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## A Stable Eimerian Assemblage in Wyoming Ground Squirrels (*Spermophilus elegans elegans*): Maintaining Viability over Winter

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**ABSTRACT:** A stable assemblage of 3 uncommon (prevalence < 20%) and 3 common (prevalence > 45%) species of *Eimeria* (Apicomplexa: Eimeriina) is known to infect Wyoming ground squirrels (*Spermophilus elegans elegans*). Several factors may contribute to the prevalence stability including host immune response, parasite-parasite interactions, and oocyst survival under ambient environmental conditions. To determine the effects of various environmental conditions on 3 of these eimerians, oocysts of 1 uncommon (*E. larimerensis*) and 2 common (*E. beecheyi* and *E. callospermophili*) species were placed into 6 treatment groups and sampled monthly over winter. Treatments included ambient temperature (sun, shade, and subsoil) and constant 8°C temperature (moistened with 2% potassium dichromate or water, or allowed to desiccate). After 6 mo, oocysts from each treatment were administered to uninfected squirrels (2 squirrels per treatment group). Although all oocysts examined appeared grossly abnormal when examined microscopically, all 12 squirrels developed patent infections of *E. larimerensis* and *E. callospermophili*, and 11 of 12 developed infections of *E. beecheyi*. All 3 eimerian species maintained viability under conditions of severe desiccation, ultraviolet radiation, and repeated freezing and thawing. It is likely that resistance to harsh environmental conditions plays an important role in the maintenance of this parasite assemblage.

**KEY WORDS:** coccidia, *Eimeria*, overwintering, transeasuality.

A stable assemblage of 6 eimerian species has been described in Wyoming ground squirrels (*Spermophilus elegans elegans* (Kennicott, 1863)) and has been found in all populations of Wyoming ground squirrels sampled to date (Shults et al., 1990; Stanton et al., 1992). Furthermore, several of these eimerian species have been found in sciurid species sympatric with the Wyoming ground squirrel (Shults et al., 1990; Seville et al., 1992). Three species (*Eimeria larimerensis* (Vetterling, 1964), *E. spermophili* (Hilton and Mahrt, 1971), and *E. bilamellata* (Henry, 1932)) occur at prevalences of less than 20% and may elicit some degree of immunity in Wyoming ground squirrels (Thomas et al., 1992). The remaining 3 species (*E. beecheyi* (Henry, 1932), *E. callospermophili* (Henry, 1932), and *E. morainensis* (Torbett, Marquardt, and Carey, 1982)) occur at prevalences of 45-75% and can repeatedly reinfect individual squirrels (Seville, 1992). Rahel (1990) proposed 3 levels of stability. At the highest level, absolute abundance of each species in a community remains constant over time. Level 2 stability is characterized by some fluctuation in absolute abundance, but relative species abundance is constant. Level 3 stability occurs when criteria for Levels 1 and 2 are not met, but the same species are always present in the community over time. According to this hierarchy, this

eimerian assemblage meets the criteria for Level 2 stability, because all 6 species are present over time and prevalences remain constant from spring emergence through the squirrels' active season (Seville, 1992). The purpose of this study was to examine oocyst survival and viability under experimental and field conditions and to determine whether or not overwinter survival of eimerian species may contribute to the stability of this host-parasite system.

Several strategies for overwintering are possible. *Eimeria* spp. may avoid harsh conditions through extraintestinal stages harbored in hosts (Fernando et al., 1987; Perry and Long, 1987; Ball et al., 1989; Ball et al., 1990) or in prolonged or arrested infections in hibernating animals (Anderson, 1971). Furthermore, harsh conditions may be moderated in microclimates beneath the soil surface (i.e., in burrows or after burial of squirrel fecal pellets by dung beetles [T. Wingert, pers. comm.]). Finally, oocysts of some eimerian species may be able to survive the severe environmental conditions encountered over winter at northern latitudes. Oocysts from ovine coccidia in Norway overwinter and provide a primary source of infection of lambs the following spring (Helle, 1970), and *E. acervulina* (Tyzzer, 1929) and *E. tenella* (Railliet and Lucet, 1891; redescribed by Fantham, 1909) oocysts have been

**Table 1. Treatment groups for overwintered oocysts.**

Treatment	Placement	Temperature	Moisture
1 Control	Refrigerator	8°C	Moistened with K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>
2 Wet	Refrigerator	8°C	Moistened with distilled water
3 Dry	Refrigerator	8°C	Not moistened
4 Sun	Shortgrass prairie	Ambient	Ambient
5 Shade	Shortgrass prairie	Ambient	Ambient
6 Buried	Shortgrass prairie	Ambient	Ambient

reported to survive exposure to a Siberian winter (Kheysin, 1972).

### Methods

#### Environmental conditions

Oocysts from *E. larimerensis*, *E. beecheyi*, and *E. callospermophili* were collected from the feces of experimentally infected Wyoming ground squirrels using a modified version of the methods of Davis (1973). These oocysts were combined with fresh feces from uninfected squirrels to produce a fecal homogenate containing 118,000 oocysts/g. Approximately 33% of these oocysts were sporulated. Forty percent of the oocysts were *E. larimerensis* (sporulation = 28%), 12% *E. beecheyi* (42%), and 48% *E. callospermophili* (35%). One-gram fecal pellets were formed and packed into 6 1-ml bioassay plates with 64 pellets/plate. One plate was used per treatment.

Oocysts were subjected to the following treatments: (1) refrigerated at 8°C, moistened with 2% potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) (control), (2) refrigerated at 8°C, moistened with distilled water (simulating moist burrow conditions), (3) refrigerated at 8°C, allowed to dry (dry burrow conditions), (4) placed on open shortgrass prairie 10 km south of Laramie, Wyoming, with direct exposure to the sun, (5) placed on shortgrass prairie but shaded from above, and (6) placed 3 cm below the soil surface to simulate burial by dung beetles (Table 1).

In October 1990, plates with pellets were placed in the refrigerator and in the field. Once a month, 8 pellets were randomly selected from each treatment. Three pellets were weighed, oven-dried for 24 hr, and reweighed to assess moisture content. The remaining 5 pellets were soaked in K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> for 24 hr before being analyzed for oocysts/g using the McMaster's technique (Whitlock, 1948). Although we had intended to measure percentage of sporulation by species throughout the study, the rapid degeneration and abnormal appearance of the internal oocyst structures made this impossible.

#### Assessing oocyst viability

After 6 mo, 8–12 of the remaining pellets from each treatment were collected and soaked in 2% K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> for 3 wk at room temperature (25–27°C), after which an inoculum was prepared from each treatment using a modified version of the methods described by Davis (1973). Because all oocysts examined were structurally abnormal, the number of viable oocysts in the inocula

could not be estimated; however, species remained identifiable by size.

The inoculum prepared from each treatment was administered to 2 uninfected squirrels via oral gavage (12 squirrels total). Although ideally, naive squirrels would have been used in the trials, we were unable to successfully raise litters of young in captivity, and due to the possibility of extraintestinal stages of coccidia in pregnant and lactating females, even presumably naive young might be exposed to coccidian infections before they are weaned. However, all squirrels had been individually housed in laboratory facilities for at least 1 yr, and sampling twice weekly revealed no patent infections for at least 3 wk prior to inoculation. Postinoculation feces were collected daily and soaked in 2% K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> for at least 24 hr before being weighed, homogenized, and analyzed for oocysts/g and species composition using the McMaster's technique. Daily oocyst production for each species was estimated by multiplying oocysts/g by the total wet fecal weight.

The Number Cruncher Statistical System (Hintze, 1990) was used to determine correlation between the number of oocysts/g in April and infection intensity; *t*-tests (significance =  $P \leq 0.05$ ) were used in the analysis of prepatent and patent periods.

### Results

#### Environmental effects

Oocysts that remained intact were identifiable to species by size throughout the sampling period. After 1 mo, approximately 70% of the oocysts were still detectable in the 2 moistened refrigerated treatments (1 and 2); Treatments 3–6 averaged 40%. Fungal growth appeared on the pellet surfaces in January (Treatment 1) and March (Treatment 2), which was associated with sizable decreases in oocysts/g. By April, oocyst counts were only 5–20% that of the original inoculum in all 6 treatments (Fig. 1).

After 2 mo, all intact oocysts observed in Treatments 2–6 exhibited gross abnormalities, including a clear crenated appearance and/or a thick, darkened oocyst wall, absence of identifiable internal structures, and, occasionally, ruptured walls (Fig. 2). By 4 mo, all oocysts observed in Treatment 1 exhibited similar abnormalities.

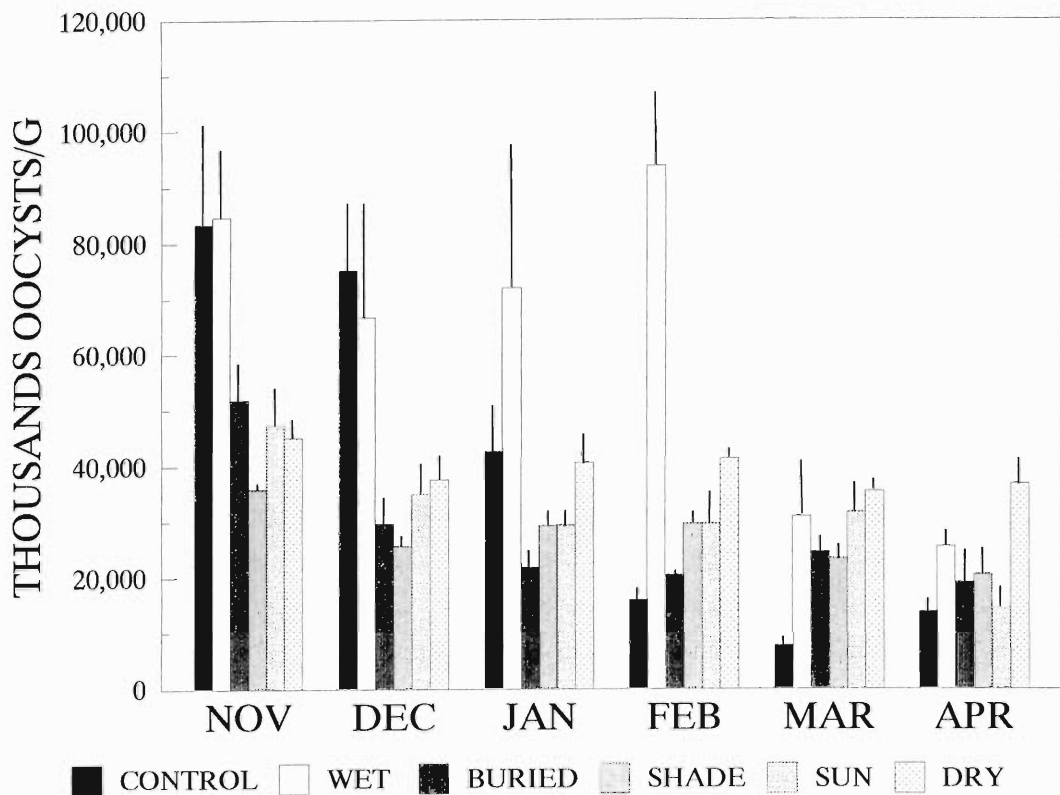


Figure 1. Mean number of oocysts/g ( $n = 5$ ) with standard error by treatment from November through April.

Treatments 1–3 remained at 8°C throughout the sampling period, but Treatments 4–6 experienced temperatures ranging from 20 to –36°C (National Oceanic and Atmospheric Administration, 1990, 1991), repeated freezing and thawing, and periodic snow cover (Table 2). Moisture content (oven-dried weight/initial weight) was highest in pellets from Treatments 1 and 2 ( $\bar{x} = 40\%$ ). Pellets from Treatments 4 and 6 averaged 32%, and those subjected to Treatments 3 and 5 averaged 10 and 9%, respectively, with moisture

content of individual pellets ranging as low as <1%.

#### Experimental infections

Despite the extreme environmental conditions and grossly abnormal appearance of all oocysts observed, all 12 squirrels inoculated with overwintered oocysts developed patent infections of *E. larimerensis* and *E. callospermophili*, and 11 of the 12 developed patent infections of *E. beecheyi*. Mean prepatent periods for *E. larimerensis*,

Table 2. Climatological data, 15 October 1990 to 15 April 1991.

Month	Temperature (°C)				Precipitation (cm)	Snowfall (cm)
	Absolute high	Mean high	Absolute low	Mean low		
Oct/Nov	20.0	10.0	–21.7	–5.0	4.0	15.7
Nov/Dec	16.1	6.1	–17.8	–6.7	0.8	7.6
Dec/Jan	6.1	–2.8	–36.1	–15.6	1.0	12.9
Jan/Feb	11.7	1.7	–27.2	–13.3	1.4	14.9
Feb/Mar	12.8	4.4	–17.7	–8.3	1.1	9.6
Mar/Apr	19.4	7.8	–17.2	–6.1	2.1	23.5

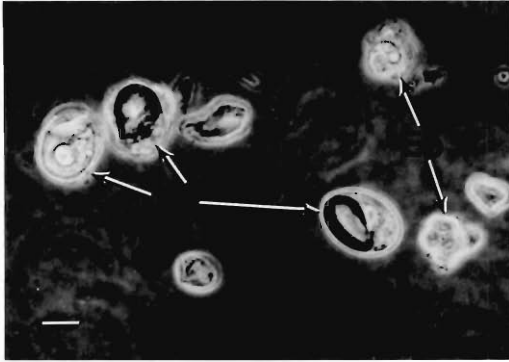


Figure 2. Oocysts of *E. larimerensis* (El) and *E. beecheyi* (Eb) showing grossly abnormal internal structures due to overwintering conditions. Scale bar = 15  $\mu$ m.

*E. beecheyi*, and *E. callospermophili* were 6.6 (SE = 0.70), 4.9 (0.21), and 5.1 (0.19) days, respectively; and patent periods were 8.2 (1.11), 4.3 (0.36), and 5.5 (0.49) days. Individual squirrels passed between 5,400 and 131,000,000 *E. larimerensis* oocysts over the course of the infections, 54,000–5,200,000 *E. beecheyi* oocysts, and 375,000–40,730,000 *E. callospermophili* oocysts.

### Species composition

In 5 of 6 treatments, relative species composition of oocyst output of infected squirrels was significantly different from that of the initial homogenate and the overwintered pellets (*t*-test,  $P \leq 0.05$ ). Additionally, the number of oocysts/g in April (overwintered) pellets did not correlate with infection intensity (total oocysts shed/squirrel) ( $R^2 = 0.24$ ). Finally, the total number of oocysts shed did not reflect any differences by treatment, and the variation in oocyst output among squirrels was extremely high. For example, 2 squirrels inoculated with oocysts from Treatment 1 differed by 3 orders of magnitude in oocyst output.

### Discussion

The relative species composition of oocysts shed by infected squirrels (output) varied considerably among hosts and did not reflect that of the oocysts in the overwintered pellets from which the inoculums were made. In multispecific infections of these 3 species, similar variation between the species composition of inoculum and output has been reported previously (Thomas et al., 1992). Squirrels initially inoculated with 1%

*E. larimerensis*, 22% *E. beecheyi*, 10% *E. bilamellata*, and 67% *E. callospermophili* shed 41% *E. larimerensis*, 5% *E. beecheyi*, 0% *E. bilamellata*, and 54% *E. callospermophili*. When the same squirrels were reinoculated with 85% *E. larimerensis*, 7% *E. beecheyi*, and 8% *E. callospermophili*, the output was 9% *E. larimerensis*, 13% *E. beecheyi*, and 78% *E. callospermophili*. Thomas et al. (1992) proposed that interspecific parasite interactions and/or partial host immunity toward *E. larimerensis* influenced oocyst production.

Although all squirrels were maintained individually in sterilized wire-mesh cages for at least 1 yr prior to this study, their complete infection histories are not known; however, host immunity toward *E. larimerensis* and interspecific parasite interactions may have influenced species composition of oocyst output. This conjecture is supported by the high variation in oocyst output among squirrels.

Because of the abnormal structure of the treated oocysts, it was impossible to determine the number of viable oocysts in the experimental infection inocula. There was no correlation between the number of oocysts/g observed in overwintered pellets (April) and infection intensity, and the latter may have been influenced by host immunity and interspecific parasite interactions. Additionally, the number of oocysts/g in April varied among treatments, and inoculum level may have affected infection intensity, with oocyst production/infective unit inversely related to dosage (Tilahun and Stockdale, 1981). Finally, oocyst walls may have broken, releasing sporozoites and infective sporozoites; therefore, the number of viable eimerian infective bodies is not necessarily correlated with the number of intact oocysts observed.

All 3 eimerian species maintained viability over all 6 treatments in numbers large enough to produce heavy patent infections. Additionally, prepatent and patent periods were not significantly different from those reported for multispecific infections using freshly sporulated oocysts of the same 3 eimerian species (Thomas et al., 1992), with the exception of the prepatent period of *E. beecheyi*, which was 1.4 days shorter in this study. The results for these 3 eimerian species differ from those of Duszynski and Conder (1977), who reported decreased oocyst production of *E. nieschulzi* oocysts exposed to radiation (15–60 k-rads) and heat (35–45°C), and Marquardt et al. (1960), who found that as little as 4 hr exposure

to sunlight, 25% or lower relative humidity, and temperatures below  $-7^{\circ}\text{C}$  are lethal to oocysts of *E. zuernii*.

Several strategies for oocyst survival over winter have been suggested: extraintestinal stages of *Eimeria* have been described in a number of hosts (e.g., mice, chickens) (Fernando et al., 1987; Perry and Long, 1987; Ball et al., 1989; Ball et al., 1990) and may overwinter in hibernating squirrels. Oocysts may also overwinter in the digestive tracts of hibernating squirrels (Anderson, 1971). Additionally, conditions may favor survival of oocysts in the fecal material buried by dung beetles. And, finally, oocysts of some eimerian species can persist overwinter both within the burrow and under harsh ambient conditions on the surface of the soil.

Transeasonality ensures that infective oocysts are readily available as soon as hosts emerge from hibernation and throughout the host's active season, thus minimizing the risk of local eimerian extinction. We propose that the maintenance of viability over winter contributes to the observed infections in the spring and the consistent prevalences throughout the active season of the squirrels, thus enhancing the stability of this eimerian guild.

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## Description of Two New Species of *Gyrodactylus* von Nordmann, 1832 (Monogenea) from Cultured Nile Tilapia, *Tilapia nilotica* (Cichlidae), in the Philippines

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**ABSTRACT:** Two new species of *Gyrodactylus* von Nordmann, 1832 (Monogenea) are described from the body surface of Nile tilapia (*Tilapia nilotica*) (Cichlidae) from fish farms in the Philippines. One species, *G. shariffi* sp. n., occurs on fish in brackish water ponds. It resembles species of *Gyrodactylus* reported from mullets (Mugilidae) in other regions of the Pacific and may represent a parasite that *T. nilotica* has acquired from mullets enzootic to coastal waters of the Philippines. The other species, *G. niloticus* sp. n., occurs on fish cultured in freshwater ponds. It resembles species of *Gyrodactylus* described from freshwater fishes (cichlids, characids, and cyprinodontids) in Africa and is believed to have been introduced into the Philippines along with shipments of Nile tilapia from stocks originating on that continent.

**KEY WORDS:** *Gyrodactylus niloticus* sp. n., *Gyrodactylus shariffi* sp. n., morphology, taxonomy.

To date, there are 348 named species of *Gyrodactylus* von Nordmann, 1832 (Monogenea) described from teleost fishes and amphibians. Only one of these, *G. plotosi* Mayes and Brooks, 1977, has been described from a host fish originating from the Philippine Archipelago (Mayes and Brooks, 1977). The only other reports of these parasites from the Philippines involve unidentified species listed only as *Gyrodactylus* sp. (see Lumanlan and Arthur, 1992).

During a study of parasites of Nile tilapia (*Tilapia nilotica*) raised at fish farms at 2 sites in the Philippines, 2 undescribed species of *Gyrodactylus* were found. The present study describes this new material as *Gyrodactylus niloticus* sp. n. and *Gyrodactylus shariffi* sp. n.

### Materials and Methods

Specimens of *Gyrodactylus* examined were collected during an extensive study of parasites of cultured Nile tilapia throughout the Philippines, the procedures of which have been described elsewhere (Natividad et al., 1986; Bondad-Reantaso and Arthur, 1990). Species of *Gyrodactylus* from 2 areas, 1 a freshwater site (culture ponds at the Bureau of Fisheries and Aquatic Resources, National Freshwater Fish Hatchery, Muñoz, Nueva Ecija Province) and the other a brackish water site (culture ponds at the University of the Philippines in the Visayas Brackishwater Aquaculture Center, Le-

ganes, Iloilo Province) are described herein. Formalin-fixed specimens were mounted individually in glycerine jelly and, when cleared, examined microscopically. Pertinent morphometric measurements were calculated from drawings prepared with the aid of a drawing tube. Enlarged photomicrographs of the marginal hooks were used to prepare the detailed drawings of the marginal hook sickle. The morphometrics follows that of Malmberg (1970). Measurements are given in micrometers ( $\mu\text{m}$ ) and are presented as the range followed by the mean  $\pm$  1 SD in parentheses.

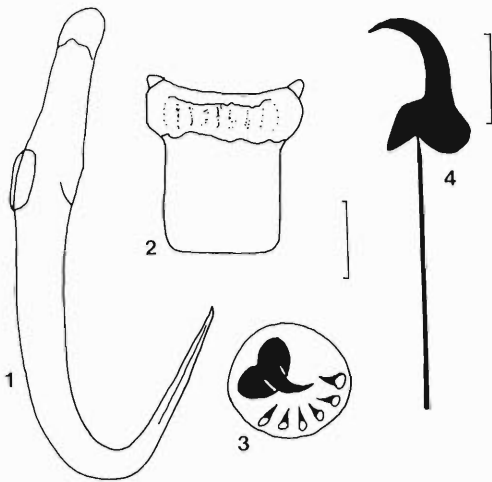
### Results

#### *Gyrodactylus niloticus* sp. n. (Figs. 1–4)

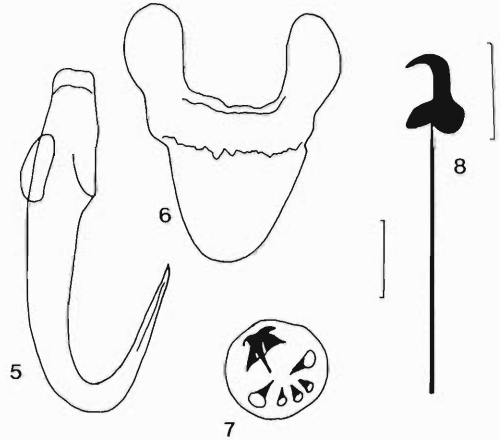
**DESCRIPTION** (based on 9 flattened specimens): Body 360–416 ( $388.7 \pm 21.1$ ) long, 64–128 ( $87.1 \pm 24.5$ ) wide at middle. Pharynx 22–39 ( $26.0 \pm 4.0$ ) ( $n = 8$ ) long, 24–35 ( $27.9 \pm 4.6$ ) wide. Penis 10–14 ( $11.4 \pm 1.9$ ) ( $n = 7$ ) in diameter, with 1 large spine and a row of 4–6 small spines. Hamuli relatively slender, 59–66 ( $61.3 \pm 2.3$ ) ( $n = 8$ ) long; root 17–25 ( $20.6 \pm 2.6$ ) ( $n = 8$ ), shaft 42–45 ( $43.8 \pm 1.0$ ) ( $n = 8$ ), point 22–28 ( $25.6 \pm 1.9$ ) ( $n = 7$ ). Ventral bar 5–7 ( $6.2 \pm 0.7$ ) long, 20–22 ( $21.1 \pm 0.9$ ) wide, with small, inconspicuous anterolateral processes 2 ( $2.0 \pm 0.0$ ) long. Ventral bar membrane almost square, 13–14 ( $13.8 \pm 0.4$ ) long. Dorsal bar 14–22 ( $20.0 \pm 2.6$ ) ( $n = 8$ ) wide. Marginal hook 23–29 ( $27.1 \pm 1.9$ ) ( $n = 8$ ) long. Sickle 7–8 ( $7.7 \pm 0.5$ ) long, 3–4 ( $3.8 \pm 0.5$ ) ( $n = 8$ ) wide proximally, 4–6 ( $4.5 \pm 0.8$ ) ( $n = 8$ ) wide distally, with slender blade. Handle 17–21

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Figures 1-4. Pertinent morphological features of *Gyrodactylus niloticus* sp. n. 1. Hamulus. 2. Ventral bar. 3. Penis. Scale bar = 10  $\mu$ m. 4. Marginal hook. Scale bar = 5  $\mu$ m.



Figures 5-8. Pertinent morphological features of *Gyrodactylus shariffi* sp. n. 5. Hamulus. 6. Ventral bar. 7. Penis. Scale bar = 10  $\mu$ m. 8. Marginal hook. Scale bar = 5  $\mu$ m.

(19.9  $\pm$  1.4) ( $n$  = 8) long, with no terminal swelling. Filament 9-11 (10.0  $\pm$  1.4) ( $n$  = 2) long.

TYPE HOST: *Tilapia nilotica*, Nile tilapia, (Cichlidae).

TYPE LOCALITY: Culture Ponds at the Bureau of Fisheries and Aquatic Resources National Freshwater Fish Hatchery, Muñoz, Nueva Ecija Province, Philippines.

LOCATION: Body surface.

TYPE SPECIMENS: Holotype (No. 84007) and paratype specimens (No. 84008) are deposited in the United States National Museum, Beltsville, Maryland.

COLLECTION DATE: November 1985.

ETYMOLOGY: This species is named after the host from which it was collected.

COMMENTS: *Gyrodactylus niloticus* sp. n. resembles numerous species of the genus (e.g., *G. cichlidarum* Paperna, 1968; *G. microalestis* Paperna, 1968; *G. cytophagus* Paperna, 1968) described from African freshwater fishes including native cichlids, characids, and cyprinodontids (Paperna, 1968). In fact, all of these species, as well as *G. niloticus* sp. n., appear to represent members of the same lineage and are characterized by possessing relatively long, narrow hamuli, a ventral bar with small anterolateral processes and an almost square posterior membrane, and marginal hooks with relatively large slender sickles. This lineage or species group has not been reported from freshwater fishes outside of the

African continent, suggesting that *G. niloticus* sp. n. arrived in the Philippines along with host shipments originating in Africa. The haptor sclerites of *G. niloticus* sp. n. resemble most closely those of *G. cichlidarum*, in both species having hamuli, ventral bars, and marginal hook sickles of similar shape and proportions. However, *G. niloticus* sp. n. is easily separated from this species by its much smaller hamuli (59-66  $\mu$ m versus 80-100  $\mu$ m in *G. cichlidarum*).

***Gyrodactylus shariffi* sp. n.**  
(Figs. 5-8)

DESCRIPTION (based on 10 flattened specimens): Body 240-383 (289.7  $\pm$  43.9) long, 48-96 (76.6  $\pm$  13.5) wide at middle. Pharynx 20-39 (26.2  $\pm$  6.0) long, 22-43 (30.7  $\pm$  6.4) wide. Penis 9-12 (10.1  $\pm$  1.1) ( $n$  = 8) in diameter, with a single large spine and a row of 5 or 6 small spines. Hamuli stout, 44-48 (45.3  $\pm$  1.6) long; root 8-14 (10.8  $\pm$  2.3), shaft 35-38 (36.5  $\pm$  1.1), point 17-21 (19.6  $\pm$  1.1) ( $n$  = 9). Ventral bar 5 (5.0  $\pm$  0.0) long, 18-22 (20.2  $\pm$  1.0) wide, with prominent, rounded anterolateral processes 7-10 (9.0  $\pm$  0.9) ( $n$  = 9) long. Ventral bar membrane 11-15 (14.0  $\pm$  1.2) long. Dorsal bar 14-20 (17.0  $\pm$  2.0) ( $n$  = 9) long. Marginal hook 18-21 (19.5  $\pm$  1.0) long. Sickle compact, 3-4 (3.8  $\pm$  0.4) long, 3-4 (3.2  $\pm$  0.4) wide proximally, 3-4 (3.2  $\pm$  0.4) wide distally. Handle 14-18 (15.8  $\pm$  1.1) long, with no terminal swelling. Filament 6-10 (7.6  $\pm$  1.3) long.

TYPE HOST: *Tilapia nilotica*.

**TYPE LOCALITY:** Culture ponds at the University of the Philippines in the Visayas, Brackishwater Aquaculture Center, Leganes, Iloilo Province, Philippines.

**LOCATION:** Body surface.

**TYPE SPECIMENS:** Holotype (No. 84009) and paratype specimens (No. 84010) are deposited in the United States National Museum, Beltsville, Maryland.

**COLLECTION DATE:** August 1985, December and January 1986.

**ETYMOLOGY:** This species is named in honor of Dr. M. Shariff, Universiti Pertanian Malaysia, Serdang, Malaysia, in recognition of his contributions to fish parasitology in Southeast Asia.

**COMMENTS:** *Gyrodactylus shariffi* sp. n. resembles most closely *G. zhukovi* Ling, 1962, described from the skin of *Mugil soiuy* in China (Ling, 1962; Zhukov, 1970). Both species belong to a lineage characterized by huge anterolateral processes of the ventral bar, relatively short stout hamuli, and marginal hooks with minute compact sickles. It appears to be a lineage that occurs on mugilid fishes in the Pacific Ocean basin because one of us (D.K.C.) has collected a similar species from *Mugil cephalus* in brackish water in coastal Peru. *Gyrodactylus shariffi* sp. n. differs from *G. zhukovi* by having much smaller hamuli (44–48  $\mu\text{m}$  versus 62  $\mu\text{m}$ ).

### Discussion

There is considerable international interest in the manner in which fish parasites are being disseminated globally by humans (Hoffman, 1970; Bauer and Hoffman, 1976; Combes and LeBrun, 1990; Arthur and Shariff, 1991; Bauer, 1991; Lumanlan et al., 1992; Kennedy, 1993; Cone et al., 1994). The present study suggests that tilapia introduced into the Philippines brought with them at least 1 species of gyrodactylid parasite (*G. niloticus* sp. n.) in addition to the 8 species of parasites previously believed to have been introduced with this fish (Bondad-Reantaso and Arthur, 1990).

The presence of *G. shariffi* sp. n. on *T. nilotica* cultured in brackish water cages suggests that Nile tilapia may have picked up the infections from wild marine fishes that also inhabit the ponds. Mulletts are plentiful in coastal waters of the Philippines (see Schroeder, 1980) and are the most likely reservoir hosts, given the known association of this particular lineage of *Gyrodactylus* with members of the Mugilidae in the Pacific Ocean.

Bondad-Reantaso and Arthur (1990), in their survey of the parasites of wild and cultured *T. nilotica* in the Philippines, showed that the assemblages of parasites are made up of those species that were apparently introduced into the region along with the original host shipments and those that have been acquired secondarily from native fishes. This scenario parallels what we suspect has happened with the viviparous monogeneans.

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### *Parasite Lives Available*

*Parasite Lives, Papers on Parasites, Their Hosts and Their Associations, to Honor J.F.A. Sprent*, edited by M. Cremin, paperback. 1986. This book contains 15 research papers on parasites, hosts, and parasite–host interactions. If you wish to obtain a copy, please send Aus\$5.00 with your request to: Mrs. E. A. Weston, Department of Parasitology, The University of Queensland, Brisbane, Queensland 4072, Australia.

### *Parasitic Case of the Month*

*Parasitic Case of the Month* now consists of 36 cases of parasitic diseases in humans. The format consists of a brief clinical description illustrated with colored projection slides. Several questions and answers are also in the descriptive material. A total of 84 slides are included. The material is intended to enhance the teaching of parasitology at all levels.

The total cost of the series, including the 84 projection slides and postage within the United States and Canada, is \$100, payable to: Herman Zaiman, P.O. Box 543, Valley City, North Dakota 58072.

## ***Dactylogyrus greenei* sp. n. (Monogenea: Dactylogyridae) from the Wedgespot Shiner, *Notropis greenei* Hubbs and Ortenburger (Pisces: Cyprinidae)**

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**ABSTRACT:** *Dactylogyrus greenei* sp. n. is described from the wedgespot shiner, *Notropis greenei* Hubbs and Ortenburger. *Dactylogyrus greenei* differs from its closest apparent relative, *D. crucis* Rogers, 1967, most notably by having a longer and more slender cirrus and larger accessory piece.

**KEY WORDS:** Monogenea, Dactylogyridae, morphology, systematics, *Dactylogyrus greenei* sp. n., *Notropis greenei*, Arkansas.

A new species of *Dactylogyrus* Diesing, 1850, is described from the wedgespot shiner, *Notropis greenei* Hubbs and Ortenburger, 1929, a cyprinid fish endemic to the Interior Highlands (Ozarks) of the eastern United States (Robison and Buchanan, 1988). This is the first report of any parasite from the wedgespot shiner.

### **Materials and Methods**

Wedgespot shiners were collected through use of a minnow seine on 4 August 1973, 5 June 1991, and 27 July 1991. They were placed in vials containing a 1:4,000 formalin solution immediately after capture; after approximately 1 hr, enough formalin was added to make a 10% solution (Putz and Hoffman, 1963). The parasites, collected from the gills of their hosts, were mounted in glycerin jelly, and observations were made with a Zeiss phase-contrast microscope. Drawings were made with the aid of a Zeiss drawing tube. Measurements, in micrometers, were made as presented by Mizelle and Klucka (1953); means are followed by ranges in parentheses. Numbering of haptoral hooks is after Mueller (1936); 4A hooks (Mizelle and Price, 1963) are considered to be ventral anchors (Kritsky and Kulo, 1992). Type specimens were deposited in the helminthological collection of the National Museum of Natural History (USNM) and the Harold W. Manter Laboratory, University of Nebraska State Museum (HWML). For comparative purposes, all original descriptions and redescriptions of North American *Dactylogyrus* species and 7 paratypes of *D. crucis* Rogers, 1967, from the collection of Dr. Wilmer A. Rogers (WAR) were examined.

### **Results and Discussion**

#### ***Dactylogyrus greenei* sp. n.**

(Figs. 1–8)

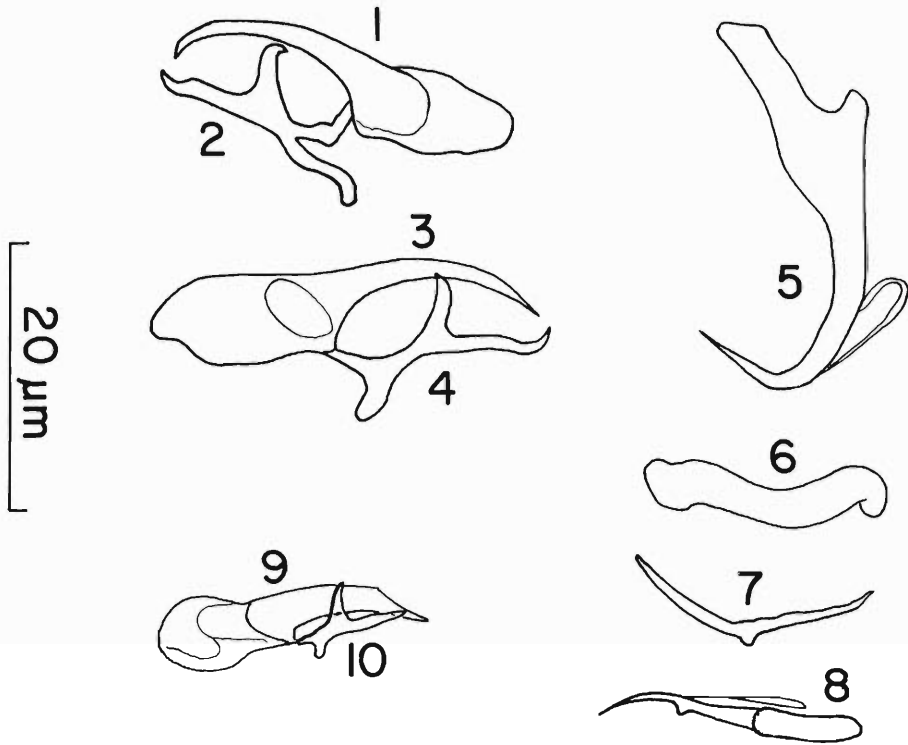
**TYPE LOCALITY:** Arkansas: Franklin Co., Mulberry River at small road to right of Redding Access, 3 km E of Cass, Arkansas River drainage.

**TYPE SPECIMENS:** Holotype, USNM 83845;

19 paratypes, USNM 83846 (2 specimens), USNM 83847 (10 specimens), USNM 83848 (2 specimens), HWML 37514 (5 specimens).

**OTHER LOCALITIES:** Arkansas: Johnson Co., Big Piney Creek at State Highway 123, ca. 0.3 km downstream on gravel road, Arkansas River drainage; Perry Co., South Fourche La Fave River at the gauging station, Arkansas River drainage.

**DESCRIPTION:** With characters of the genus as emended by Mizelle and McDougal (1970). Body with thin tegument; 249 (180–288) long, greatest width 56 (43–79). Two pairs of anterior cephalic lobes, lateral pair smaller than medial pair. Head organs not observed. Two pairs of eyes, anterior pair usually larger and farther apart than posterior pair. Pharynx circular to ovate (dorsal view), transverse diameter 18 (15–25). Gut not observed. Peduncle 16 (0–32) long, 34 (17–46) wide. Haptor 39 (32–49) long, 54 (42–70) wide. Dorsal anchor composed of solid base with short deep root, elongate superficial root, shaft curving to a sharp point; 29 (27–32) long, greatest width of base 12 (9–14). Dorsal bar 17 (14–18) long. Ventral anchor (4A) (see Kritsky and Kulo, 1992) 5 long. Vestigial ventral bar 19 (16–21) long. Fourteen hooks (7 pairs), similar in shape, with typical dactylogyrid arrangement (Mizelle and Crane, 1964). Each hook composed of solid base, slender shaft, and sickle-shaped termination provided with opposable piece. Hook lengths: No. 1, 16 (15–18); 2, 18 (15–21); 3, 23 (21–29); 4, 20 (18–25); 5, 20 (15–23); 6, 17 (14–19); 7, 18 (15–21). Copulatory complex composed of cirrus; articulated accessory piece. Cirrus bearing a gently curving slender shaft with a sharp terminus, 28 (25–29) long. Accessory piece cruciform, 15 (12–



Figures 1–10. Sclerotized parts of *Dactylogyрус* species (drawings are of the holotype unless otherwise specified). 1–8. *Dactylogyрус greenei* sp. n.: 1, 3 (USNM 83846, slide DGC 53-1), cirrus; 2, 4 (USNM 83846, slide DGC 53-1), accessory piece; 5, dorsal bar; 6, dorsal bar; 7, ventral bar; 8, hook. 9, 10. *Dactylogyрус crucis*. 9 (WAR), cirrus; 10 (WAR), accessory piece.

16) long, distal and medial processes recurved. Vagina not observed. Vitellaria distributed from pharynx region to haptor.

**REMARKS:** The closest apparent relative of *Dactylogyрус greenei* is *D. crucis*, a parasite reported from *Lythrurus ardens* (Cope), *L. bellus* (Hay), and *L. roseipinnis* (Hay) (Rogers, 1967). Both species possess a cruciform accessory piece. The major differences are as follows: the cirrus of *D. greenei* (Figs. 1, 3) is longer and more slender than that of *D. crucis* (Fig. 9); the cirrus of *D. greenei* tapers to a point distally, whereas that of *D. crucis* has a truncate opening; the accessory piece of *D. greenei* (Figs. 2, 4) is larger and has more winding processes than those of *D. crucis* (Fig. 10).

*Dactylogyрус greenei* was the only monogean found on the wedgespot shiner, occurring on 7 of 10 fish examined and averaging 3.5 individuals per fish (range 0–19). Its absence from other sympatric and syntopic cyprinid species (author's unpublished data) indicates that *D.*

*greenei* is monoxenous, parasitizing only the wedgespot shiner.

**ETYMOLOGY:** *Dactylogyрус greenei* is named after its host.

#### Acknowledgments

I thank Wilmer A. Rogers, Auburn University, for loaning paratypes of *Dactylogyрус crucis*. Gary D. Hickman, Larry L. Olmsted, and Henry W. Robison helped collect hosts.

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## 1995 Meeting Schedule

(Wednesday) 8 February 1995  
(Wednesday) 8 March 1995  
(Saturday) 6 May 1995

October 1995  
November 1995

Naval Medical Research Institute, Bethesda, MD  
Walter Reed Army Institute of Research, Washington, DC  
Joint Meeting with the New Jersey Society for Parasitology,  
at the New Bolton Center, University of Pennsylvania,  
Kennett Square, PA  
Site to be announced  
Site to be announced

## Some Digeneans (Trematoda) of the Atlantic Hawksbill Turtle, *Eretmochelys imbricata imbricata* (Testudines: Cheloniidae) from Puerto Rico

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<sup>3</sup> Caribbean Stranding Network, Department of Marine Sciences, University of Puerto Rico, P.O. Box 908, Lajas, Puerto Rico 00667-0908

**ABSTRACT:** Seven species of digeneans were collected from an Atlantic hawksbill turtle in Puerto Rico. These included 3 pronocephalids, *Pleurogonius laterouterus*, *Rameshwarotrema uterocrescens* (new host and geographic locality record), and *Diaschistorchis pandus*; 1 rhytidodid, *Rhytidodes gelatinosus*; 1 calycodid, *Calycodes caborojoensis*; 1 spirorchiid, *Amphiorchis caborojoensis*; and 1 plagiorchiid, *Enodiotrema reductum*. A list of digeneans reported from hawksbill turtles is included.

**KEY WORDS:** Digenea, *Pleurogonius laterouterus*, *Rameshwarotrema uterocrescens*, *Rhytidodes gelatinosus*, *Calycodes caborojoensis*, *Amphiorchis caborojoensis*, *Diaschistorchis pandus*, *Enodiotrema reductum*, Atlantic hawksbill turtle, *Eretmochelys imbricata imbricata*, Puerto Rico.

