ctenotus regius, Ctenotus schomburgkii,* Australia.

The genus *Ctenotus* contains 79 species of skinks that occur only in Australia, except for a single species, *Ctenotus spaldingi,* which occurs in Australia and New Guinea (Cogger, 1992). To our knowledge, the only report on the gastrointestinal helminths of lizards in this genus was by Mawson (1972), who examined *Ctenotus leae* and *Ctenotus labillardieri.* The purpose of our paper is to report the nematodes of 2 additional *Ctenotus* species, *Ctenotus regius* Storr, 1971, and *Ctenotus schomburgkii* (Peters, 1863). *Ctenotus regius* occurs in areas with sparse ground cover in central and eastern South Australia to western New South Wales, southwestern Queensland, and the southern Northern Territory; *Ctenotus schomburgkii* is found on sandy soils in association with arid scrubs and is widely distributed throughout the southern half of Western Australia, through South Australia and the southern Northern Territory, to central western New South Wales (Cogger, 1992).

Specimens from the herpetology collection of the South Australian Museum collected in South Australia at 100–300 m elevation in 1992 and 1993 were examined: 15 *C. regius,* 6 females, 9 males ($\bar{x}$ snout–vent length [SVL] = 65 ± 3.6 SD, range 57–71 mm), 15 *C. schomburgkii,* 2 females, 13 males ($\bar{x}$ SVL = 42 ± 2.9 SD, range 37–47 mm). All specimens were adults. Museum numbers and localities are given in the Appendix. While *C. regius* and *C. schomburgkii* are sympatric in parts of their ranges (Cogger, 1992), our samples were not sympatric. Lizards were originally preserved in 10% formalin and stored in 95% ethanol. Selected intact nematodes were placed in vials of 70% ethanol and deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705 (for accession numbers, see the Appendix). Terminology use is in accordance with Margolis et al. (1982).

The body was opened by a longitudinal incision from throat to vent and the gastrointestinal tract was removed by cutting across the anterior esophagus and rectum. The esophagus, stomach, small intestine, and large intestine were examined separately under a dissecting microscope. Nematodes were removed and identified using the standard glycerol wet mount procedure.

Two female *Maxvachonia chabaudi* Mawson, 1972, were found in the small intestine of 1 female (SVL = 68 mm) *C. regius.* One immature female *Skrjabinelazia* sp. was found in the small intestine of 1 male (SVL = 43 mm) *C. schomburgkii.* Encysted larvae of *Abbreviata* sp. were found in the serosa of the stomach and/or small intestine in 11 of 15 *C. regius* (73% prevalence, 6.9 ± 5.6 SD $\bar{x}$ intensity, range 1–18; 50% prevalence females, 89% prevalence males) and in 13 of 15 *C. schomburgkii* (87% prevalence, 8.9 ± 9.2 SD $\bar{x}$ intensity, range 1–34; 100% prevalence females, 85% prevalence males). *Ctenotus regius* infected with *Abbreviata* sp. averaged 66 mm SVL, range 63–71; *C. schomburgkii* averaged 46 mm SVL, range 37–47. No correlation was found between the number of *Abbreviata* sp. present and SVL for either *C. regius* or *C. schomburgkii* (correlation coefficient 0.49 and 0.30, respectively). All are new host records.

Maxvachonia chabaudi has previously been reported from the genus *Ctenotus,* namely, *C. leae* from Eyre Peninsula, South Australia, and

C. labillardieri from Pemberton, Western Australia (Mawson, 1972). No nominal species of *Skrjabinelazia* has so far been recorded from Australian hosts; in the only other report, Angel and Mawson (1968) recorded *Skrjabinelazia* sp. in the gecko *Christinus marmoratus* from Adelaide and Pearson Island, South Australia.

Species of *Abbreviata* are common parasites of mammals and reptiles but do not occur in birds (Morgan, 1946). Baker (1987) listed 58 species of *Abbreviata* known to infect reptiles. Of these, 15 (26%) are known from Australian lizards.

Roca (1993) suggested that the importance of lizards as prey can be ascertained by the prevalence of larval helminths in the lizard population; that is, prevalence of encysted larval nematodes in a lizard population indicates their degree of importance as prey because these lizards serve as intermediate hosts. Because these larvae were encysted and in relatively high prevalences, we believe the skinks to be intermediate hosts. The definitive hosts for the *Abbreviata* sp. recovered from *C. regius* and *C. schomburgkii* are likely carnivorous mammals or reptiles that feed on these skinks. One possibility might be the feral cat, *Felis catus,* which feeds on *C. regius* in southeastern Australia (Jones and Coman, 1981). Another conceivable definitive host might be varanid lizards, which also feed on *Ctenotus* sp. (Shine, 1986; James et al., 1992). More work will be required to elucidate the life cycle of these encysted *Abbreviata.*

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Appendix: South Australia Museum Catalog Numbers, Locality Data, and USNM Helminthological Collection Numbers

Ctenotus regius: SAMA R40452, 26°21′S, 135°15′E; R40502, 30°04′S, 138°17′E; R40597, 29°27′S, 134°12′E; R40789, 31°23′S, 137°03′E; R40888, 34°03′S, 139°11′E; R41274, 33°50′S, 140°56′E; R41603, 33°34′S, 139°58′E; R41786, 33°27′S, 140°19′E; R42114, 28°25′S, 136°01′E; R42322, 30°36′S, 139°32′E; R42327, 30°38′S, 139°32′E; R42501, 29°01′S, 133°16′E; R42537, 29°01′S, 133°25′E; R42540, 29°01′S, 133°25′E; 42585, 28°12′S, 133°24′E. USNM Helminthological Collection numbers: *Abbreviata* sp. 83979; *Maxvachonia chabaudi* 83978.

Ctenotus schomburgkii: SAMA R41356, 33°46′S, 139°48′E; R41402, 33°35′S, 140°40′E; R41469, 33°57′S, 139°54′E; R41475, 33°54′S, 140°12′E; R41571–41572, 41578, 33°09′S, 140°05′E; R41696, 32°38′S, 140°45′E; R41708, 32°57′S, 140°47′E; R41766, 32°49′S, 140°08′E; R42352, 30°38′S, 139°32′E; R42502, 29°01′S, 133°16′E; R42517–42518, 29°03′S, 133°19′E; R42579, 28°12′S, 133°23′E. USNM Helminthological Collection numbers: *Abbreviata* sp. 83981; *Skrjabinelazia* sp. 83980.

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Research Note

Scanning Electron Microscopy of *Geopetitia aspiculata* (Nematoda: Spirurida): Identifying Morphologic Features of the Mature Male

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ABSTRACT: *Geopetitia* spp. (Nematoda: Spirurida) are classified by the morphology of the spicules and number and distribution of genital papillae of the male. The genital papillae of the adult male *Geopetitia aspiculata* were studied with the aid of scanning electron microscopy and compared to that reported in the literature. The parasites were collected from infected birds at the Lincoln Park Zoological Gardens, Chicago, and from experimentally infected zebra finches (*Taeniopygia guttata*). The absence of spicules and the number and distribution of papillae on the ventral aspect of the posterior extremity are generally consistent with those previously reported for *Geopetitia aspiculata.* Eight pair of caudal papillae and a pair of small, lateral, subterminal papillae (phasmids) were present. No papilla was observed on the anterior portion of the circumanal cuticular inflation nor was a double subterminal papilla present.

KEY WORDS: avian, *Geopetitia aspiculata,* morphology, nematode, parasite, SEM observations, Tetrameridae.

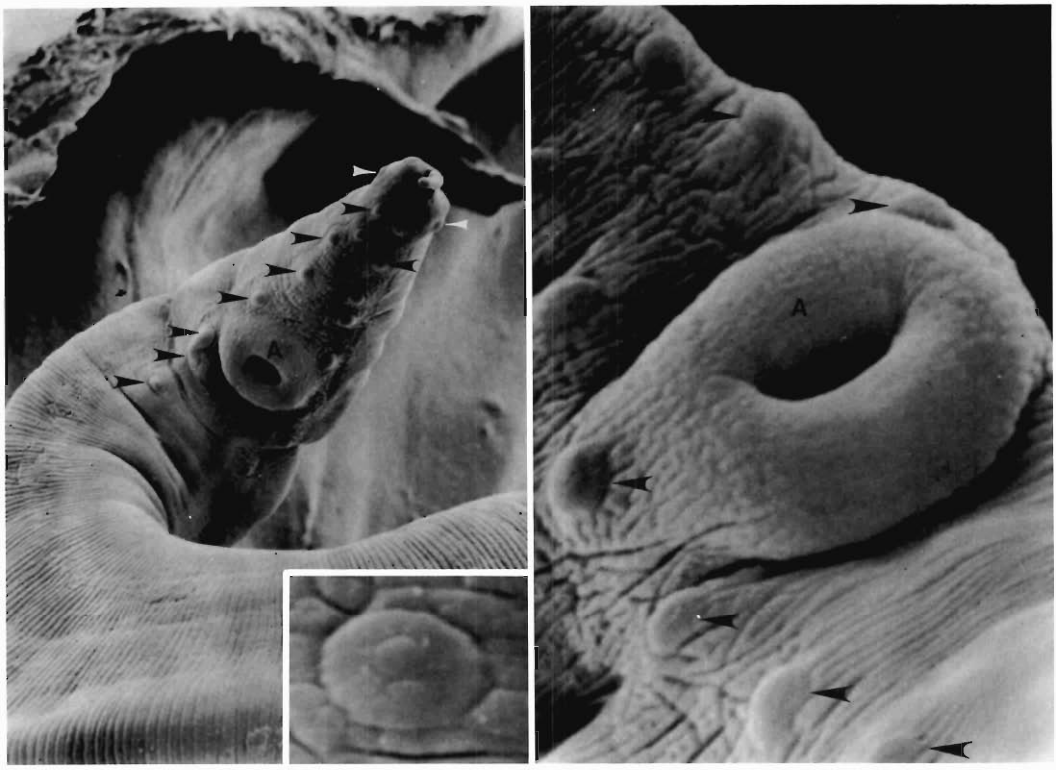
Geopetitia spp. (Tetrameridae: Geopetitiinae) are nematodes of birds (Webster, 1971; Chabaud, 1975). The genus was first described by Chabaud in 1951, and 8 species have been documented (Webster, 1971). *Geopetitia* spp. (but not *G. aspiculata*) have been reported in wild birds (Webster, 1971; Bartlett et al., 1984). Only birds in captivity have been reported to be infected with *G. aspiculata.* The natural source of parasites in captive birds is unknown. Cases have been reported only in North America at the National Zoological Park, Washington, D.C. (Webster, 1971), the Assiniboine Park Zoo, Winnipeg, Canada (Bartlett et al., 1984), and the Lincoln Park Zoological Gardens, Chicago (French et al., 1994). Barus (1968, 1971) described what he considered to be *G. aspiculata* in wild birds in Cuba; however, Barus (1968) indicated spicules were present in the male. Webster (1971) stated that it is the absence of spicules in *G. aspiculata* that readily distinguishes this species from others in the genus. Barus (1971) did not describe or give data on spicules in his second report but did include them in illustrations. It can be assumed the species described by Barus do not include *G. aspiculata,* although this has not been confirmed by reevaluation of specimens (Webster, 1971).

Identification of *Geopetitia* spp. is usually based on morphologic examination of the spicules and the number and distribution of genital papillae of the male (Webster, 1971). Females have no characteristics useful for specific differentiation of *Geopetitia* spp. (Webster, 1971; Chabaud, 1975). However, Mawson (1966) did use morphology of pseudolabia and relative position of the vulva and anus in female specimens, since no males were available, to differentiate *G. falco* and *G. chibiae.*

There are no demonstrated spicules in male *G. aspiculata* (Webster, 1971). The number and distribution of the genital papillae in the male have been described and illustrated by Webster (1971) and Bartlett et al. (1984). In Webster's (1971) description and diagrammatic illustration of genital papillae, 3 sublateral preanal pairs, 1 adanal pair, 4 postanal pairs, 1 double subterminal papilla, 1 papilla on the anterior anal lip, and subterminal phasmids were present. Bartlett et al. (1984) described and illustrated a papilla-like swelling immediately anterior to the anus and 8 pairs of caudal papillae.

The present study utilizes scanning electron microscopy (SEM) to document the morphologic features of the male used in the identification of *G. aspiculata.* The identification was confirmed as *Geopetitia* sp., "probably *G. aspiculata*" by Dr. J. Ralph Lichtenfels (USDA-ARS, National Parasite Collection, No. 69974, 27 December 1984, specimens from an orange quit, *Euneornis campestris*).

Nematode specimens of *G. aspiculata* ($n = 6$ male) were collected from naturally infected birds at the Lincoln Park Zoological Gardens, Chicago (French et al., 1994). Infected birds included 1 silver-throated tanager, *Tangara icterocephala*;



Figures 1, 2. ***Geopetitia aspiculata.*** **1. SEM of the posterior end of a mature male** ***Geopetitia aspiculata*** **from** ***Taeniopygia guttata.*** **Note the number (8) and orientation (3 pairs preanal and 5 pairs postanal) of genital papillae (black arrowheads), the lateral subterminal phasmids (white arrowheads), and the prominent circumanal cuticular inflation (A = anus). ×1,400. Inset: High magnification of the genital papillae on the male. Note the slightly raised nipplelike structure. ×5,900. 2. Higher magnification of the circumanal cuticular inflation on the male (A = anus, genital papillae [arrowheads]). The scanning electron micrograph is oriented posterior (upper left) to anterior (lower right). Note the absence of the papilla described in the literature on the anterior aspect of the cuticular inflation. Spicules are not present. ×3,560.**

2 Harris's sparrows, *Zonotrichia querula*; 2 white-spectacled bulbuls, *Pycnonotus xanthopygos*; 1 white-crested laughingthrush, *Garrulax leucolophus*; and 1 orange quit, *Euneornis campestris.* In addition, specimens (n = 5 male) were collected from experimentally infected zebra finches, *Taeniopygia guttata* (French et al., 1994).

Nematode parasites were removed from birds by sharp dissection. A pepsin digest of the parasite–tissue mass was performed to aid in the dissection of the encapsulated, tightly coiled parasites (French et al., 1994). The nematodes recovered from digestion were fixed in 2.5% phosphate-buffered glutaraldehyde for SEM. Specimens were dehydrated in standard dilutions of ethanol, critical point–dried in CO_2, mounted on aluminum stubs, sputter-coated with a thin layer of gold, and examined with an ISI-WB-6 scanning electron microscope.

