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Prevalence and Intensity of *Gyrodactylus maculosi* sp. n. (Monogenea) Parasitizing Gills of Sculpin (*Oligocottus maculosus*) in Coastal British Columbia, Canada

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ABSTRACT: *Gyrodactylus maculosi* sp. n. (Monogenea: Gyrodactylidae) is described from the gills of tidepool sculpin (*Oligocottus maculosus*) in coastal British Columbia, Canada. The parasite resembles most closely *G. cranei* Mizelle and Kritsky, 1967, and *G. pacificus* Mizelle and Kritsky, 1967, described from the Pacific tomcod (*Microgadus proximus*). The 3 species appear to represent a lineage that has radiated among neritic fishes off the coast of western North America. Members have stout hamuli with a short recurved point and long recurved root and a ventral bar devoid of anterolateral processes. The marginal hook sickle has a well-developed base with a relatively slender blade. Each species has characteristic haptoral sclerites. Host fishes were collected bimonthly from January 1988 through December 1988. Prevalence was 61–100%. Intensity was 1–598, with highest numbers of parasites recovered in June and July. In spite of intense infections there was no sign of gross pathology of the gills or of host mortality.

KEY WORDS: *Gyrodactylus maculosi* sp. n., Monogenea, *Oligocottus maculosus*, British Columbia.

During a study of parasites of sculpin (*Oligocottus maculosus* Girard) inhabiting tidepools in coastal British Columbia, a previously undescribed species of *Gyrodactylus* was found. The present study describes the new worms as *Gyrodactylus maculosus* sp. n. and reports on the parasite's seasonal occurrence and effect on the host.

Materials and Methods

Semimonthly samples of 13–16 adult *O. maculosus* were collected from tidepools located around Popham Island, Howe Sound (49°21'N, 123°29'W), British Columbia, Canada, between January and December 1988. Fish were captured by dip net during low tide and fixed immediately in 10% formalin. Standard length and weight of each fish were recorded. Gills were excised and examined microscopically for parasites. Prevalence refers to the percentage of infected fish in a sample. Mean intensity refers to the mean number of parasites per infected fish. A Kolmogorov-Smirnov test statistic revealed that the intensity data were overdispersed, approaching a negative binomial distribution. We therefore applied the nonparametric Kruskall-Wallis test when comparing grouped bimonthly intensities. We also found it appropriate to calculate median intensities, so as to minimize the influence of the overdispersed data. Ten parasites were mounted unstained in glycerine jelly and used for morphological study; 6 were measured by means of an ocular graticule. Measurements of the holotype are followed in parentheses by those of the paratypes. All measurements are in micrometers.

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Results

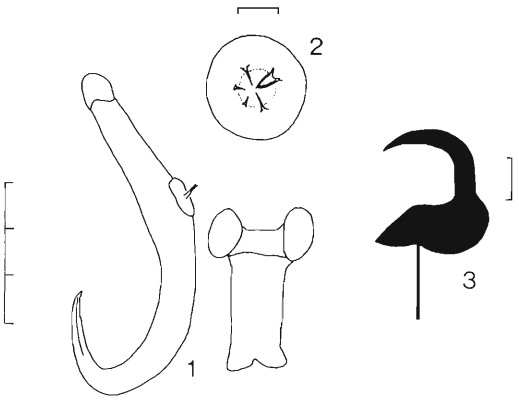
Gyrodactylus maculosi sp. n. (Figs. 1–3)

DESCRIPTION: Mounted specimens 410 (360–450) long, 100 (90–140) wide at midbody. Pharynx 40 (39–42) long, with long cellular processes. Penis (12–15) in diameter, with a single large spine and 2 pairs of small spines in single row at opening of ejaculatory duct. Hamuli robust, 67 (63–67) long; root 31 (29–31), shaft 44 (42–44), point 22 (20–23). Ventral bar dumb-bell shaped, 31 (21–25) wide, 7 (5–7) long medially. Ventral bar anterolateral processes absent. Ventral bar membrane rectangular in shape, 25 (21–25) long with posterior margin indistinct. Dorsal bar simple, without median notch. Marginal hook 36 (33–36) long. Marginal hook sickle 6 (6–7) long, 4 (4–6) wide basally, 7 (6–7) wide distally, with strongly recurved blade. Marginal hook handle consistent width along entire length and without distinct terminal swelling, 30 (30–31) long. Filament 9 (9–11) long.

TYPE HOST: *Oligocottus maculosus* Girard (adults).

TYPE LOCALITY: Popham Island, Howe Sound (49°21'N, 123°29'W), British Columbia, Canada.

SPECIMENS STUDIED: Detailed measurements were obtained from 6 specimens. The holotype (no. 82429) and 2 paratypes (no. 82430) are deposited in the USNM Helminthological Collection, Beltsville, Maryland.



Figures 1–3. Sclerites and penis of *Gyrodactylus maculosi* parasitic on the gills of *Oligocottus maculosus*. 1. Hamulus and ventral bar. Scale = 30 μm. 2. Penis. Scale = 6 μm. 3. Marginal hook sickle. Scale = 2 μm.

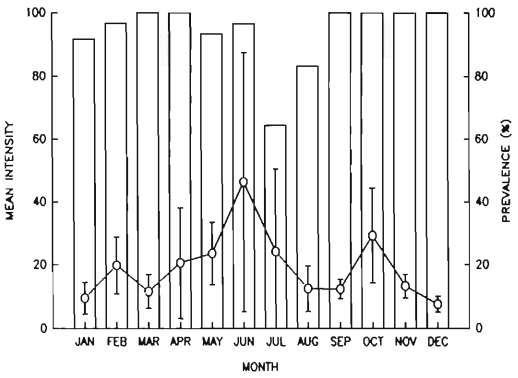


Figure 4. Changes in prevalence (histogram) and mean intensity (line graph) of *Gyrodactylus maculosi* on gills of *Oligocottus maculosus* throughout 1988. Error bars represent 95% confidence limits.

Prevalence and intensity of infection

Gyrodactylus maculosi was common on *O. maculosus* in the tidepools. Prevalence of infection was 90% or higher during all months of the year except July and August (Fig. 4). During July and August it was 61 and 82%, respectively (Fig. 4). Intensity ranged from 1 to 598. Statistical comparison of monthly intensities revealed significant seasonal changes ($P < 0.05$), with a recorded high during June 1988. The decline in prevalence during July and August corresponded with a significant ($P < 0.05$) seasonal decrease in intensity during the same period (Fig. 4).

Pathology of infection

There was no evidence of host mortality within the tidepools. Gills of infected fish appeared normal and showed no obvious gross pathology when viewed with a stereomicroscope. Intensity of infection did not correlate ($P < 0.05$) with host condition factor (weight (mg)/length (mm)^3). Intensity increased significantly with host size. A "box and whisker" plot of median intensity serves to illustrate the latter relationship by minimizing the effects of extreme values (Fig. 5).

Ecology of the host population

The sculpin collected were fish that had remained in the tidepools after tidal retreat. From January to June, samples included fish of similar length classes (Fig. 6). However, during the months of July and August, the lengths of fish sampled decreased (Fig. 6). Similarly, samples collected during November and December represented increasingly smaller fish (Fig. 6).

Discussion

Gyrodactylus maculosi resembles *G. cranei* Mizelle and Kritsky, 1967, and *G. pacificus* Mizelle and Kritsky, 1967, both species being described from tomcod (*Microgadus proximus*) inhabiting coastal waters of California (Mizelle and Kritsky, 1967). All 3 species have characteristically stout hamuli with a long recurved root and a short recurved point. The ventral bar does not have anterolateral processes and the marginal hook has a well-developed base and relatively thin blade. The 3 species likely represent a lineage that has radiated on neritic fishes of coastal waters of western North America. Similar species of *Gyrodactylus* are not known from other regions of the world. Cottid fishes from coastal regions of eastern North America historically acquired members of an entirely separate lineage of gyrodactylids involving the *groenlandicus* species group (Cone and Wiles, 1983).

The 3 species are easily identified: *Gyrodactylus maculosi* has a hamulus point that curves consistently at its base, the dorsal bar is a simple tube that inserts centrally onto the hamulus knob, the ventral bar membrane is almost rectangular, and the handle of the marginal hook has a consistent width along its length; *Gyrodactylus cranei* has a distinct angle formed at the base of the hamulus point, the dorsal bar wraps around the hamulus knob, the posterior membrane is expanded distally, and the marginal hook handle has a terminal swelling (Crane and Mizelle, 1967);

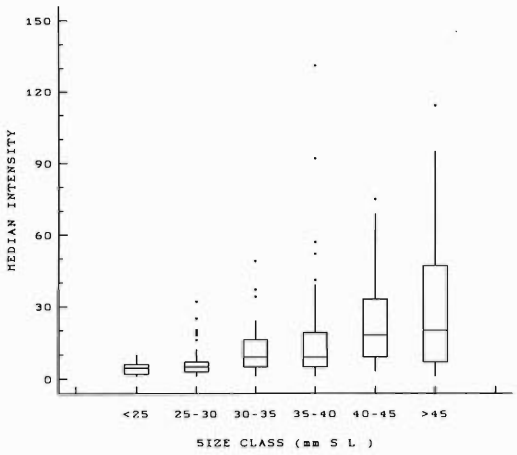


Figure 5. "Box and whisker plot" of median intensities of *Gyrodactylus maculosi* in different size classes of *Oligocottus maculosus*. The plot graphically represents a summary of data where the central line (horizontal) is the median, the box covers the middle 50% of the data between the upper and lower quartiles, the whiskers extend out to extreme values within 1.5 times the interquartile, and the points beyond the whiskers are outliers. Four omitted data points include 220, 260, 598 (35–40-mm size class), and 300 (>45-mm size class).

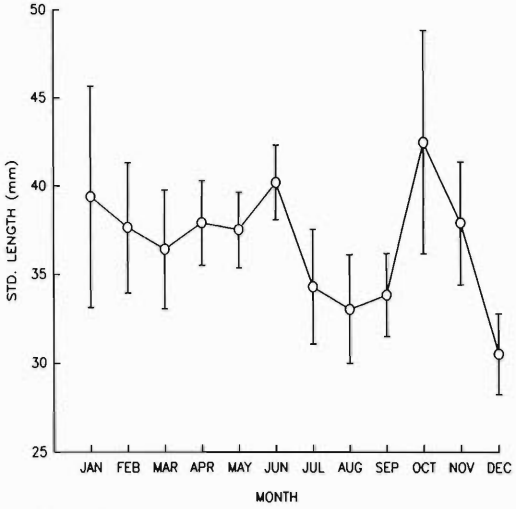


Figure 6. Mean standard length of *Oligocottus maculosus* collected from tidepools of Popham Island, Howe Sound, British Columbia, during 1988.

Gyrodactylus pacificus has relatively long hamuli (81–91 μm) and the dorsal bar is bifurcate terminally (Crane and Mizelle, 1967).

Gyrodactylus maculosi is the third species of the genus to be described from cottid fishes of the northeastern Pacific Ocean. The others include 2 species from *Leptocottus armatus* (*G. armatus* Crane and Mizelle, 1967, and *G. sculpinus* Crane and Mizelle, 1967). Neither species resembles *G. maculosus* and both normally occur on the body surface. The report (Arai, 1969) of an unidentified gyrodactylid from *O. maculosus* was likely *G. maculosi.*

Observed intensity of *G. maculosi* on *O. maculosus* is high compared to other gyrodactylids on neritic marine fishes. Kamiso and Olson (1986) observed 1–49 *Gyrodactylus stellatus* on the body of young sole (*Parophrys vetulus*) collected in Yaquina Bay, Oregon. One obvious cause for the difference is that extended aggregation of *O. maculosus* in shallow tidepools likely facilitates transmission of viviparous monogeneans. Petrushevski and Shulman (1958) also reported intense infections of *Gyrodactylus arcuatus* on sticklebacks stranded in tidepools of the White Sea. Intensities of up to 1,000 reportedly contributed to significant host mortality. In the present study we found no evidence to suggest that *G. maculosi* causes host death. In fact, our analysis failed to find any significant relationship between intensity and host condition factor, suggesting a stable host–parasite relationship even under crowded conditions of the tidepools.

Eggs of *O. maculosus* are laid late in October to late April and hatch from February to May; settlement of the larvae (10+ mm) occurs from March through the end of May and early June. Juveniles are 25–30 mm long when they are first seen in the tidepools (Craik, 1978). Our observations indicate that juvenile sculpins obtained infections shortly after establishment in the pools, presumably after contact with older, infected fishes.

The seasonal change in prevalence and intensity of *G. maculosi* near Popham Island is similar in certain respects to that reported for *G. stellatus* on *P. vetulus* in coastal Oregon (Kamiso and Olson, 1986). Young *P. vetulus* acquire infections soon after entering the estuary in spring and, by June, prevalence and intensity reach a seasonal high (98% and 10, respectively). Prevalence and intensity decreased gradually to a low in October when fish were leaving the estuary. With *G. maculosi* there is a gradual increase in intensity of infection from January to June, during the months in which we consistently sampled cohorts with a mean length of 36–40 mm. The intensity data also revealed a late summer and early winter

decline in the numbers of parasites similar to that reported for *G. stellatus* on *P. vestulus*. However, since intensity increased with host size, the observed fall oscillation in intensity is likely exaggerated because small fish were sampled. Low intensities found on the fish during the January 1988 samples suggest that a winter decline in parasite populations occurs.

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Obituary Notice

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Hapalotrema dorsopora sp. n. (Trematoda: Spirorchidae) from the Heart of the Green Turtle (*Chelonia mydas*) with a Redescription of *Hapalotrema postorchis*

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ABSTRACT: *Hapalotrema dorsopora* sp. n. from the heart of the green turtle *Chelonia mydas* is described. *Hapalotrema dorsopora* differs from all other members of the genus by having a separate dorsal uterine pore. *Hapalotrema dorsopora* most resembles *Hapalotrema mehrai* Rao, 1976, but differs, in addition to separate dorsal and ventral pores, in placement of testes and vitellaria and shape of ovary. *Hapalotrema postorchis* Rao, 1976, originally described from a single specimen, is redescribed.

KEY WORDS: Trematoda, *Hapalotrema dorsopora* sp. n., Spirorchidae, green turtle, *Chelonia mydas*, Hawaii.

From 1986 to 1988, 10 green sea turtles (*Chelonia mydas* L.) were found stranded on the islands of Lanai, Maui, and Oahu in Hawaii. The turtles were covered with neoplasms identified as fibropapillomas. Upon determination that the turtles would not survive, they were killed and examined for parasites. Eight turtles, 6 from Oahu, 1 from Lanai, and 1 from Maui, were found infected with an undescribed species of the genus *Hapalotrema* Looss, 1899.

Materials and Methods

Worms were placed in tapwater and refrigerated overnight, fixed in alcohol–formalin–acetic acid for 2 days, and then transferred to 70% ethyl alcohol for storage. Whole mounts were stained in Semichon's acetocarmine, dehydrated in a graded alcohol series, and mounted in Canada balsam. Specimens for scanning electron microscopy were critical-point dried using CO_2 as the transition fluid in a Polaron critical-point dryer and mounted on specimen stubs using conductive graphite paint (TV tube coat). Specimens were coated for 10 min at 10 mA with gold-palladium in a Technics Hummer V sputter coater and examined with an AMR 1000 at 8–20 kV. All measurements are in micrometers unless otherwise indicated and are given as a range with the mean in parentheses. Illustrations were made with the aid of a drawing tube. Voucher specimens have been deposited in the USNM Helminthological Collection.

Results

Seven species of digenetic trematodes were collected from 10 specimens of green turtles in Hawaii (Table 1). Trematodes of the genus *Hapalotrema* occurred as 35% of total worms recovered and in 80% of turtles examined.

Description

Hapalotrema dorsopora sp. n. (Figs. 1–3, 7)

Hapalotrema dorsopora sp. n. Spirorchidae Stunkard, 1921. The following description based on 10 specimens.

SPECIFIC DIAGNOSIS: Body elongate, 9.2–10.6 mm (9.9) long, maximum width 0.59–1.1 mm (0.86) at midbody. Oral sucker terminal, 420–450 (430) long by 360–370 (368) wide. Esophagus tubular, 630–720 (680) long surrounded by large gland cells at bifurcation (at 8% of body length) anterior to acetabulum. Intestinal ceca slightly sinuous, terminating posteriorly near anterior end of Y-shaped excretory vesicle. Acetabulum larger than oral sucker, discoid, covered with minute spines, pedunculate, with folded outer margin, 583–684 (610) in diameter. Peduncle 526–631 (578) long by 390–430 (410) wide. Testes numerous, 78–177 (119) long by 52–112 (78) wide, separated into pre- (47–56) and postovarian (48–54) groups filling intercecal space. External seminal vesicle 262–396 (308) long by 90–195 (139) wide, intercecal, transverse between preovarian testes group and ovary. Cirrus sac 270–338 (297) long by 60–90 (73) wide with internal seminal vesicle, ejaculatory duct and cirrus. Male genital pore median, ventral, and sinistral to ovary. Ovary oval, submedian, with irregular margins, 577–763 (665) long by 579–736 (666) wide, between testicular groups. Seminal receptacle immediately posterior to ovary. Dorsal uterine pore, median, on raised muscular pad (Fig. 7), 21 posterior to vitelline

Table 1. Digenetic trematode parasites found infecting 10 green turtles from Hawaii.

	Locality*	USNM Coll. No.	Prevalence	Mean intensity	Range
Learedius learedi	1, 3	82321	40%	20.0	4–43
Hapalotrema dorsopora	1, 2, 3	82326	80%	8.0	3–20
Polyangium linguatula	1, 2	82322	40%	3.5	1–9
Angiodictyum longum	1, 2	82323	30%	3.0	2–5
Hapalotrema postorchis	1	82324	30%	6.0	2–14
Carettacola hawaiiensis	1, 3	81897	30%	10.0	3–17
Pyelosoma cochlear	1	82327	10%	19.0	19

* 1, Oahu; 2, Lanai; 3, Maui.

reservoir. Vitellaria mostly extracecal extending from pretesticular region to posterior extremity. Uterus short with metraterm, looping posteriorly from ovary to dorsal uterine pore. Eggs ($N = 10$) elongate with single or double polar filaments, 144–196 (170) long by 20–32 (26) wide.

HOST: Green turtle, *Chelonia mydas* L.

LOCATION: Heart.

LOCALITY: Kailua Bay, Oahu, Hawaii.

HOLOTYPE: USNM Helm. Coll. No. 82326.

PARATYPE: USNM Helm. Coll. No. 82327.

ETYMOLOGY: Dorso (L) = back; pora (L) = pore.

REMARKS: The new species most resembles *H. mehrai* Rao, 1976, but differs in size (*H. dorsopora* is larger); disposition of testes (first few preovarian testes only in 2 rows in *H. mehrai,* not found in *H. dorsopora*); placement of vitellaria (begin just posterior to acetabulum in *H. mehrai,* just anterior to testes in *H. dorsopora*); ovary entire in *H. mehrai,* irregular in *H. dorsopora*; dorsal uterine pore present in *H. dorsopora,* absent in *H. mehrai.*

Hapalotrema postorchis Rao, 1976 (Figs. 4–6)

Redescription based on 10 specimens. Body elongate, 10.3–12.8 mm (11.6) long, maximum width 1.0–1.8 mm (1.3). Tegument spinose; spines lost in frozen specimens. Oral sucker terminal, small, subspherical, 230–330 (250) wide by 240–360 (275) long; pharynx absent; esophagus narrow, thin-walled, 810–870 (832) long; bifurcating at 6–8% (7%) body length, just anterior to acetabulum, into 2 slender ceca, which extend to posterior extremities. Acetabulum slightly pedunculate, spherical, larger than oral sucker, 420–910 (714) in diameter, perimeter of sucker spinose, anterior edge at 9–12% (10.5%) body length. Testes few, 15–18 (17) measuring 430–620 (496) long by 280–420 (372) wide, separated by ovary into pre- (7–11) and postovarian (6–9) groups. Cirrus pouch large, 710–900 (840) long by 240–310 (285) wide, with internal seminal vesicle, ejaculatory duct and cirrus. External seminal vesicle transverse, between preovarian testes and ovary, measuring 480–670 (590) long by 250–380 (310) wide. Genital pore sinistral at lower level of ovary. Ovary lobed, between third and fourth quarter of body, 710–870 (775) long by 470–680 (618) wide. Vitellaria inter- and extracecal beginning 638–1020 (842), postacetabulum. Uterus short with metraterm. Eggs ($N = 10$) elongate with polar filament on each end measuring 140–330 (168) × 29–41 (32).

HOST: *Chelonia mydas* L.

LOCATION: Heart.

LOCALITY: Pamban, South India, Gulf of Manar (type locality; Rao, 1976). Kaneohe Bay, Oahu, Hawaii (this study).

VOUCHER SPECIMEN: USNM Helm. Coll. No. 82324.

REMARKS: According to the original descrip-

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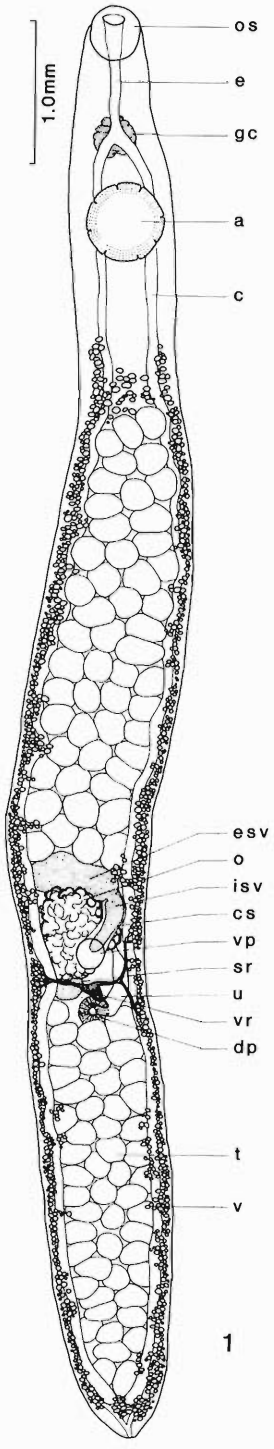
Figures 1–6. 1–3. *Hapalotrema dorsopora* sp. n. 1. Entire worm (ventral view). 2. Outline of entire worm (lateral view) showing pedunculate acetabulum, placement of dorsal and ventral openings. 3. Egg. 4–6. *Hapalotrema postorchis* redescription. 4. Entire worm (ventral view). 5. Outline of worm anterior showing slightly pedunculate acetabulum (lateral view). 6. Egg. a, acetabulum; c, cecum; cs, cirrus sac; dp, dorsal pore; e, esophagus; esv, external seminal vesicle; gc, gland cells; isv, internal seminal vesicle; o, ovary; os, oral sucker; sr, seminal receptacle; t, testis; u, uterus; v, vitellaria; vp, ventral pore; vr, vitelline reservoir.



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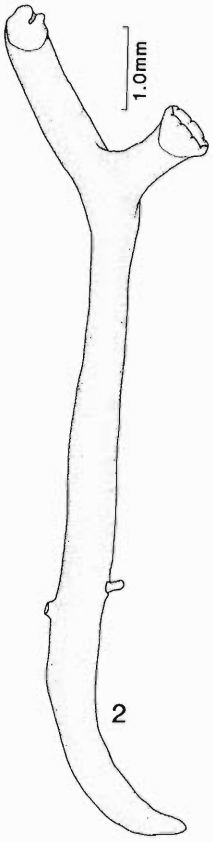

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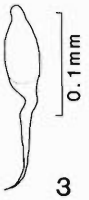

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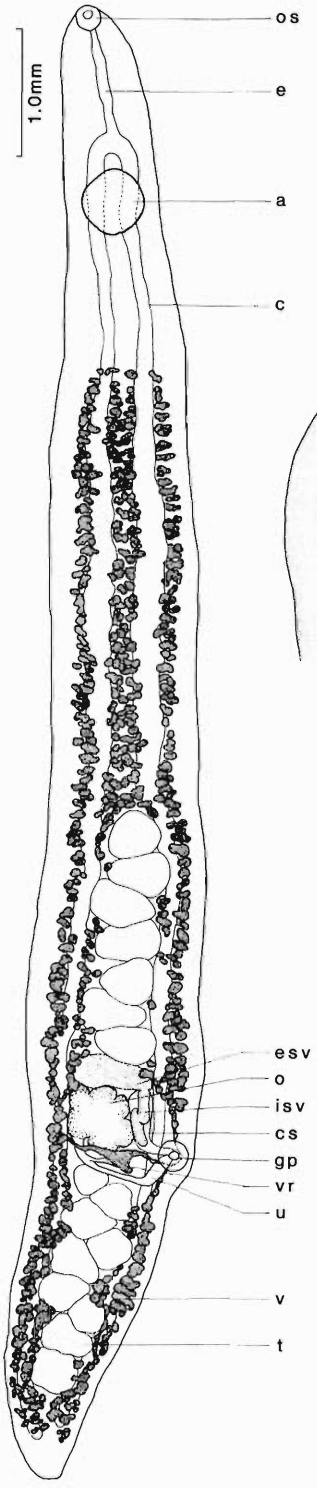

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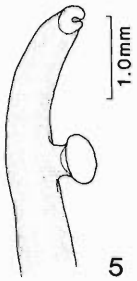

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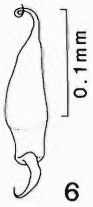

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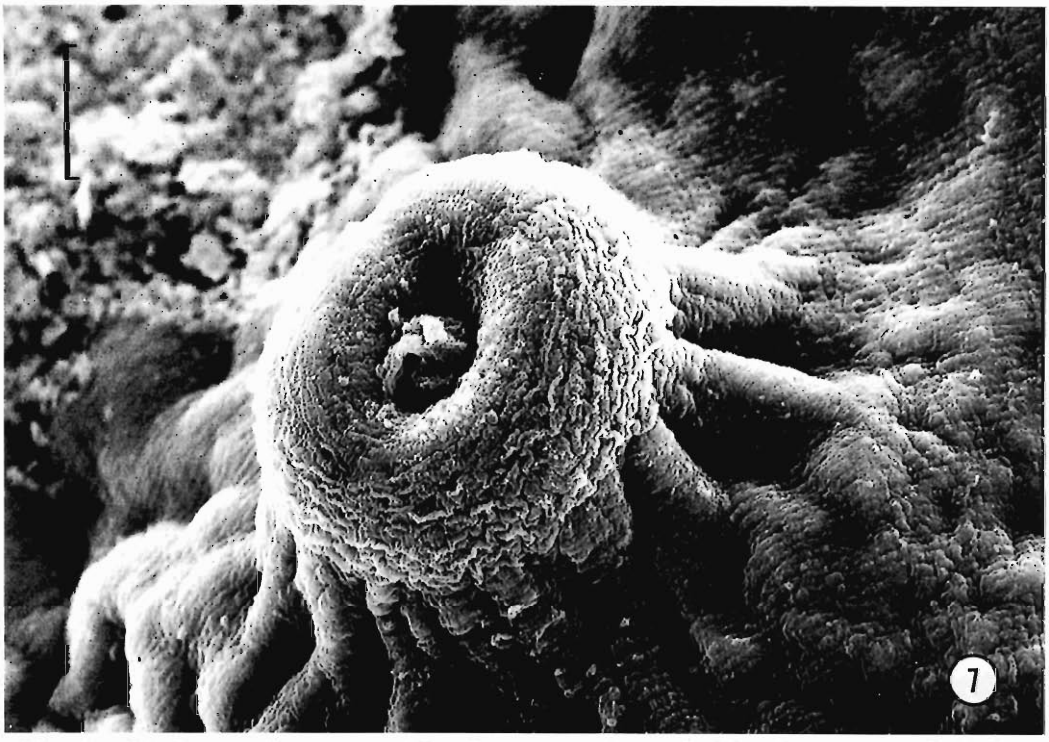

Figure 7. ***Hapalotrema dorsopora*** **sp. n. Scanning electron micrograph of dorsal uterine pore showing large, muscular-raised base. Scale bar = 30 μm.**

tion by Rao (1976), the measurements were taken from only 1 specimen. The specimen was not deposited in any collection and all attempts to examine the type material were unsuccessful. Differences seen between worms of this study and the Rao description are as follows. The acetabulum is slightly pedunculate and ringed with fine spines in the Hawaiian specimens. The total number of testes of the worm figured in the 1976 publication is shown at 19 (9 pre- and 10 postovarian). In our study, the maximum number found in any specimen examined was 18. The ceca are figured by Rao as bifurcating widely enough to circumvent the acetabulum. In the Hawaiian specimens, the ceca always continued behind the ventral sucker. Also, the vitellaria placement differs between the material in this study and those of the original description (beginning more posteriad in the Hawaiian material).

Discussion

The blood flukes of sea turtles have been reviewed recently by Smith (1972) and Glazebrook et al. (1989). The genus *Hapalotrema* is separated from other members of the subfamily Hapalotrematinae Stunkard, 1921, by its large number of testes divided by the ovary and terminal genitalia into 2 groups (Yamaguti, 1958; Skrjabin, 1964). Smith (1972) lists 5 species of *Hapalotrema* in his review (*H. loossi* Price, 1934; *H. mistroides* (Monticelli, 1896) Stiles and Hassall, 1908; *H. orientalis* Takeuti, 1942; *H. polesianum* (Ejsmont, 1927) Byrd, 1939; *H. synorchis* Luhman, 1935). Glazebrook et al. (1989) list only 3 species of *Hapalotrema* in their table "Cardiovascular flukes recovered from sea turtles (1962 to present day)." There are currently 7 recognized species of *Hapalotrema* (Smith, 1972, omitted the 2 species from India, *H. mahrai* and *H. postorchis,* both described by Rao, 1976). All *Hapalotrema* species were described from marine turtles except *H. polesianum.* This species was originally found in the freshwater turtle *Emys orbicularis* and published as *Spirhapalum polesianum* by Ejsmont (1927) and later transferred to *Hapalotrema* by Byrd (1939). Only *H. mehrai* and *H. postorchis* have previously been recorded from *C. mydas. Hapalotrema loossi, H. synorchis,* and *H. mistroides* were all found in the log-

gerhead turtle (*Caretta caretta*) in Egypt, Florida, and an unknown locality, respectively. *Hapalotrema orientalis* was found in the hawksbill (*Eretmochelys squamosa*) on the island of Okinawa, Japan.

Two genera (*Hapalorhynchus* Stunkard, 1922, and *Coeuritrema* Mehra, 1933) from freshwater turtles have dorsal genital pores. However, the size of those pores is much smaller and less muscular than that described for *H. dorsopora*. This is the first report of a spirorchid trematode with separate dorsal (uterus) and ventral (cirrus) openings.

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NEMATODE PARASITES OF VERTEBRATES: THEIR DEVELOPMENT AND TRANSMISSION, by R. C. Anderson, 1992, C. A. B. International, 600 pp. (hardback) ISBN 0 85198 799 0. Available from The University of Arizona Press, 1230 North Park Avenue, Tucson, Arizona 85719. US$142.50 (Americas only).

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Hookworms of Bobcats (*Felis rufus*) from Florida

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ABSTRACT: From 1974 to 1991, 85 bobcats (*Felis rufus* Schreber) from 17 counties in Florida were examined at necropsy for hookworms. At least 1 of 4 species of *Ancylostoma* was found in 52/85 (61%) bobcats. *Ancylostoma tubaeforme* (Zeder, 1800), *A. caninum* (Ercolani, 1859), *A. braziliense* (de Faria, 1910), and *A. pluridentatum* (Alessandrini, 1905) were represented at prevalences of 11, 18, 19, and 29%, respectively. Intensities for all hookworms combined varied from 1 to 128 ($\bar{x}$ = 17.6, SD = 28.6) with mixed infections (2 or more species) in 20 (24%) bobcats. Significantly more infected juveniles (8/10) than adults (12/35) had mixed infections (χ^2 = 5.06, 1 df, $P < 0.025$). Prevalences and intensities for all species combined did not differ significantly between sexes, ages, and regions with the exception of mean intensity of infection, which was much greater in the southern ($\bar{x}$ = 25.3) than in the northern ($\bar{x}$ = 6.3) region ($P < 0.01$). *Ancylostoma pluridentatum* was found only in bobcats from southern Florida, where its prevalence was 57%. *Ancylostoma caninum, A. tubaeforme,* and *A. braziliense* were distributed throughout the state.

KEY WORDS: bobcat, *Felis rufus,* hookworms, *Ancylostoma* spp., Florida, prevalence, intensity.

The parasites of bobcats, *Felis rufus,* have been studied in various localities in the United States (Rollings, 1945; Pollack, 1949; Progulske, 1952; Miller and Harkema, 1968; Little et al., 1971; Mitchell and Beasom, 1974; Stone and Pence, 1978; Watson et al., 1981; Fox, 1983; Tiekotter, 1985; Marchiondo et al., 1986; Heidt et al., 1988; Mensik-King, 1989). Little information has been published on the parasitic fauna of bobcats in Florida with the exception of data presented in Forrester et al. (1985) and Forrester (1992). In both references the presence of *Ancylostoma pluridentatum* in bobcats of southern Florida was discussed.

Hookworms can be highly pathogenic in domestic dogs and cats, causing rough hair coat, listlessness, weakness, poor weight gain and emaciation, edema, anemia, and death, especially in young animals (Miller, 1965, 1966; Onwuliri et al., 1981; Kalkofen, 1987). Because the bobcat is distributed throughout Florida, it may serve as a reservoir of hookworms for domestic as well as other wild carnivores such as the endangered Florida panther (*Felis concolor coryi* Bangs). The purposes of this study were to determine the species of hookworms present, their prevalence, intensity, and distribution in bobcats of Florida, and locality-, sex-, or age-related differences in those parameters.

Materials and Methods

From 1974 to 1991 bobcats were obtained as roadkills, by trapping, or by hunting from 17 counties in Florida (Fig. 1). Counties from Tampa Bay north (A–I: Leon, Wakulla, Columbia, Baker, Alachua, Levy, Flagler, Lake, and Polk) were designated as northern; those south of Tampa Bay (J–Q: Sarasota, Highlands, Glades, Lee, Hendry, Palm Beach, Collier, and Dade) were classified as southern. Carcasses were frozen until necropsy. Ages of bobcats (adult or juvenile) were based on dentition and/or size. Recovery, fixation, and preservation of helminths followed Kinsella and Forrester (1972), with the small intestine divided into sections for ease of handling. Hookworms were mounted and cleared in lactophenol or phenol–alcohol for identification and counting. Confirmation of species was impossible in some cases due to damaged or immature specimens or the inability to distinguish females of *A. tubaeforme* from *A. caninum.* Hookworms from bobcats in the previously mentioned Florida studies were reexamined for this project. The data were analyzed using the SAS system (SAS Institute, 1988) for prevalence (PROC FREQ CHISQ) and intensity (reported as mean [SD]) (PROC NPAR1WAY WILCOXON) of the hookworm species found in each region, sex, and age class. Representative specimens have been deposited in the U.S. National Parasite Collection, Beltsville, Maryland (USNM Helm. Coll. Nos. 82292–82298).

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Results and Discussion

Eighty-five bobcats, 41 from northern and 44 from southern Florida, were included in this study. Thirty were collected in 1979, 25 in 1985, 17 from 1986 to 1988; the remaining years were represented by 3 or fewer samples. There were 26 females (6 juveniles, 20 adults) and 59 males (8 juveniles, 51 adults). At least 1 of 4 species of *Ancylostoma* was found in 52/85 (61%) bobcats. In 7 bobcats, identification of all specimens could not be determined for the reasons already noted; females of *A. tubaeforme* and *A. caninum* from all bobcats were combined for analysis. Intensities varied from 1 to 128 ($\bar{x}$ = 17.6 [26.8]). Prevalences of *A. tubaeforme, A. caninum, A. braziliense,* and *A. pluridentatum* were 11, 18, 19, and 29%, respectively. The prevalence and intensity for each species of hookworm are listed in Table 1. Mixed infections (2 or more species) were seen in 20 (24%) animals. Six others had males of either *A. tubaeforme* or *A. caninum,* and females of 1 or both. Significantly more infected juveniles (8/10) than adults (12/35) had mixed infections ($\chi^2 = 4.86$, 1 df, $P < 0.05$). This could reflect increasing immunity to hookworms with age (Miller, 1965, 1966; Stone and Pence, 1978; Watson et al., 1981) or transmammary transmission. With data for all species combined, there were no differences in intensities or prevalences between the 2 age classes or sexes of hosts, nor in prevalences between northern and southern cats. However, mean intensity for all species combined varied significantly between regions (Wilcoxon $Z = 2.89$, $P < 0.01$), with northern bobcats infected with an average of 6.2 (7.7) and southern animals with 25.3 (32.1) hookworms.

While *A. tubaeforme, A. caninum,* and *A. braziliense* were found statewide, *A. pluridentatum* was present only in southern Florida, in Highlands, Lee, Hendry, Collier, and Dade counties.

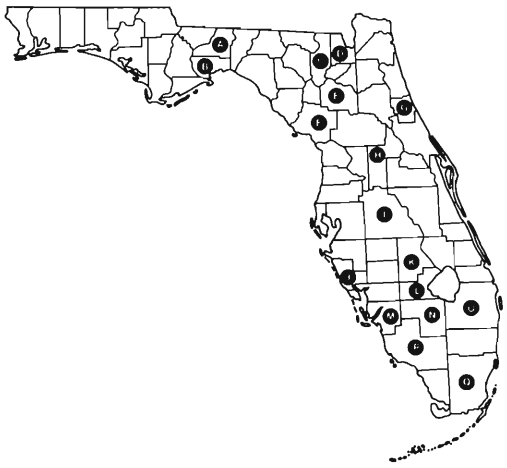

Figure 1. Collection areas for bobcats in Florida, with number collected from each county: (A) Leon (1); (B) Wakulla (5); (C) Columbia (5); (D) Baker (5); (E) Alachua (9); (F) Levy (11); (G) Flagler (1); (H) Lake (1); (I) Polk (3); (J) Sarasota (2); (K) Highlands (4); (L) Glades (2); (M) Lee (4); (N) Hendry (6); (O) Palm Beach (1); (P) Collier (21); (Q) Dade (4).

In that area, the prevalence of *A. pluridentatum* was 57% ($\chi^2 = 33.00$, $P < 0.001$) with a mean intensity of 28.6 (34.2). Prevalences and intensities for each species on a regional basis are given in Table 2. While the intensities for the other species did not vary between north and south, the prevalence of *A. braziliense* was greater in the northern region ($\chi^2 = 4.41$, $P < 0.05$).

The distribution of *A. tubaeforme, A. caninum,* and *A. braziliense* throughout the state suggests that the bobcat may serve as a reservoir of hookworm infections for other wild and domestic carnivores. Conti (1984) found all 3 species in the gray fox (*Urocyon cinereoargenteus*) of Florida, and they have been identified in samples from Florida panthers (McLaughlin and Forrester, un-

Table 1. Hookworms of 85 bobcats from 17 counties in Florida, 1974–1991.

Species of *Ancylostoma*	USNM Coll. No.	Prevalence		Intensity			Distribution*
		N	(%)	$\bar{x}$	SD	Range	
A. tubaeforme†	82295–6	9	(11)	1.9	0.78	1–3	E–I, M, P, Q
A. caninum†	82294	15	(18)	2.6	3.44	1–14	A–C, E, F, H, K, M, N, P, Q
A. braziliense	82297–8	16	(19)	4.1	3.52	1–12	B, D, E, F, I, J, M, P
A. pluridentatum	82292–3	25	(29)	28.6	34.2	1–128	K, M, N, P, Q
Ancylostoma spp.‡		24	(28)	3.2	3.63	1–16	A–K, M, N, P, Q

* Letters refer to counties in Figure 1.

† Includes only males.

‡ Includes females of *A. tubaeforme* and *A. caninum,* damaged and immature specimens.

Table 2. Prevalence and intensity of hookworms of 85 Florida bobcats by region. Values with the same letter do not differ significantly ($\alpha = 0.05$) for that species.

	Prevalence		Intensity	
	North	South	North	South
Species	*N* (%)	*N* (%)	$\bar{x}$ (SD)	$\bar{x}$ (SD)
*Ancylostoma tubaeforme**	4 (10)[a]	5 (11)[a]	2.0 (0.82)[a]	1.8 (0.84)[a]
*Ancylostoma caninum**	7 (17)[a]	8 (18)[a]	3.9 (4.78)[a]	1.5 (1.07)[a]
Ancylostoma braziliense	12 (29)[a]	4 (9)[b]	3.9 (3.15)[a]	4.8 (4.99)[a]
Ancylostoma pluridentatum	0 (0)[a]	25 (57)[b]	0[a]	28.6 (34.2)[b]
Ancylostoma spp.†	15 (37)[a]	9 (20)[a]	3.3 (3.99)[a]	3.2 (3.15)[a]

* Includes only males.

† Includes females of *A. tubaeforme* and *A. caninum*, damaged and immature specimens.

publ. data). *Ancylostoma caninum* has been found in red foxes (*Vulpes vulpes*), coyotes (*Canis latrans*), and black bears (*Ursus americanus*), and *A. tubaeforme* is known from red foxes in Florida (Crum, 1977; Conti, 1984). These hookworms are found also in domestic cats (*Felis catus*) and dogs (*Canis familiaris*) in Florida (Greiner et al., 1992; McLaughlin and Forrester, unpubl. data).

The presence of *A. pluridentatum* in Florida felids (Forrester et al., 1985) is intriguing because it has been found previously only in wild felids and domestic cats in or from Central and South America (Schwartz, 1927; Thatcher, 1971; Seesee et al., 1981; Moriena, 1983). O'Brien et al. (1990) presented genetic evidence of a recent introduction (~35 yr ago) of pumas of South or Central American origin into southern Florida. *Ancylostoma pluridentatum* may have been introduced at the same time. Its ability to infect panthers, bobcats, and domestic cats increases its potential distribution in Florida, while also augmenting the environmental egg burden. There have been no studies to determine the environmental factors limiting the range of this parasite, either in Latin America or in Florida. Also, with the exception of a preliminary study on 3 domestic kittens summarized by Forrester (1992), no information is available regarding the pathogenicity of *A. pluridentatum* to any felid species. In that study, clinical signs were noted with infections of fewer than 250 *A. pluridentatum*. Until more data are collected, it is not possible to assess the potential impact of this parasite on the endangered Florida panther, on the Florida bobcat population, on wild felids in Central and South America, or on domestic cats.

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Dentostomella tamimi sp. n. (Nematoda: Heteroxynematidae) from the Spiny Mouse, *Acomys dimidiatus,* in Saudi Arabia

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ABSTRACT: *Dentostomella tamimi* sp. n. was collected from the anterior third of the small intestine of the spiny mouse, *Acomys dimidiatus* (Cretzschmar, 1826), trapped at Hotut Beni Tamim and at Heet, Riyadh Province, Saudi Arabia. The worms are long and slender and have the largest cervical inflation in the genus *Dentostomella.* On top of this inflation, there are 4 large submedian papillae, 2 on either side, together with 2 lateral amphids, on either side between the submedian papillae. Three pairs of small, hardly discernible papillae surround the small, circular mouth opening, 1 pair dorsally and 2 pairs ventrally. The mouth opens into a shallow, triradiate buccal cavity surrounded by 3 esophageal sectors. Each sector has 2 small lateral teeth and a single, large median tooth. Females are 21.0–25.0 (22.5) mm long, 0.49–0.55 (0.52) mm wide, at the region of the vulva, which is located at about the middle of the body. Males are 14.0–16.0 (15.4) mm long and of a maximum width of 0.34–0.38 (0.354) mm. The male has a median rugose and fleshy bursa without supporting rays, and its tail bears 7 caudal papillae, 1 pair preanal, 1 pair adanal, an unpaired papilla just under the cloaca, and 1 pair subanal. The single male spicule is lightly sclerotized, with an irregular depression on its anterior end; its distal tip is bifid in ventral view. The tip of the tail in both sexes has an arrowhead appearance dorsoventrally and is more pronounced in males. This is due to the presence of 2 small, ventrolateral protuberances containing the phasmids.

KEY WORDS: *Dentostomella tamimi* sp. n., *Acomys dimidiatus,* Heteroxynematidae, oxyurid nematodes, rodent, spiny mouse.

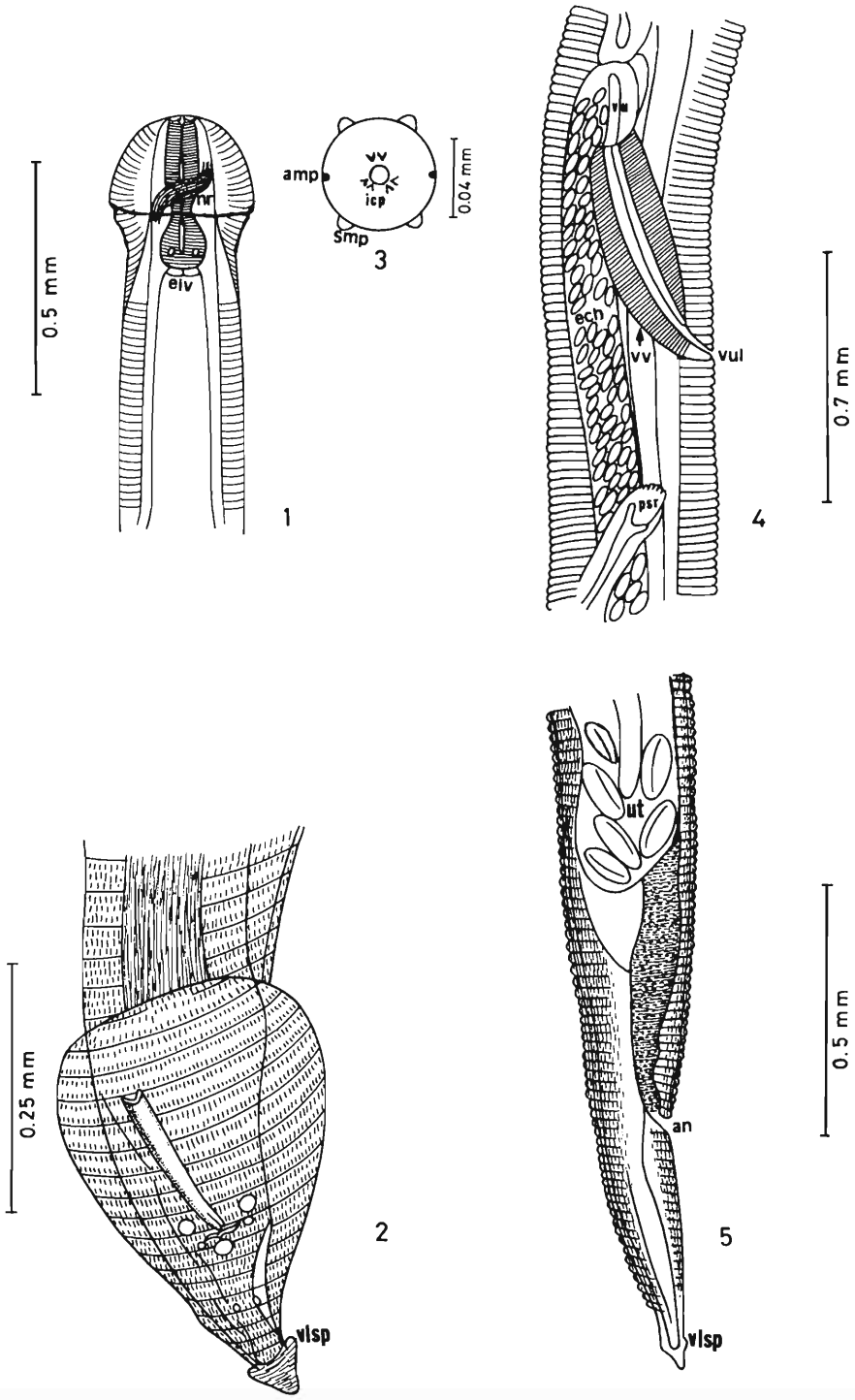
Several members of the genus *Dentostomella* Schulz and Krepkogorskaja, 1932, were described from various species of rodents including spiny mice of the genus *Acomys* Geoffroy, 1838 (Schulz and Krepkogorskaja, 1932; Myers, 1961; Chitwood, 1963; Quentin, 1975). During parasitological assessment of the indigenous fauna of Hotut Beni Tamim, Riyadh Province, Saudi Arabia, prior to the establishment of a National Park in the region, 3 female *Dentostomella* sp. were recovered from the duodenum of the spiny mouse, *Acomys dimidiatus* (Cretzschmar). Later, many males and females of the same nematode were recovered from the anterior third of the small intestine of the same host trapped at Heet, Riyadh Province. These nematodes belong to a hitherto undescribed species in the genus *Dentostomella,* which is described in the present study.

Materials and Methods

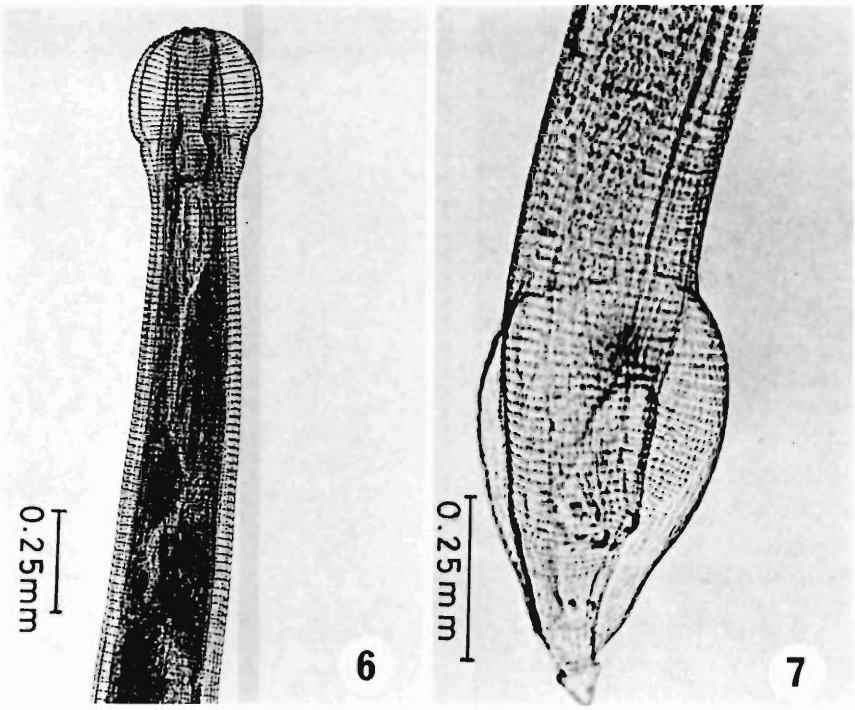
Prior to the establishment of a National Park for the endangered Nubian ibex, *Capra ibex nubiana* Cuvier, by the National Commission for Wildlife Conservation and Development (NCWCD) at Hotut Beni Tamim, a mountainous region some 300 km southeast of Riyadh, the capital of Saudi Arabia, wild animals indigenous to the region were live-trapped for the assessment of the natural fauna of the region. Six spiny mice, *A. dimidiatus,* were obtained from NCWCD for parasitological investigations. Later wild rodents were also live-trapped at Heet, another mountainous area, 30 km southeast of Riyadh. Trapped animals were identified by reference to Harrison (1972) and to Osborn and Helmy (1980). They were sexed, aged, and weighed, and their bodies and tails were measured. They were then individually housed into laboratory mouse boxes provided with sawdust for bedding. Food (laboratory mouse chow supplemented with lettuce and carrots) and water were provided ad libitum.

Animals were killed by cervical dislocation and were opened by longitudinal incision along the midventral line from the top of the rib cage to the pubis. The heart and lungs were removed into a petri dish containing normal saline (0.9% NaCl). The entire intestinal tract was then removed, measured, and cut into 3-cm segments. Each segment was separately placed into a petri dish containing normal saline, opened, and examined under a binocular dissecting microscope, and the number of nematodes per individual segment was recorded. Live nematodes were examined before they were killed in hot 70% alcohol. Then they were relaxed overnight in the refrigerator. Nematodes were cleared for study in temporary wet mounts in phenol–alcohol (80 parts melted phenol in 20 parts absolute alcohol). Some males were mounted in lactophenol or cleared in glycerine to prevent overclearing of the spicules. En face views of several specimens were mounted and studied in glycerine jelly.

The nematodes were identified by reference to Skrjabin et al. (1960), Yamaguti (1961), and Petter and Quentin (1976) and to descriptions and redescriptions of various *Dentostomella* species (Schulz and Krepkogorskaja, 1932; Myers, 1961; Chitwood, 1963; Pilitt and Wightman, 1979; Ashour and Lewis, 1982; Greve, 1985). Measurements were made with a calibrated ocular micrometer, drawings with an attached Zeiss cam-



Figures 1–5. Line drawings of male and female *Dentostomella tamimi* sp. n. 1. Ventral side of anterior end of male. 2. Ventral side of tail of male. 3. En face view of male. 4. Vulvar region of female. 5. Ventral side of tail of female. amp, amphid; an, anus; ech, egg chamber; eiv, esophageo-intestinal valve; icp, inner circle papillae; nr, nerve ring; psr, post vulvar seminal receptacle; smp, submedian papillae; ut, uterus; vlsp, ventrolateral subterminal protuberances containing the phasmids; vu, vagina uterina; vv, vagina vera.



Figures 6, 7. Photomicrographs of male *Dentostomella tamimi* sp. n. 6. Anterior end of male. 7. Tail of male.

Table 1. Measurements (in micrometers) of *Dentostomella tamimi* sp. n., *D. kuntzi, D. translucida, D. grundmanni,* and *D. legerae.*

Item	*D. tamimi* sp. n.		*D. kuntzi* Myers, 1961		*D. kuntzi* of Ashour and Lewis, 1982	
	Male	Female	Male	Female	Male	Female
Length	14–16	21–25	9–11.8	10.3–15.6	6.9–13.3	15.9–23.9
Maximum width	0.34–0.38	0.49–0.55	0.13–0.15	0.3–0.4	0.13–0.31	0.21–0.44
Width of cervical inflation	0.29–0.35	0.31–0.41	0.16*	–	0.19–0.26	0.2–0.29
Esophagus length	0.33–0.48	0.38–0.58	0.28*	0.22–0.44	0.24–0.33	0.27–0.41
Excretory pore from anterior end	1.11–1.28	1.66–1.7	1.8–2.4†		2.64	2.88
Nerve ring from anterior end	0.15–0.19	0.15–0.19	–	–	0.16–0.2	0.18–0.26
Bursa						
Length	0.6–0.76	–	0.48	–	0.31	–
Width	0.35–0.37	–	0.258	–	0.16	–
Spicule length	0.16–0.17	–	0.18–0.19	–	0.12–0.20	–
Vulva from anterior end	–	9.5–11.4	–	6–7.2	–	4.13–10.73
Vagina vera length	–	0.65–1.03	–	0.7	–	0.6
Egg						
Length	–	0.14–0.16	–	0.12–0.13	–	0.129–0.144
Width	–	0.05–0.07	–	0.03–0.04	–	0.048–0.076
Tail length	0.206–0.424	0.342–0.768	0.232	0.83–0.85	0.18–0.24	0.38–0.58

* Measured during examination of paratypes in present study.
† Authors did not differentiate between male and female.

era lucida, and photomicrographs with an attached Nikon camera.

Results

The only rodent species trapped from the rock crevices in both localities was the spiny mouse, *A. dimidiatus*. Six spiny mice (3 males and 3 females) trapped at Hotut Beni Tamim were first assessed for gastrointestinal helminths and each of the 3 males yielded a single female *Dentostomella* sp. from the anteriormost segment of the small intestine, but the female mice were free of infection. Thereafter, 75 spiny mice (40 males and 35 females) were trapped at Heet. These gave a total of 85 (56 males and 29 females) specimens of the same *Dentostomella* sp. Of these, 77 were from the anteriormost segment of the small intestine and only 8 from other parts of the jejunum. Moreover, far more male mice (85%) were found infected with the nematode than females (20%).

All of the worms collected belonged to a *Dentostomella* species that is different from any other in the genus but is somewhat more related to *Dentostomella kuntzi* Myers, 1961, than any other species. Hence, paratypes of *D. kuntzi* deposited at the United States National Museum, Maryland (USNM Helm. Coll. No. 56804) were requested and compared to the present species. This showed that the present worms are distinct and, hence, belong to an unnamed species, which is described below. All measurements are in millimeters, with means in parentheses.

Dentostomella tamimi sp. n. (Figs. 1–7; Tables 1, 2)

DESCRIPTION: Worms long and slender. Cuticle thick, transparent, coarsely and transversely striated. Striations wide, each with fine longitudinal ridges or annules. Cervical cuticle inflated, forming large, transversely striated cervical vesicle. Cervical vesicle tapering downward and extending to level of junction of esophagus with intestine, constricted at level of bulbar part of esophagus, appearing as if they were 2 instead of 1 vesicles. Worm body inside vesicle thinner than outside, tapering anteriorly (Figs. 1, 6). Cephalic end with 4 prominent submedian papillae, with 2 lateral amphids in between; all on a circle surrounding the mouth. Mouth circular, centrally located; surrounded with 3 pairs of small, hardly discernible papillae, 1 pair dorsal, 2 pairs ventrolateral (Fig. 3). Lips absent; mouth opens into triradiate, shallow buccal cavity bordered by 3 esophageal sectors; each with 3 unequal teeth; 2 small lateral, 1 large conical median tooth. Esophagus short, thick, muscular, as wide as in-

Table 1. Continued.

D. translucida Schulz and Krepkogoroskaja, 1932		*D. translucida* of Pilitt and Wightman, 1979		*D. grundmani* Chitwood, 1963		*D. legerae* Quentin, 1975	
Male	Female	Male	Female	Male	Female	Male	Female
14.2–18.3	21.8–40.6	6.14–13.14	9.63–31	4–8	13.4–17.8	7.5	21
0.664	1.08–1.36	0.374–0.53	0.375–0.996	0.18–0.33	0.6–0.8	0.3	0.8
—	—	—	—	—	—	—	—
0.33–0.35	0.4–0.44	0.294–0.319	0.344–0.432	0.23	0.33–0.35	0.27	0.31
—	3.2–5.4	2.41–3.49	1.93–4.04	—	3.7–4.1	2.32	3.6
—	0.28	0.147–0.189	0.176–0.223	0.18	0.2		0.2†
0.796–0.83	—	0.498–0.726	—	—	—	—	—
—	—	—	—	—	—	—	—
0.329–0.381	—	0.257–0.323	—	0.26	—	0.2–0.27	—
—	9.8–17.3	—	4.48–12.87	—	7.5–9.1	—	10
—	0.94–1.16	—	0.95	—	0.78	—	—
—	0.12–0.14	—	0.097–0.134	—	0.13–0.14	—	0.145
—	0.04–0.06	—	0.04–0.05	—	0.04–0.05	—	—
—	0.84–0.86	0.332–0.457	0.432–0.714	0.515	0.8	0.3–0.35	0.39

testine, constricted just below nerve ring (Fig. 1); posterior part bulbar, without armaments; esophageo-intestinal valve present (Figs. 1, 6).

FEMALE (N = 29): Length 21.0–25.0 (22.5), maximum width 0.49–0.55 (0.52) at vulvar region about middle of body (Fig. 4). Transverse cuticular striations 0.023 apart, cervical inflation 0.32–0.41 (0.363) wide. Esophagus 0.38–0.58 (0.51) long, esophageal bulb 0.09–0.14 (0.12) wide. Nerve ring and execretory pore 0.145–0.189 (0.175) and 1.66–1.70 (1.68), respectively, from anterior extremity. Vulva a transverse slit 9.5–11.4 (10.8) from anterior end, opening into thick-walled, muscular, anteriorly directed vagina vera 0.465–1.025 (0.85) in length, 0.28 maximum width (Fig. 4, Table 1). Vagina uterina anteriorly directed in part, then curves and continues posteriorly as narrow uterine tube that widens posteriorly as egg chamber (Fig. 4). Egg chamber joined by 2 posteriorly directed uteri confined to posterior part of body. Both turn forward to join egg chamber; one just above the anus and the other farther above (Figs. 4, 5). Both ovaries anterior to vulva; 2 small seminal receptacles occur between ovaries and oviduct, 1 pre- and the other postvulvar (Fig. 4). Anus crescent-shaped slit 0.342–0.768 (0.532) from arrowhead tail tip. Terminal part of tail with 2 small ventrolateral, subterminally situated, protuberances containing the phasmids; giving tail tip an arrowhead appearance in ventral view (Fig. 5). Egg oval, asymmetrical 0.14–0.16 (0.152) long, 0.05–0.07 (0.65) wide (Fig. 4, Table 1).

MALE (N = 56): Length 14.0–16.0 (15.4), maximum width 0.34–0.38 (0.345). Transverse striations 0.017 apart. Esophagus 0.33–0.48 (0.41), esophageal bulb 0.06–0.09 (0.078) wide. Nerve ring and excretory pore 0.150–0.186 (0.169) and 1.11–1.28 (1.195), respectively, from anterior end (Table 1). Tail with oblong, fleshy bursa devoid of supporting rays, 0.60–0.67 (0.645) long, 0.35–0.37 (0.362) maximum width, rugose ventrally due to transverse striae and plaquelike markings. Tail short, slightly curved ventrad, with 2 small ventrolateral, subterminal protuberances containing the phasmids (more pronounced than in females), giving tail tip arrowhead appearance in ventral view (Figs. 2, 7). Spicule single 0.16–0.17 (0.162), slightly sclerotized; upper end rounded, with uneven depression; distal tip bifid ventrally (Fig. 2). Caudal papillae 7, 1 pair preanal, 1 pair adanal, a large, unpaired papilla just below cloaca, 1 pair postanal midway between cloaca and tail tip (Figs. 2, 7).

TYPE HOST: *Acomys dimidiatus* (Cretzschmar, 1826).

LOCATION: Upper third of small intestine.

TYPE LOCALITY: Heet, Riyadh Province, Saudi Arabia.