The hawksbill sea turtle, *Eretmochelys imbricata* (L., 1776), is found in the Atlantic, Pacific, and Indian oceans. Two subspecies are recognized: *E. i. imbricata* (L.), the Atlantic hawksbill turtle, which ranges through the warmer parts of the western Atlantic Ocean; and *E. i. bissa* (Ruppell, 1835), the Pacific hawksbill turtle, which ranges through the tropical portions of the Indian and Pacific oceans (Ernst and Barbour, 1989). The hawksbills are listed as endangered throughout the range (Anonymous, 1979) and are considered highly endangered due to the lack of protected habitats and centuries of heavy exploitation for tortoise shell (Meylan, 1989). Like other endangered species, the hawksbill reached low population levels before its ecology had been adequately investigated (Carr and Stancyk, 1975).

The Caribbean Stranding Network was established for the treatment and release of rehabilitated turtles as well as the collection of biological data vital to an understanding of wild populations.

### Materials and Methods

On 11 May 1993, the Caribbean Stranding Network received 2 Atlantic hawksbills that had been speared in the neck near La Parguera, Puerto Rico. One died during the night on 23 May 1993 and was held for necropsy (Moore and Dyer, in press). The digestive tract, lungs, circulatory system, gall bladder, and urinary bladder were examined. All helminths were recovered alive in situ, fixed in hot AFA, stained in Har-

ris's hematoxylin, dehydrated, cleared in beechwood creosote, and mounted in Canada balsam.

This work was conducted under federal permits (DRN-93-01) for handling endangered species and operating a rehabilitation facility for endangered animals that were obtained and maintained by the Caribbean Stranding Network.

All measurements are in micrometers unless indicated otherwise. For comparative purposes, specimens were borrowed from the U.S. National Museum: *Amphiorchis caborojoensis* (USNM 73312, paratypes), *Pleurogonius laterouterus* (USNM 73317, paratypes), *Calycodes caborojoensis* (USNM 73321, holotype), *Enodiotrema reductum* (USNM 73334, voucher), and *Rhytidodes gelatinosus* (USNM 73336, voucher). A list of digeneans reported from hawksbill turtles is included (Table 1).

### Results and Discussion

Seven species of digeneans including 3 pronocephalids, 1 rhytidodid, 1 calycodid, and 1 plagiorchiid were found in the digestive tract and 1 spirorchiid in a blood vessel of the lung. All other tissues examined were negative for digeneans.

#### Pronocephalidae Looss, 1902

##### *Pleurogonius laterouterus* Fischthal and Acholonu, 1976

Sixteen gravid specimens were found in the large intestine. They are similar to those reported by Fischthal and Acholonu (1976) from Puerto Rico except that they are larger and the lateral body margins are smooth rather than sinuous in the midbody region. This sinuous condition in Fischthal and Acholonu's specimens (USNM

**Table 1. Digeneans reported from hawksbill turtles.**

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**Angiodictyidae**

- Angiodictyum posterovitellatum* Chattopadhyaya, 1972  
*Microsaphidium reticulare* (van Beneden, 1859) Looss, 1901  
*Octangium microrchis* Chattopadhyaya, 1972  
*Octangium sagitta* (Looss, 1899) Looss, 1902  
*Octangium travassosi* (Ruiz, 1943) Yamaguti, 1958

**Calycodidae**

- Calycodes caborojoensis* Fischthal and Acholonu, 1976

**Gorgoderidae**

- Pleisochorus cymbiformis* (Rudolphi, 1819) Looss, 1901

**Pachypsolidae**

- Pachypsolus puertoricensis* Fischthal and Acholonu, 1976  
*Pachypsolus ovalis* Linton, 1910

**Paramphistomidae**

- Schizamphistomum scleroporom* (Creplin, 1844) Looss, 1912

**Plagiorchiidae**

- Enodiotrema megachondrus* Looss, 1901  
*Enodiotrema microvitellatus* Chattopadhyaya, 1970  
*Enodiotrema reductum* Looss, 1901  
*Styphlotrema solitarium* (Looss, 1899) Odhner, 1910

**Pronocephalidae**

- Adenogaster serialis* Looss, 1901  
*Cricocephalus albus* (Kühl and van Hasselt, 1822) Looss, 1899  
*Cricocephalus americanus* Pérez Viguera, 1955  
*Cricocephalus megastomus* Looss, 1902  
*Desmogonius desmogonius* Stephens, 1912  
*Diaschistorchis lateralis* Ogura, 1936  
*Diaschistorchis pandus* (Braun, 1901) Johnston, 1913  
*Epibathra stenobursata* Fischthal and Acholonu, 1976  
*Glyphicephalus latus* Fischthal and Acholonu, 1976  
*Glyphicephalus lobatus* Looss, 1901  
*Glyphicephalus solidus* Looss, 1901  
*Metacetabulum invaginatum* Freitas and Lent, 1938  
*Pleurogonius laterouterus* Fischthal and Acholonu, 1976  
*Pleurogonius linearis* Looss, 1901  
*Pleurogonius longibursatus* Pérez Viguera, 1955  
*Pleurogonius macrophallus* (Oguro, 1936) Ruiz, 1946  
*Pleurogonius mandapamensis* Chattopadhyaya, 1972  
*Pleurogonius ozakii* Oguro, 1936  
*Pleurogonius puertoricensis* Fischthal and Acholonu, 1976  
*Pleurogonius solidus* (Looss, 1901) Ruiz, 1916  
*Pleurogonius trigonocephalus* (Rudolphi, 1809) Looss, 1901  
*Pleurogonius truncatus* Prudhoe, 1944  
*Pyelosomum parvum* Prudhoe, 1944  
*Pyelosomum posterorchis* Oguro, 1936  
*Pyelosomum solum* Chattopadhyaya, 1972  
*Rameshwarotrema uterocrescens* Rao, 1975

**Rhytidodidae**

- Rhytidodes gelatinosus* (Rudolphi, 1819) Looss, 1901  
*Rhytidodes indicus* Simha and Chattopadhyaya, 1969
-



Table 1. Continued.

## Spirorchidae

- Amphiorchis amphiorchis* Price, 1934  
*Amphiorchis caborojoensis* Fischthal and Acholonu, 1976  
*Amphiorchis indicus* Mehrotra, 1973  
*Amphiorchis lateralis* Oguro, 1938  
*Hapalotrema orientale* Takeuti, 1942  
*Hapalotrema synorchis* Luhman, 1935  
*Learedius orientale* Mehra, 1939

## Telorchidae

- Orchidasma amphiorchis* (Braun, 1899) Braun, 1901

Helm. Coll. No. 73317) may be the result either of postmortem changes or of the technique used in preparing the specimens for study. As pointed out by Ulmer (1952), morphological changes in digeneans of known age can be induced by varying the method of preparation.

**DIMENSIONS** ( $N = 16$ ): Body 3.00–4.28 (3.64) by 0.62–0.82 mm (0.75), oral sucker 88–129 (110) by 90–121 (106), esophagus 200–470 (303), postcecal space 120–180 (130), right testis 270–430 (382) by 210–330 (255), left testis 270–370 (328) by 190–300 (242), posttesticular space 110–270 (202), cirrus sac 400–720 (537) by 40–90 (51), prostatic vesicle 200–380 (274) by 50–70 (65), male genital pore 350–750 (562) from cecal bifurcation, ovary 140–220 (174) by 140–200 (160), distance from right testis 70–180 (120), anteriormost extent of vitelline follicles from anterior extremity 2,000–2,970 (2,448), Mehlis's gland 80–170 (129) by 60–130 (102), eggs 32–37 (33) excluding filaments by 15–19 (16). Six voucher specimens deposited: USNM Helm. Coll. No. 83353.

This is the second report of *P. laterouterus* in *E. i. imbricata* from Puerto Rico.

In addition to the records of *Pleurogonius* found in Yamaguti (1971), *Pleurogonius mandapamensis* Chattopadhyaya, 1972, was described from *Eretmochelys squamosa* in Mandapam, Gulf of Mannar, India; and Fischthal and Acholonu (1976) described *P. puertoricensis* and *P. laterouterus* from *E. i. imbricata* at Cabo Rojo, Puerto Rico.

***Rameshwarotrema uterocrescens* Rao, 1975**

Nine specimens of a monostome detected in the mucosa of the intestine concurred with the description of *Rameshwarotrema uterocrescens* as given by Rao (1975) on the basis of 15 mature

specimens recovered from the deeper layers of the intestine of 6 *Chelonia mydas* taken at the Gulf of Mannar, India. Measurements reported by Rao for the length (0.23–0.41 mm) and width (0.10–0.17) are in error. According to the scale given for the illustration, this specimen measures 2.03 by 0.50 mm. This is larger than our specimens.

**DIMENSIONS** ( $N = 5$ ): Body 0.67–0.94 (0.76) by 0.30–0.41 mm (0.37), oral sucker 88–92 (87) by 71–110 (86), right testis 110–220 (150) by 110–176 (129), left testis 110–189 (138) by 101–147 (124), ovary 40–70 (58) by 60–90 (72), eggs 26–30 (28) by 13–15 (14). Five voucher specimens deposited: USNM Helm. Coll. No. 83356.

This is the second report of *R. uterocrescens* in a sea turtle and represents a new host and locality record.

***Diaschistorchis pandus* (Braun, 1901)  
Johnston, 1913**

Fifty specimens were found in the stomach and 20 in the small intestine.

**DIMENSIONS** ( $N = 10$ ): Body 6.73–9.25 (8.25) by 2.25–2.62 mm (2.56), oral sucker 470–690 (597) by 520–700 (625), esophagus 280–360 (320), testis divided into several follicles arranged in 2 lateral rows, in a U-shaped manner 200–450 (286) by 240–350 (286), cirrus sac 700–950 (809) by 190–250 (216), ovary 340–400 (362) by 320–350 (345), eggs 29–39 (31) by 15–22 (18). Six voucher specimens deposited: USNM Helm. Coll. No. 83357.

In addition to the records of *Diaschistorchis* found in Yamaguti (1971), *Diaschistorchis pandus* has been reported in *Eretmochelys i. imbricata* from India by Simha et al. (1971) and Mehrotra and Gupta (1976), from Cuba by Pérez Viguera (1955), and from Cabo Rojo, Puerto

Rico, by Fischthal and Acholonu (1976). This is the second report of this monostome in *E. i. imbricata* from Puerto Rico.

#### Plagiorchiidae Ward, 1917

##### *Enodiotrema reductum* Looss, 1901

Eleven specimens were found in the small intestine. Our specimens agree with the description as given by Looss (1901) except that our specimens are slightly smaller.

**DIMENSIONS** ( $N = 6$ ): Body 1.21–1.42 (1.31) by 0.33–0.38 mm (0.34), oral sucker 128–171 (144) by 143–169 (149), acetabulum 157–160 (159) by 158–162 (160), left testis 147–187 (161) by 94–160 (151), right testis (121–191 (157) by 138–170 (153), cirrus sac 147–190 (159) by 59–84 (72), ovary 88–114 (99) by 59–88 (72), egg 31–35 (34) by 15–18 (17). Eight voucher specimens deposited: USNM Helm. Coll. No. 83354.

In addition to the records of *Enodiotrema* found in Yamaguti (1971), Groschaft et al. (1977) reported 27 specimens of *E. megachondrus* from *Eretmochelys imbricata* in Cuba. Fischthal and Acholonu (1976) found *E. reductum* in the small intestine of 3 *Eretmochelys i. imbricata* from Cabo Rojo, Puerto Rico. The present finding represents the second report of *E. reductum* in *E. i. imbricata* from Puerto Rico.

#### Rhytidodidae Odhner, 1926

##### *Rhytidodes gelatinosus* (Rudolphi, 1819) Looss, 1901

Fifty gravid specimens were recovered from the stomach and 60 from the small intestine. Our specimens are comparable to those described by Looss (1901) and Pérez Viguera (1955) from *Thalassochelys corticata* and *Eretmochelys imbricata*, respectively. While our specimens are larger than those described from *Caretta caretta* by Pratt (1914), they are smaller than those described from *Caretta caretta* and *Chelonia mydas* by Euzet and Combes (1962) and Rodriguez (1960), respectively.

**DIMENSIONS** ( $N = 20$ ): Body 9.60–16.30 (11.78) by 1.18–2.81 mm (1.99), oral sucker 250–420 (342) by 300–470 (374), acetabulum 300–400 (332) by 270–370 (331), pharynx 150–250 (201) by 160–250 (200), esophagus 180–1,690 (1,014) by 20–50 (34), cirrus sac 400–500 (498) by 270–400 (328), anterior testis 500–600 (542) by 250–450 (321), posterior testis 490–730 (621) by 280–440 (348), ovary 130–370 (298) by 120–400 (394), eggs 55–70 (59) by 27–44 (34). Sev-

enteen voucher specimens deposited: USNM Helm. Coll. No. 83355.

In addition to the records of *Rhytidodes* found in Yamaguti (1971), it has also been reported in *Chelonia mydas* from the Karachi coast, Pakistan (Bilqees, 1974), and in *E. i. imbricata* from Cabo Rojo, Puerto Rico (Fischthal and Acholonu, 1976).

This represents the second report of this species in *E. i. imbricata* from Puerto Rico.

#### Calycodidae Dollfus, 1929

##### *Calycodes caborojoensis* Fischthal and Acholonu, 1976

One gravid specimen was found in the small intestine. This species was described by Fischthal and Acholonu (1976) on the basis of a single specimen found in the small intestine of *Eretmochelys i. imbricata* from Cabo Rojo, Puerto Rico. Our specimen agrees with their description except that our specimen is smaller.

**DIMENSIONS** ( $N = 1$ ): Body 8.71 by 0.81 mm, oral sucker 320 by 400, acetabulum 400 by 290, anterior testis 200 by 180, posterior testis 300 by 210, cirrus sac 610 by 201, ovary 200 by 150, eggs 54–68 (64) by 42–47 (44). Our specimen was inadvertently destroyed but not before it was identified and measured.

This species has not been reported since its original description. The present report therefore constitutes the second report of this species in *E. i. imbricata* from Puerto Rico.

#### Spirorchiidae Stunkard, 1921

##### *Amphiorchis caborojoensis* Fischthal and Acholonu, 1976

This species was described by Fischthal and Acholonu (1976) based on 11 specimens found in the blood vessels of the lungs of 1 *Eretmochelys i. imbricata*. The single specimen found in the present study concurs with the original description.

**DIMENSIONS** ( $N = 1$ ): Body 3.42 by 0.74 mm, oral sucker 220 by 170, acetabulum 230 by 160, anterior testis 550 by 400, posterior testis 510 by 350, cirrus sac 250 by 70, ovary 165 by 290, eggs 200–235 including filaments by 38–45. Our single specimen was inadvertently discarded but not before it was identified and measured.

This species has not been reported subsequent to the original description. This constitutes the second report of *A. caborojoensis* in *E. i. imbricata* from Puerto Rico.

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***Niviventerrema yunnanensis* gen. n., sp. n.  
(Lecithodendriidae: Pleurogenetinae), from  
*Niviventer cremoriventer* (Muridae) from Yunnan  
Province of the Peoples Republic of China**

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**ABSTRACT:** During a study of helminths of mammals from Yunnan Province, southern China, 2 of 6 specimens of *Niviventer cremoriventer* (Muridae) were infected with a total of 42 specimens of an undescribed species of Lecithodendriidae (Pleurogenetinae) representing a new genus. *Niviventerrema yunnanensis* gen. n., sp. n., is most similar to *Indopleurogenes orientalis* (Pleurogenetinae) from *Rana cyanophlyctis* but differs in having an esophagus; ceca that are half long, ending immediately posterior to the testes; and a genital pore opening at level of pharynx; and in lacking a metraterm. *Niviventerrema yunnanensis* is described as the only known species in the new genus.

**KEY WORDS:** *Niviventerrema yunnanensis*, Lecithodendriidae, Pleurogenetinae, China, *Niviventer cremoriventer*, Muridae.

Of Lecithodendriidae (Lühe, 1901) Odhner, 1910, the subfamilies Allassogonoporinae Skarbilovich, 1943, Cephalophallinae Yamaguti, 1958, Gyrobascinae Macy, 1935, Posterocirrinae Yamaguti, 1971, Lecithodendriinae Looss, 1902, Leyogoniminae Dollfus, 1951, Odeningotrematinae Rohde, 1962, Pleurogenetinae Looss, 1899 (syn. Pleurogeninae Travassos, 1921), Phaneropsolinae Mehra, 1935, Prosthodendriinae Yamaguti, 1958, and Matoviinae Mas-Coma, Roset, and Mantoliu, 1985, contain species previously reported from nonchiropteran mammals. *Cephalotrema minutum* Baer, 1943, from *Neomys* sp. and *Sorex* sp. was originally assigned to the Pleurogenetinae but was placed in the Prosthogonimidae Nicoll, 1924, by Yamaguti (1971). Combes and Jourdane (1969) supported the assignment of *C. minutum* by Baer (1943) and recommended the placement of both *Cephalotrema* and *Pseudocephalotrema* Combes and Jourdane, 1969, in the Pleurogenetinae of Lecithodendriidae. Additional studies by Baysade-Dufour and Jourdane (1976) and Jourdane (1977) support this placement of *C. minutum*.

During a study of the helminths of mammals from the Yunnan Province of the Peoples Republic of China, an undescribed species of Lecithodendriidae representing a new genus of the subfamily Pleurogenetinae was found.

#### Materials and Methods

Six specimens of pencil-tailed rat, *Niviventer cremoriventer* Miller, 1900, were collected in August 1987 from Menglun, Yunnan Province, Peoples Republic of China, and examined for helminths. Trematodes were fixed in hot AFA under slight coverslip pressure, stained in Semichon's carmine, and mounted in Canada balsam or Kleermount. Specimens were sectioned by conventional paraffin technique. Measurements are in micrometers with the mean followed by the range in parentheses, unless otherwise stated. The following specimens from the USNM Helminthological Collection were examined: *Allassogonoporus marginalis* (No. 9194), *A. vesperilionis* (No. 36672), *Myotitrema asymmetricum* (No. 36673), *Pleurogenes bicolor* (No. 32876), *P. clavigerum* (No. 50468), *P. cystolobatus* (No. 59628), and *P. loossi* (No. 29746).

#### Results

Two of 6 specimens (33%) of *N. cremoriventer* (Muridae) were infected with 42 specimens of *Niviventerrema yunnanensis* gen. n., sp. n. (Lecithodendriidae: Pleurogenetinae).

#### *Niviventerrema* gen. n.

**DIAGNOSIS:** Lecithodendriidae; Pleurogenetinae. Body spinose. Oral sucker subterminal. Acetabulum preequatorial, approximately same size as oral sucker. Prepharynx short, pharynx smaller than oral sucker, esophagus moderately long, ceca half long. Testes opposite, equatorial. Cirrus

sac preacetabular, enclosing cirrus, prostate complex, and seminal vesicle. Genital pore marginal, sinistral, opening in upper half of forebody. Ovary dextral, immediately pretesticular. Seminal receptacle spherical, intertesticular. Laurer's canal present. Vitelline follicles in two symmetrical groups largely in forebody. Uterus primarily posttesticular. Eggs operculate. Excretory vesicle Y-shaped.

TYPE SPECIES: *Niviventertrema yunnanensis* sp. n.

*Niviventertrema yunnanensis* sp. n.

(Figs. 1–3)

DESCRIPTION (based on 20 adult specimens): With characteristics of genus. Body elongate, 1,350 (1,228–1,485) long by 510 (420–610) wide. Forebody 366 (320–436) long, densely spined. Oral sucker spherical, 118 (105–135) long by 117 (105–130) wide. Acetabulum 122 (112–133) long by 120 (105–135) wide. Ratio of transverse diameter of oral sucker to acetabulum, 1:1.03. Pharynx 13 (5–23) long; pharynx 58 (50–69) long by 56 (51–64) wide; esophagus 53 (26–71) long, bifurcating midway between oral sucker and acetabulum; ceca terminating midway between acetabulum and posterior extremity of body. Testes large, smooth, opposite, near midbody. Right testis 235 (180–386) long by 218 (190–265) wide; left testis 230 (170–286) long by 210 (180–245) wide. Seminal vesicle coiled, enclosed in cirrus sac, 302 (235–395) long by 70 (49–101) wide, largely preacetabular, occasionally overlapping anterior margin of acetabulum. Genital pore marginal, sinistral, opening at level of pharynx. Ovary dextral, with 3–5 lobes, 165 (102–230) long by 175 (120–235) wide, immediately pretesticular. Seminal receptacle small, 43 (30–56) long by 49 (40–63) wide, located in intertesticular region, near level of anterior margins of testes. Ootype immediately left of seminal receptacle. Vitellaria follicular, in 2 symmetrical masses, located in lateral fields between level of pharynx and anterior margins of testes, becoming almost a continuous band across forebody in larger specimens. Uterus filling most of posttesticular region of hindbody. Eggs 24 (22–26) long by 10 (7–13) wide. Excretory pore terminal, excretory vesicle long-stemmed, branches extending halfway to testes from posterior extremity.

**Taxonomic summary**

SPECIMENS DEPOSITED: Holotype USNM Helm. Coll. No. 83562; paratypes USNM Helm.

Coll. No. 83563 (5 specimens), Texas Cooperative Wildlife Coll. Ch 87–31 (5 specimens), Texas A&M University, and the University of Nebraska State Museum, HWML 37261 (5 specimens).

TYPE HOST: *Niviventer cremoriventer*.

SITE OF INFECTION: Small intestine.

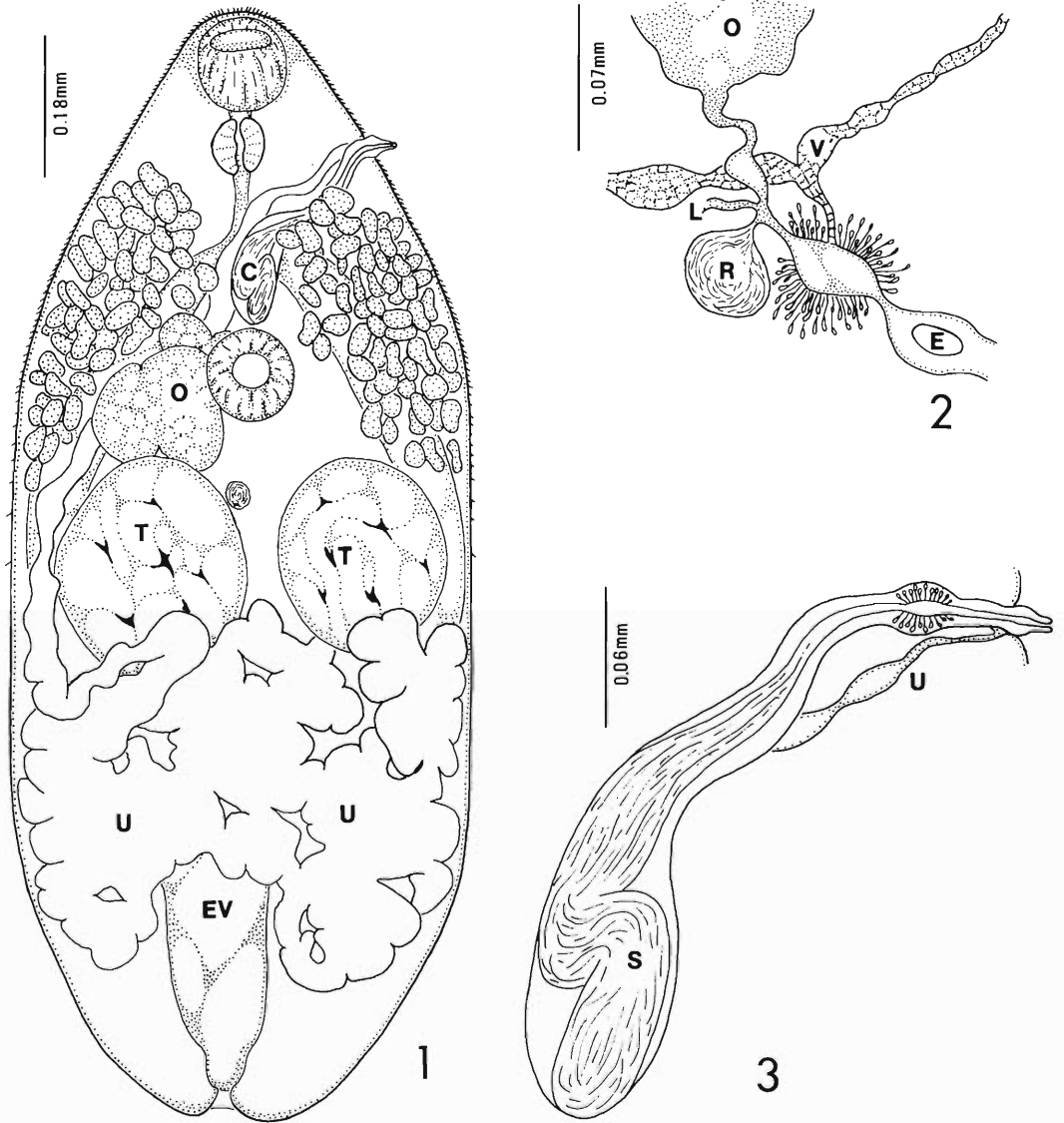
TYPE LOCALITY: Menglun, Yunnan Province, Peoples Republic of China, 21°55'N, 101°17'E.

ETYMOLOGY: The genus designation is taken from the genus of the host, *Niviventer*. The specific designation refers to the province of China where the new species was collected.

**Discussion**

Species of *Maxbraunium* Caballero and Zerecero, 1942 (Maxbrauniinae), and *Cephalotrema* Baer, 1943 (Pleurogenetinae), are the only lecitodendriids previously reported from mammals that are similar to *N. yunnanensis* in having vitelline follicles distributed in forebody from level of cirrus pouch to level of anterior margins of testes, ceca extending posteriorly beyond postacetabular testes, and a preacetabular cirrus pouch. The new species is most similar to species of *Pleurogenes* Looss, 1896, and *Indopleurogenes* Yamaguti, 1971, in having a similar distribution of vitelline follicles in forebody; symmetrical, postacetabular testes; a preacetabular cirrus pouch enclosing the seminal vesicle, prostate complex and cirrus; the genital pore opening marginally in forebody; a uterus that is mostly confined to hindbody; ceca that extend into hindbody; and a blind seminal receptacle. Srivastava (1934) described *Pleurogenes orientalis* from *Rana cyanophlyctis* in India. Yamaguti (1971) placed this species in the genus *Indopleurogenes* based on the absence of an esophagus, the tendency of vitellaria to overlap ceca, the postacetabular placement of the testes, and placement of the genital pore at level of oral sucker. *Niviventertrema yunnanensis* is more like *Indopleurogenes orientalis* (Srivastava, 1934), the only species in the genus, than species of *Pleurogenes* in having half-long ceca.

*Niviventertrema yunnanensis* differs from species of *Maxbraunium* and *Cephalotrema* in having an esophagus, a marginal rather than submedian genital pore, and a Y-shaped rather than V-shaped excretory vesicle. The new species differs from both *I. orientalis* and remaining species of *Pleurogenes* in having ceca that terminate immediately posterior to testes, instead of immediately anterior to testes (*I. orientalis*), or near



Figures 1-3. *Niviventertrrema yunnanensis* gen. n., sp. n., from *Niviventer cremoriventer*. 1. Camera lucida drawing of adult, ventral view showing cirrus apparatus (C), uterus (U), ovary (O), testis (T), and excretory vesicle (EV). 2. Composite drawing of ootype region showing ovary (O), seminal receptacle (R), Laurer's canal (L), vitelline reservoir (V), and an egg in the uterus (E). 3. Composite drawing of genital atrium region showing seminal vesicle (S), and uterus (U).

posterior extremity (*Pleurogenes* spp.), and a genital pore that opens at level of pharynx, instead of oral sucker (*I. orientalis*), or posterior to the cecal bifurcation (*Pleurogenes* spp.); and the new species lacks a metraterm. *Niviventertrrema yunnanensis* is established as the only known species in the new genus.

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## Additional Information on the Morphology of *Potamotrygonocestus magdalenensis* (Tetraphyllidea: Onchobothriidae) from the Freshwater Stingray *Potamotrygon magdalenae* in Colombia

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**ABSTRACT:** New specimens of *Potamotrygonocestus magdalenensis* were collected from *Potamotrygon magdalenae* in the Ciénega Grande near Repelón, Colombia. The description of *P. magdalenensis* is emended to include information obtained from these specimens. Scanning electron microscopy revealed blade-like microtriches on the proximal and distal bothridial surfaces as well as on the neck and strobila. The blade-like microtriches of the neck and strobila are much more sparsely arranged than those on the scolex. The former microtriches are approximately twice as large as those on the proximal bothridial surfaces. The microtriches of the proximal bothridial surfaces are approximately 2–3 times as large as those on the distal bothridial surfaces. Filiform microtriches were visible among the blade-like microtriches of the neck and strobila. Spiniform microtriches cover the distal regions of the cirrus but are less numerous on its proximal regions. The inflated base of the cirrus is entirely devoid of microtriches. Cross-sections suggest that, in its nonfused regions, the ovary is essentially bilobed. Comparison of these new specimens with type material confirmed that several details in the original description by Brooks et al. (1976) require correction. The neck microtriches are much larger than those figured by Brooks et al. (1976). The vas deferens extends from the middle of the segment to the posterior end rather than from the posterior third, and the cirrus sac overlaps the posterior region of the ovary slightly in most segments, rather than being separated from it by more than 1 length. This species shares a number of similarities with *Calliobothrium pritchardae* and *Pedibothrium* species. Stingrays of a wide range of sizes were found infected with *P. magdalenensis*. Nymphs of an unidentified burrowing mayfly species were the only food items found in the stomachs of the small stingrays, suggesting that these insects are possible intermediate hosts for this cestode species.

**KEY WORDS:** *Potamotrygonocestus magdalenensis*, Cestoda, morphology, SEM.

As part of a broader study of the phylogenetic relationships of the Onchobothriidae, fresh material of *Potamotrygonocestus magdalenensis* Brooks and Thorson, 1976, was collected to facilitate description of this species with scanning electron microscopy (SEM). This paper details the results of the SEM. These collections also allowed us to provide new data on several aspects of the internal anatomy of this cestode species as well as new data on the prevalence and intensity of this species in the freshwater stingray *Potamotrygon magdalenae* (Valenciennes, 1865).

### Materials and Methods

A scientific collecting permit was obtained from INDERENA in Bogotá, through Eduardo Del Real Martínez, Subgerente de Pesca y Fauna Terrestre. All stingrays were collected near the Estación Piscícola Repelón field station on the Ciénega Grande near Repelón, Colombia, in May 1989. Spiral intestines of a total of 34 individuals of *Potamotrygon magdalenae* were examined. The host individuals included 15 small stingrays (8–11 cm in disk width) and 19 large stingrays (19.6–29.8 cm in disk width). Six female and 8 male small individuals and 2 female and 17 male large individuals were necropsied. Small stingrays were collected using throw nets in the creek directly adjacent

to the field station. The presence of the remnant of the embryonic yolk sac in the smaller stingrays indicated that these individuals were very young. Large stingrays were purchased from fishermen using gill nets in the Ciénega Grande. The presence of embryos in most of the large females examined indicated that these hosts were sexually mature.

Ten specimens of *Potamotrygonocestus magdalenensis* were prepared as whole mounts for identification purposes. These specimens were stained in Harris' hematoxylin, dehydrated in ethanol, cleared in xylene, and mounted in Canada balsam. Twenty free segments were embedded in paraplast (Sherwood Medical Industries, St. Louis, Missouri), and serial cross-sections were cut at 10- $\mu$ m intervals with an American Optics rotary microtome. Sections were floated on water on slides coated with egg albumin, air-dried, stained in Gill's hematoxylin, counterstained with eosin, dehydrated in an ethanol series, cleared in xylene, and mounted in Canada balsam. Ten scoleces and 10 free segments were prepared for SEM. These specimens were hydrated in an ethanol series, treated with 1% osmium tetroxide overnight, dehydrated in an ethanol series, and air-dried using Peldri II (Ted Pella, Inc., Redding, California) according to the procedure given by Freidenfelds et al. (1994). Dried specimens were mounted directly on stubs using silver paint, sputter-coated with gold for 1 min (approximately 100 Å), and examined with a Coates and Welter field emission scanning electron microscope.



Illustrations were drawn with the aid of a drawing tube. All measurements are in micrometers unless otherwise stated.

### Results

Two of the 15 small stingrays and 17 of the 19 large stingrays examined were infected with *P. magdalenensis*. Each of the small stingrays was infected with 2 individuals. The mean intensity of infection for the large stingrays was  $5.4 \pm 8.2$  with a range of 1–35 individuals per host. All specimens were found in the anterior portion of the spiral intestine; the exact chamber of origin was not determined.

#### *Potamotrygonocestus magdalenensis*

Brooks and Thorson, 1976

(Figs. 1–10)

The following information emends the description of Brooks and Thorson (1976) for this species. Proximal surfaces of bothridia covered with closely spaced, long, blade-like microtriches approximately 2–3  $\mu\text{m}$  in length, which taper to a point (Fig. 2). Distal surfaces of bothridia densely covered with small, spiniform microtriches, approximately 1  $\mu\text{m}$  in length (Fig. 3). Neck covered with widely spaced, large, blade-like microtriches (Fig. 4), approximately 8  $\mu\text{m}$  in length, characterized by longitudinal striations, interspersed with numerous, small, filiform microtriches (Fig. 4). Cephalic peduncle lacking.

Distal portion of cirrus covered with narrow, spiniform microtriches, approximately 2–3  $\mu\text{m}$  in length (Figs. 6, 7); microtriches more sparsely distributed proximally. Base of cirrus characterized by inflated bulb, lacking microtriches (Fig. 6). Vas deferens extending from middle of segment (Fig. 8) to terminal genital pore. Ovary bilobed in cross-section (Fig. 9); lobes fused at ovarian bridge and at posterior extremity. Eggs single, lacking filaments. Vitellaria in single lateral bands. Segments covered with numerous, short, densely arranged microtriches interspersed with large blade-like microtriches, approximately 8  $\mu\text{m}$  in length (Fig. 5).

TYPE AND ONLY KNOWN HOST: *Potamotrygon magdalenae* (Valenciennes, 1865).

SITE OF INFECTION: Spiral intestine.

TYPE LOCALITY: Ciénega Rabón, vicinity of San Cristóbal, Bolívar, Colombia.

ADDITIONAL LOCALITY: Ciénega Grande, Estación Piscícola Repelón (new record).

MATERIAL EXAMINED: Paratypes: USNM

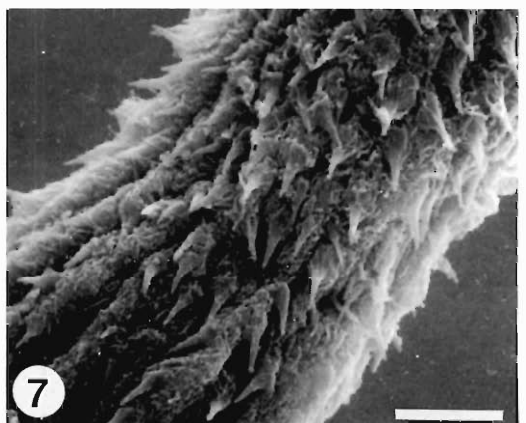
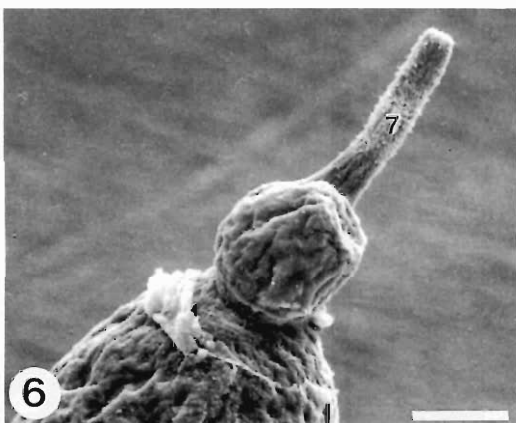
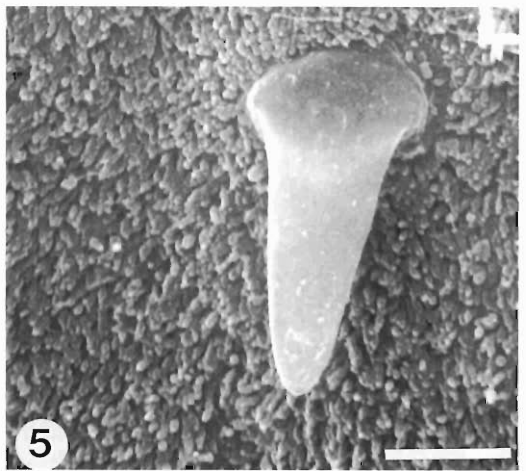
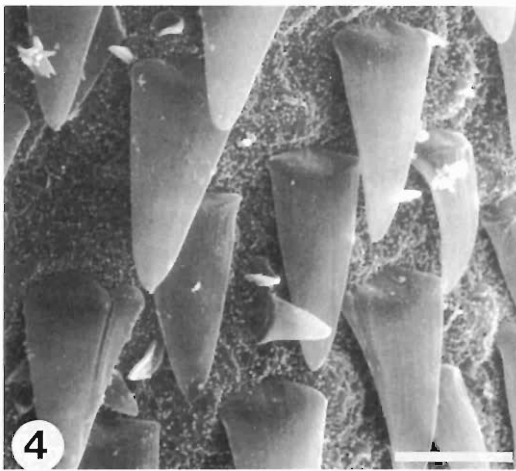
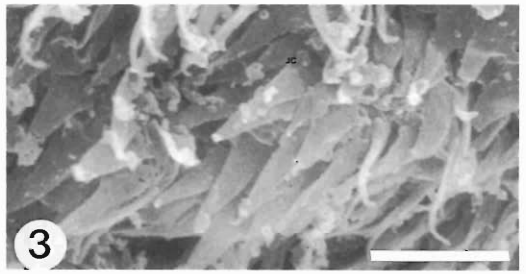
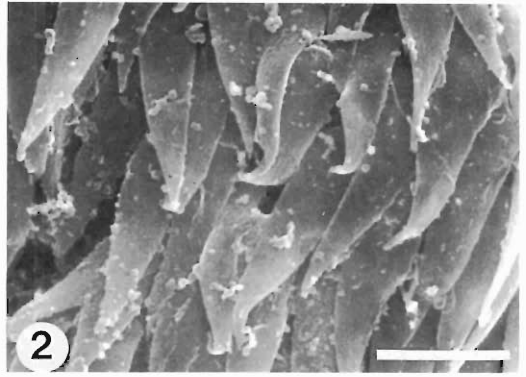
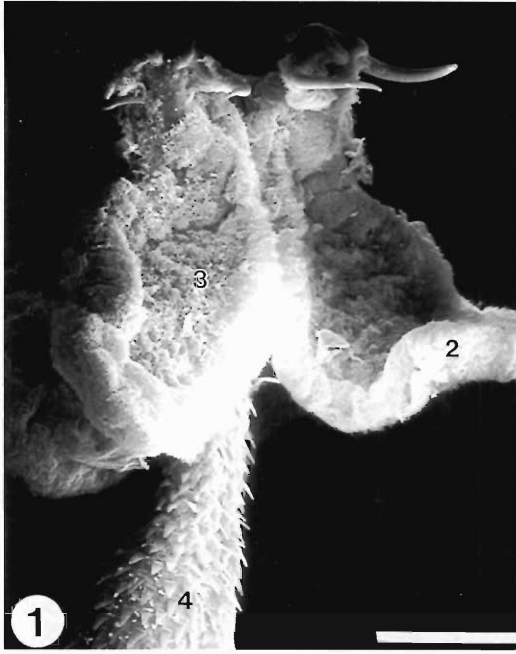
Helm. Coll. No. 73543; Univ. Neb. State Mus. H. W. Manter Lab. No. 20254. One whole mount from new locality and material illustrated in Figures 8–10 deposited at HWML (No. 37546); additional specimens retained in senior author's personal collection.

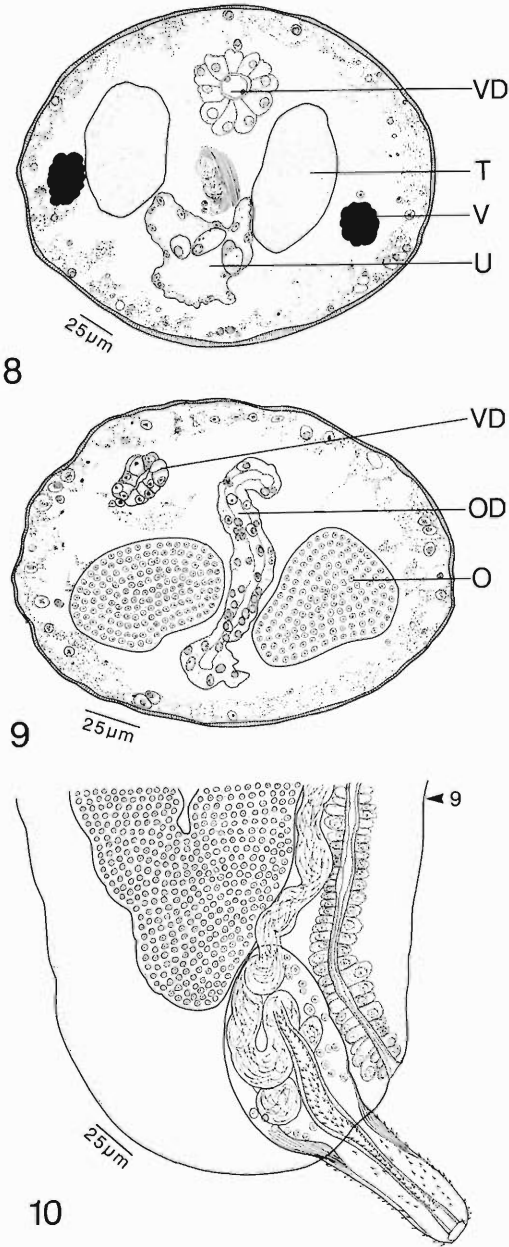
### Discussion

SEM reveals a number of similarities between the patterns of microtriches on the scolex of *Potamotrygonocestus magdalenensis* and species of *Pedibothrium* Linton, 1909, and *Calliobothrium pritchardae* Caira and Ruhnke, 1990. In all of these species, the proximal and distal bothridial surfaces exhibit blade-like microtriches, which differ somewhat in shape between the 2 surfaces. In *P. magdalenensis*, the microtriches also differ in size, with those on the proximal surfaces being much larger than those on the distal bothridial surfaces. Unlike *Pedibothrium* species, *P. magdalenensis* has no distinct scolex peduncle; microtriches are found on all external surfaces of the scolex and strobila. Our cross-sections of the ovary of *P. magdalenensis* indicate that the ovary is essentially bilobed. Its overall morphology is strikingly similar to that of *C. pritchardae*: both are inverted A-shaped, and both are basically bilobed in cross-section. This is very different from the condition of the ovary found in *Pedibothrium* species.

