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Differentiation of Late Fourth and Early Fifth Stages of *Ascaris suum* Goeze, 1782 (Nematoda: Ascaridoidea) in Swine

P. A. PILITT, J. R. LICHTENFELS, F. G. TROMBA, AND P. A. MADDEN
Animal Parasitology Institute, U.S. Department of Agriculture, Science and Education Administration, Agricultural Research, Beltsville, Maryland 20705

ABSTRACT: The morphology of late fourth and early fifth stages of *Ascaris suum* in swine was studied with light and scanning electron microscopy. Late fourth-stage larvae have dome-shaped lips, that are broadest at the base and incompletely separated laterally. The lips bear coarse, triangular denticles, irregularly spaced on the internomedial and internolateral walls. Large double papillae are located on the middle of the external lip surface. The cervical region gradually increases in width posteriorly. The late fourth-stage cuticle is marked by a coarse transverse striation and bears incomplete longitudinal ridges, creating a brickwork pattern; longitudinal alae are present. Late fourth and early fifth stages have overlapping body lengths; late fourth-stage larvae at 21–24 DAI range from 13 to 27 mm long, and early fifth stages at 23–24 DAI range from 22 to 36 mm long. Early fifth-stage lips are truncate, broadest at the level of the prominent double papillae, constricted at the base and widely separated laterally. The lips bear a single row of fine, regularly spaced denticles on the internal margins. The prominent double papillae are located slightly anterior to the middle of the external lip surface. The cervical region sharply increases in width giving a shouldered appearance. The cuticle of early fifth stages is finely striated without markings; longitudinal alae are absent.

Reliable anatomical characters and morphometrics for the late developmental stages of *Ascaris suum* are still lacking although the species has been studied by many investigators. Douvres et al. (1969) described the life cycle and the early development of *A. suum* in swine from the ingested infective egg through early fourth-stage larvae. The present report describes the structure of late fourth and early fifth stages of *A. suum* in swine.

Materials and Methods

Purebred Hampshire swine from the closed breeding herd maintained at this institute were inoculated with a single oral dose of 500 *A. suum* eggs. The dose level of 500 eggs was selected for reasons explained by Tromba (1978). The swine had been farrowed and maintained under conditions precluding previous exposure to *A. suum*. Procedures for preparation of inocula and necropsy were those reported by Douvres et al. (1969) and Madden and Tromba (1976). *Ascaris suum* in late fourth stage (includes larvae closely enveloped in a sheath), fourth ecdysis, and early fifth stage were recovered from washings of the small intestine. The nematodes were killed and fixed in Bles' fixative (Meyer and Olsen, 1971), cleared for study in phenol-alcohol, and studied in temporary wet mounts. Photomicrographs were taken with a 35-mm camera, mounted on a microscope equipped with an interference contrast attachment. Scanning electron micrographs were prepared according to the methods of Madden and Tromba (1976). Morphological terminology follows Chitwood and Chitwood (1950). Nematodes have been deposited in the U.S. National Parasite Collection as USDA Parasite Collection Nos. 68501, 68518, and 68519 for specimens collected 21, 23, and 24 days after inoculation (DAI), respectively.

Table 1. Comparison of characters useful in differentiating late fourth and early fifth stages of *Ascaris suum*.

Characters	Late fourth stages	Early fifth stages
Lips	Dome-shaped Broadest at base Incompletely separated laterally External surface rounded	Truncate Broadest at level of double papillae, lip base narrow Widely separated laterally External surface flat
Denticles	Coarse, appear triangular Irregularly spaced	Fine, may appear blunt or triangular Regularly and evenly spaced
Cervical region	Body gradually increasing in width posterior to lips	Body sharply increasing in width behind lips giving a shouldered appearance
Cuticle	Annules wide Longitudinal ridges present on annules Longitudinal alae present	Annules narrow Longitudinal ridges absent Longitudinal alae absent

Results

Nematodes were recovered from swine killed 21, 23, and 24 DAI; the pig killed 22 DAI was negative for nematodes. At 21 DAI all 14 recovered larvae were in the late fourth stage. At 23 DAI 84 of 87 recovered nematodes were in the late fourth stage; three were in the early fifth stage. At 24 DAI five of 26 recovered nematodes were late fourth-stage larvae, one was in fourth ecdysis, and 20 were in the early fifth stage. The most useful characters for differentiating late fourth and early fifth stages of *Ascaris suum* are the labial, cervical, and cuticular structures (Table 1). Ranges of measurements of late fourth stage, fourth ecdysis and early fifth stages of *A. suum* are given in Table 2.