OTHER LOCALITIES: Hotut Beni Tamim, Riyadh Province, Saudi Arabia.

SPECIMENS DEPOSITED: Holotype ♂ and allotype ♀: USNM Helminthological Collection No. 81046; Paratypes: author's collection, Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia.

ETYMOLOGY: The specific name *tamimi* is after the name of the tribe Beni Tamim, which inhabits Hotut Beni Tamim, from which the first spiny mice infected with the worm were collected.

Discussion

The genus *Dentostomella* was established by Schulz and Krepkogorskaja (1932) to include the oxyurid nematode, *Dentostomella translucida,* that they found in the large intestine of the great gerbil, *Rhombomys opimus* Lichtenstine in Kazakhstan, U.S.S.R. It was later found to be very common in great gerbils in the plateau and desert regions of the Central Asian republics of Kazakhstan and Uzbekistan (Schulz and Landa, 1935; Shleikher and Samsonova, 1954) and was more recently proven to have a wider host range among cricetid rodents (Chitwood, 1963; Danzan, 1978; Wightman et al., 1978; Pilitt and Wightman, 1979; Greve, 1985). Other species in the genus include *Dentostomella kuntzi* Myers, 1961, *Dentostomella grundmani* Chitwood, 1963, and *Dentostomella legerae* Quentin, 1975, which were described from the spiny mice, *Acomys russatus* (Wagner) and *Acomys cahirinus* (Desmarset), from the chipmunk, *Eutamias quadrivittatus* (Say), and from the large North African gerbil, *Gerbillus campestris* Levaillant, respectively (Myers, 1961; Chitwood, 1963; Quentin, 1975). Of these, *D. kuntzi* was also reported from other rodents in Egypt including *A. dimidiatus, G. campestris, Rattus rattus alexandrinus* (Geoffroy), *Rattus rattus frugivorus* (Rafinesque), and *Mus musculus* Linnaeus (Myers et al., 1962; Rifaat et al., 1969a, b; Ashour and Lewis, 1982). Moreover, both *D. translucida* and *D. kuntzi* have also been redescribed by Pilitt and Wightman (1979) and by Ashour and Lewis (1982), respectively.

All of these nematodes are unusual oxyurids in being long and, apart from *D. translucida* and

Table 2. A comparison of morphology, microhabitat, and hosts of *Dentostomella tamimi* sp. n., *D. kuntzi, D. translucida, D. grundmanni,* and *D. legerae.*

Item	*D. tamimi* sp. n.	*D. kuntzi* Myers, 1961	*D. kuntzi* of Ashour and Lewis, 1982	*D. translucida* Shulz and Krepkogorskaja, 1932	*D. grundmanni* Chitwood, 1963	*D. legerae* Quentin, 1975
Host	*Acomys dimidiatus*	*Acomys russatus, Acomys cahirinus*	*Acomys cahirinus*	*Rhombomys opimus*	*Eutamis quadrivittatus*	*Gerbillus campestris*
Microhabitat	Anterior ⅓ of small intestine	Large intestine	Anterior ⅓ of small intestine	Large intestine	Intestine	–
Cephalic inflation	Large	Large	Large	–	Small	Narrow and rounded
Mouth opening	Rounded	Rounded	Rounded	Triangular	Rounded	Rounded
Teeth per esophageal sector	3 Unequal	3 Unequal	3 Equal	5	1	3 Unequal
Vulva to anterior extremity : female body	1:2.1	1:2	1:2.2	1:2.3	1:1.9	1:2.1
Male caudal papillae						
Preanal	1 Pair	1 Pair	–	–	–	–
Adanal	1 Pair	1 Pair	1 Pair	1 Pair	1 Pair	1 Pair
Postanal	1 Pair + 1	2 Pairs + 1	2 Pairs + 1	2 Pairs + 1	3 Pairs	3 Pairs
Spicule						
Anterior end	With irregular depression	Simple	Simple	Simple	Simple	Simple
Distal end	Bifid	Simple, hooked	Simple, rounded	Bifid	Bidenticulate	Simple, rounded

D. kuntzi, which were originally described from the large intestine (Schulz and Krepkogorskaja, 1932; Myers, 1961), in inhabiting mainly the small intestine, such as *D. grundmani, D. translucida,* and *D. kuntzi* (Rifaat et al., 1969 a, b; Pilitt and Wightman, 1979; Ashour and Lewis, 1982), or the stomach, such as *D. translucida* in hamsters (Greve, 1985).

Similar to the observations of Ashour and Lewis (1982) on *D. kuntzi, D. tamimi* sp. n. also prefers the anteriormost part of the small intestine. Both worms are long and slender, have a large cervical inflation, and can easily be separated from *D. translucida, D. grundmani,* and *D. legerae,* which are shorter, stout worms devoid of the large cervical inflation (Schulz and Krepkogorskaja, 1932; Myers, 1961; Myers et al., 1962; Chitwood, 1963; Quentin, 1975; Pilitt and Wightman, 1979; Ashour and Lewis, 1982; Greve, 1985). On the other hand, *D. tamimi* can be distinguished from *D. kuntzi* in being longer and thicker and in having a much larger cervical inflation that is constricted distally to appear as if there were 2 vesicles instead of only 1. It has a larger copulatory bursa and a longer esophagus and vagina vera, and its excretory pore lies at a shorter distance from the anterior end (Table 1). Moreover, *D. tamimi* has an arrowheadlike tail tip due to the presence of 2 ventrolateral protuberances that contain the phasmids. This characteristic is not found in any other *Dentostomella* species and together with the characteristically constricted large cervical vesicle, cannot only separate *D. tamimi* from *D. kuntzi,* but can distinguish this nematode from any other species in the genus. Furthermore, the spicule of both *D. tamimi* and *D. kuntzi* is also dissimilar. That of the former has an irregular depression on its anterior end and its posterior tip is bifid. Both ends of that of the latter are simple with the distal tip either rounded (Ashour and Lewis, 1982) or hooked (Myers, 1961) (Table 2). The number and arrangement of male caudal papillae are also different in both species. *Dentostomella tamimi* has 7 papillae, 1 pair preanal, 1 pair adanal, a single larger papilla just below the cloaca, and 1 pair postanal. The male of *D. kuntzi* described by Myers (1961) has 9 papillae, 1 pair preanal, 1 pair adanal, 3 postanal papillae on a raised elevation, and 1 pair below that elevation. That described by Ashour and Lewis (1982) has 7 papillae, but 5 of these are postanal and none are preanal (Table 2). The eggs are different in both species too. Unlike that of *D. tamimi,* the egg of *D. kuntzi* is larger (Table 1) and operculated. Biologically, *D. tamimi* seems to prefer male hosts to female ones, while *D. kuntzi* infects both sexes equally (Ashour and Lewis, 1982).

Dentostomella tamimi is separated from *D. translucida, D. grundmani,* and *D. legerae,* which are stout, generally shorter worms devoid of a large, conspicuously constricted cervical vesicle. Moreover, the distinct arrowheadlike tail tip of *D. tamimi* is not found in any of these worms, nor is the irregular depression on the anterior end of the spicule. Similar to *D. tamimi,* the distal tip of the spicule of *D. translucida* is bifid, but that of *D. grundmani* is bidenticulate and that of *D. legerae* is simple and rounded (Table 2). The number and arrangement of male caudal papillae are also different in these worms. There are 7 papillae in *D. tamimi,* 1 pair of which is preanal. None of the other 3 species has preanal papillae. All have 1 pair of adanal papillae, but both *D. grundmani* and *D. legerae* have 3 pairs of postanal papillae and *D. translucida* only 5 postanal papillae (Table 2). The intestine of *D. tamimi* is as large as the esophagus (Figs. 1, 6), which is different from that of *D. translucida* and *D. grundmani,* whose intestine is much larger than the esophagus. Similar to both *D. kuntzi* and *D. legerae, D. tamimi* has 3 teeth per esophageal sector while *D. translucida* has 5 and *D. grundmani* only 1 (Table 2).

Acknowledgments

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Ultrastructure of Subventral Gland Secretory Granules in Parasitic Juveniles of the Soybean Cyst Nematode, *Heterodera glycines*[1]

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ABSTRACT: Secretory granules of the subventral esophageal glands of *Heterodera glycines* showed considerable changes in morphology soon after penetration and following initiation of syncytia in soybean roots. Within 3 hr after inoculation, the moderately dense secretory granules, usually found in subventral gland cells and their extensions, became electron-transparent except for small electron-dense residues within the secretory granule membranes. In samples taken 18 hr after inoculation and beyond, subventral gland extensions of parasitic second- and third-stage juveniles contained small, electron-dense secretory granules. The subventral glands were also characterized by the presence of moderate to very large flocculate secretion bodies within a dense matrix of rough endoplasmic reticulum, mitochondria, and Golgi apparatus. The activity of the Golgi apparatus was directly related to the formation and accumulation of condensing vesicles that appeared to merge with each other to form the larger secretory granules occurring in the subventral glands of parasitic stages of the nematode. The large flocculate secretion bodies were observed as early as 10 hr after inoculation and contrasted with the dense cytoplasm of the subventral glands observed 6 days after inoculation. The synthesis, assembly, accumulation, and transport of secretory granules within the subventral glands of the soybean cyst nematode appeared to change during parasitism.

KEY WORDS: *Heterodera glycines,* Nematoda, secretory granules, soybean cyst nematode, ultrastructure.

Subventral and dorsal glands are prominent features of the esophagus of tylenchid nematodes. Secretory granules formed in these glands are of major interest in understanding host–parasite interactions of plant-parasitic nematodes of major crop plants (Bird, 1968a, b, 1969; Rumpenhorst, 1984; Wyss et al., 1984; Hussey, 1989a; Endo, 1991). The extensions of the subventral glands in second-stage juveniles (J2) of the root-knot nematode, *Meloidogyne javanica,* accumulate secretory granules shortly before hatching and the granules change in morphology within 1–3 days after entry into the host. Meanwhile there was a 3-fold enlargement of the dorsal and subventral glands (Bird, 1967). These studies stimulated interest in esophageal gland structure–function relations in other species of endoparasitic nematodes. Changes in morphology of secretory granules in the dorsal esophageal gland occur between preparasitic and parasitic J2 stages of development in the cyst nematode, *Heterodera glycines.* Secretory granules in dorsal glands of parasitic J2 varied substantially in size and electron density from small moderately electron-dense secretory granules to large low-density secretory granules as feeding occurred (Endo, 1987). Previous work on *M. javanica* (Bird, 1967, 1975; Bird and Saurer, 1967) and in vivo observations of *Heterodera schachtii* (Wyss, 1992) emphasize that subventral glands play an active role in the parasitic behavior of these and related nematodes. This study emphasizes the changes in morphology of secretory granules of subventral esophageal glands and their sites of synthesis and modification during the infection of soybean by the soybean cyst nematode.

Materials and Methods

Preparasitic and parasitic stages of *H. glycines* in infected soybean (*Glycine max*) roots were prepared for electron microscopy by previously described procedures (Endo and Wergin, 1973; Wergin and Endo, 1976; Endo, 1978). Seedlings of susceptible and resistant cultivars were raised in vermiculite and inoculated with infective juveniles (J2) of the soybean cyst nematode. The J2 and nematode-infected root segments, sampled systematically from 3-hr through 8-day intervals after inoculation from several experiments, were fixed in buffered 3% glutaraldehyde (0.05 M phosphate buffer, pH 6.8) at 22°C for 1.5–4 hr; washed for 1 hr in 6 changes of the same buffer; postfixed in 2% osmium tetroxide in the same buffer for 2 hr; dehydrated in an acetone series; and infiltrated with a low-viscosity resin (Spurr, 1969). Silver-gray sections of selected nematodes and root tissues were cut on an ultramicrotome with a diamond knife and mounted on uncoated

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[2] Research Plant Pathologist.

75 × 300-mesh copper grids. The sections were stained with uranyl acetate and lead citrate and viewed in a Philips 301 or 400T electron microscope operating at 60 kV with 20-μm objective aperture.

Results

In this study, the ultrastructure of the subventral glands of the preparasitic J2 (Fig. 1 and inset) provides a basis for comparison of changes that take place in these organs after host penetration and establishment of second- and third-stage juveniles (J2 and J3) as parasites in susceptible and resistant root tissues.

J2 preparasitic stage

The subventral gland cells of preparasitic J2 had distinct nuclei and nucleoli and numerous Golgi bodies, mitochondria, and rough endoplasmic reticulum (RER) (Fig. 1). Sections through the Golgi apparatus in subventral glands showed secretory granules that apparently originated from stacks of cisternae (Fig. 1 inset). The matrix of the secretory granules in the gland cell was moderately electron-dense and contained a particulate electron-dense region.

J2 parasitic stage

Within 3 hr after penetration, many of the secretory granules in the gland extensions became electron-transparent except for the small electron-dense cores (Fig. 2). Secretory granules in the central region of the gland cell appeared moderately electron-dense, while others near the nucleus had lower electron density (Figs. 3–5). Golgi bodies were often located near the nucleus of the subventral gland and adjacent to the nuclear membrane (Figs. 3–5). However, Golgi bodies can occur throughout the central body of the cell (Fig. 4). Within 5 hr after inoculation of the resistant cultivar Pickett, secretory granules and related Golgi within the subventral gland were quite extensive (Figs. 6–8). As in the susceptible reaction, secretory granules in the ampullae were partially depleted of their contents (Fig. 6). There was a wide range of secretory granule morphology and content (Fig. 7) of granules accumulated in anterior region of the gland cell.

At 18 hr after inoculation of susceptible or resistant cultivars, secretory granules in the subventral gland extensions of J2 were small and electron-dense and had fine particulate contents. The electron-dense secretory granules in the ampulla of a J2 feeding on an initial syncytial cell of the resistant cultivar Bedford (Figs. 9, 10) are similar to secretory granules in subventral gland extensions and ampullae of J2 within susceptible cultivars.

The subventral glands of a parasitic J2, 2 and 3 days after inoculation, contained gland cells with nuclei, large flocculent secretion bodies, and smaller secretory granules. The electron-dense granules in the gland cell were similar in morphology to the secretory granules observed near the valves in the ampullae of the gland extension (Figs. 11, 13). Compared to these small electron-dense granules, the large flocculent secretory bodies (FSBs) within the subventral gland cell were electron-translucent and appeared to be confined to the cell body (Fig. 12). Most nematodes at this stage of development were well established at feeding sites where the nematode stylet was inserted or had access to a syncytium induced by the nematode.

At 4 days after inoculation, a nematode near a transitional stage of development, usually just prior to molt, showed subventral gland valves in open positions (Fig. 14) filled with material similar in density to contents of the secretory granules. The gland cell contained large flocculate secretion bodies, Golgi apparatus, mitochondria, and RER (Fig. 15).

J3 parasitic stage

At 5 days after inoculation, the J3 stage was recognized by the presence of the molted J2 cuticle. A section through the nerve ring of a J3 showed accumulations of subventral and dorsal gland secretory granules within their respective gland extensions (Fig. 16). The subventral gland secretory granules were relatively small and markedly different from the larger, moderately electron-dense secretory granules of the dorsal glands.

At 6–8 days after inoculation, parasitic J3 contained secretory granules in various stages of formation. At 6 days after inoculation, the subventral glands had FSBs (Fig. 17). At 8 days after inoculation, open subventral gland valves (Fig. 19) were filled with electron-dense contents that indicated the active role of the subventral glands in producing secretory granules. Similar expanded valves were observed in a J2 during host penetration at 5 hr (Fig. 18) and at the beginning of molt of a J2, 4 days after inoculation (Fig. 14).

Discussion

Defining a role for the subventral esophageal glands and their products in plant-parasitic

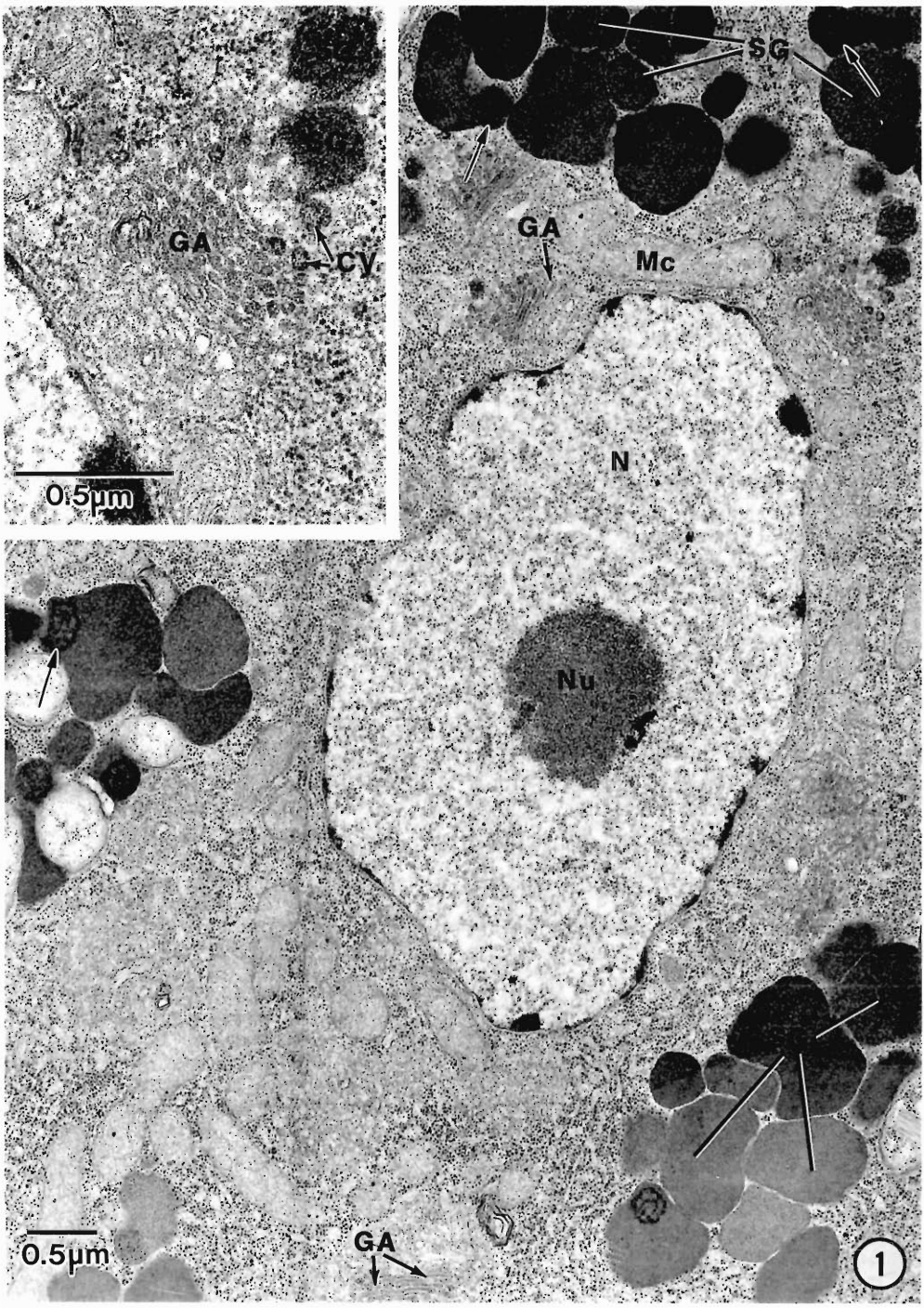
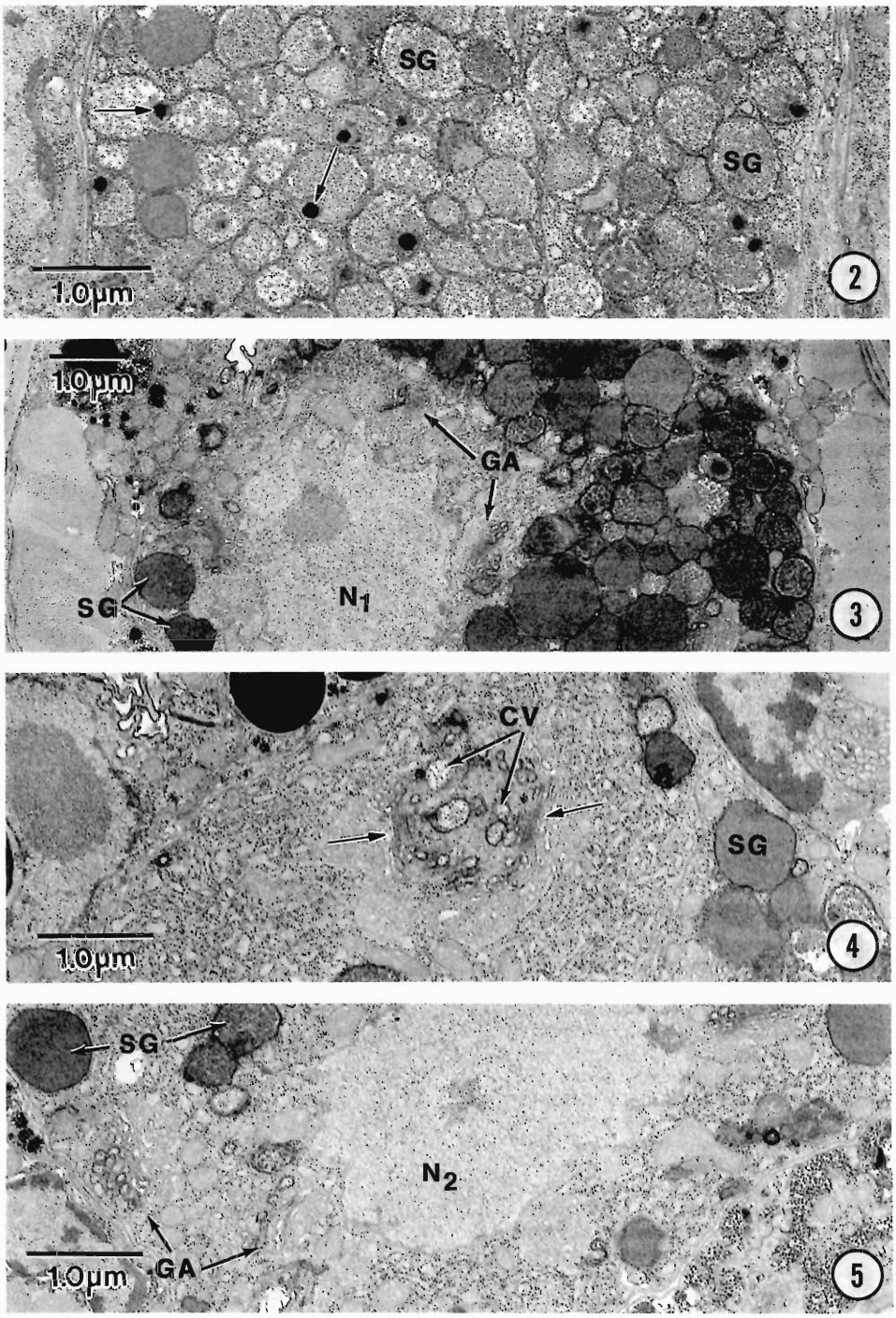
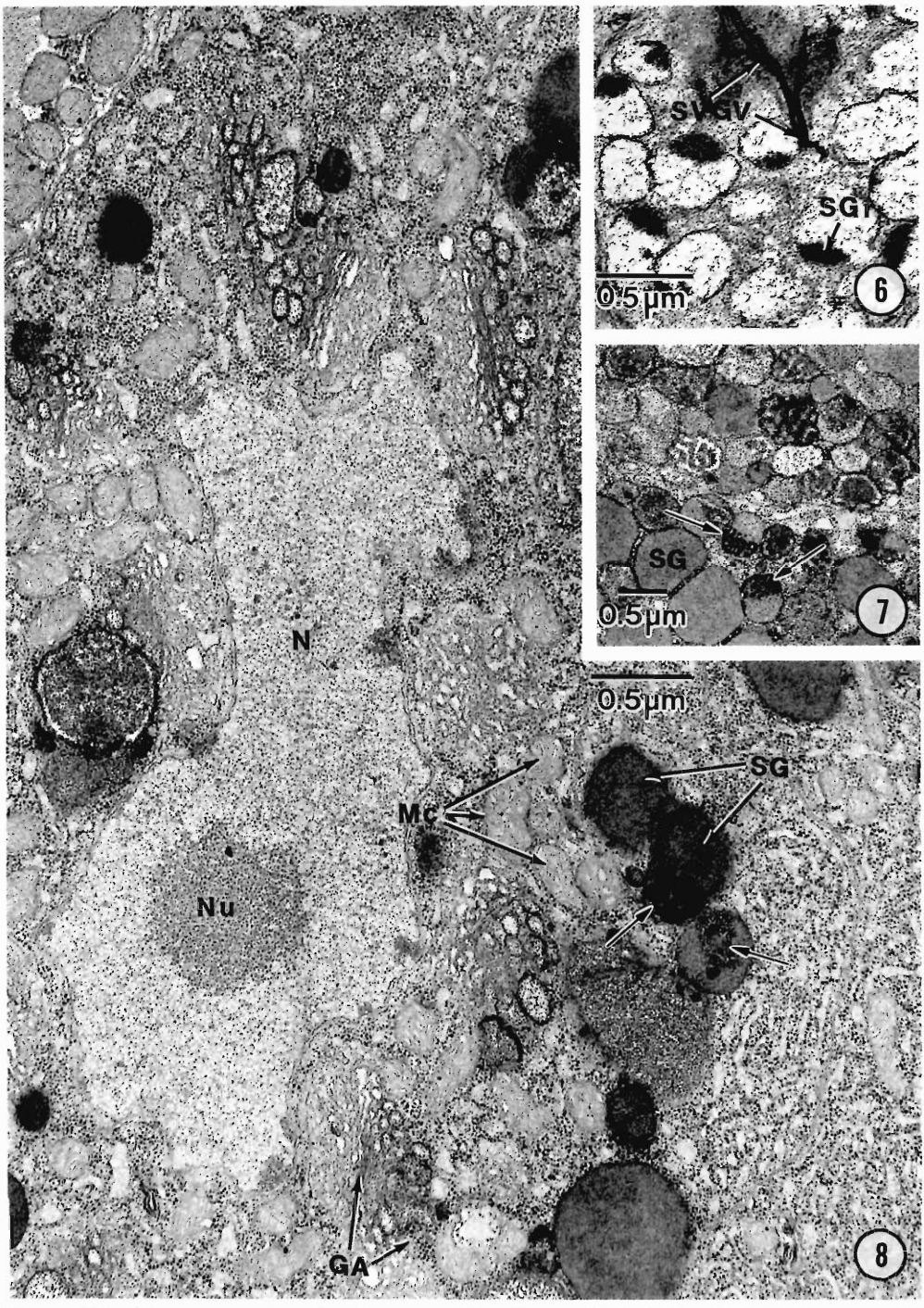


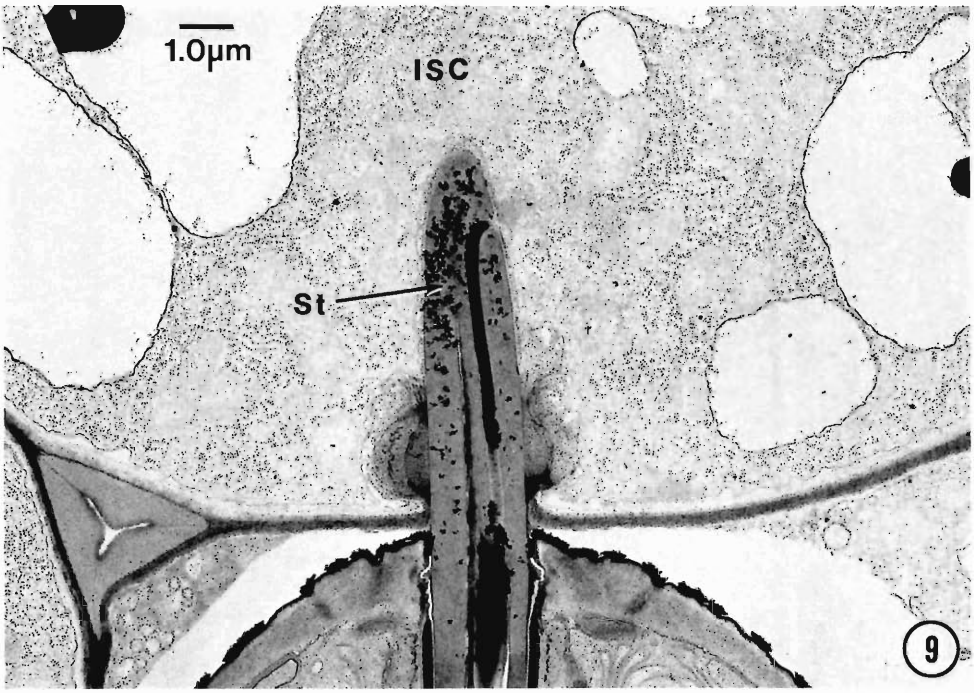
Figure 1. Longitudinal section through a subventral gland of a preparasitic J2 of *Heterodera glycines* showing electron-dense secretory granules (SG) and Golgi apparatus (GA) sites near the membrane of the gland nucleus (N) and other regions of the gland. Some secretory granules have electron-dense matrices (→). Mc, mitochondria; Nu, nucleolus. Inset shows enlargement of a Golgi apparatus (GA) and the transition from condensing vesicles (CV) to the larger secretory granules (SG).

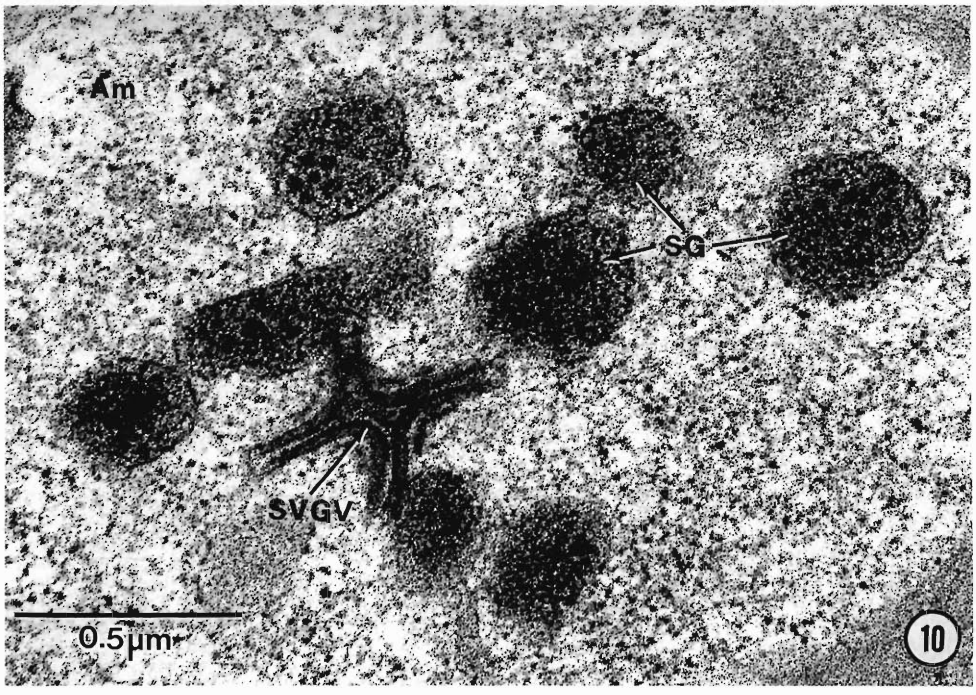


Figures 2–5. Longitudinal section through subventral gland cell extension of parasitic *Heterodera glycines* J2, 3 hr after inoculation of the susceptible soybean cultivar Lee. 2. Section near the lateroventral commissure of nerve fibers showing secretory granules (SG) of very low electron density, some with small electron-dense matrices (→). 3. Nucleus (N_1) in one of the 2 subventral gland cells surrounded by Golgi (GA) and numerous secretory granules (SG) with various levels of electron density. 4. Section showing a cluster of Golgi apparatus with trans-surface of cisternae stacks (→) facing each other. Condensing vesicles (CV) merge to form secretory granules (SG). 5. Nucleus (N_2) of the second subventral gland cell of same nematode surrounded with multiple sites of Golgi apparatus (GA) and secretory granules (SG) similar to those shown in Figure 3.

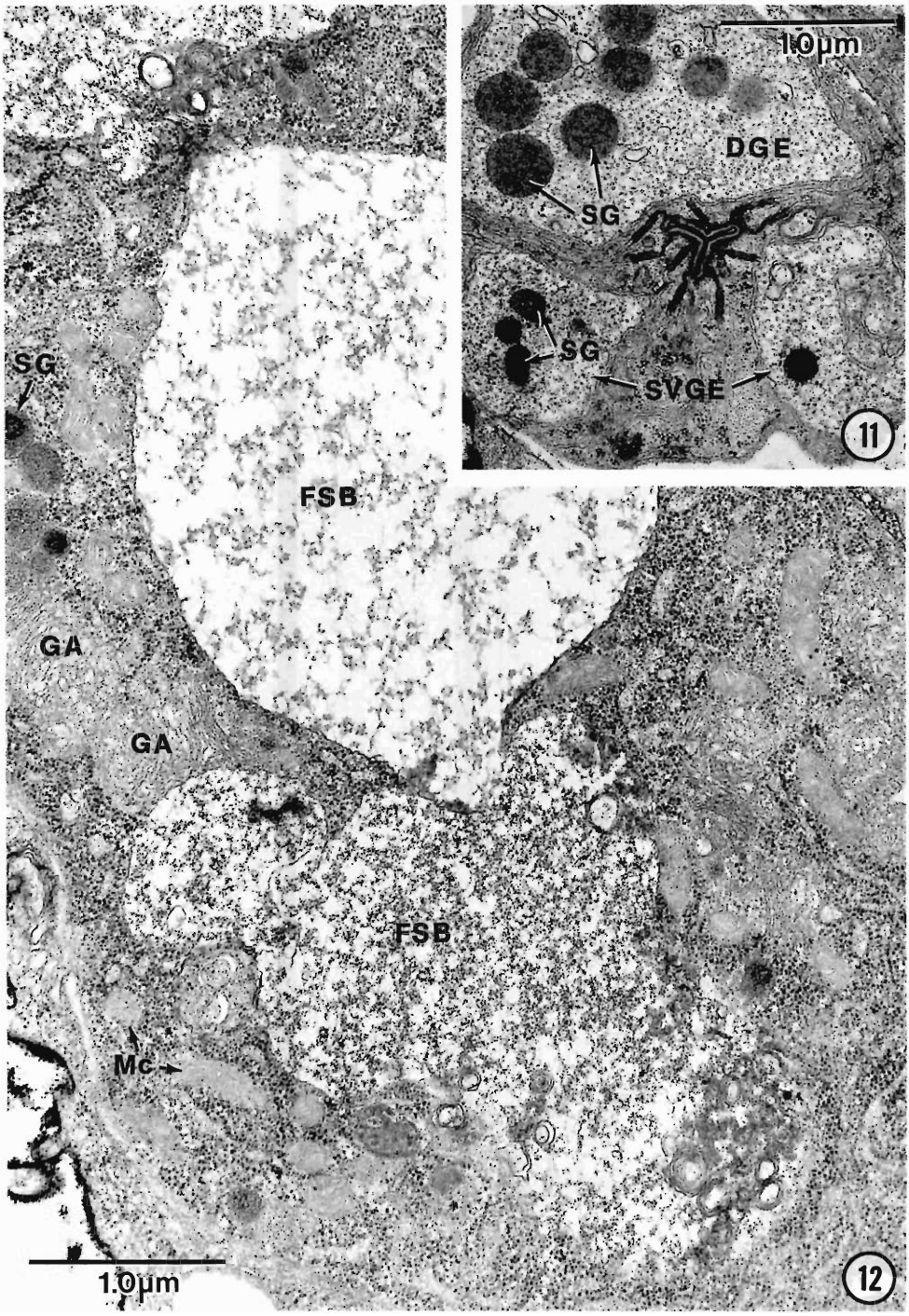


Figures 6–8. Transverse sections through sectors of the subventral gland of parasitic J2 of resistant cultivar Pickett, 5 hr after inoculation. 6. Secretory granules within ampulla are electron-translucent except for small residues of electron-dense material (SGr). SVGV, subventral gland valve. 7. Moderately dense secretory granules (SG) accumulated at anterior of gland body. Some granules contain electron-dense regions (→) within their granule matrices. 8. Section through the central region of the gland shows an enlarged nucleus (N) surrounded by Golgi (GA), secretory granules (SG), mitochondria (Mc), and a dense matrix of endoplasmic reticulum. →, electron-dense region; Nu, nucleolus.





Figures 9, 10. Longitudinal sections of J2, 18 hr after inoculation at feeding site of resistant cultivar Bedford. 9. Lateral view of extended stylet (St) within the initial syncytial cell (ISC). 10. Electron-dense secretory granules (SG) within a subventral gland ampulla (Am). SVGV, subventral gland valve.



Figures 11, 12. Transverse sections through the esophagus of a specimen 2 days after inoculation in a parasitic phase of development and established at a feeding site with access to a syncytium and feeding tube. 11. Section through gland extensions anteriad from the nerve ring shows that the secretory granules (SG) of the subventral gland extensions (SVGE) are small with dense cores, while the secretory granules of the dorsal gland extension (DGE) are larger and moderately electron-dense. 12. Secretory granules (SG) in the gland extensions appear

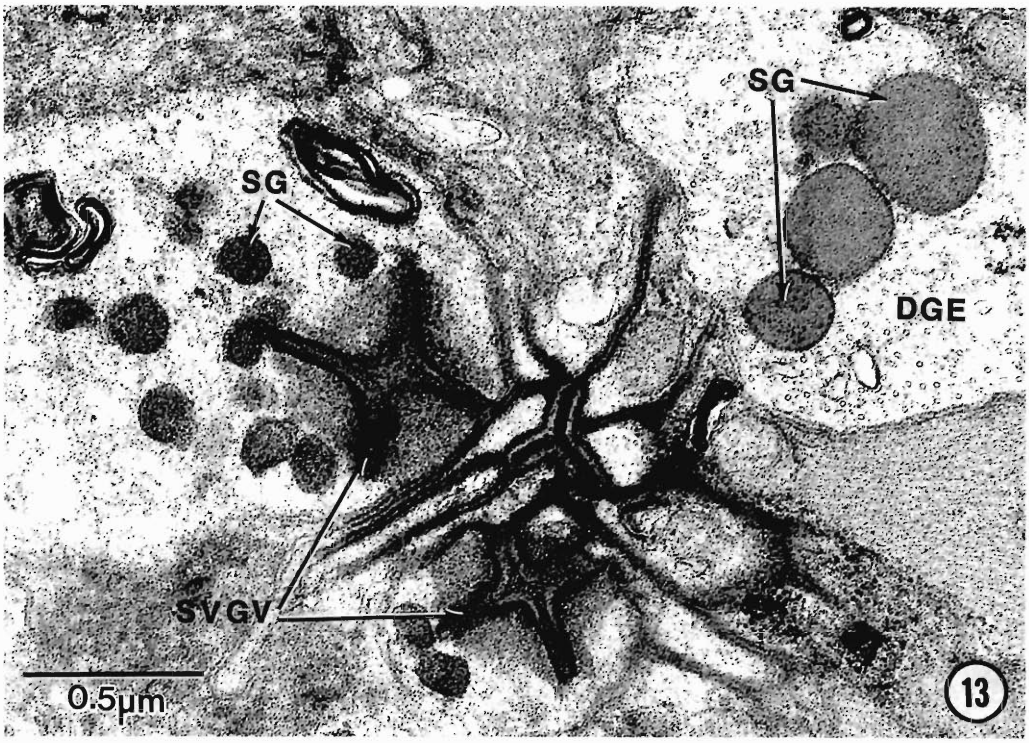
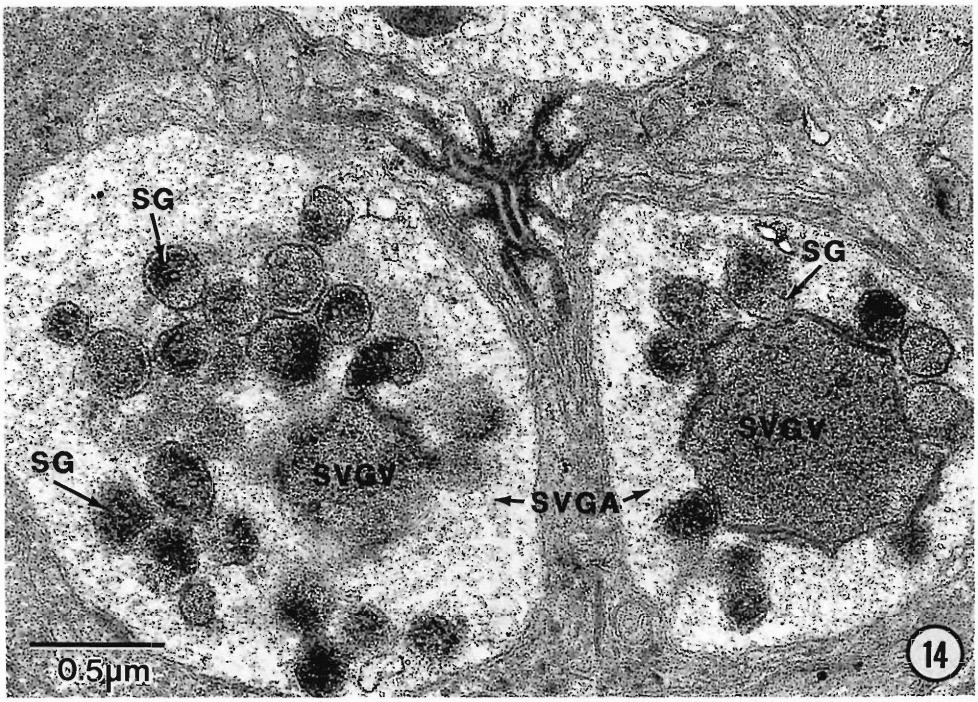


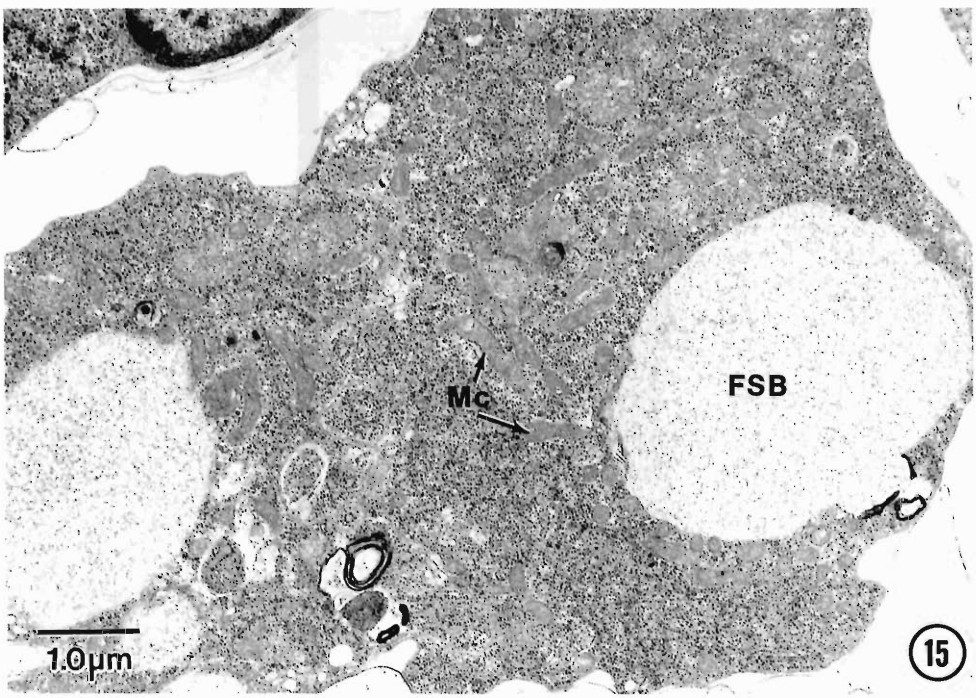
Figure 13. Transverse section of J2, 3 days after inoculation. Section shows closed subventral gland valves (SVGV) and accumulations of secretory granule (SG) contents near valves and within ampulla. DGE, dorsal gland extension.

nematodes has been based on light and electron microscope observations of the root-knot nematode, *M. javanica* (Bird, 1967, 1975). Histochemical studies by Bird and Saurer (1967) showed that the contents of the ducts of subventral glands changed their chemical composition within 2–3 days after nematode entry of the host. Bird (1967) proposed that dorsal and subventral glands play an important role in the transition from preparasitic to parasitic mode of nematode development. The subventral gland granules stained positive to periodic acid Schiff's reagent and the lumen of the esophagus became filled with material that resembled the internal contents of the granules. It was assumed the secretion(s) had an important role in the establishment of the nematode-induced giant cells (Bird, 1975). Marked chemical and morphological changes were induced by the host at the onset of parasitism (Bird, 1967). Subsequently, the subventral glands decreased in size and the dorsal gland enlarged. Stylet secretions apparently originating from secretory granules of the dorsal gland ampulla were thought to accelerate the development of the giant cells (Bird, 1968a). The change in size of the subventral gland granules reported by Bird (1975) has also been reported by Hussey and Mims (1990) in *Meloidogyne incognita*. The morphology and size of the secretory granules changed after the initiation of parasitism. An electron-dense zone developed in the periphery of the matrix of each granule that was often separated from the limiting membrane (Hussey and Mims, 1990). The electron-transparent core observed in the granules of preparasitic juveniles was absent in 7-day-old parasitic

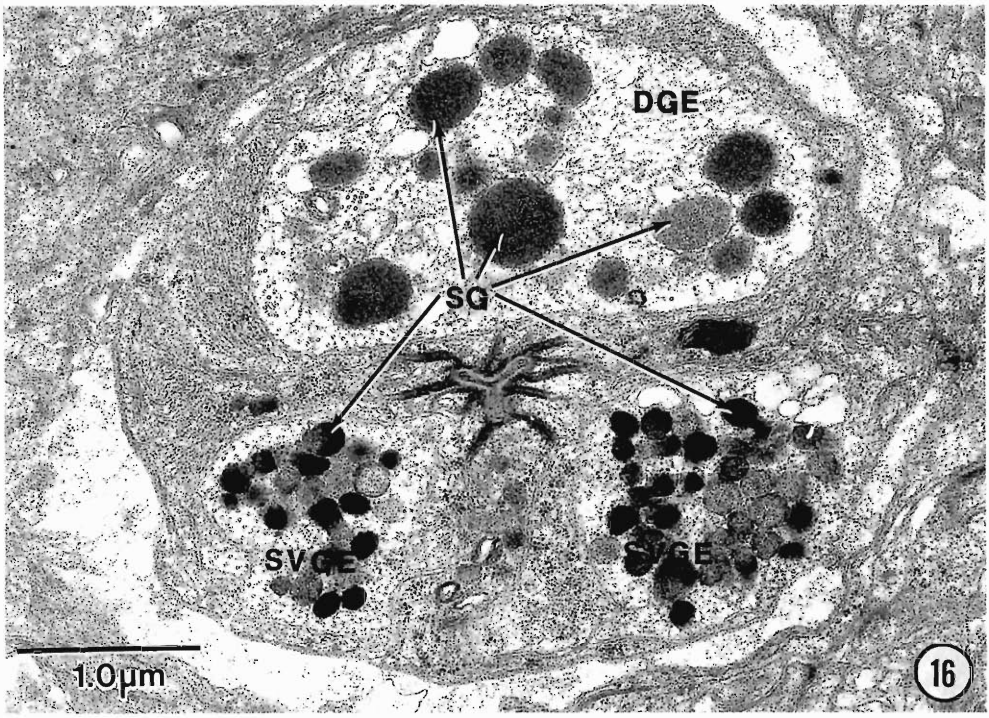
←

similar to the secretory granules of Figure 11 accumulated near a Golgi apparatus (GA) within the central body of the gland cell. The cytoplasm contains numerous sites of Golgi apparatus. As in earlier infections, flocculent secretory bodies (FSB) occupy large regions of the subventral gland. Mc, mitochondria.





Figures 14, 15. Transverse sections through a J2 at 4 days after inoculation. 14. Section shows subventral gland valves (SVGV) in open positions containing electron-dense material and secretory granules (SG) with various levels of electron density. SVGA, subventral gland ampulla. 15. A subventral gland showing electron-translucent flocculent secretory bodies (FSB) within a dense matrix of cytoplasm. Mc, mitochondria.



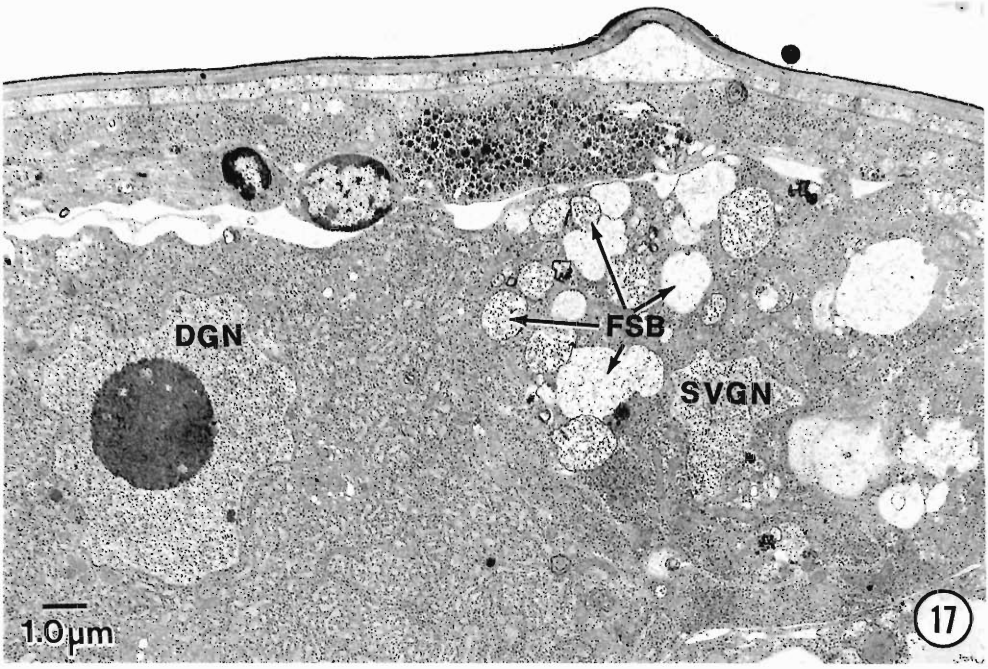
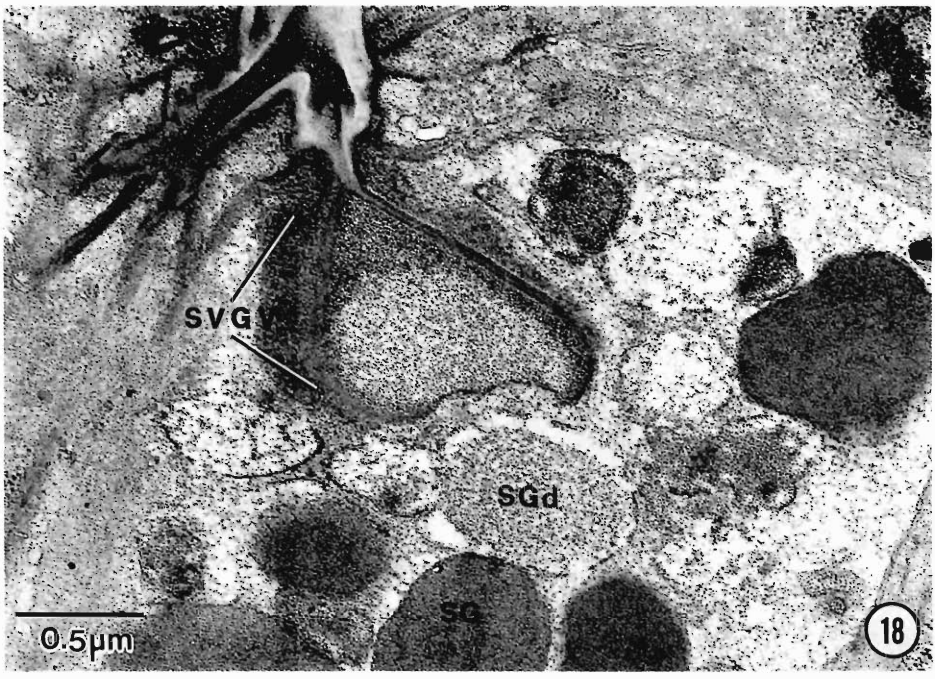
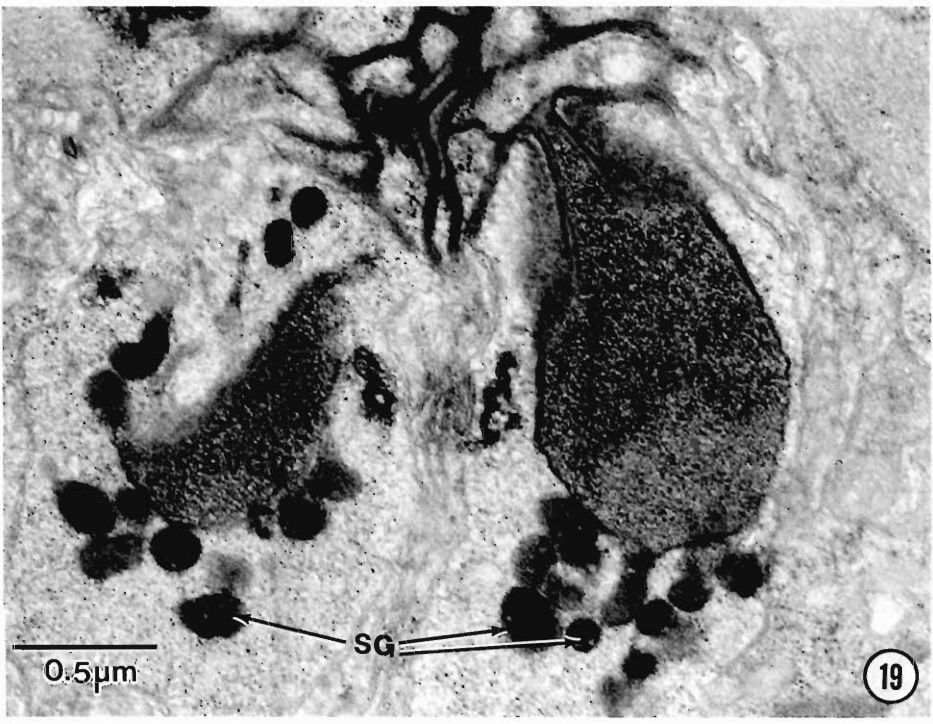


Figure 16. Transverse section through esophageal gland extensions and subventral cell of parasitic J3 at 5 days after inoculation. Gland extensions in nerve ring region showing contrast in secretory granule morphology of dorsal (DGE) and subventral gland extensions (SVGE). Secretory granules (SG) of subventral glands are considerably smaller than those of the dorsal gland and vary widely in electron density.

Figure 17. Longitudinal section through the dorsal and subventral glands of a J3 at 6 days after inoculation. Section shows nuclei of the dorsal (DGN) and of one of the subventral glands (SVGN). Moderately sized flocculent secretory bodies (FSB) with various levels of electron density are distributed throughout the subventral gland.





Figures 18, 19. Comparative sections of subventral gland valves of J2 at 5 hr and late J3 at 8 days after inoculation. 18. Longitudinal section of J2 during root penetration 5 hr after inoculation shows a secretory granule (SGd) with partially depleted contents adjacent to valve membrane. Electron-dense secretory granules (SG) in the ampullae are similar in electron density to the numerous granules in the gland extension. SVGV, subventral gland valve. 19. Open subventral gland valves (SVGV) of J3 filled with electron-dense material similar to the contents of adjacent secretory granules (SG) in the ampullae.

J2 of *M. incognita*. Subventral gland secretory granules were about three-quarters the diameter of those in the preparasitic juveniles. These authors concluded that the subventral glands that were actively synthesizing secretory components as preparasitic J2 appeared to become inactive after the nematode established a feeding site. Existing secretory granules in the gland extensions in parasitic juveniles shrank and appeared to degenerate (Hussey and Mims, 1990). In the current study, many subventral secretory granules have spherical inclusions similar to those described in preparasitic *M. incognita* J2 by Hussey and Mims (1990). Current observations suggest that after the depletion of the rather large and peripherally electron-transparent secretion granules in gland extensions of the preparasitic J2, smaller electron-dense granules are synthesized in the gland cells of the parasitic J3. The very large FSBs found in the gland cells throughout the parasitic J2 and J3 stages are distinctive because of their electron transparency. Formation of these large flocculent bodies is difficult to determine; however, many appear to result from the merging of adjacent, moderately large, electron-transparent granules. The large flocculate secretory bodies and small secretory granules apparently arise from the activities of the Golgi apparatus that are scattered throughout the cytoplasm. Alternatively, the large FSBs may be formed directly from the endoplasmic reticulum. During the synthesis and enlargement of FSBs within the gland, smaller electron-dense secretion granules could be translocated anteriad toward the gland ampulla, as Wyss (1992) observed from in vivo studies with the closely related species *H. schachtii*.

The filling and release of secretory materials in the membranous valve of the subventral glands during in vivo observations of *H. schachtii* supports the concept that the subventral glands are actively involved in the parasitic stages of cyst nematode development (Wyss and Zunke, 1986). Similar in vivo studies of the entire life-cycle of *H. schachtii* (Wyss, 1992) showed all developmental stages with distinct feeding phases of food ingestion (I), stylet withdrawal and reinsertion (II), and salivation (III). In contrast to an earlier report based primarily on a single, ideally situated J2 (Wyss and Zunke, 1986), the filling and depletion of subventral gland valves was usually difficult to detect during phase II. However, the subventral glands were still active in producing secretory granules that accumulated in the gland ampullae of parasitic J2 and J3. The consistent and repetitive feeding cycles within the molting stages emphasize the vital role of the subventral gland and its secretory granules in the life-cycle of *H. schachtii*.

A similar functional relationship can be proposed for *H. glycines* and other related cyst-forming nematodes. The structure and function of the tetraradiate end-apparatus in the dorsal gland valve of the ectoparasite *Tylenchorhynchus dubius* (Anderson and Byers, 1975) and subsequent observations on the gland valves of *Ditylenchus dipsaci* (Shepherd and Clark, 1983) and *Heterodera glycines* (Baldwin et al., 1977; Endo, 1984) have been described. The valves of subventral glands in parasitic J2 and J3 of *H. glycines* frequently contained secretory components. Wyss and Zunke (1986) proposed that after secretory fluids accumulate in the subventral gland valves of *H. schachtii,* they are released to flow into the triradiate chamber of the metacorpus and then forced backward into the intestine through the triradiate lumen of the esophagus. Thus, both the dorsal gland and the subventral glands appear to be essential components of the parasitic stages of soybean cyst nematode during infection of soybean roots. Wyss (1992) also suggested that in *H. schachtii* J2 secretions from the subventral gland granules may be used to mobilize lipid reserves while the intestine is transformed into an absorptive organ during a preparation period. This nonfeeding period for *H. schachtii* starts after initial syncytial cell selection, which lasts several hours and is characterized by a marked decrease in density of the secretory granules in the ampullae and the extensions of the 2 subventral glands. The apparent release of secretions from secretory granules in the current study 3 hr after inoculation of *H. glycines* supports the assumption that most of the secretions may be used in a preparation period that transforms the preparasitic J2 into a parasitic J2.

Recent progress has been made in developing monoclonal antibodies for various components of esophageal glands of *H. glycines* (Atkinson et al., 1988; Atkinson and Harris, 1989), *Meloidogyne incognita* (Hussey, 1989b; Hussey et al., 1990), and various other species of *Meloidogyne* (Davis et al., 1991). This technology will provide ways to understand the mechanism of parasitism by localizing the site of nematode secretions in or associated with syncytia and giant cells induced by cyst and root-knot nematodes and will

help to determine the function of secretory components synthesized in the subventral glands.

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Hemosporids (Apicomplexa, Hematozoea, Hemosporida) of Anatids from the Southern High Plains of Texas

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ABSTRACT: The structure and pattern of the hemosporid community of wintering waterfowl were examined based on thin blood smears collected from 43 cinnamon teal (*Anas cyanoptera*), 89 green-winged teal (*Anas crecca*), and 64 American wigeon (*Anas americana*) on the Southern High Plains of Texas. Thirty-two ducks (16%) were infected with *Haemoproteus nettionis, Haemoproteus greineri, Plasmodium circumflexum, Leucocytozoon simondi,* and/or microfilariae. Intensities of *H. nettionis, H. greineri,* and *P. circumflexum* ranged from <1 to 7, <1 to 28, and 1 to 28/10,000 erythrocytes, respectively. Mean intensities of *H. nettionis, H. greineri,* and *P. circumflexum* were 3.2 ± 0.8 (SE), 5.3 ± 3.0, and 6.4 ± 3.6/10,000 erythrocytes, respectively. Abundances for *H. nettionis, H. greineri,* and *P. circumflexum* were 0.2 ± 0.1, 0.2 ± 0.2, and 0.2 ± 0.2/10,000 erythrocytes, respectively. The low abundance values seemed to preclude any species interactions at the component level in this hemosporid community during the latent period. *Haemoproteus nettionis* from a cinnamon teal and *H. greineri* from American wigeon are new host records.

KEY WORDS: hemosporids, *Haemoproteus greineri, Haemoproteus nettionis, Leucocytozoon simondi, Plasmodium circumflexum,* microfilaria, ducks, *Anas* spp., American wigeon, cinnamon teal, green-winged teal.

Factors affecting survival and recruitment of waterfowl include morbidity and mortality from hemosporid blood parasites (Fallis and Bennett, 1966; Herman et al., 1975; Desser and Ryckman, 1976). Thus, waterfowl have been surveyed for Hemosporida in many breeding areas in North America (Bennett et al., 1975, 1982; Williams et al., 1977). However, there are few studies on these parasites in wintering waterfowl (Polcyn and Johnson, 1968; Kocan et al., 1979; Loven et al., 1980).