Our work indicates that several features of this species were incorrectly described by Brooks and Thorson (1976). The microtriches of the neck are much larger than those shown in the scolex illustration of this species presented by these authors (compare our Fig. 1 to their Fig. 1). The cirrus sac, at least in free segments, is not separated from the ovary as figured by Brooks and Thorson (1976) but overlaps slightly with the posterior region of the ovary, similar to the condition figured for *Potamotrygonocestus amazonensis* Mayes, Brooks, and Thorson, 1981, by Mayes et al. (1981) (see their Fig. 10). In all of the free segments of *P. magdalenensis* we examined, the vas deferens extended from the middle of the segment, rather than the posterior third as figured by Brooks and Thorson (1976). This explains why our section through the testes in the middle of the segment contained a section through the vas deferens (Fig. 8).

Our data indicate that this parasite is able to infect very young stingrays. We found adult specimens of *Potamotrygonocestus magdalenensis* in





2 stingrays with disk widths of only 9 and 9.3 cm, respectively; both stingrays still retained fairly large remnants of their embryonic yolk sacs. This size is very similar to the size of neonatal individuals of this species; one of the pups we removed from a pregnant female had a disk width of 7.5 cm. Preliminary examination of the stomach contents of the small stingrays suggests that these animals feed exclusively on nymphs of an unidentified burrowing mayfly species. This invertebrate would be a good place to begin the search for intermediate hosts of *P. magdalenensis*.

**Acknowledgments**

We are very grateful to Oscar Valencia, Director of the Estación Piscícola Repelón, for allowing us to use the facilities at the station and for making local arrangements for collection of stingrays. The trip to Colombia would not have been possible without the capable assistance of Marta Martinez-Wells, who translated all of our correspondence with Colombian officials prior to the trip and acted as translator and assisted with dissections in the field. We are indebted to Eduardo Velasco, who traveled to Bogata to facilitate the issuing of our permits. We also thank Kelly Steele for assisting with ray dissections despite her botanical inclinations and 2 anonymous reviewers for their helpful comments on an ear-

Figures 8–10. Line drawings of *Potamotrygonocestus magdalenensis*. 8. Cross-section through testes in middle of segment (voucher specimen, HWML No. 37546); note 3 eggs in uterus. 9. Cross-section through ovary posterior to ovarian bridge and anterior to fused posterior lobes of ovary (voucher specimen, HWML No. 37546). 10. Detail of terminal genitalia from whole mount of free segment (HWML No. 37546). Arrow indicates location of section through ovary shown in Figure 9. Abbreviations: O = ovary, OD = oviduct, T = testis, U = uterus, V = vitellaria, VD = vas deferens.

Figures 1–7. Scanning electron microscopy of *Potamotrygonocestus magdalenensis*. 1. Scolex. Locations at which Figures 2–4 were taken are indicated with corresponding numbers. 2. Microtriches on proximal bothridial surface. 3. Microtriches on distal bothridial surface. 4. Microtriches on neck. 5. Microtriches on mature segment. 6. Everted cirrus. Number indicates location at which Figure 7 was taken. 7. Enlarged view of cirrus microtriches. Scale bar in Figure 1 = 40 µm; scale bar in Figures 2, 3, and 5, = 2 µm; scale bar in Figure 4 = 4 µm; scale bar in Figure 6 = 50 µm; scale bar in Figure 7 = 5 µm.

lier version of this manuscript. This work was supported in part by grant IBN-9007613 from the National Science Foundation to J.N.C.

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### Early History of USDA Livestock Parasitology Available

*Animal Parasite Research in the Zoological Division, Bureau of Animal Industry, U.S. Department of Agriculture, Washington, D.C., 1923-1938*, by John S. Andrews, is a history of classical research on livestock parasites and of the development of parasitology research facilities in Beltsville, Maryland. Copies of this book are available without cost, except for \$4.00 for packaging and postage, from Dr. John S. Andrews, Jr., 4210 Quail Ridge Way, Norcross, GA 30092.

## Nematode Fauna of the Two Sympatric Rats *Rattus rattus* and *R. exulans*, in Kao District, Halmahera Island, Indonesia

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**ABSTRACT:** A comparison was made of the parasitic nematode fauna between 2 sympatric rats, *Rattus rattus* and *Rattus exulans*, in Kao District, Halmahera Island, Indonesia. A total of 29 *R. rattus* and 20 *R. exulans* were examined, and 11 nematode species were found in them. *Strongyloides venezuelensis* was recorded from *R. exulans* for the first time. A remarkable difference was observed in the prevalence of *Nippostrongylus brasiliensis*, which was quite common in *R. rattus*, whereas no adult worms were collected from *R. exulans*. It is suggested that *R. exulans* is less susceptible to *N. brasiliensis*. Some common rat parasites of the Pacific islands were not observed. This may reflect the small sample size or a depauperate fauna.

**KEY WORDS:** Nematode fauna, *Rattus* spp., Halmahera Island, Indonesia, zoogeography, ecology.

Halmahera Island belongs to the Molucca Islands of Indonesia and is located between Sulawesi and New Guinea (Fig. 1). Nothing has been reported on the parasitic helminth fauna of rats from this island. In July 1993, we had an opportunity to collect rats for parasitological examination in the Kao District, North Halmahera. This paper deals with a comparison of the nematode fauna between 2 sympatric rat species, *Rattus rattus* of the Asian variety (= *Rattus tanezumi* sensu Musser and Carleton, 1993) and *Rattus exulans*, the dispersal of which to the Pacific islands is believed to have occurred in association with human movements (see Musser and Carleton, 1993; Roberts, 1991).

### Materials and Methods

Four localities, namely, Popon, Kao, Pidiwan, and Kai, were chosen for study (Fig. 1). Kao and Popon are at less than 50 m elevation, whereas Pidiwan and Kai are at about 100 m elevation. Rodents were captured by using wire-cage live traps and plastic snap traps, baited with raw cassava or baked coconuts. Trapping sites were among bushes in palm plantation fields in Kao and Popon and forest edges in Pidiwan and Kai. Rats collected alive were euthanized by overdosing with chloroform. The lungs, alimentary canal, and liver were fixed in 10% formalin solution and then transported to the laboratory for parasite examination. The lung and liver were sliced and examined under a stereomicroscope. The alimentary canal was cut open, the mucosal surface was vigorously rubbed and washed on a sieve with an aperture size of 75  $\mu$ m, and the residues on the sieve were examined under a stereomicroscope. The stomach and esophageal walls were observed under stereomicroscope with transmitted illumination for detection of nematodes under the mucosal lining. Nematodes were rinsed in 70% ethanol

solution, cleared in a glycerol-alcohol solution, and mounted with 50% glycerol solution. Representative nematode specimens are deposited in the U.S. National Museum Helminthological Collection, Beltsville, Maryland, U.S.A., with the accession numbers USNM Helm. Coll. 84322–84338. Voucher host specimens have been deposited in the American Museum of Natural History, New York, U.S.A., with the accession numbers AMNH 267655–267680 and 267682–267703.

### Results

A total of 29 *Rattus rattus* (snout–vent length 92–216 mm [ $\bar{x}$  174  $\pm$  32 SD mm]) and 20 *Rattus exulans* (snout–vent length 90–150 [ $\bar{x}$  122  $\pm$  17 SD mm]) were examined. Ten and 7 species of nematode parasites were collected from *R. rattus* and *R. exulans*, respectively (Table 1), all in the alimentary tract. No nematodes were detected in the lungs or livers.

In *R. rattus*, *Nippostrongylus brasiliensis* (Traavassos, 1914) was the most prevalent nematode, being followed by *Strongyloides ratti* Sandground, 1925, and *Strongyloides venezuelensis* Brumpt, 1934. Concurrent infection with both *Strongyloides* species was observed in 14 (61%) of the 23 *R. rattus* in which any nematodes were detected. In 11 of the 12 rats infected with *Orientostrongylus tenorai* Durette-Desset, 1970, *N. brasiliensis* was also present. Counts of *N. brasiliensis* infections revealed that 55% of the infected *R. rattus* harbored less than 10 worms, and the rest harbored more than 60. One rat was found to be parasitized by 212 *N. brasiliensis*. In *O. tenorai* infections, more than 70% of *R. rattus* harbored less than 10 specimens. The individual with the maximum intensity of *N. brasiliensis*

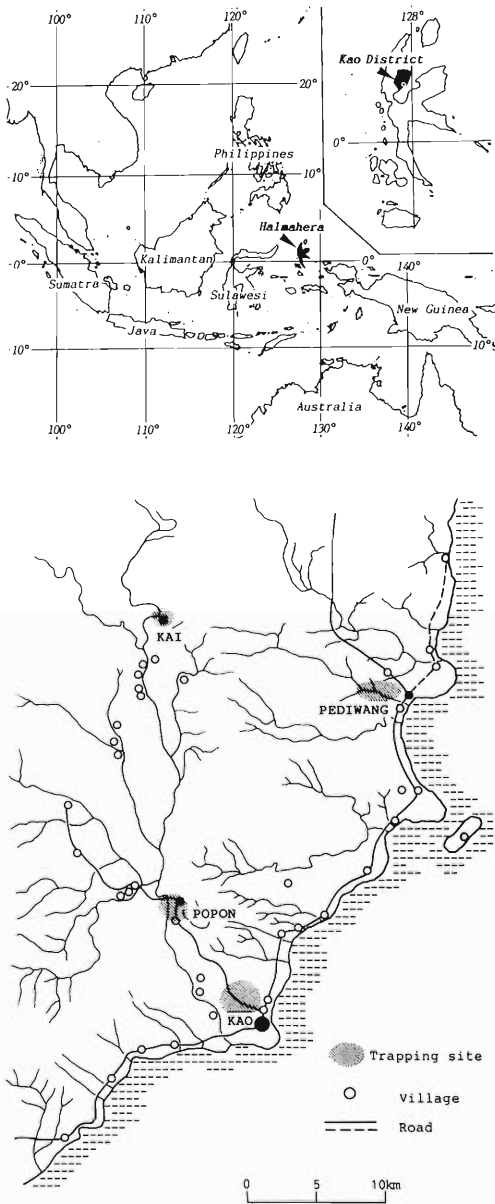


Figure 1. A map showing the survey area. Halmahera Island (above), Kao District (above right inset), and trapping sites (below).

infection also showed the highest intensity of *O. tenorai* infection, 280 worms. The worm burden profile of *Syphacia muris* (Yamaguti, 1935) showed that 9 of the 10 infected *R. rattus* were parasitized by less than 60 worms, but the remaining rat harbored ca. 1,800 individuals. The intensity of infection with adult spirurids, that

is, *Gongylonema neoplasticum* (Fibiger and Ditlevsen, 1914) and *Pterygodermatites whartoni* (Tubangui, 1931), was less than 5 in all cases. One spirurid larva, probably *Mastophorus muris* (Gmelin, 1790), was also detected.

In *R. exulans*, the most prevalent nematode was *O. tenorai*, and its prevalence was more than twice that in *R. rattus*. However, *O. tenorai* from *R. exulans* was generally smaller (males 1.39–1.65 mm long by 48–64  $\mu$ m wide, spicule length 67–74  $\mu$ m; females 2.06–2.62 mm long by 51–64  $\mu$ m wide) than that from *R. rattus* (males 1.63–2.09 mm long by 61–70  $\mu$ m wide, spicule length 70–86  $\mu$ m; females 2.53–3.31 mm long by 62–85  $\mu$ m wide). As in *R. rattus*, the intensity of infection with *O. tenorai* was less than 10 in more than 70% of *R. exulans*, and the maximum worm burden was only 56. Adult *N. brasiliensis* was not observed in *R. exulans*, although a single fourth-stage male larva of *Nippostrongylus*, probably *N. brasiliensis*, was found in 1 rat. *Strongyloides venezuelensis* was also common in *R. exulans*, being detected in over 60% of them but *S. ratti* was less prevalent, and mixed infections of the 2 species were found only in 4 individuals (20%). The prevalence of *S. muris* was much higher than in *R. rattus* (Table 1). Eleven of 15 *R. exulans* (73%) with *S. muris* infection harbored less than 60 worms, and 2 harbored ca. 600 and 800 pinworms, respectively. Only 3 spirurid larvae, presumably *P. whartoni*, were detected in 1 *R. exulans*.

Three species belonging to the subfamily Capillariinae were collected from the small intestine: *Capillaria traverae* Ash, 1962 (1 complete male), and Capillariinae gen. sp. A (1 male and 1 female each lacking anterior portion) from *R. rattus* and Capillariinae gen. sp. B (1 female lacking anterior portion) from *R. exulans* (Table 1). The male of *C. traverae*, 4.79 mm long by 43  $\mu$ m wide, was morphologically identical to the previous descriptions except that it had 2 pairs of caudal papillae, of which anterior 1 pair was quite minute and hardly discernible. Capillariinae gen. sp. A lacked lateral caudal alae, had a membranous bursa supported by 1 pair of lobular projections, an indistinct spicule with a spinose sheath in a fragmented male that was 5.1 mm long excluding the esophageal portion, and its fragmented female had a postesophageal body of 4.0 mm long, no ornamentation at the vulva, and thick-shelled eggs of 48–50 by 18–22  $\mu$ m in size. The female of Capillariinae gen. sp. B was 3.9 mm long in the postesophageal body by 45

Table 1. Prevalence of nematode parasites in *Rattus* spp. collected from Halmahera Island, Indonesia, July 1993.

Locality	Host species							
	<i>Rattus rattus</i>				<i>Rattus exulans</i>			
	Popon	Kao	Pediwan	Total	Popon	Kao	Kai	Total
No. hosts examined	9	19	1	29	3	16	1	20
Snout-vent length of hosts								
Range	112-195	92-216	214	92-216	111-126	90-150	127	90-150
$\bar{x} \pm SD$ (mm)	164 $\pm$ 28	177 $\pm$ 33		174 $\pm$ 32	119 $\pm$ 8	122 $\pm$ 19		122 $\pm$ 17
No. hosts with nematodes	3	19	1	23 (79%)	3	16	1	20 (100%)
Nematode species								
<i>Capillaria traveræ</i>		1		1 (3%)*				
<i>Capillariinae</i> gen. sp. A		1		1 (3)				
<i>Capillariinae</i> gen. sp. B								1 (5)
<i>Strongyloides ratti</i>	1	14	1	16 (55)	1	3		4 (20)
<i>Strongyloides venezuelensis</i>	1	14	1	16 (55)	1	10	1	12 (60)
<i>Syphacia muris</i>		9	1	10 (34)	2	12	1	15 (75)
<i>Nippostrongylus brasiliensis</i>	2	17	1	20 (69)	1†			1 (5)
<i>Orientostrongylus tenorai</i>	2	10		12 (41)	3	15		18 (90)
<i>Gongylonema neoplasticum</i>		2		2 (7)				
<i>Pterygodermatites whartoni</i>	1	4		5 (17)	1†			1 (5)
<i>Mastophorus muris</i>		1†		1 (3)				

\* No. hosts infected with prevalence in parentheses.

† Tentative identification due to larval stage of the worms.

$\mu\text{m}$  wide, with a prominently protruded vulval flap, and the eggs were relatively thin-shelled and 51–54 by 22  $\mu\text{m}$  in size.

### Discussion

*Strongyloides ratti*, *S. muris*, *N. brasiliensis*, *G. neoplasticum*, *P. whartoni*, and *M. muris* have been recorded as common parasites of *R. rattus* of Southeast Asia, Taiwan, southern Japan, the Philippines, and some of the Pacific islands (see Hasegawa et al., 1993, 1994). In East Asia, *S. venezuelensis* was first recorded in *Rattus norvegicus* of Okinawa, southern Japan (Hasegawa et al., 1988), and was subsequently collected from *R. rattus* on Lanyu, Taiwan (Hasegawa et al., 1994). This species is readily distinguished from *S. ratti* by having spiraled ovaries in the parasitic female, but both species seem to have been hitherto confused because of their minute sizes and frequent concurrent infection (Hasegawa et al., 1988, 1994). The prevalence of *S. venezuelensis* in *R. exulans* was much higher than *S. ratti* (Table 1). Although *Strongyloides* worms hitherto recorded from *R. exulans* of Southeast Asia and Pacific islands have been identified as *S. ratti* (Sinniah, 1979; Uchikawa et al., 1984), a detailed reexamination may reveal wider distribution of *S. venezuelensis* in this rat.

*Orientostrongylus tenorai* belongs to the subfamily Nippostrongylinae (Trichostrongyloidea: Heligmonellidae) and has been recovered from various rats of Afghanistan, India, Southeast Asia, and Taiwan (Durette-Desset, 1970; Ohbayashi and Kamiya, 1980; Ow Yang et al., 1983; Hasegawa, 1990; Hasegawa et al., 1994). Only limited records have been made on nippostrongylinae from *R. exulans*: Schacher and Cheong (1960) reported *N. brasiliensis* from Singapore and Kuala Lumpur (prevalence 0.8 and 1.2%, respectively); Sinniah (1979) found *Nippostrongylus* sp. from Peninsular Malaysia (prevalence 10.2%); Ow Yang et al. (1983) listed *O. tenorai* and *O. krishnansamy* Durette-Desset and Lim-Boo-Liat, 1974, from Malaysia (prevalence not given); and Uchikawa et al. (1984) collected Trichostrongyloidea gen. sp. from Fiji (14.3%). However, in these studies, except for Ow Yang et al. (1983), *Orientostrongylus* seemed to be not considered. Because, until recently, *O. tenorai* has often been confused with *N. brasiliensis* (see Hasegawa et al., 1994), it is highly probable that *O. tenorai* was mixed among the specimens regis-

tered as *Nippostrongylus* or Trichostrongyloidea gen. sp.

The most remarkable difference in the nematode fauna found between the 2 murine species was the prevalence of *N. brasiliensis*. This nematode was quite common in *R. rattus*, whereas only 1 fourth-stage larva was collected from *R. exulans* (Table 1). This is in sharp contrast to the prevalence of *O. tenorai*, which was common in both murines. *Nippostrongylus* infects cutaneously (Yokogawa, 1922), while *Orientostrongylus* has been shown to infect orally (Fukumoto, 1979). However, this difference seems to be insufficient to account for the observed difference in the prevalence because the sympatric murines would probably have more or less similar chances of being exposed to the skin-penetrating larvae of *N. brasiliensis* in the fields surveyed. It is therefore suggested that *R. exulans* is less susceptible to *N. brasiliensis*. The susceptibility of *R. exulans* to *N. brasiliensis* should be examined experimentally to see whether or not this is so.

The identification of a capillariid nematode is often difficult, especially when only a small number of incomplete worms are available, as in the present survey. Several capillariids have been recorded from the rats of the Pacific region, but species identification has been made only for *C. traveræ* reported from *R. norvegicus* and *R. rattus* of Hawaii and *R. exulans* from Fiji (Ash, 1962; Uchikawa et al., 1984). According to the systematics proposed by Moravec (1982), *C. traveræ* may belong to the genus *Baruscapillaria*, not to *Capillaria*, because the latter genus was defined as having a spinose spicular sheath. However, no suitable genus in his system was found to fit for the Capillariinae gen. sp. A. In the absence of a male, it is impossible to decide the genus of the Capillariinae gen. sp. B.

The nematode fauna of the rats in Kao District is depauperate compared to that on Lanyu, a much smaller island of Taiwan, where 17 nematode species have been reported from *R. rattus* (Hasegawa et al., 1994). Some common rat nematodes such as *Calodium hepaticum* (Bancroft, 1893) (= *Capillaria hepatica*), *Eucoleus bacillatus* (Eberth, 1863) (= *Capillaria bacillata*), and *Heterakis spumosa* Schneider, 1866, were not found in the present survey. The rat lung worm, *Parastrostrongylus cantonensis* (Chen, 1935) (= *Angiostrongylus cantonensis*), which is widely distributed in the Pacific region as well as in Southeast Asian countries (Alicata and Jindrak, 1970), was



not observed either. Moreover, *Globocephalus connorfilii* Lane, 1922, and *Ascarops strongylina* (Rudolphi, 1819), which are common parasites of swines but also parasitize the rats on Lanyu, were not found. However, if more rats were examined, some of these nematodes might be detected. It is also suggested that a limited opportunity for dispersal of rats to the Kao area might be a cause of the reduced nematode fauna.

#### Acknowledgments

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## ***Microfilaria sundaicus* sp. n., a *Chabfilaria*-like Parasite (Filarioidea: Onchocercidae) from the Blood of the Horseshoe Bat (*Rhinolophus affinis*) in Flores, Indonesia**

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**ABSTRACT:** *Microfilaria sundaicus* sp. n. (Filarioidea: Onchocercidae) is described from the blood of a horseshoe bat, *Rhinolophus affinis* (Chiroptera: Rhinolophidae), collected in western Flores, Indonesia. The microfilariae are similar in appearance to the genus *Chabfilaria*. They are sheathed and characterized by having a greater body length (133–177  $\mu$ m), greater tail length (15–21  $\mu$ m), and longer cephalic space (3–5  $\mu$ m) when compared to the 2 species of *Chabfilaria* described from the literature. This is the first report of a *Chabfilaria*-like microfilaria outside of South America and represents the first time a filarial parasite of this description has been found in Chiroptera.

**KEY WORDS:** Filarioidea, Onchocercidae, *Microfilaria sundaicus*, horseshoe bat, *Rhinolophus affinis*, Flores, Indonesia.

In May 1990, an adult male horseshoe bat, *Rhinolophus affinis* Horsfield, 1923, was captured using a mist net in a banana garden, in Longko village, near Ruteng (8°36'S, 120°27'E), western Flores Island, Indonesia. The island of Flores sits adjacent to Weber's Line on the eastern edge of the Oriental region separated from the Australasian region. The specimen was part of an ongoing biogeographic small mammal survey conducted by the Western Australia Museum (D. Kitchener, pers. comm.). The microfilariae are designated here as *Microfilaria sundaicus*, after the geographical region from which the host was collected.

### **Materials and Methods**

Routine thick and thin blood smears were made in conjunction with specimen processing. The blood slide was subsequently fixed with methanol and stained with Giemsa at 1:15 dilution for 15 min, revealing microfilariae described herein. The formalin-preserved bat from which the microfilariae were found was later obtained for complete necropsy. No adult worm was found; however, additional microfilariae were recovered from the body cavity. Figure 1 was drawn with the aid of a camera-lucida from examination of 10 microfilariae. All measurements are given in micrometers and are expressed as means followed in parentheses by the range.

### **Results**

#### ***Microfilaria sundaicus* sp. n. (Fig. 1)**

**HOST:** *Rhinolophus affinis*.

**LOCATION:** Microfilariae in blood, adult unknown.

**LOCALITY:** Longko village, western Flores Island, Indonesia.

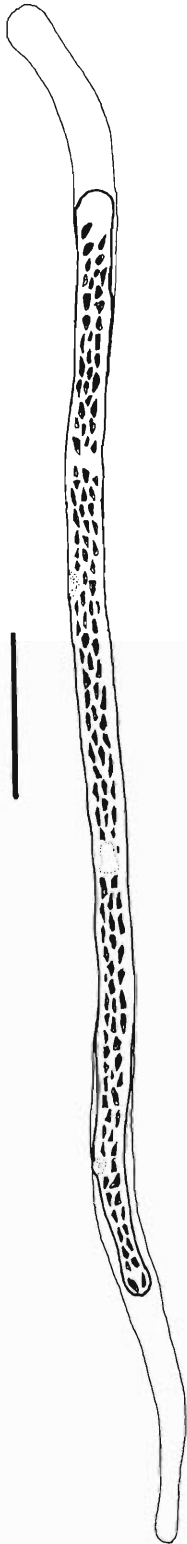
**SPECIMENS DEPOSITED:** One stained blood film (No. M32872), syntypes, deposited in the Department of Biogeography and Ecology, Western Australia Museum, Perth, Western Australia 6000.

**DESCRIPTION:** Body slender, sheathed, with both ends blunt and slightly enlarged, nuclei are distinct when stained with Giemsa. Sheath lightly stained. Body 157.5 (133–177) length by 4.9 (4–5) width at level of first cephalic nucleus, 5.4 (5–6) at nerve ring, 5.0 at excretory pore, 5.2 (5–6) at midbody, 4.0 at anal pore and 3.0 at caudal end. Distance from anterior end to nerve ring 36.5 (33–40); excretory pore, 53.0 (50–60); anterior Innenkörper 98.4 (92–105); anal pore 137.9 (118–148). Cephalic space is short 3.8 (3–5) with a ratio of length to width 0.8 (0.6–1.0):1. Innenkörper 3.7 (3–5) long.

### **Discussion**

Because the adult stage is unknown, a valid generic designation is replaced with a collective-group name for young larval Filarioidea, *Microfilaria* Cobbold, 1880. Conforming to Article 1d and special provisions provided in Articles 13b and 42b(i) in the International Code of Zoological Nomenclature (3rd ed., 1985), we propose this microfilaria as a new species within an acknowledged artificial taxon. We have chosen to publish the description as *Microfilaria sundaicus* sp. n., thus making the name taxonomically available.

Based on review of the Filarioidea found in bats and other vertebrates, the microfilariae herein differ from those previously described from



**Table 1.** Comparison microfilaria measurements of *Chabfilaria jonathani* and *Microfilaria sundaicus* sp. n.

	<i>C. jonathani</i> (Bain et al. 1983)	<i>M. sundaicus</i>
Number examined	> 10	10
Sheath	Sheathed	Sheathed
Body length	125–138*	133–177
Body width (maximum)	5–6	5–6
Cephalic space length	2	3–5
Tail length	16–18	15–24

\* All measurements in micrometers.

the Palearctic, Australasian, and Oriental zoogeographic regions (Anderson and Bain, 1976). Only *Litomosa* Yorke and Maplestone, 1926, and *Josefilaria* Moorhouse, Bain, and Wolf, 1979, have been described from bats in the Oriental region. Both genera have microfilariae possessing a pointed tail. *Chiropterotharia brevicaudata* Yeh, Symes, and Mataika, 1958, and *Microfilaria fijensis* Yeh, Symes, and Mataika, 1958, described from the fruit bat, *Pteropus hawaiiensis*, in Fiji were found unsheathed, greater in length, and having pointed tails (Yeh et al., 1958). This new microfilaria has a distinctly blunt tail and is sheathed. The Flores microfilaria most closely resembles members in the genus *Chabfilaria*, presently known only from the Americas.

*Chabfilaria* was first described by Bain et al. (1983) in which *Chabfilaria jonathani* Bain, Purnomo, and Dedet, 1983, was described and *Chabfilaria freitaslenti* (Yeh, 1957) placed in the genus. These 2 species represent the only examples of *Chabfilaria*. The genus has been described as exhibiting rudimentary characters possibly representing primitive members of the Setariinae (Bain et al., 1983). Table 1 lists some of the morphological characteristics differentiating the Indonesian microfilaria from *C. jonathani*. In general, the Indonesian specimens have a greater body, tail, and cephalic space length. The only description given for the *C. freitaslenti* microfilaria was that it was unsheathed. Although a superficial resemblance to *Chabfilaria* is noted, it remains speculative whether or not

←

**Figure 1.** *Microfilaria sundaicus* sp. n. from a horseshoe bat in Flores, Indonesia. Scale bar = 20  $\mu$ m.

this microfilaria from Indonesia has any close systematic affinities with the genus. Unfortunately, important structures of the cephalic extremity were not distinct after staining to make accurate comparisons with the description of *Chabfilaria*.

*Chabfilaria jonathani* was originally described from a 2-toed sloth, *Choloepus didactylus* (Bradyrodidae) from French Guyana, and *C. freitaslenti* from the giant anteater, *Myrmecophaga tridactyla* (Myrmecophagidae) from British Guiana. Both hosts are members of the order Edentata.

This report describes microfilariae from an insectivorous bat (order Chiroptera) indigenous to Indonesia. The microchiropteran *Rhinolophus affinis* (family Rhinolophidae) is composed of 4 subspecies that are geographically widespread and morphologically variable. Based on locality, the Flores specimen is most likely *Rhinolophus affinis princeps* K. Andersen, 1905, the intermediate horseshoe bat (Kitchener et al., 1990). *Rhinolophus a. princeps* has been described from Indonesia, India, Malaysia, and southern China. This genus of bats is restricted to the tropical and temperate regions of the Old World (Walker, 1975).

Although definitive taxonomic identification awaits the description of adult worms, we believe this finding represents a unique species based on the vertebrate host, biogeographic distribution, and morphologic differences seen in the microfilariae compared to those previously described from bats. This is the first report of a *Chabfilaria*-like microfilaria from the Oriental region and represents the first time a filarial parasite of this description has been found in Chiroptera.

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## Structural Evidence for Sensory Function in the Apical Organ of *Macracanthorhynchus hirudinaceus* (Acanthocephala)

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**ABSTRACT:** The prominent cone-shaped elevation on the apex of the proboscis of *Macracanthorhynchus hirudinaceus* is the termination for a pair of nerves from the cerebral ganglion as well as a “duct” from the sensory support cell (stützzelle). When the area posterior to the floor of this cone was examined using electron microscopy, a complex branching pattern emerged for the pair of apical sensory nerves. Each branch contained numerous ciliated structures that extended to the pit formed by the cone. Many of these cilia extended through the pit wall into the outside environment. We interpret the presence of cilia in this location as circumstantial evidence that one of the functions of the apical organ is sensory. If this is true, then these branches of the apical sensory nerves are receptors.

**KEY WORDS:** Acanthocephala, apical organ, cilia.

The function of the apical organ in Acanthocephala probably has been discussed since it was first observed. One of the earliest suggested functions was sensory. Schneider (1868) provided a description of the neuroanatomy of *Macracanthorhynchus hirudinaceus*, which included neurons that terminated in the apex of the proboscis. He indicated that these neurons probably were sensory. Leuckart (1876) described an anterior medial nerve that was located between the proboscis retractor muscles and ended at the proboscis tip in a sensory papillae. Kaiser (1893) clearly stated that the anterior medial nerve terminated in a nerve bundle. He believed this to be a sensory papillae but cautioned that the actual function was unknown. Kilian (1932) and Meyer (1933) accepted the position of these investigators as have more recent authors (Hyman, 1951; Dunagan and Miller, 1991). Nevertheless, other authors have suggested that these devices perform a different role. Hamann (1891) thought they were secretory or glandular. Bullock (1965) observed large quantities of glycoaminoglycans and cytoplasmic RNA and noted that the apical organ of *Octospiniferoides chandleri* histochemically had a strong positive aldehyde-fuchsin reaction. He considered these observations as evidence of secretory activity. It is, of course, possible that these apical proboscis structures perform more than a single function.

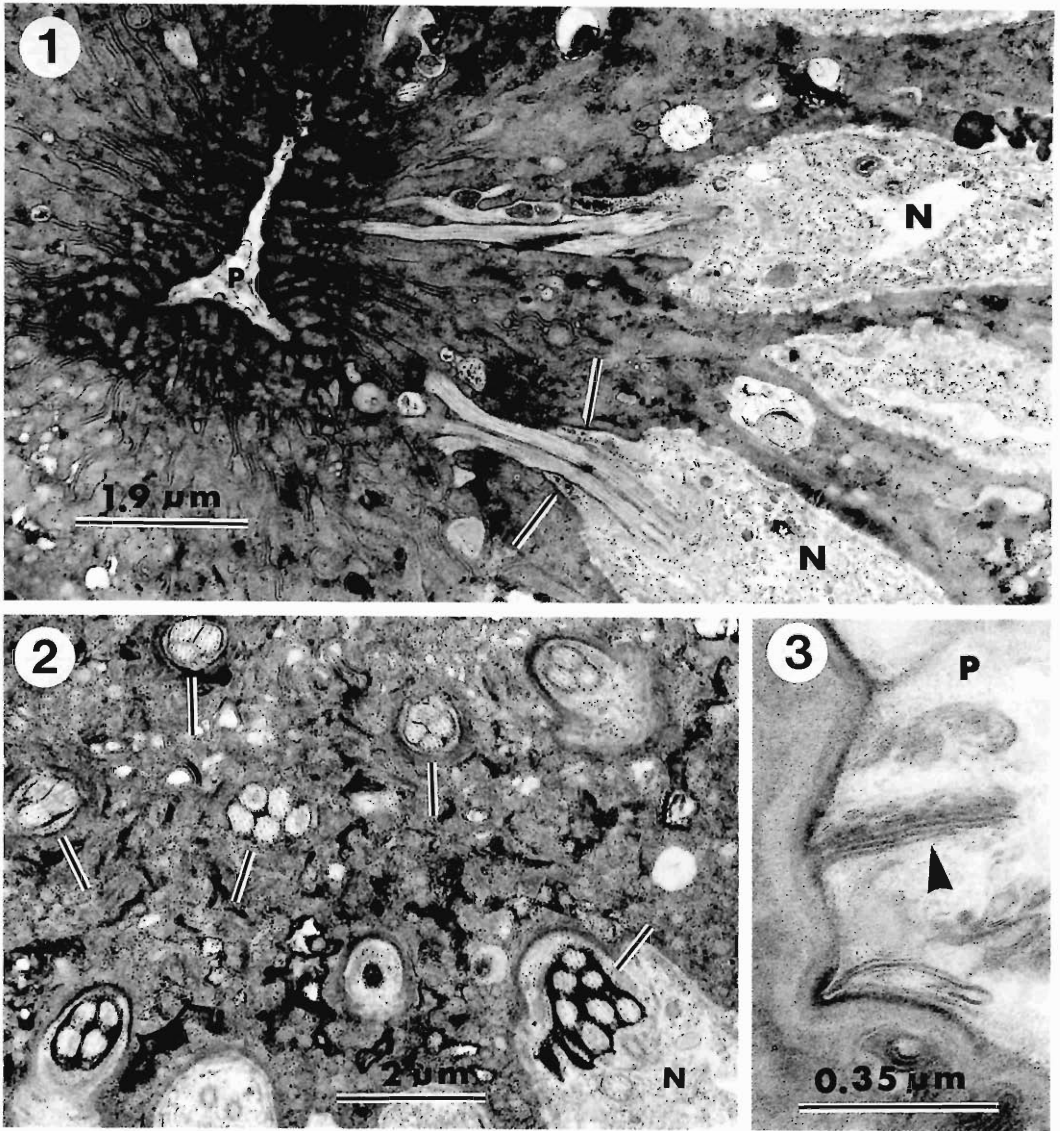
It is the purpose of this paper to describe the presence of ciliated structures in the apical organ of *M. hirudinaceus*. We believe this is circumstantial evidence of a sensory function.

### Materials and Methods

Living *M. hirudinaceus* were collected from pigs through the courtesy of Reelfoot Meat Packaging, Union City, Tennessee. Nodules with attached worms were removed from the small intestine and placed in a Dewar flask containing intestinal contents. Following transport to the laboratory, the acanthocephalans were detached from the intestine, rinsed in 30% sea water, and fixed for 1 hr at room temperature in a mixture of 2% glutaraldehyde and 0.2 mM cacodylate buffer (pH 7.2) containing 2.0 mM EGTA and 1.0 mM MgSO<sub>4</sub>. After fixation, specimens were rinsed in 0.2 M cacodylate buffer and then postfixed for 2 hr at room temperature in freshly prepared 1% OsO<sub>4</sub> and 1.5% K<sub>3</sub>Fe(CN)<sub>6</sub>. Some specimens remained in glutaraldehyde fixative for several weeks. The worms were rinsed in double-distilled water, stained with 1% aqueous uranyl acetate, dehydrated, and then infiltrated and embedded with Spurr's epoxy resin. Sections were cut, and slot grids were prepared and examined in a Hitachi H500H electron microscope. A more detailed outline of the procedures used was reported by Dunagan and Bozzola (1989).

### Results

A cross-section through the posterior portion of the cone-shaped elevation at the apex of the proboscis (Figs. 1, 3, 5) shows an open pit (P) partially filled with “debris.” The fluted margins of the pit are surrounded by radiating groups of microtubules organized in packets of cilia. These packets weave a complicated pattern (Fig. 2) as they progress toward the pit opening. The microtubules in these packets emanate from the terminal portion of branches of sensory nerves (N). As these microtubules exit the nerve, the neurocytoplasm is restricted. This results in the

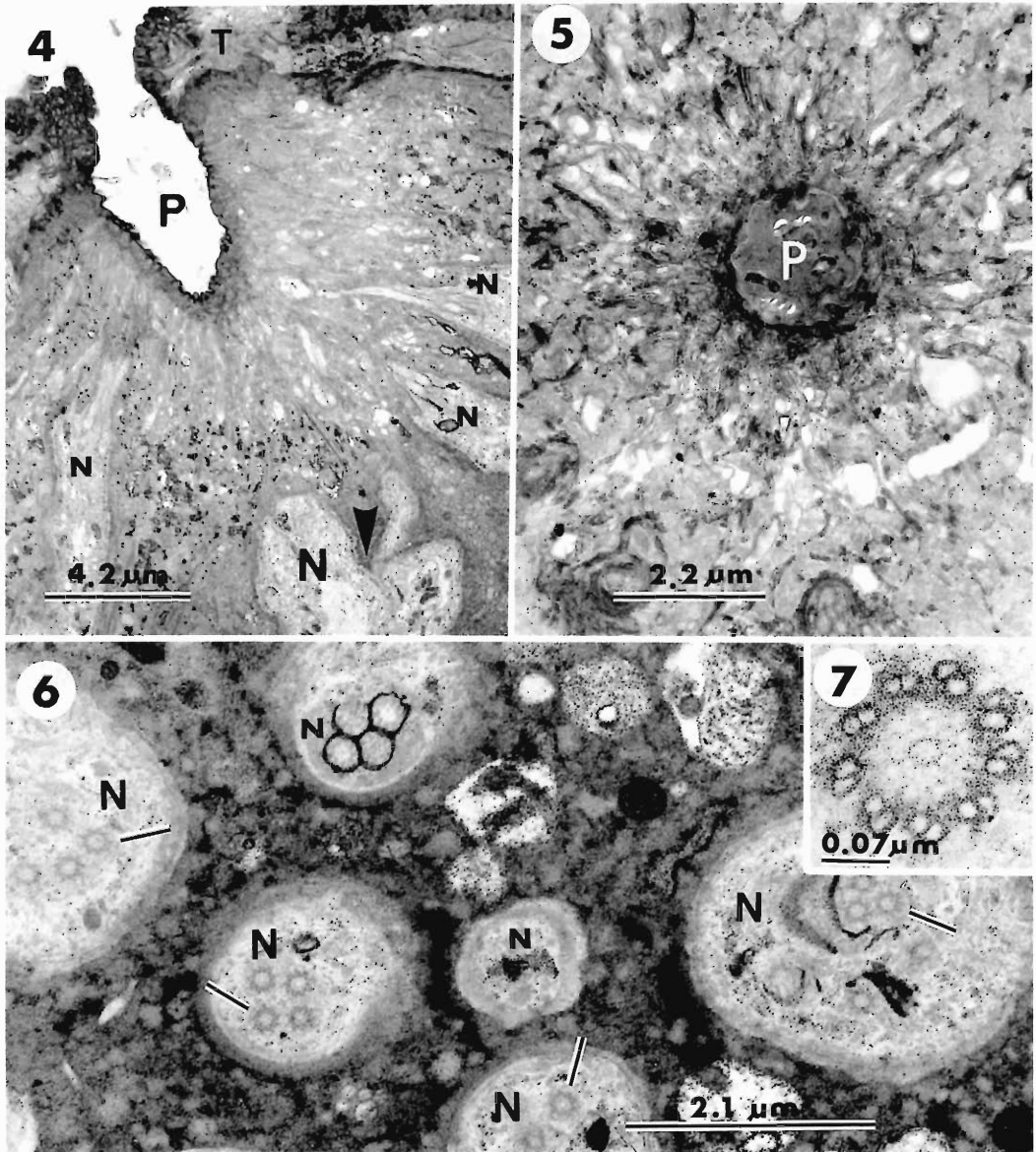


Figures 1-3. Electron micrographs of apical organ of *Macracanthorhynchus hirudinaceus* in vicinity of pit on apex of proboscis. 1. Cross-section showing sensory nerves terminating in cilia that extend to pit. Shafts denote collar formed around cilia as they leave neuron. 2. Tangential section posterior to apical pit. Shafts denote packets of microtubules. 3. Cross-section through pit of apical organ. Note that microtubules extend into pit opening. Abbreviations: N = sensory neuron, P = apical pit.

formation of a collar (Fig. 1; shafts) around the exiting tubules, following which they are outside the nerve for the remaining distance to the pit. Some of these tubules extend into the pit opening, maintaining the same general organization (Fig. 3) here as inside the apical organ. Some of the clefts in the pit wall show no cilia but, instead, appear to open to the inside of the apical organ.

Occasionally, tubelike structures (Fig. 1) can be traced to these clefts, but most sections show them disappearing in the immediate vicinity of the pit wall. Sagittal sections show (Fig. 4) that these nerve extensions enter the floor of the pit as well as its sides, but always posterior to the tegument (T).