Late fourth-stage larvae (Figs. 1–3, 7–9, 13, 14)

Three dome-shaped lips separated internomedially, internolaterally, and incompletely separated laterally (Figs. 1, 2). Dorsal lip slightly broader than subventral lips; all lips broadest at lip base; and each lip with longitudinal groove on internomedial wall. External surface of lips rounded in overall appearance (Fig. 7). Denticles on inner lip margins coarse, triangular, and irregularly spaced in single row (Fig. 2, insert). Dorsal and subventral lips bearing large double papillae of external circle in middle of externolateral lip surface (Figs. 1, 2, 8). Each subventral lip bearing small externolateral papilla (also of external circle) slightly anterior to double papilla; amphid adjacent to externolateral papilla (Fig. 2). Cervical region gradually increasing in width posterior to lips (Fig. 7). Interlabia absent. Cuticle coarsely transversely striated, annules 0.017–0.029 mm wide at base of esophagus; incomplete longitudinal ridges present on annules (Figs. 3, 9); longitudinal alae present extending from base of lips almost to tail tip.

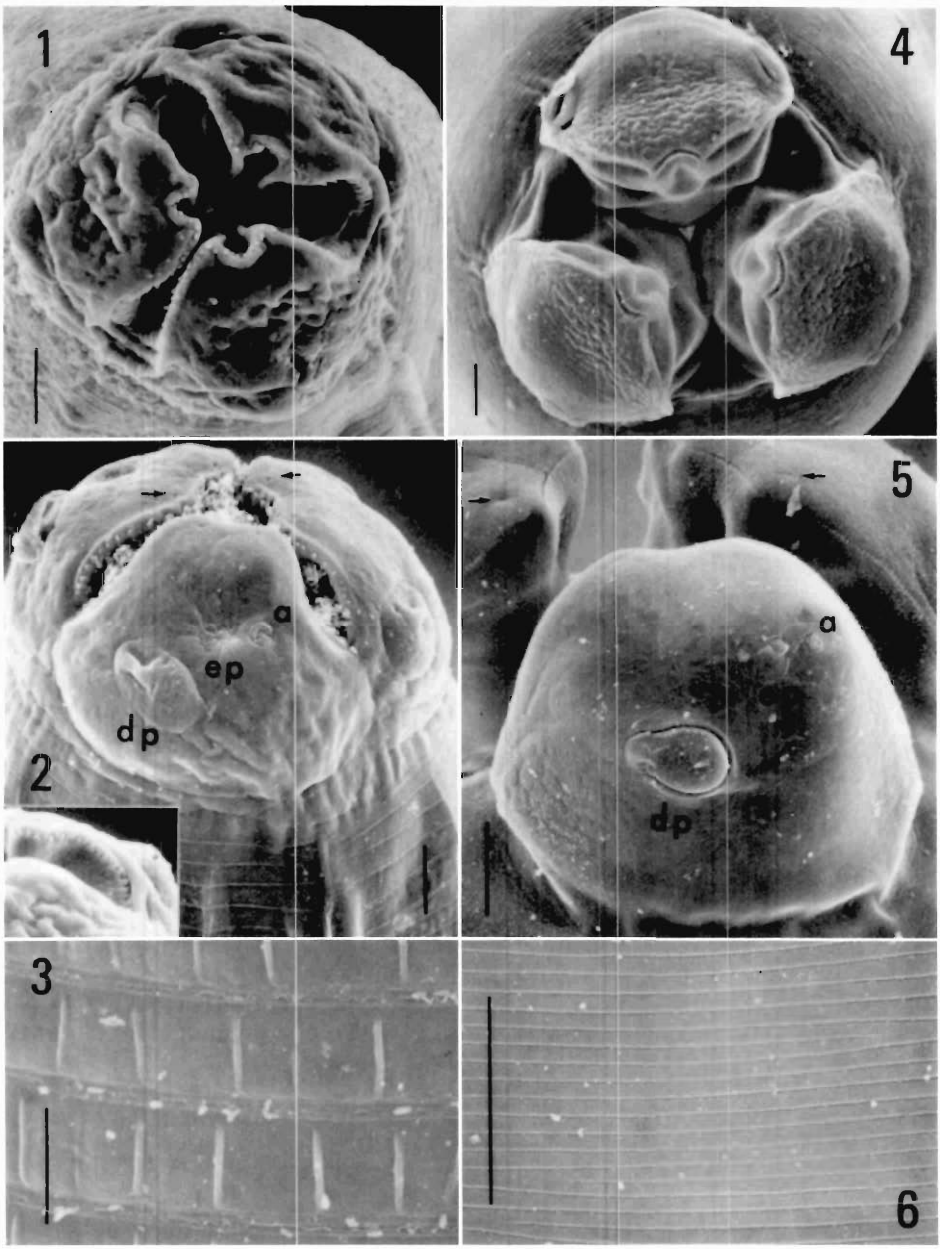
Fourth ecdysis

Single female recovered. Body emerged from fourth-stage cuticle to level of vulva. Lips characteristic of fifth stage; cuticle of anterior half of body finely striated. Posterior part of body enclosed within fourth-stage cuticle.

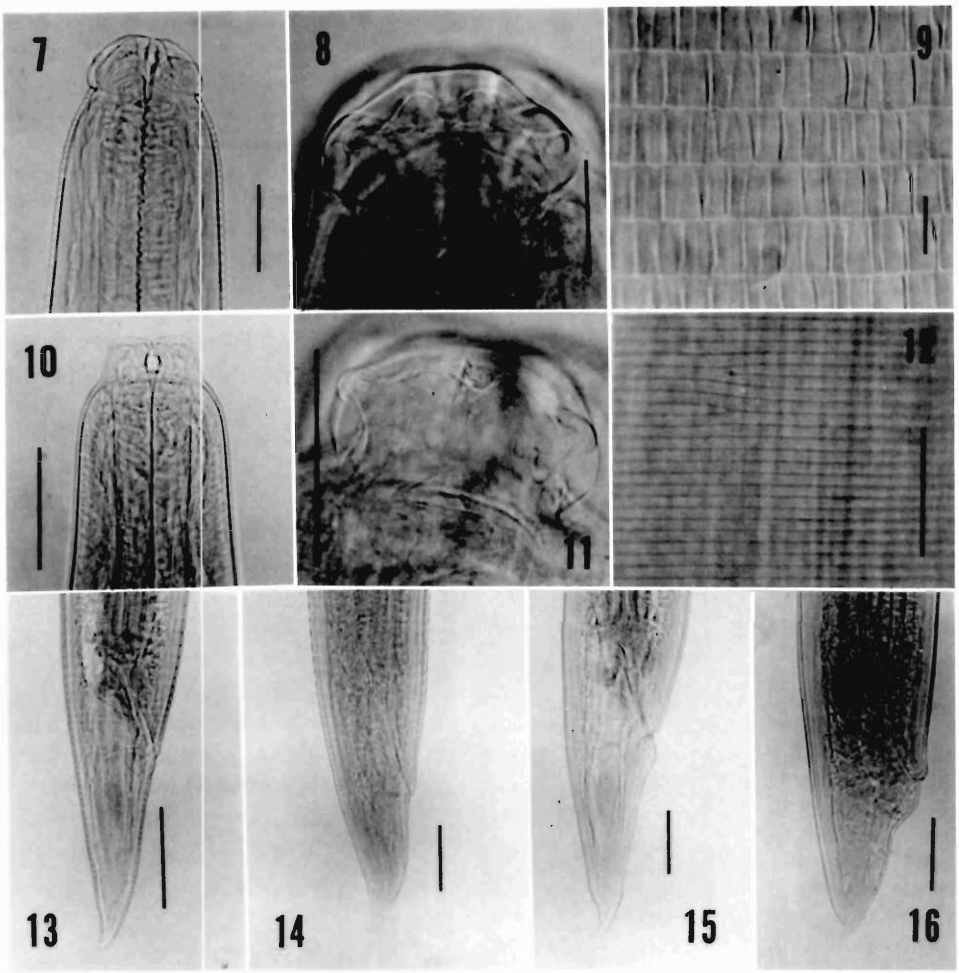
Table 2. Body measurements of late fourth stage, fourth ecdysis, and early fifth stages of *Ascaris suum* collected from experimental infections in swine.*