Light microscopic examination and measurements of sexually mature nematodes recovered from experimentally infected zebra finches have been previously described (Bartlett et al., 1984; French et al., 1994). The posterior extremity of the male was ventrally coiled, spiraled, and difficult to evaluate by SEM. Eight pairs of caudal papillae were present, 3 preanal pairs and 5 postanal approximately equidistant to the tip of the tail (Fig. 1). Some disparity was noted in the location of papillae from sample to sample. There would be misalignment of pairs of papillae and papillae would be situated such that an adanal pair would be identified on some specimens. The number of papillae (8 pairs) was always consistent. Papillae were round and slightly raised with a central, smaller, round protuberance (Fig. 1, inset). A pair of subterminal, small, stalklike papillary structures were present on the lateral

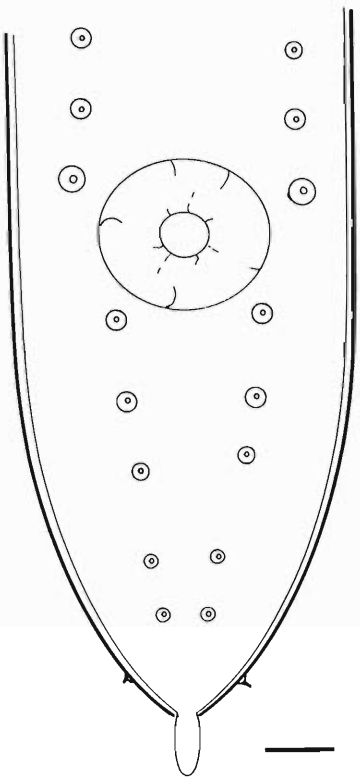


Figure 3. ***Geopetitia aspiculata*: Illustration of the ventral view of the posterior end of a mature male. There are 8 pairs of sublateral genital papillae, 1 pair of subterminal phasmids on the lateral line, and a circumanal cuticular inflation, and no spicules are present. Scale bar = 10 μm.**

aspect of the tail tip. These structures, identified as phasmids by Webster (1971), were not always visible due to inversion of the stalklike structure, thus creating a pit or inpouching. Spicules were absent. The anus was bounded circumferentially by a prominent cuticular inflation (Fig. 2). No papilla was identified on the anterior portion of the circumanal cuticular inflation in all specimens examined. The findings are summarized by illustration in Figure 3.

The nematodes recovered from birds at the Lincoln Park Zoological Gardens and used for the experimental infections were considered to be *Geopetitia aspiculata* based on morphology and previous descriptions (Webster, 1971; Bartlett et al., 1984; French et al., 1994). The lack of spicules in the males and arrangement of posterior papillae were consistent with Webster's and Bartlett's findings with the exception of the identification of a papilla anterior to the circumanal inflation (Webster, 1971) and a "papillae-like" swelling present immediately anterior to the anus (Bartlett et al., 1984). The single papilla anterior to the anus was not identified by light microscopy (French et al., 1994) or SEM in nematodes recovered from zoo specimens or experimentally infected birds. Webster (1971) also described and illustrated a double subterminal papilla that was not observed by either SEM or light microscopy in the present study and was not described by Bartlett et al. (1984). However, in general, the number of genital papillae and general distribution was constant. There are descriptive and illustrated differences in the location of the genital papillae of the male, that is, whether papillae were preanal, adanal, or postanal (Webster, 1971; Bartlett et al., 1984; French et al., 1994). The disparity was also observed by SEM. The papillae, though pedunculated in appearance with light microscopy, were morphologically nipplelike with a central protuberance. The phasmids were not described by Bartlett et al. (1984) but are difficult to see with light microscopy.

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Research Note

Characterization of Two *Steinernema scapterisci* Populations (Nemata: Steinernematidae) Using Morphology and Random Amplified Polymorphic DNA Markers

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ABSTRACT: The entomopathogenic nematode, *Steinernema scapterisci* (Rhabditida: Steinernematidae), was originally isolated from the mole cricket *Scapteriscus vicinus* (Orthoptera: Gryllotalpidae) in Uruguay. Subsequently, a population of *S. scapterisci* was isolated from the mole cricket *S. borellii* in Colon, Buenos Aires, Argentina. Because of the distance between the nematode isolates from Uruguay and Argentina and the different *Scapteriscus* species from which they were isolated, a study to examine the possible heterogeneity of *S. scapterisci* populations over space was conducted. Morphological variation was correlated with random amplified polymorphic DNA markers.

KEY WORDS: *Steinernema scapterisci*, Argentina, Uruguay, morphometrics, RAPD's, genetic variation, principal component analysis.

The entomopathogenic nematode, *Steinernema scapterisci* Nguyen and Smart, 1990 (Rhabditida: Steinernematidae), shows potential for biological control of mole crickets in the genus *Scapteriscus* Scudder in the southeastern United States (Parkman and Frank, 1992; Parkman et al., 1993, 1994). Mole crickets, accidentally introduced into North America in the early 1900's from South America (Walker and Nickle, 1981), cause extensive damage to turfgrass. *Steinernema scapterisci* initially isolated from Uruguay from *Scapteriscus vicinus* Scudder (Nguyen and Smart, 1990) was subsequently released in Florida to control mole crickets. It has become established but does not control the cricket populations (Parkman and Frank, 1992).

Stock (1992) isolated *S. scapterisci* from *Scapteriscus borellii* Giglio-Tos in Colon and Pergamino, Argentina, located in the Province of Buenos Aires approximately 500 km from the Uruguayan border. This isolate was propagated by industry (biosys, Palo Alto, California) and designated as Argentinian strain 319. We obtained the Uruguayan strain from Dr. Grover Smart, University of Florida, Gainesville, Florida. This Uruguayan isolate had been designated previously by biosys as strain 292. Because of the geographic distance between the 2 nematode isolates from Uruguay and Argentina, and because they were isolated from different *Scapteriscus* species, we conducted experiments to determine whether or not there were morphometric and DNA differences between the 2 populations.

The methods for rearing both nematode isolates were similar. We used standard in vivo culture techniques with the house cricket *Acheta domesticus* L. (Orthoptera: Gryllidae) as the host organism. First- and second-generation adults were obtained by dissecting infected house crickets 3–4 and 6–8 days, respectively, after they died. Infective juveniles were recovered when they emerged from the cadavers in a modified White trap (Woodring and Kaya, 1988), in 8–14 days. For morphometrics, nematodes were fixed in TAF and cleared in lactophenol (Gardner et al., 1994).

Quantitative measurements were made using a Leitz Ortholux II microscope with an ocular micrometer and Jandel® software or video imaging system. Standard descriptive statistics and principal component analysis (PCA) were used for analysis (SAS Institute, 1988).

Random amplified polymorphic DNA (RAPD) fragment analysis was performed to assess the extent of interpopulation genetic variation following the method of Caswell-Chen et al. (1992) and Gardner et al. (1994) with the following modifications: several thousand infective juveniles from each population collected from the modified White trap were separately washed in buffered saline (9%) 3 times. Centrifugation flotation, using 30% sugar solution, was used to further clean the nematodes, followed by 3 washes in sterile water. After washing, the infective juveniles were frozen quickly in liquid nitrogen and

Table 1. Comparison on the biometrics of males of Argentinian and Uruguayan populations of *Steinernema scapterisci*.

	Argentinian strain (n = 20)											
	Present study						Stock (1992)					
	First Generation			Second Generation			First generation			Second generation		
Character*	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range
Length (L)	1,597	131	1,355–1,800	993	153	850–1,423	1,500	250	1,000–1,900	1,000	80	989–1,300
Width (W)	133	16	108–166	73	18	55–122	130	42	90–200	65	9	57–76
Stoma L	5	0.6	4–6	4	0.5	3–5	4	0.3	4–5	3.8	1	3.5–5.5
Stoma W	6	0.7	5.5–7.5	5	0.5	4–6	5.7	1	4.5–7	5	1.2	4.5–6.5
AE-EP	85	9	67–109	64	7	53–77	65	11	60–89	63	8	54–78
AE-NR	130	14	109–172	118	12	99-143	128	10	120–140	110	10	96–129
AE-P	181	11.5	164–203	167	12	142–184	175	12	150–198	152	13	135–185
Tail L	30	3	23–35	24	3.5	17–31	23	5	19–27	23	3	19–26
Cloaca W	42	4	33–50	28	4	21–39	29	4	26–39	27	4	20–35
Mucron L	3	0.5	2–4	3	0.4	2–4	3.8	0.5	2.8–4.5	3	0.6	2.8–3.7
Testis refl.	326	30	258–368	157	20	102–197	356	39	298–395	189	18	160–215
Spicule L	91	4	83–99	74	6	63–87	80	5	72–89	77	4	74–83
Gub. L	59	7	50–75	53	6	43–67	59	4	57–70	50	4	45–59
	Uruguayan strain (n = 20)											
	Present study						Nguyen and Smart (1990)					
	First Generation			Second Generation			First generation			Second generation		
	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range
Length (L)	1,745	355	1,337–2,281	1,150	90	1,084–1,354	1,728	358	1,319–2,271	1,174	95	1,031–1,342
Width (W)	163	45	99–235	74	10	65–87	156	49	97–231	73	8	62–84
Stoma L	4.5	1	3–5	4.5	1	3–5.5	4.4	1	3–5	4.3	1	3–6
Stoma W	6.6	1.5	5.5–8	6	1	5–7.5	6.1	1	5–8	6	1.2	5–8
AE-EP	75	12	66–96	66	6	54–74	71	11	63–98	68	7	50–75
AE-NR	138	10	125–149	120	9	100–129	136	11	120–152	121	10	103–131
AE-P	190	18	162–212	170	11	140–178	187	21	164–216	168	13	138–181
Testis refl.	375	48	311–445	200	17	180–235	374	52	306–447	205	19	176–234
Tail L	22	3	20–28	23	3	20–28	25	3	21–30	25	3	22–30
Mucron L	4	0.5	2.9–4.5	3.9	0.4	3.0–4.4	4.3	0.6	3.1–4.7	3.9	0.6	3.1–4.6
Cloaca W	32	4	30–42	31	3	27–40	33	5	31–45	33	4	28–41
Spicule L	84	4	72–90	79	4	71–81	83	5	72–92	78	3	75–83
Gub. L	63	4	58–74	53	3	49–57	65	5	59–75	54	3	47–59

* Abbreviations: AE-EP = distance from tip of head to excretory pore; AE-NR = distance from head to nerve ring, AE-P = distance from head to pharynx base.

Table 2. Comparison on the biometrics of infective juveniles of Argentinian and Uruguayan populations of *Steinernema scapterisci*.

	Argentinian strain (n = 20)					
	Present study			Stock (1992)		
Character*	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range
Length (L)	524	29	467–568	530	29	500–570
Width (W)	27	2	22.5–31.5	20	3	15–25
AE-NR	78	5	69–86	89	1.1	80–97
AE-EP	38	2	34–42	36	4	34–42
AE-P	118	8	105–136	120	4	114–142
RD	0.32	0.03	0.25–0.34	0.4	0.03	0.30–0.46
RE	0.76	0.06	0.75–0.78	0.7	0.05	0.63–0.75
Tail L	48	2	45–53	49	4	47–54
	Uruguayan strain (n = 20)					
	Present study			Nguyen and Smart (1990)		
	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range
Length (L)	580	27	517–615	572	27	517–609
Width (W)	32	9	17–31	24	4	18–30
AE-NR	95	9	79–112	97	1.1	83–106
AE-EP	43	5	36–50	39	4	36–48
AE-P	125	7	111–136	127	6	113–134
RD	0.34	0.04	0.28–0.41	0.31	0.03	0.27–0.40
RE	0.8	0.09	0.64–0.98	0.73	0.06	0.60–0.80
Tail L	54	5	44–62	54	3	48–60

* Abbreviations: AE-NR = distance from tip of head to nerve ring; AE-EP = distance from head to excretory pore, AE-P = distance from head to pharynx base, RD = AE-EP/AE-P, RE = AE-EP/tail length.

stored at −80°C until processed for DNA analysis.

The frozen nematodes were transferred to a glass tissue homogenizing tube containing extraction buffer (1% sodium lauryl sulfate; 50 mM; ethylenediaminetetraacetic acid (EDTA); 100 mM Tris-HCl, pH 8; 200 mM NaCl; 50 μg/ml proteinase K), homogenized on ice at 1–2°C, and transferred to a 1.5-ml Eppendorf® tube. Extraction buffer was added to make a final volume of 300 μl. This was incubated in a water bath at 55°C for 2 hr. To remove proteins and other cellular debris, equal volumes of phenol/chloroform/isoamyl alcohol (25:24:1) were added to the tube and centrifuged at 16,000 *g* for 15 min at room temperature. The extraction procedure was repeated again, and the DNA was precipitated from the supernatant portion with 2.5 volumes of cold 95% ethanol. The precipitate was resuspended in polymerase chain reaction in (PCR) TE buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA) calibrated at 10 μg/μl and used as the DNA template for amplification using the PCR for the RAPD analysis.

Operon® primers (A-05 and A-11) 10 nucleotides in length were used for all reaction experiments with an annealing temperature of 35°C. Purified DNA from the nematode genome was subjected to the PCR, and the amplified DNA was electrophoresed on a 1.7% horizontal agarose gel. PCR products were photographed after staining with 2 μl/ml ethidium bromide for 10 min.

The isolates and/or species included on the gel were the following: *S. carpocapsae* Weiser (All strain), *S. glaseri* Steiner, *S. scapterisci* (Argentinian isolate 319 and Uruguayan isolate 292), *Heterorhabditis hawaiiensis* Gardner, Stock and Kaya, 1994, and *H. indicus* Poinar, Karunakar, and David, 1993.

Amplification products were checked for DNA contamination from the nematodes bacterial symbiont (Caswell-Chen et al., 1992), and none of the nematodes' RAPD patterns included the bacteria's DNA. Throughout this study, RAPD reactions were always duplicated and care was taken to ensure consistency in DNA banding profiles between replicates and between separate experimental runs.

PCA was performed on morphometric vari-

Table 3. Comparison on the biometrics of females of Argentinian and Uruguayan populations of *Steinernema scapterisci*.

	Argentinian strain (n = 20)											
	Present study						Stock (1992)					
	First Generation			Second Generation			First generation			Second generation		
Character*	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range
Length (L)	4,740	1,367	3,997–6,534	2,171	222	1,775–2,500	3,890	450	3,020–3,970	2,015	224	1,786–2,347
Width (W)	198	57	169–276	134	19	100–167	164	12	153–190	112	16	86–135
Stoma L	9	2	8–12	5	1	4–6	6.8	1	5–8.5	6	1.5	5–8
Stoma W	12	3	10–14	6.5	0.5	6–7.5	8.5	3	7–12	7.5	0.8	7–10
AE-EP	99	3	88–149	76	6	62–85	78	5	75–90	70	7	63–84
AE-NR	175	44	148–231	157	15	154–259	153	11	144–170	146	11	139–168
AE-P	260	65.5	230–366	206	24	198–242	215	14	198–240	210	12	196–230
V%	51	12	50–60	55	2	50–58	53	2	49–54	55	2	53–57
Tail L	63	15	55–78	54	3	47–59	42	5	30–47	47	3	39–58
Anus W	67	18	60–96	52	8	33–65	52	3	40–62	41	5	39–51
	Uruguayan strain (n = 20)											
	Present study						Nguyen and Smart (1990)					
	First Generation			Second Generation			First generation			Second generation		
	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range
Length (L)	4,247	574	3,544–5,219	2,220	231	1,996–2,545	4,162	540	3,531–5,156	2,209	223	1,841–2,530
Width (W)	184	12	161–210	150	17	100–165	179	13	159–203	123	14	94–141
Stoma L	7	1	6–9	7	1.5	6–9	7.5	1	6–9	6.7	1.4	5-9
Stoma W	10	2.5	9.5-12	9	1.1	8.5–12	10	3	9–12	8.9	0.9	8–11
AE-EP	92	4	80–98	80	7	65–90	89	5	78–94	78	6.8	66–88
AE-NR	179	11	158–195	172	12	150–187	174	13	153–194	169	12	147–184
AE-P	240	16	220–273	245	13	230–267	242	17	219–269	241	15	222–266
V%	53	2	51–54	53	2	52–59	53	2	50–54	52	2	52–60
Tail L	44	9	38–58	57	3	47–62	46	8	34–59	58	4	48–64
Anus W	56	7	46–70	45	3	50–59	58	9	41–72	47	2.8	43–52

* Abbreviations: AE-EP = distance from tip of head to excretory pore; AE-NR = distance from head to nerve ring, AE-P = distance from head to pharynx base.