Much of the "debris" in the pit (Figs. 1, 3, 5)



Figures 4–7. Electron micrographs of apical organ of *Macracanthorhynchus hirudinaceus* in area of pit on apex of proboscis. 4. Sagittal section showing that tegument forms part of pit wall. Note branch in neuron (arrowhead). 5. Cross-section through pit. Note abundance of microtubules and microfilaments that radiate from pit. 6. Cross-section of neurons below pit floor. Several neurons contain packets of microtubules. 7. Enlarged view of packet shows microtubule pattern of 9+0.

consists of fragmented microtubules and other filamentlike items. However, the pit may be largely empty (Fig. 4) or completely filled (Fig. 5). In either case, its margins never are smooth. Note that the tegument forms the lining of the pit next to its opening (Fig. 4) and that there is

a clear demarcation between the apical organ and tegument.

Detailed examination of nerve cross-sections show numerous groups of microtubules (Fig. 6). The microtubules in Figure 6 are typical of cross-sections of cilia with 9+0 doublets (Fig. 7).

### Discussion

The presoma of *M. hirudinaceus* is crowned with a cone-shaped elevation that resembles a small volcano in appearance. The historical perspective as to function recently has been reviewed (Dunagan and Bozzola, 1989, 1992). These authors have also shown that this apical organ (AO) is served by a pair of nerves from the cerebral ganglion as well as a duct from the sensory support cell (stützelle). The relationship of these 2 entities still is unclear. The apical sensory nerves (SNs) were separated from the anterior proboscis nerves (APNs) (Dunagan and Miller, 1983) when it was recognized that the paired APNs terminated in different areas. The evidence presented in this study indicates that the SNs terminate in the pit wall or the opening of the pit in the cone-shaped elevation. Part of the outermost inside surface of the pit is formed by the AO. Throughout that part of the pit formed by the AO, there are microtubules extending into the "open" area of the pit. Extended time in the fixative tends to remove these structures and is probably the reason more are not visible in the electron micrographs presented here. In addition, the lining of the pit appears to have clefts. These may be openings into the AO or sites of present or former microtubules as they penetrate the pit wall.

The presence of numerous microtubules organized into cilia that penetrate the pit wall of the AO is evidence that one function of the AO is transduction of chemical sensory information. Assuming that the site of transduction is in the cilia, they would detect chemicals in a liquid phase. A recent symposium (Corey and Roper, 1992) included several papers that pointed out the role of cilia in the reception of sensory stimuli in olfaction, photoreceptors, hair cells, etc. It is therefore clear that cilia act in signal transduction. The alternate question is what do these organized microtubules do in the pit of the AO if not serving as sensory devices? We interpret the presence and pattern of microtubules to support the hypothesis that the SN branches in *M. hirudinaceus* are dendrites of 2 receptor neurons, the soma of which lies in the cerebral ganglion.

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## Redescription of *Cavisoma magnum* (Southwell, 1927) (Acanthocephala: Cavisomidae) from the Milkfish, *Chanos chanos*, in the Philippines

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**ABSTRACT:** *Cavisoma magnum* (Southwell, 1927) Van Cleave, 1931, is redescribed from specimens collected from the intestine of adult milkfish (*Chanos chanos* (Forsskål)) caught in the Basilan Strait off the city of Zamboanga, southern Philippines. An emended definition of the genus *Cavisoma*, for which *C. magnum* is the type and only species, is also provided. This is only the second finding of *C. magnum*, which is the only acanthocephalan reported from milkfish whose specific identity has been determined.

**KEY WORDS:** Acanthocephala, *Cavisoma magnum*, redescription, fish parasites, *Chanos chanos*, Philippines.

During a survey of the parasite fauna of milkfish (*Chanos chanos* (Forsskål) (family Chanidae)) in the Philippines (see Regidor and Arthur, 1992) adult acanthocephalans were found in the intestines of adult milkfish (known locally as *sabalo*) examined at Zamboanga, Mindanao Island, Philippines. These specimens were identified as *Cavisoma magnum* (Southwell, 1927) Van Cleave, 1931, a species that until this time was known only from the original description. Southwell (1927) described *Oligoterorhynchus magnus* Southwell, 1927, from 2 perciform fishes, *Acanthurus strigosus* Bennett (syn. of *Ctenochaetus strigosus* (Bennett), the goldring surgeonfish, also known as the spintail in Southeast Asia) and *Serranus* sp., collected from the Indian Ocean off Ceylon. As the original description and illustrations of this species are inadequate by current standards, we take the opportunity to redescribe this acanthocephalan from Philippine material.

### Materials and Methods

Five adult milkfish (72-88 cm in fork length) were purchased from local fishermen at Zamboanga, Mindanao Island, Philippines, on 23 March 1987. Fish were necropsied fresh at the Bureau of Fisheries and Aquatic Resources, Regional Fisheries Training Center at Zamboanga. The intestines of 2 of the milkfish were infected with 30 and 32 acanthocephalans, respectively.

Acanthocephalans were cleaned with physiological saline, fixed with 70% ethanol, and stored in a solution of 10% glycerine in 70% ethanol for later identification. Specimens were pricked repeatedly with a dissecting needle and flattened prior to staining with Semichon's acetocarmine and mounting in Canada balsam or Per-

mount. Additional specimens were stained without flattening with Schneider's acetocarmine and mounted in Permount. Eggs were dissected from the pseudocoel for measurement. Illustrations were prepared with the aid of a drawing tube.

Identification was confirmed by comparison with 3 of the type specimens of *Oligoterorhynchus magnus* Southwell, 1927, part of a lot of unmounted specimens collected from *Serranus* sp. that was deposited in the collection of the Liverpool School of Tropical Medicine by T. Southwell. The specimens were stained unflattened with Schneider's acetocarmine and mounted in Permount.

Measurements (range followed by the mean and standard deviation in parentheses) are in millimeters unless otherwise indicated.

### Redescription

#### Genus *Cavisoma* Van Cleave, 1931

**DIAGNOSIS** (emended from Golvan, 1969): Cavisomidae Van Cleave, 1931. Medium-sized acanthocephalans. Trunk unarmed, elongate, subcylindrical. Proboscis, short, clavate, armed with longitudinal rows of hooks; posterior hooks slender, rootless; anterior hooks robust, strongly recurved, with well-developed simple roots. Double-walled proboscis receptacle attached to base of proboscis. Cerebral ganglion located near middle of receptacle. Lemnisci digitiform, of variable length, generally about equal to receptacle. Reproductive organs of male occupy only posterior one-third of trunk. Testes, 2, tandem, contiguous. Four tubular cement glands. Gonopore terminal in both sexes. Eggs fusiform, thin-shelled, with well-developed polar prolongations of middle membrane. Parasites of the digestive

tract of marine fishes. Type and only species: *Cavisoma magnum* (Southwell, 1927) Van Cleave, 1931.

***Cavisoma magnum* (Southwell, 1927)**

Van Cleave, 1931

Syn.: *Oligoterorhynchus magnus*

Southwell, 1927

(Figs. 1–6)

**GENERAL:** With the characters of the genus. Trunk subcylindrical, elongate. Neck short, often retracted. Proboscis armed with 12–13 (typically 12) rows of hooks, 8–10 hooks per row. Four basalmost hooks in each row slender, slightly curved, rootless; 5th to apical hooks more robust, recurved, with well-developed simple roots. Hooks increase in size anteriorly from basalmost hook to 6th or 7th hook, then decrease slightly to apical hook. Lemnisci approximately equal in length, typically extending to distal end of proboscis receptacle. Proboscis receptacle elongate digitiform, often with constriction at middle.

**MALES** (based on 15 flattened and 6 unflattened specimens; measurements for internal structures are from flattened specimens only):

Trunk 19.42–51.36 (35.37 ± 9.27) long. Trunk width at midbody 0.73–1.53 (1.25 ± 0.27) in flattened specimens, 0.86–1.25 (0.98 ± 0.27) in unflattened specimens. Ratio of trunk length/trunk width 24.5–34.8 in flattened specimens, 29.1–43.2 in unflattened specimens. Neck 0.25–0.40 (0.31 ± 0.08) long, 0.46–0.50 (0.48 ± 0.02) wide at base ( $n = 3$ ). Proboscis 0.91–1.05 (0.97 ± 0.06) long ( $n = 5$ ), 0.33–0.48 (0.39 ± 0.04) wide ( $n = 16$ ); armed with 12–13 (typically 12) rows of hooks, 9–10 hooks per row. Basal hooks 70–90  $\mu\text{m}$  (80.0 ± 5.8) long ( $n = 10$ ); largest hook 105–125  $\mu\text{m}$  (116.2 ± 6.5) long, its root 90–110  $\mu\text{m}$  (96.5 ± 6.3) long ( $n = 20$ ). Proboscis receptacle 1.23–3.09 (2.60 ± 0.46) long, 0.34–0.52 (0.44 ± 0.05) wide. Lemnisci 1.46–4.05 (2.60 ± 0.78) long, 0.25–0.58 (0.37 ± 0.10) wide ( $n = 13$ ). Testes 2, tandem, elongate to oval. Anterior testis 0.70–2.53 (1.42 ± 0.50) long, 0.25–2.09 (0.56 ± 0.44) wide; posterior testis 0.60–1.49 (1.04 ± 0.25) long, 0.24–0.94 (0.51 ± 0.21) wide. Vas deferens runs ventrally along cement glands, extends from posterior margin of posterior testis to proximal end of penis, often enlarged at anterior and posterior ends. Separate seminal vesicle not detected. Cement glands 4, elongate tubular, extending from near posterior margin of posterior testis to midlevel of Saeftigen's pouch

before narrowing into cement ducts. Cement glands 1.52–4.49 (2.99 ± 0.82) long, 0.06–0.27 (0.17 ± 0.06) wide. Total length of male reproductive system 6.28–11.96 (8.57 ± 1.88); ratio male system to trunk length 0.165–0.358. Saeftigen's pouch highly muscular, in specimens with noneverted copulatory bursa typically with bulbous anterior and narrow posterior portions. Total length of Saeftigen's pouch 1.28–2.85 (2.16 ± 0.39), maximum width 0.39–0.98 (0.62 ± 0.17).

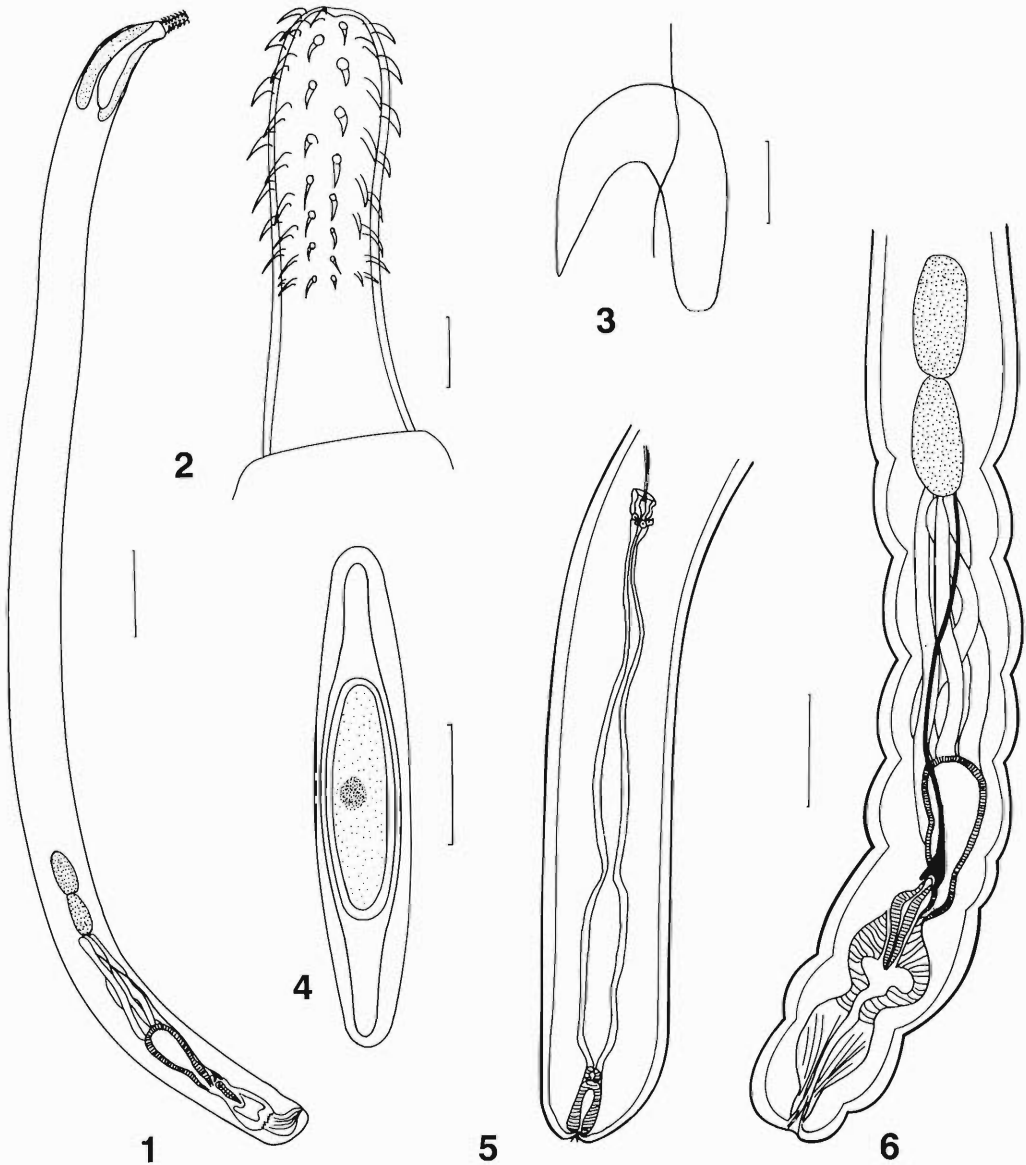
**FEMALES** (based on 16 flattened (10 mature, 6 immature) and 7 unflattened [all mature] specimens): Trunk 10.67–48.93 (30.21 ± 12.04) long (27.72–48.93 in mature specimens). Trunk width at midbody 0.54–1.89 (1.11 ± 0.44) in flattened specimens, 0.91–1.57 (1.26 ± 0.25) in unflattened specimens. Ratio of trunk length/trunk width 18.8–27.8 in flattened specimens, 27.4–36.2 in unflattened specimens. Neck 0.19–0.44 (0.25 ± 0.08) long, 0.43–0.57 (0.50 ± 0.05) wide at base ( $n = 15$ ). Proboscis 0.79–1.08 (0.95 ± 0.10) long ( $n = 11$ ), 0.32–0.43 (0.37 ± 0.03) wide ( $n = 20$ ); armed with 12 rows of hooks, 8–9 hooks per row. Basal hooks 65–95  $\mu\text{m}$  (80.5 ± 7.4) long ( $n = 22$ ); largest hooks 105–130  $\mu\text{m}$  (115.9 ± 6.6) ( $n = 16$ ) long, their roots 80–105  $\mu\text{m}$  (94.7 ± 8.3) long ( $n = 16$ ). Proboscis receptacle 1.57–3.21 (2.30 ± 0.48) long, 0.23–0.63 (0.42 ± 0.09) wide. Lemnisci 1.58–4.14 (2.72 ± 0.88) long ( $n = 14$ ), 0.13–0.44 (0.25 ± 0.12) wide ( $n = 12$ ). Eggs fusiform, thin-shelled, with well-developed polar prolongations of middle membrane, 103–121  $\mu\text{m}$  (113.4 ± 4.3) long × 15–20  $\mu\text{m}$  (17.0 ± 1.7) wide ( $n = 30$ ).

**HOSTS:** *Serranus* sp. and *Acanthurus strigosus* (syn. of *Ctenochaetus strigosus*) (goldring surgeonfish) (type host not specified by Southwell, 1927); *Chanos chanos* (milkfish).

**LOCATION:** Stomach[?] and pyloric caeca (see Southwell 1927); intestine.

**LOCALITIES:** Indian Ocean "off Negapatam, Ceylon" [=Negappattinam, India] from *Serranus* sp. and from an unspecified locality off Ceylon (Sri Lanka) from *A. strigosus* (see Southwell 1927); Basilan Strait off Zamboanga, Zamboanga del Norte Province, Mindanao Island, Philippines.

**DEPOSITION OF SPECIMENS:** Types: *Oligoterorhynchus magnus* Southwell, 1927–No. L40 (LSTM No. TA/2/01) from *Serranus* sp., and No. L41 (LSTM No. TA/2/02) from *A. strigosus*, type collection of the Liverpool School of Tropical Medicine, curated by the CAB International Institute of Parasitology, St. Albans, UK. Spec-



Figures 1–6. *Cavisoma magnum*. 1. Lateral view of male. Scale bar = 3.0 mm. 2. Proboscis. Scale bar = 150  $\mu\text{m}$ . 3. Anterior hook. Scale bar = 50  $\mu\text{m}$ . 4. Egg. Scale bar = 30  $\mu\text{m}$ . 5. Posterior of female. Scale bar = 1.5 mm. 6. Posterior of male. Same scale as Figure 5.

imens from *Chanos chanos*: Helminth Collection of the CAB International Institute of Parasitology (IIP No. 6586; 10 mounted and 11 unmounted specimens); Zoological Reference Collection, National University of Singapore (Nos. ZRC.1994.988–996; 9 mounted specimens); remainder in the personal collection of JRA.

**Remarks**

*Cavisoma magnum* was originally placed in the genus *Oligoterorhynchus* Monticelli, 1914 (family Echinorhynchidae), by Southwell (1927). Van Cleave (1931) later established a new family, Cavisomidae, and genus, *Cavisoma*, to contain *O. magnum*, as *C. magnum* (Southwell, 1927) Van

**Table 1. Comparison of mensural and meristic characters for *Cavisoma magnum* (measurements are in mm unless otherwise indicated).**

	Southwell, 1927		Original	
	Males	Females	Males	Females
<b>Trunk</b>				
Length	About 36*	About 70*	19.42–51.36	10.67–48.93
Width	1	1.5	0.73–1.53	0.54–1.89
<b>Proboscis</b>				
Length	1.1		0.91–1.05	0.79–1.08
Width	0.45		0.33–0.48	0.32–0.43
<b>Hooks</b>				
No. longitudinal rows	12			12–13
No./row	8–10			8–10
Length of largest	About 110 $\mu\text{m}$		105–125 $\mu\text{m}$	105–130 $\mu\text{m}$
Length of smallest	About 70 $\mu\text{m}$		70–90 $\mu\text{m}$	65–95 $\mu\text{m}$
Proboscis receptacle length	2.6		1.23–3.09	1.57–3.21
<b>Anterior testis</b>				
Length	1,170 $\mu\text{m}$		0.70–2.53	
Width	105 $\mu\text{m}$		0.25–2.09	
<b>Posterior testis</b>				
Length	1,030 $\mu\text{m}$		0.60–1.49	
Width	105 $\mu\text{m}$		0.24–0.94	
Cement gland length	3.25		1.52–4.49	
<b>Egg</b>				
Length		120–130 $\mu\text{m}$ *		103–121 $\mu\text{m}$
Width		22 $\mu\text{m}$		15–20 $\mu\text{m}$
Hosts	<i>Ctenochaetus strigosus</i> , <i>Serranus</i> sp.		<i>Chanos chanos</i>	
Locality	Indian Ocean off southern India and Sri Lanka		Basilan Strait, Philippines	

\* Indicated measurements were taken from largest specimens.

Cleave, 1931, the type and only species. Subsequent authors have either retained *Cavisoma* within a distinct subfamily (either Cavisominae or Cavisomatinae) within the Echinorhynchidae (Myer, 1932; Yamaguti, 1963), placed it within the family Fessisentidae (Golvan, 1969), or maintained a distinct family (Cavisomatidae) to contain it (Petrochenko, 1956). Most recently, Amin (1985) has retained Cavisomidae as a distinct family in the order Echinorhynchida to contain *Cavisoma* and 9 other genera.

The original description of *O. magnum* by Southwell (1927) is brief and rather incomplete. Measurements obtained during this study, for the most part, agree well with those given by Southwell (Table 1). Males from *C. chanos* show a considerably larger maximum size as compared to that previously reported (51.36 vs. about 36 mm in length), approaching that found for females; while the maximum size observed for females is somewhat smaller than that previously

recorded (48.93 vs. about 70 mm). Additionally, the maximum egg size for specimens from *C. chanos* is somewhat less than that previously reported (130 vs. 121  $\mu\text{m}$ ). Although the tegument of the 3 type specimens of *Cavisoma magnum* examined by us shows strong irregular external pseudosegmentation, this is much less pronounced in our Philippine material and, as noted by Golvan (1969), is probably an artifact of fixation. Southwell (1927) noted that the anterior testis of *C. magnum* was apparently fused to the posterior testis; however, in our flattened specimens, they are clearly unfused. Although this species was noted to lack a neck, a short neck is present, although it is often withdrawn in mature individuals. Southwell (1927) recorded *C. magnum* from the host's stomach and pyloric caeca, while our specimens were obtained from the intestine. His record from the stomach is probably the result of postmortem migration. Southwell (1927) noted that his material from *Serranus* sp.

was collected "off Negapatam, Ceylon." However, this city, now known as Negappattinam, is actually in Tamal Nadu, India, only about 50 km north of Sri Lanka (formerly Ceylon).

Previously, Velasquez (1976, 1977) reported the presence of unidentified acanthocephalans in *sabalo* (adult *Chanos chanos*) from Philippine waters and later (Velasquez, 1979), in apparent reference to the same material, noted that numerous worms were found in the intestine of this fish from Nasugbu, Batangas, and from Mindoro. Although she (Velasquez, 1984) subsequently referred this report to *Acanthocephalus* sp., it seems probable that this finding also involves *Cavisoma magnum*. The only other report of acanthocephalans from milkfish is that of Ruangpan and Tanomkiat (1980), who reported an unidentified species of *Acanthosentis* from the intestine of fish examined from coastal Thailand.

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## Effects of Low Temperature on the Development of *Leptorhynchoides thecatus* (Acanthocephala) in *Lepomis cyanellus* (Centrarchidae)

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**ABSTRACT:** The effect of low temperature on the development of *Leptorhynchoides thecatus* (Acanthocephala: Rhadinorhynchidae) in *Lepomis cyanellus* (Centrarchidae) was studied by infecting 28 fish with cystacanths reared in the laboratory. Fish were infected and maintained together at 12 C and 4 were examined every 2 wk from 10 to 22 wk postinfection. Trunk lengths of recovered *L. thecatus* were used in conjunction with qualitative observations of gonadal development and gametogenesis to evaluate development during the period of prepatency. The prepatent period was in excess of 22 wk in worms maintained at 12 C, nearly 3 times that of worms maintained at 21 C. Results are consistent with known seasonal patterns of recruitment and maturation and suggest that retardation of growth is a mechanism by which natural populations of *L. thecatus* overwinter in vertebrate poikilothermic hosts.

**KEY WORDS:** Acanthocephala, *Leptorhynchoides thecatus*, development, prepatent period, overwintering, green sunfish, *Lepomis cyanellus*.

Development during the prepatent period (sensu Crompton, 1985) of the fish parasite *Leptorhynchoides thecatus* (Acanthocephala: Rhadinorhynchidae) has been well studied under laboratory conditions (DeGiusti, 1949; Uznanski and Nickol, 1982; Ewald and Nickol, 1989). Such studies have revealed consistent developmental rates for worms in fishes maintained at room temperatures (20-25 C). The effects of low temperature, however, have been studied only on the viability of eggs and the rate of development in the intermediate host. DeGiusti (1949) found that eggs of *L. thecatus* stored in a refrigerator at 4 C were viable for at least 9 months and demonstrated that acanthellae in *Hyaella azteca* (Amphipoda) maintained at 13 C required twice the time needed to develop to the infective cystacanth stage as did those maintained at 20-25 C. Based on these findings, he suggested that overwintering in the environment involved both the retarded development of larval stages and the ability of eggs to withstand cold temperatures for extended periods. The role of juvenile worms in nature during the winter months is not well understood, and differences in seasonal patterns of natural infections of *L. thecatus* between localities in Wisconsin (Amin, 1988; DeGiusti, 1949) and Nebraska (Ashley and Nickol, 1989) suggest that maturation in definitive hosts may

be slowed by low temperatures as well. However, this has not been demonstrated previously. The present study examined the effect of low temperature on the development of *L. thecatus* in the green sunfish (*Lepomis cyanellus*), a host-parasite system for which laboratory and field data were readily available for comparison.

### Materials and Methods

Gravid worms of *L. thecatus* were removed from the ceca of sunfishes (*Lepomis* spp.) seined from the Elkhorn river drainage (Holt County, Nebraska) and were refrigerated in aged tap water 1-2 wk. *Hyaella azteca* and *Lepomis cyanellus* used for laboratory infections were collected in Lancaster County, Nebraska, where *L. thecatus* does not occur (Nickol and Samuel, 1983).

Eggs of *L. thecatus* were removed from the body cavity of female worms and concentrated in aged tap water such that 0.025 ml of the suspension contained approximately 100 fully embryonated eggs. The suspension was refrigerated 7 days to insure exposure of the eggs' fibrillar coat, which has been shown to enhance infection rates by causing the eggs to become entangled on vegetation (Uznanski and Nickol, 1976). Amphipods were collected with aquatic dip-nets and were counted into 11 groups of 50 individuals and held in plastic 700-ml cups to which aged tap water and an 8-cm sprig of *Elodea* sp. had been added. Approximately 600 eggs (0.15 ml of suspension) were added to each cup by pipetting the suspension slowly and evenly immediately above the floating *Elodea* sprig. The cups were left unaerated under continuous fluorescent light for 72-84 hr, after which the amphipods were transferred to aerated 9 × 30 × 17-cm plastic culture boxes containing aged tap water, clean gravel, and *Elodea*. The amphipods were maintained in the culture boxes at 21 C for 40 days after removal from the cups.

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**Table I.** Mean numbers of male, female, and total *Leptorhynchoides thecatus* from laboratory-infected *Lepomis cyanellus* maintained at 12 C.

Weeks PI* (N = 4 fish)	Number of worms†		
	Male	Female	Total
10	1.75 (±0.96), 1-3	3.00 (±1.41), 1-4	4.75 (±1.71), 3-7
12	2.75 (±1.71), 1-5	2.25 (±0.96), 1-3	5.00 (±2.45), 3-8
14	3.00 (±0.82), 2-4	3.25 (±1.26), 2-5	6.25 (±1.71), 4-8
16	2.25 (±1.50), 1-4	2.75 (±1.71), 1-5	5.00 (±1.16), 4-6
18	1.75 (±1.50), 0-3	1.50 (±1.29), 0-3	3.25 (±1.71), 1-5
20	2.75 (±0.96), 2-4	3.25 (±0.50), 3-4	6.00 (±0.82), 5-7
22	1.50 (±1.29), 0-3	2.75 (±2.87), 1-7	4.25 (±2.63), 2-8
Mean (N = 28 fish)	2.25 (±1.27), 0-5	2.68 (±1.52), 0-7	4.93 (±1.88), 1-8

\* PI = postinfection.

† Mean (±SD), range.

Twenty-eight green sunfish were held in pairs separated by a divider in 38-L aquaria. The fish were maintained at room temperature (21 C) on a diet of cockroaches (*Periplaneta americana*) and were fasted for 3 days prior to experimental infections. Cystacanths were removed from infected amphipods. Each fish was fed 10 cystacanths by pipetting the worms with a small amount of water into a number 5 gelatin capsule that was inserted into the abdominal cavity of a cockroach and then fed to a fish. Three days following ingestion of the capsule, the fish were removed from the aquaria and held together in a 190-L polyethylene tank maintained at 12 C. Beginning 10 wk postinfection (PI), 4 fish were removed from the tank every 2 wk until 22 wk PI. Each fish was killed immediately upon removal and its intestine, pyloric ceca, mesentery, and body cavity examined for the presence of parasites. Parasites recovered were refrigerated overnight in tap water, fixed in AFA, stained in Semichon's acetocarmine, dehydrated, cleared, and mounted in Canada balsam.

Overall development of male and female *L. thecatus* was examined by measuring trunk length, as this character has been shown to be influenced by the age and reproductive state of the worm (Crompton, 1985). Measurements were made from mounted specimens by using a microscope equipped with an ocular micrometer. Qualitative characterization of reproductive development was made by examining gonadal development and gametogenesis.

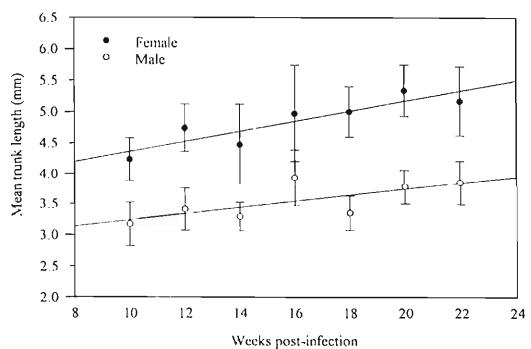
Analysis of variance (ANOVA) was used to detect differences among mean numbers and lengths of worms and for regression of worm length vs. weeks PI. Statistics were derived with SAS® using the General Linear Models procedure. ANOVA results are reported parenthetically as (*F*; *df*; *P* > *F*). Differences between means at the 95% confidence level were considered significant.

## Results

Of 2,200 amphipods exposed to eggs of *L. thecatus*, 512 survived to day 40, of which 37% harbored 1-8 (mean = 1.47) fully formed cystacanths.

Success of laboratory infections of *L. thecatus* in green sunfish was roughly 50% (~5 worms/fish) of the dosage, with approximately equal numbers of male and female worms recovered (Table I). The number of male worms ranged from 0 to 5, and female worms ranged from 0 to 7 (Table I). No significant difference in the mean number of male (0.86; 6; 0.54) or female (0.62; 6; 0.71) worms was found between weeks PI.

Over the period of 10-22 wk PI, trunk lengths ranged from 2.8 to 4.9 mm and from 3.3 to 6.1 mm in male and female worms respectively (Fig. 1). A significant positive correlation was found between trunk length and the number of weeks PI in both male (16.68; 1; 0.0001) and female (26.96; 1; 0.0001) worms.



**Figure 1.** Scatter plot of the mean trunk lengths of male and female *Leptorhynchoides thecatus* vs. weeks postinfection in experimentally infected *Lepomis cyanellus* maintained at 12 C. Lines show linear regression of data (male: slope = 0.052, intercept = 2.71,  $R^2 = 0.716$ ; female: slope = 0.085, intercept = 3.47,  $R^2 = 0.895$ ). Error bars represent the mean  $\pm$  1 SD.

At 10 wk PI, all male worms had attained sexual maturity, as indicated by the presence of spermatozoa. Female worms at this time had not begun to produce eggs; however, multiple free-floating ovaries were observed uniformly dispersed throughout the body cavity. From 4 fish in week 14, 2 of 13 female worms possessed immature eggs. In week 16, 7 of 11 female worms possessed immature eggs, and, in weeks 20 and 22, almost all female worms were filled with immature eggs. The first appearance of eggs that possessed a full complement of surrounding membranes was observed in week 22; however, they constituted only about ~0.1% of the eggs in an individual. Free-floating ovaries were observed in female worms throughout the experiment, indicating that full production and development of eggs had not been attained by 22 wk PI.

### Discussion

The method used to rear *L. thecatus* in amphipods produced results similar to those of Uznanski and Nickol (1980) in that approximately ¼ of the amphipods survived the duration of the experiment and mortality did not appear to be related to the intensity of infection.

The prepatent period of *L. thecatus* has been shown to be approximately 8 wk in green sunfish (Uznanski and Nickol, 1982) and in rock bass (*Ambloplites rupestris*) (DeGiusti, 1949) maintained at ~21 C under laboratory conditions. The present study found that in green sunfish maintained at 12 C under laboratory conditions, the prepatent period is in excess of 22 wk, demonstrating that the development of juvenile *L. thecatus* is a temperature-dependent process, as has been shown with the larval stages (DeGiusti, 1949). Uznanski and Nickol (1982) reported that 8-wk-old cecal worms reached an average trunk length of 3.7 mm and 4.9 mm in male and female worms, respectively. The present study found that the worms did not attain these lengths until almost twice that time PI (Fig. 1). DeGiusti (1949) reported that first insemination of female worms in rock bass was approximately 3.5 wk PI. Using egg production in females as an indication of fertilized versus unfertilized worms (Crompton, 1985), the time at which insemination took place in green sunfish maintained at 12 C was approximately 15 wk PI.

The longevity of *L. thecatus* is not known at present, although it may be expected to have an inverse relationship to temperature, as is true of

the prepatent period. Unfortunately, not enough is known about the relationship between the prepatent period and the longevity of acanthocephalan worms to adopt a general principle upon which estimates of lifespan can be based. What is known suggests that the prepatent period does not form a "uniform part of the acanthocephalan life history" (representing 41% of the lifespan of female *Polymorphus minutus* and 26% of female *Moniliformis moniliformis*) as described by Crompton (1985). Ashley and Nickol (1989) concluded that *L. thecatus* has a lifespan of about 1 season under natural conditions in Nebraska, with 2 generations annually. Amin (1988) reported 1 annual generation through largemouth bass (*Micropterus salmoides*) from lakes in southeastern Wisconsin, as did DeGiusti (1949) in rock bass from northern Wisconsin. The findings of the present study are consistent with a dynamic life history of *L. thecatus*, in that the effects of seasonal temperatures on the rate of development allow 2 generations to cycle through host populations annually in Nebraska, whereas in Wisconsin completion of the life cycle can occur only once annually.

The greatly increased duration of the prepatent period at low temperature also suggests a mechanism by which worms may overwinter in the definitive host in nature, during which time egg production and dispersal would be retarded until warmer temperatures in the spring. Such a mechanism would be advantageous in that it would lead to the release of eggs when aquatic vegetation and amphipods are abundant in the environment (Cooper, 1965). Thus, like the intermediate and exogenous stages of the life cycle, juvenile worms may be expected to play a role in the ability of the species to survive from one winter season to the next.

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## Parasitic Copepods on Three Species of Centrarchids from Gull Lake, Michigan

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**ABSTRACT:** The gills and buccal cavities of 101 rock bass, *Ambloplites rupestris*, 27 largemouth bass, *Micropterus salmoides*, 25 smallmouth bass, *M. dolomieu*, 63 bluegill, *Lepomis macrochirus*, 2 pumpkinseed, *L. gibbosus*, and 1 warmouth, *Chaenobryttus gulosus* (Centrarchidae), were examined for parasitic copepods from Gull Lake, Michigan, in September–October 1990 and June 1993. *Ergasilus centrarchidarum* (Copepoda: Ergasilidae), the most common species found, and *Achtheres pimelodi* (Copepoda: Lernaepodidae) were recovered from rock bass, smallmouth bass, and largemouth bass. *Ergasilus megaceros* infected rock bass and largemouth bass. *Ergasilus* spp. infected the gill filaments, whereas *A. pimelodi* attached primarily to the gill arches, rakers, and buccal cavity. The distribution of *E. centrarchidarum* and *A. pimelodi* on the gills is discussed. *Ergasilus centrarchidarum* and *A. pimelodi* are believed to have been introduced into Gull Lake around 1974.

**KEY WORDS:** *Ergasilus centrarchidarum*, *Ergasilus megaceros*, *Achtheres pimelodi*, parasitic copepods, Centrarchidae, Gull Lake, Michigan.

Esch (1971), Esch and Huffines (1973), and Esch et al. (1975) have reported on many aspects of helminths infecting several species of centrarchids in Gull Lake, Michigan. Parasitic copepods were never seen on centrarchids by these authors in 1967, 1968, 1969, 1972, and 1973 (G. W. Esch and J. R. Coggins, pers. comm.). Parasitic copepods were first seen on rock bass in Gull Lake in 1975 (H. Blankenspoor and J. Johnson, pers. comm.). During a parasitological study of rock bass from Gull Lake in 1990, 3 copepod species (*Ergasilus centrarchidarum* Wright, 1882, *Ergasilus megaceros* Wilson, 1916, and *Achtheres pimelodi* Kroyer, 1863) were found on the gills of rock bass. This study provides information on host specificity, abundance related to sex and length of the hosts, and distribution of *E. centrarchidarum*, *E. megaceros*, and *A. pimelodi* infecting rock bass, largemouth bass, and smallmouth bass in Gull Lake, Michigan.

### Materials and Methods

Centrarchids were collected in September–October 1990 and June 1993 from Gull Lake, Barry and Kalamazoo counties, southwestern lower Michigan. A description and characterization of Gull Lake can be found in Dexter (1991). Fishing gear consisted of experimental gill nets (38 m long × 1.8 m deep, with 5 equal panels of 3.8, 5.1, 6.4, 7.6, and 10.2 cm stretch mesh) and trap nets (pot with 3.8 cm stretch mesh, with a 22.9 m lead, 1.8 m deep). Fish were removed from the nets, preserved on ice, packaged, and frozen.

The buccal cavity, gill filaments, arches, and rakers

of 219 centrarchids (101 rock bass, 27 largemouth bass, 25 smallmouth bass, 63 bluegill, 2 pumpkinseed, and 1 warmouth) were examined for copepods. Total length (mm) and sex of each fish were recorded at necropsy. Gills were numbered 1–4, with gill 1 being outermost (anteriorly attached) and gill 4 being innermost (posteriorly attached). Separate gills were placed in labeled petri dishes and examined. The long gill rakers of rock bass were numbered 1–4 anterioposteriorly. *Ergasilus centrarchidarum* and *E. megaceros* were identified using information in Roberts (1970). *Achtheres pimelodi* was identified using information in and following the synonymy of Kabata (1988). The position of copepods on the gill was noted; they were then removed, counted, and preserved in 70% alcohol. Voucher specimens have been deposited in the U.S. National Parasite Collection, Beltsville, Maryland 20705: *Ergasilus centrarchidarum* (83637), *Ergasilus megaceros* (83638), and *Achtheres pimelodi* (83639).

Twenty-five gravid *E. centrarchidarum* were taken from each host species collected in June 1993. The length of the cephalothorax of each copepod was measured in micrometers under a microscope coverslip. Eggs from the egg sacs were counted to provide an indication of reproductive potential of the species at this time. The ratio of egg number to cephalothorax length was calculated for each copepod from each host species.

Prevalence is the percentage of fish infected, and mean intensity is the mean number of copepods per infected fish. Chi-square tests compared prevalence in relation to host sex, between fish species and collection times. The Kruskal-Wallis test was used to compare intensity in relation to host sex, host species, collection times, gills on the left and right sides of each fish species, and between the gills on each side. Correlation coefficients were calculated to investigate the relationship of copepod intensity and host length. All tests were performed at a significance level of  $P < 0.05$ .

**Table 1.** Prevalence and mean intensity of *Ergasilus centrarchidarum*, *Ergasilus megaceros*, and *Achtheres pimelodi* from three species of centrarchids from Gull Lake.

Copepod species	Fish species*	Date collected	Mean fish total length ± SD (range, mm)	No. exam- ined	Mean intensity	
					No. inf. (%)	± SD (maximum)
<i>Ergasilus centrarchidarum</i>	RB	Sept./Oct. 1990	163 ± 38 (90–265)	51	51 (100)	30.1 ± 18.7 (74)
	RB	June 1993	172 ± 35 (110–278)	50	49 (98)	28.7 ± 23.1 (127)
	LMB	Sept./Oct. 1990	249 ± 62 (121–304)	7	7 (100)	16.7 ± 13.8 (40)
	LMB	June 1993	260 ± 59 (183–431)	20	20 (100)	13.9 ± 10.9 (45)
	SMB	Sept./Oct. 1990	102 ± 49 (71–245)	18	5 (28)	40.0 ± 53.6 (110)
	SMB	June 1993	265 ± 85 (175–380)	7	7 (100)	55.7 ± 30.4 (96)
<i>Ergasilus megaceros</i>	RB	Sept./Oct. 1990	163 ± 38 (90–265)	51	36 (71)	12.5 ± 19.7 (86)
	RB	June 1993	172 ± 35 (110–278)	50	4 (8)	2.5 ± 1.7 (4)
	LMB	Sept./Oct. 1990	249 ± 62 (121–304)	7	3 (43)	5.7 ± 4.0 (8)
<i>Achtheres pimelodi</i>	RB	Sept./Oct. 1990	163 ± 38 (90–265)	51	22 (43)	1.7 ± 1.2 (4)
	RB	June 1993	172 ± 35 (110–278)	50	39 (78)	2.3 ± 1.3 (5)
	LMB	Sept./Oct. 1990	249 ± 62 (121–304)	7	2 (29)	1.5 ± 0.7 (2)
	LMB	June 1993	260 ± 59 (183–431)	20	12 (60)	3.3 ± 2.2 (8)
	SMB	Sept./Oct. 1990	102 ± 49 (71–245)	18	1 (6)	2
	SMB	June 1993	265 ± 85 (175–380)	7	5 (71)	4.8 ± 2.8 (9)

\* Abbreviations: RB = rock bass (*Ambloplites rupestris*), LMB = largemouth bass (*Micropterus salmoides*), SMB = smallmouth bass (*Micropterus dolomieu*).

## Results

*Ergasilus centrarchidarum* occurred on the gill filaments of rock bass, smallmouth bass, and largemouth bass in September–October 1990 and June 1993 (Table 1). The prevalence and mean intensity of *E. centrarchidarum* on each species were high and similar between dates except for smallmouth bass. Fifteen of the 18 smallmouth bass examined in 1990 were 95 mm in length or less, and only 2 were infected with *E. centrarchidarum*. The mean intensity of *E. centrarchidarum* was  $65.7 \pm 57.3$  on the remaining 3 smallmouth bass. These bass had a mean length  $\pm$  SD of  $191 \text{ mm} \pm 74.5$  (range 106–245). There were no significant correlations between intensities of *E. centrarchidarum* and host species length. No significant differences were found in the mean

intensities of *E. centrarchidarum* on gills between the left and right sides of each fish species in 1990 and 1993. There were no significant differences in the distribution of *E. centrarchidarum* between gills on the left and right sides for each host species. However, there was a trend of decreasing intensity of *E. centrarchidarum* on smallmouth bass from gills 1 through 4.

