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Hybridization of *Obeliscoides cuniculi cuniculi* (Graybill, 1923) Graybill, 1924 and *Obeliscoides cuniculi multistriatus* Measures and Anderson, 1983

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ABSTRACT: *Obeliscoides cuniculi cuniculi* (Graybill, 1923) Graybill, 1924 and *Obeliscoides cuniculi multistriatus* Measures and Anderson, 1983 were cross-bred in domestic rabbits (*Oryctolagus cuniculus*). Viable progeny were produced to the F_3 generation when female *O. c. multistriatus* were mated with male *O. c. cuniculi.* The synlophe (ensemble of enlarged longitudinal or oblique cuticular ridges) was intermediate in character. F_1 hybrids had 66 ± 3 (58–73) longitudinal cuticular ridges midbody in females and 46 ± 3 (41–51) in males. The mean number of longitudinal cuticular ridges remained stable in F_2 and F_3 hybrids, although, the range was greater. Dorsal and ventral ridges near the bursa of males (F_1, F_2, or F_3) were variable in number and position. Eggs resulting from the reciprocal mating (male *O. c. multistriatus* × female *O. c. cuniculi*) were not viable; egg shells were distorted and cleavage was unequal and ceased after a few hours. The two subspecies of *O. cuniculi* are considered incipient species.

Obeliscoides cuniculi (Graybill, 1923) Graybill, 1924 (Trichostrongyloidea) is found in the stomach of lagomorphs in North America. Measures and Anderson (1983a) distinguished two subspecies, *O. cuniculi cuniculi* (Graybill, 1923) Graybill, 1924 in cottontail rabbits (*Sylvilagus floridanus*) and *O. cuniculi multistriatus* Measures and Anderson, 1983 in snowshoe hares (*Lepus americanus*). These subspecies can be differentiated by the synlophe. Wild cottontail rabbits examined were infected only with *O. c. cuniculi* and snowshoe hares only with *O. c. multistriatus* (Measures and Anderson, 1983c). However, *O. c. cuniculi* and *O. c. multistriatus* develop experimentally in cottontail rabbits, snowshoe hares, woodchucks (*Marmota monax*), and domestic rabbits *Oryctolagus cuniculus* (Measures and Anderson, 1983b). The number and arrangement of longitudinal cuticular ridges composing the synlophe remained stable when each subspecies was passaged in these hosts (Measures and Anderson, 1983b). The purpose of the present study was to determine whether these two subspecies could interbreed.

Materials and Methods

Female New Zealand white rabbits (6–8 wk old) were used for experiments. Infective larvae of subspecies of *Obeliscoides cuniculi* were obtained as described in Measures and Anderson (1983b). Feces containing eggs of *O. cuniculi* were collected daily from rabbits with infections of approximately 1 mo duration. Feces were stored at 4°C until a sufficient amount had been collected over 3 or 4 days. Feces were then placed in aluminum trays in an incubator at 26°C, moistened daily, and turned over to break up fungal hyphae. Trays were enclosed in plastic bags to maintain humidity and reduce evaporation. After 12 days feces were placed in a Baermann apparatus and infective larvae recovered. Rabbits were infected by gastric intubation. Patent infections were determined using zinc sulfate flotation of feces.

Two rabbits were infected with approximately 2,000 infective larvae of *O. cuniculi multistriatus,* and two rabbits were infected with approximately 2,000 infective larvae of *O. cuniculi cuniculi.* Eight days postinfection rabbits were killed by cervical dislocation and worms were recovered by pepsin digestion of the stomach using a Baermann apparatus at 37°C (Measures and Anderson, 1983b). All worms were fourth-stage larvae. Males and females of each subspecies were separated, counted, and given by gastric intubation to rabbits (Table 1). Worms were kept in the pepsin digest at 37°C during the transfer procedures.

Hybrid progeny from successful crossbreeding were maintained in rabbits by passage for three generations (Table 2). Three of six rabbits infected with F_1 or F_2 hybrids were killed when infections became patent. Adult hybrids were collected, fixed, and the synlophe was examined as described in Measures and Anderson (1983a). The remaining infected rabbits were used as a source of infective larvae for subsequent passage. Eggs discharged in saline or dissected from the uteri of gravid females, and infective larvae recovered from 12-day fecal cultures, were fixed in hot glycerin alcohol (1 part glycerin to 9 parts 70% alcohol) and examined.

Fecundity of *O. c. multistriatus, O. c. cuniculi,* and F_2 hybrids (female *multistriatus* × male *cuniculi*) was determined. Gravid females were collected from domestic rabbits 36 or 37 days postinfection. Eggs were dissected from the uteri of 10 gravid females and counted. Students' *t*-test was used to test significance at $P <$

Table 1. Cross-breeding of *Obeliscoides cuniculi cuniculi* (*OCC*) and *O. cuniculi multistriatus* (*OCM*) in domestic rabbits (*Oryctolagus cuniculus*).

Rabbit no.	Number and sex of 8-day fourth-stage larvae given		Pre-patent period (days)*	Total number of worms recovered (31 days post-infection)
	OCM	*OCC*		
1	250 ♀	250 ♂	14	297
2	100 ♀	100 ♂	14	73
3	250 ♂	250 ♀	14	313
4	100 ♂	100 ♀	14	97
5 (Control)		100 ♂ & 100 ♀	14	64
6 (Control)		~100 ♂ & ~100 ♀	12	239
7 (Control)	~100 ♂ & ~100 ♀		14	174
8 (Control)	~200 ♂ & ~200 ♀		17	464

* Time for transferred 8-day four-stage larvae to mature and produce eggs detectable in feces of the host.

Table 2. Recovery of hybrid adults (female *Obeliscoides cuniculi multistriatus* × male *O. cuniculi cuniculi*) from domestic rabbits (*Oryctolagus cuniculus*).*

Generation	Time (days postinfection) of necropsy	Total no. worms recovered (% recovery)
F_1	22	61 (24)
	22	60 (24)
	22	59 (24)
	34	53 (21)
	40	40 (16)
	47	32 (13)
F_2	26	– (0)
	26	– (0)
	26	4 (2)
	27	30 (12)
	30	19 (8)
	30	38 (15)
F_3	23	111 (44)
	23	104 (42)
	23	110 (44)
	23	11 (4)
	23	53 (21)
	23	74 (30)

* All rabbits were given 250 larvae.

0.05 of measurements of worms and eggs from experimental infections.

Results

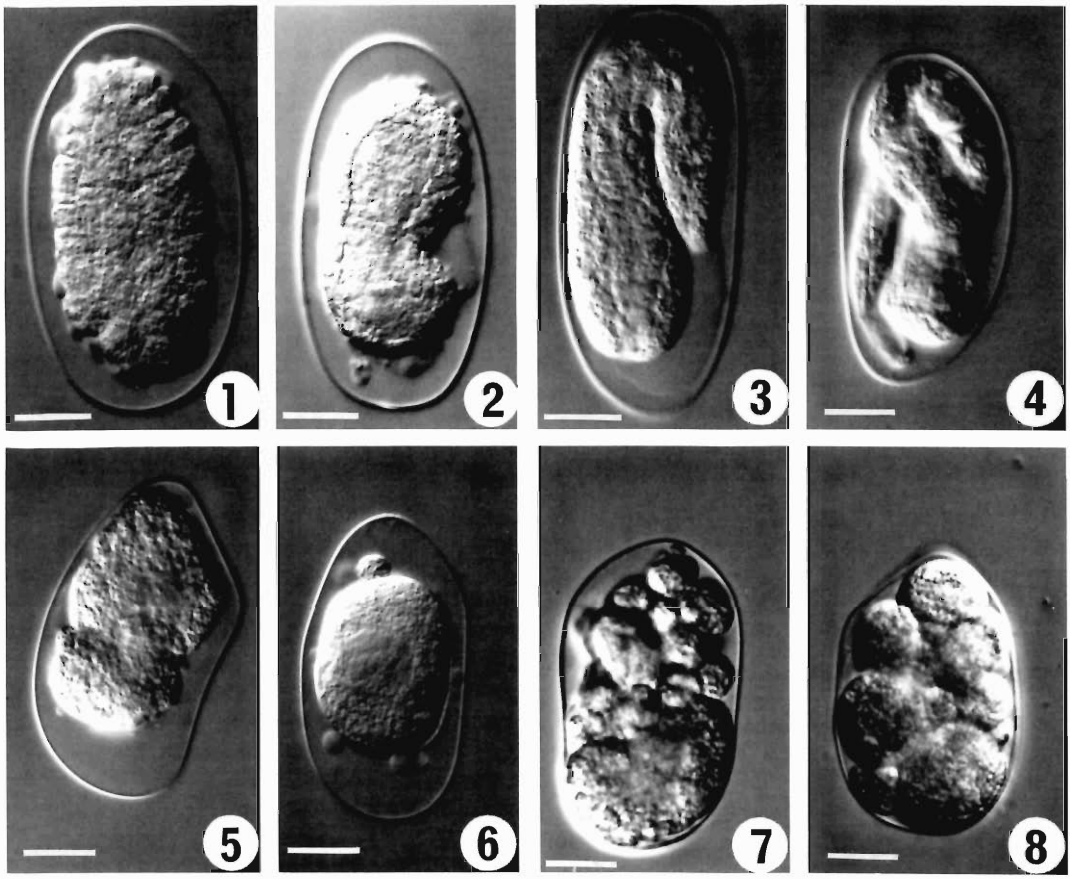
Control rabbits had patent infections 12–17 days postinfection. Examination of the synlophe of adult worms recovered from experimental and control rabbits indicated that no crosscontamination of subspecies had occurred during the infection procedures.

O. c. cuniculi males × *O. c. multistriatus* females

Rabbits had patent infections 14 days after being given fourth-stage larvae (Table 1). Sperm were observed at the junction of the uterus and oviduct in female *O. c. multistriatus*. Eggs were oval in shape, cleavage produced cells of similar size, and eggs contained first-stage larva within 23 hr at 22°C (Figs. 1–4). Progeny were viable and infective to the F_3 generation (Table 2). Eggs had polar bodies and shells. However, eggs lacking shells and polar bodies were observed in controls and in F_1 and F_2 hybrids but they were rare. Although time of necropsy varied, recovery of adult hybrids from infected rabbits appeared to have decreased at the F_2 generation and increased at the F_3 generation compared to the F_1 generation (Table 2).

Adult male and female F_1 hybrids had significantly more longitudinal cuticular ridges than *O. c. cuniculi* but these hybrids had significantly fewer ridges than *O. c. multistriatus* (Table 3). Generally ridges in hybrids (Figs. 13, 14) were prominent as in *O. c. cuniculi* (Figs. 9, 10). However, in F_2 and F_3 hybrids there were some small ridges lying between some of the larger ridges as in *O. c. multistriatus* (Figs. 11, 12). There was no consistent pattern of dorsal and ventral ridges near the bursa of hybrid males. In some specimens ridges extended to the bursa. In others, the ridges terminated at the proximal ends of spicules or terminated at a position midway between the bursa and the proximal ends of spicules. Ridges near the bursa of hybrids were often faint and discontinuous. In addition, ridges near the nerve ring in males and females and near the anus of females were also intermediate in number between those found in either *O. c. multistriatus* or *O. c. cuniculi*.

Only the synlophe distinguished hybrids from *O. c. multistriatus* and *O. c. cuniculi*. Female *O. c. multistriatus* as well as F_1 and F_2 hybrids were, however, significantly longer than female *O. c. cuniculi* (Table 4). Female F_1 hybrids were significantly longer than female F_2 hybrids. Male F_1 and F_2 hybrids were significantly longer than male *O. c. multistriatus* or *O. c. cuniculi* (Table 5), and male F_2 hybrids were significantly longer than male F_1 hybrids.



Figures 1–8. Eggs. Figures 1–4. Light micrographs of eggs of *Obeliscoides cuniculi multistriatus*. Scale bar = 20 μm. 1. Voided from oviduct of gravid female in saline. 2. Early tadpole stage after 7 hr incubation at 22°C (in water). 3. Late tadpole stage after 11 hr incubation at 22°C (in water). 4. First-stage larva in egg after 23 hr incubation at 22°C (in water). Figures 5–8. Light micrographs of eggs produced by female *Obeliscoides cuniculi cuniculi* mated with male *Obeliscoides cuniculi multistriatus*. Eggs incubated 48 hr at 22°C (in water). Scale bar = 20 μm. 5. Note abnormal shape of egg shell. 6. Note unequal cleavage of cells. 7. Note unequal cleavage of cells. 8. Note unequal cleavage of cells and abnormal shape of egg shell.

Infective third-stage larvae of *O. c. multistriatus* were significantly longer than those of *O. c. cuniculi* and those produced by F_1 and F_2 adult hybrids. F_1 larvae were significantly longer than F_2 and F_3 larvae and *O. c. cuniculi* larvae. F_3 larvae were significantly longer than F_2 larvae (Table 6).

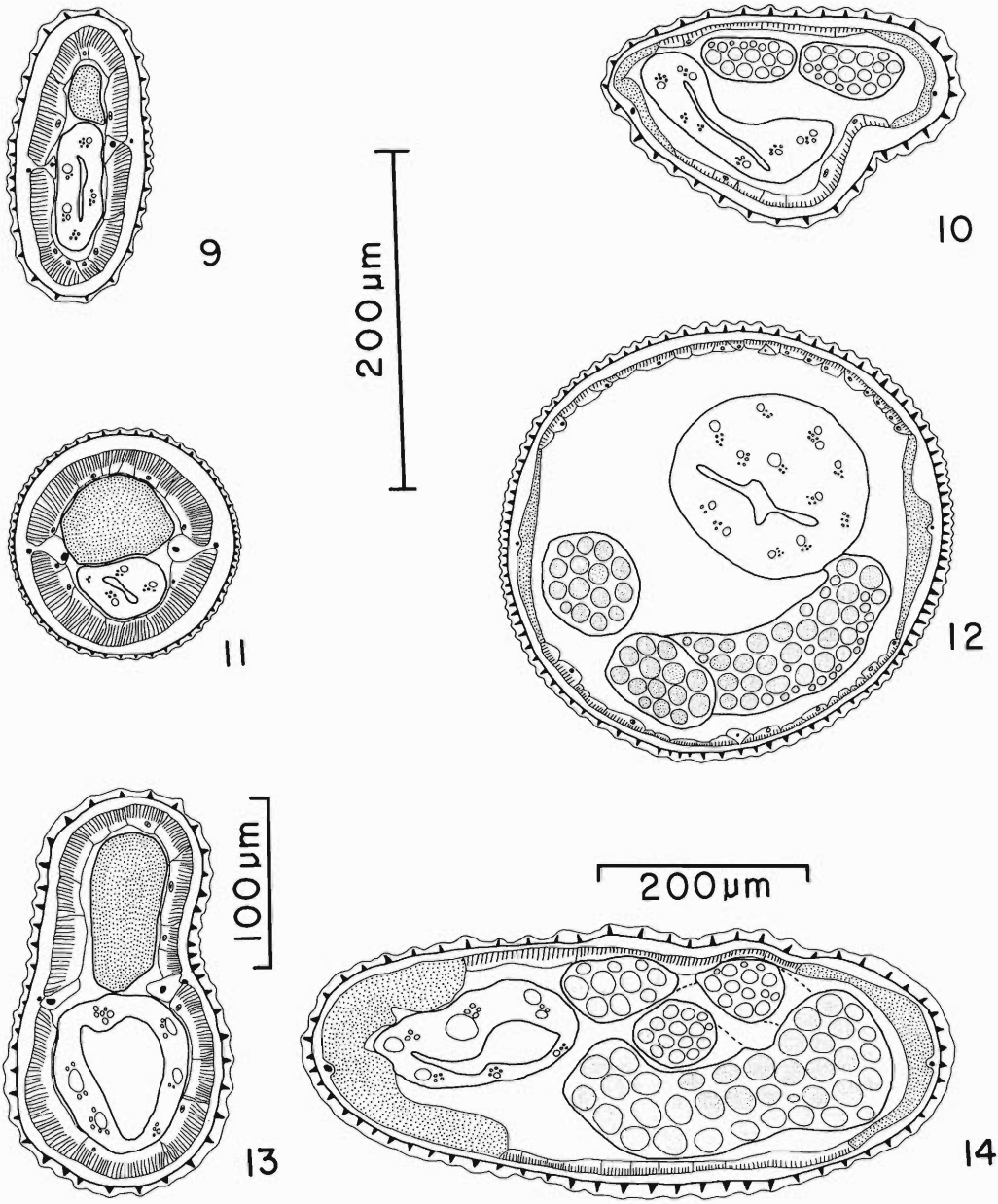
Fecal cultures containing F_2 eggs yielded fewer infective larvae after incubation than either F_1 or F_3 cultures.

O. c. cuniculi females × *O. c. multistriatus* males

Rabbits had patent infections 14 days after being given fourth-stage larvae (Table 1). Sperm were observed at the junction of the uterus and oviduct in female *O. c. cuniculi*. Eggs produced by this mating, however, failed to develop and hatch and no infective larvae were collected from fecal cultures. Eggs were abnormal in shape (Fig. 5) and cleavage was irregular and unequal (Figs. 6–8). Cleavage in eggs incubated at 22°C ceased after a few hours. Cleaving cells were either abnormally large (Fig. 6) or small (Fig. 7). In addition, many eggs lacked a shell and polar bodies.

Fecundity of the two subspecies and hybrids

O. c. multistriatus produced a significantly greater number of eggs (189 ± 68 [116–329]) than *O. c. cuniculi* (63 ± 37 [21–120]). However, F_2 hybrids produced significantly more eggs (362 ± 161 [203–668]) than *O. c. cuniculi* and



Figures 9-14. Figures 9, 10. Cross sections of *Obeliscoides cuniculi cuniculi* at midbody. Note number of longitudinal ridges (from Measures and Anderson, 1983a). 9. Adult male. 10. Adult female. Figures 11, 12. Cross sections of *Obeliscoides cuniculi multistriatus* at midbody. Note number of longitudinal ridges (from Measures and Anderson, 1983a). 11. Adult male. 12. Adult female. Figures 13, 14. Cross sections of F_1 hybrids (male *Obeliscoides cuniculi cuniculi* mated with female *O. cuniculi multistriatus*). Note number of ridges. 13. Adult male. 14. Adult female.

Table 3. Number of longitudinal cuticular ridges on *Obeliscoides cuniculi cuniculi, O. cuniculi multistriatus,* and hybrids.

Subspecies and generation	Number (mean ± SD, range) of ridges at midbody (N = no. worms)	
	Female	Male
*Obeliscoides cuniculi multistriatus (OCM)**	98 ± 10 (72–127) N = 55	64 ± 6 (53–76) N = 55
*O. c. cuniculi (OCC)**	38 ± 4 (31–46) N = 45	28 ± 3 (23–34) N = 44
OCM female × *OCC* male:		
F_1 hybrid	66 ± 3 (58–73) N = 30	46 ± 3 (41–51) N = 30
F_2 hybrid	64 ± 10 (49–90) N = 30	46 ± 7 (30–61) N = 30
F_3 hybrid	64 ± 12 (47–98) N = 30	45 ± 6 (37–65) N = 30

* Data from Measures and Anderson (1983a).

O. c. multistriatus. The small sample size and great variability of results suggests further study.

Museum specimens

Specimens of adult F_1, F_2, and F_3 hybrids have been deposited in the National Parasite Collection, United States Department of Agriculture (USNM Helm. Coll. Nos. 77708, 77709, 77710).

Discussion

Isenstein (1971) crossbred *Cooperia oncophora* (Railliet, 1898) Ransom, 1907 and *C. pectinata* Ransom, 1907 of ruminants. Male and female adult F_1 hybrids had characters intermediate between those of the two parental species. Morphology of the spicules and vulva distinguished *C. oncophora, C. pectinata,* and F_1 hybrids. Attempts to establish F_2 hybrids were unsuccessful.

LeJambre (1979) crossbred *Haemonchus contortus* (Rudolphi, 1803) and *H. placei* (Place, 1893) of sheep and cattle. Adult male F_1 hybrids (male *H. contortus* × female *H. placei*) were sterile. Adult male F_1 hybrids in the reciprocal cross were fertile but F_2 males were sterile. Females of both crosses had a low level of fertility when backcrossed with males of either parental species. The morphology of the vulva was important in distinguishing *H. contortus, H. placei,* and their hybrid combinations.

In the present study, crossing male *O. cuniculi*

Table 4. Major dimensions* of adult female *Obeliscoides cuniculi cuniculi (OCC), O. c. multistriatus (OCM),* and hybrids (*OCM* ♀ × *OCC* ♂) from domestic rabbits (*Oryctolagus cuniculus*).

Subspecies and generation	*OCM*	*OCC*	F_1 hybrid	F_2 hybrid
Time of necropsy†	31	31	22	30
Number	15	15	15	15
Length	18.3 ± 2.0 (15.0–22.1) mm	14.9 ± 1.1 (13.4–17.1 mm	19.2 ± 1.0 (17.5–20.9) mm	17.9 ± 2.0 (14.1–21.9) mm
Width‡	217 ± 20 (186–243)	174 ± 18 (143–200)	243 ± 55 (179–421)	227 ± 23 (186–279)
Nerve ring§	343 ± 31 (286–400)	341 ± 26 (279–371)	422 ± 46 (343–521)	403 ± 47 (350–479)
Excretory pore§	468 ± 51 (371–571)	498 ± 39 (450–564)	573 ± 40 (500–642)	572 ± 79 (429–715)
Deirid§	515 ± 55 (400–621)	538 ± 37 (479–614)	616 ± 40 (543–678)	598 ± 76 (450–722)
Esophagus length	870 ± 81 (542–999)	903 ± 28 (850–943)	1,030 ± 66 (907–1,130)	993 ± 96 (821–1,172)
Vulva§	13.9 ± 1.6 (10.2–17.0) mm	11.8 ± 0.9 (10.3–13.4) mm	14.5 ± 0.8 (13.1–16.1) mm	13.3 ± 1.5 (10.6–16.4) mm
Phasmids‖	66 ± 14 (43–100)	60 ± 9 (50–79)	90 ± 36 (64–157)	79 ± 12 (71–114)
Tail length	297 ± 30 (264–364)	213 ± 36 (171–300)	298 ± 29 (257–336)	292 ± 81 (221–357)

* Measurements in μm unless otherwise indicated. Values are mean ± SD (range).
† Days postinfection.
‡ At esophageal–intestinal junction.
§ From anterior extremity.
‖ From posterior extremity.

Table 5. Major dimensions* of adult male *Obeliscoides cuniculi cuniculi* (*OCC*), *O. c. multistriatus* (*OCM*), and hybrids (*OCM* ♀ × *OCC* ♂) from domestic rabbits (*Oryctolagus cuniculus*).

Subspecies and generation	*OCM*	*OCC*	F_1 hybrid	F_2 hybrid
Time of necropsy†	31	31	30	22
Number	15	15	15	15
Length	8.6 ± 0.7 (7.3–9.4) mm	8.9 ± 0.6 (7.6–10.0) mm	9.5 ± 0.7 (8.5–10.9) mm	10.4 ± 0.5 (9.0–11.1) mm
Width‡	115 ± 15 (100–157)	116 ± 8 (100–129)	140 ± 13 (114–150)	142 ± 8 (129–150)
Nerve ring§	281 ± 27 (229–336)	300 ± 15 (270–329)	317 ± 27 (271–364)	317 ± 30 (279–393)
Excretory pore§	427 ± 23 (379–479)	448 ± 27 (396–479)	452 ± 49 (386–557)	489 ± 53 (429–600)
Deirid§	445 ± 23 (400–493)	478 ± 31 (416–529)	472 ± 57 (393–592)	516 ± 52 (436–614)
Esophagus length	628 ± 50 (579–743)	700 ± 34 (636–764)	673 ± 53 (614–763)	714 ± 53 (636–807)
Spicule length	557 ± 46 (514–643)	508 ± 29 (420–550)	550 ± 41 (479–607)	579 ± 36 (500–636)
Prebursal papillae‖	498 ± 42 (421–550)	495 ± 33 (430–543)	541 ± 35 (486–607)	581 ± 69 (471–700)

* Measurements in μm unless otherwise indicated. Values are mean ± SD (range).
† Days postinfection.
‡ At esophageal–intestinal junction.
§ From anterior extremity.
‖ From posterior extremity.

Table 6. Major dimensions* of infective larvae (third-stage) from 12-day fecal cultures at 26°C and eggs discharged in saline of *Obeliscoides cuniculi cuniculi* (*OCC*), *O. c. multistriatus* (*OCM*), and hybrids (*OCM* ♀ × *OCC* ♂).

Subspecies and generation	*OCM*	*OCC*	F_1 hybrid	F_2 hybrid	F_3 hybrid
N larvae (*N* eggs)	10 (25)	10 (25)	10 (25)	6 (25)	10 (25)
Length†	697 ± 33 (622–726)	651 ± 51 (532–720)	689 ± 53 (626–780)	613 ± 15 (586–628)	665 ± 38 (609–729)
Width‡	20 ± 1 (19–21)	20 ± 1 (19–21)	20 ± 2 (17–24)	20 ± 2 (17–24)	19 ± 2 (16–20)
Nerve ring§	101 ± 12 (73–113)	100 ± 7 (90–109)	96 ± 14 (80–124)	98 ± 6 (90–104)	92 ± 11 (70–107)
Excretory pore§	131 ± 11 (112–144)	129 ± 7 (121–146)	123 ± 14 (103–147)	122 ± 12 (113–144)	123 ± 15 (96–147)
Esophagus length	152 ± 17 (126–184)	149 ± 8 (138–162)	148 ± 18 (134–181)	146 ± 10 (136–165)	145 ± 18 (107–163)
Genital primordium§	375 ± 22 (327–404)	346 ± 19 (305–380)	371 ± 21 (348–414)	337 ± 8 (324–347)	346 ± 28 (294–392)
Genital primordium length	9 ± 2 (7–11)	11 ± 2 (9–14)	11 ± 3 (7–16)	8 ± 1 (7–10)	10 ± 1 (9–11)
Tail length	61 ± 11 (31–70)	52 ± 17 (21–67)	60 ± 13 (31–73)	67 ± 8 (52–74)	72 ± 8 (60–81)
Egg length	102 ± 7 (91–111)	84 ± 6 (70–95)	90 ± 8 (70–100)	94 ± 9 (75–100)	92 ± 13 (52–120)
Egg width	49 ± 4 (41–51)	50 ± 4 (43–57)	53 ± 3 (47–60)	55 ± 4 (47–61)	50 ± 3 (47–60)

* Measurements in μm unless otherwise indicated. Values are mean ± SD (range).
† Sheath not included in length.
‡ At esophageal–intestinal junction.
§ From anterior extremity.

cuniculi and female *O. cuniculi multistriatus* produced viable progeny to the F_3 generation. The intermediate number of ridges in the first generation of hybrids and the remarkable stability in number of ridges in the second and third generations of hybrids suggests that more than one gene determines the synlophe. Interaction of these genes from each subspecies could produce the intermediate situation in hybrids. Measures and Anderson (1983b) demonstrated the stability of the synlophe of *O. c. multistriatus* and *O. c. cuniculi* in successive nematode generations. Lichtenfels (1974) has also shown that the synlophe of another trichostrongyloid, *Nippostrongylus brasiliensis,* remained stable over several nematode generations in rats, mice, and hamsters. However, when passaged in hamsters and rats for over 150 nematode generations, total body length and spicule length had changed (Lichtenfels, 1971). In the present study total body length differed between *O. c. cuniculi, O. c. multistriatus,* and hybrids.

The reciprocal mating (male *O. c. multistriatus* × female *O. c. cuniculi*) failed to produce viable progeny, even though copulation did occur and very few eggs were fertilized. Unfertilized eggs, produced when sterile male hybrids were placed with fertile female hybrids, were described by LeJambre (1979). They contained cytoplasm that had divided less equally and less frequently. Some of the unfertilized eggs had deformed shells. In the present study, nonviable eggs had been fertilized but were abnormal in shape. Cleavage in these eggs was also abnormal and similar to that observed by LeJambre in unfertilized eggs.

Foor (1967) observed that shell formation in eggs of *Ascaris lumbricoides* occurred immediately after penetration of the sperm. This is probably true of most amphimictic species of nematodes. Presence of a polar body within egg membranes indicates that a sperm nucleus has entered the primary oocyte, egg shell formation has been initiated, and the first reduction division of the oocyte has occurred. Syngamy may not occur if gametes are not compatible (gametic isolation). Nonviable eggs, observed in the present study, possessed a shell and a polar body. It is not known whether syngamy actually occurred. Failure of these eggs to develop and hatch (developmental isolation) may be due to meiotic abnormalities as LeJambre and Royal (1980) demonstrated in backcrosses of hybrids (*H. contortus* × *H. placei*). In addition, Oliver et al. (1972) and LeJambre (1979) suggested that the success of hybrids in only one direction (i.e., female *O. c. multistriatus* × male *O. c. cuniculi*) indicates that egg cytoplasm may influence the development of hybrid progeny.

Although hybrids of *O. c. multistriatus* and *O. c. cuniculi* can be produced in the laboratory, hybrids in nature have yet to be found. Bremner (1955) found naturally occurring hybrids of *H. contortus* and *H. placei.* In the present study, subspecies of *O. cuniculi* were not given a choice of mates, thus it is not known whether each subspecies would preferentially mate with its cohort. Premating isolating mechanisms may occur in nature.

Hybrid progeny produced by crossing male *O. c. cuniculi* and female *O. c. multistriatus* had reduced viability at the F_2 generation. Fewer F_2 infective larvae were recovered in fecal cultures and fewer F_2 adult hybrids were recovered from experimentally infected rabbits. This hybrid inviability was offset, somewhat, by the greater fecundity of F_2 adult hybrids.

Premating barriers to introgression may involve extrinsic isolating mechanisms consisting of geographic barriers or, in parasites, different host species may be inhabited (allopatry). The available data suggests that *O. c. cuniculi* and *O. c. multistriatus* are allopatric because they are found in different hosts (Measures and Anderson, 1983a, c). But these hosts are sympatric over part of their range in North America. Ecological separation of snowshoe hares and cottontail rabbits, as discussed by Measures and Anderson (1983c), may prevent introgression between subspecies of *O. cuniculi.* Buehler and Keith (1982) found evidence of competitive interaction between snowshoe hares and cottontail rabbits in Wisconsin. If this phenomena is common, then genetic interaction between subspecies of *O. cuniculi* is further hampered. Study of *O. cuniculi* in snowshoe hares and cottontail rabbits cohabiting a particular locale may be instructive.

Mayr (1963) defines a species as a group of actually or potentially interbreeding natural populations that are reproductively isolated from other such groups. In the present study *O. c. cuniculi* and *O. c. multistriatus* are allopatric and exhibit incipient reproductive isolation in the form of gametic or developmental isolation and hybrid inviability. Borderline cases between species and subspecies probably exist far more commonly than one would guess from a casual study of the systematic literature (Mayr, 1963).

We prefer to consider *O. c. cuniculi* and *O. c. multistriatus* as incipient species.

Acknowledgments

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Development of *Yatesia hydrochoerus* (Nematoda: Filarioidea) to the Infective Stage in Ixodid Ticks

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ABSTRACT: The arthropod host requirements and course of larval development for *Yatesia hydrochoerus* (Yates and Jorgenson, 1983), a common filaria of Colombian capybaras, are described for the first time. Development of the microfilaria to the infective stage took place within the musculature of *Amblyomma cajennense* and *A. americanum* and required 26 days under laboratory conditions. The findings of this study suggest that *A. cajennense* and perhaps other species of *Amblyomma* may serve as natural vectors of *Y. hydrochoerus*.

Yatesia hydrochoerus (=*Dipetalonema* (*Alafilaria*) *hydrochoerus* Yates and Jorgenson, 1983) and at least three additional filariae commonly infect the capybara, *Hydrochoerus hydrochaeris*, in Colombia, South America (Eberhard et al., 1976; Yates and Jorgenson, 1983). In the past few years considerable interest in capybaras has developed because of their potential as a new commercial source of high quality dietary protein (Ojasti, 1973; Fuerbringer, 1974; Rodriguez et al., 1975). The proposed husbandry of this large (27–50 kg) rodent augments the need to determine the vector requirements and life histories of these parasites.

During field studies in Colombia, hard ticks identified as *Amblyomma cajennense* were collected from freshly killed, wild capybaras. Numerous microfilariae and developing first-stage larvae of *Y. hydrochoerus* were observed in dissected specimens. Subsequently, infected capybaras were transported to our U.S. laboratory where detailed life cycle studies were undertaken. The following report summarizes our observations on the course of development and morphogenesis of the larval stages in laboratory-reared ticks.

Materials and Methods

A pair of capybaras, *H. hydrochaeris*, naturally infected with *Y. hydrochoerus* were captured alive near San Pablo, Casanare, in eastern Colombia, South America, and transported to the Delta Primate Center in Covington, Louisiana where they were housed and maintained as outlined by Crandall (1964). Laboratory-reared nymphs of *A. cajennense* and *A. americanum* were provided by the USDA Insects Laboratory in Kerrville, Texas. The ticks were placed on the infected capybaras and allowed to feed until replete and detached from the skin. During feeding, which required approximately 5 days, the ticks were confined to the host within the top portion of a 4-ml Nalgene® screw top bottle that was held in place on the shaved back of the capybara with Histo Acryl® glue and adhesive tape (modified from Gladney et al., 1970). Due to the prolonged feeding period, typical of ixodid ticks, it was impossible to know exactly when microfilariae were ingested. Therefore, we selected the time at which the engorged ticks detached themselves from the capybara as the beginning of the development period. Engorged ticks were kept in an insectary at approximately 25°C and 90% RH with 12 hr of light per day. Some ticks were dissected immediately after feeding to verify that microfilariae were ingested. Thereafter, specimens were dissected at 24-hr intervals through the 26th day post-detachment to observe and evaluate the progress of larval development. The ticks were dissected in phosphate-buffered saline (PBS) and examined for larvae with the aid of a compound microscope. All measurements were made on living specimens (at least five larvae per interval) and are expressed as micrometers (μm), the range followed by the mean in parentheses. Active second- and third-stage larvae were immobilized with 10% dimethyl sulfoxide in PBS (Lowrie and Eberhard, 1980) to facilitate measuring. Laboratory-reared *Aedes aegypti* and *Ornithodorus tartakovskyi* from our colonies, as well as locally wild-caught *Culicoides hollensis*, *Tabanus* spp., *Chrysops* spp., and *Rhipicephalus sanguineus* were also allowed to feed on the infected capybaras.

Results

Approximately 40% (221 of 563) of the laboratory-reared *A. cajennense* nymphs ingested *Y. hydrochoerus* microfilariae as they fed on the infected capybaras. Development of the microfilariae to the infective stage took place within the musculature of the tick and required 26 days.

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First-stage larvae described below were indistinguishable from those observed in naturally infected *A. cajennense* in the field. The course of larval development progressed uniformly in *A. cajennense* with no indication of aberrant development. *Amblyomma americanum* also supported development to the infective stage in 26 days. However, only 7 of the 23 nymphs that fed on the capybaras harbored developing or infective-stage larvae. Therefore, the description presented below is based only on larvae recovered from *A. cajennense*. There was no sign of larval development in *Rhipicephalus sanguineus, Ornithodorus tartakovskyi, Aedes aegypti, Tabanus* spp., *Chrysops* spp., or *Culicoides hollensis*.

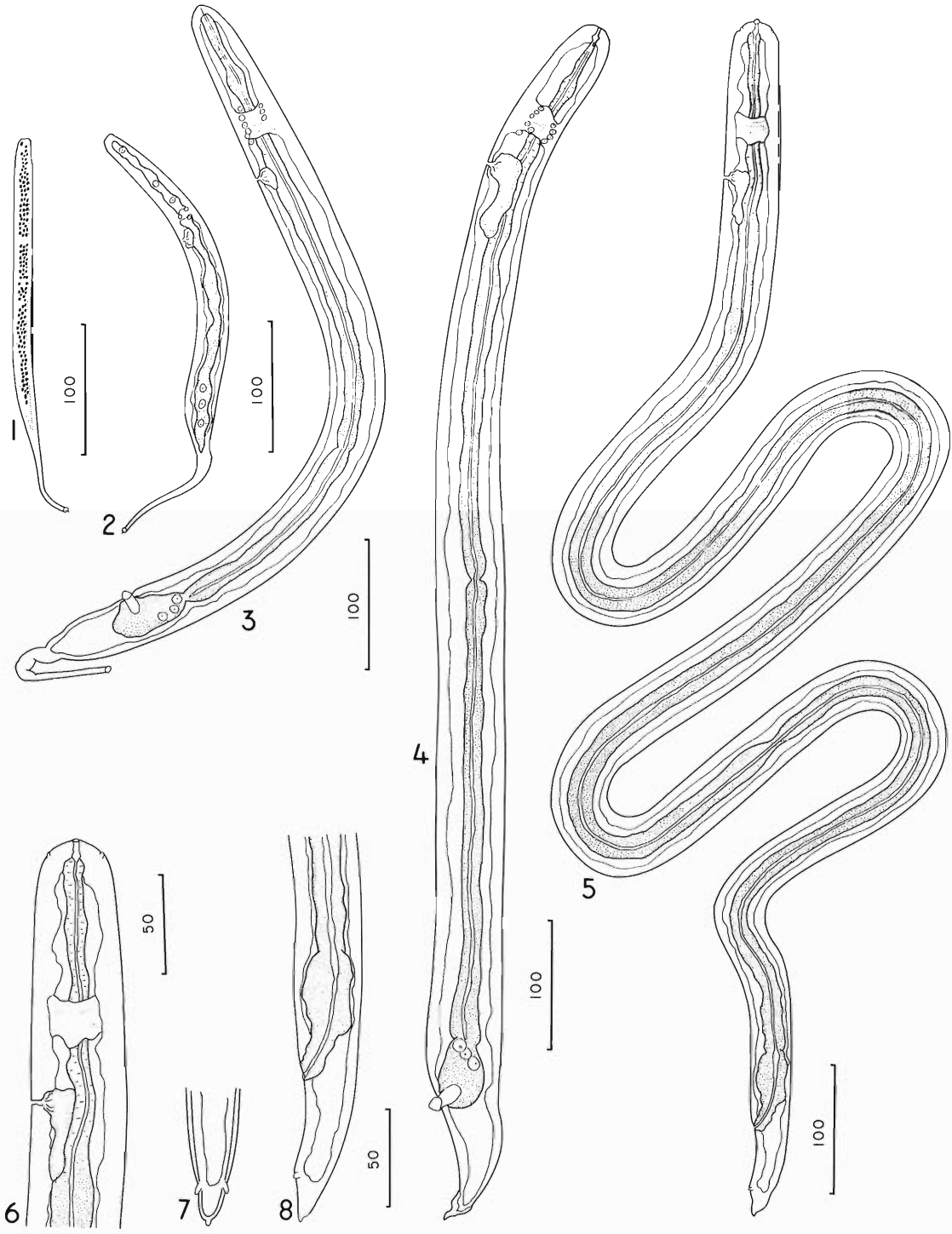
FIRST-STAGE LARVA: By the end of the first day postdetachment the microfilaria of *Y. hydrochoerus* (Fig. 1) migrated from the gut to the musculature and became quiescent. Development during the first week progressed slowly and, on the 7th day, the larva was still relatively undifferentiated (Fig. 2). No decrease in overall body length was observed during the early stage of development, and a larva dissected free of the tissues at that time was longer and more robust than the microfilaria, 355–404 (375) long × 18–23 (22) in diameter. The body wall, excretory vesicle, and cells forming the digestive tract were visible. However, the anal plug, juncture of the esophagus and intestine, and excretory cell were not distinct. Differentiation and growth proceeded slowly during the second week. A typical day 14 larva was cylindrical and 697–791 (750) long × 38–41 (40) in diameter (Fig. 3). The esophagus had a bulbous swelling just anterior to the nerve ring. The esophageal–intestinal junction and anal plug were clearly demarcated. The intestine was thick-walled with a narrow lumen and similar in diameter to the esophagus. The rectum was globular and hyaline with three prominent rectal cells. The tail was long, tubular, and gradually attenuated with a truncate tip as seen in the microfilaria. On the 14th day the cuticle appeared to be loosened at the caudal extremity of the larva signaling the beginning of the first molt that occurred by the 15th day.

SECOND-STAGE LARVA: On day 17 the second-stage larva (Fig. 4) measured 834–988 (950) long × 38–42 (40) in diameter and was similar in shape to the first-stage larva. The oral aperture opened into a clearly demarcated, cuticle-lined, buccal capsule that was diamond-shaped. The well-developed esophagus was approximately the same length as the intestine and had a short, narrow, anterior muscular portion with a bulbous swelling just anterior to the nerve ring (as seen in the first-stage larva), and a broader posterior, glandular portion. The nerve ring and excretory vesicle were easily seen. The excretory cell remained obscure, however. The esophagus and intestine were thick-walled with a narrow lumen. The rectum and the anal plug were larger than in the first-stage larva but otherwise similar. The tail was short and sharply attenuated. Development in the second-stage larva progressed more quickly than in the first-stage, the principal change being rapid growth. Between the 14th and 26th days the larva more than tripled in length (750–2,500 μm) but changed only slightly in diameter. The second molt occurred on the 26th day.

THIRD-STAGE LARVA: The infective-stage larva (Fig. 5) was long, comparatively robust, and active when freed from the tick. Ten third-stage larvae were 2,000–2,700 (2,500) long × 36–50 (44) in diameter. The body was nearly uniform in diameter throughout its length. The anterior extremity was bluntly rounded (Fig. 6) bearing at least four clearly visible papillae. The esophagus was long with distinct muscular and glandular portions. The tail was 70–82 (77) long and gently tapered with one terminal and two subterminal papillae (Figs. 7, 8). Salient anatomical features of these larvae, expressed as distance from the anterior end, were: nerve ring 70–84 (80), excretory pore 96–146 (125), base of muscular esophagus 113–166 (143), esophageal–intestinal junction 1,440–2,202 (1,812). The excretory cell and genital primordia were not observed.

Discussion

This laboratory study demonstrates that *A. cajennense* and *A. americanum* can support the development of *Y. hydrochoerus* to the infective stage. *Amblyomma cajennense* is a common ectoparasite of Colombian capybaras. Rodriguez et al. (1975) found numerous *A. cajennense* on each of 15 capybaras collected at nine different sites in Meta, Colombia. These findings, in conjunction with the senior author's field observation of *Y. hydrochoerus* larvae in *A. cajennense* removed from wild-caught capybaras in Colombia, suggest that this tick species and possibly other *Amblyomma* spp. may serve as natural vectors of the parasite. Distinctive anatomical features of *Y. hydrochoerus* infective-stage larvae that may be characteristic of the genus include



Figures 1-8. ***Yatesia hydrochoerus*** **microfilaria and larvae. 1. Microfilaria (hematoxylin and eosin stained). 2. Early first-stage larva. 3. Late first-stage larva. 4. Second-stage larva. 5. Third-stage larva. 6. Third-stage larva cephalic extremity, lateral view. 7. Third-stage larva caudal extremity, ventral view. 8. Third-stage larvae caudal extremity, lateral view. Scale units are micrometers.**

their large size, the presence of a large excretory vesicle, and at least four clearly visible cephalic papillae.

A relatively close taxonomic proximity for the genus *Yatesia* and the genus *Acanthocheilonema* (sensu Bain et al., 1982) is apparent based on morphologic criteria. Similarities in life cycle patterns of the two groups also tend to support this supposition. For example, the six *Acanthocheilonema* species for which vector requirements are known develop in fleas, lice, or ticks, and three of these develop within the musculature of ticks (Schacher, 1973).

Descriptions of developing *Acanthocheilonema* larvae are incomplete in many cases, but certain unusual life cycle features are shared by both groups. The course of development for *Acanthocheilonema* and *Yatesia* larvae is comparatively long (3–4 wk) and first-stage larvae do not undergo a decrease in length during early development (no "sausage" stage) as do most other filariae, but instead begin to increase slowly in both length and diameter (Chabaud, 1954; Nelson, 1961). *Yatesia* infective-stage larvae are readily distinguished from most other filarial species by their large size (2,000–2,700 μm long × 36–50 μm in diameter). *Acanthocheilonema dracunculoides* is the only other filaria known to us that approaches this size (2,400 μm long by 35 μm in diameter [Nelson, 1963]).

As commercial husbandry of capybaras develops, the pathogenesis, zoonotic potential, and commercial impact of *Y. hydrochoerus* can be more clearly assessed. If filaria control measures prove to be warranted, acaracidal agents currently available for veterinary applications may be effective.

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Seasonal Prevalence of *Chandlerella quiscali* (Onchocercidae: Filarioidea) in Brain of the Common Grackle (*Quiscalus quiscula versicolor*)[1]

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ABSTRACT: A high prevalence of infection with the avian filarial worm *Chandlerella quiscali* (von Linstow, 1904) was observed in common grackles (*Quiscalus quiscula versicolor*) surveyed in central Illinois. Masses of worms were recovered from the lateral cerebral ventricles of the brain, and heavy microfilaremias were observed in lung blood. Pathogenesis was limited to compression of brain tissue and inflammation was absent. The high prevalence of infection suggested an efficient vector and it was hypothesized that immature grackles were infected by association with patent adult grackles during the breeding season. The development of *C. quiscali* was studied in nestling, fledgling, juvenile, and adult grackles collected during the breeding seasons of 1977 and 1978 from two grackle colonies. Immature stages of *C. quiscali* were recovered from the cerebral ventricles of fledgling and juvenile birds on days 36 through 69 posthatching, demonstrating that young grackles were infected during the breeding season. Patent *C. quiscali* infections were first observed in juvenile birds on days 82 and 98; this yielded an estimated prepatent period of 90 days. Immature stages of *C. quiscali* were not recovered from breeding adult grackles. Microfilariae of *C. quiscali* were observed in the intestines of a presumptive midge vector (*Culicoides crepuscularis* Malloch) collected from grackle nests.

The avian filarial nematode *Chandlerella quiscali* (von Linstow, 1904) has been reported from the brain of common grackles (*Quiscalus quiscula versicolor*) during surveys in Ohio (Weckler, 1960; Robinson, 1961), Georgia (Robinson, 1955), Minnesota (Robinson, 1971), and Iowa (Odetoyinbo, 1960), but the epizootiology has been incompletely studied. During a 1976 Illinois survey, we observed all of 88 common grackles infected with *C. quiscali* (Granath and Huizinga, 1978), and heavy microfilaremias were detected in lung blood. The high prevalence of infection suggested an efficient vector, and we hypothesized that *C. quiscali* was being transmitted from adult to immature grackles during the breeding season. In this study we investigated the seasonal prevalence, pathogenesis, and vector of *C. quiscali* in immature and adult common grackles in Illinois.

Materials and Methods

Juvenile and adult grackles were collected in central Illinois with a shotgun, wire traps, and mist nets. Nestling and fledgling grackles were removed by hand from marked nests, and attendant parent grackles were collected with a shotgun. Birds were immediately placed in plastic bags and transported on ice to the laboratory for examination. Feather development, degree of skull ossification, and head lengths were used to determine age groups of birds (nestlings, mean head length = 3.7 cm; fledgling = 4.8 cm; juveniles = 5.5 cm; adults = 5.8 cm). Adult grackles were sexed by gonadal examination and plumage coloration.

Fresh lung blood smears were examined for live microfilariae of *C. quiscali* that were identified by measuring total length (170 μm), presence of a sheath, and pattern of motility. Adult and larval stages of *C. quiscali* were recovered from the lateral cerebral ventricles of grackles by meticulous dissection. The muscles and skin of nestling grackles were soaked in artificial peptic digestion fluid and dissected to find larval filarioids. Nematodes were fixed in 70% alcohol containing 10% glycerine, cleared in glycerine, and the projected image of worms was measured microscopically using a camera lucida and planimeter. Voucher specimens of *C. quiscali* are deposited in USNM Helm. Coll. No. 77363.

For study of parasite development in immature grackles, two colonies of grackles were observed during the breeding seasons of April through August 1977 and 1978. Colony 1 consisted of approximately 30 nesting sites in an artificial planting of 10–15-ft-high Scotch pine trees adjacent to a 2-acre farm pond and corn fields located at the Illinois State University farm, McLean County, Illinois. Colony 2 was composed of approximately 50 nesting sites in 25-ft-high black locust trees scattered throughout a 4-acre disturbed, oak–hickory woodlot that is traversed by an intermittent stream draining two small farm ponds and a pig lot, located at Mt. Sterling, Brown County, Illinois.

Grackles in both breeding colonies began nest-building by mid-April and egg-laying from April 28 through May 28. The incubation period of grackle eggs was 14 days. Studies by Willson et al. (1971) and Erskine (1970)

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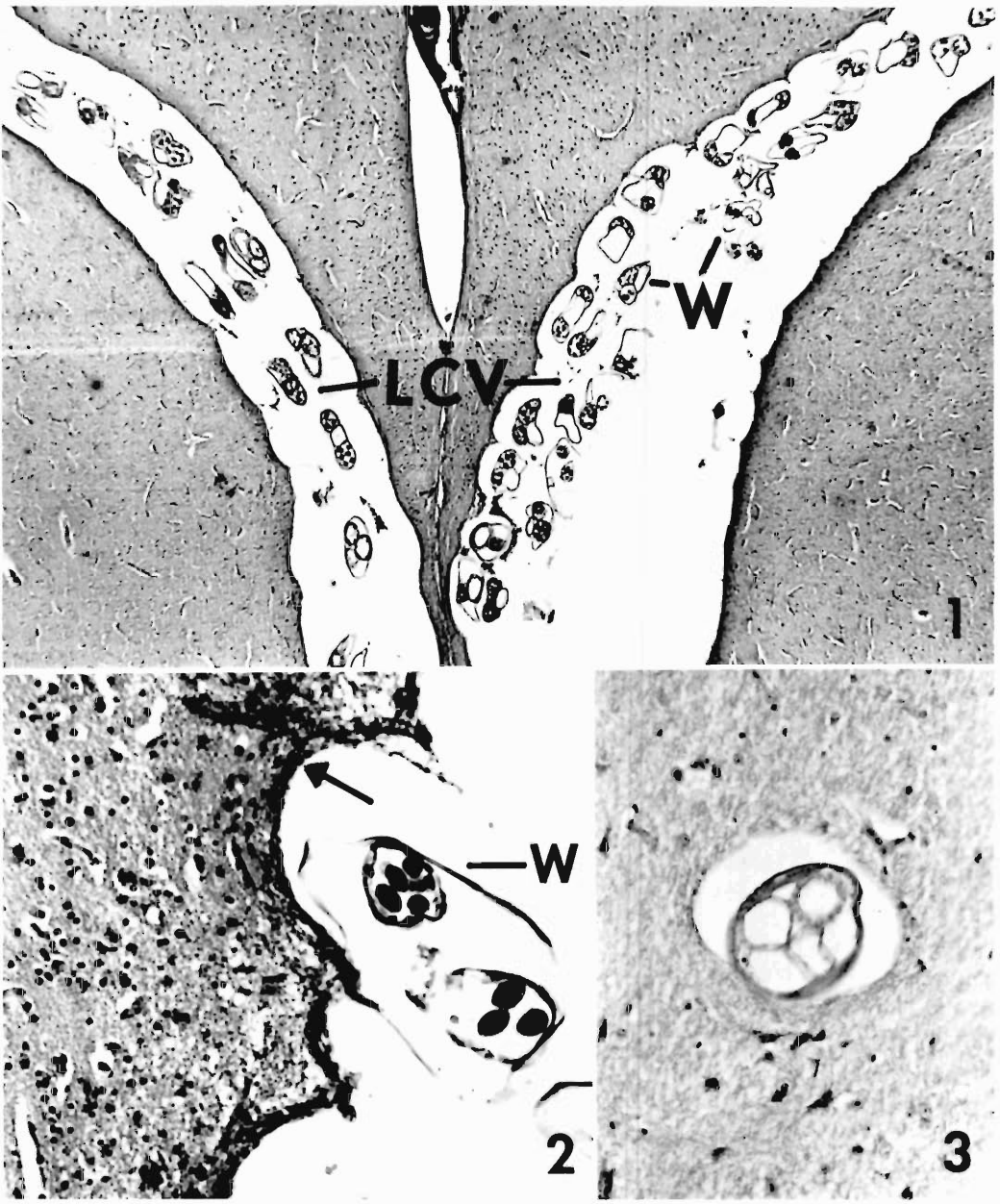

Figures 1–3. Grackles infected with *Chandlerella quiscali*. 1. Section through lateral cerebral ventricles (LCV) containing adult worms (W). Note absence of inflammatory response (×30). 2. High magnification of lateral cerebral ventricle showing compression (arrows) of brain tissue adjacent to worms (W) (×170). 3. *C. quiscali* within cerebral tissue and absence of inflammation (×180).

determined the average hatching date for common grackles in Illinois to be May 11. Because immature grackles were destroyed in nests by predators and other natural hazards, it was not possible to determine the exact ages of most nestling, fledgling, and juvenile grackles from marked nests. Therefore, the May 11 average hatching date was used as a baseline from which the age of immature birds was estimated. For example, a nestling collected on May 27 was estimated to be 16 days old, and a juvenile collected on August 17 was estimated to be 98 days old.

Grackle nests were isolated in plastic bags, and

Table 1. Seasonal prevalence of *Chandlerella quiscali* in immature and adult grackles.

Date examined	Age group*	No. birds examined	% Infected micro-filariae	% Infected adult worms	Total adult worms recovered ($\bar{x}$)	% Infected immature worms	Total immature worms recovered ($\bar{x}$)	Estimated age of birds in days†
1977 breeding season								
5/27	N	11	0	0	0	0	0	16
6/3	N	5	0	0	0	0	0	23
6/16	F	19	0	0	0	32	22 (3.7)	36
6/16	A	21	95.2	95.2	194 (9.7)	0	0	365+
7/21	J	9	0	0	0	44	14 (3.5)	41
7/18	A	2	100	100	38 (19)	0	0	365+
8/1	J	1	100	100	2 (2)	0	0	82
1978 breeding season								
5/16	N	15	0	0	0	0	0	5
5/16	A	16	87.5	93.7	92	0	0	365+
6/1	N	4	0	0	0	0	0	21
6/1	F	11	0	0	0	0	0	21
6/18	J	2	0	50	9	50	4 (4)	39
6/18	A	28	64.3	64.3	115	0	0	365+
7/21	J	3	0	0	0	100	16 (5.3)	71
8/17	J	2	100	100	9	0	0	98

* N = nestling, F = fledgling, J = juvenile, A = adult.
† Average hatching date May 11.

emerging midges were collected with an insect aspirator. Birds were washed in dilute detergent to recover ectoparasitic arthropods from feathers. Arthropods were dissected in 0.65% saline and the digestive tract and other tissues were microscopically examined for developmental stages of *C. quiscali*.

Cerebral tissues from nestling, fledgling, juvenile, and adult grackles were carefully dissected free of the skull and fixed in Bouin's fluid or neutral-buffered formalin. For in situ study of worms in selected grackles, the entire head was fixed in Bouin's and decalcified. All tissues were processed for histological study by routine paraffin sectioning (6 μm) and stained in hematoxylin and eosin.

Results

Gross pathology

When the cerebral hemispheres were separated, masses of adult *C. quiscali* were visible through the semitransparent, membranous covering of the left and right lateral cerebral ventricles. The threadlike worms were lying free within the entire length of both ventricles. There were no gross signs of lesions.

Microscopic pathology

The brains of six nestlings, five fledglings, and one juvenile grackle were sectioned, but developmental stages of *C. quiscali* were not observed in the lateral cerebral ventricles. Transverse sections of the brains of 16 adult grackles revealed worms in the left and right cerebral ventricles of 10 birds (Fig. 1). Although several nematodes were present in most sections, pathogenesis was limited to compression of brain tissue adjacent to worms (Fig. 2). Inflammation and cell-mediated responses were not apparent. In one case, a worm was observed within cerebral tissue (Fig. 3), but inflammation was not present.

Seasonal prevalence of adult grackles

During the 1977 breeding season, 22 (95.7%) of 23 adult grackles were infected with mature *C. quiscali* and circulating microfilariae (Table 1). During the 1978 season, 33 (75%) of 44 adult grackles were infected with mature *C. quiscali* and 73% had circulating microfilariae. A sample of 357 worms from 30 grackles had the following measurements: mean length of 246 females, 57.6 (±13.0) mm; mean length of 111 males, 24.8 (±5.21) mm; the female to male sex ratio was 2.21:1. Immature filarial nematodes were not recovered from adult birds.

Filarial nematode development in immature grackles

To determine the pattern of filarial nematode development, immature grackles were collected at various time intervals during the 1977 and 1978 breeding seasons. During the 1977 season,

Table 2. Immature *Chandlerella quiscali* from fledgling and juvenile grackles.

Age group*/ age bird in days†	No. worms recovered	No. worms measured‡	Immature ♂ worms $\bar{x}$ length in mm (no. measured)	Immature ♀ worms $\bar{x}$ length in mm (no. measured)	Immature worms $\bar{x}$ length in mm (no. measured)
F/36	6	3	—	19.50 (3)	—
F/36	9	4	—	17.99 (4)	—
F/36	2	1	—	16.94 (1)	—
F/36	2	2	—	—	4.69 (2)
F/36	1	1	—	22.95 (1)	—
F/38	4	3	12.70 (2)	27.72 (1)	—
J/69	8	4	18.48 (2)	29.72 (2)	—
J/69	1	1	—	—	8.86 (1)
J/71	6	4	11.54 (3)	21.25 (1)	—

* F = fledgling, J = juvenile.
† Average hatching date May 11.
‡ Damaged worms not measured.

none of 16 nestling grackles was infected on days 16 and 23 (Table 1). Six of 19 (32%) fledgling grackles contained immature *C. quiscali* in the lateral cerebral ventricles on day 41. One juvenile was infected with sexually mature *C. quiscali* and circulating microfilariae on day 82.

During the 1978 season, none of 19 nestlings (days 5, 21) and none of 11 fledglings were infected on day 21. Immature filarial nematodes were observed in the lateral cerebral ventricles of two juvenile birds on day 39 and three juvenile birds on day 71. Patent infections with adult filarial nematodes and circulating microfilariae were found in two juvenile birds on day 98 (Table 1).

C. quiscali recovered from immature grackles are listed in Tables 1 and 2. The smallest filarial nematodes recovered from the brain of a fledgling grackle averaged 4.69 mm in length on day 36. Subadult male and female filarial nematodes were recovered on days 36, 69, and 71. Sexual dimorphism was apparent with the smaller males presenting a ventrally coiled posterior and the larger females having partially developed reproductive organs. Larval filarial nematodes were not detected in the brains, muscles, or skin of 35 nestling grackles. However, developmental stages of the connective tissue nematode *Eufilaria hibleri* were observed and are reported elsewhere (Granath, 1981).

Vector relationships

The midge, *Culicoides crepuscularis,* was recovered from 13 nests during the 1977 breeding season. One to 56 midges were isolated from individual nests. A few midges were present on March 16, but peak numbers were observed on May 19. Nestling and fledgling grackles with attendant parent grackles (*C. quiscali* infected) were collected from nests. Fifty-nine *C. crepuscularis* engorged with bird blood were dissected. Ensheathed microfilariae of *C. quiscali* were observed in blood meals, but exsheathment or development of third-stage larvae was not observed in insect tissues.

Other arthropods recovered from the feathers of 34 grackles were: lice, order Mallophaga, family Philopteridae (24 birds infested, mean number per bird, 3.4, range 1–20); lice, order Mallophaga, family Menoponidae (10, 3.8, 1–6); and mites, order Acarina, family Dermanyssidae (25, 383, 1–~5,000). Representative arthropods were dissected, but developmental stages of *C. quiscali* were not found.

Discussion

The prevalence of the avian filarioid *Chandlerella quiscali* in adult common grackles is high in Illinois and other midwestern study areas (Odetoyinbo, 1960; Weckler, 1960; Robinson, 1961). This suggests an efficient vector, and Robinson (1955) proposed that avian filarial infections are transmitted from parent to nestling during the breeding season by the bite of *Culicoides* midges. Our recoveries of developmental stages from the cerebral ventricles of fledgling grackles on day 36 posthatching demonstrates that *C. quiscali* is transmitted during the breeding season in Illinois. Sexual dimorphism of the developing filarial nematodes was apparent on day 69, and

patent infections were first observed on days 82 and 98 posthatching; an estimated prepatent period of 90 days.

High adult worm densities were common in the cerebral vertricles of adult grackles, and circulating microfilariae were present during all months sampled. However, pathogenesis was limited to compression of brain tissue and inflammation was not apparent, suggesting a well-balanced host–parasite relationship. The absence of developmental stages of *C. quiscali* in patent adult grackles indicates that, following a primary infection, the adult birds become resistant to challenge infections during later breeding seasons.

Midges have been determined to be vectors of three species in the genus *Chandlerella.* Hibler (1963) showed that *Chandlerella striatospicula* Hibler, 1964 was transmitted by *Culicoides haematopotus* Malloch. Bartlett and Anderson (1980) reported development of *Chandlerella chitwoodae* Anderson, 1961 in the midges *Culicoides stilobezzioides* Foote and Pratt, and *C. travisi* Vargas. Robinson (1971) fed wild-caught midges on patent adult grackles and reported larval development of *C. quiscali* in *Culicoides crepuscularis* Malloch, *C. haematopotus,* and *C. travisi.* In our study, microfilaria of *C. quiscali* were observed in the intestines of the midge *C. crepuscularis* collected from grackle nests, and it is the presumptive vector of *C. quiscali* from adult to immature grackles.

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Evolution of the Elaphostrongylinae (Nematoda: Metastrongyloidea: Protostrongylidae) Parasites of Cervids (Mammalia)

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ABSTRACT: The phylogenetic relationships of the Elaphostrongylinae were evaluated by cladistic analysis. *Elaphostrongylus cervi* is considered the most plesiomorphic member of the subfamily and is the sister-group of *Parelaphostrongylus*. *Parelaphostrongylus*, which consists of *P. andersoni*, *P. odocoilei*, and *P. tenuis*, is monophyletic based on the presence of crura on the gubernaculum and a bifurcate gubernacular corpus. *P. tenuis* is the most plesiomorphic member of the genus and is the sister-group of a monophyletic muscleworm lineage composed of *P. andersoni* and *P. odocoilei*.

Elaphostrongylus cervi originated in the nearctic, cospeciating with *Rangifer*. The current holarctic distribution of *E. cervi* is interpreted as colonization of more primitive cervids with the retention of broad coaccommodation within the family. The meningeal worm, *P. tenuis*, originated prior to the formation of extant species of *Odocoileus*. Cospeciation of the muscleworm ancestor with the ancestor of the extant species of *Odocoileus* resulted in *P. andersoni* in *O. virginianus* and *P. odocoilei* in *O. hemionus*.

The extensive distribution of *E. cervi*, a generalist, can be attributed to its broad coaccommodation within the Cervidae, broad coaccommodation with and the ubiquitous nature of suitable molluscan intermediate hosts, and the absence of competitors in Eurasia. The distribution of *P. tenuis*, a specialist, in North America is attributed to the success of white-tailed deer and in part to the pathogenic effects of the meningeal worm in sympatric cervids. Two hypotheses for the current distribution of the species of *Parelaphostrongylus* are presented.

The Elaphostrongylinae (Protostrongylidae) comprise a small but economically important group of nematode parasites of cervids. Although commonly referred to as "lungworms," all species of the subfamily inhabit extrapulmonary sites in the definitive host. Life cycles are complex, and terrestrial gastropods are used as obligate intermediate hosts (see Anderson, 1968 for a review).

Coevolution of parasitic organisms with their hosts has been considered axiomatic. Cameron (1964) stated, "parasites have obviously evolved co-incidentally with their hosts" and that "parasite phylogeny and classification can only be interpreted in terms of host phylogeny and classification." However, Inglis (1965) stated, "evolution of most groups of nematodes has tended to occur in hosts with similar ecological requirements." Brooks (1979a) recently reexamined the concept of coevolution and identified two distinct components: *coaccommodation*, "the mutual adaptation of a given parasite species and its host through time" and *cospeciation*, "cladogenesis of an ancestral parasite species as a result of or, concomitant with, host cladogenesis." This distinction forms an important framework for the study of the evolution of host–parasite systems, as it emphasizes the historical relationship of host and parasite but does not disregard colonization as an alternative means of parasite acquisition.

Recent studies of host–parasite evolution and zoogeography have centered exclusively on the relationship between the parasite and the definitive host (Brooks, 1977, 1978, 1979b; Brooks et al., 1981; Chabaud and Bain, 1976). The role of intermediate hosts however has not been given equal attention. The following analysis of the Elaphostrongylinae includes an evaluation of the role of the intermediate and definitive hosts as they relate to the distribution and evolution of the parasite.

Materials and Methods

Specimens of *P. odocoilei* were obtained from experimentally infected mule deer (*Odocoileus h. hemionus*) as reported by Platt and Samuel (1978a, b). Specimens of *P. tenuis* were obtained from naturally infected wapiti (*Cervus elaphus canadensis*) in Pennsylvania. Type specimens of *P. andersoni* were obtained from the USNM Helm. Coll., Beltsville, Maryland. Specimens of *E. cervi* were not available, and the description by Lankester and Northcott (1979) was used as a source of morphological characters.

The direction of change of characters within the Elaphostrongylinae was determined through the use of out-group comparison (Hennig, 1966; Wiley, 1981).

As some parasitologists may not be familiar with the terminology of phylogenetic systematics, the following definitions are provided (following Wiley, 1981). Ple-

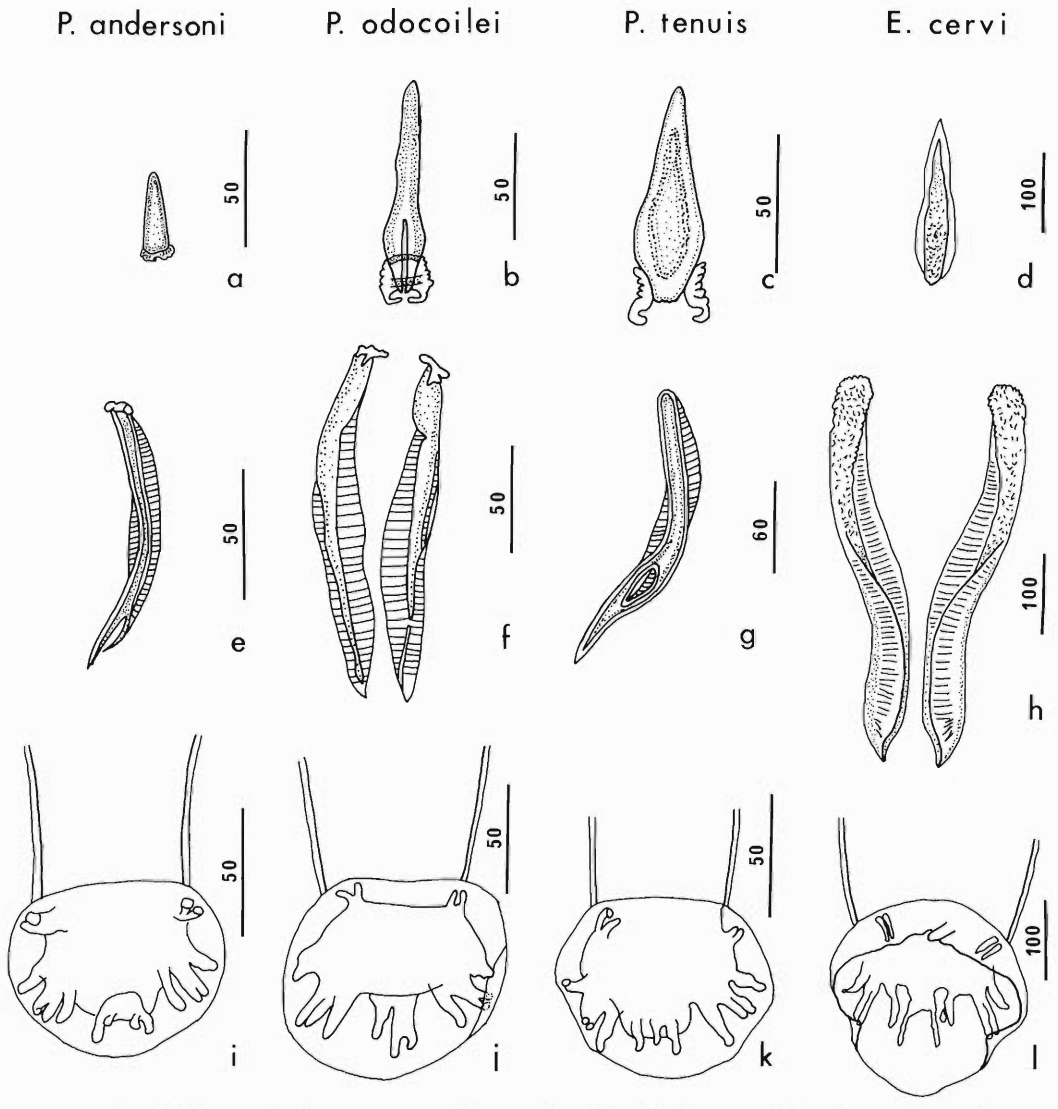

Figure 1. Male reproductive structures of the species of the Elaphostrongylinae. 1a-d, gubernacula, 1e-h, spicules. 1i-l, copulatory bursa. (Fig. b, c, e, f, i, l, and k, original; Fig. 1a, redrawn from Prestwood [1972]; Fig. 1g, redrawn from Dougherty, 1945, Parasitology 36:199-208; Fig. 1d, h, and l, redrawn from Lankester and Northcott [1979].)

siomorphic and apomorphic refer to the original and derived states, respectively, of a pair of homologous characters. Synapomorphies are "evolutionary novelties" (derived characters) shared by two or more species forming a monophyletic group. Autapomorphies are derived characters restricted to a single species. Homoplasies are structurally similar characters that are thought to be of independent origin.

As the sister-group of the elaphostrongylines is unknown, members of the Metastrongyloidea, excluding the Protostrongylidae, were employed as the out-group. Each character was assigned a 0 for the plesiomorphic state and a 1, −1, or 2 for the apomorphic state. An outline and explanation for the decision of the determination of character state polarity is presented below.

1. Crura of the gubernaculum.—The gubernaculum of the majority of the Protostrongylidae (sensu Anderson, 1978) is complex, consisting of a corpus, crura, and capitulum. The gubernaculum of the remaining metastrongylids is simple, corpus only, or absent. Two states: crura absent (0); crura present (1) (Fig. 1a–d).
2. Corpus of the gubernaculum.—The corpus is a solid

Table 1. Data matrix of the character state distribution used in the analysis of the Elaphostrongylinae.

Species	Characters							
	1	2	3	4	5	6	7	8
E. cervi	0	0	0	0	0	0	0	1
P. tenuis	1	1	0	1	0	1	0	0
P. andersoni	1	1	1	0	1	1	1	0
P. odocoilei	1	2	0	0	1	0	−1	0

piece in nonprotostrongylid members of the Metastrongyloidea. Three states; solid corpus (0); distally notched corpus (1); distally split corpus (2) (Fig. 1a–d).

3. Spicules.—The distal end of the spicules in the majority of metastrongylids is solid. Two states: solid (0); bifid (1) (Fig. 1e–h).
4. Spicular foramen.—A spicular foramen is only found in *P. tenuis.* Two states: absent (0); present (1) (Fig. 1b).
5. Shape of the dorsal ray.—The dorsal ray in the majority of nonprotostrongylids (excluding highly specialized forms with marked bursal reduction) is not compact. Two states: not compact (0); compact bulb (1) (Fig. 1i–l).
6. Branches of the dorsal ray.—The branches of the dorsal ray in nonprotostrongylids (not exhibiting bursal reduction) are terminally placed. Two states: terminal (0); ventral (1) (Fig. 1i–l).
7. Location of the dorsal ray.—The dorsal ray in nonprotostrongylids (not exhibiting bursal reduction) is terminal. Three states: terminal (0); ventral (1); dorsal (−1) (Fig. 1i–l).
8. Perityls.—Nonmovable lips are found in the majority of metastrongylids. Two states: present (0); absent (1).

A summary of the distribution of character states is given in Table 1.

Results

Two equally parsimonius cladograms can be constructed based on the distribution of the synapomorphies (Fig. 2). The first (Fig. 2a) recognizes *E. cervi* as the most plesiomorphic member of the subfamily. *Parelaphostrongylus* is recognized as a monophyletic taxon on the basis of the presence of crura and a notched or distally split gubernacular corpus (Fig. 1a–c). A muscleworm lineage consisting of *P. andersoni* and *P. odocoilei* is monophyletic based on the presence of a compact, bulbous dorsal ray (5). A single homoplasy, the ventral position of the branches of the dorsal ray (6), is shared by *P. tenuis* and *P. odocoilei.* The alternative phylogeny (Fig. 2b) also recognizes *E. cervi* as the most plesiomorphic species and *Parelaphostrongylus* as monophyletic, as described above. This phylogeny differs however in recognizing *P. andersoni* and *P. tenuis,* which are parasites of white-tailed deer, as a monophyletic group based on the position of the branches of the dorsal ray. A single homoplasy, the presence of a compact dorsal ray, is shared by *P. odocoilei* and *P. andersoni.*

Discussion

Although the two cladograms are equally parsimonius based on character distributions, the phylogeny in Figure 2a is preferred based on the location of the adult worms in the definitive host. *Elaphostrongylus cervi,* the most plesiomorphic species, has been reported from the meninges of the brain (Mitskevitch, 1964) as well as the connective tissue of the skeletal muscles (Mitskevitch, 1964; Lankester and Northcott, 1979) and occasionally deep muscle sites (Lankester and Northcott, 1979). Species of *Parelaphostrongylus* are more restricted in their site of maturation. Adult *P. tenuis* have only been found in close association with the central nervous system (CNS) (Anderson, 1968). The muscleworms are restricted to the skeletal muscles and associated connective tissue (Prestwood, 1972; Platt and Samuel, 1978b). Therefore, following the phylogeny in Figure 2a, habitat specialization is a direct process requiring no reversals or parallelisms. In the alternate phylogeny (Fig. 2b) neurotropic behavior would have been lost in the ancestral *Parelaphostrongylus* and then reappeared in *P. tenuis,* or the muscleworms lost their neurotropic behavior independently and *P. tenuis* retained the behavior as a plesiomorphic trait. Both of these scenarios require reversals or parallel evolution, hence Figure 2a is preferred.

Strict adherence to the rules of phylogenetic classification (Hennig, 1966) would result in a major change in nomenclature for the subfamily. Application of the sequencing convention (Wiley, 1979) would permit the inclusion of the four species of elaphostrongylines in a single genus, *Elaphostrongylus* Cameron, 1931. I prefer to retain the current nomenclature at the present time for the following reasons. Pryadko and Boev (1971) reduced *E. rangiferi* Mitskevitch, 1958, and *E. panticola* Liubimov, 1945, to synonyms of *E. cervi* in the absence of morphometric criteria to separate these forms. Additional investigation, however may validate one or both of these species and would require the reinstatement of *Parelaphostrongylus.* In addition, both taxa as currently defined are monophyletic based on criteria presented by Platnick (1977). Rec-

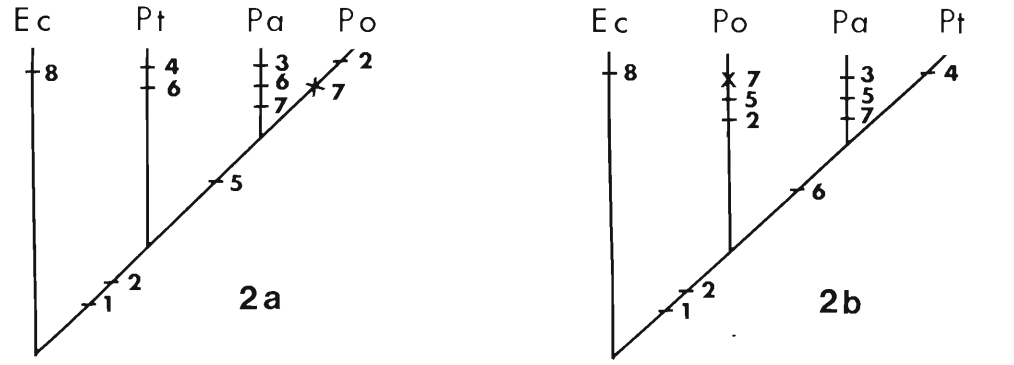

Figure 2. Cladograms of the relationships of the Elaphostrongylinae. Numbers correspond to the presence of the apomorphic character states as outlined in the text. Numbers represent character states (Table 1 and text). A line followed by a number indicates an increased change. An X indicates a decreased change (see text and Table 1) (Ec = *Elaphostrongylus cervi*; Po = *Parelaphostrongylus odocoilei*; Pa = *P. andersoni*; Pt = *P. tenuis*).

ognition of the first cladogenic event at the generic level and subsequent events at the specific level is clearly arbitrary.

Any attempt to assess the evolutionary and biogeographic history of a parasite with a complex life cycle must include an analysis of the history and distribution of the intermediate host(s). There is some disagreement as to the time of origin of the current holarctic gastropod fauna. All contemporary genera were present by the end of the Pliocene (Likhachev and Rammel'maier, 1952), however they may be substantially older (Taylor, 1960; Waldén, 1963).

The elaphostrongylines show some specificity for the intermediate host (Panin, 1964; Lankester and Anderson, 1968; Platt, 1978). A number of species in two of the four suborders (Heterauretha and Sigmaurethra) of the pulmonate order Stylommatophora, are capable of serving as suitable intermediate hosts. Therefore, it can be assumed that a modern terrestrial gastropod fauna was present when modern cervids were becoming established during the late Miocene and early Pliocene (Flerov, 1952) as suggested by Pryadko and Boev (1971). The continuity of the gastropod fauna during the late Tertiary and Pleistocene would have given the elaphostrongylines a long association with, and relatively little selection pressure from, the intermediate host. Thus, the current distribution of these nematodes must be explained as a function of the evolution and distribution of the definitive hosts.

The family Cervidae consists of 11–17 extant genera, with representatives occurring naturally on all continents except Australia and Antarctica. *Cervus* and *Mazama* will be treated sensu lato, consisting of the following: *Cervus* s.l. (=*Cervus, Dama,* and *Axis*) and *Mazama* s.l. (=*Mazama, Blastocerus, Ozotocerus, Hippocamelus,* and *Pudu*).

There have been few attempts to formally reconstruct the phylogenetic relationships of the genera of cervids. Flerov (1950) presented a general evolutionary scheme for the cervids based on paleontological, as well as neontological data that is at variance with more traditional schemes (e.g., Lydeckker, 1898; Simpson, 1945). These follow Brooke's (1878) original designation of two primary lineages based on the type of reduction of the second and fifth metacarpals. Giffin (1974) analyzed the phylogeny of the cervids using cladistic methods in a more inclusive study of the relationships of the Artiodactyla. Her work was based on a combination of gross cerebral characters as well as noncerebral characters taken from the literature. Giffin (1974) did not use the condition of the metacarpals in her analysis, and hence it serves as an independent test of that character. The results provide more support for the traditional classifications than that of Flerov (1952).

The most parsimonius hypothesis of the origin of the elaphostrongylines is a nearctic origin prior to the formation of *Rangifer* and the subsequent cospeciation of *Parelaphostrongylus* spp. with *Odocoileus* spp. (Fig. 3). *E. cervi* originated with *Rangifer,* a northern-adapted, tundra specialist. At the same time the ancestor of *O. virginianus*–

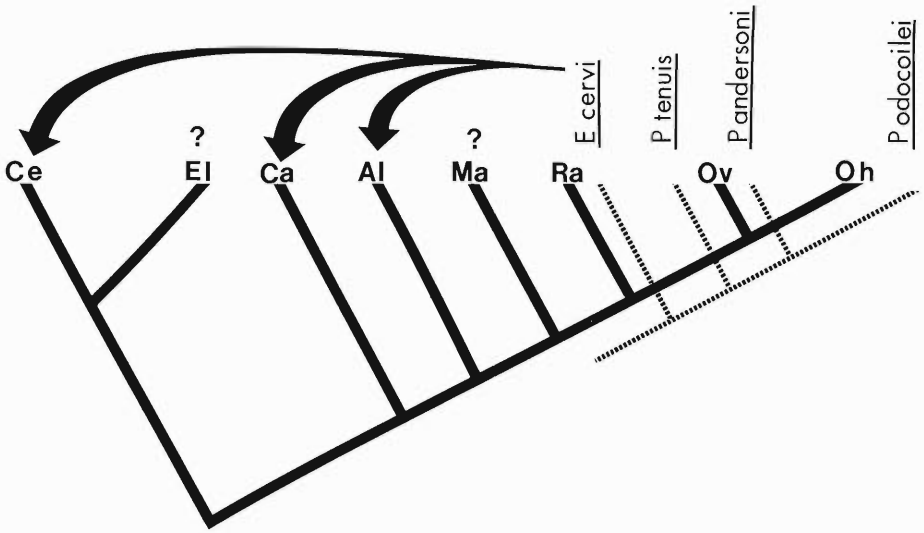

Figure 3. Correspondence between the cladogram of the Elaphostrongylinae (dashed line) and Cervidae (solid line). Arrows indicate colonization of cervids by *E. cervi* (Cervinae; Ce = *Cervus,* El = *Elaphrurus*: Odocoilinae; Ca = *Capreolus,* Al = *Alces,* Ma = *Mazama,* Ra = *Rangifer,* Ov = *Odocoileus virginianus,* Oh = *Odocoileus hemionus*). ? indicates that elaphostrongylines are not known from these genera. Cladogram of Cervidae (excluding Hydropotinae and Muntiatinae) modified from Giffin (1974).