The winter period is important because it may represent unique stresses for waterfowl (Alford and Bolen, 1977; Bennett and Bolen, 1978; Jorde et al., 1984); the additional stress of parasitemia could have a synergistic effect on survivability. Infected individuals that survive over winter and return to the breeding grounds are responsible for the perpetuation of the hemosporid vector–host cycle. Also, the migratory ability of waterfowl has particular implications for maintaining and potentially dispersing parasites between summer and winter ranges as well as along migration routes.

There is little quantitative information concerning hemosporid community structure (composition, intensity, and abundance) and pattern (prevalence and species richness) across the latent period of the hemosporid cycle because most studies represent point prevalence data that fail to demonstrate host-related patterns in hemosporid abundance (Godfrey et al., 1987). We examined the structure and pattern of intraerythrocytic hemosporid assemblages during the winter in American wigeon (*Anas americana*), green-winged teal (*Anas crecca*), and cinnamon teal (*Anas cyanoptera*) on the Southern High Plains of Texas.

Materials and Methods

Ducks were trapped at 2 sites on the Southern High Plains in Castro (34°23′N, 102°22′W) and Parmer (34°28′N, 102°55′W) counties, Texas, from October 1985 to March 1986. Blood was drawn via brachial vein puncture with a sterile lancet. Two thin blood smears were made from each bird. Each bird was banded and released.

Blood smears were fixed in methanol and stained with Diff-Quick® (Dade Diagnostics, Inc., Aguada, Puerto Rico 00602). All blood smears were examined and Hemosporida species identified and counted using the same microscope by the same observer (A.M.F.).

To determine prevalence, both blood smears were examined for 30 min for cinnamon teal and 20 min for wigeon and green-winged teal at ×1,000 magnification. A methodology has not been developed for quantifying microfilariae on thin blood smears or for quantifying hemosporids, which occur in both erythrocytes and leucocytes (*Leucocytozoon* spp.); these are reported herein only as prevalence data. Quantification of intraerythrocyte hemosporids generally followed the recommendations of Godfrey et al. (1987). Ten thousand erythrocytes were counted and examined in 100

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Table 1. Prevalence (%) of hemosporids identified from 3 species of waterfowl on the Southern High Plains of Texas from October to March 1985–1986.

Duck species	N*	*Haemoproteus nettionis*	*Haemoproteus greineri*	*Plasmodium circumflexum*	*Plasmodium* sp.	*Leucocytozoon simondi*	Microfilaria
Cinnamon teal	43	1 (2)	0 (0)	0 (0)	0	0 (0)	0 (0)
Green-winged teal	89	8 (9)	7 (8)	5 (6)	0	6 (7)	4 (4)
American wigeon	64	1 (2)	2 (3)	2 (3)	1 (2)	3 (5)	1 (2)
Total	196	10 (5)	9 (5)	7 (4)	1 (<1)	9 (5)	5 (3)

* Number of host individuals examined.

replicates of 100 erythrocytes each to provide an estimate of intraerythrocyte parasite intensity within each infected host. Counts of 10,000 erythrocytes were chosen to assure that at least >90% of infected hosts would have hemosporid intensities ≥1/10,000 erythrocytes based on the apparent intensities observed while examining the smears for prevalence.

Parasites were identified following descriptions of Garnham (1966), Williams and Bennett (1980), and Bennett et al. (1984). Identifications were confirmed by G. F. Bennett (International Centre for Avian Haematozoa, Memorial University of Newfoundland, St. John's Newfoundland, Canada A1C 5S7). Representative specimens are deposited in the International Centre for Avian Haematozoa (accession Nos. 98432 and 103691–103695).

Definitions for the terms prevalence, intensity, and abundance follow those of Godfrey et al. (1990) as modified from Margolis et al. (1982). Intensity and abundance data are presented as mean number of hemosporid individuals ± 1 SE/10,000 erythrocytes.

Results

Thin blood smears were examined from 43 cinnamon teal, 89 green-winged teal, and 64 American wigeon (Table 1). Low prevalences (≤9%) of hemosporid species precluded statistical analysis using prevalence, intensity, and abundance data to examine host-intrinsic (interspecies comparisons; intraspecies age and sex class comparisons) variables.

Thirty-two ducks (16%) were infected with *Haemoproteus nettionis, Haemoproteus greineri, Plasmodium circumflexum, Plasmodium* sp., *Leucocytozoon simondi,* and/or microfilariae. Twenty-seven percent of the green-winged teal were infected; only 9% of wigeon and 2% of cinnamon teal were infected. Ten, 9, 7, 9, and 5 ducks were infected with *H. nettionis, H. greineri, P. circumflexum, L. simondi,* and microfilariae, respectively. A *Plasmodium* sp. from one wigeon was only tentatively identified as *P.* (*Novyella*) *vaughani* (G. F. Bennett, pers. comm.). In 5 green-winged teal, 2 were infected with *P. circumflexum* and *L. simondi,* 2 with *H. nettionis* and *H. greineri,* and 1 with *H. nettionis, H. greineri,* and a microfilaria. In 2 wigeon, 1 was infected with *P. circumflexum* and *L. simondi* and 1 with *H. nettionis, H. greineri, L. simondi,* and a microfilaria.

Intensities of *H. nettionis, H. greineri,* and *P. circumflexum* ranged from <1 to 7, <1 to 28, and 1 to 28/10,000 erythrocytes, respectively. Mean intensity and abundance values for *H. nettionis, H. greineri,* and *P. circumflexum* are presented in Table 2. *Haemoproteus greineri* from American wigeon and *H. nettionis* from cinnamon teal are reported for the first time from these hosts, respectively.

Discussion

Models depicting circulation of hemosporids during the latent period show relatively low prevalences (Beaudoin et al., 1971) and intensities (Herman, 1968). Although there are no other intensity (abundance) data for hemosporids from wild waterfowl during winter, our results demonstrate that extremely low hemosporid intensities occur in the peripheral blood during the latent period. Chernin (1952) described this phenomenon in domestic ducks; he suggested that the observation of immature *L. simondi* during winter was the result of at least some schizogony occurring during this period.

Low intensities of hemosporids circulating in the peripheral blood reduce the probability of transmission on the wintering grounds. This may represent an adaptive strategy of hemosporids utilizing hosts in temperate regions as a way of conserving infective stages when vectors are not available (Allan and Mahrt, 1989; Greiner, 1991). Relapse typically occurs in late spring or summer coinciding with the emergence of vectors and an abundance of breeding ducks; this helps per-

Table 2. Intensity and abundance of hemosporids from 3 species of waterfowl on the Southern High Plains of Texas from October to March 1985–1986.

Duck species	Intensity			Abundance		
	Haemoproteus nettionis	*Haemoproteus greineri*	*Plasmodium circumflexum*	*Haemoproteus nettionis*	*Haemoproteus greineri*	*Plasmodium circumflexum*
Cinnamon teal	3.0 ± 0.0*	–†	–	0.1 ± 0.1	–	–
Green-winged teal	2.7 ± 0.8	6.0 ± 3.9	8.0 ± 5.1	0.2 ± 0.1	0.5 ± 0.3	0.4 ± 0.3
American wigeon	7.0 ± 0.0	3.0 ± 2.0	2.5 ± 1.5	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Total	3.2 ± 0.8	5.3 ± 3.0	6.4 ± 3.6	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.2

* Mean ± standard error of intraerythrocytic hemosporids/10,000 erythrocytes.
† 0/43 infected.

petuate the infection–reinfection cycle as well as producing a large pool of immunologically unchallenged ducklings. Our data support this hypothesis.

Abundance values of hemosporids during the latent period are so low that few, if any, species interactions seem probable at the component community level (as defined by Bush and Holmes, 1986). Indeed, the community ecology of the several potentially interactive hemosporid species in ducks during the spring relapse period needs to be more critically studied in terms of host, temporal, and spatial factors affecting parasite abundances. Only one previous study (Godfrey et al., 1990) has shown the effect of host and spatial factors in *Haemoproteus* spp. representing a hemosporid community in mourning doves (*Zenaida macroura*).

We found *H. greineri* in wigeon and green-winged teal; this species has not been reported previously in wintering waterfowl. Bennett et al. (1984) believed that *H. greineri* may be endemic to northern portions of the Canadian prairie provinces. This suggests that some green-winged teal and wigeon wintering or migrating through the Southern High Plains are from this region.

Herman (1951) found only 1 of 71 cinnamon teal infected with a *Plasmodium* sp. We found *H. nettionis* in 1 of 43 cinnamon teal. Perhaps these ducks are refractory to hemosporids or breed in areas with low vector densities.

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Nematodes Collected from Rodents on Uotsuri Island, Okinawa, Japan

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ABSTRACT: Nematodes parasitic in rodents on Uotsuri Island, Okinawa, Japan, were studied with special reference to the zoogeography of the hosts and parasites. *Strongyloides ratti, Capillaria bacillata, Nippostrongylus brasiliensis, Syphacia muris, Heterakis spumosa, Cyathospirura seurati, Pterygodermatites tani, Pterygodermatites whartoni,* and *Physaloptera* sp. were collected from *Rattus rattus. Heligmonoides* sp. and *Physaloptera* sp. were detected from *Apodemus agrarius. Cyathospirura seurati* was recorded for the first time from the Far East and is redescribed. *Cyathospirura dasyuridis* is synonymized with *C. seurati.* An hypothesis is presented that males of *P. tani* exhibit 2 morphological types, one of which has been regarded as *P. whartoni. Heligmonoides* sp. and *Physaloptera* sp. are presumed to have been introduced to Uotsuri Island with *A. agrarius.*

KEY WORDS: Nematoda, *Rattus rattus, Apodemus agrarius,* Uotsuri Island, Okinawa, Japan, zoogeography.

Uotsuri Island (=Uotsuri-jima) (about 4 km^2, with a maximum elevation of 363 m) is the largest island of the Senkaku Islands, which lie about 175 km north of Ishigaki Island (Fig. 1). This island has been uninhabited except for the period from the end of the last century to World War II, during which Japanese people were stationed there yearly for the collection of albatross feathers and for making dried bonitoes and stuffed specimens of marine birds (Midorima, 1984). Uotsuri Island is of mammalogical interest because of the occurrence of *Nesoscaptor uchidai,* a mole of extremely ancient origin (Abe et al., 1991), and *Apodemus agrarius,* a field mouse that seems to have arrived relatively recently (Arai and Shiraishi, unpubl.). Besides these species, 2 introduced mammals, roof rat (*Rattus rattus*) and goat (*Capra hircus*), inhabit the island (Shiraishi and Arai, 1980). The helminths of mammals on this island have not been investigated previously. Recently we had an opportunity to examine the nematodes from rodents collected on Uotsuri Island and found several species of parasitological and zoogeographical interest.

Materials and Methods

Rodents were trapped in 1979 on Uotsuri Island by the junior authors. The methods of trapping have already been reported (Shiraishi and Arai, 1980). Captured rodents were anesthetized to death with ether, and their viscera were resected and fixed in 70% ethanol. They were examined for helminths under a dissecting microscope. Collected nematodes were cleared in glycerin–alcohol or creosote for microscopical observation. Figures were made with the aid of a drawing tube on a Nikon Optiphoto microscope. Measurements are in micrometers unless otherwise stated. All specimens have been deposited in the National Science Museum, Tokyo (NSMT).

The following material was also examined for comparison: (1) *Cyathospirura* sp., 4 males and 4 females, from *Vulpes vulpes* from Frankston, Victoria, Australia, courtesy of Dr. Ian Beveridge; (2) *Cyathospirura dasyuridis* Mawson, 1968, paratypes, 1 male and 1 female, from *Dasyurops maculatus* from New South Wales, South Australian Museum AHC5194; and (3) *C. dasyuridis,* 4 males, from *Dasyurus quoll* from Tasmania, South Australian Museum AHC6987.

Results

The alimentary canals of 12 *Rattus rattus* and 2 *Apodemus agrarius* were examined. The nematodes were recorded, and their prevalence and intensity of infection are shown in Table 1. Among these species, *Strongyloides ratti* Sandground, 1925, *Capillaria bacillata* (Eberth, 1863), *Nippostrongylus brasiliensis* (Travassos, 1914), and *Heterakis spumosa* Schneider, 1866, are cosmopolitan nematodes of *R. rattus.* Description or remarks are made for other species below.

Cyathospirura seurati Gibbs, 1957

Syn. *Cyathospirura dasyuridis* Mawson, 1968, comb. n.

(Nematoda: Spiruroidea: Spiruridae) (Figs. 2–11)

GENERAL: Body small but stout (Fig. 2). Mouth grossly hexagonal, lacking labium (Figs. 3–5). Four cephalic double papillae and amphidial pores forming outer circle, and 6 minute pa-

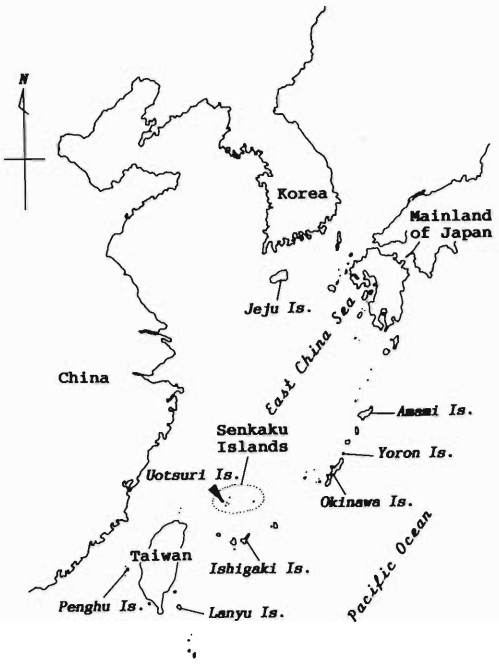


Figure 1. Geographical location of Uotsuri Island, Okinawa, Japan. Localities of adjacent areas referred to in the text are also presented.

pillae forming inner circle (Figs. 3–5). Buccal capsule well developed, thick-walled, with 8 teeth, of which dorsoventral and lateral ones larger (Figs. 3–5). Esophagus divided into anterior muscular and posterior glandular portions (Fig. 2). Nerve ring near posterior end of muscular esophagus (Fig. 2). Deirids asymmetrically positioned: right deirid posterior to nerve ring; left deirid anterior to nerve ring (Fig. 2). Narrow lateral alae commencing anterior to nerve ring and ending anterior to anus. Excretory pore at junction between muscular and glandular portions of esophagus (Fig. 2).

MALE (4 specimens): Posterior part coiled ventrad. Perianal cuticle ornamented with numerous interrupted longitudinal ridges (Fig. 6). Large caudal alae supported dorsally by numerous transverse muscle fibers present. Preanal papillae pedunculate with 4 pairs in 2 groups; unpaired midventral papilla slightly anterior to anus. Postanal papillae 6 pairs: 1 pair pedunculate, projecting midventrally immediately posterior to anus; 1 pair pedunculate at midtail; 4 minute sessile pairs grouped with phasmidial pores near tail tip (Fig. 6). Left spicule slender, filiform, with pointed tip; right spicule stout, with round tip (Fig. 7). Gubernaculum small, triangular in lateral view (Figs. 7–9). Tail conical. Measurements are presented in Table 2 compared to those of Australian material.

FEMALE (2 specimens): Body slender, tapered to both extremities. Vulva at midbody. Vagina narrow, directed dorsad and then running posteriad. Tail conical with blunt tip (Fig. 10). Eggs ellipsoidal, thick-shelled, with weak swellings at poles, containing developed larvae (Fig. 11). Measurements are presented in Table 2 compared to those of Australian material.

HOST: *Rattus rattus.*

SITE IN HOST: Stomach.

LOCALITY: Uotsuri Island, Okinawa, Japan.

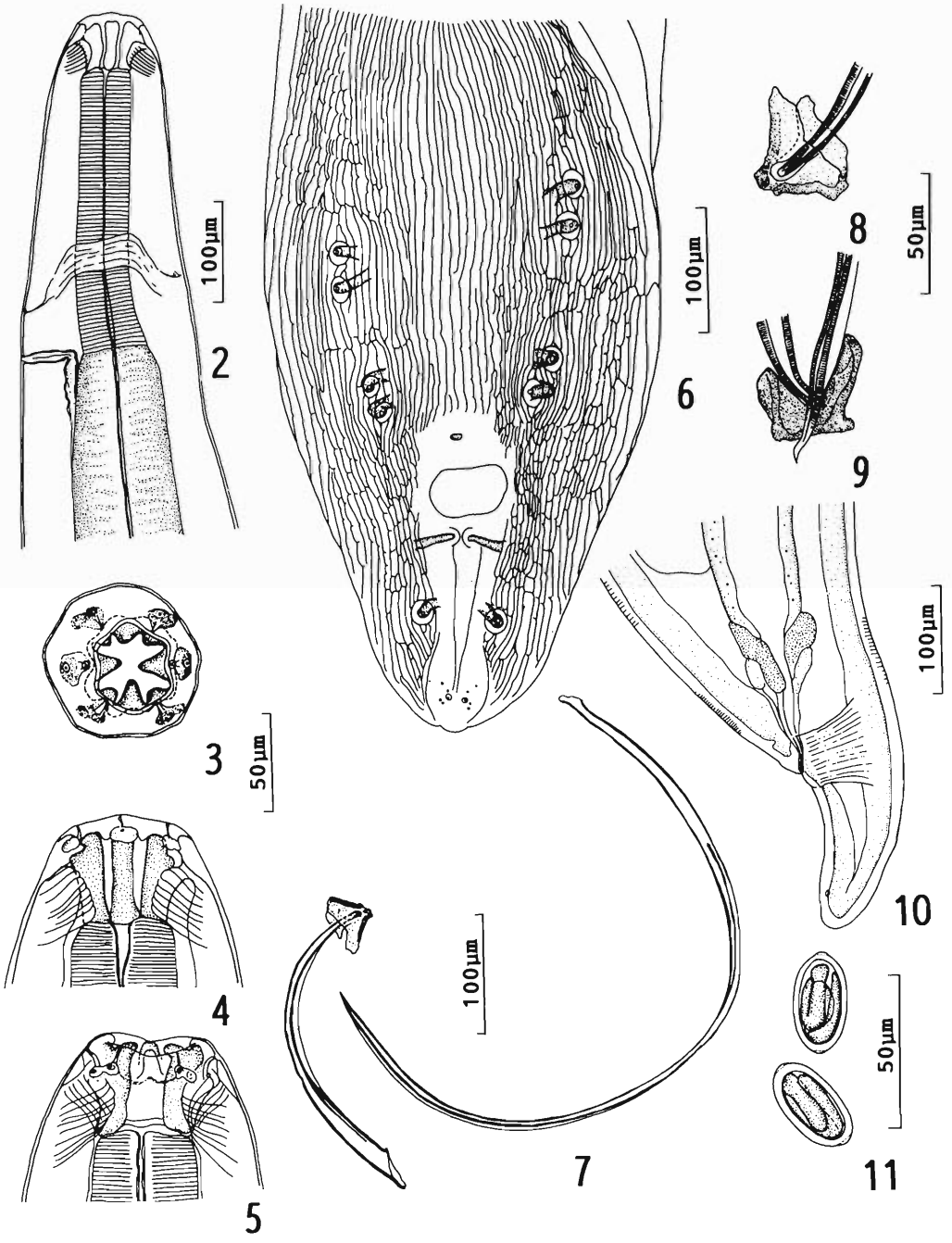
SPECIMENS: NSMT-As 2181.

REMARKS: *Cyathospirura seurati* was first described from a fennec fox, *Fennecus zerda,* in Egypt (Gibbs, 1957). However, this parasite has

Table 1. Nematode parasites collected from the rodents on Uotsuri Island, Okinawa, Japan.

Host (No. examined)	Parasite species	No. hosts infected	Intensity
Rattus rattus (12)	*Strongyloides ratti*	2	1
	Capillaria bacillata	3	—
	Nippostrongylus brasiliensis	2	1–17
	Syphacia muris	5	2–>500
	Heterakis spumosa	9	1–92
	Cyathospirura seurati	1	7
	Pterygodermatites tani	8	1–21
	Pterygodermatites whartoni	1	1
	Physaloptera sp.	5	1–6
Apodemus agrarius (2)	*Heligmonoides* sp.	2	2–10
	Physaloptera sp.*	1	1

* Third-stage larva.



Figures 2–11. ***Cyathospirura seurati*** **from** ***Rattus rattus*****. 2. Anterior part of male, left lateral view. 3–5. Anterior extremity of male, apical (3), left lateral (4), and dorsal (5) views. 6. Posterior extremity of male, ventral view. 7. Spicules and gubernaculum. 8. Gubernaculum and distal end of right spicule, left lateral view. 9. Gubernaculum and distal ends of spicules, ventral view. 10. Posterior extremity of female, left lateral view. 11. Eggs.**

Table 2. Comparison of measurements of *Cyathospirura seurati* from Japan and Australia (in micrometers unless stated otherwise).

Host Locality Specimens	*Rattus rattus* Japan Nat. Sci. Mus. NSMT-As 2181	*Vulpes vulpes* Australia Courtesy of Dr. Beveridge	*Dasyurops maculatus* Australia S. Austr. Mus. AHC 5194	*Dasyurus quoll* Tasmania S. Austr. Mus. AHC 6987
Male (No. worms measured)	(4)	(4)	(1)*	(4)
Length (mm)	6.28–8.70	7.15–8.53	10.1	7.7–10.9
Width	269–300	240–300	293	204–370
Buccal capsule length	51–59	49–63	66	63–72
Muscular esophagus				
Length	215–351	318–372	320	284–376
Width	53–74	49–59	60	49–70
Glandular esophagus				
Length (mm)	1.69–1.82	1.61–1.88	1.84	1.62–2.06
Width	129–152	103–134	180	96–160
Nerve ring†	191–254	264–280	272	268–306
Excretory pore†	194–335	324–352	384	352–420
Right deirid†	281–296	332–348	328	324–392
Left deirid†	117–170	149–168	192	176–212
Right spicule length	275–290	243–298	350	300–395
Left spicule length	720–840	693–768	995	780–1085
Gubernaculum length	38–47	37–42	42	43–49
Tail length	152–195	120–177	190	160–230
Female (No. worms measured)	(2)	(4)	(1)*	
Length (mm)	10.93–14.80	13.4–13.9	8.49	
Width	332–474	354–388	244	
Buccal capsule length	62–66	51–63	59	
Muscular esophagus				
Length	312–356	360–400	244	
Width	59–86	59–69	51	
Glandular esophagus				
Length (mm)	1.84–2.32	2.02–2.35	1.43	
Width	117–176	156–161	125	
Nerve ring†	250–281	276–304	238	
Excretory pore†	347–395	384–436	320	
Right deirid†	304–358	392–444	300	
Left deirid†	150–166	164–200	142	
Vulva (mm)†	5.23–7.48	6.03–6.90	4.40	
Tail	140–152	132–147	104	
Eggs	31–34 × 16–18	33–36 × 18–20	32–34 × 16	

* Paratypes of *Cyathospirura dasyuridis* Mawson, 1968.
† Distance from anterior extremity.

been subsequently recorded from various rodents including *R. rattus,* in Israel, Egypt, Tanzania, Formentera, and southern Spain (cf. Quentin and Wertheim, 1975; Mas-Coma and Esteban, 1983; Mas-Coma and Feliu, 1984; Gibbons et al., 1990), indicating low host specificity. The present worms are identical morphologically with the previous descriptions of *C. seurati,* although in Quentin and Wertheim (1975) the female tail is much longer (400). Gibbons et al. (1990) first noticed the faint swelling of the eggshell by scanning electron microscopic observation. They stated that the swelling was present on 1 pole. However, in the present material each pole has a swelling in most eggs. The present worms are also identical with *C. dasyuridis* from Australia. Although the distances from the anterior apex to the nerve ring, excretory pore, and deirids are somewhat longer in the Australian material (Table 2), these discrepancies may be intraspecific variations. In the previous descriptions of *C. dasyuridis,* the right spicule was slender and the left stout (Mawson, 1968; Clark, 1981). However, reexamination of the Australian material including paratypes revealed a reversed condition; i.e., the longer one is the left spicule. The swellings at poles of the eggshell were also observed in the Australian specimens.

It is thus considered that *C. dasyuridis* is a junior synonym of *C. seurati.* This is the first record of *Cyathospirura* from the Far East.

Pterygodermatites tani (Hoeppli, 1929)

Syn. *Rictularia tani* Hoeppli, 1929 (Figs. 12-17)

GENERAL: Body ornamented with paired combs subventrally. Oral opening slightly inclined dorsad, hemicircular in apical view (Figs. 12–14). Labium absent. Buccal capsule well developed, thick-walled: ventral wall with 2 large, flat teeth; lateral walls each with 3 large, round teeth; dorsal wall with 1 median tooth (Figs. 12–14). Three esophageal teeth present, 1 ventral and 2 subdorsal (Figs. 13, 14). Four large cephalic papillae, 6 small inner papillae, and amphidial pores present (Figs. 12–14). Esophagus divided into anterior muscular and posterior glandular portions (Figs. 15, 18). Nerve ring near posterior end of muscular portion of esophagus in male (Figs. 15, 18) and at middle of muscular portion of esophagus in female. Deirids at junction between muscular and glandular portions of esophagus.

MALE (2 specimens): Length 4.1–6.3 mm, width at midbody 440–442 (Fig. 15). Number of comb pairs 63–65; last several pairs small and pointed (Figs. 15, 16). Buccal depth 55–56. Muscular portion of esophagus 270–348 long by 55–59 wide; glandular portion of esophagus 1.03–1.73 mm long by 109–117 wide. Nerve ring 296, excretory pore 425, and deirids 509 from anterior extremity in male with length of 6.3 mm. Posterior end not bent ventrad (Fig. 15). Perianal region with numerous faint ridges arranged longitudinally (Figs. 16, 17). Preanal fans 3 in number, anterior 2 rudimentary, posterior 1 moderately developed (Figs. 16, 17). Tail conical, 125–160 long (Figs. 16, 17). Paired papillalike ornamentations present medially immediately anterior to anus (Fig. 17). Caudal papillae 10 pairs: 2 pairs preanal, 1 pair adanal, and 7 pairs postanal (Fig. 17). First to 4th pairs of postanal papillae set closely; 5th and 6th pairs and phasmidial pores grouped; 7th pair at tail apex (Figs. 16, 17). Spicules almost equal, simple, slightly bent ventrad: right spicule 62–78 long; left spicule 68–76 long (Figs. 16, 17).

FEMALE (8 specimens): Length 20.2–31.1 mm, width at midbody 644–900. Number of comb pairs 92–94: 42–45 pairs prevulval and 47–51 pairs postvulval; combs becoming spinelike in posterior body. Buccal depth 71–95. Muscular portion of esophagus 513–751 long by 87–119 wide; glandular portion of esophagus 2.81–4.08 mm long by 170–237 wide. Nerve ring 340–403, excretory pore 435–530, deirids 624–869, and vulva 3.33–4.84 mm from anterior extremity. Tail conical, 237–340 long. Eggs ellipsoidal, 45–47 by 29–32; thick-shelled, containing developed larvae.

HOST: *Rattus rattus.*

SITE IN HOST: Stomach and upper small intestine.

LOCALITY: Uotsuri Island, Okinawa, Japan.

SPECIMENS: NSMT-As 2182, 2183.

REMARKS: *Pterygodermatites tani* was first described from *Rattus norvegicus* in China, based on only females (Hoeppli, 1929), and subsequently redescribed by Chen (1936) and Schacher and Cheong (1960), based on males and females. The present worms agree with the previous descriptions in cephalic morphology and in having only 1 developed preanal fan and nearly equal spicules in the male. No caudal papilla was recognized in Chen (1936), and only 4 pairs of the postanal papillae in addition to 2 preanal pairs were observed by Schacher and Cheong (1960). Probably the caudal papillae were overlooked in the previous studies because of their minute size. Females of *Pterygodermatites tani* have been recorded from *R. norvegicus* on adjacent Amami and Yoron islands (Kamiya et al., 1968). *Pterygodermatites* sp., of which females are indistinguishable from *P. tani,* has been recorded from *R. rattus* on Ishigaki Island, but species identification has been withheld because its males are quite different from that of *P. tani* (Kamiya and Kanda, 1977).

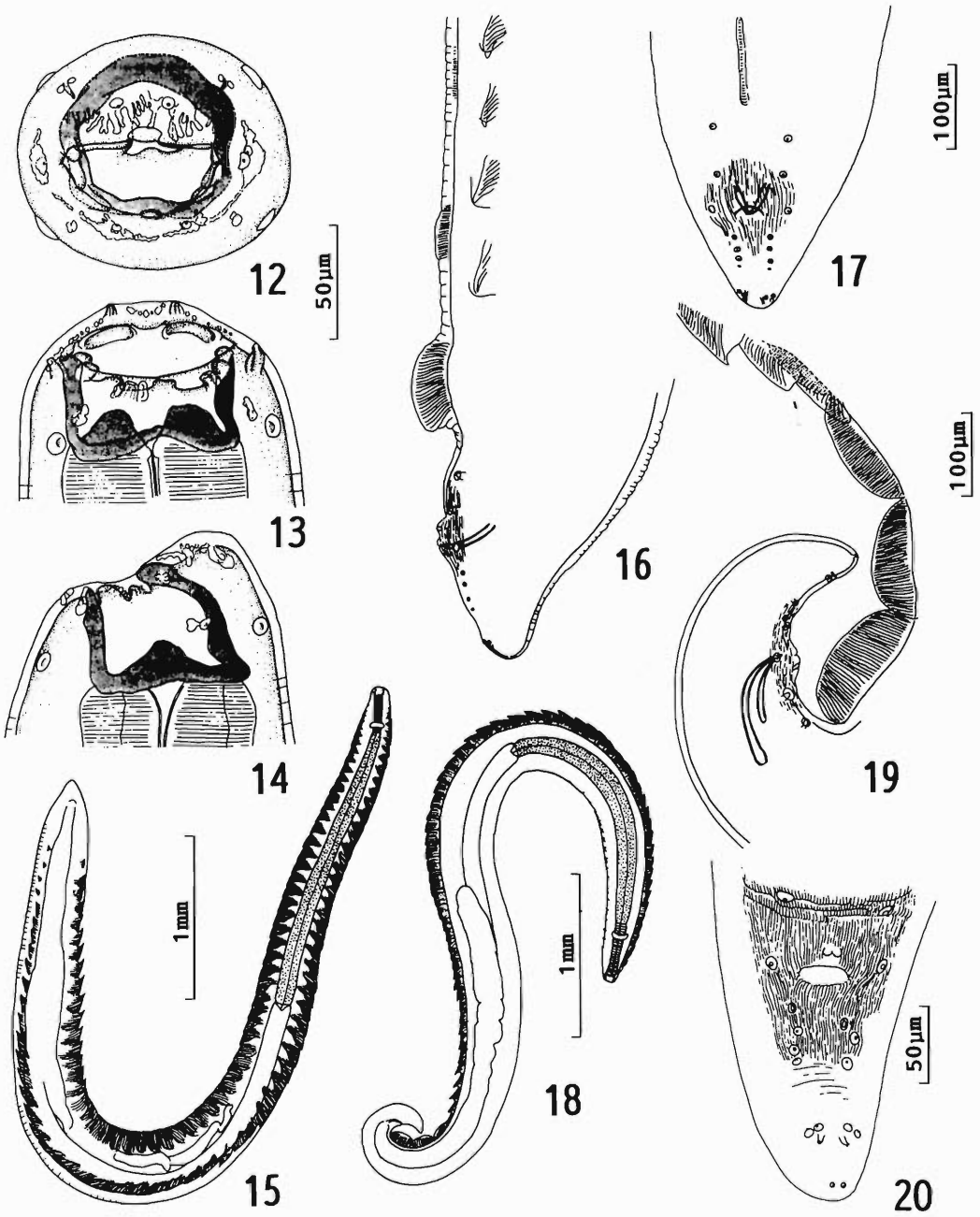
Pterygodermatites whartoni (Tubangui, 1931)

Syn. *Rictularia whartoni* Tubangui, 1931

(Nematoda: Rictularioidea: Rictulariidae) (Figs. 18–20)

GENERAL: Morphology of cephalic region was identical to that of *P. tani.*

MALE (1 specimen): Length 5.62 mm, width at midbody 400 (Fig 18). Number of comb pairs 64. Buccal depth 51. Muscular portion of esophagus 356 long by 50 wide; glandular portion of esophagus 1.52 mm long by 125 wide. Nerve ring 281, excretory pore 429, deirids 545 from anterior extremity. Posterior end bent ventrad



Figures 12–17. ***Pterygodermatites tani*** **from** ***Rattus rattus*****. 12–14. Anterior extremity of female, apical (12), dorsal (13), and right lateral (14) views. 15. Male, general view. 16, 17. Posterior extremity of male, left lateral (16) and ventral (17) views. Figures 18–20.** ***Pterygodermatites whartoni*** **from** ***Rattus rattus*****. 18. Male, general view. 19, 20. Posterior extremity of male, left lateral (19) and ventral (20) views.**

(Figs. 18, 19). Preanal fans 4 in number, posterior 3 well developed (Fig. 19). Tail conical, 192 long (Fig. 19). Perianal region with numerous faint ridges arranged longitudinally (Fig. 20). Paired papillalike ornamentations present medially immediately anterior to anus (Fig. 20). Caudal papillae 10 pairs: 2 preanal, 1 adanal, and 7 postanal (Fig. 19). First to 4th pairs of postanal papillae set closely; 5th and 6th pairs and phasmidial pores grouped; 7th pair at tail apex (Figs.

19, 20). Third papilla of postanal pairs absent on left side (Figs. 19, 20). Spicules dissimilar, simple, curved ventrad: right spicule 75 long; left 155 long (Fig. 19).

HOST: *Rattus rattus.*

SITE IN HOST: Upper small intestine.

LOCALITY: Uotsuri Island, Okinawa, Japan.

SPECIMENS: NSMT-As 2184.

REMARKS: *Pterygodermatites whartoni* was first described from only females from *R. norvegicus* in the Philippines (Tubangui, 1931), and later its male was described by Schmidt and Kuntz (1967) from a sciurid, *Sundasciurus steerii juvencus,* in the Philippines. The present male is morphologically identical to that described by Schmidt and Kuntz (1967) except that the latter has 6 postanal papillae. The last papillae at the tail tip have possibly been overlooked due to their minute sizes. Although a pair of papillalike structures at lateral sides of midtail were figured by Schmidt and Kuntz (1967), such structure was not observed in the present male. The present specimen is also identical to the males of *Pterygodermatites* sp. collected from *R. rattus* on Ishigaki Island (Kamiya and Kanda, 1977), although the unilateral double papilla described by them at level of the preanal fan was not observed.

Physaloptera sp.

(Nematoda: Physalopteroidea: Physalopteridae)

HOST: *Rattus rattus* (adult worms) and *Apodemus agrarius* (third-stage larva).

SITE IN HOST: Stomach.

LOCALITY: Uotsuri Island, Okinawa, Japan.

SPECIMENS: NSMT-As 2185, 2186.

REMARKS: The present adults from *R. rattus* are identical to those collected from *Apodemus agrarius* on Jeju Island, Korea (Hasegawa et al., unpubl.), indicating that this nematode develops to the adult stage in *Apodemus.* A detailed description of this species will be made elsewhere, on the basis of Japanese and Korean material.

Heligmonoides sp.

(Nematoda: Trichostrongyloidea: Heligmonellidae)

HOST: *Apodemus agrarius.*

SITE IN HOST: Duodenum.

LOCALITY: Uotsuri Island, Okinawa, Japan.

SPECIMENS: NSMT-As 2187.

REMARKS: The present material is identical to that recorded from *A. agrarius* at Shenyang, China (Asakawa et al., 1990); Korea, including Jeju Island; and the lowlands of Taiwan (Asakawa, pers. comm.; Hasegawa et al., unpubl.). This species resembles closely *Heligmonoides taiwanensis* Hasegawa, 1990, described from *Apodemus draco* on Mt. Alishan, Taiwan (Hasegawa, 1990). However, it differs from *H. taiwanensis* in having more cuticular ridges (28–30 in number; 24 in *H. taiwanensis*) and a less asymmetrical bursa with thinner rays. A detailed description of this species will be published elsewhere.

Discussion

Cyathospirura seurati seems to have a wide host range because it has been recorded from rodents, carnivores, and marsupials (cf. Gibbs, 1957; Mawson, 1968; Coman, 1972, 1973; Quentin and Wertheim, 1975; Gregory and Munday, 1976; Mas-Coma and Esteban, 1983; Mas-Coma and Feliu, 1984; Gibbons et al., 1990). From the Australian region this nematode has been reported as *C. dasyuridis.* Close morphological similarity between *C. seurati* and *C. dasyuridis* was noticed by Mawson (1968), who justified the latter as distinct by the host difference and geographical occurrence. However, *C. dasyuridis* was later recorded from the introduced carnivores of Tasmania and Australia (cf. Coman, 1972, 1973; Gregory and Munday, 1976). Clark (1981) presented detailed figures of the cephalic extremity and the caudal portion of *C. dasyuridis* from *Dasyurus quoll* from Tasmania, which are essentially identical to those of *C. seurati.* Beveridge (1986) presumed that the *Cyathospirura* in Australia was introduced with carnivores. This assumption may be strongly supported by the fact that *C. dasyuridis* is synonymous with *C. seurati,* as proved in this study.

Cyathospirura seurati is also closely allied to *Cyathospirura chabaudi* Gupta and Pande, 1981, which was first described in India from worms reared experimentally in pups from larvae in paratenic lizard hosts (Gupta and Pande, 1981). Gupta and Pande (1981) distinguished their species from *C. seurati* only on the basis of comparison to the description by Gibbs (1957). However, when compared to the redescription of *C. seurati* by Quentin and Wertheim (1975), the difference between the 2 species is slight except that *C. chabaudi* has a smaller body but somewhat longer spicules. It is probable that the smaller body of *C. chabaudi* is due to the younger stage of the worms, because molting larvae and immature adults as well as mature adults were also

recovered from the pups (Gupta and Pande, 1981). It is thus strongly suggested that *C. chabaudi* is a junior synonym of *C. seurati.* Unfortunately, the type specimens of *C. chabaudi* were not available for comparison in spite of every effort. If *C. chabaudi* is synonymous with *C. seurati,* it is then apparent that this spirurid is widely distributed in Africa, Eurasia, and Australia at the present time. However, its distribution in the Far East may be sporadic, because no record of a species of *Cyathospirura* has been established from adjacent areas of Uotsuri Island. The route by which *C. seurati* came to this island remains to be elucidated.

The taxonomical relationship between *P. tani* and *P. whartoni* has puzzled many researchers. Chen (1936) and Schacher and Cheong (1960) considered *P. whartoni* to be a junior synonym of *P. tani* because there was no clear distinguishable characteristic in female morphology between the 2 species. However, Schmidt and Kuntz (1967) claimed the validity of *P. whartoni* because they found a male apparently different from that of *P. tani* described previously. Kamiya and Kanda (1977) also detected *Pterygodermatites* sp. of which the female is indistinguishable from *P. tani* whereas the male is identical to *P. whartoni.* The presence of the 2 closely related species, of which females are actually indistinguishable from each other, in a rat on a small uninhabited island such as Uotsuru Island seems to be quite curious. The same situation was also observed on Lanyu Island, Taiwan, where males of both *P. tani* and *P. whartoni* were collected from *R. rattus* (Hasegawa et al., unpubl.). It is thus strongly probable that *P. tani* has 2 types of males, 1 of which has been regarded as *P. whartoni.*

A similar condition was observed in males of *Rictularia cristata* Froelich, 1802, collected from *Apodemus* spp. of China and Japan (Hasegawa and Asakawa, unpubl.). Because rictulariid males are minute, and usually much fewer in number than females, their morphology has not been described adequately in many species. The length and length ratio of the spicules have been considered as important taxonomic characters of rictulariids (cf. Quentin, 1969). If male dimorphism is a common phenomenon, then a thorough critical revision of the rictulariids may be required. Further studies may also be necessary to confirm the dimorphism of male rictulariids. In some parasitic nematodes, male dimorphism has been already reported (cf. Chabaud and Golvan, 1957; Hasegawa, 1985; Lichtenfels and Pilitt, 1989; Ainsworth, 1990), although its ecological or evolutionary significance has not yet been elucidated.

Physaloptera sp. might have been introduced to Uotsuri Island by 1 of the 2 rodent species. It seems less probable that *R. rattus* introduced this nematode because no physalopterid has been recorded from rats on the adjacent islands (cf. Kamiya et al., 1968; Kamiya and Machida, 1977), whereas the same species has been collected from *A. agrarius* on Jeju Island, Korea (Hasegawa et al., unpubl.). Moreover, Kontrimavichus and Khokhlova (1964) recorded *Physaloptera* sp. from *Apodemus* in the Far East territory of Russia, and Zhang (1985) recorded a physalopterid species from *A. agrarius* in China. It is thus suggested that *Physaloptera* sp. was introduced by *A. agrarius* and subsequently adapted to *R. rattus.* If more individuals of *A. agrarius* on Uotsuri Island are examined, mature *Physaloptera* sp. may be obtained.

Heligmonoides sp. of *A. agrarius* is considered to have arrived in Uotsuri Island with its host, because this nematode has been detected only from *A. agrarius* (Asakawa et al., 1990; Asakawa and Hasegawa, unpubl.). From other *Apodemus* species of the adjacent areas, different *Heligmonoides* species have been recorded: *H. taiwanensis* Hasegawa, 1990, from *A. draco* of Taiwan and *Heligmonoides speciosus* (Konno, 1958) from *Apodemus* spp. of the mainland of Japan (cf. Hasegawa, 1990). The close resemblance between the present *Heligmonoides* sp. and *H. taiwanensis* suggests that both nematodes were derived from a common ancestor adapted to *Apodemus.*

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Ultrastructure of the First-Stage Larvae of a *Philometra* sp. (Nematoda: Philometridae) from Freshwater Drum (*Aplodinotus grunniens*)

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ABSTRACT. An ultrastructural study of the first-stage larvae of *Philometra* sp., utilizing scanning and transmission electron microscopy, was undertaken to understand behaviors of this larval stage better. Larvae have an egg tooth present on the dorsal labial ridge and may penetrate the gut wall of the copepod host through its use. Internally, first-stage larvae were found to have a partially developed digestive system that is filled with a yolklike material and ends in storage cells. Larvae may not feed at this stage, relying instead on this material for an energy source. The caudal end of the first-stage larva consists of a concave, oval-spatulate structure abundantly supplied with muscle. Muscular contractions possibly allow the attachment to particles seen during the free-swimming phase of this larval stage.

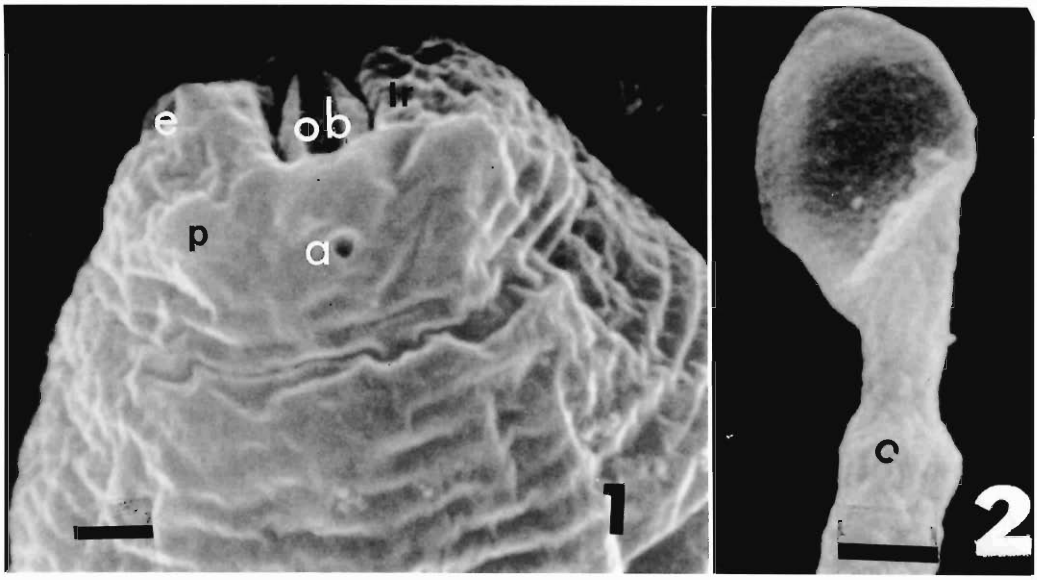
KEY WORDS: *Philometra* sp., Nematoda, morphology, ultrastructure, SEM, TEM.

Nematodes of the family Philometridae produce free-swimming first-stage larvae. From late June to early August, larvigerous females of *Philometra* sp. can be found streaming from the eyes of the freshwater drum. These worms rupture, releasing thousands of first-stage larvae into the water. The larvae attach in groups to particles and are ingested by the intermediate host, cyclopoid copepods. Once within the copepod, the larvae penetrate the copepod gut wall and establish themselves in the hemocoel. They undergo 2 molts in the hemocoel, developing to infective third-stage larvae. If the copepod is then ingested by a young freshwater drum, the larvae undergo 2 more molts, migrate to the eye of the fish, and establish themselves. Copulation occurs in the ocular orbits, and the males subsequently die. Fertilized ova within the females developed into first-stage larvae the following summer (Crites, 1980).

During its life cycle, *Philometra* sp. undergoes extensive internal and external change. Although some work has been conducted on adult philometrids using light microscopy, almost nothing is known about the structure of the larval stages. There are a few descriptions of first-stage larvae based on evidence from light microscopy, but these descriptions are incomplete and often contradictory. Crites (1980) described the first-stage larvae as having no discernable lips, an open esophageal lumen, an intestine with no discernible lumen, and a long tapering tail with an open cavity at the posteriormost end. *Philometroides*, a related genus, is described as having first-stage larvae with a dilated esophagus ending in 3 distinct cells and an intestine filled with refractile granules (Uhazy, 1976). Uhazy (1976) also mentioned that *Philometroides nodulosa* and *P. sanguinea* do not have terminal buttonlike swellings on the tail, but *P. huronensis* does. Thomas' (1929) description of *P. nodulosa* first-stage larvae included a mouth surrounded by a dorsal and a ventral lip, a thin esophagus with a visible lumen joined to an expanded intestine containing refractile granules, cement glands in the caudal region that opened to the exterior 40 μm from the distal end of the tail, and an anus situated on the ventral surface 48 μm from the posterior end. Krecker (1916) described the larvae of *Filaria cingula* (probably a species of *Philometroides*) as tapering to a sharp hairlike point at the posterior end. First-stage larvae of *Philometra ovata* are described as having a conical tail with a sharply pointed tip (Moravec, 1980).

Although *Philometra* sp. larvae are known to be extremely active, the energy source for this activity is unknown. It is uncertain whether or not this larval stage feeds. Although an anus is present, it is unknown if this structure is functional; the anus disappears in the adult.

It would seem that larval behaviors, such as clumping, penetration of the copepod gut wall, and feeding and energy storage by the first-stage larvae, are not adequately explained by present descriptions of species of *Philometra*. The objective of this study is to provide a description of the first-stage larvae using electron microscopy. The ultrastructural information provided can then be used to understand better the behaviors of this larval stage.



Figures 1, 2. First-stage larva of *Philometra* sp. from *Aplodinotus grunniens*. 1. Anterolateral view of cephalic end. a, amphidial pore; e, egg tooth; lr, labial ridges; ob, oral bars; p, pseudolabium. Scale bar = 1.1 μm. SEM. 2. Caudal end. c, cuff. Scale bar = 0.7 μm. SEM.

Materials and Methods

Small (120–160-mm) freshwater drum, *Aplodinotus grunniens,* were collected by otter trawl in Lake Erie's western basin off South Bass Island, Ohio. Infected fish were readily apparent because they exhibited popeye. This condition, characterized by protruding, reddened eyes, was seen from late June to early August in freshwater drum from the western basin. Upon dissection, 1 to several red, coiled, larvigerous female *Philometra* sp. were found in the ocular orbits of these fish. These worms rupture on exposure to lake water releasing first-stage larvae. After a short period of time to allow for any changes that might occur on release into lake water, the larvae were collected by centrifugation and fixed in 6% gluteraldehyde in Millonig's phosphate buffer according to a technique modified from Martinez-Palomo and Martinez-Baez (1977). Two percent DMSO was used to facilitate penetrance of the fixative through the cuticle of the larvae. After alcohol dehydration, the larvae were placed in 100% propylene oxide and then embedded in thin films of Spurr's (1969) low-viscosity resin. Specific larvae were excised, oriented, and reembedded onto epoxy blocks for serial sectioning.

Serial cross and longitudinal sectioning was done on an LKB NOVA ultramicrotome using glass and diamond knives. Sections showing silver interference colors (60–90 μm) were picked up on uncoated 200-mesh grids, stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963), and examined on a Zeiss 109R transmission electron microscope (TEM) operated at 80 kU.

Glutaraldehyde-fixed larvae were processed for scanning electron microscopy (SEM) using critical-point drying following alcohol dehydration. The larvae were placed on an adhesive-coated stub and coated with gold. They were scanned on a Hitachi S-500 SEM. To be sure that the head and tail structures seen using light microscopy (LM) and SEM were not artifacts of fixation, living larvae were examined under LM using Nomarsky differential interference optics.

Results

Living first-stage larvae of *Philometra* sp. have a concave, oval-spatulate posterior end that resembles a hook from the side. Anterior to this structure, the tail narrows to a small cuff. One large papillalike structure is present on the cephalic end of the larvae. No other structures were discernable with LM.

SEM of *Philometra* sp. reveals the presence of a single papillalike egg tooth located dorsally on the cephalic end of the first-stage larva (Fig. 1). There are labial ridges dorsal and ventral to the mouth and pseudolabia are present laterally (Fig. 1). Lateral amphidial pores are present on the pseudolabia. A pair of barlike structures, arranged laterally, lie within the oral opening (Fig. 1). The cuticle is striated and without ornamentation. An opening, presumably the excretory pore, is present on the ventral surface about 75–100 μm from the posterior end. The caudal area is oval-spatulate and appears to be flexible. Anterior to this structure, the tail narrows to a small cuff (Fig. 2).

Cross sections of the cephalic region, when

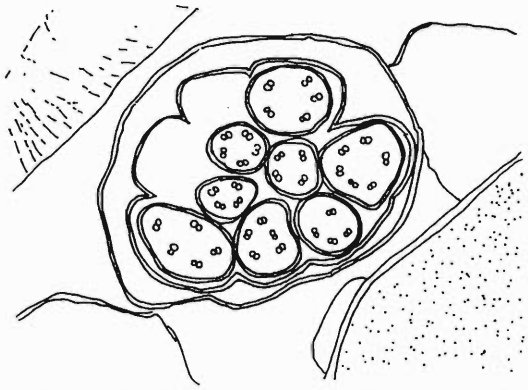


Figure 3. First-stage larva of *Philometra* sp. from *Aplodinotus grunniens*. Lateral ciliary canal drawn from an electron micrograph (TEM).

viewed using TEM, show sensory cilia associated with the amphidial pores, with 8 cilia located in a membrane-lined ciliary canal that exits anteriorly through an opening in the cuticle (Fig. 3). These cilia do not appear to possess basal bodies and are aberrant in that they show variation in microtubule pattern similar to those described for *Dirofilaria immitis* (Kozek, 1968). The egg tooth is also associated with a sensory cilium. This cilium is located primarily within the cuticle and is not contained in a ciliary canal (Fig. 4). The egg tooth appears to be cuticular and does not appear to be associated with secretory cells.

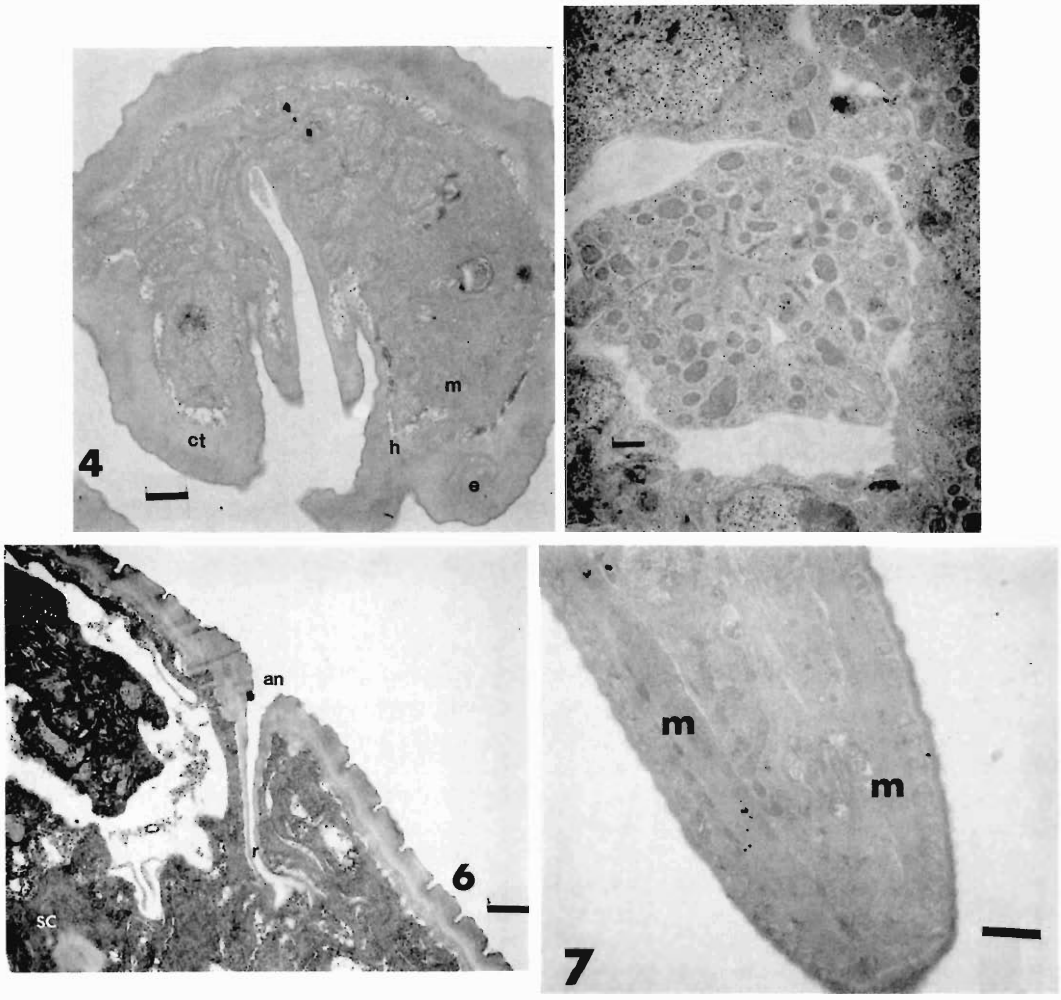
Longitudinal sections through the cephalic region show that the oral bars seen with SEM contain hypodermis and muscle and are lined with cuticle that is continuous with the external cuticle (Fig. 4). The cuticle lining the oral bars also lines the open esophagus. The lumen of the esophagus is filled with granular yolklike material. It continues into an area further posterior in the digestive tract that is not lined with cuticle and probably represents the beginning of the intestine.

Externally, a striated cuticle about 0.17 μm thick is present. Underlying the cuticle is the hypodermis, which is arranged in dorsal, ventral, and lateral cords. The hypodermis of the first-stage larvae appears to be cellular with large nuclei present, although in later stages the hypodermis is syncytial. Each lateral cord contains a honeycomblike structure that may be part of the excretory system, and there are nervous elements, consisting of tightly packed membranes, present. Fixation distortion and other fixation-related problems account for much of the inability to find definite structure in the hypodermis. The circumesophageal nerve ring was not apparent in any of the sections taken, but it is visible in LM of living and fixed larvae. Four rows of muscle cells, running anterior to posterior, are present immediately beneath the hypodermis, with 2 cells present ventrally and 2 dorsally. Each has a cytoplasmic region containing a large nucleus and a contractile region containing longitudinally arranged myofilaments typical of active muscle. The esophageal lumen of the first-stage larvae is surrounded by 3 or 4 cells arranged in a single layer (Fig. 5). The open, yolk-filled lumen of the esophagus ends in a triradiate structure that is without an apparent lumen (Fig. 5) and again opens up into a yolk-filled area that is not lined with cuticle. The digestive tract, posterior to this dilated open region, is undeveloped and closed. Three or 4 cells surround a long, narrow intestinal thread having no apparent lumen situated at the midsection of the digestive tract. Posterior to this is an area predominated by storage cells filled with granular material. Beyond the storage cells are columns of 2 or 3 cells, each containing a large nucleus and many mitochondria. Among these cells is a cuticle-lined rectum that does not appear to be connected to the digestive tract (Fig. 6) but that leads into a cuticle-lined anus. Rows of muscle are present at the caudal end (Fig. 7). No secretory tissue was found in the caudal end, nor were any pores, bristles, or other structures of attachment seen. The phasmids were not apparent in any of the sections, nor were phasmidial pores observed using SEM.

Discussion

Philometrids are ovoviviparous. The zygote develops to a vermiform stage enclosed in an egg membrane within the uterus of the female. These embryos increase in size as they develop and appear to store lipids and glycogen (Crites, 1980). First-stage larvae eventually rupture through the egg membranes and lie free in the uterus until released into the water by the bursting of the female worms (Crites, 1980).

Morphological features observed in this study for the first-stage larvae of *Philometra* sp. are an elongate oral opening containing oral bars and surrounded by 2 lateral pseudolabia bearing amphids and 2 lateral ridges, 1 bearing a dorsal egg tooth; an open lumen to the esophagus leading into an open portion of the digestive tract that is not lined with cuticle; an intestinal thread sur-



Figures 4–7. First-stage larva of *Philometra* sp. from *Aplodinotus grunniens*. 4. Oblique section through anterior end. ct, cuticle; h, hypodermis; m, muscle. Scale bar = 0.4 μm. TEM. 5. Cross section through esophageal-intestinal junction. Scale bar = 1.4 μm. TEM. 6. Oblique section through posterior region of digestive tract. an, anus; r, rectum; sc, storage cells. Scale bar = 0.7 μm. TEM. 7. Longitudinal section through posterior end. Scale bar = 1.4 μm. TEM.

rounded by cells leading into an area of storage cells; a column of cells surrounding a cuticle-lined rectum that leads into an anus; and a long tapering tail ending in an oval-spatulate structure (Fig. 8). It is apparent from SEM of the first-stage larvae that major differences, most likely related to life-style and habitat, occur between adult and larval forms. In contrast to the adult philometrids, which have 2 circles of papillae surrounding the oral opening, only 1 unpaired papilla is present on the cephalic end of the first-stage larvae. This egg tooth is common on many larvae that use copepod intermediate hosts. First-stage larvae of nematodes of the superfamily Dracunculoidea were described as having a cephalic dorsal denticle by Chitwood and Chitwood (1950). Li (1935) described the larvae of *Procamallanus fulvidraconus* as being provided with a dorsal spine for penetrating the copepod intestinal wall. Hedrick (1935), in his study on the life history of *Spiroxys contortus* (Nematoda: Spiruridae), stated that the larva penetrates the body cavity of cyclops (Copepoda) with the aid of a cuticular tooth. Furthermore, he assumes the process to be mechanical, because no glands or ducts can be seen leading to the base of the tooth. When

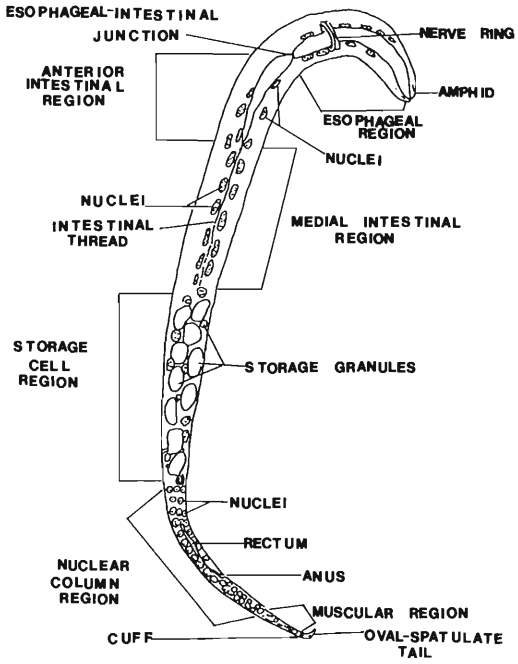


Figure 8. Diagrammatic representation of the first-stage larva of *Philometra* sp. from *Aplodinotus grunniens*.

the larva molts, this egg tooth is shed with the cuticle (Hedrick, 1935). The egg tooth of *Philometra* sp. first-stage larvae is cuticular and does not appear to be associated with any secretory cells.

Amphids, present on the adult of *Philometra* sp., are also found as early in development as the first-stage larvae. The amphids contain cilia enclosed in ciliary canals, similar to those described by Kozek (1968) in *D. immitis* (Nematoda: Filarioidea). The egg tooth also appears to be associated with a subsurface sensory cilium, indicating that it may have mechanoreceptor function beyond its supposed function in penetrating the copepod gut wall. This cilium appears to be located primarily in the cuticle and is not enclosed within a canal. Like the sensory cilia of the amphids, the cilium of the egg tooth is varied in microtubule pattern and does not appear to have a basal body. The egg tooth cilium may be used in orientation of the larva with respect to the copepod gut wall.

Mention of cuticular projections in the family Spiruridae has been made by Chitwood and Wehr (1934), but the philometrids were distinguished from the spirurids by lack of such structures. However, the microfilariae of members of the superfamily Filarioidea also have cuticular structures associated with the oral opening. These structures are described by Laurence and Simpson (1968) as a cephalic space containing an eversible hook and spines attached to an oral ring supplied with muscle for movement. These structures are lost at first molt. It is possible that larval cephalic structures of *Philometra* sp. may provide further evidence of evolutionary continuity within the order Spirurida and may provide a link with the Filarioidea. However, other larval stages need to be studied.