Three percent or less of the female *E. centrarchidarum* on each host species in 1990 had egg sacs. In 1993, 78% or more of the females on each host species had egg sacs (Table 2). The highest percentage of *E. centrarchidarum* with egg sacs occurred on largemouth bass. The cephalothorax length of females with egg sacs and egg number on each host species are shown in Table 3. Female *E. centrarchidarum* infecting rock bass had the largest mean cephalothorax length, while

**Table 2.** Numbers (percentages) of *Ergasilus centrarchidarum* and *Achtheres pimelodi* with and without egg sacs on *Ambloplites rupestris*, *Micropterus salmoides*, and *Micropterus dolomieu* from Gull Lake, June 1993.

Fish species	<i>E. centrarchidarum</i>			<i>A. pimelodi</i>		
	Total numbers	With egg sacs	Without egg sacs	Total numbers	With egg sacs	Without egg sacs
<i>Ambloplites rupestris</i>	1,404	1,097 (78)	307 (22)	90*	37 (41)	51 (57)
<i>Micropterus salmoides</i>	279	262 (94)	17 (6)	39	15 (38)	24 (62)
<i>Micropterus dolomieu</i>	390	358 (92)	32 (8)	24†	4 (17)	19 (79)

\* Two males included.

† One male included.

**Table 3.** Cephalothorax length (micrometers) and egg number of 25 *Ergasilus centrarchidarum* from each of 3 host species from Gull Lake, June 1993.

Host	Mean cephalothorax length $\pm$ SD (range)	Mean number of eggs $\pm$ SD (range)	Ratio of mean egg number/mean cephalothorax length (range)
<i>Ambloplites rupestris</i>	344.7 $\pm$ 15.6 (318.4–378.8)	189.0 $\pm$ 42.6 (119–270)	0.5484 $\pm$ 0.1189 (0.3430–0.7700)
<i>Micropterus salmoides</i>	329.3 $\pm$ 16.9 (298.2–354.6)	155.0 $\pm$ 34.4 (91–215)	0.4724 $\pm$ 0.0994 (0.2660–0.6210)
<i>Micropterus dolomieu</i>	335.6 $\pm$ 20.3 (294.2–386.9)	207.2 $\pm$ 35.5 (146–254)	0.6186 $\pm$ 0.1070 (0.4070–0.8100)

individuals from smallmouth bass had the largest mean egg number and mean egg number-to-mean cephalothorax length ratio.

*Ergasilus megaceros* occurred on the gill filaments of rock bass and largemouth bass with highest infection values on the rock bass in 1990 (Table 1). Less than 1% of the females on hosts in 1990 and only 1 individual (10%) on rock bass in 1993 had egg sacs.

*Achtheres pimelodi* infected rock bass, largemouth bass, and smallmouth bass in 1990 and 1993 (Table 1). Infection values were highest on each host species in 1993. Correlation coefficients between the intensity of *A. pimelodi* and host species length on each date were not significant. In 1990, 21% of the *A. pimelodi* on rock bass had egg sacs. The numbers of female *A. pimelodi* with egg sacs on largemouth and smallmouth bass were not kept in 1990. The percentage of *A. pimelodi* with egg sacs was highest on rock bass in 1993 (Table 2). The prevalences and mean intensities of *A. pimelodi*, *E. centrarchidarum*, and *E. megaceros* were not significantly different between host sexes.

*Achtheres pimelodi* was attached to the gill arch,

raker, filament, and buccal cavity of the hosts (Table 4). The number of *A. pimelodi* was highest on the arch and rakers of gill 1 and decreased through gill 4 on each host species. *Achtheres pimelodi* was commonly attached to the buccal cavity of largemouth bass. The total number of *A. pimelodi* on long rakers 1, 2, 3, and 4 for all the gills of rock bass were 30, 10, 6, and 4, respectively. Two male *A. pimelodi* were attached to 1 female on a rock bass and 1 male was attached to 1 female infecting a smallmouth bass.

Sixty-three bluegill ( $\bar{x}$  length  $\pm$  SD = 146 mm  $\pm$  37.4, range 85–230 mm), 2 pumpkinseed ( $\bar{x}$  length = 185 mm), and 1 warmouth (length = 190 mm) examined in June 1993 were negative for copepods.

## Discussion

Fish host species for *Ergasilus centrarchidarum*, *E. megaceros*, and *Achtheres pimelodi* are similar but vary by location. Tedla and Fernando (1969) found *E. centrarchidarum* infecting rock bass and smallmouth bass but not pumpkinseed from Lake Ontario. Cloutman and Becker (1977) reported that 151 bluegill from Arkansas were negative for *E. centrarchidarum* while largemouth bass and spotted bass, *Micropterus punctulatus*, were infected. Burris and Miller (1972) found *E. centrarchidarum* on 9 species of centrarchids including bluegill from several North Carolina locations. Hanek and Fernando (1978a, b) discussed the host–parasite relationships of *E. centrarchidarum* infecting rock bass and pumpkinseed from Lake Ontario. Miller et al. (1982) found *E. centrarchidarum* on largemouth bass, black crappie (*Pomoxis nigromaculatus*), white crappie (*P. annularis*), and white catfish (*Ictalurus catus*), from North Carolina; while 287 bluegill, 124 green sunfish (*Lepomis cyanellus*), and 23 pumpkinseed were negative. Davis and

**Table 4.** Distribution of *Achtheres pimelodi* on the arches and rakers of different gills and filaments and in the buccal cavity of 3 species of centrarchids from Gull Lake, June 1993.

Site	<i>Ambloplites rupestris</i>	<i>Micropterus salmoides</i>	<i>Micropterus dolomieu</i>
Arch or raker of gill			
1	66	9	13
2	13	8	2
3	5	7	2
4	3	1	0
Gill filament	1	0	1
Buccal cavity	0	14	5

Miller (1989) found *E. centrarchidarum* on bluegill, pumpkinseed, and white crappie in North Carolina. The absence of *E. centrarchidarum* on bluegill from Gull Lake in 1993 is noteworthy because bluegill, rock bass, and bass were caught in the same nets together on the same dates.

Tedla and Fernando (1969) considered the rock bass to be the preferred host of *E. centrarchidarum* in Lake Ontario based on cephalothorax length and egg number. Using the same characteristics, *E. centrarchidarum* from rock bass in Gull Lake had the largest mean cephalothorax length, while females from smallmouth bass in June 1993 had the largest mean egg number and mean egg number-to-cephalothorax length ratio. The mean intensities of *E. centrarchidarum* also were highest on smallmouth bass. Therefore, rock bass and smallmouth bass are preferred hosts for *E. centrarchidarum* in Gull Lake. Largemouth bass are also appropriate hosts for *E. centrarchidarum* based on prevalence and mean intensity. The results of this study and of Tedla and Fernando (1969) suggest that *E. centrarchidarum* has seasonal preferred hosts or its preferred hosts vary between locations.

The mean cephalothorax lengths and egg numbers of *E. centrarchidarum* in the present study differ from values reported by Tedla and Fernando (1969) and for egg number by Cloutman and Becker (1977). Female *E. centrarchidarum* in our study were collected in June, whereas Tedla and Fernando (1969) collected specimens in August, which may explain why their specimens attained a larger mean size than ours. Egg production and number of *E. centrarchidarum* may depend on an optimum water temperature range (Cloutman and Becker, 1977). Therefore, egg number may vary among studies because optimum water temperature ranges occur in different months at different locations. More *E. centrarchidarum* with egg sacs were found on hosts in June 1993 than in September–October 1990 in Gull Lake. Similarly, Cloutman and Becker (1977) found that *E. centrarchidarum* laid eggs in June–September and females did not have egg sacs during the cold months.

*Ergasilus megaceros* has been found in the nasal fossae and on the gill filaments of ictalurids, catostomids, and cyprinids, primarily occurring east of the Mississippi River. Fish from these groups were not examined from Gull Lake. Although *E. megaceros* infected rock bass and largemouth bass from Gull Lake, few females had egg sacs indicating that centrarchids may not

be preferred hosts. Rock bass and largemouth bass are new host records, and Michigan is a new state record for *E. megaceros*. This species, to our knowledge, has not been reported from Canada.

*Achtheres pimelodi* has been reported from the gills of many fish species: largemouth bass and spotted bass by Becker et al. (1966) and Cloutman and Becker (1977) in Arkansas, rock bass and pumpkinseed by Hanek and Fernando (1978a) in Ontario, and bluegill by Davis and Miller (1989) and largemouth bass by Miller et al. (1982) in North Carolina. The prevalences of *A. pimelodi* on hosts from Gull Lake in June 1993 are the highest reported to date. In the present study, *A. pimelodi* primarily attached to the first 2 long gill rakers of arch 1, buccal cavity, and rarely filaments. Gill filaments of centrarchids may not provide a stable attachment for the bulla of *A. pimelodi* and may not be able to support the adult.

The fish community in Gull Lake has changed somewhat over the past 60 yr due to the stocking of species. Bluegill, largemouth bass, yellow perch (*Perca flavescens*), emerald shiner (*Notropis atherinoides*), brown trout (*Salmo trutta*), lake trout (*Salvelinus namaycush*), splake (hybrid, *Salvelinus namaycush* × *Salvelinus fontinalis*) were stocked sporadically between 1930 and 1966 (Dexter, 1991). It is unlikely that *E. centrarchidarum* and *A. pimelodi* were introduced with these fishes since these copepods were not found by Esch and coworkers. The stocking of lake trout, Atlantic salmon (*Salmo salar*), and rainbow trout (*Oncorhynchus mykiss*) into Gull Lake after 1964 (Dexter, 1991) probably did not play a role in the introduction of *E. centrarchidarum* and *A. pimelodi* since they have not been reported from salmonids. Assuming these copepod species were not present earlier, they may have been introduced into Gull Lake around 1974 by the release of infected bass by anglers or by the movement of waterfowl with attached copepods. Perhaps not enough time has passed for *E. centrarchidarum* and *A. pimelodi* to have established host-parasite relationships with bluegill, thus explaining their absence on this fish species in Gull Lake.

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Research Note

Neotropical Monogenoidea. 22. Variation in *Scleroductus* Species (Gyrodactylidae, Gyrodactylidae) from Siluriform Fishes of Southeastern Brazil

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**ABSTRACT:** Specimens of *Scleroductus* are reported from the external surface of 4 siluriform catfish, *Glanidium melanopterum* Ribeiro (Auchenipteridae), *Parauchenipterus striatulus* (Steindachner) (Auchenipteridae), *Pimelodella* sp. (Pimelodidae), and *Rhamdia quelen* (Quoy and Gaimard) (Pimelodidae), collected from the Rio Guandu near Rio de Janeiro, State of Rio de Janeiro, Brazil. These reports represent new host and locality records for *Scleroductus* species in the Neotropics. Morphometric variability of the haptor anchors and hooks of specimens from respective hosts and additional diagnostic characters for the genus are presented.

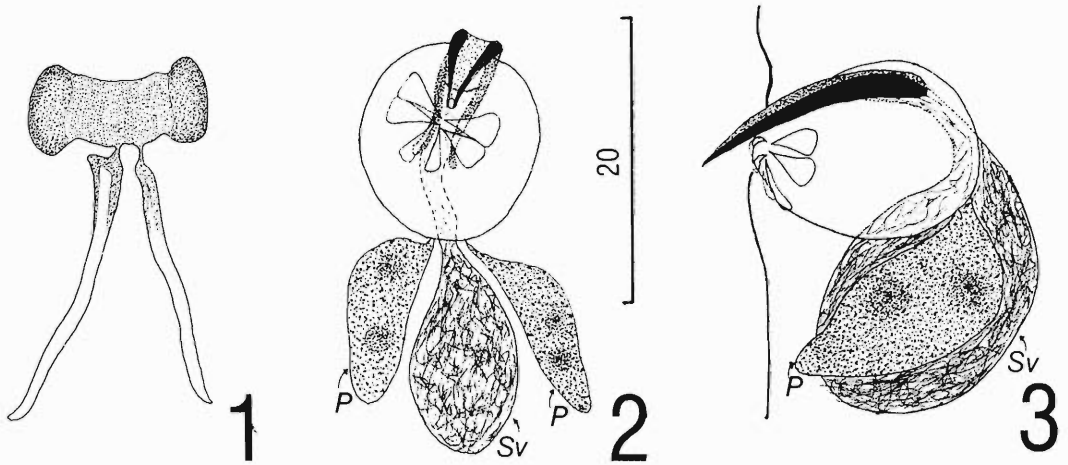
**KEY WORDS:** Monogenoidea, Gyrodactylidae, Brazil, morphometric variability, *Scleroductus* species, *Glanidium melanopterum*, *Parauchenipterus striatulus*, *Pimelodella* sp., *Rhamdia quelen*.

Jara and Cone (1989) proposed *Scleroductus* (Gyrodactylidae) for the viviparous *S. yuncensi* Jara and Cone, 1989, from the external surface of the pimelodid catfish, *Pimelodella yuncensis* Steindachner, in Peru. The genus was characterized by species possessing a bulbous male copulatory organ armed with a terminal spinous ring and 2 sclerotized ribs originating within the ejaculatory duct.

As part of a survey of neotropical Gyrodactylidae, specimens attributable to *Scleroductus* were found on the external surfaces of 4 species of catfish (Siluriformes): *Glanidium melanopterum* Ribeiro (Auchenipteridae), *Parauchenipterus striatulus* (Steindachner) (Auchenipteridae), *Pimelodella* sp. (Pimelodidae), and *Rhamdia quelen* (Quoy and Gaimard) (Pimelodidae). Fish hosts were caught by throw or gill net from below a rapids on the Rio Guandu near Rio de Janeiro, State of Rio de Janeiro, Brazil, between 1 January 1991 and 13 June 1991 (all new host and locality records). Individual or pooled specimens of each host species were placed in containers containing a 1:4,000 formalin solution for removal of parasites (Putz and Hoffman, 1963).

After 1 hr, the vials were vigorously shaken, and sufficient formalin was added to increase the concentration to 5%. Some helminths were mounted unstained in Hoyer's or Gray and Wess's medium for study of sclerotized structures. Other specimens, stained with Gomori's trichrome (see Kritsky et al., 1978) or Mayer's carmine, were mounted in permount for determination of internal anatomy. In addition, 2 paratypes of *Scleroductus yuncensi* (USNM 80630) were examined. Measurements, all in micrometers, include the average followed by the range and number (*n*) of structures measured in parentheses; dimensions of the anchor are shown in Figure 7. Helminths collected during the survey were deposited in the collections of the University of Nebraska State Museum (HWML 37317, 37318, 37319, 37320), the U.S. National Museum (USNM 83733, 83734, 83735, 83736), and the Instituto Oswaldo Cruz (*Scleroductus* sp. from *Glanidium melanopterum*; IOC 33154, 33146, 33147, 33148, 33149; from *Rhamdia*: 33150, 33151, 33152, 33155; from *Parauchenipterus*: 33153; from *Pimelodella*: 33145, 33156).

Although general morphology is similar to that of *Gyrodactylus* spp., *Scleroductus* appears justified based on the derived male copulatory organ. The elongate spine originating within the copulatory bulb and serving as a distal conduit for sperm appears to be a synapomorphy for the genus (Figs. 2, 3). Jara and Cone (1989) indicated that 2 ribs (each representing thickened lateral margins) of the spine originate within the ejaculatory duct, but stained specimens we studied show that the spine lies along the duct. The thick edges of the spine are united medially by a thin sclerotized membrane; they are fused distally (external to the bulb) to form a short tube through which sperm are apparently ejected. The male copulatory organ has 6–8 overlapping spinelets



Figures 1-3. Superficial bar and terminal male genitalia of *Scleroductus* sp. from *Rhamdia quelen*. 1. Superficial bar. 2. Male genitalia (ventral). 3. Male genitalia (lateral). All figures are drawn to the 20  $\mu$ m scale. Abbreviations: P = prostate, Sv = seminal vesicle.

that comprise the "horseshoe-shaped spinous ring" described by Jara and Cone (1989); 2 prostates and a single seminal vesicle empty via short ducts into the base of the copulatory bulb (Fig. 2).

In addition to characteristics provided in the generic diagnosis of *Scleroductus* by Jara and Cone (1989), we note the following from specimens collected in Brazil: Eyes absent; cephalic glands unicellular, comprising bilaterally paired groups of prepharyngeal, pharyngeal, and post-pharyngeal dorsal glands and single bilaterally paired group of posteroventral glands (anteroventral glands not observed) (see Kritsky, 1978); pharynx composed of 2 tandem subhemispherical bulbs; testis postgerminal. *Scleroductus* species appear specific to siluriform fishes of the Neotropics.

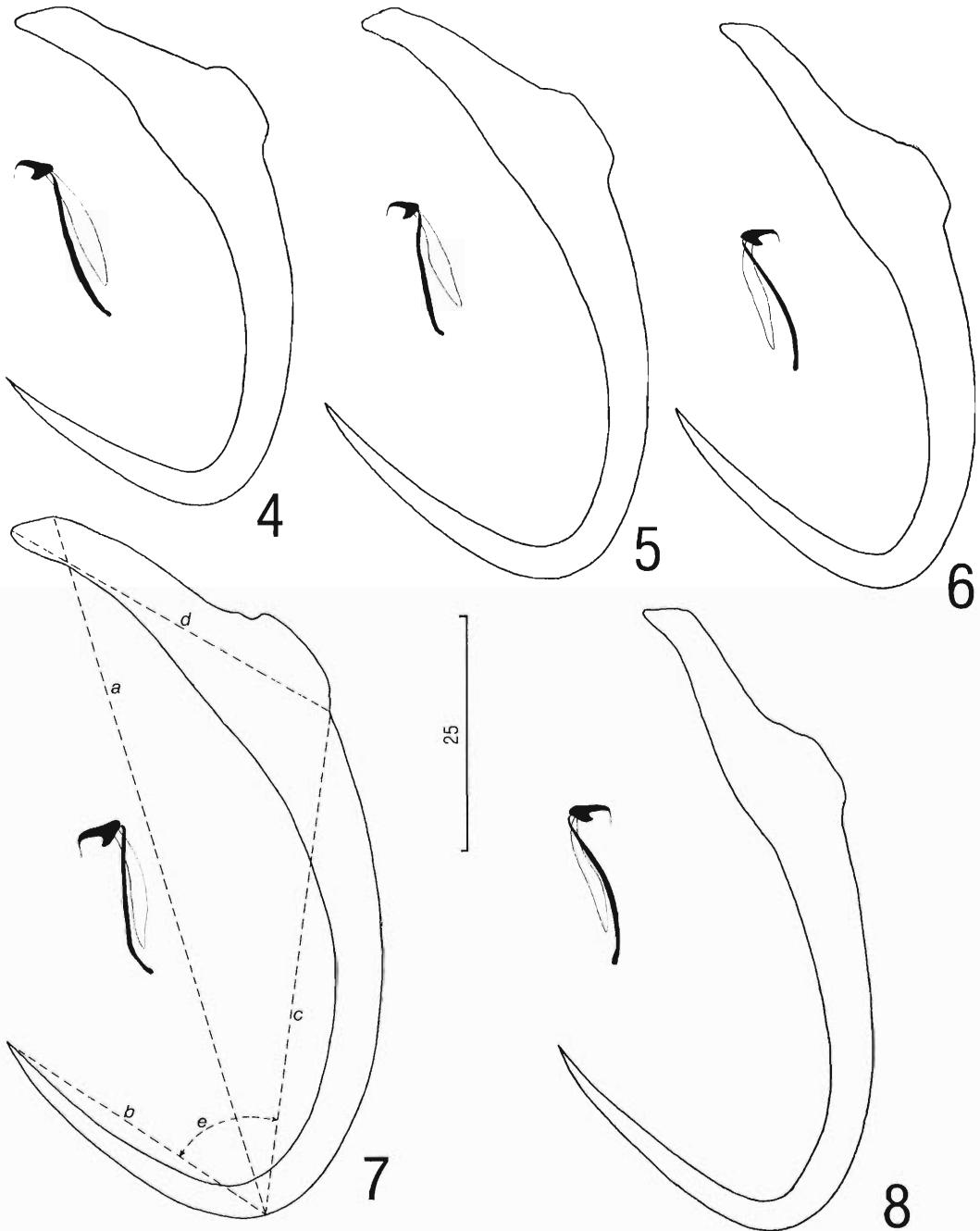
Morphology of the anchors, hooks, and bars of specimens collected from respective Brazilian hosts and that of *S. yuncensi* are strikingly similar (Figs. 1, 4-8). The deep bar is variably bent and rod-shaped. The superficial bar is platelike with expanded terminations; its shield is represented by 2 bilaterally flattened posterior projections that parallel the shafts of the anchor (Fig. 1). The ventral anchor possesses an elongate superficial root, a basal knob, a delicate evenly curved shaft, and elongate point and lacks a basal fold (Fig. 4-8). The hooks have an extrahamular distribution in the haptor (Kritsky and Mizelle, 1968); each possesses a delicate shank, elongate fine

point, a ventrally sloping shaft, and small base with upright truncate toe and rounded heel; the hooklet is usually flexed ventrally. Some of these features, particularly those of the anchors and shield of the superficial bar, may also provide diagnostic synapomorphic characters for the genus.

Comparative measurements of the anchors and hooks of specimens from respective hosts, including those of *S. yuncensi*, are provided in Table 1. Greatest variability in size among specimens from respective hosts is seen among the anchors, with those of specimens from *Rhamdia quelen* occupying the lower limit and those of *S. yuncensi* the upper limit. ANOVA followed by the Tukey test for significance ( $P < 0.05$ ) indicate existence of 3 forms based on dimensions of the anchor (except point length and angle). The anchors of worms from *R. quelen* are different (smallest) from those off *Pimelodella* sp., *Glanidium melanopterum*, and *Parauchenipterus striatulus*, and the anchors of both groups are different from those of *S. yuncensi* (largest).

Because of the morphologic redundancy of the anchor, bar, hooks, and male copulatory organ of specimens from Brazil and Peru, we have chosen not to assign our specimens to *S. yuncensi* or to describe them as new species. It is unknown whether the observed variations in morphology and size are of specific value or are results of influences of host and/or environmental factors. In any case, if our specimens are indicative of





Figures 4–8. Anchors and hooks of *Scleroductus* species from 5 siluriform hosts. 4. *Scleroductus* sp. from *Rhamdia quelen* (Brazil). 5. *Scleroductus* sp. from *Glanidium melanopterum* (Brazil). 6. *Scleroductus* sp. from *Pimelodella* sp. (Brazil). 7. *Scleroductus yuncensi* from *Pimelodella yuncensis* (Peru). 8. *Scleroductus* sp. from *Parauchenipterus striatulus* (Brazil). All figures are drawn to the 25  $\mu\text{m}$  scale. Figure 7 shows measurements taken for the anchors: a = total length, b = length of point, c = length of shaft, d = length of base, e = angle point/shaft.

**Table 1. Comparative measurements of *Scleroductus* species from Brazilian and Peruvian siluriform hosts.**

	Hosts									
	<i>Glanidium melanopterum</i> N		<i>Rhambdia quelen</i> N		<i>Parauchenipterus striatulus</i> N		<i>Pimelodella yuncensis</i> * N		<i>Pimelodella</i> sp. N	
Anchor										
Length	65 (64-68)	8	58 (54-60)	13	64 (60-70)	17	78-79	2	65 (60-70)	20
Shaft	44 (42-45)	8	39 (38-41)	11	43 (37-45)	16	56	2	43 (39-45)	20
Point	31 (30-33)	8	26 (24-27)	12	31 (26-33)	16	35	2	31 (29-33)	20
Base	30 (29-32)	8	28 (26-29)	12	31 (28-33)	16	40 (39-42)	2	31 (30-33)	19
Angle	60 (50-74)	8	68 (64-71)	11	61 (53-76)	14	67 (63-70)	2	62 (55-68)	19
Hook										
Length	17 (16-18)	5	18 (17-21)	9	17 (16-19)	12	17	1	18 (16-20)	15
Hooklet	5	5	5	11	5-6	13	5-6	2	5-6	17

\* Specimens measured from this host are the 2 paratypes of *Scleroductus yuncensis*.

the variability among species in the genus, specific determination based on morphometrics will be difficult.

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**Research Note**

**The Component Parasite Community of Three Sympatric Toad Species, *Bufo cognatus*, *Bufo debilis* (Bufonidae), and *Spea multiplicata* (Pelobatidae) from New Mexico**

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**ABSTRACT:** The component parasite communities in 3 sympatric species of toads from New Mexico were examined. *Bufo cognatus* ( $N = 36$ ) harbored the cestode *Distoichometra bufonis* and the nematodes *Aplectana itzocanensis*, *Rhabdias americanus*, and larvae of *Physaloptera* sp. *Bufo debilis* ( $N = 49$ ) harbored *D. bufonis*, *A. incerta*, *A. itzocanensis*, *R. americanus*, and larvae of *Physaloptera* sp. *Spea multiplicata* ( $N = 31$ ) harbored *D. bufonis*, *A. incerta*, *A. itzocanensis*, and larvae of *Physaloptera* sp. The highest prevalence (69%, 34/49) was recorded for *A. incerta* in *B. debilis*. The greatest mean intensities (20) were recorded for *A. incerta* in *B. debilis* and *R. americanus* in *B. cognatus*. New Mexico is a new locality record for each of these species of helminths. The helminth component communities are depauperate and conform to the pattern of isolationist communities. The helminth compound community for these sympatric species of toads encompasses 5 species.

**KEY WORDS:** helminth community, Cestoda, *Distoichometra bufonis*, Nematoda, *Aplectana incerta*, *Aplectana itzocanensis*, *Physaloptera* sp., *Rhabdias americanus*, Bufonidae, *Bufo cognatus*, *Bufo debilis*, Pelobatidae, *Spea multiplicata*.

Parasite community structure is hierarchical: a parasite infrapopulation represents all members of a single species of parasite within an individual host; a parasite infracommunity includes all of the infrapopulations within an individual host; a component parasite community represents all of the infracommunities within a given host population; and a compound parasite community consists of all the helminth infracommunities within a community of host species (Root, 1973; Esch et al., 1975; Bush and Holmes, 1986; Holmes and Price, 1986). Caswell (1978) and Hanski (1982) have introduced the concept of core and satellite species at the component community level; core species are those that occur with relatively high prevalences and intensities of infection, whereas satellite species occur with less prevalence and are relatively less numerous. Holmes and Price (1986) developed

a set of theoretical considerations that predict that helminth infracommunities span a continuum ranging from isolationist to interactive. Isolationist communities are predicted when the colonization abilities of parasites are limited; interactive communities are predicted when the colonization abilities of the parasites are high.

Using this approach, we examined the component parasite communities in 3 sympatric toad species. This system is particularly advantageous for the study of helminth community organization as the hosts are locally abundant, diminishing the risks of serious impact by sampling. The great plains toad, *Bufo cognatus* Say, 1823, has a geographic range extending from extreme southern Canada to San Luis Potosí, Mexico, from near sea level to 2,440 m; the green toad, *Bufo debilis* Girard, 1854, ranges from southeast Colorado and southwest Kansas to Zacatecas Mexico and southeast Arizona to east Texas from sea level to above 1830 m; and the New Mexico spadefoot, *Spea multiplicata* (Cope, 1863), ranges from southwest Utah and southern Colorado to Guerrero and Oaxaca, Mexico, and western Arizona to western Oklahoma and western Texas from near sea level to around 2,470 m (Stebbins, 1985). The purpose of this paper is to examine helminth species overlap in a community of sympatric hosts.

Thirty-six *B. cognatus* (mean snout–vent length [SVL]  $\pm$  SD = 57 mm  $\pm$  14, range 35–85 mm); 49 *B. debilis* (SVL = 39 mm  $\pm$  4, range 31–52 mm), and 31 *S. multiplicata* (SVL = 43 mm  $\pm$  5, range 32–50 mm) were collected 11 km on the road to Corralitos Ranch off Interstate 10, Exit 127, ca. 3 km W Las Cruces, Doña Ana County, New Mexico (32°17'N, 107°00'W, elevation 1,350–1,400 m), 4–6 August 1992. All toads were deposited in the Natural History Museum of Los Angeles County (LACM): *B. debilis* (LACM

**Table 1. Prevalence, intensity, and range of helminths from *Bufo cognatus*, *Bufo debilis*, and *Spea multiplicata* from New Mexico.**

Parasite species	<i>Bufo cognatus</i>		<i>Bufo debilis</i>		<i>Spea multiplicata</i>	
	Prevalence	$\bar{x}$ intensity (range)	Prevalence	$\bar{x}$ intensity (range)	Prevalence	$\bar{x}$ intensity (range)
<b>Cestoidea</b>						
<i>Distoichometra bufonis</i>	39%	4 (1-10)	51%	2 (1-11)	10%	2 (1-3)
<b>Nematoda</b>						
<i>Aplectana incerta</i>	—	—	69%	20 (1-68)	16%	4 (1-6)
<i>Aplectana itzocanensis</i>	50%	12 (1-69)	63%	9 (1-35)	39%	7 (1-31)
<i>Physaloptera</i> sp.	22%	3 (1-9)	2%	1	3%	8
<i>Rhabdias americanus</i>	14%	20 (1-67)	6%	2 (1-5)	—	—

140514-140562); *B. cognatus* (LACM 140563-140598); and *S. multiplicata* (LACM 140599-140629).

Toads were 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 lungs, esophagus, stomach, small intestine, large intestine, and bladder of each toad were examined separately. Each helminth was removed and placed on a microscope slide in a drop of undiluted glycerol. A coverslip was added, and the slide was set aside until the helminth became transparent. Each helminth was identified using this glycerol wet-mount method. Representative cestodes were stained with hematoxylin and mounted in balsam for further examination.

One cestode, *Distoichometra bufonis* Dickey, 1921, and 4 nematodes, *Aplectana incerta* Caballero, 1949, *Aplectana itzocanensis* Bravo Hollis, 1943, *Rhabdias americanus* Baker, 1978, and third-stage *Physaloptera* sp. were found. Lesions or scars attributable to parasitic infection were not observed. Terminology use is in accordance with Margolis et al. (1982). Prevalence and mean intensity of infection varied across host species (Table 1). Selected intact specimens were placed in vials of 70% ethanol and deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland, U.S.A.: *B. cognatus*: *Distoichometra bufonis* (83295), *Aplectana itzocanensis* (83296), *Physaloptera* sp. (83294), *Rhabdias americanus* (83660); *B. debilis*: *D. bufonis* (83297), *A. incerta* (83298), *A. itzocanensis* (83299), *Physaloptera* sp. (83362), *Rhabdias americanus* (83361) and *S. multiplicata*: *D. bu-*

*fonis* (83302), *A. incerta* (83303), *A. itzocanensis* (83304), *Physaloptera* sp. (83301).

Twenty-eight of 36 (78%) *B. cognatus* harbored helminths: 6 of 7 males (86%), 11 of 15 females (73%), and 10 of 14 juveniles (71%). A total of 394 helminths were found: 54 *D. bufonis*, 216 *A. itzocanensis*, 25 third-stage *Physaloptera* sp. and 99 *R. americanus*. *Aplectana itzocanensis* had the highest prevalence (50%); *Rhabdias americanus* had the greatest mean intensity (20). There was no significant difference in prevalence of infection among male, female, and juvenile toads: *D. bufonis* (3, 5, and 6 infected male, female, and juvenile toads, respectively; chi-square = 0.14, 2 df,  $P > 0.05$ ), *A. itzocanensis* (4, 8, and 6; chi-square = 0.16, 2 df,  $P > 0.05$ ), *Physaloptera* sp. (2, 5, and 1; chi-square = 2.06, 2 df,  $P > 0.05$ ), or *R. americanus* (1, 2, and 2; chi-square = 0, 2 df,  $P > 0.05$ ). Of the infected toads, 15 were infected by a single species of helminth (6 with *D. bufonis*, 6 with *A. itzocanensis*, 2 with *Physaloptera* sp., and 1 with *R. americanus*), 9 were infected by 2 species (4 with *A. itzocanensis* and *Physaloptera* sp., 3 with *D. bufonis* and *A. itzocanensis*, 1 with *A. itzocanensis* and *R. americanus*, and 1 with *D. bufonis* and *Physaloptera* sp.), and 4 were infected by 3 species (3 with *D. bufonis*, *A. itzocanensis*, and *R. americanus*, 1 with *D. bufonis*, *A. itzocanensis*, and *Physaloptera* sp.). Mean intensity for total helminth load was 11 (1-43). There was no correlation between total number of helminths and SVL ( $r = 0.6$ ).

Forty-eight of 49 (98%) *B. debilis* harbored helminths: 37 of 38 male toads (97%) and 11 of 11 females (100%). A total of 1,024 helminths were found: 57 *D. bufonis*, 674 *A. incerta*, 285 *A. itzocanensis*, 1 *Physaloptera* sp., and 7 *R.*

*americanus*. *Aplectana incerta* had the highest prevalence (69%) and greatest mean intensity (20). There was no significant difference in prevalence of infection between male and female toads: *D. bufonis* (18 males and 6 females infected; chi-square [Yate's correction] = 0.30, 1 df,  $P > 0.05$ ), *A. incerta* (28 and 6; chi-square = 0.33, 1 df,  $P > 0.05$ ), *A. itzocanensis* (23 and 6; chi-square = 0.05, 1 df,  $P > 0.05$ ) and *R. americanus* (2 and 1; chi-square = 0.17, 1 df,  $P > 0.05$ ); a single male was infected with *Physaloptera* sp. Of the infected toads, 14 were infected by a single species of helminth (7 with *A. incerta*, 4 with *A. itzocanensis*, and 3 with *D. bufonis*); 22 were infected by 2 species (9 with *A. incerta* and *A. itzocanensis*, 7 with *D. bufonis* and *A. incerta*, 4 with *D. bufonis* and *A. itzocanensis*, and 2 with *A. itzocanensis* and *R. americanus*); 12 were infected by 3 species (11 with *D. bufonis*, *A. incerta*, and *A. itzocanensis* and 1 with *D. bufonis*, *A. itzocanensis*, and *Physaloptera* sp.). There was no correlation between total number of helminths and SVL ( $r = 0.27$ ). *Aplectana incerta* and *A. itzocanensis* cooccurred in 16 *B. debilis*; in 10 (63%) of these cooccurrences, *A. incerta* had the higher intensity.

Seventeen of 31 (55%) *S. multiplicata* harbored helminths: 5 of 10 males (50%) and 12 of 21 females (57%). A total of 115 helminths were found: 6 *D. bufonis*, 21 *A. incerta*, 80 *A. itzocanensis*, and 8 *Physaloptera* sp. *Aplectana itzocanensis* had the highest prevalence (39%) and mean intensity (7). There was no significant difference in prevalence between male and female toads: *D. bufonis* (1 male and 2 females infected; chi-square [Yate's correction] = 0.01, 1 df,  $P > 0.05$ ), *A. incerta* (1 and 4; chi-square = 0.30, 1 df,  $P > 0.05$ ), *A. itzocanensis* (3 and 9; chi-square = 0.21, 1 df,  $P > 0.05$ ); a single male was infected by *Physaloptera* sp. Of the infected toads, 14 were infected by a single species of helminth (9 with *A. itzocanensis*, 3 with *A. incerta*, 1 with *Physaloptera* sp., and 1 with *D. bufonis*), 2 with 2 species (1 with *A. incerta* and *A. itzocanensis* and 1 with *D. bufonis* and *A. itzocanensis*), and 1 with 3 species (*D. bufonis*, *A. incerta*, and *A. itzocanensis*). There was no correlation between total number of helminths and SVL ( $r = 0.11$ ). *Aplectana incerta* and *A. itzocanensis* cooccurred in 2 *S. multiplicata*.

None of the parasites found in this study were unique to *B. cognatus*, *B. debilis*, or *S. multiplicata*; however, *B. debilis* is a new host record for

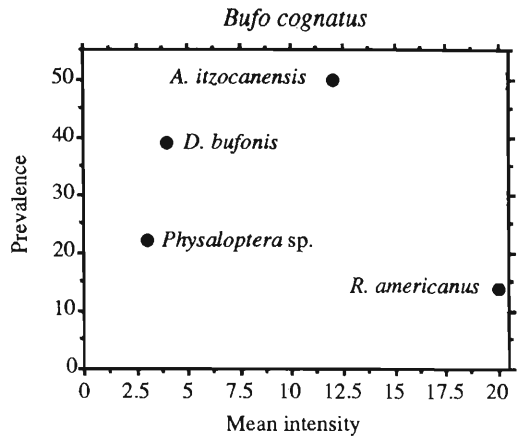


Figure 1. Scattergram of prevalence versus mean intensity of helminths from *Bufo cognatus*. Core species appears in the upper-right quadrant.

*A. incerta*, *A. itzocanensis*, *Physaloptera* sp., and *R. americanus*, and *S. multiplicata* is a new host record for *A. incerta* and *Physaloptera* sp. New Mexico is a new locality record for each of these species of helminths. In each host species, the composite helminth community is depauperate with more than 55% of the individual helminths belonging to a single species. The compound helminth community for these sympatric toads is limited to 5 species.

Because core species are defined as those species that occur with relatively high prevalence and mean intensity, whereas satellite species occur with less frequency and are relatively less numerous than core species (Caswell, 1978; Hanski, 1982), we constructed a scatter plot of prevalence and mean intensity in order to categorize members of the component parasite community (Figs. 1–3). As these plots give equal weight to prevalence and mean intensity, we would expect core species to appear in the upper-right quadrant of the graph and satellite species to appear in the other quadrants. Several authors (Bush and Holmes, 1986; Stock and Holmes, 1987; Kennedy and Bakke, 1989) consider core species as those that have prevalences higher than 70%. Roca and Hornero (1992) defined core species in depauperate reptile communities as those species with prevalences greater than 30%. For each host, we defined a single core species: *Aplectana itzocanensis* for *Bufo cognatus* and *Spea multiplicata*, and *A. incerta* for *B. debilis*.

Three species of *Aplectana* are known from

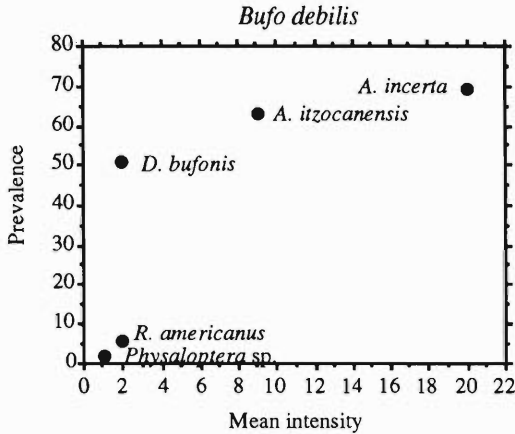


Figure 2. Scattergram of prevalence versus mean intensity of helminths from *Bufo debilis*. Core species appears in the upper-right quadrant.

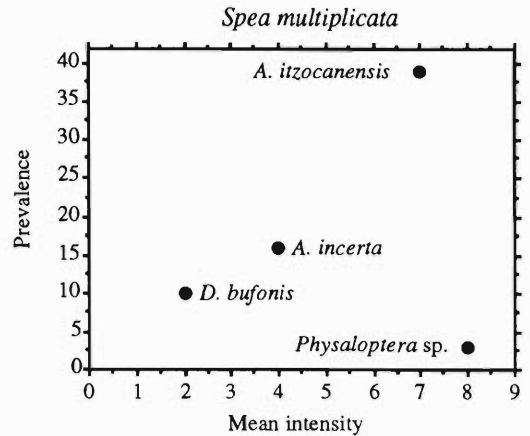


Figure 3. Scattergram of prevalence versus mean intensity of helminths from *Spea multiplicata*. Core species appears in the upper-right quadrant.

North America. *Aplectana incerta* was originally described by Caballero y C. (1949) from *Bufo marinus* from Chiapas, Mexico, and has been redescribed from type specimens by Baker (1985). It is also known from *Scaphiopus couchii* from Arizona (Goldberg and Bursley, 1991). *Aplectana itzocanensis* was originally described by Bravo H. (1943) from *Spea* (= *Scaphiopus*) *multiplicata* and *B. marinus* from Puebla, Mexico. It was also redescribed by Baker (1985) from *Bufo woodhousii woodhousii*. It has been reported in *B. marinus* from Costa Rica (Brenes and Bravo Hollis, 1959) and Veracruz, Mexico (Caballero Deloya, 1974), as well as *B. alvarius* and *B. cognatus* from Arizona (Goldberg and Bursley, 1991) and *S. couchii* from Arizona (Tinsley, 1990). The third North American species, *A. hoffmani*, originally described by Bravo H. (1943) from *S. multiplicata*, is considered by Baker (1985) to be synonymous with *A. itzocanensis*. Thus, the colonization abilities of species of *Aplectana* appear to be rather limited. Although the life histories of American species of *Aplectana* have not been studied, Chabaud and Brygoo (1958) studied *Aplectana courdurieri* and showed that infection in adult toads is acquired when larvae are swallowed by tadpoles and retained through metamorphosis or when larvae are accidentally swallowed by adult toads.

Of the satellite helminth species, infection by *Distoichometra bufonis* and *Physaloptera* sp. occurs through the ingestion of insects while *Rhabdias americanus* infects the host by skin penetration. *Rhabdias americanus* is known only from

species of *Bufo*; *Distoichometra bufonis* is known from species of *Bufo*, *Scaphiopus*, and *Spea* (see Baker, 1987). To our knowledge, no cases of parasitism of toads by adult physalopterans have been reported, although they are commonly found in reptiles, birds, and mammals (Anderson, 1992). Goldberg et al. (1993) listed herpetiles infected by larval physalopterans only (infection by adults is unknown in these species); *B. debilis* and *S. multiplicata* should be added to that list. Because development to adult stages does not occur and because these larvae do not encyst as would be expected in paratenic hosts, we consider infection by larval physalopterans to be incidental, a byproduct of prey ingestion, and unimportant in the study of helminth community dynamics of toads.

In conclusion, our results confirm that the helminth communities of the 3 toad species are depauperate and conform with the pattern of isolationist communities. We would predict that any other species of toad in this study area would harbor parasites from the compound community.

We thank Paul Hyder (New Mexico State University) for assistance in collection of specimens.