O. hemionus, a temperate and subtemperate generalist (Brokx, 1972), originated harboring the ancestor of *Parelaphostrongylus* spp.

Alternatively, the elaphostrongylines could be regarded as eurasian in origin (Pryadko and Boev, 1971). This would require strict cospeciation of the parasite and host with subsequent extinction of the parasites of *Cervus* s.l. through *Mazama* s.l. preceding or as a result of colonization of these hosts by *E. cervi.* There is no evidence to support these extinctions, nor is the hypothesis testable in any way. A second alternative involves the survival of *E. cervi* in the more primitive cervids and recognizing *E. cervi* as ancestral to *Parelaphostrongylus.* However, since *E. cervi* is not plesiomorphic for all characters examined it does not meet the minimum requirements of an ancestor. The presence of *E. cervi* or an undescribed species of *Elaphostrongylus* in South American cervids (*Mazama* s.l.) would falsify the nearctic origin of the group, due to the geographic isolation of the hosts and the unlikely possibility of long-distance dispersal of the nematode to the southern hemisphere.

The second speciation event gave rise to the neurotropic specialist *P. tenuis,* which exhibits narrow coaccommodation and is the ancestor of the muscleworm lineage (Figs. 2, 3). The vicariant event responsible for this speciation event is unknown. It was not however, a response to speciation in the host lineage (Fig. 3). Speciation in the muscleworm lineage followed the separation of *Odocoileus* into a western population, *O. hemionus,* and an eastern form, *O. virginianus,* as described by Brokx (1972). Vicariance could have been accomplished by the formation of the midwestern plains during the Pliocene (Clements and Chaney, 1937) or by a later reestablishment of the plains during the Pleistocene (Blair, 1958).

The current distribution of elaphostrongylines can be explained as a function of dispersal (mobilism), coaccommodation, and possibly ecologic factors. Overlap between sister-taxa is indicative of mobilism (Croizat et al., 1974). *Elaphostrongylus cervi* has an extensive distribution in Europe and Asia (see Kontramavichus et al., 1976). Naturally occurring infections of *E. cervi* have been reported from reindeer in Newfoundland (Lankester, 1976; Lankester and Northcott, 1979), which is evidence for a holarctic distribution. *Parelaphostrongylus* is known only from North America (Anderson, 1972). *P. tenuis* has been reported from a wide variety of

locations in eastern North America (Anderson, 1956; Prestwood and Smith, 1969; Gilbert, 1973) and as far west as western Manitoba (Bindernagel and Anderson, 1972) and eastern Oklahoma (Carpenter et al., 1972; Kocan et al., 1982). The distribution of *P. andersoni* has been well documented in the southeastern United States (Prestwood et al., 1974). Pybus and Samuel (1981) have reported this species from white-tailed deer in southeastern British Columbia. *P. odocoilei* has been reported from central California (Hobmaier and Hobmaier, 1934; Brunetti, 1969), west-central Alberta (Platt and Samuel, 1978a), and Vancouver Island, British Columbia (Platt, unpubl.).

The current holarctic distribution of *E. cervi* could be attributed to the colonization of more plesiomorphic cervids (e.g., *Alces, Capreolus,* and *Cervus*) (Fig. 3) as a result of a period of westward mobilism of *Rangifer,* broad coaccommodation within the Cervidae, and the apparent absence of competitors in Europe and Asia. Natural infections have been reported from five genera of cervids (Anderson, 1968; Kontramavichus et al., 1976; Kotrlá and Kotrlý, 1977). Larvae identical to those described for other elaphostrongylines, as well as other protostrongylids, have been reported from caribou in Ontario and Manitoba (Lankester et al., 1976), suggesting that *E. cervi* is sympatric with *P. tenuis* in some parts of its range. White-tailed deer show no ill effects of infection with *E. cervi* in limited experimental trials (Lankester, 1976) or under field conditions (Kotrlá and Kotrlý, 1977). Although white-tailed deer can harbor infections of *E. cervi,* competition from *P. tenuis,* which is extremely pathogenic to reindeer, could have resulted in competitive exclusion and may have prevented the introduction of *E. cervi* into populations of *O. virginianus.*

The absence of neurotropic elaphostrongylines in mule deer from western North America may be explained by a low tolerance of the proto-*hemionus* population for CNS-inhabiting species. Experimental infections of *P. tenuis* in mule deer resulted in paralysis and death of the host (Anderson et al., 1966; Tyler et al., 1980). The absence of CNS-inhabiting species could be further tested by experimentally infecting mule deer with *E. cervi.* The prediction being that mule deer would succumb to the infection or at least show signs of CNS disturbance, thereby reducing host fitness. This would have prevented sympatry between mule deer and wapiti where *E. cervi* was present and the subsequent spread of *E. cervi* into areas occupied only by *O. hemionus.*

The current pattern of distribution of muscleworm species is considerably more difficult to explain. The recent report of *P. andersoni* from British Columbia (Pybus and Samuel, 1981) occupying an area of overlap with *P. odocoilei* indicates a period of mobilism for *P. andersoni* associated with the expansion of the range of white-tailed deer. The timing of this movement is unclear. Krämer (1972) hypothesized that the white-tailed deer in British Columbia represent an autochthonous population that survived the last glacial period in western refugia.

The meningeal worm, *P. tenuis,* has not been reported from white-tailed deer in western Canada (British Columbia, Alberta, and western Saskatchewan) despite intensive investigation (Bindernagel and Anderson, 1972; Bindernagel, 1973; Samuel and Holmes, 1974). There are two possible explanations for the absence of *P. tenuis* from western North America. The first involves two separate periods of mobilism from white-tailed deer; one, reaching the west coast and, preceding the last glaciation, which resulted in deer harboring *P. andersoni* but not *P. tenuis,* and a later period of mobilism of white-tailed deer infected with *P. tenuis.* If this scenario is correct, *P. tenuis* may eventually reach cervid populations in and west of the Rocky Mountains, as suggested by Bindernagel and Anderson (1972).

A more parsimonius hypothesis may relate to differences in biological valence between the muscleworm species and *P. tenuis.* Shostak and Samuel (1979) demonstrated significant lowered infectivity of the first-stage larvae of *P. tenuis* to gastropods after exposure to freezing conditions. Larvae of *P. odocoilei* did not show a similar decline in infectivity. Larvae of *P. andersoni* have not been tested, however this species would be expected to have a response similar to its sister-species, *P. odocoilei,* and to have the capacity to survive freezing conditions. This would result in an ecological limitation to the spread of *P. tenuis,* but not *P. andersoni,* in accord with the general hypothesis proposed by Samuel and Holmes (1974). They stated that, "some ecological feature, possibly associated with drier conditions" prevented the westward spread of *P. tenuis.* This would predict that *P. tenuis* has reached the westernmost limits of its range and cannot move farther west without significant ecological changes

or human intervention. Recent ecological studies (Kocan et al., 1982) provide circumstantial evidence for this hypothesis. Single or multiple periods of mobilism of white-tailed deer would be consistent with this scenario.

Success of the host(s) may also play a prominent role in determining the distribution of the parasite. This is exemplified by the wide distribution of *P. tenuis* and *P. andersoni* in North America. Range expansion of these species is directly related to the success of the white-tailed deer and the wide availability of suitable intermediate hosts. The case of *P. tenuis,* which exhibits narrow coaccommodation and is pathogenic to ecologically similar hosts, is particularly intriguing.

Barbehenn (1969) proposed a "germ warfare" theory of species diversity as a mechanism for the maintenance of a competitively inferior host in the presence of a superior competitor. Coevolution of the host–parasite complex would produce a more successful competitor than the host alone. Barbehenn (1969), citing the work of Karns (1966), recognized *Pneumostrongylus* (=*Parelaphostrongylus*) *tenuis–O. virginianus* as a possible example of a host–parasite complex that could adversely affect sympatric ungulates. Investigations in Maine (Gilbert, 1973, 1974) and Ontario (Saunders, 1973) have provided circumstantial evidence for the reduction of moose (*Alces alces*) populations in areas where they are sympatric with white-tailed deer that have a high prevalence of infection of *P. tenuis.* The pathologic effects of *P. tenuis* infections in moose (Anderson, 1964; Kurtz and Schlotthauer, 1966) support his hypothesis. Kelsall and Telfer (1973) suggested that *P. tenuis* may have had a role in restricting the range of moose. Experimental infections of *P. tenuis* in reindeer have also proved lethal (Anderson, 1971) and Dauphiné (1975) hypothesized that the failure of reindeer (*Rangifer tarandus*) to establish in Nova Scotia was a result of the acquisition of *P. tenuis* from resident white-tailed deer.

Wapiti (*Cervus elaphus*) were widely distributed in the northern and central United States following the last glacial period (Hall and Kelson, 1959), however they are currently restricted to higher elevations in western North America and northern habitats where white-tailed deer do not typically occur. This tremendous reduction in range may be associated with the expansion of the *O. virginianus–P. tenuis* complex. The pathogenic effects of *P. tenuis* in wapiti have been well documented (Carpenter et al., 1973; Trainer, 1973).

The role of parasites in altering the range of host organisms can only be approached circumstantially, as presented above. Range extension is undoubtedly a complex process involving a variety of factors. Climate and concomitant changes in vegetation during the Pleistocene have been used to explain significant shifts in the distribution of a variety of mammals (Blair, 1958); however, the possibility of a host–parasite complex acting as a selective agent must be considered where the evidence warrants.

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Mode of Entry of First-Stage Larvae of *Parelaphostrongylus odocoilei* (Nematoda: Metastrongyloidea) into Four Species of Terrestrial Gastropods

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ABSTRACT: The mode of entry of the first-stage larvae of the metastrongyloid nematode, *Parelaphostrongylus odocoilei,* was examined for four species of terrestrial gastropods: two slugs, *Deroceras laeve* and *Deroceras reticulatum,* and two snails, *Vitrina limpida* and *Zonitoides arboreus.* Gastropods were exposed to first-stage larvae in glass fingerbowls lined with moist filter paper, killed at 30-min intervals, and serially sectioned. Direct penetration of the ventral epithelium was the predominant mode of entry into *D. laeve* and *V. limpida.* Direct penetration and ingestion occurred at similar rates in *Z. arboreus. Deroceras reticulatum* was an unsuitable intermediate host for *P. odocoilei* based on low levels of larval entry.

Nematodes of the superfamily Metastrongyloidea typically require an intermediate host, generally a gastropod. First-stage larvae (L1's) are passed in the feces of the definitive host and must gain entry into the intermediate host where two molts occur to the infective stage (L3). The principal modes of entry of L1's of various Metastrongyloidea into gastropods are passive entry via ingestion presumably with food (e.g., Harris and Cheng, 1975; Yousif and Lammler, 1977), active penetration of the body surface, usually the foot (e.g., Hobmaier and Hobmaier, 1934; Kassai, 1958; Švarc and Zmoray, 1974), or both (Cheng and Alicata, 1965).

The present study was undertaken to determine the mode of entry of L1's of *Parelaphostrongylus odocoilei,* a parasite of mule deer (*Odocoileus hemionus* subspp.), into four species of terrestrial gastropods. Three of the gastropod species examined occur in Jasper, Alberta (Platt, 1980), an area enzootic for *P. odocoilei. Deroceras laeve* (Müller, 1774) is the primary intermediate host in the area, and *Zonitoides arboreus* (Say, 1816) is also capable of harboring larvae infective to deer (Platt, 1978). *Vitrina limpida* Gould, 1850 is abundant in Jasper (Platt, 1980) but is not known as an intermediate host, and *Deroceras reticulatum* (Müller, 1774) was used for comparative purposes and to determine its suitability as a potential laboratory host.

Materials and Methods

Vitrina limpida was collected in Jasper National Park, Alberta, Canada. Because artificial digestion of over 100 of these individuals revealed no metastrongyloid larvae, it was assumed that individuals used in the experimental trials were free of *P. odocoilei. Zonitoides arboreus* was collected in Elk Island National Park, Alberta, where *Parelaphostrongylus* spp. are not known to occur (Bindernagel, 1973). Specimens of *Z. arboreus* were dissected and were negative for nematodes. All *Deroceras* were laboratory reared.

First-stage larvae of *P. odocoilei* were collected from feces of experimentally infected mule deer as described by Platt and Samuel (1978). Approximately 30,000 L1's of *P. odocoilei* were placed on a moistened disc of Whatman No. 1 filter paper in each of four 12.4-cm glass fingerbowls. Larvae were introduced in a latticelike fashion to ensure their even distribution on the substrate. Sufficient water was added to completely moisten the paper, but not flood it.

Fifteen to 23 gastropods of each species were placed in the four bowls. The gastropods were permitted to move freely, but if they began to climb the sides of the fingerbowl they were gently replaced at the center of the dish. Five to eight individuals of each gastropod species (Table 1) were removed 30, 60, and 90 min postexposure (MPE), killed in boiling water, fixed in 10% neutral-buffered formalin, and prepared for histological examination. Gastropods were serially sectioned at 7 μm and stained in Whipf's polychromatic stain (Vetterling and Thompson, 1971).

Entry of nematode larvae into gastropods was scored as an index. Preliminary observations indicated that an individual larva was present in an average of eight consecutive sections. All sections of larvae were counted in every seventh section of mollusc and tabulated according to location (foot or viscera). An index of entry for the foot (IEF) and viscera (IEV) was calculated based on the mean number of nematode sections per section of snail examined.

Comparisons of larval entry were made using the Kruskal-Wallis test for changes within the foot or viscera over time and to assess differences in the IEV and IEF between species of gastropods. Wilcoxon's signed rank test was used to compare differences between the foot and viscera at the same time interval. Statistical analyses were executed on a VAX 11/750 digital computer.

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Table 1. Index of entry of the first-stage larvae of *Parelaphostrongylus odocoilei* into four species of terrestrial gastropods.

Gastropod species	Minutes postexposure	*N*	Index of entry	
			Foot	Viscera
Vitrina limpida	30	8	1.27*	0.015
	60	8	3.85*†	0.16†
	90	7	4.64*	0.10
Zonitoides arboreus	30	6	0.41	0.41
	60	5	0.53	0.52
	90	7	0.93	0.77
Deroceras laeve	30	5	0.35*	0
	60	5	0.47*	0
	90	5	1.40*†	0
Deroceras reticulatum	30	7	0.03	0
	60	8	0.05	0.04
	90	7	0.06	0

* Significant difference between columns ($P < 0.05$), Wilcoxon signed rank test.

† Significantly greater than the preceding value within the column ($P < 0.05$), Kruskal-Wallis test.

Results

The entry of L1's of *P. odocoilei* were distinct for each of four species of gastropods (Table 1). Larvae of *P. odocoilei* were present in the foot of all species and in the foot and viscera of *V. limpida* and *Z. arboreus* at 30 MPE. Larvae were not observed in the viscera of *D. laeve*. The IEF for *D. laeve* was similar at 30 and 60 MPE, but increased significantly from 60 to 90 MPE (Table 1). Larval entry was poor in the slug, *D. reticulatum*.

There were significantly fewer larvae in the viscera than in the foot of *V. limpida* (Table 1). Significant increases in the IEF and IEV of *V. limpida* occurred between 30 and 60 MPE. Significant changes were not observed in or between the IEF and IEV of *Z. arboreus* over or at any specific time, respectively.

Discussion

There are two pathways for entry of first-stage larvae of metastrongyloid nematodes into terrestrial gastropods. These are an active mode, by direct penetration of the epithelium, and a passive mode, by ingestion, with subsequent penetration of the gut and migration to the site of development.

Ingestion of larvae was not demonstrated in *D. laeve*, the primary host of *P. odocoilei* in Alberta (Platt, 1978). The number of larvae in the viscera of *V. limpida* was minimal compared to the number in the foot. Thus, a feeding response was not initiated or was minimal by *D. laeve* or *V. limpida*, respectively, under the current design. A feeding response was stimulated in *Z. arboreus*, a natural host of *P. odocoilei* in Alberta (Platt, 1978). The indexes for foot and viscera in this species were virtually identical. There was no histological evidence for the escape of larvae from the gut of *V. limpida* or *Z. arboreus*, however the time of the trials may have been too short for this to occur. Ingestion, as a viable mode of entry, appears to depend on a specific chemical stimulus provided by the larvae or substrate.

Field studies have identified terrestrial pulmonates (Stylommatophora) as the intermediate hosts for *Parelaphostrongylus* (Mitskevich, 1964; Panin, 1964; Lankester and Anderson, 1968; Platt, 1978). Our results indicate that direct penetration is probably the primary mode of entry for these nematodes under natural conditions, with ingestion occurring only if a feeding response is stimulated.

Results of the penetration trials suggest that *Vitrina limpida* should be considered as a primary candidate for natural transmission of *P. odocoilei*, but only preinfective-stage larvae were found in 11 of 1,514 *V. limpida* examined from Alberta (Samuel, Platt and Knispel, unpubl.). By contrast, the prevalence of infection in *D. laeve* and *Z. arboreus* was 5.3% (135 of 2,564) and 0.8% (7 of 861), respectively, and both harbored infective larvae.

Direct penetration of larvae into a terrestrial intermediate host such as *D. laeve* should be more advantageous for transmission than ingestion. Penetration only requires contact and some stimulus for penetration on the part of the larvae. Ingestion, on the other hand, would require that larvae consistently encounter and remain on material regularly ingested by the mollusc. Food preference in pulmonates may be highly selective (Runham, 1975) and, thus, could severely limit the incidence and prevalence of infection. There is no good evidence, of which we are aware, that terrestrial gastropods are attracted to or preferentially ingest feces, which is the most likely site of infection if ingestion is the primary mode of entry. At the present time, acquisition of elaphostrongyline infections by terrestrial gastropods, under field conditions, is wholly conjectural due to the lack of information concerning the location and behavior of these larvae in the terrestrial environment.

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Heliconema psammobatidus sp. n. (Nematoda: Physalopteridae) from a Skate, *Psammobatis lima* (Chondrichthyes: Rajidae), Taken in Chile

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ABSTRACT: *Heliconema psammobatidus* sp. n. is described from a skate, *Psammobatis lima*, taken in Chile. This nematode is distinguished from its closest relatives in the genus (*H. heliconema, H. brevispiculum*) by the number of tessellated longitudinal ridges in the male (14, as opposed to 20), the spicule ratio (1:4.2, as opposed to 1:20 or 1:2.4), and the protruding conical vulva of the female.

In August 1970 24 sexually mature nematodes (2 male, 22 female) were recovered from the spiral valve of a skate, *Psammobatis lima* (Poeppig, 1835) taken near San Antonio, Chile. The worms were killed and fixed in 10% formalin and later transferred to 70% glycerine–alcohol. The nematodes are new to science and are described herein. The figures were drawn with the aid of a drawing tube and Polaroid prints obtained with a Zeiss Ultraphot microscope. All measurements are given in micrometers, unless otherwise indicated.

Heliconema psammobatidus sp. n. (Figs. 1-12)

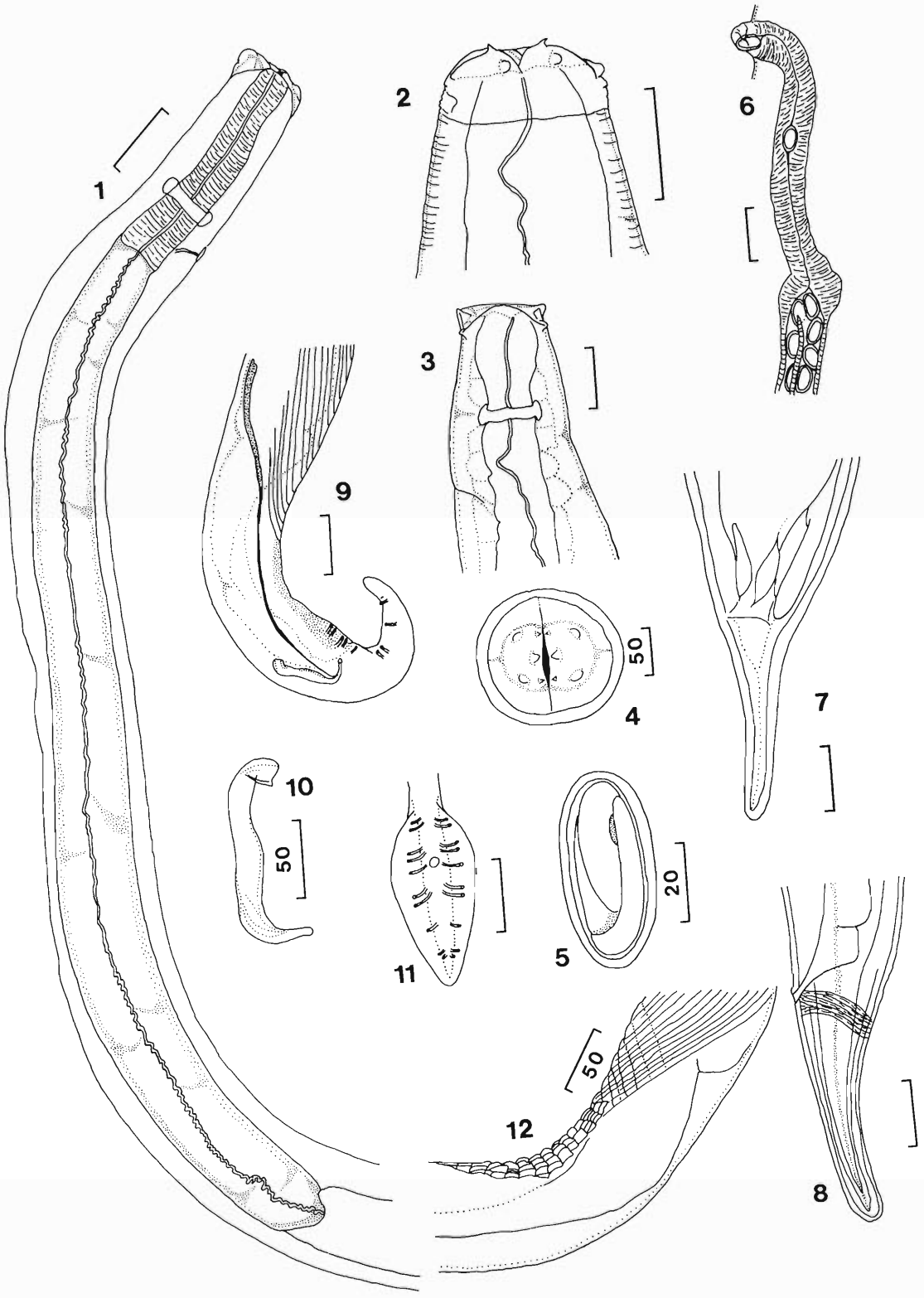
DESCRIPTION: Creamy/white worms with cuticle in anterior region reflected to form cephalic collar. Mouth with two lateral lips, each bearing a median conical tooth and a small subdorsal and subventral tooth (Figs. 2, 4). Esophagus with short muscular region, followed by large, posterior glandular portion. Nerve ring in posterior half of muscular esophagus, with excretory pore near junction of muscular/glandular portions of esophagus. Cervical papillae not observed.

MALE (based on two mature specimens: mean [range]): body length 10.3 mm (9.0–11.5), maximum width 235.0 (220.0–250.0); 43.5 (40.9–46.0) times longer than wide; whole body tightly coiled. Transverse cuticular striations 9.9–11.6 apart. Esophagus, total length 2,089.5 (1,732.0–2,447.0), anterior muscular region 347.0 (317.0–377.0), posterior glandular region 1,742.5 (1,415.0–2,070.0) (Fig. 1). Nerve ring 258.0 (216.0–300.0) from cephalic end. Excretory pore located 311.5 (248.0–375.0) from anterior end, 32–75 (53.5) posterior to nerve ring. Spicules dissimilar, unequal in length (Fig. 9); left spicule narrow, tapering to a fine point distally, 589.5 (558.0–621.0) long; right spicule, short, wide, with blunt proximal and distal ends, 141.0 (131.0–151.0) (Fig. 10) long; spicule ratio 1:4.2 (1:4.1–4.3). Gubernaculum absent. Caudal alae not united anteriorly, supported by 10 symmetrical pairs of pedunculate papillae; four precloacal pairs in two groups of two pairs each; six postcloacal pairs, in four groups of one, two, one, and two pairs (Fig. 11). Phasmids not observed. Longitudinal, somewhat spiral, tessellated ridges in approximately 14 rows, extending anteriorly a short distance from midpoint of left spicule; resulting in a scalelike appearance at posterior end of this region (Fig. 12). Tail flexed ventrad; distance from cloaca to tip of tail 209.5 (184.0–235.0).

FEMALE (based on 22 mature specimens): body length 15.2 mm (6.9–20.5), maximum width (usually in posterior third of body) 410.0 (300.0–600.0); 37.5 (19.3–58.7) times longer than wide;

→

Figures 1-12. ***Heliconema psammobatidus*** **sp. n. 1. Anterior end of male (holotype); 2. dorsal view of lips of female; 3. lateral view of anterior end of female; 4. en face view of anterior end of female to show lips, teeth and cephalic collar; 5. egg with larva; 6. vagina and associated structures of female, including conical vulvar region; 7, 8. tail of two females; 9. posterior end of male to show spicules, papillae, and longitudinal ridges; 10. right spicule of male; 11. disposition of pedunculate papillae on caudal alae and relationship to cloaca; 12. tessellated, longitudinal ridge region of male. (Scale bars are in micrometers and represent 100 μm, except where indicated.)**



body not tightly coiled. Transverse cuticular striations 14–23 apart. Esophagus total length 2,665.0 (1,987.0–3,381.0); distinction between anterior muscular region (502.5 [423.0–635.0] long) and posterior glandular region (2,405.8 [1,826.0–2,921.0] long) not as marked as in males. Nerve ring 230.7 (193.0–262.0) from cephalic end, in middle third of anterior muscular esophagus (Fig. 3). Deirids not seen. Excretory pore posterior to nerve ring, 287.5 (216.0–350.0) from antèrior end (Fig. 3). Vulva conical, protruding (Fig. 6), and situated 5.9 mm (3.6–8.5), or 42.6 (32.0–52.0) percent of body length, from cephalic end. The position of the vulva in three specimens <10 mm long was more posteriorly placed (49, 51, and 52% of the body length from the cephalic end) than in larger (>10 mm) specimens. Vagina muscular, uterus didelphic (Fig. 6), amphidelphic, one ovary associated with each uterine sac. Eggs (50 measured) 50.3 (48–53) long by 23 (23) wide, containing larvae (Fig. 5). Tail blunt (Figs. 7, 8), normally curved slightly dorsad; cloaca to tip of tail 348.2 (271.0–409.0).

HOST: *Psammobatis lima* (Poeppig, 1835).

LOCALITY: San Antonio, Chile.

HOLOTYPE: (Male) USNM Helm. Coll. No. 77628.

ALLOTYPE: (Female) USNM Helm. Coll. No. 77629.

PARATYPES: (1 male, 13 females) USNM Helm. Coll. No. 77630.

ETYMOLOGY: The nematode is named for the host from which it was taken.

REMARKS: Yamaguti (1961) recognized one subfamily, Physalopterinae, within the family Physalopteridae, and placed members of this subfamily that occur in fishes in four genera (*Heliconema, Paraleptus, Proleptus, Pseudoproleptus*). Chabaud (1975), however, listed three subfamilies within the Physalopteridae, with the parasites of fishes falling in the Proleptinae (genera *Heliconema, Paraleptus, Proleptus, Bulbocephalus*). This latter scheme is at variance with the generic makeup of the subfamily Physalopterinae as recognized by Specian et al. (1975) (genera *Heliconema, Paraleptus, Proleptus, Pseudoproleptus, Dogielina, Neoleptus*). The reasons for the latter discrepancies are due to the fact that: (1) Chabaud (1975) felt that *Dogielina*, although seeming to belong to the Physalopteroidea, was not known well enough to classify; (2) Le Van Hoa and Lien-Huong (1969) transferred *Pseudoproleptus* to the Cystidicolidae; (3) Le Van Hoa et al. (1972) clarified the taxonomic position of *Bulbocephalus*, placing it near *Heliconema*; (4) the works by Chabaud (1975) and Specian et al. (1975) appeared in print at the same time, so that the former was not aware of *Neoleptus* created by the latter.

The present specimens have the following characters: (a) pedunculate papillae, (b) a vulva that is located in the middle third of the body, and (c) unequal, dissimilar spicules, placing them in the genus *Heliconema* Travassos, 1919, which was reviewed by Ogden (1969). The occurrence of a member of this genus in a skate is of interest due to the fact that previous hosts for the genus have usually been anguilliform fishes or snakes. The genus characteristic of skates is *Proleptus* Dujardin, 1854, with Dailey and Carvajal (1976) having recovered *Proleptus acutus* Dujardin, 1854 from the skate *Rhinobatos planiceps* taken in Chilean waters.

Heliconema psammobatidus differs from other known species in that the male has approximately 14 tessellated longitudinal ridges in the last quarter of the body, with the females having a protruding, conical, vulva. *H. heliconema* and *H. brevispiculum* have females with prominent "vulvar ridges," but the males have 20 tessellated longitudinal ridges and their spicule ratios (right/left: 1:20 and 1:2.4, respectively) differ from the present species (1:4.2). Fusco and Palmieri (1980) list four species additional to the above (*H. ahiri, H. baylisi, H. longissima, H. serpens*) in their key, and note that in these latter four the females have indistinct "vulvar ridges" and males have 8–12 tessellated longitudinal ridges. Specian et al. (1975) transferred *Proleptus urolophi* Johnston and Mawson, 1951 to *Heliconema*, in view of the extreme anterior placement of the vulva, and noted that this species has an unusual arrangement of cephalic teeth. The present specimens differ from *H. urolophi* in virtually all measurements, e.g., short spicule 131–151 as opposed to 180–200 (given as 2 mm [2,000] in Johnston and Mawson (1951)); long spicule 558–621 compared with 950–1,200; eggs 48–53 × 23 against 40 × 20, and in the position of the vulva. It should be noted that *H. urolophi* was originally described from a stingray, *Urolophus testaceus*, by Johnston and Mawson (1951).

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A New Peruvian *Hexamermis* sp. (Nematoda: Mermithidae) Parasite of Corn Rootworms, *Diabrotica* spp.

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ABSTRACT: A new mermithid nematode parasite in the genus *Hexamermis* was recovered from *Diabrotica* spp. collected in Peru. It is currently being evaluated as a potential biological control agent for introduction into our North American *Diabrotica* populations. Parasitism often exceeded 50% of the adult beetles collected. Field and laboratory studies indicated the nematode apparently has a wide host range including: corn rootworms, Colorado potato beetles, Mexican bean beetles, and beneficial coccinellids.

Diabrotica Dejean is a largely neotropical genus of chrysomelid beetles, a few representatives of which have colonized temperate North America. Among these are several species of economic importance, perhaps the most consequential pest being *D. virgifera virgifera* LeConte, the western corn rootworm. There is evidence that temperate *D. virgifera* is a relatively recent introduction from the tropics. In the tropics it is attuned to an annual cycle of wet and dry but continuously warm seasons; the mechanism adaptive for dormancy in warm, dry seasons apparently serves the species in the temperate climate (Krysan et al., 1977). It follows therefore that a logical place to search for natural enemies of *Diabrotica* is in wet/dry tropics; in January and February of 1979 through 1982, such searches were undertaken in the Cuzco Department of Peru. We report the discovery of, and provide biological information on a new nematode parasite of *Diabrotica* currently being evaluated as a potential agent for introduction into North America. It will be described in a future publication.

Cuthbert (1968) reported the effect of a similar mermithid nematode parasite *Filipjevimermis* of the banded cucumber beetle, *Diabrotica balteata* LeConte, in a sweet potato field in Charleston, South Carolina. He found that 50–100% of the grubs in the soil were parasitized by a mermithid and indicated that few larval *Diabrotica* developed to maturity in that area during late August and September due to this nematode parasite.

Nickle (1974, 1981) has pointed out that nematode parasites of insects have the most potential on soil- and water-dwelling insects. These habitats are more difficult and expensive for conventional insecticides and certain other biologicals such as hymenopterous parasites.

In order to make field releases of this nematode in North America, the potential host range of the nematode had to be determined. Very little is known about the host range of coleopteran mermithids in the literature. We know that dipteran mermithids vary in their host specificity from being species specific, to genus specific, to a wide multigeneric host range.

Materials and Methods

Peruvian studies and collections

The 1979 and 1980 expeditions were conducted under a cooperative agreement between the USDA, Beneficial Insect Introduction Laboratory and the Department of Entomology, University of Maryland, to investigate the potential for the biological control of the Mexican bean beetle, *Epilachna varivestis* Mulsant and *Diabrotica* pests in the United States by means of imported natural enemies collected in the higher altitudes of Peru. The explorations conducted in 1981 by personnel of Department of Entomology, University of Maryland were sponsored by the USDA, Office of International Cooperation and Development (OICD). In 1982, OICD sponsored an exploration by USDA entomologists to investigate the potential for biological control and development of host plant resistance to *Diabrotica* in selected agricultural crops.

The headquarters for the 4-yr foreign studies was established in the city of Cuzco, Peru. Collections of *Diabrotica* were made in the surrounding countryside (approximate radius was 100 mi) during January–February 1979, and January 1980, 1981, and 1982. *Diabrotica* adults were collected from alfalfa, beans, corn, and cucurbit plants with a sweep net or by aspirating them directly from the plant. To survey for nematodes in the field, beetles were squeezed to cause the nematodes to pop out through the abdomen (Fig. 1). The adult *Diabrotica* collected were separated in the laboratory into morphologically similar groups and placed

in cages. The cages were ½–1-gallon plastic ice cream containers with bottoms removed and replaced with 16-mesh screen. The lids were clear plastic. The beetles were fed a dry diet (Guss and Krysan, 1973) and water or slices of domestic squash. The nematodes that emerged passed through the screen into moist sterile sand under the cage. Cages were in a room having exposure to natural light and at room temperature (approx. 20°C). The nematodes that were recovered either from the soil or adult beetles were placed in petri dishes with a moist mixture of sterile sand and Spanish moss. The dishes were turned over daily to prevent the nematodes from sticking to the surface of the dishes. The mixture in the dishes was examined periodically for adequate moisture content.

Nematodes collected from the soil were found in the same field where large populations of *Diabrotica* (50%+) were found parasitized by the nematode. The top soil was examined very carefully for nematodes and the depth of the soil was recorded where the first and last nematodes were collected. The size of the pits dug for nematodes varied, but generally they were 2′ × 3′. The location, elevation, soil depth, and host plants were recorded. Other organisms including beneficial coccinellids (isopods [sowbugs]) that were in the same field were collected and examined for nematodes.

Transfer of the nematode to southern corn rootworm, Beltsville, Maryland

Diabrotica undecimpunctata howardi Barber, southern corn rootworm (SCR), were reared by the method of Jackson and Davis (1978) from a colony maintained by the methods of Branson et al. (1975). Nematode eggs were obtained from either the nematodes collected from soil from the Peruvian alfalfa field or from matured nematodes from the postparasitic stages that had emerged from caged beetles. The postparasitic nematodes were transferred to sterile sand in the laboratory until eggs were laid. Infective-stage preparasitic nematodes were hatched from the eggs by the addition of water. Ten infective-stage nematodes were placed in water in a deep-well slide under a stereoscopic microscope and a 1st or 2nd SCR larva was held with a pair of forceps partially submerged until parasitized.

Caged field release studies, Beltsville, Maryland

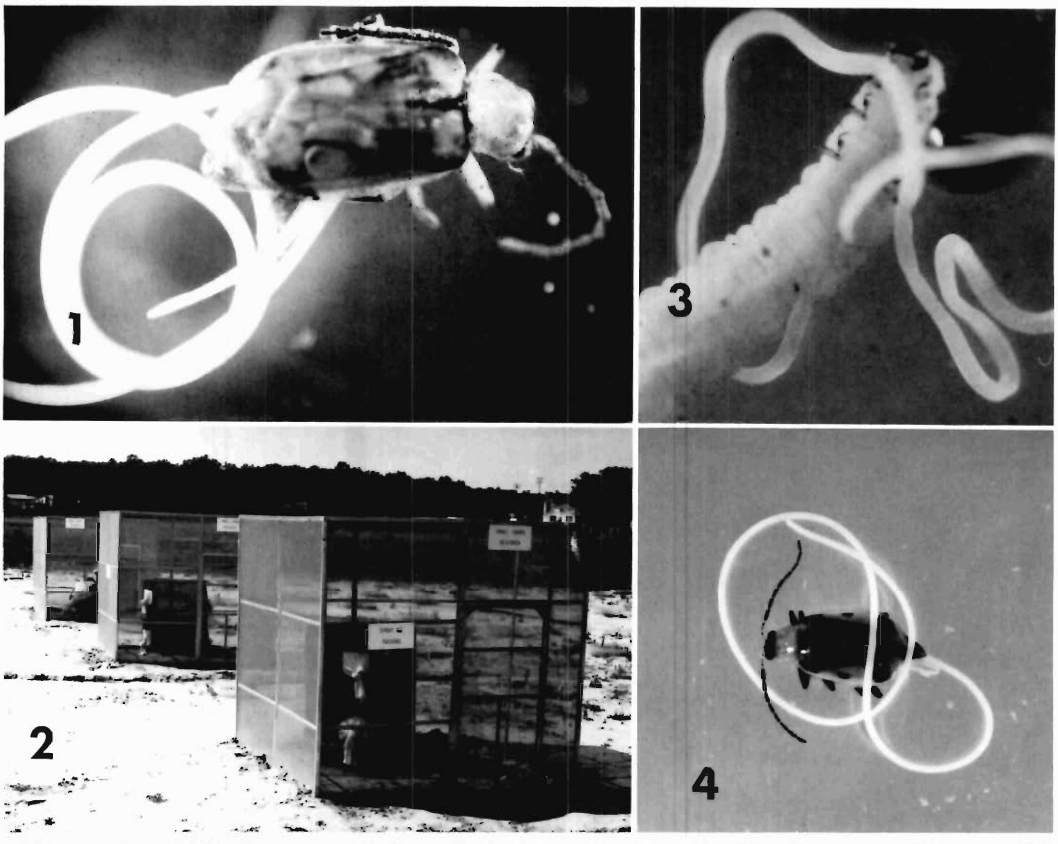
Permission was obtained from the state of Maryland and APHIS to bring into the United States the nematode parasite without soil and without the living Peruvian *Diabrotica*. Also, permission was obtained to allow a caged field release at Beltsville in a caged area around a nematode containment pit (Fig. 2). Each of three pits was surrounded by a plexiglass sleeve, 39 inches in diameter set 24 inches deep in the soil. Corn, fava beans, and potatoes were planted in the soil of the pits. Nematodes and SCR were added at various times. An inner cage was constructed over the pit and plants were placed inside to attract the insects. It was made of heavy tomato cage wire covered with nylon mosquito netting. Sleeves were sown on the inside cages to allow access to the insects and plants. Another sturdy 6′ × 6′ × 6′ screened aluminum cage was constructed around the inner cage to contain any parasitized insects that may have escaped from the inner cage. Earthworms, Mexican bean beetles (*Epilachna varivestis* Mulsant), Colorado potato beetles (*Leptinotarsa decemlineata* (Say)), and four species of coccinellids including *Harmonia axyridis* Pallas, and *Harmonia conformis* (Boisduval) which are arboreal species, and *Hippodamia convergens* Guérin-Méneville and *Cryptolaemus montrouzieri* Mulsant were exposed to the nematode in the soil in the outdoor cages.

Results

Field studies in Peru

The nematode was first discovered during a survey of beetles for parasites in 1979. *Diabrotica speciosa vigens* Erickson adults collected from beans, corn, and alfalfa near Curahausi and Limatambo contained the first nematodes: 118 nematodes emerged from 4,229 *D. speciosa vigens* beetles. Subsequent efforts concerning this nematode were focused on alfalfa and cucurbit fields in the area of Limatambo. Initially, 50% of 30 beetles collected from alfalfa at Limatambo in 1979 were parasitized by the nematodes. This high degree of parasitism was further substantiated in the laboratory when the remaining 970 adult beetles were examined and 500 contained the nematode. Over the 4-yr period, sampling indicated infection rates of 5–90% for *D. speciosa vigens* in the Limatambo area.

Because nematodes collected at the beginning of the field studies in Peru were mostly in the postparasitic juvenile stage that required an obligatory diapause prior to maturing to adult stage, adult nematodes were collected from the soil to obtain infective stages for immediate use in culturing the nematode in the laboratory. The depth at which the nematodes were found in the soil varied from year to year; in a wet year, they were found at depths of 1–3 inches and in a dry year they were found at a depth of 16 inches.



Figures 1–4. ***Diabrotica speciosa.*** **1. Adult *Diabrotica speciosa* from Peru showing postparasitic mermithid nematode parasite, *Hexamermis* sp. emerging from its body cavity. 2. Caged field release site showing the outside 6′ × 6′ × 6′ aluminum cage and the inside cage over the nematode containment pit. 3. Larva of southern corn rootworm with the exotic Peruvian mermithid nematode emerging from its body cavity. 4. Adult southern corn rootworm (*D. undecimpunctata howardi*) showing the exotic Peruvian mermithid nematode emerging from its body cavity.**

During the 4 yr that nematodes were collected, approximately 1,500 were shipped to the United States for further evaluation at the USDA Beneficial Insect Introduction Laboratory and Nematology Laboratory, Beltsville, Maryland.

Several tachinid flies emerged from adult *Diabrotica* that were later identified by C. W. Sabrosky (USDA, Systematic Entomology Laboratory) as *Celatoria* sp. near *diabroticae* (Shimer). Forty-four adult beneficial coccinellids, *Hippodamia convergens,* were collected at Limatambo from squash and alfalfa plants. These beetles were dissected and 31 (70%) were infected with a mermithid nematode. We are not sure these nematodes were the same species as those from *Diabrotica* as they appeared to be morphologically different. An additional trip to Peru is scheduled for January 1984 to identify this nematode. At the same site, 20 isopods (sowbugs) were collected of which four (20%) contained mermithids (species not determined).

Successful transfer to southern corn rootworm

The southern corn rootworm was used as a laboratory and caged field release host for the exotic *Hexamermis* sp. nematode. The SCR is easily reared in the laboratory and is native to the Maryland area. In water in a deep-well slide the infective-stage nematodes penetrated the body cavity directly through the body wall of the *Diabrotica* larvae, often after a period of attachment of 30–120 sec. The infected insect was then placed on sprouted corn. It was found that when a SCR larva became infected at an early stage, the nematode emerged and killed the insect before it pu-

pated (Fig. 3). Growth from a 1-mm-long infective-stage nematode to the postparasitic mermithid stage (5 cm long) was found to take 7 days at room temperature. When late instar larval SCR were parasitized, the postparasitic nematode did not emerge until the insect became an adult (Fig. 4). Each nematode became a female and laid eggs. No male nematodes have been found, and they apparently are not needed by this species for reproduction. It took 1–3 mo for the postparasitic nematodes to become egg-laying females after emerging from their host at room temperature. The eggs hatched in 12–15 days after the addition of water.

Caged field release studies: effect of the exotic nematode on beneficial coccinellids and other organisms

In order to eventually evaluate the effectiveness of the nematode against *Diabrotica* in the field, host specificity studies had to be conducted under quarantine conditions to determine the potential hazards of releasing the exotic nematode. In 1981, 310 *Harmonia conformis* and 248 *Harmonia axyridis* were released at intervals during the summer. During this time, 95 adult coccinellids (*Harmonia* spp.) were removed from the cages and dissected. Only one *H. axyridis* and two *H. conformis* were parasitized by mermithid nematodes. Approximately 50% of the *Diabrotica* released in the cages were parasitized by the nematode. Surface soil samples taken at intervals through the summer indicated the presence of infective stages of the nematode during the time the other beneficial insects and earthworms were exposed. In the laboratory eight *H. axyridis* larvae were parasitized by the nematode. All of the parasitized larvae died in the pupal stage.

In 1982, 255 *Hippodamia convergens* (lady beetles) and 30 *Cryptolaemus montrouzieri* (lady beetles) were released during the summer in the field cages. Soil samples taken during the same time showed the presence of the nematode. One of the 27 *C. montrouzieri* adults removed from the cage was infected by the nematode. None of the 66 *Hippodamia convergens* collected from the cage were found to be parasitized by mermithids. A search of the North American literature revealed only one report (Christie, 1936) of a mermithid (*Agamermis decaudata*) being found on entomogenous lady beetles (*Ceratamegeilla fuscilabris* Mulsant).

Twelve earthworms were taken from the nematode containment site and dissected. None contained mermithid nematodes.

Larvae of the Colorado potato beetle, western and southern corn rootworm, and Mexican bean beetle, all of which are phytophagous insects, were readily infected with this corn rootworm mermithid nematode in the laboratory, and the parasite grew to its normal size in these pest insects.

Discussion

A mermithid nematode parasite of the genus *Hexamermis* discovered in Peru was found to parasitize more than 50% of the *Diabrotica* populations in alfalfa and corn fields. The first importation and caged field release studies in the U.S. show that the parasite can overwinter in the Beltsville area, has a fairly wide coleopteran host range, and can parasitize the southern and western corn rootworms, Colorado potato beetle, Mexican bean beetle, and some beneficial coccinellids. Life cycle studies were undertaken in the laboratory using SCR larvae. In the first caged release field studies 50% of the *Diabrotica* spp. tested were infected at Beltsville. Colorado potato beetles and Mexican bean beetles were parasitized in the laboratory and a few (CPB) adults were found to be parasitized in the cages. Since this nematode apparently has a broad host range, a protocol for the importation and release of such exotic nematodes is being developed in cooperation with APHIS, state of Maryland, and USDA officials. Studies are underway to identify this nematode.

Acknowledgments

We gratefully acknowledge the assistance of J. J. Jackson, ARS, Brookings, South Dakota, for supplying SCR eggs and larvae during the first 2 yr of this project. The authors gratefully acknowledge the financial assistance of the Small Farms Research Project in the purchase of the cages and the hire of summer help.

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Immunoparasitology Symposium

The Third International Immunoparasitology Symposium will be held June 19–21, 1985 in Lincoln, Nebraska. The symposium will be technique/approach oriented. Topics covered will stress immunodiagnosis, parasite modulation of host immune responses, separation and characterization of substances of parasite origin, immune modulations, and genetic engineering/biotechnology for vaccination against parasites. Protozoans, arthropods, cestodes, trematodes, and nematodes will be addressed. Several new systems will be demonstrated. Poster presentations by participants are encouraged.

Proceedings of the meeting will be available to participants at registration. Attendance will be limited to 300 persons. A fee of $75.00 ($50.00 for graduate students) will cover registration costs, proceedings, and lunches for each day. For more complete information, including speakers and topics, and registration forms, contact Dr. Gary L. Zimmerman, Symposium Chairman, College of Veterinary Medicine, Oregon State University, Corvallis, Oregon 97331 (telephone (503) 754-2927 or 2141).

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Daubaylia olsoni sp. n. (Daubayliidae: Rhabditida) from the Leech, *Dina anoculata,* in California

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ABSTRACT: The new species, *Daubaylia olsoni,* is described from the predaceous leech, *Dina anoculata,* in Southern California. This is the first report of a *Daubaylia,* a typical genus of snail parasites, from a member of the order Annelida.

Size, shape of the male and female tail, spicules and gubernaculum, and the elongate spermatheca are characters that separate this species from previously described *Daubaylia.* This report extends the range of *Daubaylia* into Western North America.

Species of *Daubaylia* are known as parasites of snails throughout the world. The present report records for the first time a member of this genus from a predaceous leech belonging to the Annelida. Discovery of a genus of traditional snail parasites in another host phylum raises some interesting questions concerning the host specificity of *Daubaylia* species.

Materials and Methods

Specimens of *Daubaylia* removed from a preserved leech, *Dina anoculata* Moore, were collected and submitted to the present author by Andrew C. Olson, Jr., and Richard M. Gadler, both of the Department of Zoology at San Diego State University. They were encountered during a study of the gut contents of *D. anoculata.*

The infected leech was collected on October 2, 1982 from Laguna Meadow pool, Mount Laguna in San Diego County, California.

The nematodes were originally recovered from the intestine where they were associated with partially digested gut contents; however, a reexamination of the infected leech revealed the possibility of a coelomic infection, because nematodes were also found in the body cavity.

Results

The nematodes removed from *D. anoculata* were found to represent a new species of *Daubaylia* and are described below. In the quantitative portion of the description, all measurements are given in micrometers unless otherwise specified. The number following the character is the average value and the figures in parentheses represent the ranges of the characters. The species is named in honor of Dr. Andrew Olson, Jr., who noted the significance of this association.

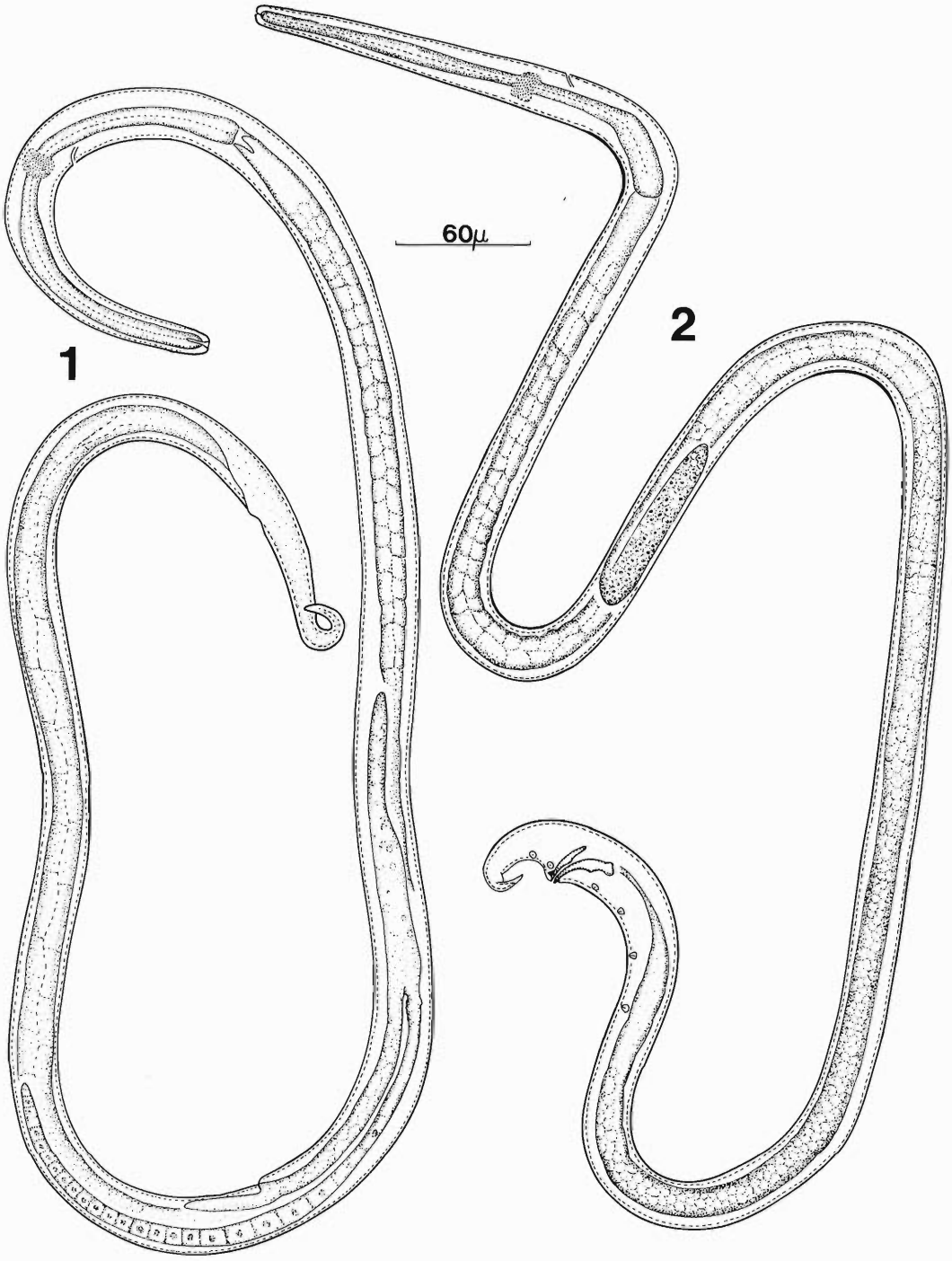
Daubaylia olsoni sp. n.

Daubaylia Chitwood and Chitwood (1934); Daubayliidae (Chitwood and Chitwood) Poinar, 1977; Rhabditoidea (Oerley) Travassos; Rhabditida (Oerley) Chitwood. Relatively slender nematodes; cuticle smooth, six lips either partially or completely fused; amphids dorsolateral, small; stoma partially collapsed at base, leaving a small anterior vestibule; glottoid apparatus lacking; pharynx elongate, composed of a slender cylindrical corpus and isthmus and a glandular basal bulb lacking a valve; ovary single, tip reflexed, usually past vulva; testis single, reflexed; spicules paired, similar, separate; gubernaculum present; bursa absent; genital papillae present. The family contains the single genus *Daubaylia.*

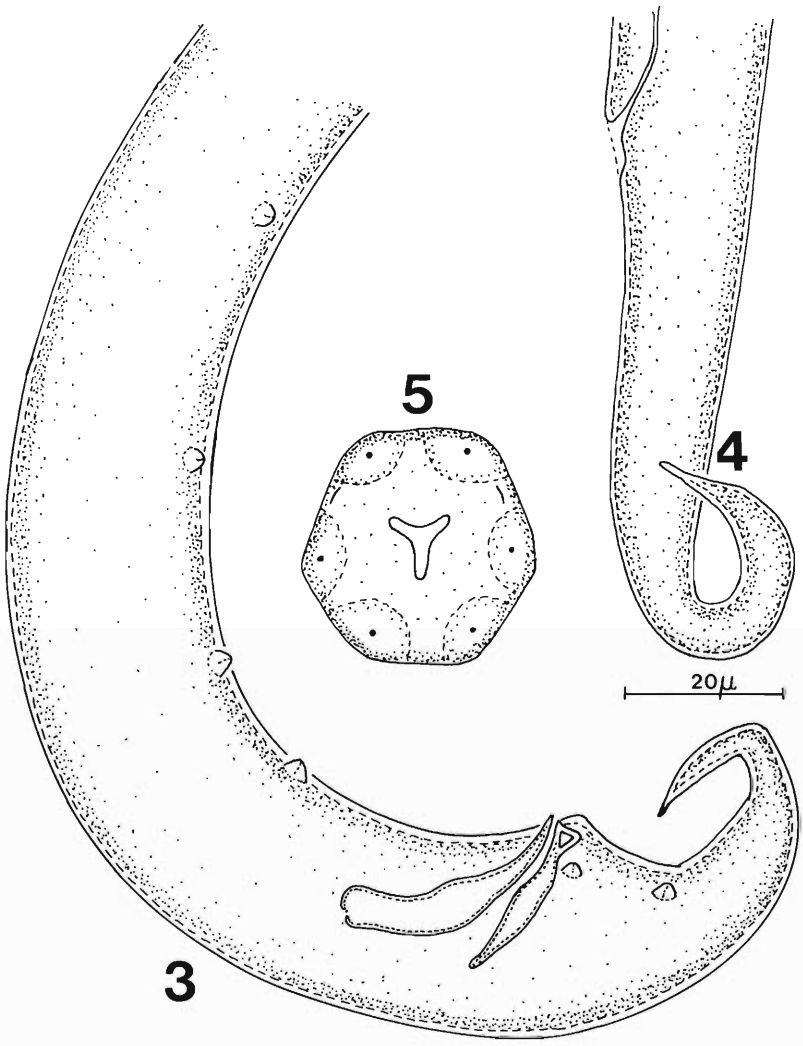
DAUBAYLIA OLSONI: With characters as presented in the above generic description. Lips fused, amphids indistinct, stoma collapsed at base leaving a small anterior vestibule; excretory pore opens slightly below nerve ring; pharynx cylindrical and elongate, with a nonvalvated basal glandular bulb; postuterine sac present, generally longer than body width at vulva; elongate saclike spermatheca at junction of oviduct and uterus; female tail bent dorsally 180° or more; spicules paired, similar, separate; gubernaculum with a posterior (dorsal) v-shaped structure at the tip; male tail with six pairs of genital papillae arranged in two ventrolateral rows; four pairs preanal, one pair adanal, and one pair postanal.

FEMALE ($N = 10$): Length, 1.29 (1.02–1.63) mm; greatest width, 24 (20–29); distance from head to nerve ring, 115 (104–133); distance from head to excretory pore, 130 (120–149); length of pharynx, 206 (179–237); length of tail, 97 (78–107); length of postvulvar uterine sac, 39 (26–64); % vulva, 55 (45–60); eggs were 50 × 22 "in utero."

MALE ($N = 4$): Length, 1.30 (1.20–1.42) mm; greatest width, 21 (20–22); distance from head to nerve ring, 126 (118–131); distance from head



Figures 1, 2. Adults of *Daubaylia olsoni*. 1. Female. 2. Male.



Figures 3-5. ***Daubaylia olsoni.*** **3. Lateral view of male tail. 4. Lateral view of female tail. 5. "En face" view of female.**

to excretory pore, 141 (131–147); length of pharynx, 217 (203–224); length of tail, 49 (48–50); length of spicules, 29 (26–32); length of gubernaculum, 18 (17–20).

TYPE LOCALITY: Laguna Meadow pool, Mount Laguna, San Diego County, California.

TYPE HOST: *Dina anoculata* Moore (Hirudinea; Erpobdellidae).

TYPE SPECIMENS: Holotype (female) and allotype (male) deposited in the nematology collection at the University of California, Davis.

DIAGNOSIS: *D. olsoni* is a medium-sized *Daubaylia.* The females are smaller in length than those of *D. dewiti* Schuurmans-Stekhoven (1956), *D. potomaca* Chitwood and Chitwood (1934), and *D. elegans* Honer and Jansen (1961). The tail of females of *D. olsoni* is longer than that of *D. dewiti* and shorter than that of *D. elegans.* In addition, the 180° or greater dorsal bend of the tail separates females of *D. olsoni* from those of *D. helicophilus* Poinar and Richards (1979), *D. elegans, D. malayanum* Sullivan and Palmieri (1978), and *D. seistanensis* Baylis and Daubney (1922). The presence of a long, saclike spermatheca may be diagnostic for *D. olsoni* because none of the previously described species were noted to possess this structure.

Males of *D. olsoni* are larger in length than

those of *D. helicophilus, D. seistanensis,* and *D. malayanum,* and smaller than those of *D. potomaca* and *D. dewiti.* In addition, the gubernaculum of *D. olsoni* possesses a wedge-shaped structure on the dorsal ridge of the apical portion, a structure absent in all previously described species except *D. malayanum.* However, the latter species has spicules shaped differently from those of *D. olsoni.* The strongly curved tail tip and presence of six genital papillae also separate *D. olsoni* from most other males of *Daubaylia.*

The rarity of eggs in the uteri of female *D. olsoni* may indicate infrequent egg development coupled with rapid oviposition. This phenomenon was also noted for *D. elegans* (Honer and Jansen, 1961).

Discussion

All previous species of *Daubaylia* have been recovered from planorbid snails where they occurred in the mantle cavity and internal tissues. Only rarely have they been found in the mollusc's intestinal tract.

Morphologically, members of *Daubaylia* represent moderately specialized obligate parasites. The only detailed biological studies on this genus were conducted on *D. potomaca* (Chernin et al., 1960; Chernin, 1962) and revealed the interesting fact that a specialized infective stage is lacking. According to the above authors, infection is initiated by gravid females that leave the parasitized host and make contact with a healthy one. Laboratory studies showed that parasite–host contact was passive and made when a snail crawled over a gravid female nematode. At this time the nematode entered the slime and somehow arrived in the snail tissues, presumably by passing through the mantle cavity, although this action was never observed.

Such a nonspecific type of infection process is surprising with the specialized morphology of the parasite. However, if a similar biology occurred with *D. olsoni,* then it is possible to see how leeches and possibly other freshwater invertebrates could become infected with *Daubaylia* nematodes.

Adult and juvenile nematodes were recovered from the infected leech, but because the leeches had first been killed and preserved, it was difficult to establish the exact location of the parasites (e.g., body cavity, intestine, or both).

Since *D. anoculata* is predaceous and stomach contents revealed the presence of whole *Daphnia,* ostracods, and amphipods, it is conceivable that snails are also a source of nourishment. If an infected snail were ingested, could the nematodes survive and develop inside the leech's intestine and possibly enter the tissues? An examination of the pond indicated the presence of the planorbid snail, *Planorbella tenuis.*

Moreover, a *D. olsoni* infection was noted in specimens of this snail that were dissected. Because there was no indication that the parasitized leech had recently ingested snail tissue and all stages (juveniles and adults) of *D. olsoni* appeared normal in the leech, it is concluded that both snails and leeches were infected in this habitat.

Previous reports of nematode parasites of leeches were made by Schuberg and Schroder (1904) and Pereira (1931), who reported the presence of *Myenchus* in the tissues of leeches in Germany and Brazil, respectively. The genus *Myenchus* is a member of the order Tylenchida and is quite distinct from *Daubaylia.*

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A Support Cell to the Apical and Lateral Sensory Organs in *Moniliformis moniliformis* (Acanthocephala)

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ABSTRACT: A large cell containing five nuclei is described adjacent to the proboscis sheath and ventral to the middle or anterior part of the cerebral ganglion in *Moniliformis moniliformis*. Four tubelike ducts or processes exit the body of this cell identified as the sensory support cell. The two posterior extensions penetrate the proboscis sheath and join the ventral proboscis retractor muscles. These processes gradually move together as they extend anteriorly and fuse into a single process slightly anterior to the cerebral ganglion. This single duct continues anteriorly surrounded by proboscis retractor muscles and eventually terminates in the apical organ. Each of the two anterior processes joins the ventral anterior medial nerve. Parts of the nerve and each duct move laterally as they extend anteriorly and terminate in the lateral sensory organ.

The discovery (Miller and Dunagan, 1983) of a multinucleated cell ventral to the cerebral ganglion in *Macracanthorhynchus hirudinaceus* suggested that a similar cell might occur in other species. That this was not an isolated event was also suggested by Harada's (1931) original report of a "stutzzelle" near the cerebral ganglion of *Bolbosoma turbinella*. In each of these species, this support cell had extensions that terminated in the lateral sensory organs in *B. turbinella* and both lateral and apical sensory organs in *M. hirudinaceus*. The former is a member of the Palaeacanthocephala, and the latter belongs to the Archiacanthocephala.

This paper describes a sensory support cell in *Moniliformis moniliformis* (=*M. dubius*) and compares this cell with the two similar cells that have previously been described.

Materials and Methods

Moniliformis moniliformis was raised in Sprague Dawley rats from intermediate stages provided by Dr. Jane Starling of University of Missouri at St. Louis. Adult worms were fixed, embedded, stained, and sectioned according to routine techniques (Dunagan and Miller, 1976). However, prior to fixation, the worms were placed overnight into distilled water in a refrigerator. This hyposmotic solution causes the body and praesoma to swell. This influx of water tended to separate internal structures, which in this case were the receptacle protrusor muscles, from the cerebral ganglion. Although this disrupted the normal relationships between components, it also enabled one to clearly view the sensory support cell (SSC) that otherwise was so tightly packed between the receptacle protrusors, proboscis sheath, and cerebral ganglion, that it was difficult to differentiate from other surrounding structures.

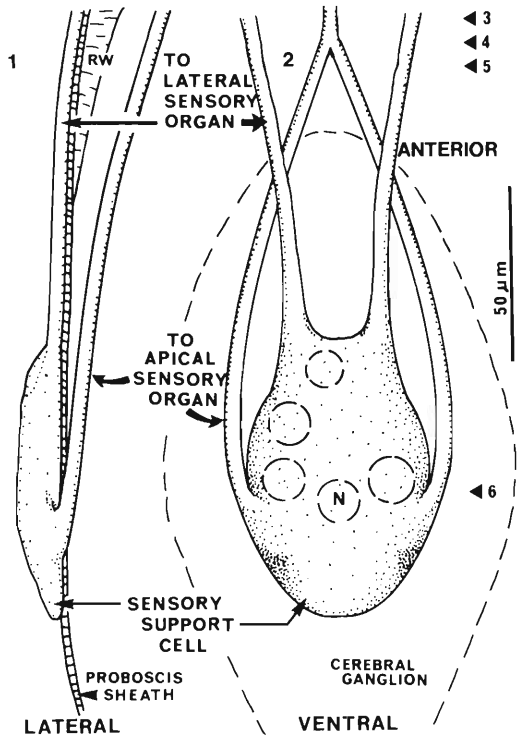
The ventral surface was determined according to Hyman (1951, p. 5) who used the position of the cerebral ganglion as the determining factor. Her identification of surfaces conformed to earlier usage by German authors (Hamann, 1891; Kaiser, 1893; Rauther, 1930; etc.).

Results

The position, size, and shape of the sensory support cell varied somewhat from specimen to specimen presumably depending on the size of the worm and the technique used to prepare the material for study. Isosmotic washes and fixation maintained the SSC pressed between the receptacle protrusors and the proboscis sheath that flattened the cell and caused less taper along its longitudinal axis. The SSC was located adjacent to the proboscis sheath and along its ventral surface in the vicinity of the middle to upper third of the cerebral ganglion (Figs. 1, 2). A typical measurement at maximum dimensions was 80 μm long × 55 μm wide × 14 μm thick. The lateral view (Fig. 1) of the SSC indicates that the thickness varied throughout but primarily at the anterior and posterior extremities.

The SSC cell body contains five nuclei of approximately the same size. Three of these nuclei are visible in Figure 6. The level of this section is indicated on Figure 2. Note that each nucleus has a prominent round nucleolus.

Arising from the SSC soma are four tubelike extensions (Fig. 2). The two posterior lateral processes penetrate the proboscis sheath and associate with the origin of the ventral part of the proboscis retractor muscles (Fig. 7). The ducts move to the medial surface of these muscles, and as they extend in an anterior direction, they gradually move together until they fuse into a single tube (Fig. 3). This sequence of events is shown in Figures 3–5 whose representative level of sectioning has been indicated on Figure 2. Figure 4



Figures 1-2. Diagrammatic representations of the sensory support cell from *Moniliformis moniliformis*. Abbreviations: N, nucleus. 1. Lateral view. 2. Ventral view.

shows the posterior ducts adjacent to each other but prior to fusion. After fusion, this single duct continues anteriorly enclosed by the proboscis retractor muscles until it penetrates the apical sense organ. It is accompanied by the apical sensory nerves (medial pair) and the anterior proboscis nerves (outer pair). The latter do not penetrate the apical organ but service muscles in the apical region.

The anterior extensions of the SSC (Fig. 7) join a large group of nerves from the anterior part of the cerebral ganglion. Each of these anterior ducts along with several nerves extend anteriorly, gradually moving laterally in the process. Each of the anterior extensions terminates in a lateral sensory organ.

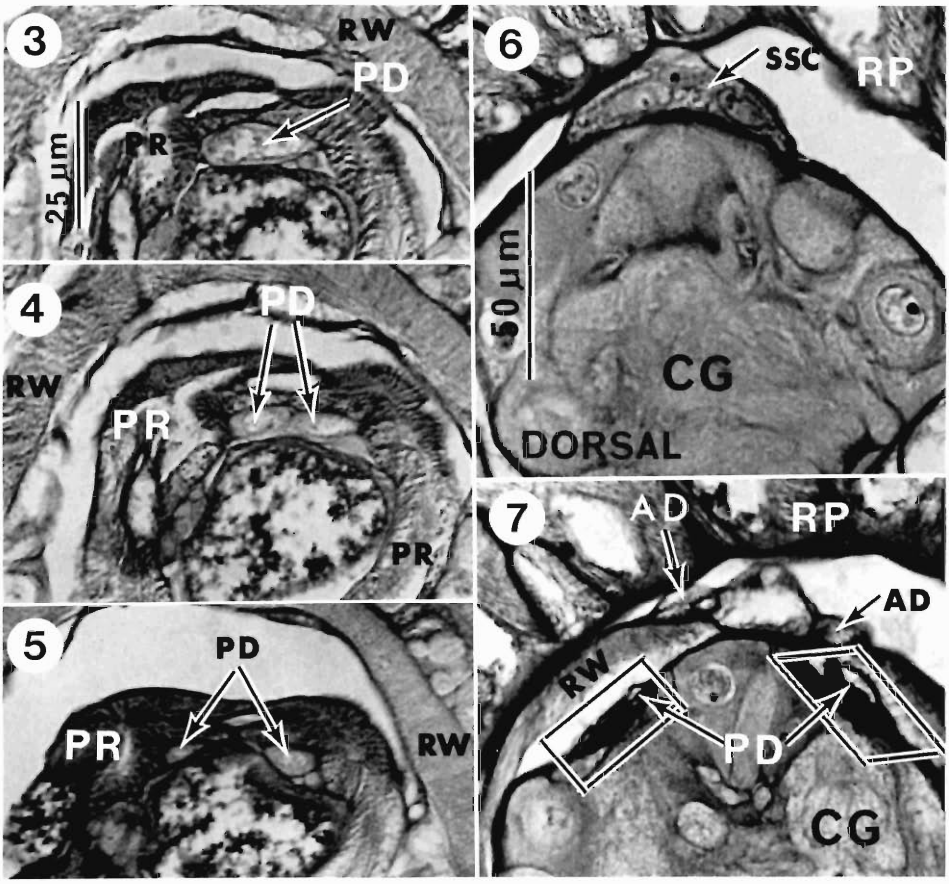
Discussion

The sensory support cell of *M. moniliformis* has previously appeared in published drawings or photographs. Kaiser (1893) published a diagram (plate VIII, fig. 34) of a cross section through the cerebral ganglion of *M. moniliformis*. He illustrated a structure ventral to the ganglion labeled "Markraum" that showed two nuclei. This is the same structure that we now identify as the SSC. Likewise, we published photographs of the cerebral ganglion of *M. moniliformis* in which the SSC may be seen as a cap on the convex surface of the CG (Dunagan and Miller, 1975, fig. 5-C; 1976, fig. 5).

Previous descriptions of this type of cell were made by Harada (1931) and Miller and Dunagan (1983). Harada described this cell in *Bolbosoma turbinella* as anterior to the ganglion in the space between the dorsal proboscis retractors and the proboscis sheath. We described the cell in *M. hirudinaceus* as ventral to the ganglion on the external surface of the midventral longitudinal receptacle muscle. We do not believe that the sensory support cell (Stützzelle) is dorsal in one species and ventral in another but that this is a problem of misinterpreting surfaces. Our directions are based on Hyman's (1951) statement that the cerebral ganglion in Acanthocephala is nearest the ventral surface. Her illustration (fig. 9) of a cross section of the proboscis through the cerebral ganglion (after Luehe, 1912) clearly labeled the structures on this surface as ventral. On that basis, the sensory support cell in *M. hirudinaceus* and *M. moniliformis* is ventral to the ganglion. Harada (1931, p. 171) stated that the ganglion in *B. turbinella* was almost in the center of the proboscis sheath. However, his description of the ganglion clearly shows that his designation of dorsal surface corresponds to our designation of ventral surface. Thus, the support cell is located on the same side of the cerebral ganglion in each of the three species so far described.

Harada described the support cell as "U"-shaped and consisting of two cells joined at their base with anterior extensions that serviced the lateral sensory organs. However, he indicated (p. 175) that nerve fibers did not penetrate these organs and that the extensions of the support cell did not always terminate in a papilla but rather were sometimes flattened without penetrating the surrounding muscle layers. Neither of these observations are consistent with the morphology of the support cell in *M. hirudinaceus* or *M. moniliformis*. The latter have a single multinucleated cell that services the apical and lateral sense organ and that is also serviced by neurons.

In 1943, von Haffner (p. 81) indicated that a median nerve extended from the cerebral gan-



Figures 3-7. Cross sections of sensory support cell and its extensions. Abbreviations: AD, anterior duct from lateral anterior margins of support cell. CG, cerebral ganglion. PD, posterior duct from posterior half of support cell. PR, proboscis retractor muscle. RP, receptacle protrusor muscle; RW, receptacle wall muscle. SSC, sensory support cell. Position of certain sections indicated on Figure 2. 3-5. The fusion of the PD into single process. 6. The position of the SSC. 7. The relative positions of AD and PD following the separation of the AD from the soma of the SSC. Scale in Figure 3 applies to Figures 4, 5. Scale in Figure 6 applies to Figure 7.

glion to the anterior end of *O. thumbi* where it formed a "Endaufknäuelung." He later (p. 82) stated that the median nerve divided into two branches upon entry into the "Muskelplatte," which from his drawing we would interpret as the posterior part of the apical organ. This same nerve was described by von Haffner (1943, p. 86) as originating from two separate nerves in the "vorderen Fläche des Ganglion." These two nerves then united to form the single median nerve. We believe that von Haffner may have made the same mistake in identifying the median nerve that we and others (Kaiser, 1893; Kilian, 1932; etc.) have made. His description matches our earlier discovery (Miller and Dunagan, 1983) that the anterior medial nerve in *M. hirudinaceus* was actually the fused posterior extensions of the sensory support cell. Indeed, von Haffner (1943, p. 89) compared his description of the median nerve in *O. thumbi* with the median nerve in *M. hirudinaceus* indicating that their organization and location were of the same type. This reinforces our belief that a reexamination of *O. thumbi* would show that the previously described branches of the median nerve do not enter the cerebral ganglion, but are extensions of a cell ventral to but in close proximity to the cerebral ganglion. If this is true, then von Haffner's conclusion that the median nerve branched upon entering the apical sense organ and formed the "Endaufknäuelung" or terminal ball of coiled nerves will also need to be reconsidered. Fur-

thermore, his description of the four motor nerves that accompany the median nerve may also need revision. He described them as passing over the external surface of the apical organ to service the muscles associated with the proboscis hooks. If they follow the *M. hirudinaceus* model as he suggested, the medial pair of these nerves enters the apical organ and the lateral pair divides near the posterior terminus of the apical organ and extends over its external surface before further divisions occur.

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Variability and Redescription of *Acanthocephalus dirus* (Acanthocephala: Echinorhynchidae) from Freshwater Fishes in North America

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ABSTRACT: Examination of various *Acanthocephalus dirus* (Van Cleave, 1931) populations from the Mississippi River drainage system in Mississippi, Kentucky, Illinois, West Virginia, Wisconsin, and from Ohio revealed it to be the most variable species of the genus from North American freshwater fishes. These findings are substantiated by morphometric comparisons, stratified histograms, discriminant function analysis, and cluster analysis. Some populations previously reported as *A. jacksoni* Bullock, 1962, from Kentucky, Illinois, and Ohio are reassigned to *A. dirus*. Variability in males and females is reported. The number of proboscis hooks per row was relatively high in southern populations from Mississippi (11–13) but became more variable in the more northern populations having rows with as few as six and eight hooks in males and females, respectively. The taxonomic implications of the expanded diagnosis of the *A. dirus* populations include the designation of *A. jacksoni* and *A. parksidei* Amin, 1975, as junior synonyms of *A. dirus*.

Since the description of *Echinorhynchus dirus* by Van Cleave (1931) from Mississippi and its subsequent assignment to the genus *Acanthocephalus* by Van Cleave and Townsend (1936), the taxonomic status of this species has not been evaluated. It was occasionally reported in some fish-parasite surveys from various Mississippi River drainage system localities, e.g., Bangham and Venard (1942). Its ecology in its crustacean and/or fish hosts was treated by Seidenberg (1973), Camp (1977), and Camp and Huizinga (1980).

Van Cleave's (1931) brief and largely incomplete description of a localized population from the upper Mississippi River exposed only a limited degree of the variation in this species that turns out to be the most variable of the genus *Acanthocephalus* from North American freshwater fishes. In taxonomic studies of the other North American species of the same genus, erection of new species was largely justified based on differences in such characters as the distribution patterns of proboscis hooks from those of *A. dirus*, sensu Van Cleave, 1931 (with "11–13" hooks per row). The elucidation in this paper of the full range of variability of the *A. dirus* populations studied from various Mississippi River drainage localities poses important taxonomic questions. For example, the number of proboscis hooks per row of 6–13 in males and 8–14 in females is sufficient to call for a reappraisal of the systematics of the whole genus from North American freshwater fishes. In this paper, the variability of *A. dirus* is documented, the synonymy of *A. jacksoni* Bullock, 1962, and *A. parksidei* Amin, 1975, with it is proposed, and the species is redescribed.

Materials and Methods

This report is primarily based on the study of 775 *Acanthocephalus* worms (376 males, 399 females) from Mississippi, Kentucky, Illinois, West Virginia, Wisconsin, and Ohio. All collections were taken from waters presently connected with the Mississippi River drainage system except the Ohio site where the connection ceased to exist during the late Wisconsin glaciation about 14,000 years ago; see footnotes (Table 1). Other materials examined that were determined to be *A. dirus* include two specimens from Ewell's (1953) collection from *Cottus bairdi* and *Salmo irideus* (given to me by W. L. Bullock) in central Tennessee that were communicated by Riser (1954, pers. comm. *in* Bullock, 1962), two specimens from Bangham's (1972) collection from *Amia calva* (labeled 1145 P-1-B, dated 8-6-1957 and loaned by G. L. Hoffman) in western Lake Erie, 40 specimens from *Moxostoma erythrurum* and *M. macrolepidotum* (collected by Skelly during 1977–1979) in the Kankakee River, Will County, Illinois, and a few specimens from Page's (1974, 1976) collection from *Etheostoma* spp. (loaned by D. F. Oetinger) in Illinois and Kentucky. The *Acanthocephalus* sp. material from Kentucky suckers reported by Combs et al. (1977) as "very closely related to *A. jacksoni*, as reported by White (1974) from the Kentucky River, and *A. dirus* (Bullock, pers. comm.)" was not available for examination but may be *A. dirus*.

Sketches of individual specimens were made to scale with the aid of a microprojector and used in subsequent morphometric comparisons. The following taxonomic characteristics were effective in comparisons among populations studied. The number of proboscis hooks per row (of two adjacent rows), length of largest proboscis hook (measured only in profile), mean number of hooks on the proboscis, ratio of body width to body length (exclusive of proboscis and bursa; provides a

Table 1. Variations in *Acanthocephalus dirus* populations studied from various locations on the Mississippi River drainage system.