Table 4. PCA eigenvectors.

	Males first generation			Males second generation		
Variables*	PC I	PC II	PC III	PC I	PC II	PC III
LLENGTH	0.295839	0.351143	0.055837	**0.458753**	0.104773	−0.257540
LWIDTH	0.295271	0.321798	0.031507	0.312456	0.154789	0.057423
LSTL	−0.134271	0.213577	0.541455	−0.095463	−0.09874	0.321456
LSTW	−0.009748	0.344268	**0.588545**	−0.014537	0.214568	0.231114
LAEEP	−0.040713	**0.451225**	−0.466217	0.317662	0.222009	−0.546420
LAENR	0.191067	0.266521	−0.311030	0.038308	0.449822	0.089736
LAEPH	0.242651	0.407037	0.022556	0.304647	0.052375	**0.510891**
LTAILL	**0.376394**	−0.134926	0.000515	0.127584	0.477967	−0.160825
LMUCL	**0.374418**	−0.120605	0.015726	−0.007926	**0.595884**	0.420361
LWANUS	−0.375566	0.160077	−0.043911	0.411356	−0.188966	0.386077
LTREF	−0.374313	0.141142	0.014545	0.284663	−0.244254	−0.024976
LSPICL	−0.283503	0.300227	−0.186097	0.388296	0.082999	−0.080399
LGUBL	0.258314	−0.016155	0.068581	0.420412	−0.221052	0.08559
	Females first generation			Females second generation		
	PC I	PC II	PC III	PC I	PC II	PC III
LLENGTH	0.316118	−0.256909	0.338031	0.268249	0.437956	−0.249719
LWIDTH	0.32629	−0.350363	−0.152237	0.237282	0.423147	−0.077213
LSTL	0.350094	0.183427	−0.277429	0.419417	−0.299813	0.151857
LSTW	0.299056	0.390519	−0.303922	0.447163	−0.241607	0.252938
LAEEP	0.328718	−0.097695	0.246767	0.195385	**0.517416**	−0.084405
LAENR	0.269963	−0.478798	0.259357	0.421995	0.035068	0.012652
LAEPH	**0.371723**	−0.055152	−0.053824	**0.461802**	−0.149729	−0.028579
LTAILL	0.361324	0.294745	−0.119113	0.013954	0.406327	0.492631
LWANUS	0.348978	0.103314	−0.089645	0.237042	−0.105532	0.012395
LVUL	0.100745	**0.534519**	**0.734808**	−0.099867	0.115204	**0.770519**
	Infective juveniles					
	PC I	PC II	PC III			
LLENGTH	**0.457758**	−0.054397	−0.099386			
LWIDTH	−0.065448	−0.272419	**0.923116**			
LAEEP	**0.416861**	−0.105271	−0.117848			
LAENR	0.441831	0.301795	0.168456			
LAEPH	0.374406	−0.277930	−0.061180			
LRA	0.243034	**0.503654**	0.277628			
LRB	0.045611	**0.633476**	0.083024			
LTAILL	0.419901	−0.297427	0.089444			

* See text for definition of acronyms. Boldface indicates dominant eigenvector.

ables representing mensural data of the pooled males and females of first- and second-generation and infective juveniles from the Argentinian and Uruguayan populations (Tables 1–3). Eigenvectors of all the characters of the infective juveniles, male and female first generations and male and female second generations contributing to the 3 principal components (PC I, PC II, PC III) are presented in Table 4.

Within the first-generation males, variables have relatively small values in PC I; the negative values indicate negative covariation of those characters with the other character values. PC II is influenced most by the distance from head to excretory pore (LAEEP) and the distance from head to pharynx base (LAEPH), whereas PC III is mainly influenced by the stoma width (STW). PC I of second-generation males is influenced by the total length (LLENGTH), whereas PC II and III are most influenced by the length of the tail (LTAILL) and the distance from head to nerve ring (LAENR), respectively.

Eigenvectors of the variables of first- and second-generation females show that PC I and PC III are dominated by the distance from head to pharynx base (LAEPH) and V% (LVUL), respectively, whereas PC II is influenced by V% (LVUL) in first-generation females and the dis-

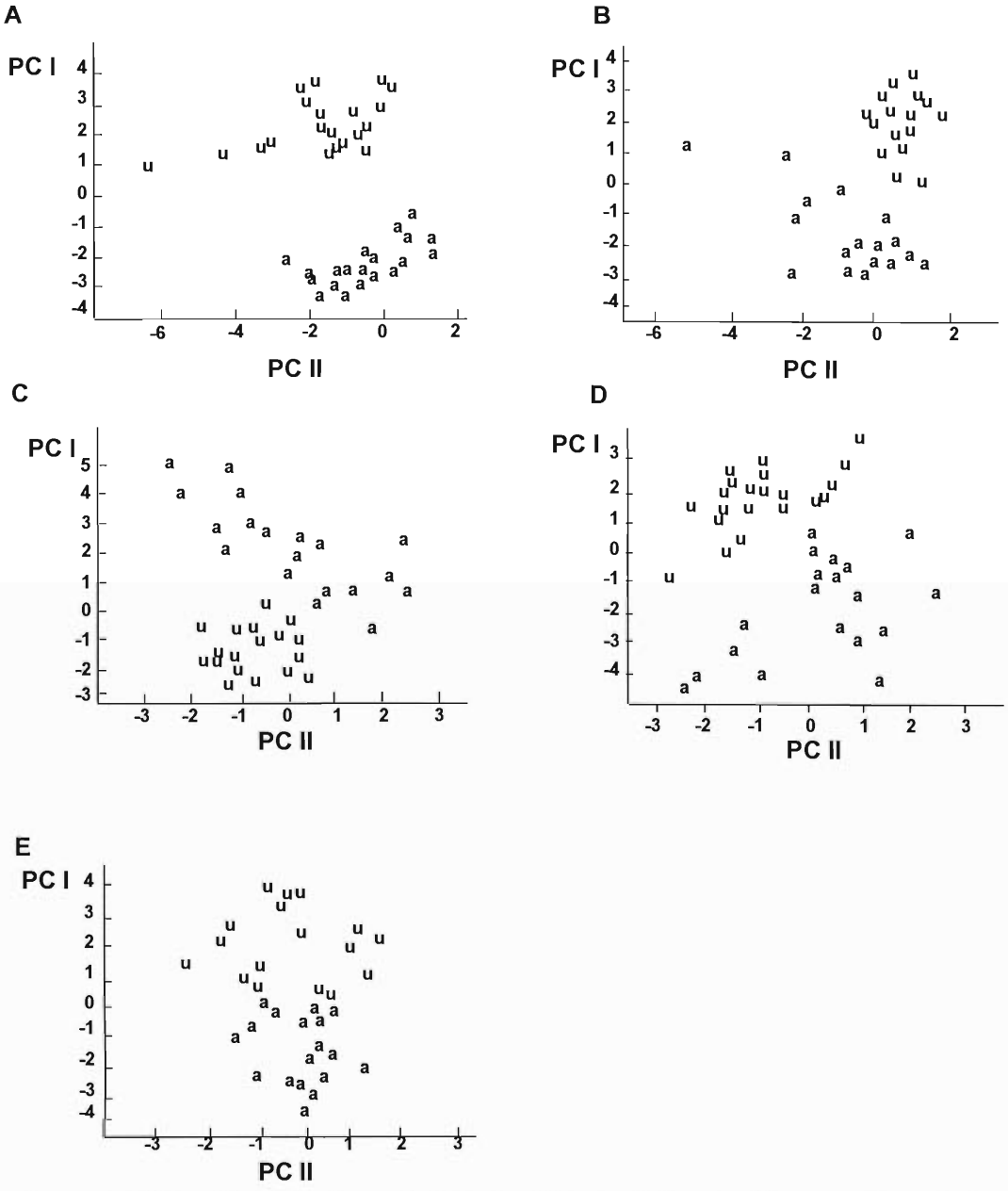

Figure 1. Scatter plots of PCA showing the clustering of the Argentinian (a) and Uruguayan (u) populations of *Steinernema scapterisci* by means of PC I and PC II of the matrix of the morphometric characters of each nematode stage. A. Males first generation. B. Males second generation. C. Females first generation. D. Females second generation. E. Infective juveniles.

tance from head to excretory pore (LAEEP) in second-generation females.

Within the infective juveniles, all variables have positive values except the width (LWIDTH), which indicates that this character has a negative covariance with the rest of the variables in the data set. It appears to show that PC II is dominated by ratio A (LRA) and ratio B (LRB) and PC III is mostly influenced by width (LWIDTH).

Results generated by the statistical analysis us-

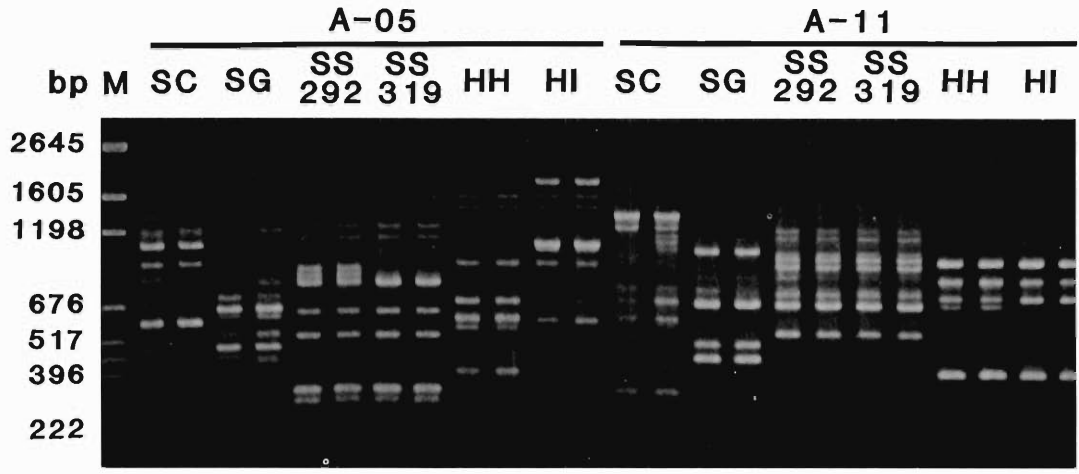

Figure 2. RAPD fragments from isolates of 4 species/isolate of *Steinernema* and 2 species of *Heterorhabditis*. For each presumptive species/isolate, the sample was duplicated on the gel to check consistency; thus, there are 2 lanes on the gel for each species/strain, except for the molecular size standard in the first lane, M. From left to right: bp = base pairs; operon primer A-05: M = lane 1, the molecular size RAPD standard; SC = lanes 2 and 3, *Steinernema carpocapsae*; SG = lanes 4 and 5, *S. glaseri*; SS 292 = lanes 6 and 7, *S. scapterisci* from Uruguay; SS 319 = lanes 8 and 9, *S. scapterisci* from Argentina; HH = lanes 10 and 11, *Heterorhabditis hawaiiensis*; HI = lanes 12 and 13, *H. indicus*; operon primer A-11; lanes 14–25, same sample order as in operon primer A-05.

ing PCA show that there are significant quantitative morphological differences between the Uruguayan and Argentinian populations, which are illustrated by scatter plots of PC I vs. PC II (Fig. 1A–E). It is evident that, given the variables used in the analysis, PCA provided good separation of the individuals of these 2 populations.

Analysis of the RAPDs (using operon primer A-05) showed that there were some differences in the band patterns between the Argentinian and Uruguayan populations of *S. scapterisci*.

The differences observed were between the range of 676 and 1,198 base pairs of the molecular size standard marker (Fig. 2). No differences could be demonstrated using operon primer A-11.

Even though a minor variation in the band patterns was generated by 1 of the markers when comparing the 2 populations, the analysis of genetic variation using RAPDs is well suited for use in population genetics and studies of biodiversity (Waugh and Powell, 1992).

This study shows that there is significant heterogeneity in *S. scapterisci* populations in space.

Careful examination of these nematodes should reveal further heterohomogeneity in the morphological and genetic characteristics in different populations. Thus, in our study, the combination of molecular techniques and classical morphological studies was a useful tool to evaluate the biodiversity of steinernematids and may have useful application for determining differences in pathogenicity against insect pests.

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Research Note

Description and Host Relationships of Cystacanths of *Polymorphus spindlatus* (Acanthocephala: Polymorphidae) from Their Paratenic Fish Hosts in Peru

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ABSTRACT: Cystacanths of *Polymorphus spindlatus* Amin and Heckmann, 1991, were collected from the body cavity of 4 species of killifish in the genus *Orestias* in Lake Titicaca, Peru, where adults were originally described from night herons. Infections were more common in the livers of *O. agassi* from open waters than from other hosts. Attachment structures of cystacanths were similar in size to those of adults but trunk, trunk spines, lemnisci, and reproductive structures were smaller than the same structures in adults. Cystacanths were encapsulated on the liver surface within hyaline envelopes and caused host hepatic tissue necrosis, disruption of hepatic envelope, and edematous liver cells.

KEY WORDS: *Polymorphus spindlatus,* Acanthocephala, cystacanth description, paratenic hosts, *Orestias* spp., killifish, Peru.

Since the description of *Polymorphus spindlatus* by Amin and Heckmann (1991), considerable effort has been invested in exploring additional host systems associated with the life history of this acanthocephalan. This report describes the anatomy of the cystacanth of *P. spindlatus* in its paratenic fish host system at Lake Titicaca, where adults were initially collected from the definitive host, black-crowned night heron, *Nycticorax nycticorax* (Linnaeus, 1753). This report also describes host–parasite relationships at the histopathological level as well as in relation to host habitats and sites and frequency of infection.

Fish were captured between February and July 1991 and 1993 by gill nets and seines from Lake Titicaca in 2 locations. The first location was Puno Bay where adults were previously collected (Amin and Heckmann, 1991) and the other was the open deeper waters of the lake. Of 304 adult fishes of the genus *Orestias* examined fresh, 64 (21%) were infected with an average of 5.2 cystacanths per infected fish (range 1–8) in liver and intestinal serosa (Table 1). Thirty-three cystacanths were processed for microscopical examination, of which 26 specimens were morphometrically studied. The remaining material was sectioned in situ. Methods of processing both sets of samples are the same as those described by Amin and Heckmann (1991).

Ten males and 16 females were measured. Measurements are in micrometers unless otherwise noted; the range is followed by the mean in parentheses. Width measurements refer to maximum width. Body (trunk) length does not

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Table 1. Prevalence and mean intensity of cystacanths of *Polymorphus spindlatus* infecting 2 body cavity sites of *Orestias* spp., Lake Titicaca, Peru, 1991-1993.