The oral opening of the first-stage larvae of *Philometra* sp. is elongate and contains oral bars. The oral bars open externally into the oral opening. These structures are not found in the adult, although adult structure is disputed. The purpose of the oral bars is unknown. The mouth of the first-stage larva of *Philometra* sp. opens into a cuticle-lined esophagus. The cuticular lining is continuous with the cuticle of the oral bars, suggesting that these are the ends of a buccal capsule. The esophageal lumen is filled with yolk, apparently a remnant of the yolk supplied to the embryo before the egg membranes are laid down. The cuticular lining ends halfway down the digestive tract, indicating that at least part of the intestine is also developed. Taylor (1960) described the intestine of the filarid *Dirofilaria immitis* as starting as a solid mass of cells that later form a cavity through the center. Development of *Philometra* sp. appears to be similar. Posterior to this open area is a column of cells surrounding an intestinal thread similar to the pharyngeal thread, or long narrow tube that the gut develops around, described by McLaren (1972) for various filarid species. However, while the pharyngeal thread is lined with cuticle anteriorly where the esophagus will form, the intestinal thread is not lined with cuticle anywhere along its length, indicating that the esophagus has already differentiated but the intestine, which is not lined with cuticle, has not—a somewhat more advanced stage of development than has been described for the filarids.

The posterior end of the intestinal area is filled with storage cells. Histochemical staining of this area shows these cells to be periodic acid–Schiff positive, indicating the presence of glycogen, and Sudan B-black positive, indicating the presence of lipids (Crites, 1980). Von Brand (1966) indicated that granules in the digestive tract provide

an endogenous energy source for the larvae. Given the large amount of stored energy within the larvae themselves, and the lack of a completely differentiated digestive system, it is likely that the larvae do not feed at this stage. An anus and a rectum are present, although by the time the third larval stage is reached the rectum has already atrophied (Crites, 1980). In larvigerous females, the anus is absent and the rectum has degenerated to a strand that connects the end of the intestine to the body wall (Crites, 1980), indicating that secretion of solid wastes probably does not take place at this stage either, although the adult females do feed on red blood cells (Crites, 1980). Whether the anus and rectum are ever functional in the philometrids, or they are only present vestigially as part of the developmental sequence, is unknown.

Philometra sp., as a member of the class Secernentia, should have phasmidial pores opening near the anus. Phasmids were not seen on any of the first-stage larvae examined and are possibly not present until a later developmental stage. Phasmids are present on adult philometrids and have been described for the larvae of *Philometra obturans* (Moravec, 1980).

Crites (1980) describes clumping behavior of the first-stage larvae and indicates that although larvae are capable of attachment by their tails, no glands could be ascertained. Neither SEM nor TEM produced evidence of cement glands, bristles, spines, or secretory cells in the caudal end of the first-stage larvae of *Philometra* sp. However, rows of muscle were found, indicating that the oval-spatulate area of the tail may function as a sucker or grasping organ. No mention has been made of such organs in any other larval stages studied, nor are suckers and muscular grasping organs a common adaptation of nematodes.

From a behavioral standpoint, clumping by larvae may increase the probability of their being encountered by copepods. This would be important to an organism that is relying on a finite amount of stored energy, and it is indicated by Stromberg and Crites (1974) that copepods are more strongly attracted to larvae that are moving. Larvae continue to be active for up to 27 days depending on temperature, but infectivity decreases rapidly in this time (Crites, 1980). Activity of the larva and ability to penetrate the copepod gut wall are significantly correlated, indicating that larval activity is important to gut wall penetration (Stromberg and Crites, 1974). This is substantiated by the lack of visible secretory structures associated with the egg tooth of the first-stage larvae.

This study provides a more complete description of the first-stage larva of *Philometra* sp. and provides a basis for the understanding of some of the larval behavior. There is also the possibility that this description of the larvae will help in assigning evolutionary relationships among the spirurids and provide a better understanding of the relationship between the philometrids and filarids. It does point to the importance of knowing larval structure and poses questions about the developmental processes occurring in the other larval stages and in the adults of *Philometra* sp. and related genera.

Acknowledgments

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Vertical Transmission of Parasites

Saturday, 1 May 1993

University of Pennsylvania, New Bolton Center, Kennett Square, PA
Annual Joint Meeting with the New Jersey Society for Parasitology

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U.S. Department of Agriculture
Beltsville, MD 20705
Protozoans

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Dr. D. Bruce Conn
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St. Lawrence University
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Cestodes

J. Helminthol. Soc. Wash.
60(1), 1993, pp. 55–61

Parasites of Parr and Lake Age Chinook Salmon, *Oncorhynchus tshawytscha,* from the Pere Marquette River and Vicinity, Michigan

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ABSTRACT: Changes in the parasite fauna of chinook salmon, *Oncorhynchus tshawytscha* (Salmonidae), were determined by examining 360 fish of various ages at different times and localities from the Pere Marquette River and Lake Michigan, Michigan. Four parasite species infected chinook salmon parr from the Pere Marquette River, with *Acanthocephalus dirus* being most common. Ten parasite species infected age 0 salmon from Lake Michigan. In these fish, prevalence and mean intensity of *A. dirus* decreased and another acanthocephalan, *Echinorhynchus salmonis,* was found in increasing numbers in older salmon from Lake Michigan. Eight parasite species infected age 1 fish from Lake Michigan and 7 infected adult fish that had returned to the river to spawn. Cestodes were found only in chinook salmon from Lake Michigan and in mature adults that had returned to the river to spawn. Parasites infecting parr from the river were not found in salmon in the lake except for *A. dirus* in one fish. Changes that take place in the parasite fauna of chinook salmon from the Pere Marquette River and Lake Michigan are related to fish age, diet, and movements. Additional information on movements of salmonids and changes in their parasites is provided by the examination of 156 steelhead, *Oncorhynchus mykiss,* and 67 coho salmon, *Oncorhynchus kisutch,* of various ages at different times and localities from the Pere Marquette River.

KEY WORDS: *Oncorhynchus tshawytscha, Oncorhynchus kisutch, Oncorhynchus mykiss,* Salmonidae, fish migration, parasites, survey, Pere Marquette River, Lake Michigan, Michigan.

Several publications, listed and (or) summarized by Margolis (1970) and Margolis and Arthur (1979) as well as Haderlie (1953), Pennell et al. (1973), Olson (1978), and Jennings and Hendrickson (1982) have studied parasites of salmon from Canada and the northwestern United States. These studies as well as those of Dogiel (1966) and Dogiel et al. (1970) have documented changes in the parasite fauna of anadromous salmonids during their lifetime. These changes in the parasite fauna are related to differences in diet and to movements of salmon from freshwater to the marine environment and back to freshwater.

Although the parasites of salmon, *Oncorhynchus* spp., have been studied in the Great Lakes (Collins and Dechtiar, 1974; Lankester and Smith, 1980; Amin, 1985; Muzzall and Peebles, 1986; Dechtiar and Christie, 1988; Dechtiar et al., 1988; Dechtiar and Lawrie, 1988; Muzzall, 1989), investigations on changes in the parasite fauna of parr and lake age salmonids in relation to their movements and diet have not been done. The present study identifies parasites acquired by parr in the Pere Marquette River, Michigan, and examines the changes in the parasite faunas of salmonids migrating from the river to Lake Michigan and then returning to the river as mature adults. Emphasis is placed on the parasites of chinook salmon, *Oncorhynchus tshawytscha,* with supplemental information provided on parasites of steelhead, *Oncorhynchus mykiss,* and coho salmon, *Oncorhynchus kisutch.*

Materials and Methods

The Pere Marquette River is located in west-central lower Michigan. It flows east to west for more than 160 km through Lake and Mason counties into Pere Marquette Lake, which empties into Lake Michigan at Ludington. A total of 360 chinook salmon were collected in May, August, and September 1983, July and August 1989, and May–October 1990. Fish were collected by angling, electrofishing, and drift nets from 3 localities in the Pere Marquette River, Weldon Creek (a tributary of the Pere Marquette River), and Lake Michigan in close proximity to the mouth of the river (Fig. 1). One hundred fifty-six steelhead and 67 coho salmon from various localities were also examined for parasites. Age categories of salmonids were based on known length–age relationships (R. Elliott, unpubl.). Salmon were not aged by scale analysis. Salmonids were described as: parr (ages 0 and 1) inhabiting the Pere Marquette River that had not moved to Lake Michigan, lake age fish (ages 0 and 1) that had entered Lake Michigan, or adults (ages not determined) that had returned to the river to spawn. Salmon parr collected in the Pere Marquette River system are a result of natural reproduction (Carl, 1982).

Approximately 300 salmonids were examined within 24 hr of collection. Others were killed and frozen or

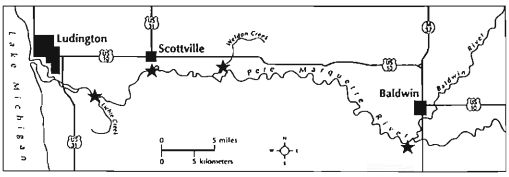

Figure 1. Map of the Pere Marquette River and sampling localities (*, sampling localities).

preserved in 15% formalin for later examination. In the necropsy of parr, the entire fish was examined. Total length (mm) and sex of most fish were recorded. The digestive tract, associated viscera, gills, and in some cases, eyes of adults were examined. Helminths and copepods were counted and processed using conventional techniques. Prevalence is the percentage of fish infected, and mean intensity is the mean number of worms per infected fish. Parasites considered to be of river origin and of lake origin are those acquired by salmonids in the Pere Marquette River system and in Lake Michigan, respectively. Voucher specimens of the following parasites have been deposited in the U.S. National Museum (USNM) Helminthological Collection: *Cyathocephalus truncatus* (USNM# 82310), *Diphyllobothrium* sp. (USNM# 82311), *Eubothrium salvelini* (USNM# 82312), *Proteocephalus* sp. (USNM# 82313), *Capillaria salvelini* (USNM# 82314), *Cystidicola farionis* (USNM# 82315), *Haplonema hamulatum* (USNM# 82316), *Spinitectus gracilis* (USNM# 82317), *Acanthocephalus dirus* (USNM# 82318), *Echinorhynchus salmonis* (USNM# 82319), *Ergasilus luciopercarum* (USNM# 82320). *Crepidostomum cooperi* was not retained by the author and therefore not deposited.

Results

A total of 219 (61%) chinook salmon were infected with 14 parasite species, regardless of age and collecting locality. The 3 protozoan species and *Ergasilus luciopercarum* Henderson, 1926 infected the gills. *Cyathocephalus truncatus* (Pallas, 1781), *Eubothrium salvelini* (Schrank, 1790), *Proteocephalus* sp., *Capillaria salvelini* (Polyansky, 1952), and *Spinitectus gracilis* Ward and Magath, 1917 were found in the pyloric ceca or anterior intestine. *Acanthocephalus dirus* (Van Cleave, 1931) and *Echinorhynchus salmonis* (Müller, 1784) occurred throughout the intestine. *Cystidicola farionis* Fischer, 1798 infected the swim bladder. *Diphyllobothrium* sp. was found encysted and unencysted on the surface of the pyloric ceca and in the stomach wall, liver, and spleen. *Haplonema hamulatum* Moulton, 1931 infected the anterior intestine. There was no significant difference in prevalence (chi-square analysis) and intensity (Student's t-test) with respect to host gender ($P > 0.05$).

Twenty-six age 0 parr (mean length = 58 mm) collected in May and August 1983 in the Pere Marquette River, downriver from the Baldwin River junction, were negative for parasites. One *Spinitectus gracilis* infected 1 of 7 age 1 parr (mean length = 133 mm) from this locality in August 1983. Parasites were not found in 27 age 0 parr (mean length = 91 mm) collected in August 1983 from Weldon Creek. Four parasite species infected age 0 parr collected downriver of Lichte Creek (Table 1). *Acanthocephalus dirus* had the highest prevalence, and *S. gracilis* had the highest mean intensity. Age 1 parr from this locality had a higher prevalence and mean intensity of *A. dirus* than did age 0 parr. Percentages of chinook salmon parr infected by age were: age 0 (37%), age 1 (50%).

Ten parasite species infected lake age 0 chinook salmon from Lake Michigan. *Acanthocephalus dirus* had a lower prevalence and mean intensity in these fish in July and August 1989 compared to parr in the river. One age 0 fish from Lake Michigan in September 1990 harbored *A. dirus*. It was not found in lake age 1 fish in September and October 1990 nor in adults that returned to the river to spawn. *Echinorhynchus salmonis* first appeared in age 0 fish from Lake Michigan in July 1989. Prevalence and mean intensity increased in older, larger lake age 0 fish collected in September and October 1990 and in lake age 1 fish from June through August 1990. Eight parasite species infected age 1 fish from Lake Michigan and 7 species infected mature adults. Cestodes were found only in age 0 fish in October and November 1990, in age 1 fish from the lake, and in adults. Percentages of chinook salmon infected in Lake Michigan by age were: age 0 (64%), age 1 (69%). All adults from the river were infected. A total of 13 parasite species infected chinook salmon in Lake Michigan and adults on their spawning run in the river. However, only 4 species infected parr in the river. Parasites infecting salmon in the lake and adults returning to spawn were not found in parr in the river and parasites infecting parr from the river were not found in salmon in the lake except for *A. dirus* in one fish.

Steelhead and coho salmon were examined for parasites at different times and localities from the Pere Marquette River system (Table 2). Ten parasite species (1 Monogenea, 1 Digenea, 3 Cestoda, 1 Nematoda, 2 Acanthocephala, 2 Protozoa) infected steelhead (Table 3). Fourteen (11%)

Table 1. Prevalence (P) and mean intensity (MI) of parasites in *Oncorhynchus tshawytscha* of various ages from different localities in the Pere Marquette River and Lake Michigan.

Locality (date collected):	PMR*, downriver of Lichte Creek (5/90, 6/90)		PMR, downriver of Lichte Creek (5/90)		LM†, near mouth of PMR (7/89, 8/89)		LM, near mouth of PMR (9/90, 10/90)		LM, near mouth of PMR (6/90, 7/90, 8/90)		PMR, south of Scottville (9/83)	
Number examined (fish age mean length in mm):	89 (0 parr, 84)		11 (1 parr, 129)		76 (0, 154)		34 (0, 275)		10 (1, 332)		80 (adult, length not measured)	
Parasite	P	MI ± 1 SD (range)	P	MI ± 1 SD (range)	P	MI ± 1 SD (range)	P	MI ± 1 SD (range)	P	MI ± 1 SD (range)	P	MI ± 1 SD (range)
Protozoa												
Epistylis sp.	1	–	–	–	–	–	–	–	–	–	–	–
Trichodina sp.	6	–	18	–	1	–	–	–	–	–	–	–
Trichophyra sp.	–	–	–	–	3	–	12	–	20	–	–	–
Cestoda												
Cyathocephalus truncatus	–	–	–	–	–	–	6	1	20	2.0 ± 1.4 (1–3)	5	1
Diphyllobothrium sp.‡	–	–	–	–	–	–	9	1	10	1	70	4.7 ± 5.9 (1–31)
Eubothrium salvelini	–	–	–	–	–	–	–	–	10	3	11	3.2 ± 2.4 (1–9)
Proteocephalus sp.‡	–	–	–	–	–	–	3	1	10	1	15	1.9 ± 1.4 (1–5)
Nematoda												
Capillaria salvelini	–	–	–	–	–	–	–	–	–	–	23	6.4 ± 10.2 (1–42)
Cystidicola farionis	–	–	–	–	3	2	–	–	20	2.5 ± 2.1 (1–4)	–	–
Haplonema hamulatum‡	–	–	–	–	–	–	–	–	–	–	3	1
Spinitectus gracilis	6	4.0 ± 4.8 (1–12)	18	1.5 ± 0.7 (1–2)	4	1.3 ± 0.6 (1–2)	–	–	–	–	–	–
Acanthocephala												
Acanthocephalus dirus	51	2.7 ± 3.1 (1–14)	55	4.5 ± 2.5 (2–8)	9	2.6 ± 2.5 (1–8)	3	11	–	–	–	–
Echinorhynchus salmonis	–	–	–	–	46	10.1 ± 20.2 (1–112)	100	23.5 ± 40.4 (1–241)	100	216.1 ± 123.2 (25–429)	100	302.5 ± 463.8 (4–2,643)
Copepoda												
Ergasilus luciopercarum	–	–	–	–	1	1	4	1	10	1	–	–

* PMR, Pere Marquette River.

† LM, Lake Michigan.

‡ Larval or immature helminth, no footnote indicates gravid helminths found.

Table 2. Numbers and mean lengths of parr and adult *Oncorhynchus mykiss* and *Oncorhynchus kisutch* examined from different localities in the Pere Marquette River system.

Species	Locality*	No. examined	Mean length (mm) ± 1 SD	Dates collected
Oncorhynchus mykiss	BRJ	44 P†	104 ± 45	8/83, 9/83
	WC	79 P	108 ± 46	8/83, 9/83
	WCJ	25 A	–	9/83
	LC	8 P	193 ± 15	6/90, 7/90
Oncorhynchus kisutch	WC	61 P	91 ± 10	8/83, 9/83
	WC	3 A	–	9/83
	S	3 A	–	9/83

* BRJ, downriver of Baldwin River Junction; WC, Weldon Creek; WCJ, downriver of Weldon Creek Junction; LC, downriver of Lichte Creek; S, Scottville.

† P, parr; A, adults.

of 131 parr and 24 (96%) of 25 adults harbored parasites. Four parasite species infected parr. Of the infected parr, 10 had external parasites only. Six helminth species, 5 of which were of lake origin, infected adults that had returned to spawn. *Echinorhynchus salmonis* had the highest prevalence and mean intensity in adult steelhead and coho salmon. Four parasite species (3 Cestoda, 1 Acanthocephala), all of lake origin, infected adult coho salmon that returned to the river to spawn. All 61 coho salmon parr from Weldon Creek were negative.

Discussion

Fourteen parasite species were found in chinook salmon, 10 in steelhead, and 4 in coho salmon from the Pere Marquette River system and Lake Michigan. Eleven (69%) of the 16 parasite species found in *Oncorhynchus* spp. are endoparasites, most of which inhabit the digestive tract. With few exceptions, most endoparasites are acquired by salmonids through feeding on infected intermediate hosts. *Echinorhynchus salmonis* and the cestodes (except for *Cyathocephalus truncatus*) infected adult chinook salmon, coho salmon, and steelhead. Adult chinook salmon and steelhead shared *Cystidicola farionis.*

Four parasite species infected chinook salmon parr, and 4 species (3 of which occurred on the gills) infected steelhead parr from the river. None of 61 coho salmon parr was infected. Based on numbers, stomach content analyses indicated that parr of *Oncorhynchus* spp. from Weldon Creek and the downriver localities were feeding primarily on terrestrial organisms (isopods, millipedes, slugs, spiders), and a few amphipods and mayfly larvae. Collins and Dechtiar (1974) and Muzzall and Peebles (1986) reported a similar lack of parasites in parr in tributaries of the Great Lakes. Parts of the Pere Marquette River have been treated with the lampricide, 3-trifluoromethyl-4-nitrophenol (TFM). Merna (1985) reported that a portion of the Baldwin River treated with TFM had reduced numbers of aquatic benthos when compared to control areas. The use of TFM in the Pere Marquette River system may have reduced the number of possible intermediate hosts or even eliminated some species.

Chinook salmon and coho salmon were successfully introduced into the Great Lakes in 1967 and 1966, respectively. The comparably low number of parasite species in chinook salmon parr and the lack of parasites in coho salmon parr in the present study may be due to the fact that these species have not been in the Pere Marquette River system long enough to establish a variety of host–parasite relationships or, because they spend such a short time in this river.

The present study demonstrates that changes that take place in the parasite fauna of chinook salmon of various ages, from various localities and from discontinuous years in the Pere Marquette River and Lake Michigan are related to fish age, diet, and movements. Parr, collected downriver from the Baldwin River and Weldon Creek, harbored only 1 *S. gracilis.* As fish became older (larger), moved into the larger stretches of the river (Lichte Creek area), and their diet became more diverse, they were infected with 4 species of parasites. In the Lichte Creek area, *A. dirus* was the most common parasite in age 0 parr. Gut content analyses indicated that parr began to feed on isopods, which are known intermediate hosts for *A. dirus* (see Amin et al., 1980; Muzzall, 1984). *Spinitectus gracilis* became more abundant in parr at this locality, and *A. dirus* was most common in age 1 parr.

Table 3. Parasties of 131 parr and 25 adult *Oncorhynchus mykiss* and 6 adult *Oncorhynchus kisutch* from different localities in the Pere Marquette River system.

Parasite	Host	Locality*	Prevalence	Mean intensity ± 1 SD (range)
Monogenea				
Gyrodactylus sp.	*O. mykiss* P†	BRJ	1	—
Digenea				
Crepidostomum cooperi	*O. mykiss* A	S	4	1
Cestoda				
Diphyllobothrium sp.‡	*O. kisutch* A	S	50	2.7 ± 1.2 (2–4)
	O. mykiss A	WCJ	4	1
Eubothrium salvelini	*O. kisutch* A	S	33	2.0 ± 1.4 (1–3)
	O. mykiss A	WCJ	48	13.8 ± 16.0 (2–44)
Proteocephalus sp.	*O. kisutch* A	S	83	29.2 ± 46.4 (5–112)
	O. mykiss A	WCJ	4	1
Nematoda				
Cystidicola farionis	*O. mykiss* A	WCJ	1	2
Acanthocephala				
Acanthocephalus dirus	*O. mykiss* P	LC	2	2.7 ± 1.5 (1–4)
Echinorhynchus salmonis	*O. kisutch* A	S	100	91.2 ± 89.1 (7–218)
	O. mykiss A	WCJ	96	28.1 ± 9.2 (2–336)
Protozoa				
Epistylis sp.	*O. mykiss* P	BRJ	3	—
Trichodina sp.	*O. mykiss* P	BRJ, WC, LC	6	—

* Locality where parasite infected fish: BRJ, downriver of Baldwin River Junction; S, Scottville; WCJ, downriver of Weldon Creek Junction; LC, downriver of Lichte Creek Junction; WC, Weldon Creek.

† P, parr; A, adult.

‡ Larval helminth.

Larger, older chinook salmon in their Lake Michigan phase acquired a richer and more varied parasite fauna than parr in the Pere Marquette River. Reasons for this include piscivory that begins in the oldest age 0 fish as well as a greater volume of food ingested, including intermediate hosts and more fish transport hosts (Hnath, 1969). Similar results have been seen in adult salmon in their marine phase along the west coast (Margolis, 1965; Olson, 1978). In age 0 chinook salmon from Lake Michigan in July and August 1989, *A. dirus* decreased in intensity, *E. salmonis* became most common, and the number of parasite species increased. Gut content analyses indicated that salmon from Lake Michigan were feeding on the amphipod *Pontoporeia affinis,* which is a known intermediate host for *E. salmonis* (see Amin, 1978). Cestodes (*C. truncatus, Diphyllobothrium* sp., *E. salvelini, Proteocephalus* sp.) and nematodes (*C. salvelini, C. farionis*) infected lake age 0 fish in September and October 1990, lake age 1 fish, and adults that had returned to the river to spawn. These cestodes and nematodes, as well as *E. salmonis* and *E. luciopercarum,* are considered to be of Lake Michigan origin. As adult chinook salmon and coho salmon move into the Pere Marquette River to spawn, their parasites accompany them and die with their hosts. A large number of *E. salmonis* and their eggs are lost when infected salmon die. The mean number of *E. salmonis* in spawning adult chinook salmon is lower than the mean number (330) in adults from Lake Michigan (Muzzall, 1989). Perhaps, the *E. salmonis* populations begin to die off as adult salmon quit feeding before the spawning run. The absence of parasites of lake origin in salmon parr indicates that necessary intermediate hosts are absent in the river.

Data from parr and adult coho salmon and steelhead also demonstrate that the parasite faunas change and become more varied as salmon become larger and move to Lake Michigan. None of 61 coho salmon parr from Weldon Creek was infected. All adult coho, however, harbored at least 1 of 4 helminth species of lake origin (*Diphyllobothrium* sp., *E. salvelini, Proteocephalus* sp., *E. salmonis*). Steelhead parr from the upriver localities were infected with external parasites (*Gyrodactylus* sp., *Epistylis* sp., *Trichodina* sp.),

whereas *A. dirus* and *Trichodina* sp. infected parr downriver of Lichte Creek. Adult steelhead were infected only with parasites of lake origin except for *C. cooperi,* which infrequently infect brown trout, *Salmo trutta,* in the river (unpubl.). Adult steelhead may not die after spawning, so their parasites may return with them to Lake Michigan.

It is beyond the scope of this paper to postulate on how useful parasites may be in the future in helping to understand the biology of the salmon in the Great Lakes and on specific effects that they may have upon the survival of their salmon hosts. However, some general comments can be made. First, none of the parasites found in chinook salmon parr in the Pere Marquette River survives throughout the life cycle of the fish. Thus, the parasites found in parr in this study do not appear to have any potential for use as biological tags (see Mackenzie, 1983 for a review of this subject). In addition, *E. salmonis,* which infected most chinook salmon in Lake Michigan and all those returning to spawn, with heavy intensity in some, is known to be detrimental to its host when it is abundant, as reported by Bauer (1953 *in* Petrochenko, 1956), Petrushevski and Kogteva (1954 *in* Collins and Dechtiar, 1974), Petrochenko (1956), Bauer (1961), and Petrushevski and Shulman (1961). Thus, *E. salmonis* may be a health concern to salmon in Lake Michigan and returning adults.

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Annotated List of Metazoan Parasites Reported from the Blue Whale, *Balaenoptera musculus*

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ABSTRACT: A list of all metazoan parasites reported from blue whales, *Balaenoptera musculus* (L.), indicates that of 18 endohelminth species and 8 ectoparasites or epizoa reported worldwide only 2 (*Tetrabothrius schaeferi* Markowski, 1955 and *Crassicauda tortilis* Skrjabin, 1959) are restricted to blue whales. All other reported parasites have been found in other species of mysticetes, odontocetes, or pinnipeds. The acquisition of parasites by blue whales, which have a moderate diversity of parasites, is probably restricted by their highly specialized euphausid diet. Two parasites of blue whales, *Anisakis simplex* (Rudolphi, 1809) and *Diplogonoporus balaenopterae* (Lonnberg, 1892), are known zoonotic pathogens.

KEY WORDS: parasites, blue whale, *Balaenoptera musculus,* zoonoses, epizootiology.

Whales harbor a variety of parasites, some of which have zoonotic potential. An understanding of the epizootiology and population dynamics of parasites of whales is essential to understand their effects on fish, fisheries, and whale populations themselves. Early parasitological studies of whales, mostly mysticetes taken during commercial whaling, were conducted primarily in Antarctica and the North Pacific Ocean and consisted of descriptions of new species, reports of new host and geographic distributions, and some observations of lesions. The blue whale, *Balaenoptera musculus* (Linnaeus, 1758), was hunted from 1883 to 1966 and is currently officially protected by international agreement (Small, 1971).

With increasing interest in marine mammals by scientists and environmental groups it was considered worthwhile to assemble the literature on parasites of blue whales. Parasite–host lists for marine mammals have been published by Margolis (1954), Delyamure (1955), Margolis and Dailey (1972), Dailey and Brownell (1972), and Arvy (1982). In the present paper reports of parasites of blue whales were obtained from the primary literature where possible. Localities given are as precise as possible; however, most early whalers and some biologists did not specify exact locations of their catches.

An examination of reported metazoan parasites of blue whales reveals that they harbor members of most major parasitic phyla (Table 1). All epizoa have been reported from other whales with none being unique to blue whales (Arvy, 1977). Epizoa such as *Odontobius ceti* and *Balaenophilus unisetus* are commensals on the baleen, while *Conchoderma virgatum* has only been found attached to *Pennella balaenopterae. Pennella balaenopterae* attaches to the skin and blubber of blue whales. The lamprey, *Entosphenus tridentatus,* and remora are predatory vertebrates that do not remain permanently attached to whales. The epizoa of cetaceans have been reviewed by Arvy (1982).

Most endoparasites reported from blue whales are also found in pinnipeds or other whales (mysticetes and odontocetes). Except for *Tetrabothrius schaeferi* and *Crassicauda tortilis,* blue whales do not possess any unique helminths. The taxonomy of *Crassicauda* with many species described only from cephalic and posterior extremities is still problematic (see Lambertsen, 1985; Raga and Balbuena, 1990).

Blue whales are euphausid or "krill" specialists (Nemoto, 1959). The few reports of fish in the stomach of blue whales (Klumov, 1963; Lockyer 1981; Yochem and Leatherwood, 1985) have been considered as accidental ingestions with negligible epizootiological significance for blue whales.

The life cycle of most endoparasites of marine mammals is poorly known. Gastric nematodes such as *Anisakis simplex* are believed to use euphausids as intermediate or paratenic hosts (Smith, 1983), and this is the likely source of infection for blue whales. However, it has not been established whether fish are obligate second intermediate hosts for *A. simplex. Pseudoterranova decipiens* is a gastric parasite of seals, and blue whales may acquire larvae from infected pelagic crustaceans. Evidence, however, suggests transmission of *P. decipiens* involves benthic in-

Table 1. Reported metazoan parasites of blue whales (*Balaenoptera musculus*).

Ectoparasites/epizoa	Locality*	Reference†
Copepoda		
Balaneophilus unisetus Aurivillius, 1879	H	20, 21
Pennella balaenopterae Koren and Danielssen, 1877	HID	21, 28, 33, 37
Cirripedia		
Xenobalanus globicipitis Steenstrup, 1851	HD	20, 21, 37
Coronula reginae Darwin, 1854	D	5, 31, 37
Conchoderma auritum (Linnaeus, 1767)	ABD	9, 14–18, 25, 37
C. virgatum (Spengler)	AD	14, 37
Nematoda		
Odontobius ceti Roussel de Vauzème, 1834	DEGH	4, 10, 21, 23
Amphipoda		
Cyamus balaenopterae Barnard, 1931	AH	1, 2, 12
C. bahamondei Buzeta, 1963	H	30
Cyamus sp.	D	9, 18, 37
Pisces		
Entosphenus tridentatus (Richardson, 1836)	H	20, 21
Remora australis (Bennett, 1840)	H	20, 21,29, 35
Remora sp.	HL	11, 22
Cestoda		
Diplogonoporus balaenopterae (Lonnberg, 1892)	BEF	8, 10, 13
Priapocephalus grandis (Nybelin, 1922)	ABDEFKM	4, 6, 8, 10, 13
Tetrabothrius affinis (Lonnberg, 1891)	ABDEFM	4, 6, 8, 10, 13, 31
T. wilsoni (Leiper and Atkinson, 1914)	ABE	8, 10, 13
T. schaeferi Markowski, 1955	BEF	8, 10, 13
Tetrabothrius sp.	D	5, 8, 31
Digenea		
Ogmogaster antarcticus (Johnston, 1931)	E	6, 10
O. plicatus (Creplin, 1839)	DK	4, 6, 10, 32, 34
Nematoda		
Anisakis simplex (Rudolphi, 1809)	DEKN	4, 6, 8, 10
Anisakis sp.	G	23
Crassicauda crassicauda (Creplin, 1829)	BDEHK	4, 6, 7, 8, 10, 21, 36
C. tortilis Skrjabin, 1959	G	23
C. boopis (Baylis, 1920)	B	7, 8
Pseudoterranova decipiens (Krabbe, 1878)	E	6, 10
Acanthocephala		
Bolbosoma turbinella (Diesing, 1851)	DEGJK	4, 6, 10, 19, 26, 27
B. balaenae (Gmelin, 1790)	DEKO	4, 6, 10, 26
B. brevicolle (Malm, 1867)	ABCDEK	3, 4, 6, 8, 10
B. hamiltoni (Baylis, 1929)	BE	3, 4, 6, 8, 10
B. nipponicum Yamaguti, 1939	GH	21, 23
B. paramuschiri Skrjabin, 1959	G	23
Bolbosoma sp.	H	20

* A, Southeast Atlantic Ocean; B, South Atlantic Ocean; C, Southwest Atlantic Ocean; D, Antarctic waters; E, Arctic waters; F, Southern Ocean; G, Northwest Pacific Ocean; H, Northeast Pacific Ocean; I, Northeast Atlantic Ocean; J, Northwest Atlantic Ocean; K, North Atlantic Ocean; L, North Indian Ocean; M, North Pacific Ocean; N, Southeast Pacific Ocean; O, Tasman Sea.

† 1, Barnard (1931); 2, Barnard (1932); 3, Baylis (1929); 4, Baylis (1932); 5, Cockrill (1960); 6, Delyamure (1955); 7, Gibson (1973); 8, Gibson and Harris (1979); 9, Kakuwa et al. (1953); 10, Klumov (1963); 11, Leatherwood et al. (1984); 12, Margolis and Dailey (1972); 13, Markowski (1955); 14, Nilsson-Cantell (1930); 15, Nilsson-Cantell (1939); 16, Nishiwaki and Hayashi (1950); 17, Nishiwaki and Oye (1951); 18, Ohno and Fujino (1952); 19, Porta (1908); 20, Rice (1963); 21, Rice (1978); 22, Rice and Caldwell (1961); 23, Skrjabin (1959); 24, Yochem and Leatherwood (1985); 25, Clarke (1966); 26, Petrochenko (1958); 27, Measures (1992); 28, Turner (1905); 29, Follett and Dempster (1960); 30, Berzin and Vlasova (1982); 31, Rees (1953); 32, Skrjabin (1969); 33, Quidor (1910); 34, Jagerskiold (1891); 35, Carl and Wilby (1945); 36, Baylis (1920); 37, Mackintosh and Wheeler (1929).

vertebrates and fish (McClelland, 1990). Both of these species can infect humans (Jackson, 1975; Margolis, 1977). The life cycles of *Crassicauda* spp. that inhabit the fascia, cranial sinuses, mammary glands, and urogenital system in whales are unknown. Life cycles are probably heteroxenous, involving an arthropod, such as a crustacean, intermediate host as do most spirurids. Lambertsen (1986), however, suggested that a direct life cycle may be involved.

Acanthocephalans in the intestines of whales are not particularly host specific. A number of species of acanthocephalans have been reported in the same species of whale, but an individual whale host usually has only one species of acanthocephalan in its intestine (Petrochenko, 1956). Acanthocephalans with aquatic life cycles use arthropod intermediate hosts such as amphipods, ostracods, or other crustaceans. Shimazu (1975) reported juvenile *Bolbosoma caenoforme* in 2 species of euphausids collected in the North Pacific Ocean. *Bolbosoma* sp. can infect humans but this is considered rare (Beaver et al., 1983; Tada et al., 1983).

The life cycles of the intestinal notocotylids *Ogmogaster plicatus* and *O. antarcticus* are unknown and the latter has also been reported in pinnipeds. *Diplogonoporus balaenopterae* found in the intestines of whales presumably has a typical pseudophyllidean life cycle (crustacean first intermediate host, fish second intermediate host). *Diplogonoporus balaenopterae* has been involved in almost 100 human cases of infection in Japan. It is likely acquired by eating raw fish or squid, but plerocercoids of *D. balaenopterae* in fish or squid have not been reported (Oshima and Kliks, 1986). Copepods were shown to be first intermediate hosts (Kamo et al., 1973). The biology of the intestinal tetrabothriids *Priapocephalus grandis* and *Tetrabothrius* spp. is unknown. Crustaceans, cephalopods, and/or teleosts are suggested as intermediate and/or paratenic hosts of tetrabothriids. However, two-host cycles (blue whales acquiring larval cestodes from their crustacean prey) may occur (see Hoberg, 1987).

Blue whales are migratory and this vagility could contribute to the acquisition of a great diversity of parasites. As shown here blue whales have a moderate diversity of parasites. Their acquisition is probably restricted by the specialized feeding habits of blue whales. However, the occurrence of *D. balaenopterae* in blue whales suggests that they ingest more than the occasional accidental fish (second intermediate host?). Blue whales may ingest up to 4 tons of krill per day during the feeding season (Lockyer, 1981). Because fish such as capelin and herring also feed on swarms of krill, it is not surprising that blue whales may ingest some of these fish during feeding. This may also explain reports of *Anisakis* sp. and *Pseudoterranova* sp. if fish are obligate hosts.

Quantitative data on helminths of blue whales are not available in the literature, which is not surprising given the dimensions of the gastrointestinal tract; the intestine may be up to 150 m long! However, future studies should be directed to determinations of intensities, particularly with a view to understanding the population dynamics of zoonotic parasites such as species of *Anisakis* and *Diplogonoporus*.

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The Coccidia of the World: a Central Clearing House

We are attempting to assemble a complete collection of the World's literature on the coccidia (Family Eimeriidae) of both invertebrate and vertebrate animals on a computer data base. Descriptive data on all oocyst and life cycle stages will be entered and cross-referenced by species, host(s), locality, author, and perhaps other parameters. The data will then be compiled by host group in whatever way seems most useful (e.g., invertebrate hosts by Phylum; vertebrate hosts by Family). Once established, the data base can be added to and archived in appropriate places (e.g., the U.S. National Parasite Collection, Beltsville, MD) on a regular basis (e.g., each decade), and it can be made available to workers in the field for the cost of reproducing and mailing computer disks or hard copy. We would appreciate receiving a copy of any and all published papers in your reprint collection in which new species of coccidia are described or redescribed. Of most value are copies of old papers that have appeared in specialty journals or in non-English journals with limited circulations. Please mail reprints to: Dr. Donald W. Duszynski, at the address below. Also, please continue to send copies of new papers as they are forthcoming. Any constructive suggestions you may have in this regard are welcomed. We thank you in advance for your cooperation.

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Plagiorchis elegans: Requirements for Metacercarial Development to Infectivity, and Conditions Required for Excystment

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ABSTRACT: The temperatures and times required for the development of *Plagiorchis elegans* (Digenea: Plagiorchiidae) metacercariae from encystment to infectivity were studied in an experimental host, *Aedes aegypti* (Diptera: Culicidae). The ability of metacercariae to excyst was evaluated in vitro and was equated with infectivity to the definitive host. Development of metacercariae to infectivity followed a sigmoidal curve at temperatures between 15 and 30°C. Rates of development increased significantly with temperature. At 15°C metacercariae first excysted 72 hr postinfection (PI); at 30°C this occurred as early as 12 hr PI. Excystment reached 80% after 132 hr at 15°C and 60 hr at 30°C. A minimum of 8 hr of contact with the host was required for subsequent development to infectivity in vitro. Excystment of infective metacercariae was temperature dependent. Less than 6% of metacercariae excysted at ≤30°C, whereas ≥80% excysted at ≥37°C. Such temperature requirements may explain in part why adult *P. elegans* are principally parasites of homeothermic animals.

KEY WORDS: *Plagiorchis elegans*, Digenea, Trematoda, metacercaria, *Aedes aegypti*, excystment, in vitro.

Cercariae of *Plagiorchis elegans* (Rudolphi, 1802) actively penetrate their insect intermediate hosts and then undergo morphological and physiological changes from free-swimming cercariae to encysted, relatively inactive metacercariae (Lackie, 1975). A variety of environmental factors may affect the subsequent development of the parasite toward infectivity to the vertebrate definitive host. Metacercariae must be adapted to survive intermediate host defense mechanisms as well as the digestive system of the definitive host. In the intestinal tract of the definitive host, metacercariae must respond to 1 or many of a wide range of stimuli, excyst, and attach to the gut lining (Lackie, 1975). This requires that the parasite has reached a stage of development that allows it to receive and respond quickly to such stimuli.

The present study determined the temperature and time requirements for development of *P. elegans* metacercariae to infectivity in an experimental insect host, *Aedes aegypti* (L.) and also determined the conditions and temperature requirements for excystment of infective metacercariae in vitro.

Plagiorchis elegans

The behavior and development of the various stages of *P. elegans* have been described by Macy (1960), Blankespoor (1977), and Genov and Samnaliev (1984). Cercariae of species of the genus *Plagiorchis* are released from the molluscan first intermediate hosts and penetrate a number of aquatic insects as well as crustaceans (Williams, 1963). Cercariae attach to the host cuticle and penetrate by means of a stylet and histolytic enzymes (Bock, 1984; Taft, 1990) and then encyst as metacercariae in the hemocoel. The time required for metacercariae to reach infectivity is reported as 4–5 days for *Plagiorchis noblei* (Blankespoor, 1974) and 3 days for *P. elegans* (Genov and Samnaliev, 1984). Metacercariae excyst and transform into adults in the intestine of the definitive host.

Materials and Methods

Groups of 200 fourth instar *A. aegypti* were maintained in plastic containers containing 300 ml tap water and fed ground Tetramin® fish food ad libitum. A 16:8 light : dark regime was maintained in each temperature-regulated incubator. Infected snails, *Stagnicola elodes* (Say), were placed in the dark to induce cercarial emergence. Eight hours later approximately 1,000 cercariae were introduced to each container of mosquitoes, which were maintained at 15, 20, 25, 30, or 35°C. Twenty minutes postexposure the mosquitoes were transferred to containers of clean water to prevent subsequent infection and maintained at the respective temperatures.

At 12-hr intervals postexposure, 20 metacercariae were dissected from each group of larvae, placed in an artificial excystment medium, MBEM (0.015 M $NaHCO_3$, 0.015 M NaCl, 0.5 g/l bile salts [50:50 Na cholate : Na deoxycholate] (Sigma B-8756), pH 7.5), modified after Bock (1986), and incubated at 37°C. The number of successfully excysted metacercariae was recorded 2 hr following incubation. This was repeated 5 times at each temperature. For the purpose of this study,

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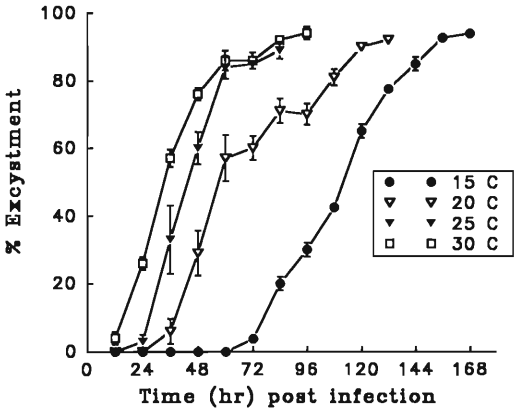

Figure 1. Excystment of *Plagiorchis elegans* metacercariae removed from the insect host at various times postinfection. Data are presented as mean % excystment ± SE.

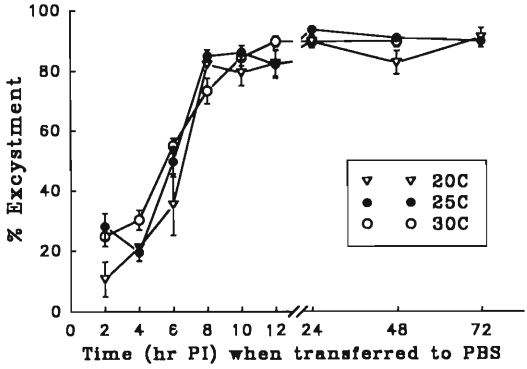

Figure 2. Excystment of *Plagiorchis elegans* metacercariae removed from the mosquito host at various times postinfection and maintained in PBS until they reached the age required for 80% excystment as determined from Figure 1. Data are presented as mean % excystment ± SE.

a successful excystment was considered to produce a mobile juvenile digenean. Those partially emerged or still bound to the cyst wall were considered nonviable.

In order to test the in vivo contribution of the host to the development of the parasite, mosquitoes were infected as above and maintained at 20, 25, and 30°C. Metacercariae were dissected from mosquitoes at 2-hr intervals postinfection for the first 12 hr, and subsequently at 12-hr intervals. Groups of 20 such metacercariae were transferred to phosphate-buffered saline (PBS) and maintained at the same temperature as the mosquito host from which they had been removed. Media were replaced daily. This procedure was repeated 5 times at each temperature. At the time when in vivo incubation at the particular temperature would consistently provide >80% excystment of metacercariae in vitro (as determined by the first experiment), metacercariae were transferred from PBS to MBEM at 37°C. All metacercariae maintained at the same temperature were excysted at the same age postinfection. The number of metacercariae successfully excysting within 2 hr following the transfer was recorded.

To test the range of temperatures that will induce excystment of metacercariae, mosquitoes were infected as above, and the metacercariae allowed 6 days at 20°C to reach infectivity. Groups of 20 infective metacercariae were removed from the hosts and were placed in MBEM at temperatures ranging from 15 to 45°C. The number of successful excystments was recorded at 15-min intervals for 2 hr. This was repeated 5 times at each temperature.

The effect of temperature alone on excystment was studied by placing groups of 20 metacercariae in PBS or RPMI medium at 37°C for 2 hr. Unexcysted metacercariae were subsequently placed in MBEM at 20°C for 2 hr. Those that still had not excysted were then incubated in MBEM at 37°C for 2 hr.

Statistical analysis was done using SYSTAT 5.0 software using the Mann–Whitney *U*-test, Tukey's multiple comparison tests, and Student's *t*-test. The level of significance (α) was set at 0.05.

Results

The effect of temperature on metacercarial development within the insect host was significant. There was an inverse relationship between temperature and time required to reach infectivity. The first successful excystment at 15°C occurred after 72 hr, compared to 36, 24, and 12 hr at 20, 25, and 30°C, respectively. Levels of excystment reached 80% after 132, 108, 60, and 60 hr at 15, 20, 25, and 30°C, respectively. The development of metacercariae at each temperature, as measured by the ability to excyst in MBEM, followed a sigmoidal curve (Fig. 1).

Metacercariae that received ≥8 hr of host contact showed no significant difference in their rate of development to infectivity compared with metacercariae maintained entirely in the larval hosts. However, regardless of temperature, a minimum of 8 hr of host–parasite contact was required to ensure development to 80% excystment levels (Fig. 2). Some metacercariae with fewer than 8 hr of host contact did excyst successfully, but levels of excystment were significantly lower (Mann–Whitney *U*-test, $P < 0.05$). Host–parasite contact in excess of 8 hr did not significantly increase levels of excystment (Mann–Whitney *U*-test, $P > 0.05$).

The excystment of infective metacercariae was temperature dependent. Less than 6% of the metacercariae excysted at temperatures ≤30°C. There was no significant difference (Tukey HSD, $P > 0.05$) between levels of excystment at temperatures ≥37°C (Fig. 3). Levels of excystment

at 35°C were intermediate and significantly different from those at ≤30 and ≥37°C (Tukey HSD, $P < 0.05$) (Fig. 3).

Elevated temperatures alone were insufficient in inducing excystment. When infective metacercariae were placed in PBS or RPMI at temperatures between 37 and 40°C, <5% of the metacercariae excysted. Such unexcysted metacercariae failed to excyst when transferred to MBEM at 20°C. However, subsequent transfer to MBEM at 37°C, resulted in >80% successful excystment within 2 hr (Table 1). Significant differences ($P < 0.05$) existed between groups containing MBEM at 37°C and all other groups (Student's t-test, $P < 0.05$).

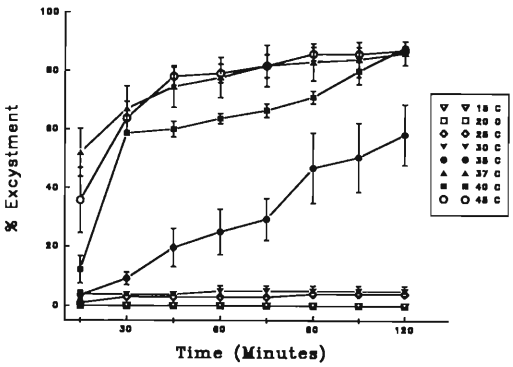

Figure 3. Excystment of infective *Plagiorchis elegans* metacercariae following 2 hr incubation in excystment medium at various temperatures. Data are presented as mean % excystment ± SE.

Discussion

Whether metacercarial development is directly affected by temperature or is mediated through the physiology of the insect host was not addressed in this study. Reduced temperatures in the presence of an adequate food supply slow the development of mosquito larvae (Christophers, 1960) and thus may also affect developing parasites. The developmental curves of metacercariae in mosquitoes were sigmoidal and parallel at the various temperatures (Fig. 1), differing only in the lag phase.

The contribution of the host per se to metacercarial development is unknown. After only 8 hr within the host, metacercariae are able to develop to infectivity in nonnutritive PBS. Although overall development of metacercariae is temperature dependent (Fig. 1), there is a temperature-independent phase of metacercarial development; the first 8 hr of host contact are required by all metacercariae independent of ambient temperature (Fig. 2). There are several explanations for this, all related to the uptake of essential nutrients from the host: (1) The metacercariae may have exhausted reserves during penetration and encystment and may require 8 hr to restore nutrient levels required for subsequent development. (2) Uptake of specific compounds may require 8 hr to be completed, or metacercariae may take up nutrients only following the completion of cyst wall formation. (3) The cyst walls produced by the parasite or the third cyst layer of host origin may impede the rapid influx of nutrients and thus impair parasite development. (4) Encapsulation and melanization of the cyst by the insect host may interfere with nutrient uptake. All of the above suggest a nutrient dependency on the host during the first 8 hr following encystment.

Some successful excystment occurs when cysts are removed from the insects earlier than 8 hr postinfection. However, excystment of these metacercariae is characteristically and significantly less than those that have received >8 hr of host contact (Fig. 2). Smyth and Halton (1983) state that metacercariae can absorb nutrients during the process of growth and differentiation. What nutrients cross the cyst wall, either actively

Table 1. Excystment of infective *Plagiorchis elegans* metacercariae exposed for 2 hr to each of a sequence of media and temperatures.

Sequence of conditions	% Excystment*	Sequence of conditions	% Excystment	Sequence of conditions	% Excystment
PBS 37°C	2.0 ± 1.2	RPMI 37°C	1.0 ± 1.0		
↓		↓			
MBEM 20°C	2.0 ± 1.2	MBEM 20°C	1.6 ± 0.3	MBEM 20°C	1.2 ± 0.2
↓		↓		↓	
MBEM 37°C	84.0 ± 2.9	MBEM 37°C	85.2 ± 2.1	MBEM 37°C	86.2 ± 4.1

* Cumulative mean % excystment ± SE.

or passively, is not known. The completion of the 2-layered cyst wall may limit the influx of some compounds and reduce uptake (Bock, 1988), and Taft (1990) suggests the third cyst layer of host origin may have a similar effect. The speed with which these layers, particularly the third, are deposited may vary with the location of the cyst and the occurrence of other host-mediated factors (Taft, 1990). When the deposition of this third layer of cyst wall is delayed or absent, metacercariae may obtain nutrients more effectively and may reach infectivity with <8 hr of host contact.

Within the range of temperatures used, metacercariae showed a distinct pattern of development, the most visible of which is an increase in the size and optical density of the excretory vesicle. The cyst walls may restrict the elimination of excretory products. Alternatively, by retaining these products, the parasite may avoid stimulating host defense mechanisms. The size and conspicuousness of this vesicle can be used as an indicator of the age and infectivity of metacercariae.

Excystment of metacercariae may be passive or active, and parasites that are ingested by their hosts as inactive stages may play an active role in their own excystment (Lackie, 1975). The metacercariae of species of *Plagiorchis* excyst intrinsically. Bock (1986, 1989) found that metacercariae of *Plagiorchis* sp. 1 became very active when exposed to an artificial excystment medium. The juveniles egested stored cecal fluid against the inner walls of the cyst and actively emerged through this area. This "explosive expulsion" of juveniles has been reported by other authors (Howell, 1970; Bass and LeFlore, 1984). The internal pressure of the metacercariae aids in rapid excystment and allows early attachment by the parasite to the intestine (Bock, 1989). Bile and bile salts (a component of MBEM) stimulate muscular activity in a number of species (Lackie, 1975). This implies that the metacercariae are mature and are ready to contribute actively to their liberation under appropriate conditions. Some metacercariae excyst to produce immobile, nonviable juveniles that differ morphologically from normal individuals. This occurs most commonly when metacercariae normally approach infectivity and is characterized by a poorly developed excretory vesicle. Although such metacercariae may not have developed fully, they nevertheless respond to the excystment stimulus. The ability to excyst may precede the ability to survive in a postexcystment environment.

In many digenean species, excystment is temperature dependent and may be inhibited by incubation at inappropriate temperatures (Dixon, 1966). Similarly, *P. elegans* metacercariae showed essentially no excystment in MBEM at temperatures <35°C. However, when transferred to MBEM at 37°C, >80% of the worms excysted within 2 hr. High temperature alone did not induce excystment. There were significant differences in the levels of excystment in those groups that included exposure to MBEM at 37°C and all other groups (Table 1). A suitable temperature and medium must be present simultaneously to ensure significant levels of excystment. Threshold temperatures for excystment exist for many digeneans, and metacercariae will not excyst in vitro unless the temperature approximates that of the definitive host (Thompson and Halton, 1982; Asanji and Williams, 1975). Temperature differences of as little as 4°C may influence excystment levels (Fried and Huffman, 1982).

Bock (1986) concluded that although pretreatment by passage through the stomach was not required for excystment, contact with gastric juices enhanced the effects of bile. Because *Plagiorchis* sp. 1 excyst at temperatures as low as 21°C, Bock (1986) chose the speed of excystment as a criterion of successful excystment. In contrast, *P. elegans* metacercariae did not excyst at temperatures <30°C.

The present study defines the temperature–time relationship for the development of metacercariae of *P. elegans* to infectivity within an insect intermediate host. We have shown a temperature-independent obligatory period of host–parasite contact followed by a temperature-dependent development period to obtain infective metacercariae. The physiological interactions between intermediate host and parasite, including nutrient uptake, remain unclear. Active excystment of infective metacercariae requires both appropriate temperatures (≥37°C) and an activating stimulus.

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Internal Parasites from the Marten (*Martes americana*) in Eastern Washington

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ABSTRACT: Helminths were recovered from 37 of 42 (88%) American martens (*Martes americana*) collected from northeastern Washington in December of 1990. *Capillaria putorii* was detected in the stomachs of 36 (86%) martens, *Mesocestoides lineatus* in the small intestines of 14 (33%), and *Trichinella spiralis* in the tongues of 2 (5%). Prevalence of *M. lineatus* was significantly greater ($P < 0.05$) in juveniles than adults, and simultaneous infections with both helminths was significantly greater ($P < 0.05$) in juveniles than adults. Histologic examination of the tongues revealed *Sarcocystis* sp. in 4 of 42 (10%) martens. *Capillaria putorii* is reported for the first time in martens in Washington. The number of helminths from martens in northeastern Washington was fewer than reported from western Washington.

KEY WORDS: Marten, *Martes americana, Capillaria putorii, Mesocestoides lineatus, Trichinella spiralis,* helminths, *Sarcocystis,* Washington.

Helminths in martens (*Martes americana*) have been reported in western Washington (Hoberg et al., 1990) and other regions of North America (Holmes, 1963; Butterworth and Beverley-Burton, 1980, 1981; Poole et al., 1983; Scranton, 1986). In these previous reports from different regions of North America, helminth species diversity, prevalence, and intensity have varied greatly. We report the recovery of 3 species of helminths during necropsy examinations of 42 martens from northeastern Washington, including the first report of *Capillaria putorii* in martens in Washington, and compare the helminth recovery in this study with a recent study conducted in western Washington.

Materials and Methods

Forty-two marten carcasses (22 females, 20 males, 19 juveniles, 16 adults, 7 age undetermined) were obtained by a trapper from Pend Oreille County (48°7′30″ to 49°00′N, 117°2′ to 117°26′W) in northeastern Washington. The martens were trapped in December 1990, skinned, and the carcasses frozen before transport to Washington State University for examination. Carcasses were thawed at room temperature (21°C) prior to necropsy. At necropsy each animal was weighed and the sex determined. Tongues were removed and fixed in 10% buffered formalin. An approximately 1-cm^3 piece of each tongue was embedded in paraffin, sectioned at 6 μm, stained with hematoxylin and eosin, and examined microscopically at 40× for the presence of parasites. Heads were removed and placed in a solution of bleach and hot water to facilitate the removal of flesh from the skull. The skulls were examined to determine the age of the animals (juvenile vs. adult) as described by Taber (1971) and Strickland et al. (1982). Trachea, lungs, liver, kidneys, and the gastrointestinal tract were removed for examination. Lungs and livers were cut into approximately 1-cm sections and viewed for parasites using a light magnifying lens. Kidneys were cut longitudinally and examined grossly. The gastrointestinal tract was divided into 3 sections: stomach, small intestine, and large intestine. Each section was cut open, the mucosa scraped and the contents preserved in separate jars of 10% formalin and examined for parasites using a dissecting microscope at 15×. Parasites were counted and stored in 10% formalin. A representative sample of nematodes and cestodes as mounted in CMCP-9AF mounting medium (Masters Chemical Company, Inc., 520 Bonnie Lane, Elk Grove, Illinois 60007) for identification. The cestodes were stained in Semichon's acetic carmine prior to mounting. Representative specimens have been deposited in the U.S. National Museum Parasite Collection (Beltsville, Maryland 20705) as follows: *C. putorii,* No. 82299; *Mesocestoides lineatus,* No. 82300.

One-way analysis of variance tests were used to compare helminth intensity and prevalence between age classes and sexes to identify significant differences ($P < 0.05$).

A fecal sample was collected from the large intestine of 33 martens (9 animals had no feces present in the gastrointestinal tract) and examined microscopically for the presence of parasite eggs using a sugar flotation centrifugation technique. Differences in prevalence and intensity of parasites between sexes and age classes were compared.

Results and Discussion

Helminths were recovered from 37 of 42 (88%) martens. *C. putorii* (Rudolphi, 1819) and *M. lineatus* (Goeze, 1782) were the only gastrointestinal helminths recovered (Table 1). Prevalence of *M. lineatus* and simultaneous infections with both helminths were significantly greater ($P < 0.05$) in juveniles than adults. No other significant differences were observed in prevalence or

Figure 1. ***Sarcocystis*** **sp. in the tongue of a marten. Note the thin wall (large arrow) of the nonseptate sarcocyst, and the muscle cell (small arrow). Scale bar = 20 μm.**

intensity between age classes or sexes. When present, *C. putorii* was always detected in the stomach, occasionally in the small intestine (8 martens) and rarely in the large intestine (2 martens). The mean intensity of *C. putorii* in the stomach, small intestine, and large intestine was 55.4, 4.0, and 1.5 worms, respectively. *Capillaria putorii* occurs in the stomach and intestine of the marten, ferret (*Mustela putorius furo*), mink (*Mustela vison*), short-tailed weasel (*Mustela erminea*), raccoon (*Procyon lotor*), fisher (*Martes pennanti*), and striped skunk (*Mephitis mephitis*) (Levine, 1980; Butterworth and Beverley-Burton, 1981).

The cestode *M. lineatus* was found exclusively in the small intestine. *Mesocestoides* sp. has previously been reported in martens in Washington (Hoberg et al., 1990). Among the 37 martens from which helminths were recovered, 84% had only *C. putorii,* 3% had only *M. lineatus,* and 35% were simultaneously infected with both parasites. No helminths were found in the trachea, lungs, liver, or kidneys of any of the martens.

Histologic analysis of the tongues revealed *Trichinella spiralis* (Owen, 1835) larvae in 2 martens, one containing 3 larvae and the other 2 larvae. Cysts of *Sarcocystis* sp. were found in the tongues of 4 martens. Mean size of 32 sarcocysts was 63.9 × 49.9 μm (range, 24–132 × 24–79 μm), and the mean intensity was 15 (range, 2–30) sarcocysts per tongue. Cysts were nonseptate with thin walls (Fig. 1). To our knowledge, this is the first report of the occurrence of *Sarcocystis* sp. cysts (sarcocyst) in marten. The presence of sarcocysts in a carnivore host is atypical. Generally, sarcocyst formation occurs in an intermediate herbivorous host (asexual) and oocyst production (sexual) in a definitive carnivore host. Sarcocysts have occasionally been detected in carnivores. Kirkpatrick et al. (1986), Everitt et

Table 1. Prevalence and intensity of helminths of martens from Pend Oreille County, Washington, U.S.A.

Helminth	All hosts ($N = 42$)	Males ($N = 20$)	Females ($N = 22$)	Juveniles ($N = 19$)	Adults ($N = 16$)
Capillaria putorii	86 (2–477) 56*	85 (4–89) 40	86 (2–477) 72	84 (2–87) 34	94 (9–477) 73
Mesocestoides lineatus	33 (1–43) 10	40 (1–43) 11	27 (1–19) 10	58 (1–43) 12	19 (1–10) 5
C. putorii and *M. lineatus*	31 (NA)† NA	35 (NA) NA	27 (NA) NA	53 (NA) NA	19 (NA) NA
C. putorii or *M. lineatus*	88 (NA) NA	90 (NA) NA	86 (NA) NA	89 (NA) NA	94 (NA) NA
Trichinella spiralis	5 (2–3) 3	10 (2–3) 3	0 (0) 0	0 (0) 0	13 (2–3) 3

* Percent prevalence (range in intensity) mean intensity.
† Not applicable.

al. (1987), Fiori and Lowndes (1988), and Edwards et al. (1988) found *Sarcocystis* in tissues of domestic cats, and Hill et al. (1988) detected sarcocysts in the muscles of a dog in addition to 2 domestic cats. Sarcocysts have been reported in raccoons (Seneviratna et al., 1975; Kirkpatrick et al., 1987; Snyder et al., 1990) and in sylvatic felids including cougars (*Felis concolor*) and bobcats (Felis rufus) (Greiner et al., 1989; Anderson et al., 1992). It has been suggested that sarcocyst formation in carnivores is associated with an immunocompromised host, yet in their survey of the prevalence of sarcocysts in Florida bobcats, Anderson et al. (1992) found the infected animals to be healthy and in good physical condition.

Fecal samples from 33 martens were analyzed for the presence of parasite eggs. *Capillaria* sp. eggs were present in 64% of the samples and coccidian oocysts in 6%. No other parasites were detected.