Characteristics		Sources of material studied									
		Mississippi Van Cleave (1931)	Mississippi Van Cleave (type)[1]	Kentucky Gleason[2]	Kentucky White[3]	Illinois Camp[4]	Illinois Seidenberg[5]	Illinois Schmidt[6]	W. Virginia Huffman[7]	Wisconsin Amin[8]	Ohio Muzzall[9]
Body length (BL):	MM	4.0–6.0[10]	2.44–5.04	2.24–4.04	2.28–4.56	2.56–4.92	2.80	2.72–3.80	2.32–4.16	2.48–5.76	2.84–4.48
		—	(3.51) 5	(2.80) 20	(3.48) 10	(3.72) 88	(2.80) 1	(3.30) 9	(3.08) 20	(3.69) 202	(3.62) 21
	FF	10.0–20.0	12.08–16.8	3.60–13.36	2.40–15.12	5.76–15.95	8.72–12.04	8.80–9.20	4.16–11.6	6.56–15.12	5.80–15.52
		—	(12.67) 6	(5.78) 60	(7.70) 17	(10.14) 87	(10.27) 7	(9.00) 3	(7.67) 25	(9.64) 175	(9.87) 19
Body width (BW):	MM	0.45	0.52–0.68	0.48–0.76	0.48–0.92	0.48–0.80	0.60	0.48–0.72	0.44–0.68	0.44–0.96	0.52–0.76
		—	(0.57) 5	(0.60) 20	(0.67) 10	(0.63) 81	(0.60) 1	(0.64) 9	(0.55) 20	(0.63) 202	(0.62) 21
	FF	0.7	0.56–1.20	0.52–1.16	0.48–1.24	0.48–1.32	0.92–1.44	0.80–1.00	0.56–1.16	0.52–1.20	0.52–1.20
		—	(0.76) 7	(0.85) 60	(0.78) 16	(0.81) 81	(1.14) 7	(0.88) 3	(0.70) 25	(0.77) 178	(0.80) 19
Mean BW/BL:	MM[11]	—	(16.9%)	(21.9%)	(19.4%)	(17.0%)	(21.4%)	(19.4%)	(18.0%)	(17.4%)	(17.3%)
	FF[11]	—	(6.6%)	(15.4%)	(11.7%)	(8.0%)	(11.1%)	(9.8%)	(9.3%)	(8.0%)	(8.2%)
Proboscis (P) length:	MM	—	420–700	378–574	322–448	434–588	602	504–588	336–546	434–742	364–560
		—	(555) 3	(462) 19	(406) 9	(522) 64	(602) 1	(538) 5	(443) 15	(583) 83	(474) 21
	FF	—	630–714	490–714	476–714	578–812	588–770	630–658	490–700	588–882	490–742
		—	(667) 6	(613) 58	(571) 12	(664) 76	(638) 7	(644) 2	(577) 18	(726) 106	(585) 19
Proboscis (P) width:	MM	—	168–196	112–182	126–196	112–196	140	126–154	112–168	112–238	98–168
		—	(182) 3	(140) 19	(158) 10	(150) 70	(140) 1	(140) 6	(142) 17	(172) 153	(142) 21
	FF	—	168–266	168–238	140–210	154–266	210–266	196–252	196–238	140–308	168–238
		—	(226) 6	(194) 58	(191) 13	(211) 83	(228) 7	(228) 3	(213) 19	(246) 140	(194) 19
No. hooks (H)/row:	MM	11–13	11–12	8–12	7–10	9–12	—	12	6–11	7–12	7–11
		—	(11.5) 3	(10.0) 15	(8.4) 6	(10.4) 24	—	(12.0) 1	(8.6) 7	(10.3) 39	(9.3) 18
	FF	11–13	11–13	9–14	8–12	9–13	9–11	—	8–11	9–13	8–11
		—	(12.3) 5	(11.1) 51	(9.8) 8	(10.4) 48	(9.8) 5	—	(9.1) 15	(10.8) 83	(9.2) 17
No. hook (H) rows:	MM	15–16	19–20	13–16	12–14	11–16	14	14	12–16	11–16	13–18
		—	(19.5) 2	(13.9) 19	(13.4) 7	(13.9) 76	(14.0) 1	(14.0) 4	(14.1) 15	(13.5) 146	(15.0) 21
	FF	15–16	16–18	13–17	14–19	12–18	13–16	15–17	14–17	13–18	13–18
		—	(17.3) 3	(15.1) 60	(14.9) 12	(15.0) 76	(14.9) 7	(16.0) 3	(15.3) 19	(14.9) 130	(15.5) 19
Mean no. H on P:	MM	—	(224)	(137)	(115)	(142)	—	—	(123)	(142)	(140)
	FF	—	(213)	(168)	(147)	(156)	(146)	—	(138)	(163)	(144)
Largest PH length:	MM	48–60	45–51	35–51	45–54	45–61	54	54–61	42–54	45–74	42–64
		—	(48) 3	(40) 19	(49) 8	(53) 73	(54) 1	(54) 4	(49) 14	(58) 93	(53) 21
Largest PH length:	FF	72	54–70	42–64	45–70	58–80	61–74	64–70	51–74	58–90	61–80
		—	(64) 6	(50) 56	(59) 11	(68) 65	(66) 7	(67) 2	(62) 19	(74) 112	(70) 19

Table 1. Continued.

Characteristics		Sources of material studied									
		Mississippi Van Cleave (1931)	Mississippi Van Cleave (type)[1]	Kentucky Gleason[2]	Kentucky White[3]	Illinois Camp[4]	Illinois Seidenberg[5]	Illinois Schmidt[6]	W. Virginia Huffman[7]	Wisconsin Amin[8]	Ohio Muzzall[9]
P receptacle L:	MM	–	602–1,022	644–812	462–812	560–994	532	644–868	420–812	406–1,134	490–798
		–	(795) 5	(726) 19	(667) 9	(805) 82	(532) 1	(754) 8	(643) 20	(749) 85	(644) 21
	FF	–	770–1,442	420–1,190	588–1,400	728–1,302	742–1,288	798–1,105	630–1,036	560–1,680	490–1,148
		–	(1,141) 7	(898) 56	(816) 12	(1,034) 84	(998) 7	(994) 3	(833) 22	(1,047) 53	(771) 19
P receptacle W:	MM	–	196–252	154–224	140–266	126–252	196	154–210	154–266	140–280	140–224
		–	(230) 5	(177) 19	(201) 9	(196) 84	(196) 1	(182) 8	(196) 20	(201) 85	(179) 21
	FF	–	280–322	168–280	196–252	154–308	280–350	238–252	168–252	168–322	140–266
		–	(292) 7	(224) 57	(228) 10	(246) 83	(316) 7	(245) 2	(214) 22	(255) 54	(205) 19
Lemnisci length:	MM	2/3 recept.	532–1,078	434–882	280–812	448–924	336	350–770	294–770	196–868	420–756
		–	(812) 5	(574) 34	(619) 16	(680) 102	(336) 1	(580) 12	(517) 36	(601) 322	(587) 35
	FF	2/3 recept.	1,120–1,120	392–1,246	602–994	644–1,526	742–1,260	980	602–980	294–1,246	350–1,372
		–	(1,120) 2	(808) 73	(787) 9	(931) 84	(921) 10	(980) 1	(791) 34	(799) 237	(806) 22
Lemnisci width:	MM	–	42–140	70–238	56–182	70–182	168	56–196	56–182	70–308	98–266
		–	(92) 5	(124) 34	(118) 16	(106) 95	(168) 1	(109) 9	(127) 36	(159) 321	(197) 35
Lemniscus width:	FF	–	112–196	98–280	56–182	56–238	98–210	210	70–238	98–364	112–280
		–	(154) 2	(165) 72	(115) 9	(136) 79	(157) 10	(210) 1	(119) 34	(200) 236	(179) 22
Ant. testis L (ATL):		–	392–938	350–616	420–854	462–938	Monorchid	518–742	420–574	504–1,008	532–938
		–	(689) 5	(462) 18	(668) 10	(707) 67	–	(621) 9	(546) 18	(744) 184	(732) 21
Ant. testis W (ATW):		–	252–378	210–420	280–560	238–546	–	294–448	224–434	195–686	280–434
		–	(328) 5	(284) 18	(402) 10	(367) 65	–	(367) 9	(321) 18	(346) 186	(318) 21
Mean ATL/BL:		–	(18.9%)	(16.6%)	(19.2%)	(18.9%)	–	(18.8%)	(17.8%)	(20.6%)	(20.2%)
Mean ATW/BW:		–	(56.5%)	(46.9%)	(60.8%)	(58.7%)	–	(57.5%)	(58.6%)	(55.2%)	(51.4%)
Posterior testis L:		–	378–910	322–672	518–784	406–938	–	462–728	420–574	518–924	532–826
		–	(627) 5	(450) 16	(652) 10	(694) 64	–	(613) 9	(501) 18	(698) 178	(672) 21
Posterior testis W:		–	224–378	224–392	238–518	266–644	–	252–434	210–392	196–616	266–420
		–	(305) 5	(291) 17	(381) 10	(361) 65	–	(351) 9	(316) 18	(351) 186	(325) 21
No. cement glands (CG):		–	5–6	5–6	5–11	5–8	–	6–7	4–9	0–11	4–10
		–	(5.70) 3	(5.95) 20	(6.55) 9	(6.16) 83	–	(6.33) 6	(6.20) 20	(6.16) 190	(6.33) 21
CG length:		–	196–560	126–350	126–378	140–476	–	154–350	126–350	112–392	168–476
		–	(316) 8	(214) 39	(267) 18	(246) 166	–	(232) 12	(224) 40	(235) 373	(301) 42
CG width:		–	168–308	126–280	98–294	84–294	–	126–210	84–238	84–322	126–308
		–	(229) 8	(177) 39	(211) 18	(179) 166	–	(170) 12	(171) 40	(191) 373	(214) 42
Male reproductive system L/BL:		–	70–81	63–81	61–79	64–82	–	70–78	63–77	59–89	64–82
		–	(73%) 5	(70%) 18	(72%) 8	(73%) 45	–	(73%) 9	(72%) 20	(74%) 67	(73%) 21

Table 1. Continued.

Characteristics	Sources of material studied									
	Mississippi Van Cleave (1931)	Mississippi Van Cleave (type)[1]	Kentucky Gleason[2]	Kentucky White[3]	Illinois Camp[4]	Illinois Seidenberg[5]	Illinois Schmidt[6]	W. Virginia Huffman[7]	Wisconsin Amin[8]	Ohio Muzzall[9]
Egg length:	90–108	61–74	54–74	67–93	51–93	ovarian balls	ovarian balls	ovarian balls	51–74	64–90
	—	(68) 11	(64) 13	(81) 11	(65) 54	—	—	—	(62) 2	(75) 30
Egg width:	12–15	6–16	8–13	10–16	8–13	—	—	—	10–10	10–16
	—	(11) 11	(11) 13	(13) 11	(10) 54	—	—	—	(10) 2	(13) 30

[1] From *Aplodinotus grunniens, A. simer,* and *Ictiobus* sp.; USNM Helm. Coll. nos. 37598–37600.

[2] From *Hypentelium nigricans* and *Moxostoma erythrurum* in Casper River (Warren Co.) and Drakes Creek (Simpson Co.), tributaries of the Ohio River. Similar material was recently examined from *Etheostoma squamiceps* in Casper River.

[3] From *Catostomus commersoni* in Wilgreen Lake, Kentucky River and Otter Creek (Madison Co.) and Boone Creek (Fayette Co.), tributaries of the Kentucky River; reported as *A. jacksoni* by White (1974) and White and Harley (1973, 1974).

[4] From *Semotilus atromaculatus* and *Lepomis cyanellus* in Sugar Creek at Normal (McClean Co.), tributary of the Illinois River; reported by Camp (1977) and Camp and Huizinga (1980).

[5] From *Hybopsis* sp. in Mud Creek (McLean Co.), a tributary of the Illinois River; obtained from W. L. Bullock.

[6] From *Cottus bairdi* in Pecumsangan Creek (LaSalle Co.), a tributary of the Illinois River; reported as *A. jacksoni* by Schmidt et al. (1974).

[7] From *Notropis cornutus, Esox americanus* and isopods (*Lirceus fontinalis*) in Twelvepole Creek at Shoals (Wayne Co.), a tributary of the Ohio River.

[8] From *Amia calva, Esox lucius, Cyprinus carpio, C. commersoni, Ictalurus melas, I. natalis, I. punctatus, Roccus chrysops, Ambloplites rupestris, L. cyanellus, L. gibbosus, L. macrochirus, Pomoxis nigramaculatus, Perca flavescens,* and *Stizostedion vitreum* in Tichigan Lake (Racine Co.) on the Fox River, a tributary of the Mississippi River.

[9] From *S. atromaculatus, N. umbratilis, N. spilopterus. C. carpio, Carassius auratus, Notemigonus crysoleucas,* and *L. cyanellus* in Jackson Cutoff (Wood Co.); reported as *A. jacksoni* by Muzzall and Rabalais (1975a, b).

[10] Range (mean) *N.*

[11] These ratios were 20–22% in male and 11% in female *A. dirus* from *C. bairdi* and *Salmo irideus* in central Tennessee as computed from Ewell (1953).

measure of robustness in body form), ratio of testis length and width to body length and width (provide a numerical value of the relative size of testes compared to body size; the anterior testis was arbitrarily chosen since both testes in individual males are usually of equal size), and the ratio of reproductive system length (distance from anterior margin of anterior testis to posterior end of body excluding bursa) to body length (indicates the relative space occupied by reproductive structures in the male body cavity; monorchid males were excluded). The above characteristics together with other features including body and testis size, number of proboscis hook rows, and host and geographical distribution were important in distinguishing species of *Acanthocephalus*.

Data on 767 specimens of *A. dirus* from the Mississippi River drainage and Ohio (Table 1) were subjected to computer analysis in search of morphometric heterogeneity among the geographically different collections. The Seidenberg material was represented by the more complete collection of Camp from a close tributary of the Illinois River in Illinois. Data were analyzed on a DEC-System 10 computer. A total of 23 variables were measured on the worms, and from these an additional 15 variables, consisting mostly of ratios, were synthesized.

Data were first analyzed using BMDP7D, which prints stratified histograms, in order to see which variables contained the most information regarding differences between the collections. Combinations of these variables were then run through SPSS Multiple Stepwise Discriminant Function Analysis, BMPD2M Cluster Analysis of Cases and BMDPKM K-Means Clustering. The morphometric variables compared included: body length (BL), body width (BW), BW/BL, proboscis length (PL), proboscis width (PW), PW/PL, PL/BL, PW/BW, number of proboscis hook rows (PHR), number of hooks per row (HPR), total number of hooks (TNH), HPR/PHR, maximum hook length (MHL), MHL/PL, MHL/HPR, proboscis receptacle length (PRL), proboscis receptacle width (PRW), PRW/PRL, PL/PRL, lemniscus I length (LIL), lemniscus I width (LIW), LIIL, LIIW, LL/PRL, anterior testis length (ATL), anterior testis width (ATW), ATL/BL, ATW/BW, posterior testis (PT) length, PT width, cement gland (CG) number and dimensions of largest and smallest glands, and CG length/testes length, male reproductive system length/BL. Two cement glands (the largest and smallest) from each male were measured. All measurements are in micrometers with means in parentheses, except body dimensions (in mm).

Results and Discussion

I.—The Mississippi River populations of *A. dirus*

STATISTICAL ANALYSES: All *Acanthocephalus* populations referred to above (Table 1) are regarded as representing *A. dirus* based on the following evidence.

1. CONVENTIONAL TAXONOMY: Morphometric similarities are readily discernible from Table

Table 2. Discriminant function analysis of male *Acanthocephalus dirus* using three variables (body width, number of proboscis hook rows, maximum hook length).

Actual group	Group designation	*N*	Predicted group membership, *N* (%)							
			1	2	3	4	5	6	7	8
Ohio (Muzzall)	1	21	11 (52.4)	0 (0.0)	1 (4.8)	1 (4.8)	2 (9.5)	1 (4.8)	3 (14.3)	2 (9.5)
Mississippi (Van Cleave)	2	1	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
W. Virginia (Huffman)	3	14	2 (14.3)	0 (0.0)	7 (50.0)	3 (21.4)	0 (0.0)	0 (0.0)	1 (7.1)	1 (7.1)
Kentucky (Gleason)	4	19	0 (0.0)	0 (0.0)	2 (10.5)	15 (78.9)	0 (0.0)	2 (10.5)	0 (0.0)	0 (0.0)
Illinois (Schmidt)	5	3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	1 (33.3)	1 (33.3)
Kentucky (White)	6	7	0 (0.0)	0 (0.0)	2 (28.6)	0 (0.0)	1 (14.3)	4 (57.1)	0 (0.0)	0 (0.0)
Wisconsin (Amin)	7	95	8 (8.4)	0 (0.0)	7 (7.4)	0 (0.0)	14 (14.7)	4 (4.2)	53 (55.8)	9 (9.5)
Illinois (Camp)	8	63	13 (20.6)	0 (0.0)	10 (15.9)	1 (1.6)	12 (19.0)	9 (14.3)	11 (17.5)	7 (11.1)

Table 3. Cross tabulation of computer-generated clusters versus the geographical origin of eight *Acanthocephalus dirus* populations using four variables (body width, proboscis length, hook number, maximum hook length).

Collections	Sex	Clusters						
		1	2	3	4	5	6	7
Ohio (Muzzall)	MM	0	0	1	4	5	11	0
	FF	0	0	1	1	5	7	4
Mississippi (Van Cleave)	MM	0	0	2	0	1	0	1
	FF	0	1	1	1	5	0	0
W. Virginia (Huffman)	MM	0	1	3	1	11	2	2
	FF	1	0	4	1	9	0	10
Kentucky (Gleason)	MM	0	0	0	3	9	8	0
	FF	6	4	3	2	23	19	3
Illinois (Schmidt)	MM	0	1	1	4	2	1	0
	FF	0	0	0	0	2	1	0
Kentucky (White)	MM	0	0	1	3	3	3	0
	FF	1	0	1	2	3	4	7
Wisconsin (Amin)	MM	6	49	31	32	24	34	30
	FF	1	66	39	34	25	6	8
Illinois (Camp)	MM	0	12	14	26	13	20	3
	FF	10	15	19	9	12	11	12

1. To the extent that such similarities may express some degree of genetic homogeneity, they may be regarded in a phenetic sense particularly those characters that express biological processes, e.g., reproductive, metabolic, etc., that are undoubtedly under the genetic control of worm genomes (see Conclusions).
2. STRATIFIED HISTOGRAMS: These were produced for all variables for the purpose of visually comparing the collections; see Table 1. Group means and spread of cases were similar in all collections except those from Kentucky (Gleason) that showed some indications of uniqueness (see Conclusions) and where clinal traits, e.g., number of proboscis hooks per row, were noted.
3. DISCRIMINANT FUNCTION ANALYSIS: Several combinations of variables of both males and females were used for discriminant function analysis. An example of a scatterplot of males from eight *A. dirus* populations using a combination of six variables that shows near ideal overlap of clusters with minimal euclidean distances among centroids is presented in Figure 1. Eight other scatterplots made using different combinations of variables showed correspondingly definite overlap of population clusters somewhat similar to that presented in Figure 1. The total percentage of grouped cases correctly classified ranged between 32 and 67%. Disregarding the Gleason collection, the "best" group per run varied from one run to another and no consistent grouping pattern emerged. An example of a run with grouped cases correctly classified totaling 43.95% is shown in Table 2.
4. CLUSTER ANALYSIS: The possible presence of natural clusters among the various *A. dirus* collections was investigated using BMDPKM K-Means clustering. Differing combinations of variables were used to force the specimens into 2, 5, and 7 clusters. In none of these runs were there any indications of assignment of specimens to clusters corresponding to their geographical origin. The two largest collections from Illinois (Camp) and Wisconsin (Amin) were almost invariably dispersed more or less uniformly among the clusters, e.g., Table 3.

Acanthocephalus dirus has been reported as *A. jacksoni* by Page (1974), Schmidt et al. (1974), White (1974), White and Harley (1973, 1974) and Muzzall and Rabalais (1975a, b). Specimens could have conveniently been assigned to *A. jacksoni,* which has 7–10 proboscis hooks per row (Bullock, 1962), as distinct from *A. dirus,* with a reported 11–13 such hooks on its proboscis (Van Cleave, 1931). The fact that a rela-

tively high hook count in *A. dirus,* sensu Van Cleave, 1931, appears to be characteristic of southern populations, and that the range of variability in this character includes rows with as few as six hooks in males and eight in females (Table 1) in the more northern populations would potentially place all the New England (*A. jacksoni*) populations in *A. dirus* if this character alone is used.

In all *A. dirus* males and/or females studied, each had at least 11 proboscis hooks per row. The Kentucky (Gleason), Illinois (Camp), and Wisconsin specimens were the most variable in this character and had maximum overlap with Van Cleave's reported 11–13 hooks per row, i.e., 8–12, 9–12, and 7–12 in males and 9–14, 9–13, and 9–13 in females, respectively; a decidedly *A. dirus* trait. The mean number of proboscis hooks was also comparable, i.e., 137, 142, and 142 in males and 168, 156, and 163 in females, in the same order (Table 1). Body form, relative size of anterior testis and male reproductive system, and length of largest proboscis hooks of these three populations compared well with those from Mississippi. Van Cleave's and Gleason's female specimens represented opposite extremes of variation in body form; mean body width/body length being 6.6 and 15.5%, respectively. This ratio in all other females varied between 8.0 and 11.7%. In the Gleason male specimens, the relative width of the anterior testis was somewhat low (46.9%) compared to that in other populations studied (51.4–60.8%, Table 1).

The populations from Kentucky (White), Illinois (Seidenberg, Schmidt), West Virginia, and Ohio belong to *A. dirus* because of their cylindrical body form, at least some proboscis hook rows with more than 10 hooks each, comparably moderate length of largest hooks, and relatively large size of testes and male reproductive system. Furthermore, the Ohio collection was from a tributary of the Maumee River near its outlet into western Lake Erie from which *A. dirus* has been previously reported by Venderland (1968) and Bangham (1972). The Venderland material, from *Aplodinotus grunniens* was not available for examination but that of Bangham included one male from *Amia calva* that had 15 rows of proboscis hooks each with 13 hooks, the longest of which reached 48.

UNUSUAL VARIATIONS IN THE MISSISSIPPI RIVER POPULATIONS OF *A. DIRUS*: Unusual structural variations observed in 376 male and 393 female

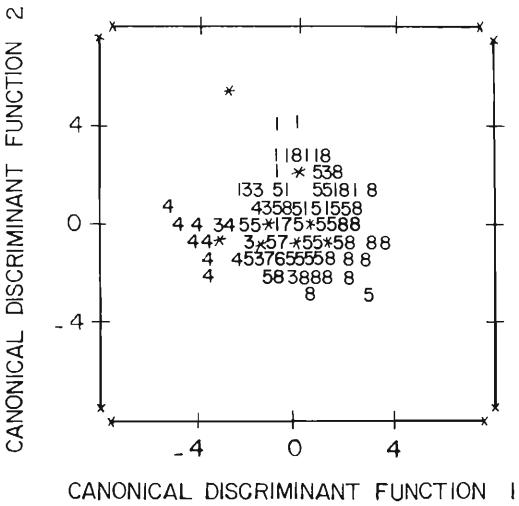

Figure 1. A discriminant function analysis scatterplot of males from eight Mississippi River *A. dirus* populations using the following six variables: body width, number of rows of proboscis hooks, maximum hook length, proboscis receptacle length, anterior testis length, and anterior testis width/body width. Symbols used and sample size: 1: Ohio (Muzzall, 21), 2: Illinois (Van Cleave, 1), 3: West Virginia (Huffman, 14), 4: Kentucky (Gleason, 19), 5: Illinois (Camp, 49), 6: Illinois (Schmidt, 3), 7: Kentucky (White, 7), 8: Wisconsin (Amin, 48). * = group centroids.

A. dirus (including 21 males and 19 females from Ohio) are reported for the first time. Five males and 12 females had body wall blisters like those previously described in *A. parksidei* by Amin (1975b, 1982) and in *Echinorhynchus salmonis* by Amin and Redlin (1980) resulting from glycogen–phospholipid metabolic dysfunction (Lester and Wright, 1978). Rows of proboscis hooks were incomplete anteriorly or posteriorly in three males and 12 females. Each incomplete row usually alternated with two other incomplete rows (one on each side of it) on the opposite end of the proboscis. One male and three females had a displaced, extra, or missing hook each. Usually one or two hooks pointing anteriorly were noted at the posterior end of the proboscides of 11 males and nine females. One male from Ohio had five anteriorly oriented hooks in one row and another male from Illinois (Camp) had at least 24 such hooks on its proboscis. One hook on a female proboscis from Kentucky (White) appeared branched at its middle. Hook-mediated self-inflicted scars as reported in *A. parksidei* by Amin (1975b) were rarely encountered. One male and one female from Wisconsin had min-

Table 4. Morphometric comparisons between *Acanthocephalus dirus* and its junior synonyms *A. jacksoni* and *A. parksidei*.

Characteristics		*A. dirus*	*A. jacksoni* Bullock (1962)[1]	*A. jacksoni* (type)[2]	*A. parksidei* Amin (1975a)[3]
Body length (BL):	MM	2.24–6.00 (3.60) 376	2.90–5.50	2.20–4.52 (3.20) 54	2.28–4.24 (2.97) 141
	FF	2.40–20.00 (9.00) 399	7.00–14.30	5.56–11.40 (7.90) 71	4.00–14.64 (8.23) 210
Body width (BW):	MM	0.44–0.96 (0.62) 369	0.54–1.50	0.52–1.32 (0.67) 54	0.32–0.64 (0.45) 141
	FF	0.48–1.44 (0.79) 396	0.68–0.93	0.48–1.24 (0.91) 71	0.32–1.20 (0.65) 210
BW/BL	MM	11.0–31.0 (17.7)	—	13.0–36.0 (21.5)	10.0–25.0 (16.9)
	FF	5.4–18.0 (9.5)	—	6.0–18.0 (14.0)	6.0–14.0 (7.9)
Proboscis (P) length:	MM	322–742 (526) 220	310–620	378–672 (519) 45	406–630 (510) 132
	FF	476–882 (661) 304	460–850	560–868 (688) 66	476–728 (612) 193
Proboscis (P) width:	MM	98–238 (160) 300	120–240	140–224 (186) 45	98–168 (138) 132
	FF	140–308 (222) 348	140–300	210–392 (258) 67	140–252 (192) 193
No. hooks (H)/row:	MM	6–13 (9.9) 113	7–10 (8.3) 167	7–10 (8.5) 30	8–11 (9.3) 142
	FF	8–14 (10.6) 232	7–10 (8.9) 130	7–10 (8.9) 55	8–11 (9.6) 206
No. hook (H) rows:	MM	11–20 (13.8) 293	11–16 (13.4) 167	12–16 (13.5) 45	12–16 (13.7) 238
	FF	12–19 (15.0) 329	12–18 (14.3) 130	12–17 (14.3) 64	12–19 (15.0) 279
No. H on P:	MM	90–234 (139.5)	(111)	96–150 (115)	96–168 (127)
	FF	112–211 (159.5)	(127)	96–160 (127)	112–178 (144)
Largest PH length:	MM	35–74 (54) 234	41–84 (66) 153	45–80 (67) 44	42–64 (52) 133
	FF	42–90 (66) 297	55–103 (83) 144	58–99 (83) 68	54–77 (66) 197
P receptacle length (PRL):	MM	406–1,134 (746) 250	400–1,300	420–1,232 (860) 50	364–728 (552) 139
	FF	420–1,680 (964) 263	400–1,610	588–1,484 (1,079) 70	350–980 (612) 188
P receptacle width (PRW):	MM	126–280 (195) 252	—	154–308 (229) 50	126–224 (167) 139
	FF	140–350 (240) 261	—	168–378 (290) 70	140–280 (199) 188
Lemnisci length:	MM	196–1,078 (609) 563	0.5–slightly > PRL	308–1,078 (592) 74	252–910 (499) 137
	FF	294–1,526 (828) 472	0.75–slightly > PRL	588–1,316 (855) 71	280–896 (572) 187
Lemnisci width:	MM	42–308 (146) 552	—	42–322 (123) 74	42–182 (110) 137
	FF	56–364 (174) 465	—	56–280 (160) 71	56–252 (137) 187
Anterior testis L (ATL):		350–1,008 (701) 332	380–790	308–700 (533) 50	392–924 (613) 133
Anterior testis W (ATW):		195–686 (345) 332	300–430	168–434 (287) 50	196–462 (279) 133
ATL/BL %:		10.5–30.0 (19.8)	—	12.0–23.0 (17.4)	15.0–31.5 (21.8)
ATW/BW %:		28.0–93.0 (55.6)	—	20.0–71.0 (44.0)	32.0–88.0 (61.8)
Posterior testis L:		322–924 (667) 321	300–700	210–686 (488) 48	280–770 (544) 133

Table 4. Continued.

Characteristics	*A. dirus*	*A. jacksoni* Bullock (1962)[1]	*A. jacksoni* (type)[2]	*A. parksidei* Amin (1975a)[3]
Posterior testis W:	196–644 (346) 331	200–500	168–406 (293) 48	182–448 (273) 133
No. cement glands (CG):	0–11 (6.17) 352	4–11 (6.17) 211	4–10 (5.94) 48	0–12 (5.91) 1,801
CG length:	112–560 (241) 698	—	126–588 (267) 98	98–420 (204) 380
CG width:	84–322 (188) 698	—	112–420 (185) 98	84–280 (156) 380
Male reproductive system L/BL (%):	59–89 (73) 193	ca. 50%	48–78 (65) 48	66–90 (76) 64
Egg length:	51–108 (69) 121	56–90	54–96 (71) 110	61–109 (83) 166
Egg width:	6–16 (11) 121	16	10–19 (14) 110	9–16 (12) 166

[1] Additional data on proboscis hooks were derived from figures 1 and 2 (Bullock, 1962); fresh eggs in saline measured 65–103 by 14–19.

[2] Measurements made from USNM Helm. Coll. nos. 37479–37481, 59634 and other types in Bullock's collection; these data were used in the statistical analyses and mostly in the redescription.

[3] The *A. parksidei* population from southwestern Lake Michigan (Amin, 1977) was typical of that from the Pike River (Amin, 1975a, table 1) particularly in the number of proboscis hooks per row (8–8 to 10–11, mean 9.3; $N = 44$ in males and 8–8 to 11–11, 9.2; 65 in females) and maximum hook length (45–58, 52; 47 in males, and 54–80, 65; 95 in females). The number of hook rows on the proboscis was 12–16, 13.8; 38 in males and 12–19, 15.3; 79 in females, which was comparable to that of *A. parksidei* from the Pike River as verified through many additional collections (12–16, 13.6; 200 in males and 13–17, 14.9; 200 in females). The full range of variation in this character is documented in this table.

iature hooks. Lemnisci were frequently at least slightly subequal. Lobed lemnisci included two lobes each. Specimens with multiple lemnisci usually had three, but six were observed in one female from Wisconsin. A total of 17 males and 19 females showed lemnisci abnormalities. In 15 monorchid males, the single testis was usually larger than either testis of normal specimens. The anterior testis of one male from Wisconsin was atrophied. Most of the above variations are comparable to those previously reported for *A. jacksoni* by Bullock (1962) and *A. parksidei* by Amin (1975b).

A most unusual proboscis abnormality is reported here for the first time. A few males and females from various hosts in Wisconsin had two proboscides each, one within the other. This condition was usually associated with proboscis receptacle abnormalities and will be described in a later publication. The number of cement glands varied between 0 and 11 (mean 6.15) with 35% (5% of 20 males from Kentucky [Gleason] to 48% of 21 males from Ohio) of 351 males having other than six cement glands each. This percentage is comparable to the 37% reported in *A. jacksoni* by Bullock (1962) but not the 10% in *A. parksidei* by Amin (1975b).

Presently, available information is not sufficient to develop the concept that the prevalence of anomalies among populations (or species) may be of taxonomic importance, or to document its precedence. However, only when this information becomes available will the exploration of such concepts be possible.

The possible effect of host species on *A. dirus* variation was investigated in the Wisconsin population where sufficient numbers of specimens were obtained from two or more host species. The largest number of worms was collected from three centrarchid hosts (*Lepomis cyanellus,* 24 males, 20 females; *L. gibbosus,* 34, 27; *L. macrochirus,* 19, 29) and one ictalurid fish (*Ictalurus natalis,* 78, 61). Analysis of data (in a manner similar to that summarized in Table 1) did not show marked differences within the centrarchid fish worms or between them as a group and the specimens from *I. natalis.*

II.—The non-Mississippi River populations of *A. dirus*

A summary of the morphometric characteristics of the Mississippi River populations of *A. dirus* as well as those of the more northern and geographically isolated *A. parksidei* and *A. jack-*

soni is presented in Table 4. *Acanthocephalus jacksoni* was originally distinguished from *A. dirus,* sensu Van Cleave, 1931 (see Introduction), by having fewer proboscis hooks per row (7–10), longer hooks (41–84 in males, 55–103 in females), and smaller eggs (56–90 by 16 in body cavity of fixed females, 65–103 by 14–19 fresh in saline) (Bullock, 1962). *Acanthocephalus parksidei* was also distinguished from *A. dirus* by having a smaller body: 2.28–4.24 (2.97) by 0.32–0.64 (0.45) in males, 4.00–14.64 (8.23) by 0.32–1.20 (0.65) in females, fewer proboscis hooks per row (8–11), and rows of hooks (10–14 in males, 10–16 in females), smaller hooks in females (54–77), and smaller eggs 61–109 (83) by 10–16 (12) (Amin, 1975a). See Table 4, footnote 3.

Morphometric comparisons among these three entities (Table 4) clearly show that these distinguishing characteristics practically fall within the normal range of variation of *A. dirus* from the Mississippi River drainage as documented above (Table 1). Although the more slender *A. parksidei* and robust *A. jacksoni* have a distinctly narrower range of variation in the number of proboscis hooks per row with the first species having a relatively smaller proboscis and the second with relatively larger proboscis hooks (might also be affected by host differences) and smaller testes and male reproductive system compared to *A. dirus,* these differences are not regarded as sufficient to retain the specific status of either species. It is thus proposed that *A. jacksoni* and *A. parksidei* be designated as junior synonyms of *A. dirus.* Additional evidence supporting this proposal includes (1) Stratified histograms that were produced for all variables showed that, except for the characteristic population features listed above, species means and spread of cases largely coincided in all other variables. (2) Cluster analysis of males and females was separately made to determine if the specimens could be objectively grouped into clusters representing the species as designated (*A. parksidei, A. jacksoni, A. dirus*). Several combinations of variables were used in this hierarchical analysis. No single combination of variables was able to simultaneously sort out *A. dirus* vs. *A. jacksoni* vs. *A. parksidei,* even though one of each of these entities formed a largely homogeneous cluster in most runs probably reflecting some population distinctiveness (above).

Redescription

Acanthocephalus dirus (Van Cleave, 1931) Van Cleave and Townsend, 1936

The following redescription is based on the *A. dirus* material examined from the Mississippi River as well as those of *A. dirus* from New England (=*A. jacksoni*) and *A. dirus* from Wisconsin and Lake Michigan (=*A. parksidei*) (Table 4). It includes some of Van Cleave's (1931) and Bullock's (1962) range values when not represented in my measurements.

GENERAL: With characteristics of the genus *Acanthocephalus,* worms cylindrical and elongate. Trunk and all shared structures larger in females than males. Proboscis long, cylindrical, and with nearly parallel sides. Proboscis hooks somewhat fewer per row and larger in more northern locations. Largest hooks near middle of proboscis, all hooks rooted; roots simple, unmodified. Proboscis receptacle double-walled, relatively longer and wider than proboscis. Lemnisci variable in length and somewhat shorter than proboscis receptacle, often at least slightly subequal and infrequently multiple or irregularly formed.

MALES: Body often slightly wider near middle; 2.20–6.00 mm (3.41) long × 0.32–1.50 mm (0.58) wide. Body width/length 10.0–36.0% (17.0). Proboscis 310–742 (520) long × 98–240 (156) wide; armed with 11–20 (13.7) rows of 6–13 (9.1) hooks each; number of hooks on proboscis 90–234 (125). Largest hooks 35–84 (57) long. Proboscis receptacle 364–1,300 (697) long × 126–308 (190) wide. Lemnisci 196–1,078 (588) long × 42–322 (137) wide. Testes spherical to elongate ellipsoidal, about equal in size and usually contiguous. Anterior testis 308–1,008 (662) long × 168–686 (322) wide. Anterior testis length/body length 10.5–31.5% (19.4); anterior testis width/body width 20.0–93.0% (55.5). Posterior testis 210–924 (617) long × 168–644 (322) wide. Cement glands spherical to clavate, 0–12 (5.97) per male; 98–588 (231) long × 84–420 (177) wide. Reproductive system length/body length 48–90% (72).

FEMALES: Body often slightly wider anteriorly; 2.40–20.00 mm (8.65) long × 0.32–1.44 (0.76) wide. Body width/length 5.4–18.0% (8.8%). Proboscis 460–882 (647) long × 140–392 (216) wide; armed with 12–19 (14.9) rows of 8–14 (9.8) hooks each; number of hooks on proboscis 112–211

(146). Largest hooks 42–103 (70) long. Proboscis receptacle 350–1,680 (852) long × 140–378 (232) wide. Lemnisci 280–1,526 (765) long × 56–364 (163) wide. Eggs (in whole mounts) 51–109 (75) long × 6–19 (12) wide.

Conclusions

The reported Mississippi River populations of *A. dirus* showed considerable homogeneity in the anatomical characteristics studied (Table 1). Unusual individual variations were generally comparable to those previously described from the more northern *A. dirus* populations in Wisconsin and New England.

Although the above populations were mostly obtained from waters connected to the Mississippi River, they were geographically widely separated. Distinct population characteristics that may be geographically related were evident principally in the Kentucky (Gleason) material. Specimens in this population, mostly from *Hypentelium nigricans* (all 20 males and 58 females), had somewhat more robust bodies, smaller testes, and correspondingly smaller testicular proportion relative to body dimensions, and relatively shorter proboscis hooks compared to other populations studied (Table 1). The peculiarities of this population entail its recognition as an incipient species. It is not regarded as sufficiently separate from other Mississippi River populations of *A. dirus* described, particularly using routine parasitological criteria, e.g., proboscis hook distribution patterns, to warrant independent specific status at this time. Other parasites collected from the same hosts and in the same waters have been shown to possess characteristics distinct from those common to the same species elsewhere, e.g., the acanthocephalan *Pomphorhynchus bulbocolli* by Gleason and Huffman (1981).

Geographical factors appear to be related to certain variations in characteristics of proboscis hooks. Largest hooks were progressively longer in the more northern populations (Table 1), apparently a clinal trait. Host differences may also influence hook length. The relatively large number of hooks per row (11–13) in the Mississippi population reported by Van Cleave (1931) appears to be characteristic of the upper Mississippi River population that he believed "to be restricted to the extreme south." The more northern populations were more variable and included specimens with rows having as few as six hooks in males and eight in females. Geographical variation in the same characteristic was also observed in other acanthocephalan populations. Bullock (1962) observed a larger number of proboscis hooks per row in *A. jacksoni* from New Hampshire than from Massachusetts. Similarly, Lincicome and Van Cleave (1949) reported more, but relatively smaller, hooks on the proboscis of *Leptorhynchoides thecatus* from Canada than from the United States.

Acanthocephalus dirus is apparently the most variable species of this genus from North American freshwater fishes. The expanded diagnosis of *A. dirus* described in this paper was documented through the study of an extensive series of specimens from various geographical populations. Satisfactory assignment of specimens to *A. dirus* can be more readily achieved when variability is considered. This becomes particularly evident when certain important but overlapping taxonomic characteristics, e.g., the number of proboscis hooks per row, are considered. The new diagnosis of *A. dirus* further calls for a reevaluation of the status of other parasite members of this genus in North America and of taxonomic characteristics useful for distinguishing them.

Taxonomically important characteristics that might be construed as expressing relatedness among these acanthocephalans include size and shape of body, size of proboscis, and distribution of proboscis hooks, and size of male reproductive system relative to body dimensions. These taxonomic characteristics may be regarded as phenetic manifestations of the genomes of individual worms or species particularly when the biological processes that they express are considered. For example, proboscis and hook characteristics practically reflect survivability inasmuch as effective attachment in the intestine of the proper host allows it. Characteristics of the male reproductive system may be regarded as a measure of the reproductive potential of the species. Body size and shape are expressions of biological mass and surface area reflecting aspects of metabolic activities such as absorption of nutrients, circulation, etc. In females, body cavity space represents the maximum carrying capacity of sex cells and is thus regarded as an additional important measure of reproductive potential. Accounting for this by counting the

maximum possible number of ovarian balls or eggs would be impractical.

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TENTATIVE MEETING SCHEDULE
HELMINTHOLOGICAL SOCIETY OF WASHINGTON
1984-1985

10 October	Uniformed Services University of the Health Sciences, Bethesda, MD (At Naval Medical Research Institute)
14 November	Animal Parasitology Institute, Beltsville, MD
12 December	Plant Protection Institute, Beltsville, MD (*with* Oxford Biological Laboratory)
16 January	National Institutes of Health, Bethesda, MD
13 February	Naval Medical Research Institute, Bethesda, MD (*with* Food and Drug Administration)
13 March	Walter Reed Army Institute of Research, Washington, DC (*with* Armed Forces Institute of Pathology)
17 April	School of Hygiene and Public Health, Johns Hopkins University, Baltimore, MD
11 May	New Bolton Center, University of Pennsylvania, Kennett Square, PA

Proc. Helminthol. Soc. Wash.
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Interspecific Variability in the Genus *Acanthocephalus* (Acanthocephala: Echinorhynchidae) from North American Freshwater Fishes, with a Key to Species

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ABSTRACT: Three species of the genus *Acanthocephalus* are known from North American freshwater fishes, i.e., *A. dirus* (Van Cleave, 1931), *A. tahlequahensis* Oetinger and Buckner, 1976, and *A. alabamensis* Amin and Williams, 1983. The distinctiveness of each species is documented through the study of intra- and interspecific morphometric variability. The results of the morphometric analysis are presented in stratified histograms. Findings indicate that *A. dirus,* the largest and most widely distributed species, is also the most variable. Of the two smaller southern species, *A. alabamensis* (like *A. dirus*) has a cylindrical body and large testes compared to body size, whereas *A. tahlequahensis* has a spindle-shaped body and smaller testes relative to body size, among other features. A key aiding in the identification of these species is included.

The expanded diagnosis of *Acanthocephalus dirus* (Van Cleave, 1931) by Amin (1984) from populations in the Mississippi River drainage, Wisconsin–Lake Michigan (=*A. parksidei*), and New England (=*A. jacksoni*) necessitated reconsideration of species relationships within this genus from North American freshwater fishes. Interspecific variations in morphometric characteristics among these species are documented and a key encompassing the most significant taxonomic features is provided.

Materials and Methods

Data derived from the redescription of *Acanthocephalus dirus* by Amin (1984) and the original descriptions of *A. tahlequahensis* Oetinger and Buckner, 1976, and *A. alabamensis* Amin and Williams, 1983, are used for comparison. In addition, five male and seven female *A. dirus* from a Michigan river draining into Lake Michigan and loaned by P. M. Muzzall were also examined. Specimens were examined and data analyzed as previously described by Amin (1984). All measurements are in micrometers except body dimensions (in mm).

Results and Discussion

The extent of the interspecific variability and the distinctiveness of each of the species is expressed by the results of histogram analysis. Stratified histograms were produced for all variables for the purpose of visually comparing the three species. Species means and spread of cases did not coincide in most variables including all taxonomically important features used in the key. Differences were particularly distinct in 20 variables summarized in Figure 1. Results of the cluster analysis were inconclusive because *A. tahlequahensis* and *A. alabamensis* were present in small numbers.

Acanthocephalus dirus appears to exhibit the widest range of variation in practically all characteristics. Unusual variations and/or abnormalities in body wall, proboscis hooks, lemnisci, male gonads, and cement glands were comparable in *A. dirus* populations from New England, Wisconsin–Lake Michigan, and the Mississippi River drainage as reported by Bullock (1962) and Amin (1975, 1984), respectively. Lobed lemnisci were reported in *A. tahlequahensis* by Oetinger and Buckner (1976), incomplete rows of proboscis hooks and body wall blisters in *A. alabamensis* by Amin and Williams (1983), and cement gland numbers other than six in both species.

The three species of *Acanthocephalus* fall into two presumably natural groups by size. *A. dirus* includes larger forms that are more variable and widely distributed than the two other species. Variability of its three major geographically isolated populations is well documented. The Wisconsin–Lake Michigan population more closely resembles *A. dirus* from the Mississippi River even though individuals in the former population are slightly smaller than the latter in all characteristics except egg and proboscis hook length. Both populations have comparably cylindrical body form (except for the somewhat more robust individuals of the Mississippi River *A. dirus* population from south-central Kentucky) and similarly large ratio of male reproductive system to body length (table 4, Amin 1984). Length of the largest proboscis hook and the number of pro-

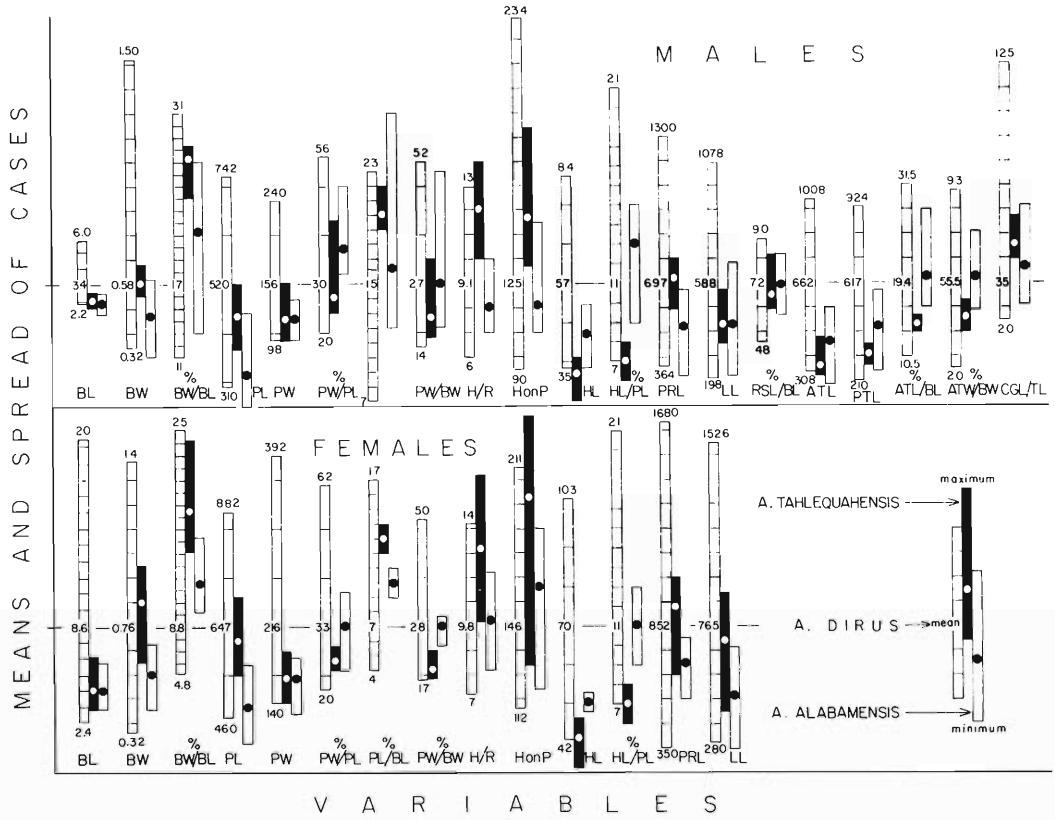

Figure 1. Summary of stratified histograms contrasting means of 20 variables and spread of cases in male and female *A. dirus, A. tahlequahensis,* and *A. alabamensis.* Legend at lower right. Symbols used: body length, BL; body width, BW; body width/body length, BW/BL; proboscis length, PL; proboscis width, PW; proboscis width/proboscis length, PW/PL; proboscis length/body length, PL/BL; proboscis width/body width, PW/BW; number of proboscis hooks per row, H/R; total number of hooks on the proboscis, H on P; maximum hook length, HL; maximum hook length/proboscis length, HL/PL; proboscis receptacle length, PRL; lemnisci length, LL; length of male reproductive system/body length, RSL/BL; anterior testis length, ATL; posterior testis length, PTL; anterior testis length/body length, ATL/BL; anterior testis width/body width, ATW/BW; cement gland length/anterior testis length, CGL/TL. Only bars within each of the variables are to same scale.

boscis hook rows are almost identical. Individuals of the more northern *A. dirus* from New England, on the other hand, have more robust bodies, smaller ratio of male reproductive system to body length, and distinctly longer proboscis hooks. The *A. dirus* populations from Wisconsin–Lake Michigan and New England characteristically exhibit a narrower range of variation in the number of proboscis hooks per row.

In species of the second group, *A. tahlequahensis* and *A. alabamensis,* the body and all other structures are considerably smaller than in *A. dirus. A. alabamensis* is similar to *A. dirus* from the Mississippi River and Wisconsin–Lake Michigan in its cylindrical body form and the relative size of anterior testis and male reproductive system compared to its body dimensions. Relative dimensions of the spindle-shaped body of *A. tahlequahensis* and its relatively smaller male reproductive structures compared to body size are more comparable to those of *A. dirus* from New England. In addition, the length of the proboscis and proboscis receptacle, the total number of hooks on the proboscis and of proboscis hook rows are significantly smaller and the largest proboscis hooks are relatively longer in *A. alabamensis* than in *A. tahlequahensis.*

A key to species of genus *Acanthocephalus* from North American freshwater fishes is presented

below. See Amin (1984, Conclusions) for discussion of the taxonomic characteristics used in this key. Mean values are in parentheses.

1. Small forms; males 1.4–2.8 and females 3.3–6.7 mm long. Proboscis hooks short; largest hooks 27–51 long in males and 35–54 in females. Testes small; anterior testis 252–560 by 210–308 2
   Large forms; males 2.2–6.0 and females 2.4–20.0 mm long. Probiscis hooks medium or large; largest hooks 35–84 long in males and 42–103 in females. Testes large; anterior testis 308–1,008 by 168–686. In fishes from the Mississippi River drainage system, Great Lakes basin, and New England ..... *A. dirus*
2. Body spindle-shaped; width 24–28% (26) of length in males and 15–24% (18) in females. Testes small compared to body; anterior testis length and width 13–15% (14) and 35–48% (41) of body length and width. Proboscis 385–520 (450) long. Proboscis hooks 10–14 (11.9) per row in males and 10–16 (13) in females; longest hooks 27–38 (33) in males and 35–46 (42) in females. In fishes from Oklahoma ..... *A. tahlequahensis*
   Body cylindrical; width 13–27% (22) of length in males and 10–16% (12) in females. Testes large compared to body; anterior testis length and width 16–28% (20) and 45–77% (58) of body length and width. Proboscis 266–462 (334) long. Proboscis hooks 7–10 (8.0) per row in males and 8–12 (10.1) in females; longest hooks 35–51 (44) in males and 48–54 (51) in females. In fishes from Alabama ..... *A. alabamensis*

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Parasites of the Black-Tailed Prairie Dog (*Cynomys ludovicianus*) from Eastern New Mexico

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ABSTRACT: Fifty black-tailed prairie dogs from three prairie dog towns in eastern New Mexico harbored one nematode (*Subulura* sp.) and one acanthocephalan (*Moniliformis clarki*) species. One tick (*Ornithodoros turicata*) and one mite (*Proctolaelaps* sp.) species were recovered, whereas three species of fleas (*Opisocrostis hirsutus, Pulex simulans, Echidnophaga gallinacea*) were present. All represent new distribution records, and *Subulura* sp., *O. turicata, Proctolaelaps* sp., and *E. gallinacea* represent new host records. Male hosts had higher mean flea and nematode intensities. Male and female hosts in the lightest weight class had greater abundance of fleas. Greater flea intensities may be associated with reproductive cycles of host.

There are few studies on parasites of the black-tailed prairie dog in North America (Buscher and Tyler, 1975; Tyler and Buscher, 1975; McKenna et al., 1977). Noteworthy is that there are few helminth species reported from this host (Vetterling, 1962). Trematodes have not been reported. Only five cestode species are reported (Buscher and Tyler, 1975, *Raillietina* sp. and *Raillietina salmoni*; Vetterling, 1962, *Cladotaenia circi, Cladotaenia globifera,* and *Hymenolepis* sp.). *Moniliformis clarki,* the only acanthocephalan reported from this host, was recovered in South Dakota prairie dogs (Vetterling, 1962).

Adults (*Trichostrongylus texanus,* Rodenberg and Pence, 1978; *Physaloptera getula, Hepaticola hepatica,* and *Spiroptera* sp., Vetterling, 1962) and eggs (*Physaloptera massino,* Vetterling, 1962) of five nematode species have been reported from this host. Normally their prevalence is very low (Vetterling, 1962; Buscher and Tyler, 1975; McKenna et al., 1977), which contrasts sharply with the findings of Rodenberg and Pence (1978). They observed a 60% (9/15) prevalence in prairie dogs from Texas.

Ectoparasites reported from this host include mites (*Euschoengastoides hoplai,* Loomis, 1971), ticks (*Ixodes* sp., McKenna et al., 1977), sucking lice (*Neohaematopinus marmotae,* Spencer, 1966; *Hoplopleura acanthopus,* McKenna et al., 1977) and fleas. Fleas from this host include *Pulex simulans* (Smit, 1958; Hubbard, 1968), *Opisocrostis hirsutus* (Hubbard, 1968; Tyler and Buscher, 1975; McKenna et al., 1977), *Opisocrostis bruneri, Opisocrostis tuberculatus cynomuris,* and *Thrassis fotus* (McKenna et al., 1977).

The present study was initiated to (1) supplement existing information about parasite distributions, (2) compare our results with other published information, and (3) determine if parasite distributions and intensities differ among three prairie dog towns located near Portales, New Mexico.

Materials and Methods

Fifty prairie dogs were collected from three prairie dog towns near Portales, Roosevelt County, New Mexico, in May–June and September–November 1981. The towns were identified as preserve (P), Crow's (C), and dairy (D). Town P is located approximately 10 mi NE of the other two towns. It is characterized as a short-grass prairie dominated by blue grama (*Bouteloua gracilis*), side-oats grama (*Bouteloua curtipendula*), purple three-awn (*Aristida purpurea*), and sand dropseed (*Sporobolus cryptandrus*) with the predominant shrub being sand sage (*Artemisia filifolia*). Town C was being grazed by cattle but not extensively and is dominated by blue grama and side-oats grama grasses with the dominant shrub being honey mesquite (*Prosopis glandulosa*). Town D is a nearly barren, caliche flat characterized by a low (2″–4″), sparse stand of kochia weed (*Kochia scoparia*) and is also grazed by cattle.

Animals were trapped using 4-inch steel jaw traps that were checked twice daily at 7 A.M. and 7 P.M. Prairie dogs were removed from the traps using a grasping snake stick and immediately placed in large, heavy gauge plastic bags containing cotton soaked in chloroform. Death occurred by thoracic compression. Each bag containing a single carcass was immediately tied, labeled, and placed in a freezer within 2 hr of capture.

Frozen carcasses were later weighed, sexed, and skinned. The feet, tail, and linings of ears and nostrils were also removed along with the hide and placed in a jar of 25% KOH solution and allowed to dissolve at room temperature. In approximately 18–24 hr the solution was strained through a series (850-, 600-, 420-, 300-, 212-, and 149-μm openings) of sieve screens. Recovered ectoparasites were stored in 70% ethyl alcohol. Fleas were transferred from 70% ethanol to 100% ethanol and cedarwood oil and mounted in euparal.

Table 1. Helminths and arthropod parasites from *Cynomys ludovicianus*, in eastern New Mexico.†

Taxon	Scientific name	Prevalence	
		No. infested/ no. examined	Percent
Acanthocephala	*Moniliformis* (S.I.)‡ *clarki*	1/52	2
Nematoda	**Subulara* sp. (S.I.)	2/52	4
Acarina	**Ornithodoros* (SK) *turicata*	2/52	4
	**Proctolaelaps* (SK) sp.	5/52	10
Siphonaptera	*Opisocrostis* (SK) *hirsutus*	49/52	94
	Pulex simulans (SK)	44/52	85
	**Echidnophaga* (SK) *gallinacea*	20/52	38

* New host record.
† All represent new distribution records.
‡ S.I., small intestine; SK, skin.

Ticks and mites were identified by Gary Maupin (CDC, Fort Collins).

Internal organs, body cavities, and gastrointestinal contents were examined using a stereomicroscope. Nematodes were fixed in glacial acetic acid, stored in 70% ethanol with 5% glycerine by volume, and identified in glycerine wet-mounts following alcohol evaporation. Acanthocephala were fixed in A-F-A, stored in 70% ethanol, stained in Celestine blue B, and mounted in Canada balsam. Representative specimens are deposited in the USNM Helminthological Collection, Beltsville, Maryland (Nos. 77418 and 77417). All data were analyzed using one-way ANOVA and chi-square analysis.

Results

Fifty black-tailed prairie dogs were collected: 15 from town P (May 13–June 10), 19 from town D (September 24–October 14), and 16 from town C (October 17–November 22).

Trematodes and cestodes were not recovered. There was no attempt to recover protozoa. One acanthocephalan (*Moniliformis clarki*) was found in a male host from town P, and two additional male hosts yielded one and six nematodes (*Subulura* sp.) from towns P and C, respectively. No endoparasites were recovered from prairie dogs in town D. Helminthic prevalences ranged from 0% in town D, to 12% (2/17) in town P, and 6% (1/16) in town C.

Mites and ticks were not recovered from hosts in town C. Two ticks (Table 1) (*Ornithodoros turicata*; both nymphs) were collected from separate hosts in town P, whereas 23 mites (Table 1) (*Proctolaelaps* sp.) were recovered from five separate hosts in town D.

Unlike fleas, which were so numerous ($\bar{x}$ = 19.66 fleas/prairie dog), there were no lice harbored by these hosts. Three species of fleas (*Opisocrostis hirsutus, Pulex simulans,* and *Echidnophaga gallinacea*) were observed and *O. hirsutus* was consistently the most common in each of the towns (Table 2).

Pulex simulans was the only species that showed significant differences ($0.05 > P > 0.01$) in sex ratio among the three different towns. In each town *P. simulans* females were always more abundant than males. Significant differences

Table 2. Comparison of flea intensities and prevalence, by species, taken from three prairie dog towns.

Town	Average no./dog	*O. hirsutus*		*P. simulans*		*E. gallinacea*	
		No. fleas	% fleas	No. fleas	% fleas	No. fleas	% fleas
P	39	383	65	122	21	80	14
C	19	210	68	94	30	7	2
D	4.5	52	60	31	36	4	5

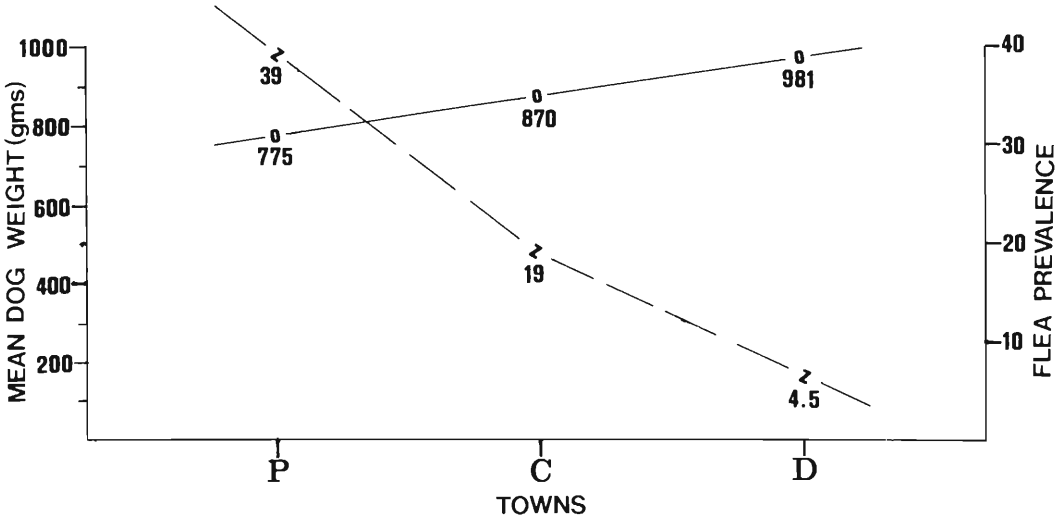

Figure 1. Comparison of flea prevalence and mean prairie dog weight from each of three prairie dog towns.

($0.01 > P > 0.001$) were also obtained when the difference in flea intensity per host sex and host weight class was tested. The weight difference in the heaviest and lightest prairie dog was 540 g, which, if tripartitioned, creates 180-g weight classes. Among males of the different weight classes (580–760 g; 760–940 g; 940–1,120 g) the lightest group was always most heavily parasitized with the heaviest weight class ranking second in terms of flea intensities. Lightest females were always most heavily parasitized, followed in turn by medium and heavy weight classes, respectively. Finally, an inverse relationship is evident when comparisons are drawn between mean prairie dog weight and the relative density of fleas per prairie dog from each town (Fig. 1). There were no significant differences in the distribution of fleas by sex according to host sex nor was there any correlation as to flea sex and weight class of host.

Discussion

Of the parasites recovered from the black-tailed prairie dog in eastern New Mexico only *M. clarki, O. hirsutus,* and *P. simulans* are previously reported from this host. *Moniliformis clarki* is the only acanthocephalan from this host. It has only been recovered once previously (Vetterling, 1962). According to Hubbard (1968), *O. hirsutus* and *P. irritans* (=*P. simulans,* Smit, 1958) are common ectoparasites throughout this host's range. *Subulura* sp., *Ornithodoros turicata, Proctolaelaps* sp., and *E. gallinacea* represent new host and distribution records.

A consistent observation of published reports is the conspicuous lack of diversity and abundance of helminths in the black-tailed prairie dogs (Vetterling, 1962; Buscher and Tyler, 1975; McKenna et al., 1977; Rodenberg and Pence, 1978). However, Rodenberg and Pence (1978) observed a 60% prevalence (9/15) and intensities of 4–68 nematodes (*Trichostrongylus texanus*) per host. *Trichostrongylus texanus* has been recovered from this host only in Texas (Dikmans, 1937; Rodenberg and Pence, 1978).

This is the second report of mites (Loomis, 1971) and ticks (McKenna et al., 1977) collected from the black-tailed prairie dog. *Proctolaelaps* sp. and *Ornithodoros turicata* both represent new host and distribution records.

Eleven species of fleas have been reported from the black-tailed prairie dog (Hubbard, 1968; McKenna et al., 1977). *Opisocrostis hirsutus* appears to be the most abundant species on this host. McKenna et al. (1977) observed that *O. hirsutus* outnumbered the least common flea species 85 to 1. Our observed ratios confirm the dominance of this species, but with less extreme variation. The flea species (*O. hirsutus, P. simulans, E. gallinacea*) were in a collective ratio of 7:3:1 in the present study.

Quantitative differences in flea intensities and distributions indicated that not only were there significant differences in abundances but there

were also notable differences in intensities of species across towns. For example, if numerical ratios are calculated from intensities presented in Table 2, then significant ratio differences appear to exist among towns P (5:1.5:1), C (30:13:1), and D (13:8:1).

The lower mean weights of prairie dogs in town P could be seasonal because prairie dogs may encounter substantial weight losses during the winter months of dormancy. The onset of spring initiates host reproduction and would doubtfully enhance significant weight gains. Increased numbers of fleas in town P suggested that correlations exist between host and flea reproductive cycles (Rothschild, 1965). Fifty-three percent (8/15) of the hosts in town P were in the lower weight group (580–760 g), and all were collected during May 13 through June 10. Young prairie dogs are born during the months of March–May (Walker, 1968); since egg-laying among fleas would probably commence during the first 5 wk (Stark, 1959) of this period, there would be adequate time for resident fleas to produce new generations of adults because their life cycles are only 3–5 wk in duration (Stark, 1959).

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A Seasonal Survey of Metazoan Parasites of Arctic Cisco (*Coregonus autumnalis*) from Alaskan Arctic Coastal Waters

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ABSTRACT: A seasonal survey of metazoan parasites of arctic cisco, *Coregonus autumnalis,* from the Alaskan Beaufort Sea coastal area revealed only three species: two cestodes and one acanthocephalan. The most common cestode, *Bothrimonus sturionis,* had high prevalence in summer samples, but was absent in winter samples. The other two parasite species, a metacestode stage of a *Diphyllobothrium* species and the acanthocephalan *Neoechinorhynchus tumidus,* had lower prevalence and apparently were not seasonal. None of the parasites found occurred in numbers that appeared detrimental to host fishes.

The family Coregonidae includes the most important subsistence and commercial species in coastal waters of the western Beaufort Sea. Parasites of northern coastal or shallow water marine fish species, including coregonids, have been studied in Siberian and European waters, and in eastern waters of North America (Sandeman and Pippy, 1967; Threlfall and Hanek, 1970; Hicks and Threlfall, 1973; reviews in Bauer, 1970 and Lawler, 1970); however, there is little information available on metazoan parasites of fishes in northern Alaskan coastal waters. As part of a study of trophic and ecological relationships of nearshore fish species in the western Beaufort Sea a seasonal survey of the most important commercial and subsistence species, the arctic cisco (*Coregonus autumnalis*), was undertaken to provide information on its metazoan parasites.

Arctic cisco enter coastal brackish waters of the Beaufort Sea shortly after spring break-up (usually in June) of river systems where the populations have overwintered. During the short open water season they feed in shallow coastal waters on epibenthic crustaceans, principally mysids and to a lesser extent, amphipods (Craig and Haldorson, 1981). By freeze-up in late September or early October they return to overwintering sites in river drainages. The Colville River system is apparently the major overwintering habitat for this species in the western Beaufort Sea, and is possibly a major spawning site, although spawning locations for arctic cisco have never been identified in Alaskan waters.

Materials and Methods

Samples of arctic cisco were collected by gill net under the ice in the Colville River delta (70°25′N, 150°07′W) in November 1976 and 1977, and three times during the 1977 open water season in nearby (about 25 mi) Simpson Lagoon (70°30′N, 149°12′W). The fresh or frozen fish were examined for external and encysted parasites. The viscera and gills were preserved in 10% formalin and examined later.

Voucher specimens of the parasite species were deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705 (Nos. 77592–77594).

Results and Discussion

No parasites were found externally, encysted in the flesh, or on the gills. The viscera were infected with three species: an adult cestode (*Bothrimonus sturionis* Duvernoy, 1842), a cestode plerocercoid (metacestode) stage (*Diphyllobothrium* sp.), and an adult acanthocephalan (*Neoechinorhynchus tumidus* Van Cleave and Bangham, 1949). The prevalence and intensity of infections for all samples are presented in Table 1.

The number of parasite species found in this survey was surprisingly low. Other surveys of coregonid parasite faunas by Hicks and Threlfall (1973) found 12 and 13 species in *Coregonus clupeaformis* and *Prosopium cylindraceum,* which is slightly lower than the 15 and 18 species Arthur et al. (1976) report for the same species, respectively. Watson and Dick (1979) reported 19 and 18 species in *C. clupeaformis* and *Coregonus artedii,* two species from which Leong and Holmes (1981) reported 16 and 15 species. The other surveys were conducted on freshwater populations where the parasite species were dominated by copepod-vectored cestodes, mollusc-generated metacercaria, and other amphipod-vectored parasites. The low number of parasite species encountered in this study may be due in part to dietary differences (i.e., arctic cisco feed mainly on mysids), a yearly cycle of anadromy, or reduced species diversity in higher latitudes.

Table 1. Infection rates (prevalence) and average number of parasites per infection (intensity) for three parasites of arctic cisco from the Colville River Delta and adjacent marine waters, with mean lengths in cm (L) and standard deviations (SD) of cisco.

			Prevalence/intensity		
Date	*N*	L̄/SD	*Bothrimonus sturionis*	*Diphyllobothrium* sp.	*Neoechinorhynchus tumidus*
11/76	25	337/18.5	0/0	12/10	36/1.8
6/77	25	356/14.2	12/1.0	12/3.3	40/3.2
7/77	25	357/17.7	92/35.6	28/2.6	52/4.3
9/77	25	358/13.7	84/5.7	28/3.1	44/3.4
11/77	21	322/30.0	0/0	10/1.0	33/2.7

The adult tapeworm, *Bothrimonus sturionis,* occurred in the lumen of pyloric caeca and intestine. Burt and Sandeman (1969) synonymized *B. sturionis* and *Diplocotyle olrikii,* and that convention will be followed in this report. *B. sturionis* is a widely distributed, circumarctic species (Sandeman and Burt, 1972), and has been reported from arctic char in Alaskan waters (Mudry and McCart, 1976). Bauer (1970) reports that in Russia this species infects fish in marine waters, but perishes when the host migrates into fresh water. The pattern of infection in this study (Table 1) coincides with that observation. Fish collected in brackish nearshore waters during mid- to late summer were highly infected (84–92%), in contrast to those fish collected in early winter under Colville River ice (0% infected). The intensity was highest in July (35.6); these were small tapeworms about 1 cm in length. By early September the average length had increased to 3–4 cm and the intensity had decreased to 5.7 worms per infection. The temporary nature of *B. sturionis* infections has also been noted by Sandeman and Burt (1972), and the generality of these observations suggests that Dick and Belosevic's (1981) use of this parasite as a marker for sea-run arctic char is inappropriate. Lawler (1970) reported that *D. olrikii* may retard fish growth and cause general deterioration, but apparently this occurs at higher intensity than found in this study. The assumption that all samples were part of the same migratory population is supported by tagging studies, by the absence of arctic cisco in nearshore marine habitats during winter, and by similar size frequencies in all samples (Craig and Haldorson, 1981).

A second tapeworm, *Diphyllobothrium* sp., occurred in the metacestode stage on the stomach walls, pyloric caeca walls, and in mesenteric tissue. Most specimens measured 2–3 mm in diameter. Bauer (1970) reports similar parasites in Russian populations, and suggests that they mature in fish-eating birds. In addition, he reports the occurrence of *D. dendriticum,* a species capable of infecting man, in Russian coregonid populations.

The acanthocephalan (*Neoechinorhynchus tumidus*) was found in the lumen of intestines, usually attached to the intestine wall. Adult parasites were 0.5–1.0 cm in length. Bauer (1970) has reported this species infecting riverine coregonids in eastern Russia, and it has been reported from four freshwater coregonid species in Alaska (Schmidt, 1965). Acanthocephalan (*Echinorhynchus* sp.) infections have been reported to cause deterioration in coregonid fishes when intensities of over 250 per fish occur; however, infections of less than 30 have shown little effect (Bauer, 1970). It seems unlikely, therefore, that intensity levels in these samples (Table 1) were high enough to be detrimental to the hosts.

The intensity levels of parasites found in this study do not appear to significantly harm host fishes. The fish feed and increase in weight and fat content during the open water season. Fulton's condition factor was calculated for the sample of fish taken at the end of the open water season in September 1977. There was a very weak correlation ($r = 0.06$) of total parasite numbers and condition factor in that sample.

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L. Jensen and M. Moser identified the cestodes, and A. Moles identified the acanthocephalan. P. Craig provided support and encouragement. The study was funded by the Bureau of Land Management through a cooperative agreement with NOAA under the Outer Continental

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Helminth Parasites of Capelin, *Mallotus villosus*, (Pisces: Osmeridae) of the North Atlantic

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ABSTRACT: Capelin (*Mallotus villosus*) from the North Atlantic (Newfoundland waters, Grand Banks and Icelandic waters) were examined for helminths. The following were recorded: Monogenea—*Gyrodactyloides petruschewskii, G. andriaschewi,* and *Laminiscus gussevi*; Digenea—*Derogenes varicus, Hemiurus levinseni,* and *Lecithaster gibbosus* (*D. varicus* and *H. levinseni* are new host records); Cestoidea—*Eubothrium parvum* (adult), *Diphyllobothrium* sp(p)., plerocercoids (new host record[s]), other larval pseudophyllideans, and a larval tetraphyllidean; Acanthocephala—*Echinorhynchus gadi* (new host record); Nematoda—*Anisakis simplex, Contracaecum* sp., and *Hysterothylacium* sp. (all third-stage larvae).

Capelin, *Mallotus villosus* (Müller), is known to be an important food source for many marine fishes, particularly cod (Winters and Carscadden, 1978), as well as marine mammals (Sergeant, 1963, 1973). In recent years, however, the development of large commercial capelin fisheries in the North Atlantic, in both Newfoundland and Icelandic waters, as well as in the Barents Sea has led to a decline in the number of available fish.

Despite the importance of capelin in the North Atlantic ecosystem and as a commercially valuable species, little is known about its parasite fauna, and most studies that cover the whole spectrum of helminth parasites have been done by Soviet authors on fish taken in the Barents (Polyanskĭ, 1955) and Bering seas (Zhukov, 1960, 1963). Larval nematodes and one cestoidean species are the only helminths that have been previously reported from capelin from the North Atlantic (Templeman, 1948; Winters, 1970).

Thus, the purpose for the present study was to make a comprehensive collection of the helminth parasites of capelin from the Northwest Atlantic in order to provide a guide to identification. Quantitative data and complete listings of the geographic localities will be published separately, together with a discussion of their potential use in stock discrimination.

Materials and Methods

Capelin collected from various localities in the Northwest Atlantic from November 9, 1979 to July 2, 1981 were mostly taken in the spawning season (May–July) of each year: offshore samples were caught from Environment Canada research vessels, using either an otter or a midwater trawl; inshore samples in purse seines, and beach-spawning samples by castnet or dipnet.

For the purpose of obtaining helminths for identification, capelin (mostly inshore samples) were examined while fresh. Other animals (mostly offshore samples) were fast-frozen as soon as possible after capture.

Fish were examined using standard helminthological procedures; helminths collected and location within the host were recorded. Monogenea were fixed and prepared as described by Pálsson and Beverley-Burton (1983); specimens of *Lecithaster gibbosus* and adult Cestoidea were fixed in hot AFA (alcohol-formaldehyde-acetic acid) (80–90°C) and stained in aceto-carmine in the routine preparation of whole mounts. Other Digenea, Acanthocephala, and larval Cestoidea, all from frozen fish, were fixed in cold AFA and stained as above. Nematodes, also from frozen fish, were preserved in 70% ethanol with 5% glycerol, cleared by evaporation of the ethanol, and mounted in glycerol.

Measurements were made and drawings prepared with the aid of a camera lucida and are expressed, unless otherwise stated, in mm with the mean followed by the range in parentheses.

Representative specimens of the Monogenea described by Pálsson and Beverley-Burton (1983) have been deposited previously in the United States National Museum Helminthological Collection, Beltsville, Maryland (Nos. 77100–77102). Voucher specimens of the remaining helminths have also been deposited in the above collection (Nos. 77658–77669).

Results

Monogenea: Gyrodactylidae

Gyrodactyloides petruschewskii Bykhovskiĭ, 1947

GEOGRAPHIC LOCALITIES: Northwest Atlantic (Newfoundland waters, Middle Cove 47°39′N, 52°41′W) and Icelandic waters (63°40′N, 22°28′W).

LOCATION ON HOST: Gills.

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REMARKS: Originally described from capelin taken in the Barents Sea by Bykhovskiĭ (1947), *G. petruschewskii* was redescribed by Pálsson and Beverley-Burton (1983). It has been found on capelin in the Okhotsk Sea (Bykhovskiĭ and Polyanskiĭ, 1953) and Bering Sea (Zhukov, 1960); additional hosts—herring (*Clupea harengus pallasi*) see Zhukov (1960) and Kulachkova (1974); Dolly Varden (*Salvelinus malma*) see (Zhukov, 1960).

***Gyrodactyloides andriaschewi* Bykhovskiĭ and Polyanskiĭ, 1953**

GEOGRAPHIC LOCALITIES: As above.

LOCATION ON HOST: Gills.

REMARKS: Originally described from capelin taken in the Barents and Okhotsk seas by Bykhovskiĭ and Polyanskiĭ (1953), *G. andriaschewi* was redescribed by Pálsson and Beverley-Burton (1983). It has been found on capelin in the Bering Sea (Zhukov, 1960).

***Laminiscus gussevi* (Bykhovskiĭ and Polyanskiĭ, 1955) Pálsson and Beverley-Burton, 1983**

GEOGRAPHIC LOCALITIES: As above.

LOCATION ON HOST: Gills.

REMARKS: This species was originally described from capelin in the Barents Sea by Bykhovskiĭ and Polyanskiĭ (1953) and was reported from capelin in the Bering Sea (Zhukov, 1960). Pálsson and Beverley-Burton (1983) redescribed *gussevi* and transferred it from *Gyrodactyloides* to the newly proposed genus *Laminiscus*.

Digenea: Hemiuridae

***Derogenes varicus* (Müller, 1784) Looss, 1901**

GEOGRAPHIC LOCALITIES: Northwest Atlantic (Grand Banks 44°22′N, 49°57′W and Newfoundland waters, Middle Cove 47°39′N, 52°41′W).

LOCATIONS IN HOST: Stomach and intestine.

REMARKS: *D. varicus* has not previously been reported from capelin, thus, this is a new host record. The specimens from capelin are morphologically similar to those described by Odhner (1905). Measurements (body 1.35 [0.79–1.92] long × 0.34 [0.25–0.45]; transverse diameter of oral and ventral suckers 0.14 [0.10–0.18] and 0.27 [0.18–0.35] respectively; eggs 52 [47–57] μm by 31 [28–34] μm) are similar to those provided by Rees (1953) and Overstreet and Hochberg (1975).

***Hemiurus levinseni* Odhner, 1905**

GEOGRAPHIC LOCALITIES: Northwest Atlantic (Newfoundland waters, Placentia Bay 46°20′N, 54°50′W and Bonavista Bay 48°31′N, 53°30′W).

LOCATION IN HOST: Stomach.

REMARKS: *H. levinseni* has not previously been reported from capelin, thus, this is a new host record. The specimens from capelin are morphologically similar to those described by Odhner (1905). Measurements (body 0.99 [0.74–1.26] long by 0.25 [0.12–0.42]; transverse diameter of oral and ventral suckers 0.13 [0.10–0.18] and 0.12 [0.09–0.18] respectively; eggs 24 [22–26] μm by 13 [10–14] μm) are less than those presented by Odhner (1905) and Linton (1940), but most fall within the ranges given.

***Lecithaster gibbosus* (Rudolphi, 1802) Lühe, 1901**

GEOGRAPHIC LOCALITY: Northwest Atlantic (Newfoundland waters, Conception Bay 47°24′N, 53°08′W) and Icelandic waters.

LOCATIONS IN HOST: Stomach and intestine.

REMARKS: Because of considerable variation in internal anatomy, there is difficulty in differentiating *L. gibbosus* from *L. confusus* Odhner (1905). In addition, the morphometrics of the two species are similar. Odhner (1905) characterized the two species as follows:

L. gibbosus—seminal vesicle dorsal to and not extending beyond the ventral sucker posteriorly. Lobes of ovary round (about as wide as long), lobes of vitellarium elongate. Eggs 25–27 μm long.

L. confusus—seminal vesicle extending beyond, or located completely posterior to, ventral sucker. Lobes of ovary elongate, similar in shape to the lobes of the vitellarium. Ovary smaller than vitellarium. Eggs 15–17 μm long.

In the present specimens (which had not been frozen) the lobes of the ovary were rounded, the lobes of the vitellarium were elongate (up to twice as long as wide) and the seminal vesicle extended behind the ventral sucker: a combination of characters which, according to Odhner (1905), does not occur. Overstreet (1973), who dismissed the location of the seminal vesicle as a significant diagnostic character, recognized both *L. gibbosus* and *L. confusus,* but Naidenova (1974), who considered the differentiation between the species unreliable, reduced *L. gibbosus* and *L. confusus* to subspecies: *L. gibbosus gibbosus* with eggs over 20 μm long from northern waters and *L. gibbosus confusus* with eggs less than 20 μm long from

southern waters. However, the separation of the two taxa on the basis of egg size has been found problematic as the range of egg lengths of the present material (19–22 μm) and that of *L. confusus* (15–23 μm) published by Overstreet (1973) overlap.

Therefore, at the present time we regard *L. gibbosus* and *L. confusus* as valid species and, based on the shape of the lobes of the ovary and vitellarium, the specimens from capelin (which measured 1.38 [0.70–2.00] long × 0.42 [0.21–0.64] wide; transverse diameter of oral and ventral suckers 0.13 [0.07–0.19] and 0.21 [0.11–0.34] respectively; eggs 21 [19–22] μm × 14 [12–15] μm) are designated as *L. gibbosus*.

L. gibbosus has been reported from capelin in the Bering Sea (Zhukov, 1963), as was *L. confusus* from the Barents Sea by Isaichikov (1933, cited by Polyanskiĭ, 1955).

Cestoidea: Pseudophyllidea

Amphicotylidae

***Eubothrium parvum* Nybelin, 1922**

GEOGRAPHIC LOCALITIES: Northwest Atlantic (Newfoundland waters, Conception Bay 47°30′N, 53°05′W; Bonavista Bay 48°31′N, 53°30′W and Trinity Bay 47°39′N, 53°31′W).

LOCATION IN HOST: Intestine.

REMARKS: The morphology of the specimens studied is similar to that described by Wardle and McLeod (1952) and Sekhar and Threlfall (1970) based on *E. parvum* from capelin taken in Norwegian waters and from cunner (*Tautogolabrus adspersus*) caught in Newfoundland waters respectively. The scolex morphology resembles that described by Andersen (1979) for specimens from capelin taken in Norwegian waters.

Measurements of the present material (Table 1) are mostly greater than those presented by Sekhar and Threlfall (1970) for two specimens from cunner both of which contained only a small number of eggs. In contrast, the gravid proglottides of the capelin parasites contained many eggs that may be an indication that cunner is not such a suitable host for *E. parvum* as capelin.

Templeman (1948) reported *E. parvum* from capelin in Newfoundland waters.

Diphyllobothriidae

***Diphyllobothrium* sp. Plerocercoid I**

GEOGRAPHIC LOCALITIES: Northwest Atlantic (Newfoundland waters, Conception Bay 47°30′N, 53°05′W and Bonavista Bay 48°31′N, 53°30′W) and Icelandic waters (63°40′N, 22°30′W).

LOCATIONS IN HOST: Stomach wall (encysted) and lumen of stomach.

REMARKS: Larvae measured 6.48 (4.85–12.54) long, and 0.41 (0.30–0.58) in maximum width across scolex, which has two well-developed bothria. No trace of internal organs was visible.