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Research Note

Gastrointestinal Helminths of Three Introduced Anoles:  
*Anolis bimaculatus leachi*, *Anolis grahami*, and *Anolis roquet* (Polychridae)  
from Bermuda

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**ABSTRACT:** *Anolis bimaculatus leachi* ( $N = 4$ ), *Anolis grahami* ( $N = 59$ ), and *Anolis roquet* ( $N = 11$ ) from Bermuda were examined for gastrointestinal helminths. *Anolis bimaculatus leachi* harbored *Parapharyngodon cubensis* and larvae of *Abbreviata* sp.; *Anolis grahami* harbored *Atractis scelopori*, *Parapharyngodon cubensis*, and *Protrellus aurifluus* (a parasite of cockroaches); *Anolis roquet* harbored *Atractis scelopori* and larvae of *Abbreviata* sp. *Anolis bimaculatus leachi* is a new host record for *Abbreviata* sp.; *Anolis grahami* is a new record for *Protrellus aurifluus*; *Anolis roquet* is a new host record for *Atractis scelopori* and *Abbreviata* sp.

**KEY WORDS:** Nematoda, *Atractis scelopori*, *Parapharyngodon cubensis*, *Protrellus aurifluus*, *Abbreviata* sp., *Anolis bimaculatus leachi*, *Anolis grahami*, *Anolis roquet*, Polychridae.

The terrestrial herpetofauna of Bermuda is limited to 1 endemic skink, *Eumeces longirostris* Cope, 1861, and 3 introduced anoles, *Anolis bimaculatus leachi* Duméril and Bibron, 1837, *Anolis grahami* Gray, 1845, and *Anolis roquet* Lacépède, 1788. Each anole originated from a different West Indian island: *Anolis bimaculatus leachi* is native to Antigua and Barbuda; *Anolis grahami* is native to Jamaica; and *Anolis roquet* is native to Martinique (Schwartz and Henderson, 1991). However, only the introduction of *Anolis grahami* is documented. It was deliberately introduced from the Kingston area of Jamaica in September 1905 in an attempt to control the fruit fly *Ceratitis capitata*, and by 1909 it was well established in Bermuda (Wingate, 1965). Apparently, there are no accounts of helminths from *Eumeces longirostris* or *Anolis roquet*, but Bundy et al. (1987) reported helminths of *Anolis grahami* from Jamaica and Dobson et al. (1992) reported helminths of *Anolis bimaculatus leachi* from Antigua. The purpose of this note is to report the helminth faunas of *Anolis bimaculatus leachi*, *Anolis grahami*, and *Anolis roquet* from Bermuda.

Four *Anolis bimaculatus leachi* (mean snout-vent length [SVL] = 79.3 mm  $\pm$  20.7 SD, range 65–110 mm) were collected at Warwick Pond, vicinity Middle Road, Warwick Parish (69°49'N, 32°16'W), 18 August 1992. Fifty-nine *Anolis grahami* (mean SVL = 58.9 mm  $\pm$  11.1 SD, range 22–76 mm) were collected at the Bermuda Biological Station for Research, St. George's Parish (64°42'N, 32°22'W), 14–18 August 1992. Eleven *Anolis roquet* were collected (mean SVL = 57.6 mm  $\pm$  8.9 SD, range 49–71 mm). Six were collected at the Bermuda Maritime Museum, Sandy's Parish (64°52'N, 32°18'W), 17 August 1992; 5 were collected at Long Bay, Sandy's Parish (64°52'N, 32°22'W), 18 August 1992. All specimens were collected by hand-held noose and preserved in 10% formalin. The abdominal wall was slit to allow rapid penetration of fixative. The body was opened by a longitudinal incision from throat to vent and the gastrointestinal tract was removed by cutting across the esophagus and rectum. The esophagus, stomach, and small and large intestine were examined separately under a dissecting microscope. Nematodes were removed and identified utilizing the standard glycerol wet-mount procedure.

Helminth fauna for these anoles were limited to 2 monoxenous nematodes, *Atractis scelopori* (Gedoelst, 1919) and *Parapharyngodon cubensis* (Barus and Coy Otero, 1969) Barus, 1973. Also found were encysted larvae of *Abbreviata* sp. and *Protrellus aurifluus* (Chitwood, 1932) Chitwood, 1933, presumably a pseudoparasite in anoles since it is commonly found as a parasite of cockroaches (see Skryabin et al., 1951). Data on infection prevalence, intensity, and location are given in Table 1. *Anolis bimaculatus leachi* is a new host record for *Abbreviata* sp. *Anolis grahami* is a new record for *Protrellus aurifluus*. *Anolis roquet* is a new host record for *Atractis scelopori* and *Abbreviata* sp. Voucher specimens were deposited



in the U.S. National Parasite Collection, Beltsville, Maryland 20705: *Anolis bimaculatus leachi*; *Abbreviata* sp. (larvae, 83868); *Parapharyngodon cubensis* (83867). *Anolis grahmi*; *Atractis scelopori* (83864); *Parapharyngodon cubensis* (83865). *Protrellus aurifluus* (83866). *Anolis roquet*; *Abbreviata* sp. (larvae, 83870); *Atractis scelopori* (83869). Anoles were deposited in the herpetology collection of the Natural History Museum of Los Angeles County (LACM): *Anolis bimaculatus leachi* (140346–140349); *Anolis grahmi* (140361–140420); and *Anolis roquet* (140350–140360).

The nematodes reported here are shared with other herptile species. *Parapharyngodon cubensis* is widely distributed in the Caribbean (see Baker, 1987; Bundy et al., 1987; Dobson et al., 1992), where it has been reported from 21 other anole species as well as amphisbaenid, gekkonid, teiid, and tropidurid lizards and colubrid snakes. *Atractis scelopori* is broadly distributed in the Caribbean, Mexico, and Central and North America (see Baker, 1987), where it has been recorded from 22 other anole species as well as gekkonid, polychrid, teiid, and tropidurid lizards. Encysted larvae of *Abbreviata* sp. have been reported from 4 other anole species as well as gekkonid, teiid, and tropidurid lizards from Cuba (Coy Otero and Barus, 1979) and a eleuthero-dactylid frog from Bermuda (Goldberg et al., 1995). The *Abbreviata* sp. larvae were encysted in organ surfaces, which suggests to us that these herptiles were paratenic hosts rather than definitive hosts. No adult *Abbreviata* sp. has been reported from any species of *Anolis* (see Baker, 1987).

Because no parasite list exists for the endemic skink *Eumeces longirostris*, it is not known whether the preceding nematodes were previously in Bermuda or they arrived with the introduced anoles. It is of interest to note that both *Atractis scelopori* and *Parapharyngodon cubensis* have previously been reported in *Anolis grahmi* from Jamaica (Bundy et al., 1987). Thus, it is conceivable that these nematode species were present in the introduced *Anolis grahmi*.

Both *Anolis bimaculatus leachi* and *Anolis roquet* are sympatric with *Anolis grahmi*; therefore, it would not be unexpected that contact between these different anole species or soil contaminated with their feces would promote infection by the monoxenous nematodes *Atractis scelopori* and *Parapharyngodon cubensis*. Species of *Abbreviata*, heteroxenous nematodes, are com-

Table 1. Helminths from *Anolis bimaculatus leachi*, *Anolis grahmi*, and *Anolis roquet* from Bermuda.

Nematode	<i>Anolis bimaculatus leachi</i> (N = 4)			<i>Anolis grahmi</i> (N = 59)			<i>Anolis roquet</i> (N = 11)		
	Prevalence	$\bar{x}$ intensity (range)	Location*	Prevalence	$\bar{x}$ intensity (range)	Location	Prevalence	$\bar{x}$ intensity (range)	Location
Oxyurida									
<i>Parapharyngodon cubensis</i>	75%	4.7 (1–9)	c	10%	1.5 (1–3)	c	—	—	—
Ascaridida									
<i>Atractis scelopori</i>	—	—	—	19%	40.9 (2–138)	b, c	18%	6.0 (5–7)	c
<i>Protrellus aurifluus</i>	—	—	—	2%	2	b	—	—	—
Spirurida									
<i>Abbreviata</i> sp. (larvae)	50%	1.5 (1–2)	a	—	—	—	9%	7	a

\* Abbreviations: a = stomach; b = small intestine; c = large intestine.

mon parasites of mammals and reptiles but do not occur as parasites in birds (Morgan, 1946). Roca (1993) suggested that the importance of lizards as prey can be ascertained by the prevalence of larval helminths in the lizard population. More work will be required to elucidate the life cycle of these encysted *Abbreviata* and to determine whether or not the anoles are prey items in any mammals of Bermuda.

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#### Research Note

### Helminths of an Introduced Population of the Giant Toad, *Bufo marinus* (Anura: Bufonidae), from Bermuda

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**ABSTRACT:** Forty-five giant toads, *Bufo marinus*, from Bermuda were examined for helminths. Two nematode species were found: *Aplectana* sp. (87% prevalence) and *Rhabdias fuelleborni* (71% prevalence). One toad harbored the trematode *Mesocoeilium monas* (2% prevalence). Bermuda represents a new distributional record for *M. monas* and *R. fuelleborni*.

**KEY WORDS:** Trematoda, *Mesocoeilium monas*, Nematoda, *Aplectana* sp., *Rhabdias fuelleborni*, *Bufo marinus* (Bufonidae).

The giant toad, *Bufo marinus* (Linnaeus, 1758), originally ranged from southern Texas to central Brazil but has since been introduced into the Caribbean Islands, Hawaii, Fiji, Philippines, Taiwan, Ryukyus, New Guinea, Australia, and many Pacific islands (Frost, 1985). Specimens from Guiana were introduced into Bermuda about 1885 (Wingate, 1965). The purpose of this

note is to report the helminths harbored by *B. marinus* from Bermuda. Helminths infecting other populations of *B. marinus* have been summarized by Speare (1990).

Forty-five adult *B. marinus* (mean snout-vent length = 122.4 mm  $\pm$  2.5 SE, range 81–155) were hand-collected on the grounds of the Bermuda Biological Station for Research, St. George's Parish (64°42'N, 32°22'W), Bermuda, 14–18 August 1992. The sample consisted of 27 males and 18 females. The toads were fixed in neutral-buffered 10% formalin. The body was opened by a longitudinal incision from throat to vent and the gastrointestinal tract was removed by cutting across the esophagus and rectum; the lungs, liver, and urinary bladder were also examined. Each organ was examined separately under a dissecting microscope. Helminths were placed on a glass slide in a drop of glycerol and covered with a coverslip. They were allowed to clear overnight before identification was attempted utilizing a compound microscope. Terminology usage is in accordance with Margolis et al. (1982). Selected helminths were deposited in the U.S. National Parasite Collection (USDA, Beltsville, Maryland): *Mesocoelium monas*, 83794; *Aplectana* sp., 83795; *Rhabdias fuelleborni* adults, 83796, *R. fuelleborni* larvae, 83797. All toads were deposited in the herpetology collection of the Natural History Museum of Los Angeles County, LACM 140191–140235.

Forty of 45 (89%) *B. marinus* harbored helminths: 24 of 27 males (89%), and 16 of 18 females (89%). The trematode *Mesocoelium monas* (Rudolphi, 1819) Freitas, 1958, was found in the small intestine of 1 female toad (prevalence 2%; intensity 23.0). *Aplectana* sp. (females only) were found in the small and large intestines of 39 toads (prevalence 87%, mean intensity = 20.6  $\pm$  4.3 SE, range 1–109, 802 nematodes); 23 males (85%) and 16 females (89%) were infected. *Rhabdias fuelleborni* Travassos, 1926 (larvae and hermaphroditic adults), were found in 32 toads (prevalence 71%, mean intensity = 9.1  $\pm$  2.5 SE, range 1–53, 291 nematodes). Of these, 26 toads (17 males, 65%; 9 females, 35%) harbored hermaphroditic adults in the lungs (prevalence 58%, mean intensity = 10.5  $\pm$  3.0 SE, range 1–52, 272 nematodes) and 9 toads (7 males, 26%; 2 females, 11%) had larvae in the stomach, small or large intestine (prevalence 20%, mean intensity = 2.1  $\pm$  5.1 SE, range 1–8, 19 nematodes). There was no significant difference in prevalence of infection by nematode species between male and fe-

male toads (*Aplectana* sp., chi-square = 0.01, 1 df,  $P > 0.05$ ; *R. fuelleborni*, chi-square = 0.44, 1 df,  $P > 0.05$ ).

Williams (1959) published the results of the only previous parasitological investigation of *B. marinus* from Bermuda. Thirty-three of 40 toads (83%) harbored *Rhabdias sphaerocephala* in the lungs; a maximum of 50 nematodes were found. Ten of 40 toads (25%) harbored *Aplectana velardi*; a maximum of 130 nematodes were found. Although we question the identity of the species, prevalence and maximum number for *Rhabdias* in these 2 reports are similar: 71% with a maximum of 53 (our study) versus 83% with a maximum of 50 (Williams, 1959). Prevalence of *Aplectana* in our study is more than triple that reported by Williams (1959); 87% compared to 25%; however, maximum numbers are similar: 109 (our study) compared to 130. Since *Rhabdias* and *Aplectana* are monoxenous, these differences may simply reflect the patchiness of infective larvae. Williams (1960) experimentally infected *B. marinus* with *Rhabdias* larvae recovered from fresh toad feces.

Species of *Rhabdias* are lung parasites of amphibians and reptiles (Anderson, 1992). Those previously reported from *Bufo marinus* include *R. fuelleborni*, Brazil (Kloss, 1971) and Guatemala (Caballero y C., 1954, = *B. horribilis*), and *R. sphaerocephala*, Mexico (Bravo H. and Caballero y C., 1940), Costa Rica (Brenes and Bravo Hollis, 1959), Bermuda (Williams, 1960), Jamaica (Colam, 1971), and Brazil and Paraguay (Kloss, 1971, 1974). Baker (1987) considers *R. sphaerocephala* to be a European species and that American records of its occurrence need confirmation. The species of *Rhabdias* in this study lack the anterior body wall swelling and cuticular inflation as well as the anterior esophageal swelling of *R. sphaerocephala* and is somewhat shorter. It best fits the description of *R. fuelleborni* as given by Travassos (1926a). This report represents a new distributional record for *R. fuelleborni*.

Forty nominal species of *Aplectana* occur as intestinal parasites of amphibians and reptiles (see Baker, 1987). Males are known for all of these species. Barus (1972) described 2 species of *Aplectana* from *Eleutherodactylus* spp. from Cuba. Males were not found for either species, but 1 was named *Aplectana cubana*; the other was not named. Because species identification in *Aplectana* cannot be determined in the absence of males, Baker (1987) considered *A. cubana* as

a species incertae sedis. Goldberg et al. (1995) reported a similar *Aplectana* sp. in *Eleutherodactylus johnstonei* from Bermuda; that is, males were absent. Individuals of *Aplectana* reported herein are larger (2.10–2.80 mm) than those from *Eleutherodactylus johnstonei* (1.70–2.00). However, when intensities in *B. marinus* are greater than 100 individuals, maximum *Aplectana* length is limited to about 2 mm. Individual nematodes from *B. marinus* frequently contained more eggs than did those from *E. johnstonei*. Otherwise, no morphological differences were noted in specimens of *Aplectana* from the 2 amphibian species. In both nematodes, the reproductive systems were didelphic and prodelphic; eggs were 63–74  $\mu\text{m} \times$  40–51  $\mu\text{m}$  and in many cases contained a coiled larva. Most likely the same species of *Aplectana* infects both *B. marinus* and *E. johnstonei* in Bermuda. However, with the absence of males, species identification is not possible. Size differences are likely due either to crowding, a well-described phenomenon in helminths (Morgan, 1942), or related to size differences between the 2 hosts; our Bermuda *B. marinus* sample is approximately 5 times larger than our *E. johnstonei* sample (Goldberg et al., 1995). Other species of *Aplectana* have been reported from *B. marinus*: *A. hoffmanni* from Costa Rica (Brenes and Bravo Hollis, 1959); *A. incerta* from Mexico (Caballero y C., 1949); *A. itzocanensis* from Costa Rica by Brenes and Bravo Hollis (1959) and Mexico (Caballero Deloya, 1974); and *A. vellardi* from Brazil (Travassos, 1926b).

Freitas (1963) synonymized 19 species of the genus *Mesocoelium* from a wide variety of amphibians and reptiles with *M. monas*. The list of synonymized species was expanded to 23 by Nasir and Diaz (1971), who, in the process, recognized only 4 species. *Mesocoelium monas* has been recovered from *B. marinus* from widely separated geographical regions such as Brazil, Columbia, Costa Rica, Hawaii, Paraguay, Puerto Rico (Nasir and Diaz, 1971), Jamaica (Wong and Bundy, 1985), and American Samoa (Goldberg and Bursey, 1992). This is the first report of *M. monas* from the North Atlantic islands.

The herpetofauna of Bermuda is limited to 1 endemic skink, *Eumeces longirostris*, 3 introduced anoles, *Anolis bimaculatus leachi*, *A. grahami*, and *A. roquet*, and 2 eleutherodactylid frogs, *Eleutherodactylus gossei* and *E. johnstonei* and *B. marinus* (Wingate, 1965). Whether the helminths of Bermuda *B. marinus* were acquired

from contact with sympatric herptiles or may have been present in the introduced toads is not known.

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### Research Note

## Gastrointestinal Helminths of *Eleutherodactylus johnstonei* (Leptodactylidae) from Bermuda

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**ABSTRACT:** Eighty-four leptodactylid frogs, *Eleutherodactylus johnstonei*, were collected in August 1992 from St. George's Parish, Bermuda, and examined for gastrointestinal helminths. Forty-five (54%) harbored 1 or more species of nematode: 18 with *Aplectana* sp. (21% prevalence, mean intensity  $6.6 \pm 2.2$  SE), 25 with *Parapharyngodon garciae* (30%,  $1.5 \pm 0.2$  SE), and 3 with larval physalopterans (4%,  $1.7 \pm 0.7$  SE). *Eleutherodactylus johnstonei* represents a new host record for each of these species of nematodes.

**KEY WORDS:** Nematoda, *Aplectana* sp., *Parapharyngodon garciae*, physalopteran larvae, *Eleutherodactylus johnstonei*, Leptodactylidae.

The leptodactylid frog, *Eleutherodactylus johnstonei* Barbour, 1914, is known from Anguilla, Antigua, Barbados, Barbuda, Grenada, Guadeloupe, Jamaica, Montserrat, Nevis, Saba,

St. Barthélemy, St. Christopher, St. Eustatius, St. Lucia, St. Martin, and St. Vincent and is found from sea level to 853 m (Schwartz and Henderson, 1991) on these Caribbean islands. *Eleutherodactylus johnstonei* was accidentally introduced into Bermuda, probably from Jamaica, about 1886 (Pope, 1917). To our knowledge, there are no previous reports of helminths from this frog. The purpose of this report is to report the gastrointestinal helminths of *E. johnstonei* from Bermuda.

Eighty-four *E. johnstonei* (mean snout-vent length =  $24.6 \text{ mm} \pm 0.4$  SE, range 11–31) were hand-collected on the grounds of the Bermuda Biological Station for Research, St. George's Parish (64°42'N, 32°22'W), Bermuda, 14–18 August

Table 1. Records of *Aplectana* spp. in eleutherodactylid frogs.

Host	<i>Aplectana</i>	Locality	Reference
<i>Eleutherodactylus acmonis</i>	<i>Aplectana cubana</i> *	Cuba	Coy Otero and Ventosa, 1984
<i>E. albipes</i>	<i>Aplectana cubana</i> *	Cuba	Coy Otero and Ventosa, 1984
	<i>Aplectana hamatospicula</i>	Cuba	Coy Otero and Ventosa, 1984
<i>E. coqui</i>	<i>Aplectana</i> sp.	Puerto Rico	Schmidt and Whittaker, 1975
<i>E. cuneatus</i>	<i>Aplectana</i> sp.	Cuba	Barus, 1972
	<i>Aplectana cubana</i> *	Cuba	Barus, 1972
	<i>Aplectana cubana</i> *	Cuba	Coy Otero and Ventosa, 1984
	<i>Aplectana hamatospicula</i>	Cuba	Coy Otero and Ventosa, 1984
<i>E. dimidiatus</i>	<i>Aplectana</i> sp.	Cuba	Barus, 1972
	<i>Aplectana cubana</i> *	Cuba	Barus, 1972
	<i>Aplectana cubana</i> *	Cuba	Coy Otero and Ventosa, 1984
	<i>Aplectana hamatospicula</i>	Cuba	Coy Otero and Ventosa, 1984
<i>E. eileenae</i>	<i>Aplectana</i> sp.	Cuba	Barus, 1972
	<i>Aplectana cubana</i> *	Cuba	Coy Otero and Ventosa, 1984
<i>E. gundlachi</i>	<i>Aplectana cubana</i> *	Cuba	Coy Otero and Ventosa, 1984
	<i>Aplectana hamatospicula</i>	Cuba	Coy Otero and Ventosa, 1984
<i>E. johnstonei</i>	<i>Aplectana</i> sp.	Bermuda	This paper
<i>E. klinikowskii</i>	<i>Aplectana cubana</i> *	Cuba	Coy Otero and Ventosa, 1984
	<i>Aplectana hamatospicula</i>	Cuba	Coy Otero and Ventosa, 1984
<i>E. locustus</i>	<i>Aplectana</i> sp.	Puerto Rico	Schmidt and Whittaker, 1975
<i>E. pinarensis</i>	<i>Aplectana cubana</i> *	Cuba	Coy Otero and Ventosa, 1984
<i>E. planirostris</i>	<i>Aplectana cubana</i> *	Cuba	Coy Otero and Ventosa, 1984
<i>E. richmondi</i>	<i>Aplectana</i> sp.	Puerto Rico	Schmidt and Whittaker, 1975
<i>E. sierramaestrae</i>	<i>Aplectana cubana</i> *	Cuba	Coy Otero and Ventosa, 1984
	<i>Aplectana hamatospicula</i>	Cuba	Coy Otero and Ventosa, 1984
<i>E. symingtoni</i>	<i>Aplectana cubana</i> *	Cuba	Coy Otero and Ventosa, 1984
	<i>Aplectana hamatospicula</i>	Cuba	Coy Otero and Ventosa, 1984
<i>E. turquinensis</i>	<i>Aplectana cubana</i> *	Cuba	Coy Otero and Ventosa, 1984
<i>E. zeus</i>	<i>Aplectana hamatospicula</i>	Cuba	Coy otero and Ventosa, 1984
<i>E. zugi</i>	<i>Aplectana cubana</i> *	Cuba	Barus, 1972

\* *Species incertae sedis*, only females are known.

1992. The sample consisted of 28 males and 56 females. The frogs were sacrificed by an overdose of ethanol, and the abdominal wall was slit to allow rapid penetration of fixative and then immersed in 10% formalin.

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 utilizing the standard glycerol wet mount procedure.

Seventeen of 28 (61%) males and 28 of 56 (50%) females were parasitized. Prevalence and mean intensity are used in accordance with Margolis et al. (1982). Since no male *Aplectana* was found, identification to species was not possible. *Aplectana* sp. (21% prevalence, mean intensity  $6.6 \pm 2.2$  SE) and *Parapharyngodon garciae* Schmidt and Whittaker, 1975 (30% prevalence, mean intensity  $1.5 \pm 0.2$  SE), were found in the

small and/or large intestines, while larval physalopterans (4% prevalence, mean intensity  $1.7 \pm 0.7$  SE) were found in the coelom. There was no significant difference in prevalence of infection between male and female frogs (chi-square = 0.25, 1 df,  $P > 0.05$ ). Four male frogs (14%) harbored *Aplectana* sp. only; 14 female frogs (25%) harbored *Aplectana* sp. only; 12 male frogs (43%) harbored *P. garciae* only; 13 female frogs (23%) harbored *P. garciae* only; 1 male frog (4%) harbored larval physalopterans; and 1 female frog (2%) harbored larval physalopterans. Only 1 female frog harbored 2 species of nematodes, *Aplectana* sp. and larval physalopterans.

Likewise, there was no significant difference in prevalence of infection by nematode species between male and female frogs (*Aplectana* sp., chi-square = 0.62, 1 df,  $P > 0.05$ ; *P. garciae*, chi-square = 2.86, 1 df,  $P > 0.05$ ; larval physalopterans, not tested). These helminths, to our knowledge, represent the first nematodes recovered from *E. johnstonei* and are new host records.

Selected nematode specimens were placed in 70% alcohol in glass vials and deposited in the U.S. National Parasite Collection, Beltsville, Maryland 20705: *Aplectana* sp. (83567); *Parapharyngodon garciae* (83569); larval physalopterans (83568). All frog specimens were deposited in the herpetology collection of the Natural History Museum of Los Angeles County, LACM 140695–140778.

None of the parasites found in this study are unique to *E. johnstonei*. *Aplectana* spp. were previously reported from 16 species of *Eleutherodactylus* (Table 1). *Parapharyngodon garciae* was first described from *Eleutherodactylus antillensis* and *Eleutherodactylus portoricensis* from Puerto Rico by Schmidt and Whittaker (1975) and, until this report, represented the only host species. Barus (1972) found a physalopteran larva in a stomach cyst in *Eleutherodactylus cuneatus* from Cuba (1 of 46, 2%) which he identified as *Abbreviata* sp. Measurements of the physalopteran larvae found in our current study on *E. johnstonei* are similar to those reported by Barus (1972). These larvae were encysted on organ surfaces, which suggests to us that *E. johnstonei* were paratenic hosts only. No adult *Abbreviata* sp. has been reported from any species of *Eleutherodactylus*; however, Baker (1987) reported *Physalopteroides valdesi* in *Eleutherodactylus dimidiatus* and *Eleutherodactylus turquinensis*.

The genus *Eleutherodactylus* contains over 400 species, which are primarily neotropical in distribution (Zug, 1993). Schwartz and Henderson (1991) list 124 species from the Caribbean. There are currently reports of helminths from only 23 eleutherodactylid species (Baker, 1987) or approximately 6% of the genus. These average  $2.5 \pm 0.3$  SE (range 1–6) helminth species per eleuth-

erodactylid species. Clearly, helminthological surveys of many more species of *Eleutherodactylus* are needed before there can be discussion of helminth diversity within this very large genus.

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**Research Note**

**Metazoan Parasites of the Graybelly Salamander,  
*Eurycea multiplicata griseogaster* (Caudata: Plethodontidae), from  
Arkansas**

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**ABSTRACT:** Fifty graybelly salamanders, *Eurycea multiplicata griseogaster* Moore and Hughes, 1941, were collected between December 1988 and 1993 from 7 counties of Arkansas and examined for metazoan parasites. Seven (14%) were infected with 1 or more parasites, including 1 (2%) with *Brachycoelium salamandrae* (Frölich, 1789) Dujardin, 1845, 3 (6%) with *Desmognathinema nantahalaensis* Baker, Goater, and Esch, 1987, 2 (4%) with *Fessisentis vanceavei* (Moore and Hughes, 1943) Nickol, 1972, and 2 (4%) with larval *Hannemania* sp. Oudemans, 1911. New host and distributional records are reported for some of these parasites of *E. multiplicata griseogaster*. In addition, the cave salamander, *Eurycea lucifuga* Rafinesque, 1822, is documented as a new host for *D. nantahalaensis*.

**KEY WORDS:** *Eurycea multiplicata griseogaster*, graybelly salamander, *Eurycea lucifuga*, cave salamander, Amphibia, Caudata, Plethodontidae, *Brachycoelium salamandrae*, *Desmognathinema nantahalaensis*, *Fessisentis vanceavei*, *Hannemania* sp.

The graybelly salamander, *Eurycea multiplicata griseogaster* Moore and Hughes, 1941, is a small plethodontid that ranges from southcentral Missouri and extreme southwestern Kansas to adjacent Arkansas and westward to northeastern Oklahoma (Conant and Collins, 1991). This salamander inhabits cave-fed springs and cool Ozarkian streams where it hides beneath rocks, logs, and debris in seepage areas. The biology of *E. multiplicata griseogaster* was summarized in a species account by Dundee (1965) and Ireland (1970, 1976) reported on the natural history and ecology of this salamander in Arkansas. However, little is known about its helminth parasites (Malewitz, 1956; Buckner and Nickol, 1978; McAllister et al., 1991), and some data are only available from unpublished theses (Bouchard, 1953; Fogle, 1960; Saltarelli, 1977). This note reports some new host and locality records for metazoan parasites of *E. multiplicata griseogaster* from Arkansas.

Fifty larval, neotenic, and adult ( $\bar{x} \pm SE$  snout-vent length [SVL] =  $45.5 \pm 1.1$ , range 35–55 mm) graybelly salamanders were collected alive by dipnet or hand between December 1988 and December 1993 from the following counties (sample sizes in parentheses): Conway (11), Franklin (2), Jackson (2), Pope (9), Van Buren (4), Washington (20), and White (2) counties of Arkansas. Salamanders were considered sexually mature at SVL's of  $\geq 37$  mm (Ireland, 1976). They were kept moist and cool in plastic collecting bags on ice and returned to the laboratory within 24 hr. Salamanders were sacrificed by overdose with an aqueous solution of tricaine methanesulfonate (TMS-222). Methods for salamander processing and preparation and staining of parasites follow McAllister and Upton (1987). Voucher specimens of salamanders are deposited in the Arkansas State University Museum of Zoology (ASUMZ 19223–19238, 19242–19253). Specimens of parasites are deposited in the U.S. National Parasite Collection, Beltsville, Maryland 20705, as follows: *Brachycoelium salamandrae* (USNM 83520), *Desmognathinema nantahalaensis* (USNM 83522–83524), *Fessisentis vanceavei* (USNM 83519), and *Hannemania* sp. (USNM 83521).

Seven (14%) of the *E. multiplicata griseogaster* harbored metazoan parasites, including 1 (2%) with *Brachycoelium salamandrae* (Frölich, 1789) Dujardin, 1845, 3 (6%) with *Desmognathinema nantahalaensis* Baker, Goater, and Esch, 1987, 2 (4%) with *Fessisentis vanceavei* (Moore and Hughes, 1943) Nickol, 1972, and 2 (4%) with larval *Hannemania* sp. Oudemans, 1911. Of the 7 infected salamanders, only 1 (14%) was multiply infected. In addition, none of the *E. multiplicata griseogaster* were found to be passing coccidian oocysts in the feces, and the blood was



negative for intraerythrocytic hematozoans or trypanosomes.

The single specimen of the plagiorchid trematode, *Brachycoelium salamandrae*, was found in the small intestine of an adult female *E. multiplicata griseogaster* (SVL 47 mm, ASUMZ 19247) collected from a stream north of Morrilton, Conway County. This represents a new host record for *B. salamandrae*. Winter et al. (1986) reported *B. storeriae* Harwood, 1932, from the Caddo Mountain salamander, *Plethodon caddoensis* and the Fourche Mountain salamander, *P. fourchensis* in Arkansas. In addition, Rosen and Manis (1976) reported *B. ambystomae* Couch, 1966, and *B. elongatum* Cheng, 1958, from the spotted salamander, *Ambystoma maculatum*, and the Ouachita dusky salamander, *Desmognathus brimleyorum*, in Arkansas, respectively. Other *Eurycea* spp. have been reported as hosts of *B. salamandrae*, including the northern 2-lined salamander, *E. bislineata*, from New York (Fischthal, 1955a), Pennsylvania (Fischthal, 1955b), Massachusetts (Rankin, 1945), and North Carolina (Mann, 1932; Rankin, 1937), the longtail salamander, *E. longicauda*, from Illinois (Landewe, 1963) and North Carolina (Mann, 1932; Rankin, 1937), and the cave salamander, *E. lucifuga*, from Kentucky (Castle et al., 1987) and Illinois (Landewe, 1963). However, because of difficulty in determining specific identity due to morphological variation and the crowding effect, Dyer and Brandon (1973) and Dyer and Peck (1975) have reported *Brachycoelium* sp. from *E. lucifuga* in Illinois and Alabama and Tennessee, respectively.

*Brachycoelium* spp. are the most common flukes encountered in salamanders (Dyer, 1983). However, the continual recognition of numerous species of *Brachycoelium* (Parker, 1941; Cheng, 1958; Cheng and Chase, 1961; Couch, 1966; Dunbar and Moore, 1979; Sellers et al., 1981) or a single species, *B. salamandrae* (Rankin, 1938; Rabalais, 1970), for North American salamanders is not without controversy (see Dyer and Brandon, 1973). Until an exhaustive revision of this morphologically variable genus is complete, we suggest adopting a conservative approach and report only *B. salamandrae* in North American salamanders.

Thirty-five specimens of the seuratoid nematode, *Desmognathinema nantahalaensis*, were found in the small intestine of 3 salamanders, including a larva (35 mm SVL) with 15 specimens and an adult (47 mm SVL) with 10 spec-

imens from the Morrilton site and a neotene (45 mm SVL) with 10 specimens from Savoy Cave, Washington County. In addition, we recently found 5 *D. nantahalaensis* in another host, a single larval cave salamander, *Eurycea lucifuga* (SVL = 30 mm, ASUMZ 19192), from a stream outside of Blowing/Cushman Cave, Independence County, Arkansas (unpubl. obs.). Baker et al. (1987) originally described *D. nantahalaensis* from the blackbelly salamander, *Desmognathus quadramaculatus* (type host), and seal salamander, *D. monticola*, from North Carolina. Interestingly, sympatric species of *Desmognathus* are not present in habitats where the infected graybelly salamanders were collected. Further, it is not known why there appears to be an apparent disjunct pattern in distribution of this parasite, but it could be explained by lack of survey data for salamanders from other regions of North America. *Eurycea lucifuga* and *E. multiplicata griseogaster* are new hosts and Arkansas a new locality for *D. nantahalaensis*.

Fifteen specimens of the acanthocephalan, *Fessisentis vanceavei*, were found in the small intestine of 2 adult salamanders (41 mm SVL, 5 specimens, ASUMZ 19244; 47 mm SVL, 10 specimens, ASUMZ 19245) collected from Van Buren County. Hughes and Moore (1943) originally described *F. vanceavei* from the Oklahoma salamander, *Eurycea tynerensis*, in Cherokee County, Oklahoma. Later, *F. vanceavei* was reported in *E. multiplicata griseogaster* from Cherokee County, Oklahoma (Malewitz, 1956), and Madison and Benton counties, Arkansas (Saltarelli, 1977; Buckner and Nickol, 1978) and in the dark-sided salamander, *E. longicauda melanopleura* (Cope, 1893), from Benton County (Saltarelli, 1977). Fogle (1960), in an unpublished thesis, reported an unknown "acanthocephalan" from *E. multiplicata griseogaster* in northwestern Arkansas. However, since voucher specimens were apparently not deposited, it is unknown whether this acanthocephalan was *F. vanceavei* or another taxon.

Larval intradermal mites, *Hannemanina* sp., infested 2 adult salamanders (43 and 46 mm SVL) collected from Petit Jean State Park and the Morrilton site, Conway County. Unengorged and partially engorged larvae were encapsulated on the neck, appendages, and toes by host dermal connective tissue. Specific identity was not possible since only larvae were found. The graybelly salamander is a new host of *Hannemanina* sp.

*Hannemanina dunnii* Sambon, 1928, has been

reported from Rich Mountain salamanders, *P. ouachitae*, southern redback salamanders, *P. serratus*, *P. caddoensis*, *P. fourchensis*, and *D. brimleyorum* in Arkansas (Dunn and Heinze, 1933; Pope and Pope, 1951; Duncan and Highton, 1979; Winter et al., 1986). Indeed, at least 2 additional species of *Hannemania*, namely, *H. eltoni* Sambon, 1928, and *H. multifemorala* Loomis, 1956, are known from Arkansas amphibians (see Loomis, 1956).

In summary, metazoan parasites of *E. multiplicata griseogaster* appear to be typical of helminths from other plethodontid salamanders (Rankin, 1937; Goater et al., 1987; Aho, 1990) and exhibit little or no host specificity. Interestingly, Saltarelli (1977) reported an unknown species of *Paraquimperia* Baylis, 1934, from *E. multiplicata griseogaster*. However, this identification is doubtful and most probably represents *D. nantahalaensis* (reported herein), a related species in the subfamily Quimperinae Gendre, 1828. Members of the genus *Paraquimperia* are mainly parasitic in eels and have not been reported previously from amphibians (see Moravec, 1967).

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**Research Note**

**Hemogregarines (Apicomplexa) and *Falcaustra chelydrae* (Nematoda) in an Alligator Snapping Turtle, *Macrolemys temminckii* (Reptilia: Testudines), from Arkansas**

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**ABSTRACT:** Two alligator snapping turtles, *Macrolemys temminckii* (Harlan, 1935), were collected from 2 counties in Arkansas and examined for gastrointestinal and hemoparasites. One of the turtles was found to be harboring 3 different hemogregarine forms in erythrocytes, and kathlaniid nematodes, *Falcaustra chelydrae* Harwood, 1932, were recovered from the rectum. This represents the first definitive report with quantitative information and photomicrographs of hemogregarines in *M. temminckii*. In addition, we document *F. chelydrae* in *M. temminckii* for a second time with Arkansas representing a new geographic distribution record for the parasite.

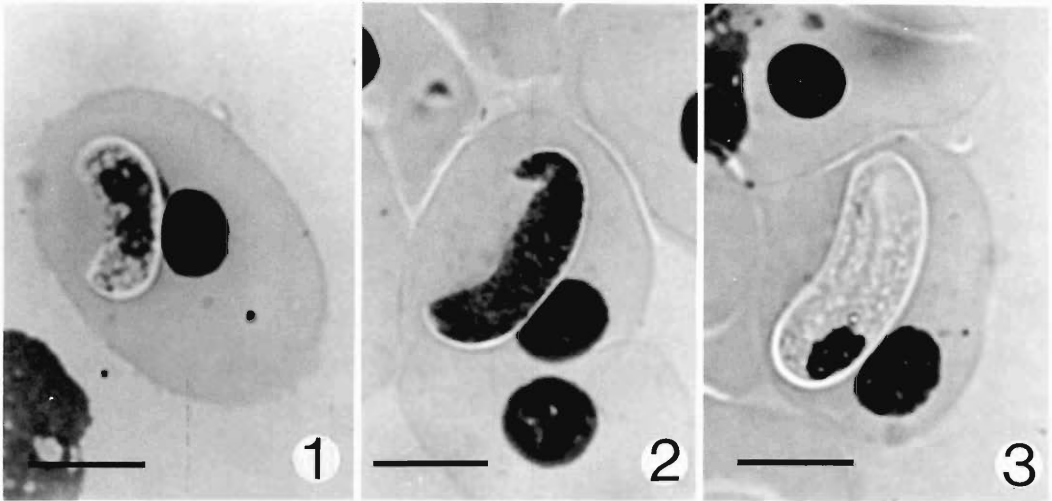
**KEY WORDS:** Apicomplexa, *Falcaustra chelydrae*, Nematoda, gamonts, hemogregarines, *Macrolemys temminckii*, alligator snapping turtle, Testudines, Chelydridae, Arkansas.

The alligator snapping turtle, *Macrolemys temminckii* (Harlan, 1835), is the largest North American freshwater turtle; males may attain weights of more than 140 kg (Lovich, 1993). This turtle is restricted to river systems of the United States that drain into the Gulf of Mexico and is distributed widely from Oklahoma, Kansas, Illinois, and Indiana south to Texas and east to Florida (Conant and Collins, 1991). The species is economically important, has been exploited for years as a food resource and may be disappearing from much of its range (Pritchard, 1989).

Lovich (1993) summarized biological information on *M. temminckii*; however, little is known about its helminth fauna (Ward and Hopkins, 1931; Mackin, 1936; Cahn, 1937; Ernst and Ernst, 1977), and even less information is available on the protozoan parasites of this turtle (Ernst and Ernst, 1979; Upton et al., 1992). The purpose of this note is to provide quantitative information and photomicrographs of hemogregarines from this host and to report a new locality record for a nematode parasite of this turtle.

During early September 1993 and mid-April 1994, 2 adult male *M. temminckii* (carapace length = 51 and 53 cm) were collected alive, 1 from an Oxbow Lake off the Ouachita River in the vicinity of Camden (33°35'N, 92°50'W), Ouachita County, and 1 from Village Creek in the vicinity of Swifton (35°49'N, 91°07'W), Jackson County, Arkansas. The turtles were transported to the laboratory and overdosed with sodium pentobarbital (Nembutal®, Abbott Laboratories, North Chicago, Illinois) by intracardial injection for examination of gastrointestinal and hemoparasites. Blood samples were obtained from heavily anesthetized turtles by tail-clipping, and thin films were methanol-fixed and stained with Giemsa or Wright's. Immediately following euthanasia, the gastrointestinal tract from the esophagus to anus was removed and examined for helminths. Rectal contents and feces were collected, placed in 2.5% (w/v) aqueous potassium dichromate, and processed for coccidia using previously described methods (Upton et al., 1992). Nematodes were preserved in 70% ethanol and examined using a glycerol wet mount. Measurements were made on a total of 55 intraerythrocytic parasites (15–20/form) using a calibrated ocular micrometer under a ×100 oil immersion lens and are reported in micrometers as means ± SD followed by the ranges in parentheses. Mean length and width measurements were tested for statistical significance ( $P \leq 0.05$ ) using 1-way ANOVA followed by a posteriori Student-Newman-Keuls (SNK) procedure for multiple comparisons (SAS Institute, 1988).

Host vouchers are deposited in the Arkansas State University Museum of Zoology as ASUMZ 19261 and 19544. Voucher specimens of parasites are deposited in the U.S. National Parasite



Figures 1–3. Three different hemogregarine forms in erythrocytes of *Macroclmymys temminckii* from Arkansas. 1. Small form resembling a macrogamont. 2. Medium form resembling a microgamont. 3. Large form resembling an immature erythrocytic gamont. Scale bar 10.0  $\mu\text{m}$ .

Collection (USNM), Beltsville, Maryland 20705, as follows: hemogregarines (USNM 83473), *Falcaustra chelydrae* (USNM 83475).

One turtle (Ouachita County specimen, ASUMZ 19261) was found to be harboring parasites, including 3 different hemogregarine forms and a kathlaniid nematode, *Falcaustra chelydrae* Harwood, 1932. Although coccidia have been reported previously in *M. temminckii* from Arkansas (Upton et al., 1992), both turtles examined in this study were negative.