Fish species	Fish infected/ fish examined (%)	Cystacanths (mean/infected fish)	
		Liver	Intestinal serosa
O. agassi	47/161 (29)	4	2
O. luteus	15/132 (11)	1	2
O. mulleri	1/3 (33)	3	1
O. olivaceous	1/8 (13)	1	2

include neck, proboscis, or male bursa (which was never extruded). Proboscis hook counts involved at least 2 complete and adjacent rows of hooks; the largest 2 hooks in perfect profile of each specimen were measured.

Male and female cystacanths were deposited at the U.S. National Museum, Beltsville, Maryland, Helminthological Collection, No. 84237 and the University of Nebraska State Museum Manter Laboratory Collection, No. 37935.

Of the 43 native species of killifish, genus *Orestias,* known from the Lake Titicaca basin (Parenti, 1984), at least 15 are endemic to the Altiplano and Lesser Lake Titicaca, Peru–Bolivia (Lauzanne, 1982). Four species from the latter population were examined for parasites (Table 1). All species appear to maintain stable reproductive populations throughout the year and show no seasonal variation (Loubens and Sarmiento, 1985; Loubens, 1989). The most frequently and heavily infected species, *O. agassi* Valenciennes, 1846, is also the most abundant and commercially important species in the lake. It inhabits the pelagic zone and is plentiful in the entire lacustrine plant belt. It has a varied diet (Loubens et al., 1984; Loubens and Sarmiento, 1985). *Orestias luteus* Valenciennes, 1846, and *O. olivaceous* Garman, 1895, are perimacrophytic benthos feeders that are also plentiful in the vegetation belt (Loubens et al., 1984; Loubens, 1989). *Orestias mulleri* Valenciennes, 1846, typically inhabits the benthic part of the medium depth area of the lake (Loubens et al., 1984). No intestinal parasites were detected.

Worms infected liver capsules more frequently than the intestinal serosa (Table 2). The lower prevalence of infection in *O. agassi* and *O. luteus* from Puno Bay compared to the open deeper waters (Table 2) may reflect heavier predation on infected Bay fishes by the definitive host.

The following description of male and female cystacanths of *P. spindlatus* (Figs. 1, 2) from their fish paratenic hosts is based on a complete morphometric study of 10 males and 16 females.

GENERAL DESCRIPTION: Spindle-shaped trunk and proboscis. Proboscis hooks similar in size in both sexes but double-walled proboscis receptacle and clavate lemnisci larger in females than in males. Proboscis with 16–18 rows of 11–13 hooks each; largest hooks at expanded center. Lemnisci shorter than proboscis receptacle. Anterior trunk spines very small, in 5–8 circles. Hypodermal nuclei prominent and extend into zone of trunk spines. Male and female reproductive systems not well developed but posterior invaginations large; reproductive openings terminal.

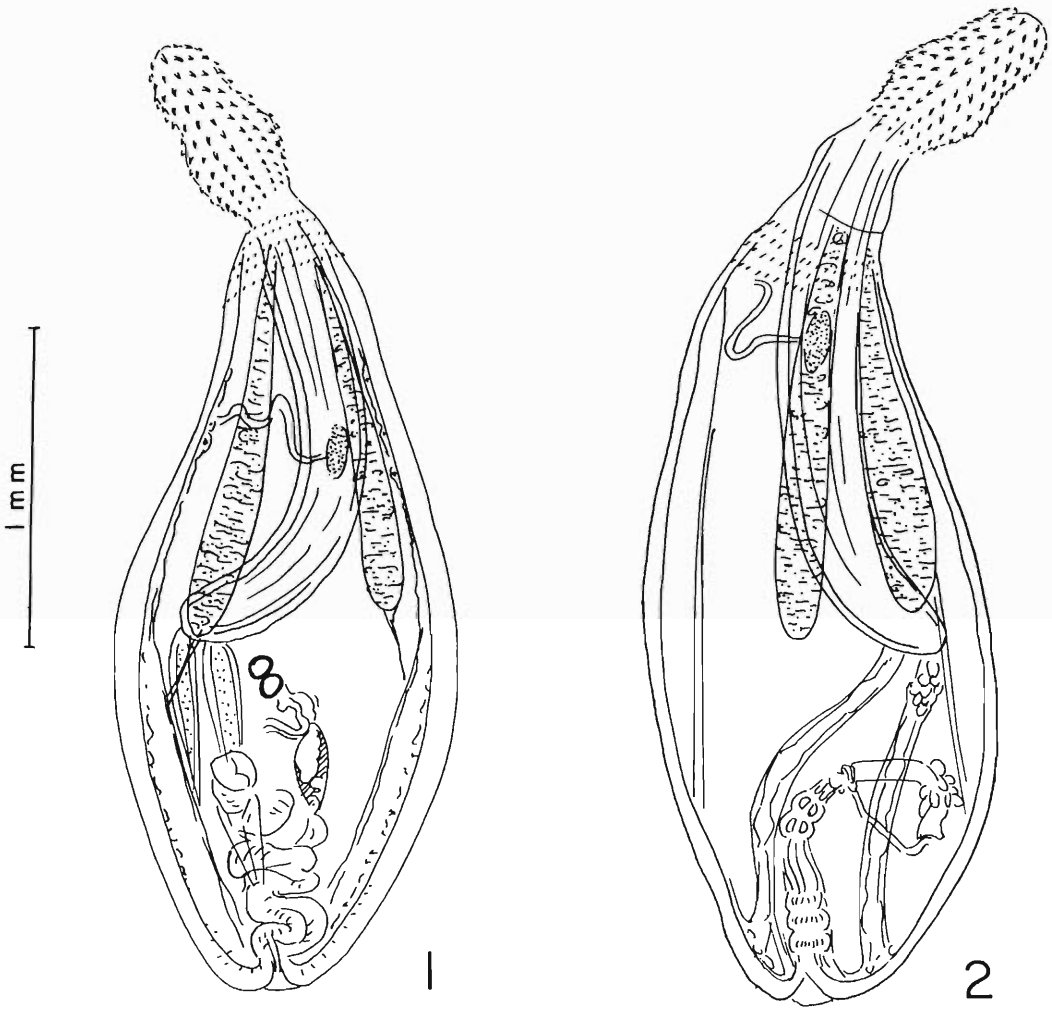
DESCRIPTION OF MALES: Trunk 1.92–2.56 (2.26) mm long by 0.77–1.12 (0.98) mm wide. Proboscis 672–770 (707) long by 266–308 (285) wide. Largest proboscis hooks 54–64 (59) long. Proboscis receptacle 1,120–1,512 (1,370) long by 238–280 (252) wide. Lemnisci 840–1,190 (1,032) long by 70–266 (161) wide. Very small, ovoid-spheroid, contiguous, nearly oblique testes in ligament sac; anterior testis 56–98 (79) long by 58–84 (68) wide and posterior testis 70–91 (80) long by 56–84 (71) wide. Posterior end of reproductive structures and bursal muscles unusually enlarged and never extruded.

DESCRIPTION OF FEMALES: Trunk 1.61–3.01 (2.38) mm long by 0.77–1.16 (1.01) mm wide. Proboscis 616–780 (709) long by 220–332 (292)

Table 2. The effect of locality on infection of body cavity sites of *Orestias agassi* and *O. luteus* with cystacanths of *Polymorhpus spindlatus*.

Fish species	Puno Bay		Open deep waters		Total
	Intestinal	Hepatic	Intestinal	Hepatic	
O. agassi	0/50 (0)*	5/50 (10)	4/50 (8)	34/50 (68)	43/100 (43)
O. luteus	0/50 (0)	3/50 (6)	1/50 (2)	9/50 (18)	13/100 (13)
Total	0/100 (0)	8/100 (8)	5/100 (5)	43/100 (43)	56/200 (28)

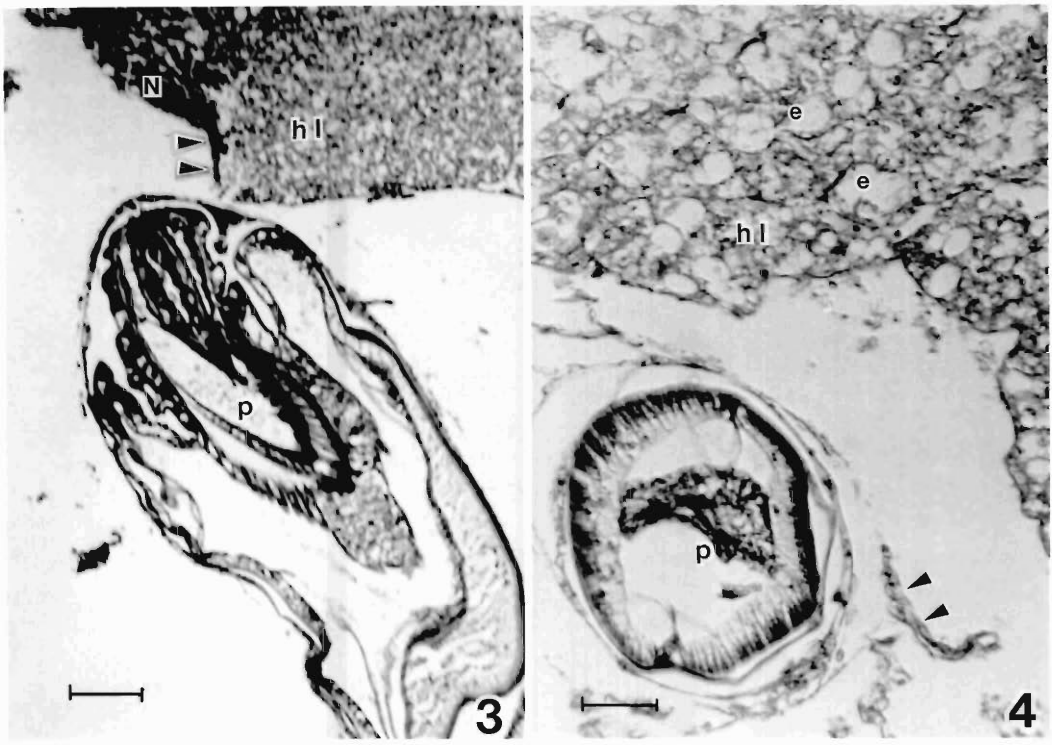
* Fish infected/fish examined (%).



Figures 1, 2. Cystacanths of *Polymorphus spindlatus* from liver surface of their paratenic hosts of the genus *Orestias*. 1. Male cystacanth. 2. Female cystacanth.

wide. Largest proboscis hooks 54–64 (57) long. Proboscis receptacle 1,330–1,680 (1524) long by 252–336 (274) wide. Lemnisci 902–1,470 (1245) long by 98–280 (183) wide. Uterus and uterine bell small but distinct, and vagina asymmetrical and greatly enlarged. Only very few and minute ovarian balls may be present in ligament sacs.

The pattern of proboscis armature and trunk spines in cystacanths of *P. spindlatus* is the same as that of the adults. The trunk is about half as long as that of the adults, and there is less sexual dimorphism in the cystacanths than in the adults. The proboscis and proboscis hooks are practically identical in size and shape to those of the adults. The proboscis receptacle, however, is distinctly longer than in adults of both sexes. Growth in size of trunk and lemnisci as well as development of the reproductive structures appear to be slower than those of other structures. Cystacanths are clearly not precocious and must undergo marked reproductive development in the definitive host. Developmental priorities are apparently placed on attachment structures to secure successful establishment in the fish-eating avian definitive host, *N. nycticorax*. The state of development of *P. spindlatus* in the intermediate host is unknown, but it would be interesting to see whether or not the degree of development of attachment structures is comparable to that observed in the paratenic hosts. All worms exam-



Figures 3, 4. Histopathology of *Polymorphus spindlatus* cystacanths in the liver of *Orestias agassi*. 3. Parasite (p) adjacent to host liver tissue (hl) causing necrosis (n) and liver capsule separation (double arrowheads). 4. A cross-section of the trunk of a parasite (p) next to host liver (hl). Note the disruption of the liver capsule with necrotic cells (arrowheads). The encasement of the cystacanth in a hyaline envelope is visible. Edematous hepatocytes (e) are seen within hepatic lobules (hl) (trichrome stain). Scale bars = 500 μm.

ined were in excellent condition and were probably viable upon recovery from their hosts. *Orestias* spp. clearly serve as an indispensable link in the natural infectious cycle of *P. spindlatus* between the intermediate crustacean host and the piscivorous definitive host.

Other cystacanth features that differ significantly from those of adults include the minute size of trunk spines, the marked enlargement of vaginal and bursal invaginations, and the lemnisci being shorter than the proboscis receptacle. The neck is not as well developed, as indicated in the original description of adults.

Cystacanths were often found beneath the liver capsule of *Orestias* spp. (Fig. 3). Worms do not appear to move into the hepatic lobules but remain on the surface. Encapsulation often involved formation of a hyaline envelope (Fig. 3). The trunk of the specimen in Figure 4 was also encapsulated adjacent to hepatic lobules of *Orestias*. Damage to the hepatocytes immediately next to cystacanths included necrosis (Fig. 3), edematous liver cells (Fig. 4), and disruption of the capsule surrounding the liver (Fig. 4). Parasites were readily detachable by breaking the dense collagenous connective tissue capsule surrounding them. All worms sectioned had retracted proboscides.

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Research Note

Histopathology of *Oligacanthorhynchus tortuosa* (Oligacanthorhynchidae) Infection in the Virginia Opossum (*Didelphis virginiana*)

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ABSTRACT: *Oligacanthorhynchus tortuosa,* a common acanthocephalan of the Virginia opossum (*Didelphis virginiana*) in North America, has been reported to be associated with large, nodule-like lesions at points of attachment of the proboscides. Three lesions resulting from the attachment of individuals of *O. tortuosa,* 1 each from 3 infected opossums, were prepared for histological examination to further characterize histopathologic changes elicited by this parasite. Histologically, lesions involved the mucosa, submucosa, and muscularis. The proboscides were contained within abscesses characterized by necrotic debris interspersed with many pycnotic nuclei. The abscesses were approximately 1.4 mm in diameter and were surrounded by regions of dense connective tissue (collagen), approximately 142 μm wide. The bands of dense connective tissue were surrounded by regions of active fibroblast and fibrocyte proliferation, approximately 169 μm wide, in which evidence of collagen synthesis was observed. Both longitudinal and smooth muscle layers of the muscularis had been completely destroyed in the area of the lesion. Absence of polymorphonuclear leukocytes were indicative of chronic lesions. Histopathologic changes elicited by *O. tortuosa* include chronic inflammatory response to mechanical trauma resulting from injury caused by the proboscis with subsequent fibrosis and nodule formation.

KEY WORDS: histopathology, *Oligacanthorhynchus tortuosa, Didelphis virginiana,* opossum, Acanthocephala.