***Diphyllobothrium* sp. Plerocercoid II**

GEOGRAPHIC LOCALITIES: Northwest Atlantic (Grand Banks 45°28′N, 49°53′W and Newfoundland waters, Middle Cove 47°39′N, 52°41′W).

LOCATION IN HOST: Intestine.

REMARKS: Larvae measured 1.05 and 1.00 long and 0.31 and 0.27 in maximum width across scolex, which has two shallow bothria. No trace of internal organs was visible.

Records of *Diphyllobothrium* spp. plerocercoids from marine fish are rare and the only previous record from the Atlantic appears to be that of Andersen (1977) for the blue whiting (*Micromestius poutasson*) taken on the Faeroe Banks. Delyamure (1955) listed 10 *Diphyllobothrium* spp. occurring as adults in marine mammals of the North Atlantic, of which nine parasitized seals. Thus, *Diphyllobothrium* spp. pleocercoids I and II in the present study are probably larvae of a marine mammal parasite.

Other larval Pseudophyllidea (Fig. 1)

GEOGRAPHIC LOCALITIES: Northwest Atlantic (Newfoundland waters, Conception Bay 47°30′N, 53°05′W and Notre Dame Bay 49°40′N, 54°46′W).

LOCATIONS IN HOST: Stomach and intestine.

REMARKS: Larvae measured 504 (255–680) μm long × 268 (143–387) μm in maximum width; terminal sucker of scolex 87 (71–99) μm long × 129 (84–152) μm wide. The larvae are similar morphologically and morphometrically to those found in cunner in Newfoundland waters by Sekhar and Threlfall (1970) that were designated as pseudophyllideans.

Larval Tetraphyllidea (Fig. 2)

GEOGRAPHIC LOCALITY: Northwest Atlantic (Newfoundland waters, Trinity Bay 47°39′N, 53°31′W).

LOCATIONS IN HOST: Stomach and intestine.

REMARKS: Larvae measured 719 (502–866) μm long × 254 (190–289) μm wide; apical sucker of scolex 70 (56–83) μm wide; bothria 147 (124–178) μm long × 98 (64–125) μm wide with an-

Table 1. Comparative measurements (in micrometers) of *Eubothrium parvum* Nybelin, 1922 from fish taken in the northwest Atlantic (Newfoundland waters).

	Sehkar and Threlfall (1970)	Present study*
Scolex:		
Total length	340 & 500	736 (526–890)
Maximum width	470 & 520	595 (371–783)
Width of apical plate	250	302 (216–350)
Mature proglottides:		
Length	180–430	346 (267–433)
Width	500–1,750	2,119 (1,583–2,800)
Ovary:		
Length	200	200 (133–250)
Width	550	739 (557–1,183)
Cirrus sac:		
Length	180–250	225 (166–285)
Width	140–160	113 (101–139)
Eggs:		
Length	27–40	39 (36–42)
Width	15–20	26 (24–29)
Number of specimens	2	10
Host	*Tautogolabrus adspersus*	*Mallotus villosus*
Locality	Newfoundland waters	Newfoundland waters

* Mean, followed by range in parentheses.

terior loculus 68 (51–89) μm long × 88 (62–112) μm wide and posterior loculus 79 (66–97) μm long × 98 (64–125) μm wide. These specimens are morphologically and morphometrically similar to tetraphyllid larvae found in cunner from Newfoundland waters by Sekhar and Threlfall (1970) and designated as *Scolex pleuronectis bilocularis* (G. R. Wagener, 1854) Southwell, 1925. Along the Atlantic coast of Canada, similar tetraphyllid larvae have been reported from a number of other fishes (Ronald, 1958; Gaevskaya and Umnova, 1977); in the Barents Sea they have been previously found in capelin (Polyanskiĭ, 1955).

Acanthocephala: Echinorhynchidae

Echinorhynchus gadi Zoega in O. F. Müller, 1776

GEOGRAPHIC LOCALITIES: Northwest Atlantic (Grand Banks 45°28′N, 49°53′W and Newfoundland waters, Middle Cove 47°39′N, 52°41′W).

LOCATION IN HOST: Intestine.

REMARKS: *E. gadi* has not previously been reported from capelin, thus, this is a new host record.

The present identification is based on the number and arrangement of hooks (20 rows with 11–12 hooks per row) on the proboscis: the worms are immature and smaller than the mature worms measured by Golvan (1969). However, they are morphometrically similar to the immature *E. gadi* from *Oncorhynchus* sp. in British Columbian waters measured by Ekbaum (1938).

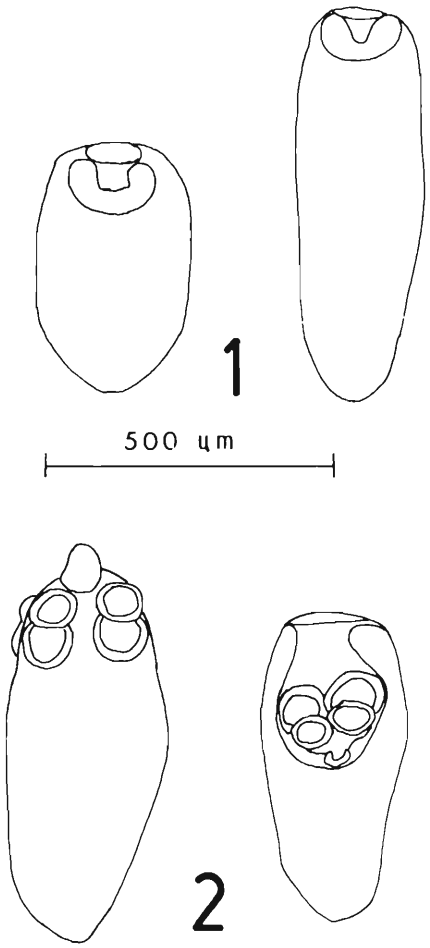
Nematoda (all third-stage larvae): Anisakidae

Anisakis simplex (Rudolphi, 1809) Dujardin, 1845

GEOGRAPHIC LOCALITY: Northwest Atlantic (Newfoundland waters, Trinity Bay 47°36′N, 53°33′W), Icelandic waters (63°40′N, 22°30′W).

LOCATIONS IN HOST: Body cavity, encysted on viscera or free.

REMARKS: The identity of larval *Anisakis* sp. I (Berland, 1961) in the North Atlantic was established as *Anisakis simplex* by Pippy and van Banning (1975). *Anisakis simplex* larvae from the North Atlantic were described by Grainger (1959) and Beverley-Burton et al. (1977) and from the Pacific by Cannon (1977). The morphology



Figures 1, 2. Plerocercoids. 1. Pseudophyllidean plerocercoids. 2. Tetraphyllidean plerocercoids.

of the specimens from capelin is similar to that of the above descriptions and the measurements of the larvae (total length 21.11 [15.37–24.71] × 0.47 [0.34–0.56]; anterior end to nerve ring 0.22 [0.15–0.35]; esophagus 1.72 [1.40–2.23] long; ventriculus 0.79 [0.54–0.99] long; tail 0.10 [0.08–0.13] long) conform with previously published data.

Larvae of *A. simplex* have been reported from fishes throughout the world (Polyanskiĭ, 1955; Berland, 1961; Koyama et al., 1969; Cannon, 1977) and Margolis and Arthur (1979) list a total of 53 host species from the Atlantic and Pacific coasts of Canada alone. *Anisakis* sp. larvae have previously been reported from capelin in the Barents and the Bering seas (Polyanskiĭ, 1955 and Zhukov, 1963, respectively) and Templeman et al. (1957) identified *Anisakis* sp. type larvae from this host taken in Newfoundland waters.

Contracaecum sp.

GEOGRAPHIC LOCALITY: Northwest Atlantic (Newfoundland waters, Trinity Bay 47°36′N, 53°33′W), Icelandic waters (63°40′N, 22°30′W).

LOCATIONS IN HOST: Body cavity, encysted on viscera or free.

REMARKS: The larvae morphologically resemble *Contracaecum* sp. larvae from *Gadus callarias* and *Cyclopterus lumpus* as described by Berland (1961). Berland later (1963) concluded that third-stage larvae of *Contracaecum* and *Phocascaris* are probably indistinguishable, but Myers (1957a, b) found *Contracaecum osculatum* (Rudolphi, 1803) Baylis, 1920 to be common in harp seals (*Pagophilus groenlandicus*) from the Magdalen Islands, Quebec, as well as in seals from the eastern Canadian Arctic, whereas *Phocascaris* sp. was relatively rare. Thus, the larvae found in the present study seem more likely to be *Contracaecum* sp., are designated as such, and measure: 9.98 (2.46–19.49) long × 0.35 (0.15–0.50); anterior end to nerve ring 0.18 (0.11–0.32); esophagus 0.78 (0.39–1.35) long; ventriculus 0.06 (0.02–0.09) long; intestinal caecum 0.53 (0.23–0.94) long; ventricular appendix 0.77 (0.40–1.31) long; tail 0.15 (0.09–0.18) long.

Hysterothylacium sp.

GEOGRAPHIC LOCALITY: Northwest Atlantic (Grand Banks 46°41′N, 51°56′W), Icelandic waters (63°40′N, 22°30′W).

LOCATIONS IN HOST: Body cavity, encysted on viscera or free.

REMARKS: Morphologically these larvae resemble the third-stage larvae described by Berland (1961) as *Contracaecum aduncum* (=*Hysterothylacium aduncum* (Rudolphi, 1802) Deardorff and Overstreet, 1981). Although *H. aduncum* has been reported (both as third-stage larvae in the body cavity and viscera and as adults in the gut) from a large number of species of fish from Canadian waters it is questionable if all the specific identifications are valid especially as Hartwich (1975) noted that, in fishes from the North Sea and Baltic, more than one species of *Hysterothylacium* may be present. Thus, the larvae (which measure 10.69 [8.16–13.22] long × 0.25 (0.18–0.37); anterior end to nerve ring 0.26 [0.18–0.37]; anterior end to excretory pore 0.32 [0.22–0.45]; esophagus 1.23 [0.83–1.65] long; ventriculus 0.07 [0.05–0.12] long; intestinal cae-

cum 0.64 [0.46–0.85] long; ventricular appendix 0.68 [0.45–1.13] long; tail 0.15 [0.08–0.22]) are designated as *Hysterothylacium* sp.

Templeman (1948) found nematode larvae in capelin in Newfoundland that were identified as *Contracaecum clavatum* (synonym of *H. aduncum*), but later Templeman (1968) referred to these larvae as *Contracaecum* sp.

Polyanskiĭ (1955) recorded larval *H. aduncum* from the body cavity of capelin in the Barents Sea and found specimens in the intestine, but it is not clear if these specimens were adults or larvae.

Discussion

The parasite fauna of capelin is mostly composed of nonhost specific helminths: the three digenean species and the three species of larval nematodes have all been reported from a number of fishes (Margolis and Arthur, 1979). In addition, the larval cestodes are probably not host specific, as larval Tetraphyllidea have been found in various hosts including both fishes (Ronald, 1958; Sekhar and Threlfall, 1970) and squid (Brown and Threlfall, 1968). Of the three monogeneans, *G. andriaschewi* and *L. gussevi* are probably host specific as they have not been reported from fishes other than capelin and the cestode *E. parvum* appears to be also highly host specific.

As capelin is a major component of the diet of many marine mammals (Sergeant, 1963, 1973), it is probably an important intermediate or paratenic host of parasites, such as *Diphyllobothrium* spp., *A. simplex* and *Contracaecum* sp., that mature in cetacean or pinniped hosts.

The digenean fauna of capelin is qualitatively sparse. Other than *Lecithaster* spp. the only species previously reported from capelin is *Pseudopentagramma petrowi* (Layman, 1930) found by Margolis and Ching (1965) in capelin from the Pacific coast of Canada.

In the present study, no parasitic crustaceans were found. The only previous record is that of Fröiland (1974) who found one specimen of the copepod *Haemobaphes cyclopterina* (Fabricius) on capelin from the Barents Sea.

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Ecology of Helminth Parasitism of Bobwhites in Florida[1]

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ABSTRACT: Between 1971 and 1977, examination of 483 bobwhites, *Colinus virginianus* (L.), from six localities in Florida revealed 13 species of helminths, including 10 nematodes, 2 cestodes, and 1 trematode. The number of species per infected host varied from 1 to 7 (mean, 2.2) with 64 birds free of helminths. The cestodes (*Raillietina* spp.) and four species of nematodes (*Trichostrongylus tenuis* (Mehlis, 1846) Railliet and Henry, 1909, *Cyrnea colini* Cram, 1927, *Heterakis bonasae* Cram, 1927, and *Dispharynx nasuta* (Rudolphi, 1819) Railliet, Henry, and Sisoff, 1912) were considered to be characteristic components of the helminth fauna due to their prevalence, intensity, and distribution. The total number of nematodes per infected bird ranged from 1 to 341 (mean, 11.1). Simpson's index was low (0.19) indicating an equitable distribution of species on a statewide basis; however, helminths of bobwhites from the central and southern parts of the state had a tendency towards increasing dominance. Indices of similarity were fairly high, indicating similarities of the helminth faunas of bobwhites from various collection areas throughout the state. There was no effect of host sex, host age, or interaction of sex and age on the prevalence of the parasites. Four species (*T. tenuis, C. colini, H. bonasae,* and *A. galli*) showed significant differences ($P < 0.05$) in prevalence from year-to-year. There was no relationship between the sex of the bobwhites and intensity of infection for any of the parasites, and an age relationship was found only in the significantly higher intensities of *H. bonasae* in juveniles than in adult birds. There were significant year-to-year changes in intensity of *C. colini.*

The bobwhite, *Colinus virginianus* (L.), is one of Florida's most important upland game birds (Murray and Frye, 1964). Two recognized subspecies occur in Florida; the Florida bobwhite (*C. v. floridanus*) and the Eastern bobwhite (*C. v. virginianus*) (American Ornithologists' Union, 1957) (Fig. 1). Although there are numerous publications on the parasites of bobwhites (Kellogg and Calpin, 1971; Kellogg and Doster, 1972), our knowledge of the helminths of bobwhites in Florida is limited to studies that have been conducted in one area (Leon County) in northern Florida (Kellogg and Prestwood, 1968; Davidson et al., 1980, 1983). The present paper is concerned with the prevalence and intensity of helminths in bobwhites in other parts of Florida and includes a 5-yr study of year-to-year changes in the helminth fauna of a population of bobwhites in southern Florida.

Materials and Methods

A total of 483 bobwhites was collected from six counties in Florida (Fig. 1) between March 1971 and February 1977. The birds were killed with shotguns or were live-trapped in modified Stoddard box traps. Age classifications (adults or juveniles) were accomplished by plumage analysis (Haugen, 1957). Collections in five of the areas (localities 1–5 on Fig. 1) were made during various times of the year. A large series ($N = 381$) was obtained from area 5 (Charlotte County) over a 5-yr period. For this collection, from 30 to 120 birds were obtained each year during the months of November through February.

Carcasses, or in some cases gastrointestinal tracts, were frozen until they could be processed. The techniques described by Kinsella and Forrester (1972) were employed to recover, fix, preserve, and stain helminths. Representative specimens have been deposited in the U.S. National Parasite Collection (Beltsville, Maryland) as USNM Helm. Coll. Nos. 77385–77395 and 77518.

The data on prevalence were analyzed as a linear model using FUNCAT from the Statistical Analysis System (SAS) (Helwig and Council, 1979). This was a multiway contingency analysis incorporating simultaneous tests of age, sex, and year of collection. Square-root transformations of intensity data were analyzed using the General Linear Models procedure and least

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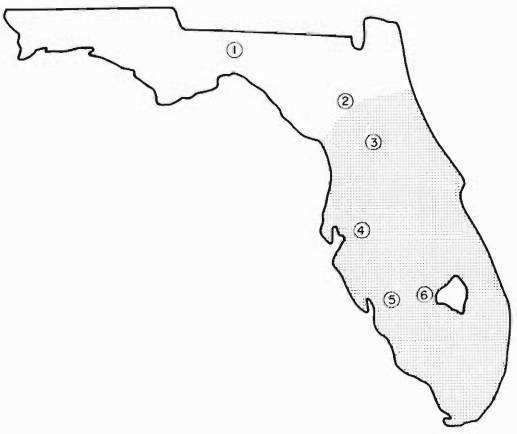


Figure 1. Collection areas for bobwhites in Florida. Numbers of birds collected from each area are as follows: (1) Leon County ($N = 21$); (2) Alachua County ($N = 33$); (3) Marion County ($N = 14$); (4) Hillsborough County ($N = 3$); (5) Charlotte County ($N = 381$); (6) Glades County ($N = 31$). The stippled portion of the map indicates the range of the Florida bobwhite; the clear area shows the range of the Eastern bobwhite.

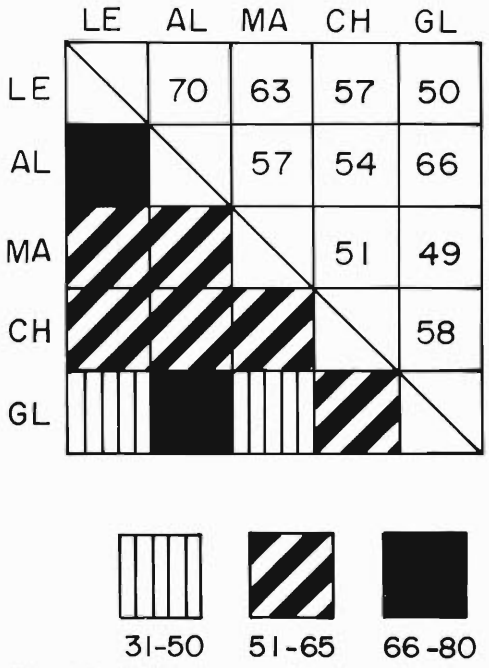


Figure 2. Trellis diagram of indices of similarity for helminth faunas of bobwhites from five localities in Florida. Abbreviations refer to county names as given in Table 2.

squares means option of SAS. Significance is taken at $P < 0.05$. Indices of similarity and diversity were prepared following Holmes and Podesta (1968).

Results and Discussion

Thirteen species of helminths (10 nematodes, 2 cestodes, and 1 trematode) were found. In Table 1 the site, prevalence, and intensity (means, medians, and ranges) of each species are presented. All the helminths with the exception of *Ascaridia galli* and *Zonorchis petiolatum* have been reported previously from bobwhites (Kellogg and Doster, 1972).

The number of species per infected host varied from 1 to 7 (mean, 2.2), with 64 birds free of helminths. The cestodes (*Raillietina* spp.) and four species of nematodes (*Trichostrongylus tenuis, Cyrnea colini, Heterakis bonasae, Dispharynx nasuta*) were considered characteristic components of the helminth fauna due to their prevalence, intensity, and distribution (Tables 1, 2). Because trematodes occurred in only one bird and because of the lack of intensity data for the cestodes, the subsequent analyses are confined to data on nematodes that represented the most common taxon. Of the 4,557 specimens obtained from all the bobwhites, 36% were *T. tenuis* and 32% were *C. colini*; *H. bonasae* and *D. nasuta* constituted 13% and 1% of the total worms encountered, respectively. The total number of nematodes per infected bird ranged from 1 to 341 (mean, 11.1).

Discounting Hillsborough County for which only a very small number of birds ($N = 3$) were examined, three nematodes (*T. tenuis, C. colini* and *H. bonasae*) were more prevalent in bobwhites from the northernmost county (Leon) than in other areas (Table 2). Similarly, higher prevalences of *T. pattersoni* also were apparent in northern counties (Leon and Alachua). *Ascaridia galli,* however, occurred commonly in Charlotte County in southern Florida and was completely absent from four of the other areas. The only other remarkable differences among the remainder of the less commonly found nematodes were the occurrence of the spirurid, *Cheilospirura spinosa,* in Leon and virtually no other county, and the unusually high prevalence of the filariid, *Aproctella stoddardi,* in Alachua County.

Simpson's index was low (0.19) indicating an equitable distribution of helminths across samples on a statewide basis, but when this index was calculated on a county basis it became evi-

Table 1. Location, prevalence, and intensity of infection of helminths of 483 bobwhites collected in Florida, 1971-1977.

Parasite	Site*	Prevalence (%)	Intensity†		
			Mean	Median	Range
Nematoda					
Cyrnea colini Cram, 1927	PR, GI	71.6	4.2	3.0	1–26
Ascaridia galli (Schrank, 1788) Freeborn, 1923	SI	28.6	2.7	2.0	1–17
Trichostrongylus tenuis (Mehlis, 1846) Railliet and Henry, 1909	CE	18.2	18.7	4.0	1–297
Heterakis bonasae Cram, 1927	CE	14.5	8.6	2.5	1–56
Tetrameres pattersoni Cram, 1933	PR	12.2	2.4	2.0	1–10
Aproctella stoddardi Cram, 1931	BC	7.0‡	2.6	2.0	1–17
Dispharynx nasuta (Rudolphi, 1819) Railliet, Henry, and Sisoff, 1912	PR	6.6	2.0	1.5	1–5
Strongyloides sp.	SI	6.4	3.9	3.0	1–18
Spirurid larvae	PR	3.1	1.5	1.0	1–4
Capillaria obsignata Madsen, 1945	SI	2.7	1.2	1.0	1–4
Cheilospirura spinosa Cram, 1927	PR, GI	1.9	2.0	1.0	1–4
Cestoda					
Raillietina spp.§	SI	21.3	—	—	—
Trematoda					
Zonorchis petiolatum (Railliet, 1900) Denton and Byrd, 1951	SI	0.2	2.0	2.0	2

* PR = proventriculus; GI = gizzard; SI = small intestine; CE = ceca; BC = body cavity.

† Number of worms/infected bobwhite.

‡ Actual prevalence may have been higher because only viscera were obtained from a large sample of the bobwhites from Charlotte County.

§ Specimens in poor condition due to freezing and recovery techniques; at least two species; intensity not determined.

dent that helminths of bobwhites from the central and southern parts of the state had a tendency towards increasing dominance compared to the helminths of birds in the northern localities (Table 2). A similar trend was noted in the helminth faunas of mourning doves (*Zenaida macroura*) in Florida (Forrester et al., 1983). Indices of similarity were calculated comparing faunas of bobwhites of each county (except Hillsborough) with every other county, and a trellis diagram was constructed (Fig. 2). The least similarity (49) existed between bobwhites from Marion and Glades counties, but most values were high. When our data on the helminths of 21 bobwhites in Leon County were combined with the data published by Davidson et al. (1980) on 185 bobwhites from the same county and compared with our data from 381 bobwhites in Charlotte County, an index of similarity of 48 was obtained. Although similarity values might suggest a north to south decrease in similarity (Figs. 1, 2), there was no significant correlation of geographical distance between samples and similarity values between samples (Spearman's rank: $r = -0.28$, $P > 0.05$). Furthermore, there does not appear to be any greater similarity within subspecies than between subspecies (Fig. 2). In short, similarity in the helminth fauna of bobwhites from Florida is high despite the varied collection areas and the two subspecies of hosts involved. Most of that high similarity is attributable to the four characteristic nematode components.

The effects of host age, host sex, and year on prevalence and intensity of the five species of nematodes for which we have suitable data were examined for the 381 bobwhites from Charlotte County (Cecil Webb Wildlife Management Area) in southern Florida. There was no significant effect of host sex or host age on the prevalence of any of the helminths, nor was there any interaction of age and sex on prevalence. Four of the species showed significantly different preva-

Table 2. Helminths of bobwhites from six localities in Florida.

	County*					
	Le	Al	Ma	Hi	Ch	Gl
Sample size	21	33	14	3	381	31
Simpson's index	0.16	0.15	0.30	0.31	0.24	0.24
% Hosts infected with:						
Cyrnea colini	95	61	43	0	76	39
Ascaridia galli	0	3	0	0	36	0
Trichostrongylus tenuis	76	58	29	67	12	10
Heterakis bonasae	90	9	7	100	12	0
Tetrameres pattersoni	33	36	0	0	10	3
Aproctella stoddardi	19	45	0	0	2†	26
Dispharynx nasuta	10	18	0	33	4	19
Strongyloides sp.	10	3	0	0	7	0
Spirurid larvae	0	15	0	0	3	0
Capillaria obsignata	5	0	0	0	3	0
Cheilospirura spinosa	38	0	7	0	0	0
Raillietina spp.	57	33	57	33	18	10
Zonorchis petiolatum	0	0	0	0	1	0

* Le = Leon County, Al = Alachua County, Ma = Marion County, Hi = Hillsborough County, Ch = Charlotte County, Gl = Glades County.

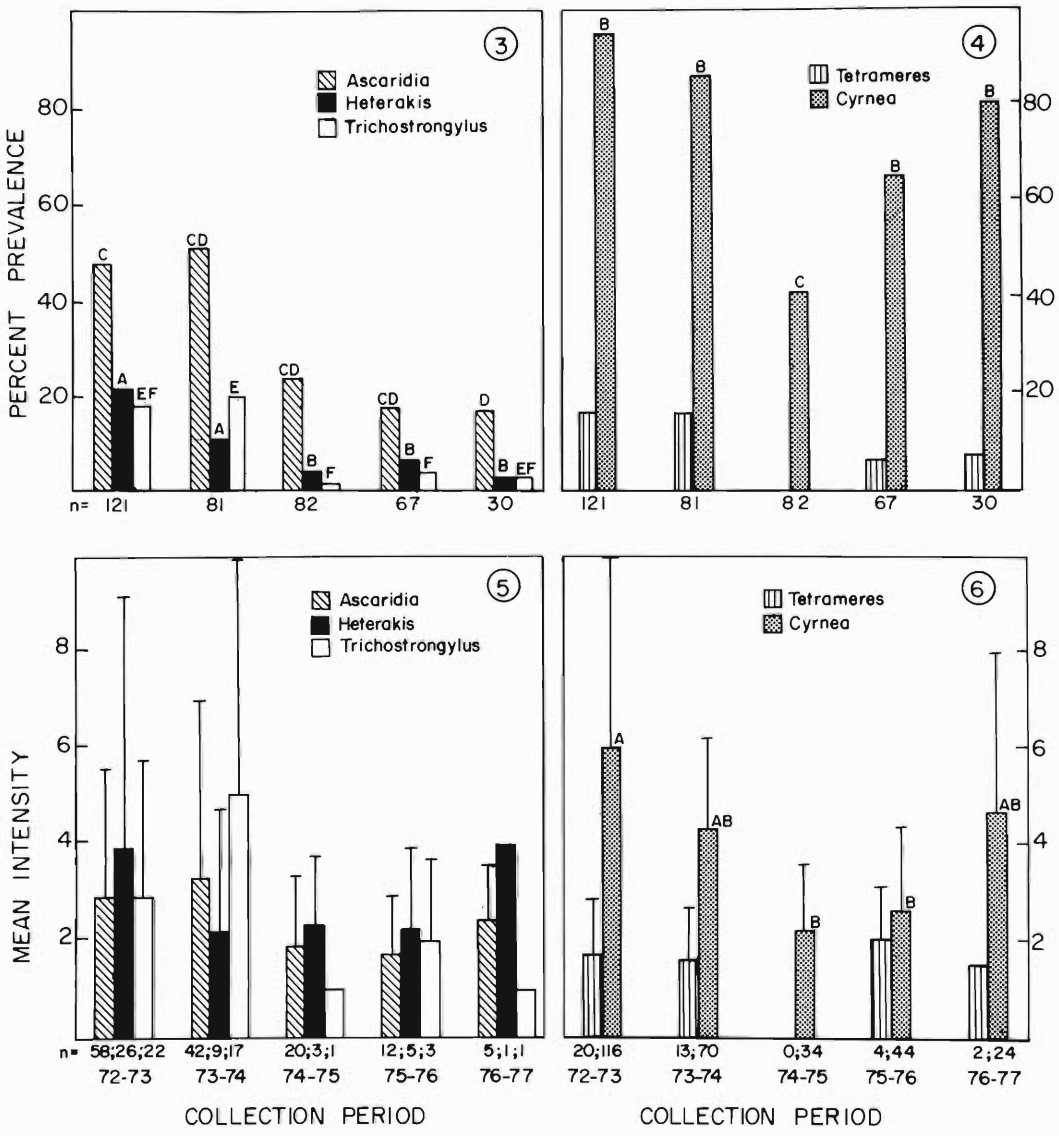
† Actual prevalence may have been higher because only viscera were obtained from a large sample of the bobwhites from this county.

lences from year-to-year (Figs. 3, 4). All five species showed decreases in prevalence during the 1974–1975 season. The prevalences for four of the species remained low for the next 2 yr; only for *C. colini* did the prevalences return to the levels they were in the previous years.

There was no relationship between the sex of the bobwhites and intensity of the infections. The intensity of *H. bonasae* was significantly higher in juveniles than in adult birds, but there was no such effect for the other helminths. Only *C. colini* showed significant year-to-year changes in intensity, particularly a major decrease in 1974–1975 from 2 yr earlier, although *T. pattersoni, T. tenuis,* and *A. galli* showed decreases as well in 1974–1975 (Figs. 5, 6).

We can offer no explanation for the decreased prevalence and intensity values for the years 1974–1975. Analysis of weather records for the 5-yr period (U.S. Department of Commerce, 1972–1977) yielded no clues as to what might have caused the observed changes. It is possible that the density of the bobwhite populations may have had an effect. During the 1973–1975 hunting seasons, the harvest of bobwhites was the lowest of the 5-yr period suggesting that the bobwhite population was low for those 2 yr. This might indicate that the prevalence and intensity of the nematodes in question were regulated by density-dependent mechanisms.

The pathogenic effects of the helminths of bobwhites are not well known. Although the present investigation was not designed to determine the impact of these parasites on populations of bobwhites, a few comments on nematode infections should be made. Davidson et al. (1980) reported a few cases of proliferation of the gizzard lining due to *C. colini,* ulceration of the proventriculus due to *D. nasuta,* and enlarged proventriculi due to large numbers of *T. pattersoni. Trichostrongylus tenuis* is known to be pathogenic to red grouse (*Lagopus lagopus scoticis*) in Great Britain when present in large numbers (Wilson, 1982). Its effect on bobwhites is unknown. Levine (1980) analyzed the literature on the pathogenesis of *A. galli* and concluded that it is the "most serious helminth parasite of poultry in many areas." The effects include decreased weight gains (Chubb and Wakelin, 1963), splenomegaly, hepatomegaly, atrophy of the thymus, anemia, and leukocytosis (Sadun, 1950), and considerable damage to the intestine resulting in hemorrhagic enteritis or chronic catarrhal enteritis (Ackert, 1931). Adult *A. galli* are known to perforate the intestine and cause peritonitis (Levine, 1980). Similar effects may occur in bobwhites,



Figures 3-6. Year-to-year variation in prevalence and mean intensity of five species of nematodes in bobwhites from the Cecil Webb Wildlife Management Area in Charlotte County, Florida. Vertical lines represent standard deviations for intensity data. For each species of parasite, dissimilar letters above bars indicate values that are significantly different between certain years. Letters in common or bars with no letters indicate no significant differences. *N* = sample sizes for prevalence data (Figs. 3, 4) and for mean intensity (Figs. 5, 6). Sample sizes for intensity data differ for each parasite during a given year because they are based on numbers of parasites per infected bobwhite, which varies for each parasite.

but this is unknown at the present time. *Heterakis bonasae* is not known to be pathogenic to bobwhites (Davidson et al., 1983) and does not appear to transmit *Histomonas meleagridis* (Davidson et al., 1978).

Recently, Davidson et al. (1982) published an overview of the importance of parasitism in bobwhites in the southeastern U.S. Their analyses were based on extensive studies done by the Southeastern Cooperative Wildlife Disease Study

since 1963, including a 12-yr parasite-monitoring study in one area in north-central Florida (Leon County). They concluded that parasitism in bobwhites is usually subclinical and that the common helminths of bobwhites have limited pathogenicity. Thus, although there are helminths in bobwhites that are pathogenic and cause overt disease in some individual birds, they may have a minimal impact on populations as a whole.

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Parasites of Trout from Four Lotic Localities in Michigan

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ABSTRACT: Trout (281 brown trout, *Salmo trutta*; 227 brook trout, *Salvelinus fontinalis*; and 24 rainbow trout, *Salmo gairdneri*, Salmonidae) were collected from four lotic environments in the lower peninsula of Michigan and examined for parasites between July 1981 through September 1982. Fifty-four percent of the fishes harbored one or more of the following parasites: *Crepidostomum cooperi, C. cornutum, Acanthocephalus dirus, Neoechinorhynchus cristatus, N. saginatus, Pomphorhynchus bulbocolli, Salmincola edwardsii, Eubothrium* sp., *Proteocephalus* sp., *Cucullanus* sp., and *Rhabdochona* sp. All infected the digestive tract except *S. edwardsii*, which parasitized the gills and external surface of brook trout. *Acanthocephalus dirus* and *S. edwardsii* were relatively common and their effects on trout are discussed.

Several studies have been performed on different species of trout from inland aquatic environments of Michigan (Metzelaar, 1928; Shetter 1936, 1968; Leonard and Leonard, 1946; Cooper, 1952, 1953; Latta, 1963; Alexander and Shetter, 1969). The Michigan Department of Natural Resources has conducted parasitological investigations, primarily of protozoa of trout (Allison, 1958; Allison and Latta, 1969; Maclean and Yoder, 1970; Yoder, 1972; Allison et al., 1977). Extensive parasitological investigations on Michigan trout have not, however, been published. This study reports on the parasites of rainbow trout, *Salmo gairdneri*; brown trout, *Salmo trutta*; and brook trout, *Salvelinus fontinalis* from four inland aquatic environments of the lower peninsula of Michigan.

Materials and Methods

Trout were collected by electrofishing from Fish Creek, between Colby and Pakes roads (R.5W.–6W., T.10N., S.11), Montcalm County; Honey Creek at Conservation Road and McCabe Avenue (R.10W., T.7N., S.23), Kent County; the Pine River, between Kendaville and Lake Montcalm roads (R.4W., T.11N.–12N., S.6), Gratiot County; and the Rogue River, 12 Mile Road and Summit Avenue (R.10W.–11.W., T.9N., S.25), Kent County, Michigan from July 1981 through September 1982. Trout are stocked in the Pine and Rogue rivers, whereas natural populations of brook and brown trout occur in Fish Creek, and Honey Creek has a natural population of brook trout.

Trout were either brought to the laboratory alive and necropsied within 24 hr, or were fixed in 10% formalin at the sampling area and examined later. The gills, external surface, digestive tract, and, occasionally, the muscles, were examined for parasites. All parasites found were processed using standard parasitological techniques. Prevalence is the percentage of fish infected in a given sample and mean number is the number of parasites per host in a given sample. The value following the mean is the standard error. Ecotypes of most specimens were deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland. Accession numbers are listed in Table 1. Remaining specimens have been retained in the author's collection.

Results

Totals of 281 brown trout, *Salmo trutta*; 227 brook trout, *Salvelinus fontinalis*; and 24 rainbow trout, *Salmo gairdneri* were collected. The number and mean total length (mm) of trout examined by species and locality were: brown trout (Rogue River—141, 164 ± 4.5; Fish Creek—120, 147 ± 6.6; Pine River—20, 185 ± 5.6); brook trout (Honey Creek—124, 140 ± 5.1; Fish Creek—79, 134 ± 5.9; Pine River—1, 288); rainbow trout (Rogue River—22, 172 ± 9.9; Pine River—2, 184 ± 46.5). Included in the 227 brook trout were 23 that were negative for parasites, collected from two tributaries of the Rogue River.

Eleven species of parasites from Fish and Honey creeks, and the Pine and Rogue rivers are listed in Table 1. Of the 532 trout examined, 288 (54%) were infected with at least one species of parasite and 144 (27%) were infected with intestinal helminths. One hundred two (36.3%) of 281 brown trout, 18 (7.9%) of the 227 brook trout, and all 24 rainbow trout examined were infected with intestinal helminths. Irrespective of trout species, five species of parasites were found in or on hosts from Fish Creek, three in Pine River hosts, three in Rogue River hosts, and two in or on Honey Creek trout. Irrespective of locality, eight species of parasites infected brook trout; four infected brown trout and two infected rainbow trout. A maximum of three species of helminths were recovered from a single fish, a 288-mm-long brook trout from the Pine River.

The trematode fauna was represented by *Crepidostomum cooperi* and *C. cornutum* (Allocreadiidae); these species occurred in the pyloric ceca and anterior intestine of all three species of trout. Immature *Eubothrium* sp. was found in the pyloric ceca, and *Proteocephalus* sp. occurred only in the rectum (Table 1). *Cucullanus* sp. (Cucullanidae) and *Rhabdochona* sp. (Rhabdochonidae) infected the middle portion of the brook trout intestine. Of the four acanthocephalan species found, immature *Neoechinorhynchus cristatus* and *N. saginatus* (Neoechinorhynchidae) occurred in the rectum of Fish Creek trout. The degree of infection was low for these eight parasitic species.

Gravid *Pomphorhynchus bulbocolli* (Pomphorhynchidae) occurred in the posterior intestine of Pine and Rogue River trout. In two brown trout from the Pine River, the proboscides and bulbs of several *P. bulbocolli* penetrated the intestinal wall and embedded in the swim bladder. When the gut was removed, the proboscides stayed and the worms remained attached to the swim bladder. The swim bladders of these fish were dark and the bladder walls appeared thicker in the areas where the worms were attached.

Acanthocephalus dirus (Echinorhynchidae) infected 64% of the brown and 96% of the rainbow trout examined from the Rogue River. This species was attached throughout the gut of its trout hosts between the pyloric ceca and the rectum. On one occasion, six *A. dirus* were attached in the stomach of a 165-mm-long brown trout. The maximum number of *A. dirus* in a single trout was 123.

Nondigested isopods, *Caecidotea intermedius,* from Rogue River trout were dissected and 2 of 60 isopods examined were infected with *A. dirus.* Subsequent examination of more *C. intermedius* from the Rogue River showed that 12 (4.7%) of 257 and 17 (12.9%) of 132 isopods examined in December 1981 and April 1982, respectively, were infected with *A. dirus.* The mean numbers of *A. dirus* in isopods in these periods were 1.3 ± 0.2 and 1.1 ± 0.1.

Seventy-six percent and 63% of the brook trout examined from Honey and Fish creeks, respectively, were infected with the gill louse, *Salmincola edwardsii* (Copepoda: Lernaeopodidae). Mean and range values on the infected 76 Honey Creek and 25 Fish Creek brook trout were 3.5 ± 0.41 (1–28) and 5.0 ± 1.56 (1–32), respectively. There was no significant difference in the prevalence or mean number of *S. edwardsii* on brook trout between localities or on female and male brook trout from each locality.

Gill lice were found on the gills, the inner surface of the opercula (usually on the lower half), the branchial rim, the pectoral and pelvic fins (usually on the base), and infrequently on the dorsal and adipose fins. The distribution of gill lice on brook trout in four arbitrary length classes from Honey and Fish creeks is shown in Table 2. In Honey Creek hosts ≤99 mm long, 70% of the gill lice found were on the opercula; the percentages of gill lice on 100–149-mm-long trout decreased on the opercula and increased on the gills and pectoral and pelvic fins. The percentage of gill lice on the gills of 150–199-mm-long fish increased to 23.6%, the percentage on the opercula decreased, and the percentages on the pectoral and pelvic fins remained similar. In fish over 200 mm long, 89% of the gill lice were found on the gills. Although the number of brook trout infected in each length class from Fish Creek was small, a similar infection pattern was also evident. The number of gill lice significantly increased with increase in host length ($r = 0.76$, $P > 0.01$, Honey Creek; $r = 0.67$, $P > 0.01$, Fish Creek).

The percentages of the total number of gill lice on Honey Creek trout were similar and largest on gills and opercula and lower on the pectoral and pelvic fins. On Fish Creek hosts, a significantly larger number of gill lice occurred on the gills when compared to the number found on all areas combined ($\chi^2 = 3.90$, $P > 0.05$). The mean numbers of gill lice on the four gills were not significantly different.

The muscles of 73 brown trout, 88 brook trout, and 13 rainbow trout were examined for parasites; all were negative.

Discussion

Many parasites encountered in this survey occurred only sporadically (or were immature) in each piscine population. This could not be due to the lack of food (intermediate hosts) because the guts of fish were full of amphipods, isopods, mayfly larvae, and other aquatic and terrestrial organisms. The richness of the helminth fauna of the digestive tract was comparable to that reported in other studies of trout parasites. Richardson (1936) found two species of intestinal helminths from 39 brook trout in two lakes of Quebec, Canada. Choquette (1948) examined 210 brook trout from 42 lakes and rivers from seven different drainage systems of Quebec, Canada,

Table 1. Parasites and their prevalence in trout collected from four Michigan localities.

Parasite	USNM No.	Host (locality)*	Prevalence
Trematoda			
Crepidostomum cooperi Hopkins, 1931	77620	*Salvelinus fontinalis* (FC)	3 (79–2)§
Crepidostomum cornutum (Osborn, 1903)	77621	*Salmo trutta* (PR)	15 (20–3)
		Salvelinus fontinalis (PR)	100 (1–1)
Cestoda			
Eubothrium sp.		‖ *Salvelinus fontinalis* (HC)	2 (140–3)
Proteocephalus sp.		‖ *Salmo trutta* (FC)	3 (120–3)
		‖ *Salvelinus fontinalis* (FC)	8 (79–6)
Nematoda			
Cucullanus sp.		‖ *Salvelinus fontinalis* (PR)	100 (1–1)
Rhabdochona sp.		‖ *Salvelinus fontinalis* (FC)	4 (79–3)
Acanthocephala			
†*Acanthocephalus dirus*	77622	*Salmo trutta* (RR)	64 (141–90)
(Van Cleave, 1931)	77623	*Salmo gairdneri* (RR)	96 (22–21)
Neoechinorhynchus cristatus Lynch, 1936	77624	‡‖ *Salmo trutta* (FC)	1 (120–1)
Neoechinorhynchus saginatus Van Cleave and Bangham, 1949	77625	‡‖ *Salvelinus fontinalis* (FC)	3 (79–2)
Pomphorhynchus bulbocolli (Linkins, 1919)	77626	*Salmo gairdneri* (PR)	100 (2–2)
		Salmo trutta (PR)	25 (20–5)
		Salmo gairdneri (RR)	5 (22–1)
Copepoda			
Salmincola edwardsii (Olsson, 1869)	77627	*Salvelinus fontinalis* (HC)	76 (124–94)
		Salvelinus fontinalis (FC)	63 (79–50)

* FC = Fish Creek, PR = Pine River, HC = Honey Creek, RR = Rogue River.
† New state record.
‡ New host record.
§ Percent of fish infected (number of fish examined − number infected).
‖ Immature parasites. No prescript in host (locality) column indicates adult parasites.

and found seven species of helminths in the digestive tract. Bangham (1951), working with 291 cutthroat trout (*Salmo clarki*) and 141 brook trout in the upper Snake River drainage and in Yellowstone Lake, Wyoming, found five species of helminths in the digestive tract. Heckman (1971) found three species of intestinal helminths in 263 cutthroat trout examined from Yellowstone Lake and its tributaries. Fox (1962) found four species in 69 rainbow trout collected from two Montana lakes. Becker (1967), in an extensive study of 1,405 salmonids from six Washington lakes, reported four species of intestinal helminths. Lockard et al. (1975) found three species of intestinal nematodes in 306 brown trout collected from 16 Montana streams. Leong and Holmes (1981) reported four species of intestinal helminths in 35 lake trout (*Salvelinus namaycush*) from Cold Lake, Alberta. Generally, in all these studies, except for Choquette (1948) and Fox (1962), one or two species is quite common in its abundance in the digestive tract and the other helminth species are not.

Of the parasitic species found in the present study, *A. dirus* and *S. edwardsii* deserve discussion. Although the mean number of *A. dirus* was not calculated, it infected 111 (68%) of the trout examined from the Rogue River. Michigan is a new state record for *A. dirus,* and it appears that this species is well established in the Rogue River because the isopod intermediate host, *C. intermedius,* is very common; gravid *A. dirus* were also found in white suckers, *Catostomus commersoni.* Some trout harboring more than 100 worms appeared emaciated and the head appeared large for the size of the fish. Bullock (1963) demonstrated that *A. jacksoni* caused complete destruction of the fish intestinal mucosa, malnutrition, and emaciation.

Salmincola edwardsii is widespread through-

Table 2. Distribution of *Salmincola edwardsii* on brook trout in four arbitrary length classes (mm).

Length class	No. of hosts	Distribution of *Salmincola edwardsii* recovered					
		Gills	Opercula	Branchial rim	Pectoral fins	Pelvic fins	Dorsal and adipose fins
Honey Creek hosts:							
<99	24	2 (4.5)*	31 (70.4)	3 (6.8)	5 (11.4)	1 (2.3)	2 (4.5)
100–149	27	10 (10.8)	38 (41.3)	1 (1.1)	19 (20.6)	23 (25.0)	1 (1.1)
150–199	16	13 (23.6)	17 (30.9)	3 (5.4)	12 (21.8)	9 (16.4)	1 (1.8)
200>	9	65 (89.0)	3 (4.1)	—	2 (2.7)	3 (4.1)	—
Total	76	90 (34.1)	89 (33.7)	7 (2.7)	38 (14.4)	36 (13.6)	4 (1.5)
Fish Creek hosts:							
<99	9	—	12 (92.3)	—	1 (7.7)	—	—
100–149	3	3 (18.8)	8 (50.0)	3 (18.8)	1 (6.2)	1 (6.2)	—
150–199	9	15 (48.4)	9 (29.0)	1 (3.2)	4 (12.9)	2 (6.5)	—
200>	4	55 (85.9)	6 (9.4)	—	—	3 (4.7)	—
Total	25	73 (58.8)	35 (28.2)	4 (3.2)	9 (7.3)	3 (2.4)	—

* Number of *S. edwardsii* recovered (percentage of total recovered).

out trout waters of North America (Hoffman, 1967). The numerical data presented in the present study are of females that were attached to brook trout by their bullae. The mean numbers of gill lice in the present study are much lower than those found by Allison and Latta (1969) in a lentic environment, but similar to those reported by Cope (1958) working with *Salmincola* sp. on cutthroat trout in lotic environments. The higher mean number of gill lice on Fish Creek brook trout than on Honey Creek hosts may be due to the fact that infected brook trout from Fish Creek were larger than those from Honey Creek. The higher number of gill lice on larger hosts may be due to a longer exposure period, more surface area to attach to, and/or a change in brook trout behavior as they increase in length. Fasten (1921) reported that larval *S. edwardsii* occur near the surface of the water throughout the day, but at night they sink down near the bottom of the stream. Larger brook trout frequently occur in slow-deep areas and they tend to be bottom-oriented during darkness, making them more accessible to gill lice infections. Fasten (1921), Savage (1935), Allison and Latta (1969), and Black (1982) also reported an increase in gill lice on larger brook trout.

The decrease in the number of lice on the fins of brook trout as they increase in length may also be caused by a change in the brook trout's behavior and/or habitat. Larger trout have extensive contact with bottom materials and older fish during nest-building and other spawning activities, which may cause gill lice to be removed (Cope, 1958). If this occurs, it is suggested that female brook trout should have a significantly lower mean number of *S. edwardsii* than males because they "undergo more contact" during spawning activities than do males; however, a significant difference was not found. This increase in the number of *S. edwardsii* on the gills may be due to an increase in the thickness (toughness) of the epidermis in older brook trout, thus decreasing the number of gill lice that attach to and/or remain on the opercula and fins. Therefore, the gill filaments of larger trout provide the most suitable substrate for attachment. Similarly, Kabata and Cousens (1977), working with laboratory infections, found that the fins, fin bases, and skin of young sockeye salmon (*Oncorhynchus nerka*) were most frequently infected with *S. californiensis,* and that the gills were one of the major attachment sites of *S. californiensis* on adult sockeye salmon in nature.

In the present study, the distal portions of several gill filaments infected with gill lice showed extreme hyperplasia and clubbing. The left or right operculum of five infected trout was folded underneath itself, exposing a portion of the gills. In these exposed portions, filaments were not present on the arches resulting in "crypting" as reported by Friend (1941). Gill lice on the inner surface of the opercula caused hyperplasia of adjacent gill filaments; also, gills of some trout exhibited hyperplasia and clubbing even though the gill louse was not found in these areas, which

may have been the result of a previous louse infection. Kabata and Cousens (1977), who reported several types of mechanical injury inflicted by *S. californiensis* on sockeye salmon, stated (p. 202) ". . . response is such as to become sometimes deleterious to the fish (e.g. in gill injuries)." Although Allison and Latta (1969) found no relationship between *S. edwardsii* and mortality of brook trout in lentic environments of Michigan, Leitritz and Lewis (1976), in referring to the gill louse infecting rainbow trout and steelhead, stated (pp. 149–150) that "heavy infestation debilitates fish, provides a route for secondary infection, and when on the gills, makes respiration more difficult." Although *S. edwardsii* appears to debilitate its host in many ways in nature, it may not play a direct role in their mortality.

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The Role of *Gyraulus parvus* as an Intermediate Host for Avian Schistosomes[1]

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ABSTRACT: *Gyraulus parvus* was found to be the natural intermediate host for three species of avian schistosomes in southwestern Michigan. Of 5,635 snails examined for patent infections during the summers of 1979–1981, 143 (2.5%) shed cercariae. Snails 4–7 mm in diameter were more likely to be infected (17%) than smaller ones, 1–3 mm in diameter (0.8%). The highest overall infection rates occurred in July of 1980 and May of 1981. Patent infections of a fourth species were established in laboratory-reared *G. parvus*. This study shows that *G. parvus* does play a significant role as an intermediate host for avian schistosomes. Furthermore, at least three of the four species can cause swimmer's itch.

Schistosome cercarial dermatitis, commonly known as swimmer's itch, is currently a problem of increasing concern in Michigan (Wall, 1976). It is caused by cercariae of avian and rodent schistosomes that accidentally penetrate human skin. Three families of freshwater gastropods are known to serve as intermediate hosts for avian schistosomes: Planorbidae, Lymnaeidae, and Physidae. Members of the latter two families are more commonly infected in southern Michigan.

Gyraulus parvus is a small planorbid snail that based on the literature, appears quite insignificant as an intermediate host for avian schistosomes. Brackett (1940) found it to host two species in Wisconsin but did not determine their infection rate or seasonal prevalence of infections. Relationships between seasonal changes in snail size and infection rate have not been documented for *G. parvus* as they have for *Physa integra*, another intermediate host of avian schistosomes (Kulesa et al., 1982). This paper reports a comparable study of a large natural population of *G. parvus* at the Kellogg Bird Sanctuary of Michigan State University.

Materials and Methods

From June through August of 1979 and 1980 and from May through September of 1981, snails were collected at the Kellogg Bird Sanctuary of Michigan State University, in northeastern Kalamazoo County, Michigan. Wintergreen Lake and several adjacent ponds that support large and diverse populations of waterfowl were chosen as study areas. The major collection site was a small, cement-walled channel connecting Wintergreen Lake to the middle ponds. Snails of all sizes were randomly collected by hand from the wall, sticks, and filamentous algae.

After measuring the largest diameter of each snail, 10 large to 25 small ones were placed in 3-inch finger bowls of lake water. Each bowl was checked daily for cercariae for at least 3 days. If cercariae were present, snails from that bowl were isolated individually in small vials to identify the infected ones. Those not shedding cercariae were returned to the collecting site. Generally, two collections were made each week.

Cercariae were first examined alive and then fixed in 3–5% hot formalin and stained with neutral red and methylene blue. The different cercarial species were readily distinguished by differences in size, morphology, and behavior. In addition, all species were established in laboratory-reared snails exposed to miracidia from naturally infected birds.

Results

Of 5,635 *Gyraulus parvus* examined during the three summers, 143 (2.54%) contained patent infections of avian schistosomes. The overall infection rates for 1979, 1980, and 1981 were 1.56%, 6.52%, and 1.50%, respectively. The highest rate (33%) was found in 5-mm snails. Below 5 mm, it decreased in relation to snail size (Table 1).

Changes in infection rates during each of the three summers were varied. In 1979 and 1981, the highest infection rate was early in the season. In 1980, there was a peak in infection rate in mid-July (see Fig. 1). Overall, the highest infection rates (averaged over 2-wk periods) were in July of 1980 (13.6%) and May of 1981 (13.2%) with sample sizes of 243 and 38, respectively.

A comparison of mean snail size for collections made from May through September 1981 showed that the largest snails were found in May. Snail sizes in June and July were smaller but increased

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Table 1. Percent of *Gyraulus parvus* infected with schistosomes, shown by snail size and by year of collection.

Size (mm)	No. examined (no. infected)				% Infected
	1979	1980	1981	Total	
7	0 (0)	0 (0)	1 (0)	1 (0)	0.0
6	3 (0)	0 (0)	10 (2)	13 (2)	15.4
5	26 (4)	23 (6)	39 (19)	88 (29)	33.0
4	120 (11)	297 (48)	99 (15)	516 (74)	14.3
3	489 (7)	610 (20)	1,235 (9)	2,334 (36)	1.5
2	667 (0)	220 (1)	1,508 (1)	2,395 (2)	0.1
1	104 (0)	4 (0)	180 (0)	288 (0)	0.0
Total	1,409 (22)	1,154 (75)	3,072 (46)	5,635 (143)	
% Infected	1.56	6.52	1.50	2.54	

slightly in August and September. In 1979 and 1980, collections were only made from June through August, therefore, such a pattern was not apparent.

Cercariae obtained from naturally infected *G. parvus* represent three species of avian schistosomes. Of these, two possessed long tail-stems; one longer than the other. The third species was characterized by a short tail. Finally, a fourth type was recovered from laboratory-reared snails that were exposed to miracidia obtained from naturally infected ducks. It is interesting to note that no natural infections of the latter species were found.

Cercariae of three species of avian schistosomes isolated from *G. parvus* during this study were capable of eliciting a skin reaction in humans known as swimmer's itch. One of the authors (DLD) readily obtained lesions in the laboratory when exposed to cercariae of all but one species.

Discussion

The overall patent infection rate of 2.54% observed in this study is considerably higher than in a similar study on another intermediate host for avian schistosomes. Kulesa et al. (1982) reported only 0.19% of 26,775 *Physa integra* to be infected with bird schistosomes representing two species. The higher rate reported here for *Gyraulus parvus* may in part be due to the large number of birds that frequent the study area, particularly during spring and fall migrations. Brackett (1940) noted that larger *G. parvus* were more likely to be infected with schistosomes, but he did not monitor infection rates. The results of the present study support his observation.

Seasonal differences in snail size reflect the reproductive cycle of *G. parvus*. The average snail size is highest in May, before die-off of mature adults leaves the population dominated by young, hatching in late spring. Marked increases in size were not observed during the remainder of the summer. The life span of *G. parvus* in southwestern Michigan thus seems to be just over a year. This life cycle is very similar to that reported for *Lymnaea catascopium* (=*Stagnicola emarginata angulata*) and *Physa parkeri,* two other snails that serve as intermediate hosts for avian schistosomes in Michigan (Cort et al., 1940 and 1941, respectively).

Although the prevalence of infection differed considerably during each of the three summers, it is difficult to find a plausible explanation for these variations. Factors that may be important include: changing weather patterns and water levels and therefore fluctuations in the density of one or both host species.

A high percentage of the snails collected in May were found to be infected with schistosomes. It is not known whether these snails overwinter with the infection or become infected immediately after the ice melts (usually in March). However, it seems unlikely that infections found in early May could have resulted from exposure after the ice melted because cold temperatures would delay development of the sporocysts. It thus seems more likely that snails overwinter with infections.

Brackett (1940) described two species of avian schistosomes from *G. parvus* collected in Wisconsin: namely, *Cercaria elongata* and *C. gyrauli.* Based on morphological and behavioral features, the four species of avian schistosomes recovered from the same species of planorbid snail in Michigan are not similar to those de-

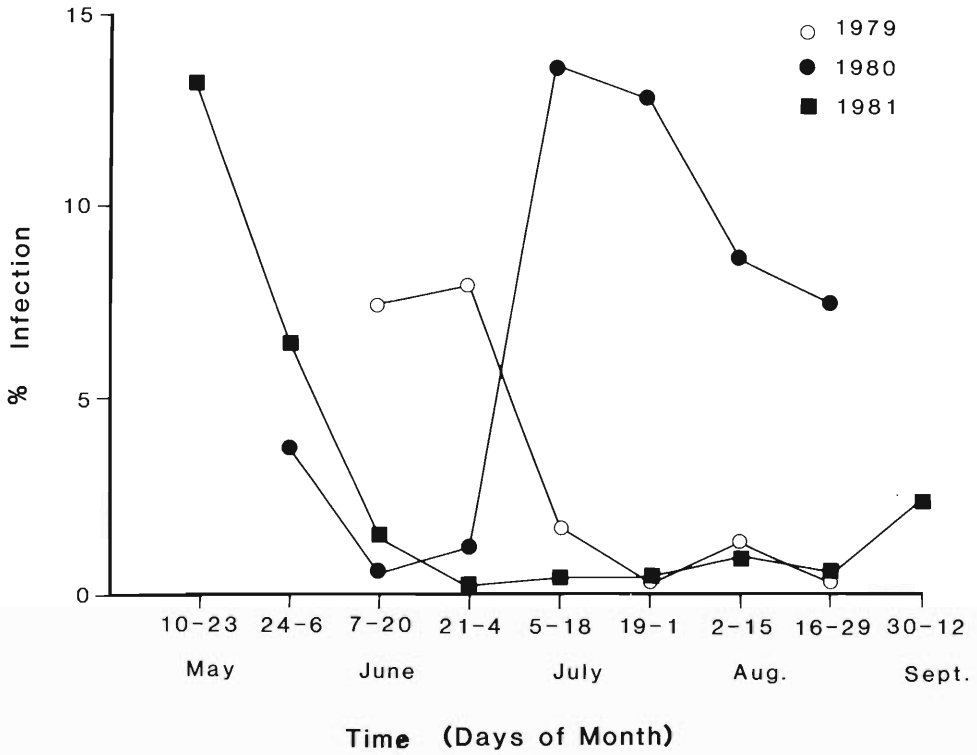

Figure 1. Changes in percent infection of *Gyraulus parvus* during each of the three summers (1979-1981).

scribed by him. Therefore, at least six species of avian schistosomes are now known to cycle through *G. parvus*.

Data from this study indicate that *G. parvus* plays a more significant role as intermediate host for avian schistosomes than has been previously recognized. Because this snail is common and widely distributed in many aquatic habitats and because it harbors dermatitis-producing cercariae, it must be more closely considered in the future as the causative agent for outbreaks of swimmer's itch.

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Aspidogastrid Trematodes from Freshwater Mussels in Missouri with Notes on the Life Cycle of *Cotylaspis insignis*

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ABSTRACT: From 590 freshwater mussels of 32 species and 2 subspecies examined between September 1975 and August 1979 from Missouri rivers and lakes, *Aspidogaster conchicola* was found in 213 (23 species), *Cotylaspis insignis* in 50 (16 species and 1 subspecies), and *Cotylogaster occidentalis* in 4 individuals (3 species). New hosts reported for *A. conchicola* are *Ligumia subrostrata, Pleurobema sintoxia,* and *Venustachoncha ellipsiformis ellipsiformis. Cotylaspis insignis* is reported for the first time from *V. ellipsiformis ellipsiformis.* This is the first report of *Cotylogaster occidentalis* from Missouri, where it is found for the first time in *Lampsilis ventricosa, Quadrula pustulosa,* and *Actinonaias ligamentina ligamentina. Cotylaspis insignis* eggs maintained in stream water at 20–21°C produced bioculate cotylocidia after 21–23 days. Observations on intensity and frequency of infections by *C. insignis* and *A. conchicola* suggest that development and transmission of the former are more successful in lentic than lotic environments. Since the review by Hendrix (1968), published reports have additionally placed *A. conchicola* in Washington, Massachusetts, Ohio, Louisiana, Wisconsin, and Oklahoma, and *C. insignis* in Ohio, Louisiana, North Carolina, and Minnesota. *Cotylogaster occidentalis* has previously been reported from Iowa, Ohio, Kentucky, Michigan, Minnesota, and Ontario.

Aspidogastrids most commonly parasitizing North American freshwater molluscs are *Aspidogaster conchicola* von Baer, 1827, *Cotylaspis insignis* Leidy, 1858, and *Cotylogaster occidentalis* Nickerson, 1902. Hendrix (1968) reviewed distribution of *A. conchicola* and *C. insignis,* and more recent reports place the former in Washington (Pauley and Becker, 1968), Massachusetts (Michelson, 1970), Ohio (Stromberg, 1970), Louisiana (Vidrine, 1973), Wisconsin (Williams, 1978) and Oklahoma (Nelson et al., 1975), and the latter in Ohio (Stromberg, 1970), Louisiana (Vidrine, 1973), North Carolina (McDaniel and McDaniel, 1972), and Minnesota (LoVerde and Fredericksen, 1978). *Cotylogaster occidentalis* has been found in *Lampsilis siliquoidea* from Iowa (Kelley, 1926), *Goniobasis* sp. from Lake Erie (Dickerman, 1948) and Kentucky (Whittaker and Kozel, 1975), *Anodonta grandis* and *Ligumia nasuta* from Michigan (Wootton, 1966), *L. siliquoidea* from Minnesota (Fredericksen, 1972), and *Elliptio complanata* from Ontario (Ip et al., 1982).

In a brief report on parasites of Missouri unionids, Utterback (1916) listed *A. conchicola* from *Leptodea* (*Lasmonos*) *fragilis* and *C. insignis* from *Anodonta grandis.* The present study expands knowledge of aspidogastrid distribution in Missouri and relates it to transmission ecology of *A. conchicola* and *C. insignis.*

Materials and Methods

Mussels were collected by SCUBA or dip net from the Marais des Cygnes River at Rich Hill west of Route 71, the Osage River at Osceola (east of Route 13) and Iconium (at Route CC), the Sac River at Blackjack north of Route W, the Niangua River between Bennett Springs and Eldridge, the Bates County drainage ditch 5.2 km northeast of Foster, the North Fork of the White River between Dora and Sycamore, White Rock Lake at Pleasant Valley, the Lake of the Ozarks north of Route 54 (Camdenton), and Swope Park lagoon in Kansas City, Missouri. Stream velocities were measured with a Teledyne Gurley Current Meter. Unionid pericardial cavity, nephridia, intestine, and visceral mass exterior were examined, and a fine jet of water at moderate pressure facilitated removal of *A. conchicola* from deep pericardial and nephridial tissue folds. Mussel shells were deposited in the Ohio State University Museum of Zoology collection. *Aspidogaster conchicola* were placed in 10% formalin, and *C. occidentalis* were heat-fixed under gentle coverslip pressure, stained with acetocarmine, and mounted in Gum Damar. Gravid *C. insignis* maintained in stender dishes containing filtered stream water spontaneously extruded eggs that were isolated and similarly maintained at 20–21°C to observe development. Voucher specimens of the trematodes have been deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705 (Nos. 77714–77716).

Results

Examination of 590 mussels representing 32 species and 2 subspecies from nine localities in Missouri showed aspidogastrids widely distributed, but not ubiquitous. *Aspidogaster conchicola* occurred in 213 individuals of 23 species, and *Cotylaspis insignis* infected 50 individuals of 16 species and 1 subspecies (Table 1). Current velocity and comparative infection prevalence and intensity relationships are represented in Table 2. *Cotylogaster occidentalis* (four mature and

Table 1. Presence of *Aspidogaster conchicola* and *Cotylaspis insignis* in Missouri unionids.

Host species	Locality	*A. conchicola* No. examined†	*A. conchicola* No. infected‡	*A. conchicola* Intensity Mean§	*A. conchicola* Intensity Range‖	*C. insignis* No. infected	*C. insignis* Intensity Mean	*C. insignis* Intensity Range
Actinonaias ligamentina carinata (Barnes)	OR	21	1	1.0	—	1	1.0	—
	SR	4	0	—	—	0	—	—
Actinonaias ligamentina ligamentina (Lamarck)	OR	2	0	—	—	0	—	—
Alasmidonta marginata (Say)	OR	1	0	—	—	0	—	—
	SR	1	0	—	—	0	—	—
Amblema plicata (Say)	OR	103	53	2.5	1–19	1	1.0	—
Anodonta grandis (Say)	BCDD	2	2	6.0	2–10	1	18.0	—
	SPL	4	3	3.0	1–4	4	13.8	1–27
	WRL	14	0	—	—	0	—	—
Anodonta suborbiculata (Say)	LO	2	0	—	—	0	—	—
Arcidens confragosus (Say)	OR	2	0	—	—	0	—	—
Cyclonaias tuberculata (Rafinesque)	OR	10	2	3.5	3–4	1	1.0	—
	SR	3	3	2.3	2–3	0	—	—
Elliptio dilatata (Rafinesque)	OR	37	5	1.2	1–2	0	—	—
Fusconia flava (Rafinesque)	OR	59	3	4.0	1–8	0	—	—
	SR	6	0	—	—	1	1.0	—
Lampsilis reeviana brevicula (Call)	NFWR	3	0	—	—	2	2.5	1–4
	NR	8	0	—	—	0	—	—
Lampsilis teres teres (Rafinesque)	SR	1	1	5.0	—	1	1.0	—
Lampsilis ventricosa (Barnes)	OR	9	2	2.5	2–3	3	1.0	1–1
	SR	8	0	—	—	5	2.8	1–6
	NFWR	3	0	—	—	2	1.0	1–1
Lasmigona complanata (Barnes)	OR	2	0	—	—	0	—	—
	BCDD	2	1	1.0	—	0	—	—
Leptodea fragilis (Rafinesque)	MCR	6	2	1.5	1–2	0	—	—
Ligumia recta (Lamarck)	OR	4	0	—	—	1	2.0	—
Ligumia subrostrata (Say)	BCDD	3	2*	2.0	1–3	2	1.0	1–1
	SPL	8	8*	4.3	1–8	4	2.0	1–3
	WRL	8	0	—	—	0	—	—
Magnoniais nervosa (Utterback)	OR	37	27	4.0	1–13	0	—	—
Obliquaria reflexa (Rafinesque)	OR	31	5	2.0	1–6	0	—	—
	SR	5	0	—	—	0	—	—
Plagiola lineolata (Rafinesque)	OR	19	0	—	—	0	—	—
	SR	1	0	—	—	0	—	—
Pleurobema sintoxia (Rafinesque)	OR	4	2*	2.5	1–4	0	—	—
	SR	1	1*	7.0	—	0	—	—
Potamilus alata (Say)	OR	33	24	6.8	1–32	8	2.0	1–5
	SR	3	3	4.0	1–8	2	3.5	2–5
Potamilus ohiensis (Rafinesque)	OR	1	1	143.0	—	0	—	—
	SR	1	1	1.0	—	0	—	—
Ptychobranchus fasciolaris (Rafinesque)	NFWR	1	0	—	—	1	6.0	—
Quadrula metanevra (Rafinesque)	OR	16	6	1.7	1–3	0	—	—
	SR	1	0	—	—	0	—	—
Quadrula pustulosa (Lea)	OR	28	16	1.9	1–4	2	1.0	1–1
	SR	9	2	2.0	1–3	0	—	—
Quadrula quadrula (Rafinesque)	OR	5	5	8.2	1–17	0	—	—
	SR	6	5	9.0	3–18	0	—	—
Strophitis undulatus undulatus (Say)	OR	4	0	—	—	0	—	—
	SR	1	1	1.0	—	0	—	—
Tritogonia verrucosa (Rafinesque)	OR	31	24	5.9	1–21	4	1.0	1–1
Truncilla donaciformis (Lea)	OR	1	0	—	—	0	—	—
	MCR	3	0	—	—	0	—	—

Table 1. Continued.

Host species	Locality	*A. conchicola*				*C. insignis*		
		No. examined†	No. infected‡	Intensity		No. infected	Intensity	
				Mean§	Range‖		Mean	Range
Truncilla truncata (Rafinesque)	OR	4	0	—	—	0	—	—
	SR	1	0	—	—	0	—	—
Uniomerus tetralasmus (Say)	SPL	3	1	1.0	—	2	1.0	1–1
	SR	1	0	—	—	0	—	—
Venustaconcha ellipsiformis ellipsiformis (Conrad)	SR	2	1*	1.0	—	1*	2.0	—
Venustaconcha ellipsiformis pleasii (Marsh)	NFWR	1	0	—	—	1	3.0	—

* New host record.

† Total number of each host species examined.

‡ Total number of hosts of each species found to be infected.

§ Average worm burden per infected individual examined (mean intensity).

‖ Minimum and maximum infection intensity.

BCDD = Bates County drainage ditch; LO = Lake of the Ozarks; MCR = Marais des Cygnes River; NFWR = North Fork of the White River; NR = Niangua River; OR = Osage River; SPL = Swope Park lagoon; SR = Sac River; WRL = White Rock Lake.

two immature) infected the intestines of 2 of 17 *Lampsilis ventricosa* (Sac and Osage rivers), 1 out of 2 *Actinonaias ligamentina ligamentina* (Osage River), and 1 of 28 *Quadrula pustulosa* (Osage River); all are new hosts for this worm that was previously unreported from Missouri.

Four of six *C. insignis* eggs completed development to produce bioculate cotylocidia (0.129 × 0.043 mm) that began active movement within eggs after 21–23 days. Mild coverslip pressure caused opercular detachment from the egg's aboral end. Cotylocidia moved by body elongation and contraction or swam by beating posteriorly placed ciliary tufts.

Discussion

Collection site ecology

Field areas studied displayed various ecological characteristics and histories important to distribution of aspidogastrids and their hosts. Harman (1972) demonstrated that greatest aquatic molluscan diversity is attained in habitats displaying maximum benthic substratum diversity, and according to Fischthal (1953) this relationship can be extended to parasites of aquatic organisms as well. In the present study the Osage and Sac rivers were most favorable for unionid and also aspidogastrid diversity, with *A. conchicola, C. insignis,* and *C. occidentalis* represented from various of the 27 unionid species encountered. Large size and moderate velocities of these rivers provided adequately large surface areas of various benthic substratum types (mud, sand, fine and coarse gravel) to support diverse and dense unionid populations.

Impacts of impoundment on a fluvial mussel population and its parasites was demonstrated at the Lake of the Ozarks that was formed by damming the Osage River downstream from the Iconium and Osceola localities presently examined. Habitat diversity reduction by siltation and particularly water level manipulation by floodgate adjustment resulted in severe unionid population decline. Local residents reported a mussel die off in the winter of 1976 after a water level drop of 4 m that exposed most of the lake's prime mussel habitat to freezing and desiccation. In three such areas examined by the author only two living (uninfected) *Anodonta suborbiculata* were found, both with growth lines indicating they parted from their fish hosts after water level was restored. Numerous large and empty shells of species known as aspidogastrid hosts (*Quadrula quadrula, Leptodea fragilis, Potamilus alata,* and *A. suborbiculata*) were found in bottom mud, attesting to a previously more favorable situation for aspidogastrid host populations.

Aspidogastrids were not observed in White Rock Lake unionids that were probably introduced into this recently manmade lake as uninfected juveniles parasitizing stocked gamefish. The isolated nature of this lake makes natural establishment of aspidogastrids in it improbable.

Table 2. Comparison of water velocity to mean intensity and prevalence of *Aspidogaster conchicola* and *Cotylaspis insignis* infections in localities endemic to both worms.

Locality	Water velocity (mps)	Mean intensity		Prevalence	
		Aspidogaster	*Cotylaspis*	*Aspidogaster*	*Cotylaspis*
Swope Park lagoon	0.00	3.67	6.50	0.80	0.66
Bates County drainage ditch	0.01	3.40	6.66	0.71	0.42
Sac River	0.30	4.50	2.42	0.31	0.17
Osage River	0.34	4.71	1.45	0.37	0.04

Swope Pake lagoon was also manmade, but differed from White Rock Lake by connecting with a natural stream that probably provided infected mussels or transmission stages of *A. conchicola* and *C. insignis*. Poor water circulation, small size, and substratum homogeneity (mud and silty sand) limited mussel diversity and population size in the Bates County drainage ditch. The Marais des Cygnes and Osage rivers that this ditch connects undoubtedly were the source of mussels and aspidogastrids to this area.

The North Fork of the White and Niangua rivers provided similar habitats of cold, clear, fast-flowing water, and few mussels were collected in these streams in spite of several kilometers of underwater visual search. Niangua River mussels were probably too widely spaced for effective aspidogastrid transmission to occur, whereas the unionids found in the North Fork of the White River were located in a quiet, 4 × 6-m^2 pocket protected from the stream's channel. The origin of *C. insignis* (in six out of eight mussels) in such an isolated population is difficult to explain without detailed evaluation of upstream factors but reciprocal infection probably occurred among individuals living in this small area.

Transmission ecology of *C. insignis* and *A. conchicola*

Unionids examined in the present study show *C. insignis* to have a lower prevalence than *A. conchicola,* as was also noted in Illinois, Iowa, and Pennsylvania (Kelley, 1899), Tennessee (Hendrix, 1968), Ohio (Stromberg, 1970), and Oklahoma (Nelson et al., 1975). These observed differences in infection prevalence are probably strongly influenced by differences in transmission ecology between the two trematode species. These differences will now be examined.

The life cycle of *A. conchicola* has been determined as direct, with transmission occurring by unhatched eggs (Huehner and Etges, 1972, 1977; Bakker and Davids, 1973), whereas that of *C. insignis* has received confusing reports. Fredericksen (1978) reported the *C. insignis* cotylocidium as nonciliated, presumably having misinterpreted Osborn's (1903) description of a "very young individual" as being that of a larva. Osborn (1903) remarked that the life cycle of *C. insignis* may involve an intermediate host because his attempts to induce development of eggs in fresh water were unsuccessful. Present results show that *C. insignis* eggs develop spontaneously in fresh water to produce a free-swimming, ciliated larva after about 3 wk at 20°C. Transmission is probably achieved by larval entry into the host's incurrent siphon. The required developmental period of *C. insignis* eggs allows them to accumulate on the benthos near the original host population if water currents are absent or weak, thereby enhancing the probability of successful transmission. Conversely, swift currents would be expected to transport *C. insignis* eggs and larvae over a wide area, reducing their local concentration, survival, and infection prevalence and frequency. It is therefore not surprising that the present study finds *C. insignis* infections most prevalent and intense in quiet water and decreasing as host habitat water velocity increases (Table 2). The affinity of *C. insignis* for species of *Anodonta* noted by Stromberg (1970) and others may therefore result largely from the quiet water habitat in which these hosts are found.

In contrast to *C. insignis,* the transmission stage of *A. conchicola* is immediately infective, can survive for about 2 wk in water (Huehner and Etges, 1977), is protected from physical damage by an egg shell, and moves from host to host only by outside influences such as water current and host movement. *Aspidogaster conchicola* infection was two to three times as intense as that of

C. insignis in habitats of moderate current flow as was similarly reported from Oklahoma unionids by Nelson et al. (1975). The high prevalence of *A. conchicola* in lentic water observed in the present study (Table 2) probably resulted from host crowding or movement. High current speed, however, appears to be detrimental to *A. conchicola* transmission. Williams (1978) found no *A. conchicola* in potentially host unionids in a fast-flowing portion (1.25 mps) of the St. Croix River, whereas greatest prevalence (56%) occurred in a "lenticlike" area. More definitive understanding of *A. conchicola* and *C. insignis* transmission ecology is complicated by probable differences in fecundity between these worms as well as by lack of continuity in sampling techniques and host habitat description. Further laboratory and field investigations must be conducted before greater understanding of this system can be attained.

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The Life History of *Lutztrema monenteron* (Price and McIntosh, 1935) Travassos, 1941 (Trematoda: Dicrocoeliidae)

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ABSTRACT: *Lutztrema monenteron* was recovered from the gall bladder of the following passerine birds: *Turdus migratorius, Toxostoma rufum, Mimus polyglottos, Catharus guttata* (new host record), and *Sialia sialis* from Augusta, Georgia. The land mollusks, *Anguispira alternata, Deroceras reticulatum, Lehmannia poirieri,* and *Ventridens intertexus* served as experimental first intermediate hosts. Slimeballs containing longicercous xiphidiocercariae were released 62–93 days after mollusks ingested eggs of *L. monenteron.* The milliped *Oxidus gracilis* served as a suitable experimental secondary host; this is the first report of a dicrocoeliid using this group of arthropods.

Lutztrema monenteron (Price and McIntosh, 1935) Travassos, 1941 is a common dicrocoeliid trematode in the gall bladder and bile ducts of many passerine birds of North America and Europe. Life history studies have shown that 10 species of Stylommatophora can serve as experimental molluscan hosts: *Bulimulus alternatus mariae* (Albers), *Polygyra texasiana* (Moricana), *Practicollela berlandieriana* (Moricana) and *Deroceras reticulatum* (Muller) by Denton (1941); *Vallonia pulchella* (Muller), *Anguispira alternata* (Say), *Deroceras laeve* (Muller), *Retinella indentata* (Say), and *Triodopsis multilineata* (Say) by Villella (1961); and *Allogona ptychophora* (Brown) by Carney (1966).

This paper reports the first life history to be demonstrated in the genus *Lutztrema,* and the first record of a milliped serving as the second intermediate host of a dicrocoeliid trematode.

Materials and Methods

Specimens of *Lutztrema monenteron* were obtained mainly from the American robin, *Turdus migratorius* (L.), in the Augusta, Georgia area. Adult flukes were placed in distilled water and stored at 10°C to provide eggs for infection experiments. Others were fixed in FAA or Bouin's fluid and stained with Harris' hematoxylin or borax carmine for whole mounts.

Land mollusks from the Augusta, Georgia area were used to establish laboratory colonies that were maintained in terraria at room temperature (20–24°C). After fasting for 2 days laboratory-reared mollusks were exposed to infection by feeding them eggs of *L. monenteron* teased from the anterior uterus of adults and mixed with oatmeal or placed on lettuce. These mollusks were kept at room temperature on moistened paper towels in glass culture dishes and fed oatmeal and lettuce until they shed slimeballs containing cercariae or until they were crushed to obtain developmental stages. Fully developed cercariae were obtained by placing a slimeball in distilled water until they were released. Developmental stages were studied as living specimens stained with neutral red and nile blue, or as stained whole mounts.

For use as second intermediate hosts, millipeds *Oxidus gracilis* (Koch) were maintained in laboratory colonies kept in terraria with autoclaved soil and fed oatmeal. Development of metacercariae in millipeds was studied as before for intramolluscan stages of the parasite. To test their infectivity, metacercarial cysts of known age were dissected from millipeds in 0.4% saline, placed in gelatin capsules and force-fed to 5–9-wk-old robins. Periodically, exposed birds were autopsied to determine whether infection had occurred and to follow development of the metacercariae to adult flukes.

Measurements in micrometers are given as means, followed or not by ranges in parentheses. Drawings were made with a camera lucida and supplemented with free-hand details from living and preserved materials. Representative specimens of life cycle stages (daughter sporocyst, cercariae, metacercariae, juvenile, and adult) of *L. monenteron* have been deposited at the U.S. National Parasite Collection, Beltsville, Maryland (USNM Helm. Coll. Nos. 77670–77676, respectively).