The species richness of the helminth community of martens in this study was less than reported previously from Washington by Hoberg et al. (1990). Nine species of helminths were identified in that study of 78 martens from 2 regions of western Washington (southern Cascades and northern Cascades) both located approximately 165 km or more west and south of the present study. They reported 48% of the martens from the southern Cascades ($N = 64$) had multiple helminth infections with a maximum of 4 species per host, and 21% from the northern Cascades ($N = 14$) had multiple infections with a maximum of 3 species per host. Although fewer species of parasites were identified in our study, the percentage of martens with multiple infections (35%) is equal to that reported by Hoberg et al. (1990) with both study regions combined. The 88% prevalence of helminth infection we report is very similar to the approximately 85% identified by Hoberg et al. (1990) when all 78 martens are considered. Nematodes were uncommon, intensity levels low, and *C. putorii* was not detected in martens from the southern and northern Cascade range of Washington (Hoberg et al., 1990). We found relatively high numbers of *C. putorii* present in martens from northeastern Washington (Table 1), with 9 martens having more than 50 worms and 4 with more than 100. The intensity of *M. lineatus* was similar to the mean intensity of *Mesocestoides* sp. reported by Hoberg et al. (1990) for the northern Cascades (10), but lower than the southern Cascades (20). The 5% prevalence of *T. spiralis* we detected in tongues was much lower than the 31% and 50% Hoberg et al. (1990) reported in diaphragms of martens from the southern and northern Cascades, respectively. The large difference in prevalence of *T. spiralis* between the 2 studies may be due in part to the different tissues and techniques utilized to detect *T. spiralis* larvae.

The martens in our study were trapped in a relatively small geographic area, probably comprising a specific population. This may explain the lower number of helminth species recovered in this study relative to that previously reported by Hoberg et al. (1990), the lower prevalence of *T. spiralis* and the high number of martens infected with *C. putorii,* a species not identified in the previous report. This may suggest a difference in prey availability and/or prey selection among martens in different regions of the state, resulting in a greater or lesser exposure to infection of specific parasites. Two small mammals, a red-backed vole, *Clethrionomys gapperi,* and a red squirrel, *Tamiasciurus hudsonicus,* were identified in the stomach contents of 2 martens. Their role in the transmission of parasites was not investigated.

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Ultrastructure of Cuticular Exudates and Related Cuticular Changes on Juveniles in *Heterodera glycines*[1]

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ABSTRACT: Fibrillar exudates formed on the cuticle surface of parasitic *Heterodera glycines* second-stage juveniles (J2) during feeding on soybean roots. Accumulation of cuticular exudates was correlated with the fibrillar and porous nature of the epicuticle, exocuticle, and endocuticle. The apparent source of the exudates was the hypodermis, where coalesced secretory vesicles were assembled by Golgi bodies and transferred to the inner surface of the apical membrane of the hypodermis. Products of the secretory vesicles were apparently released into a secretion accumulation zone at the base of the endocuticle by some mechanism and then extruded through and onto the cuticle surface. Golgi bodies occurred in large expanded regions of the hypodermis, especially in the hypodermal cords, where prominent nuclei and other cellular components were located. During ecdysis of the J2 cuticle and early stages of third-stage juvenile (J3) cuticle formation, fine reticulate material accumulated at the secretory–excretory pore. Concurrently, moderately electron-dense material occurred in the invaginated cephalic region and in the space extending between the molted J2 cuticle and the entire J3 body.

KEY WORDS: cuticle, cuticular exudations, exudations, *Heterodera glycines*, ultrastructure.

Thick cuticular exudates on the surface of adult females of the sugarbeet cyst nematode, *Heterodera schachtii* Schmidt, 1871, were named "subcrystalline layer" by Schmidt (1871, 1872). The layer was thought to be a by-product that was produced by an outside organism such as a fungus (Brown et al., 1971). This concept has been altered because exudates are produced on the cuticle of the same species feeding on host plants grown under monoxenic culture conditions (Zunke, 1985). Recent ultrastructural studies of second- (J2) and third-stage juveniles (J3) of *H. schachtii* support the concepts that the exudate is produced by the nematode alone and that the cuticle is a relatively porous structure providing continuity between secretory granules in the hypodermis and fibrillar exudates on the cuticle surface (Endo and Wyss, 1992).

This study was initiated to elucidate formation of cuticle exudations in the soybean cyst nematode, *Heterodera glycines* Ichinohe, 1952, and compare the results to those obtained for *H. schachtii*.

Materials and Methods

Infective and parasitic stages of *H. glycines* in infected soybean (*Glycine max*) roots were prepared for electron microscopy by previously described procedures (Endo and Wergin, 1973; Wergin and Endo, 1976). Briefly, seedlings of the susceptible cultivar Lee were raised in vermiculite and inoculated with infective J2 of races 3 and 4 of the soybean cyst nematode. Nematode-infected root segments from several experiments were periodically sampled within 5 hr to 7 days after inoculation (DAI). Infective J2 and root samples were fixed in buffered 3% glutaraldehyde (0.05 M phosphate buffer, pH 6.8) at 22°C for 1.5 hr, washed for 1 hr in 6 changes of the same buffer, postfixed in 2% osmium tetroxide in the same buffer for 2 hr, dehydrated in an acetone series, and infiltrated with a low-viscosity medium (Spurr, 1969). Silver-gray sections were cut on an ultramicrotome with a diamond knife and mounted on uncoated 75×300-mesh copper grids. The sections were stained with uranyl acetate and lead citrate and viewed in a Philips 301 or 400T electron microscope operating at 60 kV with a 20-μm objective aperture.

Results

The cuticle of infective J2 of *H. glycines* consists of 3 distinct zones: an outer epicuticle, an underlying exocuticle, and a striated endocuticle in contact with or adjacent to the hypodermis (Figs. 1, 2). The epicuticle is thin with closely arranged electron-dense fibrillar striations. The exocuticle is moderately electron-dense with fine fibrillar strands that appear to traverse the epicuticle and show continuity through a flocculent intermediate zone with the striated endocuticle (Fig. 2). Hemidesmosomes are arranged circumferentially and usually in pairs under each cuticle annulus (Figs. 1, 2). The hemidesmosomes connect the base of the endocuticle to the basal la-

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

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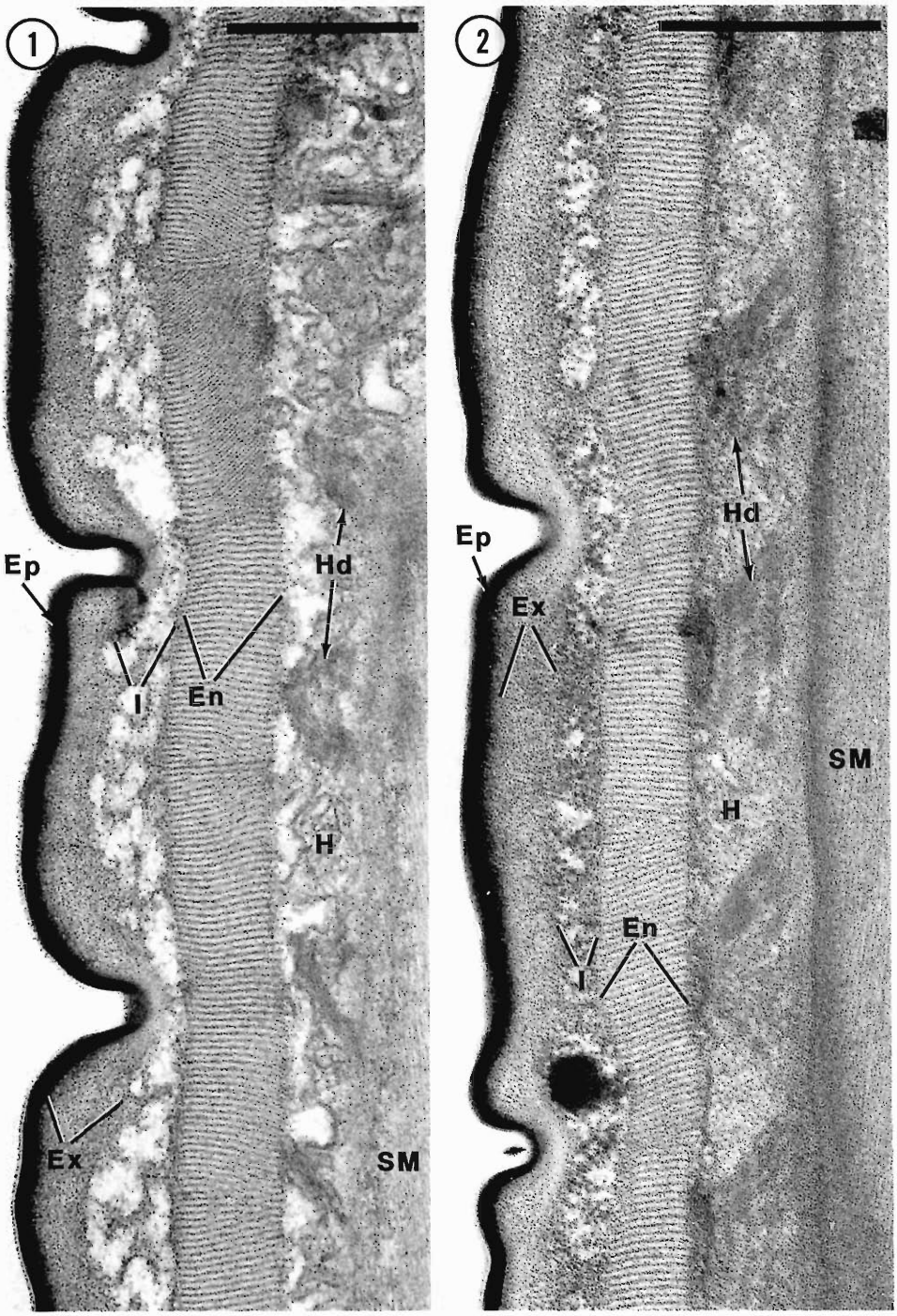
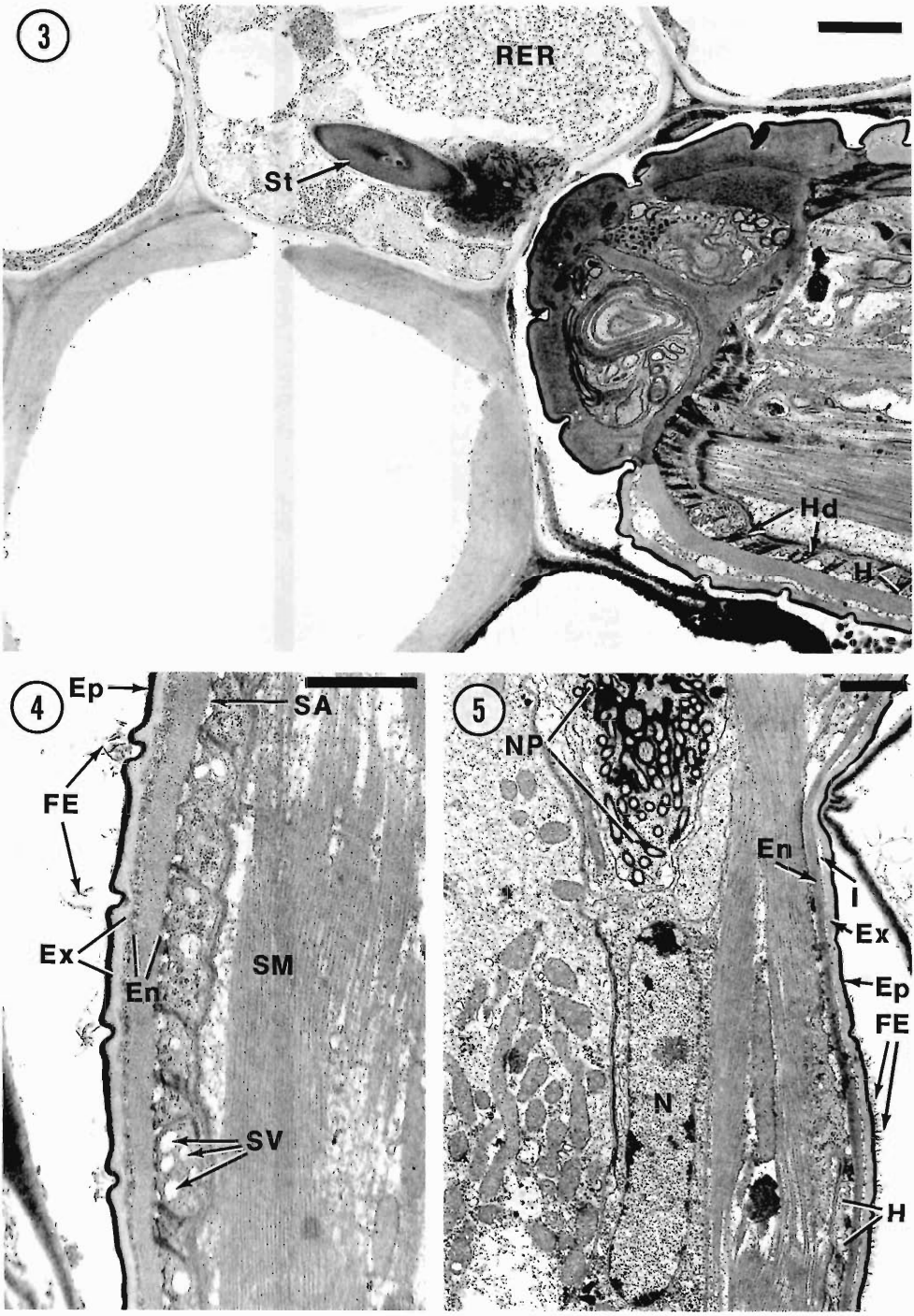


Figures 1, 2. Longitudinal sections through infective and parasitic J2 of *Heterodera glycines* 5 hr after inoculation of soybeans roots. 1. Section of infective J2 posteriad from lip region showing rounded annulations of cuticle with flocculent intermediate zone (I) between the exocuticle (Ex) and endocuticle (En). Hemidesmosomes (Hd) appear in pairs under each annulation. Ep, epicuticle; SM, somatic muscles. 2. Fibrils within exocuticle (Ex) of J2 appear similar in dimensions to striae of endocuticle (En). Hemidesmosomes (Hd) appear uniformly stretched between endocuticle and the somatic muscles (SM) near the amphidial cell region. H, hypodermis. Bars = 0.5 μm.



Figures 3–5. Cuticle exudates and related secretory vesicles in hypodermis of *Heterodera glycines* J2, 1 day after inoculation. 3. Lip region of J2 with stylet (St) extended into initial syncytial cell located adjacent to metaxylem vessel. Hemidesmosomes (Hd) stretched within expanded hypodermis (H). RER, rough endoplasmic reticulum. 4. Longitudinal section near the stylet base shows fibrillar exudations (FE) concentrated at annulations of cuticle. Electron-transparent secretory vesicles (SV) occur throughout cytoplasm of hypodermis. En, endocuticle;

mella of the somatic musculature in the intercordal regions of infective (Fig. 1) and parasitic J2 (Fig. 2). The hypodermis adjacent to the somatic muscles is narrow in these regions but increases in width and volume as it extends into the body cavity at the dorsal, ventral, and lateral cord sectors. In hemidesmosome-free areas, the base of the endocuticle is either directly contiguous with the apical membrane of the hypodermis or in contact with secretory vesicle deposition sites that are especially evident after host penetration and initiation of feeding. At 5 hr after inoculation, fibrillar continuity between the zones of the cuticle is present, but no exudations from the cuticle surface are apparent (Fig. 2).

Day 1

Initial signs of cuticle exudations are present in specimens located at feeding sites where syncytial cells have been stimulated (Fig. 3). While exudations are obvious at the annulations (Fig. 4), some specimens show similar exudations directly on the annulus surface (Fig. 5). The intermediate zone between the exocuticle and the endocuticle is electron-transparent and appears similar to the zone observed in infective J2 (Fig. 1). The expanded underlying hypodermis contains electron-transparent secretory vesicles, whose products presumably enter the secretion accumulation sites that lie between the membrane of the hypodermis and the base of the endocuticle (Fig. 4).

Day 2

Transverse or longitudinal sections through the anterior of parasitic J2 show moderate accumulations of fibrillar exudations on the cuticle surface (Figs. 6, 7). Fibrillar exudates extend through the endocuticle, exocuticle, and epicuticle (Fig. 7, inset). The intermediate zone between the exocuticle and endocuticle shows increased density but retains some of the flocculent features observed in the preparasitic J2 (Fig. 2). Fibrillar exudations are oriented perpendicular to the body surface (Figs. 6, 7), except where the body is appressed against the host tissue. Within the hypodermis, Golgi bodies assemble secretory vesicles that coalesce and appear as electron-transparent secretory granules close to the apical membrane of the hypodermis. The contents of the secretory granules may be released into a secretion accumulation zone located between the hypodermis and the endocuticle (Fig. 7).

Day 3

Fibrillar exudates of the cuticle surface of parasitic J2 at 3 DAI are relatively dense (Figs. 8, 9). Fibrillar continuity between the exudates and the various regions of the cuticle is discernible. The hypodermis associated with the cuticle at 3 DAI contains electron-transparent secretory vesicles that apparently arise from Golgi bodies (Fig. 9).

Day 4

At 4 DAI, parasitic J2 in early stages of molting show electron-dense accumulations between the J2 cuticle and the outer boundary of the J3 body (Figs. 10, 11). During molting, the dissolution of portions of the most anterior region of the J2 results in the separation of the J2 cuticle with the attached stomatal wall and stylet cone from the newly formed boundaries of the J3 (Fig. 10). The electron-dense zone under the cuticle at the anterior of the J2 merges with an accumulation of similar material found in the invaginated anterior of the J3. Thus, the former apical membrane of the J2 hypodermis becomes the precursor of the J3 cuticle (Fig. 11). During early stages of molt, the hypodermis of the J3 contains electron-dense and -transparent secretory vesicles associated with earlier exudate formation and molting (Fig. 11).

Day 6

At 6 DAI, the J2 cuticle becomes detached from the body surface of the J3 and the moderately electron-dense material separating the J2 cuticle and the J3 body (Figs. 12–14). All zones of the J2 cuticle and fibrillar exudations remain intact (Figs. 13, 14). In contrast to the coarse, moderate to electron-dense depositions in the invaginated anterior (Fig. 12) and the narrow space between the molting J2 cuticle and the J3

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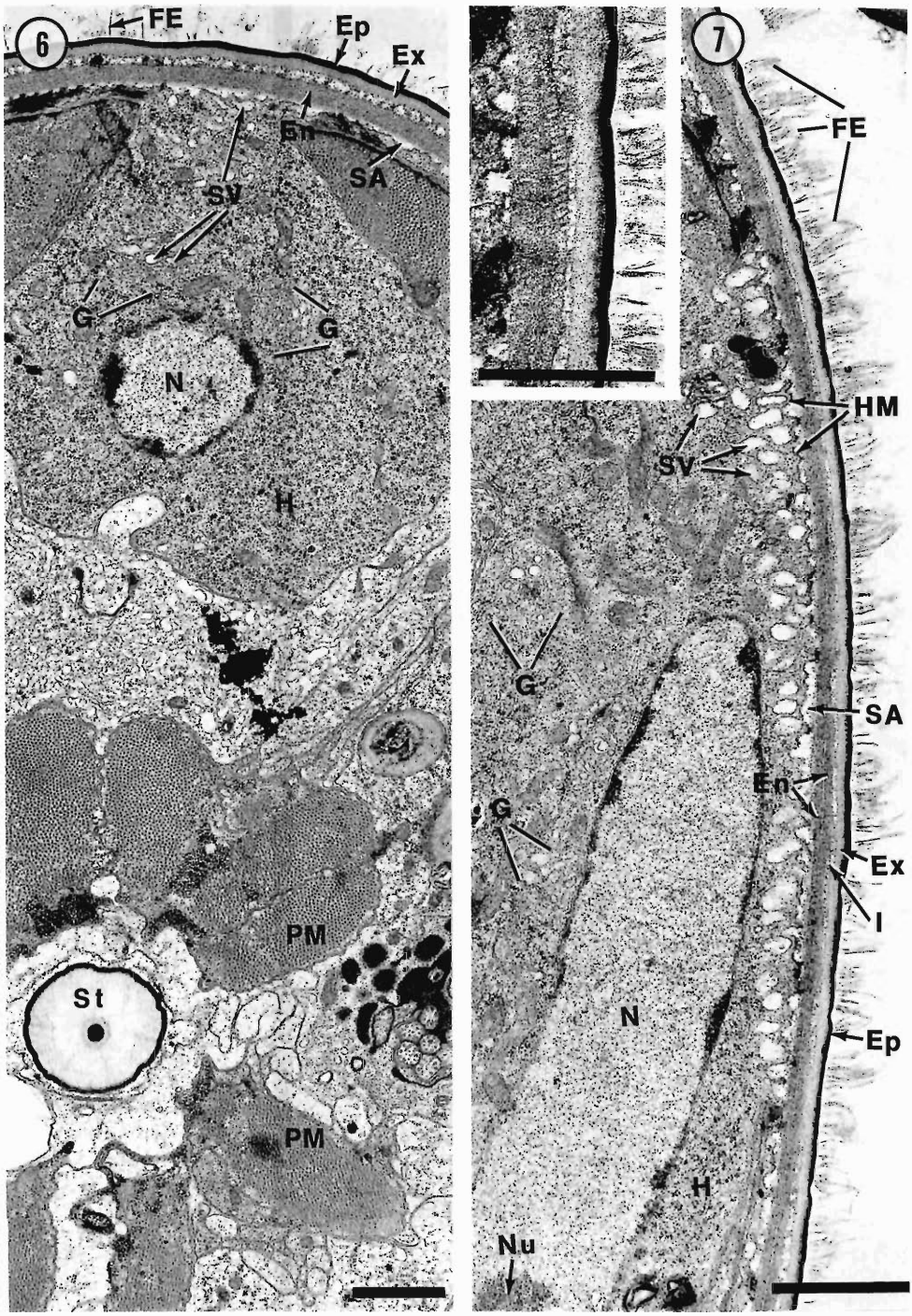
Ep, epicuticle; Ex, exocuticle; SA, secretion accumulation zone; SM, somatic musculature. 5. A third specimen shows fibrillar exudations (FE) along annules of cuticle. H, hypodermis; N, nucleus; NP, nerve process. Bars = 1.0 μm.



Figures 6, 7. ***Heterodera glycines*** **J2, 2 days after inoculation. 6. Transverse section in region of stylet shaft shows sites of Golgi (G) and secretory vesicles (SV) in hypodermis (H). Vesicles tend to accumulate near apical membrane of hypodermis, where their contents are apparently transferred to secretion accumulation zone (SA) between plasmalemma and base of endocuticle (En). Fibrillar exudates (FE) accumulate on surface of epicuticle (Ep). Ex, exocuticle; N, nucleus; PM, protractor muscles; St, stylet. 7. Fibrillar exudates (FE) present on cuticle surface near stylet region of longitudinal section of parasitic J2. Section through hypodermal chord shows**

body surface (Fig. 14), a fine reticulate material is present at the duct terminus of the secretory–excretory (S-E) gland (Fig. 13). Electron-dense depositions on the outer surface of the J3 hypodermal membrane constitute the very early stages of J3 cuticle formation. The area of low electron density between the trilaminar hypodermal membrane and the adjacent electron-dense depositions accounts for the bilayered appearance of the initial stage of the J3 cuticle (Fig. 14, inset).

In other specimens at 6 DAI, the J2 cuticle is completely separated from the J3 body. Although the various zones of the J2 cuticle are discernible, deterioration of the exocuticle and endocuticle has taken place (Figs. 15–18). Some variation in the rate of development existed among the J3 specimens observed at 6 DAI. However, most of the nematodes had completed the molting process and new stylet components were well developed. The original electron-dense material accumulated during early stages of molt at about 4 DAI has become electron-transparent and diffuse. The material is distributed throughout the space formed by the expanded deteriorating J2 cuticle and developing J3 body (Figs. 15–18). Prior to the complete separation of the J2 cuticle, the fine particulate material that accumulated near the outlet of the S-E duct at 5 DAI appears diffuse and retains sections of the S-E duct wall of the J2 apparently extruded during molting (Figs. 16, 17). At sites distant from the base of the S-E gland pore, less concentrated material was distributed between the J2 cuticle and the J3 body surfaces. These accumulations occurred at the time when the epicuticle was being formed and at later stages when all zones of the J3 cuticle were deposited, including the exocuticle, endocuticle, and basal layer that adjoins the hypodermis. At these later stages of J3 cuticle development, an S-E gland cell shows sections of the J2 duct wall lying within sections of a J3 duct wall (Fig. 16).

Day 7

Within 7 DAI, the J2 cuticle is disintegrated with only the epicuticle and parts of the stylet cone discernible (Figs. 19, 20). The same cuticle zones are present as in the J2, and an additional basal layer appears. The J3 stylet, supported by a stomatal wall, appears to be in a developmental stage preparatory to host penetration (Fig. 19). However, since feeding has not commenced, cuticular exudations are lacking. The cephalic region of the J3 has a stylet formed (Fig. 19) well beyond the initial components that usually occur in the invaginated anterior of early stages of J3 development. The cuticle of the anterior region is highly convoluted and of low electron density (Fig. 19). In the dorsal gland region of the J3, the cuticle has a relatively thick flocculent basal layer (Fig. 21).

Discussion

The occurrence of exudates on the surface of the sugarbeet cyst nematode, *H. schachtii,* while feeding on root tissues of *Raphanus sativus* var. *oleiformis* under monoxenic cultural conditions (Zunke, 1985), established that cuticle exudates are produced in the absence of an external factor such as a fungus. Such a microorganism was proposed as a symbiont for the formation of a subcrystalline layer observed on adult females of *H. schachtii* by Schmidt (1871, 1872) and on other species including *H. mani, H. avenae,* and *H. trifolii* (Brown et al., 1971). The subcrystalline layer on the cuticle surface of the latter 3 adult cyst nematodes consisted of even-numbered saturated fatty acids and their calcium salts; the layer was considered to be a metabolic product of an unidentified fungus that had fed on the secretions of the nematodes. The subcrystalline layer was proposed to function as a barrier to potential pathogens and predators (Brown et al., 1971). The cuticle of a parasitic J2 of *H. glycines* was reported to have a uniform layer of fibrillar material that was oriented perpendicular to its surface (Endo, 1987). Fibrillar exudates on the cuticle of sedentary J2 of *Globodera rostochiensis* emerged from annulations (Forrest et al., 1989).

Recent ultrastructural studies of *H. schachtii* in *Brassica* sp. roots by Endo and Wyss (1992) showed that cuticle exudates occurred within 1 day after inoculation. Three to 4 days later, ex-

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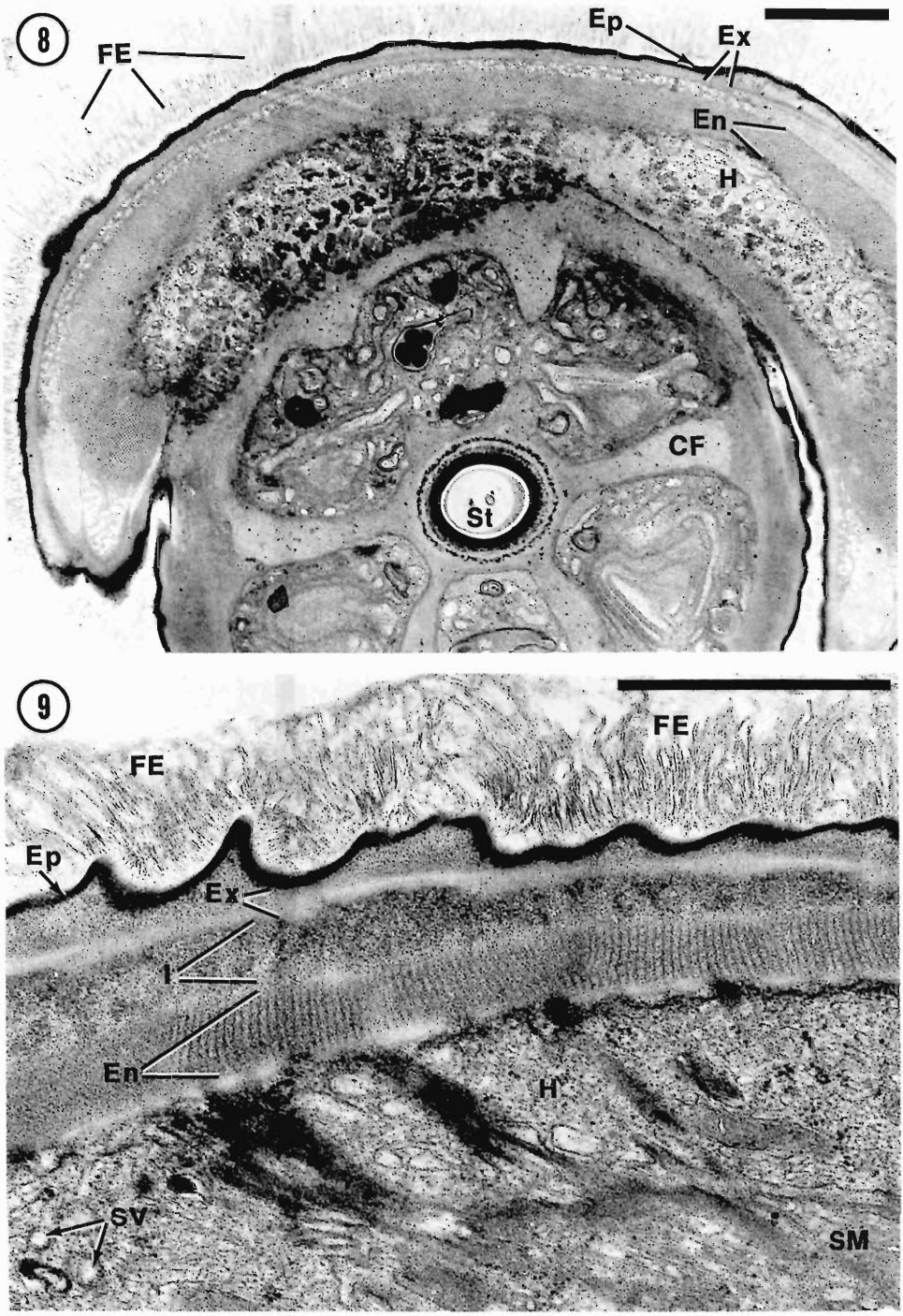
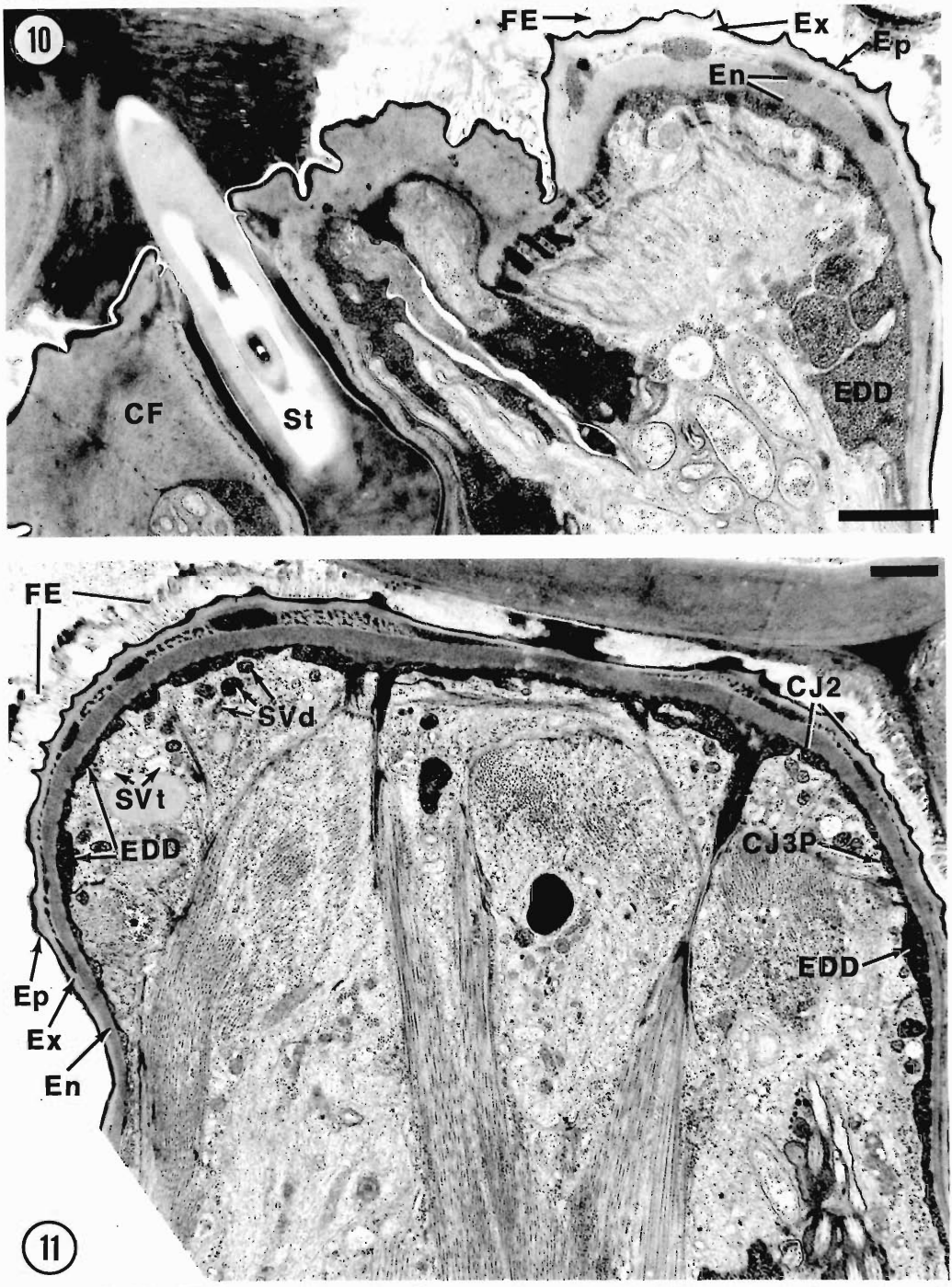
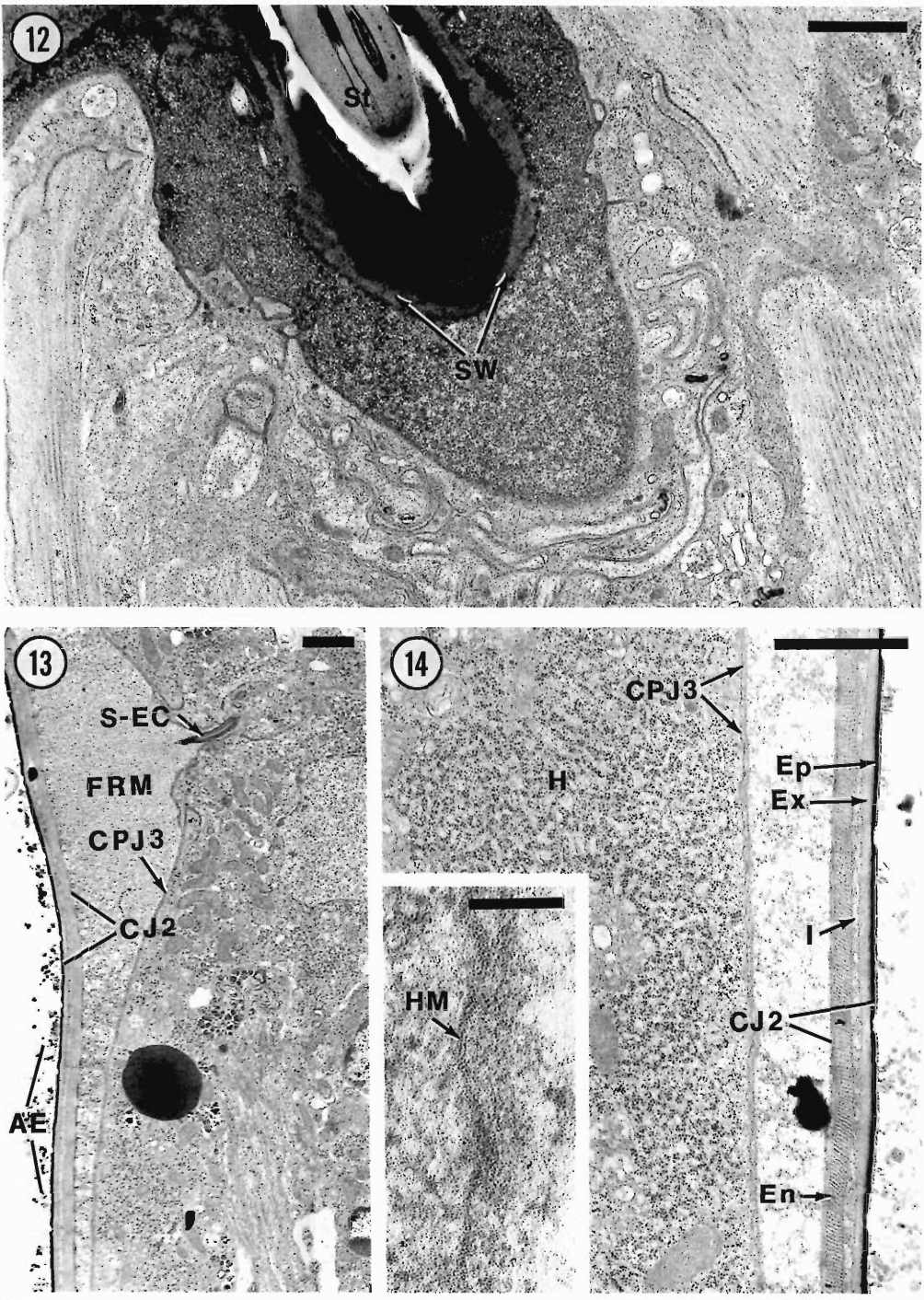
elongated nucleus (N) and extensive accumulation of secretory vesicles (SV) near Golgi (G) and apical membrane (HM) of the hypodermis (H). En, endocuticle; Ep, epicuticle; Ex, exocuticle; I, intermediate zone; Nu, nucleolus; SA, secretion accumulation zone. Bars = 1.0 μm. Enlargement shows fibrillar exudate continuity with portions of the cuticle. Bar = 0.5 μm.



Figures 8, 9. Exudates on cuticle of parasitic J2, 3 days after inoculation. 8. Cross-section immediately posteriad from cephalic region shows fibrillar exudates (FE) on cuticle surface. CF, cephalic framework; En, endocuticle; Ep, epicuticle; Ex, exocuticle; H, hypodermis; St, stylet. 9. Longitudinal section near the cephalic region of J2 shows dense accumulations of fibrillar exudates (FE) over entire cuticle. En, endocuticle; Ep, epicuticle; Ex, exocuticle; H, hypodermis; I, intermediate zone; SM, somatic musculature; SV, secretory vesicles. Bars = 1.0 μm.



Figures 10, 11. Cuticle separation and early stages of second molt of *Heterodera glycines*, 4 days after inoculation. 10. Longitudinal section shows electron-dense deposits (EDD) occurring between J3 endocuticle and developing outer boundaries of J3. Stylet cone (St) retained within the stomatal wall and cephalic framework (CF). Residual fibrillar exudates (FE) present on second-stage cuticle. En, endocuticle; Ep, epicuticle; Ex, exocuticle. 11. Tangential section below that of Figure 10 shows outlines of transparent (SVt) and electron-dense (SVd) secretory vesicles near apical membrane of hypodermis. Residual fibrillar exudate (FE) adheres to J2 cuticle. CJ2, cuticle of J2; CJ3P, cuticle of CJ3 primordium; EDD, electron-dense deposits; En, endocuticle; Ep, epicuticle; Ex, exocuticle. Bars = 1.0 μm.



Figures 12–14. Ecdysis of parasitic *Heterodera glycines* J2, 6 days after inoculation. 12. Entire anterior mid-central portion of J2 has deteriorated. Stylet cone (St) and stomatal wall (SW) are retained in contact with J2 cuticle. Tissues at base of invaginated anterior is site of J3 stylet initials (Endo, 1985). 13. Longitudinal section through secretory–excretory gland showing accumulation of fine reticulate material (FRM) concentrated at the pore. Remnants of fibrillar exudates, amorphous cuticular exudates (AE) adhere to J2 cuticle. Outer boundary

tensive accumulations were present on the annuli and, to a limited extent, near the annulations. The exudations appeared as fibrils that extended through the endocuticle, intermediate zone, exocuticle, and epicuticle. Endo and Wyss (1992) proposed that secretion vesicles, assembled at many Golgi sites in the hypodermis of *H. schachtii,* coalesced and formed large electron-translucent vesicles in the cytoplasm. These vesicles appeared to migrate toward the cuticle, where they would contact the plasmalemma and transfer their contents to an accumulation site by exocytosis or a similar mechanism.

Based on light microscopic (Zunke, 1985; Wyss and Zunke, 1986; Wyss et al., 1986; Wyss, 1992) and ultrastructural (Endo and Wyss, 1992) observations on feeding and cuticle exudations in *H. schachtii,* the cuticular fibrillar material of *H. glycines* can also be designated as cuticular exudate. Although video-enhanced contrast light micrographs of in vivo activity of *H. glycines* in host roots are not available, the exudations appear to correlate with the feeding periods of the nematode. For example, exudations occurred on the cuticle of parasitic J2 at 3 DAI, when stylet tips of these specimens were observed in syncytia containing feeding tubes (Endo, 1987, 1991). The abundance of secretory granules and the presence of numerous sites of Golgi activity in the hypodermis are indicative of active metabolism of the nematode. In an ultrastructural study of cuticle formation in *Meloidogyne javanica,* Bird and Rogers (1965) showed that at the start of molting the hypodermis becomes thickened and filled with ribosomelike granules that are probably associated with the formation of a new cuticle. Recently, it was observed that *H. glycines* grown in monoxenic culture in the absence of any other organism produced exudations 15 DAI (Endo, unpubl.).

Information is lacking on the sequence of events that influence the formation and localization of secretory vesicles in the hypodermis as they accumulate below the endocuticle and pass through other zones of the cuticle to emerge as exudates. Visualization of sites of synthesis and movement of secretory vesicles may be enhanced by utilizing labeling techniques such as fluorescence microscopy and autoradiography. In monoxenic cultures of various cruciferous plants, exudates from *H. schachtii* failed to show carbohydrate binding of fluorochrome-conjugated lectins; however, some binding may have been associated nonspecifically with fatty acids. Aumann et al. (1991) concluded that exudates may function as a protective layer for the cuticle and mask recognition by root tissues.

Involvement of the S-E gland in ecdysis was previously reported in the animal parasitic species, *Phocanema decipiens* (Davey and Kan, 1968), and in plant parasitic species (Nakasono, 1973; Bird, 1984). Observations of *Rotylenchulus reniformis* showed swelling in the region of the excretory pore of the J2 during the start of the second molt and seemed to implicate the excretory system in the early stages of molting. The excretory or S-E system (Nelson et al., 1983; Bird and Bird, 1991) was also implicated at a later stage after the final molt, when the duct wall had thickened and appeared to extrude material under the shed cuticles. The material was suggested to be remnants of the cuticular lining of the duct or associated with physiological functions related to molting (Bird, 1984). In the same species, Nakasono (1973) had observed dilations in the excretory gland at the final molt. Wright and Perry (1991) failed to observe changes or activities in the excretory system during molting of *Aphelenchoides hamatus.* Instead, just prior to ecdysis, the metacorpus was very active.

The transition from J2 to J3 of the soybean cyst nematode is accompanied by fundamental changes in the accumulation of electron-dense material in the invaginated anterior region of the developing J3 (Endo, 1985). During molt, the boundary of the electron-dense zone merges with

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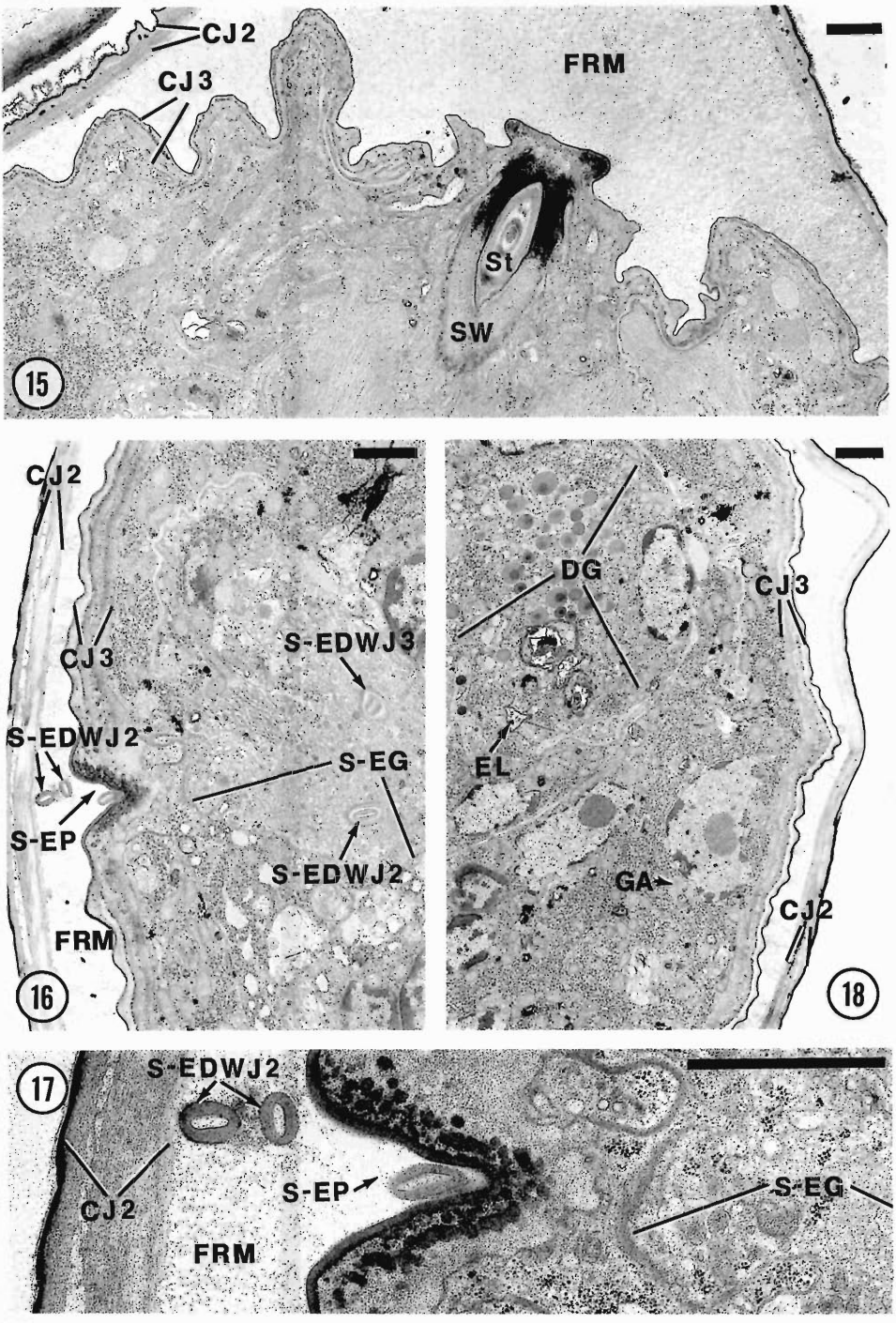
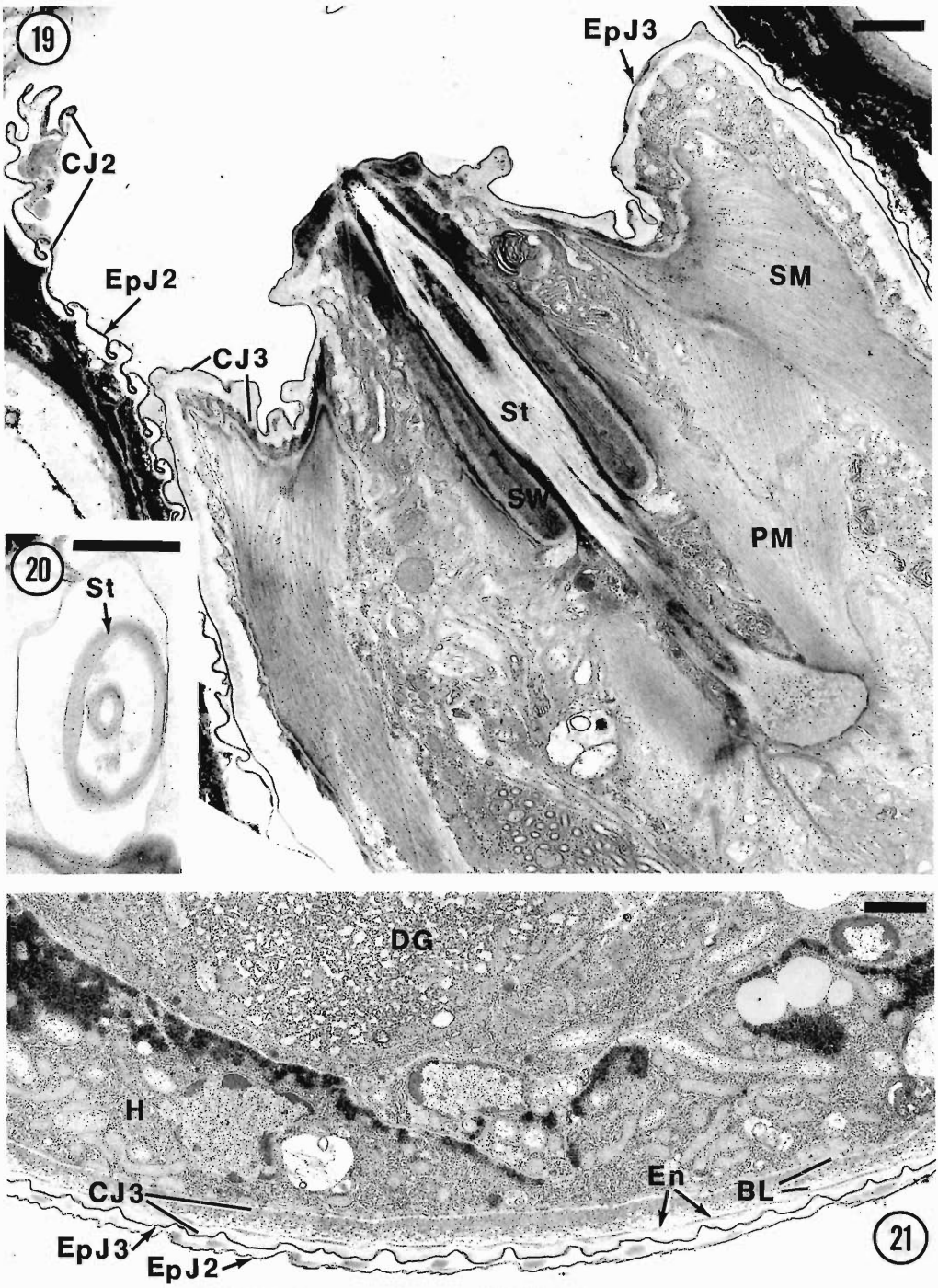
of J3 consists of trilaminar membrane from which short fibrillar projections appear to form on a primordial J3 cuticle (CPJ3). CJ2, cuticle of J2; S-EC, secretory–excretory canal. 14. Cuticle of J2 (CJ2) separated from J3 at some distance away from the secretory–excretory pore. Longitudinal section through apical membrane of hypodermis reveals a trilaminar membrane with short projections similar in appearance to striae at the base of the J2 endocuticle. Space between the J2 cuticle and the J3 body is filled with reticulate material but is dissimilar to the fine reticulate material accumulating near the secretory–excretory canal pore. CPJ3, cuticle primordium of J3; En, endocuticle; Ep, epicuticle; Ex, exocuticle; H, hypodermis; I, intermediate zone. Bars = 1.0 μm. Inset shows cuticle primordium of J3. HM, hypodermal membrane. Bar = 0.1 μm.



Figures 15–18. Longitudinal sections of J3 showing newly formed stylet, secretory–excretory pore site, and deteriorated J2 cuticle, 6 days after inoculation. 15. In contrast to earlier stages of J3 (Figs. 12–14) stylet (St) formation is well advanced and stylet is surrounded by thick stomatal wall (SW); J3 cuticle (CJ3) is multizoned. CJ2, cuticle of J2; FRM, fine reticulate material. 16. Section through secretory–excretory pore (S-EP) and gland (S-EG) shows segments of J2 secretory–excretory gland duct walls (S-EDWJ2) within the newly formed J3 duct wall (S-EDWJ3) or remnants of J2 duct wall accumulated between the J2 (CJ2) and J3 cuticles (CJ3). FRM, fine reticulate material. 17. Section of specimen in Figure 16 at higher magnification and different level shows part of secretory–excretory canal within the invaginated J3 cuticle which forms the secretory–excretory pore (S-EP). CJ2, cuticle; FRM, fine reticulate material; S-EDWJ2, secretory–excretory gland duct wall of J2; S-EG, secretory–excretory gland. 18. Sector of J3 shows well developed cuticle (CJ3) and detached convoluted *J2 cuticle (CJ2)* with signs of deterioration. DG, dorsal gland; EL, esophageal lumen; GA, Golgi apparatus. Bars = 1.0 μm.



Figures 19–21. Longitudinal and cross-sections of a J3 of *Heterodera glycines,* 7 days after inoculation. 19. Developing anterior of J3 with stylet (St) supported by stomatal wall (SW). Thick cuticle of J3 (CJ3) contrasts with deteriorated J2 cuticle (CJ2) consisting mainly of the epicuticle (EpJ2). EpJ3, epicuticle of J3; PM, protractor muscles; SM, somatic muscles. 20. Extreme anterior of J3 in Figure 19 shows nematode with residue of stylet cone (St). 21. Cross-section near dorsal gland (DG) region shows J3 cuticle (CJ3) with interrupted bands of narrow striated endocuticle (En) and thick basal layer (BL). Remaining J2 epicuticle (EpJ2) adjacent to J3 cuticle (EpJ3). H, hypodermis. Bars = 1.0 μm.

the widening zone between the J2 cuticle and the J3 body, which is bounded initially by the membranes of the hypodermal cells. Work is needed to determine the relationship between the reticulate material formed near the S-E terminus and the accumulated electron-dense material within the invaginated anterior of a developing J3. Because advanced stages of the J3 show J2 duct wall remnants within sectors of the J3 duct wall, morphology of the duct wall of the S-E canal is apparently related to the developmental stage of the J3 cuticle.

Acknowledgments

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Observations in Horses on the Effects of Ivermectin Treatment on Strongyle Egg Production and Larval Development

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ABSTRACT: Two field tests were completed on naturally infected horses ($N = 8$ treated and 2 nontreated) using a single dose of ivermectin liquid or paste formulation at the therapeutic dose rate (200 μg/kg body weight) per os to evaluate the effect of treatment on counts of strongyle eggs and larvae per gram of feces. Drug activity for each test was monitored by egg and larval counts at posttreatment intervals of 4 hr for 36 or 60 hr and thereafter at longer intervals. Fecal cultures indicated that only small strongyle larvae were present. Horses ($N = 4$), treated with the ivermectin liquid, had negative egg counts at 50 or 60 hr in the 2 tests. Larval counts were negative by 28 or 56 hr. Paste-treated horses ($N = 4$) had egg count values of 10 at 50 hr in 1 test and 0 at 72 hr in the other test. Larval counts were 0 at 32 and 48 hr in the 2 tests. Effect of ivermectin on larval development was also evidenced by presence of second-stage larvae and sluggish third-stage larvae during the posttreatment period. The egg count observations indicate that horses should be isolated for at least 3 days after treatment with ivermectin to minimize chances of contamination of pasture with small strongyle eggs. However, the isolation or quarantine period may be even shorter because development of infective larvae ceased during the 24–48-hr posttreatment interval.

KEY WORDS: ivermectin, horses, small strongyle, egg, larvae, counts.

Owners and operators of horse farms with good parasite control programs are particularly concerned about worm infections in transient horses because of potential pollution of pastures with feces containing eggs of internal parasites. Therefore, these horses are routinely isolated and treated with antiparasitic compounds upon arrival at the farm. The question arises as to how soon after treatment with ivermectin can transient horses be commingled with other animals on a good parasite control program so there is minimal chance of contamination of pastures with strongyle eggs. One study indicated that passage of strongyle eggs in feces of ponies is nil 4 days after treatment with ivermectin (DiPietro et al., 1990).

The current research was done with ivermectin to determine the earliest time interval after treatment that minimal strongyle contamination of pasture may occur and whether the liquid or paste formulation produces this effect quicker.

Materials and Methods

Test horses and time of tests

Two field tests (A and B) were completed in 10 horses ($N = 5$/test). Most of the horses had been on the farm for several years and were on the same parasite control program of almost exclusive use of ivermectin at approximately 8-wk intervals during recent years. Parasite infections were naturally acquired. Adult female mixed grade or Thoroughbred horses were used.

The horses were kept in individual box stalls during the tests and were fed mixed timothy hay ad libitum. An exception was that predominantly red clover was fed the first 12 hr in Test B. It was replaced with timothy because it apparently caused some diarrhea, particularly in the nontreated control horse. Test A was completed 18–20 January 1991 and Test B between 26 August and 18 November 1991.

Drug formulation and administration

For each of the 2 tests, 4 horses were treated once with ivermectin (2 with the liquid formulation and 2 with the paste formulation) and 1 horse was nontreated. Commercial preparations of ivermectin (Eqvalan; Merck, Rahway, New Jersey) liquid (1.0%) and paste (1.8%) were administered per os into the back of the mouth, at the therapeutic dose rate of 200 μg/kg of body weight. Individual dose rates were calculated and the drug was measured into small (6 or 12 cc) plastic syringes for administration. Before treatment, the mouth of each horse was examined to ensure no cuds of feed were present. After each treatment, the horses were observed for approximately 3 or 4 min to verify dose retention.

Parasitologic procedures

Rectal fecal samples for determinations of egg and larval counts were collected at the time of treatment. Also, they were taken every 4 hr posttreatment for 36 hr, with an additional collection at 50 hr in Test A and every 4 hr posttreatment until 60 hr, with single collections at 72 and 104 hr, and at 1, 2, 4, 6, 8, 10, and 12 wk in Test B.

The fecal samples were processed for worm egg and larval counts within approximately 1 hr of collection. A modified Stoll method was used to determine egg

Table 1. Data (means) on strongyle eggs per gram of feces in horses in 2 tests (A and B) of activity of ivermectin (200 μg/kg) liquid or paste formulations administered per os.

Time post-treatment	Strongyle eggs per gram of feces					
	Test A			Test B		
	Ivermectin			Ivermectin		
	Liquid ($N = 2$)	Paste ($N = 2$)	Nontreated ($N = 1$)	Liquid ($N = 2$)	Paste ($N = 2$)	Nontreated ($N = 1$)
Hours						
0	425	230	230	2,555	2,290	2,180
4	275	255	250	4,350	2,165	3,080
8	235	240	410	4,665	3,875	1,500
12	300	295	350	3,475	800	2,080
16	340	310	270	4,285	1,900	1,350
20	220	310	740	2,100	870	1,230
24	145	335	610	1,690	1,420	1,070
28	60	185	200	1,180	210	470
32	30	55	320	1,180	210	760
36	5	45	290	ND	185	220
40	ND	ND	ND	735	85	170
44	ND	ND	ND	430	40	220
48	ND	ND	ND	55	40	220
50/52	0	10	260	35	10	140
56	ND	ND	ND	25	0	180
60	ND	ND	ND	0	10	20
72	ND	ND	ND	0	0	70
104	ND	ND	ND	0	0	20
Weeks						
1	ND	ND	ND	0	0	230
2	ND	ND	ND	0	0	750
4	ND	ND	ND	0	0	380
6	ND	ND	ND	0	0	350
8	ND	ND	ND	5	5	560
10	ND	ND	ND	35	20	510
12	ND	ND	ND	280	35	610

ND = not determined.

counts expressed as eggs per gram of feces (epg) (Lyons et al., 1976). The larval counts, expressed as larvae per gram of feces, were derived from individual 50 g fecal cultures by a previously described method (Drudge et al., 1979).

Results

Observations on strongyle egg (Table 1) and larval (Table 2) counts (means), before and after treatment with ivermectin, are summarized for the tests. Only small strongyle larvae were found in larval counts. Small strongyle egg and larval values were relatively equal for the animals at the beginning of each test.

Liquid formulation

Strongyle egg counts (means) rapidly declined in treated horses about 24 hr after treatment. Negative counts were found at 50 hr in Test A and at 60 hr in Test B. Reappearance of positive egg counts occurred at 8 wk after treatment in Test B.

Third-stage larvae (L_3) were sluggish from 16 through 24 hr and absent by 28 hr in Test A. Second-stage larvae (L_2) were found at 4 hr and 16 through 32 hr. For Test B, negative larval (L_3) counts were found at 48 hr through 6 wk, with the exception of 52 hr. Sluggish L_3 were observed at 16–52 hr, except at 48 hr. First appearance of L_2 was at 12 hr and, except at 52 hr, were found through 56 hr. At 8 wk, larval (L_3) counts were again positive.

Paste formulation

The egg counts (means) were quickly reduced after about 24 hr, but a mean epg of 10 was recorded at 50 hr in Test A. In Test B, zero counts

Table 2. Data (means) on small strongyle larvae per gram of feces in horses in 2 tests (A and B) of activity of ivermectin (200 μg/kg) liquid or paste formulations administered per os.