In blood smears, 3 distinct morphological and statistically different types of hemogregarine forms were observed (Figs. 1–3; Table 1). One form was small, resembling a macrogamont, and had a lightly staining cytoplasm, often curved in shape with a centrally located nucleus (Fig. 1).

An intermediate form resembling a microgamont, the least commonly observed, had a basophilic cytoplasm, centrally located nucleus, and a curved tail (Fig. 2). Hemogregarines from turtles are known to exhibit sexual dimorphism in the gamont stage (Siddall and Desser, 1992). Further, Paterson and Desser (1976) noted that gamonts with a slightly recurved tail are microgamonts that divide into microgametes in the intestines of leeches. In size, both micro and macrogamonts were similar to those of *Haemogregarina balli* Paterson and Desser, 1976, described from the common snapping turtle, *Chelydra serpentina* (Linnaeus, 1758), in Ontario, Canada (Paterson and Desser, 1976). The most common form was large and stout and resembled an immature (nondividing) erythrocytic meront

Table 1. Measurements of 3 hemogregarine forms found in erythrocytes of *Macroclmymys temminckii*.

Morphological type	Length ( $\mu\text{m}$ )* $\bar{x} \pm \text{SD}$ (range)	Width ( $\mu\text{m}$ )† $\bar{x} \pm \text{SD}$ (range)
Small form ( $N = 20$ )	12.3 $\pm$ 1.2 (10.4–14.4)	4.6 $\pm$ 0.6 (4.0–5.4)
Medium form ( $N = 15$ )	19.0 $\pm$ 1.2 (16.0–20.8)	4.8 $\pm$ 0.5 (4.0–5.6)
Large form ( $N = 20$ )	32.6 $\pm$ 2.2 (28.0–36.8)	4.0 $\pm$ 0.6 (3.2–4.8)

\* The means of all lengths were significantly different (ANOVA,  $F = 774.73$ ,  $P < 0.00001$ ). All critical values for within mean length comparisons were significantly different at  $P < 0.05$  by SNK procedure ( $q = 16.83$ – $54.83$ ).

† The means of all widths were significantly different (ANOVA,  $F = 11.87$ ,  $P < 0.0005$ ). Critical values for within mean width comparisons were significantly different at  $P < 0.05$  by SNK procedure for small to large ( $q = 4.98$ ) and medium to large forms ( $q = 6.53$ ) but not significantly different for small to medium forms ( $q = 1.92$ ).

with an eccentric nucleus and a curved, tapered tail that was often folded on itself (Fig. 3). This latter form was similar to immature meronts described in detail by Siddall and Desser (1992). Although we have refrained from designating a genus for these hemogregarines at this time, the parasite most probably represents a species of *Haemogregarina* (sensu stricto) or a species of *Hepatozoon*. Indeed, the presence of erythrocytic meronts suggests the former, as the latter does not undergo erythrocytic merogony. Telford (1984) cautioned that hemogregarines cannot be consistently distinguished based solely on their erythrocytic stages.

Hemogregarines have been extensively studied and reported from *C. serpentina* from various states and southern Ontario, Canada (e.g., Desser, 1973; Ernst and Ernst, 1979; Strohle and Christensen, 1984; Siddall and Desser, 1991, 1992). Our report for the first time provides documentation with quantitative information and photomicrographs of hemogregarines in the related chelydrid, *M. temminckii*. In a report on peripheral blood components, Powell and Knesel (1993) reported intraerythrocytic parasites in *M. temminckii* from Louisiana without further comment. In addition, McAllister and King (1980) found 4 different hemogregarine forms in red-eared sliders, *Trachemys scripta elegans* (Wied, 1838), from Arkansas. These forms tended to be smaller and were morphologically different, and photomicrographs of the hemogregarines did not resemble those from *M. temminckii*.

One hundred fifty-seven *Falcaustra chelydrae* were recovered from the rectum of *M. temminckii*. These nematodes fit descriptions of the parasite provided by Harwood (1932) and Mackin (1936). The species was originally described from *C. serpentina* in Texas (Harwood, 1932) and, although reported from *M. temminckii* once previously, the locality was unspecified (Mackin, 1936). However, other turtles reported by Mackin (1936) to be infected with *Falcaustra* spp. originated from areas within the range of *M. temminckii* in southeastern Oklahoma and Illinois. Therefore, we believe our survey to be the first definitive report of *F. chelydrae* from Arkansas. *Falcaustra chelydrae* infects numerous North American freshwater turtles from various localities in the United States and Ontario, Canada (see Baker, 1987), including the adjacent states of Oklahoma (Mackin, 1936; Williams, 1953;

McKnight, 1958) and Tennessee (Reiber, 1941; MacDonald and Litchford, 1974). A related species, *Falcaustra affinis* (Leidy, 1856) Harwood, 1932 (syn. *F. concinnae* Mackin, 1936), was reported by Rosen and Marquardt (1978) from *T. scripta elegans* in Arkansas.

In summary, we have provided new information on 2 endoparasites of *M. temminckii*. However, since there still appears to be a paucity of parasite data on alligator snapping turtles, we suggest that future ecological and natural history studies incorporate survey information from *M. temminckii*, in order to develop a more complete understanding of its host-parasite relationships.

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Research Note

***Hexametra boddaertii* (Nematoda: Ascaridae) in the Sidewinder, *Crotalus cerastes* (Crotalidae), from California**

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**ABSTRACT:** Examination of 40 sidewinder rattlesnakes, *Crotalus cerastes*, revealed the presence of the nematode *Hexametra boddaertii* (prevalence 5%, mean intensity 3.5) in the small intestine. This is a new host record and the first report of a natural infection by the genus *Hexametra* in California.

**KEY WORDS:** Nematoda, Ascarididae, *Hexametra boddaertii*, Reptilia, Viperidae, *Crotalus cerastes*.

The sidewinder, *Crotalus cerastes* Hallowell, 1854, ranges from southern Nevada and extreme southwestern Utah into northeast Baja California, northwest Sonora to southcentral Arizona from below sea level to around 1,830 m (Stebbins, 1985). It is sympatric with a number of snakes: predominantly, coachwhip, *Masticophis flagellum* (Shaw); gopher snake, *Pituophis catenifer* (Blainville); glossy snake, *Arizona elegans* Kennicott; and western shovelnose snake, *Chionactis occipitalis* (Hallowell); but also western blind snake, *Leptotyphlops humilis* (Baird and Girard); rosy boa, *Lichanura trivigata* Cope; spotted leafnose snake, *Phyllorhynchus decurtatus* (Cope); western patchnose snake, *Salvadora hexalepis* (Cope); common kingsnake, *Lampropeltis getula* (Linnaeus); longnose snake, *Rhinocheilus lecontei* Baird and Girard; night snake, *Hypsiglena torquata* (Günther); lyre snake, *Trimorphodon biscutatus* (Duméril, Bibron and Duméril); speckled rattlesnake, *Crotalus mitchelli* (Cope); and Mojave rattlesnake, *Crotalus scutulatus* (Kennicott).

*Hexametra boddaertii* (Baird, 1860) Kreis, 1944, was originally described from a single female specimen taken from a South American colubrine snake, *Mastigodryas boddaerti* (Senzten) Stuart. Baylis (1916, 1920) redescribed the specimen and placed the species in the genus *Polydelphis* Dujardin, 1845, because the specimen had more than 2 uterine branches. Kreis (1944) subsequently transferred the species to the genus *Hexametra*, a genus created by Travassos

(1920) to house ascaridoids with 6 uterine branches. In 1978, Sprent reviewed the genus and placed *Ascaris quadrangularis* Schneider, 1866, *Polydelphis hexauterina* Skrjabin, 1916, and *Hexametra quadricornis* (Wedl, 1861) sensu Araujo, 1969, in synonymy with *H. boddaertii*. Sprent (1978) also examined specimens of *Hexametra* in the collection of the U.S. National Parasite Collection and found that they were *H. boddaertii*; he therefore concluded that all specimens of *Hexametra* from the Western Hemisphere represented a single species. However, specimens collected from *Crotalus horridus* Linnaeus and *Agkistrodon piscivorus leucostoma* (Troost) from Louisiana were described by Bowman (1984) as *Hexametra leidyi*. These 2 species, *H. boddaertii* and *H. leidyi* represent the genus in North American snakes.

This note reports the presence of *H. boddaertii* in *Crotalus cerastes* from California. This finding represents a new host and locality record and what we believe to be the second report of an infection by this parasite in a wild population of North American snakes.

Forty *Crotalus cerastes* (21 male, 19 female, mean snout–vent length [SVL]  $49.0 \pm 6.0$  cm, range 34.1–61.4 cm) were collected in the Kelso Dunes 3.5 km south of Kelso, San Bernardino County, California, elevation 650–700 m, 34°59'N, 115°57'W, in September 1991 for use in a doctoral dissertation (Secor, 1992). The snakes were subsequently searched for endoparasites. The body cavity was opened ventrally and the gastrointestinal system and body cavity examined. Nematodes were cleared in glycerol for microscopic examination. Five of the 40 specimens were deposited in the herpetology collection of the Los Angeles County Museum of Natural History (LACM 140779–140783).

Two of 40 (5% prevalence) *Crotalus cerastes* harbored nematodes: 3 adult, white cylindrical



ascarid nematodes tapering both anteriorly and posteriorly but with greater thickness in the posterior half of the body were found in the small intestines of LACM 140779 (female, 61.4 cm SVL); LACM 140781 (female, 54.7 cm SVL) harbored 4 larval ascarids on visceral fat bodies within the coelom. The male specimen measured 90 mm, width at midbody 2 mm; both female specimens measured 130 mm, width at vulva 2.4 mm; larvae measured 2.4–2.7 mm in length. Each possessed 3 equally sized lips, a dorsal and 2 lateroventral, with rounded angles, slightly wider than long. In the adults, the anterior border of each lip was serrated with tiny teeth (male, 98 teeth; females, 83, 87 teeth); the denticles did not reach the base of the lip. A ventriculus was absent; there were no esophageal or intestinal caeca. The intestine had a dark brown coloration that remained throughout the length of the body. The testes were irregularly coiled, reaching 39 mm from the posterior end. The cloacal orifice was 0.25 mm from the terminus. The tail of the male was short, in the form of a cone and provided with 2 pairs of ventral and 4 pairs of sublateral papillae. There were 50 pairs of precloacal papillae. The spicules were equal, well sclerotized, and 1.25 mm in length and tapered to a point distally. The vulva was located midbody; the rest of the female reproductive system was in the posterior half of the body. The vagina was 4.2 mm in length and directed posteriorly; the unbranched portion of the 6 branched uterus was 3.25 mm. The anterior ovarian branches approached the vulva but did not extend anterior of it, and posteriorly they did not reach the anus. The eggs were subspherical, 79–85  $\mu\text{m}$  by 71–82  $\mu\text{m}$ . (U.S. National Museum Helminthological Collection, Beltsville, Maryland 20705, Accession No. 83512: *Hexametra boddaertii*; 83512: ascarid larvae).

The adult nematodes were identified by utilizing 3 differential characters that Bowman (1984) established for separating *H. boddaertii* from *H. leidy*: the number and distribution of the denticles on the lips and the size of the eggs. That is, in *H. boddaertii* the dentigerous ridge extends to the level of the anterior margin of the double papilla whereas in *H. leidy* the dentigerous ridge extends to the base of each side of the lip; *H. boddaertii* has fewer denticles (79–103) than *H. leidy* (141–216); and the egg size of *H. boddaertii* is smaller (72–86  $\mu\text{m}$  by 65–82  $\mu\text{m}$ ) than that of *H. leidy* (84–96  $\mu\text{m}$  by 74–89  $\mu\text{m}$ ). Thus, LACM 140779 harbored 1 male and

2 female *Hexametra boddaertii* in the small intestines.

The genus *Hexametra* contains 7 species, 4 from lizards and 3 from snakes (see Baker, 1987). Sprent (1978) considered all ascarids with 6 uterine branches from Old World snakes to belong to *H. quadricornis* and synonymized 15 species of nematodes with *H. quadricornis*; likewise, all ascarids with 6 uterine branches from New World snakes were considered to belong to *H. boddaertii* and he synonymized 3 species with *H. boddaertii*. The range of *H. boddaertii* in North America cannot be determined because all but 1 of the specimens in the U.S. National Parasite Collection were from zoo animals; the only noncaptive host was a “rattlesnake” collected in central Florida (Bowman, 1984). The other species of *Hexametra* from snakes is *H. leidy* described by Bowman (1984) from *Crotalus horridus* and *Agkistrodon piscivorus leucostoma* from Louisiana. Bowman (1984) reported 1 additional occurrence of *H. leidy*; that was in a western rattlesnake, *Crotalus viridis* (Rafinesque), that had been housed in the San Diego zoo. *Hexametra boddaertii* is the first species of the genus to be reported from the western United States and only the second nematode to be reported from *Crotalus cerastes*. *Thubunaea cnemidophorus* Babero and Matthias, 1967, was the first nematode, possibly a pseudoparasite, i.e., a secondarily ingested parasite of prey, to be reported from *Crotalus cerastes* (see Babero and Emerson, 1974).

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### Research Note

## Lack of Transmammary Transmission of *Strongyloides stercoralis* from a Previously Hyperinfected Bitch to Her Pups

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**ABSTRACT:** Larvae were not found by Baermann examination of the gastrointestinal tract or parenteral tissues of 7-day-old pups whelped by a bitch previously hyperinfected with *Strongyloides stercoralis* and either suckled on the bitch or fed an artificial milk diet. In sharp contrast, experimentally infected comparable pups yielded larvae when examined by the same technique. Additionally, although the bitch transiently shed larvae in the feces prior to whelping, larvae were not found in filtered aliquots of the bitch's milk from day 1 to day 7 after whelping, whereas they were recovered from filtered aliquots of *S. stercoralis*-seeded milk samples. These results suggest that transmammary and placentational transmission of the parasite does not occur in dogs.

**KEY WORDS:** *Strongyloides stercoralis*, transmammary transmission, canine model.

*Strongyloides stercoralis* is a nematode parasite with the ability to replicate within its host allowing for accumulation of adult and larval forms, both in the gastrointestinal tract and in extraintestinal sites. It infects humans, primates, and dogs and is capable of causing serious disease leading to death in all of these hosts. The most severe manifestations of strongyloidiasis are hyper- and disseminated infection in which precociously developing third-stage larvae autoinfect the host, leading to massive numbers of, respectively, enteral and parenteral forms. *Strongyloides stercoralis*-infected dogs treated with

corticosteroids have been shown to develop both hyper- and disseminated infections with the parasite (Grove et al., 1983; Schad et al., 1984; Gentile et al., 1986; Mansfield and Schad, 1992) providing a good model for the study of the human disease. Infections by species of strongyloides are usually acquired by either of 2 routes: by direct penetration of the skin in unsanitary surroundings or by transmammary transmission.

Because transmammary transmission occurs in several other *Strongyloides* species, it has been suspected to occur in *S. stercoralis*, but this was never proven. Vertical transmission of other *Strongyloides* species in animals has been well documented, including *Strongyloides ransomi* in pigs (Moncol and Batte, 1966; Batte and Moncol, 1968), *Strongyloides westeri* in horses (Lyons et al., 1969), *Strongyloides papillosus* in sheep and cattle (Lyons et al., 1970), and *Strongyloides ratti* in rats (Katz, 1969; Zamirdin and Wilson, 1974). Additionally, reports have described the presence of infective larvae in the milk of *Strongyloides* spp. (*Strongyloides papillosus* group) (Grove, 1989) and *Strongyloides fulleborni*-infected women (Brown and Girardeau, 1976). Vertical transmission of *S. fulleborni* also has been shown to occur in primates (Wong and Conrad, 1978) and is suspected in humans in Papua, New Guinea (Ashford and Barnish, 1989). Conflict exists, however, in distinguishing the species of *Strongyloides* and, in particular, in differentiating *S. stercoralis* from *S. fulleborni* in both human and primate infections. The identity of

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the single larva discovered in the Brown and Girardeau (1976) study was challenged (Grove, 1989) and claimed to be an autoinfective *S. stercoralis* larva. The possible occurrence of vertical transmission of *S. stercoralis* has greater implications for the host than in the other host-parasite associations because once an infection is acquired the parasite can increase its numbers within the host by autoinfection.

Transmammary transmission has been demonstrated in rats infected with *S. stercoralis* during pregnancy (Wilson et al., 1982). It is epidemiologically more relevant, however, to examine this phenomenon in previously infected females with cryptic or chronic infections because the majority of humans become infected early in life (Grove, 1989). Because we had a well-defined, laboratory-maintained strain of *S. stercoralis*, we undertook to test the hypothesis that the parasite can be transmitted vertically from chronically infected mother to offspring in dogs.

A laboratory-reared beagle bitch, negative for nematode parasites by repeated fecal flotation and Baermann examinations, was infected with 2,500 third-stage, infective larvae of *S. stercoralis* by subcutaneous injection. She developed a patent infection that was enhanced by prednisolone treatment (3.3 mg/kg, once a day). During the prednisolone treatment, the bitch was anesthetized with Thiopental (2.2 mg/kg intravenously, to effect), an endotracheal tube was passed, and 10 ml of sterile saline was flushed into and aspirated out of the lungs. The saline was examined at 10 $\times$  magnification for the presence of larval stages of the parasite. The prednisolone was discontinued by tapering the dose gradually. Larval shedding ceased after 2 wk. After 4 mo with no steroid treatment, the bitch cycled and was bred by artificial insemination to a uninfected beagle dog. She conceived. At 35 days of gestation the 3 times/wk, fecal Baermann examinations were resumed. The bitch's temperature was recorded twice daily beginning at 50 days of gestation. When her temperature dropped to 37°C, she was watched closely for whelping. The bitch's perineal region was scrubbed with an iodine preparation to kill any existing *S. stercoralis* larvae, and she was prevented from having contact with her fecal material. To minimize the possibility of accidental reinfection of the bitch or infection of the pups by skin penetration, the bitch was housed in a stainless steel cage that was washed daily in a

cage washer at 82°C. Six pups were received on sterile towels. Three pups were placed with the bitch for nursing, and 3 were taken to a separate incubator and fed a canine milk substitute (Esbilac, Borden Co., Hampshire, Illinois). From day 1 to day 7 after whelping, the bitch was milked once a day from all teats, a minimum of 10 ml of milk filtered through a 0.45- $\mu$ m filter and the filter examined under 30 $\times$  magnification for the presence of larvae of *S. stercoralis*. Bovine milk samples containing known quantities of third-stage larvae (10, 100, 1,000) were filtered by identical means and examined microscopically as controls. At 1 wk of age, all pups were sacrificed with an overdose of barbiturate anesthesia. All tissues except for the skin and gastrointestinal tract were examined during necropsy, divided into organ systems, minced, and soaked in phosphate-buffered saline (PBS) on screens (Baermann examination) for parasite recovery. The gastrointestinal tract was slit longitudinally and hung in graduated cylinders containing PBS and the skin was soaked in a flat pan of PBS, after parallel partial-thickness slicing of the subcutaneous side of the skin (Bianco et al., 1980). All preparations were incubated at 37°C for 3 hr, the tissue removed, and the solid material sedimented and searched for larvae of the parasite. Feces taken from the rectum of the pups during necropsy was subjected to Baermann examination. Three 10-day-old purpose-bred, parasite-naive control pups were preanesthetized with Inno-var® (0.4 mg/9–22.5 kg fentanyl and 50 mg/9–22.5 kg droperidol, subcutaneous), anesthetized with 2% halothane delivered via mask with oxygen, and infected by inoculation of approximately 1,500 third-stage larvae through a ventral midline abdominal incision directly into the exteriorized distal ileum. The pups were allowed to recover from anesthesia and were examined by identical means at approximately 4 days after infection to test our ability to recover migrating larvae.

The bitch developed a patent infection in approximately 17 days, shed larvae in the feces for a 30-day period, and then ceased to shed larvae as determined by 3 times/wk fecal Baermann examinations (Fig. 1). After 3 consecutive negative Baermann examinations, the dog was given oral prednisolone. Seven days after commencing drug treatment, the dog shed first-stage larvae of the parasite in the feces, the numbers of which continued to increase over a 6-wk period (Fig.

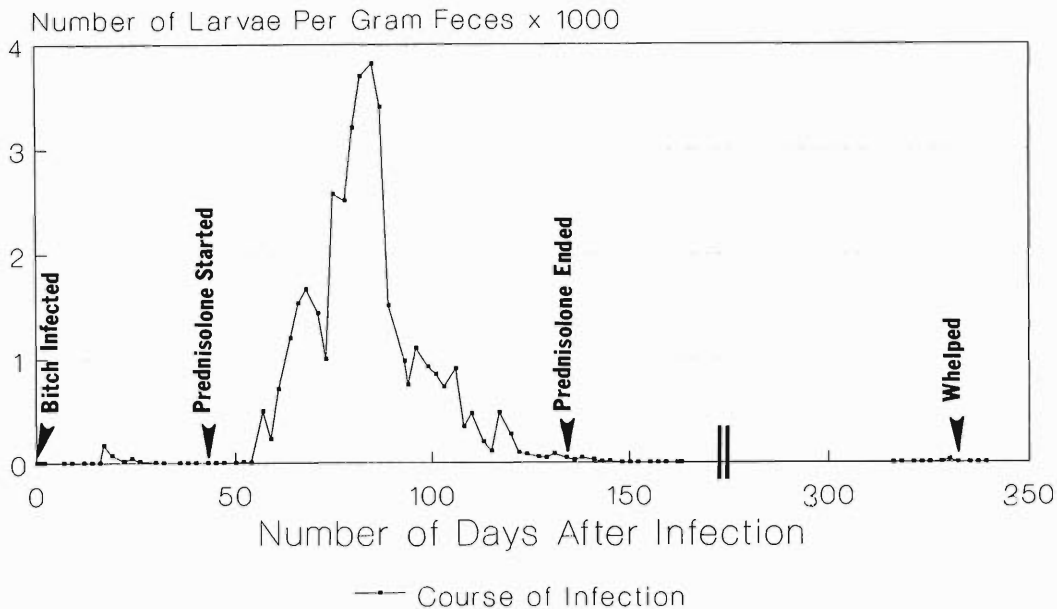


Figure 1. The course of infection of a *Strongyloides stercoralis*-infected bitch treated with oral prednisolone to induce hyperinfection.

1). At the peak of larval shedding, the bitch excreted 3,800 larvae/g in a 50.0-g sample. This exceeded the maximum first-stage larval yield for *Strongyloides* spp., which may be determined by multiplying the infective dose by the maximum number of eggs/adult/day (estimated max-

Table 1. Number of *Strongyloides stercoralis* parasites recovered by quantitative Baermann examination from 7-day-old pups whelped by previously hyperinfected bitch and control pups infected with 1,500 third-stage larvae. Experimental pups were either separated from the bitch at whelping or allowed to suckle on the bitch.

	Pup number		
	1	2	3
Group 1—No suckling			
Skin	0	0	0
Gastrointestinal tract	0	0	0
All other organs	0	0	0
Group 2—Suckling			
Skin	0	0	0
Gastrointestinal tract	0	0	0
All other organs	0	0	0
Group 3—Control*			
Skin	0	0	2
Gastrointestinal tract	99	30	220
All other organs	4	0	2

\* Ten-day-old pups were experimentally infected with 1,500 third-stage *Strongyloides stercoralis* larvae.

imums of 40) (Triantaphyllou and Moncol, 1977). This confirmed the occurrence of hyperinfection, because a greater number of shedding adult females would have been required to produce the observed number of fecal larvae than the number given in the infective dose. Additionally, 2 third-stage larvae were recovered by tracheal wash, indicating the presence of the parasite outside the gastrointestinal tract after the prepatent period, suggesting active larval migration, although simple aberrant larval location cannot be ruled out.

The bitch shed first-stage larvae in the feces intermittently at low levels (1–10 larvae/g) in the 2 wk prior to whelping but ceased to shed larvae from the day of whelping and thereafter (Fig. 1). Milk was produced by the bitch from the day of whelping until the conclusion of the experiment. The milk was examined for the presence of larvae from day 1 to day 7 after whelping. No larvae were found in the milk of the bitch at any time during the study, whereas control milk samples seeded with larvae were judged positive by the filter screening procedure. No larvae were found in the feces, gastrointestinal tract, skin, or internal organs of any of the pups whelped by the bitch (Table 1). In contrast, larvae were recovered from control pups experimentally infected with the parasite and examined by identical means (Table 1).

Transmammary or transplacental transmission of *S. stercoralis* in humans has not been documented conclusively at this time. Our results suggest that vertical transmission of the parasite does not occur in dogs. The bitch described in this study harbored a patent infection of *S. stercoralis*. She developed a hyperinfection of the parasite during corticosteroid therapy as judged from fecal larval recoveries higher than possible from the infecting dose alone and the presence of third-stage larvae in the lungs after the prepatent period. Despite the chronic-active nature of her infection, the bitch failed to transmit any detectable larvae to her offspring, either through the placenta or through the milk. The 3 pups allowed to suckle on the bitch served as a test for transmission of larvae via the transmammary route, whereas the 3 pups fed an artificial milk replacer served as a test for transmission of larvae via the transplacental route. Although recovering larvae from milk is difficult (Ashford and Barnish, 1989), the use of pups to suckle milk, and thereby collect any larvae present and concentrate them in their guts, should maximize recoveries. Transmammary transmission of *Strongyloides* spp. may depend on the general susceptibility of the host and the timing of the infection. Sows transmit *S. ransomi* to their offspring in the milk from tissue stores (Moncol and Batte, 1966; Batte and Moncol, 1968), but rats must be infected during gestation to pass *S. ratti* in the milk (Zamirdin and Wilson, 1974; Wilson et al., 1982).

The lack of transmammary passage of *S. stercoralis* from this experimental bitch to her pups casts doubt on the speculation that transmammary transmission of this parasite occurs in humans or other natural hosts. However, because this experiment involved only 1 dog, the results are merely suggestive, but they should serve as a preliminary study on which additional studies of vertical transmission in *S. stercoralis*-infected animal models (dog and primate) could be based.

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## Research Note

# Parasites of Feral Horses from the Namib Desert, Namibia

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**ABSTRACT:** A quantitative study of the internal parasites from 3 horses revealed the presence of 4 nematode and 1 larval fly species. Additionally, 1 tick species was recovered. This horse population has ranged freely for more than 80 yr in the Namib Desert, Namibia. All 3 of the horses were infected with *Habronema muscae*, *Oxyuris equi*, *Probstmayria vivipara*, *Strongylus edentatus*, *Gasterophilus pecorum*, and *Hyalomma marginatum rufipes*.

The low population density of horses and few numbers and species of helminths are discussed in terms of a diminished helminth community. Further, the dynamics as an isolationist rather than interactive community are discussed.

**KEY WORDS:** nematodes, stomach bots, ticks, horses, survey, Namibia.

Eighty years ago a small group of domestic horses were released into the Namib Desert of Namibia. According to circumstantial evidence, this population of horses descended from military stock bred for the army and police in German South West Africa before World War 1 (Van der Merwe, 1984) but exact numbers of the original group are unknown (Van der Merwe, 1994, pers. comm.). Since then, they have roamed an isolated area of 400 square mi on the gravel plains of the Namib Desert (Coulson, 1987). Their habitat forms part of a restricted mining area, and they have been protected from all but natural predators. Two watering points were provided in recent years. As a result of this protection and the provision of water, the horses numbered 400 in 1991 (Ministry of Wildlife Conservation and Tourism, Namibia, 1991, pers. comm.).

To prevent overpopulation, horses were either translocated or removed randomly by culling. The latter provided an opportunity to collect various biological specimens including samples for quantitative examination of internal and external parasites.

The 2 vegetation types of this area (26–28°E, 14–18°S) are “desert and succulent steppe” and “semi-desert and savanna transition” (Giess, 1971). Grazing consists predominantly of *Stipagrostis* spp. on calcareous soil (Meyer, 1988). The mean annual expected rainfall in this region is 50–100 mm (Department of Water Affairs, South West Africa/Namibia, 1977, unpubl. data).

In April 1991, 3 adult horses were randomly selected and sacrificed, each with a cervical shot. These horses included 2 stallions between 2–5 yr of age and 1 mare of 4 yr. Condition scoring for the horses, based on a 0–10 scale according to Henneke et al. (1983), ranged between 4 and 5. The postmortem examination and collection of internal parasites followed the methods of Malan et al. (1981a, b). The cranial mesenteric arteries were examined for larval stages of *Strongylus vulgaris*. For ease of transport, modifications to these methods included formalin fixation of arteries and gut walls rather than freezing.

Macroscopic lesions were noted in the cecal and colonic walls of the large intestine. Specimens of affected tissues were collected and preserved in 10% buffered formalin. These were processed in paraffin wax, sectioned at 6  $\mu$ m, and stained with hematoxylin and eosin. Histopathologically there was a prominent submucosal edema and tissue eosinophilia in the lamina propria, which was consistent with migration, but no parasites were observed.

Accession numbers for specimens deposited in the U.S. National Parasite Collection (USNM) (Beltsville, Maryland 20705, U.S.A.) are as follows: *Habronema muscae* (USNM 83514), *Oxyuris equi* (USNM 83515), *Strongylus edentatus* (USNM 83516), *Gasterophilus pecorum* (USNM 83517), and *Hyalomma marginatum rufipes* (USNM 83518). Nematodes, fly larvae, and ticks were identified according to Lichtenfels (1975), Zumpt (1965), and Howell et al. (1983), respectively.

The prevalences, mean abundances and intensities, and ranges of parasites for each of 4 nem-

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**Table 1. Numbers of parasites in 3 horses from the Namib Desert, Namibia.**

Parasite	Prevalence (%)	Mean abundance and intensity	Range
<b>Nematodes</b>			
<i>Habronema muscae</i>	100	142	18–284
<i>Oxyuris equi</i>	100	222	1–658
<i>Probstmayria vivipara</i>	100	616,762	80,110–1,255,670
<i>Strongylus edentatus</i>	100	9	2–23
<b>Diptera</b>			
<i>Gasterophilus pecorum</i> * (third instar)	100	796	450–982
<b>Ticks</b>			
<i>Hyalomma marginatum rufipes</i>	100	3	1–5

atodes, 1 oestrid fly species, and 1 tick species recovered from the hides of the horses are listed in Table 1.

The species of internal parasites reported here concur with those known from elsewhere in the horse (Zumpt, 1965; Lichtenfels, 1975; Krecek et al., 1989). However, the diversity of species in the present study was considerably less if compared to other reports. Twenty-six nematode and 3 *Gasterophilus* sp. were recovered from horses in South Africa (Krecek et al., 1989) and 14 nematode species from 3 Hartmann's mountain zebras studied in 2 areas in Namibia (Scialdo-Krecek et al., 1983). The present study reports an overall higher prevalence in all horses examined, that is 100% for all nematode and fly parasites, than has been found previously in southern Africa. Two exceptions are the nematodes *Probstmayria vivipara* and *Crossocephalus viviparus*, which were present in all of the zebras in a previous study (Scialdo-Krecek et al., 1983). In addition, these parasites were more abundant than in the zebra studies, while *O. equi* and *S. edentatus* occurred in larger numbers than in the previous horse study (Krecek et al., 1989). The smaller diversity of strongyle species, limited to only *S. edentatus*, could be attributed to minimal availability of grazing. Migration of infective larvae from feces to herbage and their subsequent ingestion is the principle means of transmission (Levine, 1980). Furthermore, the lack of moisture in the environment probably limits the development of these parasites and their intermediate hosts (i.e., *Musca* spp. are the intermediate hosts for *H. muscae*). Finally, the stocking rate of horses in the area was low (i.e., 400 horses in 400 square mi) and may have further reduced transmission.

It is significant that horses were infected with 6 different parasites in their semi-desert environment. The life cycles of these parasites are largely mediated by environmental factors (i.e., moisture levels). In spite of extreme conditions, these parasites continue to exist, suggesting extreme survival limits.

Stable high population densities at a host's epicenter of origin tends to support larger numbers of more numerous species of helminths (a well-developed helminth community), whereas the unstable lower population densities of a host species at the periphery of its range may support fewer numbers and species of helminths (diminished or no helminth community) (Radomski et al., 1991). This concept may partially explain the lack of species diversity and low numbers of helminths in these horses from the Namib since larger numbers and more numerous species of helminths exist where this host is more numerous.

When our data is considered in terms of helminth infracommunity dynamics (Pence, 1990), a number of criteria suggest that this is an isolationist community versus an interactive community. Criteria that support this are low probability of colonizing the horse host, low vagility, and 2 direct versus 1 indirect life cycles (additionally, *Probstmayria vivipara* is probably direct). Further, niche utilization includes low mean species diversity and low number of high density species. According to Radomski et al. (1991), invasion of a host species into a new locality may be more successful if the colonizing species is free from specific pathogenic parasites infecting it in the original habitat. Certainly the success of this horse in its desert habitat has been enhanced by the absence of helminth parasites.

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Research Note

## Intestinal Helminths of Capybara (*Hydrochaeris hydrochaeris*) from Bolivia

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**ABSTRACT:** During May to September (dry season) 1993, a total of 8 species of helminths were collected from 41, wild-caught Capybara (*Hydrochaeris hydrochaeris*) in 9 localities in eastern Bolivia. These include 3 cestodes (*Monoecocestus hagmanni*, Janicki, 1904; *M. macrobursatum*, Rego, 1961; *M. hydrochoeri*, Baylis, 1928), 3 nematodes (*Habronema clarki*, Foster and Chitwood, 1937; *Viannella hydrochoeri*, Travassos, 1920; *Protozoophaga obesa*, Diesing, 1851), and 2 trematodes (*Taxorchi schistocotyle*, Fiscoeder, 1901; *Hippocrepis hippocrepis*, Diesing, 1850). This report represents new localities for all helminth species recovered.

**KEY WORDS:** Capybara, *Hydrochaeris hydrochaeris*, helminth parasites, Bolivia, new locality.

The capybara, *Hydrochaeris hydrochaeris*, is the largest living rodent in the world and has existed in South America (except Chile) since the upper Pliocene (Patterson and Woods, 1982). Capybara are semiaquatic herbivores and rarely are found feeding more than 500 m from water (Azcarate, 1980). They occupy a wide array of habitats, from forested riverside to open savannas, and brackish mangrove swamps (Mones and Ojasti, 1986). In this study, all animals were taken east of the Andes, from the northern city of Magdalena to Palca del Tuna in southern Bolivia.

Four recent surveys dealing with the intestinal parasites of the capybara currently exist. Rego (1961) wrote a revision of the cestode genus *Monoecocestus* from existing material at the Oswaldo Cruz Helminthological Institute collection. He revised 2 species (*M. hagmanni*, *M. hydrochoeri*) and described 1 new species (*M. macrobursatum*) during this study. Mayaudon (1980) looked at 18 samples from the capybara, 7 from those he necropsied and 11 from previously collected material sent to the University of Venezuela (Maracay) parasitology laboratory. He lists 7 parasites (2 trematodes, 1 cestode, 4 nematodes) and their prevalence from each host. In 1983, Mones and Martinez summarized the existing bibliographical information on capybara

parasites, and a total of 54 endoparasites and 30 ectoparasites belonging to 15 families were reported. A report by Draghi (1992) for the Argentinean experimental farm lists a total of 7 species of helminths (1 cestode, 3 trematodes, 3 nematodes) recovered in 3 areas of northeastern Argentina (San Roque, Mercedes, San Martin).

The present study examined 41 *H. hydrochaeris* from 9 localities in eastern Bolivia (Department of Beni, Santa Cruz and Chuquisaca). All animals were necropsied in the field and the entire gastrointestinal tract removed from esophagus to anus. Each section (stomach, small intestine, large intestine, cecum, colon) was secured by ligature and placed separately in buckets and slit lengthwise for analysis. After opening, each section was washed and all visible worms collected. Nematodes were fixed in glacial acetic acid, stored in 70% EtOH and cleared in lactophenol. Cestodes and trematodes were relaxed in tap water, fixed in 10% formalin, stained in Semichon's acetic carmine, and mounted in Canada balsam.

Two museum skins and skeletons of *H. hydrochaeris* were donated to the Natural History Museum "Noel Kempff Mercado" in Santa Cruz, Bolivia, and voucher specimens of all helminths were deposited in the U.S. National Parasite Collection, USDA, ARS, Biosystematic Parasitology Laboratory, Beltsville, Maryland (Table 1).

Eight species of helminths were found in the present study (Table 1). Nematodes having the highest to lowest prevalence ranged from 100% in *Protozoophaga obesa* to 2% in *Habronema clarki* and *Viannella hydrochoeris*.

Only 1 genus of cestode (*Monoecocestus*) was found during this study, of which *M. macrobursatum* was the second most common parasite recovered, with *M. hagmanni* and *M. hydrochoeri* being of equal prevalence. The trematode *Hippocrepis hippocrepis* was the third most prevalent worm found, while *Taxorchi schistocotyle*

**Table 1. Prevalence of infection with helminths in the Capybara from Bolivia.**

Parasite	Prevalence	Range of worms/host	$\bar{x}$	USNM Helm. Coll. No.
<b>Cestoda</b>				
<i>Monoecocystus hagmanni</i> Janicki, 1904	5/41 (12%)	1-15	10	83958
<i>M. hydrochoeri</i> Baylis, 1928	5/41 (12%)	1-9	5	83962
<i>M. macrobursatum</i> Rego, 1961	14/41 (34%)	1-30	20	83960 and 83961
<b>Nematoda</b>				
<i>Habronema clarki</i> Foster and Clitwood, 1937	1/41 (2%)	—	10	83956
<i>Viannella hydrochoeri</i> Travassos, 1920	1/41 (2%)	—	5	83954
<i>Protozoophaga obesa</i> Diesing, 1951	41/41 (100%)	Massive*		83955
<b>Trematoda</b>				
<i>Taxorchis schistocotyle</i> Fischeoeder, 1901	5/41 (12%)	1-20	10	83959
<i>Hippocrepis hippocrepis</i> Diesing, 1850	8/41 (20%)	1-15	10	83957

\* Too numerous to count.

equaled *M. hydrochoeri* and *M. hagmanni* in percentage infected. The range of worms per host varied from 1,000+ in *P. obesa* to a single worm for the cestodes and trematodes. In *H. clarki* and *V. hydrochoeri*, only single infections were found, consisting of 10 and 5 worms, respectively.

Although more than 80 parasites have been previously reported from the South America capybara (Mones and Martinez, 1983), this is the first report from Bolivia. *Protozoophaga obesa* has been previously reported from Argentina as infecting the stomach (Draghi, 1992). This was not found as a site of infection during the present study. We found *P. obesa* free in the lumen of the cecum while always attached to the mucosal wall in the intestines. Draghi (1992) also reported the cestode *M. macrobursatum* as occurring in the unusual site of the stomach; our study did not confirm this finding as all cestodes were found only in the intestine.

The only other report on prevalence of intestinal helminths from the capybara was by Mayaudon (1980). He examined 7 animals from Venezuela and found only 4 of the parasites reported in this study (*V. hydrochoeri*, 42.8%; *P. obesa*, 100%; *H. hippocrepis*, 42.8%; *T. schistocotyle*, 100%). In all cases he found heavier infections (except *P. obesa*) than were found in Bolivian populations. He also reported finding the cestode *Monoecocystus decrescens* (Diesing, 1876) Fuhrmann, 1932, in 5 of 7 animals examined. This parasite was not found in our Bolivian study. It must be stated that the Mayaudon study was conducted on a site where capybara are raised

for propagation purposes. In contrast, all 41 animals in this study were taken in the wild.

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## Research Note

# Endoparasites of Beaver (*Castor canadensis*) from Kansas

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**ABSTRACT:** A survey was conducted to determine the endoparasite fauna of *Castor canadensis* populations in 3 counties in northeast and eastcentral Kansas. During the 1990 trapping season, 63 skinned beaver carcasses were provided by local trappers for parasitological examination. Six species of parasites were found: 2 protozoans, *Eimeria sprehni* Yakimoff, 1934, and *E. causeyi* Ernst, Cooper, and Frydendall, 1970; 1 digene, *Stichorchis subtriquetrus* (Rudolphi, 1814); and 3 nematodes, *Travassosius americanus* Chapin, 1925, *Dracunculus* sp., and *Baylisascaris* sp. This is the first report of *Dracunculus* sp. and *Baylisascaris* sp. from North American beavers.

**KEY WORDS:** beaver, *Castor canadensis*, *Eimeria sprehni*, *Eimeria causeyi*, *Stichorchis subtriquetrus*, *Travassosius americanus*, *Dracunculus* sp., *Baylisascaris* sp., Kansas, histopathology, eosinophilic granuloma.