Observations

Adult (Fig. 1)

The adult of *Lutztrema monenteron* is well known from the descriptions in the studies of Price and McIntosh (1935), Mettrick (1958), Binder (1971), and Rietschel (1971). Therefore, that stage is not redescribed here. It was found in the gall bladders of 35 of 52 robins examined from the Augusta, Georgia area during their migration period (January–March). *Lutztrema mo-*

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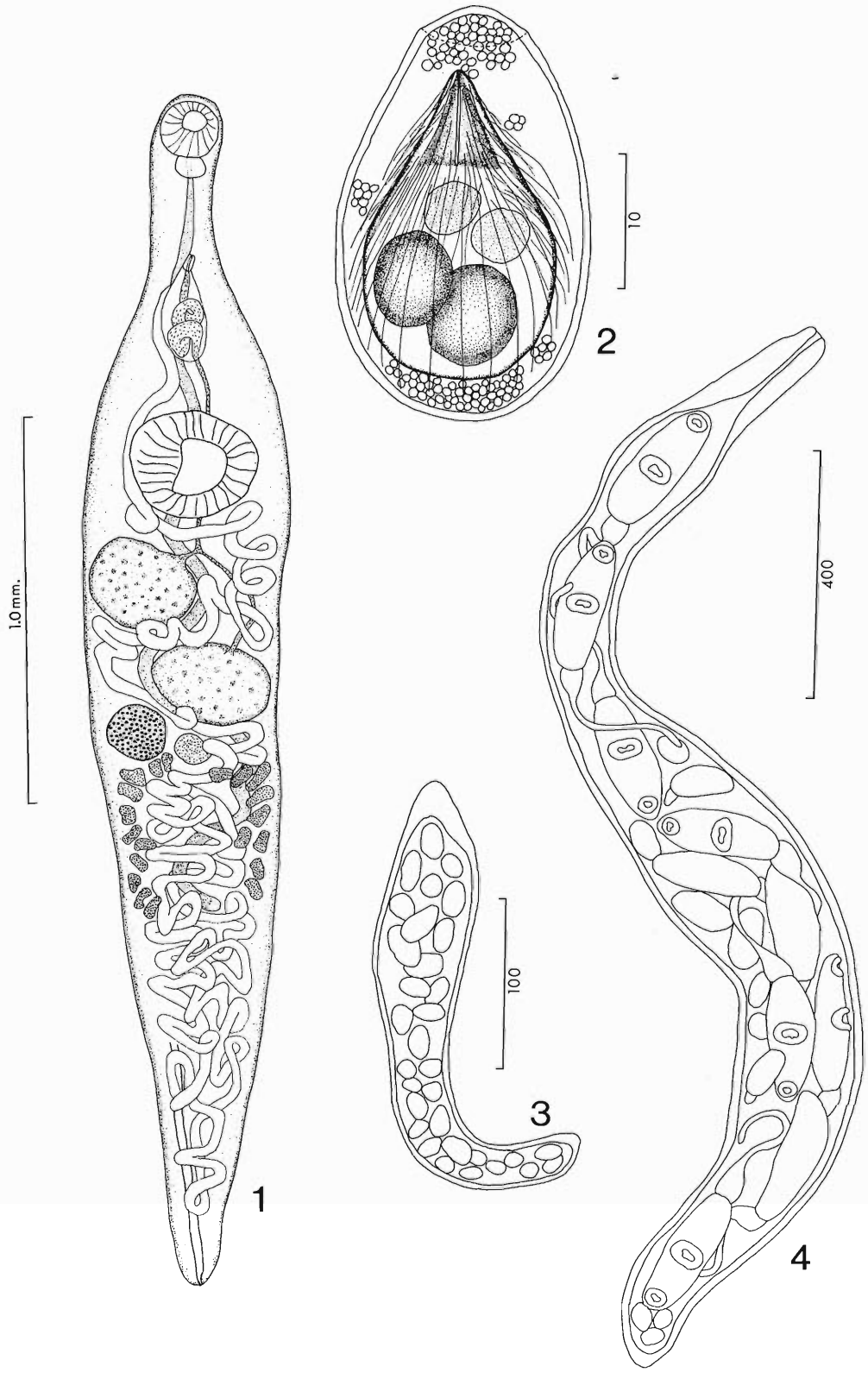

nenteron was also recovered from 1 of 19 brown thrashers, *Toxostoma rufum* (L.); 1 of 3 mockingbirds, *Mimus polyglottos* (L.); 1 of 2 hermit thrushes, *Catharus guttata* (Pallas), new host record; and the one eastern bluebird, *Sialia sialis* (L.), that was examined.

Eggs and miracidium (Fig. 2)

Eggs from the distal uterus 32 (30–34) by 21 (20–22), operculate, yellowish to dark brown, fully embryonated, with stylet end of miracidium toward operculum. Fecal examination after 24–48 hr showed that hatching occurred in all nine species of terrestrial mollusks fed eggs. Larval development ensued in the slugs *Deroceras reticulatum* (Muller) and *Lehmannia poirieri* (Mabille) and the snails *Anguispira alternata* (Say) and *Ventridens intertextus* (Binney). *Anguispira alternata* proved to be most suitable for laboratory maintenance. Free miracidium in the gut contents active, elongated oval to pyriform, 25 by 14; anterior half ciliated. Apical gland occupying narrow anterior body region, surrounding stylet. Two large germinal cells posterior to apical gland, staining with acetic-orcein (La Cour, 1941). Posterior to them, two large refractile vesicles 6–8 in diameter. Penetration of host gut wall probably near digestive gland where developing parasite occurs within 24 hr after egg ingestion.

Sporocyst (Figs. 3, 4)

By 4 wk, the infection has become a complex lobulated mass of parasite and host tissue, between lobes of the digestive gland. Daughter sporocysts removed from the mass, sacculate, 148 (142–175) by 82 (73–93), containing germinal balls. At 6 wk, daughter sporocyst (Fig. 3) 273 (248–298) by 79 (68–93) with pointed anterior end; containing elongating cercarial embryos. At 7th through 9 wk, migration of daughter sporocysts between the lobes of digestive gland begins. At 9 wk, sporocysts 708 (557–1,190) by 96 (47–155), containing 3–5 fully developed cercariae and several embryos of various sizes of development. At 14 wk, sporocyst (Fig. 4) 1,749 (1,490–2,140) by 171 (149–204) containing 8–10 fully developed cercariae besides embryos.

Cercariae and slimeball (Figs. 5–8)

Cercaria semitransparent; body elliptical flattened dorsoventrally, 266 (218–326) by 79 (71–87); tail long, sinuous, bulbous anteriorly, 385 (299–476) by 43 (37–46) in maximum width. Tegument aspinose, with presumed sensory papillae on ventral, lateral, and dorsal surfaces. Mouth and cup of the acetabular opening with surrounding fringe of cilialike projections. Oral sucker subterminal, 46 by 43. Prepharynx absent, pharynx 9.3–15.5 in diameter. Esophagus short; intestine a single cecum visible only anterior to ventral sucker. Stylet 17–23 long, 5.5–6.0 wide, 4.5–5.0 high dorsoventrally; with blunt dorsolateral wings and ventral keel; ending in a sharp anterior point (Fig. 7). Ventral sucker slightly postequatorial 47 by 48; with thin internal anterior–posterior ridge. Two groups of unicellular glands with ducts opening near stylet; a preacetabular group composed of two masses each with 5 small penetration glands; a postacetabular group composed of 12 large glands filling space in hindbody not occupied by excretory bladder (Fig. 8), narrowing anterior to ventral sucker to become two bundles of 6 ducts each running anteriorly in lateral region of forebody. Postacetabular glands are much easier seen and differentiated in cercariae removed from daughter sporocyst or sectioned daughter sporocysts (Fig. 8) than in cercariae removed from slimeballs where they are barely visible. Excretory system typical of dicrocoeliid cercariae; with 24 flame cells in 2[(2+2+2) + (2+2+2)]. Excretory bladder in posterior one-third of body; simple, tubular, with thick wall of cuboidal cells and posterior excretory pore. Genital primordia anterior and posterior to ventral sucker connected by strand of cells over dorsal side of ventral sucker. Tail attached to body by 6–8 slender peripheral tissue strands and very thin tegument.

Cercariae released from daughter sporocysts migrate to the mollusk's respiratory chamber where up to several hundred become incorporated in each slimeball formed. The mucuslike slimeball has central region occupied by the cercariae tangled together by their long, sinuous tails. Periodically, the slimeballs are released through

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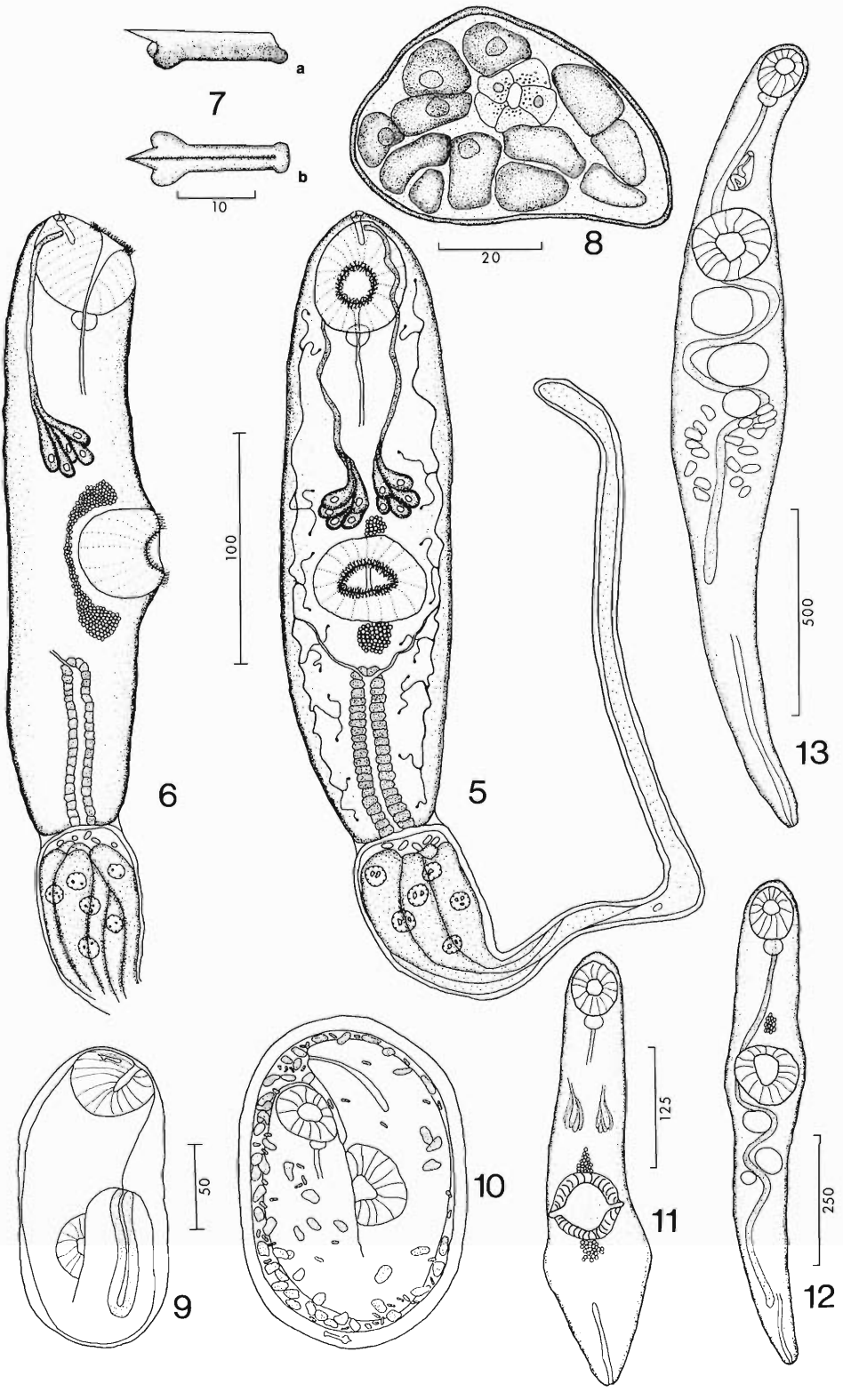
Figures 1–4. ***Lutztrema monenteron.*** **1. Adult of 82 days, ventral view. 2. Unhatched egg containing miracidium. 3. Six-week-old daughter sporocyst. 4. Fourteen-week-old daughter sporocyst.**



the host's respiratory pore. Release of slimeballs in *Anguispira alternata* infection began as early as 62 days postinfection and required up to 93 days, in other molluscan hosts. Their size, shape, and number seem to depend less on host species involved than on intensity of the infection. Heavier infection produces more slimeballs that may be larger and more variable in size and shape. They vary from spherical, with a diameter of 400–500, to sausage-shaped and up to 2,000 long by 150 in width.

Slimeball release is usually more frequent at night when the host is most active or after it is washed with cool tap water. Over an 8-day period, five experimentally infected mollusks released 79 slimeballs from 5:00 PM to 9:00 AM, with lights in the room off; 25 were released from 9:00 AM to 5:00 PM with lights on. Slimeball production continued as long as the host lived; one snail released slimeballs into the 324th day of infection before dying 3 days later.

Metacercariae (Figs. 9–11)

Millipeds, *Oxidus gracilis* (Koch), readily ingested slimeballs containing cercariae when they were presented to them. In the field, millipeds were found with terrestrial mollusks and seemed to be feeding on the mucus released from them. Experimentally, 63% of the millipeds offered slimeballs were found to contain metacercariae of *L. monenteron*. Infection of the milliped is evidenced by black scars where cercariae penetrated the inner cuticle lining of the esophagus, and to a lesser extent, the midgut. One hour after feeding on slimeballs, millipeds had cercarial bodies in their hemocoel and fat body. Many were quiescent and bent ventrally on themselves. Within 24 hr after ingestion, encystment began with the formation of a very delicate transparent membrane enclosing the worm (Fig. 9).

During the first week, the cyst wall begins to thicken and cells sloughed off the excretory bladder lining are seen in cyst lumen. The wall continues to thicken during the second week, and the stylet is shed, usually to become embedded in the cyst wall. After 42 days, cyst measured 196 (140–231) by 155 (120–177), with a wall thickness of 2.6–4.1. The cyst fluid contains more cells from the denuded excretory bladder that is thin-walled and tubular (Fig. 10). Metacercariae teased from 45–85-day cysts measured 484 (334–511) by 104 (96–118) in maximum width and expansion of hindbody (Fig. 11). Sucker opening lacks the cilialike fringe present in cercariae, and the ventral sucker has small lateral auricles.

That the cyst wall is laid down largely from the inside, presumably by the metacercaria, is suggested by the fact that the thin initial layer remains externalmost as the wall thickens. Metacercariae were found throughout the milliped from the head to the last body segments, but often more cysts were present in more posterior body segments. Metacercariae within the wall of the esophagus had much thinner cyst walls than did those of the same age found elsewhere in the milliped. At 50 days metacercariae were able to infect a young robin. They may become infective before that time; feeding experiments using younger metacercariae were not performed because of the limited supply of avian hosts.

Juvenile (Figs. 12–13)

Juveniles recovered from the gall bladder of robins 12 hr after ingestion of encysted metacercariae, averaged 410 by 54; 15 days, 760 by 121; 20 days, 918 by 146 (Fig. 12); 50 days, 1,874 by 276 (Fig. 13); and at 82 days, 3,875 by 518 (Fig. 1). Ratios of pre- to postacetabular length of juveniles at these ages, except 12 hr, were 1:1.6, 1:2, 1:3, and 1:4, respectively. Both suckers increased in size with maturation of the juveniles. The width ratio of oral to ventral sucker in the metacercariae is 1:1.6 with reduction of the lateral auricles on the ventral sucker; the ratio drops to 1:1.4 in the 15–20-day-old flukes. It then increases to 1:1.5 in the 50-day-old fluke and to 1:1.7–2.2 in the 82-day-old fluke.

The digestive system is distinct in young flukes. The cecum terminates approximately ⅙ the body length from the posterior end of 15–20-day-old juveniles, ¼ that length at 40 days, and ⅓ at 50 days. Differentiation of reproductive structures

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Figures 5–13. ***Lutztrema monenteron.*** **5. Cercaria from slimeball, ventral view (postacetabular glands omitted). 6. Cercaria from slimeball, lateral view. 7. Cercarial stylet, a. lateral view, b. ventral view. 8. Cross section of mature cercaria at level of excretory bladder, showing 12 large postacetabular glands. 9. Metacercaria 48 hr old. 10. Metacercaria 88 days old. 11. Metacercaria of 82 days, dissected from cyst. 12. Juvenile of 20 days, ventral view. 13. Young adult of 50 days, ventral view. Uterus and eggs omitted for clarity.**

begins with the formation of a pair of small spherical testes evident in 15-day-old juveniles. Some 15-day-old juveniles also show the ovary as a small spherical structure. Vitellaria appear as small refractile groups of cells posterior to the ovary. The cirrus sac and associated structures remain undifferentiated until about the 20th day. At 50 days, the reproductive system is functional, with eggs in uterus and sperm in seminal receptacle, but topography of the adult reproductive system is not attained until about the 82nd day of development. At that age, fluke had fully embryonated eggs in the distal end of the uterus, and eggs were present in the bile fluids of the host.

Remarks

The life cycle of *Lutztrema monenteron* is very similar to that of *Brachylecithum* species and *Dicrocoelium dendriticum* with each having mother and daughter sporocysts, longicercous cercariae that are released from molluscan hosts in slimeballs, and metacercarial development in arthropods. It is of interest to note that the intramolluscan period before slimeball release varies widely with these dicrocoeliids whether in differing hosts or in like hosts. With *L. monenteron* developing in *Anguispira alternata,* it is 62 days; for *Dicrocoelium dendriticum* in *Cionella lubrica* 3 mo or longer (Krull and Mapes, 1952), and *Brachylecithum stunkardi* in *Allogona ptychophora* 498 days (Carney, 1974). Using *A. ptychophora* for three other dicrocoeliids, *L, monenteron, B. myadestis,* and *B. mosquensis,* Carney (1966, 1970, 1972) reported first slimeball release in 148, 196, and 252 days, respectively. With *L. monenteron* infection in other mollusks, slimeball release ranged from 62 to 148 days at room temperature, which may have varied sufficiently to account for this. Kingston (1963) found the time of *B. orfi* slimeball releases to be influenced by environmental temperatures.

Dicrocoeliids are known to use only arthropods as second intermediate hosts. To the list of arthropods, the life cycle of *L. monenteron* adds a new group, the polydemid millipeds, which may prove to host other dicrocoeliids for metacercarial development.

In the laboratory *L. monenteron* could cycle twice a year since development in the mollusk, milliped, and avian hosts each requires no more than 2 mo. The principal definitive host of *L. monenteron* in North America is the American robin, a migratory species. It is suggested that because the molluscan and milliped intermediate hosts occur in both summer and winter ranges of the robin, *L. monenteron* is cycling in both ranges and possibly along the migratory routes.

Acknowledgments

It is my pleasure to acknowledge Dr. J. F. Denton whose direction made this study possible. With Dr. Denton's death in April 1978, we have lost an invaluable source of expertise in dicrocoeliid taxonomic and life history research, an outstanding ornithologist, and a friend.

I am grateful to Dr. J. P. E. Morrison, Division of Mollusks, Smithsonian Institution, who identified the molluscan hosts, and Dr. R. E. Crabill, Division of Myriopoda and Arachnida, Smithsonian Institution, who identified the milliped host.

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Studies on Larval Trematodes Infecting Freshwater Snails in Pakistan. IX. Virgulate Xiphidiocercariae[1,2]

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ABSTRACT: Two new virgulate xiphidiocercariae parasitizing *Lymnaea auricularia* and *L. rufescens* in Pakistan are described. *Cercaria nudicauda* sp. n. is characterized by a partly spined body and an unspined tail; stylet with indistinct shoulders; virgula organ comprised of two oval bodies, overlapping mesially; two pairs of penetration glands; a V-shaped excretory bladder and the flame cell formula 2[(2+2) + (1+1)] = 12. *C. microvirgula* sp. n. has a spinose body and tail; stylet with prominent shoulders; reniform virgula organ; four pairs of penetration glands; a V-shaped excretory bladder, with thick syncytial walls; and the flame cell formula 2[(1+1+1) + (1+1)] = 10. *Cercaria constrictiformis* nom. n. for *C. buckleyi* Khan and Haseeb, 1979 nec. Vercammen-Grandjean, 1960 is proposed.

Only three nonvirgulate xiphidiocercariae are previously known from Pakistan: cercaria of *Ganeo micracetabulus* (Bhutta and Khan, 1974) Bhutta and Khan, 1974; *Cercaria constrictiformis* nom. n. (=*C. buckleyi* Khan and Haseeb, 1979 nec. Vercammen-Grandjean, 1960), and *C. chilyaensis* Ahmed and Khan, 1967. This paper describes two new virgulate xiphidiocercariae.

Materials and Methods

Snails collected in the field were isolated individually in 3″ × 1″ glass tubes and the water was examined hourly and the following morning. The snails that had not shed cercariae were maintained in glass aquaria at 20–25°C and fed boiled or dried lettuce. These snails were reexamined at weekly intervals up to 3 wk, and those not shedding were dissected, crushed, and examined for nonemergent infections.

Cercariae were fixed without pressure in hot (70–80°C) 5% formalin and stained with aceto-alum carmine (Southwell, 1930). Living specimens were studied as unstained temporary mounts, refrigerated (2–3 hr) to facilitate visualization of the excretory system.

Camera lucida drawings from fixed specimens were supplemented free-hand from observations on living cercariae. Measurements from at least 10 fixed specimens are presented in micrometers as ranges followed by means in parentheses. The type specimens are deposited in the author's (DK) collection.

Observations

Cercaria nudicauda sp. n. (Fig. 1)

HOST: *Lymnaea auricularia* (Gray).

LOCALITY: A privately owned lake near Ghazanfargarh in Muzaffargarh District (May 19, 1977).

PERCENTAGE OF INFECTION: 0.3% (1/298).

Cercaria

Body oval to oblong, dorsoventrally flattened, spined anteriorly only as far as pharyngeal level. Oral sucker large, its posterior half containing virgula organ comprised of two oval bodies, overlapping mesially. Stylet with fine tip, indistinct shoulders; shaft slightly broader anteriorly than at base; its walls with maximum reinforcement at anterior end, thinning gradually posteriorly and disappearing on last third of shaft. Prepharynx lacking, pharynx moderately developed. Intestinal bifurcation immediately postpharyngeal; ceca widen as they proceed backward to terminate near posterior end of body. Penetration glands two pairs, in posterior third of body. Ventral sucker aspinose, smaller than oral sucker, located about two-thirds of body length from anterior end. Excretory bladder V-shaped, with cornua reaching posterior level of ventral sucker. Main excretory ducts highly convoluted anterolateral to ventral sucker where each duct divides into an anterior collecting duct, receiving capillaries from two pairs of flame cells and a posterior collecting duct, joined by only one pair of flame cells, the flame cell formula being 2[(2+2) + (1+1)] = 12. Caudal pockets absent. Tail simple, unspined, shorter than body. Measurements: body 109–126 (117) × 54–71 (58); tail 74–129 (97) × 17–20 (19); oral sucker 23–37 (32) × 29–34 (30); virgula organ 17–20 (18) × 26–29 (26); ventral sucker 20–34 (25) × 20–23 (22); excretory bladder 17–23 (20) × 20–26 (23); stylet length 17; breadth at shoulder 5–6 (6), at base 4–5 (4).

[1] Part of a thesis submitted by the senior author to the University of the Punjab, Lahore, Pakistan for the degree of Doctor of Philosophy.

[2] Supported by Pakistan Science Foundation Grant P-PU/BIO (9).

Behavior

The cercariae emerge in swarms at night. They are poor swimmers, float passively most of the time, and show no geotactic or phototactic response.

Sporocyst

The sporocysts are long, well developed, and without a discernible birthpore. Each contained six or seven germ balls and three or four immature to fully formed cercariae. The largest sporocyst measured 136 × 25. They inhabit the hepatopancreas of the snail.

Cercaria microvirgula sp. n. (Fig. 2)

HOST: *Lymnaea rufescens* (Gray).

LOCALITY: Small, temporary ponds on Lahore-Qasur road (May 10, 1978).

PERCENTAGE OF INFECTION: 0.4% (1/226).

Cercaria

Body oval, dorsoventrally flattened, covered with spines. Oral sucker well formed, with reniform virgula organ in posterior half. Stylet with a fine tip, prominent shoulders, and broader rounded posterior end. Oral sucker followed by well-developed pharynx. Esophagus short but broad. Intestinal bifurcation immediately preacetabular. Intestinal ceca embrace ventral sucker and terminate just posterior to it. Ventral sucker immediately postequatorial, smaller than oral sucker. Penetration glands four pairs, ducts opening near tip of stylet. Excretory bladder V-shaped, thick walled. Main excretory ducts form a few coils at paracetabular level, where each divides into an anterior collecting duct receiving capillaries from three flame cells, and a posterior collecting duct draining two flame cells, making the flame cell formula: $2[(1+1+1)+(1+1)] = 10$. Caudal pockets absent. Tail simple, without finfold, nearly as long as body and covered by minute, posteriorly directed spines. Measurements: body 109–143 (128) × 57–71 (68); tail 71–129 (103) × 18–23 (19); oral sucker 31–34 (33) × 29–34 (31); ventral sucker 17–23 (21) × 20–23 (22); excretory bladder 14–20 (17) × 20–26 (21); stylet length 17–20 (18); breadth at shoulder 6, at base 5.

Behavior

The cercariae emerge in small numbers at any time but in large numbers only during early afternoon. They are poor swimmers and show a slight tendency to aggregate near the bottom of the container. Resting periods of 5–10 sec alternate with swimming periods of 3–8 sec. At rest, the body and tail are in line, with the tail contracted. A phototactic response was not observed.

Sporocyst

The sporocysts are oval and without a discernible birthpore. Each contained three or four germ balls and two or three developing cercariae. Living sporocysts measure 100–325 × 25–50. They were found in the hepatopancreas of the snail.

Discussion

Lühe (1909) divided Xiphidiocercariae (Diesing, 1855) into four groups: Cercariae Microcotylae, Cercariae Virgulae, Cercariae Ornatae, and Cercariae Armatae. Cercariae Virgulae accommodated the forms with a characteristic virgula organ, a ventral sucker smaller than the oral, three to six pairs of penetration glands, excretory bladder V-shaped, oval or reniform and tail without a finfold. Sewell (1922) established two subgroups of Cercariae Virgulae: Virgula subgroup for cercariae of small size (body less than 0.2 mm), tail usually smaller than the body, acetabulum smaller than the oral sucker (usually about half), virgula organ well developed or reduced, excretory bladder oval or reniform, esophagus and ceca absent; and Paravirgula subgroup for cercariae with a V-shaped excretory bladder and with esophagus and ceca. Hall (1960) presented an extensive definition of Cercariae Virgulae and listed specific characters. Since then, several other diagnostic features have been employed for species identification (Khan, 1961; Probert, 1965; Etges et al., 1969; Nasir and Diaz, 1973). For a discussion on the classification of Xiphidiocercariae see Nasir (1972).

Cercaria of *Acanthatrium hitaensis* Koga, 1953 (Ito, 1964); *Cercaria* A Takahashi, 1928 (Ito, 1964); *Cercaria mucobuccalis* Faust, 1924, and *Cercaria tetrasolenata* Faust, 1924 are virgulate xiphidiocercariae that, like *Cercaria nudicauda,* possess two pairs of penetration gland cells. Although cercaria of *Acanthatrium oregonense* Macy, 1939 as described by Knight and Pratt (1955) has two pairs of penetration gland cells, Burns (1961) has described three pairs. In the former description there is no mention of flame cell formula and in the latter, it is: $2[(3+3+3)+(3+3)] = 30$. Also, there are differences in measurements noted by these authors. It is quite pos-

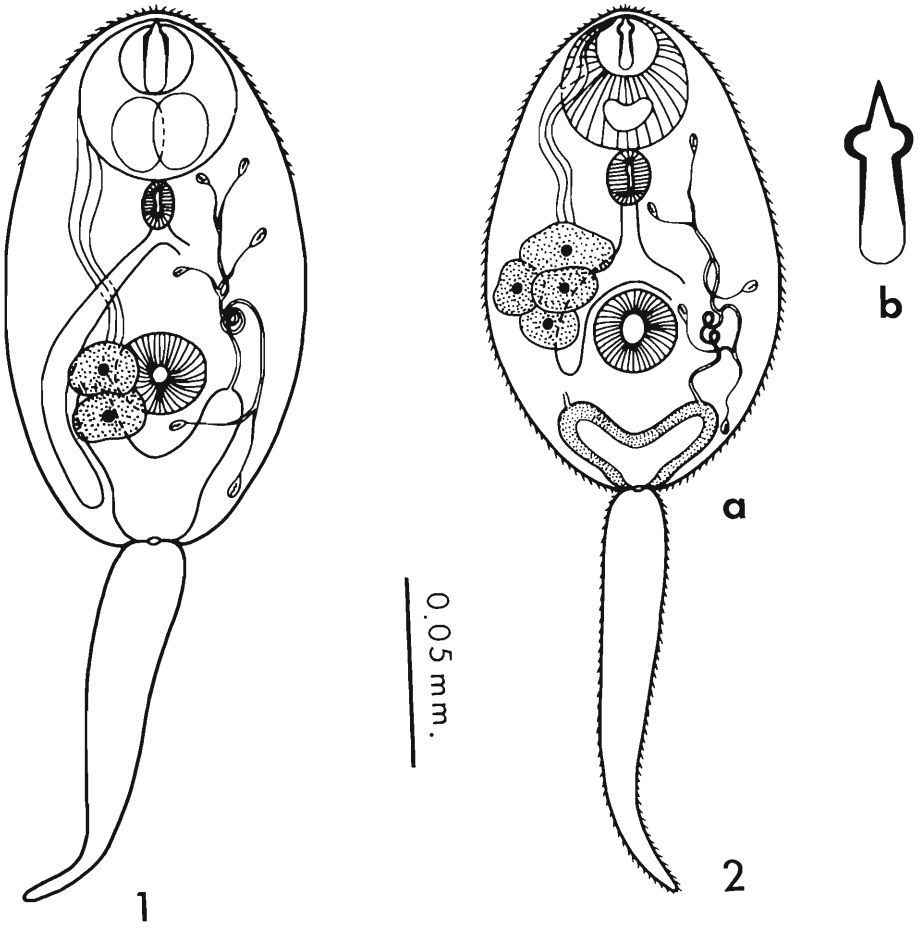

Figure 1. ***Cercaria nudicauda*** **sp. n. Entire specimen; flame cells shown on one side and penetration glands on the other side only; musculature of oral sucker not drawn. 2.** ***Cercaria microvirgula*** **sp. n. 2a. Entire specimen; flame cells or penetration glands shown on one side only. 2b. Stylet at a higher magnification.**

sible that they were dealing with different species, and thus a comparison of the present form with the cercaria of *A. oregonense* would appear doubtful.

Cercaria naukuchiensis Malaki and Sing, 1962 has two pairs of penetration glands, both the body and tail spined, no trace of gut beyond pharynx, and a flame cell formula: 2[(3+3) + (3+3)] = 24. However, in its drawing (Malaki and Sing, 1962, fig. 3, p. 140) there have been shown to be two nonoverlapping bean-shaped bodies that possibly represent the virgula organ but there is no mention of this feature in the text. A caudal excretory duct(?) has also been shown in the same figure. Furthermore, they have compared this form with other nonvirgulate xiphidiocercariae.

The cercaria of *Acanthatrium hitaensis* and *Cercaria* A differ from *C. nudicauda* in the shape and reinforcements of the stylet, size, and shape of the virgula organ and form of the excretory bladder. In addition, they lack digestive tract beyond pharynx. *Cercaria mucobuccalis* and *C. tetrasolenata* have a flame cell formula: 2[(1+1) + (1+1)] = 8; a different virgula organ, stylet, and excretory bladder. The former has both the body and tail spined and postacetabular penetration gland cells, whereas the latter is aspinose and the penetration glands are preacetabular.

There are 24 virgulate xiphidiocercariae that, like *Cercaria microvirgula,* have four pairs of penetration glands. These are: cercariae of *Cephalophallus obscurus* Macy and Moore, 1954 (Macy and Moore, 1954); *Loxogenes liberum* Seno, 1907 (Ito, 1964); *Pleurogenes claviger*

(Grabda-Kazubska, 1971); *Pleurogenes japonicus* Yamaguti, 1936 (Ito, 1964); *Pleurogenoides medians* (Ždárská, 1963); *Cercaria astrachanica* XI Ginetzinskaya and Dobrovolski, 1968; *C. geddesi* Ameel, 1939; *C. halli* Etges et al., 1969; *C. helvetica* VIII Dubois, 1929; *C. helvetica* IX Dubois, 1929 (Wesenberg-Lund, 1934); *C. kawa* Fain, 1953; *Cercaria* sp. XII Kerala Mohandas, 1977; *C. lahtinensis* Probert, 1965; *C. levantina* 15 Lengy and Gold, 1978; *C. leyteensis* no. 4 Ito, Yasuraoka, Santos, and Blas, 1977; *C. nodulosa* V. Linst. (Wesenberg-Lund, 1934); *C. octoglandulata* Pike, 1967; *C. siamensis* Ito, Papasarathorn, and Tongkoom, 1962; *C. stenophvenezua* Nasir and Diaz, 1973; *C. tabitha* Faust, 1921; *C. tarda* Khan, 1961 (Pike, 1967); *C. virgula* Fil. (Wesenberg-Lund, 1934); *Xiphidiocercaria* sp. III Ostrowski de Nunez, 1975 and *Xiphidiocercaria* type D Ryšavý, Ergens, Groschaft, Yousif, and El Hassan, 1975.

All of these can be differentiated from *C. microvirgula* on the basis of one or more of the following diagnostic features: number, nature of contents, and disposition of penetration glands; absence or presence and length of intestinal ceca; flame cell formula; size and shape of virgula organ; size; spination; shape, size, and reinforcement pattern of stylet; form of excretory bladder; sucker ratio; and cercarial behavior.

Knight and Pratt (1955) have described three pairs of penetration glands for the cercaria of *Allassogonoporus vespertilionis* Macy, 1940, whereas Burns (1961) has described four pairs. The flame cell formula as given in the latter description is: $2[(2+2+2) + (2+2+2)] = 24$. Moreover, measurements given in the two descriptions are different, thus, it appears doubtful that the authors were working with the same species.

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Thermal Effects on *Schistosoma mansoni* Irradiation-Attenuated Vaccine Production and Administration

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ABSTRACT: Studies were conducted on variable thermal conditions for *Schistosoma mansoni* nonirradiated cercarial penetration and worm burdens and for irradiation-attenuated vaccine production and administration. A broad range of temperatures (20–35°C) yielded no significant difference in nonirradiated cercarial penetration but worm burdens were greatest with cercariae administered at 30°C. Irradiation attenuation of cercariae for vaccine production was best conducted at 20°C, whereas 30°C was optimal for percutaneous vaccine administration.

The most effective immunization approach in schistosomiasis has employed live highly irradiated cercariae (Taylor et al., 1976; Minard et al., 1978; Miller and Smithers, 1980; Stek et al., 1981a, b). However, optimal physical conditions for vaccine preparation and administration have not been established. These physical conditions may, to some extent, explain variable results obtained by different laboratories.

Temperature is a factor that might be expected to influence immunization because several studies have shown that temperature changes influence longevity and infectivity in normal cercariae (Kuntz, 1947; DeWitt, 1965; Stirewalt and Fregeau, 1965; Purnell, 1966; Chappell and Coles, 1973; Ghandour, 1976; Christensen et al., 1979; Lawson and Wilson, 1980). This project was planned to first investigate thermal effects on infectivity with the nonirradiated cercarial strain employed in our laboratory. The second and third objectives were to establish optimal temperatures for irradiation attenuation of cercariae and for percutaneous administration of the live vaccine.

Materials and Methods

Animal model

NMRI (NIH/NmriCV) mice, 6 wk of age at the beginning of each experiment, were used in these studies. Animals for each experiment were from the same batch and were kept under identical conditions. Individual subsets in each experiment employed equally divided male and female mice.

Cercariae

The parasite used was *Schistosoma mansoni* (Puerto Rican strain) maintained in Swiss mice and *Biomphalaria glabrata* snails. Cercariae were collected from snails and administered percutaneously by 30 min tail immersion within 3 hr of emergence for immunization and within 2 hr for challenge infections.

Effect of temperature on nonirradiated cercarial penetration and worm loads (experiments 1 and 2)

Two consecutive experiments employing the same design were conducted to assess the effect of 5 selected temperatures (20, 25, 30, 35, and 40°C) on the penetration rate of normal nonirradiated cercariae and on subsequent worm burdens. Five groups of 20 mice (10 female and 10 male) were challenged by percutaneous exposure in this fashion at the 5 temperatures, respectively. Cercariae in aged tap water (189 ± 12 cercariae/mouse for experiment 1 and 187 ± 10 cercariae/mouse for experiment 2) were pipetted into plastic exposure tubes. These tubes were held in racks in water baths adjusted to the various test temperatures. Cercarial bodies that had lost their tails were also counted. Once temperature equilibration was obtained, mice held in plastic holders were arranged over the tubes to permit tail immersion exposure to the cercariae. After a 30-min percutaneous exposure period, mice were removed and the suspension from each tube counted for whole cercariae and bodies in order to calculate the penetration percent using the following formula:

$$\% \text{ penetration} = \frac{c^1 - [c^2 + (b^2 - b^1)]}{c^1} \times 100$$

where c^1 = mean number of cercariae before exposure;
c^2 = mean number of cercariae after exposure;
b^1 = mean number of bodies before exposure;
b^2 = mean number of bodies after exposure.

Eight weeks after percutaneous infection, mice in both experiments were perfused via the hepatic portal vein for worm recovery after the method of Yolles et al. (1947). Briefly, mice were sacrificed by intraperitoneal injection of 0.3 ml of a sodium pentobarbitol (50 mg/ml) heparin (100 units/ml) solution. The hepatic portal vein was torn and each mouse perfused via the dorsal aorta with citrated saline (1.5% sodium citrate and 0.85% sodium chloride) and the worms

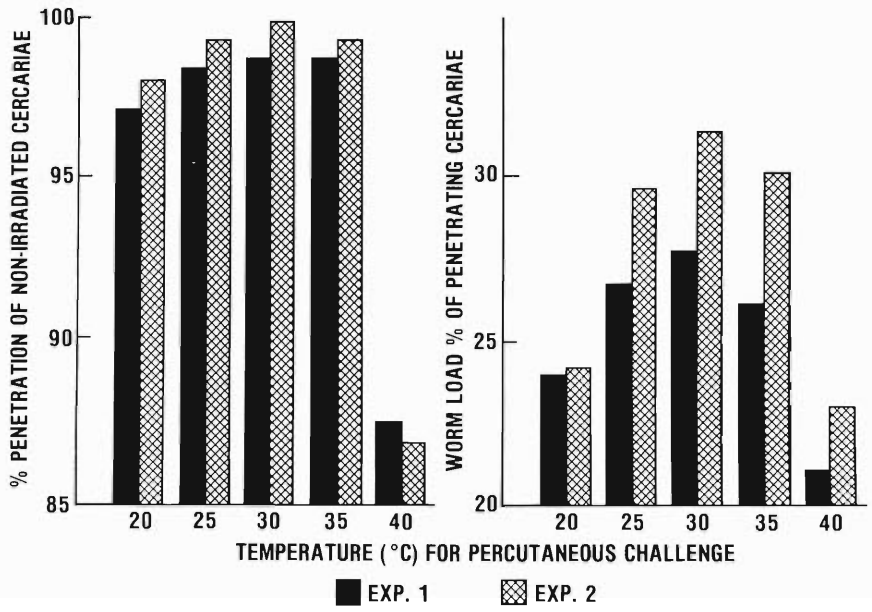

Figure 1. Effect of temperature on nonirradiated cercarial penetration and worm loads.

collected over a mesh sieve for counting. The worm load percentage of penetrating cercariae was calculated using the following formula:

$$\text{worm load \% of penetrating cercariae} = \frac{w}{p} \times 100$$

where w = mean number of worms recovered by perfusion;
p = mean number of penetrating cercariae.

Effect of temperature during irradiation attenuation and percutaneous immunization on irradiated cercarial penetration and vaccine efficacy (experiments 3 and 4)

Two consecutive experiments employing the same design were conducted to assess the effect of five selected temperatures (20, 25, 30, 35, and 40°C) on irradiated cercarial penetration and vaccine efficacy. Each experiment used five groups of 20 mice (10 female and 10 male) that were immunized percutaneously at the five temperatures with cercariae irradiated at those respective temperatures. A sixth group of 20 mice (10 female and 10 male) was held as nonimmunized controls.

The temperatures were maintained during irradiation attenuation and percutaneous immunization. Cercarial suspensions (approximately 3,000/ml) for experiments 3 and 4 were equally apportioned into five 15-ml polystyrene vials. These vials were then inserted into the appropriate water bath. Following attainment of temperature equilibration, vials were transferred to styrofoam insulating sleeves and irradiated with 50 Krads of gamma rays at 1.8 Krads/min from a cesium-137 source (Gammator M, Isomedic Inc.) at the National Institutes of Health, Bethesda, Maryland. Immediately postirradiation the internal vial temperatures were recorded. The vials were then placed in their respective water baths and the requisite number of irradiated cercariae (approximately 900/mouse) were introduced into plastic exposure tubes and suspended with a sufficient quantity of temperature-equilibrated aged tap water to cover the subsequently introduced mouse tails. Tubes containing irradiated cercariae were maintained at the five specific temperatures for the 30-min percutaneous exposure period. The mouse tail immersion method described above was used to conduct the percutaneous immunization that occurred within 3 hr of cercarial shedding from snails. Following exposure, as had been done prior to exposure, whole cercariae and body counts were made and the percent penetration obtained using the formula previously given.

All mice in the six groups for each experiment were challenged 4 wk postimmunization. Challenge infections employed normal cercariae administered percutaneously at room temperature (25°C) by tail immersion (193 ± 7 cercariae/mouse in experiment 3 and 177 ± 14 cercariae/mouse in experiment 4). Worms were recovered by perfusion 8 wk postchallenge as described for experiments 1 and 2 to measure the worm burden and calculate percent reduction.

Effect of temperature during irradiation on attenuated cercarial penetration and vaccine efficacy (experiment 5)

In this experiment the cercariae for vaccine production were irradiated at two temperatures (20 and 30°C), whereas percutaneous immunization and subsequent normal cercarial challenge infections were conducted

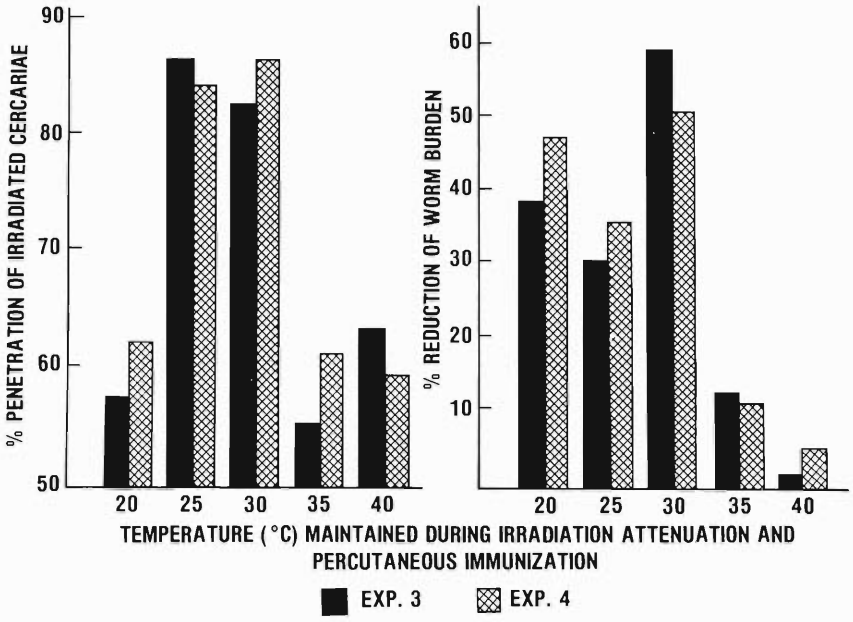

Figure 2. Effect of temperature on irradiated cercarial penetration and vaccine efficacy.

at 30°C. Two groups of 20 mice (10 female and 10 male) were immunized percutaneously at 30°C with cercariae irradiated at the two temperatures (20 and 30°C). A third group of 20 mice (10 female and 10 male) was held as nonimmunized controls.

Cercariae in aged tap water (approximately 3,000/ml) were equally aliquoted into two 15-ml polystyrene vials. The vials were then inserted into the appropriate water bath. Following attainment of temperature equilibration, vials were transferred to styrofoam insulating sleeves and irradiated with 50 Krads of gamma rays as previously described. Immediately postirradiation and prior to preparation of the exposure tubes, the internal vial temperatures were recorded. The vial was then placed in a 30°C water bath and the requisite number of irradiated cercariae (approximately 900/mouse) were pipetted into plastic exposure tubes and suspended with a sufficient quantity of 30°C equilibrated aged tap water to cover the subsequently introduced mouse tails. Tubes containing the irradiated cercariae were maintained at 30°C for the 30-min percutaneous exposure period. The mouse tail immersion method and the calculation of the percent penetration were done as previously described.

All mice in the three groups (two immunized and one nonimmunized) were challenged 4 wk postimmunization. Challenge infections employed normal cercariae administered percutaneously at 30°C by tail immersion (185 ± 23 cercariae/mouse). Worms were recovered by perfusion 8 wk postchallenge to measure the worm burden and calculate percent reduction.

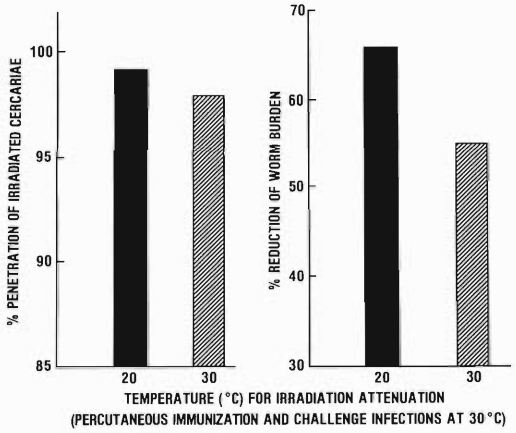

Figure 3. Effect of temperature during irradiation on attenuated cercarial penetration and vaccine efficacy.

Results

Effect of host sex on cercarial penetration

Using Student's *t*-test, no significant difference was found in the percentage penetration or worm recoveries between female and male mice in each group for each experiment; therefore, the cumulative results from the total number of mice for each group are represented (Figs. 1, 2, and 3).

Table 1. Internal vial temperatures for the cercarial suspensions pre- and postirradiation.

Experiment 3		Experiment 4	
Preir-radiation	Postir-radiation	Preir-radiation	Postir-radiation
20°C	24°C	20°C	24°C
25°C	25°C	25°C	26°C
30°C	29°C	30°C	29°C
35°C	32°C	35°C	30°C
40°C	34°C	40°C	32°C

Effect of temperature on nonirradiated cercarial penetration and worm loads

Employing the Student-Newman-Keuls multiple range test at the 0.05 level to define homogeneous subsets for experiments 1 and 2, no significant variation in cercarial penetration was noted for the 20–35°C-temperature groups. However, a significantly lower percent penetration was recorded for 40°C in both experiments (Fig. 1). The greatest worm loads, as a percent of penetrating cercariae, were noted at 30°C for both experiments with the temperatures of 25–35°C inclusive for each experiment defined as a homogeneous subset. The lowest worm load percent of penetrating cercariae was recorded for 40°C for both experiments (Fig. 1).

Effect of temperature on irradiated cercarial penetration and vaccine efficacy

Although the vials that held the cercariae during irradiation were insulated, internal vial temperature changes were noted to have occurred. The greatest changes were recorded for the lower and upper temperature groups (Table 1).

Employing the Student-Newman-Keuls multiple range test at the 0.05 level, two homogeneous subsets were defined for experiments 3 and 4. The highest percent penetration for irradiated cercariae was recorded at 25 and 30°C for both experiments. In experiment 3 the highest percent penetration (86.3%) was recorded at 25°C, whereas in experiment 4 the highest percent penetration (85.8%) was at 30°C (Fig. 2). The other homogeneous subset included the 20, 35, and 40°C groups that revealed significantly lower percent penetration than obtained at 25 or 30°C.

The greatest vaccine efficacy as measured by percent reduction of worm burdens (59% in experiment 3 and 50.1% in experiment 4) was obtained with the cercariae irradiated at 30°C and maintained at that temperature during percutaneous immunization. Using the Student-Newman-Keuls multiple range test, three homogeneous groups were defined in experiment 3 that corresponded to the high (30°C), middle (20 and 25°C), and low (35 and 40°C) percent reductions of worm burden. In experiment 4, three homogeneous groups were also defined but they varied somewhat in comparison with the previous experiment. The highest percent reduction groups were 20 and 30°C, the middle group was 25°C, and the low groups were as before, 35 and 40°C (Fig. 3).

Because the most potent vaccine in terms of effect per cercaria appeared to be the cercariae irradiated at 20°C, although the greatest degree of penetration occurred at 30°C, it was decided to conduct an experiment in which the cercariae were irradiated at 20 and 30°C while percutaneous immunization and challenge infections were conducted at 30°C. Employing Student's *t*-test, no significant difference was noted between the penetration rate of cercariae irradiated at 20 and 30°C where the exposure temperature was controlled at 30°C. The vaccine efficacy, as determined by percent reduction of worm burden, was greatest with the 20°C irradiation-attenuated cercariae (66.7%) and varied significantly (*t*-test) from the worm burdens obtained from mice immunized with the cercarial vaccine irradiated at 30°C (54%) (Fig. 3).

Discussion

The results obtained in experiments 1 and 2 indicate that penetration of *S. mansoni* nonirradiated cercariae was not significantly affected by temperature changes between 20 and 35°C, but decreased significantly at 40°C. The worm load, as a percent of penetrating nonattenuated cercariae, peaked at 30°C. The results are similar to other studies on the effect of temperature on cercarial infectivity and worm burdens at temperature extremes (DeWitt, 1965; Stirewalt and Fregeau, 1965; Purnell, 1966; Chappel and Coles 1973; Ghandour, 1976; Christensen et al., 1979; Lawson and Wilson, 1980).

The penetration rate of irradiated cercariae as seen in experiments 3 and 4, however, differed from those obtained with nonirradiated cercariae. Highest penetration percentages were at 25 and 30°C with significantly lower percentages on either side of those temperatures. A biphasic curve was noted in the percent reduction of worm bur-

dens for both experiments, with two peaks at 20 and 30°C. The greatest reduction in worm burdens was found using cercariae irradiated and percutaneously administered at 30°C as a vaccine. This is the same temperature at which the greatest worm burdens were obtained with non-irradiated cercarial infections. However, because the percent penetration was 22–29% lower at 20°C than at 25 or 30°C, the immunizing cercariae irradiated at 20°C were of greater vaccine potency per cercaria than those irradiated at 30°C.

The followup experiment, which investigated two temperatures of irradiation attenuation while employing 30°C for percutaneous immunization and challenge infections, showed that there was no significant difference in the penetration rate for cercariae irradiated at 20 and 30°C when the penetration temperature was held constant at 30°C. However, greater worm burden reduction was seen with the 20°C-irradiated cercarial vaccine compared with the 30°C-irradiated cercarial vaccine.

Variability of vaccine efficacy from laboratory to laboratory may be attributed, at least partially, to variations in the physical conditions at the different laboratories during cercarial irradiation attenuation and percutaneous immunization. The data on temperature clearly indicate that investigators should carefully record and report those conditions so that uniformity of procedures could be established. In addition, because many areas of the world endemic for schistosomiasis have average daily temperatures in excess of those optimal for vaccine production and administration as shown in these studies, temperature control and monitoring will be essential for vaccine investigations conducted in these areas.

In conclusion, these studies indicate that 20°C was the optimal temperature for irradiation attenuation, whereas 30°C appeared to be the best temperature at which to conduct percutaneous immunizations. Investigations continue to firmly establish optimal temperatures. Further evaluations of thermal and other physical, chemical, and temporal factors are planned to establish optimal conditions for vaccine production and administration.

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An Analysis of Swimming by the Cercariae of *Fasciola hepatica* Using High Speed Cinematography

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ABSTRACT: The locomotion of the cercariae of *Fasciola hepatica* was studied by cinematography. The rate and pattern of the swimming stroke were determined and compared with other species of cercariae whose locomotion has been analyzed.

In digenetic trematodes, cercarial tails show a wide range of structure and function. In most cases they are used for swimming, but in a number of species they are used for other purposes that appear to enhance the completion of the life cycle. The tails of swimming species vary from those of the pigmented, ornate "bait" cercariae of the Azygiidae (Cable, 1965) to the simple furcocercous and leptocercous cercariae (e.g., *Fasciola*). Observations on swimming habits are almost a requisite for the description of a new species of cercaria, hence there are many reports on swimming, but in-depth studies of cercarial swimming are relatively rare.

The cercariae of *Fasciola hepatica* swim briefly before encysting on a substrate. In the laboratory the majority encyst at the meniscus where the water meets the glass, plants, or cellophane; however, a number encyst elsewhere. Generally, these cercariae swim for only a short time after emergence.

Materials and Methods

A Red Lake Laboratories camera (Model Locam 162-5AC) was mounted on a heavy-duty drill press stand. Magnification of the specimen was provided by a Zeiss dissecting microscope. A microscope lamp (American Optical, Mod. 11144, 7.5 volts) was used for illumination by means of a substage mirror. The film used was 16-mm Eastman 4X negative film 7224 developed in D-76 for 10 min at 25°C. Cinematographic records were taken at 125 and 333 frames/sec (fps); the latter speed was adequate for the analysis of tail movement. Limiting factors were: (1) the speed of the film in fps, (2) the magnification, (3) the amount of light from the lamp, and (4) the ASA rating of the film.

Additional photographs were taken of swimming cercariae, using a 35-mm camera (Exakta VX2a) with a lens for macrophotography (Steinheil Macro-S-Quinon) and an electronic flash (Bauer E201) for illumination.

Eggs of *F. hepatica* were obtained by sedimentation from bile taken from infected cattle livers provided by the Beeville Packing Co., Beeville, Texas. The eggs were incubated at room temperature; hatching was stimulated by a change of water and exposure to bright light. The snail host was *Fossaria bulimoides* collected locally; individual snails were exposed to 5–8 miracidia for 4 hr and then placed in culture cages containing growing algae.

For photography, freshly emerged cercariae were mounted in a hanging drop preparation sealed with petroleum jelly. Their emergence could be stimulated at any time of the day by placing the snails in fresh, aerated, well water. Untimely encystment was frequent during these studies; freshly emerged cercariae readily encyst in pipettes and on slides within a few minutes.

The temperature at the start of the experiment was 23°C; it must have risen during the brief period of bright illumination. However, it was 15 min after mounting before the hanging drop preparation deteriorated due to condensation on the bottom of the well.

Results

An analysis of swimming involves separating the process into its components, the most obvious of which are tail and body movements. To study them, the cinematographic image of the cercaria was projected a frame at a time onto a surface for drawing. For each frame the cercaria was drawn with the body in the same relative position so that the change in tail position in successive frames could then be interpreted as a function of time (Fig. 1). To study body movement, the tail was drawn in the same relative position for each frame so that changes in body position could be interpreted as a function of time (Fig. 2).

At rest, the cercarial body is cordiform or elongate, but as swimming commences, it becomes transversely ellipsoidal in shape. Here, the swimming stroke is described entirely with reference to the body as the cercaria swims in a plane parallel to the surface of the water.

The tail lashes in a sinuous manner with a movement to an "S" to the right and another movement to the left constituting the fundamental swimming unit (Fig. 1).

The swimming stroke passes through six, distinctive, intergrading, tail shapes that are described here for the convenience of discussion:

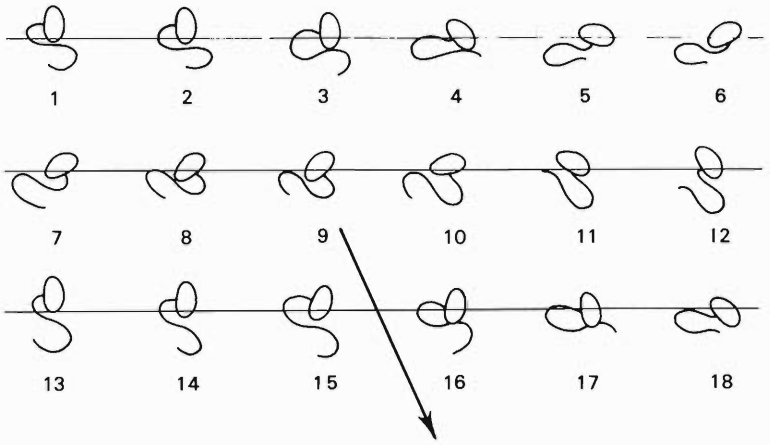

Figure 1. A cercaria of *Fasciola hepatica* drawn from projected images in 18 successive cinematographic frames taken at 333 frames/sec. Arrow indicates the direction of movement of the cercaria in 0.054 sec.

(1) the right hand "S" (Fig. 1, No. 2); (2) the left hand "S" (Fig. 1, No. 9); (3) the right hand loop (Fig. 1, No. 11); (4) the left hand loop (Fig. 1, No. 6); (5) the right lateral "S" (Fig. 1, No. 13); and (6) the left lateral "S" (Fig. 1, No. 6). The rates required for various components of the stroke can be derived from Figure 1.

In a continuum of movement from the right hand "S," the base of the tail appears to pull the tail downward with a movement toward the other side of the body with the remainder of the tail following to form the left hand loop (Fig. 1, Nos. 3–5). Movement continues anteriorly until the tail forms a left lateral "S" (Fig. 1, Nos. 1–8). The whole process is reversed with the movement of the tail to the right (Fig. 1, Nos. 8–16). The duration of one stroke averages about 0.054 sec (range 0.045–0.066 sec) or about 20 Hz (strokes/sec).

Variation is also observed in symmetry of the tail in relation to the body (Fig. 1, Nos. 7–9 and 14–16). In some strokes the tail barely reaches the transverse axis of the contracted body on one side, but it is carried beyond that point on the other side. Without that side-to-side difference in amplitude of the stroke, it appears that no change in direction would take place.

During the swimming stroke, the body turns as if pivoted near the base of the tail (Fig. 1). Figure 2 shows angular movement of the body as a function of time; the time interval between the angles shown is 0.003 sec. During the left hand phase of the stroke, the body movement is counterclockwise (Fig. 1, Nos. 8–16, Fig. 2A). During the right hand part of the stroke, the body moves clockwise (Fig. 1, Nos. 1–7, Fig. 2B). The rate of angular rotation appears to be compensatory depending on the part and rate of the tail stroke involved. It is slowest during transition of the stroke from one side to the other (Fig. 2B). The faster angular movement of the body at other phases of the swimming stroke is shown in that figure and can be seen in Figure 1, Nos. 5, 10, and 11.

When swimming, the cercaria of *F. hepatica* lashes the tail at a rate of 20 Hz, which is too rapid for the human retina to perceive the nature of the stroke, however some directional movement is noted. To study that directional movement, a cercaria was drawn from 18 successive frames of cinematographic film using the margin of each frame and the base of the tail as reference points. A study of those drawings revealed that a large amount of body and tail movement propelled the cercaria an exceedingly short distance, about $\frac{2}{5}$ the width of the cercarial body in about 0.054 sec. The direction of movement shown here is about 66° from the vertical axis of the body as seen in the first frame (Fig. 1, No. 1). It should be noted that no two positions in this series are identical; the asymmetrical lashing of the tail probably accounts for the net movement sideways.

To determine whether the confinement of the hanging drop (about 0.05 ml) modified swimming behavior, 48 photographs were taken of

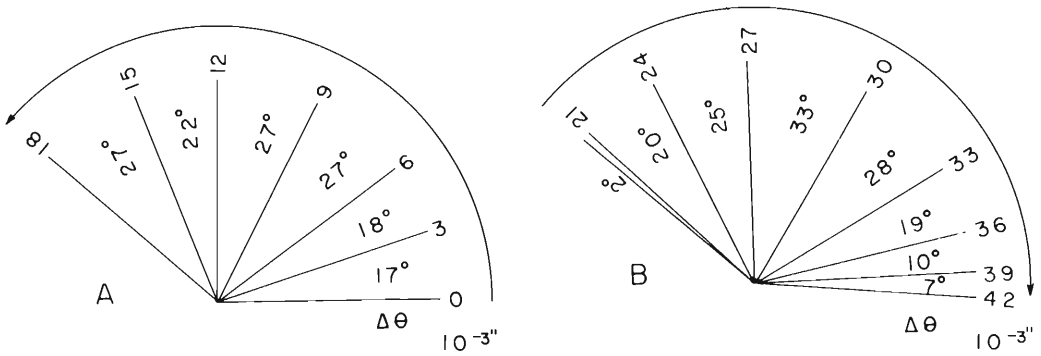

Figure 2. The angular counterclockwise (A) and clockwise (B) movement of the cercarial body of *Fasciola hepatica* in successive cinematographic frames drawn with the tail in the same relative position. $\Delta\theta$ indicates the angular change during a 0.003-sec time interval.

cercariae swimming in a large volume (60 ml) of water. Movement was arrested on film by using an electronic flash. Other than the cercariae being free to move longer distances, no differences in their swimming behavior could be discerned.

Discussion

In the life cycle of *F. hepatica,* the cercariae encyst in the open on various substrates. Upon emergence in the laboratory, cercariae swim up and most of them encyst on the glass near the meniscus. What significance that tendency has for the dispersal of *F. hepatica* cercariae is not clear. Their swimming appears "aimless" or random, but movement that leads to the encystment of most of the cercariae in one area is the result of behavioral mechanisms. The short swimming period before encystment may have selective value by: (1) reducing predation on cercariae, (2) requiring a small energy storage, and (3) by taking advantage of the ephemeral nature of the hosts' aquatic habitat in North America. Factors that stimulate the cercariae to encyst are not known.

The form of the tail wave in *Cryptocotyle lingua* is unique for species studied to date in that the tail forms a loop or a "360° arc" that moves down the length of the tail alternately from one side of the body to the other. This motion propels the cercariae in a "complicated helical path" (Chapman and Wilson, 1973). The tail wave in *Echinoparyphium* sp. is similar to that seen in *F. hepatica* in that the tail is drawn up alongside of the body in a plump "S" shape (Graefe and Burkert, 1972), but only four frames were drawn precluding a complete comparison of this species. In the case of *Himasthla secunda* the tail oscillates from side to side forming a tight "U" alongside of the body (Chapman and Wilson, 1973). The cercaria of *Parorchis acanthus* forms a tail wave resulting in a tight "U" in the basal half of the the tail and a more open "U" in the distal half as the tail oscillates (Rees, 1971). Not one of the simple-tailed cercariae have a tail wave exactly like that seen in *F. hepatica* cercariae.

The rates of tail oscillation have been given in strokes per unit time at temperatures ranging from 4°C (Chapman and Wilson, 1973) to 28°C (Haas, 1974). Low temperature was used to slow swimming activity to a more manageable rate for analysis. In general, the tails of furcocercariae oscillate faster than do those of cercariae with simple tails. Rates given for three furcocereous species are: *Transversotrema partialense* 30 Hz at 24°C (Bundy, 1981), *Diplostomum spathaceum* 55 Hz at 28° (Haas, 1974), and *Schistosoma mansoni* 22.5 Hz, no temperature given (Graefe et al., 1967). In contrast, the rates of simple-tailed cercariae are: *Himasthla secunda* 4 Hz at 5°C (Chapman and Wilson, 1973), *Parorchis acanthus* 3 Hz at 22°C (Rees, 1971) and *Echinoparyphium* sp. 10 Hz, no temperature given (Graefe and Burkert, 1972). *F. hepatica* cercarial tails oscillate at a rate much faster than those reported for other simple-tailed cercariae (20 Hz at 23° C. Most interesting is the case of *Cryptocotyle lingua* in which the shed tail continues to beat at an undiminished rate and in a tail wave similar to that seen in the intact cercaria giving the impression that the tail activity is myogenic

(Chapman and Wilson 1973). The severed cercarial tails of *F. hepatica* appear to be moribund.

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Effects of *Echinostoma revolutum* (Trematoda) Adults on Various Dimensions of the Chicken Intestine, and Observations on Worm Crowding

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ABSTRACT: Day-old domestic chicks were fed 100 metacercarial cysts of *Echinostoma revolutum* to determine the effects of adult worms on various dimensions of the chicken intestine and the effects of crowding on adult worms. Worms were recovered from the ileum, rectum, cloaca, bursa of Fabricius, and ceca at 1, 2, and 3 wk postinfection. Significant differences were not seen in the intestinal length or weight of infected versus control chickens. The cecal body area of infected chickens was significantly less than that of controls. The body area of worms from crowded sites (>25 worms/site) was significantly less than that of worms from noncrowded sites (1–10 worms/site). Worms from noncrowded sites contained about twice the number of eggs than those from crowded sites.

Echinostoma revolutum occupies numerous sites in the intestine of the domestic chicken including the ileum, rectum, cloaca, cecum, and the bursa of Fabricius (Beaver, 1937; Senger, 1954; Fried and Weaver, 1969; Fried and Butler, 1978; Fried and Alenick, 1981). Little information is available on the effects of this parasite on various dimensions of the chicken intestine such as weight, length, and area.

Although recent studies have been concerned with the effects of worm crowding on *Zygocotyle lunata* and *Philophthalmus gralli* in domestic chickens (Fried and Nelson, 1978; Nollen, 1983), similar studies on *E. revolutum* are not available. Our study examines the effects of *E. revolutum* grown in domestic chickens for up to 3 wk on various dimensions of the chicken intestine, and also examines the effects of worm crowding in the intestine.

Materials and Methods

Metacercarial cysts of *E. revolutum* were removed from the kidneys of experimentally infected *Physa heterostropha* snails (Fried and Weaver, 1969). Cysts were treated in 3% $NaHCO_3$ and then fed, 100 ± 20 cysts per chick, to day-old White Leghorn chicks (Fried and Butler, 1978). Control chicks were treated identically except they were not fed cysts. Infected and control chicks were placed in the same electrical brooder cage, fed ad libitum on a commercial mash diet (Fried and Butler, 1978) from 12 hr postexposure to 12 hr prior to necropsy, and necropsied at 1, 2, and 3 wk postinfection. At necropsy the intestine from duodenum to cloaca was removed and weighed. Each cecum was removed at the ileo–cecal junction, weighed, and then measured (length × maximum width) to determine cecal area as described by Fried and Nelson (1978) for studies on *Z. lunata*. The intestine and ceca were opened longitudinally and the location of worms in each site determined. Worms were fixed in hot alcohol-formalin-acetic acid, stained in Gower's carmine, and prepared as whole mounts. Body area measurements were made on fixed worms (length × midacetabular width) following the model of Berntzen and Macy (1969). Attempts to measure live worms as described for *P. gralli* by Nollen (1983) were abandoned since *E. revolutum* curled badly in cold water or Locke's.

Student's *t*-test was used to determine significant differences between experimental and control chicks. The following parameters were examined: intestinal weight, intestinal length, cecal weight, and cecal area. The *t*-test was also used to examine significant differences in the body area of worms from noncrowded (1–10 worms/site) versus crowded sites (>25 worms/site) as described by Nollen (1983).

Results

Infectivity data along with intestinal weights and length from infected versus control chickens are summarized in Table 1. Of 45 exposed chicks, 25 (56%) became infected. Infectivity was highest at 2 wk with worms recovered from 9 (69%) of 13 chicks. From about 4,500 cysts fed to 45 chicks, 529 (12%) worms were recovered with an average of 21 worms/infected chicken. Significant differences were not seen in intestinal weight or length between infected versus control chickens at all ages.

Four of nine (44%) and three of seven (43%) chickens necropsied at 2 and 3 wk, respectively, had flukes in the ceca (Table 2). Cecal weights in chickens with worms in the ceca were not significantly different than cecal weights of control chickens. However, the mean area of infected ceca was less than the area of ceca from control chickens at 2 ($P < 0.05$) and 3 ($P < 0.10$) wk.

Table 1. Summary of *Echinostoma revolutum* infectivity data and observations on intestinal length and weight in experimental versus control chicks.

No. of chicks used	No. and (%) of infected chicks	No. and (%) of worms recovered	Mean chick intestinal length ± SD (cm)	Mean chick intestinal weight ± SD (g)
Experiment A (chicks 1 wk old* at necropsy)				
18	9 (50)	180 (10)	84.9 ± 11.1	11.1 ± 1.2
8†	— —	— —	80.8 ± 9.7	10.1 ± 1.3
Experiment B (chicks 2 wk old* at necropsy)				
13	9 (69)	272 (21)	90.2 ± 11.6	14.8 ± 4.4
10†	— —	— —	95.2 ± 8.7	16.6 ± 3.5
Experiment C (chicks 3 wk old* at necropsy)				
14	7 (50)	77 (6)	97.8 ± 10.0	22.5 ± 3.6
13†	— —	— —	97.2 ± 14.6	19.2 ± 4.5

* Age of chick at necropsy = age of worms.
† Uninfected controls.

Distribution data of worms in various intestinal sites are presented in Table 3. At 1 wk worms were located mainly in the lower ileum, and at 2 wk they were about equally distributed between the lower ileum and the cloaca–rectum. At 3 wk worms were mainly in the cloaca–rectum.

Measurements of worms from different sites are presented in Table 3. At 1 wk the body area of worms from the lower ileum was significantly larger than that of rectal worms. At 2 wk the body area of cecal worms was significantly less than that of worms from the ileum, rectum, and cloaca. At 3 wk the body area of bursal worms was significantly less than that of worms from the ileum, ceca, rectum, and cloaca.

Observations on the effects of worm crowding are summarized in Table 4. At all ages the body area of worms from crowded sites was significantly less than that of worms from noncrowded sites.

Week-old worms were preovigerous, whereas 2- and 3-wk-old worms were ovigerous. At 2 wk worms from crowded sites contained about 80–100 eggs/worm, whereas those from noncrowded sites contained about 160–200 eggs/worm. At 3 wk worms from crowded sites contained about 250 eggs/worm, whereas those from noncrowded sites contained about 500 eggs/worm. Eggs from both sites appeared identical and attempts to embryonate them were not made.

Discussion

Infectivity data in our study were less consistent with similar studies on *E. revolutum* by Fried and Weaver (1969) and Fried and Butler (1978), but were similar to Senger (1954) who reported

Table 2. Observations on cecal weight and area in chicks with cecal infections of *Echinostoma revolutum* versus control chicks.

No. and (%) of chicks with cecal infections	No. of worms recovered in ceca*	Mean cecal weight ± SD per cecum (mg)	Mean cecal area ± SD per cecum (cm^2)
Experiment B (chicks 2 wk old at necropsy)			
4 (44)	13	901 ± 151	4.9 ± 0.13
— —	—	895 ± 253	6.3 ± 0.17
Experiment C (chicks 3 wk old at necropsy)			
3 (43)	5	1,226 ± 141	6.1 ± 0.84
— —	—	1,205 ± 349	7.5 ± 2.50

* Each infected cecum had 1–5 worms.
Ten and 13 control chicks used in Experiments B and C, respectively.

Table 3. Distribution of *Echinostoma revolutum* in chick intestinal sites and observations on worm body area.

Site of worms	No. and (%) of chicks with infections in the site		No. and (%) of worms recovered from site		No. of worms measured	Mean worm body area ± SD (mm^2)
Experiment A (chicks 1 wk old at necropsy)						
Ileum	7	(88)	139	(17)	10	1.25 ± 0.35
Cecum	1	(13)	1*	(<2)	—	—
Rectum	4	(50)	15	(2)	6	0.82 ± 0.14
Cloaca	0	(0)	0	(0)	—	—
Bursa	0	(0)	0	(0)	—	—
Experiment B (chicks 2 wk old at necropsy)						
Ileum	5	(71)	104	(15)	8	3.07 ± 0.64
Cecum	4	(29)	13	(2)	5	2.12 ± 0.70
Rectum	3	(43)	63	(9)	23	2.99 ± 0.60
Cloaca	3	(43)	45	(6)	12	3.13 ± 0.86
Bursa	1	(14)	1	(<1)	1	3.88
Experiment C (chicks 3 wk old at necropsy)						
Ileum	1	(17)	6	(1)	5	5.31 ± 0.87
Cecum	3	(50)	5	(1)	2	6.59 ± 1.10
Rectum	4	(67)	11	(2)	6	5.45 ± 1.30
Cloaca	4	(67)	35	(6)	21	4.17 ± 1.10
Bursa	2	(33)	6	(1)	6	3.64 ± 0.31

* Specimen curled and was not measured.

61% infectivity compared to our 56%. Many factors influence infectivity of *E. revolutum* in the domestic chicken including age and pretreatment of metacercarial cysts, and host-gut emptying time. Unpublished work by one of us (B.F.) indicates that the use of preselected cysts as done by Hays et al. (1972) with *Fasciola hepatica* enhances infectivity of *E. revolutum* in the domestic chicken. Cyst preselection was not used in our study.

Fried and Nelson (1978) noted that ceca infected with *Z. lunata* weighed less than those from control chicks. The intestinal and cecal weights of chickens infected with *E. revolutum* were not significantly different from uninfected controls. However, cecal area was smaller in infected versus control chicks.

Considerable differences were seen in the body area of worms of the same age, but from different sites. However, body area measurements of worms in particular sites with 1–10 worms were significantly greater than those from the same site with over 25 worms. These observations are in accord with previous studies on the crowding effect of *Z. lunata* by Willey (1941) and Fried and Nelson (1978), and on *P. gralli* by Nollen (1983).

Nollen (1983) made no comparison in the number of eggs produced in crowded versus uncrowded conditions in *P. gralli.* In *E. revolutum* under crowded conditions the number of eggs in the uterus was about one-half that of worms in uncrowded sites.

As worms aged they tended to move more posteriad in the gut. This observation is in accord with previous studies on *E. revolutum* by Senger (1954), Fried and Weaver (1969), and Fried and Alenick (1981).

Table 4. Body area measurements of worms from noncrowded (NC) versus crowded (C) sites in six chicks.

Site	No. of worms in site	No. of worms measured	Mean body area ± SD (mm)
Experiment A (chicks 1 wk old at necropsy)			
Ileum (NC)	3	3	1.54 ± 0.02
Ileum (C)	55	6	1.34 ± 0.36
Experiment B (chicks 2 wk old at necropsy)			
Rectum (NC)	9	8	3.26 ± 0.52
Rectum (C)	50	11	2.97 ± 0.59
Experiment C (chicks 3 wk old at necropsy)			
Cloaca (NC)	3	3	5.95 ± 0.03
Cloaca (C)	28	17	3.98 ± 0.85

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Aspidogastrid (Trematoda) Parasites of Bivalve Molluscs in Western West Virginia

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ABSTRACT: Five hundred bivalve molluscs (22 species) from 32 localities in western West Virginia were examined for aspidogastrid trematodes. Seven species were infected by *Aspidogaster conchicola* von Baer: *Amblema plicata, Anodonta grandis, Lampsilis radiata luteola, Pleurobema cordatum, Potamilus alatus, Quadrula pustulosa,* and *Tritogonia verrucosa.* Five species served as hosts for *Cotylaspis insignis* Leidy: *A. grandis, L. r. luteola, L. ventricosa, Q. quadrula,* and *Strophitus undulatus.* Prevalence was low with 47 (9.4%) individual bivalves parasitized by *A. conchicola* and 14 (2.8%) by *C. insignis. P. alatus* carried the greatest parasite load (mean intensity = 39.5 *A. conchicola*).

Hendrix (1968) stated that although *Aspidogaster conchicola* von Baer, 1827 and *Cotylaspis insignis* Leidy, 1857 are common parasites of freshwater mussels in the United States, their geographical distribution is incompletely known. No published accounts regarding these trematodes in West Virginia exist. Indeed, the mussel fauna in many streams of West Virginia is poorly known (Ralph Taylor, pers. comm.). Thus the aim of this work was to survey the western portion of the state, and determine the prevalence and intensity of aspidogastrid infections in as many bivalve species as possible.

Materials and Methods

Five hundred bivalve molluscs representing 22 species were taken from 32 collection sites in western West Virginia between May 17, 1981 and July 18, 1982, and examined for aspidogastrid trematodes (Fig. 1). Bivalves were collected by hand (with the exception of Site 27 where a clam brail was employed) and brought to the laboratory, in buckets with water from the collection site, within 3 hr of capture. Here they were maintained in aerated holding tanks with stream water, along with a sand and gravel substrate, for no longer than 3 days before necropsy. The soft parts were exposed by severing the adductor muscles with a scalpel; the mantle cavity, foot, gills, kidney, and pericardial region were then examined under a dissecting microscope. Trematodes were flattened under the weight of a cover glass then killed and fixed in 10% formalin at room temperature. Worms were subsequently rinsed in distilled water (10 min), stained in dilute Semichon's acid carmine prepared in 70% ethanol, cleared in methyl salicylate, and mounted in Kleermount®.

Ecological terms follow the definitions of Margolis et al. (1982).

Voucher specimens have been deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705, as: *Aspidogaster conchicola* (No. 77883), and *Cotylaspis insignis* (No. 77884).

Results

Two Aspidogastrid species, *Aspidogaster conchicola* and *Cotylaspis insignis,* were found in 10 of the 22 species examined (Table 1). Seven species served as hosts for *A. conchicola,* whereas five species harbored *C. insignis.* Only *Anodonta*

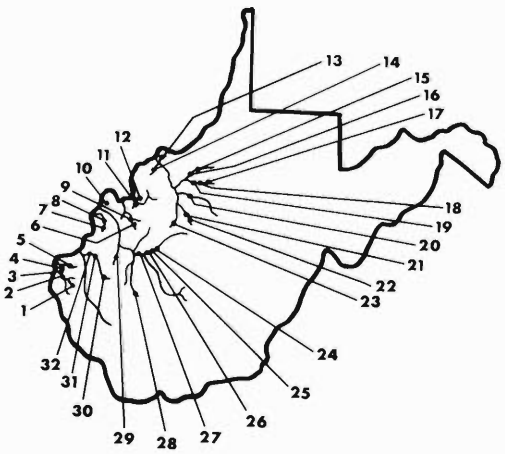

Figure 1. Map of West Virginia showing approximate location of collection sites. OHIO RIVER DRAINAGE: Twelvepole Creek (1–4); Beech Fork of Twelvepole Creek (5); Mill Creek (6 and 9); Sixteen Mile Creek (7 and 8); McClintic Wildlife Station Pond #6 (10); Crooked Fork of Sandy Creek (11); and Sandy Creek (12). LITTLE KANAWHA RIVER DRAINAGE: Worthington Creek (13); Tygarts Creek (14); North Fork Hughes River (15 and 16); South Fork Hughes River (17–19); Little Kanawha River (20); Sandstone Creek (21); West Fork Little Kanawha River (22); and Spring Creek (23). KANAWHA RIVER DRAINAGE: Elk River (24–26); Kanawha River (27); Coal River (28); and Hurricane Creek (29). GUYANDOTTE RIVER DRAINAGE: Mud River (30–32).

Table 1. Prevalence (P) and intensity (I) of mature *Aspidogaster conchicola* and *Cotylaspis insignis* in bivalves collected from four West Virginia river drainages.

	River drainage and parasite species									
	Ohio		Little Kanawha				Kanawha		Guyandotte	
	Aspidogaster conchicola		*Aspidogaster conchicola*		*Cotylaspis insignis*		*Cotylaspis insignis*		*Aspidogaster conchicola*	
Bivalve species/collection no.	P	I	P	I	P	I	P	I	P	I
Actinonaias carinata (Barnes, 1823)/3817*			0/6	—	0/6	—				
Amblema plicata (Say, 1817)/3819			7/25	14.0	0/25	—				
Anodonta grandis (Say, 1829)/3816	1/2	20.0					3/3	14.3		
Anodontoides ferruscianus (Lea, 1834)/3815	0/8	—								
Corbicula fluminea (Müller, 1774)	0/17	—					0/2	—	0/9	—
Elliptio dilatata (Rafinesque, 1820)/3820			0/6	—	0/6	—				
Fusconaia flava (Rafinesque, 1820)/3813	0/18	—	0/2	—	0/2	—	0/1	—		
Fusconaia maculata (Rafinesque, 1820)/3814			0/2	—	0/2	—				
Lampsilis radiata luteola (Lamarck, 1819)	12/98	15.8	5/146	1.2	6/146	2.0	0/13	—	1/29	75.0
Lampsilis ventricosa (Barnes, 1823)/3810	0/12	—	0/2	—	0/2	—	1/1	4.0		
Lasmogonia complanata (Barnes, 1823)/3805	0/6	—	0/1	—	0/1	—	0/4	—		
Lasmogonia costata (Rafinesque, 1820)/3806	0/1	—	0/9	—	0/9	—	0/1	—		
Obovaria subrotunda (Rafinesque, 1820)/3812			0/2	—	0/2	—				
Pleurobema cordatum (Rafinesque, 1820)									1/1	11.0
Pleurobema sintaxia (Rafinesque, 1820)/3821			0/1	—	0/1	—				
Potamilus alatus (Say, 1817)/3811	11/17	39.5	0/3	—	0/3	—				
Ptychobranchus fasciolaris (Rafinesque, 1820)/3818			0/2	—	0/2	—				
Quadrula pustulosa (Lea, 1831)/3809	2/9	2.0								
Quadrula quadrula (Rafinesque, 1820)/3808	0/1	—	0/1	—	0/1	—	3/3	4.3		
Sphaerium sp. of Scopoli, 1777	0/7	—								
Strophitus undulatus (Say, 1817)	0/13	—	0/2	—	0/2	—	1/1	5.0		
Tritogonia verrucosa (Rafinesque, 1820)/3807	2/8	6.5	1/1	2.0	0/1	—			4/4	29.8
Totals	28/217		13/211		6/211		8/29		6/43	

* Marshall University Malacology Collection number.

grandis and *Lampsilis radiata luteola* were parasitized by both aspidogastrids.

Overall prevalence rates were low. Of the 500 bivalves examined only 47 (9.4%) were parasitized by *A. conchicola,* and 14 (2.8%) by *C. insignis. A. conchicola* was found at 15 different sites, whereas *C. insignis* was confined to three sites on forks of the Hughes River (Little Kanawha River drainage), and Hurricane Creek (Kanawha River drainage).