Oligacanthorhynchus tortuosa, a common acanthocephalan of the Virginia opossum (*Didelphis virginiana*) in North America, has been reported to be associated with large, nodule-like lesions at points of attachment of proboscides. *Oligacanthorhynchus tortuosa* is represented by large worms with females achieving lengths of up to 350 mm (Richardson, unpubl. data). The globular proboscis bears 6 spiral rows of 6 hooks each and has a length of 0.22–0.23 mm and width of 0.23–0.29 mm (Van Cleave, 1953). Leidy (1850) reported a specimen of *O. tortuosa* as having the anterior 3 lines of its length buried in an oval tumor, 4 lines in diameter, in the mesentery of an opossum. Based on this statement, Van Cleave (1924) concluded that the worm had penetrated the intestinal wall, entered the body cavity, and attached to the mesentery. Feldman et al. (1972) reported severe ulcerative lesions evoked at points of attachment of unidentified acanthocephalans from opossums. Brief description and a photomicrograph (Feldman et al., 1972) suggest that these specimens were *O. tortuosa.* Richardson et al. (1992) reported 2 poorly developed individuals of *O. tortuosa* from the small intestine of a raccoon (*Procyon lotor*) that caused "severe lesions" at points of attachment; however, histological examination was not conducted. The only histological examination of lesions caused by *O. tortuosa* was conducted by Babero (1957), who reported elevated nodules over the serosal surface of the small intestines of 2 Illinois opossums having a base diameter of 2–7 mm. He reported the nodules to have a bright red appearance due to congestion of intestinal blood vessels. Histologically, lesions reported by Babero (1957) resulted in complete mechanical destruction of the mucosal and submucosal layers with some focal atrophy and necrosis of the muscularis. He further noted limited leukocytic infiltration and some pigment deposition. Babero (1960) examined opossums from Georgia

infected with *O. tortuosa* in which no such hemorrhagic lesions were observed.

The purpose of this investigation was to further characterize lesions resulting from *O. tortuosa* infections in the Virginia opossum.

Three lesions resulting from the attachment of individuals of *O. tortuosa,* 1 each from 3 opossums, were prepared for histological examination. Material was obtained from opossums collected in the course of a survey of Acanthocephala of opossums from Arkansas, results of which were reported by Richardson (1993). All lesions examined were caused by mature worms. Immediately after sacrificing the opossum, the small intestine was examined for nodules on the serosal surface, then removed and longitudinally dissected. Mature worms were severed so as to leave the proboscis intact and undisturbed in the lesion. The lesion along with normal tissue immediately surrounding the lesion was excised and placed directly into Bouin's fixative.

After fixing in Bouin's solution for 24 hr, tissues were stored in 70% ethanol. Tissues were dehydrated by treating in a graded series of ethanol. Tissues were cleared in toluene, infiltrated and embedded in paraffin blocks, and sectioned at a thickness of 5–7 μm using a rotary microtome. Ribbons were attached to slides with albumin and stained following Masson's trichrome technique as described by Luna (1968). Two percent light green was substituted in place of aniline blue to enhance staining of connective tissue. Stained sections were mounted in Canada balsam and examined using light microscopy.

Worms were restricted to the anterior ½ of the small intestine, with most occurring in the anterior ⅓. Grossly, hard, white nodules were apparent on the serosal surface of the intestine corresponding to points of worm attachment. Proboscides were firmly embedded in the intestinal wall resulting in nodule formation; however, there was no apparent evidence of hemorrhage as observed grossly by Babero (1957). At the base, nodules ranged from approximately 1 to 5 mm in diameter.

Lesions involved the mucosa, submucosa, and muscularis; however, the serosa was intact and apparently unaffected (Fig. 1). Proboscides were contained within abscesses characterized by necrotic debris interspersed with many pycnotic nuclei, particularly abundant around the periphery of abscesses. Small aggregations of collagen were present. No evidence of recent hemorrhage was observed. Abscesses, which were approximately 1.14 mm in diameter, were surrounded by regions of dense connective tissue (collagen), approximately 142 μm wide. These regions of dense connective tissue, which appeared to have effectively contained the abscesses, were interspersed with small numbers of fibrocytes and fibroblasts. Spaces were noted between strands of collagen, many of which appeared to be lymphatics. Bands of dense connective tissue were surrounded by regions of active fibroblast and fibrocyte proliferation, approximately 169 μm wide, in which evidence of collagen synthesis could be observed. Within these regions, occasional plasma cells, lymphocytes, and mast cells were observed (approximately 1/0.133 mm^2 [×40 field]). These areas were infiltrated with many blood vessels and lymphatics. Lumina of arterioles were occluded by contraction of smooth

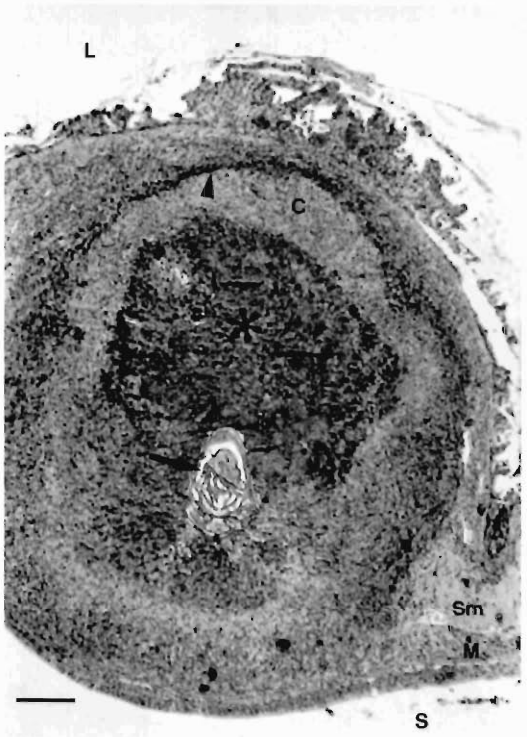

Figure 1. Photomicrograph of a cross-section of a lesion elicited by *Oligacanthorhynchus tortuosa* in the intestine of a Virginia opossum (*Didelphis virginiana*) showing proboscis (arrow), necrotic abscess (asterisk), ring of collagen (C), region of active fibrocyte proliferation (arrowhead), muscularis (M), normal submucosa (Sm), serosal side (S), and luminal side (L). Scale bar = 200 μm. Figure 1 appeared in *Foundations of Parasitology,* 5th edition, Wm. C. Brown, Publishers, and is used here with permission of the company.

muscle in the arteriole wall. Abscesses along with bands of connective tissue resulted in drastic enlargement of the submucosa to over 7 times its normal width, resulting in formation of the grossly observable nodule. Width of the true submucosal region of unaffected tissue was approximately 240 μm, whereas that of affected regions was 1.87 mm. In the regions of the lesions, both longitudinal and smooth muscle layers of the muscularis had been completely eroded; however, the serosa remained intact and appeared to be unaffected. No evidence of hypertrophy of any of the muscular layers in the vicinity of the lesions was observed. The mucosa and muscularis mucosae appeared to be intact on lumenal sides of the lesions except for entry points of worms, with thinning of the muscularis mucosae as an apparent result of stretching.

Histological series from which these data were obtained were deposited in the Harold W. Manter Laboratory, University of Nebraska, Lincoln, Nebraska, and given accession Nos. HWML 37832–37834.

Absence of polymorphonuclear leukocytes was indicative of chronic lesions. Observable pathology may be solely accounted for by mechanical damage resulting in subsequent fibrous nodule formation. These findings corroborate the synopsis given by Nicholas (1967), who summarized typical pathology of acanthocephalan infection as traumatic injury as a result of the proboscis penetrating deeply into the gut wall leading to an inflammatory response with cellular infiltration and the eventual formation of a dense fibrous nodule around the proboscis. Severe pathological manifestations associated with acanthocephalan infections often appear to be a result of peritonitis caused by perforation of the serosa by the proboscis (Stunkard, 1965; Schmidt, 1972). Apparently this phenomenon was observed by Leidy (1850) for *O. tortuosa.* This is likely considering the extent of mechanical damage found in the present study, including complete destruction of the muscularis.

Lesions examined in this study were similar to those described by Nelson and Nickol (1986) from domestic swine experimentally infected with *Macracanthorhynchus hirudinaceus,* which also had nodule formation as a result of an increase in size of the submucosa. Nelson and Nickol (1986) interpreted the preponderance of monocytes and lymphocytes, fibrosis, and neovascularization as evidence of a chronic lesion.

Extensive fibrosis suggests that frequent movement of the worm does not occur after it has established and embedded its proboscis into the intestinal wall, unlike *Moniliformis moniliformis* in rats, which was found to attach superficially, penetrating only the mucosa and tunica propria, with no fibrosis (Taraschewski et al., 1989). Taraschewski et al. (1989) interpreted these lesions as evidence that worms frequently changed their sites of attachment.

Pathologic changes induced by *O. tortuosa* in the opossum were similar, with notable exceptions, to those elicited by *M. hirudinaceus* and *M. ingens,* which also belong to the family Oligacanthorhynchidae, in swine and raccoons, respectively (Nelson and Nickol, 1986). Nelson and Nickol (1986) reported extensive eosinophil proliferation and hypertrophy of the muscularis in raccoons infected with *M. ingens* and congregations of tissue macrophages, monocytes, and plasma cells in swine infected with *M. ingens.* Such a pronounced cellular response was not associated with *O. tortuosa* infections in the opossum. Additionally, hypertrophy of the muscularis was not observed. Instead, the muscularis was virtually completely destroyed and had been replaced by connective tissue. Both of these differences may possibly be accounted for by duration of infection. The observations of Nelson and Nickol (1986) were based on lesions from a raccoon infected with *M. ingens,* which was killed 63 days postinfection, and swine killed 3, 7, and 14 days after experimental infection with *M. ingens.* The duration of infection was not given for *M. hirudinaceus* in swine. It is possible that the reduced cellular response and pronounced erosion of the muscularis with subsequent fibrosis in the present study, which are characteristic of chronic inflammation (McCutcheon, 1948), was a result of increased duration of infection. The lack of hemorrhage, as described by Babero (1957), is further evidence of the chronicity of the lesions.

Pronounced absence of polymorphonuclear leukocytes suggests lack of a specific immune response to the presence of *O. tortuosa* with the resultant inflammatory response and subsequent fibrosis appearing to adequately contain the infection. Absence of polymorph proliferation suggests a low degree of pathogenicity elicited by this parasite. One opossum examined was infected with 99 *O. tortuosa,* mostly adults, and exhibited no overt signs of illness. Histopathologic changes elicited by *O. tortuosa* may be generalized as a chronic inflammatory response to

mechanical trauma resulting from injury caused by the proboscis, with subsequent fibrosis and nodule formation, not unlike the inflammatory response elicited by an inanimate irritating body as described by McCutcheon (1948).

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Research Note

Some Trematode, Nematode, and Acanthocephalan Parasites of Rainbow Trout, *Oncorhynchus mykiss,* Introduced into Chile

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ABSTRACT: The gastrointestinal tracts of 211 adult *Oncorhynchus mykiss* (Walbaum, 1792) were examined for trematode, nematode, and acanthocephalan parasites from 8 lakes in southern Chile (between 41°05′ and 39°03′ south latitude). The parasites *Derogenes patagonicus, Acanthostomoides apophalliformis, Camallanus corderoi, Hysterothylacium* sp., *Acanthocephalus tumescens,* and *Acanthocephalus* sp. were present. *Camallanus corderoi* and *D. patagonicus* were present in 8 and 6 lakes, respectively. In 6 lakes, the prevalence was higher for *C. corderoi* compared to 2 other lakes. In 3 different lakes, the mean intensities were higher for *D. patagonicus* (Lakes Rupanco, Puyehue, and Maihue) and *C. corderoi* (Lakes Ranco, Colico, and Caburga) with respect to other species. Acanthocephalans were infrequent in rainbow trout, except for *A. tumescens* in Lake Villarrica.

KEY WORDS: Trematoda, *Derogenes patagonicus, Acanthostomoides apophalliformis,* Nematoda, *Camallanus corderoi, Hysterothylacium* sp., Acanthocephala, *Acanthocephalus tumescens, Acanthocephalus* sp., Salmonidae, *Oncorhynchus mykiss,* prevalence, intensity.

The successful introduction of salmonids into Chile was carried out from Hamburg in 1905 (Golusda, 1927), and their production in fish hatcheries has undergone an important development since 1981 (Alvarado et al., 1990). Research on the parasitic helminths of wild salmonids is important because of zoonotic implications, damage to fish tissues, potential risk of transmission to fish hatcheries, and impact on the tourist activity in the lake region of southern Chile (Wetzlar, 1979; Torres et al., 1989). This note presents information on the prevalence and mean intensity of 6 helminth species in the gastrointestinal tract of rainbow trout, from 8 lakes in southern Chile, where no previous records have been made of these parasite groups.

Between 1989 and 1990, 211 adult rainbow trout (Salmonidae), were examined. They were caught with 5-, 10-, and 20-mm mesh gill nets in the following lakes (geographic locality/number of fish/standard length in centimeters [$\bar{x} \pm$ SD]): Todos los Santos (41°05′S, 72°15′W/14/30 ± 6.2), Rupanco (40°46′S, 72°30′W/27/32.3 ± 7.3), Puyehue (40°36′S, 72°26′W/30/29.2 ± 6.5), Maihue (40°15′S, 72°02′W/33/28.1 ± 7.4), Ranco (40°11′S, 72°22′W/30/27.2 ± 7.4), Villarrica (39°13′S, 72°06′W/29/29.4 ± 5.2), Caburga (39°06′S, 71°45′W/27/33.8 ± 4.7), and Colico (39°03′S, 71°59′W/21/29.4 ± 7.6). Dead fish were kept at 4°C and examined within 72 hr of collection. Procedures of host necropsy, fixation, staining, and/or clearing of parasites followed those of Torres et al. (1990a, 1992). The definitions of prevalence, mean intensity, and locality adhere to Margolis et al. (1982). Representative helminths were deposited in the Collection of the Institute of Parasitology, Universidad Austral de Chile: *Derogenes patagonicus* (IPUAT 233–236), *Camallanus corderoi* (IPUAT 237–241), *Acanthocephalus tumescens* (IPUAT 242–243), *Acanthocephalus* sp. (IPUAT 244), *Hysterothylacium* sp. (IPUAT 245), and *Acanthostomoides apophalliformis* (IPUAT 246).

The prevalence and mean intensity of the 6 helminth species in rainbow trout from 8 lakes are given Table 1. The taxa consisted of 2 trematode species, *Derogenes patagonicus* (Szidat, 1956) and *Acanthostomoides apophalliformis* Szidat, 1956; 2 nematode species, *Camallanus corderoi* Torres, Teuber, and Miranda, 1990, and *Hysterothylacium* sp.; and 2 acanthocephalan species, *Acanthocephalus tumescens* (Linstow, 1896) and *Acanthocephalus* sp. *Derogenes patagonicus* was found in the stomach, whereas the other species occurred in the intestine. All helminth species are first records for rainbow trout in the lakes studied.

The specimens of *D. patagonicus, Acanthocephalus* sp., and *A. tumescens* were represented by adults, sometimes gravid worms, and juveniles. The specimens of *C. corderoi* were adults and fourth-stage larvae (L4s). The only specimens of *A. apophalliformis* and *Hysterothylacium* sp. were a gravid adult and a male, respectively.

Three helminth taxa of various assemblages were present in rainbow trout from 6 of the 8 lakes, with the exception of Lakes Caburga and

Puyehue, where 1 and 2 species, respectively, were recorded. *Camallanus corderoi* was the only parasite infecting rainbow trout from all 8 lakes, whereas *D. patagonicus* was found in 6 lakes.