Anatomical feature	Late fourth stages 21–24 DAI		Fourth ecdysis 24 DAI	Early fifth stages 23–24 DAI	
	Males (19)	Females (13)	Female (1)	Males (11)	Females (10)
Total length	15.30–24.12 (18.99)	13.38–27.00 (20.33)	27.18†	21.96–35.64 (26.43)	24.30–34.92 (28.60)
Width (midbody)	0.31–0.51 (0.40)	0.32–0.53 (0.45)	0.51	0.41–0.56 (0.48)	0.50–0.62 (0.54)
Esophagus‡	1.32–1.88 (1.65)	1.41–2.01 (1.71)	2.03	1.18–2.35 (2.00)	1.94–2.39 (2.10)
Excretory pore‡	0.35–0.46 (0.42)	0.40–0.48 (0.43)	0.38	0.38–0.50 (0.42)	0.40–0.48 (0.43)
Spicule length	0.11–0.25 (0.18)	—	—	0.19–0.34 (0.24)	—
Vulva‡	—	9.02–14.63 (11.42)	15.22	—	12.91–18.01 (15.07)
Tail length	0.17–0.24 (0.21)	0.27–0.35 (0.31)	0.36	0.20–0.30 (0.24)	0.33–0.44 (0.39)
Width of annule§	0.017–0.029 (0.022)	0.021–0.029 (0.024)	0.002	0.002–0.003 (0.002)	0.002–0.003 (0.002)

* Ranges (and averages) in millimeters.
† Length of female in fourth ecdysis does not include the sheath.
‡ Structures were measured from the anterior end.
§ Annule was measured in area at base of esophagus.



Figures 1–6. *Ascaris suum*, scanning electron micrographs of the labial and cuticular structure of late fourth and early fifth stages. Scale bars 25 μm. Figures 1–3. Late fourth stages. 1. En face view, showing lip denticles and incomplete lateral lip separation. 2. Dome-shaped subventral lip, showing large double papilla (dp), externolateral papilla (ep), amphid (a), and the internal circle of cephalic papillae (at arrows). Insert: Enlargement of dorsal lip, showing triangular shape of denticles in lateral view. 3. Cuticle (just anterior to excretory pore), showing the brickwork pattern of incomplete longitudinal ridges. Figures 4–6. Early fifth stages. 4. En face view, showing wide lateral lip separation. 5. Truncate subventral lip, showing prominent double papilla (dp), externolateral papilla (ep), amphid (a), and the internal circle of cephalic papillae (at arrows). 6. Cuticle (posterior to lips), showing fine transverse striae and annules without markings.



Figures 7–16. ***Ascaris suum*, interference-contrast light micrographs of the labial and cuticular structure of late fourth and early fifth stages. Scale bars 100 μm in Figures 7–8, 10–11, 13–16; scale bar 25 μm in Figures 9, 12. 7. Late fourth-stage anterior extremity, dorsal view, showing gradual increase in width from lip base. 8. Dome-shaped dorsal lip of late fourth-stage larva, showing two large double papillae. 9. Coarsely striated cuticle of late fourth-stage larva (near base of esophagus), showing incomplete longitudinal ridges. 10. Early fifth-stage anterior extremity, dorsal view, showing shouldered appearance from narrowed lip base. 11. Truncate dorsal lip of early fifth stage, showing two prominent double papillae. 12. Finely striated cuticle of early fifth stage (near base of esophagus). 13. Late fourth-stage female tail. 14. Late fourth-stage male tail. 15. Early fifth-stage female tail. 16. Early fifth-stage male tail.**

Early fifth stages (adults) (Figs. 4–6, 10–12, 15, 16)

Lips truncate, broader than tall, separated internomedially, internolaterally, and widely separated laterally (Figs. 4, 5). All lips broadest at level of prominent double papillae; narrowed at base. External surfaces of lips relatively flat in overall view (Fig. 10). Denticles on internal lip margins fine, triangular to blunt, and regularly and evenly spaced in single row (Fig. 5). Dorsal and subventral lips bearing prominent double papillae of external circle slightly anterior to middle of

lip surface (Figs. 4, 5, 11). Subventral lips each bear an externolateral papilla of external circle anterior to large double papilla and porelike amphid next to externolateral papilla (Fig. 5). Cervical region sharply increases in width at base of lips giving shouldered appearance (Fig. 10). Cuticle very finely striated, annules 0.002–0.003 mm wide at base of esophagus; annules without ridges (Figs. 6, 12); longitudinal alae absent.