A total of 973 adult *A. conchicola* individuals were recovered from the 47 parasitized bivalves for a mean intensity of 20.7. The heaviest *A. conchicola* infection was recorded for *Potamilus alatus.* Only 77 adult *C. insignis* individuals were recovered from 14 parasitized bivalves for a mean intensity of 5.5. The heaviest *C. insignis* infection was found in *A. grandis.*

Four immature forms of *A. conchicola* were found in each of two *L. r. luteola* individuals from Site 5 in July 1981. The following May, large numbers (>100) of immature *A. conchicola* individuals were recovered from each of six *L. r. luteola* individuals from Sites 16, 17, and 19. These were the only recovery records for immature aspidogastrids.

Discussion

Two surveys, comparable to the present one in terms of total sample size and number of mussel species in common, have been done in neighboring Ohio (Stromberg, 1970; Huehner and Etges, 1981).

Huehner and Etges (1981) examined 334 individual mussels (13 species) for *Aspidogaster conchicola* and found a prevalence of 42.8%, a figure considerably higher than the 9.4% in the present study. There were other notable differences. For example, *Lampsilis ventricosa, Lasmogonia complanata,* and *Quadrula quadrula* from West Virginia were not parasitized by *A. conchicola,* whereas the prevalence rates for those mussels in Ohio were 31.5%, 41.5%, and 55.6%, respectively. Moreover, *Lampsilis radiata luteola* and *Tritogonia verrucosa,* which were not infected by *A. conchicola* in the Huehner and Etges study, were parasitized in western West Virginia (6.3% and 53.8%, respectively). Prevalance for *A. grandis* was similar in both surveys (48.3% in Ohio vs. 50.0% in West Virginia).

Stromberg (1970) examined 374 mussels (37 species) and reported overall prevalence rates of 23.8% for *A. conchicola,* and 9.6% for *C. insignis.* Since Stromberg calculated prevalence rates for mussel genera, only limited comparisons can be made with host species of other studies. Still, he demonstrated that *Quadrula* (at 73.3%) and *Anodonta* (at 65.4%) exhibited the highest prevalance rates for *A. conchicola,* whereas only *Anodonta* (at 73.1%) was frequently infected by *C. insignis.*

Aspidogaster conchicola has also been the dominant aspidogastrid in other mussel parasite surveys (Kelly, 1899; Hendrix, 1968; Nelson et al., 1975), although Hendrix and Short (1965) and Flook and Ubelaker (1972) found higher prevalence rates for *C. insignis* in bivalves.

Characteristically, the number of aspidogastrids per infected bivalve is low. Kelly (1899) reported a low mean intensity of 1.0 for *L. luteolus* to a high mean of 18.0 for *L. ventricosus.* Mean intensities of *A. conchicola* and *C. insignis* in Oklahoma mussels were 16.9 and 6.4, respectively (Nelson et al., 1975). Stromberg (1970) reported high mean intensities of 14.4 *A. conchicola* in *Protoptera,* and 12.7 *C. insignis* in *Anodonta.* Huehner and Etges (1981) reported a high mean intensity of 19.0 for *A. conchicola* in *A. grandis,* and a low mean of 3.0 in *Actinonaias carinata.* Thus, mean intensities for infected bivalves from West Virginia, with the possible exception of *P. alatus,* generally agree with those reported by other writers. Still, heavy aspidogastrean infections can occur. Najarian (1955) reported a mean of 97.5 *C. insignis* in six *A. grandis* from a stream with very little water flow in Tennessee (Hendrix, pers. comm.). Nelson et al. (1975) reported a range of 400–1,545 *A. conchicola* from five infected *Potamilus purpuratus.* Factors influencing the intensity of these infections are poorly known leading one to conclude that relationships between freshwater bivalves and their aspidogastrid parasites warrant further study.

Acknowledgments

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Sureshia trychopea sp. n. (Cestoda: Dilepididae) from White-Throated Swifts, *Aeronautes s. saxatalis,* (Aves: Apodiformes) with Notes on Dilepidine Cestodes Having a Vaginal Sclerite

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ABSTRACT: *Sureshia trychopea* sp. n. (Cestoda: Dilepididae) is described from the white-throated swift, *Aeronautes s. saxatalis* (Woodhouse) (Aves: Apodiformes) in Colorado. It differs from other members of the genus in the number and morphology of rostellar hooks and by having 25–31 testes. The generic diagnosis of *Sureshia* Ali and Shinde, 1967, is emended; *Mehdiangularia* Shinde, 1969, is considered a junior synonym of *Neoliga* Singh, 1952; and the following new combinations are proposed: *S. macropterygis* (Heubscher, 1937) comb. n. (formerly *Anomotaenia macropterygis*), *S. depressa* (v. Siebold, 1836) comb. n. (formerly *A. depressa*), *Neoliga affinis* (Shinde, 1968) comb. n. (formerly *S. affinis*), *N. alii* (Shinde, 1968) comb. n. (formerly *S. alii*), and *N. swifti* (Shinde, 1969) comb. n. (formerly *Mehdiangularia swifti*).

In 1975–1976, seven white-throated swifts, *Aeronautes s. saxatalis* (Woodhouse) were collected during their spring migration near Clifton, Colorado, and examined for parasites. Six swifts were infected with a new species of *Sureshia* Ali and Shinde, 1967 (Dilepidinae). Study of these specimens prompted a review of the dilepidine cestodes possessing a vaginal sclerite. This resulted in the revision of the generic diagnosis of *Sureshia,* the description of *S. trychopea* sp. n., the proposal of five new combinations, and the placement of *Mehdiangularia* Shinde, 1969, in synonomy with *Neoliga* Singh, 1952.

Materials and Methods

After removal from the gut, live cestodes were flattened under coverslip pressure and fixed in 10% formalin. The tapeworms were stained in Semichon's aceto-carmine, dehydrated, cleared in beechwood creosote, and mounted in permount. Cross sections were made using standard histological techniques. Drawings were made with the aid of a microprojector or camera lucida. Measurements are in micrometers unless otherwise stated; the mean is followed by the range in parentheses. Type specimens are in the USNM Helminthological Collection (Nos. 75152 holotype, 75153 paratypes [8], 75154 fragments) and the University of Nebraska State Museum, Harold W. Manter Laboratory (UNSM) (No. 20912 paratype, fragments).

Sureshia Ali and Shinde, 1967

EMENDED GENERIC DIAGNOSIS: Dilepididae, Dilepidinae. Rostellum armed with two rows of similar hooks. Strobila spined or not. Genital pores irregularly alternate. Genital ducts passing dorsal to longitudinal excretory canals. Testes numerous, lying dorsal to ovary. Ovary bilobed, with narrow isthmus; lobes branched. Vaginal sclerite present. Seminal receptacle preovarian, central. Uterus saccate, lobed. Vitellaria ventral, compact, lying posterior to ovarian isthmus. Parasites of Apodiformes.

TYPE SPECIES: *S. micropusia* Ali and Shinde, 1967, from *Apus affinis,* Aurangabad (Maharashtra), India.

REMARKS: The original generic diagnosis of *Sureshia* and the description of the type species, *S. micropusia* by Ali and Shinde (1967) have several inconsistencies. The diagnosis states that the hooks of the two rostellar rows are of equal size, whereas the text indicates that they are nearly the same length. The diagnosis indicates that the genital pores regularly alternate, but their figure 3 clearly shows a specimen in which the pores irregularly alternate. These authors indicate in their text that the genital ducts passing dorsal to longitudinal excretory canals is a generic characteristic, whereas their figure 4 shows the genital ducts passing between the longitudinal excretory canals. In addition, the authors did not include the nature of the seminal receptacle, vitellaria, or uterus in their original description.

Two related species, *Anomotaenia depressa* (v. Seibold, 1836) and *A. macropterygis* Huebscher, 1937, both collected from the same host (*Apus affinis*) and having vaginal sclerites and similar internal anatomy, are precluded from *Sureshia*

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as originally defined. Ali and Shinde (1967) limited *Sureshia* to species having up to 23 testes and lacking body spines. However, *A. depressa* has 30–40 testes (Burt, 1969) and *A. macropterygis* possesses neck and body spines (Dollfus, 1958). After examining specimens of *A. depressa, Pseudangularia* sp., and *Neoliga* sp., we do not consider these characteristics sufficient to exclude *A. depressa* and *A. macropterygis* from *Sureshia,* and our emended generic diagnosis accommodates these species. We define *Sureshia* for species having a vaginal sclerite, irregularly alternating genital pores, genital ducts passing dorsal to the longitudinal excretory canals, and a rostellum armed with two rows of hooks.

Sureshia trychopea sp. n. (Figs. 1-5)

HOST AND LOCALITY: White-throated swift, *Aeronautes s. saxatalis* (Woodhouse); 6.5 km south of Clifton (Mesa Co.), Colorado (May 1975; July 1976).

LOCATION: Small intestine.

DESCRIPTION: Largest strobila 28.3 mm long, maximum width 1.4 mm. Scolex 255 (190–310) long, 216 (160–270) wide ($N = 8$). Suckers delicate, subovate, 162 (130–195) long, 98 (75–127) wide ($N = 7$). Rostellum stout, 107 (67–176) long, 109 (91–125) wide ($N = 8$). Rostellar sac 134 (75–156) long ($N = 7$), extending to near level of posterior margin of suckers. Rostellar hooks in two circles of 15 each; each hook with elongate handle, short guard, stout blade; blade and guard of anterior hooks longer than those of posterior; anterior hook 45 (43–50) long ($N = 9$), posterior hook 42 (41–45) long ($N = 9$). Neck short; proglottids 47–84 in number, craspedote, apolytic. Genital pores lateral, lying near anterior margin. Reproductive systems protandrous. Genital atrium simple. Testes 25–31, subspherical, extending to level of anterior margin of ovary (in mature segments); each 74 (45–104) in diameter ($N = 7$). Vas deferens extending anteriorly from median field, coiled in anterior portion of proglottid and within cirrus pouch, terminating in muscular sphincter at base of cirrus. Seminal vesicle absent. Ejaculatory duct delicate. Cirrus eversible, armed with two zones of minute spines. Cirrus pouch 347 (260–429) long, 109 (73–143) wide ($N = 7$); constricted in gravid proglottids. Ovary median, dendritic, with 12–18 aporal and 8–11 poral branches. Vitellarium lobate, variable, ventral. Vagina opening dorsoposterior to cirrus in genital atrium, with delicate sclerotized wall. Vaginal sclerite dumbbell shaped, 61 (55–67) long ($N = 9$). Seminal receptacle 249 (150–351) long, 114 (85–150) wide ($N = 7$), lying anterior to ovarian isthmus. Uterus an egg-filled sac with delicate wall, numerous internal trabeculae. Ventral osmoregulatory canals frequently enlarged, with simple anastomosis near posterior margin of each proglottid.

Remarks

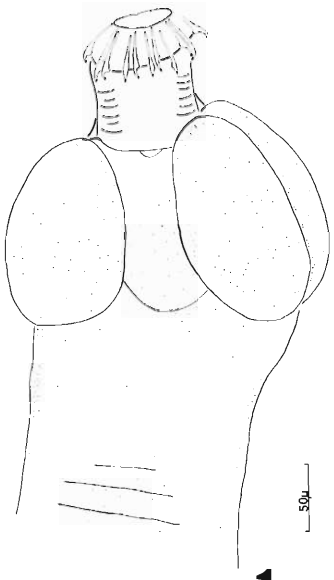
Sureshia trychopea sp. n. is most similar to *S. micropusia* Ali and Shinde, 1967, from which it differs by having rostellar hooks in two rows of 15 each (two rows of 13 each in *S. micropusia*), 25–31 testes (17–23 in *S. micropusia*), and two zones of cirral spines (one zone in *S. micropusia*).

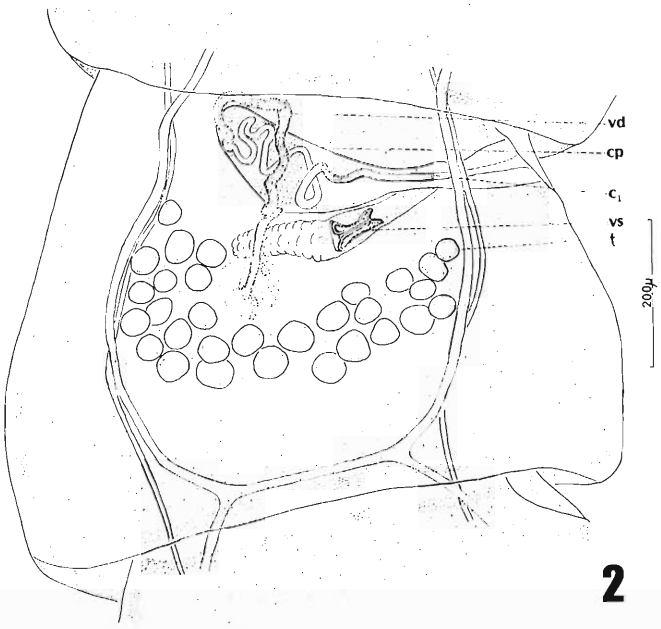
In *S. trychopea,* the vaginal sclerite can be distinguished early in the development of the proglottid. It is first discernible, after the appearance of the cirrus pouch, as a scoop-shaped structure with rounded aporal and flattened poral ends. At this time, the sclerite is open along its entire ventral length. In more mature proglottids, the edges of the sclerite curl ventrally, meet, and fuse along the midventral surface to form the characteristic dumbbell.

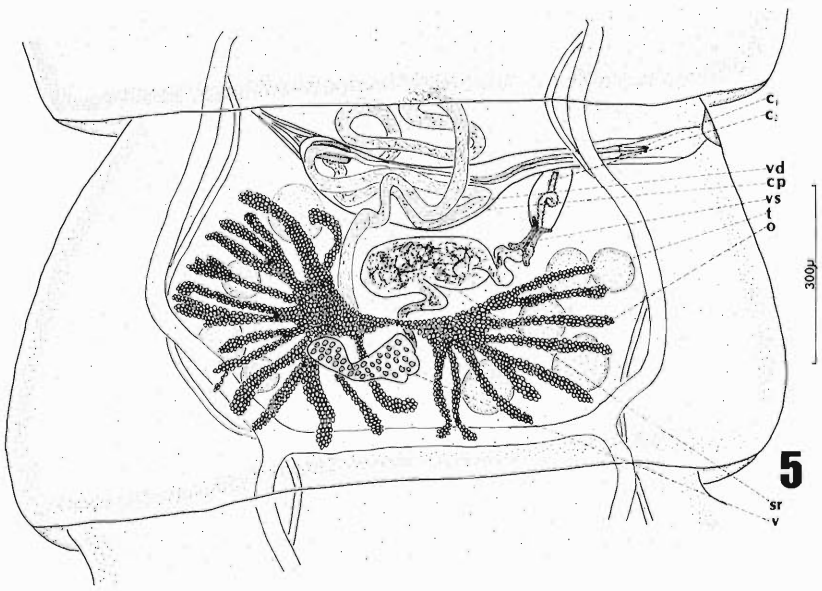
In several proglottids, the vagina contains the terminal portion of a cirrus apparently lost by the partner during copulation (Fig. 5), hence, the specific name from Greek (*trycho* = consume + *peos* = penis). In these instances, the tip of the cirrus is firmly held in the vaginal sclerite by the distal zone of spines. When the cirrus has been lost from the male system, the cirrus pouch is contracted to a compact subspherical mass located in the midanterior portion of the proglottid. This condition was also observed in specimens of *Pseudangularia* sp. from *Apus affinis* from Taiwan (specimens courtesy of Dr. Schmidt) and in specimens of *Anomotaenia depressa* from *A. affinis* from Borneo (specimens courtesy of Dr. Gibson).

Discussion

Delipidine cestodes with a vaginal sclerite are represented in the following genera: *Anomotaenia* Cohn, 1900, *Mehdiangularia* Shinde, 1969, *Neoangularia* Singh, 1952, *Pseudangularia* Burt, 1969, *Sureshia* Ali and Shinde, 1967, and *Neoliga* Singh, 1952. All reports of species with vaginal sclerites are from apodiform birds of the Old World.







Figures 1–5. ***Sureshia trychopea*** **sp. n. 1. Scolex. 2. Immature proglottid (ventral). 3. Anterior hook. 4. Posterior hook. 5. Mature proglottid (ventral). Abbreviations: c_1, cirrus; c_2, consumed cirrus; cp, cirrus pouch; o, ovary; sr, seminal receptacle; t, testis; v, vitellarium; vd, vas deferens; vs, vaginal sclerite.**

Matevosyan (1963) synonymized *Neoliga* Singh, 1952, with *Liga* Weinland, 1857. However, we retain the former genus for species having a vaginal sclerite, two rows of rostellar hooks, regularly alternating genital pores, and genital ducts passing dorsal to the osmoregulatory canals as originally described by Singh (1952). Species of *Liga* do not possess a vaginal sclerite.

Shinde (1969) erected *Mehdiangularia* for his new species, *M. swifti,* collected from *Apus affinis* in India. The new genus was distinguished from *Neoliga* by possessing a single row of rostellar hooks. However, Shinde (1969) states that the hooks are not regularly arranged in a circle and his figure 1 shows two rows. Also, Shinde's (1969) figure 2 clearly shows hooks of two different sizes. Therefore, we consider *Mehdiangularia* a synonym of *Neoliga,* and propose *N. swifti* (Shinde, 1969) comb. n.

Our redefinition of *Sureshia* (see emended diagnosis) precludes retention of *S. affinis* Shinde, 1968, and *S. alii* Shinde, 1968, in this genus. These species are characterized, in part, by having regularly alternating genital pores and a double crown of rostellar hooks. Because these characters are diagnostic for *Neoliga,* as defined above, we propose the following new combinations: *N. affinis* (Shinde, 1968) comb. n., and *N. alii* (Shinde, 1968) comb. n.

Burt (1969) redescribed the proglottids of *Anomotaenia depressa* (v. Siebold, 1836), and Dollfus (1958) reported on the morphology of *A. depressa* and *A. macropterygis* Heubscher, 1937. Both of these species possess two rows of rostellar hooks, vaginal sclerites, and irregularly alternating genital pores. These characters are diagnostic for *Sureshia* as defined above. Thus *S. depressa* (v. Siebold, 1836) comb. n. and *S. macropterygis* (Heubscher, 1937) comb. n. are proposed.

Dollfus (1958) described *Anomotaenia brachycolopos* and *S. depressoides* from the intestine of *Apus pallidis brehmorum* from Morocco. Although both species possess vaginal sclerites, *A. brachycolopos* has irregularly alternating genital pores and *A. depressoides* regularly alternating genital pores. Because information is not complete regarding the rostellar armature, these species cannot be assigned at the generic level and are considered incertae sedis.

Acknowledgments

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Cerebral Coenuriasis in Domestic Cats in Wyoming and Alaska[1]

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ABSTRACT: Cerebral coenuriasis was diagnosed in three domestic cats from Wyoming (two) and Alaska (one). Measurements of hooks and hook numbers from these larval cestodes indicate they were probably *Taenia* (*Multiceps*) *serialis* (Gervais, 1847) Baillet, 1863. Only two cases of feline coenuriasis previously have been reported.

Cerebral coenuriasis in the domestic cat has been reported twice (Georgi et al., 1969; Hayes and Creighton, 1978). This is a report of three additional cases of coenuri in brains of domestic cats from Wyoming and Alaska.

A 4-yr-old domestic short-haired male cat (WC1) from western Wyoming was examined in September of 1980. The only clinical sign was protrusion of the third eyelids to cover approximately one-half the surface of the eye; rectal temperature was 38.8°C. Six weeks later the animal was reexamined; signs included incoordination, balance and position deficits, visual impairment, and head pulled to the left side. The affected animal was euthanized.

The second Wyoming case (WC2) occurred in a 6-mo-old domestic long-haired cat presented in March of 1983 with slight dehydration, nystagmus, and apparent blindness. Temperature, pulse and respiration rates, ophthalmic examination, and a complete blood count were normal. Fecal flotation and direct smears were negative for evidence of parasites. Opisthotonus began 1 wk later and the cat was euthanized.

Another domestic cat, a 1-yr-old male, from the vicinity of Anchorage, Alaska was examined in 1976; epileptiform seizures and severe depression were noted; the animal was euthanized.

Materials and Methods

These animals were necropsied and brains removed for examination; brains and cysts found therein were preserved in 10% buffered formalin. Brains were cut into coronal slices and processed by standard histologic techniques. Masson's trichrome stain was used on selected brain sections. Photomicrographs of sectioned areas of the brain and coenuri were made using a 35-mm Zeiss SLR mounted on the microscope.

En face preparations of the hook-bearing region of the rostellum were made in Hoyer's fluid from scolices dissected from the inner walls of coenuri. Photomicrographs of the hooks were made and hooks were measured using a calibrated filar micrometer. Total length was defined as the length of the line from the end of the handle to the tip of the blade ignoring the arc of curvature.

Coenuri from four rabbits were available for comparison with the feline parasites. Two cases of leporine coenuriasis were in domestic rabbits. Several coenuri were from the subcutaneous tissues over the scapula of a rabbit from near Laramie, Wyoming in 1975 and a single coenurus was from the retroperitoneal tissues along the lumbar muscles of a rabbit from Walden, Colorado in 1983. A coenurus was removed from the body cavity of a white-tailed jackrabbit (*Lepus townsendi*) collected near Baggs, Wyoming in 1981 and a case of pulmonary coenuriasis was found in a snowshoe hare (*Lepus americanus*) south of Fairbanks, Alaska in 1981.

Results

In WC1, a 2-cm diameter cavity in the left cerebral hemisphere was covered by a superficial, yellowish-white membrane. Eleven multilobulate coenuri (Fig. 1) were recovered. The coenuri ranged in size from about 1 mm up to 20 mm in diameter. Some were single and subspherical (10 × 5 mm) or trapezoidal (6 × 5 mm) and showed the beginning of fingerlike outgrowths or "buds" at their polar ends or from the acute angles on their margins. Other coenuri consisted of parent coenuri each of which had numbers of exogenously budded daughter coenuri. In one such coenurus, the parent measured 10 × 8 mm and, in addition to nascent buds, had four large buds connected to it by narrow pedicles; the larg-

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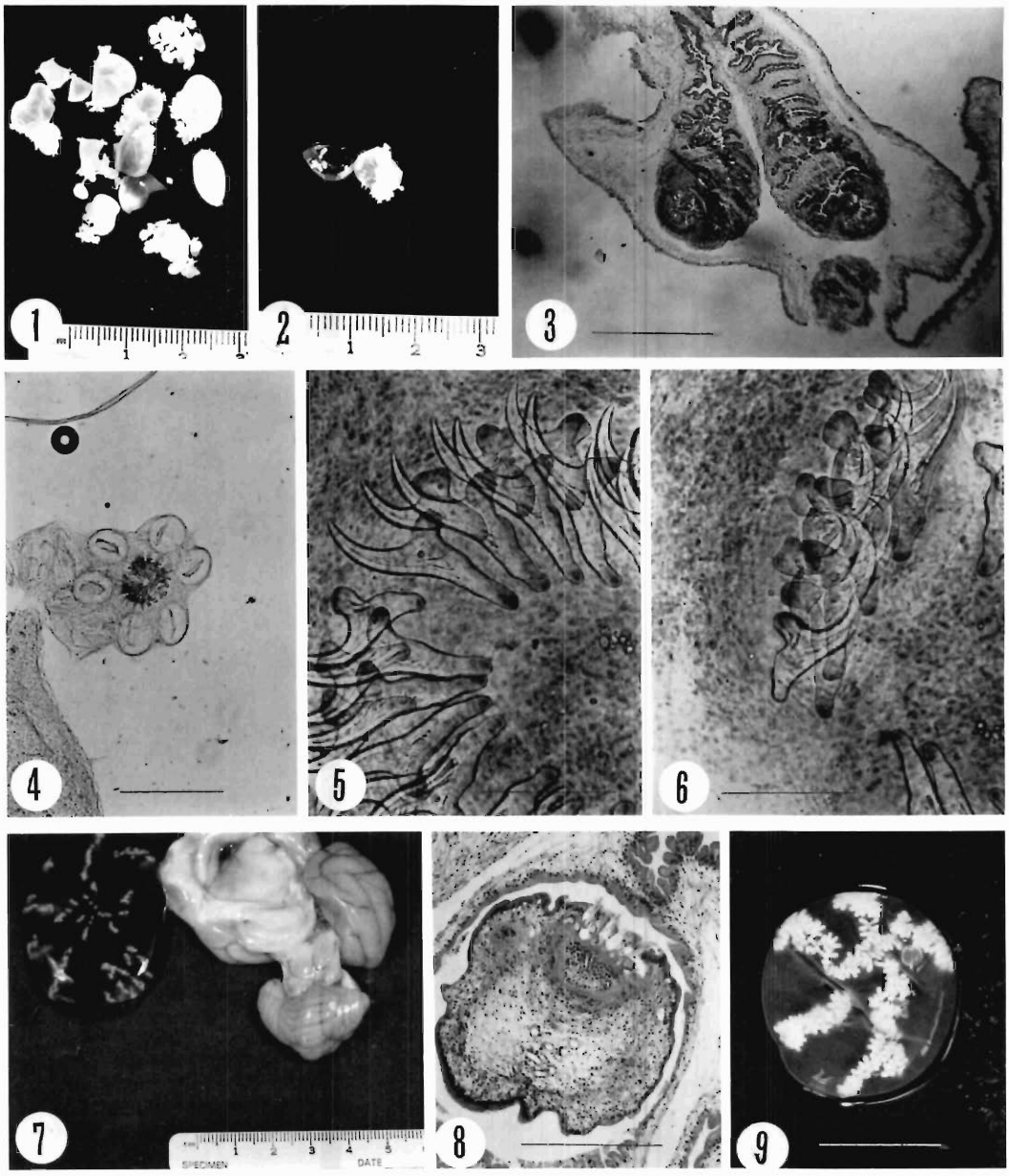

Figures 1-9. Coenuri from cat and jackrabbit brains. 1. Coenuri from brain of 4-yr-old domestic cat (Wyoming Case 1). 2. Single coenurus from WC1 showing larval scolices. 3. Section through scolex of cerebral coenurus, cat; WC1. Scale = 1 mm. 4. Scolex with six suckers from abdominal coenurus, white-tailed jackrabbit. Scale = 1 mm. 5. Rostellar hooks, en face preparation, from cerebral coenurus from cat, WC1. Focus on large hooks. Scale = 100 μm. 6. Rostellar hooks, en face preparation, from cerebral coenurus from cat, WC1. Focus on small hooks. Scale = 100 μm. 7. Coenurus and brain of 6-mo-old domestic cat (Wyoming Case 2). 8. Section through scolex of cerebral coenurus, cat; Alaska. Scale = 0.5 mm. 9. Coenurus from body cavity of white-tailed jackrabbit. Scale = 2 cm.

est of these daughter coenuri was 7 × 3.5 mm. Another parent coenurus, measuring 7 × 5.5 mm had six daughter buds many showing bizarre branching. Two parent coenuri, measuring about 10 × 7 mm, had 27 and 17 small coenuri; these small coenuri measured from 1 to 5 mm. Other coenuri showed similar patterns of budding daughters. Scolices were infrequent and were lacking in some. Where present, they were single or arranged in groups of two to three with sometimes as many as five (Fig. 2). They extended inward within the coenurus, were globular, and were attached to the inner wall of the coenurus by narrow pedicles (Fig. 3).

One scolex with four suckers seen in en face preparation had 30 hooks in two circles of 15 large hooks alternating with 15 small hooks. Another scolex with six suckers was clearly abnormal (see Fig. 4 for similar scolex from white-tailed jackrabbit); 34 hooks were in two circles of 17 large and 17 small hooks. Suckers, somewhat flattened, measured 0.250–0.295 mm. The large hooks measured 142–156 μm (Table 1) and were sinuous with an open blade terminating in a sharp point (Fig. 5); guards were short; the dorsal aspect of the handles was smooth or sometimes sinuous and often showed a bulge before joining the guards. Small hooks measured 95–106 μm (Table 1) and resembled the large hooks except in size and the truncated handle (Fig. 6). Guard lengths were approximately equal in both long and short hooks. Sections of scolices through the region of the rostellum usually showed only two suckers, the other two not being in the plane of section. Sections of hook handles, guards, and blades were seen, although not all parts of any single hook were visible. The outer margin of the introverted scolex was not well defined, whereas the inner cuticle (=tegument) was thick and convoluted (Fig. 3). Regions in the scolex with lacunae surrounded by refractile bounding membranes were interpreted as the former sites of calcareous corpuscles.

Sections of cerebral cortex from WC1 showed a cystic space that had previously contained the coenuri. Adjacent neuropil was slightly compressed, collagen fibers and a few epithelial-like cells were present, and there was an infiltrate of lymphocytes, plasma cells, and macrophages. Many vessels in the area were cuffed by mononuclear inflammatory cells.

The left cerebral hemisphere of WC2 contained a single large coenurus. A thin membrane covered the cyst dorsally within the brain. Cerebral gyri were flattened and there was coning of the cerebellum into the foramen magnum. The coenurus was 5.5 × 4.6 × 0.4 cm when removed from the brain, the scolices were linearly arranged along the inner wall, and there were no daughter coenuri (Fig. 7). For measurements of hooks from WC2 see Table 1.

Microscopic features of the brain were similar to those in WC1 except that collagen did not occur in the cyst wall and adjacent areas of malacia were more extensive. Moderate nonsuppurative meningitis was present.

In the Alaskan case, a 2-cm diameter fluid-filled cyst was in the left occipital cortex. An irregular 1-cm diameter mass within the cystic space had convoluted germinal epithelium from which numerous scolices with fully developed rostellar hooks had arisen (Fig. 8). Large hooks measured 127–137 μm and small hooks about 90 μm (Table 1). Microscopic changes in the brain were similar to WC2.

Scolices in coenuri from the rabbits were arranged linearly and were single or grouped in clusters (Fig. 9). Some were typical with four suckers whereas others showed sucker abnormalities as noted previously (Fig. 4). Numbers of hooks and their measurements from the domestic rabbit and wild hare coenuri are shown in Table 1.

Discussion

There are only two previous reports of cerebral coenuriasis in cats. Georgi et al. (1969) reported the first case in a female domestic cat in New York State; they recovered an extensive coenurus from the left cerebral hemisphere. Clinical signs included vestibular disturbance, left head tilt, circling to the left, nystagmus, and ataxia. Examination of scolices from the coenurus revealed abnormal suckers (six to eight) in some cases; most of the hooks were undeveloped or abnormal. Photographs of some apparently normal hooks show both long and short hooks of a typical taeniid type. Georgi et al. (1969) declined to identify the parasite beyond saying that it was a typical coenurus of *Taenia* (*Multiceps*) probably *serialis* although the immature state of the material precluded specific identification.

Hayes and Creighton (1978) reported the second case of a polycephalic cestode larva in the brain of a castrated male domestic short-haired cat. Personality changes, ataxia, marked extensor

Table 1. Comparative sizes (in μm) of rostellar hooks from coenuri of *Taenia* (*Multiceps*) spp.

	Present study: coenuri from brain of domestic cats		*Taenia* (*Multiceps*)‡ *serialis*		*Taenia* (*Multiceps*)‡ *serialis brauni*		*Taenia* (*Multiceps*)‡ *multiceps*		Coenurus: hares and domestic rabbits	
	L*	S†	L	S	L	S	L	S	L	S
	WC1								Domestic, WY	
Larval hooks										
$\bar{x}$	152	102	156	111	145	108	167	135	148	98
Range	142–156	95–106	145–170	95–125	139–150	102–114	157–177	109–136	135–157	88–105
N	24								27	19
Adult										
$\bar{x}$			165	113	137	96	169	126		
Range			154–175	95–125	125–150	91–102	157–177	98–136		
Number of hooks	30–34		28–34		22–30		22–30		23–34	
	WC2								Domestic, CO	
Larval hooks										
$\bar{x}$	139	103							156	102
Range	130–143	98–105							153–159	96–108
N	15								10	
Number of hooks	30–32								28	
	AK								Jack rabbit, WY	
Larval hooks										
$\bar{x}$	127–137	ca. 90							147	103
Range									140–150	98–110
N									15	
Number of hooks	Not given								28–30	
									Snow-shoe hare, AK	
Larval hooks										
$\bar{x}$									132	88
Range									118–141	77–93
N									15	
Number of hooks									25–31	

* L = large hook sizes.
† S = small hook sizes.
‡ Verster, 1969.

rigidity on falling, left circling, intermittent positional vertical nystagmus, slowed left pupillary reflex, exaggerated bilateral knee-jerk reflexes, and crossed extensor reflexes in all limbs were the predominant signs seen before euthanasia. On necropsy, significant lesions were restricted to the brain; a rounded cavity, 1.5–2 cm, was centered in the left parietal lobe. The cavity contained a coenurus containing about 60 scolices clustered in two main regions of the cyst wall and distributed linearly. Scolices were white and measured up to 0.5 mm long. Scolices were immature and hooks were underdeveloped. These authors speculated that the species in question was *T.* (*M.*) *serialis* (Gervais, 1847) Baillet, 1863.

Our identification of the tapeworm in the present cases as *T.* (*M.*) *serialis* must remain tentative inasmuch as the material available for examination consisted only of the larval cysts with scolices and hooks. It is clear that the parasite is a taeniid tapeworm, and because of the nature of the larval cysts, coenuri showing exogenous budding and multiple scolices can be assigned to the genus *Taenia* subgenus *Multiceps*. The number of hooks and their sizes place this form closest to *T.* (*M.*) *serialis* or to *T.* (*M.*) *s. brauni* known from dogs and other canids, rodents, and primates in Africa and North America (Sandground, 1937; Verster, 1969).

Larval *T.* (*M.*) *serialis* is a parasite of lagomorphs and rarely rodents (Wardle and McLeod, 1952) where coenuri are typically found under the skin and between the muscles, particularly of the hind legs. Adults are found in carnivores, usually canids, but have been reported in cats (Lewis, 1927). Larval stages have been recovered from wild and domestic rabbits in Wyoming and from a hare in Alaska (Honess and Winter, 1956; Dau and Barrett, 1981; Kingston and Honess, 1982; present paper).

Since adult *T.* (*M.*) *serialis* are typically parasites of canids the probability of autoreinfection in the present case is remote. Sources of infection might include tapeworm egg contamination of water, herbage, or a shared litter box. Usually carnivores are protected against larval infection owing to the inability of the eggs to hatch; some unusual digestive condition would have been necessary for the eggs to hatch in the gut of these cats. Reports of *Taenia* (*Multiceps*) causing a fatal ascites in a dog (Voge and Berntzen, 1963) and of *Echinococcus* larvae (hydatid cysts) developing in a dog (Abduladze, 1970) demonstrate the rare occurrence of taeniid eggs hatching in the gut of their usual definitive hosts.

Human infection with larval *Taenia* (*Multiceps*) spp. is infrequent with about 50 cases reported mostly from tropical Africa (Hermos et al., 1970; Orihel et al., 1970). Infection with *T.* (*M.*) *serialis* has been reported in connective tissue and muscle in man in North America (Orihel et al., 1970) and, tentatively, once in the brain of man (Faust, 1949 in Abuladze, 1970). Coenuri identified as *T.* (*M.*) *multiceps* have been found in brain and spinal cord in man and caused neurologic disease and death (Hermos et al., 1970) and "gid" in sheep (Jensen and Swift, 1982) in North America. However, there are no authenticated reports of this parasite having been found in sheep in North America since the 1920's (Becklund, 1970; Jensen and Swift, 1982).

Voucher specimens of the materials discussed in this article have been deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland: Nos. 77683 (cat, Wyoming) and 77684–77686 (rabbits, Wyoming and Colorado).

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Alcataenia pygmaeus sp. n. (Cestoda: Dilepididae) from the Whiskered Auklet, *Aethia pygmaea,* in the Western Aleutian Islands, Alaska, with a Comment on the Genera *Alcataenia* and *Rissotaenia*

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ABSTRACT: *Alcataenia pygmaeus* sp. n. is described from whiskered auklets, *Aethia pygmaea* (Gmelin), from the western Aleutian Islands, Alaska. It is distinguished from its congeners by the overall small size of the strobila and a combination of other characters. The rostellum is armed with 34–38 hooks that measure 41–49 μm in length. The cirrus sac is relatively short, 101–174 μm in length, and reaches or just crosses the poral osmoregulatory canals. There are 36–50 testes located entirely posterior to the female organs. The scolex is deeply embedded in the mucosal tissue of the duodenum of the host. This is the first dilepidid to be recorded from *Aethia pygmaea.* In addition the genus *Rissotaenia* Spasskaia and Kolitolova, 1972 was found to be identical to *Alcataenia* Spasskaia, 1971 and is suppressed as a synonym.

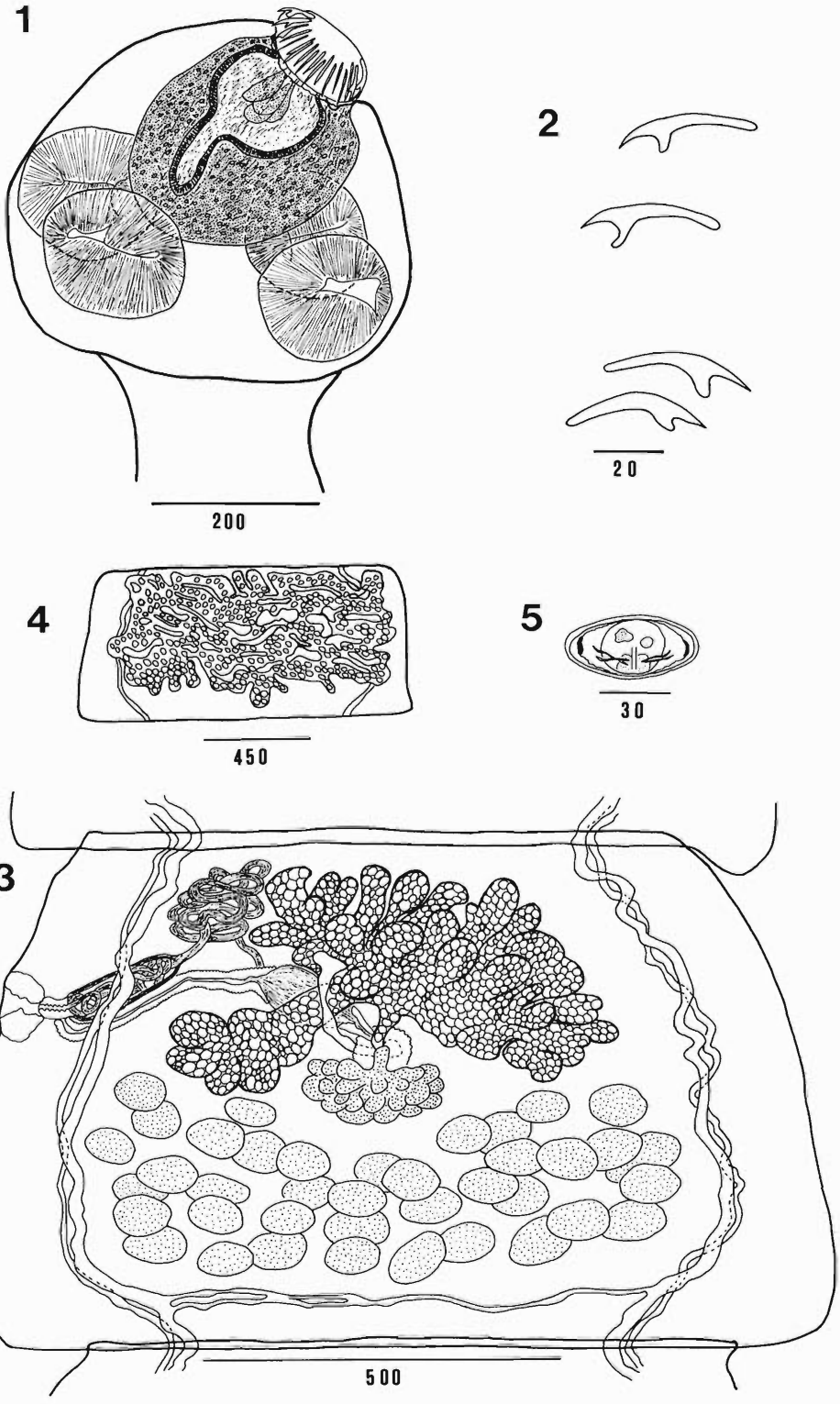
Dilepidid cestodes representing a previously undescribed species of *Alcataenia* Spasskaia, 1971 have been collected from whiskered auklets, *Aethia pygmaea* (Gmelin) (Charadriiformes: Alcidae), in the western Aleutian Islands, Alaska. Immature cestodes were recovered from three birds collected in 1974, and several mature specimens and a single gravid specimen were found in four of five birds collected in 1982. This is the first dilepidid cestode to be described from birds of the genus *Aethia.* In addition only one other cestode, *Diorchis pelagicus* Hoberg, 1982, typically occurs in seabirds of this genus (Hoberg, 1982).

Materials and Methods

In the field, cestodes from auklets were either fixed in boiling 10% formalin (1974) or killed with boiling water prior to fixation with 10% buffered formalin (1982). Portions of the host intestine with worms in situ were also preserved. Cestodes were stained in Semichon's acetic carmine and prepared as whole mounts. Two specimens, fixed in situ, were prepared as 15-μm paraffin sections stained in hematoxylin–eosin. All measurements are in micrometers unless stated otherwise; ranges of measurements are followed by mean values in parentheses. The following description is based on 20 specimens mounted entire, and 2 specimens sectioned in situ.

Alcataenia pygmaeus sp. n. (Figs. 1-5)

DESCRIPTION: Strobila weakly craspidote, up to 16 mm long and with 76 proglottids. Segments wider than long: immature, 200–413 long by 448–590 wide; mature, 531–731 long by 672–1,191 wide; gravid, 879 long by 2,109 wide. Neck short, 156–212 wide at base of scolex. Scolex ($N = 20$) 354–495 (452) in transverse diameter. Suckers ($N = 42$) 153–203 (180) in greater diameter. Rostellum ($N = 12$) strongly muscular, 232–389 (284) long by 120–229 (193) at apex; armed with 34–38 (36) ($N = 15$ scolices) hooks in two regularly alternating rows, measuring 41–49 (43) ($N = 80$). Blade 15.1–20.1 (17.9) long, handle 20.9–30.2 (25.5) long; blade : handle ratio 1:0.54–0.94. Rostellum with minimum of four glandular regions terminating near center of rostellar pad. Rostellar sac 217–261 in diameter containing mass of densely staining tissue with prominent nuclei surrounding base of rostellum. Dorsal excretory canals 4–7.5 in diameter; ventral canals 11–21, connected by transverse canal 6–10 in diameter near posterior border of segment. Occasionally ventral canals joined by multiple anastomoses. Genital pores irregularly alternating. Genital atrium ($N = 14$) muscular, 73–102 long by 58–73 wide, situated in anterior ⅓ of proglottid. Genital ducts passing dorsally to osmoregulatory canals. Genital anlagen visible by 10th segment; testes by about 19th; ovary by about 30th; fully mature by 40th; and first ova appear in uterus about 50th segment. Cirrus sac ($N = 30$) cylindrical, just reaching across poral osmoregulatory canals, 101–174 (130) long by 23–49 (36); containing coiled vas deferens. Cirrus ($N = 11$) unarmed, 14–26 (21) in diameter. Vas deferens highly coiled, dorsal to ovary up to 17 in diameter. Testes ($N = 30$ segments) 36–50 (42) in number; ($N = 50$) 61–81 (71) long by 44–70 (55) situated in two layers entirely posterior to female organs. Vagina ($N = 20$) thick-walled,



14.5–29 in diameter with lumen 3–6; maximum length 213–348 in mature and pregravid segments; entering genital atrium posterior to cirrus sac. Seminal receptacle ($N = 15$) pyriform, 87–174 (137) long by 29–75 (51), situated dorsal to ovary; proximal end with strongly developed sphincter. Ovary highly lobed, with two wings; smaller poral wing joined to larger antiporal wing by narrow isthmus; situated in anterior ½ of proglottid ventral to other female organs except uterus; overall length ($N = 15$) 118–236 (191) by 413–719 (612) in width. Vitelline gland ($N = 12$) lobed, 58–94 (82) long by 131–247 (192) wide. Mehlis gland ($N = 10$) 58–93 (70) in diameter, situated dorsal to vitelline gland. Uterine stem arising from dorsal surface of Mehlis gland, passing to ventral surface of ovary. Uterus initially develops as a flat, coarse, highly lobed reticulum that has extended beyond osmoregulatory canals when first ova appear; eventually assuming a sacculate form when fully gravid. Eggs numerous, ovoid, elongate, outer envelope ($N = 6$) 69–87 (74) long by 46–56 (52); embryophore ($N = 50$) 44–56 (49) long by 22–28 (24), containing ovoid oncosphere ($N = 50$) 23–28 (25) long by 17–22 (20). Walls of embryophore 1.0–2.5 thick. Embryonic hooks ($N = 35$) 12.7–13.9 (13.2) for lateral pairs; 11.6 for median groups.

TYPE HOST: *Aethia pygmaea* (Gmelin), whiskered auklet (Charadriiformes: Alcidae).

LOCALITY: Buldir Island, Alaska (lat. 52°21′N; long. 175°56′E).

HABITAT: Duodenum, deeply embedded in the mucosa.

SPECIMENS: Holotype: USNM Helm. Coll. No. 77546, collected by D. J. Forsell, July 1982. Paratype 1: No. 77547, an early mature specimen collected July 1982. Paratypes 2 and 3: No. 77548, collected July 1974. A supplementary specimen, also listed under No. 77548, is a single worm sectioned in situ.

ETYMOLOGY: The species name *pygmaeus* is derived from the Latin "pygmaea" for "dwarfish" indicating the small size of members of this species. In addition *pygmaea* is the specific name of the type and only known host for this species of cestode.

Systematics and taxonomy

The genus *Alcataenia* Spasskaia, 1971 was established for three species of dilepidid cestodes from Alcidae (Charadriiformes), viz., *A. campylacantha* (Krabbe, 1869) from *Cepphus* spp. (type); *A. armillaris* (Rudolphi, 1810) and *A. meinertzhageni* (Baer, 1956) from *Uria* spp. (Spasskaia, 1971). It was distinguished from *Anomotaenia* Cohn, 1900 on the basis of the distribution of rostellar hooks (in *A. campylacantha* every third hook is in the anterior row); a flat, coarse reticular uterus; a strongly muscular genital atrium; mature eggs that lack polar filaments; and apparent ecological and phylogenetic specialization of the host-group. The genus *Rissotaenia* Spasskaia and Kolitolova, 1972 was established for two species of dilepidids from Laridae, viz., *R. larina* (Krabbe, 1869) (type) and *R. dominicanus* (Railliet and Henry, 1912) [Odening (1982) would also include *R. micrantha* (Krabbe, 1869)] (Spasskaia and Kolitolova, 1972). It was distinguished from *Alcataenia* on the basis of the uterus, a reticulum composed of a series of large loops, and from *Anomotaenia* by having eggs without filaments; a muscular genital atrium; and ecological and phylogenetic relationships of the host-group. The genus *Anomotaenia* in charadriiform birds was thought to represent cestodes that had a reticulate uterus of a form different from that reported for either *Alcataenia* or *Rissotaenia*; a weakly developed genital atrium; and eggs with polar filaments (Spasskaia and Spasskii, 1978).

Based on recent studies of cestodes from alcids and larids in the North Pacific Ocean and Bering Sea, and on a reexamination of Krabbe's (1869) type series of *A. campylacantha, A. socialis* (a synonym of *A. armillaris*), *R. larina,* and *R. micracantha,* Baer's (1956) types of *A. meinertzhageni* and additional specimens of *A. armillaris, A. campylacantha, R. larina,* and *R. micracantha* redescribed by Baer (1956) it was apparent that the uterus was identical in structure in those taxa that represent *Alcataenia* and *Rissotaenia.* As the distribution of hooks on the rostellum of *A. campylacantha* has taxonomic

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Figures 1-5. ***Alcataenia pygmaeus*** **sp. n. 1. Scolex. 2. Rostellar hooks. 3. Ventral view of mature segment. 4. Early development of the uterus showing reticular structure. 5. Oncosphere contained within thick-walled embryophore. All scales in micrometers.**

significance only at the specific level, there are no morphological differences that can be used to differentiate these two genera. Supposed ecological isolation of the primary host-groups, although not an acceptable taxonomic character, is also not constant because at least two species, *A. armillaris* and *R. larina* have the same species of euphausiid crustacean as an intermediate host (Shimazu, 1975). Consequently *Rissotaenia* is suppressed as a synonym of *Alcataenia*. Odening (1982) already pointed out the similarity of *A. meinertzhageni* to species placed in the genus *Rissotaenia*.

The genus *Alcataenia* contains seven nominal taxa: *A. campylacantha* (type), *A. armillaris, A. meinertzhageni,* and *A. pygmaeus* sp. n. from alcids, and *A. larina* comb. n., *A. micracantha* comb. n. sensu Odening (1982), and *A. dominicanus* comb. n. sensu Odening (1982) from larids. *Alcataenia* is considered valid pending completion of a more detailed analysis of this genus and its relationships to *Anomotaenia* (Hoberg, in prep.). Morphological characteristics defined by Spasskaia and Spasskii (1978) for *Anomotaenia* indicate that it is probably distinct from *Alcataenia*.

Alcataenia pygmaeus sp. n. is clearly distinguished from all other species of *Alcataenia* by its small size (16 mm), consistently smaller organs, and relatively few segments (76) when gravid. Representatives of species of *Alcataenia* are generally large, with several hundred segments, and range in length from 40–80 mm for *A. larina,* 47–65 mm for *A. dominicanus,* 80–120 mm for *A. micracantha,* about 80 mm for *A. meinertzhageni,* 50–60 mm for *A. campylacantha,* and greater than 42 mm for *A. armillaris* (Schiller, 1951; Baer, 1956; Odening, 1982). The cirrus sac, 101–174 μm in length, is shorter than that in *A. armillaris* (204–274), *A. meinertzhageni* (160–182), *A. micracantha* (up to 216), *A. dominicanus* (155–266), and *A. larina* (>240). The number of testes (36–50) is greater than that reported for *A. micracantha* (13–18), *A. campylacantha* (15–20), and *A. dominicanus* (up to 28); about equal to that reported for *A. armillaris* (33–40) and *A. larina* (up to 40); and less than the number reported for *A. meinertzhageni* (53–56). The rostellar hooks are similar in form to those of other species in the genus. However, *A. pygmaeus* has 34–38 hooks that measure 41–49 μm. In this respect it is most similar to *A. armillaris* that has 20–25 hooks 35–46 μm in length in specimens from the North Atlantic, and 20–22 hooks 40–45 μm in length for specimens from the western North Pacific (Krabbe, 1869; Baer, 1956; Spasskaia and Kolitolova, 1971). *A. pygmaeus* is also clearly distinguished from the remaining species of *Alcataenia* in which the maximum number of hooks in representatives of any taxon is 30.

Pathology

The scolex of specimens of *A. pygmaeus* is characteristically deeply embedded in the mucosal tissue of the duodenum of its host. Among pelagic seabirds, this situation has been reported for three other species of cestodes, viz., *A. armillaris* and *A. meinertzhageni* in *Uria* spp. (Krabbe, 1869; Baer, 1956) and *Parorchites zederi* (Baird, 1853) in penguins, *Aptenodytes forsteri, Pygoscelis adeliae, P. papua,* and *P. antarctica* (Fuhrmann, 1921; Ippen et al., 1981). In infections with *A. pygmaeus,* the cestodes are embedded individually rather than occurring in groups as has been reported in other avian species. The lesions associated with *A. pygmaeus* are also not as extensive. Lesions associated with infections of other species of *Alcataenia* or *Parorchites* are often massive and may cause a noticeable swelling on the serosal surface of the intestine. With infections of *Parorchites,* the intestine may be perforated and adhesions may develop (Hoberg, pers. obs.). On the mucosal surface there is often a fibrous collar that surrounds the neck of the cestode or group of cestodes at the point of insertion. In *A. pygmaeus,* there was no evidence of infection on the serosal surface, and the cestodes were never surrounded by a fibrous collar at the point of insertion. The scolex was enclosed in a deep cavity lined with a thin wall of fibrous connective tissue. The scolex sometimes extended to the depth of the muscularis mucosae. The surrounding tissue was densely infiltrated with lymphoid cells, predominately lymphocytes and plasma cells. Heterophils and histiocytes were occasionally present. The lesions were extensive; infiltration and fibrosis extended through the muscularis mucosae into the muscularis externa and was accompanied by some erosion of the intestinal epithelium. The lesions appear to be of a chronic nature and were similar to those reported by Baer (1956) for *A. meinertzhageni.*

Discussion

This is the second record of cestodes from the whiskered auklet. It is of interest that *A. pygmaeus* was not found in a sample of 19 crested auklets, *Aethia cristatella* collected from localities in the western Aleutian Islands where the

two avian species are sympatric. Additional specimens of this ecologically similar auklet were examined from St. Lawrence (20 birds) and St. Matthew Islands (11) in the Bering Sea where whiskered auklets do not occur. Of the 50 crested auklets examined from all localities, seven were infected with two other species of *Alcataenia* but not *A. pygmaeus*. Specimens of least auklets, *Aethia pusilla* from St. Lawrence (26) and St. Matthew Islands (24) were not infected with any species of dilepidid cestode. However, Smetanina (1979) found specimens of *Alcataenia armillaris* infecting least auklets near Vladivostok, USSR. Previous studies indicated that whiskered and crested auklets share the hymenolepidid cestode *Diorchis pelagicus* Hoberg, 1982 only where they co-occur in the western Aleutian Islands. This suggests that *A. pygmaeus* has a limited geographic distribution, and is probably a host-specific parasite of the whiskered auklet.

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Sporulation of *Eimeria tenella* (Coccidia) Oocysts Revealed by Scanning Electron Microscopy[1]

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ABSTRACT: Using scanning electron microscopy, we studied the process of sporulation of *Eimeria tenella* oocysts. By means of a procedure involving double sticky tape, we opened oocysts at hourly intervals until sporulation was complete. The cytoplasm of freshly passed oocysts consisted largely of amylopectin granules. Changes during sporulation consisted of contraction of the cytoplasmic mass from the oocyst wall, the appearance of a matrix embedding the amylopectin granules, formation of an outer surface over the cytoplasm, and cytokinesis that resulted in the formation of four sporoblasts. The sporoblasts became triangular, their edges thickened, and they then elongated into sporocysts within the oocyst. We did not see the formation of sporozoites.

Using light and interference microscopy, Canning and Anwar (1968) and Wagenbach and Burns (1969) presented detailed descriptions of the structural changes occurring in sporulating *Eimeria tenella* oocysts, but the process of sporulation has not been followed by scanning electron microscopy. In this study we examined the cytoplasmic changes occurring in sporulating *E. tenella* oocysts by fixing and processing oocysts at 1-hr intervals for viewing and study with an AMR 1000A scanning electron microscope.

Materials and Methods

We performed two trials, one with strain LS-18 (Merck & Co.) and the other with Lilly-65 (Eli Lilly Co.). In both trials we obtained oocysts by scraping the ceca of infected White Leghorn chicks, approximately 4 wk of age, 7 days (LS-18) or 8 days (Lilly-65) postinfection. We homogenized the cecal cores in a Waring blender for 1 min, followed by pepsin digestion according to Rikimaru et al. (1961). We then brought the contents to a total volume of 2 liters with tap water adjusted to a pH of 7.5 and allowed the free oocysts to settle overnight at 4°C. After removing the supernatant fluid by aspiration, we centrifuged the oocysts at 200 *g* for 10 min and resuspended them in 100 ml of 0.5% $K_2Cr_2O_7$. To enhance sporulation, we bubbled the solution with air at approximately 22°C until sporulation was complete (40–48 hr). Because the oocysts could begin sporulation immediately upon removal from the chicks, we withdrew 2-ml aliquots at hourly intervals from the start of pepsin digestion until sporulation was completed at 48 hr. We centrifuged aliquots at 200 *g* for 10 min, resuspended the sediments in double distilled water, centrifuged again at 200 *g* for 10 min, and resuspended the sediment in 3.0% glutaraldehyde in sample tubes at 4°C.

We held the oocysts in glutaraldehyde for a minimum of 35 min and a maximum of 3 days. After fixation each aliquot of oocysts was drawn into a 5-ml syringe through a 4-inch 14-gauge cannula that was then replaced with a 13-mm syringe filter containing a type HA 0.45-μm-pore membrane (Millipore Corp.). Throughout the fixing process the oocysts remained on the filter membrane as the fluids were discharged through the syringe. To change fluid without altering the oocysts or rupturing the membrane from reverse flow, we removed the filter containing the oocysts, reaffixed the cannula, and aspirated the next fluid into the syringe. We expelled the fluid through the filter and oocysts again.

Two 5-min rinses in double distilled water followed glutaraldehyde fixation. Postfixation was with 1.0% OsO_4 for 10 min followed by three 5-min double distilled water rinses. We dehydrated the samples by suspension for 10 min in 30%, 50%, 70%, 75%, 80%, 85%, 90%, 95%, and three 100% ethanol treatments.

We excysted sporozoites according to Doran and Farr (1962) as just described.

After dehydration, we removed the filter membranes containing the oocysts from the filters and critical-point dried them with a 10-min exchange period for the CO_2. We mounted these membranes on pin-type mounts, oocyst side up and then took 2 cm^2 of double sticky tape (3M Co.), formed a loop, and holding the tape with forceps we gently touched it to the surface of the filter. The oocysts that adhered to the loop were fractured by compression between the loop and another 2-cm^2 segment of double sticky tape mounted on a pin-type stubmount. We peeled the top tape from the bottom one, inverted it, and mounted it next to the bottom tape segment. We sputter-coated all mounts with 200 Å of gold-palladium for 4 min and then examined them in the scanning electron microscope. The filter membrane mounts provided a check on oocyst population and condition before fracturing. We took photographs with Polaroid type 55 positive-negative film.

Results

We verified the results of Nyberg and Knapp (1978a, b) that oocyst structure was not signifi-

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cantly affected by the glutaraldehyde within a period of several days. The wall of the freshly passed oocysts appeared smooth with no micropyle evident (Fig. 1). After sporulation, in some instances internal features were perceptible (though not clearly distinguishable) when higher voltages were applied (Fig. 17). The cytoplasm of freshly passed oocysts consisted largely of numerous granules, approximately 0.67 μm ($\pm$0.5 nm) long, identified by Wang et al. (1975) as amylopectin (Figs. 2, 3). Initial changes during sporulation consisted of a contraction or shrinkage of the cytoplasmic mass (Fig. 3) away from the oocyst wall, and the appearance of a matrix embedding some of the amylopectin granules (Fig. 4), although in some oocysts many internal granules remained separate and free from one another. We also noted short fiberlike structures throughout the granules (Fig. 4). As sporulation progressed, the outer surface of the cytoplasm formed a distinct but discontinuous cover that separated the cytoplasm clearly from its oocyst wall (Figs. 5, 6).

The first indication of cytokinesis was evident after approximately 20–22 hr. Cytokinesis was complete with the final separation into individual sporoblasts (Fig. 9) that soon appeared as rather uniform spheres (Fig. 10).

The individual sporoblasts then became more flattened and triangular (Fig. 11) after approximately 24 hr of sporulation, followed by thickening of their outer edges (Fig. 12) and the formation of distinct membranes (Fig. 13). Many large pores were present at this stage of development (Figs. 11, 13). As the sporozoites developed, the sporoblasts thickened (Fig. 14) and elongated into sporocysts (Fig. 15) with smooth, firm walls (Fig. 16). The oocyst walls remained smooth within and without (Figs. 6–9, 11, 14), and were sufficiently thin and translucent that individual sporocysts (Fig. 17) could be seen under high voltage electron microscopy. We did not see stieda bodies clearly although we noted projections (Figs. 15, 17) that corresponded closely in location and shape with these structures. When we excysted intact sporulated oocysts (Fig. 17), typical sporozoites (Fig. 18) emerged.

Discussion

The stages of sporulation of *Eimeria tenella* described in this paper corresponded well with the description of sporulation in *E. maxima* by Canning and Anwar (1968) and with that of Wagenbach and Burns (1969) for *E. tenella,* except that certain internal structures revealed by phase microscopy were not apparent by scanning electron microscopy. Scanning EM showed surface structures more readily than light microscopy and contributed an interesting and different view of the remarkable process of sporulation. We did not see distinct nuclei, chromosomes, or micropyles, nor did we observe the meiotic spindle reported by Canning and Anwar (1968) and Wagenbach and Burns (1969).

Because we sporulated the oocysts at approximately 22°C, sporulation was more prolonged than that reported by Canning and Anwar (1968) and Wagenbach and Burns (1969). We noted a synchrony of events to some extent but not all oocysts sporulated at the same rate and assigning specific changes in development to specified hours of sporulation was difficult.

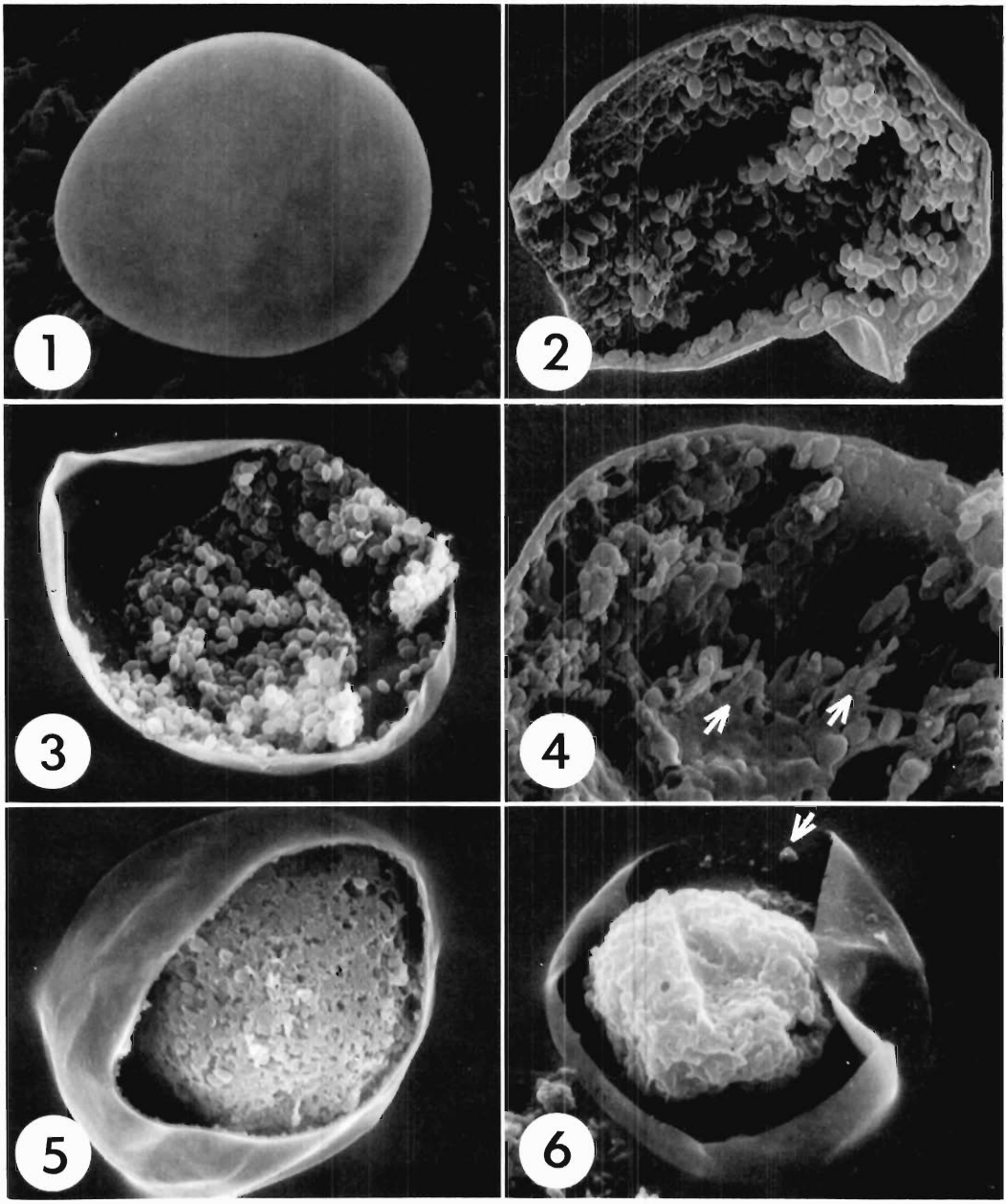
The granules present in the early oocysts corresponded in shape and size to the amylopectin granules reported by Wang et al. (1975), which gradually diminished in number during sporulation and provided an energy source for the process.

The nature and source of the embedding matrix (Fig. 3) was unclear, but it may have been the lipid or lipid complex that Wagenbach and Burns (1969) separated during high-speed centrifugation. The significance of the fibrous, rodlike structures (Fig. 4) was not clear and we could only speculate they might be associated with the amylopectin granules or perhaps be integrated into the envelope covering the sporoblasts and sporocysts yet to form. Canning and Anwar (1968) described filamentous chromosome material at approximately this time in sporulation, but the structures we saw were not enclosed within a nuclear membrane (Fig. 4).

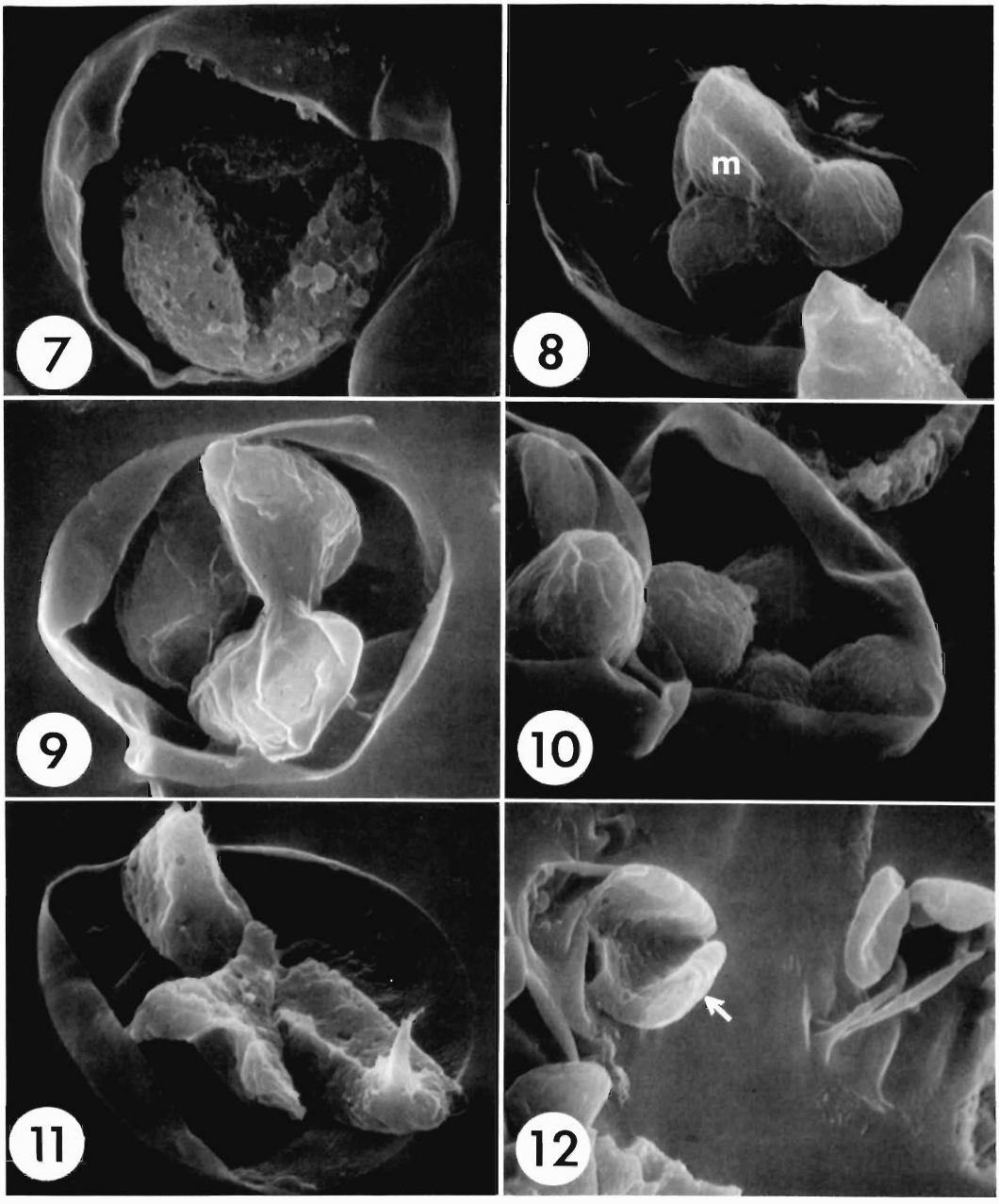
We saw no cytoplasmic vesicles as described by Wagenbach and Burns (1969) on the surface of the zygote, but during early cytokinesis we noted the appearance of porelike structures (Figs. 6, 7) that were soon obliterated by the membranes of the sporoblasts. Pores reappeared again in the triangular stage (Figs. 11, 13), but their significance was not apparent.

Cytokinesis appeared first as an invagination of the cytoplasmic mass (Fig. 6), progressing rather rapidly (Figs. 7–9) to completion (Fig. 10). In late cytokinesis distinct overlying shroud or membrane covered the developing sporoblasts (Figs. 8–10) and may have been incorporated into their walls.

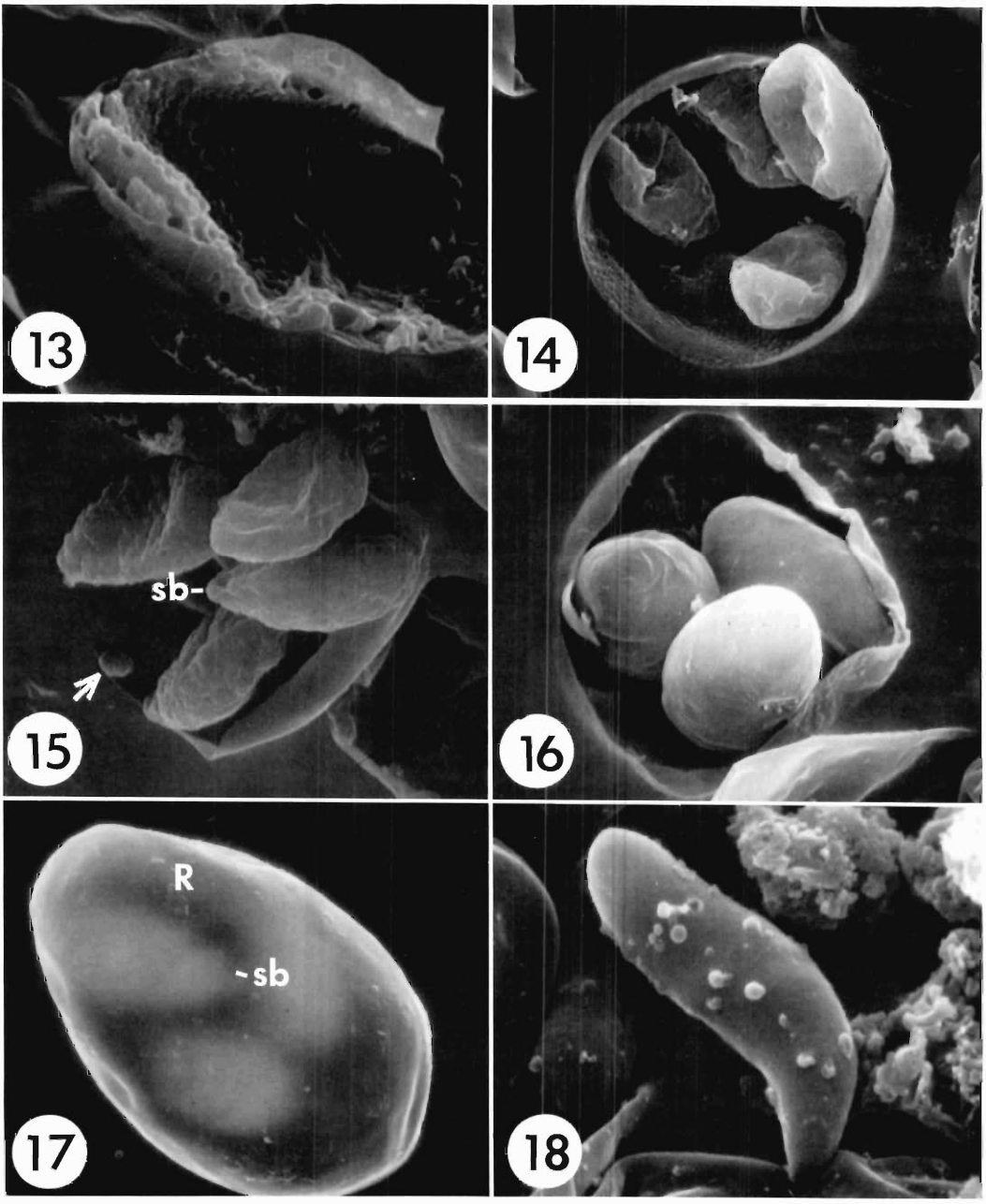
One of the more confusing issues that arose



Figures 1–6. Scanning electron micrographs of *E. tenella* undergoing sporulation. 1. Freshly passed intact oocyst before being opened. 0 hr, ×3,628. 2. Freshly passed oocyst, broken open, containing cytoplasm with amylopectin granules throughout. 0 hr, ×4,700. 3. Oocyst showing cytoplasm shrinking away from oocyst wall. 2 hr, ×5,260. 4. Oocyst showing amylopectin granules embedded in cytoplasmic matrix containing numerous fiberlike projections (arrows). 4 hr, ×11,000. 5. Oocyst showing progressive development of a distinct porous surface of the cytoplasmic mass, separating it from the wall of the oocyst. 8 hr, ×5,500. 6. Early cytokinesis showing the invagination of the cytoplasmic mass. The surface material is more dense with distinct openings. Note polar body (arrow). 12 hr, ×3,628. The time in hours of sporulation is approximate for all figures.



Figures 7-12. Scanning electron micrographs of *E. tenella* undergoing sporulation. 7. Oocyst in intermediate cytokinesis. Note pores in cytoplasm. 13 hr, ×4,346. 8. Late cytokinesis with four sporoblasts forming. Note distinct overlying membrane (m). 14 hr, ×5,250. 9. Cytokinesis nearly complete into sporoblasts. Membrane is still evident. 20 hr, ×4,440. 10. Sporoblasts have separated completely in oocyst on left. Note membrane over sporoblasts in oocyst on right, perhaps incomplete cytokinesis. 22 hr, ×5,360. 11. Triangular sporoblasts. Narrow end may become stieda body. Distinct membrane not apparent. 24 hr, ×3,900. 12. Triangular sporoblast with primordial sporozoites (arrow) forming from thickening periphery. 25 hr, ×3,430. The time in hours of sporulation is approximate for all figures.



Figures 13–18. Scanning electron micrographs of *E. tenella* undergoing sporulation. 13. Sporoblast showing numerous pores. 26 hr, ×3,870. 14. Two of the four sporoblasts are beginning to thicken. Note inner wall of oocyst. 28 hr, ×3,430. 15. Sporoblasts have elongated to sporocysts. End with stieda body (sb) can be recognized. Note polar body (arrow). 28 hr, ×2,681. 16. Three of four mature sporocysts, completely formed with smooth walls. 30–36 hr, ×4,151. 17. Three of four sporocysts, one with stieda body (sb), showing through the oocyst wall. 38 hr, × 3,604. 18. Excysted sporozoite. ×8,365. The time in hours of sporulation is approximate for all figures.

during this study was the appearance and disappearance of a distinct membrane around the sporoblasts. We did not see membranes form but one must have been present throughout cytokinesis (Figs. 6, 7), not just at its completion (Figs. 8, 9). The distinct membrane apparent in Figures 8 and 9 disappeared at the triangular stage (Fig. 11) and was either replaced by or incorporated into another membrane for each specific sporoblast (Fig. 13), preceding the formation of the walls of the sporocysts (Figs. 14–16).

The transition from the spherical sporoblasts to the flatter triangular form (Fig. 11) was rapid, followed by a pronounced thickening of its edges (Fig. 12), which apparently were destined to become the sporozoites. The more pointed end of the triangle perhaps will become the stieda body.

Our methods did not reveal the process of sporozoite maturation except that the sporozoite primordia appeared to be the distinct peripheral folds seen in the late triangle stages.

In two instances we saw what we interpreted to be polar bodies (Figs. 6, 15). Throughout sporulation the internal wall of the oocysts appeared smooth.

As the sporoblasts matured to sporocysts, they elongated with a distinct anterior end appearing as the stieda body (Fig. 15). Viable motile sporozoites emerged (Fig. 18) following excystation in vitro.

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The Parasitic Crustaceans of Fishes from the Brazilian Amazon. 9. *Ergasilus callophysus* sp. n. (Copepoda: Cyclopoida) from *Callophysus macropterus*

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ABSTRACT: *Ergasilus callophysus* sp. n. (Copepoda: Cyclopoida) is described from the gills of a fish, *Callophysus macropterus* (Lichtenstein), taken in the Amazon river near Manaus, Amazonas, Brazil. The new species has a curved pectinate seta on the first exopod that indicates a relationship to two previously described Amazonian species, namely: *E. bryconis* Thatcher, 1981 and *E. jaraquensis* Thatcher and Robertson, 1982. The new species has toothlike sensilla on the second and third antennal segments, however, which the other two species lack. *E. callophysus* sp. n. also bears a superficial resemblance to *E. cerastes* Roberts, 1969, but differs from this species in having a curved pectinate seta on the first exopod, a long fifth leg that reaches to the second abdominal segment, a reduced maxillule without setae, bifid mandible and palp, and a long third abdominal segment.

Up to the present time, the following three species of *Ergasilus* have been reported from the Brazilian Amazon: *E. bryconis* Thatcher, 1981; *E. leporinidis* Thatcher, 1981; *E. jaraquensis* Thatcher and Robertson, 1982; and *E. colomesus* Thatcher and Boeger, 1983. Additionally, Cressey and Collette (1970) identified some specimens from a needlefish (Belonidae) taken at Gurupá, Pará, Brazil as belonging to *E. orientalis* Yamaguti, 1939. In view of the high degree of host specificity found in Amazonian ergasilids by Thatcher and Boeger (1983), the presence here of a Japanese species seems doubtful. The present paper presents the description of the fifth verified Amazonian species.

Materials and Methods

Living ergasilids were killed and fixed in 70% alcohol. The methods used in their preparation and study were those explained in Thatcher (1981) and in Thatcher and Robertson (1982). Color determinations were made with reference to Smithe (1974). Drawings were made with the aid of a camera lucida and measurements with an ocular micrometer. All measurements are expressed in micrometers.

Systematic Section

Ergasilidae Nordmann, 1832

Ergasilus callophysus sp. n. (Figs. 1–10)

HOST: *Callophysus macropterus* (Lichtenstein); Siluriformes.

SITE: Gill filaments.

MALE: Unknown.

LOCALITY: Amazon River, near Manaus, Amazonas, Brazil.

HOLOTYPE (female): Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas, Brazil, No. PA-237-1.

PARATYPES: INPA, Nos. PA-237-2 to PA-237-7; Museu de Zoologia da Universidade de São Paulo, S.P., Brazil, Nos. 3016, 3017; and U.S. Nat. Mus. No. 204987.

ETYMOLOGY: The generic name of the host was used as the species name.

SPECIES DIAGNOSIS (based on 22 specimens studied and 10 measured; Tables 1 and 2): Cephalothorax tapering posteriorly; rounded anteriorly; head fused with first two thoracic segments (Fig. 1). Eye prominent, bifurcate posteriorly, color cobalt blue (Color 68 of Smithe, 1974). Body pigmentation ventrally located, smalt blue (Color 69); pigment distribution indicated by dark areas in Figures 1, 2, 5–7, and 9.

Antennae (Figs. 2, 5; Table 2). Antennule of six segments, bearing simple setae, principally on anteroventral face; setal formula = 1-11-4-4-2-7; total = 29. Prehensile antenna four-segmented; basal segment subtriangular; second segment elongate, with toothlike sensillum located about midway on anterior margin; third segment elongate, slightly curved, with toothlike sensillum near distal end; segment 4 (claw) elongate, curved and sharply pointed, with simple sensillum distally on medial margin: ratio of segmental lengths = 1:2.5:2:1.

Thorax (including genital segment) of five free segments (Fig. 1). Segment 6 bearing vestigial fifth legs. Genital segment subrectangular (Fig. 6).

Abdomen (Fig. 6) three-segmented; first two segments with pigmentation. Each uropod with

Table 1. Measurements (μm) of 10 adult females of *Ergasilus callophysus* sp. n.