In the majority of lakes, the prevalence was higher for *C. corderoi* compared to Lakes Rupanco and Todos los Santos. The mean intensities were higher for *D. patagonicus* in Lakes Rupanco, Puyehue, and Maihue compared to 5 other lakes and *C. corderoi* in Lakes Ranco, Caburga, and Colico. The mean intensity of *A. tumescens* was highest in Lake Villarrica.

Camallanus corderoi was described in perch trout, *Percichthys trucha,* in the Valdivia River basin, Chile (Torres et al., 1990b). Later, it was recorded in wild-introduced salmonids, *O. mykiss,* and brown trout, *Salmo trutta,* in the same basin (Torres et al., 1991a) and in rainbow trout cultured in Lake Puyehue (Torres et al., 1993). Analysis of the diet of wild salmonids suggests that the transmission of *C. corderoi* is augmented by the frequent consumption of other autochthonous plankton-eating fishes (especially *Galaxias* spp.), which harbor L4s and immature adults (Torres et al., 1991a). *Derogenes patagonicus* was described by Szidat (1956) in *P. trucha* in the Argentinian Patagonia. In Chile, *D. patagonicus* has been recorded in *O. mykiss* and *S. trutta* in Lakes Yelcho (43°16′S, 72°15′W) and Tagua Tagua (41°39′S, 72°09′W) (Torres et al., 1992).

Acanthocephalus tumescens was described by Linstow (1896) from the Patagonian pejerrey, *Atherinichthys microlepidotus,* from Argentina. Simultaneous infections by *A. tumescens* and *Acanthocephalus* sp. were not observed in rainbow trout. *Acanthocephalus tumescens* occurred only in rainbow trout from Lakes Maihue, Ranco, and Villarrica. The mean intensity of *Acanthocephalus* spp., in general, was low, except in Lake Villarrica, where *A. tumescens* had a mean intensity of 22.2.

Acanthocephalus tumescens and *Acanthocephalus* sp. have been recorded in rainbow trout, brown trout, puye, and southern smelt, *Aplochiton taeniatus,* in Lake Yelcho (Torres et al., 1992). *Acanthocephalus* sp. has also been recorded in *P. trucha* from Lake Tagua Tagua (Torres et al., 1992).

The presence of *A. apophalliformis* and *Hysterothylacium* sp. in rainbow trout from Lakes Rupanco and Ranco, respectively, seems to be "accidental" by low prevalence. *Hysterothylacium* sp. is recorded for the first time in rainbow trout from Chile. Torres et al. (1992) reported that this species had a low intensity (1–3) in brown trout and perch trout in Lakes Yelcho and Tagua Tagua, respectively.

Acanthostomoides apophalliformis was described by Szidat (1956) in perch trout in Lake Pellegrini, located in the Argentinian Patagonia. In Chile, it was recorded in perch trout from Lake Tagua Tagua with a prevalence of 45% and a mean intensity of 38. Their metacercariae have been observed in the liver of puye and juvenile southern smelt in Lakes Yelcho and Tagua Tagua (Torres et al., 1992).

The 6 helminth species infecting rainbow trout in the present study have been found previously

Table 1. Prevalence and mean intensity of helminth parasites from *Oncorhynchus mykiss* in 8 lakes from southern Chile.

Lake Helminth taxon	No. infected fishes (% prevalence)	Mean intensity (maximum)
Caburga (27)*		
Camallanus corderoi	2 (7)	10.5 (17)
Colico (21)		
Derogenes patagonicus	2 (10)	2.5 (3)
Camallanus corderoi	6 (29)	8.3 (27)
Acanthocephalus sp.	3 (14)	2.3 (5)
Maihue (33)		
Derogenes patagonicus	5 (15)	15.4 (58)
Camallanus corderoi	9 (27)	5.9 (25)
Acanthocephalus tumescens	5 (15)	1.8 (3)
Puyehue (30)		
Derogenes patagonicus	10 (33)	82.8 (280)
Camallanus corderoi	14 (47)	23.6 (101)
Ranco (30)		
Camallanus corderoi	6 (20)	9.8 (31)
Acanthocephalus tumescens	4 (13)	5.8 (10)
Hysterothylacium sp.	1 (3)	1.0
Rupanco (27)		
Derogenes patagonicus	13 (48)	24.7 (85)
Acanthostomoides apophalliformis	1 (4)	1.0
Camallanus corderoi	12 (44)	12.3 (89)
Todos los Santos (14)		
Derogenes patagonicus	1 (7)	1.0
Camallanus corderoi	1 (7)	1.0
Acanthocephalus sp.	4 (29)	1.5 (2)
Villarrica (29)		
Derogenes patagonicus	1 (3)	2.0
Camallanus corderoi	16 (55)	10.9 (49)
Acanthocephalus tumescens	6 (21)	22.2 (120)

* Number of fishes examined.

in autochthonous Chilean fishes (Torres et al., 1990a, b, 1992). These fishes act as reservoirs from which infections have apparently developed in introduced salmonids. In regard to salmonid breeding in the lakes of southern Chile, the presence of parasites in introduced and autochthonous fishes should be considered. This parasitological knowledge should precede initiation of intensive salmon breeding activities in limnetic ecosystems in order to assess potential risk factors to fish populations.

Prevalence, mean intensity, and pathology by *Diphyllobothrium latum* and *Diphyllobothrium dendriticum* have been reported in rainbow and brown trouts in lakes from southern Chile (Torres et al., 1991b). Infection by *D. latum* has been reported in humans associated with consuming raw and smoked salmonids in Chile (Torres et al., 1989).

Acknowledgments

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Research Note

Morphological Observations on Third-Stage Larvae of *Anisakis simplex* A (Anisakidae: Nematoda) from Adriatic and Ionian Waters

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ABSTRACT: Additional morphology on third-stage larval specimens of *Anisakis simplex* A (Rudolphi, 1809, det. Krabbe, 1878) infecting *Merluccius merluccius* L. and *Sardina pilchardus* Walb. from the Adriatic and Ionian seas (Southern Italy) is described and illustrated. Particular attention (light, scanning electron microscope observations, and histological studies) was given to illustrate head structures such as papillae, oral and amphidial openings, excretory pore, boring tooth, excretory system, rectal glands, and tail.

Larvae collected in Mediterranean waters were morphologically similar, and morphometrics fit well (with considerable overlap in most measurements) with the previous descriptions of *A. simplex* A (type I larvae) reported from Australian, Canadian, Japanese, North Sea, northeast Atlantic, and New Zealand waters, confirming its cosmopolitan geographical distribution.

KEY WORDS: *Anisakis* larvae, Mediterranean Sea, *Merluccius merluccius, Sardina pilchardus,* SEM morphology.

Seventeen species of the genus *Anisakis* Dujardin, 1845 (Nematoda: Ascaridata), have been studied in detail by Davey (1971), showing that spicules, postanal papillae, form of ventriculus, vulva position, and lips shape are the main char-

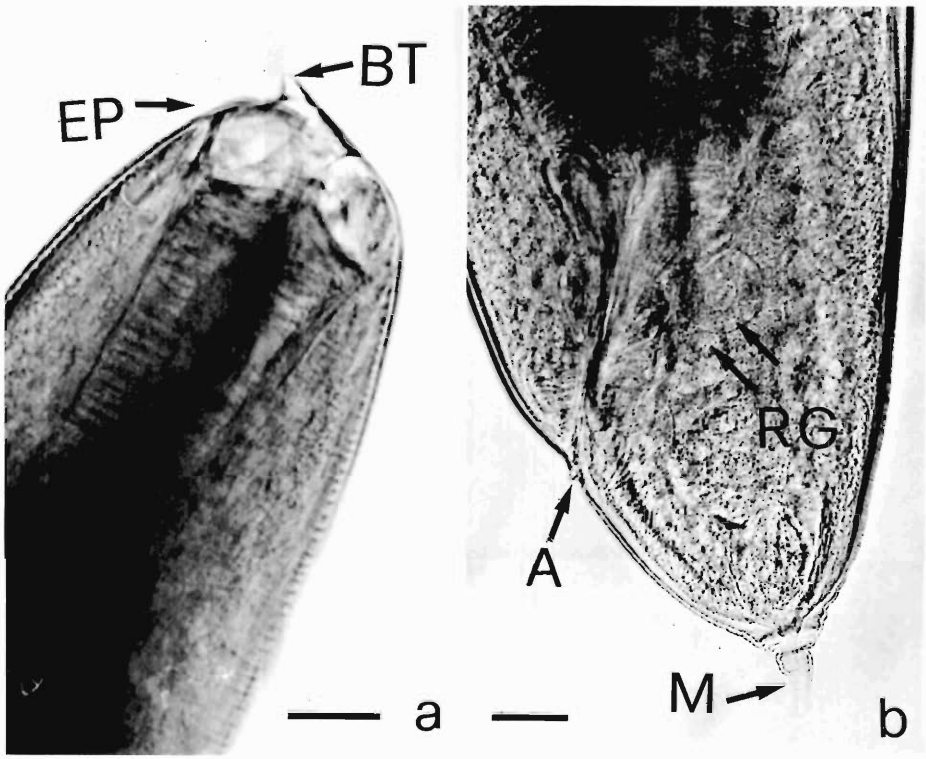

Figure 1. ***Anisakis simplex*** **larvae (L3). a) Anterior extremity, lateral view (BT = boring tooth, EP = excretory pore). b) Posterior extremity (A = anus, M = tail spine [mucron], RG = rectal glands). Scale bar = 25 μm.**

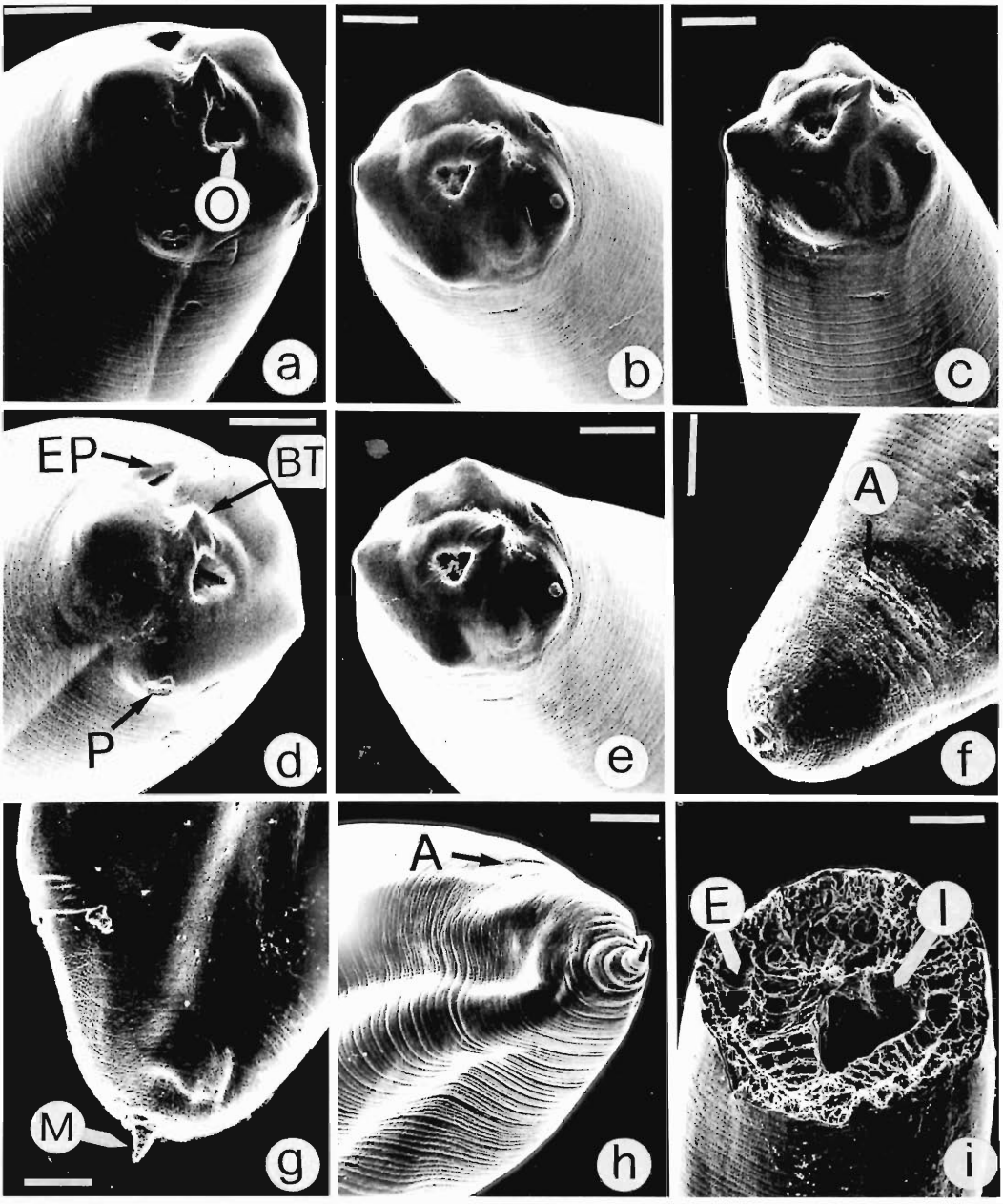

Figure 2. SEM morphology of Mediterranean *Anisakis simplex* larvae (L3). a–e) Anterior end en face view or profile showing oral (O) opening, papilla (P), and boring tooth (BT). f–h) Ventral and lateral view of the posterior end (A = anus, M = tail spine). i) Cross-section at 10% of body length showing the intestine (I) and the excretory cell (E). Scale bars: a–h = 40 μm; i = 100 μm.

acters, in order of importance, for identifying adult specimens of *A. simplex* (Rudolphi, 1809, det. Krabbe, 1878), *A. typica* Diesing, 1860, and *A. physeteris* Baylis, 1923. Morphological illustration of larval stages is less extensive because of their uncertain identification and the difficulties in following their life cycle.

Last year in Southern Italy, extensive alarm for public health was raised by the occurrence of *Anisakis* sp. larvae found in the peritoneal cavity

Table 1. Measurements of *Anisakis simplex* A larvae from *Merluccius merluccius* and *Sardina pilchardus* (n = 25).

	$\bar{x}$ ± SD	Range
Body length (mm)	21.60 ± 3.47	15.00–27.50
Body width (mm)	0.41 ± 0.05	0.34–0.51
Esophagus length (mm)	2.65 ± 0.29	2.06–3.08
Ventriculus length (mm)	0.70 ± 0.08	0.59–0.92
Ventriculus width (mm)	0.24 ± 0.05	0.15–0.32
Tail (mm)	0.11 ± 0.01	0.09–0.14
Anal body width (μm)	119.00 ± 17.66	69.33–150.67
Tail's mucron (μm)	25.69 ± 4.15	17.33–32.00
Boring tooth (μm)	9.35 ± 2.41	5.50–14.50
a	52.91 ± 5.10	42.86–59.46
b	8.15 ± 1.05	6.80–11.16
c	199.93 ± 43.49	133.3–288.9
c′	0.95 ± 0.21	0.78–1.79

of *Merluccius merluccius* and *Sardina pilchardus*, common fish species of the South Adriatic and Ionian seas.

The third-stage larval (L3) *Anisakis* nematode population from Mediterranean waters was identified as *A. simplex* A (type I larvae; Berland, 1961) from ventriculus dimensions and the presence of the tail spine (mucron), according to the key suggested by Pippy and Van Banning (1975).