Discussion

The shape of the lips, denticular structure, shape of the cervical region, and cuticular structure differ markedly between the stages (Table 1). The denticles in fourth-stage lips can be seen by focusing through the lip surface to the denticular ridge, however, denticles in fifth-stage lips are not easily seen by light microscopy. Madden and Tromba (1976) found that denticle size is directly related to the size and age of the nematodes and that denticles appear triangular to blunt, depending upon the angle of view. The "brickwork" pattern of fourth-stage cuticle (Douvres et al., 1969) is not present on the anteriormost annules (Fig. 2) but begins slightly anterior to the excretory pore (Fig. 3) and continues to the tail tip. Fifth-stage cuticle is finely striated and the annules are narrow without markings. A similar change in cuticular structure was reported (Pilitt et al., 1979) between fourth and early fifth stages of *Parascaris equorum* (Goeze, 1782). In both *A. suum* and *P. equorum,* late fourth stages have a brickwork-patterned cuticle, but early fifth stages have a finely striated cuticle without markings. However, this change in cuticle from fourth to fifth stage is not seen in all ascaridoids. One example, the genus *Sulcascaris* (parasitic in molluscs as larvae, in turtles as adults), retains a brickwork pattern in the cuticle of the fifth stage (Lichtenfels et al., 1978).

Additional characters for separation of both stages and sexes include vulva location and tail length. Douvres et al. (1969), using the reproductive system, body length, and tail characters were unable to separate the sexes in early fourth stage. The reproductive system of early fourth stages is still limited to a multicellular genital primordium. In late fourth-stage females the prepatent vulva is located slightly posterior to midbody, beneath the fourth-stage cuticle. The vagina separates into two posteriorly directed uteri just posterior to the vulva. The patent vulva of the early fifth-stage female is located about midbody. The female reproductive tract is easier to locate in the fourth stage than in the early fifth stage. Late fourth-stage female tails (Fig. 13) are longer than fourth-stage male tails (Fig. 14). Genital papillae and developing spicules can be seen in males under the fourth-stage cuticle. In some fourth-stage males the weakly sclerotized spicules may become overcleared in phenol-alcohol. Tails of early fifth-stage females (Fig. 15) are longer than tails of fifth-stage males (Fig. 16). Males have two equal spicules and genital papillae; caudal alae are absent.

All nematodes described in this study were from a single series of swine inoculated simultaneously. The fourth ecdysis occurred between 22 and 25 DAI in these infections. In other experimental inoculations of swine at this institute, the fourth ecdysis of *A. suum* has been observed at 23 DAI. Roberts (1934) proposed that the fourth ecdysis occurred in the small intestine of swine between 21 and 29 DAI; two of his 29 DAI larvae were in fourth ecdysis (17.5 mm and 22.5 mm long, respectively). Schwartz (1959) suggested that the natural elimination of

ascarid larvae from swine between 21 and 30 days corresponded to the beginning of the fourth molt.

The body lengths of late developing stages overlap and if used alone are unreliable for separation of stages (Table 2). Roberts (1934) relied on body lengths to identify larval ascarids. He also used anatomical characters to separate stages of development, but frequently the characters he used were common to more than one larval stage. Douvres et al. (1969), in their description of early and middle developmental phases of *A. suum* in swine, also reported that body lengths often overlapped between stages; however, they presented additional characters of head, lip, and other body features for the separation of the different phases and stages of development. The most useful characters presented here for differentiating late fourth and early fifth stages are labial and cuticular structure (summarized in Table 1).

Literature Cited

Chitwood, B. G., and M. B. Chitwood. 1950. Introduction to Nematology (Reprinted, 1974). Univ. Park Press, Baltimore, Maryland. 334 pp.

Douvres, F. W., F. G. Tromba, and G. M. Malakatis. 1969. Morphogenesis and migration of *Ascaris suum* larvae developing to fourth stage in swine. J. Parasitol. 55:689–712.

Lichtenfels, J. R., J. W. Bier, and P. A. Madden. 1978. Larval anisakid (*Sulcascaris*) nematodes from Atlantic molluscs with marine turtles as definitive hosts. Trans. Am. Microsc. Soc. 97:199–207.

Madden, P. A., and F. G. Tromba. 1976. Scanning electron microscopy of the lip denticles of *Ascaris suum* adults of known ages. J. Parasitol. 62:265–271.

Meyer, M. C., and O. W. Olsen. 1971. Essentials of Parasitology. Wm. C. Brown Co. Publishers, Dubuque, Iowa. 305 pp.