	Length	Width
Body (less caudal setae)	800–900 (860)	200–280 (239)
Cephalothorax	410–480 (441)	200–280 (239)
Free thoracic segments:		
3	65–88 (78)	173–225 (192)
4	70–88 (78)	130–163 (142)
5	50–68 (60)	73–125 (107)
6	25–33 (29)	75–98 (81)
7 (genital)	63–70 (66)	68–105 (86)
Abdominal segments:		
1	20–25 (23)	60–65 (63)
2	15–20 (17)	53–60 (57)
3	23–28 (25)	48–55 (52)
Uropods	28–43 (38)	20–23 (21)
Leg 5	68–88 (75)	—
Eggs sac	360–470 (384)	50–70 (61)
Caudal filament	230–340 (295)	—

Table 2. Antennal measurements (μm) of 10 adult females of *Ergasilus callophysus* sp. n.

	Length	Width
Antenna I (antennule)	130–170 (148)	20–30 (28)
Antenna II (prehensile)		
Segment 1	70–100 (91)	50–70 (56)
Segment 2	210–230 (225)	40–50 (47)
Segment 3	170–200 (188)	30–50 (38)
Segment 4	90–110 (102)	20

one long, one medium, and two short setae; with few spinules ventrally (Fig. 4).

Mouthparts (Fig. 10). Mandible two-segmented; bifid terminally; terminal segment bristled: palp bifid and bristled: maxillule simple, without setae: maxilla bifid and bristled.

Legs (Figs. 7–9; Table 3). Leg 1 (Fig.7); endopod two-segmented, exopod three-segmented; first endopodal segment laterally pectinate and with one plumose medial seta; terminal endopodal segment laterally pectinate and with two large rasplike spines and five short plumose seta: first exopodal segment with single posterolateral spine; second segment with a single medial plumose seta; terminal segment with two large rasplike spines, one curved pectinate seta, and four plumose setae. Legs 2 and 3 (Fig. 8) closely similar, all rami three-segmented; endopodal segment 1 serrate and sparsely pilose laterally and with a single plumose setae medially; endopodal segment 2 serrate laterally and with two plumose seta medially; endopodal segment 3 pectinate laterally and with four plumose setae medially; exopodal segment 1 pilose medially and with a single posterolateral spine; exopodal segment 2 pectinate laterally and with a single plumose seta medially; exopodal segment 3 pectinate laterally and with one large rasplike spine and six plumose setae. Leg 4 (Fig. 9); endopod of three segments and exopod of two; endopodal segment 1 pilose laterally and with a single plumose seta medially; endopodal segment 2 with two plumose setae medially; endopodal segment 3 with one large spine and three plumose setae; exopodal segment 1 pilose medially and with one posterolateral spine; exopodal segment 2 with one large spine and four plumose setae. Leg 5 (Fig. 6) of two long simple setae; dorsal seta longer; extends posteriorly to level of second abdominal segment.

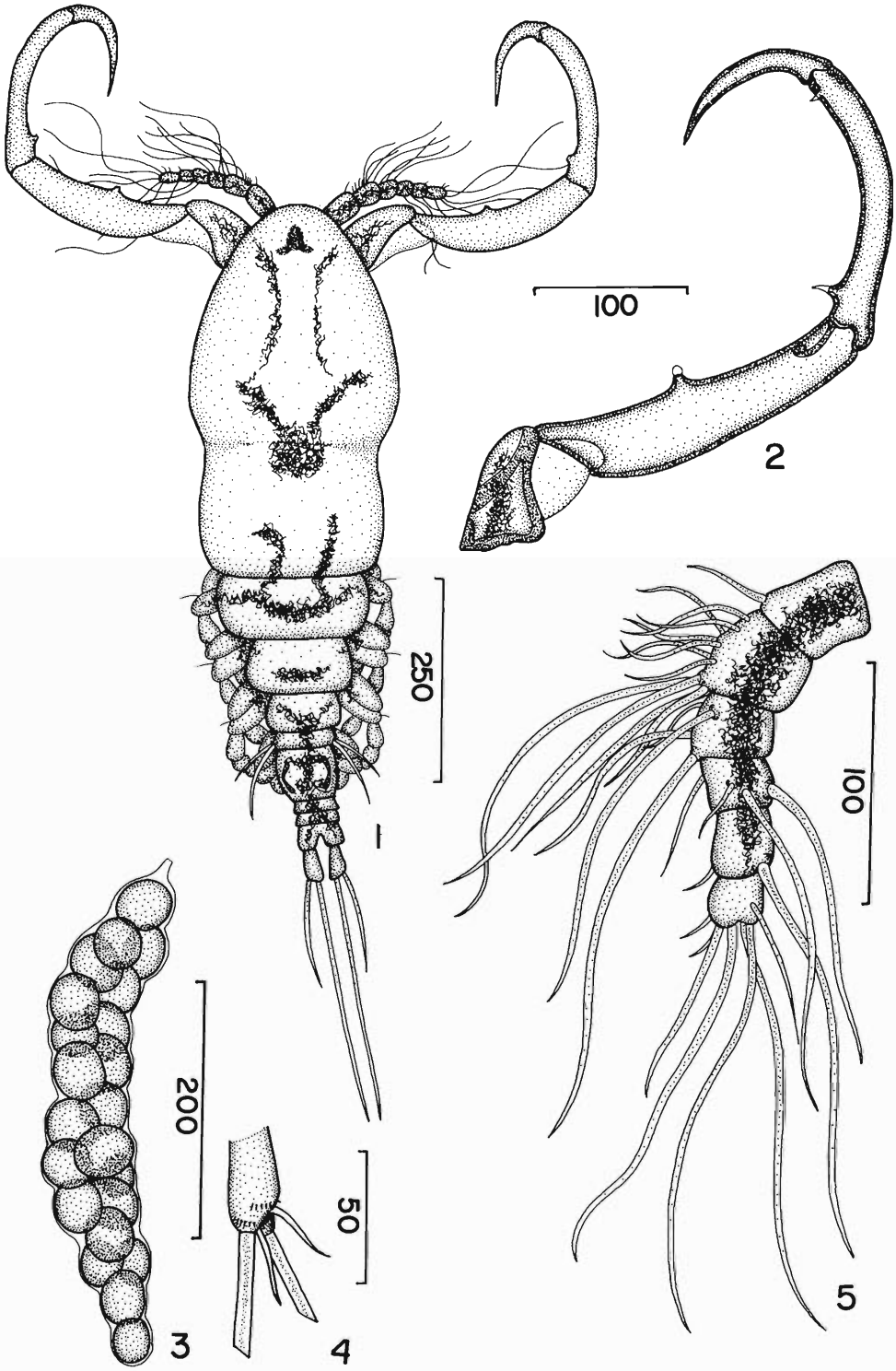
Egg sac (Fig. 3) elongate, with 20–50 spherical eggs.

Discussion

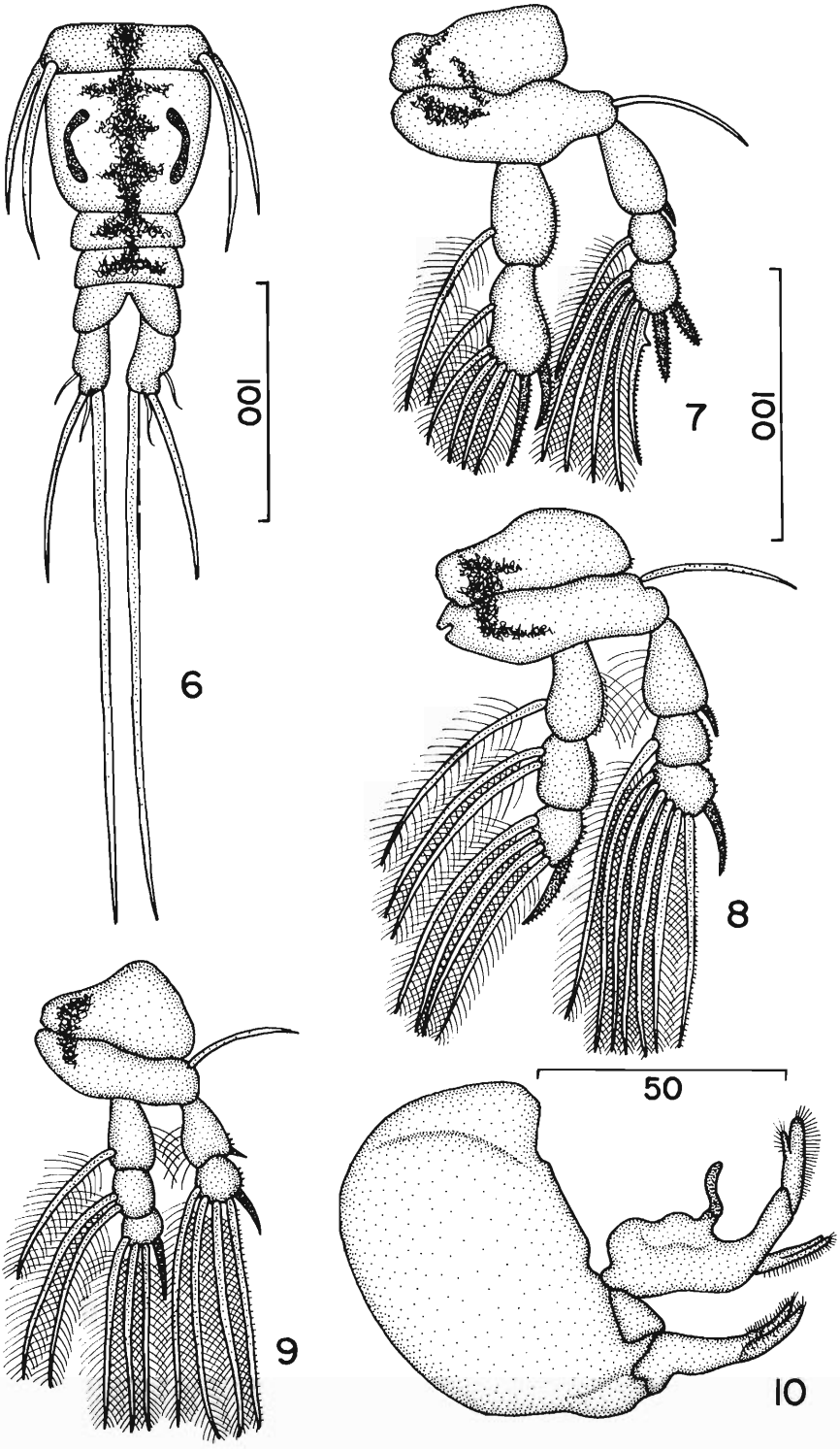
Ergasilus callophysus sp. n. has a curved and pectinate seta on the first exopod that suggests a relationship to the Amazonian species, *E. bryconis* Thatcher, 1981 and *E. jaraquensis* Thatcher and Robertson, 1982. The projecting, toothlike sensilla on the second and third segments of the prehensile antenna of *E. callophysus* sp. n. serve to separate it from the other two. Among North American ergasilids, *E. cerastes* Roberts, 1969, shows some similarity to *E. callophysus* sp. n. but these species can be distinguished by the following characters: (1) The new species has a curved pectinate seta on the first exopod, which *E. cerastes* lacks. (2) The fifth leg of *E. callophysus* sp. n. is long and reaches to the second abdominal segment, whereas that of *E. cerastes*

Table 3. Relationships of spines to setae on the legs of *Ergasilus callophysus* sp. n.

	Endopod	Exopod
Leg 1	0-1, II-5	I-0, 0-1, II-5
Leg 2	0-1, 0-2, I-4	I-0, 0-1, I-6
Leg 3	0-1, 0-2, I-4	I-0, 0-I, I-6
Leg 4	0-1, 0-2, I-3	I-0, I-4



Figures 1–5. ***Ergasilus callophysus*** **sp. n. (female). 1. Dorsal view. 2. Antenna. 3. Egg sac. 4. Ventral view of uropod. 5. Antennule.**



Figures 6–10. ***Ergasilus callophysus*** **sp. n. (female). 6. Genital segment, abdomen, and uropods. 7. Leg 1. 8. Leg 2 (=Leg 3). 9. Leg 4. 10. Mouthparts.**

is shorter, reaching only to the middle of the genital segment. (3) The maxillule of *E. cerastes* is provided with two setae which are lacking in the new species. (4) In *E. callophysus* sp. n., the mandible, palp, and maxilla are all bifid, whereas in *E. cerastes* only the maxilla is bifid. (5) The third abdominal segment of the new species is longer than that of *E. cerastes*.

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Research Note

Effect of Different Concentrations of Formalin on the Preservation of *Trichostrongylus retortaeformis* (Zeder, 1800) (Nematoda)

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Three to five percent formalin (1.2–2.0% formaldehyde) is widely recommended as a preservative for helminths (Anon., 1971, MAFF Tech. Bull. 18:1285–1291) but recent reports have cast doubts on the safety of this chemical (Perera and Petito, 1982, Science 216:1285–1291). Boag (1982, Nematol. Medit. 10:115–118) indicated that the recommended concentration of formalin and the preservative T.A.F. (triethanolamine formalin) Courtney (Polley and Miller, 1955, Pl. Dis. Repr. 39:570–571) could be much reduced without any adverse affects on the morphology or morphometrics of plant parasitic nematodes. This research note reports the results of reduced concentrations of formalin on the gastrointestinal nematode *Trichostrongylus retortaeformis*.

To reduce morphological variability due to the age of the host etc. (Lancaster, 1968, Vet. Rec. 82:674–675) adult female *T. retortaeformis* were collected from a single freshly killed wild European rabbit *Oryctolagus cuniculus* (L.). One hundred and twenty randomly selected nematodes were removed from the gastrointestinal contents and batches of 10 were allocated to 7.5 × 2.5-cm tubes that contained 5 ml of the following range of different concentrations of formalin: 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19, 0.10, and 0.05%. After 4 mo at 20°C the nematodes were taken from the tubes, examined, and measured.

Table 1. Effect of formalin on the morphometrics of *Trichostrongylus retortaeformis*

Parameter measured	Concentration of formalin* 3.1%	0.78%	SED
Total length (μm)	794	788	29.3
Maximum width (μm)	95	93	5.2
Tail length (μm)	111	108	3.3
Tail width at anus (μm)	37	37	1.1
Position of vulva†	77	77	1.0
Length of uterine eggs (μm)	76	73	1.8
Width of uterine eggs (μm)	43	43	1.1

* Data for each concentration are the means of 10 nematodes.

† Length of anterior end to vulva expressed as a percentage of the total length.

The results indicated that the nematodes were well preserved for up to 4 mo at concentrations as low as 0.78% formalin (0.3125% formaldehyde). Table 1 compares the effect of formalin at the 0.78% with that of 3.12% formalin. Nematodes in concentrations of formalin of 0.39% or less decomposed. No significant differences were observed in the body length or width, position of the vulva, size of nematode eggs, or in any other morphological characters used to identify *T. retortaeformis*.

This study has indicated that when gastrointestinal nematodes are removed from the intestinal contents they can be stored in concentrations of formalin below that presently recommended. The results suggest that concentration of formalin could be reduced to 1% (i.e., 0.4% formaldehyde) without any deleterious effects on the specimens but with the advantage that much of the discomfort experienced when working with this chemical is alleviated. The reduction in the concentration of formaldehyde fumes would also decrease any health risk associated with the chemical.

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Research Note

Response of *Nippostrongylus brasiliensis* (Nematoda) to Bile

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Bile from the host may influence the development and establishment of intestinal parasites. Jorgenson (1980, Exp. Parasitol. 49:106–115) reported that bile increased the motility of the infective larvae of the cattle lungworm, *Dictyocaulus viviparus,* but reduced movement of preinfective helminths. Various bile salts produced differential effects, but detergents caused no significant effects. Larvae of *Nematospiroides dubius* were activated also by bile (Sukhdeo and Croll, 1981, Int. J. Parasitol. 11:157–162). Additionally, surgical redirection of the bile flow altered the establishment of *N. dubius.* In contrast, bile inhibited the development and reproduction of *Trichinella spiralis* (Jacqueline et al., 1981, Ann. Parasitol. 56:395–400).

Despite the extensive use of *Nippostrongylus brasiliensis* as a model organism, no information is available about the influence of host bile, and studies of other nematodes have concerned the effects of bile on larval or preadult stages. Accordingly, this study examined the locomotor responses of the sexes of adult *N. brasiliensis* to bile during in vivo and in vitro assays.

In vitro responses of *N. brasiliensis* to bile from the mouse host were tested according to bioassay procedures for pheromone (Bone et al., 1977, J. Parasitol. 63:364–367). Gall bladders were removed from uninfected animals and ruptured in 900 μl of Tyrode's solution. Various numbers of gall bladders were used per unit volume to prepare different concentrations. Twenty-microliter doses were added to bioassay chambers to examine locomotion of male and female *N. brasiliensis* toward or away from the chemical source. Twenty replicates were done for each dose.

Similar procedures were used to determine the nematode's response to known bile components. A range of dosages from 10^{-7} to 10^{-2} M were examined for cholic acid, deoxycholic acid, lithocholic acid, dehydrocholic acid, taurocholic acid (sodium salt) taurodeoxycholic acid (sodium salt), taurochenodeoxycholic acid (sodium salt), and chenodeoxycholic acid (Sigma).

Additionally, the location of adult *N. brasiliensis* in animals with ligated bile ducts was determined. One hundred helminths (1:1 sex ratio) were surgically injected at a site that represented 10% of the pyloric–caecal length (Glassburg et al., 1981, J. Parasitol. 67:898–905). Injections of worms were done immediately after ligation of the bile duct. Animals were sacrificed after 24, 48, 72, or 96 hr to recover *N. brasiliensis* from segments of the intestine (Glassburg et al., 1981, loc. cit.). Control animals were anesthetized and the bile duct was exposed, but no ligature was applied prior to injection of the nematodes. Data

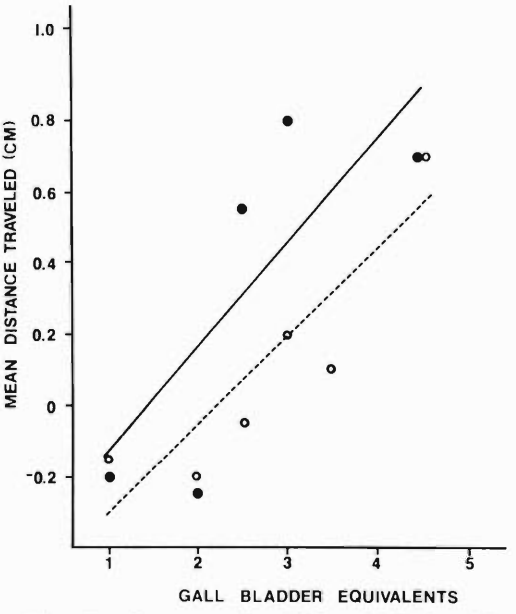

Figure 1. Responses of male (●, solid line) and female (○, broken line) *N. brasiliensis* to concentrations of mouse bile during in vitro assay ($r = 0.75, 0.92$; MSE = 0.22, 0.13, respectively).

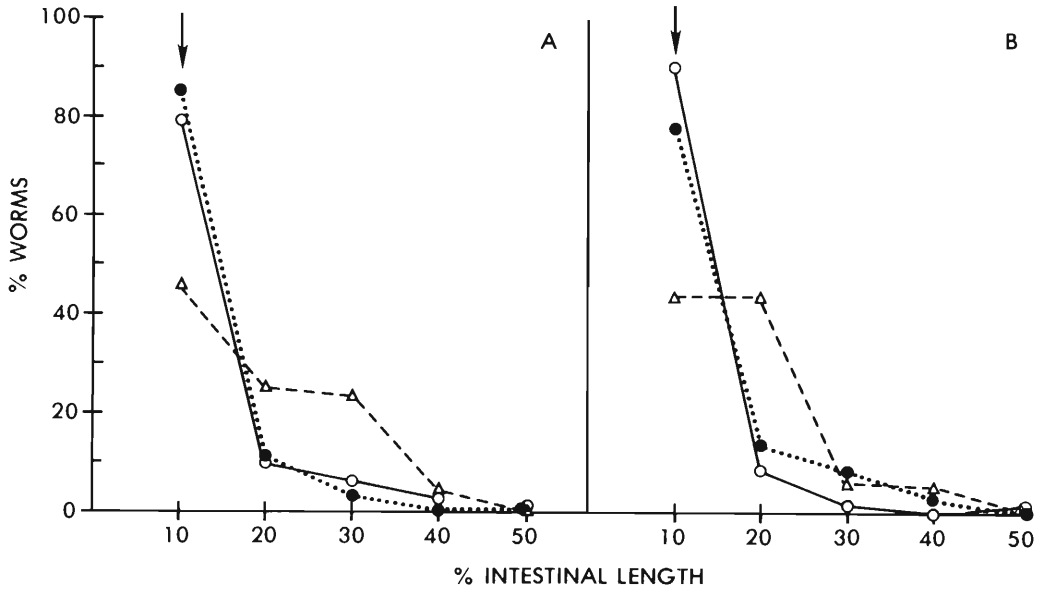

Figure 2. Establishment of *N. brasiliensis* in the mouse intestine after 24 (●), 48 (○), and 72 (△) hr in control (A) and bile duct-ligated (B) animals. Arrow indicates site of injection.

were analyzed by linear regression or the Kolmagorov-Smirnov test for two samples. The 0.05 probability level was considered significant.

The responses of *N. brasiliensis* to solutions from mouse gall bladder are given in Figure 1. Increased concentrations of bile caused linear increases in the locomotion of male and female nematodes (r = 0.75, 0.92, respectively). Responses to 2.5 and 5 gall bladder equivalents were different from zero and the control for male and female worms, respectively (MSE = 0.22, 0.13). Thus, both sexes are attracted to whole bile or some component of bile.

The responses of both sexes of *N. brasiliensis* to known bile components were not linear and were often erratic. Therefore, the responses of *N. brasiliensis* to the tested, single components of bile, were not comparable to those observed for whole bile from the host.

The timed recovery of *N. brasiliensis* from control and bile duct-ligated animals is shown in Figure 2. No significant difference was found in the distribution of the total helminths after 24, 48, or 72 hr in ligated versus control animals. However, the absence of *N. brasiliensis* in animals 96 hr after ligation of the bile duct prohibited distributional analysis. *N. brasiliensis* remained in the anterior aspect of the intestine at this period in the controls. This loss of helminths in animals indicates an influence on the nematode's distribution after ligation of the bile duct. Residual bile in the intestine may be involved in the establishment of *N. brasiliensis* for up to 72 hr. Alternatively, some unknown change in intestinal physiology in ligated animals may be responsible.

These results indicate that the sexes of adult *N. brasiliensis* are attracted to bile during in vitro and in vivo tests. However, the attractive component remains unknown. The nematode may respond to selected mixtures of bile components based on their responsiveness to solutions of mouse bile. Alternatively, the unique bile acids of rodents, α- and β-muricholic acid (Hsia, 1971, pp. 95–120, *in* P. P. Nair and D. Kritcheusky, eds., The Bile Acids. Plenum, N.Y.) may be involved, but were not available commercially for testing. Specific effects of certain bile salts are known for the tapeworm *Echinococcus granulosus* (Smyth and Haslewood, 1963, Ann. N.Y. Acad. Sci. 113:234–260). Thus, bile or some specific component may be involved in the site selection of *N. brasiliensis* in the anterior aspect of the intestine.

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Research Note

Cuticular Abnormality of *Oesophagostomum radiatum* (Nematoda) Fourth-Stage Larvae Grown In Vitro

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Recently, Douvres (1983, J. Parasitol., 69:570–576) described a roller culture system (F-48) for the development of infective larvae of *Oesophagostomum radiatum,* the nodular worm of cattle, to numerous young adult males and females. However, in some cultures, the in vitro development of *O. radiatum* larvae resulted in numerous fourth-stage larvae (L4) that were ostensibly normal, but one or more L4 that had an abnormality of the body wall cuticle (Douvres, pers. obs.). Live or dead L4 with the cuticular abnormalities were readily recognized in cultures by the presence of bleb-, vesicle-, or blister-like (BVBL) swellings on the cuticle surface. These cultures, which were prepared and handled aseptically, contained media with high concentrations of two antibiotics and an antimycotic. The cultures exhibited no evidence of contamination by fungi or bacteria (Douvres, 1983, loc. cit.). The current studies describe this abnormal cuticular structure.

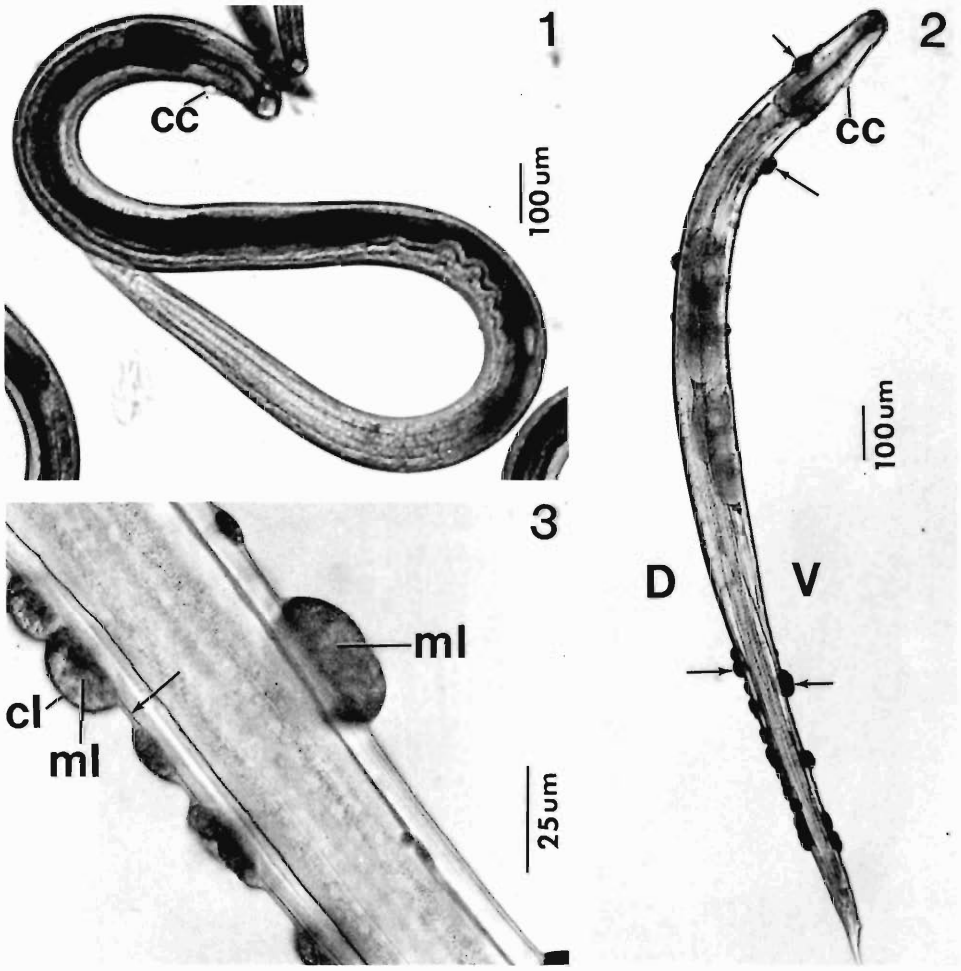
Methods for growing advanced stages of *O. radiatum* from exsheathed infective larvae in roller culture system F-48 were as described previously (Douvres, 1983, loc. cit.). In cultures begun with 40,000–160,000 larvae, development was examined in 27 cultures from five cultivation trials, for periods of 14–51 days.

Development from exsheathed infective larvae to young adult males and females was obtained in the five cultivation trials. This stage was attained in 11 cultures; and in the others development was limited to L4 or to fourth molt (4M).

The examination of extant and terminated cultures showed that larvae in third stage, third molt, L4, 4M, and young adults had body wall cuticles that were ostensibly normal. However, in 14 of the 27 cultures, development of larvae to normal L4 (Fig. 1), which occurred as early as day 9, was followed by development to L4 with an abnormal cuticle (Fig. 2) that occurred as early as day 14 in one culture and at various intervals from days 19 to 51 in other cultures. In most cultures, the abnormal L4 remained alive throughout the cultivation period. In the five trials, only two cultures had dead abnormal L4. Abnormal L4 were found in one or all cultures within the same trial. The number of abnormal L4 varied among cultures within the same trial and between trials. For example, among 10 cultures of a trial only one had abnormal L4 on day 14 and these comprised 0.5% of the population. In another trial of three cultures on day 27, abnormal L4 constituted 2% of the culture. In other trials, cultures had 1–28 abnormal L4 per culture.

Light and scanning electron microscopy were used to examine abnormalities of the body wall cuticle of *O. radiatum* L4 grown in vitro. For microscopy, live abnormal L4 recovered from terminated cultures were freed of media, by washing 4–6 times in Earle's balanced salt solution (without phenol red) and then treated as follows. For light microscopy, larvae were studied alive and after fixation in hot 5% buffered formalin. Specimens floating freely in saline (live larvae) or formalin under a cover slip (to prevent distortion) were used to obtain body measurements. For scanning electron microscopy, larvae were fixed in 3% glutaraldehyde in 0.05 M phosphate buffer, pH 6.8 at 22°C. Chemical fixation for 2–24 hr was followed by dehydration in an ethanol series. The larvae were then transferred in 100% ethanol to a critical point drier where they were coated with 20–30 nm of gold–palladium in a Technics Hummer sputtering device. The coated specimens were viewed and photographed with a Hitachi HHS-2R or 430 scanning electron microscope operating at 10 or 15 kV.

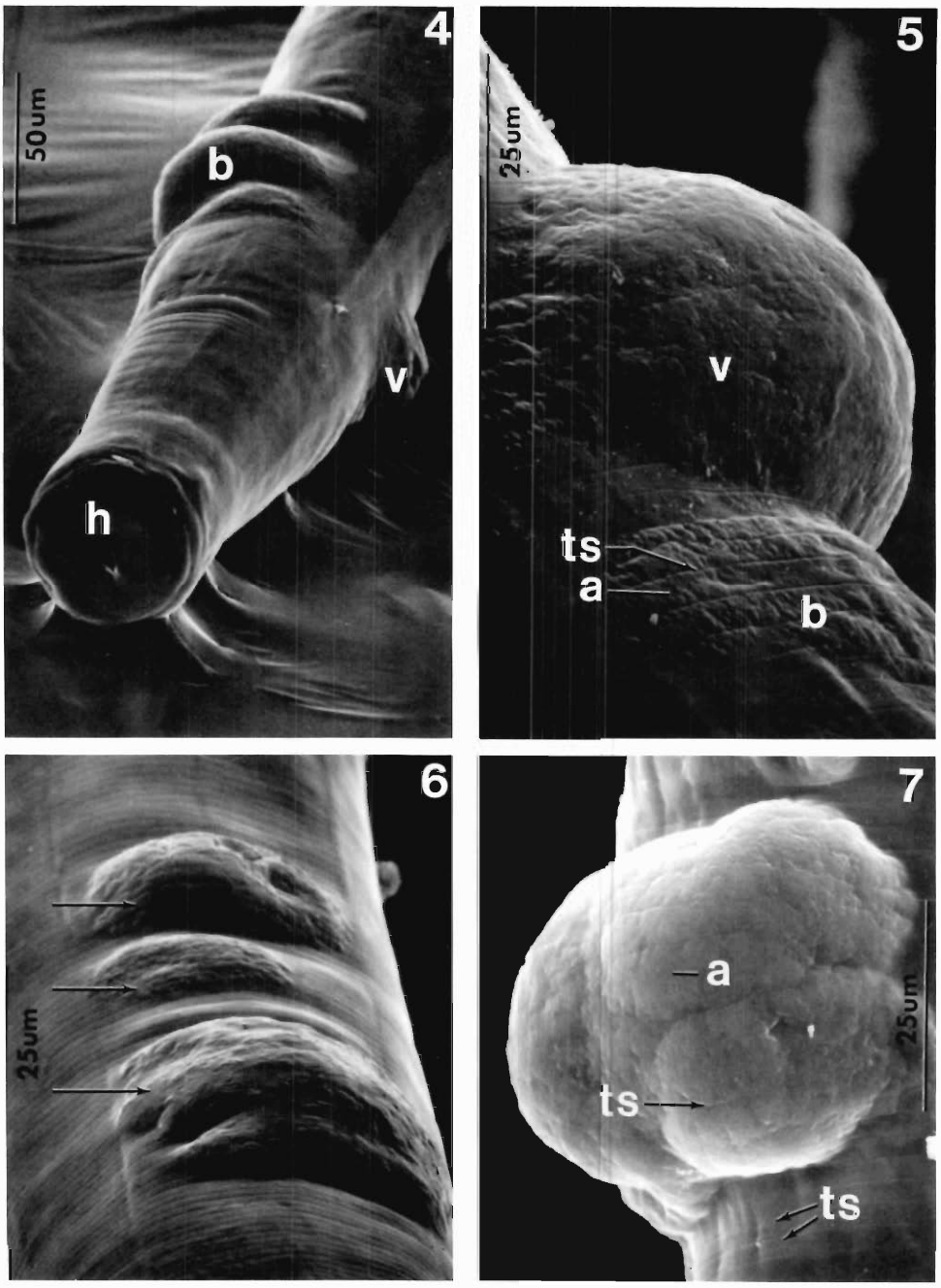
The L4 of *O. radiatum* has a body wall cuticle that is about 2–3 μm thick and consists of three major layers: outer cortical, median (=matrix), and inner fiber layers (Douvres and Thompson, 1973, J. Parasitol. 59:417–424). A characteristic cuticular inflation or collar is located in the cervical region, from the base of head to the excre-



Figures 1-3. ***Oesophagostomum radiatum,*** **light micrographs of live, fourth-stage larvae grown in vitro. 1. Whole mount of normal mid-fourth stage showing that body cuticle surface is unaffected by abnormal swellings. Note the characteristic bulge of cervical cuticular inflation or collar (cc) at the level of the excretory pore. 2. Whole mount of abnormal early fourth stage showing that the body cuticle is marred by bleb-, vesicle-, and blister-like swellings (arrows) on its ventral (V) and dorsal (D) surfaces. Note the characteristic bulge of the cervical cuticular collar (cc). 3. Posterior region of early fourth stage showing that abnormal swellings are part of the body wall cuticle layers in that the swellings that are filled with dark granular material specifically affect the median layer (ml) and are externally and internally bordered by the cortical (ml) and fiber (arrow) layers, respectively.**

tory pore (Figs. 1, 2). The cuticle surface is marked by complete transverse striae (=grooves) and annules between the striae that are 2.5–2.7 μm wide. With light microscopy, these markings are not readily visible; therefore, the body cuticle has a smooth appearance in normal L4 (Fig. 1), and with one exception, in abnormal L4 (Figs. 2, 3). Figures 2 and 3 show that the body cuticle of abnormal L4 exhibits BVBL swellings. These swellings, which specifically affect the median layer, are bordered externally and internally by the cortical and fiber layers, respectively (Fig. 3). The swollen part of the median layer appears to be filled with dark and granular materials (Fig. 3). One or more of the swellings can be found along the body of the abnormal L4.

With scanning electron microscopy, the cuticular surface of abnormal L4 shows the presence



Figures 4–7. ***Oesophagostomum radiatum,*** **scanning electron micrographs of body cuticle of abnormal fourth-stage larvae grown in vitro. 4. Anterior extremity, from head (h) to near base of esophagus, showing blister- (b) and vesicle-like (v) swellings on ventral and dorsal surfaces of cuticle, respectively. 5. Ventral surface of cuticle (midbody) showing that vesicle- (v) and blister-like (b) swellings are externally covered by cortical layer, which is marked by transverse striae (ts) and annules (a). 6. View of midbody cuticle showing contrasting differences between abnormal area with blister-like swellings (arrows) and unaffected areas with normal transverse striations. 7. Ventral surface of cuticle (midbody) showing a large vesicle-like swelling that appears rough, pitted, and compartmentalized. The latter is due to distortions of transverse striae (ts) and annules (a) of the cortical layer that externally covers the swelling. Arrows point to normal appearance of striae and annules in unaffected area of cuticle.**

of transverse striae and annules and the BVBL swellings (Figs. 4–7). The abnormal swellings are externally covered by the cortical layer that shows the transverse striae and annules (Figs. 5–7). The BVBL swellings appear to be rough, pitted, or compartmentalized (Figs. 4–7). The latter is undoubtedly due to distortions of the striae and annules.

Insofar as we know, a cuticular abnormality of *O. radiatum* L4 has not been previously described for this or any other stage for this nematode.

Jackson and Rudzinska (1972, J. Invertebr. Pathol. 19:405–408) described an abnormal "bubbling" of the body wall cuticle in developmental stages of axenically cultured *Neoplectana glaseri,* a nematode parasite of insects. They attributed the abnormality to excessive elaboration or overgrowth of cuticle. Other investigators who found similar abnormalities in the body wall cuticle of nematodes attributed them to bacteria or viruses (Ibrahim, 1967, Proc. Helminthol. Soc. Wash. 34:18–20; Anderson et al., 1973, J. Parasitol. 59:765–769; Ibrahim and Hollis, 1973, J. Nematol. 5:275–281; Anderson et al., 1978, Proc. Helminthol. Soc. Wash. 45:219–225; Bird, 1980, pp. 213–336, *in* B. N. Zuckerman, ed., Nematodes as Biological Models). Cuticular abnormalities caused by bacteria are described as circular "knobby" surface lesions in *Stephanurus dentatus,* the swine kidney worm (Anderson et al., 1973, loc. cit.) and as filamentous, flat, cratered, and proliferate surface lesions in *Strongylus edentatus,* a parasite of horses (Anderson et al., 1978, loc. cit.). The viral-caused swarming disease of *Tylenchorhynchus martini,* a plant parasitic nematode, affected the cuticle by causing partial dissolution of cortical and matrix layers and irregular cracking of cortical sublayers in sublateral areas of the body (Ibrahim, 1967, loc. cit.; Ibrahim and Hollis, 1973, loc. cit.).

We have described a cuticular abnormality for *O. radiatum* L4 grown in vitro that closely resembles the abnormal cuticular "bubbling" of the in vitro-grown *N. glaseri* reported previously by Jackson and Rudzinska (1972, loc. cit.). However, they reported that cuticular "bubbling" occurred on all developmental stages, from infective larvae to adults of *N. glaseri;* whereas, we found that the cuticular abnormality of *O. radiatum* affected only larvae in L4. Because the larvae with the abnormal cuticle occurred in aseptic cultures and appeared to exhibit normal movement and development from early to late phases of L4, the blistering does not appear to be associated with a pathogen. Further study with transmission electron microscopy may help to determine the nature of this condition.

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Research Note

A Monodelphic *Ascaris suum*

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During dissections of female *Ascaris suum* Goeze, 1782 for developmental studies, a specimen was found to possess only one uterus, oviduct, and ovary (i.e., monodelphic). This is a departure from the usual didelphic condition. Paraffin sections of the monodelphic uterus were cut at about 5 cm from the vulva and stained with hematoxylin and eosin. Equivalent sections were prepared from a didelphic specimen for comparison. Fertilized eggs were removed from the monodelphic specimen and cultured at 30°C for 21 days in 0.05 M H_2SO_4 (see Fairbairn, 1961, Can. J. Zool. 39:153–162) to determine their viability. There were no differences in the size and location of the gonads. The *vagina uterina* narrowed smoothly down to the single uterus, with no rudiment of a second uterus. Histological sections showed no sign of a longitudinal septum in the lumen of the uterus, as could occur from the

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fusion of two uteri. Fertilized eggs developed normally.

Monodelphic *A. suum* have been reported before by Harms (1906, Zool. Anz. 30:487–488), McDermott and Feman (1962, J. Parasitol. 48: 506), Schewiakoff (1894, Zentralbl. Bakteriol. Parasitenkd. 15:473–476), and Sowinska (1975, Przegl. Zool. 19:270–273). Harms and Schewiakoff found no septum either. McDermott and Feman noted a possible rudiment of the second uterus at about 11 mm from the vulva. Harms also found a monodelphic specimen of *Parascaris equorum* Goeze, 1782. The lack of a septum, and the possible presence of a rudiment, suggest that monodelphy results from the suppression of the growth of the second uterus.

Observations of *A. suum* with three uteri (tridelphic) are more numerous (Balss, 1906, Zool. Anz. 30:485–487; Chandler, 1924, J. Parasitol. 10:208; Monaco and Mizelle, 1953, J. Parasitol. 39:222; Pollak, 1954, J. Parasitol. 40:480; Hass and Todd, 1959, J. Parasitol. 45:300; Harpur, 1975, J. Parasitol. 61:881; Sowinska, 1975, loc. cit.; Long et al., 1982, Trans. Amer. Microsc. Soc. 101:105–106). These two anomalies are the only departures from the usual condition in *A. suum.*

Chandler (1924, loc. cit.) suggested that polydelphy is a "deep-seated tendency" of ascarids that shows permanent expression in *Polydelphis.* One problem with this concept is that *Polydelphis* spp. have four or six uteri, not three. "Polydelphy" is too broad, and may refer to more than one developmental process. Another problem with Chandler's suggestion is that it does not account for monodelphic ascarids. Belogurov and Belogurova (1979, Zool. Zh. 58:1730–1733) noted that the most frequent developmental anomalies in nematodes involve the genital system. They suggested that monodelphy and polydelphy arose from a didelphic ancestral state. The phylogenetic significance (i.e., teratology, atavism, etc.) of these conditions has yet to be determined.

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Research Note

Natural Infections of *Parelaphostrongylus odocoilei* (Nematoda: Protostrongylidae) in Several Hosts and Locations

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Parelaphostrongylus odocoilei (Hobmaier and Hobmaier, 1934) is a nematode parasite common in the skeletal muscles of mule deer (*Odocoileus hemionus hemionus*) in Alberta (Samuel, 1978, unpubl. rpt. to Parks Canada). It was reported also from black-tailed deer (*O. h. columbianus*) and California mule deer (*O. h. californicus*) in California (Hobmaier and Hobmaier, 1934, Proc. Soc. Exp. Biol. Med. 31:509–514; Brunetti, 1969, Calif. Fish and Game 55:307–316). Experimental infections have been reported in moose (*Alces alces*) (Platt and Samuel, 1978, Exp. Parasitol. 46:330–338; Pybus and Samuel, 1980, Proc. N. Am. Moose Conf. and Workshop 16:152–170).

Parelaphostrongylus odocoilei can be pathogenic in individual mule deer (Brunetti, 1969, op. cit.; Pybus, 1983, Ph.D. Thesis, Univ. Alberta, Edmonton, Alberta. 185 pp.) and has the potential to affect the population dynamics in free-ranging mule deer (Pybus, 1983, op. cit.). It can cause severe local myositis in moose (Pybus and Samuel, 1980, op. cit.).

First-stage larvae indistinguishable from those

of *P. odocoilei* (and a few other closely related nematodes) have been collected from the feces of a variety of cervid and bovid populations in western Canada (Pybus and Samuel, 1981, J. Wildl. Manage. 45:537–542; Pybus and Samuel, unpubl.). In this paper we report the recovery of *P. odocoilei* from two new hosts and two new geographic locations.

BLACK-TAILED DEER: In June 1977, 16 fecal samples were collected from black-tailed deer habitat on Vancouver Island, British Columbia (50°N, 126°W). Nine of these samples (56%) contained dorsal-spined larvae (mean intensity 0.7 ± 0.6 larvae/g). Two laboratory-reared snails, *Triodopsis multilineata,* exposed to these larvae were digested 37 days later in pepsin-HCl at 37°C. The third-stage larvae recovered were administered to a 9-wk-old black-tailed deer raised at the University of Alberta Biomedical Research Centre without access to snails. The deer was killed 262 days later. The necropsy methods were as described in Pybus (1983, op. cit.). Two hand-raised fawns were treated similarly, excluding exposure to larvae.

Twenty-nine adult *P. odocoilei* were recovered (27 from the skeletal muscles of the back and thighs and 2 from the gastrocnemius and latissimus dorsi muscles) from the experimentally infected fawn. All worms were associated with focal hemorrhage and localized tissue damage. No worms were found in the control fawns.

HYBRID: In 1980, seven fecal samples were collected from September to December from a yearling, hybrid, female white-tailed deer/mule deer in Jasper National Park (JNP), Alberta (53°N, 118°W) (see Wishart, 1980, J. Mammal. 61:716–720). All fecal samples collected from this deer contained dorsal-spined larvae. Intensity ranged from 75 to 171 larvae/g ($\bar{x} = 129$). The deer was captured and transported to the research center. Electrophoretic patterns of serum albumen also indicated a hybrid condition (see McClymont et al., 1982, J. Wildl. Manage. 46:540–544). Daily fecal samples were collected until the doe was killed. The carcass was examined as that of the black-tailed deer. At necropsy, three adult *P. odocoilei* were recovered from the right vastus muscles. The nematodes were not associated with any noticeable tissue damage.

MOUNTAIN GOAT: In February 1982, an 8-yr-old male mountain goat (*Oreamnos americanus*) was found in a weakened condition near Newhalem, Washington (48°N, 121°W). The animal died and was sent to Washington State University. At necropsy, areas of gross hemorrhage in skeletal muscles and lungs were removed and teased apart or fixed in 10% buffered formalin for histologic sectioning. Additional lesions were fixed in formalin and sent to the University of Alberta for collection and identification of adult worms.

The goat was thin and had no visible body fat. Gross lesions included extensive hemorrhage throughout the skeletal muscles. Approximately 65 worms (later identified as *P. odocoilei*) were adjacent to the hemorrhages. Dorsal-spined larvae were found in the fecal sample taken during the necropsy. A few *Protostrongylus rushi* were recovered from the bronchioles.

Hemorrhages were seen adjacent to adult worms throughout the muscle sections. Adult nematodes were present beneath the perimysium and between individual myocytes but were not associated directly with any inflammatory response. Lesions in the lung sections were indicative of verminous pneumonia.

In June 1982, a 7-yr-old female mountain goat was killed on the highway in JNP and transported to Edmonton, Alberta for necropsy. The methods were as indicated above for the black-tailed deer.

The goat was in excellent body condition as indicated by extensive fat deposits throughout the carcass. Six adult *P. odocoilei* were found in the backstrap muscles. Worms were coiled in pairs (one male, one female) in connective tissue between the muscle bundles. Local hemorrhage (5–15 mm diameter) was associated with each pair of worms. Dorsal-spined larvae and larvae of *Protostrongylus* spp. (2.8 larvae/g) were present in the feces in a ratio of 2:1, respectively.

Gross and histologic examinations indicated a mild verminous pneumonia. No adult worms were found in the bronchioles but *Protostrongylus stilesi* was recovered from the lung parenchyma. Foci of granulomatous inflammation around dorsal-spined larvae were scattered throughout all lobes.

Specimens of *P. odocoilei* from each host were deposited in the National Museum of Natural Sciences, Ottawa, Ontario, accession #NMCIC(P) 1983-0027–0030.

Parelaphostrongylus odocoilei has been reported previously only from cervids. The establishment of a natural infection in a bovid indi-

cates that the host specificity is not as narrow as previously reported. Also, unidentified adult nematodes have been seen in the skeletal muscles of two additional mountain goats in Washington (Foreyt, pers. obs.) and dorsal-spined larvae have been observed in 184 of 336 (55%) fecal samples of mountain goats in Alberta, central British Columbia, and Washington (Pybus, Samuel, and Foreyt, unpubl.). Further investigation is necessary to determine the identity of the worm(s) in these goat populations.

The presence of *P. odocoilei* on Vancouver Island (Platt, pers. comm.; present study) and in Washington extends the known distribution of *P. odocoilei*. It may be distributed throughout northwestern North America, perhaps, in a variety of hosts.

These findings have important implications for wildlife managers and wildlife parasitologists throughout northwestern North America. They provide important information concerning the host specificity of *Parelaphostrongylus* spp. In addition, the goat harboring a natural infection of 65 worms was weak and emaciated but the extent to which the parasite may have contributed to this condition is not known. The potential of *P. odocoilei* to be pathogenic and its ability to infect various hosts, suggests that a search for *P. odocoilei* should be included when examining any big game animals in this region.

The authors gratefully acknowledge the assistance of Parks Canada and the Alberta Fish and Wildlife Division for assistance in obtaining animals. R. A. McClymont (Alberta Fish and Wildlife Division) conducted the electrophoretic study. Dr. C. W. Leathers (Washington State University) evaluated the histopathology of the Washington goat.

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An Austrian Mermithid Nematode Parasite Offers Biological Control of the Colorado Potato Beetle, *Leptinotarsa decemlineata* (Say)

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The Colorado potato beetle (CPB) is a serious pest of potatoes, tomatoes, and other crops in the northeastern United States; it does not have many parasites and predators and has become resistant to many of our chemical pesticides. No mermithid nematode parasites have been found in this insect thus far in the U.S.A. Recently however, Kaiser (1972, Mermithidae [Nematoda] als Parasiten des Kartoffelkäfers [*Leptinotarsa decemlineata* Say] in der Steiermark, Ph.D. Thesis, University of Graz, Austria) has reported on a new species of *Hexamermis* from CPB in Austria. Apparently, after the CPB was introduced into Europe and spread eastward, one of the native mermithids began to parasitize it, often up to 60% of the population. This nematode has also been reported in Poland and the USSR. No insect parasitic nematode has ever been introduced into the United States for biological control release. Attempts have been made to set up

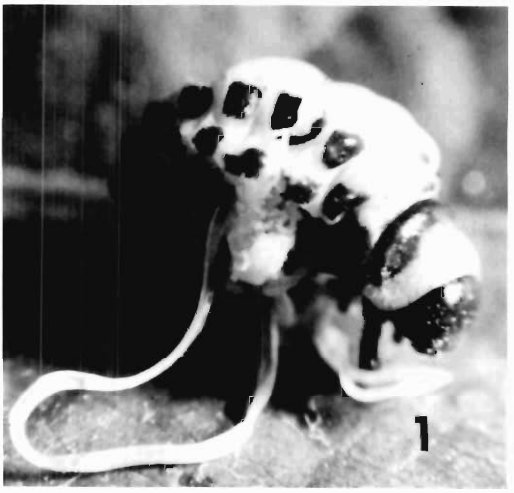

Figure 1. Larval stage of the Colorado potato beetle with a mermithid nematode parasite *Hexamermis* sp. (Photo courtesy of Steve Brown.)

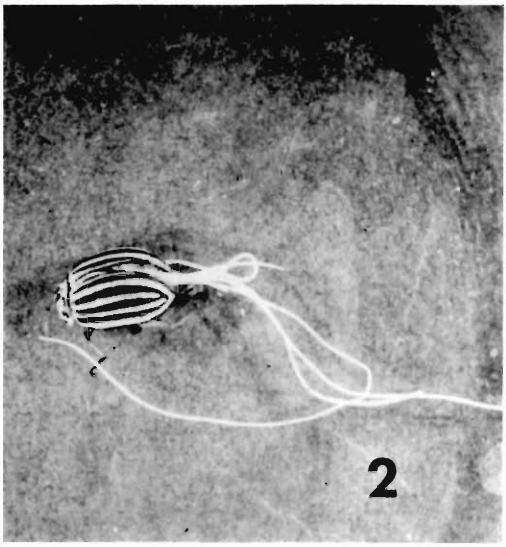

Figure 2. Adult Colorado potato beetle with mermithid nematode parasites (*Hexamermis* sp.) emerging from its body cavity. (Photo courtesy of Helmut Kaiser.)

a protocol for the introduction of exotic nematodes for release into the United States.

Approximately 250 of these mermithid nematodes were obtained from Dr. H. Kaiser in 1980 and 1981. Permission was obtained from APHIS, the State of Maryland, and the USDA for this importation and for the controlled caged field release of this *Hexamermis* sp. from Austria at the Beltsville Agricultural Research Center. The mermithid nematode adults and larvae were released on June 4, 1980 and June 3, 1981 in three nematode containment pits 1 m in diameter surrounded by two cages (Nickle et al., 1984, Proc. Helminthol. Soc. Wash. 51:212–216). No nematodes were placed in these soil pits after June 3, 1981. Potato and tomato plants were planted in the soil and about 100 CPB eggs were added each spring to each of the three cages.

The *Hexamermis* sp. was found to overwinter and establish in the caged field test at BARC. Soil samples (approximately two tablespoons) taken each week from the containments pits showed the presence of infective stage mermithid nematodes that had hatched from eggs in all three cages from June 1 until the first frost. In the summer of 1982, 20 of 30 larval CPB (Fig. 1) and 5 of 8 adult CPB (Fig. 2) that were dissected from the cages were found to be parasitized by this nematode. Our observations and dissections indicate that CPB larvae are very susceptible to the nematode infection when entering the soil to pupate. Often, dissections revealed small mermithid parasites in the adult beetles just after they emerge from the soil. Young larval CPB were capable of being infected in the laboratory. This stage in nature often falls to the ground and they are then vulnerable to infection as they crawl along the soil surface, especially after a rain. The infective stage nematode is also able to climb a short distance up the plant and infect the CPB.

This *Hexamermis* sp. does not infect earthworms and so far the only beneficial organisms that were parasitized were 2 of the 38 adult lady beetles (*Harmonia* sp. and *Hippodamia* sp.) that were placed in the cage release and forced to hibernate in the soil pit.

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Research Note

Occurrence of *Elaeophora poeli* (Vryburg, 1897) (Nematoda) in the Philippine Water Buffalo (*Bubalus bubalis* Linn.)

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Elaeophora poeli occurs in the aorta of buffaloes in many countries especially India, Malaya, and Indonesia (Mohan, 1968, Vet. Bull. 38: 735–756). It is commonly observed in carabaos, the Philippine water buffalo, at slaughter. Carabaos, being beasts of burden, are killed for food only when aged and when weakened or incapacitated by injury or disease.

Five hundred carabaos, 179 females and 321 males, part of the kill for 6 days at the Manila City abattoir were examined for the parasite. Three hundred twenty-seven or 65.4% of the animals examined had lesions in the thoracic aorta attributable to *E. poeli.* Affected carabaos were 127 (71%) of 179 females and 200 (62%) of 321 male carabaos. This indicates a high prevalence of elaeophoriasis in the Philippine water buffalo. The high rate of infection may be associated with the abundance of possible bloodsucking vectors throughout the year in most parts of the country. Soulsby (1965, Textbook of Veterinary Clinical Parasitology. F. A. Davis Co., Philadelphia. 1,120 pp.) stated that some bloodsucking arthropod likely serves as intermediate host.

Aortic lesions consisted primarily of nodules that varied from 5 to 15 mm in diameter and from 1 to 13 mm in height (Fig. 1). Affected aortas had roughened and corrugated intima and fibrin tags and some also had crateriform depressions 7–10 mm in diameter and 2–3 mm in depth. Although a few carabaos had as many as 10 nodules in various stages of development, most animals had 2–5 predominantly along the dorsal aspect of the aorta near the origin of the intercostal arteries. Similar intimal lesions but smaller nodules were described in affected Indian water buffaloes (Prasad and Bhalla, 1977, Indian Vet. J. 54:97–101). In the present study, parasites and organized fibrin tags were invariably attached to the nodules. This is an important differential feature from nodules induced by *Onchocerca armillata* (Jubb and Kennedy, 1970, Pathology of Domestic Animals, 2nd ed., Vol. 1. Academic Press, New York. 593 pp.). The nodules of *O. armillata* encompass both male and female parasites and protrude on both the internal and ex-

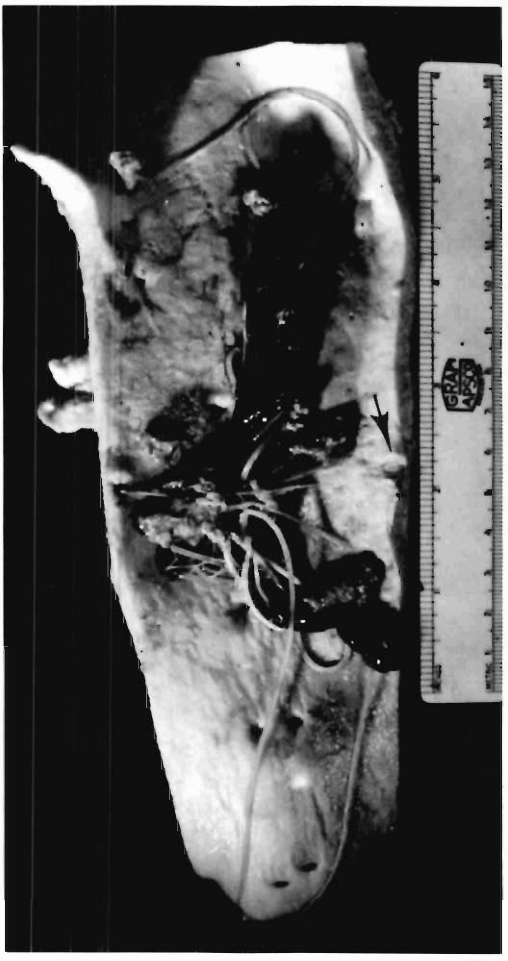

Figure 1. Thoracic aorta from a Philippine water buffalo (carabao) containing nodules with attached female *Elaeophora poeli* and clotted blood. Note roughened intima and crateriform depression (arrow).

ternal wall of the aorta (Chodnik, 1958, Ann. Trop. Med. Parasitol. 52:145–148; Cheema and Ivogli, 1978, Vet. Pathol. 15:495–505). Parasites were never found lying free in the lumen of aortas infected with *O. armillata* (Al-Zubaidy, 1973, Trans. R. Soc. Trop. Med. Hyg. 67:436). With *E. poeli,* the male resides within the nodules, whereas the anterior portion of the female is fixed in the nodule, and the rest of the body swings free in the lumen of the vessel. In this survey, the female varied from 110 to 250 mm in length with a maximum width of 2.5 mm, whereas the males were 55–60 mm long and about 0.5 mm wide. Microfilariae and embryonated eggs were easily teased out of the females. Morphologic features of the parasite, gross lesions induced, and predilection site are similar to those reported for *E. poeli.*

Lapage (1962, Monnig's Veterinary Helminthology and Entomology, 5th ed. Williams and Wilkins Co., Baltimore. 600 pp.) stated that infected animals show no clinical signs. Microfilariae have not been associated with skin lesions comparable to those produced by *E. schneideri* in sheep as described by Jensen and Seghetti (1955, J. Am. Vet. Med. Assn. 127:499–505). Soulsby (1965, op. cit.) recorded no evidence that *E. poeli* has pathogenic significance. However, in this study many parasite nodules were sometimes found circumscribed along the aortic wall so that the patent lumen of the aorta was reduced to about a third of its usual size. The nodules, together with the attached parasites and obturating thrombi, conceivably could cause obstruction to blood flow and lead to heart failure. Thrombosis and embolism may also occur from detached pieces of regressing nodules and dead parasites. These factors may reduce the endurance and usefulness of severely affected animals. Further studies are needed to determine the developmental cycle of the parasite and its pathogenic effects in the Philippine water buffalo. Voucher specimens of *E. poeli* from *Bubalus bubalis* have been deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland (No. 77680).

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Research Note

The Survival of Protoscolices of *Echinococcus multilocularis* (Cestoda) at Constant Temperatures

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Echinococcus multilocularis, the causative agent of multilocular or alveolar hydatid disease, is primarily boreal in distribution, occurring naturally in foxes, coyotes, and various species of rodents (Rausch, 1967, Ann. Parasitol. 42:19–63). In the contiguous United States, the tapeworm is endemic to several north-central and mountain states (Leiby et al., 1970, J. Parasitol. 56:1141–1150; Kritsky et al., 1977, Am. J. Trop. Med. Hyg. 26:1046–1047; Eastman and Worley, 1979, J. Parasitol. 65:34), and in 1979 an autochthonous case of alveolar hydatid disease in a human was identified in Minnesota (Gamble et al., 1979, J. Am. Med. Assoc. 241:904–907).

Echinococcus granulosus is also considered to be a public health problem in several states in the United States (Pappaioanou et al., 1977, Am. J. Trop. Med. Hyg. 26:732–742; Schantz et al., 1977, Am. J. Trop. Med. Hyg. 26:121–126; Crellin et al., 1982, Am. J. Epidemiol. 116:463–474). In efforts to understand the distribution of *E. granulosus* and the potential for increased transmission, researchers have studied the impact of temperature extremes upon the infective stages.

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Andersen and Loveless (1978, J. Parasitol. 64: 78–82) reported that protoscolices of *E. granulosus* in intact hydatid cysts of ovine origin could survive 16 days when stored at 1°C or 10°C. Colli and Williams (1972, J. Parasitol. 58:422–426) showed that eggs of *E. granulosus* could survive 24 hr at −30°C with no loss of infectivity to laboratory rodents, and Sweatman and Williams (1963, Parasitology 53:339–390) were able to maintain eggs in viable condition for 7.5 mo at −6°C.

Although considerably less research has been done on the bionomics of *E. multilocularis*, Schiller (1955, J. Parasitol. 41:578–582) found that eggs of this species were still infective to red-backed voles when the tapeworms were stored for 42 days within carcasses of arctic foxes at −26°C. To our knowledge, no reports are yet available on the survivability of *E. multilocularis* protoscolices. The purpose of our study was to determine the survival of this larval stage when stored at constant temperatures.

Cotton rats that had been experimentally infected with an isolate of *E. multilocularis* (originally received through the courtesy of Dr. Robert L. Rausch, School of Public Health, University of Washington, Seattle, Washington, and then maintained via secondary perpetuation in our laboratory) were the source of test material used in our experiments. Rats were killed in an ether jar and used as follows: the first treatment group consisted of intact carcasses containing a 3–5-mo cystic growth of *E. multilocularis*, the second treatment group consisted of 1-g samples of cystic material removed from similar source rats and transferred to 100 × 15-mm polystyrene petri dishes containing 0.85% NaCl, and the third treatment group consisted of approximately 1,000 free protoscolices stored in 1 ml of 0.85% NaCl in stoppered, 65 × 10-mm pyrex test tubes. Sufficient numbers of each of the three treatment types were then placed in appropriate temperature storage cabinets to permit an evaluation of survivability of protoscolices within each sample after 1, 2, 4, 8, 16, 32, and 64 days storage and at constant temperatures of −10, 0, 5, 10, 20, 25, 30, 40, and 50°C. Survival was determined by subjecting the protoscolices to trypsin, pancreatin, and sodium taurocholate (Smyth, 1967, Parasitology 57:111–133) and then examining them with a compound microscope for evagination, motility, and, in questionable cases, the presence of flame-cell activity.

Table 1 shows the percent survival of the protoscolices of *E. multilocularis* when stored within the three treatment groups described above. Protoscolices did not survive for even 1 day at the temperature extremes of −10 or 50°C, regardless of the method of storage. These two temperature extremes tested in our laboratory approximate those that occur under normal field conditions in our region. In 1976, the average monthly minimum temperature at soil surface under a short pasture grass cover was −6.1°C during the month of December, and the average monthly maximum temperature during August of that year at the same site was 52.1°C (Bullick and Andersen, 1978, Gt. Basin Nat. 38:369–378). The results on survival of protoscolices in our study suggest that these average monthly temperature extremes (with shorter periods within each month of lower and higher daily extremes) would definitely decrease the survival of *E. multilocularis* protoscolices if exposed to natural conditions in our region.

Protoscolices survived as long as 64 days when stored within intact carcasses at both 0 and 5°C (43 and 53%, respectively); however, it is important to note that the protoscolices were not in a frozen condition when stored at 0°C. Protoscolices within carcasses and within the excised 1-g cyst samples were shown to freeze at approximately −3°C, whereas those in 1 ml 0.85% NaCl froze at −1°C. The ability of the organisms to survive as long as 64 days at 0 or 5°C illustrates the potential for transmission over extended time periods during seasons when such temperature ranges might exist in nature. Also, such survival capabilities as noted for the protoscolices at either 0 or 5°C suggest that protoscolices might be stored for several weeks in a refrigerator and still be valuable for certain experimental studies in the laboratory or as inoculum in the perpetuation of secondary echinococcosis in laboratory rodents.

At 10°C only 8% of the protoscolices survived for 32 days and none at 64 days. Survival at the storage temperature of protoscolices within excised 1-g cyst samples was 70% at 16 days and 0% at 32 days. These levels agree well with those reported for *E. granulosus*, wherein 22% survived in excised hydatid cysts of sheep origin for 16 days and 0% at 32 days (Andersen and Loveless, 1978, loc. cit.).

At constant storage temperatures near to that of normal laboratory working conditions, the maximum survival time of *E. multilocularis* pro-

Table 1. Percent survival of protoscolices of *Echinococcus multilocularis* stored in carcasses of *Sigmodon hispidus*, excised multilocular hydatid cysts, or in 1 ml 0.85% NaCl at constant temperatures.

Temperature (°C)	Medium	Days							
		1	2	4	8	16	32	64	128
−10	Intact carcass	0							
	Cyst	0							
	Saline	0							
0	Intact carcass	100	100	92	98	76	88	43	0
	Cyst	100	100	100	35	0			
	Saline	91	67	0					
5	Intact carcass	100	100	100	100	95	94	53	0
	Cyst	100	100	93	68	70	0		
	Saline	100	74	83	0				
10	Intact carcass	95	97	100	100	100	8	0	
	Cyst	100	98	100	100	70	0		
	Saline	90	67	10	3	1	0		
20	Intact carcass	100	100	100	100	23	0		
	Cyst	100	100	97	0				
	Saline	100	100	100	0				
25	Intact carcass	100	100	94	0				
	Cyst	100	100	100	0				
	Saline	100	100	95	0				
30	Intact carcass	100	100	0					
	Cyst	100	90	0					
	Saline	100	93	0					
40	Intact carcass	78	0						
	Cyst	80	0						
	Saline	83	0						
50	Intact carcass	0							
	Cyst	0							
	Saline	0							

toscolices was 16 days (23%) at 20°C, 4 days (94%) at 25°C, and 2 days (100%) at 30°C. Bacterial overgrowth was apparent at these warmer conditions and probably had a deleterious effect upon the parasite. At 40°C the maximum survival time was only 1 day, with survival of 78, 80, and 83% within intact carcasses, in excised cyst material, and in 1 ml 0.85% NaCl, respectively.

In general, our results illustrate the marked adaptation of *E. multilocularis* for perpetuation under natural field conditions, and underscore the potential for an increased distribution of the parasite into more temperate zones. Indeed, further spread of *E. multilocularis* in the United States now seems likely (Wilson and Rausch, 1980, Am. J. Trop. Med. Hyg. 29:1340–1355), and the ability of the protoscolices to survive for more than 2 mo at temperatures just above freezing could facilitate increased transmission. In addition to the transmission cycle in the wild, *E. multilocularis* has been reported to occur infrequently in domestic cats and house mice (Leiby and Kritsky, 1972, J. Parasitol. 58:1213–1215), which may maintain this cestode in urban areas. Also, sled dogs are known to be important in rodent capture and subsequent transmission to humans in some regions of Alaska (Rausch, 1972, Arch. Env. Hlth. 25:246–252). We suggest, therefore, that individuals living in areas where *E. multilocularis* may occur have their dogs and cats treated at least annually with an echinococcocidal compound and also use precautionary measures to prevent them from having ready access to all rodents in that region.

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Research Note

Abnormal Cysticerci (Cestoda) in the Human Brain

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It is generally thought that the cysticercus of *Taenia solium* eventually becomes calcified. The brief case report that follows illustrates an unusual deviation from what has been considered to be the norm.

In 1981, an old woman (precise age unknown) was found dead on a street in Mexico City and was taken to the city morgue. On autopsy, large numbers of cysticerci were found in and on the surface of the brain. Several of these were fixed in 10% formalin and sent by Dr. Enrique Navarete Cadena to the Parasitology Laboratory.

The cysticerci measured about 1.8 cm in diameter and appeared to have several compartments (Fig. 1). Histologic sections stained with hematoxylin and eosin showed a scolex in one of these (Fig. 2). Sections also showed a very weak uptake of stain by the tissue. The subtegumental layer of cells had all but disappeared (Fig. 3) and the cyst wall was much attenuated. Also, contrary to expectations, very few calcareous corpuscles were seen.

The normal histology of *Taenia solium* cysticerci has been described by Voge (1963, J. Parasitol. 49:85–90) and by Šlais (1970, The Morphology and Pathogenicity of the Bladder Worms. Dr. W. Junk, N.V. Publishers, The Hague). Both authors described the prominent subtegumental layer of cells and also the flame cells and other structures in the parenchyma. In the specimens described here all these features were not observed, indicating degeneration and death of the larvae without calcification, but with enlargement in size due to expansion of the outer wall, consisting only of fibrous and acellular material. This could represent the stage before calcification of the larvae. Whether or not the cysticerci might have died before the host, resulting in the changes described here, cannot be determined.

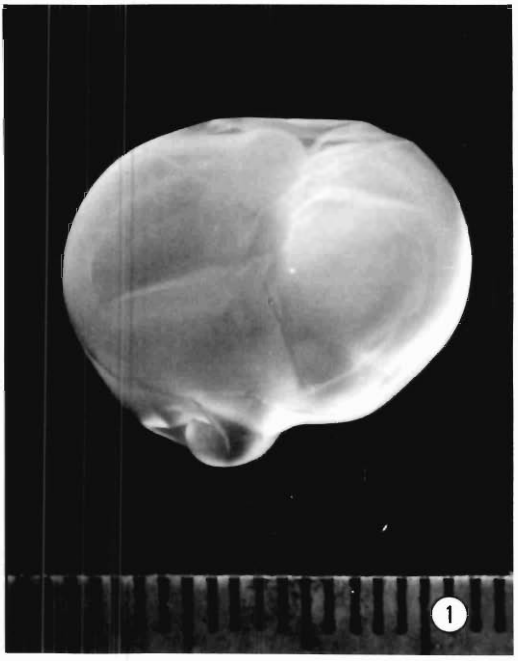

Figure 1. A large cysticercus from human brain that appears to have several compartments.

The authors are grateful to Dr. Marietta Voge for advice with the manuscript, Zane Price for preparing the photographs, and to Dr. A. M. Zaragoza for technical assistance.

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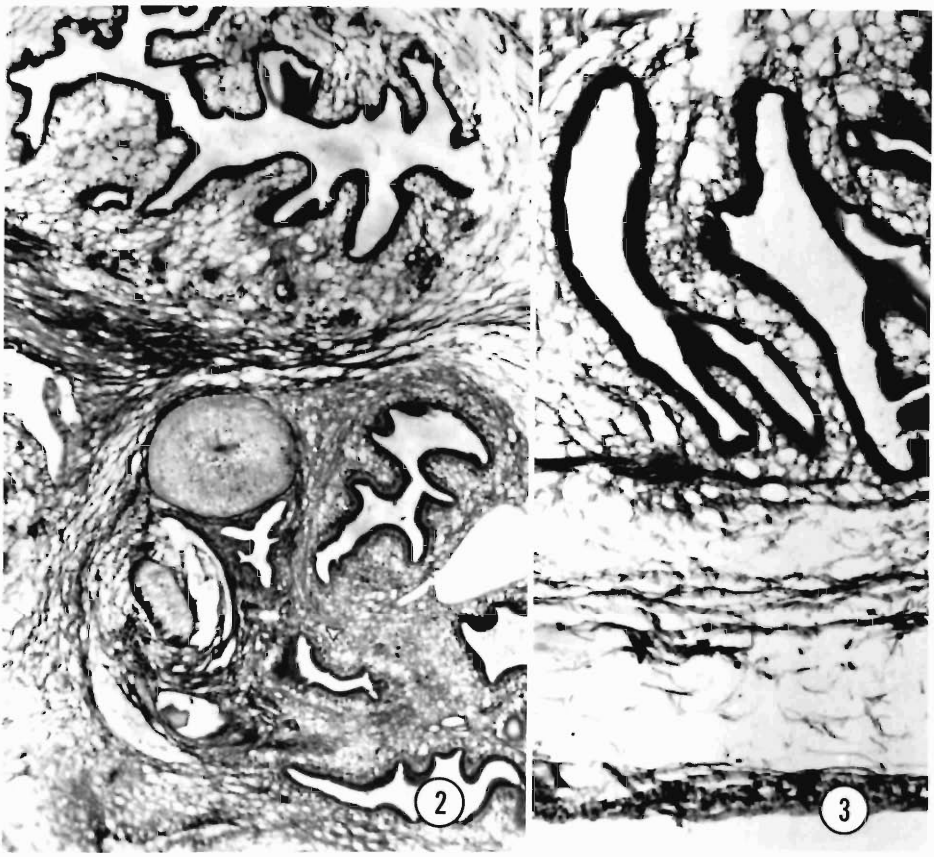

Figures 2, 3. Histologic sections of cysticerci. 2. Photograph of a section through cysticercus showing what appear to be two sections of the curved neck region adjacent to the sucker area. Advanced degeneration prevented detailed study. 3. Section through bladder wall showing loss of subtegumental cells as well as loss of cells in parenchyma.

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The Genus *Spirorchis* MacCallum, 1919 and Family Spirorchiidae Stunkard, 1921 (Trematoda)

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The genus *Spirorchis* was erected by G. A. MacCallum, ([1919] Zoopathologica, New York Zoological Society, Vol. I, No. 3, p. 92), but the author omitted the specific name intended for the species. The report was based on three specimens from the intestine of *Clemmys insculpta* (=*Chelopus insculptus*).

Ward (1921, J. Parasitol. 7:114–128) described a new species of blood flukes from turtles under the name *Proparorchis artericola.* The account was based on specimens in the Department of Zoology of the University of Illinois. For the new genus, *Proparorchis,* he erected a new subfamily, Proparorchiinae. He recognized the genus *Spirorchis,* 1919 as a related form, proposed the specific name *innominata* for the parasite, and included *Spirorchis* in the subfamily Proparorchiinae. Looss (1902, Zool. Jahrb. Syst. 16:411–894) had erected the genus *Hapalotrema* to receive the species *Distoma constrictum* Leared, 1862, a parasite of the vascular system of the marine turtle *Thalassochelys corticata* (=*Caretta caretta*). This species was included (Odhner 1912, Zool. Anz. 41:54–71) in the subfamily Liolopinae, family Harmostomidae. Ward removed *Hapalotrema* from the subfamily Liolopinae and included it with Proparorchiinae in a new family Proparorchiidae. Ward (1921, loc. cit., p. 124) stated, "I have endeavored as yet unsuccessfully to get for examination one of the specimens on which MacCallum's description is based." In 1920, I was told by Dr. MacCallum that the slides were misplaced and apparently lost. But after the publication of the paper by Ward, he found the specimens and sent them to me.

Stunkard (1921, American Museum Novitates, No. 12, issued July 15, 1921) described blood-flukes from turtles that he had collected and studied as a graduate student in the Department of Zoology at the University of Illinois, and others collected later in the New York and New Jersey area. Study of the specimens of *Spirorchis* loaned by Dr. MacCallum disclosed errors in the description. After corrections and revision of the genus, it was apparent that certain of the blood-flukes belonged in *Spirorchis.* Further, it was apparent that *Proparorchis* was a synonym. In response to an inquiry, Dr. C. W. Stiles, in a letter dated March 1, 1920, stated, "According to the rulings of the International Commission, a generic name may be valid even though no specific name is published with it. The first specific name that is published after the generic name becomes type of the genus." The suppression of *Proparorchis* as a synonym invalidated the subfamily and family names, Proparorchiinae and Proparorchiidae. Accordingly, Stunkard erected and characterized the subfamily Spirorchinae (revised spelling Spirorchiinae) and the subfamily Hapalotreminae (revised Hapalotrematinae) to contain the genus *Hapalotrema.* The two subfamilies were included in the family Spirorchidae (revised Spirorchiidae).

MacCallum (1921, Zoopathologica, Vol. I, No. 6, dated August 1921) described a new species, *Spirorchis emydis.* The account begins, "Family—Spirorchiidae, Subfamily—Spirorchiinae. Host—*Emys blandingi,* Blanding's turtle. Habitat—lung. Locality—Ohio, U.S.A. In a paper on *Telorchis* and other trematodes (Zoopathologica, New York Zool. Soc., 1918, I:81) I described a worm from the intestine of *Chelopus insculptus,* giving it the name *Spirorchis.* This same worm had been found by me previously (in 1912) in the lung of *Chrysemys picta.* Unfortunately, through an error the specific name *eustreptos,* which was intended, was omitted. Ward in his paper of March (Journal of Parasitology), without consulting me, and without studying the form, suggests the specific name *innominata,* but that name is not acceptable and I maintain the name *Spirorchis eustreptos.*"

This account was the first time MacCallum mentioned subfamily Spirorchiinae or family Spirorchiidae, and the publication bears the date August 1921. The actual date of publication is unknown. At that time, The New York Zoolog-

ical Society published monthly lists of its publications. The issue of Zoopathologica, No. 6, was not included in the November 1921 list and apparently was not available at that date. It was included in the January 1922 list. In any event, it was published after the paper by Stunkard issued July 15, 1921, which has priority.

MacCallum (1922, Anat. Rec. 23:114) reported on North American blood-flukes. In an abstract, without descriptions or names, he listed collections of blood-flukes from various turtles from 1912 to 1921.

Stunkard (1923, Bull. Amer. Mus. Nat. Hist. 48:165–251, issued Oct. 8, 1923) reviewed the literature on the blood-infesting trematodes of North American turtles. New genera and new species were described. He redescribed the species designated as *Spirorchis eustreptos* by MacCallum and figured the specimen labeled by him as type under the name *Spirorchis innominata* Ward, 1921. That was an error; Ward implied, but did not make the combination, *Spirorchis innominata.*

MacCallum (1926, Ann. d. Parasitol. 4:97–105) repeated the record of his collections of blood-flukes as reported in 1922. He noted that he (1921, loc. cit.) had erected the genus *Spirorchis,* but the intended specific name, *eustreptos,* was omitted. Now he redescribed *Spirorchis eustreptos* MacCallum, 1921 and *Spirorchis emydis* MacCallum, 1921, and briefly described *Spirorchis picta* n. sp., *Spirorchis chelydrae* n. sp., and *Spirorchis blandingi* n. sp. Referring to the account by Ward (1921, loc. cit.) he declared, "Récemment (Journal of Parasitology, 1921, VII, 123), et sans autre avis, Ward suggéra comme nom d'espèce le nom d'*innominata*; mais comme cette suggestion n'a pas été publiée en même temps qu'une description du ver, il parait préférable de laisser le nom prévu pour cette espèce, type du genre *Spirorchis.* Les noms de Spirorchiidae et de Spirorchiinae désigneront la famille et la sous-famille."

Yamaguti (1971, Synopsis of Digenetic Trematodes of Vertebrates, Keigaku, Tokyo 1,074 pp.) and others for 50 years recognized the validity of the family Spirorchiidae Stunkard, 1921 and subfamily Spirorchiinae Stunkard, 1921.

However, Yamaguti (1975, A Synoptical Review of Life Histories of Digenetic Trematodes of Vertebrates. Keigaku, Tokyo 590 pp.) credited the names Spirorchiidae and Spirorchiinae to MacCallum, 1921. It is obvious that Yamaguti was unaware that the publication of the family and subfamily names by Stunkard antedated that of MacCallum, 1921. Apparently, Yamaguti was impressed by the argument of MacCallum (1921, loc. cit.; 1926, loc. cit.) and his insistence on the restoration of *eustreptos* as type of the genus *Spirorchis.* Actually, MacCallum was correct because, according to Stiles, "The first specific name that is published after the generic name becomes the type of the genus." MacCallum's (1921, loc. cit.) publication of the combination, *Spirorchis eustreptos,* validated the name. But credit for authorship of the family and subfamily names claimed by MacCallum cannot be accepted.