Orecchia et al. (1986), Nascetti et al. (1986), and Beverley-Burton et al. (1977) have proposed the use of multilocus electrophoresis to provide diagnostic characters for the identification of larvae of the *Anisakis simplex* complex from the Mediterranean Sea and northeast Atlantic. Nascetti et al. (1986) also suggested, on the basis of biochemical data, to synonymize *A. simplex* A with *A. pegreffi* (already synonymized with *A. simplex* by Davey [1971]). Orecchia et al. (1989) reported later the occurrence of larvae of *Anisakis* sp. from Italian waters, identifying them as *A. simplex* A (type I larvae) and *A. physeteris* (type II larvae), using biochemical keys, without giving morphometrical features.

Here a morphological and morphometrical illustration of the Mediterranean population of *A. simplex* A is presented and compared to those reported from Australia, the North Sea, New Zealand, and Japan (Brunsdon, 1956; Koyama et al., 1969; Pippy and Van Banning, 1975; Smith, 1983; Hurst, 1984).

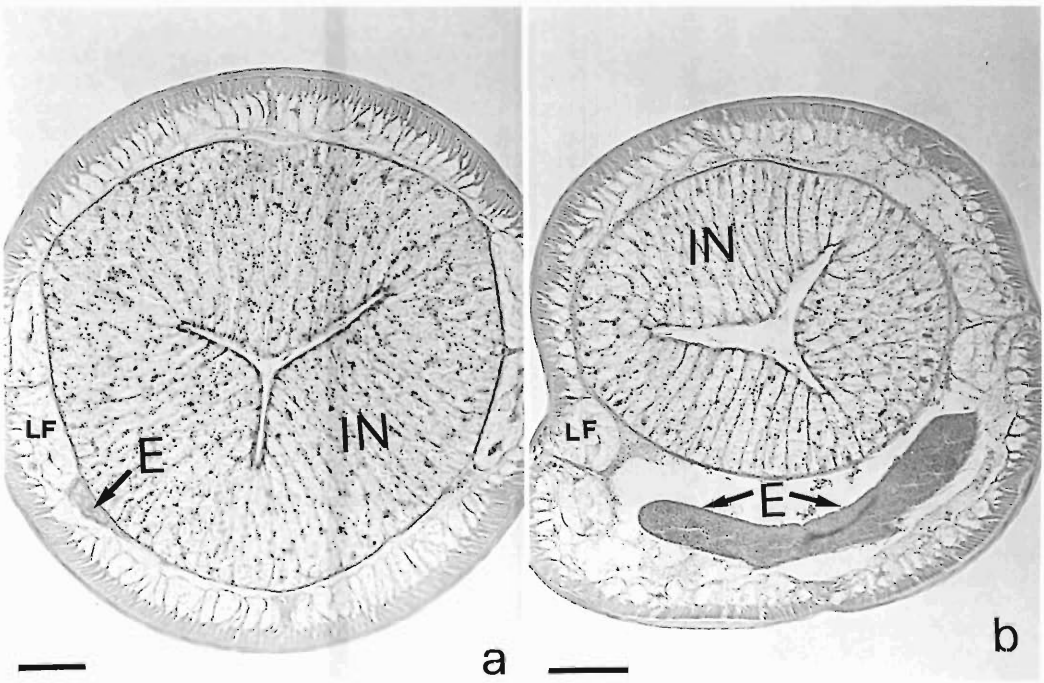

Figure 3. Cross-sections at 8% (a) and 35% (b) of body length of Mediterranean *Anisakis simplex* larvae showing the different shape and size of the excretory cell (E) within the body. IN = intestine, LF = lateral fields. Scale bar = 40 μm.

Table 2. Measurements of *Anisakis simplex* A larvae from teleosts.

Locality	Authority	*n*	BL (mm)		%BW/BL		%EL/BL		%VL/BL		%TL/BL	
			$\bar{x}$ ± SD	Range	$\bar{x}$ ± SD	Range	$\bar{x}$ ± SD	Range	$\bar{x}$ ± SD	Range	$\bar{x}$ ± SD	Range
North Sea	Pippy and Van Banning, 1975	20–24	19.7	16.1–22.5	2.48 ± 0.48	—	13.3* ± 2.41	—	—	—	0.66 ± 0.14	—
	Punt, 1941	22–56	20.4	13.3–30.0	1.78 ± 0.39	—	12.2* ± 2.02	—	—	—	0.53 ± 0.15	—
Northeast Atlantic	Smith, 1983	30	22.6	—	—	—	—	—	4.10	—	—	—
Australia	Brundson, 1956	10	20.6	15.9–25.0	2.19 ± 0.12	—	9.84 ± 1.05	—	3.05 ± 0.27	—	0.54 ± 0.07	—
	Cannon, 1977	60	19.7	14.6–24.6	—	—	—	—	—	—	—	—
	Hurst, 1984	50	20.3 ± 3.04	14.0–26.0	2.14 ± 0.21	—	9.99 ± 1.50	—	3.43 ± 0.50	—	0.59 ± 0.12	—
Chile	Torres et al., 1978	7	28.2	19.7–39.3	2.13	—	8.34	—	3.84	—	0.51	—
Japan	Koyama et al., 1969	139	28.4	19.0–36.0	1.58	—	7.79	—	3.92	—	0.40	—
	Shiraki, 1974	9	28.4	23.0–31.7	1.73 ± 0.26	—	7.2 ± 0.26	—	4.59 ± 0.94	—	0.40 ± 0.05	—
Mediterranean Sea	This study	25	21.6 ± 3.47	15.0–27.5	1.91 ± 0.20	1.68–2.33	12.5* ± 1.47	8.96–14.70	3.30 ± 0.40	2.84–4.00	0.52 ± 0.12	0.35–0.75

* Esophagus including ventriculus.

Material and Methods

Specimens were collected during spring 1992 from the peritoneal cavity of *Merluccius merluccius* and *Sardina pilchardus* in various localities of the South Adriatic and Ionian seas, with a prevalence of 39 and 31% and a range of intensity of 1–25 and 1–6 for *M. merluccius* and *S. pilchardus,* respectively (n = 100 of each species). Our data regarding prevalence and intensity for *M. merluccius* are quite close to those reported by Orecchia et al. (1989).

Nematodes for light microscope studies were fixed in 4% formaldehyde solution and mounted permanently in dehydrated glycerine following Seinhorst's (1959) method. Specimens for scanning electron microscopy (SEM) were processed by Eisenback's (1985) method and observed with a JEOL 50-A stereoscan. Glycerine-infiltrated specimens were also used for SEM observations.

For histological studies, specimens were fixed in Bouin's solution, dehydrated in ethanol, and embedded in Histowax. Transverse (cross) sections were cut at 5 μm and stained with hematoxylin–eosin.

A comparison of all previous descriptions of populations of *A. simplex* A from the North Sea, northeast Atlantic, Australia, New Zealand, and Japan to those of the present study was also made, using the following morphometrical parameters: BW/BL (body width/body length), EL/BL (esophagus length/body length), VL/BL (ventriculus length/body length), and TL/BL (tail length/body length), expressed as a percentage according to Hurst (1984). Body ratios were also calculated (Siddiqi, 1986): a (body length/body width), b (body length/esophagus length), c (body length/tail length), and c′ (tail length/body width at anus).

Specimens of the Mediterranean population of *A. simplex* A L3 are deposited in the collection of the Museum National d'Histoire Naturelle, Paris, France, and several Bouin's fluid-fixed specimens are deposited in our institute.

Results

L3s (N = 25) obtained from either fish species are morphologically and morphometrically identical (Table 1). The cuticle is 12–16 μm thick, usually with distinct striations mainly at the anterior and posterior body extremities (Figs. 1, 2). In en face view (SEM observation), a triangular oral opening is visible between trilobed lateral lips; a prominent V-shaped projecting boring tooth (3–9 μm long) is located ventrally to the mouth. The excretory opening, seen by light microscope below the boring tooth on the ventral side, is revealed by SEM as an oval lateral slit (Fig. 2). Rectangular to circular outlines of papillae could be seen on each of the lateroventral lips (Fig. 2). Cross-sections of the excretory cell along the body of larva show that it has different shapes and dimensions at different body levels (Fig. 3) and is always contiguous to hypodermal cells, the alimentary canal, and sometimes the

somatic musculature. At the pharynx level, it has an oval section, a well-visible central duct and appears full of eosinophilic granulation. At mid-body, it increases enormously, its lobes enveloping the ventral portion of the intestine; posteriorly it becomes narrower and ends just before the anal opening. The short tail (Table 1) ends with a distinct, not reflexed, mucron (Fig. 2). There are 3 rectal glands: 2 are dorsal and 1 is ventral (Fig. 1b), well visible on glycerine-mounted specimens.

REMARKS: The L3 *Anisakis* larvae from the Adriatic and Ionian seas are very close (with considerable overlaps) to the type I larvae found by Brunsdon (1956) in 54 New Zealand fishes and fit very well with all the previous descriptions (Berland, 1961; Koyama et al., 1969; Table 2) of this larval stage.

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Book Review

Parasites of Puerto Rican Freshwater Sport Fishes, by Lucy Bunkley-Williams and Ernest H. Williams, Jr. Puerto Rico Department of Natural and Environmental Resources, San Juan, PR, and Department of Marine Sciences of the University of Puerto Rico, Mayaguez, PR. 1994. 168 pages.

Parásitos de Peces Recreativos en Agua Dulce de Puerto Rico, Lucy Bunkley-Williams and Ernest H. Williams, Jr. Puerto Rico Departamento de Recursos Naturales y Ambientales, San Juan, PR, y Departamento de Ciencias Marinas de Recinto Universitario de Mayaguez, Mayaguez, PR. 1995. 190 paginas.

This compact book not only covers the freshwater fish parasite fauna in detail but also discusses the interaction of the unnatural mix of parasites and hosts from an almost completely introduced fish fauna. The interesting and important issues involved with introducing parasites along with their exotic fish hosts is addressed not only in the Discussion but with many of the individual parasite descriptions. Most of the fishes introduced to Puerto Rico have also been introduced to many other locations around the world, but their concomitantly introduced parasites have received little attention. This is the first work that addresses the complex problems of an exotic mix of parasites interacting with native and exotic fishes and parasites.

Puerto Rico essentially lacks a native freshwater fish fauna. Fishery biologists, aquaculturists, and aquarists have imported a diverse fauna. Puerto Rico has thus become a testing ground for interactions of parasites that may serve as a lesson or warning for other geographic areas. The authors formulate 10 intriguing "exotic parasite ecology hypothesis" that may apply to similar situations elsewhere. They also present methods to prevent further exotic parasite introductions. They suggest that the variety of exotic hosts, exotic parasites, and available niches will drive some interesting parasite evolution for which their book should serve as a baseline.

The authors believe that the freshwater fishes of Puerto Rico are remarkably free of some of the more destructive exotic diseases (e.g., *Argulus japonicus, Lernaea cyprinacea, Lironeca symmetrica,* channel catfish virus disease, *Edwardsiella ictaluri,* lymphocystis) and that Puerto Rico could avoid introducing these and others in the future. They suggest methods for avoiding these diseases that may apply to other areas of the world.

Three local parasites appear to limit the numbers of Mozambique tilapia. This fish has become a pest in some tropical/subtropical areas around the world. The authors suggest that these parasites may be developed into effective controls for this fish. They found that the simplified parasite mix on some imported fishes provided opportunities to more easily study parasite species that are difficult to understand within diverse parasite mixes of their native habitats.

The book contains a discussion of each parasite ($N = 100$) including an illustration, synopsis of importance, diagnostic characters, records in Puerto Rico, geographic range, life history, location in host, size, host specificity, damage to host, detection, significance to sport fishing, preparation for study, treatment, and comments. Illustrations of whole specimens of many of these important parasites have not appeared elsewhere. Each major parasite group (Protozoa [protozoans], Chlorophyta [green algae], Oomycota [fungus], Monogenea [gillworms], Digenea [flukes], Cestoidea [tapeworms], Nematoda [roundworms], Acanthocephala [thorny-headed worms], Hirudinea [leeches], Copepoda [copepods], Brachiura [fish lice], Isopoda [isopods], Acarina [mites], Pentastomida [tongueworms], and Mollusca [glochidia]) is defined, discussed, and characterized. The taxonomy of locally occurring examples is shown in an internal contents for each group. The structures and anatomy of a generalized gillworm, fluke, roundworm, thorny-headed worm, and isopod are figured and labeled. Nonparasitic fish diseases encountered during the study are also briefly detailed. Methods for sending specimens and a data form are provided. The number and variety, origin and evolution, fish kills, and significance to sport fishing of freshwater fish parasites are discussed. Fish parasites in humans are briefly discussed. The host-disease checklist includes small drawings of each fish host ($N = 63$). The bibliography is partially annotated and includes background references useful for further parasitological and fish kill work. Maps of the reservoirs, lagoons, and major rivers are provided. No other reference on Puerto Rico illustrates all of the freshwater fish fauna, and none includes all bodies of water.

The text is written for a popular audience without sacrificing scientific content. It is sprinkled

with enough fascinating parasite and fishery items of interest to maintain a general reader's interest (e.g., importing the life cycle of a native parasite, aquaculture developing and spreading the most hardy and adaptive parasites, the success and abundance of the largemouth bass in the absence of its most damaging parasites, fish parasites with the ability to infect and kill humans, how the public can help to protect their natural environment). The diversity of parasites in the study is rather high with most phyla of fish parasites represented. Each major group is succinctly introduced, making this book useful as a general introduction to fish parasites. Few popular treatments of this topic are available in English, and I am aware of no others in Spanish.

Sportfish restoration is usually a dry, scientific business relegated to gray-literature reports. It is refreshing to see a research project written not only for the scientific community but also for the very sportfishermen who supported the research. A Spanish-language edition will be available by the time this review is published. The fish-parasitology research and the book were supported by Sportfish Restoration Funds. The book was reviewed for the U.S. Fish and Wildlife Service by Dr. John Grizzle, Co-Editor of the *Journal of Aquatic Animal Health.* Copies of the English or Spanish editions may be requested from the Department of Marine Sciences, University of Puerto Rico, P.O. Box 908, Lajas, Puerto Rico 00667.

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Willis A. Reid, Jr. receiving the 1994 Anniversary Award from J. Ralph Lichtenfels at the Anniversary Dinner meeting, November 9, 1994.

Presentation of the 1994 Anniversary Award of the Helminthological Society of Washington to Willis A. Reid, Jr., 9 November 1994

As Chairperson of the Awards Committee, consisting also of Nancy D. Pacheco and Harley G. Sheffield and representing the Executive Committee, it is my pleasure to present the 1994 Anniversary Award.

The highest honor bestowed by the Helminthological Society of Washington is its Anniversary Award. Our Constitution stipulates that the Anniversary Award can be given for outstanding contributions to parasitology, an exceptional paper presented at a meeting of the Society or published in its Journal, or outstanding service to the Society.