	Small strongyle larvae per gram of feces*					
	Test A			Test B		
	Ivermectin			Ivermectin		
Time post-treatment	Liquid (N = 2)	Paste (N = 2)	Nontreated (N = 1)	Liquid (N = 2)	Paste (N = 2)	Nontreated (N = 1)
Hours						
0	168	142 (1)	210 (10)	920	1,590	1,060
4	283 (5)	133†	80	3,920	950	1,020
8	215	497 (1)	210	750	1,150	640
12	397	135	160 (10)	268 (46)	81† (15)	550
16	14† (15)	280	25	17† (33)	50† (5)	135
20	16† (7)	36† (2)	240	15† (11)	34† (14)	290 (25)
24	2† (2)	0 (15)	220	2† (102)	27† (33)	50 (5)
28	0 (3)	2† (1)	35	8† (10)	28† (8)	180
32	0 (1)	0 (9)	370 (10)	2† (16)	6† (4)	ND
36	0	0 (1)	240	4† (12)	18† (5)	215
40	ND	ND	ND	2† (11)	3 (5)	105
44	ND	ND	ND	1† (2)	3†	125
48	ND	ND	ND	0 (2)	0	165
50/52	0	0 (1)	240	2†	0	80
56	ND	ND	ND	0 (1)	0	70
60	ND	ND	ND	0	0	30
72	ND	ND	ND	0	0	40
104	ND	ND	ND	0	0	15
Weeks						
1	ND	ND	ND	0	0	160 (10)
2	ND	ND	ND	0	0	535
4	ND	ND	ND	0	0	370
6	ND	ND	ND	0	1	425
8	ND	ND	ND	3	4	565
10	ND	ND	ND	26	39	405
12	ND	ND	ND	203	23	560

* Values in parentheses are for second-stage larvae.
† Most were sluggish.
ND = not determined.

were observed at 56 hr and thereafter, except at 60 hr, through 6 wk. Positive egg counts returned at 8 wk.

Larval (L_3) counts (means) were negative after 28 hr in Test A. Sluggish L_3 in Test A were seen at 4, 20, and 28 hr. L_2 were found at 0, 8, 20–36, and 50 hr. In Test B, zero counts were first observed at 48 hr. L_3 were sluggish at 12 through 44 hr, except at 40 hr. L_2 were observed at 12–40 hr. Reappearance of positive larval counts was at 6 wk.

Both formulations

Egg and larval counts after treatment for the treated paired horses were similar in Test A. For Test B, 1 liquid-treated and 1 paste-treated horse had negative counts much sooner (32 and 24 hr, respectively) than the other pair (60 and 72 hr, respectively).

Nontreated control horses

The egg and larval counts were essentially uniform throughout Test A. These values in Test B began a dramatic decline by 8 hr but began rebounding by 1 wk.

Discussion

The strongyle egg and larval data indicate a general similarity in the activities of the 2 formulations, even though it had been reported (Asquith et al., 1987) that the liquid formulation of ivermectin reaches peak blood plasma concen-

trations 9–10 hr faster than the paste formulation in horses.

There was a similar pattern for the posttreatment decreases in the egg and larval counts in the present tests. Larval counts declined more rapidly than the egg counts. Also, a prominent characteristic of larval counts was the appearance of L_2 and sluggishly moving L_3 within a few hours after treatment, with a shift to a predominance of L_2. The reduced mobility of the larvae (L_3) and the increase in numbers of L_2 are attributed to the effect of ivermectin. Even though eggs and larvae persisted for several hours after treatment, infectivity of larvae soon after treatment was not ascertained and may be questionable.

In Test B, the diet of clover during the first 12 hr may have caused diarrhea, particularly in the nontreated control horse. This probably caused a dramatic decrease in egg and larval counts in this horse. A subsequent increase in these counts occurred after replacing clover with timothy hay.

It is apparent that, based on egg count data only, a 3-day isolation period of transient horses after ivermectin treatment should minimize strongyle transmission. However, larval count data indicate detrimental effect on larvae a few hours after treatment and, except for 1 of 8 horses, complete development of L_3 ceased before 48 hr posttreatment. The exceptional horse had a low larval count at 52 hr.

Until more research is completed, it would seem prudent to isolate transient horses 2–3 days after ivermectin treatment before turning them out to pasture. As previously mentioned, another study on posttreatment strongyle egg production suggested a 4-day isolation period after treatment with a paste formulation of ivermectin (DiPietro et al., 1990, and pers. comm., 1991).

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Prevalence and Intensity of *Thelandros magnavulvaris* and *Omeia papillocauda* (Nematoda) in Two Species of Desmognathine Salamanders from West Virginia

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ABSTRACT: Two species of intestinal nematodes are reported from desmognathine salamanders of the Fernow Experimental Forest, Tucker County, West Virginia. *Thelandros magnavulvaris* (Oxyuroidea: Oxyuridae) was recovered from 49.5% of 107 *Desmognathus monticola* and 14.3% of 119 *D. ochrophaeus*, respectively. *Omeia papillocauda* (Seuratoidea: Quimperiidae) was present in 22.4% of the D. monticola and 4.2% of the *D. ochrophaeus* individuals sampled. Mean intensities of 1.0 for *T. magnavulvaris* in *D. ochrophaeus* and 2.5 for *O. papillocauda* in *D. monticola* were recorded. Infected individuals of both salamander species were significantly larger than their uninfected counterparts.

KEY WORDS: *Desmognathus monticola, Desmognathus ochrophaeus*, salamanders, *Omeia papillocauda, Thelandros magnavulvaris*, Nematoda, West Virginia.

Although parasites of desmognathine salamanders have been studied by several investigators, most notably Rankin (1937), Fischthal (1955a, b), Dunbar and Moore (1979), and Baker et al. (1987), little is known about salamander parasites in West Virginia. The purpose of this study, therefore, was to report on the prevalence rates, mean intensities, and sex ratios of nematodes found in West Virginian populations of the Appalachian seal salamander, *Desmognathus monticola* Dunn, and the mountain dusky salamander, *Desmognathus ochrophaeus* Cope.

Materials and Methods

The present study was done in conjunction with an examination of the food preferences of *Desmognathus monticola*, the most aquatic species of this genus in West Virginia, and *Desmognathus ochrophaeus*, a smaller terrestrial species. These salamanders were collected from the Fernow Experimental Forest, which is managed under the auspices of the USDA Forest Service, Northeastern Forest Experiment Station at Parsons, Tucker County, West Virginia.

Totals of 107 *D. monticola* (56 males and 51 females) and 119 *D. ochrophaeus* (62 males and 57 females) were collected from May to October 1989. Salamanders were killed and fixed in 3% formalin, washed in water, and then transferred to 70% ethanol for storage. Each salamander was subsequently measured for snout–vent length and then dissected. Sex was determined for each host individual upon dissection, and then gut contents were examined for nematodes. Nematodes recovered were stored in 70% ethanol. Selected nematode individuals were later dehydrated in an ethanol series, cleared in methyl salicylate, and mounted in Permount®. Nematode species diagnoses were made using the information presented by Rankin (1937), Schad (1963), and Baker et al. (1987). Voucher specimens have been deposited in the U.S. National Parasite Collection (Beltsville, Maryland 20705): *Thelandros magnavulvaris* (82397) and *Omeia papillocauda* (82396).

Because there were no significant differences in *T. magnavulvaris* prevalences between the sexes for either host species ($\chi^2 = 0.009$, df = 1, $P > 0.05$ for *D. monticola*, and $\chi^2 = 0.035$, df = 1, $P > 0.05$ for *D. ochrophaeus*), infection data on males and females for both host species were combined. Prevalences of *O. papillocauda* were considered too low for a reliable Chi-square analysis between host sexes.

Results

Thelandros magnavulvaris (Rankin, 1937) Schad, 1960, and *Omeia papillocauda* Rankin, 1937, were recovered from the intestines of *Desmognathus monticola* and *D. ochrophaeus* collected at the Fernow Experimental Forest, West Virginia, in 1989. Prevalences and intensity of infections are shown in Table 1. While 14 *D. monticola* harbored dual infections, none of the *D. ochrophaeus* was found infected concurrently with both nematode species (Tables 2, 3).

Statistical comparisons of mean intensities between the 2 host species were not made because of the relatively small number of nematodes (only 27) recovered from *D. ochrophaeus*. In *D. monticola*, where suitable numbers of nematodes were available, there was no statistical difference in the mean intensity levels between *T. magnavulvaris* and *O. papillocauda* (Table 1).

Host length was an important indicator of infection, since infected individuals in both host species were significantly larger than their uninfected counterparts (Tables 2, 3).

All 139 *T. magnavulvaris* recovered from both host species were females. Of the 70 *O. papil-*

Table 1. Prevalence and mean intensity of *Thelandros magnavulvaris* (T) and *Omeia papillocauda* (O) in *Desmognathus monticola* and *Desmognathus ochrophaeus* from the Fernow Experimental Forest.

Desmognathus monticola				*Desmognathus ochrophaeus*			
Prevalence		Intensity ($\bar{x} \pm 1$ SD)		Prevalence		Intensity ($\bar{x} \pm 1$ SD)	
T	O	T	O	T	O	T	O
53/107*	24/107†	2.3 ± 1.4‡	2.5 ± 1.9‡	17/119*	5/119†	1.0 ± 0	2.0 ± 0.7

* $\chi^2 = 31.11$, df = 1, $P < 0.05$.
† $\chi^2 = 15.15$, df = 1, $P < 0.05$.
‡ $t = 0.515$, df = 75, $P > 0.05$.

locauda recovered from both host species, only 6 were males for a male : female sex ratio of 1:11.7.

Discussion

Prevalences of *Thelandros magnavulvaris* in *D. monticola* (49.5%) and *D. ochrophaeus* (14.3%) from West Virginia (Table 1) compared favorably to the 46.5 and 13.9% reported by Dunbar and Moore (1979) for the same 2 host species, respectively, from Tennessee. Thus, we are in agreement with Dunbar and Moore that the more aquatic salamanders (i.e., *D. monticola*) are far more likely to be infected with *T. magnavulvaris.* There is one other record of *T. magnavulvaris* from West Virginia (at Cooper's Rock, some 65 km north of the present study site) (Schad, 1963). Here, 6 of 25 (24.0%) green salamanders, *Aneides aeneus,* were infected by this oxyuroid. Rankin (1937) noted "large numbers" of these female oxyuroids from desmognathine, and other salamander species from North Carolina, but did not cite specific numbers of infected versus uninfected hosts. Conversely, *T. magnavulvaris* was not mentioned in a more recent study of desmognathine salamanders from North Carolina (Baker et al., 1987), nor was this oxyuroid found in a sample of 57 northern dusky salamanders, *Desmognathus fuscus,* from New York (Fischthal, 1955b). Eighteen of 178 *D. fuscus* from Pennsylvania were, however, infected by *T. magnavulvaris* (Fischthal, 1955a), as were 119 of 442 *D. fuscus* from Illinois (Dyer et al., 1980) and 48 of 171 red-backed salamanders, *Plethodon cinereus,* from Michigan (Muzzall, 1990). Only Dyer et al. (1980) segregated male from female hosts for the purpose of comparing prevalences between the sexes. A Chi-square value, calculated from their Table 1, showed no significant difference in the *T. magnavulvaris* prevalences between male and female *D. fuscus* (i.e., $\chi^2 = 1.47$, df = 1, $P > 0.05$), a finding compatible with our observations.

In the present study *Omeia papillocauda* was recovered from 22.4 and 4.2% of the *D. monticola* and *D. ochrophaeus* examined, respectively (Table 1). While Baker et al. (1987) cited a higher prevalence (8.4%) for this seuratoid from *D. ochrophaeus,* their recorded prevalence of 20.0% for *D. monticola* was comparable to that found at Fernow. Dunbar and Moore (1979) found no *O.*

Table 2. Snout-vent lengths (SVLs; in millimeters) for infected and uninfected *Desmoganthus monticola* individuals. Mean SVL based on sample number given in n_a. SVL not available for some individuals in host sample (n_b).

	Thelandros magnavulvaris infected	*Omeia papillocauda* infected	Dual infections	Uninfected
n_a	33	9	12	38
n_b	6	1	2	6
Mean SVL		52.9*†		44.8*

* $t = 3.75$, df = 90, $P < 0.05$.
† Calculated for entire n_a sample of 54.

Table 3. Snout-vent lengths (SVLs; in millimeters) for infected and uninfected *Desmognathus ochrophaeus* individuals. Mean SVL based on sample number *n*.

	Thelandros magnavulvaris infected	*Omeia papillocauda* infected	Dual infections	Uninfected
n	17	5	0	97
Mean SVL		35.9*†		34.2*

* $t = 2.26$, df = 117, $P < 0.05$.
† Calculated for the entire *n* sample of 22.

papillocauda (=*O. chickasawi*) in 36 *D. ochrophaeus,* but 17 of 43 (39.5%) *D. monticola* were infected, the highest verifiable prevalence recorded to date for this seuratoid.

Mean intensity of *T. magnavulvaris* infections in salamanders is characteristically low. Schad (1963) recovered 18 *T. magnavulvaris* from 6 infected green salamanders, while Fischthal (1955a) and Muzzall (1990) recorded mean intensities of 1.6 and 1.9 for their respective host samples. Thus, the mean of 2.3 for *D. monticola* in the present study (Table 1) cannot be considered unusual. No previous data are available for mean intensity of *O. papillocauda* infections. In the present study, there were no significant differences in mean intensity levels between *T. magnavulvaris* and *O. papillocauda* in *D. monticola* (Table 1).

All 139 *T. magnavulvaris* recovered from salamanders in this study were females. The recovery of only females for this nematode species is nearly universal (Rankin, 1937; Walton, 1940; Fischthal, 1955a; Dyer and Peck, 1975; Dunbar and Moore, 1979). Males are known, however, since Schad (1963) redescribed this oxyuroid species on the basis of 5 females and 5 males and Dyer et al. (1980) recovered 5 males from 442 *D. fuscus* in Illinois. Muzzall (1990) added that ". . . female *T. magnavulvaris* were much more common than males in red-backed salamanders." *Omeia papillocauda* populations in the present study were also heavily female-biased, with a female : male sex ratio of 11.7:1.0. While previous investigators have recovered males of *O. papillocauda* (Rankin, 1937; Walton, 1940; Baker et al., 1987), precise sex ratios for this species are not cited by those investigators.

Infected individuals, of both host species, are significantly larger than their uninfected counterparts (Tables 2, 3). The reasons for this are not clear. For example, it is easy to conclude, in the case of *D. monticola,* that larger host individuals with their larger intestinal tracts, provide more habitat for these relatively large nematodes. That argument, however, is weakened considerably when one considers that the smaller, uninfected *D. monticola* individuals are still considerably larger than the infected *D. ochrophaeus* hosts. This added to the fact that dual infections occurred in *D. monticola* but not in *D. ochrophaeus* demonstrates that the infection dynamics between these nematode species and their desmognathine hosts are still not clearly understood.

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Larval Parasitic Nematodes Infecting Marine Crustaceans in Eastern Canada. 1. Sable Island, Nova Scotia

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ABSTRACT: Sable Island, an emergent sand bar located near 44°N, 66°W, 290 km east of Halifax, Nova Scotia, is a useful site to investigate the life cycle of the sealworm, *Pseudoterranova decipiens*, due to the large number of seals that haul out there, and the presence of sealworm in enclosed ponds on the island. Totals of 5,000 amphipods (*Gammarus oceanicus* and *G. setosus*) and 3,448 mysids (*Neomysis americana*) collected from Wallace Lake, Sable Island, in August 1991 were digested enzymatically to compare levels of infection of sealworm between the 2 invertebrate groups. Three third-stage sealworm larvae and 3 larvae of *Paracuaria adunca* were found in mysids, whereas amphipods were uninfected. Four sealworm larvae also were found in 1,408 intertidal sand hoppers (*Americorchestia megalophthalma*) collected from Sable Island beaches in August 1990 and 1991. One nematode was recovered by digestion of 753 amphipods and the other 3 from dissections of 655 amphipods. Results of the present and other studies indicate that mysids may be more important than amphipods in the transmission of sealworm to fish hosts.

KEY WORDS: sealworm, *Pseudoterranova decipiens*, *Paracuaria adunca*, life cycle, mysid, *Neomysis americana*, amphipod, *Americorchestia megalophthalma*, Sable Island.

Life cycles of parasitic nematodes in the marine environment are generally poorly understood. The sealworm, *Pseudoterranova decipiens* (Krabbe, 1878) (Nematoda: Ascaridoidea), is of economic importance because its large reddish-brown third-stage larva in the flesh of groundfish is esthetically distasteful to consumers. In 1985, the annual cost of sealworm removal and downgrading of infected commercial fish fillets in Atlantic Canada was estimated to be $25 million (U.S.) (Malouf, 1986).

Definitive hosts of *P. decipiens* are marine mammals, especially seals, and larval stages are reported from over 60 species of groundfish in North Atlantic and adjacent waters (McClelland et al., 1990). The early part of the life cycle, however, is not fully understood. Laboratory evidence suggests that meiofaunal crustaceans, such as harpacticoid copepods, are necessary for early development of the parasite or, at least as a transfer host, while a macroinvertebrate is required for transmission to fish (McClelland, 1990). Macroinvertebrates that have proven susceptible in the laboratory include amphipods, mysids, isopods, cumaceans, mud shrimp, polychaetes, and gastropods (McClelland, 1990). Natural infections were observed in the mysids (*Mysis* and *Erythrops* spp. (Scott and Black, 1960), the polychaete *Lepidonotus squamatus* (Val'ter and Popova, 1974), and the amphipods *Caprella septentrionalis* (Val'ter, 1978), *Marinogammarus obtusatus* (Val'ter, 1987), *Gammarus lawrencianus*, and *Unciola irrorata* (McClelland, 1990).

More recently, Marcogliese (1992a) found *P. decipiens* infecting the mysids, *Neomysis americana*, but lacking in amphipods, *Gammarus oceanicus*, collected from brackish ponds on Sable Island, Nova Scotia. While nematodes were not detected in 2,364 amphipods and 1,462 mysids examined microscopically live or by dissection, 4 larval sealworms were found in 3,950 mysids subjected to pepsin digest in a Baermann apparatus. The nematodes *Paracuaria adunca* (Creplin, 1846) and *Cosmocephalus obvelatus* (Creplin, 1825) (Nematoda: Acuarioidea) were also recovered from mysids by enzymatic digestion. As amphipods were not examined by the seemingly more efficient digestion technique, it was premature to conclude that mysids were more heavily infected than amphipods in Sable Island ponds. In the present study, both amphipods and mysids from Sable Island ponds were screened for parasitic nematodes by enzymatic digestion to permit a more valid comparison of nematode infection levels in the 2 crustaceans. Intertidal sand hoppers, *Americorchestia* (=*Talorchestia*) *megalophthalma*, from the beaches of Sable Island, also were examined by dissection and digestion for nematodes.

Materials and Methods

Sampling area

Sable Island is an emergent sand bar 35 km long and 1.5 km wide located near 40°N, 66°W, 290 km east of Halifax, Nova Scotia, on the region of the continental shelf known as the Scotian Shelf. The island is the site

of the largest breeding colony of gray seals (*Halichoerus grypus*) in eastern North America and is also frequented by harbor seals (*Phoca vitulina*). Both phocids are definitive hosts for *P. decipiens* (Scott and Fisher, 1958). Harbor seals often bask on the edge of Wallace Lake, a brackish pond approximately 2 km long and 0.5 km wide located on the south beach of the island. Larval sealworms were found in three-spined sticklebacks (*Gasterosteus aculeatus*) and four-spined sticklebacks (*Apeltes quadracus*) collected from Wallace Lake and surrounding ponds (Marcogliese, 1992b), indicating that the sealworm life cycle can progress at least as far as the fish host in these ponds. Because they are inhabited by few species of invertebrates (Wright, 1989), these ponds provide a simplistic ecosystem where the life cycle of *P. decipiens* and other marine parasites can be investigated in situ.

Sampling and laboratory protocol

Totals of 5,000 amphipods (approximately 80% *G. oceanicus* and 20% *G. setosus*), 3,448 mysids (*N. americana*), and 335 isopods (*Chirodotea coeca*) were collected with dip nets from Wallace Lake (43°55.7′N, 59°58.8′W) in August 1991. A total of 1,408 sand hoppers (*Americorchestia megalophthalma*) was collected with dip nets from exposed beaches of the intertidal area on the north side of the island (43°56′N, 60°01.5′W) at low tide in August 1990 and 1991. Dip nets were used in collection of megalops larvae of the crab *Cancer irroratus* from a shallow water area formed between the beach and a sand bar on the south side of the island during low tide. Sand hoppers (N = 655), isopods (N = 88), and megalops (N = 170) were fixed in cold 5% glycerol in 70% ethanol and subsequently examined for parasites by dissection with a stereomicroscope. All other invertebrates were sorted, counted, and digested in 7 g pepsin, 4 ml concentrated HCl, and 6 g NaCl in 1,000 ml water at room temperature in a modified Baermann apparatus equipped with a 1-mm sieve. The filtrate was examined with a stereomicroscope periodically over 24 hr, and nematodes found were fixed in hot 5% glycerol in 70% ethanol. Nematodes were measured and identified using a Leitz Diaplan compound microscope equipped with a calibrated ocular micrometer.

Results

Nematodes were not detected by dissection of 170 megalops larvae of *C. irroratus*, by digestion of 5,000 *Gammarus* spp., nor by dissection (N = 88) or digestion (N = 247) of 335 isopods (*C. coeca*). Three sealworm larvae, *Pseudoterranova decipiens*, and 3 *Paracuaria adunca* larvae were recovered by digestion of 3,448 mysids (*N. americana*) from Wallace Lake. Four sealworm larvae were recovered from sand hoppers (*A. megalophthalma*), 1 sealworm by digestion of 753 amphipods in 1991, and 3 from dissection of 655 sand hoppers collected in 1990. Infection levels of sealworm in mysids vs. *Gammarus* spp. from Wallace Lake are not independent (G-test with Williams correction, $P < 0.05$), suggesting that they depend on the type of host.

Seven third-stage larvae of *P. decipiens* found in *N. americana* and *A. megalophthalma* possessed lip primordia, an apical boring tooth, an excretory pore ventral at the base of the lip primordia, a cecal ligament, and a tail mucron. Lengths or positions of characteristic structures are shown in Table 1. Measurements correspond to those of wild-caught and lab-reared *P. decipiens* (McClelland, 1990).

Two third-stage larvae of *P. adunca* from *N. americana* were 2.144 and 3.136 mm in length. Lengths of the buccal cavities were 0.080 and 0.112 mm; the muscular esophagi, 0.279 and 0.415 mm; the glandular esophagi, 0.594 and 0.978 mm; and the tails, 0.071 and 0.087 mm. Nerve rings were 0.096 and 0.135 mm, and excretory pores 0.138 and 0.183 mm from the anterior end. Measurements correspond to those of molting second-stage and third-stage larvae (Anderson and Wong, 1982).

Representative specimens of *P. decipiens* from *N. americanus* (#CMNP1992-0019) and *A. megalophthalma* (#CMNP1992-0020 and -0021), and those of *P. adunca* (CMNP1992-0022) have been deposited in the Canadian Museum of Nature (P.O. Box 3443, Station D, Ottawa, Ontario, Canada K1P 6P4).

Discussion

Larval *Pseudoterranova decipiens* were previously described from the mysid *N. americana* from Wallace Lake, Sable Island (Marcogliese, 1992a). Sealworm abundance (A = no. of nematodes recovered/no. of hosts examined) in the 1991 sample of mysids was 0.0009 (N = 3,448), similar to that recorded in a 1990 sample (A = 0.001; N = 3,950). Natural infections of larval sealworm also were found in the mysids *Mysis* sp. and *Erythrops* sp. from the Bras d'Or Lakes, Nova Scotia (Scott and Black, 1960). These results may tenuously be extrapolated to the open ocean, where mysids may play an important role in transmitting sealworm to fish. In a comparative study of diets of 3 species of flatfish, single third-stage larval sealworms were found in the cephalothorax of 2 *Mysis mixta* from the stomach of American plaice (*Hippoglossoides platessoides*) from Sable Island Bank (Martell, 1992).

The fact that nematodes were not found by digestion of 5,000 *Gammarus* spp. herein, nor by microscopic examination of 2,364 amphipods

Table 1. Characteristic dimensions (in millimeters) of *Pseudoterranova decipiens* from the mysid *Neomysis americana* collected in Wallace Lake in 1991 and from the amphipod *Americorchestia megalophthalma* sampled from Sable Island beaches in 1990 and 1991. Total length (L), lengths of the preventriculus (Pre), ventriculus (Ven), intestinal cecum (I.C.) and tail, and distance of nerve ring (N.R.) from the anterior end.

Host	Length (position) of structure					
	L	Pre	Ven	I.C.	N.R.	Tail
Neomysis americana	8.960	1.054	0.697	0.572	0.231	0.122
	7.392	0.889	0.648	0.445	0.221	0.116
	6.528	0.787	0.483	0.220	0.195	0.071
Americorchestia	4.160	0.648	0.358	—	0.189	0.067
megalophthalma	5.888	0.660	0.420	0.154	0.179	0.118
	4.480	0.681	0.415	—	0.179	0.107
	3.936	0.572	0.333	—	0.156	0.096

the previous year (Marcogliese, 1992a), indicates that amphipods of the genus *Gammarus* may not be hosts of sealworm in Wallace Lake, or that infection levels in these crustaceans were so low that they were undetectable with the sample sizes employed. However, gammaridean amphipods cannot be ruled out as intermediate hosts. Natural sealworm infections were reported in *Marinogammarus obtusatus* (Val'ter, 1987), *Gammarus lawrencianus,* and *Unciola irrorata* (McClelland, 1990). Moreover, 4 sealworms were found in 1,408 sand hoppers collected from beaches on Sable Island. These beaches are frequented by a gray seals, the most important definitive hosts of *P. decipiens* in the Northwest Atlantic (McClelland et al., 1983). The proximity of infected seals to these amphipods undoubtedly promotes contact between infective stages of the parasite with these intertidal amphipods.

The 3 smallest sealworm larvae, all from *A. megalophthalma,* did not possess an intestinal cecum, but the absence of an intestinal cecum in smaller third-stage *P. decipiens* is not unusual (McClelland and Ronald, 1974). While cecal length is not a reliable taxonomic characteristic, the ratio of the length of the ventriculus to that of the combined length of the preventriculus and ventriculus can reliably be used to distinguish *P. decipiens* from *Anisakis* sp. (McClelland and Ronald, 1974). Templeman et al. (1957) state that this ratio is 0.31–0.41 in sealworm. In nematodes from the present study, this ratio ranged from 0.35 to 0.42. Furthermore, even though the cecum appeared to be lacking at times, all nematodes possessed a cecal ligament, which is not found in *Anisakis* sp. Within *P. decipiens,* 3 sibling species have been distinguished based on genetic evidence, but only Type B has been found in Canadian waters south of Labrador (Paggi et al., 1991).

Marcogliese's (1992a) identification of third-stage *Paracuaria adunca* from the mysid *N. americana* was the first record of this nematode in a marine invertebrate. Mature *P. adunca* are cosmopolitan parasites of piscivorous birds (Wong and Anderson, 1982) and probably occur in herring gulls (*Larus argentatus*) and other fish-eating fowl common on Sable Island. Paratenic hosts on the island include three-spined and four-spined sticklebacks and the nine-spined stickleback, *Pungitius pungitius* (Marcogliese, 1992b).

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Larval Parasitic Nematodes Infecting Marine Crustaceans in Eastern Canada. 2. Passamaquoddy Bay, New Brunswick

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ABSTRACT: Four species of crustaceans were collected from Passamaquoddy Bay and vicinity and examined for parasitic nematodes using a pepsin digest in a modified Baermann apparatus. Sealworm (*Pseudoterranova decipiens*) and *Ascarophis* sp. were identified in a sample of 170 mysids (*Mysis stenolepis*) from Brandy Cove, New Brunswick. Eight *Paracuaria adunca* were found in 340 *M. stenolepis* from St. Croix Island, and 11 *P. adunca* also were found in 1,047 *Marinogammarus obtusatus* from Mohawk Island, new host records for this nematode. One specimen of *Tetrameres* sp. was also found in *M. obtusatus*.

KEY WORDS: sealworm, *Pseudoterranova decipiens, Paracuaria adunca, Ascarophis* sp., *Tetrameres* sp., mysid, *Mysis stenolepis,* amphipod, *Marinogammarus obtusatus,* Passamaquoddy Bay, Bay of Fundy.

Recent surveys show that infection levels of larval sealworm *Pseudoterranova decipiens* (Krabbe, 1878) (Nematoda: Ascaridoidea) are increasing in eastern Canada groundfish (McClelland et al., 1983a, b, 1987, 1990), and that infection levels in American plaice (*Hippoglossoides platessoides*) from the Bay of Fundy are among the highest in eastern Canada (McClelland et al., 1987). Passamaquoddy Bay, near the mouth of the Bay of Fundy, is home to large numbers of harbor seals (*Phoca vitulina*), definitive hosts of sealworm (Scott and Fisher, 1958).

As part of an ongoing study of the life cycle of *P. decipiens,* invertebrates from Passamaquoddy Bay, New Brunswick, were screened for nematode parasites, using a pepsin digest technique in a modified Baermann apparatus (Marcogliese, 1992). This bay was chosen as sampling site because of the high levels of sealworm in fish residing there, and because of the accessibility of seal haul-out areas, where heavily infected intermediate hosts might be found. The digestion technique permitted rapid screening of large numbers of invertebrates for parasitic nematodes, thus providing insight into the life cycles of various nematode species.

Materials and Methods

Invertebrates were collected with a 6-m beach seine with a 1-mm mesh or by hand near seal haul-out areas in Passamaquoddy Bay, New Brunswick, in May 1991. We seined 340 and 170 mysids (*Mysis stenolepis*) from St. Croix Island and Brandy Cove, respectively. Brandy Cove (45°05′N, 67°05′W) is situated near the mouth of the St. Croix River, which divides New Brunswick and Maine, and St. Croix Island (45°07.8′N, 67°08′W) is located 6.6 km upstream. At low tide, 1,047 amphipods (*Marinogammarus obtusatus*) were gathered from under rocks on Mohawk Island, a rocky outcropping frequented by harbor seals, and 300 amphipods (*Corophium volutator*) were picked by hand from an intertidal beach on Long Island. Mohawk Island (45°02.3′N, 66°54.3′W) is situated on the Fundy side of Letite Passage, one of the channels separating Passamaquoddy Bay from the Bay of Fundy. Long Island (45°08.8′N, 66°57.5′W) is located on the northeast side of the head of Passamaquoddy Bay in Digdequash Harbor. Sand shrimp (*Crangon septemspinosus*) ($N = 353$) were collected by beach seine on St. Croix Island.

Crustaceans were sorted by species, counted, and placed in a modified Baermann apparatus containing 7 g pepsin, 4 ml concentrated HCl, and 6 g NaCl and 1,000 ml H_2O solution and fitted with a 1-mm sieve. The filtrate was examined periodically over a 24-hr period with a stereomicroscope, and all nematodes found were fixed in hot 5% glycerol in 70% ethanol. Nematodes were measured and identified using a Leitz Diaplan compound microscope equipped with a calibrated ocular micrometer.

Results

Digestion of 170 *Mysis stenolepis* collected from Brandy Cove yielded 1 third-stage sealworm larva and 1 larval *Ascarophis* sp. (Spirurida: Habronematoidea). Eight larval *Paracuaria adunca* (Nematoda: Acuarioidea) were found in the 340 *M. stenolepis* collected on St. Croix Island. Eleven larval *P. adunca* and 1 larval *Tetrameres* sp. (Spirurida: Habronematoidea) were found in the 1,047 *M. obtusatus* collected on Mohawk Island. No nematodes were found in 300 *Corophium volutator* from Long Island, nor in 353 *Crangon septemspinosus* from St. Croix Island.

The sealworm from *M. stenolepis* possessed lip primordia, a boring tooth, an excretory pore

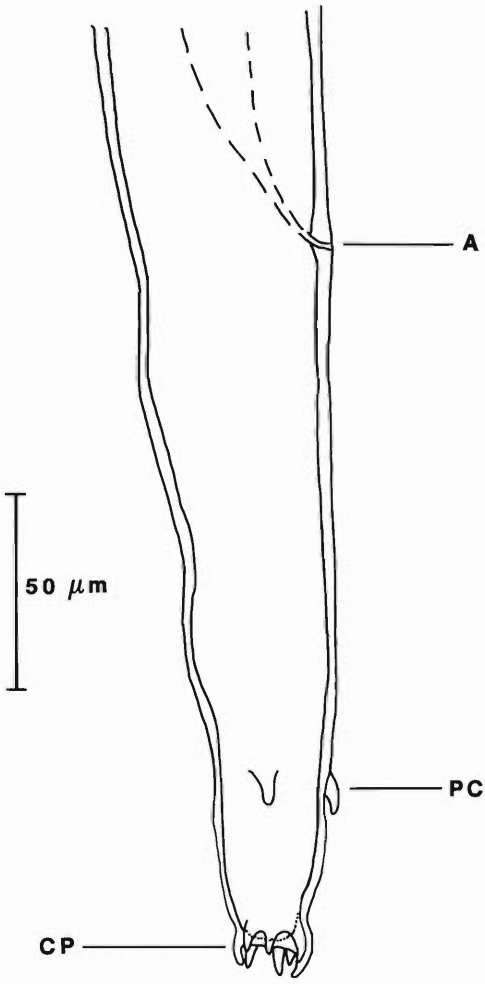

Figure 1. Diagram of posterior end of *Tetrameres* sp. found in *Marinogammarus obtusatus* collected from Mohawk Island, Bay of Fundy. A, anus; CP, caudal papillae; PCP, precaudal papillae.

at the anterior end, an intestinal cecum attached to the body wall at its distal end by a cecal ligament, and a tail mucron. The nematode was 2.784 mm in length, the preventriculus, ventriculus, and intestinal cecum, 0.406, 0.241, and 0.058 mm in length, respectively. The nerve ring was 0.132 mm from the anterior end, and the tail was 0.087 mm long. Measurements correspond to those of third-stage *P. decipiens* in McClelland (1990).

The single *Ascarophis* sp. from *M. stenolepis* was characterized by 2 lateral pseudolabia each bearing conical protuberances, 4 cephalic papillae, and 2 lateral amphids. This nematode measured 2.304 mm in length, the muscular esophagus, the glandular esophagus, and the tail, 0.151, 0.563, and 0.128 mm in length, respectively. The buccal cavity, nerve ring, and excretory pore were 0.067, 0.109, and 0.164 mm from the anterior end, respectively.

Table 1. Characteristic mean dimensions and standard error of 7 *Paracuaria adunca* from the mysid *Mysis stenolepis* and 11 *P. adunca* from the amphipod *Marinogammarus obtusatus* collected in Passamaquoddy Bay, New Brunswick in May 1991. Measurements of buccal cavity, nerve ring, and excretory pore reflect distance from anterior end. Ranges of measurements in millimeters are in parentheses.

	Length (position) of structure	
Host	*M. stenolepis*	*M. obtusatus*
Total length	2.642 ± 0.427 (1.984–3.040)	3.142 ± 0.302 (2.624–3.712)
Buccal cavity	0.097 ± 0.006 (0.087–0.106)	0.106 ± 0.008 (0.093–0.122)
Nerve ring	0.117 ± 0.005 (0.109–0.125)	0.127 ± 0.007 (0.116–0.138)
Excretory pore	0.159 ± 0.015 (0.141–0.177)	0.183 ± 0.011 (0.157–0.196)
Muscular esophagus	0.326 ± 0.024 (0.289–0.358)	0.368 ± 0.033 (0.302–0.425)
Glandular esophagus	0.886 ± 0.138 (0.691–1.067)	1.032 ± 0.065 (0.953–1.130)
Tail	0.082 ± 0.006 (0.071–0.092)	0.090 ± 0.006 (0.083–0.100)

The specimen of *Tetrameres* sp. from *M. obtusatus* possessed 8 cuticular caudal papillae arranged in a circle and 2 papillae 0.039–0.045 mm from the posterior end (Fig. 1). The nematode was 1.760 mm in length, the muscular esophagus, the glandular esophagus, and the tail, 0.218, 0.466, and 0.141 mm in length, respectively. The buccal cavity, nerve ring, and excretory pore were 0.013, 0.138, and 0.176 mm from the anterior end, respectively.

All specimens of *P. adunca* possessed prominent triangular pseudolabia, interlabia, and 2 pairs of cephalic papillae. Measurements of *P. adunca* are presented in Table 1.

Representative specimens of *P. adunca* from *M. stenolepis* (#CMNP1992-0024) and *M. obtusatus* (#CMNP1992-0025) have been deposited in the Canadian Museum of Nature (P.O. Box 3443, Station D, Ottawa, Ontario, Canada K1P 6P4), as have the single specimens of *P. decipiens, Ascarophis* sp., and *Tetrameres* sp. (#CMNP1992-0023, -0026, and -0027, respectively).

Discussion

This is the first irrefutable record of a natural larval sealworm infection in the mysid, *Mysis stenolepis*. Sealworm was tentatively reported in other mysids previously, including *Mysis mixta, M. stenolepis,* and *Erythrops erythrophthalma* (Scott and Black, 1960), and confirmed in *Neomysis americana* (Marcogliese, 1992) and *Mysis mixta* (Martell, 1992). *Mysis stenolepis* was shown to be susceptible to *Pseudoterranova decipiens* infection in the laboratory, but nematodes were subsequently destroyed by a hemolytic response (McClelland, 1990).

Larval *P. decipiens* also has been found in the gammaridean amphipod, *Marinogammarus obtusatus,* in the White Sea (Val'ter, 1987), but the parasite was not found in 1,047 *M. obtusatus* collected directly from a seal haul-out area in the Bay of Fundy. Possibly greater numbers of amphipods would have to be examined in order to detect the parasite. Larval sealworm has been found in a second amphipod species, the beach flea, *Americorchestia megalophthalma,* collected close to haul-out areas on Sable Island (Marcogliese, 1993), but strong tidal currents in the Bay of Fundy may carry eggs and free-living second-stage larvae away from seal haul-outs. The only infected crustacean, *M. stenolepis,* was collected in Brandy Cove, a populated area avoided by seals. Presence of infected mysids in this cove could be attributed to passive dispersal of eggs and infective stages by tidal currents.

Ascarophis is a cosmopolitan genus composed of 23 species, all of which infect a wide variety of marine and freshwater fish (Ko, 1986). Adults of a single ascarophid (Ko, 1986), *Ascarophis arctica* Poljansky, 1952, have been identified from 3 species of fish, the ocean pout (*Macrozoarces americanus*), the winter flounder (*Pleuronectes americanus*), and the rock gunnel (*Pholis gunnelus*) in Passamaquoddy Bay (Appy, 1981). Larval *Ascarophis* spp. are found primarily in decapods, including shrimp, lobsters, crabs, and hermit crabs (Uspenskaja, 1960; Uzmann, 1967; Petter, 1970; Poinar and Kuris, 1975; Poinar and Thomas, 1976; Owens, 1987), but *A. pacificus* has been described from the amphipods *Anisogammarus kygi, A. tiuschovi,* and *A. ochotensis* and the isopod *Idothea ochotensis* (Tsimbalyuk et al., 1970). An unusual monoxenous form (*A. arctica*?) was found in *Gammarus ocenicus* in the White Sea (Val'ter et al., 1987).

Paracuaria adunca and *Tetrameres* sp. parasitize birds as adults. Mature *P. adunca* are cosmopolitan in piscivorous birds (Wong and Anderson, 1982), and infective stages can be found in crustaceans inhabiting both freshwater (Anderson and Wong, 1982) and marine (Marcogliese, 1992) environments. The mysid (*Mysis stenolepis*) and amphipod (*Marinogammarus obtusatus*) are new intermediate host records for this acuarioid nematode. In general, measurements of *P. adunca* correspond to third-stage larvae except that total lengths of 3 of those from *M. stenolepis* and 10 from *M. obtusatus* were longer than those measured by Anderson and Wong (1982). However, all larvae were smaller than the fourth-stage larvae as indicated by Anderson and Wong (1982). The genus *Tetrameres* contains many species, 2 of which, *T. crami* Swales, 1933, and *T. fissispina* (Diesing, 1861), are widespread in North America (Wong et al., 1990). *Tetrameres crami* is known only from freshwater amphipods (*G. fasciatus, Hyalella azteca*) (Swales, 1936), but marine and freshwater invertebrates, including amphipods, isopods, cladocerans, ostracods, insects, flatworms, and oligochaetes are reported as intermediate hosts for *T. fissispina* (McDonald, 1969). Third-stage larvae of *T. crami,* with 9 papillae surrounding a central papilla on the posterior end (Swales, 1936), and *T. fissispina,* having a ring of 10 papillae (Garkavi, 1949), differ from our specimen of *Tetrameres,* which has a circle of 8 caudal papillae. However, the number of caudal papillae may not be a reliable taxonomic character, as third-stage larvae of *T. cardinalis* Quentin and Barre, 1976, possess 5–8 papillae (Quentin and Barre, 1976).

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Anoplocephaloides dentatoides sp. n. from the Gray Red-backed Vole, *Clethrionomys rufocanus bedfordiae,* in Hokkaido, Japan

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ABSTRACT: Small, wedge-shaped cestodes, *Anoplocephaloides dentatoides* sp. n., were found from the terminal ileum of gray red-backed voles, *Clethrionomys rufocanus bedfordiae,* in Hokkaido, Japan. Prevalence in 48 yearling voles was 33% at 1 locality (43°12′N, 141°27′E) in the early summer of 1989. Morphologically, the new species most closely resembles the European form or population of *Anoplocephaloides dentata* (Galli-Valerio, 1905) Rausch, 1976, which is known principally from *Microtus* spp. in western Eurasia but differs from the latter in the following points: larger cirrus sac, which does not substantially cross the poral excretory canals, female organs situated more centrally in segments, and smaller eggs. In addition, these 2 species apparently differ in host specificity; *A. dentatoides* appears limited to voles of the genus *Clethrionomys,* whereas *A. dentata* is primarily a parasite of *Microtus* spp. (a genus of vole not currently known to occur in Hokkaido, Japan) and has only been incidentally reported from red-backed voles in the Palaearctic.

KEY WORDS: *Anoplocephaloides dentatoides* sp. n., *Clethrionomys rufocanus bedfordiae,* taxonomy, Cestoda, Japan.

The genus *Anoplocephaloides* Baer, 1923 emend. Rausch, 1976, is composed of diverse groups of species that have a fundamental similarity in the developmental pattern of the uterus, but that are distinguished based on the form of the strobila, host range, and geographical distribution (Rausch, 1976; Genov and Georgiev, 1988). The group "infrequens" proposed by Genov and Georgiev (1988) consists of species having a small, wedge-shaped strobila and parasitizing rodents of the family Arvicolidae in the Holarctic and Geomyidae in the Nearctic; it includes *A. dentata* (Galli-Valerio, 1905) Rausch, 1976, *A. infrequens* (Douthitt, 1915) Baer, 1923, *A. troeschi* (Rausch, 1946) Rausch, 1976, *A. lemmi* (Rausch, 1952) Rausch, 1976, and *A. kontrimavichusi* Rausch, 1976.

Of that group, *A. dentata* is the only species considered to have a distribution limited to the Palaearctic, whereas other species are endemic to North America or have holarctic ranges. The former species occurs exclusively in *Microtus* spp. (*M. nivalis, M. subterraneus, M. guentheri, M. arvalis,* and *M. oeconomus*). Infrequently, however, this species has been recorded from voles of the genera *Clethrionomys* and *Apodemus* (Zarnowski, 1955; Gubanov and Fedorov, 1970; Murai, 1974; Tenora and Murai, 1980; Tenora et al., 1986).

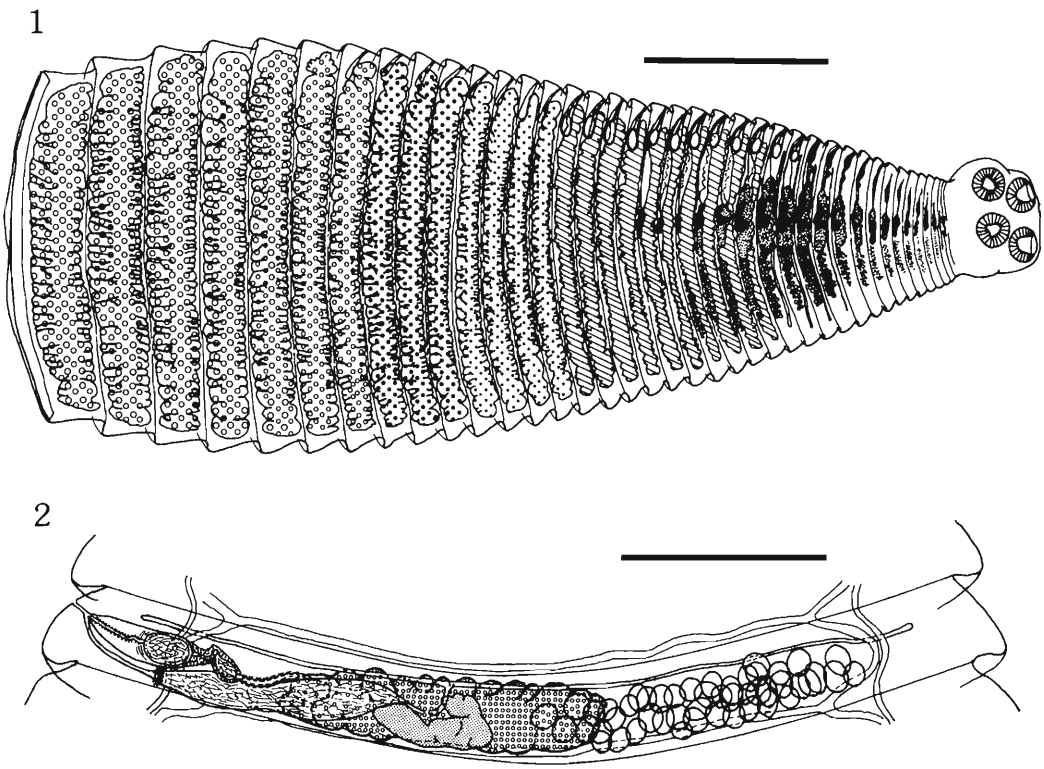
We found a high prevalence of small, wedge-shaped cestodes of the genus *Anoplocephaloides* in gray red-backed voles, *Clethrionomys rufocanus bedfordiae,* in Hokkaido, Japan, where no *Microtus* spp. occur. Morphologically, these cestodes most closely resembled *A. dentata,* but some important differences existed. Thus, we describe the specimens as *A. dentatoides* sp. n. and discuss the differences from *A. dentata.*

Materials and Methods

Gray red-backed voles were collected in a wind shelter-belt on the Ishikari Plain (Tohbetsu; 43°12′N, 141°27′E), Hokkaido, Japan, on 28 May and 5 July 1989. For age determination of the host, second and third molars were collected at necropsy, and the shape and root ratio were evaluated according to the method of Abe (1976). After removal from the host, cestodes were relaxed in water, lightly pressed at fixation in hot 10% formalin, stained with Semichon's carmine, and mounted by conventional technique. Drawings were made with the aid of a camera lucida. The asymmetric situation of female organs in segments was represented as an index $A:B$, where A was the distance between the poral segmental margin and the center of vitelline gland and B was the width of that segment. Egg capsules and oncospheres were measured using both mounted specimens and egg suspension from gravid segments preserved in formalin. All measurements given below are in millimeters with the range followed by the mean and standard deviation ($N = 12$) in parentheses.

Results

The voles examined consisted of 48 yearlings >9 (±2) mo old and 13 juveniles <2 mo old. Small, wedge-shaped cestodes were found in the



Figures 1, 2. ***Anoplocephaloides dentatoides*** **sp. n. from the gray red-backed vole,** ***Clethrionomys rufocanus bedfordiae.*** **1. Whole view of specimen. Scale bar = 2.0 mm. 2. Last mature segment, dorsal view. Scale bar = 0.50 mm.**

terminal ileum (1–2 cm above the ileocecal valve) of 16 yearling voles (6 of 17 males and 10 of 31 females). The cestodes had passed into the cecum of voles, which died in captivity. The prevalence was slightly lower in July (28.6% of 14 voles) than in May (35.3% of 34 voles). The intensity ranged from 1 to 3 (9, 3, and 4 voles with 1, 2, and 3 cestodes, respectively); in all, 27 specimens were collected. The following description was based on 12 complete specimens having gravid segments, in which apolysis had occurred.

Description

Anoplocephaloides dentatoides sp. n. (Figs. 1, 2)

Strobila wedge-shaped, 7.38–15.28 (10.93 ± 2.70) long. Maximum width 3.46–4.60 (4.08 ± 0.38), attained in the last postmature and/or first gravid segments. All segments wider than long; length : width ratio of mature, postmature, and gravid segments ranging around 1:8.4–16.4, 1:8.0–14.5, and 1:3.8–12.7, respectively. Segmental margins serrate. Scolex 1.080–1.380 (1.282 ± 0.086) wide, slightly wider than beginning strobila; suckers round, 0.304–0.384 (0.351 ± 0.022) in diameter. Neck not discernible. Anlagen of genital organs visible in strobila immediately posterior to scolex, where external segmentation not evident. In segments following these immature ones, genital organs developed rapidly. Total number of segments 28–47 (39.50 ± 5.30), including 4–6 (4.50 ± 0.91) immature, 9–13 (10.92 ± 1.73) premature and mature, 12–21 (17.5 ± 2.43) postmature, and 3–9 (6.58 ± 2.07) gravid segments. The last mature segment was the 13th–18th (15.42 ± 1.44) from the first immature segment. Genital ducts passing dorsally across the longitudinal excretory canals. Dorsal canals situated lateral to ventral canals in approximately the same plane. Genital pores unilateral, either sinistral or dextral, near middle of segmental margin. Cirrus sac elongated, slightly extending mediad across the ventral excretory canal; maximum dimensions 0.224–0.416 (0.328 ± 0.048) by 0.082–0.136 (0.110 ± 0.021), attained in the 4th–12th postmature segments. Cir-

rus densely covered with minute spines. Internal seminal vesicle begins to fill in the last mature segments, increasing in size posteriad. External seminal vesicle, smaller than the internal one, extending mediad from the proximal end of cirrus sac. Testes spherical, 0.052–0.076 in diameter, distributed dorsally from the midline of segment laterad to the antiporal ventral excretory canal, slightly overlapping antiporal part of ovary; approximately 50 in number, arranged 3–4 deep. Vagina a thick-walled tube, opening in the genital atrium posteroventrally to orifice of male duct, connected around the dorsal excretory canal to seminal receptacle. Seminal receptacle markedly elongated, extending mediad beyond poral margin of vitelline gland; beginning to fill in the last mature segment, attaining maximum dimensions of 0.400–0.616 (0.545 ± 0.069) by 0.100–0.176 (0.143 ± 0.027) in anterior postmature segments. Lobated ovary transversely elongated, extending mediad beyond midline; situated in poral ⅖–⅗ fields of segment. Vitelline gland bilobed, situated dorsally over middle part of ovary near posterior margin of segment. An index of asymmetrical location of the female organs in segment was 0.332–0.440:1 (0.392 ± 0.028) in the last mature segment. Ovary and vitelline gland attaining maximum dimensions in the last mature and/or first postmature segments, thereafter disappearing rapidly; maximum width of ovary 0.536–0.992 (0.760 ± 0.125); maximum width of vitelline gland 0.272–0.536 (0.353 ± 0.073). Uterus first visible as transverse tube, extending beyond the longitudinal excretory canals bilaterally. Egg capsules approximately spherical, with well-developed pyriform apparatus; 0.031–0.034 in diameter, with oncospheres 0.008 in greater diameter, in mounted specimens; egg capsules 0.033–0.039 in diameter, with oncospheres 0.011–0.012 by 0.010–0.011, in formalin-preserved specimens.

TYPE HOST: *Clethrionomys rufocanus bedfordiae.*

TYPE LOCALITY: Hokkaido, Japan (Tohbetsu; 43°12′N, 141°27′E).

SITE IN HOST: Terminal ileum.

SPECIMENS DEPOSITED: Holotype and paratypes in the National Science Museum, Tokyo (NSMT-Pl 4127). Dates for collection of the specimens: 28 May and 5 July 1989.

ETYMOLOGY: This new species closely resembles *A. dentata* not only in morphology but also in the host specificity (closely related rodents of the family Arvicolidae) and Palaearctic distribution, so it is named *A. dentatoides.*

Remarks and Discussion

Of the "infrequens" group of *Anoplocephaloides* mentioned previously, 3 species, *A. dentata, A. troeschi,* and *A. infrequens,* resemble each other in morphology. They had been confused until Rausch (1976) comprehensively redefined the systematics and taxonomic status of these *Anoplocephaloides* species. *Anoplocephaloides dentata* and *A. troeschi* are common cestodes of various *Microtus* spp. in Eurasia (Tenora and Murai, 1980; Genov and Georgiev, 1988) and North America (Rausch, 1952), respectively, while *A. infrequens* is a parasite of the pocket gophers, *Geomys bursarius* and *Thomomys talpoides,* of the family Geomyidae in North America (Rausch, 1976). Specimens of *Anoplocephaloides dentatoides* sp. n. resemble these 3 species in morphology (Table 1). *Anoplocephaloides baeri* Rausch, 1976, having a small strobila of parallel margins occurs also in Hokkaido, Japan, but is a parasite of murid rodents, *Apodemus argenteus* (Rausch, 1976). This species is distinct in morphology from *A. dentatoides* (see Table 1). Asakawa et al. (1983) suggested incidental infections of gray red-backed voles (3 of 175 voles) with *A. baeri* in Hokkaido. The brief description of their materials agrees with *A. dentatoides* rather than *A. baeri* in the following points: the form of strobila (wedge-shaped, not of parallel margins), total segment number (35), sizes of suckers (0.363–0.386 mm in diameter), and eggs (0.033–0.038 mm). Considering that they examined damaged material, we suspect that their tapeworms might be *A. dentatoides.*

Anoplocephaloides dentatoides can be distinguished from *A. infrequens* by fewer segments, larger scoleces, and more rapid maturation. The testes of *A. dentatoides* are distributed antiporally between the ventral excretory canal and the midline of the segment, whereas in *A. infrequens* the testes have a somewhat broader distribution and extend porally beyond the midline. Similarly, *A. dentatoides* can be distinguished from *A. troeschi* by wider strobilae, larger scoleces, longer cirrus sacs, and slower maturation. As in *A. infrequens,* the testes of *A. troeschi* extend porally beyond the midline of the segments. The ovary and vitelline gland of *A. troeschi* are situated almost in the center of the segment. Based on these morphological differences as well as the host range

Table 1. Comparison of morphological features (in millimeters) of *Anoplocephaloides dentatoides* sp. n. with some species of *Anoplocephaloides* having small strobilae.

	A. dentatoides sp. n.	*A. dentata*		*A. troeschi*	*A. infrequens*	*A. baeri*
Genus of main host:	*Clethrionomys*	*Microtus*	*Microtus*	*Microtus*	*Geomys* and *Thomomys* (Geomyidae)	*Apodemus*
Geographical distribution	Japan (Hokkaido)	Western Eurasia (Europe)	Eastern Eurasia (Northeastern Siberia)	North America	North America	Japan (Hokkaido)
Reference	The present work	Genov and Georgiev, 1988	Rausch, 1976	Rausch, 1976	Rausch, 1976	Rausch, 1976
Whole length	7.38–15.38 (av. 10.9)	7.0–12.2 (av. 8.5)	5.5–8.0 (av. 6.2)	6.5–11 (av. 8.4)	10.5 and 18	11–12 (av. 13.4)
Maximum width	3.46–4.60 (av. 4.08)	2.30–4.33 (av. 3.25)	2.0–2.5 (av. 2.4)	1.5–3.5 (av. 2.6)	3 and 3.5	1.9–2.7 (av. 2.2)
Number of segments	28–47 (av. 39.5)	33–45 (av. 37.8)	27–35 (av. 32)	31–47 (av. 38)	60 and 73	41–74 (av. 49)
Last mature segment	13–18 (av. 15.4)	13–15	9–12 (av. 11)	8–16 (av. 12)	17 and 20	16–19 (av. 18)
Scolex width	1.080–1.380 (av. 1.282)	0.912–1.280 (av. 1.128)	0.650–1.2 (av. 1.0)	0.500–0.976 (av. 0.711)	0.580 and 0.672	0.806–0.975 (av. 0.885)
Number of testes	ca. 50	41–67 (av. 49.7)	ca. 50	35–50	50–60	80–90
Maximum length of cirrus sac	0.224–0.416 (av. 0.328)	0.175–0.325 (av. 0.249)	0.350	0.230	0.360	0.323
Distribution of testes	Midline of segment, slightly overlapping antiporal part of ovary, to antiporal excretory canals			Antiporal margin of vitelline gland to antiporal excretory canals	Middle of vitelline gland to antiporal excretory canals	Poral margin of ovary to antiporal excretory canals
Egg	0.033–0.039	0.040–0.053 × 0.040–0.050 (av. 0.047 × 0.044)	0.039–0.051 × 0.032–0.049 (av. 0.042 × 0.038)	0.034–0.058 × 0.032–0.049 (av. 0.042 × 0.038)	0.039–0.044 × 0.034–0.041 (av. 0.041 × 0.036)	0.045–0.057 (av. 0.050)

and geographical distribution, *A. dentatoides* shares more similarities with *A. dentata* than with *A. infrequens* or *A. troeschi.*

Two morphological forms of *A. dentata* have been known since Spasskii (1951) distinguished its European (Caucasian) and Siberian forms on the basis of different number of testes, 30 or fewer in the former and approximately 50 in the latter. Additionally, *A. dentata* from Siberia has smaller strobilae and longer cirrus sacs. Rausch (1976) considered that these 2 forms probably represented distinct species, and, thence, *A. dentata* was generally regarded as a composite species. Recently, it became apparent that the number of testes in *A. dentata* from Europe and Siberia was comparable (Tenora and Murai, 1980; Genov, 1984; Tenora et al., 1986; Genov and Georgiev, 1988), although these forms can apparently be differentiated by other morphological characters.

As evident in Table 1, *A. dentatoides* can be easily distinguished from *A. dentata* from Siberia. Although *A. dentatoides* and *A. dentata* from Europe share many similarities, some important differences exist between them in the maximum length of the cirrus sac, the relationship of the cirrus sac with the excretory canals, the location of female organs, and egg size.

In their detailed comparative study, Genov and Georgiev (1988) stated that the cirrus sac of *A. dentata* from Europe substantially crossed the excretory canals. The cirrus sac of *A. dentatoides,* albeit apparently larger than that of *A. dentata* from Europe, attains but does not substantially overlap the excretory canals. The female organs of *A. dentatoides* are situated more centrally in the segment than *A. dentata* from Europe. Genov and Georgiev (1988) described that the ovary of *A. dentata* from Europe reached up to the cirrus sac porally and to the middle of the segment antiporally, when fully developed. On the other hand, the ovary of *A. dentatoides* was situated more centrally, extending antiporally beyond the midline of the segment. The index of asymmetric situation of female organs of *A. dentata* from Europe is as follows: 0.310–0.351:1 (av. 0.330), 0.303–0.372:1 (av. 0.337), and 0.281–0.378:1 (av. 0.337) for specimens from *M. nivalis, M. subterraneus,* and *M. guentheri,* respectively (Genov and Georgiev, pers. comm.). That index was 0.332–0.440:1 (0.392 ± 0.028, $N = 12$) in the present specimens. These figures support the finding that the female organs of *A. dentatoides* are situated more centrally than those of *A. dentata,* although the ranges are overlapped considerably and Erhardova and Rysavy (1955) described centrally located ovaries of *A. dentata* from *M. arvalis* and *C. glareolus.* More data are needed as to this point. Genov and Georgiev (1988) reported variations in the size of eggs of *A. dentata* obtained from different *Microtus* spp., and Rausch (1952) reported that both size range and average size can differ considerably from 1 locality to another in a related species, *A. troeschi.* Without considering these possibilities, the present specimens have apparently smaller eggs than *A. dentata* from Europe.

The 2 species, which most closely resemble each other, *A. dentatoides,* and the European form of *A. dentata,* are separated geographically, and the area of distribution of the Siberian form of *A. dentata* is interposed. This may support the recognition of *A. dentatoides* as a previously undescribed species. Another support is the fact that *A. dentatoides* apparently is host-specific. *Anoplocephaloides dentatoides* were obtained from gray red-backed voles from Hokkaido, Japan, where *Microtus* spp. are not currently known to occur. As already mentioned, *A. dentata* is a common cestode in various *Microtus* spp. in the Eurasian continent, but infrequent in voles of the genus *Clethrionomys* there. Its prevalence is as follows: 2.5% of 80 *C. glareolus* (Zarnowski, 1955), 2.0% of 324 *C. glareolus* (Tenora, 1967), 0.3% of 295 *C. glareolus* (Murai, 1974), 1.1% of 532 *C. rufocanus* (Haukisalmi et al., 1987), and 0.3% in 1,750 *C. rutilus* (Gubanov and Fedorov, 1970). Rausch, who carried out an extensive field survey in northeastern Siberia, could not find *Anoplocephaloides* species in *C. rufocanus* and *C. rutilus,* but commonly did in *Microtus* spp. (pers. comm.). These findings imply that *A. dentata* occur only accidentally in voles of the genus *Clethrionomys.* On the other hand, the prevalence shown in the present study is high enough to consider that voles of the genus *Clethrionomys* are the true hosts of *A. dentatoides.*

Geographical distributions of *A. baeri* and *A. dentatoides,* both of which appear to be host-specific, have been recorded only from Hokkaido, Japan, and we are greatly interested in whether or not these cestodes occur also in the neighboring area of the Eurasian continent.