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Research Note

Quantitation of *Fasciola hepatica* Egg Counts in Sheep

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Helminth infections in ruminants are routinely diagnosed by fecal egg counts. Although many studies have correlated counts of eggs per gram of feces (epg) with numbers of nematodes, few have correlated epg with numbers of trematodes. Investigations of epg production and quantitative diagnosis of *Fasciola hepatica* in Australian sheep found that approximately one *F. hepatica* produced 33 epg (Happich and Boray, 1969, Aust. Vet. J. 45:329–331). The present paper reports

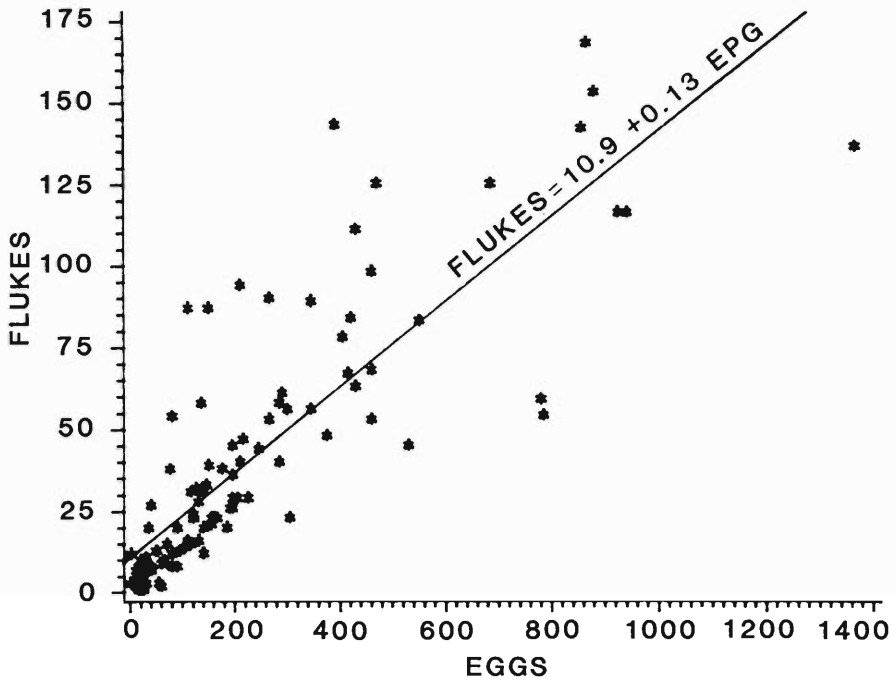

Figure 1. Plotted eggs-per-gram (epg) counts for populations of *Fasciola hepatica* recovered from livers of sampled sheep.

a study on quantitative diagnosis of experimental fascioliasis in sheep by epg counts using a combined screening-sedimentation technique.

Observations were made and data collected on 104 untreated Polled Dorset sheep, usually less than 1 yr old but up to 8 yr old, that had been infected with varying numbers of *F. hepatica* cysts. Fecal samples were collected from the sheep before necropsy, epg counts were made, and after necropsy the number of flukes was counted. The infections varied in length of patency, but generally were 13–20 wk postinoculation of cysts. An egg counting technique devised by the writer (reported as pers. comm. by Rew et al., 1978, J. Parasitol. 64:290–294) was used with a modification of screen sizes from 153 and 53 μm to 102 and 53 μm. A single epg count was made for each sheep and recorded along with the number of flukes recovered from the liver. Data were analyzed for linear regression of epg vs. number of flukes, and a regression equation for estimate of fluke numbers based on epg counts was computed.

Regression analysis of epg vs. number of flukes was positive and highly significant with an F value of 227, and a correlation coefficient of 0.83. Figure 1 presents graphically the observations with a regression line and equation. The regression equation for estimating numbers of flukes correlated with epg counts based on the observations of this study is number of flukes = 10.9 + 0.13 epg.

The use of this equation for estimation of number of flukes based on epg has proved to be a close approximation of fluke burdens and a valuable tool when checked against actual experimental infections at the Animal Parasitology Institute. A group of 25 sheep different from those on which the regression analysis was made was inoculated with *F. hepatica* cysts. After about 16 wk fecal samples were collected, egg counts made, and the sheep necropsied for worm counts. Predicted worm counts calculated with the regression equation, egg counts, and the actual recovered worm counts are presented in Table 1. Below 50 epg the predicted worm numbers were low and over 300 epg the predicted numbers were slightly higher. In both cases the predictions were a fair approximation.

The numbers of flukes in the sheep in the study for the regression analysis were low with relatively few counts above 100/sheep; fluke counts

Table 1. Comparison of experimental actual *Fasciola hepatica* counts with predictions based on regression equation (flukes = 0.13 epg + 10.9).

epg	Actual fluke count	Predicted fluke count
15	25	13
45	39	17
50	27	17
50	27	17
60	19	19
75	20	21
85	19	22
90	22	23
100	34	24
100	38	24
125	16	27
130	30	28
135	28	28
145	64	30
160	40	32
220	40	40
235	61	41
245	42	43
280	47	47
320	41	53
325	49	53
510	70	77
525	70	79
535	50	80
Average	38	36

ranged from 1 to 168. The epg in the study ranged from 15 to 1,365 with average epg per fluke of 6.75 and a range of 1.26–25.0. The infections reported in sheep by Happich and Boray (1969, loc. cit) were considerably larger, with numbers of flukes ranging from 1 to 350. The epg counts in the Australian study ranged from 6 to 9,320 with average epg per fluke ranging from 12 to 33. The variance in epg between the Australian observations and those in this study may be the result of an Australian strain of *F. hepatica* with greater fecundity. Other factors affecting epg counts could include fecal sampling methods, fecal sample size, counting techniques, and diet of animals.

The data of Happich and Boray suggest a curvilinear regression in infections above 100 flukes with the epg counts per fluke decreasing, possibly as a result of crowding. In the study presented herein there were only 11 observations with more than 100 flukes, and a flattening of the epg curve with greater numbers of flukes did not appear.

Estimation of numbers of flukes present in an infection, whether experimental or natural, is important, and the use of the regression equation provides a tool for approximation.

Appreciation is expressed to Peter Eeg for contribution of data.

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Research Note

In Vitro Maintenance and Tracking of *Echinostoma revolutum* (Trematoda) Adults

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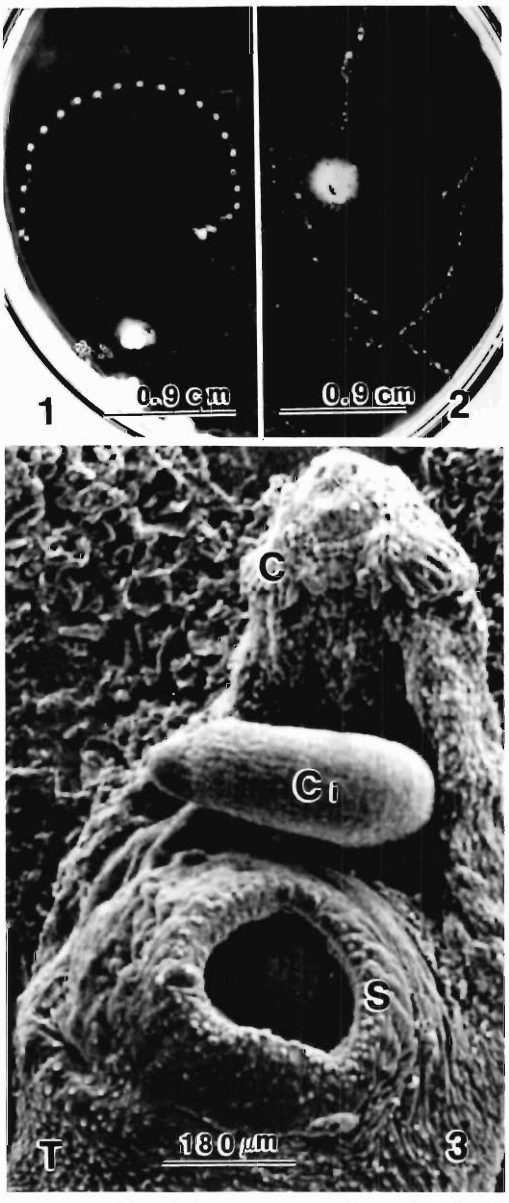
In vitro tracking of nematodes has been reported for *Caenorhabditis elegans* by Ward (1973, Proc. Nat. Acad. Sci. 70:817–821; 1978, *in* Taxis and Behavior. Chapman Hall, London). Studies on tracking of adult trematodes are not available although Fried and Pallone (1983, Proc. Helminthol. Soc. Wash. 50:350–351) attempted to observe tracking of *Echinostoma revolutum* in viscous agar.

The purpose of this study was to develop a medium to observe tracking of *E. revolutum* adults maintained in vitro.

E. revolutum adults, 13–15-days-old, from experimentally infected domestic chickens (Fried and Weaver, 1969, Proc. Helminthol. Soc. Wash. 36:153–155) were washed rapidly in three changes of sterile Locke's solution and a final wash of 1,000 units/ml penicillin and 1,000 μg/ml streptomycin (Gibco, Diagnostics Laboratories, Madison, Wisconsin) in Locke's solution for 15 min. This antibiotic concentration has been used as a wash for hermaphroditic digeneans (Austin and Fried, 1972, Proc. Helminthol. Soc. Wash. 39: 262–263). The worms were used within 15 min



Figures 1-3. ***Echinostoma revolutum*** **in vitro. 1. Photograph of one-half of a Petri dish 12 hr postinoculation showing distinct acetabular tracks of a single worm. 2. Photograph of one-half of a Petri dish 48 hr postinoculation showing tracks of a single worm. 3. SEM of a worm maintained 48 hr in culture; C = cephalic collar; Ci = cirrus; S = sensory papillae on the acetabulum; T = tegumentary spines.**

of antibiotic treatment. Tracking was studied in 5.5-cm Petri dishes containing a 1:1 mixture of 0.8% agar (Difco Laboratories, Detroit, Michigan) plus Dulbecco's modified Eagle's medium (DME) (Gibco) supplemented with 10% fetal calf serum (DME-10) (Gibco). To prepare this medium 2.5 ml of the DME-10 was placed in the dish followed by 2.5 ml of the agar solution poured at 42 ± 1°C and the mixture was allowed to gel for 5–7 min at 21°C. In 30 trials two worms were positioned 2 cm apart in each dish and in 15 trials single worms were placed in the center of the dish. The cultures were maintained in a high humidity incubator at 37°C with 6–7% CO_2 and observations were made at various intervals up to 48 hr postinoculation of worms. In cultures with two worms, 48 of 60 *E. revolutum* migrated to the periphery of the dish whereas in single worm cultures 14 of 15 worms migrated to the periphery.

Worms in contact or within 5 mm of each other were considered paired (Fried and Roberts, 1972, J. Parasitol. 58:88–91). We observed pairing in only 5 of the 30 double worm cultures, and this pairing was considerably less than reported previously for *E. revolutum* using a Locke's overlay and an agar substratum (Fried et al., 1980, J. Parasitol. 66:1014–1018). Fried and Pallone (1984, Z. Parasitenk. 70:297–302) also observed reduced pairing of *E. revolutum* when the defined medium NCTC 135 was substituted for the Locke's overlay.

Worms migrated at the surface or within 2 mm below the surface of the 0.8% agar: DME-10 medium. Worm tracks (Figs. 1, 2) were produced mainly by acetabular impressions in the medium, although some impressions of cephalic and tegumentary spines (not visible in Figs. 1, 2) were noted. Tracking was noted within 4 hr postincubation, but became more evident at 12 hr. All worms survived up to 48 hr at which time the cultures were terminated. Worms occasionally laid eggs in the medium; the cirri of some worms were protruded (Fig. 3), but cross-copulation was never observed. Media combinations that did not yield tracking were: 0.8% agar : DME-10 (0.5:1); 0.8% agar : DME-10 (0.75:1); and an 0.8% agar substratum with a DME-10 overlay.

Bacterial contamination was noted in some of the cultures and routine microbiological testing revealed the presence of *Escherichia coli, Bacillus cereus* and *Bacillus* sp.

Some worms maintained in culture for 48 hr

were examined by scanning electron microscopy (SEM) (Fried and Fujino, 1983, Int. J. Parasitol. 13, in press). These worms showed tegumentary damage anterior to the acetabulum, although the cephalic collar, tegumentary spines and sensory papillae were observed (Fig. 3).

Nollen and Nadakavukaren (1974, Exp. Parasitol. 36:123–130) reported the presence of *Escherichia coli* on the tegument surface of *Megalodiscus temperatus*. Our high magnification SEM's failed to show this bacterium associated with the surface of *E. revolutum*.

In summary, worm tracking was observed in an 0.8% agar : DME-10 (1:1) medium and worms survived in this medium up to 48 hr postinoculation.

We thank Ms. Lynn Steele, Infectious Disease Research Laboratory, Wilmington Medical Center, Wilmington, Delaware for aid in bacteriological identification.

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Research Note

Notes on the Life Cycle of *Dictyangium chelydrae* (Digenea: Microscaphidiidae)

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As part of a year-long study on the population biology of the hydrobiid snail *Amnicola* (*Cincinnatia*) *peracuta*, over 7,000 snails were collected from Beaver Pond Branch, Livingston Parish, Louisiana, and examined for larval digeneans. Five shed a bioculate, monostome cercaria, that proved to be the larva of the microscaphidiid *Dictyangium chelydrae* Stunkard, 1943, a parasite of the common snapping turtle *Chelydra serpentina*. The life cycle has not previously been reported, therefore we provide brief descriptions of the redia, cercaria, and metacercaria.

Three small *C. serpentina* (shell length: 5.5–12.5 cm) reared from eggs, and three large (shell length: 20.0–31.5 cm) wild-caught turtles served as experimental hosts. Prior to their use, wild-caught turtles were held for 8 mo during which fecal examinations were negative for *D. chelydrae* eggs. All turtles were maintained on frozen crayfish. Small turtles were fed 10–20 metacercariae by stomach tube; for large turtles, metacercariae were placed under the exoskeleton of crayfish that were offered to each turtle in a separate aquarium. All turtles were killed and necropsied 70 days postexposure.

Trematodes were pipetted into steaming AFA, stained with Van Cleave's hematoxylin, and mounted in Permount. All measurements are in micrometers with ranges followed by means in parentheses.

GERMINAL SACS: Sporocysts were not observed. Redia (Fig. 2) 600–700 (672) long by 85–95 (88) wide, in hepatopancreas and gills of host. Procruscula and birth pore absent. Mouth terminal. Prepharynx 5 long. Pharynx 28–32 (30) by 33–37 (35). Gut 200–250 (219) long, thin-walled (5), lined with cuboidal epithelium; containing brown finely granular material. Gut followed by one or two eyespotted cercarial embryos and other developmental stages.

CERCARIA (FIG. 1): Cercaria monostomate, bioculate. Body 350–460 (425) by 210–260 (232). Tegument unspined, 7–8 thick. Tail 600–650 (620) by 76–84 (80) at base; its attachment dorsally subterminal. Oral sucker protrusible, 122–128 (125) by 108–115 (112), with two anteroventral lappets. Pharynx absent. Ceca slightly sinuous extend to level of excretory pore. Eyespots 39–42 (40) by 25–28 (27), dorsal to and partially overlapping oral sucker posterolaterally. Penetration glands absent. Cystogenous glands dorsal,

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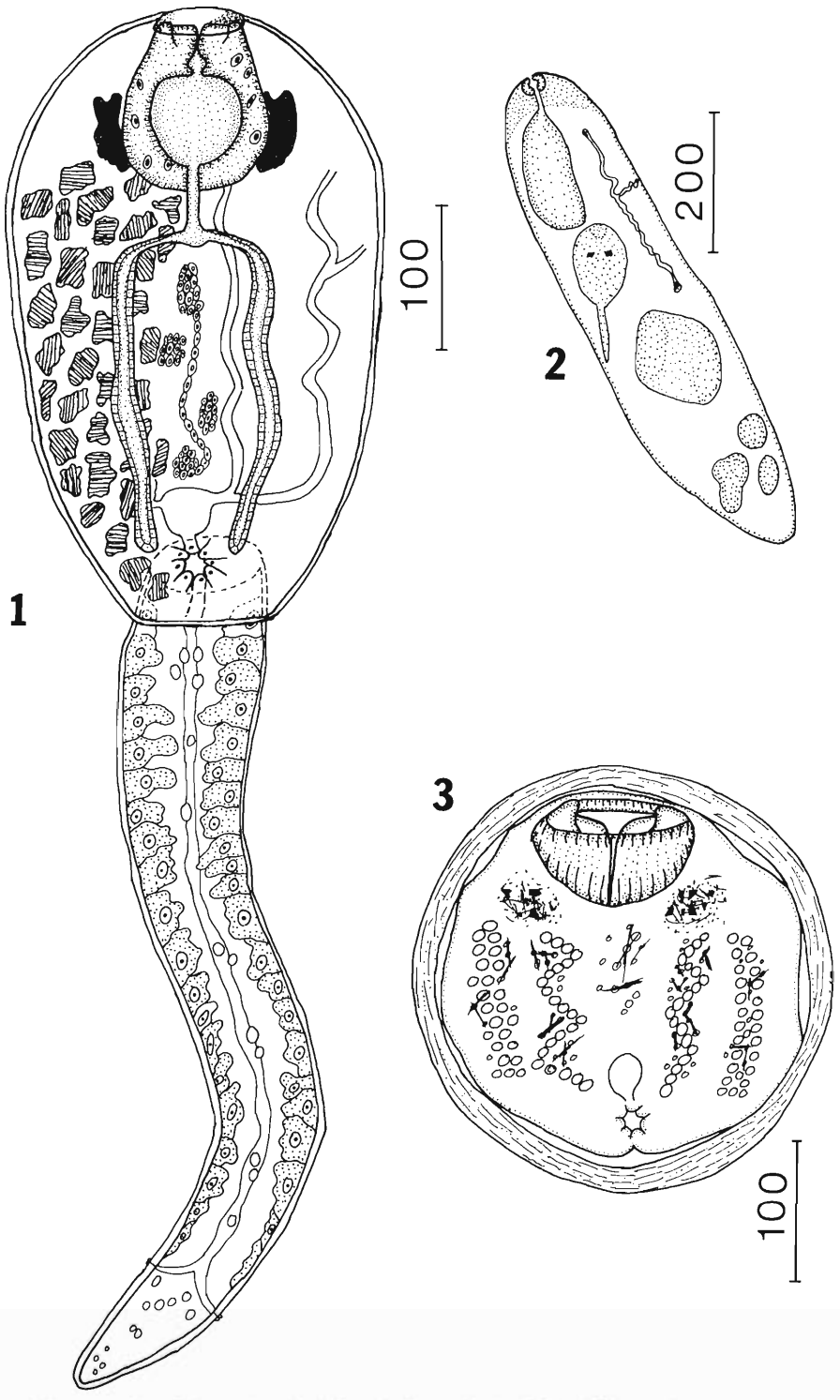

Figures 1–3. ***Dictyangium chelydrae.*** **1. Cercaria. 2. Redia. 3. Encysted metacercaria.**

from eyespots to posterior end of body. Primordia of terminal genitalia, testes, and ovary arranged as in adult: testes anterior to ovary, oblique; ovary median, connected with terminal genitalia just posterior to cecal bifurcation by a chain of nuclei. Excretory system consists of a thin-walled bladder that receives a short primary duct formed by the confluence of two secondary ducts (the medial pair of secondary ducts may represent a lymphatic system). Each secondary duct extends anteriorly to level of oral sucker. Bladder opens from body through a dorsal, subterminal pore, surrounded by a ring of darkly staining cells. From the pore, a single excretory tube extends nearly the length of the tail to bifurcate and open laterally at small pores.

METACERCARIA (FIG. 3): Shed cercariae encyst on substrate within half an hour. Cyst hemispherical, 300 in diameter, its wall 4–7 thick. Metacercariae freed from cysts 310–470 (407) by 130–260 (204). Oral sucker 100–130 (106) by 102–120 (111). Cystogenous glands absent. Eyespots diffuse.

ADULT: No specimens were recovered from the small snapping turtles that were fed metacercariae. Two of the large ones yielded three and five specimens. A single worm was recovered from a naturally infected *Chrysemys floridana* from Beaver Pond Branch. Our specimens agreed with the original description of *D. chelydrae* (Stunkard, 1943, J. Parasitol. 29:143–150).

VOUCHER SPECIMENS: *A. peracuta* from Beaver Pond Branch, Louisiana Delaware Museum of Natural History, Greenville, Delaware, DMNH #154181. *D. chelydrae*—USNM Helm. Coll., Beltsville, Maryland, #78005-78008.

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Research Note

Glycogen and Protein Content in Adult *Echinostoma revolutum* (Trematoda)

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Although a histochemical study of glycogen in *Echinostoma revolutum* adults (Fried and Kramer, 1968, J. Parasitol. 54:942–944) is available, an analysis of glycogen content is not. Various studies have reported the glycogen and protein content in trematodes (Smyth, 1976, Introduction to Animal Parasitology. John Wiley & Sons, New York; Cornford et al., 1982, J. Parasitol. 68:1010–1020), but none are available on *E. revolutum*. Glycogen and protein content in preovigerous (7-day-old worms) and ovigerous (14-, 21-, and 28-day-old worms) adults of *E. revolutum* are reported in this note.

Metacercarial cysts of *E. revolutum* were fed about 100 cysts/chick to day-old domestic chicks; chicks were given food and water ad libitum until necropsy at 7, 14, 21, and 28 days postinfection (Fried and Butler, 1978, J. Parasitol. 64:175–177). Worms were removed rapidly from the intestine, washed in three changes of Locke's solution, blotted dry and weighed. Worms were digested in 1 ml 30% KOH for glycogen analysis or homogenized in 1 ml of 0.25 M sucrose in 6 mM ethylenediamine tetraacetic acid (EDTA) for protein analysis. Worm glycogen content was isolated and analyzed as described by Montgomery (1957, Arch. Biochem. 67:378–386). Protein content was determined using the method of Bradford (1976, Anal. Biochem. 72:248–254) with slight modifications as described in the Bio-Rad (Richmond, California) protein assay kit. Reagent grade glycogen (Fisher Scientific Co., Boston, Massachusetts) was used to make glycogen standards and bovine serum albumin, fraction V (Sigma Chemical Co., St. Louis, Missouri) was used to make protein standards.

At each age beyond 7 days, 10 worms were analyzed for glycogen content and another 10 for protein content. Each analysis of the 7-day-old worms was done with 10 groups of 3 worms each.

Table 1. Glycogen and protein content in adult *Echinostoma revolutum* at 7, 14, 21 and 28 days.*

Age (days)	Wet weight (mg)	Dry weight (mg)	Protein (% dry wt)	Glycogen (% dry wt)
7	0.28 ± 0.03	0.13 ± 0.03	25.5 ± 4.1	45.4 ± 7.0
14	4.00 ± 0.63	1.43 ± 0.22	28.0 ± 3.5	17.4 ± 8.9
21	5.28 ± 0.57	1.68 ± 0.19	31.8 ± 1.9	36.8 ± 7.0
28	7.52 ± 0.70	2.31 ± 0.22	28.7 ± 4.1	22.1 ± 4.1

* Mean values ($N = 10$) ± SD.

Additional worms were used to determine dry weights at each age. These worms were dried at 100°C until a constant weight was achieved, and weights were recorded.

The homogenate of a single 3-wk-old worm was divided into two equal volumes. An aliquot of a glycogen solution containing 0.5 mg of glycogen was added to one of the homogenates and an equal volume of water was added to the other. The amount of glycogen in each homogenate was then determined and the difference between the two was used to calculate the percent recovery of glycogen. Analysis of the worm homogenate plus added glycogen showed a 91.8% glycogen recovery.

The age of the echinostomes was known and chicks were maintained on a controlled diet until just prior to necropsy. Our 7-day-old worms were preovigerous, whereas the 14-, 21-, and 28-day-old worms were ovigerous (Senger, 1954, Exp. Parasitol. 3:491–496). The possible effects of worm crowding were avoided by using worms from hosts that contained fewer than 20 worms at necropsy. The results of this study are presented in Table 1.

For *E. revolutum,* the glycogen percentage was highest in preovigerous worms and became reduced in ovigerous worms. Glycogen analyses of numerous species of adult trematodes have indicated that glycogen, measured as percent dry weight/fluke, varies from about 1 to 29% (von Brand, 1973, Biochemistry of Parasites, 2nd ed. Academic Press, New York). The average percent glycogen in our study, 30.4%, is over the upper limit of this range. As discussed by Smyth (1976, loc. cit.), factors such as worm age and maturity, and the nutritional condition of the host, must be known for glycogen content values to have any meaning.

Protein content remained relatively constant in both preovigerous and ovigerous worms. Protein analyses of numerous species of adult trematodes have indicated that protein, as precent dry weight/fluke, varies from about 43 to 66% (von Brand, 1973, loc. cit.). The average percent protein in our study, 28.5%, did not fall within the above-mentioned range. The values cited by von Brand were obtained by multiplying the experimentally determined nitrogen content by 6.25. As discussed by von Brand, this procedure may lead to spurious values if an organism contains considerable nonprotein nitrogen.

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Research Note

Prevalence of Black Spot (*Neascus pyriformis*: Trematoda: Diplostomatidae) of Fishes in Brule Creek, South Dakota

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Duru et al. (1981, Proc. Helminthol. Soc. Wash. 48:177–183) reported the black spot trematode *Neascus pyriformis* Chandler, 1951, from 7 of 10 fish species in Brule Creek, South Dakota. In the present study we examined changes in prevalence and range of intensity in all seven hosts collected in fall 1978, early summer 1979, and fall 1979, and changes in the relative density of the metacercarial cysts in certain host species.

Brule Creek is a small meandering stream in southeastern South Dakota. The study area was a 125-m section just above a bridge in the SE corner Section 35, Township 93N, Range 50W; this area is about 9 km from the stream's confluence with the Big Sioux River. Fish were collected using a knot-mesh minnow seine (2.95 m × 1.2 m with a mesh size of 6.3 mm, bar measure) and killed and preserved in 10% formalin. The total length of each fish was measured and the fully developed cysts in the skin and fins counted. The cysts are readily visible because of melanocytes present in the outer portion of the 'host cyst' (see Duru et al., 1981, loc. cit.). The three collection periods for all seven hosts were September–October 1978 (9/10-78), June–July 1979 (6/7-79), and September–October 1979 (9/10-79). The 9/10-79 period was subdivided into early September (E9-79), late September (L9-79), and October (10-79) for those hosts collected in larger numbers. All fish collected in each period were included in the samples.

The Statistical Analysis System (SAS) (Barr et al., 1979, SAS User's Guide. SAS Institute Inc., Raleigh, North Carolina. 494 pp.) was used for all statistical procedures. All computations were carried out on the IBM 3031 Computer at the University of South Dakota. Prevalence is the number of infected fish (expressed as percentage) per sample; relative density is the mean number of cysts per fish in a sample; and range of density gives the minimum and maximum number of cysts per sample (Margolis et al., 1982, J. Parasitol. 68:131–133).

The following approach was used to compare the relative density of the cysts for the different collection periods within a host species. All cyst counts were transformed to the square root of the counts and host lengths squared. Groups of 10 fish were selected at random from each sample and means calculated for these groups (Dixon and Massey, 1969, Introduction to Statistical Analysis. McGraw-Hill Co., New York. 638 pp.). The means were used in place of the single observations in the analysis of covariance procedure. With this approach, compared to the use of single observations, a better distribution of the residuals was obtained, no significant difference in regression line slopes of length vs. cyst number for samples within a species occurred, and plotted data showed no distinct nonlinearity. The greatest variance ratio for the samples within a species was 3:1.

A total of 8,553 fishes, belonging to 10 species and three families, from Brule Creek were examined for *Neascus pyriformis*. Seven species (fathead minnow, *Pimephales promelas*; creek chub, *Semotilus atromaculatus*; plains minnow, *Hybognathus placitus*; stoneroller, *Campostoma anomalum*; common shiner, *Notropis cornutus*; red shiner, *N. lutrensis*; white sucker, *Catostomus commersoni*) of two families were infected with the metacercarial cysts. The prevalence of *Neascus pyriformis* in all seven hosts was greater in 9/10-79 than in 9/10-78 and greater in 9/10-79 than in 6/7-79 for five hosts (Table 1). If samples containing less than 10 fish are eliminated from consideration, the prevalence for 9/

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Table 1. Prevalence, relative density and range of intensity of *Neascus pyriformis* in fishes from Brule Creek.

Species*	Coll.† period	No. exam.	No. inf. (%)	Rel. density $\bar{x} \pm SD$	Range of intensity	Host length (cm) $\bar{x} \pm SD$
Pimephales promelas Cyprinidae	9/10-78	1,193	545 (45.7)	1.77 ± 3.95	1–47	5.15 ± 0.82
	6/7-79	175	121 (69.1)	3.97 ± 6.66	1–50	6.28 ± 0.46
	E9-79	1,379	1,360 (98.6)	8.69 ± 7.69	1–96	5.27 ± 0.79
	L9-79	154	152 (98.7)	11.77 ± 8.28	1–47	5.62 ± 0.78
	10-79	472	472 (100)	13.69 ± 12.23	1–108	5.42 ± 0.77
	9/10-79	2,005	1,984 (99.0)	10.10 ± 9.25	1–108	5.77 ± 0.82
Semotilus atromaculatus Cyprinidae	9/10-78	274	212 (77.8)	4.47 ± 7.11	1–57	7.58 ± 3.96
	6/7-79	156	81 (51.9)	6.62 ± 22.97	1–222	7.10 ± 4.51
	E9-79	286	286 (100)	16.90 ± 17.33	1–139	6.71 ± 4.32
	L9-79	53	53 (100)	32.60 ± 31.39	2–152	8.66 ± 5.14
	10-79	110	109 (99.1)	25.59 ± 28.45	1–143	6.96 ± 4.35
	9/10-79	449	448 (99.8)	20.88 ± 23.11	1–152	7.00 ± 4.46
Notropis cornutus Cyprinidae	9/10-78	15	3 (20.0)	0.40 ± 0.91	1–3	6.60 ± 1.14
	6/7-79	7	6 (85.7)	2.40 ± 1.27	1–4	8.24 ± 2.00
	E9-79	405	362 (89.4)	4.19 ± 5.73	1–78	5.07 ± 1.01
	L9-79	182	179 (98.4)	6.95 ± 5.61	1–50	5.25 ± 0.55
	10-79	653	626 (95.9)	7.18 ± 6.73	1–78	5.62 ± 0.91
	9/10-79	1,240	1,167 (94.1)	6.16 ± 6.41	1–78	5.39 ± 0.94
Campostoma anomalum Cyprinidae	9/10-78	22	15 (68.2)	1.27 ± 1.32	1–5	6.84 ± 0.63
	6/7-79	27	13 (48.1)	2.15 ± 4.55	1–21	7.13 ± 2.40
	9/10-79	102	102 (100)	11.90 ± 8.86	1–40	8.12 ± 1.70
Notropis lutrensis Cyprinidae	9/10-78	9	6 (66.7)	0.78 ± 0.67	1–2	4.54 ± 0.83
	6/7-79	2	2 (100)	3.50 ± 0.71	3–4	4.2 ± 0.42
	9/10-79	20	20 (100)	29.60 ± 26.12	2–111	4.33 ± 1.21
Hybognathus placitus Cyprinidae	9/10-78	159	76 (47.8)	1.09 ± 1.64	1–10	7.26 ± 0.44
	6/7-79	1	1 (100)	2	2	6.00
	9/10-79	10	9 (90.0)	26.40 ± 34.69	6–121	7.42 ± 1.12
Catostomus commersoni Catostomidae	9/10-78	46	25 (54.3)	0.87 ± 1.07	1–5	11.35 ± 4.26
	6/7-79	66	28 (42.4)	0.62 ± 0.89	1–4	13.36 ± 4.32
	9/10-79	69	53 (76.8)	3.72 ± 4.72	1–21	14.31 ± 4.95

* The following species were not infected with black spot; number examined are enclosed in parentheses. Bigmouth shiner, *Notropis dorsalis* (Cyprinidae), 9/10-78 (393), 6/7-79 (323), 9/10-79 (1,004); sand shiner, *N. stramineus* (Cyprinidae), 9/10-78 (243), 6/7-79 (96), 9/10-79 (224); johnny darter, *Etheostoma nigrum* (Percidae), 9/10-78 (6), 6/7-79 (12), 9/10-79 (203).

† 9/10-78 = September–October 1978; 6/7-79 = June–July 1979; 9/10-79 = September–October 1979; E9-79 = early September 1979; L9-79 = late September 1979; 10-79 = October 1979.

10-78 vs. 9/10-79 samples increased by 22.0% (creek chub) and 75.1% (common shiner), and for 6/7-79 vs. 9/10-79 samples by 29.9% (fathead minnow) and 59.9% (stoneroller). The analysis of covariance showed homogeneous regressions of cyst number on fish length across collection period for those species tested (fathead minnow, creek chub, common shiner) and significant period effects for each species (Table 2). Because of small sample size, the red shiner, plains minnow, white sucker, and stoneroller were eliminated from the covariance analysis and tests with common shiners were limited to fall 1979.

Neascus pyriformis is adapted to the Cyprinidae in Brule Creek, because six of the seven infected species of this study were cyprinids. However, judging from prevalence, relative density, and range of intensity, certain cyprinids are more suitable hosts than others, and two cyprinids were never infected although large numbers were examined (Table 1).

Nothing is known of the life history of *Neascus pyriformis*. Because this species is considered closely related to *Uvulifer ambloplitis* (Hughes, 1927) and certain workers (Chandler, 1951, Am. Midl. Nat. 45:711–721; Dubois, 1969, Rev. Suisse Zool. 76:3–31) suspect *U. semicircumcisus* Dubois and Rausch, 1950, to be the adult, one might expect planorbid snails as first intermediate hosts and kingfishers as final hosts. Although kingfishers were present in the collection area, no planorbid snails were found. Only phys-

Table 2. Comparison of relative densities of *Neascus pyriformis* in fishes from Brule Creek.

Species	Collection period				
	9/10-78	6/7-79	E9-79	L9-79	10-79
Pimephales promelas	0.98*	0.61	2.83	3.02	3.09
Semotilus atromaculatus	1.59	1.45		4.73	4.57
Notropis cornutus			3.83		
			1.96	2.56	2.34

* Means underscored by the same line are not significantly different at the 0.05 level. These means are square root relative densities adjusted for fish length squared.

id snails were collected in this area, but none was infected with trematode larvae.

Brule Creek is a typical prairie creek with alternating riffles and pools; the bottom substrate consists of clay and silt with a thin gravel or rubble covering. All seven fish species infected with *Neascus pyriformis* are primarily pool dwellers. In contrast, the three fish species not infected prefer riffles or gravelly runs of the stream. This suggests that the snail host may be a pool dweller. Berra and Au (1978, Ohio Acad. Sci. 78:318–322) noted that pool-dwelling fish species were more heavily infected with *U. ambloplitis* than were riffle dwellers, and related this to the snail host's preference for quiet water.

The substantial increase in prevalence and range of intensity for all seven hosts from fall 1978 to fall 1979 apparently resulted from increased parasite recruitment. This recruitment was not manifested until fall 1979, because if host length is taken into account, no increase in the relative density of the cysts was evident from fall 1978 to early summer 1979 (Table 2).

The significant increases in the relative density of cysts from early (E9-79) to late (L9-79) September, with no increase from L9-79 to October (10-79), may indicate that parasite recruitment was occurring in late summer 1979 but had ended by early September (Table 2). The intensity increases undoubtedly resulted from earlier infections, since Hoffman and Putz (1965, Trans. Am. Fish. Soc. 94:143–151) first noted pigmented cysts of *U. ambloplitis* in experimentally infected fish 24 days postinfection.

The water level of Brule Creek was higher in 1979 than in 1978 because of increased rainfall and spring runoff. This higher level may have increased snail host habitat and ultimately enhanced the rate of black spot recruitment. A density-independent factor such as rainfall may be a primary factor in regulating black spot infrapopulations in fishes of small streams such as Brule Creek in South Dakota. The alternating dry and wet periods occurring in this state may be of more importance than density-dependent factors. This could result in a pattern of cyclic fluctuations in parasite numbers persisting over a period of years. Such a population, however, would be considered unstable and local extinction could result from a drastic change in this climatic parameter (Kennedy, 1977, pp. 63–109, *in* Esch (ed.), Regulation of Parasite Populations. Academic Press, New York).

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Research Note

Prevalence of *Cryptosporidium* Infections, and Their Relation to Diarrhea in Calves on 12 Dairy Farms in Maryland

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Cryptosporidium has been reported worldwide as a possible cause of neonatal calf diarrhea and deaths, in individual calves and in group outbreaks (Morin et al., 1976, Can. J. Comp. Med. 40:228–240; Pohlenz et al., 1978, J. Am. Vet. Med. Assoc. 172:452–457; Snodgrass et al., 1980, Vet. Rec. 106:458–459; Tzipori et al., 1980, Vet. Rec. 107:479–480; Howerth, 1981, J. S. Afr. Vet. Assoc. Sept:251–253; Jerrett et al., 1981, Austral. Vet. J. 57:434–435). In Idaho dairy herds where *Cryptosporidium* is considered to be a significant pathogen in neonatal calves (Anderson, 1982, Cal. Vet. 36:9–10) a survey was conducted on 12-day-old calves (Anderson, 1982, J. Am. Vet. Med. Assoc. 181:484–485). At least one *Cryptosporidium*-positive calf was found on 56% of the 73 farms surveyed, and 38.7% of all fecal samples (284) were positive. Jungman (1983, Monats. Vet. 38:299–300) found 51% of 172 calves infected with *Cryptosporidium*; 45% of those infected had acute diarrhea.

Because some calves purchased from dairy farms for studies at the Animal Parasitology Institute have developed diarrhea containing *Cryptosporidium* oocysts during their first 2 weeks of life and shed oocysts for approximately 4–7 days, a survey was conducted in the spring of 1983 to determine the prevalence of *Cryptosporidium* on 12 randomly chosen farms within 60 miles of Beltsville, Maryland. The study was limited to heifer calves because bulls were usually sold within a week of birth.

Management systems varied from farm to farm. All farmers reported giving colostrum to newborn calves followed by either whole milk, milk plus replacer, or milk replacer alone. Many farmers fed antibiotics in the milk or milk replacer and some also gave injectable vitamins and iron shortly after calves were born. Housing conditions varied from fenced and separated fiberglass hutches on well-drained ground, to small pens with plywood partitions within a barn, or to sheds with several calves. Cleaning varied from none, to sporadic, to removal of bedding and manure at 2–3-day intervals. None of the farms reported significant calf losses during the past 2

Table 1. Prevalence of *Cryptosporidium* oocysts in feces of neonatal calves in Maryland in the spring of 1983.

County	Farm	No. of fecal samples	No. of positive samples	No. of calves examined	No. of calves positive	No. of calves with diarrhea	No. of calves with both diarrhea and *Cryptosporidium*
Anne Arundel	A	23	3	12	3 (25%)	0	0
Baltimore	B	24	1	12	1 (8.3%)	2	0
	C	24	10	12	9 (75%)	7	6
Carroll	D	29	6	18	6 (33.3%)	1	0
	E	14	0	12	0 (0%)	0	0
Howard	F	25	2	17	2 (11.8%)	0	0
	G	22	2	11	1 (9.1%)	1	0
Montgomery	H	28	14	16	10 (62.5%)	2	2
	I	2	0	2	0 (0%)	0	0
	J	7	0	6	0 (0%)	1	0
Prince George's	K	11	2	6	2 (33.3%)	0	0
	L	24	2	12	2 (16.7%)	2	0
Total 6	12	233	42 (18.0%)	136	36 (26.5%)	16 (11.8%)	8 (5.9%)

yr; some had undiagnosed diarrhea and one had *Escherichia coli*-related diarrhea in "older" calves.

Usually, two direct fecal samples were taken from each calf at approximately 7 and 14 days of age; 136 calves provided 233 samples. Time and travel distances precluded daily sampling. Diarrhea was characterized by unformed fluid to watery feces, as opposed to semisolid unformed feces normally found in young calves. Feces in individual plastic jars were stored at 5°C for 1–5 days until examined in the laboratory. One to 5 ml of each specimen was diluted 1:20 in water, filtered through cheesecloth and 15 ml of the filtrate was centrifuged at 450 *g* for 10 min. The supernate was decanted and the pellet mixed with Sheather's sugar solution and centrifuged at 30 *g* for 10 min. A small quantity of the feces–Sheather's mixture was then aspirated from the surface of the flotation with a Pasteur pipette and a drop of this mixture placed on a microscope slide with a coverslip and examined with phase-contrast microscopy for the presence of *Cryptosporidium* oocysts. The actual number of oocysts per gram of feces was not determined, but oocyst counts ranged from 1 to 300 per slide examined.

On 9 (75%) of the 12 farms, from 8.3 to 75% of the calves examined were shedding *Cryptosporidium* oocysts and on 3 (25%) of the farms none of the calves (20) were positive. Of a total of 136 calves examined 36 (26.5%) and *Cryptosporidium* and 16 (11.8%) had diarrhea. Only 8 calves (5.9%) had diarrhea and *Cryptosporidium* concurrently (Table 1).

Because calves were examined on only 1 or 2 days during their first 3 wk of life it is likely that dairy farms in general may have more animals that become infected with *Cryptosporidium* than were found in this survey. In this survey there appeared to be no relationship between management systems, number of infected calves, and numbers of oocysts shed.

Because only 5.9% of the calves examined had both diarrhea and *Cryptosporidium* infection, and because these infections were not found to be associated with any severe or prolonged clinical manifestations, *Cryptosporidium* could not be considered a significant pathogen within the confines of this survey.

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Research Note

Use of Monoclonal Antibodies to Locate *Eimeria* Sporozoites (Protozoa) in Intestinal Sections

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Preliminary experiments exploring the effects of exogenous compounds on the invasion of avian intestinal epithelial cells by *Eimeria* sporozoites indicated the need for a rapid, positive procedure for locating the parasites in sections of intestinal tissue. The sporozoites were difficult to find using standardly employed staining procedures such as hematoxylin and eosin, and attempts to count the intracellular sporozoites were time consuming. Additionally, because the sporozoites were so difficult to find, especially in the interior of the villi, the counts lacked the accuracy demanded in the invasion studies. Monoclonal antibodies that have been produced against *Eimeria* sporozoites reacted brightly and specifically with air-dried sporozoites, when followed by a fluorescein-conjugated goat antimouse IgG (whole molecule) (Danforth, 1982, J. Parasitol. 68:392–397). Therefore, a protocol was developed for using these monoclonal antibodies to locate sporozoites in intestinal sections.

For the tissue sections, turkeys and chickens were inoculated with 2×10^7 oocysts of *E. meleagrimitis, E. adenoeides, E. acervulina,* or *E. tenella* and killed 1, 2, 3, and 4 hr postinoculation. Samples of intestine (~2 cm long) from the

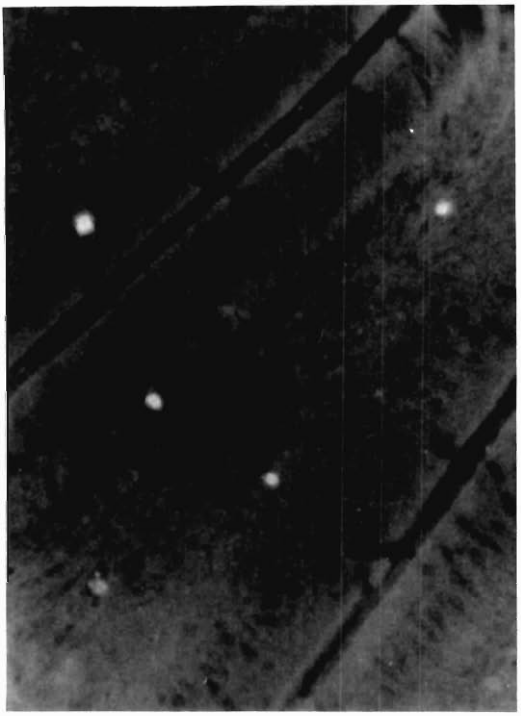

Figure 1. ***Eimeria meleagrimitis*** **sporozoites in intestinal tissue sections labeled with indirect fluorescent antibody procedures using monoclonal antibody 1209 (×400).**

duodenum, jejunum, and cecum were fixed in Carnoy's solution and embedded in paraffin. Sections, 4 μm thick, were cut from these samples and mounted on microscope slides.

Immediately prior to use in the indirect fluorescent antibody (IFA) procedure, the intestinal sections were placed in xylene for 10 min to remove the paraffin, air-dried, and washed in phosphate buffered saline (PBS, pH 7.8) for 10 min. The excess PBS was blotted from the slides and the IFA procedure was run. The sections were exposed to the cross-reactive monoclonal antibody, 1209 (originally produced against *E. acervulina*), for 30 min at 37°C, and washed with PBS for 10 min at room temperature. They were then incubated with a 1:16 dilution of fluorescein-conjugated antimouse IgG (Sigma Chemical Co., St. Louis, Missouri) containing 125 μg/ml Evans blue dye. After 30 min at 37°C, the slides were washed in PBS for 10 min at room temperature, mounted with coverslips in buffered glycerol (pH 8.1), and examined with a Zeiss ultraviolet epifluorescence microscope.

A number of antibodies that react with different areas of the sporozoite were tested for use in this protocol. Only those antibodies that specifically label the refractile bodies of air-dried sporozoites also labeled the sporozoites in intestinal tissue. However, in the tissue sections, the fluorescence was not confined to the refractile bodies. Fixation and paraffin-embedding may have caused a movement of antigenic determinants from the refractile body to the cytoplasm of the sporozoites. It is also possible that the treatment of the tissue may have exposed additional antigenic determinants that reacted with the monoclonal antibodies. As a result, the sporozoites in the tissue sections exhibited a general internal fluorescence with slightly stronger reaction at the periphery (Fig. 1). The intestinal tissue, counterstained by the Evans blue, appeared dark red under UV light, and provided a stark contrast to the green, fluorescing sporozoites. Only dark red counterstained tissue was seen in sections from uninoculated birds. Thus, by the use of monoclonal antibodies and IFA techniques, *Eimeria* sporozoites can be positively and rapidly identified in sections of intestinal tissue.

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Research Note

Eye Anomalies in the Leech, *Dina anoculata,* (Erpobdellidae)

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The type locality of *Dina anoculata* Moore, 1898 was mountains of San Diego County in California, and as the name suggests, it was described as without "pigmented eyes" (Moore, 1898, Proc. U.S. Nat. Mus. 21:543–563). Since 1955, additional collections have been made from the Laguna Meadow area (elevation 1,652 m) of Mount Laguna, San Diego County, where Big Laguna Lake, a relatively permanent body of water, exists and which possibly was the original collection locality. Freshly preserved specimens collected in 1973 were sent to R. T. Sawyer who identified them as *D. anoculata* and confirmed the presence of pigmented eyes (pers. comm.). Later, Klemm (1982, Environmental Monitoring and Support Laboratory Report 600/3-82-025. U.S. Environmental Protection Agency, Cincinnati, Ohio. 177 pp.) modified the description of *D. anoculata* to read "Eyes absent or if present, 3 pairs in separate labial and buccal groups." In all of the living worms we have examined, eyes have been present and this report details their distribution patterns.

Leeches for this study were collected from the muddy bottom of a pond 120 m south of Big Laguna Lake between October 2, 1982 and January 14, 1983 and held in 6-cm deep water in a 26 × 18-cm aerated aquarium with 1 cm of small gravel on the bottom until examined between January and March 1983. Initially, worms were relaxed with menthol crystals, drawn between the fingers to remove mucus, straightened and placed between damp paper towelling in a tray

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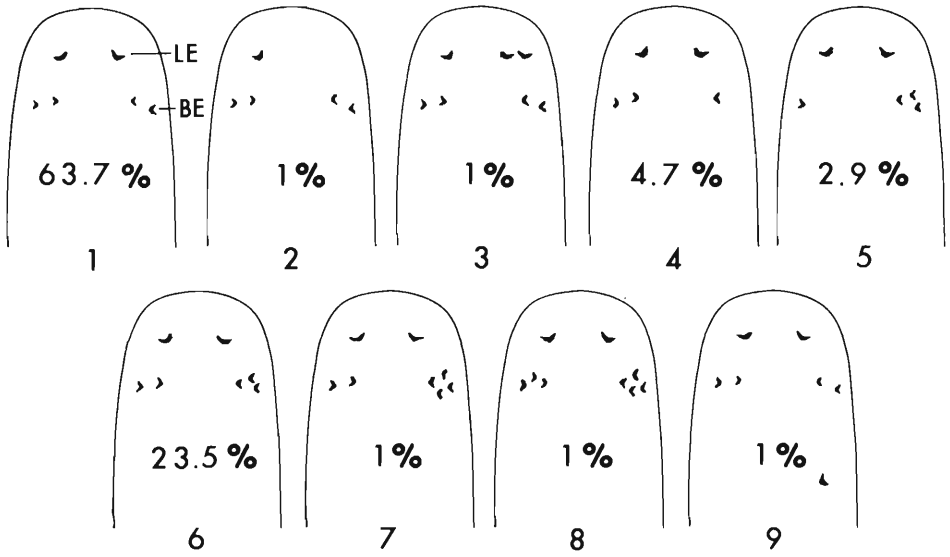

Figures 1-9. Eye distribution patterns and the prevalence of each, expressed as percentages, in 102 *Dina anoculata*. 1. Standard arrangement with one labial and two transversely positioned buccal eye pairs. 2. One labial eye absent. 3. Two labial eyes on one side. 4. One buccal eye absent. 5. One buccal eye absent on one side and three on opposite side. 6. Three buccal eyes on one side. 7. Four buccal eyes on one side. 8. Three buccal eyes on one side and four on opposite side. 9. One eye behind the two buccal eyes on one side. LE, labial eye; BE, buccal eye.

and flooded with buffered 10% formalin, and measured and examined for pigmented eyes. However, 24 hr later, crescent-shaped eyes that we had seen in the living worms were difficult to observe, even after clearing the worms in lactophenol. Consequently, living worms were used for this study. They were relaxed with menthol crystals, then manipulated under a dissecting microscope to determine eye distribution before being fixed and measured. When it occurred, asymmetry of eyes was random. Thus, in the tabulation no distinction was made between right and left.

Eye distribution patterns were determined for 102 worms 13–52 mm long. The nine observed patterns and prevalence of each, expressed as percentages, are shown in Figures 1–9. The most common pattern includes one labial pair (larger anterodorsal pair) and two buccal pairs (behind the anterodorsal pair) transversely arranged as in Figure 1. Because 63.7% had that pattern in this study, it is designated to be the standard. The other patterns are considered to be anomalies, of which only one occurred in more than 5% of the worms.

Amin (1978, Proc. Helminthol. Soc. Wash. 45: 272–275) documented the number and position of eyes of *D. lineata* and found that eyes were rarely absent. He recognized two categories: one with the buccal eyes transversely arranged and a second, in which all eyes behind the labial eyes are arranged in two longitudinal rows with the distance between eyes widening posteriorly. Although that latter category was not observed in our worms, one did have a single eye behind the usual transversely arranged buccal pairs (Fig. 9). Our results suggest that in the mountains of San Diego County, *D. anoculata* has pigmented eyes, usually one labial pair and two transversely arranged buccal pairs. Because no worm was found without eyespots, apparently the previous reports of *D. anoculata* lacking eyes were based on specimens in which eye pigmentation had been lost as a result of preservation and/or clearing.

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Research Note

Gastrointestinal Parasitism in Pygmy Killer Whales

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In 1976, White (Florida Sci. 39:31–36) reported several hundred nematodes (*Filocapsularia* sp.) and several tetrabothriid cestodes from the stomach of a pygmy killer whale (*Feresa attenuata* Gray, 1874) that was found on the east coast of Florida. Several years later, Forrester et al. (1980, J. Mammal. 61:356–360) reported parasites from three pygmy killer whales that stranded in Florida. They found nematodes (*Stenurus globicephalae* Baylis and Daubney, 1925) and trematodes (*Nasitrema lanceolata* Neiland, Rice and Holden, 1970) in the cranial sinuses, cestodes (*Tetrabothrius forsteri* Krefft, 1871) in the small intestine, tetraphyllidean cestode larvae in the liver, and pseudaliid nematodes (unidentified) in the lungs. The present report summarizes the gastrointestinal parasites collected from three adult male pygmy killer whales (total lengths 199 cm, 201 cm, and 208 cm) that stranded at Panama City Beach, Bay County, Florida on January 8, 1981.

The forestomach, fundic stomach, and intestine of each whale were examined. Parasites were collected with fine mesh screens, preserved, and later identified. Voucher specimens have been deposited in the USNM Helminthological Collection, Beltsville, Maryland 20705. The three species of parasites found were: *Pholeter gastrophilus* (Kossack 1910) (No. 77366); *Anisakis typica* (Diesing 1860) (No. 77367); and *Trigonocotyle* sp. (No. 77368).

The discovery of the trematode, *P. gastrophilus,* constitutes a new host record. One whale contained a single *P. gastrophilus* in the fundic

stomach; a second whale contained three specimens encysted in the fundic stomach and duodenum. The nematode, *Anisakis typica,* constitutes another new record for this host, though not unexpected because this parasite is common in cetaceans from warm and tropical waters (Davey, 1971, J. Helminthol. 45:51–72). Specimens of *A. typica* were found in the fore- and fundic stomach in all three whales. Intensities were similar, with 51, 145, and 166 worms collected from each whale. Specimens of *Trigonocotyle* sp. also were found in all three whales, and they were the most abundant parasite (6,600, 7,200, and 14,500 estimated total worms from each whale via dilution count procedure). They mostly were concentrated in the first 4 m of the intestine (av. total length approx. 15 m). Not only is the discovery of *Trigonocotyle* sp. in pygmy killer whales reported for the first time, but it appears that the specimens also may represent an undescribed species.

We wish to correct an error that was made by Forrester et al. (1980, op. cit.) a few years earlier. Since our work and their work on pygmy killer whales was conducted in the same laboratory, we had access to the material collected from their study. Whereas they deposited a few specimens of *Tetrabothrius forsteri* from a male whale in the U.S. National Parasite Collection, we discovered a jar containing the 2,328 specimens from a female whale that also were identified as *T. forsteri.* However, the latter specimens were unlike the deposited specimens, but identical to the *Trigonocotyle* sp. found in our study. Some of these specimens have been added to the USNM Helminthological Collection (No. 77679).

We gratefully acknowledge Daniel K. Odell of the University of Miami for obtaining the gastrointestinal tracts in addition to supplying pertinent data. Florida Agricultural Experiment Stations Journal Series No. 4361.

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Research Note

Helminths of Foxes and Coyotes in Florida

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Twenty-six gray foxes (*Urocyon cinereoargenteus*), four red foxes (*Vulpes vulpes*), and three coyotes (*Canis latrans*) were collected from 1972 to 1981. Most were road-kills, but a few were shot or live-trapped. Most of the foxes (21 gray, 2 red) were collected in Alachua County in north-central Florida; the remaining were from five other counties in the central part of the state. Two coyotes were from northwestern Florida (Escambia and Gadsden counties); the third coyote was collected in central Florida (Lake County). The animals were frozen and later examined at necropsy. Necropsy techniques for the recovery and identification of parasites followed the procedures as described by Kinsella and Forrester (1972, Proc. Helminthol. Soc. Wash. 39:173–176). Voucher specimens were deposited in the U.S. National Parasite Collection in Beltsville, Maryland and given the accession numbers 77800–77823.

Table 1 shows the site, prevalence, and intensity for each species of helminth. Mean, median, and range values are presented for intensity. Twenty-two species were found including 13 Nematoda, 4 Acanthocephala, 4 Cestoda, and 1 Trematoda. The number of helminth species per infected host varied from 1 to 7 (mean 4, median 3) with one gray fox free of helminths. The total number of helminths per infected animal ranged from 3 to 302 (mean 50, median 23). The greatest number of any single species of helminth occurred in two gray foxes, both of which harbored over 200 specimens of *Molineus barbatus* in their small intestine. This trichostrongyloid was the most prevalent parasite. It occurred in 73% of the animals examined, and comprised 41% of

Table 1. Location, prevalence, and intensity of helminths of gray foxes, red foxes, and coyotes in Florida, 1972–1981.

Parasite	% Prevalence (intensity, mean; median; range)*		
	Gray fox $N = 26$	Red fox $N = 4$	Coyote $N = 3$
Trematoda			
Alaria marcianae (3)†	19 (27; 37; 1–55)	0	33 (2; 2; 2)
Cestoda			
Taenia pisiformis (3)	4 (3; 3; 3)	0	67 (13; 13; 1–24)
Taenia sp. (3)‡	8 (2; 2; 1–2)	0	33 (8; 8; 8)
Taenia sp. (3)§	0	25 (1; 1; 1)	0
Mesocestoides sp. (3)‖	12 (3; 1; 1–8)	0	0
Spirometra mansonoides (3)	4 (1; 1; 1)	0	0
Acanthocephala#			
Unidentified (3)	4 (1; 1; 1)	0	33 (1; 1; 1)
Centrorhynchus wardae (3)	4 (1; 1; 1)	0	0
Pachysentis canicola (3)	4 (1; 1; 1)	0	0
Moniliformis moniliformis (3)	4 (8; 8; 8)	0	0
Nematoda			
Molineus barbatus (3, 5)	77 (30; 7; 1–224)	75 (5; 5; 1–9)	33 (49; 49; 49)
Physaloptera rara (1, 2, 3)	69 (11; 10; 1–49)	50 (27; 27; 13–40)	33 (25; 25; 25)
Ancylostoma tubaeforme (3)	77 (11; 5; 1–48)	50 (10; 10; 5–14)	0
Trichuris vulpis (4, 5)	23 (9; 2; 1–43)	50 (4; 4; 2–6)	67 (11; 11; 9–12)
Spirurid larvae (1)	15 (6; 1; 1–19)	25 (14; 14; 14)	0
Spirocerca lupi (1)	12 (1; 1; 1)	25 (2; 2; 2)	0
Dirofilaria immitis (6)	8 (3; 3; 1–5)	25 (1; 1; 1)	33 (9; 9; 9)
Capillaria aerophila (7)	4 (2; 2; 2)	50 (2; 2; 1–2)	33 (1; 1; 1)
Ancylostoma caninum (3)	4 (6; 6; 6)	25 (4; 4; 4)	33 (2; 2; 2)
Ancylostoma braziliense (3)	4 (4; 4; 4)	0	0
Capillaria plica (8)	0	0	33 (2; 2; 2)
Strongyloides stercoralis (3)	4 (71; 71; 71)	0	0
Anafilaroides pararostratus (7)	0	25 (2; 2; 2)	0
Toxocara canis (3)	0	25 (3; 3; 3)	0

* Intensity = number of worms/infected animal; values ≥0.5 were rounded to next highest integer.

† Numbers in parentheses indicate site in host: (1) esophagus; (2) stomach; (3) small intestine; (4) cecum; (5) large intestine; (6) heart; (7) lungs; (8) urinary bladder.

‡ Hooks on scolices unsuitable for definitive species identification; specimens closely resemble *T. macrocystis*.

§ No scolices, only few unidentifiable segments available.

‖ Unidentified to species because of the uncertain taxonomic status of members of this genus.

All specimens were immature, and none had fully evaginated proboscises. The unidentified specimens belonged to either the genus *Oncicola* or *Macracanthorhynchus*.

the total number of parasites. *Ancylostoma tubaeforme* and *Physaloptera rara* were the next most prevalent helminths (both 67%), and they comprised 15% and 17%, respectively, of the total number of parasites. Although prevalent, the mean intensities for *M. barbatus, A. tubaeforme,* and *P. rara* in the 26 gray foxes examined were not high (30, 11, and 11, respectively). The remaining helminths occurred infrequently and usually in low numbers (Table 1).

The presence and high prevalence of *A. tubaeforme* in both the gray and red foxes was most unusual. This species is generally considered a hookworm of felids, but foxes and cats ingest similar prey including small rodents such as the cotton rat (*Sigmodon hispidus*) that may serve as paratenic hosts (Norris, 1971, J. Parasitol. 57: 998–1009). Wild rodents commonly preyed upon by foxes in Florida may be better adapted as paratenic hosts for *A. tubaeforme* than either *A. braziliense* or *A. caninum,* the two hookworms less commonly found in foxes in this study. Alternately, the high occurrence of *A. tubaeforme* may result from greater contact of foxes (or paratenic hosts) with wild felids such as bobcats (*Felis rufus*) or feral cats (*Felis domesticus*) than

with feral dogs (*Canis familiaris*) or coyotes, the latter of which are rare in Alachua County. By contrast, a high prevalence (71%) of hookworms identified as *A. caninum* was reported from gray foxes in other southeastern states (Miller and Harkema, 1968, Proc. Helminthol. Soc. Wash. 35:118–125).

Two gray foxes, one red fox, and one coyote were infected with the heartworm, *Dirofilaria immitis*. Only female heartworms from the coyote contained microfilariae. The overall prevalence (12%) was typical of the findings of many other studies on wild canid populations (Simmons et al., 1980, J. Wildl. Dis. 16:225–228). The role of foxes and coyotes in the epizootiology of dirofilariasis is uncertain. Simmons et al. (1980, op. cit.) stated that wild canids should not be considered as reservoir hosts for heartworms because some studies have shown that populations of foxes with a low occurrence of heartworms exist in areas where heartworms are prevalent in local dog populations. Although this may be true for foxes, coyotes appear to be quite suitable as potential reservoir hosts of canine heartworm as reported by Weinmann and Garcia (1980, J. Wildl. Dis. 16:217–221).

Taenia pisiformis appears to be a common helminth of gray foxes, reported from 70% of 543 foxes in Illinois (Dyer and Klimstra, 1982, Trans. Ill. State Acad. Sci. 75:289–295), 57% of 112 foxes in Texas (Buechner, 1944, J. Mammal. 25: 185–188), 88% of 17 foxes in Mississippi (Ward, 1947, J. Parasitol. 33:23–24), and 38% of 24 foxes in North Carolina, South Carolina, and Georgia (Miller and Harkema, 1968, op. cit.). That only 12% of the gray foxes in Florida were infected with *Taenia* spp. (*T. pisiformis* and a species resembling *T. macrocystis*) suggests that gray foxes in this area do not prey as heavily upon rabbits as in other localities. However, foxes are omnivores that probably exhibit variations in their parasitic faunas as a result of the seasonal and geographical availability of certain food items, especially intermediate hosts (Dyer and Klimstra, 1982, op. cit.). Data from a small survey such as this should be interpreted with caution in the absence of better seasonal and geographical data.

Appreciation is expressed to P. P. Humphrey, G. W. Foster, and R. M. Anderson for examining foxes and coyotes. Special thanks are due B. B. Nickol for identifying the Acanthocephala, R. L. Rausch for identifying the species of *Taenia,* J. R. Lichtenfels for assistance with the species of *Ancylostoma,* and D. J. Forrester, C. H. Courtney, E. C. Greiner, and M. D. Young for their helpful suggestions in the preparation of this note. Supported in part by grants number 977-G, 1270, and 1270-G from the Florida Game and Fresh Water Fish Commission. A contribution of Federal Aid to Wildlife Restoration, Florida Pittman-Robertson Project W-41. Florida Agricultural Experiment Stations Journal Series No. 4776.

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Research Note

Helminth Parasites of *Anolis carolinensis* (Reptilia: Lacertilia) from Southeastern Louisiana

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The green anole, *Anolis carolinensis,* is a common iguanid lizard throughout the southeastern United States. As a diurnal, primarily arboreal species, it feeds mostly on insects and other arthropods (Schmidt and Inger, 1957, Living Reptiles of the World. Hanover House, Garden City, New York. 287 pp.).

Between November 1981 and October 1983, 543 anoles (398 ♂, 145 ♀) were examined for parasitic helminths. Anoles were hand-collected at night by flashlight within a 20-mi radius of La Place, Louisiana and shipped the next day by air freight to our laboratory (pers. comm., The Snake Farm, La Place, Louisiana 70068). The snout–

Table 1. Helminths collected from *Anolis carolinensis* in southeastern Louisiana.

Helminth	Site* in host	No. (%) hosts infected	Intensity	
			Mean ± SE	Range
Nematoda				
Oswaldocruzia pipiens	SI	54 (9.9)	1.3 ± 0.09	1–4
Cosmocercoides sp.	LI	6 (1.1)	3.8 ± 2.23	1–16
Camallanus sp. larvae	SI	3 (0.6)	3.0 ± 1.63	1–7
Unidentified free larvae	BC; SI	9 (1.7)	1.0 ± 0.00	1
Unidentified encysted larvae	P	8 (1.5)	1.3 ± 0.15	1–2
Cestoda				
Mesocestoides lineatus tetrathyridia	BC; M; F	2 (0.4)	174.0 ± 115	10–338
Oochoristica anolis	SI	2 (0.4)	27.0 ± 17.0	3–51
Proteocephalid plerocercoids	BC	1 (0.2)	4.0 ± —	4
Trematoda				
Allopharynx multispinosa	SI	2 (0.4)	3.5 ± 1.77	1–6
Unidentified plagiorchioids	SI	2 (0.4)	1.0 ± 0.00	1
Acanthocephala				
Unidentified cystacanths	M	4 (0.7)	1.5 ± 0.43	1–3

* Site abbreviations: BC = body cavity, F = muscle fascia, LI = large intestine, M = mesenteries, P = peritoneum of stomach, SI = small intestine.

vent length of each anole was measured upon examination ($\bar{x}$ = 53.42 mm; SD = 8.13); the mean for this study was within the normal range for adults of this species. Each anole was killed by decapitation and a thorough search for helminths was made of all viscera, muscles, and subcutaneous tissues under a dissecting microscope. Nematodes and acanthocephalans recovered were fixed in Travassos' solution and mounted unstained in glycerol gelatin. Cestodes and trematodes were fixed in AFA, stained with Semichon's aceto-carmine and mounted in damar.

Of 543 anoles examined, 82 (15%) were infected with at least one helminth; this included 62 (16%) of the males and 20 (14%) of the females. Of the 82 anoles infected, 72 (88%) were infected by 1, 9 (11%) by 2, and 1 (1%) by 3 helminth species. A total of 11 helminth species was found (Table 1).

Oswaldocruzia pipiens Walton, 1929 was by far the most common helminth in the present study. Sellers (1971, J. Parasitol. 57:355) reported *O. pipiens* from 1 of 200 anoles collected in northern Louisiana. The present report is a new host record for *Cosmocercoides* sp., a common nematode genus in many North American amphibians and reptiles. *Camallanus* sp. larvae also are reported here for the first time from *A. carolinensis,* which probably is an accidental or paratenic host.

Oochoristica anolis Harwood, 1932 was first described from a single specimen collected by Harwood (1932, Proc. U.S. Natl. Mus. 81:1–66) from 1 of 30 anoles in Texas; it was reported from 2 of 200 anoles in northern Louisiana (Sellers, 1971, op. cit.) and from 2 of 50 anoles from La Place, Louisiana (Carter and Etges, 1973, J. Parasitol. 59:1140–1141). The *Mesocestoides lineatus* (Goeze, 1782) tetrathyridia reported here are from the same population reported by Conn et al. (1984, J. Parasitol. 70:68–77). Specific identification of these tetrathyridia was made from experimentally obtained adults. The large number of tetrathyridia in one lizard apparently resulted from ingestion of a large number of infective-stage worms, because they showed no sign of asexual proliferation during many intraperitoneal transfers between experimental hosts. This observation is not surprising, because asexually proliferative tetrathyridia are encountered only rarely in field collections. The proteocephalid plerocercoids reported here were identified by their prominent apical organs and large scoleces, but otherwise were morphologically similar to the tetrathyridia. Harwood (1932, op. cit.) reported an immature adult *Proteocephalus* sp. from the intestine of 1 of 30 anoles in Texas.

The present report is the first for the plagiorchiid fluke, *Allopharynx multispinosa* (Bennett, 1935), since its original description from anoles in Baton Rouge, Louisiana (Bennett, 1935, J. Parasitol. 21:83–90). The unidentified plagiorchioid trematodes reported here were very small (<1.0 mm long) and were damaged during processing so that more definite identification was not possible.

Helminths previously reported from *A. carolinensis,* but not found in the present study, include *Urotrema wardi* Pérez Vigueras, 1940, reported from Florida by Sellers (1971, op. cit.) and *Alloglyptus crenshawi* Byrd, 1950, reported from Georgia by Byrd (1950, Trans. Am. Microsc. Soc. 69:280–287).

Some specimens collected in the present study were deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705, with the following accession numbers: *O. pipiens* (78127), *Cosmocercoides* sp. (78128), *Camallanus* sp. (78129), *M. lineatus* adult (78124), tetrathyridia (78123), *A. multispinosa* (78125), cystacanth (78126).

We thank John L. Crites of Ohio State University for verifying the identifications of the nematodes.

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Research Note

Helminths of Desert Cottontail Rabbits (*Sylvilagus auduboni* (Baird)) Inhabiting Prairie Dog Towns in Eastern New Mexico

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The desert cottontail, *Sylvilagus auduboni,* is a common mammal of the southwestern United States and is found in close association with the black-tailed prairie dog (*Cynomys ludovicianus*). Fourteen desert cottontails were collected from three active prairie dog towns (towns P, D, and C) near Portales, Roosevelt Co., New Mexico (see Pfaffenberger et al., 1984, Proc. Helminthol. Soc. Wash. 51, in press). The rabbits were infected with one species of Nematoda (*Dermatoxys veligera* (Rudolphi, 1819) Schneider, 1866) and two species of Cestoda (*Raillietina retractilis* Stiles, 1925 and *Taenia pisiformis* (Bloch, 1780) Hall, 1919).

Necropsy was done with the aid of a stereomicroscope. All body cavities, internal organs, and gastrointestinal contents were examined. Nematodes were placed in acetic acid, cleared in a 70% ethanol/5% glycerin mixture followed by pure glycerin, and then stored in FGA.

Cestodes were relaxed in cold distilled water, fixed in AFA, stained with either Semichon's aceto-carmine or Celestine blue B, dehydrated with absolute ethanol, cleared in methyl salicylate, and mounted in Canada balsam. Cysticerci were fixed in AFA, cleared in methyl salicylate, and stored in FGA. Representative specimens are deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705 (nos. 77824–77826).

The results of this study are shown in Table

Table 1. Helminths of desert cottontails inhabiting prairie dog towns in eastern New Mexico.

Helminth	Town	Prevalence No. infected/ no. examined	Intensity Range (mean)
Nematoda			
*Dermatoxys veligera**	P, C, D	4/14	1–19 (6.3)
Cestoda			
*Raillietina retractilis**	P, C, D	6/14	1–26 (9.5)
Taenia pisiformis†	C	3/14	5–15 (10)

* Small intestine.
† Intestinal mesenteries and liver.

1. Nine (64%) of 14 hosts were infected with helminths. Of those, single species infections existed in six (43%) hosts; three (21%) with *R. retractilis,* two (14%) with *D. veligera,* and one (7%) with *T. pisiformis.* Multispecies infections occurred in three (21%) hosts; one (7%) was infected with both *R. retractilis* and *T. pisiformis.* A second host (7%) was infected with *D. veligera, R. retractilis,* and *T. pisiformis,* and the remaining host (7%) was infected with *D. veligera* and *R. retractilis.*

Previous reports of helminths from *S. audubboni* are: Stiles (1896, U.S. Nat. Mus. Proc. 19: 145–235); Herman and Jankiewicz (1943, J. Wildl. Mgt. 7(4):395–400); Voge (1954, Amer. Midl. Nat. 54(2):413–417); Stock (1961, Univ. Utah Anthro. Pap. 55:91–94); Buscher (1975, Proc. Okla. Acad. Sci. 55:103–107); and Buscher and Tyler (1975, Proc. Okla. Acad. Sci. 55:108–111). As well as the above-mentioned species, these authors reported the Nematoda *Obeliscoides cuniculi* (Graybill, 1923) and *Nematodirus leporis* Chandler, 1924. Additional cestodes reported were *Cittotaenia variabilis,* Stiles, 1895, *Raillietina* (*Raillietina*) *loeweni* Bartel and Hansen, 1964, *Raillietina* (*Raillietina*) selfi sp. n. Buscher, 1975, undescribed *Raillietina* sp., and unidentified cestode cysticerci.

Pfaffenberger et al. (1984, op. cit.) reported the presence of Acanthocephala and Nematoda among resident prairie dogs. The results reported herein, for desert cottontails from these same locations, seem to indicate that helminth overlap between black-tailed prairie dogs and desert cottontails is nonexistent in eastern New Mexico. This is in contrast to the findings of Buscher and Tyler (1975, op. cit.) who observed limited overlap in the helminth fauna of these same inhabitants in Oklahoma prairie dog towns.

We wish to thank Dr. Danny B. Pence for his help in identifying the cysticerci. This study was supported in part by funds from the Llano Estacado Center for Advanced Professional Studies and Research.

Obituary Notice

MARIETTA VOGE
July 7, 1918–July 2, 1984

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MINUTES

Five Hundred Fifty-Seventh Through Five Hundred Sixty-Fourth Meetings

557th Meeting: Uniformed Services University of Health Sciences, Bethesda, Maryland, 14 October 1983. The Anniversary Award was presented to Leon Jacobs. J. R. Lichtenfels announced that the Proceedings will be published in a two column format beginning with the January issue. The following slate of officers was proposed: President, S. S. Hendrix; Vice-President, W. A. Reid, Jr.; Corresponding Secretary-Treasurer, M. D. Ruff; Recording Secretary, R. P. Eckerlin. LTC Bryce C. Redington presided over the following papers: "Partial protection against murine schistosomiasis mansoni by inoculation with purified glycoprotein antigens from adult worms," Eugene Hayunga; "Studies on a knobless clone of *Plasmodium falciparum* in Colombian owl monkeys," Susan Langreth; "A new approach in design of antimalarials selectively toxic to the parasite," Leonard Schaibel.

558th Meeting: Animal Parasitology Insitute, USDA, Beltsville, Maryland, 4 November 1983. The slate of officers nominated at the 557th meeting was elected unanimously. A moment of silence was observed in honor of the deceased Leo Jachowski. Frank Tromba was the recipient of the Life Membership Award. Harry Herlich presided over the following papers: "Review of sarcocystosis in food and companion animals," Ronald Fayer; "Review of toxoplasmosis in domesticated animals," J. P. Dubey.

559th Meeting: Plant Protection Institute, USDA, Beltsville, Maryland, 2 December 1983. M. D. Ruff reported that the Executive Committee had accepted the following recommendations of the Business Advisory Commitee: 1) authorization for the publication of up to 400 pages in Volume 51 (1984) and up to 450 pages in Volume 52 (1985) of the Proceedings, 2) page charges to authors will be increased to $40.00 per page of the Proceedings, 3) the current sale of back issues of the Proceedings will terminate and the following charges implemented, Volumes 1–36 @ $6/volume, Volumes 37–49 @ $12/volume and Volumes 50 and 51 @ $30/volume except members may purchase these two volumes at the membership rate (currently $15). Outgoing President M. N. Lunde passed the gavel to the newly elected President Sherman S. Hendrix. Raymond V. Rebois presided over the following papers: "The effects of an azosteroid on sterol metabolism in *Caenorhabditis elegans,*" David J. Chitwood; "Comparative attraction studies of the soybean cyst nematode, *Heterodera glycines,*" Robin N. Huettel; "Parasites of salmon, revisited," Jeff W. Bier.

560th Meeting: National Institutes of Health, Bethesda, Maryland, 13 January 1984. A proposal from the Membership Committee to amend the Constitution to establish an emeritus membership was read. A vote will be taken at the next meeting. Franklin A. Neva presided over the following papers: "Target antigens of transmission blocking immunity in malaria," Nirbhay Kumar; "A role for calcium-calmodulin in *Giardia lamblia* catabolism," M. de Lourdes Munoz; "Parasite antigen specific human T-cell lines: influence on the regulation of the immune response," Thomas Nutman; "Single gene defects in IgM antibody responses and protective immunity induced by an attenuated schistosome vaccine," Rodrigo Correa-Oliveira.

561st Meeting: Uniformed Services University of Health Sciences, Bethesda, Maryland, 10 February 1984. M. D. Ruff presented the Treasurer's report. Editor J. R. Lichtenfels reported that the new double column format has permitted more manuscript pages to be printed on fewer journal pages of the Proceedings thus completely eliminating the backlog of manuscripts. An amendment to the Society Constitution creating a new emeritus class of membership was passed unanimously. Aaron Rosenfield and Richard L. Beaudoin presided over the following papers: "First occurrence of epizootic sarcomas in Chesapeake Bay soft clam populations," C. A. Farley, S. V. Otto and C. A. Reinisch; "*Haematractidium scombri* an unusual hemoparasite of the mackerel," S. A. MacLean; "An amplified ELISA for malarial sporozoites," F. W. Carson; "Cross-reactivity and cross-protection in murine malaria," M. Sedegah.

562nd Meeting: Walter Reed Army Institute of Research, Washington, D.C., 9 March 1984. President S. S. Hendrix announced the resignation of K. Darwin Murrell as Executive Committee Member-at-large. The Society financial records were audited by P. A. Pilitt and M. L. Rhoads and they were found to be in order. LTC Willis A. Reid, Jr. presided over the following papers: "Leishmaniasis in Baringo District, Kenya," L. K. Lightner, J. Githure and R. Beach; "Identification of *Leishmania* by DNA hybridization with kinetoplast DNA cloned into *E. coli* plasmids," P. R. Jackson, J. M. Lawrie, J. M. Stiteler and W. T. Hockmeyer; "Quantitative in vitro assessment of antifolate antimalarials," W. K. Milhous; "Identification of possible protective *Leishmania donovani* surface antigens," W. R. Ballou, R. M. Crawford and W. T. Hockmeyer.

563rd Meeting: Johns Hopkins University, School of Hygiene and Public Health, Baltimore, Maryland, 13 April 1984. President S. S. Hendrix made available copies of the first announcement of the Sixth International Congress of Parasitology to be held in August 1986 in Brisbane, Australia. Everett L. Schiller presided over the following papers: "Diagnostic tests for dog heartworm infection employing excretory-secretory antigens," C. Ladouceur; "Nematode infections of the harbor porpoise: a new finding and its behavioral implications," D. Ketten and D. Shirazian; "Sex pheromones in *Schistosoma mansoni,*" D. Shirazian and E. L. Schiller.

564th Meeting: The University of Pennsylvania, New Bolton Center, Kennett Square, Pennsylvania, 12 May 1984. The Honorary and Life Membership Committee nominated A. James Haley for Life Membership. The attending members approved the nomination. The symposium title was "Molecular aspects of parasitology." Mike Phillips presided over the following papers: "Gene rearrangements and membrane attachment: a perspective of current research on the variant antigens of African trypanosomes," George A. M. Cross; "Molecular and immunologic studies on metacyclic variants of African trypanosomes," Klaus Esser; "Molecular studies on red cell membranes and implications of *Plasmodium* penetration," Harvey Rubin.

The following 36 new members were elected at the meetings indicated: *557th:* John M. Aho, Brian Boag, Goran Bylund, Nuzhat Nafesa Handoo, Jorgen W. Hansen, M. A. Haseeb, Michael B. Hildreth, Edward H. Michelson, Peter Nanson, Paz Maria Salazar. *558th:* Benjamin N. Tuggle. *559th:* Suzan M. Bandoni, Paul F. Basch, Roy G. Taylor. *560th:* David M. Hudson, Glenn Kietzmann, Jr., G. Gnana Mani, Alan A. Marchiondo, Franklin A. Neva, Edith Trott. *561st:* Joe Conti, Donald Heyneman, Rokkam Madhavi, Dieter Sturhan, Barbara A. Wilson. *562nd:* Isabella Akinbah Quakyi. *563rd:* Moses Fon Asanji, Frederick Carson, Gary N. Fritz, Tom Kass, F. A. Khan, Lee S. Monroe. *564th:* Fatorma K. Bolay, Pauline Jolly, Paul M. Tefft, David P. Thrush.

Report on the Brayton H. Ransom Memorial Trust Fund

Balance on hand, 1 January 1983	$6,731.48
Receipts: Net interest received in 1982	1,071.85
	$7,803.33
Disbursements: Grant to the Helminthological Society of Washington for 1983	50.00
Publication support	360.00
Publication of B. H. Ransom Biography	196.58
	$ 606.58
On hand, 31 December 1983	$7,196.75

HARLEY G. SHEFFIELD
Secretary-Treasurer

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Gilbert F. Otto

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AUTHOR INDEX FOR VOLUME 51

ANNIVERSARY AWARD RECIPIENTS

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

HONORARY MEMBERS

* George R. LaRue	1959	Justus F. Mueller	1978
Vladimir S. Ershov	1962	John F. A. Sprent	1979
* Norman R. Stoll	1976	Bernard Bezubik	1980
Horace W. Stunkard	1977	Hugh M. Gordon	1981

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	May Belle Chitwood	1975
* Albert Hassall	1931	Carlton M. Herman	1975
* Charles W. Stiles	1931	Lloyd E. Rozeboom	1975
* Paul Bartsch	1937	Albert L. Taylor	1975
* Henry E. Ewing	1945	David R. Lincicome	1976
* William W. Cort	1952	Margaret A. Stirewalt	1976
* Gerard Dikmans	1953	* Willard H. Wright	1976
* Jesse R. Christie	1956	* Benjamin Schwartz	1976
* Gotthold Steiner	1956	Mildred A. Doss	1977
* Emmett W. Price	1956	* Everett E. Wehr	1977
* Eloise B. Cram	1956	Marion M. Farr	1979
* Gerald Thorne	1961	John T. Lucker, Jr.	1979
* Allen McIntosh	1963	George W. Luttermoser	1979
Edna M. Buhrer	1963	John S. Andrews	1980
* Benjamin G. Chitwood	1968	* Leo A. Jachowski, Jr.	1981
Aurel O. Foster	1972	Kenneth C. Kates	1981
Gilbert F. Otto	1972	Francis G. Tromba	1983
* Theodor von Brand	1975	A. James Haley	1984

* Deceased.

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