The recipient of the 1994 Anniversary Award, Dr. Willis A. Reid, Jr., qualifies in all of these categories. He has been one of the pillars of the Society for many years and, we hope, for many more. Willis was elected to membership in this Society on 16 December 1965 after receiving his Master's degree under Bill Coil at the University of Kansas in Lawrence. The title of his thesis was "Hemiurid Trematodes of Formosan Marine Fishes." He also assumed the duties of Parasitologist, Walter Reed Army Institute of Research, in 1965, where he worked on schistosomiasis and filariasis until 1967 when he moved to North Carolina State University, where he worked for his Ph.D. under Reinard Harkema. The title of his 1970 dissertation was "The Histochemistry of *Procyotrema marsupiformis* Harkema and Miller, 1959 (Trematoda: Diplostomatidae)."

At this point we look back a few years to find what launched Willis into a career in the military and parasitology. Working for his B.S. at North Carolina State College, Willis had the good fortune to have Grover C. Miller as Academic Advisor. For those of us who know the long history of parasitology at North Carolina State with the Miller and Harkema team, we understand how Willis got interested in this subject. Indeed, Willis confirmed that after taking Miller's Invertebrate Zoology and Harkema's Parasitology courses, he informed Harkema that he wanted to be a parasitologist. But Willis also became interested in the Army at North Carolina. He was a Distinguished Military Graduate, which meant that he was entitled to a Regular Army commission. Willis declined in favor of a Reserve Commission and arranged to have it delayed so he could go to graduate school. Bill Coil offered Willis a Research Assistantship at the University of Kansas (to work on some of

Bob Kuntz's parasite collections) but with the stipulation that he spend the summer of 1963 as Coil's Research Assistant at the Duke University Marine Laboratory in Beaufort, North Carolina. It was a fortunate time for Willis because, that summer, he met his future wife, Janet Warner, then a student at Duke University, now Dr. Janet Warner Reid, copepod systematist, Research Associate, at the Smithsonian Institution's Museum of Natural History and current president of the Biological Society of Washington. Willis and Jan raised 2 children, Blake Dietrich Reid (a physics graduate, now in the Peace Corps in Zimbabwe) and Alexander Nathan Reid (an engineering major at McGill University in Montreal). After completing his Master's degree and entering the Army, Willis competed successfully for an Army program under which he went back to North Carolina State for his Ph.D. as an Army officer.

Willis has had a long and distinguished career in the Medical Service Corps after finishing graduate school in 1970, including duty at the 4th Army Area Lab at Fort Sam Houston, San Antonio, Texas (1971); Chief, Department of Parasitology, Long Binh, Vietnam, where he supervised the entire Parasitic Diseases Diagnostic Laboratory for U.S. forces in Vietnam (1971–1972); Assistant Chief, Schistosomiasis Research Unit, Walter Reed Army Institute of Research (WRAIR), Washington, D.C. (1972–1977); Chief, Anti-Schistosome Drug Testing Section and later the Commander of the U.S. Army Medical Research Unit, Brasilia (1978–1982); and Chief, Department of Parasitology, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D.C. (1982–1990). During the years 1972–1990, Willis and his associates produced and managed at WRAIR, an unequaled antischistosomal drug development program. From 1990 to 1993, Willis served as Administrator, Research Health Sciences and Chief, Office of Research and Technology Applications, at Walter Reed. In his last 3 years of active Army service, Willis developed the most comprehensive and successful technology transfer program in the Army today. Willis retired from the Army in 1993 and is currently Consultant to the Walter Reed Program on Technology Transfer.

During his postings in Brasilia and Washington, Willis was also an Adjunct Professor at the University of Brasilia School of Medicine and at the Uniformed Services University of the Health Sciences in Bethesda, Maryland. At these universities and in his leadership positions, Willis was a mentor for numerous young scientists and officers; an activity that he enjoyed and one in which he excelled.

During his distinguished career Willis published 25 research papers and one patent, in addition to his heavy administrative and service responsibilities. His honors and awards included the Order of Military Medical Merit (1990); Legion of Merit (1993); Bronze Star Medal (1972); two Meritorious Service Medals (1978 and 1982); Republic of Vietnam Cross of Valor (1972); and Certificate for Outstanding Volunteer Service, Montgomery County Schools.

Willis served this Society as President in 1985 and in numerous other capacities. He hosted regular spring meetings at Walter Reed for many years and organized the first student paper competition for the society in 1989 and the second competition in 1991.

Willis, your distinguished record as a parasitologist and your long service to the Helminthological Society of Washington has brought credit and honor to this Society and we now present to you our highest honor—the 1994 Anniversary Award!

J. R. Lichtenfels
Chair
Awards Committee

Acceptance of the 1994 Anniversary Award by Willis A. Reid, Jr.

Thank you, Ralph, for that glowing introduction. Ladies and Gentlemen of the Society, I am told that it is customary for the Recipient of the Anniversary Award to make some BRIEF acceptance remarks. I think that most of you know that using the name "Willis Reid" and "brief remarks" in the same sentence is really an oxymoron, like "military intelligence," or—as some of you might

prefer—"deafening silence." But I can assure you that, having actually written out these remarks, and promising myself that I will not diverge extemporaneously, I *WILL* be brief.

First, I want to express my appreciation to the Anniversary Award Committee for selecting me from what I am sure was a long and distinguished list of candidates and to the Society for approving the Committee's recommendation.

I've thought long and hard about a theme for this acceptance and I kept coming back to two simple words—"influence" and "service." Influence not in its political connotation of string pulling and lobbying, but in the connotation of the impact that all of one's professional associations over his or her career has had on the shaping of that career. I am humbled, therefore, in the realization that, while I am accepting this recognition for myself, I am also accepting it for all of those people who influenced whatever small accomplishments I might have made. Foremost among these was the late Dr. Reinard Harkema, who in both 1962 and 1970 put me on the parasitology path and who in 1963 told me in no uncertain terms that the Chemical Corps was definitely *not* an appropriate Army branch selection and insisted that I immediately go to the ROTC office and switch to the Medical Service Corps (he, I might add, was a veteran to the Sanitary Corps and the Army Medical School (now the WRAIR) and was a Colonel in the Army Reserve).

Doc Harkema and Grover Miller at N.C. State, and later Bill Coil at the University of Kansas, started a whole parade of professional associations that influenced what I am today. The list could fill the HelmSoc directory, but I would like to name just a few: the late Elvio Sadun, John Bruce, Norm Wilkes, and Myron Radke, Dale Wykoff, Jim Burke—all of whom were my colleagues and bosses early in my career. It is interesting that many of these earlier Army parasitologists (including Doc Harkema) were, themselves, mentored and *influenced* by none other than George W. Hunter III. Then, there were all of my contemporary colleagues and associates—both past and present—both in and out of the Army parasitology sphere, and all of those Division of ET and Walter Reed people who supported me and made life so interesting in the 1980's and who have contributed their influential ingredients to this broth who stands here—and is being brief.

The final ingredient of influence—and the real spice of the concoction—comes from all of those outstanding professional associations with whom I've have the pleasure and honor of working over these years. I won't even attempt to name them all, but most of them were or are members of HelmSoc and, if you look in a mirror and around this room, you have a better than even chance of seeing many of them.

My remarks concerning "Service" will be even briefer. I have always held that membership in professional societies was a responsibility and duty of the science professional, for it was only through those memberships and associations that scientists could expand their horizons beyond their own limited fields of endeavor. It follows that the membership of these societies influence (there's that word again) the organization's directions and policies through providing services to that organization—service in the form of holding Society officer positions, service on committees and subcommittees, service on editorial boards, and in a whole raft of volunteer positions. Quite often, we join these organizations only to receive the Journal and to read the papers presented therein without having to go to the library. But I will have to be honest with you, my association (and service) over the years with the Helminthological Society of Washington has not been just a duty to my profession—it has been, and continues to be—downright fun! And the fact that you have honored me in this way just for having fun is all the more humbling.

Thank you.

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MINUTES

Six Hundred Forty-First Through Six Hundred Forty-Fifth Meetings

641st Meeting: USDA, Animal Parasitology Institute, Beltsville, MD, 12 October 1994. Dr. Mark Jenkins presided over the business meeting and Dr. Darwin Murrell presided over the scientific session. The following two papers were presented: "Regulation of immunity and immunopathology by IL-12 in murine schistosomiasis," by Dr. Thomas Wynn; and "Interleukin-4 can cure gastrointestinal nematode infections in immunocompetent and immunodeficient mice," by Dr. Joseph Urban. The slate of officers for 1995 was presented: Joan E. Jackson, President; Susan Fricke-Meyer, Vice President; Harley G. Sheffield, Secretary-Treasurer; Michael J. Bangs, Recording Secretary.

642nd Meeting: Uniformed Services University of the Health Sciences, Bethesda, MD, 9 November 1994. The Anniversary Dinner Meeting and program were presided over by President Mark Jenkins. Recognizing the achievements in parasitology of Purnomo of the U.S. Naval Medical Research Unit No. 2 and the University of Indonesia, he was presented with a Certificate of Honorary Membership by Willis Reid on behalf of the Society. Willis Reid, chair of the Life and Honorary Committee, presented Life Memberships to Dr. Louis S. Diamond and Dr. Mary Lou Pritchard. Dr. J. Ralph Lichtenfels, chair of the Awards Committee, presented the 1994 Anniversary Award to Dr. Willis Reid. The Keynote Speaker for the evening was Purnomo, who spoke on human and animal filariasis in Indonesia. The slate of officers for 1995 was elected and installed: Eileen Jenkins, President; Susan Fricke-Meyer, Vice President; Harley G. Sheffield, Corresponding Secretary-Treasurer; and Michael J. Bangs, Recording Secretary. Support for the Anniversary Dinner Meeting was provided by the American Society of Parasitologists.

643rd Meeting: Naval Medical Research Institute, Bethesda, MD, 8 February 1995. Joan Jackson presided over the business meeting and Eileen Franke presided over the scientific session. Two presentations were given covering recent investigations within the NMRI Malaria Program: "Protection against malaria by immunization with plasmid DNA encoding circumsporozite protein," by Dr. Martha Sedegah, and "Field sites for malaria vaccine trials: baseline studies," by Dr. Walter Weiss.

644th Meeting: Walter Reed Army Institute of Research, Washington, DC, 8 March 1995. Both the business and scientific meetings were presided over by Joan Jackson. Dr. Dennis Kyle spoke on "In vitro drug susceptibility of admission and recrudescent isolates of *Plasmodium falciparum* for evaluating antimalarial drug efficacy" and Dr. Maurice Iwu spoke on "Reinventing drugs so others might live: natural product drug development for orphan parasite diseases."

645th Meeting: New Bolton Center, University of Pennsylvania, Kennett Square, PA, with the New Jersey Society of Parasitologists, 6 May 1995. Dr. Gerhard A. Schad presided over the scientific program, which consisted of three presentations on modern malaria for non-malariologist: Dr. Akhil Vaidya spoke on "Mating behavior of malarial parasites," Dr. Harvey Rubin discussed "Regulation of DNA synthesis in *Plasmodium falciparum,*" and Dr. Theodore Taraschi spoke on "The parasitophorous duct: a target for drug and vaccine development." Support for the meeting was provided by SmithKline Beecham Animal Health and the Laboratory of Parasitology, University of Pennsylvania.

The following new members were elected at the respective meetings—641st: Stephen Simicik; 643rd: Vagn Flyger, Douglas Gill, and Claudia Portes Santos; and 645th: Prema Arasu, Ahmed Kamal Dyab, William E. Moser, Jason D. Smith, and Deborah R. Sullivan.

Respectfully submitted,

Michael J. Bangs
Recording Secretary

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KEY WORD AND SUBJECT INDEX FOR VOLUME 62

ANNIVERSARY AWARD RECIPIENTS

*Edna M. Buhrer	1960	*Horace W. Stunkard	1977
*Mildred A. Doss	1961	Kenneth C. Kates	1978
*Allen McIntosh	1962	*Everett E. Wehr	1979
*Jesse R. Christie	1964	*O. Wilford Olsen	1980
*Gilbert F. Otto	1965	*Frank D. Enzie	1981
*George R. LaRue	1966	Lloyd E. Rozeboom	1982
*William W. Cort	1966	Leon Jacobs	1983
*Gerard Dikmans	1967	Harley G. Sheffield	1984
*Benjamin Schwartz	1969	A. Morgan Golden	1985
*Willard H. Wright	1969	Louis S. Diamond	1986
Aurel O. Foster	1970	Everett L. Schiller	1987
Carlton M. Herman	1971	Milford N. Lunde	1988
*May Belle Chitwood	1972	J. Ralph Lichtenfels	1989
*Elvio H. Sadun	1973	A. James Haley	1990
E. J. Lawson Soulsby	1974	Francis G. Tromba	1991
David R. Lincicome	1975	Thomas K. Sawyer	1992
Margaret A. Stirewalt	1975	Ralph P. Eckerlin	1993
*Leo A. Jachowski, Jr.	1976	Willis A. Reid, Jr.	1994

HONORARY MEMBERS

*George R. LaRue	1959	Hugh M. Gordon	1981
*Vladimir S. Ershov	1962	E. J. Lawson Soulsby	1990
*Norman R. Stoll	1976	Roy C. Anderson	1991
*Horace W. Stunkard	1977	Louis Euzet	1992
*Justus F. Mueller	1978	John C. Holmes	1993
John F. A. Sprent	1979	Purnomo	1994
Bernard Bezubik	1980		

CHARTER MEMBERS 1910

*W. E. Chambers	*Philip E. Garrison	*Maurice C. Hall	*Charles A. Pfender
*Nathan A. Cobb	*Joseph Goldberger	*Albert Hassall	*Brayton H. Ransom
*Howard Crawley	*Henry W. Graybill	*George F. Leonard	*Charles W. Stiles
*Winthrop D. Foster			

LIFE MEMBERS

*Maurice C. Hall	1931	*Mildred A. Doss	1977
*Albert Hassall	1931	*Everett E. Wehr	1977
*Charles W. Stiles	1931	Marion M. Farr	1979
*Paul Bartsch	1937	John T. Lucker, Jr.	1979
*Henry E. Ewing	1945	George W. Luttermoser	1979
*William W. Cort	1952	*John S. Andrews	1980
*Gerard Dikmans	1953	*Leo A. Jachowski, Jr.	1981
*Jesse R. Christie	1956	Kenneth C. Kates	1981
*Gotthold Steiner	1956	Francis G. Tromba	1983
*Emmett W. Price	1956	A. James Haley	1984
*Eloise B. Cram	1956	Leon Jacobs	1985
*Gerald Thorne	1961	*Paul C. Beaver	1986
*Allen McIntosh	1963	*Raymond M. Cable	1986
*Edna M. Buhrer	1963	Harry Herlich	1987
*Benjamin G. Chitwood	1968	Glenn L. Hoffman	1988
Aurel O. Foster	1972	Robert E. Kuntz	1988
*Gilbert F. Otto	1972	Raymond V. Rebois	1988
*Theodor von Brand	1975	Frank W. Douvres	1989
*May Belle Chitwood	1975	Thomas K. Sawyer	1989
Carlton M. Herman	1975	*J. Allen Scott	1990
Lloyd E. Rozeboom	1975	Judith H. Shaw	1990
*Albert L. Taylor	1975	Milford N. Lunde	1991
David R. Lincicome	1976	Everett L. Schiller	1991
Margaret A. Stirewalt	1976	Harley G. Sheffield	1991
*Willard H. Wright	1976	Louis S. Diamond	1994
*Benjamin Schwartz	1976	Mary Hanson Pritchard	1994

*Deceased.

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