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Research Note

A Coprological Survey of Parasites of Wild Muriquis, *Brachyteles arachnoides,* and Brown Howling Monkeys, *Alouatta fusca*

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ABSTRACT: One hundred twenty-eight fecal examinations from 57 muriquis or woolly spider monkeys, *Brachyteles arachnoides,* and 62 fecal samples from 9 brown howling monkeys, *Alouatta fusca,* that coexist in the Atlantic forest in southeastern Brazil were examined for evidence of parasite infections. Hosts from 4 sites, 2 in Minas Gerais (EBC and FE) and 2 in São Paulo (FBR and PECB), showed differences in parasite prevalence and diversity. Neither muriquis nor brown howling monkeys from site 1 (EBC) revealed parasites. Monkeys from site 2 (FE) had only eggs presumed to be *Strongyloides cebus* and those from site 3 (FBR) had only *Trypanoxyuris brachytelesi.* Eggs and larvae presumed to be *Strongyloides cebus, Trypanoxyuris brachytelesi, Graphidiodes berlai,* and an unidentified digenean were found at site 4 (PECB). The 4 study sites differ substantially in size (40–37,000 ha), degree of human disturbance, climate, plant species composition, and muriqui densities. Contrary to expectations, muriquis from the largest and least-disturbed forest (with the lowest population density) had the highest prevalence and diversity of parasitic infection. A variety of factors, including vegetation and climatic differences, could explain this paradoxical pattern. However, the fact that PECB is the site least affected by human disturbance also suggests that the complex ecological web involved in parasitic transmission has been disrupted at the other sites. Our findings reaffirm the importance of using parasites as ecological indicator species in studies of wild primates and suggest that management plans which involve translocations must be sensitive to the potential effects of parasites on naive hosts.

KEY WORDS: muriqui, Brazil, woolly spider monkey, *Brachyteles arachnoides,* brown howling monkey, *Alouatta fusca, Trypanoxyuris brachytelesi, Strongyloides cebus,* survey.

The Atlantic forest of southeastern Brazil is one of the world's most devastated tropical ecosystems with only isolated forest patches remaining (Fig. 1). Relatively little information has been published about parasitism in the muriqui or woolly spider monkey (*Brachyteles arachnoides*) and the sympatric brown howling monkey (*Alouatta fusca*), both endangered primate species from the Brazilian Atlantic forest. Deane et al. (1969) reported both *B. arachnoides* and *A. fusca* hosting *Plasmodium brasilianum* and *Plasmodium simium.* Other reports for *B. arachnoides* include *Entamoeba hartmannii, Trypanoxyuris brachytelesi,* and *Graphidiodes berlai* (Travassos, 1943; Hugot, 1985; Milton, 1985). Diesing (1851) reported a cestode, *Mathevotaenia megastoma* (=*Taenia megastoma*), and a nematode, *Dipetalonema gracile* (=*Filaria gracilis*) from *B. arachnoides.*

In conjunction with an ecological study of muriquis by one of the authors (K.B.S.), fecal samples were examined for parasite products (eggs, larvae, cysts, and oocysts) from 4 separate sites in the Atlantic forests of southeastern Brazil. All sites differ in size, elevation, vegetation, rainfall, and degree of human disturbance (Table 1). Two of the sites are in the state of Minas Gerais: Estação Biologica de Caratinga and Fazenda Esmeralda; 2 are in the state of São Paulo: Fazenda Barreiro Rico and Parque Estadual Carlos Botelho.

The Estação Biologica de Caratinga (EBC) is a privately owned, 800-ha forest located at 19°50′S, 41°50′W, on Fazenda Montes Claros near the city of Caratinga, Minas Gerais. Altitudes in the forest range from 320 to 680 m above sea level. Annual rainfall is between 1,000 and 1,200 mm, with most of the rain falling from October through April (Strier, 1987a). Selective logging and natural disturbances such as fires and tree falls have resulted in only about 30% primary forest remaining. Secondary forest, good regenerating forest, scrub, and bracken comprise the rest (see Strier, 1987b).

Fazenda Esmeralda (FE) is also a privately owned farm that supports a 40-ha forest at about 100 m above sea level. It is located at 20°13′S, 42°39′W, about 170 km east of the city of Belo Horizonte, Minas Gerais. Vegetation and climatic conditions are quite similar to those at

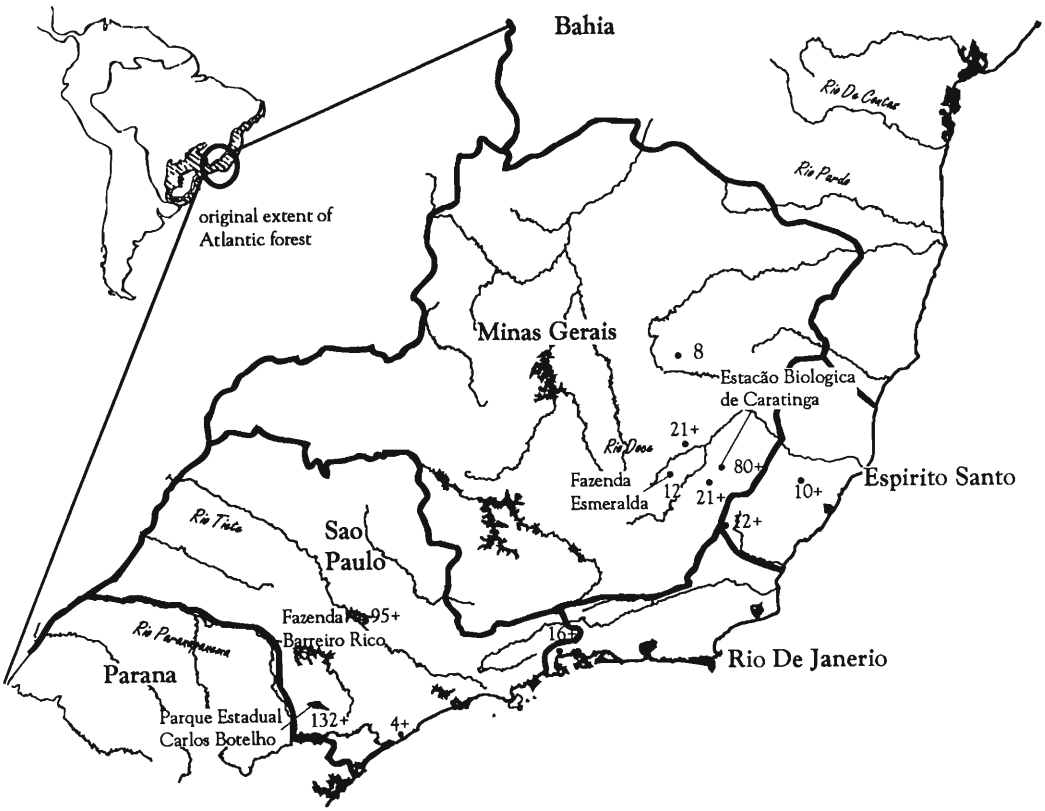

Figure 1. Atlantic forest remnants with *Brachyteles arachnoides* populations indicating minimum known numbers of individual muriquis at each site. Map modified from Mittermeier et al. (1987), Hatton et al. (1984), and Strier (pers. obs.).

EBC (Fonseca, 1983). At both sites some selective logging continues, although the smaller size of the forest at FE means that any disturbance has a greater overall effect.

Fazenda Barreiro Rico (FBR) is a large privately owned cattle ranch with 3,259 ha of forest remaining. FBR is flat and at an altitude of 480 m above sea level. It is located at 22°40′S, 48°11′W, near the city of Piracicaba, São Paulo. Annual rainfall at FBR is slightly greater than at the Minas Gerais forests, averaging 1,263 mm (Milton, 1984). Some selective logging, especially around the edges, has disturbed this primary and secondary forest.

Parque Estadual Carlos Botelho (PECB) is a large, 37,432-ha forest located at 24°44′–24°15′S, 47°46′–48°10′W, near the cities of São Miguel Arcanjo in the north and Sete Barros in the south. PECB is the wettest of the 4 sites, with annual rainfall between 1,475 and 2,189 mm. Altitudes vary from 30 to 970 m above sea level. The forest was designated and protected as a state park in 1982. It consists of primary forest and has not been subjected to selective logging. It is the least disturbed of the forests discussed here.

Because both the muriqui and brown howling monkey are highly endangered, only fecal samples as noninvasive indices of parasitic infection were permitted. Between June 1989 and April 1991, 128 fecal samples were collected from 57 muriquis, *Brachyteles arachnoides,* from the 4 sites described above and 62 fecal samples from 9 brown howling monkeys, *Alouatta fusca,* occurring sympatrically with *B. arachnoides* at EBC. Fresh fecal samples were collected during the course of systematic behavioral observations on recognized individuals at EBC and PECB and during the course of a live capture–release project at FE and FBR. Feces were preserved immediately in 10% buffered formalin in FeKal (Trend Scientific, Inc., St. Paul, Minnesota 55112) or Para-Pak (Meridian Diagnostics, Inc., Cincinnati, Ohio 45244) plastic transport vials. The presence of parasite products was determined us-

Table 1. Prevalence of parasitic infections in 4 populations of *Brachyteles arachnoides* in Brazil.

Sites	EBC	FE	FBR	PECB	Total
Estimated no. of muriquis*	80	12	95	132–500	–
Size in hectares	800	40	3,259	37,000	–
Density (no. of muriquis/ha†)	0.1	0.3	0.03	0.01–0.03	–
Level of disturbance (1 = most, 4 = least)	2–2.5	1	2.5–3	4	–
Trypanoxyuris brachytelesi (no. positive/no. examined) (%)	0/31	0/9	1/2 (50%)	1/15 (7%)	2/57 (3.5%)
Strongyloides cebus (no. positive/no. examined) (%)	0/31	8/9 (89%)	0/2	7/15 (47%)	15/57 (26%)
Graphidiodes berlai (no. positive/no. examined) (%)	0/31	0/9	0/2	12/15 (80%)	12/59 (21%)
Unidentified digenean (no. positive/no. examined) (%)	0/31	0/9	0/2	1/15 (7%)	1/57 (1.75%)
Total no. positive muriquis	0/31	8/9	1/2	13/15	22/57 (39%)

* These numbers are based on Mittermeier et al. (1987) and updated for EBC by Strier (1991) and for FE by Lemos de Sá (pers. comm.)

† Density comparisons were based on the number of animals and the total area of each site because all parts of the sites are assumed to be equally available to these large active monkeys.

EBC = Estação Biologica de Caratinga, FE = Fazenda Esmeralda, FBR = Fazenda Barreiro Rico, PECB = Parque Estadual Carlos Botelho.

ing Trend Scientific's CON-trate system®, a formalin/ethyl acetate centrifugation technique (Long et al., 1985). Since adult helminths were not collected, identification of parasitic products must remain tentative. However, when egg shape and size and larval structures closely matched previously described parasites from these host species, identification of the parasite to the species level was presumed correct.

No parasites were detected at the EBC site in either the 31 *B. arachnoides* or the 9 *A. fusca* individuals sampled. FE had only *Strongyloides cebus*. FBR had only *Trypanoxyuris brachytelesi*. PECB showed *Graphidiodes berlai, Strongyloides cebus, Trypanoxyuris brachytelesi,* and an unidentified digenean egg. Six of the 15 monkeys had 2 or more parasites (see Table 1).

Both eggs and larvae presumed to be *Graphidiodes berlai* (Strongylida: Trichostrongylidae) were found in 80% (12 of 15) of the animals at PECB only. Worms of the genus *Graphidiodes* have been reported only from *B. arachnoides* and cavimorph rodents (Travassos, 1943). The eggs from our samples were relatively large. Travassos (1943) reported uterine eggs 68–76 × 45–60 μm while the eggs observed in these fecal samples (N = 10) measured 81–84 × 51–77 μm.

Both larvated eggs and larvae of what is believed to be *Strongyloides cebus* (Rhabditida: Strongyloididae) were found in 15 of 24 animals from FE and PECB. Larvated eggs in feces measured 44–62 × 35–43 μm (average 57 × 40 μm; N = 10). Little (1966) described the eggs of *S. cebus* as in early cleavage when passed in the feces of the common spider monkey, *Ateles geoffroyi. Brachyteles arachnoides,* however, has a more elongated intestinal tract than *A. geoffroyi,* and its proportionally longer gut passage rates could account for the advanced development of these eggs and larvae. This parasite is presumably transmitted by fecal contamination rather than skin penetration by third larval stages. However, one investigator, at a different site (MDS in Costa Rica), noted surprisingly little opportunity for fecal contamination of branches in mantled howling monkeys, *Alouatta palliata,* because the feces usually fall directly to the ground or into the substory without contaminating troop pathways. Another possible source of infection is contamination by filariform larvae during human handling in capture and transport procedures although this is considered doubtful. Although some of the muriquis at FE have been captured and handled, muriquis at PECB and both muriquis and howling monkeys at ECB were never handled.

Eggs and larvae similar to *Trypanoxyuris brachytelesi* (Oxyurata: Oxyuridae) were seen in only 2 of 57 muriquis. We suspect a much higher level of infection. Bogitsh and Cheng (1990) reported human pinworm eggs were only found in the feces of about 5% of cases. A similar survey

in Costa Rica (Stuart et al., 1990) showed only 22% of fecal samples from mantled howling monkeys, *Alouatta palliata,* with *Trypanoxyuris minutus* eggs or larvae, although direct observation indicated 100% of animals over 4 wk of age were infected.

A single very small (27 × 17 μm), light golden, operculated egg was observed in 1 specimen from PECB. Whether this represents a spurious parasite or a dicrocoelid similar to *Controrchis biliophilus* observed in other platyrrhine monkeys (Stuart et al., 1990) is unknown.

The results of the comparative data on *Brachyteles arachnoides* indicate an unusual pattern of parasite distribution. Intraspecific variation in parasite infections has been associated with population densities and climatic factors in other primates. Comparative studies of both chimpanzees, *Pan troglodytes* (McGrew et al., 1989), and mantled howler monkeys, *Alouatta palliata* (Stuart et al., 1990), indicate that higher prevalences of parasitic infections occur in primates inhabiting more humid environments than among those in drier areas. The muriqui data described here are consistent with this pattern, as the population from the forest with the highest annual rainfall and highest humidity, PECB, had the highest prevalence of infection.

Data from both chimpanzees and mantled howler monkeys also indicate that the prevalence of parasites is higher in populations occurring at higher densities, presumably because of the greater opportunities for infectious transmission. The muriqui data, however, deviate from this relationship in important ways. Although PECB is the largest forest included in the study, it supports the lowest muriqui density (0.03 individual/ha). Yet, *B. arachnoides* from this site had the highest prevalence and diversity of parasite species found. The smallest forest in the study, FE, has the highest muriqui density (0.30 individual/ha). While FE had the highest proportion of infected individuals, only 1 species of parasite, *Strongyloides cebus,* was detected. EBC, in contrast, has the second-highest muriqui density (0.10 individual/ha), yet no parasites were detected in any of the individuals sampled at this site. These results are interesting because a much larger number of *B. arachnoides* was sampled across different seasons at EBC than at any of the other sites, and because no parasites were detected in the fecal samples from the small number of sympatric *A. fusca* individuals examined from the same site. The sample size at the FBR site was too small to be considered in analysis of differences between sites.

It is possible that differences in the degree of habitat disturbance at the 4 sites may be responsible for the unusual patterns of variation in parasitic infection across populations. The fact that *Brachyteles arachnoides* from the largest, least-disturbed forest at PECB had the greatest number of species of parasites while no parasites were detected from those in the moderately disturbed forest at EBC suggests that the complex ecological relationships between parasites and their primate hosts may have been disrupted by human interference.

The results of this study reinforce the idea that parasites are valuable ecological indicator species. Such assessments may be particularly important for primates because many species, such as *Brachyteles arachnoides* and *Alouatta fusca,* are highly adaptable and may be able to survive in disturbed areas. Understanding the effects of altered parasite–host relationships in endangered primate species such as these will be important in evaluating management plans for surviving populations. The evidence for pronounced intraspecific variation in parasite species composition and prevalence of infection in *B. arachnoides* must be considered in any translocation or reintroduction projects in order to avoid introducing unfamiliar parasites into naive hosts.

Permission to conduct research in Brazil and transport fecal samples was granted by CNPq. Field collections at EBC and PECB, and the purchase of analyses kits were financed by NSF grants BNS 8619442 and BNS 8958298 and the University of Wisconsin–Madison. R. Lemos de Sá provided an opportunity for K.B.S. to collect samples at FE and provided the samples from FBR; O. Carvalho, Jr., collected the samples from PECB. We are grateful to the Instituto Florestal and especially Bento Vieira de Moura Netto, director of PECB, and Gustavo da Fonseca of Universidade Federal de Minas Gerais for their collaboration and support. The Undergraduate Research Council of the University of North Carolina–Asheville supported S.P. for her senior research. Research instrumentation was financed by North Carolina Board of Science and Technology grant 89SE01.

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Research Note

Subcutaneous Helminths of the Raccoon (*Procyon lotor*) in Southern Florida

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ABSTRACT: Examination of the subcutaneous tissues of 54 raccoons (*Procyon lotor*) from southern Florida revealed the presence of spargana of 1 cestode, *Spirometra mansonoides* (prevalence 40.7%), and adults of 3 species of nematodes, *Dracunculus insignis, Dirofilaria tenuis,* and *Dipetalonema procyonis* (prevalences 16.7, 14.8, and 1.9%, respectively).

KEY WORDS: Cestoda, *Spirometra mansonoides,* Nematoda, *Dracunculus insignis, Dirofilaria tenuis, Dipetalonema procyonis,* raccoon, *Procyon lotor,* Florida.

The raccoon, *Procyon lotor,* is distributed throughout southern Canada, the continental United States, Mexico, and Central America. Previous studies on the parasite fauna of this mammal throughout its range have resulted in some helminthological information for raccoons in Florida (Harkema and Miller, 1964; Schaffer et al., 1981; Telford and Forrester, 1991; Forres-

Table 1. Prevalence and intensity of subcutaneous helminths of 54 raccoons in southern Florida, 1990-1991.

Species of helminth	Prevalence		Intensity	
	No. infected	%	$\bar{x}$	Range
Spirometra mansonoides	22	40.7	3.9	1–16
Dracunculus insignis	9	16.7	1.0	—
Dirofilaria tenuis	8	14.8	2.3	1–4
Dipetalonema procyonis	1	1.9	1.0	—

ter, 1992). The incidental discovery of infections of *Dracunculus insignis* in 2 endangered Florida panthers (*Felis concolor coryi*) in 1989–1990 (Forrester, 1992) triggered an interest in the subcutaneous parasites of raccoons in southern Florida since this parasite has been found in raccoons in other parts of the southeastern United States (Harkema and Miller, 1964; Schaffer et al., 1981). The carcasses of 54 raccoons collected for an environmental contaminant study (Roelke et al., 1991) within Florida panther habitat became available for study. The purpose of this report is to describe the prevalence and intensity of the subcutaneous helminths in these raccoons.

The raccoons were collected between April 1990 and March 1991 from Collier (N = 16), Dade (N = 25), and Monroe (N = 13) counties. Forty (85%) of the raccoons were obtained during March to June, 6 in October, and 1 each in January and February. Complete necropsies were performed including examination of the subcutaneous tissues of the skinned carcasses. Helminths were teased out of the tissues with forceps. Nematodes were fixed and preserved in 70% glycerine–alcohol and later were mounted in lactophenol for identification. The cestodes were fixed and preserved in AFA solution. A sample of skin and subcutaneous tissue from 1 raccoon with a lesion on its forelimb was fixed in 10% neutral buffered formalin, sectioned at 6 μm, and stained with hematoxylin-eosin using standard procedures. Representative specimens were deposited in the U.S. National Parasite Collection (Beltsville, Maryland 20705): *Dracunculus insignis* (male), USNM Helm. Coll. No. 82195; *Dracunculus insignis* (female), No. 82196; *Dirofilaria tenuis* (female), No. 82197; *Dirofilaria tenuis* (male), No. 82198; *Dipetalonema procyonis* (female), No. 82199; and *Spirometra mansonoides* (sparganum), No. 82200.

Prevalence and intensity for each species of helminth found are given in Table 1.

Spirometra mansonoides occurs commonly in bobcats (*Felis rufus*) and Florida panthers in Florida (Forrester et al., 1985; Forrester, 1992). The raccoon may be a source of infection for these felids, especially the Florida panther, since part of their diets consists of raccoons (Maehr and Brady, 1986; Maehr et al., 1990).

In other areas, the prevalence of *Dracunculus insignis* in raccoons has been observed to be higher than found in the present study. Prevalences varying from 50 to 83% were reported in raccoons from Virginia, Texas, and Ontario (Chandler, 1942; Crichton and Beverley-Burton, 1977; Schaffer et al., 1981). These differences may have been due to variations in food habits and the season of the year when samples were obtained.

The prevalence of *Dirofilaria tenuis* was lower than has been reported previously for the same geographic region by Isaza and Courtney (1988), i.e., 15 vs. 45%. In the present study, prevalences were determined by locating adults in subcutaneous tissues, whereas Isaza and Courtney (1988) used the presence of microfilariae in the peripheral blood as their criterion for infection. Our technique may have resulted in an underestimation of the actual prevalence.

Mansonella llewellyni, a subcutaneous nematode found in previous raccoon studies in Florida (Telford and Forrester, 1991), was not observed in this study. Telford and Forrester (1991) stated that there was a north to south decrease in the prevalence of *M. llewellyni* (50 and 79% in 2 groups of raccoons from Duval County in northern Florida, and 5% in Hillsborough County in central Florida). The absence of the parasite in animals examined in the present study in southern Florida provides additional evidence that such a southerly decline exists.

In all but 1 case, the presence of subcutaneous helminths did not cause grossly obvious lesions, indicating that infections with these helminths at the intensities observed may not be detrimental to the host. The single lesion found on the

forelimb of 1 raccoon appeared to contain a dead, mineralized specimen of *Dracunculus insignis*. When examined by histopathology, deep dermal and subcutaneous inflammation and fibrosis were found to be present surrounding small mineralized foci. This is consistent with the description given by Crichton and Beverley-Burton (1977) of the inflammation occurring during absorption of a dead *D. insignis* following its larvigerous stage.

These helminth infections in southern Florida may be more significant as zoonoses than as disease problems in wildlife. *Dirofilaria tenuis,* for example, has been reported to cause subcutaneous and conjunctival nodules in humans. These human infections are especially common in southern Florida and are associated with high prevalences of infected raccoons (Isaza and Courtney, 1988).

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Research Note

Gastrointestinal Helminths of the Tree Lizard, *Urosaurus ornatus* (Phrynosomatidae)

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ABSTRACT: The gastrointestinal tracts of 205 *Urosaurus ornatus* were examined for helminths: 117 from Aguirre Spring, New Mexico; 73 from Doña Ana Mountains, New Mexico; and 15 from southern Arizona. *Spauligodon giganticus* was the most prevalent helminth (prevalence 24.8%, mean intensity 5.6) and occurred in all 3 samples. The Aguirre Spring sample had significantly higher prevalences of *S. giganticus* (38.5%) than the other 2 samples. In addition, third-stage larvae of *Physaloptera* sp. (prevalence 4.2%, mean intensity 3.5) and *Oochoristica* sp. (prevalence 4.2%, mean intensity 1.6) and tetrathyridia of *Mesocestoides* sp. (prevalence 3.2%, mean intensity 103.8) were recovered from the New Mexico samples. The finding of *Mesocestoides* sp. within skeletal muscle in 1 specimen is noteworthy because it demonstrates that this parasite can migrate out of the body cavity. All findings represent new host records.

KEY WORDS: *Urosaurus ornatus,* Phrynosomatidae, cestode, *Oochoristica* sp., *Mesocestoides* sp., nematode, *Spauligodon giganticus, Physaloptera* sp., prevalence, intensity.

The tree lizard, *Urosaurus ornatus* (Baird and Girard, 1852), is one of the most abundant lizards in the American Southwest. It is an oviparous lizard that occupies a wide variety of habitats from riparian environments to dry, rocky areas (Dunham, 1982). It ranges from southwestern Wyoming to southern Sinaloa and northern Coahuila, Mexico, and from southeastern California to central Texas occurring from sea level to 2,770 m (Stebbins, 1985). To our knowledge there are 3 previous reports of helminths from *U. ornatus*: Walker and Matthias (1973) and Specian and Ubelaker (1974) recovered the nematode *Parathelandros texanus*; Benes (1985) found cestode tetrathyridia. This report contains helminth prevalence and intensity data for populations of *U. ornatus* from New Mexico and Arizona.

Two hundred five *Urosaurus ornatus* were examined: 117 from Aguirre Spring Recreation Area and 73 from Doña Ana Mountains, Doña Ana County, New Mexico; and 15 from Pima, Pinal and Cochise counties, Arizona. By ANOVA, there was no significant difference in snout–vent length for the 3 populations ($P > 0.05$).

Aguirre Spring Recreation Area is a relatively mesic environment located at 1,700 m elevation on a northeast slope of the Organ Mountains (32°22′N, 106°33′W, Doña Ana County, New Mexico). Boulders tend to be clumped, resulting in discontinuous habitats for *U. ornatus*. Most lizards are found on boulders or in trees or bushes overhanging boulders. In this habitat, the lizards are both saxicolous and arboreal. The Doña Ana Mountains have a more xeric environment. The major collecting site (Mount Summerford) lies on a southwest-facing slope at 1,475 m elevation (32°30′N, 106°49′W) in Doña Ana County, New Mexico. Some lizards were collected from neighboring mounts. These sites are strewn with cubic-meter-sized and larger, lichen-covered boulders. Shade-creating vegetation is mostly lacking. The lizards at these localities are strictly saxicolous. Infected specimens from Arizona were from Kitt Peak, Baboquivari Mountains (31°95′N, 111°59′W, elevation 1,885 m), Pima County, Arizona. Here the topography consists of granite boulders and outcroppings. In this habitat *U. ornatus* is arboreal.

Specimens from New Mexico were collected 1985–1986; Arizona specimens were collected 1967–1969. Specimens were preserved in 10% formalin and stored in 95% ethanol prior to examination. The body cavity was opened and the gastrointestinal tract was excised by cutting across the anterior esophagus and the rectum. The esophagus, stomach, small intestine, and large intestine were examined separately. The body cavity was examined for cestode tetrathyridia. Each helminth was removed and identified utilizing a glycerol wet mount and then returned to alcohol storage. Selected cestodes were stained with hematoxylin and mounted in balsam. After

dissection, all lizards were deposited in the herpetology collection of the Natural History Museum of Los Angeles County (Aguirre Spring 139449–139565; Doña Ana Mountains 139566–139638; Arizona 139438–139448, 139639–139642). Representative helminths in vials of alcohol were deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705: *Spauligodon giganticus* (82180–82182), *Physaloptera* sp. (82184), *Mesocestoides* sp. (82185), and *Oochoristica* sp. (82183).

Two species of nematodes, *Spauligodon giganticus* (Read and Amrein, 1953) Skrjabin, Schikhobalova, and Lagodovskaja, 1960, and *Physaloptera* sp., and 2 species of cestodes, *Mesocestoides* sp. and *Oochoristica* sp., were found. Location of infection, prevalence, and intensity (by host sex) are shown in Table 1. All of these helminths represent new host records for *U. ornatus*.

The most prevalent helminth was *S. giganticus,* which was found in lizards of all 3 populations. For all samples, prevalence was 24.8% and mean intensity 5.6. There was no significant difference for prevalence of *S. giganticus* infection between males and females at Aguirre Spring (Kruskal-Wallis statistic, $P > 0.05$). Because of low prevalences, tests of significant difference between males and females in the other 2 samples were not done.

Third-stage larvae of *Physaloptera* sp. were recovered from the New Mexico populations only (prevalence 4.2%, mean intensity 3.5) as were *Oochoristica* sp. (prevalence 4.2%, mean intensity 1.6) and tetrathyridia of *Mesocestoides* sp. (prevalence 3.2%, mean intensity 103.8). Tetrathyridia had lodged in skeletal musculature of the forelimbs, chest, and tail of a single Aguirre Spring female, making it impossible to obtain an accurate count.

The prevalence of infection of *S. giganticus* at Aguirre Spring was significantly different from the other sites (Chi-square = 25.7, $P < 0.001$); however, mean intensity of infection was not significantly different at the 3 sites (Kruskal-Wallis statistic, $P > 0.05$). The Aguirre Spring site is more moist, with greater vegetation and shade than the Doña Ana sites. In addition, the population density of *U. ornatus* at Aguirre Spring is much higher than the other sites. Boulders are found in clusters, and there is often 1 dominant male lizard and several subordinate males around the periphery of a boulder cluster as well as sev-

Table 1. Prevalence, mean intensity, and site of infection for helminths from *Urosaurus ornatus*.

Helminth	Site	Prevalence (%)						Mean intensity (range)					
		Aguirre Spring		Doña Ana		Arizona		Aguirre Spring		Doña Ana		Arizona	
		F (N = 86)	M (N = 31)	F (N = 35)	M (N = 38)	F (N = 7)	M (N = 8)	F	M	F	M	F	M
Cestoda													
Mesocestoides sp.	Body cavity*	2.3	6.5	2.9	2.6	0	0	43.5 (1–86)	109 (50–167)	110	106	0	0
Oochoristica sp.	Small intestine	7.0	3.2	2.9	0	0	0	1.8 (1–4)	2	1	0	0	0
Nematoda													
Spauligodon giganticus	Large intestine	38.3	38.7	8.6	0	14.2	25.0	6.6 (1–29)	3.8 (1–14)	2.3 (1–4)	0	1	6 (3–9)
Physaloptera sp.	Stomach	5.8	0	2.8	5.3	0	0	3.0 (1–9)	0	1	6 (1–11)	0	0

* One case of skeletal musculature involvement.

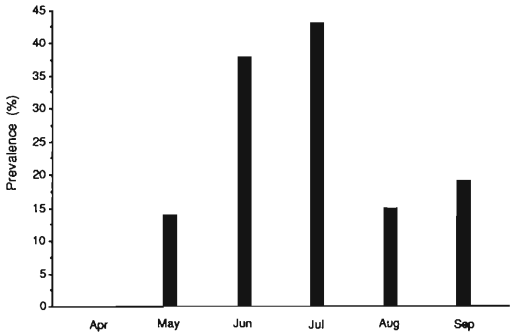

Figure 1. Prevalence of *Spauligodon giganticus* by month in the Aguirre Spring population, Doña Ana County, New Mexico.

eral females in each dominant male's territory (Zucker, 1989). At the Doña Ana Mountains, where granite boulders dominate the terrain, lizards are less dense with generally only 1 male and 1 female in overlapping territories (N. Zucker, pers. obs.). Male territories often do not lie adjacent to each other. *Urosaurus ornatus* is uncommon in the Baboquivari Mountains, where it is restricted to oak trees. It apparently cannot compete with the larger Yarrow's spiny lizard, *Sceloporus jarrovii,* which occupies the rocks.

When infection in the Aguirre Spring population was examined by month (Fig. 1), there was an increase in prevalence May through July and then a sharp decrease in August. *Spauligodon giganticus* has a direct life cycle with infection spread by fecal contamination (Telford, 1971). In a *S. jarrovii* population, sympatric with the Baboquivari Mountain population of *U. ornatus,* Bursey and Goldberg (1992a) found monthly prevalences of *S. giganticus* to range from 86 to 100%. Infections occurred in all seasons but with no annual pattern of infection. *Sceloporus jarrovii* has been observed to lick the ground (DeFazio et al., 1977) and is thought to obtain environmental cues through this behavior. Substrate licking plays an important role in the epidemiology of *S. giganticus* (see Goldberg and Bursey, 1992). There are no reports of substrate licking in *U. ornatus*; however, this lizard frequently "snout-wipes." That is, with the back of the lizard arched upward, the tip of the snout is rubbed back and forth on the substrate several times (Delahunt, 1976). It is possible that eggs of *S. giganticus* are ingested when *U. ornatus* performs this behavior. Infection may occur soon after *U. ornatus* becomes active in the spring with more lizards acquiring infection over time. Bursey and Goldberg (1992a) estimated that 91–98 days were required for completion of the *S. giganticus* life cycle. It is likely that the August drop in monthly prevalence (Fig. 1) represents the completion of the life cycle by the first generation of nematodes. The prevalence seen in August and September would represent infection by the second generation, which would mature during the lizard hibernation period. *Spauligodon giganticus* did not appear in the Doña Ana Mountains population of *U. ornatus* until July; only 3 lizards of that population were infected. It is plausible that the difference in infection rates of the New Mexico samples may result primarily from differences in density of the lizard populations. The greater density of *U. ornatus* at Aguirre Spring leads to more substrate fecal contamination. This increases chance of egg ingestion and results in higher prevalence and intensity of *S. giganticus* infection.

Other phrynosomatid lizards reported as hosts for *S. giganticus* are *Callisaurus draconoides* by Telford (1970); *Sceloporus graciosus* by Telford (1970) and White and Knapp (1979); *S. jarrovii* by Goldberg and Bursey (1990b); *Sceloporus occidentalis* by Telford (1970), Pearce and Tanner (1973), White and Knapp (1979), and Lyon (1986); *Sceloporus orcutti* by Telford (1970) and Goldberg and Bursey (1991); *Sceloporus undulatus* by Pearce and Tanner (1973); and *Uta stansburiana* by Telford (1970).

Physaloptera spp. occur in the stomach of a number of terrestrial vertebrates with such regularity that the genus is commonly called "the stomach worm." Telford (1970) found this helminth to be the most common nematode recovered from southern California lizard species. It is of interest that only third-stage larvae of *Physaloptera* were found in the New Mexico populations of *U. ornatus.* Third-stage but not adult *Physaloptera* sp. have been found in a number of other species of lizards.

Eight species of *Oochoristica* have been recovered from North American lizards (Bursey and Goldberg, 1992b). The species found in *U. ornatus* is apparently undescribed. It differs from the previously described species in length, proglottid number, scolex width, sucker measurements, and number of testes.

Tetrathyridia of *Mesocestoides* sp. are most commonly seen in the body cavity. The occurrence of tetrathyridia in North American lizards has been compiled by McAllister (1988) and Goldberg and Bursey (1990b). While there have

been reports of tetrathyridia in the liver, heart, and mesenteries (McAllister, 1988), this is apparently the first report of skeletal muscle invasion and suggests that migration out of the body cavity is possible when large numbers of tetrathyridia are present. Tetrathyridia of *Mesocestoides* sp. were recovered from the sympatric Baboquivari Mountain (Kitt Peak) *S. jarrovii* population by Goldberg and Bursey (1990a).

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Research Note

Surgical Implantation of *Echinostoma caproni* Metacercariae and Adults into the Small Intestine of ICR Mice

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ABSTRACT: Encysted and excysted metacercariae or adults of *Echinostoma caproni* were surgically implanted into the small intestine of ICR mice. Worm recovery at 10 days postimplantation from mice that had received either 25 encysted or 25 excysted metacercariae was 93%. Worm recovery at 7 days postimplantation from mice that had previously received 25 7-day-old worms was 64%. Worms recovered from surgically implanted hosts showed similar growth and development patterns to those obtained from hosts fed cysts by stomach tube.

KEY WORDS: *Echinostoma caproni,* ICR mouse, metacercariae, surgical implantation.

Recent studies have demonstrated that laboratory mice are excellent experimental hosts for the intestinal trematode, *Echinostoma caproni,* with infection rates of 100% and worm recoveries of 45–95% (Odaibo et al., 1988; Hosier and Fried, 1991). These studies used 25 encysted metacercariae per mouse, and cysts were administered by stomach tube. Less information is available on the surgical implantation of echinostome metacercariae or adults into the small intestine of mice. Christensen et al. (1986) surgically implanted excysted metacercariae of *E. caproni* (referred to as *E. revolutum* in that paper) into the mouse duodenum to examine host immunogenic effects on that larval stage. Nollen (1990) surgically transplanted radioisotopically labeled adults of *E. caproni* into the mouse small intestine to study the reproductive physiology of these worms. We have not found reference to studies on the surgical implantation of echinostome cysts into the mouse gut. The present study was undertaken to determine the efficacy of implanting encysted and excysted metacercariae, and young adults of *E. caproni,* into the small intestine of ICR mice.

Encysted metacercariae were removed from experimentally infected *Biomphalaria glabrata* snails and fed by stomach tube (25 cysts/host) or implanted surgically (25 cysts/host) into the mouse small intestine as described below. Excysted metacercariae were obtained following metacercarial excystation in an alkaline trypsin–bile salts solution (Fried and Emili, 1988) and implanted surgically. Adults, 7 days old, were removed from donor hosts and implanted surgically (25/host) into the mouse duodenum as follows.

Mice were fasted for 12 hr before surgery. Under sodium pentobarbital anesthesia (60 mg/kg body weight), a 10–15-mm incision was made in the upper abdomen. The stomach and first 20–25 mm of the small intestine were pulled through the incision, and a 3–5-mm slit was made in the stomach wall. *Echinostoma caproni* adults or metacercariae were drawn into polyethylene tubing (0.86 mm inner diameter) attached to a 1-ml tuberculin syringe, and the tubing was inserted into the slit and gently manipulated past the pyloric valve into the first 10–15 mm of the duodenum. Adults or metacercariae were injected with 0.1–0.2 ml Locke's solution. The incisions were closed with 5/0 sutures.

At necropsy, mice were anesthetized lightly with ether and killed by cervical dislocation, and the small intestine was examined for worms. Mice receiving larvae either orally or surgically were necropsied 10 days postinfection. Mice receiving adults were necropsied 7 days postimplantation. Worms recovered from mice were in the lower part of the small intestine, usually 20–40 cm from the pylorus. They were fixed in hot alcohol–formalin–acetic acid, stained in carmine and mounted in Permount, and body area measurements were made (Hosier and Fried, 1991).

The results of the experiments are summarized in Table 1. There was no difference in worm recovery or worm body area among mice that received encysted metacercariae by stomach tube (group A), encysted metacercariae by surgery (group B), or excysted metacercariae by surgery (group C, ANOVA, $P > 0.05$). Of the 20 fixed and stained worms from groups A, B, and C, 19 were ovigerous and 1 from group B was preovigerous. The infectivity, growth, and development

Table 1. Summary of transplantation experiments with *Echinostoma caproni* larvae and adults in ICR mice.

Group*	No. of infected mice†	Mean number ± SE of worms recovered per host‡	No. of worms measured	Mean ± SE of body area (mm^2) of worms
A	5	20.4 ± 1.7	10	2.3 ± 0.19
B	5	22.6 ± 1.3	5	1.70 ± 0.17
C	5	22.8 ± 1.7	5	2.02 ± 0.21
D	2	16	6	3.10 ± 0.26

* All mice received 25 worms as follows: A, cysts fed by stomach tube; B, cysts implanted surgically; C, excysted metacercariae implanted surgically; and D, 7-day-old adults implanted surgically.

† All mice exposed to larvae or adults became infected.

‡ Worms were recovered 10 days postinfection except in D, where recoveries were made at 7 days postimplantation.

studies showed that surgical implantation of either encysted or excysted metacercariae is feasible for studies on *E. caproni* in ICR mice. Encysted metacercariae of this species can excyst in vivo without passage through the stomach. Fried and Emili (1988) reported that encysted metacercariae of this species can be excysted in vitro in the absence of acid-pepsin pretreatment. Surgical implantation of 7-day-old adults into the mouse gut resulted in a worm recovery rate of 64% at 7 days postimplantation. These 14-day-old worms (7 days in donor plus 7 days in recipient) were ovigerous with a mean body area of 3.1 mm^2 (see D, Table 1). Ten worms grown for 7 days in mice were preovigerous with a mean body area of 0.85 ± 0.07 mm^2. Data from Hosier and Fried (1991) showed that 30 worms grown for 14 days in mice were ovigerous with a mean body area of 5.2 ± 0.4 mm^2. The difference in body area (Student's t-test, $P < 0.05$) of the 14-day-old worms grown in our study compared to that seen in Hosier and Fried (1991) may reflect some retardation of growth in worms transplanted to a new host. The surgical implantation procedure described herein is suitable for echinostome adults as well as metacercariae.

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Research Note

Endoparasites of Western Slimy Salamanders, *Plethodon albagula* (Caudata: Plethodontidae), from Arkansas

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ABSTRACT: Thirty-seven western slimy salamanders, *Plethodon albagula* Grobman, 1944, collected between December 1988 and March 1992 from 10 counties of Arkansas, were examined for endoparasites. Fourteen (38%) were infected with 1 or more parasites: 2 (5%) with *Cepedietta michiganensis* (Woodhead, 1928) Corliss, de Puytorac, and Lom, 1965, 4 (11%) with an isosporan, 10 (27%) with *Cylindrotaenia americana* Jewell, 1916, 4 (11%) with *Batracholandros salamandrae* (Schad, 1960) Petter and Quentin, 1976, and 1 (3%) with an acanthocephalan cystacanth. In addition, 3 (8%) salamanders harbored an intraerythocytic inclusion, *Cytamoeba bactifera* Labbé, 1894, thought by some to represent a protozoan of undetermined taxonomic status. This note represents only the second time an isosporan has been reported from salamanders of the world. With the exception of *C. michiganensis*, new host and distributional records are documented for these parasites of *P. albagula*.

KEY WORDS: *Isospora* sp., *Cylindrotaenia americana, Plethodon albagula,* Plethodontidae, Caudata, *Batracholandros salamandrae,* acanthocephalan cystacanth, *Cepedietta michiganensis, Cytamoeba bactifera.*

The western slimy salamander, *Plethodon albagula* Grobman, 1944, is a large plethodontid that ranges from southern Missouri southward through Arkansas and eastern Oklahoma; disjunct populations occur on the Balcones Escarpment of southcentral Texas (Dixon, 1987; Conant and Collins, 1991). This taxon was once recognized as a subspecies of the northern slimy salamander, *P. glutinosus* (Green, 1818), but has been distinguished biochemically from it as well as 14 other species of the complex (Highton et al., 1989). Although much is known about parasites of eastern members of the *P. glutinosus* complex (Rankin, 1937a, b; Fischthal, 1955; Cheng, 1958, 1960; Powders, 1970; Dunbar and Moore, 1979; and others), little is known regarding parasites of *P. glutinosus*-like salamanders found west of the Mississippi River. Byrd (1937) and Rabalais (1970) reported *Brachycoelium salamandrae* (Frölich, 1789) Dujardin, 1845, from *P. glutinosus* in Louisiana. Winter et al. (1986) found the protozoan *Cepedietta michiganensis* (Woodhead, 1928) Corliss, de Puytorac, and Lom, 1965, and unidentified immature oxyuroids in a small sample of *P. glutinosus* (=*albagula*) from Arkansas. To our knowledge, nothing else has been published on its parasites. Herein, we report several endoparasites from *P. albagula.*

Between December 1988 and March 1992, 37 juvenile and adult ($\bar{x}$ ± SEM snout–vent length [SVL] = 53.9 ± 0.2, range 23–86 mm) *P. albagula* were collected by hand from the following locations (sample sizes in parentheses): Garland (7), Grant (6), Jackson (1), Newton (6), Perry (2), Polk (2), Pope (2), Saline (1), Stone (7), and White (3) counties of Arkansas and examined for endoparasites. Methods for salamander necropsy, coccidial isolation, and preparation and staining of blood smears and helminths follow McAllister and Upton (1987). Voucher specimens of salamanders are deposited in the Arkansas State University Museum of Zoology (ASUMZ 13136–37, 15462–67, 18019–21, 18273–75, 18277–79, and 18371–79). Specimens of parasites are deposited in the U.S. National Parasite Collection, Beltsville, Maryland 20705, as follows: *Cepedietta michiganensis* (USNM 82341), *Cylindrotaenia americana* (USNM 82217), *Batracholandros salamandrae* (USNM 82343), and acanthocephalan cystacanth (USNM 82342).

Fourteen (38%) of the *P. albagula* were infected, including 2 (5%) with *Cepedietta michiganensis,* 4 (11%) with an *Isospora* sp., 10 (27%) with *Cylindrotaenia americana* (Jewell, 1916), 4 (11%) with *Batracholandros salamandrae* (Schad, 1960) Petter and Quentin, 1976, and 1 (3%) with an acanthocephalan cystacanth. In addition, 3 (8%) salamanders harbored *Cytamoeba bactifera* Labbé, 1894.

Heavy infections of the astomatous ciliate *Cepedietta michiganensis* were found in the gall

bladder and small intestine of 2 adult *P. albagula* (male, 66 mm SVL, ASUMZ 18375; female, 78 mm SVL, ASUMZ 18371) collected in March 1992 from Grant and Garland counties, respectively. Winter et al. (1986) reported this ciliate from a single *P. albagula* from the western Ouachita Mountains of Arkansas. In addition, these same authors reported *C. michiganensis* from *Plethodon fourchensis* and *P. ouachitae.* There appears to be little host specificity for *C. michiganensis,* as the following ambystomatid and plethodontid salamanders have also been reported as hosts: *Ambystoma jeffersonianum, A. opacum, Desmognathus fuscus, D. monticola, Eurycea bislineata, E. longicauda, Hemidactylium scutatum, P. cinereus, P. jordani, P. glutinosus,* and *Pseudotriton montanus* (see Powders, 1967).

The 4 salamanders passing isosporan oocysts in the feces were collected in May 1989 and March 1992 from Pope, Grant, and Garland counties. Unfortunately, not enough oocysts completed sporulation to determine specific identification of the coccidian. Doran (1953) described *I. jeffersonianum* from the Jefferson salamander, *Ambystoma jeffersonianum* (Green, 1827) in north-central (Bemidji, Beltrami County) Minnesota; however, *A. jeffersonianum* is not known to occur in Minnesota (Conant and Collins, 1991). The type host is most likely the blue-spotted salamander, *A. laterale,* or a hybrid of the *A. laterale–jeffersonianum* complex (see Lowcock et al., 1987). Ambystomatids and plethodontids belong to separate salamander families and are unrelated phylogenetically; therefore, it is doubtful the isosporan reported herein is the same species reported from *A. jeffersonianum.* Thus, additional specimens will need to be recovered in order to describe the new species.

A total of 51 *C. americana* (mean intensity = 5.1, range 1–15) was recovered from the small intestine of 10 *P. albagula* (5 males, 5 females, 66.3 ± 3.8, 44–86 mm SVL, ASUMZ 18019–21, 18371–75, 18378–79) collected in December 1991 and March 1992 from Garland, Grant, and White counties. McAllister (1991) provided a summation of the amphibians and reptiles of the world reported to be hosts of *C. americana.* In North America, the parasite has been found in various hosts from 17 states ranging from Washington, Oregon, and California east to Maine and south to Florida and Texas. Of the 9 species of salamanders listed as hosts, 8 are plethodontids, including the related *P. glutinosus* from eastern Tennessee (Dunbar and Moore, 1979). Winter et al. (1986) reported unknown nematotaeniids from *P. caddoensis, P. fourchensis, P. ouachitae, P. serratus,* and *Desmognathus brimleyorum* from western Arkansas, although none of the 5 *P. albagula* they examined was infected with cestodes. Thus, *P. albagula* represents a new host, and Arkansas a new locality, for *C. americana.*

Sixteen oxyurid nematodes, *Batracholandros salamandrae* (mean intensity = 4.0 ± 1.4, range 3–6), were found in the rectum of 4 *P. albagula* (2 males, 2 females, 64.0 ± 7.2, 44–78 mm SVL, ASUMZ 19371, 18373–75) collected during March 1992 from Garland, Grant, and Stone counties. This parasite was originally described from *Aneides hardii* in New Mexico (Schad, 1960). Other salamander hosts include *P. neomexicanus* from New Mexico (Panitz, 1967), *P. elongatus, P. stormi,* and *P. vehiculum* from Oregon (Panitz, 1969), and *D. ochrophaeus, E. bislineata, P. glutinosus, P. richmondi,* and *P. ruber* from Tennessee (Dunbar and Moore, 1979). The related *Batracholandros magnavulvaris* (Rankin, 1937) Petter and Quentin, 1976, was reported from *P. glutinosus* from North Carolina (Rankin, 1937b) and, although not yet found in *P. albagula,* was reported from 5 other salamanders in Arkansas (Winter et al., 1986).

A single acanthocephalan cystacanth was recovered from the mesenteries of an adult female *P. albagula* (68 mm SVL, ASUMZ 18374) collected in March 1992 from Stone County. Cysts of *Acanthocephalus acutulus* Van Cleave, 1931, have been reported from *P. glutinosus* as well as numerous other salamanders from North Carolina (Rankin, 1937a).

Blood smears of 3 adult salamanders (2 males, 1 female, 66–75 mm SVL, ASUMZ 18375, 18378–79) revealed the intraerythrocytic organism *Cytamoeba bactifera.* Rankin (1937a) reported *C. bactifera* from *P. glutinosus* in North Carolina as well as *Notophthalmus viridescens, D. fuscus, D. phoca, D. quadramaculatus, P. ruber, E. guttolineata, A. maculatum, A. opacum,* and *P. cinereus.* In addition, Lehmann (1961) reported on the morphology and prevalence of *C. bactifera* in salamanders from California.

The taxonomic status of *C. bactifera* is currently unknown. Ayala (1978) considered *C. bactifera* to be a bacterium or virus, whereas cytochemical studies by de Sousa and Freire (1975) suggested it may actually represent a rickettsial agent or chlamydian. More recently, Bovee (1985) recognized this organism as belonging to the sub-

phylum Sarcodina, class Lobosea, family Entamoebidae.

In summary, this note represents only the second time an isosporan has been reported from salamanders of the world and, with the exception of *C. michiganensis,* new host and distributional records are documented for other parasites of *P. albagula.* It is not surprising that the majority of parasites reported herein for *P. albagula* are shared with *P. glutinosus,* given the 2 hosts are closely related phylogenetically and inasmuch all show little host specificity.

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Research Note

The Asian Fish Tapeworm, *Bothriocephalus acheilognathi*, in Fishes from Nevada

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ABSTRACT: A total of 73 fish representing 5 species from the Muddy River and cooling pond of the Moapa Power Plant in Nevada was examined for *Bothriocephalus acheilognathi* (Yamaguti, 1934) (Asian fish tapeworm) and other parasites. Three of 14 *Cyprinella lutrensis* (Baird and Girard, 1853) from the Muddy River and 10 of 11 *Gila robusta* (Baird and Girard, 1853) from the cooling pond of the Moapa Power Plant in Nevada were infected with *Bothriocephalus acheilognathi*. From 79 fish examined from 4 bait fish shops in the Las Vegas, Nevada, area 3 of 38 *Notemigonis crysoleucas* (Mitchill, 1814) were infected with *B. acheilognathi* but none of the 41 *Pimephales promelas* (Rafinesque, 1820) was infected. The bait fish were originally from fish farms in California, Arkansas, and Missouri. *Cyprinella lutrensis* is a new host and the Muddy River in Clark County, Nevada, is a new locality for the Asian fish tapeworm. The finding of *B. acheilognathi* in bait fish obtained from stores in the Las Vegas, Nevada, area represents a potential source for its introduction into new areas.

KEY WORDS: *Bothriocephalus acheilognathi*, Asian fish tapeworm, Nevada.

The Asian fish tapeworm *Bothriocephalus acheilognathi* (Yamaguti, 1934) has been introduced into the United States through shipments of grass carp *Ctenopharyngodon idella* (Valenciennes, 1844), which were brought into this country from China to control aquatic vegetation (Hoffman and Schubert, 1984). The Asian fish tapeworm has spread from its initial introduction in the southern part of the United States to the western part due to infected fish introductions (Heckmann et al., 1986). *Bothriocephalus acheilognathi* is considered one of the most dangerous pseudophyllidean cestodes for cultured carp in Europe (Hoffman and Schubert, 1984).

The Asian fish tapeworm in fishes from the Virgin River in Utah and Nevada was first reported in 1986 (Heckmann et al.) and later confirmed in other species of fish (Heckmann et al., 1987). The objectives of this study were to expand the knowledge of the range of *B. acheilognathi* infection in fishes from Nevada and to determine the potential sources of infection.

Fish were obtained from the cooling pond, Moapa Power Plant, and the Muddy River in Clark County, Nevada, through the help of fisheries biologists. Fish were also obtained from bait shops in the Las Vegas, Nevada, area. Each fish was weighed and measured and then checked for parasites using standard methods (Heckmann et al., 1986). The abdominal cavity was opened ventrally, and the digestive system was removed and placed in a saline solution. The intestine was examined using a binocular dissecting microscope. Cestodes were excised from the intestinal tract, enumerated, fixed in AFA, stained with Semichons carmine, mounted on glass slides, and identified using standard keys and by comparison to known specimens of *B. acheilognathi* in the senior author's collection.

A total of 73 fish representing 5 different species from the Muddy River and from the cooling pond of the Moapa Power Plant was examined. Three of 14 (21%) *Cyprinella lutrensis* (Baird and Girard, 1953) and 10 of 11 (91%) *Gila robusta* (Baird and Girard, 1953) were infected with *B. acheilognathi*. None of the 17 *Gambusia affinis* (Baird and Girard, 1953), 26 *Poecilia mexicana* (Steindachner, 1863), and 5 *Crenichthyes baileyi* (Gilbert, 1893) was infected.

From 4 bait shops in the Las Vegas, Nevada, area 79 bait minnows of 2 species were purchased and examined. Three of 38 (8%) *Notemigonus crysoleucas* (Mitchill, 1814) were infected with *B. acheilognathi*, but none of 41 *Pimephales promelas* (Rafinesque, 1820) was infected. These bait minnows, commonly used for bait by fishermen in Lake Mead, Nevada, originate from commercial ponds in either California, Arkansas, or Missouri. The Muddy River drains into Lake Mead. In the western United States, *B. acheilognathi* has been found previously in *N. crysoleucas, P. promelas, C. idella, G. affinis, G. robusta, Rhinichthys osculus* (Girard, 1856), *Lepidomeda mollispinis* (Miller and Hubbs, 1960), *Plagopterus argentissimus* (Cope, 1874), and *Ptychocheilus lucius* (Girard, 1856) (Heckmann et al.,

1986). *Cyprinella lutrensis* is a new host for *Bothriocephalus acheilognathi.* This report expands the range of the Asian fish tapeworm to include the Muddy River in Nevada and suggests a potential for its spread by way of infected baitfish.

Two whole mounts of *Bothriocephalus acheilognathi* have been deposited with the Harold W. Manter collection, University of Nebraska State Museum, voucher numbers HWML 35094 and HWML 35095. Two slides with sections of fish intestine infected with *B. acheilognathi* were also deposited with the same collection, voucher numbers HWML 35096 and HWML 35097.

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Research Note

Acanthocephala of the Virginia Opossum (*Didelphis virginiana*) in Arkansas, with a Note on the Life History of *Centrorhynchus wardae* (Centrorhynchidae)

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ABSTRACT: *Centrorhynchus wardae* was collected from the small intestines in 2 of 8 Virginia opossums (*Didelphis virginiana*) examined from Van Buren County, Arkansas, representing a new host record. Because all reports of this acanthocephalan have been of immature specimens collected from mammals, it appears that these represent aberrant infections. The diverse array of mammalian hosts is indicative of the low degree of host specificity exhibited by this parasite. *Oligacanthorhynchus tortuosa* was found in 10 of 15 opossums examined from Van Buren, Washington, and Yell counties in Arkansas, representing a new geographic distribution record for this parasite in the opossum.

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

The small intestines of 15 Virginia opossums (*Didelphis virginiana*) were examined postmortem for acanthocephalan infections between January and November 1991. The opossums were live-trapped in Van Buren, Washington, and Yell counties in Arkansas. Two species of acanthocephalans were found, *Centrorhynchus wardae* Holloway, 1958, and *Oligacanthorhynchus tortuosa* (Leidy, 1850) Schmidt, 1972.

Centrorhynchus wardae was found in 2 of 8 opossums from Van Buren County, Arkansas, constituting a new host record for this parasite. One male worm and 1 female worm, and a single female worm, respectively, were found in 2 adult male opossums. All worms were immature. Voucher specimens were deposited in the Harold W. Manter Laboratory, University of Nebraska, Lincoln (accession No. HWML 35091). The only previous report of *C. wardae* from Arkansas was by Richardson et al. (1992), who reported 3 specimens of *C. wardae* from 2 of 30 raccoons (*Procyon lotor*) examined from Van Buren County. The opossums and raccoons hosting *C. wardae*

were all collected within a 1-mile radius. The geography of this collection site and the surrounding area was described by Richardson et al. (1992).

Although extensive helminthological surveys have been conducted throughout the southeastern United States, only 10 specimens of *C. wardae* have been reported prior to this study. *Centrorhynchus wardae* was originally described by Holloway (1958), who examined 5 immature worms from the alimentary canal of a spotted skunk (*Spilogale putorius*) from Giles County, Virginia. In addition to those reported by Richardson et al. (1992), single immature specimens have been reported from the small intestine of a gray fox (*Urocyon cinereoargenteus*) in Florida (Conti, 1984) and an aquatic, green frog (*Rana clamitans*) from Pocahontas State Park, Chesterfield County, Virginia (Campbell, 1968).

The specimens recovered in this study conform to the original description of *C. wardae* by Holloway (1958) with regard to the number of longitudinal rows of hooks (34–36); however, slight discrepancies were found in the number of hooks per row, in the number of the large, anterior hooks, and in the size of the thorns at the base of the proboscis. The description of *C. wardae* states that the first 5 hooks are more sturdy and recurved than the posterior ones. In the specimens found in this study, the first 4 or 5 anterior most hooks were larger (57.6 μm $\pm$ 7.6 μm [$\bar{x} \pm$ SE]; range = 40–70 μm; $N = 26$) and more recurved than the posterior ones (42.9 μm $\pm$ 7.8 μm; range = 29–62 μm; $N = 66$). This arrangement is characteristic of *C. conspectus* described by Van Cleave and Pratt (1940). The 17 or 18 hooks per row possessed by the specimens in this study also conform to the description of *C. conspectus* (16–19). *Centrorhynchus wardae* is reported to have 18–20 hooks per row. Aside from the noted exceptions, the specimens recovered in this study most closely resemble *C. wardae*; however, the resemblance of this species to *C. conspectus* warrants mention. The original description of *C. wardae* was based on 5 specimens and should probably be expanded to accommodate 4 or 5 larger, anterior hooks and 17–20 hooks per row. Clearly, the most salient character that distinguishes immature *C. wardae* from *C. conspectus* is the number of longitudinal rows of hooks.

Ward (1940) recovered 11 encysted juvenile acanthocephalans of the genus *Centrorhynchus* from the intestinal wall of a water snake (*Nerodia sipedon*). Her description of these immature worms closely resembles both *C. wardae* and *C. conspectus*. She reported 28–33 longitudinal rows of 18–22 hooks, the first 4 being larger than the posterior ones. These ranges overlap with both *C. conspectus* and *C. wardae*. Unfortunately, critical taxonomic assessment of these 2 species will be possible only when mature specimens of *C. wardae* become available.

All evidence indicates that individuals of the genus *Centrorhynchus* utilize birds exclusively as their natural definitive hosts; however, they have been reported from a number of mammals throughout the world (Van Cleave, 1953). Van Cleave (1953) suggested that these were accidental infections obtained when mammals ingest infected birds or second intermediate hosts. Successful experimental infections of rats with *C. spinosus* (normally a parasite of birds) fed cystacanths taken from a garter snake (second intermediate host), suggest a low degree of host specificity (Read, 1950). The reports of *C. wardae* from 4 families of mammals and 1 amphibian enhance this view. Van Cleave (1953) concluded that the limitation of birds as the normal definitive hosts for *Centrorhynchus* spp. was a result of feeding habits rather than any mammalian physiological condition inimical to the establishment of the worms. The low prevalence of *C. wardae* in mammals suggests that these animals represent accidental hosts for this parasite and probably do not make a significant contribution to its transmission. All reported specimens of *C. wardae* were immature, further supporting the concept of mammals as aberrant hosts for *Centrorhynchus* spp. Although the normal definitive host for *C. wardae* is not presently known, it is probably a bird.

Oligacanthorhynchus tortuosa, a common acanthocephalan of the opossum throughout its range in North America, was found in 10 of 15 opossums examined. This represents a new geographic distribution record for this parasite in the opossum. Voucher specimens have been deposited at the Harold W. Manter Laboratory (accession No. HWML 35092). Among infected opossums, the mean intensity ($\pm$SE) was 16.8 ($\pm$29.3) with a range of 1–99. Seven of 10, 3 of 5, and 2 of 2 opossums from Van Buren, Washington, and Yell counties, respectively, were infected.

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Research Note

Piscicolaria reducta (Hirudinea: Piscicolidae) from Fishes in a Subtropical Florida Stream

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ABSTRACT: The leech *Piscicolaria reducta* was found on the fins of 39 of 114 fishes collected from Blackwater Creek, Florida, from October 1982 to July 1983. Six species of fish were infested: *Pomoxis nigromaculatus, Erimyzon sucetta, Tilapia aurea, Lepomis gulosus, L. auritus,* and *L. punctatus.* The former 5 species and *Micropterus salmoides,* taken in a Hillsborough River collection, represent new host records. For the commonly collected fish species, prevalence (81.8%) and mean intensity (4.4) of *P. reducta* was highest on *L. gulosus,* followed by *L. auritus* (32%, 1.6) and *L. punctatus* (30.6%, 1.7). Considering all fish species, the caudal fin was the most common infestation site (49.4%), followed by the dorsal (23.1%), anal (13.2%), pectoral (9.9%), and pelvic fins (4.4%). This differential distribution on fins may be caused by differences in fin surface areas and movement and placement of fins associated with fish movements.

KEY WORDS: Hirudinea, *Piscicolaria reducta,* prevalence, intensity, survey, freshwater fish, Florida.

Piscicolaria reducta Meyer, 1940, is a widely distributed freshwater leech, reported from 19 states in the United States and 1 province in Canada east of the Rocky Mountains. It has low host specificity and has been found on 19 species in 6 genera and 4 families of fishes (Table 1). The purposes of this paper are to report an extension of the geographical range of *P. reducta* into Florida, report 6 new host records, and describe the prevalence and intensity of this parasite for a fish community in a small stream in west central Florida.

Blackwater Creek (28°10′N) is a tributary of the Hillsborough River and drains land in Hillsborough and Polk counties, Florida. The study site (Sec. 9, R. 21E., T. 27S., Hillsborough Co., Florida) was 4–12 m wide, consisted of a series of shallow (1–2 m deep) pools connected by riffles and contained patches of submergent vegetation (*Egeria densa, Potamogeton illinoensis, Vallisneria neotropicalis*). Riffle substrate consisted of boulders and gravel, whereas sand, silt, and leaves covered the bottom of pools. Fish collections were made in October 1982 and January, February, April, and July 1983. Fish were collected by electroshocking (mean operating time per col-

Table 1. Fishes of North America previously reported to be hosts of *Piscicolaria reducta*.