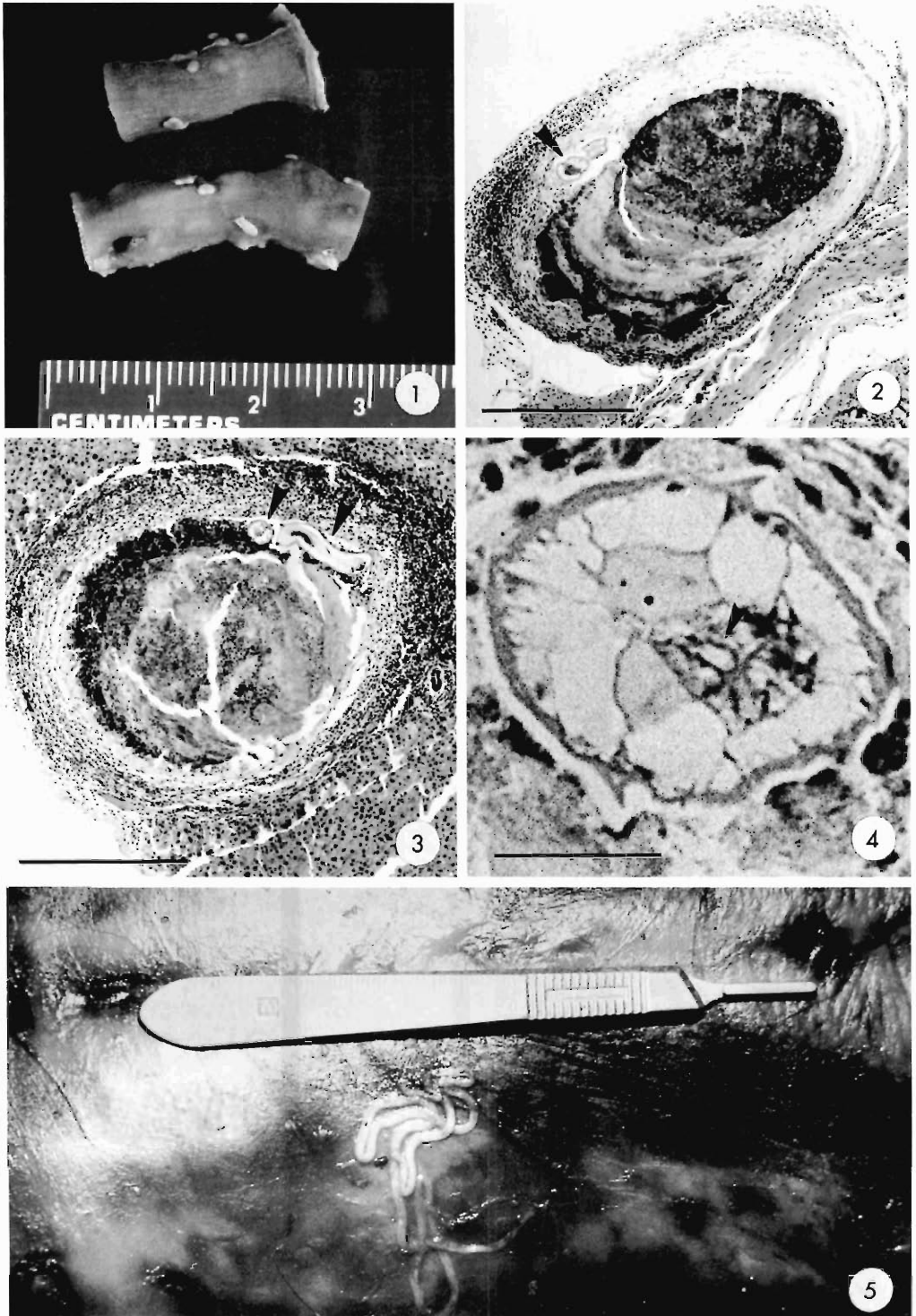
The beaver, *Castor canadensis* Kuhl, is the largest member of the order Rodentia found north of Panama (Hall, 1981), with exceptional individuals weighing as much as 44 kg (Bee et al., 1981). Beaver have one of the most extensive and varied distributions of any North American mammal and inhabit most of the continental United States, Canada, and Alaska, from as far north as the Brooks Range and south to the states of Nuevo Leon and Tamaulipas, Mexico (Hall, 1981; Nowak and Paradiso, 1983).

Reports of the parasite fauna of beaver date to 1669 (see Lawrence and Graham, 1955, for review). However, the majority of reports (21/29), where the collection site of the host is given, have been made above 40°N latitude. Thus, little information is available on the protozoa or helminths of beavers from the mid- to southern areas of its range. Studies conducted below 40°N latitude are from Alabama (Ernst et al., 1970), Colorado (Olsen, 1949), Louisiana (Bennett and Humes, 1939), Mississippi (Foil and Orihel, 1975), Texas (Fedynich et al., 1986), and Virginia (Ogburn-Cahoon and Nettles, 1978). To the best of our knowledge, no comprehensive

study has been undertaken to examine the parasite fauna of beavers from the central United States. Consequently, the following study was initiated to determine the endoparasites of various beaver populations from northeastern and eastcentral Kansas.

Between 2 February and 18 March 1990, 63 skinned beaver carcasses were received at the Department of Veterinary Diagnostic Investigations, Kansas State University. Trappers acquired animals from 5 different sites from 3 counties: 6 animals from Lyon County (along a section of the Neosho River between 38°24'N, 96°09'W, and 38°22'N, 96°06'W), 2 from Pottawatomie County (Cedar Creek between 39°17'N, 96°33'W and 39°16'N, 96°32'W), and 55 from 3 localities in Riley County (the Kansas River between 39°05'N, 96°42'W, and 39°07'N, 96°39'W; the Blue River between 39°30'N, 96°39'W, and 39°32'N, 96°37'W; and Wildcat Creek between 39°13'N, 96°42'W, and 39°12'N, 96°42'W). Carcasses were presented within 48 hr of trapping, with none having been frozen prior to examination. Upon receipt, all specimens were sexed and weighed, and a total length measurement was taken. Each carcass was examined grossly for the presence of any tissue-dwelling parasites, obvious lesions, or deformities. Although necropsies were performed on all 63 animals, tissues were collected from the first 42 only and placed in 10% buffered neutral formalin for later histologic examination.

Gastrointestinal tracts were removed intact from all 63 beavers. Viscera were separated into stomach, small intestine, cecum, large intestine, and rectum. The mucosal surface of the duodenum from 20 animals was scraped with a scalpel blade, and the scrapings were examined as squash preparations for *Giardia* sp. using Nomarski interference contrast optics (Upton et al., 1991). A fecal sample was then removed from the rectum



Figures 1-5. *Baylisascaris* sp. larvae and *Dracunculus* sp. from Kansas beavers. 1. Formalin-fixed sections of small intestine with nodules, each nodule containing a single *Baylisascaris* sp. larva. 2. Photomicrograph of

of 59 animals and placed individually in 2.5% (w/v) aqueous potassium dichromate ( $K_2Cr_2O_7$ ) solutions for protozoal examination. Gut sections were split lengthwise and the contents washed into separate containers, after which mucosal scrapings of the wall of each section were collected and added to the contents of each respective container. The combined mucosal scrapings and free gut contents were then washed, using cool tap water, through a graded series of sieves, with exclusion sizes of 2 mm, 850  $\mu m$ , and 425  $\mu m$ , respectively (Fisher Scientific, St. Louis, Missouri). Contents of each sieve were examined grossly and with the aid of a dissecting microscope, and any parasites seen were removed and placed in tap water. All nematodes were fixed in hot alcohol-formalin-acetic acid (AFA) and then stored in 70% ethanol. Trematodes were allowed to relax and die in water and then were fixed and stored in AFA. Voucher specimens of helminths recovered in this study have been deposited in the U.S. National Helminthological Collection (Beltsville, Maryland 20705, U.S.A.): *S. subtriquetrus*, 82813; *T. americanus*, 82814; *Dracunculus* sp., 82816; *Baylisascaris* sp., 82815. Paraffin blocks of liver and small intestine containing *Baylisascaris* sp. larvae have been deposited in the Armed Forces Institute of Pathology (Washington, D.C. 20306, U.S.A.): 2401031.

Feces were examined following flotation in a modified Sheather's sugar solution (specific gravity 1.30). Samples positive for coccidian oocysts were placed in petri dishes in a thin layer of 2.5%  $K_2Cr_2O_7$  and allowed to sporulate for 1 wk at room temperature (23°C). Samples were subsequently reexamined using Nomarski interference contrast microscopy. All oocysts were measured within 10 days following sporulation.

Of the 63 beavers examined, 57 (91%) harbored 1 or more species of 6 parasites. These included 2 protozoa (parasite followed by number of hosts infected/percentage of hosts infected), *Eimeria sprehni* (16/25%) and *Eimeria causeyi* (3/5%); 1 digene, *Stichorchis subtriquetrus* (56/89%); and 3 nematodes, *Travassosius amer-*

*icanus* (23/35%), *Dracunculus* sp. (2/3%), and a larval *Baylisascaris* sp. (1/2%). No trophozoites or cysts of *Giardia* sp. were seen in any of the duodenal or fecal samples examined. The majority, 49 (86%) of the 57 animals positive for parasites, were found to be infected by 1 (23/37%) or 2 (26/41%) parasite species. Of the 8 animals remaining, 7 (11%) had 3 parasites and 1 (2%) had 4 different species. Interestingly, of all 57 infected animals, 56 (98%) harbored *S. subtriquetrus*. The exception was a single animal infected only with coccidian *E. sprehni*. Of the 19 animals shedding coccidian oocysts, 18 (95%) had monospecific infections, with *E. sprehni* and *E. causeyi* accounting for 16 and 2 infections, respectively. One animal shed oocysts of both *Eimeria* species.

An adult 18-kg female taken from Cedar Creek, Pottawatomie County, had numerous small (1–2 mm), white raised nodules scattered over the serosal surface of the liver, small intestine, and colon (Fig. 1). One nodule was removed and compressed between 2 glass slides as a squash preparation. Microscopic examination under low power (100 $\times$ ) revealed a vigorously undulating nematode larva. Histopathological examination of sections of small intestine and liver containing nodules showed demarcated and slightly encapsulated masses (Figs. 2, 3). The center of each nodule was eosinophilic, caseous material with abundant cellular debris. Peripheral to the caseous center was a layer of macrophages with some multinuclear cells. The outer layer of inflammation had numerous eosinophils with a few neutrophils, macrophages, lymphocytes, plasma cells, and fibroblasts. Cross-sections and oblique sections of a nematode larvae at the interface of the cellular layer and caseous core could be seen (Fig. 4). Microscopic and gross morphological characteristics of these larvae, as described by Bowman (1987), led to a tentative diagnosis of *Baylisascaris* sp. Positive identification of the larva as *Baylisascaris* sp. was made by Dr. Kevin R. Kazacos of Purdue University (West Lafayette, Indiana).

Two female beavers, 1 5.5 kg and the other 14

←

intestinal lesion caused by the presence of *Baylisascaris* sp. larva (arrowhead). H&E stain. Bar = 350  $\mu m$ . 3. Photomicrograph of liver lesion caused by the presence of *Baylisascaris* sp. larva (arrowheads). H&E stain. Bar = 350  $\mu m$ . 4. Higher magnification of *Baylisascaris* sp. larva from liver showing the lateral alae and characteristically laterally compressed intestinal lumen (arrowhead). H&E stain. Bar = 20  $\mu m$ . 5. Female *Dracunculus* sp. in situ on musculature of the lateral abdomen.

kg, both taken from the same locality (Wildcat Creek, Riley County), were found to harbor 4 and 1 fertile adult female *Dracunculus* sp., respectively. Lengths in millimeters, from 3 intact female nematodes, were as follows: 220, 210, and 185. In the smaller beaver, parasites were located bilaterally under the latissimus dorsi muscle. The single *Dracunculus* sp. present in the other animal was visible through the fascia of the external abdominal oblique muscle of the lateral abdomen (Fig. 5). Because only female worms were found, we were unable to make a specific identification.

Neither *Baylisascaris* sp. or *Dracunculus* sp. has been reported previously from North American beaver. The only reference to *Baylisascaris* sp. in beaver appears to be that of Kelly and Innes (1966). They reported the impossibility of raising infant beavers, *C. canadensis*, to maturity at the Dublin Zoological Gardens in Dublin, Ireland. They described clinical signs of motor weakness and progressive incoordination in an entire litter within 2 wk of birth. Histopathological examination of the brain of an affected individual revealed areas of patchy encephalitis and perivascular cuffing as well as cross-sections of 2 nematode larvae, identified only as "ascarids." A photomicrograph of a cross-section of 1 of the larvae clearly shows the intestinal lumen to be laterally compressed, a key character for the identification of the larvae as *Baylisascaris* sp. (Bowman, 1987).

Sprent (1968) and Kazacos and Boyce (1989) have listed several North American species of the genus *Baylisascaris* that may cause larval migrants in various avian and mammalian hosts. Among them are *B. columnaris* in skunks, *B. devosi* in fishers and martens, *B. melis* in badgers, *B. procyonis* in raccoons, and *B. transfuga* in bears. Larval identification beyond the generic level is impossible for *Baylisascaris* spp. The 2 most likely possibilities for the larvae found in this particular beaver are *B. columnaris* or *B. procyonis*, as both striped skunks (*Mephitis mephitis*) and raccoons (*Procyon lotor*) are common throughout the state (Bee et al., 1981).

Various mammals have been reported as definitive hosts for *Dracunculus* spp. in the United States and Canada (Muller, 1971; Crichton and Beverley-Burton, 1974). With 2 exceptions, the muskrat (*Ondatra zibethicus*) and the opossum (*Didelphis* sp.), all reported hosts have been of the order Carnivora. With our findings, the beaver becomes the second naturally occurring rodent

host for this parasite. While no exact figures are available for Kansas, the occurrence of *Dracunculus insignis*, particularly in raccoons, is a relatively common finding in North America (Crichton and Beverley-Burton, 1977; Tumilson et al., 1984) and has also been reported from dogs in the state (Ewing and Hibbs, 1966; Veatch and McKown, 1990). A specific determination cannot be made until males are collected.

The earliest report of coccidia in beaver from North America comes from Morley (1934), who reported oocysts in the large intestine of a beaver from Pennsylvania. During the same year, Yakimoff (1934) described and named *E. sprehni* from a captive North American beaver. To our knowledge, no other studies reporting coccidia in *C. canadensis* exist or were published until Ernst et al. (1970) gave a redescription of *E. sprehni* and provided a description of a new coccidian, *Eimeria causeyi*. To date, coccidia from only 4 regions in North America, Alabama (Ernst et al., 1970), Pennsylvania (Morley, 1934), Washington (Frost et al., 1980), and Kansas (this study), have been reported. We feel that the paucity of reports come not from the rarity of the parasite but, rather, from the lack of investigation.

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**Research Note**

**Nonproliferous Tetrathyridia of *Mesocestoides* sp. (Eucestoda: Mesocestoididea) in *Ptenopus garrulus maculatus* (Sauria: Gekkonidae) from Namibia, South West Africa, with a Summary of the Genus from Old World Lizards**

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**ABSTRACT:** A new host and geographic distribution record is reported for tetrathyridia of *Mesocestoides* sp. in 1 of 12 (8%) specimens of barking geckos, *Ptenopus garrulus maculatus*, from Namibia, South West Africa. The infected host had numerous tetrathyridia encapsulated in the liver and mesenteries. Further examination failed to demonstrate morphological features indicative of asexual proliferation in these tetrathyridia. A summary is provided of the Old World lizards thought to be hosts of tetrathyridia of *Mesocestoides* sp.

**KEY WORDS:** Eucestoda, Mesocestoididea, tetrathyridia, *Mesocestoides* sp., lizards, geckos, Sauria, Gekkonidae, *Ptenopus garrulus maculatus*, survey, prevalence, intensity.

Tapeworms of the genus *Mesocestoides* are parasites that, in the tetrathyridial (metacestode) stage, infect a variety of amphibians and reptiles. In Old World lizards, metacestodes thought to represent *Mesocestoides* sp. have been reported from the families Agamidae, Anguillidae, Chamaeleonidae, Gekkonidae, Lacertidae, and Scincidae (e.g., Witenberg, 1934; Hughes et al., 1941a, b). Two recent papers summarize reports of *Mesocestoides* sp. tetrathyridia from North American lizards (McAllister, 1988; Goldberg and Bursley, 1990). However, not since the compendium of Hughes et al. (1941a, b, 1942) have summations been provided on this tapeworm in Old World lizard hosts. Herein, we provide a new host and geographic distribution record for *Mesocestoides* sp., along with a summary of the Old World lizards previously reported to harbor this metacestode.

Twenty-two nocturnal geckos, including 12 barking geckos, *Ptenopus garrulus maculatus* (A. Smith, 1849), 4 sand geckos *Chondrodactylus angulifer* Peters, 1870, 4 Bibron's geckos *Pach-*

*yodactylus bibronii* (A. Smith, 1845), and 1 each of *Pachydactylus mariquensis* A. Smith, 1849, and spotted thick-toed gecko, *Pachydactylus punctatus* Peters, 1854, were collected alive by hand in early October 1987 in flat (elevation 540 m), sandy habitat in the vicinity of Keetmanshoop, Keetmanshoop District, Namibia, South West Africa (26°36'S, 18°08'E) and examined for tetrathyridia. Specimens were killed by intraperitoneal injection of T-61 euthanasia solution, fixed in 10% neutral-buffered formalin, and stored in 70% ethanol. Stomachs were removed from preserved geckos for dietary analysis (Bauer et al., 1989) and, at the same time, if encapsulated tetrathyridia were noted, tissue was embedded in paraffin blocks, sectioned at 10  $\mu$ m, stained with hematoxylin and eosin or Mallory's azan trichrome, and mounted in Permount®.

Representative specimens of *Mesocestoides* sp. tetrathyridia have been deposited in the U.S. National Parasite Collection, U.S. Department of Agriculture, Beltsville, Maryland 20705 as USNM Helm. Coll. No. 82906. Voucher specimens of geckos are deposited in the herpetological collection of the California Academy of Sciences, San Francisco (CAS).

Only 1 (5%) of the 22 individual geckos were infected; 1 of 12 (8%) *P. garrulus maculatus* (adult female, snout-vent length = 55 mm, CAS 167747). An undetermined (not all quantified) number of encapsulated tetrathyridia were found in the liver (Fig. 1) and mesenteries of this host. On further examination, these tetrathyridia were found to represent *Mesocestoides* sp. Each thin host-derived capsule contained either a single tetrathyridium or 2–3 tetrathyridia (Fig. 1). Infected tissues had minimal inflammatory re-



Table 1. Old World lizards reported or thought to be hosts of tetrathyridia of *Mesocestoides* sp.

Family/species*	Locality	Reference
<b>Agamidae</b>		
<i>Agama bibroni</i>	not given	Frank, 1981
<i>Stellio caucasi</i>	Soviet Union†	Sharpilo, 1976; Annaev, 1978
<i>S. erythrogaster</i>	Soviet Union†	Annaev, 1978
<i>S. stellio</i>	Middle East	Witenberg, 1934
<i>Trapelus sanguinolentus</i>	Soviet Union†	Annaev, 1978
<b>Anguidae</b>		
<i>Anguis fragilis</i>	Poland	Lewin, 1990
<i>Pseudopus apodus</i>	Soviet Union†	Annaev, 1978
<b>Chamaeleonidae</b>		
<i>Bradypodion pumilum</i> ‡	South Africa	Burrage, 1973
<i>Chamaeleo brevicornis</i> §	Madagascar	Brygoo, 1963
<i>C. dilepis</i>	Kenya	Baylis, 1937
<i>C. fischeri</i>	Tanzania	Baylis, 1937
<i>C. oustaleti</i>	Madagascar	Brygoo, 1963
<b>Gekkonidae</b>		
<i>Ptenopus garrulus</i>	Namibia	This paper
<i>Tarentola annularis</i>	Egypt	Meggitt, 1927a
<i>T. delalandii</i>	Canary Islands	Roca et al., 1987
<i>T. mauritanica</i>	Algeria	Crety, 1887; Joyeux and Baer, 1932
<i>Tenuidactylus caspius</i>	Soviet Union†	Annaev, 1978
<i>T. fedtschenkoi</i>	Soviet Union†	Annaev, 1978
<b>Lacertidae</b>		
<i>Acanthodactylus boskianus</i>	Syria	Witenberg, 1934
<i>Eremias arguta</i>	Soviet Union†	Sharpilo, 1976
<i>E. velox</i>	Soviet Union†	Annaev, 1978
<i>Lacerta agilis</i>	Germany	von Linstow, 1878
<i>L. laevis</i>	Middle East	Witenberg, 1934
<i>L. saxicola</i>	Soviet Union†	Sharpilo, 1976
<i>L. schreiberi</i>	Spain	Roca and Ferragut, 1989; Roca et al., 1990
<i>L. viridis</i>	France	Valenciennes, 1844; Henry, 1927; Joyeux and Baer, 1933, 1936
<i>L. vivipara</i>	France	Leuckart, 1874; Joyeux and Baer, 1936
<i>Ophisops elegans</i>	Soviet Union†	Sharpilo, 1976
<i>Podarcis bocagei</i>	Spain	Roca et al., 1989
<i>P. hispanica</i>	Spain	Roca et al., 1989
<i>P. muralis</i>	Spain	Garcia-Adell and Roca, 1988
<i>P. pityusensis</i>	Spain	Roca and Hornero, 1991, 1992
<i>Psammodyromus hispanicus</i>	Spain	Roca et al., 1986; Roca and Lluch, 1988
<b>Pygopodidae</b>		
<i>Lialis burtonis</i>	Australia	Hill, 1895
<b>Scincidae</b>		
<i>Chalcides ocellatus</i>	Morocco	Dollfus, 1958
<i>Mabuya carinata</i>	Burma	Meggitt, 1927b

\* Some of these hosts were originally reported to be infected with *Cisticercoideum*, *Cysticercoides*, *Cysticercus*, *Dithyridium*, *Piestocystis*, or *Tetrathyridium*; reassignments as tetrathyridia made by Witenberg (1934) and Hughes et al. (1941a).

† Specific localities in states of the former Soviet Union not known.

‡ Burrage (1973) reported unknown cestode capsules and metacestodes that may represent *Mesocestoides* sp.

§ Brygoo (1963) reported unknown tetrathyridia that may represent *Mesocestoides* sp.



Figure 1. Three tetrathyridia of *Mesocestoides* sp. encapsulated in the liver of *Ptenopus garrulus maculatus* from Namibia, South West Africa.

sponse or compression. None of the tetrathyridia exhibited morphological evidence of asexual proliferation such as large numbers of tetrathyridia per capsule, multiple scoleces, supernumerary suckers or buds (see Specht and Voge, 1965). Tetrathyridia lacked an apical organ and armed rostellum but possessed prominent suckers, calcareous corpuscles, and a deep invagination canal. They appeared similar histologically to New World *Mesocestoides* sp. tetrathyridia from xantusid (Goldberg, 1985), phrynosomatid (McAllister, 1988; Goldberg and Bursey, 1990; McAllister et al., 1992), and teiid (McAllister et al., 1991b, c) lizards.

About 36 species of Old World lizards representing 19 genera within 7 families are thought to be naturally infected hosts of tetrathyridia of *Mesocestoides* sp. (Table 1). Although footnotes explain inclusion of some hosts in the table, many of these lizards were reported originally to be

infected with metacestodes allocated to other genera (i.e., *Cisticercoideum*, *Cysticercoides*, *Cysticercus*, *Dithryridium*, *Piestocystis*, or *Tetrathyridium*). Reassignment to tetrathyridia and therefore *Mesocestoides* was made by Witenberg (1934) and Hughes et al. (1941a). However, inclusion of these records here is based on the published opinions of these authors because voucher specimens of hosts and/or parasites are not available. As in snakes (Conn and McAllister, 1990; McAllister et al., 1991a), metacestodes that resemble tetrathyridia but belong to different taxa occur in lizards and may be confused with *Mesocestoides* sp. tetrathyridia. Therefore, some of these records may not be valid for the genus.

In conclusion, our report represents the first time *Mesocestoides* sp. tetrathyridia have been found in *P. garrulus maculatus* and the country of Namibia, South West Africa. McAllister et al. (1991a) recently reported *Mesocestoides* sp. tetrathyridia from neighboring Cape Province, South Africa, in a Namib tiger snake, *Telescopus beetzi* (Barbour, 1922). Interestingly, as has been reported for other amphibians and reptiles (McAllister, 1988; McAllister and Conn, 1990), overall prevalence of infection in lizards appears to be low for *Mesocestoides* sp., whereas intensity of infection is usually rather high. Further documentation of additional host records are warranted, in order to gain a better understanding of the biology and distribution of this enigmatic cestode.

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### Research Note

## Ectopic *Moniliformis moniliformis* from a Laboratory-Infected Rat, *Rattus norvegicus*

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**ABSTRACT:** An 11.3-cm gravid female *Moniliformis moniliformis* was removed from the greater omentum of a female outbred Sprague-Dawley rat 5 mo post-infection. Within the omentum, the worm was isolated in a host connective tissue tunnel in which there were inflammatory reactions and abscess formation. Eggs released by the worm elicited granulomatous reactions. Because lymphocytes were abundant throughout the omental tissue, with large areas of perivascular infiltration, it is suspected that the eggs of *M. moniliformis* in this extraintestinal site have antigenic components capable of stimulating a cell-mediated delayed hypersensitivity reaction.

**KEY WORDS:** Acanthocephala, ectopic, granuloma, *Moniliformis moniliformis*, omentum.

Necropsy of a laboratory-reared 9-mo-old outbred female Sprague-Dawley rat, fed 20 cystacanths of *Moniliformis moniliformis* (Bremer, 1811) Travassos, 1915, at age 4 mo, revealed a tumorous mass posterior to the stomach (Fig. 1). The approximately 2.5- $\times$ -1- $\times$ -1-cm mass appeared to be contained completely within a diverticulum of the greater omentum (Fig. 1). Examination revealed the presence of a worm looped throughout the mass (Fig. 1). The worm was removed intact and transferred to tapwater, in

which it exhibited slight, very sluggish motility. It was maintained in several changes of tapwater over a period of 2 days before fixation in alcohol-formalin-acetic acid and later processing as a whole mount stained with Mayer's carmalum. The remainder of the mass was fixed in neutral-buffered 10% formalin, postfixed in 70% ethanol, washed in running tapwater overnight, and processed for paraffin sectioning at 10  $\mu$ m. Sections were stained with Harris's hematoxylin and eosin (Luna, 1968) as well as May-Grünwald stain, the periodic acid-Schiff reaction (PAS), Verhoeff's elastica stain, and Weigert's differential stain for fibrin (Thompson, 1966).

The parasite, an adult female *M. moniliformis*, was approximately 11.3 cm long. It did not become fully distended, nor did the proboscis evaginate, as expected after 2 days in tapwater. This may have been due to injury of the worm during its removal, or it might have been a reflection of reduced viability as a result of the host's response. In the whole-mount specimen, the tegument appeared friable and in some areas seemed to be sloughing. However, the ligament sacs of

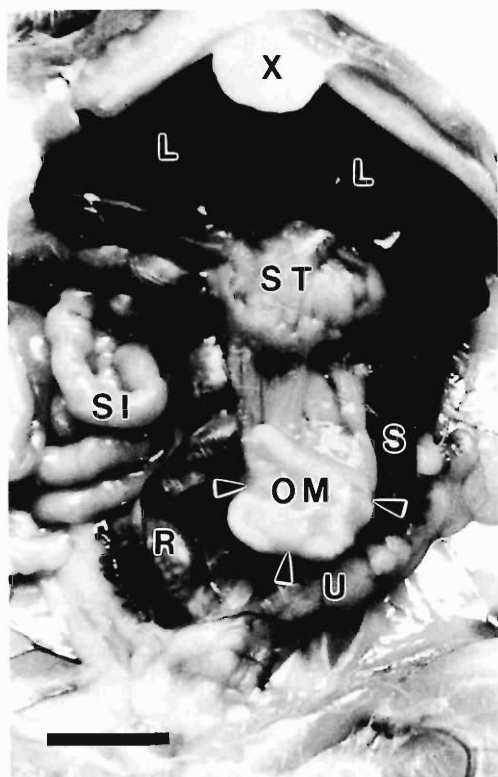


Figure 1. Photograph of ventral view of abdominal viscera and omental mass (pointers) containing *Moniliformis moniliformis*. Abbreviations: L = liver, OM = omental mass, R = rectum, S = spleen, SI = small intestine, ST = stomach, U = uterus, X = xiphoid process of sternum. Scale bar = 16 mm.

the worm were filled with fully developed, apparently viable eggs.

Histology of the mass was consistent with that of omentum. There were abundant white fat cells, blood vessels, and mononuclear cells. Mononuclear cells appeared to be mainly lymphocytes—frequently plasma cells, and occasional macrophages. Perivascular infiltration of lymphocytes was common throughout the sections. There were occasional eosinophils, and mast cells were found in clusters. A portion of the pancreas was incorporated in the diverticulum of the omentum. Whereas the majority of the tissues of the mass showed little postmortem necrosis, the acinar cells showed a degree of cloudy swelling with somewhat pycnotic nuclei. Whether this was the result of changes associated with the presence of *M. moniliformis* or truly postmortem necrosis could not be established; however, the

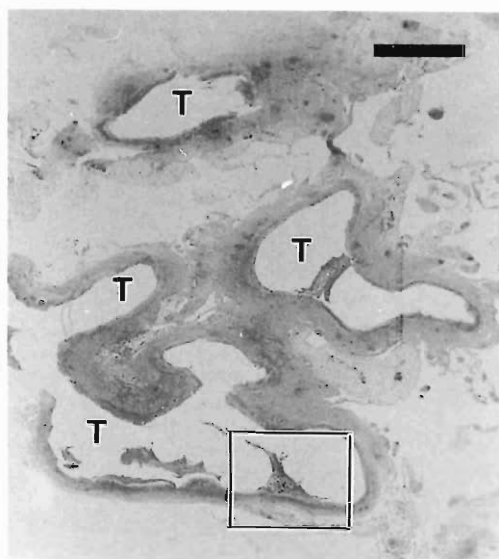


Figure 2. Macrophotograph of hematoxylin and eosin-stained paraffin section of omental mass. Area outlined by rectangle shown at higher magnification in Figure 3. Abbreviations: T = tunnel that contained the female *Moniliformis moniliformis*. Scale bar = 2 mm.

latter is most likely because approximately 30 min lapsed from the time of death until fixation of the mass.

The most striking pathological feature of the

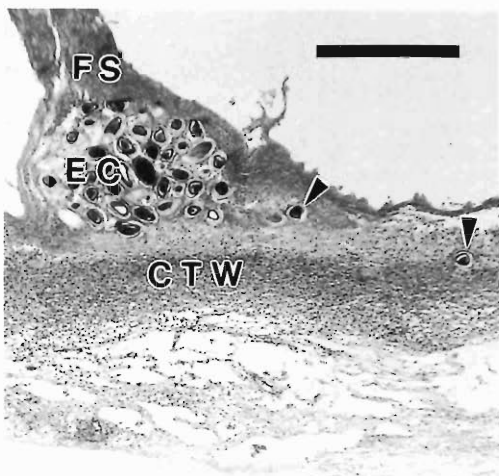
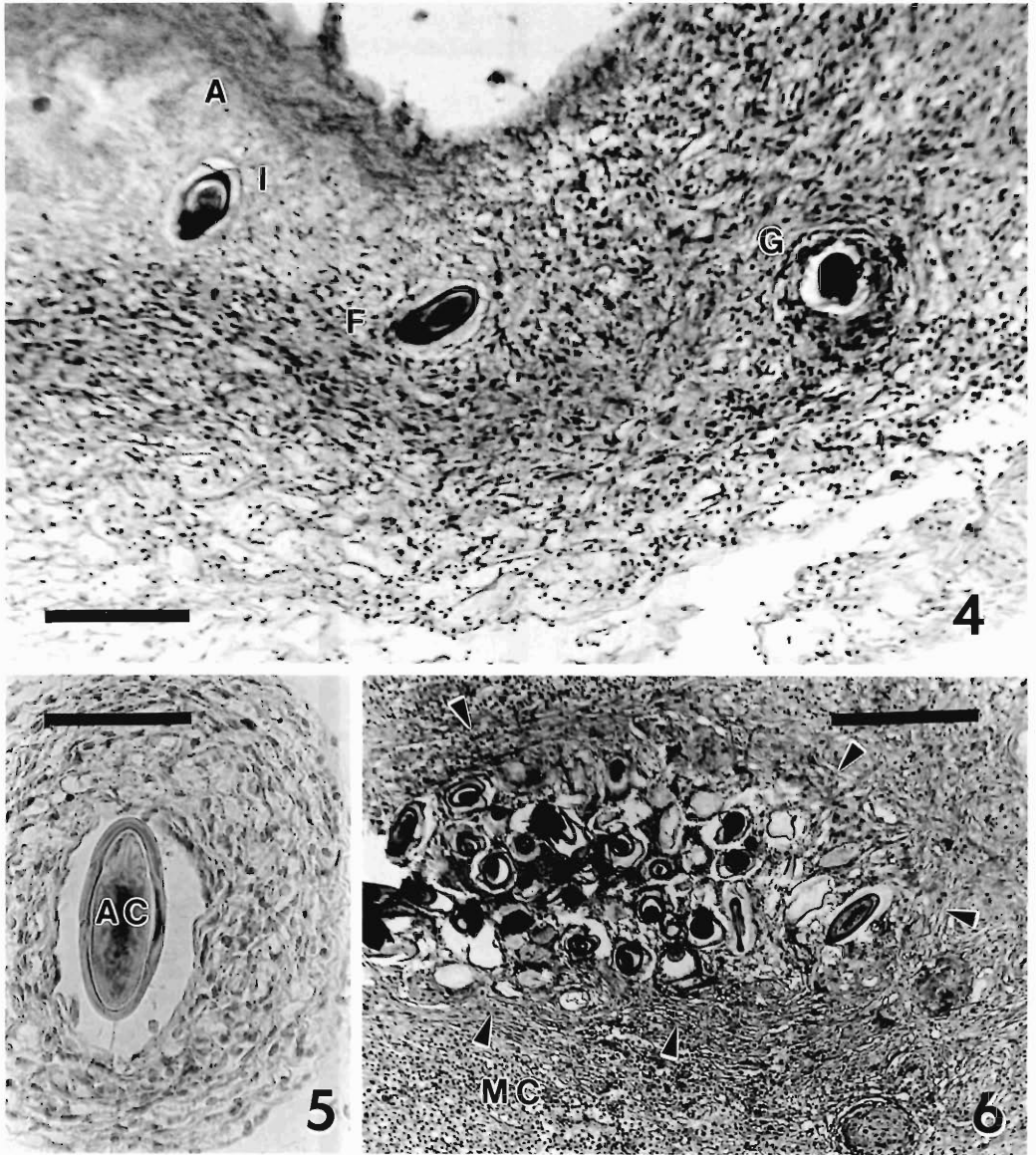


Figure 3. Photomicrograph of region noted in Figure 2 with fibrinoid substance protruding into the tunnel and a sequestered clump of *Moniliformis moniliformis* eggs. Pointers indicate individual eggs in stages of granulomatous reactions. Abbreviations: CTW = connective tissue wall of worm tunnel, EC = egg clump, FS = fibrinoid substance. Scale bar = 0.4 mm.



Figures 4–6. Photomicrographs of granulomatous reactions to *Moniliformis moniliformis* eggs. 4. Three eggs in different degrees of granuloma formation (Verhoeff stain). Scale bar = 0.2 mm. 5. Longitudinal section of a large granuloma containing a fully formed acanthor (hematoxylin and eosin stain). Scale bar = 0.08 mm. 6. A clump of eggs in the fibrous stage of granulomatous reaction. Pointers indicate areas of fibers (Verhoeff's stain). Scale bar = 0.2 mm. Abbreviations: A = region of abscess reaction, AC = acanthor, F = fibrous stage, G = completed granuloma, I = inflammatory stage, MC = mononuclear cells.

*M. moniliformis*-associated mass was the presence of a tunnel (Fig. 2), the site occupied by the worm, throughout the sections. The tunnel was surrounded by connective tissue, rich in collagen fibers. Within the tunnel there were occasional accumulations of numerous, apparently dead

polymorphonuclear leukocytes (PMN's) peripheral to large areas of a PAS-positive fibrinoid substance (Fig. 3). Thus, the worm appeared to have evoked a fibrino-purulent inflammatory host response.

Embedded among the PMN's and in the fi-

brinoid material are *M. moniliformis* eggs occurring singly and in small clumps (Figs. 3–5). As eggs occurred closer to the connective tissue margin of the tunnel (Fig. 3), they were subjected to granulomatous reactions. Such granulomatous reactions were most obvious for single, isolated eggs (Fig. 4, 5) but also were seen in response to clumps of eggs (Fig. 6). All stages of granuloma development (abscess formation, inflammatory stage, and fibrous stage) described by Hirata et al. (1993) for eggs of *Schistosoma japonicum* in the livers of mice were identified in sections of the omental mass containing *M. moniliformis*.

Several studies have demonstrated granulomatous reactions to acanthocephalans. Abe (1973) described the response to acanthocephalan proboscides in the submucosa of the pyloric cecum of rainbow trout. Taraschewski (1989) studied the reaction to dead, disintegrating *Acanthocephalus anguillae* in the peritoneal cavities of goldfish and carp. However, the present report is the first description of granulomatous reactions to acanthocephalan eggs. Inflammatory responses resulting in granuloma formation can be classified as either foreign body granuloma or infectious (or hypersensitivity) granuloma (Kellermeyer and Warren, 1970). Characteristic of each response are the development rate of the granuloma, the types of cells involved, and a variety of biochemical and immunologic determinations (Kellermeyer and Warren, 1970). Although the rate at which granuloma to *M. moniliformis* eggs develop is not known, the large accumulations of lymphocytes indicated that the response could have been cell-mediated delayed hypersensitivity, but experimental verification is necessary.

This is the first report of a gravid adult female *M. moniliformis* occupying an extraintestinal site in its definitive host. Varute and Patil (1971) described *Moniliformis dubius* (= *M. moniliformis*) in 6 of 30 moderate to heavily infected rats as causing perforations of the ileum with worms protruding into the abdominal cavity. The small intestine of the female rat in the present report contained no other worm and there was no gross sign of perforation damage along the wall of the small intestine. Because no ectopic male *M. moniliformis* was found and the female was gravid, it seems likely that the worm moved from the small intestine after insemination (as early as 16 days postinfection [Crompton, 1974]). A fixed, turgid female *M. moniliformis* at 15 days post-

infection is approximately 30 mm long and 0.7 mm wide (D. F. Oetinger and J. Essepian, unpubl. data). A worm this size could have moved to the pancreas and subsequently the omentum via a pancreatic duct, as has been reported (Popp and Schuster, 1989) for *Filicollis anatis* in its mallard host *Anas platyrhynchos*. However, Crompton (1974) indicated that full patency of a female *M. moniliformis* may require contact with 1 or more males over a period of 5 wk. A 50-day-old female *M. moniliformis* is approximately 162 mm long and 1.85 mm wide (D. F. Oetinger and J. Essepian, unpubl. data), probably too large to move through a rat pancreatic duct.

That ectopic *M. moniliformis* has not been reported from rats previously may reflect a phenomenon that is more common in female hosts but has not been noted because most experimental work with moniliformids has been conducted with male rats. Those researchers who use female animals should be aware of the possibility that extraintestinal movement may explain the greater intestinal loss rate of worms from female rats such as reported by Crompton and Walters (1972).

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62(1), 1995, p. 102

## Electronic Directory of Parasitologists

Electronic communications provide a rapid means of obtaining information from or sending information to colleagues involved in parasitological research. Thus, I am in the process of building a centralized directory of parasitologists that provides electronic mail (e-mail) addresses, as well as telephone and FAX numbers and research interests. The directory currently contains such information for more than 400 individuals from more than 40 countries. If you are interested in being included in the directory, please send me a message at one of the addresses listed below, and I will send you additional information.

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## In Memoriam

MAYBELLE HUTSON CHITWOOD  
(1908–1994)

MayBelle Hutson Chitwood, Life Member of the Helminthological Society of Washington and world authority on the structure and classification of nematodes, died peacefully in her sleep at the home of her daughter in Nichols, Iowa, September 19, 1994. She had Alzheimer's disease. MayBelle, as she was known to many, was born in Lubbock, Texas September 17, 1908. Her formal education, which included numerous institutions and a Bachelor of Arts from the University of Maryland in 1958, was not what distinguished her. Rather she was distinguished by her extraordinary knowledge of nematode structure which was gained from working side by side with her husband Benjamin G. Chitwood, first as students at Rice, continuing throughout their marriage from 1927 to 1952, and subsequently as an independent researcher until her retirement in 1976. She received an Honorary Doctor of Science Degree from Northern Michigan University in 1977.

MayBelle came to Washington when her husband joined the Bureau of Plant Industry, Department of Agriculture in 1928. He transferred to the Bureau of Animal Industry in 1931. B.G. and MayBelle worked long hours into the night to produce the classic, **Introduction to Nematology**, first published from 1937 to 1950 and reprinted in 1974. It is still an unparalleled source of information on nematodes. The decade 1937–1947 was spent on Long Island working on nematodes of ornamental plants and potatoes. The Chitwoods returned to Beltsville in 1947 to work on root-knot nematodes.

MayBelle raised 2 children (Marie D. Chapman of Nichols, Iowa and Edward M. Chitwood of Longwood, Florida) while participating as an unpaid consultant on many of her husband's projects at Beltsville, Maryland and Long Island, New York. When MayBelle and B. G. Chitwood ended their marriage in 1952, she began a paid career with the Agricultural Research Service at Beltsville. Although she lacked an advanced degree, MayBelle was recognized for her peerless knowledge of nematodes and was promoted from technician to the rank of Senior Scientist. Working with Allen McIntosh, Frank Douvres, and later training new recruit Ralph Lichtenfels, MayBelle was always willing to help a steady stream of colleagues and students identify nematodes. She truly loved her work and called her nematodes, "my children." Many of her colleagues, including this writer, learned most of what they know about nematodes by sitting at MayBelle's elbow and peering into her microscope on command.

After completing her recently reprinted landmark manual **Identification of Parasitic Metazoa in Tissue Sections**, MayBelle retired from Government service in 1973. She established a Primate Parasite Registry at the University of California at Davis from 1973 to 1976. She was President of the Helminthological Society of Washington in 1967, the recipient of the Anniversary Award in 1972 and elected Life Member in 1975. MayBelle published more than 50 research papers, including the 2 definitive contributions mentioned above, while carrying a heavy load of service identifications and curating the U.S. National Parasite Collection. She made significant contributions to the identification of *Capillaria philippinensis*, *Angiostrongylus cantonensis* and numerous others, many of which were published only after consultation with MayBelle.

She always told me that she would be remembered about as long as it took water to level when an object was removed from it. For once she was wrong. Her contributions were so great that scientists all over the world still, after more than 40 years, use the **Introduction to Nematology** as a bible and the **Identification of Parasitic Metazoa in Tissue Sections** is regularly requested by veterinary pathologists after 22 years. Sadly, during her working years this highly respected scientist, who helped so many, felt unappreciated. To honor her memory, I request that you take the time to write a note of praise to a colleague whom you admire.

J. Ralph Lichtenfels  
USDA, ARS, Beltsville, Maryland 20705-2350

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*Benjamin Schwartz	1976	Mary Hanson Pritchard	1994

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