Host	Locality	Reference
Cypriniformes		
Cyprinidae		
Notemigonus crysoleucas	Maine	Meyer, 1954
Notropis atherinoides	Kentucky	White and Crisp, 1973
Siluriformes		
Ictaluridae		
Ictalurus melas	Kansas	Harms, 1959, 1960
	Georgia	Booth and Aliff, 1978
I. punctatus	Kansas	Harms, 1959, 1960
	Oklahoma	Nagel, 1976
	Kansas	Wetzel, 1982
Perciformes		
Centrarchidae		
Lepomis cyanellus	Michigan	Klemm, 1972
L. macrochirus	New Jersey	Meyer, 1946
	Wisconsin	Petty and Magnuson, 1974
L. punctatus	Georgia	Booth and Aliff, 1978
Percidae		
Etheostoma blennioides	Kentucky	Bauer and Branson, 1975
	Tennessee	Bauer, 1976
	West Virginia	Murray et al., 1977
E. caeruleum	Kentucky	Bauer and Branson, 1975
	Minnesota	Erickson, 1976
	Kentucky	Kozel and Wittaker, 1982
E. stigmaeum	Kentucky	Bauer and Branson, 1975
E. virgatum	Kentucky	Bauer and Branson, 1975
E. zonale	Kentucky	Bauer and Branson, 1975
	Minnesota	Erickson, 1976, 1978
Percina aurantiaca	Tennessee	Bauer, 1976
P. copelandi	Tennessee	Bauer, 1976
P. caprodes	Kentucky	White and Crisp, 1973
	Kentucky	Bauer and Branson, 1975
	Tennessee	Bauer, 1976
	Ohio	White, 1977
	West Virginia	Schramm et al., 1981
P. evides	Kentucky	Bauer and Branson, 1975
	Minnesota	Erickson, 1976
	Tennessee	Bauer, 1976
P. maculata	Kentucky	Bauer and Branson, 1975
	Minnesota	Erickson, 1976
	Tennessee	Bauer, 1976
P. phoxocephala	Illinois	Meyer, 1940; Page and Smith, 1971
	Minnesota	Erickson, 1976
P. sciera	Illinois	Page and Smith, 1970
*	Canada, Ontario	Klemm, 1972
*	Minnesota	Klemm, 1977
	New York	
	Pennsylvania	
*	Arkansas	Klemm, 1982
	Louisiana	
	Massachusetts	

* No hosts cited.

lection—23.0 min), transported alive in individual containers to the laboratory, and measured (standard length to nearest millimeter). Within 2 hr of capture, the external surface, gills, and mouth of each fish were examined for leeches using a dissecting microscope. Leeches were removed, narcotized gradually in increasing concentrations of ethanol, fixed in 10% formalin, and then transferred to 70% ethanol. Representative specimens of *P. reducta* were deposited as

Table 2. Prevalence (%) and mean intensity of *Piscicolaria reducta* on fishes from Blackwater Creek, Florida.*

Fish species	Mean length (mm) ± 1 SD (range)	No. examined	No. infected (prevalence)	Mean intensity ± 1 SD (range)
Erimyzon sucetta	110.0 ± 49.5 (75–145)	2	1 (50.0)	3.0
Lepomis auritus	91.8 ± 17.4 (46–121)	25	8 (32.0)	1.6 ± 1.3 (1–5)
Lepomis gulosus	76.5 ± 18.9 (56–105)	11	9 (81.8)	4.4 ± 2.4 (1–8)
Lepomis punctatus	78.7 ± 17.8 (41–125)	62	19 (30.6)	1.7 ± 1.3 (1–6)
Pomoxis nigromaculatus	100.0	1	1 (100.0)	2.0
Tilapia aurea	76.0 ± 25.1 (50–100)	3	1 (33.3)	1.0

* Fishes on which *P. reducta* was not found: *Amia calva,* 1; *Aphredoderus sayanus,* 1; and *Ictalurus natalis,* 8.

accession No. USNM 151916 in the Hirudinea Collection, National Museum of Natural History, Washington, D.C. 20560.

The terms prevalence and mean intensity follow the definitions of Margolis et al. (1982). The following statistical tests were performed: correlation and Chi-square analyses. Correlation analysis was used to ascertain relationships between prevalence and intensity of infestation and host size. It was also used to determine relationships between host fin size and type and intensity of infection. The distribution of leeches on host fins was examined using Chi-square analysis. Values were considered statistically significant at $P < 0.05$.

Piscicolaria reducta was found on 6 of 9 species of fishes collected in Blackwater Creek (Table 2) as well as on the largemouth bass, *Micropterus salmoides,* taken from a single collection at a downstream site in the Hillsborough River in July 1984. All infested species represent new host records with the exception of *Lepomis punctatus.*

Thirty-nine (34%) of the 114 fish examined were infested with 1 or more leeches. Prevalence and mean intensity for each host are given in Table 2. Among the 3 most commonly collected fish species, *P. reducta* exhibited the highest prevalence (81.8%) and mean intensity (4.4) on the warmouth, *Lepomis gulosus.* Parasite loads were low for all hosts with 54% of infested fish harboring only 1 leech, 15% had 2 leeches, 13% had 3, and 18% had 4 or more leeches.

For other studies in which relatively large numbers of hosts (15+) were examined, prevalences of *P. reducta* varied greatly, ranging from 3% for the channel catfish, *Ictalurus punctatus,* and black bulkhead, *Ictalurus melas,* in Kansas (Harms, 1959) to 70% for the banded darter, *Etheostoma zonale,* in Minnesota (Erickson, 1976, 1978). Mean intensities and ranges reported previously were similar to those found in the present study (Harms, 1959; Page and Smith, 1970, 1971; Bauer and Branson, 1975; Bauer, 1976; Erickson, 1976; Booth and Aliff, 1978; Kozel and Whittaker, 1982). *Piscicolaria reducta* is a host generalist that appears to exhibit low infestation intensities.

To examine the relationship of prevalence of *P. reducta* to fish length, the 2 most commonly collected species were assigned separately to 10-mm length classes except for the largest and smallest individuals (excluded due to small sample size). Sample sizes of *L. gulosus* were too small to test statistically. No significant correlation existed between prevalence and fish size for either *L. punctatus* or *L. auritus.* There was no significant correlation between leech numbers and individual lengths for each of the 3 species of *Lepomis.* Page and Smith (1970) reported that *P. reducta* was more numerous on larger than smaller *Percina sciera.*

Piscicolaria reducta was found only on the fins, generally with its anterior end pointing toward the posterior end of the fish. Considering all fish species, leeches were most commonly located on the caudal fins, followed by the dorsal, anal, pectoral, and pelvic fins (Table 3). Individual host species followed this distributional pattern with minor variations. This difference in leech distribution was significant for *L. punctatus* and *L. gulosus* (Chi-square test = 20.8, 23.5, $P = 0.0001$). Number of leeches on the fins of *L. auritus* were too small to test statistically. Most previous studies have reported this leech infesting the fins only, usually the caudal fin (Page and Smith, 1970, 1971; Bauer and Branson, 1975; Bauer, 1976; Erickson, 1976; Schramm et al., 1981). However, other sites of infestation have been reported: bases of fins and ventral part of head (Harms, 1959; Page and Smith, 1971), gills (Klemm, 1972), and isthmus and mouth (Klemm et al., 1979).

Table 3. Infestation sites of *Piscicolaria reducta* on fishes from Blackwater Creek, Florida. Number of leeches is given with percentage of occurrence in parentheses.

	L. punctatus	*L. gulosus*	*L. auritus*	Others	Total
Pectoral fins	3 (9.4)	5 (12.5)	1 (7.7)	0	9 (9.9)
Pelvic fins	0	0	4 (30.8)	0	4 (4.4)
Dorsal fin	13 (40.6)	8 (20.0)	0	0	21 (23.1)
Anal fin	4 (12.5)	6 (15.0)	1 (7.7)	1 (16.7)	12 (13.2)
Caudal fin	12 (37.5)	21 (52.5)	7 (53.8)	5 (83.3)	45 (49.4)

The differential distribution of *P. reducta* on fins of fishes from Blackwater Creek appears to relate, in part, to differences in fin surface areas. Surface areas were calculated for the fins of 10 *L. punctatus* and 10 *L. gulosus* and the percentages of total fin area were as follows: dorsal, 30.3, 26.5; caudal, 26.0, 25.9; pectorals, 16.7, 19.2; anal, 13.6, 13.5; and pelvic, 13.4, 14.9; respectively. For *L. punctatus,* a significant positive correlation existed between percentage fin area and the number of leeches attached to fins ($r = 0.96$, $P = 0.01$). Larger fins, with greater surface areas, supported more leeches than smaller fins. Although leeches on *L. gulosus* followed this same trend, its correlation was not significant.

Movement and placement of fins during various locomotor activities of fish may also affect the attachment sites of *P. reducta.* Based on percentage surface area, the caudal fins of *L. gulosus* supported more leeches and the dorsal fins fewer leeches than would be expected. The caudal fin may provide the largest, most stable area for attachment since it is the only fin that is not collapsible. Observations of these species of sunfish indicate that individuals periodically collapse the anterior spinous part of the dorsal fin, increasing disturbance and reducing the surface area available for leech attachment. Few infestations occurred on the pectoral or pelvic fins of the species of *Lepomis.* The pelvic fins of these species are often pressed against the body and the pectoral fins are either held in a similar posture or are in near constant motion. Both activities could deter leech attachment.

Among species of *Lepomis,* there was a significant positive correlation between individuals harboring 2 or more *P. reducta* and the number of different fin types involved ($r = 0.72$, $P = 0.002$). More than 1 fin type was infested for 15 of 16 fish, which were parasitized by 2 or more leeches. These preliminary data indicate that spatial partitioning may be involved in the distribution of this leech on its hosts in Blackwater Creek. However, further investigations are needed to resolve this aspect of the ecology of *P. reducta.*

We thank Dr. Donald J. Klemm for confirmation of the identification of *P. reducta,* Dr. Raymond A. Schlueter for fish identifications and assistance in the field, and Mr. Robert Thomas for permission to collect on his property. This study was supported by a University of Tampa Faculty Development Grant to the senior author.

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Research Note

Eubothrium salvelini (Cestoda: Pseudophyllidea) in Brook Trout, *Salvelinus fontinalis,* from West-central Lower Michigan

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ABSTRACT: *Eubothrium salvelini* infected 39 of 166 brook trout collected in 1983 and 19 of 23 brook trout collected in 1991 from Sweetwater Creek in lower Michigan. The mean intensity of *E. salvelini* was higher in brook trout in 1983 than in 1991. The intensity of *E. salvelini* significantly increased with host length in 1983. Gravid *E. salvelini* infected the pyloric ceca. Sweetwater Creek flows into the Pere Marquette River, which empties into Lake Michigan. The occurrence of *E. salvelini* in brook trout in this small creek and its absence in resident brown trout and young salmon in the Pere Marquette River is discussed. The occurrence of *E. salvelini* in brook trout in North America is summarized.

KEY WORDS: *Eubothrium salvelini,* Cestoda, prevalence, intensity, *Salvelinus fontinalis,* brook trout, geographical distribution, Michigan.

The biology, systematics, and geographical distribution of the species in the genus *Eubothrium* Nybelin, 1922, have been discussed by Kennedy (1978), Andersen (1979), Andersen and Kennedy (1983), and Ching and Andersen (1983). During a parasitological survey of trout in west-central lower Michigan, brook trout, *Salvelinus fontinalis* (Salmonidae), were found to be infected with *Eubothrium salvelini* (Schrank, 1790). This note reports on the host–parasite relationships of *E. salvelini* infecting wild brook trout in a small Michigan creek and summarizes the occurrence of *E. salvelini* in brook trout in North America.

One hundred sixty-six ($\bar{x}$ ± SD total length = 111 ± 34, range 52–200 mm) brook trout and 23 (157 ± 16, 113–188) brook trout were collected by electrofishing in August to September 1983 and October 1991, respectively, from Sweetwater Creek. This creek (maximum width = 4.8 m, depth = 0.9 m) is located 4 km east of Branch, Michigan, in Lake County. One (139 mm) brown trout, *Salmo trutta,* and 25 (76 ± 9, 61–95 mm) slimy sculpins, *Cottus cognatus* (Cottidae), were also collected from this creek in October 1991. Fish were brought to the laboratory alive and examined within 36 hr or frozen. Cestodes from fresh fish were relaxed in distilled water, killed in AFA, stained with Mayer's paracarmine, cleared in xylene, and mounted in Canada balsam. Prevalence is the percentage of fish infected, and mean intensity is the mean number of worms per infected fish. Representative specimens of *E. salvelini* have been deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705, as USNM 82383.

The prevalence of *E. salvelini* in brook trout from Sweetwater Creek was higher in 1991 than in 1983, while mean intensity was higher in 1983 than in 1991 (Table 1). The difference in prevalence may involve the length of brook trout examined. Trout infected with *E. salvelini* in 1983 and 1991 had mean total lengths of 133 ± 29, 74–200 mm, and 155 ± 16, 113–188 mm, respectively. One hundred three (87%) of the 118 trout, 120 mm in length or less, examined in 1983 were not infected with *E. salvelini.* Gravid *E. salvelini* occurred in brook trout in every month sampled. The scolex of *E. salvelini* was found in the distal end of the pyloric cecum. Two or more worms never occurred in the same cecum. A significant correlation existed between the intensity of *E. salvelini* and host length in 1983 (Table 1). There were no significant differences in prevalence (Chi-square analysis) and intensity (Student's *t*-test) with respect to host gender ($P > 0.05$). Nongravid *E. salvelini* occurred throughout the intestine of 5 (20%) slimy sculpins. Mean intensity was 2.6 ± 2 (1–6). The single brown trout was not infected with *E. salvelini.*

Sweetwater Creek flows into the Pere Marquette River, which empties into Lake Michigan. Adult chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*Oncorhynchus kisutch*), and steelhead (*Oncorhynchus mykiss*) infected with *E. salvelini* in Lake Michigan migrate into the Pere Marquette River to spawn (Muzzall, 1993). *Oncorhynchus* spp. do not spawn in Sweetwater Creek. Resident brown trout and young chinook salmon, coho salmon, and steelhead from the Pere Marquette River were negative for *E. salvelini.* The presence of *E. salvelini* in adult *Oncorhynchus* spp. in Lake Michigan, its absence in resident brown trout and young salmon in the Pere Marquette River, and its presence in brook trout in Sweetwater Creek indicate that the appropriate intermediate host(s) are present in Lake Michigan and Sweetwater Creek but absent in the Pere Marquette River.

Table 1. Occurrence of *Eubothrium salvelini* in *Salvelinus fontinalis* from Sweetwater Creek in August and September 1983 and October 1991.

Year	Number examined	Prevalence	Mean intensity ± 1 SD (range)	r^2
1983	166	24	3.5 ± 4.0 (1–16)	0.537*
1991	23	83	2.2 ± 1.4 (1–6)	0.347

* $P < 0.01$.

Eubothrium salvelini is widespread throughout North America and infects a variety of salmonids (Hoffman, 1967; Margolis and Arthur, 1979). In the United States, *E. salvelini* has been found in brook trout from Michigan (Cooper, 1918), the present study, New York (Hunter and Hunter, 1930), and Maine (Meyer, 1954). Harrietta, Michigan, where Cooper's study occurred, is approximately 48 km northeast of Sweetwater Creek and is in another drainage system.

In Canada, *E. salvelini* has been found in brook trout from British Columbia (Mudry and Anderson, 1977), Ontario (MacLulich, 1943; Bangham and Venard, 1946), James Bay (Wardle, 1932, 1933), Quebec (Richardson, 1936, 1937; Lyster, 1940; Choquette, 1948; Fantham and Porter, 1948; Worley and Bangham, 1952; Hanek and Molnar, 1974; Black, 1981; Albert and Curtis, 1991), New Brunswick (Frimeth, 1987a, b), Labrador (Hicks and Threlfall, 1973; Chinniah and Threlfall, 1978), and Newfoundland (Sandeman and Pippy, 1967; Threlfall and Hanek, 1970; Cone and Ryan, 1984; Marcogliese and Cone, 1991a, b). Ching and Andersen (1983) suggested that the *E. salvelini* found in northern squawfish, *Ptychocheilus oregonensis,* from northwestern Canada by Kuitunen-Ekbaum (1933) and Bangham and Adams (1954) were *E. tulipai.*

Brook trout primarily occur in northeastern North America from the Atlantic seaboard south

to Cape Cod, in the Appalachian Mountains, west in the upper Mississippi and Great Lakes drainages to Minnesota, north to Hudson Bay (Scott and Crossman, 1973). Interestingly, *E. salvelini* has been found in brook trout in many more localities in Canada than in the United States. Could this indicate that *E. salvelini* (or its intermediate host) is more common in Canada than in the United States, or the lack of thorough parasitological studies on brook trout in the United States?

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Research Note

The Presence of *Udonella ophiodontis* in Washington and of *U. caligorum* in British Columbia

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ABSTRACT: The symbiotic flatworm, *Udonella ophiodontis*, was found on copepods, *Lepeophtheirus pravipes* Wilson, 1912 from lingcod, *Ophiodon elongatus* Girard, and on *L. hospitalis* Fraser, 1920 from starry flounder, *Platichthys stellatus* (Pallas) in Washington. The more commonly reported species, *U. caligorum*, was found on *Lepeophtheirus bifidus* Fraser, 1920 and *L. parviventris* Wilson, 1905 on rock sole, *Lepidopsetta bilineata* (Ayers, 1855), and on *L. hospitalis* on starry flounder in British Columbia.

KEY WORDS: *Udonella ophiodontis, Udonella caligorum*, copepod hosts, British Columbia, Washington.

On the theme of "Big fleas have littler fleas," the genus *Udonella* is of particular interest to parasitologists because its members are symbionts on copepods, which are themselves parasites of marine fishes. The systematic position of *Udonella* has been controversial with placement among the monogeneans, in a separate class, or within an order of the Turbellaria as discussed by Beverly-Burton (1984). There are no records of these symbionts from ectoparasitic copepods on fishes of the Canadian Pacific coast of North America (Beverly-Burton, 1984). The lack of records is surprising because Fraser (1920) named the caligid copepod *Lepeophtheirus hospitalis* "on account of the species being host to so many parasites." Fraser commented on the scores of copepods which he found that were covered with parasites and the numerous infected copepods on fishes in the Vancouver Island area. Two species of *Udonella* have been reported in Washington in waters adjacent to British Columbia. Kay (1945) described a new species, *Udonella ophiodontis* from *Lepeophtheirus* sp. from the buccal cavity of lingcod, *Ophiodon elongatus* Girard. Schell (1972) described the early development of *U. caligorum* from eggs taken from *L. hospitalis* on starry flounder, *Platichthys stellatus*

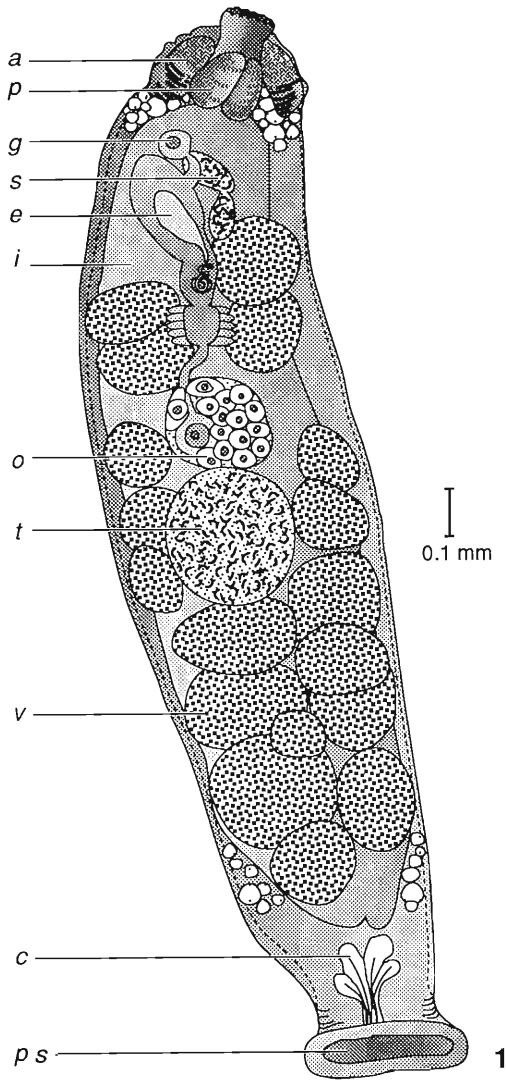

Figure 1. Whole mount, ventral view, of *Udonella ophiodontis* from the copepod *Lepeophtheirus pravipes* from lingcod, *Ophiodon elongatus* in Washington. U.S.N.M. Helminthol. Coll. No. 82442. Abbreviations: a, anterior haptor; c, caudal glands; e, egg; g, genital pore; i, intestine; o, ovary; p, pharynx; ps, posterior sucker; s, seminal vesicle; t, testis; v, vitellarium.

(Pallas). The purpose of this study is to compare the morphology of the 2 species and verify their presence in Pacific waters.

Specimens of *Udonella* spp. were studied from the authors' collections from Washington and British Columbia, the U.S.N.M. Helminthological Collections, and the Harold W. Manter Laboratory Collections. Specimens collected at Friday Harbor, Washington, were fixed in AFA (alcohol–formalin–acetic acid), stained in hematoxylin, and mounted in Permount. When specimens were taken from copepods preserved in 70% alcohol, they were flattened and cleared for study using 95% acetic acid and 5% formalin. Measurements are given in μm unless stated otherwise.

Three specimens of *Udonella* sp. collected from caligid copepods from Washington were compared with 4 specimens on a slide labeled *Udonella socialis,* collected by Linton in 1910 from Dry Tortugas, Florida, U.S.N.M. Helminthol. Coll. No. 8537. Price (1938) considered *U. socialis* to be a synonym of *U. caligorum.* Specimens from this slide were used as the basis for Price's description of *U. caligorum.* The morphology of the specimens from Washington was quite different from *U. caligorum* and similar to the description of *Udonella ophiodontis* by Kay (1945). The most striking difference was the size of the ovary in relation to the testis, which was smaller in *U. ophiodontis* and much larger in *U. caligorum.* The anterior end of *U. ophiodontis* had fewer surface annulations, proportionately smaller anterior haptors, smaller, rounder pharynx, a submarginal genital pore, a bilobed seminal vesicle, and clumped arrangement of caudal glands (Fig. 1). Measurements of 6 specimens of a species of *Udonella* collected from *L. hospitalis* from Washington (H.W.M.L. No. 23888) and figures of the adult and developing stages described by Schell (1972, 1985) as *U. caligorum* matched the description of *L. ophiodontis.* Differences between the 2 species are summarized in Table 1. There may also be differences in the proportion of the gonads in the development and maturation stages. Price (1938) observed that young worms had a testis that was larger than the ovary, a condition verified in young specimens on the slide. Mature specimens had gonads reversed in size with the ovary larger than the testis in *U. caligorum.* Schell (1972) observed differences in the process of gonad maturation in what he called *U. caligorum* in that both testis and ovary appeared at the same time, and in the mature state the testis was larger than the ovary.

New and corrected records

Udonella ophiodontis

1. *Lepeophtheirus pravipes* Wilson, 1912 from the buccal cavity of lingcod, *Ophiodon elongatus,* collected from Friday Harbor, Washington, by Ching, June 1957.

Table 1. Summary of differences between *Udonella ophiodontis* and *U. caligorum*.*

	U. ophiodontis	*U. caligorum*
Body length (mm)	2–2.8	1.1–1.4
Body width	303–743	155–262
Pharynx	164–205 × 131–164 (N = 3)	150–152 × 85–95
Genital pore	Submarginal	On left margin
Testis (transverse diameters)	189–286	76–95
Ovary (transverse diameters)	135–270	133
Ratio of testis/ovary	1:0.7–1:0.9	1:1.7
Egg	147–155 × 74 (N = 2)	133 × 42
Caudal glands	Clumped	2 separate groups
Posterior sucker	245–327	187–210

* Ranges of *U. ophiodontis* based on measurements of 9 specimens unless noted otherwise; measurements of *U. caligorum* taken from Price (1938). All measurements in μm unless stated otherwise.

2. *Lepeophtheirus hospitalis* Fraser, 1920 from *Platichthyes stellatus,* collected near San Juan Island, Washington, by Schell (1972).

Udonella caligorum

1. *Lepeophtheirus bifidus* Fraser, 1920; prevalence of 64/195 or 32.8%.

2. *Lepeophtheirus parviventris* Wilson, 1905; prevalence of 1/26 or 3.9%. *Lepeophtheirus bifidus* and *L. parviventris* were collected from 12 of 31 *Lepidopsetta bilineata* (Ayers, 1855), from Bamfield, British Columbia, Canada, by Leighton in 1980. None of 10 *L. hospitalis* on the fish host was infected including 6 in coinfections with *Udonella*-infected *L. bifidus.*

3. *Lepeophtheirus hospitalis* Fraser, 1920 on *Platichthyes stellatus,* Tsawwassen, British Columbia, Canada, by Leighton in 1982.

Although the presence of *U. caligorum* has been documented, recent searches for other species in British Columbia have been unsuccessful. *Lepeophtheirus breviventris* was collected from lingcod in March 1989, and September 1991, and except for 1 egg attached to 1 copepod, 78 were free of udonellids. Twelve *Lepeophtheirus pravipes* from the fall sampling were also negative.

The report of *U. caligorum* and description of a new species, *Udonella murmanica,* by Kornakova and Timofeeva (1981) appear to differ considerably from the European and North American concepts of the type species. The measurements and figures of both species show much larger, robust flatworms with gonads in the proportions described for *U. ophiodontis.* The clumped arrangement of the caudal glands is also what we have observed for *U. ophiodontis.* The figures and descriptions lack precise information on the anterior surface annulations, anterior suckers, nature of the vitellarian follicles, and nature of the seminal vesicle. Kornakova and Timofeeva (1981) have suggested that *U. caligorum* is a species complex. From the evidence presented here, there are at least 2 species in northeast Pacific waters. We suggest from our data on prevalences that there may be complex interactions between the 2 species, their copepod hosts and their fish hosts, which dictate their distribution and which may be influenced by seasonal and other physical factors. Schell (1972) and our data showed that several species of ectoparasitic copepods may be present on fish but only 1 or 2 harbor worms of the genus *Udonella.* One caligid copepod such as *L. hospitalis* may be host for 2 species as was found in Washington and British Columbia or be negative depending on the abundance of other species of *Lepeophtheirus* present on the fish.

Mary Lou Pritchard kindly measured specimens of *Udonella ophiodontis* deposited in the Manter Laboratory by Schell. Claudia Hand collected caligid copepods from lingcods; Bob Bandoni, Andy Lamb, and Suzanne Spohn provided lingcods for examinations.

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Research Note

Endoparasites of the Bird-voiced Treefrog, *Hyla avivoca* (Anura: Hylidae), from Arkansas

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ABSTRACT: Sixty-one juvenile and adult bird-voiced treefrogs, *Hyla avivoca* Viosca, 1928, were collected from 8 counties of central and southern Arkansas and examined for endoparasites. Thirteen (21%) of the frogs were found to be infected with 1 or more parasites, including 13 of 13 with *Tritrichomonas augusta,* 10 of 13 (77%) with *Opalina* sp., 4 of 13 (31%) with *Nyctotherus cordiformis,* 1 of 61 (2%) with *Megalodiscus temperatus,* 3 of 61 (5%) with *Cylindrotaenia americana,* 4 of 61 (7%) with *Batracholandros bassii,* 2 of 61 (3%) with *Abbreviata* sp., and 5 of 61 (8%) with *Oswaldocruzia* (*Oswaldocruzia*) *pipiens.* All represent new host records for the respective parasites.

KEY WORDS: *Abbreviata* sp., Anura, *Batracholandros bassii,* bird-voiced treefrog, *Cylindrotaenia americana, Hyla avivoca,* Hylidae, intensity, *Megalodiscus temperatus, Nyctotherus cordiformis, Opalina* sp., *Oswaldocruzia pipiens,* prevalence, survey, *Tritrichomonas augusta.*

The bird-voiced treefrog, *Hyla avivoca* Viosca, 1928, is a small anuran that ranges within the Mississippi River and Gulf Coast drainage systems from extreme southern Illinois to South Carolina, Georgia, and Florida westward to parts of Arkansas and Oklahoma (Smith, 1966; Conant and Collins, 1991). The species generally inhabits permanent wooded swamps comprised of tupelo-cypress, birch, and buttonbush. Although much is available on the natural history of related hylids, the biology of *H. avivoca* is not well-known (Trauth and Robinette, 1990; Jamieson et al., 1993), and only 1 report has been published on parasites of this frog. Reiber (1941) reported *Oswaldocruzia waltoni* Ingles, 1936 from *H. avivoca* from Reelfoot Lake, Tennessee, a species inquirenda according to Baker (1987). We report the identity and prevalence of endoparasites infecting *H. avivoca* from southern and central Arkansas.

During May and June 1990 and again between May and July 1991, 61 juvenile and adult *H. avivoca* (58 males, 3 females, mean ± snout–vent length [SVL] = 36.3 ± 0.5, range 31–48 mm) were collected by hand in swampy habitat from 8 counties in central (35°05′N, 92°26′W) and southern (33°19′N, 92°32′W) Arkansas. Of these, a subset of 13 frogs (all males, 36.4 ± 0.7, 33–41 mm SVL) were collected during July 1991 from Conway (N = 6) and Lafayette (N = 7) counties and examined for protozoans, and all 61 were examined for helminths. Frogs were examined within 48 hr of capture. Detailed methods for examining and processing hosts and preparing and staining parasites are identical to those provided by McAllister et al. (1989).

Voucher specimens of parasites are deposited in the U.S. National Museum Parasite Collection (USNM), USDA, Beltsville, Maryland 20705. Voucher specimens of hosts are deposited in the Arkansas State University Museum of Zoology

(ASUMZ 16003–16015, 16080–16081, 16409–16411, 17733–17738, 17767–17769, 17800–17813, 17815–17821, 17920–17932).

All 13 *H. avivoca* examined for protozoans harbored at least 1 species, and 13 of 61 (21%) were infected with helminths (Table 1). None of the frogs was infected with apicomplexan or trypanosomal parasites in the blood, the intestinal contents and feces were negative for coccidia, and the gallbladder did not contain myxozoans.

The cosmopolitan flagellate, *Tritrichomonas augusta* Alexeieff, 1911, was commonly found in the colon and rectum of *H. avivoca*. This protozoan has been reported previously from numerous anurans, including the canyon treefrog, *Hyla arenicolor*; green treefrog, *H. cinerea*; mountain treefrog, *H. eximia*; squirrel treefrog, *H. squirella*; gray treefrog, *H. versicolor*; spring peeper, *Pseudacris* (syn. *Hyla*) *crucifer*; Pacific chorus frog, *P.* (*H.*) *regilla*; spotted chorus frog, *P. clarkii*; Brimley's chorus frog, *P. brimleyi*; and the western chorus frog, *P. triseriata* (Walton, 1964; McAllister, 1991).

Endocommensal *Opalina* sp. not identifiable to species were observed in the colon of *H. avivoca*. *Opalina hylaxena* Metcalf, 1923, has been previously reported from closely related *H. versicolor* from Michigan, Massachusetts, Indiana, and Georgia (Metcalf, 1923). In addition, an *Opalina* sp. was reported by Metcalf (1923) from Cope's gray treefrog, *H. chrysoscelis* in Texas; however, he noted that 3 *H. chrysoscelis* from Hot Springs, Arkansas, were negative for opalinids. Other opalinids have been reported from other species of *Hyla* (see Carini, 1937; Metcalf, 1940).

The ciliate *Nyctotherus cordiformis* Ehrenberg, 1838, also infected the colon of *H. avivoca*. This ciliate has been reported previously from *H. versicolor* (Walton, 1947) and numerous other hylids (Walton, 1964; McAllister, 1987).

A single paramphistomatid digenean, *Megalodiscus temperatus* (Stafford, 1905) Harwood, 1932, was found in the cloaca of an adult male *H. avivoca* from an unnamed slough near Blackwell, Conway County. Other hylid hosts include *H. chrysoscelis* from Nebraska (Brooks, 1976), *H. cinerea* from Texas (Harwood, 1932), *H. eximia* from Mexico (Bravo-Hollis, 1941), and *P. crucifer* from Michigan (Najarian, 1955). Brooks (1976) provided a summary of the amphibians reported to harbor this worm.

Adult nematotaeniid cestodes, *Cylindrotaenia americana* Jewell, 1916, were recovered from the small intestine of 3 *H. avivoca* from an unnamed slough near Blackwell and Cox Creek Lake in Grant County; mean intensity was 3.3 ± 1.5 (range 1–6) worms. A total of 7 hylids, 3 of which are in the genus *Hyla*, have been reported previously as hosts of *C. americana*, including the european hylid, *H. arborea* from Czechoslovakia, *H. arenicolor* from Utah, and *H. squirella* from Texas (see McAllister, 1991).

Table 1. Parasites found in *Hyla avivoca* from Arkansas.

Parasite	USNM Helminth Collection Number	Prevalence*
Protozoa		
Mastigophora		
Tritrichomonas augusta	82024–82025	13/13 (100%)
Opalinata		
Opalina sp.	82024	10/13 (77%)
Ciliophora		
Nyctotherus cordiformis	82025	4/13 (31%)
Platyhelminthes		
Trematoda		
Megalodiscus temperatus	82023	1/61 (2%)
Cestoidea		
Cylindrotaenia americana	82021–82022	3/61 (5%)
Nematoda		
Abbreviata sp.	82066	2/61 (3%)
Batracholandros bassii	82065	4/61 (7%)
Oswaldocruzia pipiens	82020	5/61 (8%)

* Number infected/number examined (%).

A single pharyngodontid nematode, *Batracholandros bassii* (Walton, 1940) Petter and Quentin, 1976, was found in the colon of 4 frogs (3 males, 1 female) collected from Calion Lake in Union County, Cox Creek Lake in Grant County, and an unnamed slough near Blackwell. The species was described originally by Walton (1940) as *Pharyngodon bassii* from Cuban treefrogs, *Osteopilus septentrionalis*. Since then, additional frogs have been listed as hosts, including Puerto Rican crested toads, *Peltophryne* spp., bullfrogs, *Rana catesbeiana*, and tropical frogs, *Eleutherodactylus* spp. (Barus and Moravec, 1967; Barus, 1972, 1973; Coy Otero and Ventosa, 1984).

Two third-stage *Abbreviata* sp. were found in the stomach of each of 2 frogs (male and female) collected from Calion Lake and an unnamed

slough near Blackwell. Numerous authors have reported physalopteroid larvae from North American frogs (see McAllister and Freed, 1992). *Abbreviata* (syn. *Physaloptera*) *ranae* (Walton, 1931) Morgan, 1941, thought to be a valid species from frogs, has been designated as species inquirenda by Baker (1987).

Five specimens of *Oswaldocruzia* (*Oswaldocruzia*) *pipiens* Walton, 1929, were recovered from the small intestine of 5 *H. avivoca* (3 males, 2 females) collected from the aforementioned locales and Lake Erling, Lafayette County. This nematode is a common parasite of amphibians and reptiles ranging from Canada to southern Texas, including *P. crucifer, P. streckeri, P. triseriata, H. cinerea,* and *H. versicolor* (Baker, 1977, 1987; McAllister, 1987).

In summary, several new host records are reported for parasites of *H. avivoca* from Arkansas. Unfortunately, prevalence of infection could not be compared between the sexes due to a paucity of females. However, *H. avivoca* is infected by a parasite fauna typical of that reported previously for other hylid frogs. This is interesting given that the diet of frogs reported herein, unlike other species of *Hyla,* consisted primarily of *Cremastogaster* ants (Jamieson et al., 1993). Therefore, interpretation of the ecological relationships of *H. avivoca* parasites and potential intermediate hosts will have to await additional study of bird-voiced treefrogs collected outside the breeding season along with sympatric congeners. Indeed, these parasites may not be typical of this treefrog population in general, as there was a conspicuous absence of ground-dwelling arthropods in their diet compared to other species of *Hyla* (Jamieson et al., 1993).

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The Helminthological Society of Washington 629th Meeting (With Trustees of the Brayton H. Ransom Memorial Trust Fund)

University of Maryland—October 14, 1992

Introductory Comments

"Ladies and Gentlemen:

I think it would be appropriate, since this meeting is with the trustees of the Ransom Memorial Trust Fund, to first tell you who the trustees are. There are currently the usual 5 regular Trustees and 2 Trustees Emeritus. The regular ones are: Ralph Lichtenfels; Robin Huettel; Nancy Pacheco; Harley Sheffield, Secretary-Treasurer; and Morgan Golden, Chairman. The Trustees Emeritus are: Aurel Foster and Gilbert Otto, both of whom stepped down earlier this year. However, we quickly elected them to their present emeritus status so that we could continue to benefit from their wisdom, valuable opinions, and sometimes even lively discussions on matters of business before the trustees.

We are very pleased to have this opportunity to meet with the Helminthological Society of Washington tonight. We hope to (1) give you a better insight about Brayton H. Ransom (who he was, what he did, etc.); (2) provide some details about the Ransom Memorial Trust Fund (when established, objectives, etc.); and (3) pay tribute to two of our outstanding colleagues who have given long and dedicated service to the Ransom Memorial Trust Fund and, I should add, to the Helminthological Society of Washington and the science of parasitology also."

A. MORGAN GOLDEN, Chairman
Ransom Trustees

History of Brayton H. Ransom and the Memorial Trust Fund

When Chairman Morgan Golden recently asked me to serve as a trustee of the Ransom Fund, I decided that I should find out more about Ransom himself as well as the purpose of the trust fund. I would like to share what I learned with you this evening. (A special thanks to Morgan Golden who supplied copies of all his material on Ransom and to HelmSoc archivist Pat Pilitt and custodian of back issues Ralph Lichtenfels for early Society information.)

I found much of the following information on Dr. Ransom in his obituary published in *Science* in 1925 and written by Maurice C. Hall. Brayton Howard Ransom was born in Missouri Valley, Iowa, on March 24, 1879, and educated in the public schools of Bancroft, Nebraska. He attended the University of Nebraska and received a B.S. in 1899, an M.A. in 1900 and a Ph.D. in 1908. He was a fellow in zoology at the University of Missouri from 1900 to 1901 and at the University of Nebraska from 1901 to 1902. In 1902, he came to Washington as assistant in zoology in the Hygienic Laboratory of the Public Health and Marine Hospital Service and the following year succeeded Dr. Charles Wardell Stiles in charge of the Zoological Laboratory of the Bureau of Animal Industry. At 25, he was the youngest to hold this position. In 1906, he was made chief of the laboratory and, at that time, it became the Zoological Division. Under Dr. Ransom's leadership, the Zoological Division developed to great importance in the organization of the Bureau of Animal Industry contributing to the solution of many important practical and purely scientific problems pertaining to parasitology and related subjects and thereby achieving a worldwide reputation. In the solution of these problems, Dr. Ransom played the most conspicuous role; his own researches in parasitology constituted an enviable record of scientific accomplishment. His work dealt largely with the morphology, taxonomy, and life history of parasitic worms and with the practical application of facts to the prevention of parasitic diseases in man and in domestic animals.

He did monographic systematic work on parasites and established basic facts in the life histories of such important parasites as *Ascaris*, *Haemonchus* and *Strongyloides*. He first found in the United States many of our economically important parasites and contributed to our

knowledge of the true pathological conditions or causes in the case of infestations with *Davainea echinobothrida, Cooperia punctata, Syngamus trachea,* and *Ascaris lumbricoides.* He developed measures for the control of stomach worms in sheep and originated and developed the famous swine sanitation system popularly known as the McLean County System. He also developed the basic USDA regulations for the control of parasites, especially trichinae and cysticerci, through the meat inspection service, and established some of the fundamental facts on which dipping for cattle ticks is based. His bibliography of over 160 titles represents a quarter century of productive work. He was not a dabbler and his most prominent characteristics were his extreme thoroughness and carefulness. An obituary in the *Journal of the Washington Academy of Sciences,* vol. 15, 1925, states, "Richly endowed with a healthy scientific curiosity, with resourcefulness, thoroughness and the ability to apply himself unswervingly to the solution of baffling problems, he brought these qualities to bear on his work, his writings being thoughtful, finished, and scholarly productions. His many charming personal qualities, his unassuming dignity, his thoughtful consideration for the feelings of others, his high sense of justice, and his frankness are reflected in his scientific papers which are singularly free from personal criticism, from unwarranted conclusions, and are liberal in acknowledging the contributions of other scientific workers."

As an executive, Dr. Ransom was a man of vision in his attitude toward his problems and was just, considerate, and generous toward his associates in the laboratory. Under his supervision the Zoological Division had a steady and healthy growth from the time he took charge and, at the time of his death, Dr. Ransom had a technical staff of 6 associates in Washington and 4 technical associates in charge of as many field projects at various places in the United States.

Dr. Ransom died in 1925 after a short illness. He was only 46 years old, a comparatively young man, but in the short space of that brief lifetime he had crowded more of valuable achievement than most of us may hope for in what Hall (1925) called "the biblical allotment of three score years and ten." In the scope comprehended in his investigations and in his grasp of the broad field of veterinary parasitology, he was quite unusual. His death was a personal loss to all his staff. They were devoted to his interests, deeply concerned when his health and life were imperiled, and maintained throughout a high morale consistent with the obligations imposed by his kindly treatment and intelligent supervision.

In a centenary biographical note on Ransom, Aurel O. Foster (1980) pointed out that he had met every chief of the Zoological Division except Ransom and had gathered much of his information from Ransom's colleagues who emphasized their special and personal high regard for him as a colleague, friend, and supervisor. He learned that Ransom, despite achievements and rewards that normally bespeak personal fulfillment and modest internal satisfaction, lived with the tragic personal burden of a disease contracted during college hazing which eventually led to his death.

Ransom's sound counsel and scientific achievements were widely recognized among scientific groups. He was the U.S. delegate to several international congresses. He was on the editorial boards of the *American Journal of Tropical Medicine* and the *Journal of Parasitology* which he helped establish. He was a member of at least 14 scientific societies in this country as well as being a foreign correspondent of two European societies. He served as president of the American Microscopical Society and secretary-treasurer of the American Society of Tropical Medicine. He was one of the founders of the American Society of Parasitologists in 1925 and served on its first council. As chief of the Zoological Division, he became Honorary Assistant Custodian of the Helminthological collections of the U.S. National Museum. In recognition of his work on ascarids he received the Gold Medal of the Seaman's and Tropical Diseases Research Association of Kobe, Japan.

Dr. Ransom was one of the 13 charter members of the Helminthological Society of Washington in 1910. He was elected the 3rd president of the Society in 1917 and served through 1920. He was the first representative of HelmSoc to the Washington Academy of Sciences from 1922 to 1925.

The Brayton H. Ransom Memorial Trust Fund

Following Dr. Ransom's death, the Helminthological Society of Washington deemed it fitting that his memory should be perpetuated because of his valuable contributions to the study and control of parasites. A committee composed of Charles Wardell Stiles, Maurice Hall, Eloise Cram, and W. W. Cort was appointed to solicit

funds. Friends and colleagues residing in all parts of the world contributed a total of $1,020.93 to create a memorial in honor of Dr. Ransom. Contributors also made suggestions as to the form that this memorial should take.

In October, 1935, a second committee composed of Gotthold Steiner, chairman, Paul Bartsch, Eloise Cram, Gerard Dikmans and Gilbert Otto was appointed to set up the trust. HelmSoc appointed Eloise Cram, Gerard Dikmans, Gilbert Otto, E. W. Price and Gotthold Steiner as trustees and the money was turned over to them. They were instructed to use the income from the fund but in no case was the principal to be used.

The use of the income was left to the discretion of the trustees as long as that use fulfilled the general purpose of perpetuating the memory of Dr. Ransom and the advancement of the science of parasitology and related sciences. Suggested possibilities included presenting prizes at intervals, either in the form of money or medals, publishing worthy writings, subsidizing in part or in whole a journal or other publication, disseminating knowledge, or granting scholarships.

The first use of the interest from the Ransom Fund was, appropriately, token support of a struggling new journal, namely the *Proceedings of the Helminthological Society of Washington.* The cover of our journal carries the words: "Supported in part by the Brayton H. Ransom Memorial Trust Fund". These first appeared on Vol. 6, No. 1 in January of 1939. (A little bit of trivia: Vol. 1, No. 1, of the *Proceedings* was published in 1934 and, at that time, the subscription price was $1.00 and the foreign rate was $1.25.) This support has continued and increased, not only for the *Proceedings* itself, now the *Journal,* but also to aid in publication of meritorious manuscripts by authors lacking institutional or other backing. In 1960, the trustees established a commemorative award in recognition of excellence and achievement in parasitology, designated the Brayton Howard Ransom Memorial Award. It has been awarded only once, to James H. Turner, at a banquet on October 8, 1960, on the occasion of the Fiftieth Anniversary of HelmSoc. The award was presented by Gilbert Otto who reviewed the life of Ransom and the trust fund at that time. The Ransom Fund also, on one occasion, loaned money to a needy parasitologist who temporarily signed over the cash value of a life insurance policy as collateral.

In 1936, there was $1,299.91 in the Ransom Fund which included the original contributions plus interest of $290.73 minus expenses of $11.75. In 1956, there was more than $1,700 and $55.00 was distributed. By 1983, when Morgan Golden published an updated report, there was $6,100.00 and distributions for that year were $156.00. Last year, $500.00 was distributed: $50.00 for the HelmSoc Journal, $50.00 for annual membership in the American Association for Zoological Nomenclature, and $400.00 to help pay page charges in the Journal for 3 scientists. As of January 1, 1992, there was over $12,500 in the Ransom Fund.

At a recent HelmSoc executive committee meeting, Journal editor Ralph Eckerlin reported an increase in requests for Ransom Fund support because of the withdrawal of funds for page charges by some U.S. universities. While the purpose of tonight's meeting was not to solicit donations for the Ransom Fund, in the present economic circumstances of lower interest rates and increased need, any contributions would be welcome. On our dues request statements, there is now a line for contributions to the Ransom Fund.

The Brayton H. Ransom Memorial Trust Fund has been in existence for 56 years. During that time, there have been a total of 13 trustees, 5 serving at a time. Ten of the 13 trustees have resigned. The average length of time served by these 10 was 24 years with the shortest term 8 years and the longest, 56 years. There have been 4 chairmen: Gotthold Steiner for 30 years, Gilbert Otto for 17 years, Aurel Foster for 6 years, and Morgan Golden for the past 13 years. There have been 5 secretary-treasurers: Eloise Cram for 28 years, Aurel Foster for 19 years, Lloyd Rozeboom for 1 year, Morgan Golden for 5 years, and Harley Sheffield for the past 13 years.

In the spring of 1992, trustees Morgan Golden, Chairman, Ralph Lichtenfels and Harley Sheffield reluctantly accepted the resignations of Gilbert Otto and Aurel Foster as trustees and promptly elected them as trustees emeriti. Gilbert Otto had served as a trustee since 1936, 56 years, and Aurel Foster since 1953, 39 years. Subsequently, Robin Huettel and I were elected to replace them; an impossible task. I have been overwhelmed to learn of the dedication of those men and women who contributed to and set up the Brayton H. Ransom Memorial Trust Fund originally, as well as that of the trustees who have served over the years.

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NANCY D. PACHECO

RECOGNITION OF AUREL OVERTON FOSTER

Dr. Aurel Foster (left) receiving the Certificate of Appreciation from Dr. Morgan Golden.

Thank you, Morgan.

Ladies and gentlemen, honored guests, tonight we honor Dr. Aurel O. Foster primarily for his long-term service to the Brayton H. Ransom Memorial Trust Fund. But we also take this opportunity to celebrate some of his other outstanding contributions to the broader parasitological community.

For the preparation of my remarks, the published text of the 1970 Helminthological Society Anniversary Award to Dr. Foster by Buddy Diamond was an excellent reference.

Dr. Foster was born in Marathon, New York, September 25, 1906. He received an A.B. and an M.A. from Wesleyan College in 1929 and 1930. He moved to Johns Hopkins School of Hygiene where he studied under W. W. Cort and met his longtime friend, Dr. Gilbert F. Otto, who was then an instructor at Hopkins. Dr. Foster married his lovely wife Margie in 1931. They celebrate 61 years together this year. Dr. and Mrs. Foster are the parents of Jeanne and Richard and have 4 grandchildren.

Dr. Foster's research at Johns Hopkins used the dog and cat hookworm as a model of hookworm disease. One of his earliest papers, "The effect of diet on hookworm infestation in dogs", appeared in *Science* in 1931. Dr. Foster received a Doctor of Science degree from Johns Hopkins in 1933.

In 1934 he moved to the Gorgas Memorial Laboratory in Panama where for 5 years he studied parasites of horses. The scientific team that went to Panama in 1934 included Dr. Foster's longtime friend, Dr. Lloyd E. Rozeboom. During the 5-year period in Panama, Dr. Foster apparently established a world record for the number of horses and mules necropsied for worms. His papers on helminths of horses to this day provide the most complete study of natural infections of equines. The specimens he collected are in the U.S. National Parasite Collection and will continue to provide information to parasitologists of future generations.

Dr. Foster left Panama in 1939 to join the Bureau of Animal Industry, the precursor of the Agricultural Research Service (ARS). From 1940 to 1960 Dr. Foster's research centered in parasite control. He became Leader of Chemotherapy Investigations, and in 1960 was appointed Director, Beltsville Parasitological Laboratory. From 1960 until he retired in 1971, Dr. Foster was probably the most influential parasitologist in the country. Leader of 44 scientists and 75 support staff members at Beltsville, he was also responsible for national leadership of ARS parasitology programs in Auburn, Tifton, Albuquerque, Las Cruces, and Pullman. He was the last parasitologist to have leadership responsibility for the entire ARS parasitology program.

During this period he became President of the American Society of Parasitologists (ASP) in 1959 and President of the Second International Congress of Parasitology in Washington in 1970. After the International Congress, Dr. Foster and his co-honoree tonight, Dr. Otto, who was Secretary General of the Congress, were able to pre-

sent to the American Society of Parasitologists $27,500 resulting from a $2,000 investment by ASP. This was the largest ever donation to the Stoll-Stunkard Endowment Fund. At the time (1974) it almost doubled the value of the fund. This fund is now worth more than $200,000 and many ASP functions are possible only because of it. This is a little-known contribution of Drs. Foster and Otto to parasitology.

In 1971, Dr. Aurel Overton Foster had it all! He was at the top of his profession, and with no mountains left to climb, he retired. When some of us asked him what he would do next, he said, "I'm going to Disney World!" Actually, he said he would become a male hooker, but there is no evidence that Mrs. Foster has allowed that to happen.

Dr. Foster's long association with HelmSoc goes back to his days in graduate school at Johns Hopkins. While at Beltsville he was a strong supporter, serving in every capacity except Editor and receiving every award HelmSoc can bestow on a member.

My personal impression of the man relates to my observation of the questions that he has for almost every speaker at HelmSoc presentations, or at a dissertation defense (Dr. Foster sat on my examination panel at Maryland), or in discussions with colleagues or visiting scientists. 1) He always has a penetrating question; and, 2) His questions are not designed to demonstrate his knowledge, but are superbly phrased and designed to provoke thought and further research in the pursuit of knowledge.

I suggest that one of the reasons for Dr. Foster's success as a scientist and administrator is his ability to ask the right questions!

Tonight we honor Dr. Foster for 39 years of service to the Brayton H. Ransom Memorial Trust Fund. He served as a Trustee (1953–1992), Secretary-Treasurer (1956–1973) and Chairman (1973–1979). Dr. Foster, along with his friend and co-honoree, Dr. Otto, developed and nurtured the Trust Fund and made possible its contributions to parasitologists (and especially the Helminthological Society of Washington) that Nancy described to you.

Dr. Aurel Overton Foster, for this and your many other outstanding accomplishments and contributions to Parasitology, we salute you!

J. R. LICHTENFELS

RECOGNITION OF GILBERT F. OTTO

Dr. Gilbert Otto (left) receiving the Certificate of Appreciation from Dr. Morgan Golden.

On this occasion of honoring Dr. Gilbert Otto for his dedicated service to the Ransom Memorial Trust Fund, I am happy to present to you a brief summary of Gilbert's background. If Dr. Foster hadn't been likewise honored it would be more appropriate, and certainly more entertaining, to have him talking about Gilbert, his longtime friend and colleague. Some of you may recall that Dr. Foster presented the Helminthological Society Anniversary Award to Gilbert in 1965. I have taken the liberty of using, in my talk, some of his comments from that occasion.

Gilbert was born in Chicago on December 16, 1901. It didn't take long for his parents to realize that he was a gifted child. At a very early age he learned to spell the family name. But that wasn't enough for Gilbert. Unlike other children, he learned to spell it backwards as well! He later learned that he got "Toot" when *he spelled* it inside out!

His collegiate training began at Kalamazoo College, in Michigan, where he majored in mathematics with minors in French and chemistry. From there he went to Kansas State University and studied zoology and entomology receiving his M.S. degree in 1927. Gilbert finished his formal training at Johns Hopkins University where he received a Doctor of Science degree in 1929

with a major in parasitology and minors in biostatistics and bacteriology. Following graduation he accepted a position as instructor at Hopkins and remained there until 1953. During his tenure, he progressed through Assistant Professor and Associate Professor and served for 7 years as Assistant Dean of the School of Hygiene and Public Health.

From 1953 to 1966, Gilbert was employed by Abbott Laboratories. He began as Head of the Parasitology Department, became Director of Agricultural and Veterinary Research and then Assistant to the Vice-President and the Director of Personnel where he was in charge of professional education and recruitment. In 1966 he came to the University of Maryland as Professor of Zoology and, upon retirement, became a Senior Research Associate there.

In the short time allotted to me it is impossible to cover the many facets of Gilbert's career in parasitology but I will try to point out some of them most closely related to this audience.

Many of us first became acquainted with Gilbert through the HelmSoc. With the exception of Aurel Foster, there are probably few, if any, in this audience who knew Gilbert when he served as President of the organization in 1935. He was appointed to the Editorial Committee of the *Proceedings* in 1934 and, with the exception of the years 1977–1980, he served continuously up to the present time. For 13 years, starting in 1952, Gilbert was the Editor of the *Proceedings*. I didn't dig deeply in the HelmSoc records but would not be surprised to find that Gilbert was on or involved with every HelmSoc committee that has ever been appointed. His advice, while not always unanimously popular, has been instrumental in guiding the Society over the years.

Gilbert's editing skills have been utilized by others as well. He was Associate Editor and Editor of the *Proceedings of the American Heartworm Society* between 1974 and 1989, a society in which he was a charter member and held the offices of Secretary-Treasurer and President. He also served as Editor of the Section on Parasitology in *Biological Abstracts*.

The American Society of Parasitologists, the American Society of Tropical Medicine and Hygiene, and the American Mosquito Control Association have all greatly benefited from his services in many offices and committees. In all, Gilbert is a member or fellow in 21 national and international scientific societies.

His talents have been recognized by numerous organizations. He has served as a consultant for the Food and Drug Administration, the Navy, the Army, the Public Health Service, World Health Organization, and the National Academy of Science among others.

Gilbert's publication list is long. He has written articles or chapters for at least 14 books and has published well over 100 papers in a number of areas of parasitology and entomology. He has received many honorary memberships and awards. In 1986, he was the Meritorious Awardee of the Council of Biology Editors and, more recently, he received the American Association of Veterinary Parasitologists Distinguished Veterinary Parasitologist Award which was presented to him in Boston on August 3, of this year.

All of these memberships, consultancies and awards were not given because Gilbert is a friendly, personable guy who can come up with a good joke on any occasion but rather because he has been a diligent researcher who has made highly significant contributions in many important areas of parasitology related to human and animal health. His personal interests include epidemiology of hookworm and *Ascaris* infections, biology and control of heartworm of dogs, immunity to helminth infection, development and evaluation of chemotherapeutic agents against parasites of man and domestic animals, and epidemiology and control of "elephantiasis" in humans. At HelmSoc meetings, and the annual meetings of the Parasitology and Tropical Medicine Societies, I have always been impressed to see Gilbert asking incisive questions of authors making presentations in widely spread areas of parasitology demonstrating his broad interests. Such broadness was a trait of parasitologists in years past when molecular genetics and gene mapping were fantasy subjects. In his presidential address before the American Society of Parasitologists in 1958, Gilbert spoke on the ecology of parasitism. He questioned whether we adequately consider the parasite in its ecological relations. While discussing amebiasis, leucocytozoan, filariasis, hookworm, echinococcus, and trichinosis he pointed out that an understanding of the ecology of the parasite and the resulting parasitism required an appreciation of many facets including the ecology of the host and how parasite and host influence each other. This kind of perspective guided Gilbert through the years in his research and his association with students. Although I haven't commented on his specific

achievements, you can be assured that they are numerous and outstanding.

Our objective this evening is to honor Gilbert for his work as a trustee of the Brayton H. Ransom Memorial Trust Fund. As has been pointed out by Nancy Pacheco, Gilbert was appointed to a committee to set up a trust as a memorial to Dr. Ransom. He must have done a good job in this capacity because he was then appointed as a trustee of the fund and remained in that position until this year when his resignation was reluctantly accepted. He was President of the Trust Fund for 17 of his 56 years as a trustee. Gilbert's ability to look ahead and his financial acumen have served the fund well. For many years, the income from the fund was not sufficient to allow much initiative in support of parasitology. When interest rates (and our income) increased in recent years, Gilbert contributed significantly to the development of a policy whereby the fund could support publication charges for persons publishing in the *Proceedings* (now the *Journal*) of the HelmSoc who had no institutional support for page charges. While this is a single item, it is indicative of the many ways in which Gilbert has benefited the fund.

So, it is with great pleasure that I, on behalf of the trustees of the Brayton H. Ransom Memorial Trust Fund, express our greatest appreciation to Gilbert F. Otto for his long and valued service.

HARLEY G. SHEFFIELD

Old friends

The Helminthological Society of Washington

Application for Membership

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

The Recording Secretary
Helminthological Society of Washington
Post Office Box 368
Lawrence, Kansas 66044

Print name: _____ Date of birth: _____

Mailing address: _____
_____
_____

Degree and year received: _____

Present position: _____

Field of interest: _____
_____

Signature of applicant: _____ Date: _____

Signature of sponsor: _____
(a member)

ANNIVERSARY AWARD RECIPIENTS

* Edna M. Buhrer	1960	* Leo A. Jachowski, Jr.	1976
Mildred A. Doss	1961	* Horace W. Stunkard	1977
* Allen McIntosh	1962	Kenneth C. Kates	1978
* Jesse R. Christie	1964	* Everett E. Wehr	1979
* Gilbert F. Otto	1965	* O. Wilford Olsen	1980
* George R. LaRue	1966	Frank D. Enzie	1981
* William W. Cort	1966	Lloyd E. Rozeboom	1982
* Gerard Dikmans	1967	Leon Jacobs	1983
* Benjamin Schwartz	1969	Harley G. Sheffield	1984
* Willard H. Wright	1969	A. Morgan Golden	1985
Aurel O. Foster	1970	Louis S. Diamond	1986
Carlton M. Herman	1971	Everett L. Schiller	1987
May Belle Chitwood	1972	Milford N. Lunde	1988
* Elvio H. Sadun	1973	J. Ralph Lichtenfels	1989
E. J. Lawson Soulsby	1974	A. James Haley	1990
David R. Lincicome	1975	Francis G. Tromba	1991
Margaret A. Stirewalt	1975	Thomas K. Sawyer	1992

HONORARY MEMBERS

* George R. LaRue	1959	Bernard Bezubik	1980
Vladimir S. Ershov	1962	Hugh M. Gordon	1981
* Norman R. Stoll	1976	E. J. Lawson Soulsby	1990
* Horace W. Stunkard	1977	Roy C. Anderson	1991
Justus F. Mueller	1978	Louis Euzet	1992
John F. A. Sprent	1979		

CHARTER MEMBERS 1910

* W. E. Chambers	* Philip E. Garrison	* Maurice C. Hall	* Charles A. Pfender
* Nathan A. Cobb	* Joseph Goldberger	* Albert Hassall	* Brayton H. Ransom
* Howard Crawley	* Henry W. Graybill	* George F. Leonard	* Charles W. Stiles
* Winthrop D. Foster			

LIFE MEMBERS

* Maurice C. Hall	1931	* Benjamin Schwartz	1976
* Albert Hassall	1931	Mildred A. Doss	1977
* Charles W. Stiles	1931	* Everett E. Wehr	1977
* Paul Bartsch	1937	Marion M. Farr	1979
* Henry E. Ewing	1945	John T. Lucker, Jr.	1979
* William W. Cort	1952	George W. Luttermoser	1979
* Gerard Dikmans	1953	* John S. Andrews	1980
* Jesse R. Christie	1956	* Leo A. Jachowski, Jr.	1981
* Gotthold Steiner	1956	Kenneth C. Kates	1981
* Emmett W. Price	1956	Francis G. Tromba	1983
* Eloise B. Cram	1956	A. James Haley	1984
* Gerald Thorne	1961	Leon Jacobs	1985
* Allen McIntosh	1963	Paul C. Beaver	1986
* Edna M. Buhrer	1963	Raymond M. Cable	1986
* Benjamin G. Chitwood	1968	Harry Herlich	1987
Aurel O. Foster	1972	Glenn L. Hoffman	1988
* Gilbert F. Otto	1972	Robert E. Kuntz	1988
* Theodor von Brand	1975	Raymond V. Rebois	1988
May Belle Chitwood	1975	Frank W. Douvres	1989
Carlton M. Herman	1975	Thomas K. Sawyer	1989
Lloyd E. Rozeboom	1975	* J. Allen Scott	1990
* Albert L. Taylor	1975	Judith H. Shaw	1990
David R. Lincicome	1976	Milford N. Lunde	1991
Margaret A. Stirewalt	1976	Everett L. Schiller	1991
* Willard H. Wright	1976	Harley G. Sheffield	1991

* Deceased.

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