Pilitt, P. A., J. R. Lichtenfels, and P. A. Madden. 1979. Differentiation of fourth and early fifth stages of *Parascaris equorum* (Goeze, 1782) Nematoda: Ascaridoidea. Proc. Helminthol. Soc. Wash. 46:15–20.

Roberts, F. H. S. 1934. The large roundworm of pigs, *Ascaris lumbricoides* L., 1758. Its life history in Queensland, economic importance and control. Yeerongpilly Anim. Health Stn. Bull. 1:1–81.

Schwartz, B. 1959. Experimental infection of pigs with *Ascaris suum*. Am. J. Vet. Res. 20:7–13.

Tromba, F. G. 1978. Effect of ultraviolet radiation on the infective stages of *Ascaris suum* and *Stephanurus dentatus* with a comparison of the relative susceptibilities of some parasitic nematodes to ultraviolet. J. Parasitol. 64:245–252.

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Transmammary Transmission of *Strongyloides venezuelensis* (Nematoda) in Rats[1]

THOMAS J. NOLAN[2] AND FRANK F. KATZ
Department of Biology, Seton Hall University, South Orange, New Jersey 07079

ABSTRACT: Sprague-Dawley-derived dams were inoculated subcutaneously with 4,000 infective filariform larvae suspended in water. The inoculations were given once from 10 hours to 9 days postpartum. Nurslings were killed at intervals after the inoculations and their tissues were squashed between glass slides for microscopic examination. Larvae were first found in the nurslings 3 days after the inoculation. There was no extraintestinal migration in these animals but suckling rats inoculated orally had the usual heart-lung phase of the life cycle. A few larvae were recovered from mammary tissue of nursing dams. About 50 times as many worms were recovered from the nurslings as from their dams.

Several species of parasitic nematodes can be transmitted by the transmammary route (Stone and Smith, 1973). Among the members of the genus *Strongyloides,* infection of the newborn by this means has been demonstrated for *S. ransomi* in pigs (Moncol and Batte, 1966; Stewart et al., 1969, 1976), *S. westeri* in equines (Lyons et al., 1973, 1977) and *S. ratti* in rats (Katz, 1969; Zamirdin and Wilson, 1974; Wilson et al., 1976a, b, 1978a). Larvae of *S. papillosus* have been recovered from the milk of goats and sheep (Moncol and Grice, 1974), sheep and cattle (Lyons et al., 1970), and buffaloes (Chauhan et al., 1974), and those of *S. fuelleborni* from human milk (Brown and Girardeau, 1977).

The objective of this study was to investigate the possibility of transmammary transmission of *Strongyloides venezuelensis* and, if it does occur, to elucidate both the migratory pathway and the biological significance.

Materials and Methods

The strain of *S. venezuelensis* used in this study was isolated from a rat trapped in Tel Aviv by Dr. Guta Wertheim of the Hebrew University, Jerusalem, Israel, and has been maintained serially by the author since 1964.

Charcoal-fecal cultures were made from infected stock animals and served as the source of the filariform larvae for the experimental work. Cultures were baermannized and the larvae in 0.075 ml (Stoll pipet) samples of the suspension were counted. The volumes of the larvae in water were adjusted to obtain the desired inoculum size.

Using a 1-ml tuberculin syringe with a 25 gauge needle, mother rats were inoculated subcutaneously in the interscapular region with 4,000 filariform larvae in 0.05 ml. After the injection, the rats were kept separate from their litters for 30 min to allow any worms which might have seeped from the injection site time to penetrate the skin. Cages were cleaned daily or every other day to prevent contamination of the young. Water and Purina Rodent Laboratory Chow® were provided ad libitum to all animals.

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[1] A portion of a thesis submitted by T.J.N. in partial fulfillment of the requirements for the degree of Master of Science in the Department of Biology.

[2] Present address: Department of Zoology, Rutgers University, New Brunswick, New Jersey 08903.

Table 1. ***Strongyloides venezuelensis*** **recovered from nurslings transferred from infected dams to uninfected dams at various times (hours) after inoculation.**

Time of transfer	Neg.	Pos.
5–75	17	0
76	0	1
77	1	0
81–120	0	8
Not transferred, remained with		
infected dams	0	3
uninfected dams	6	0

In experiments 1 through 4, the rats were Charles River CD strain while in experiment 5, the rats were CAMM SD/BR strain. The matings and litters were the first for these animals.

Nurslings transferred from one mother rat to another were marked by cutting off one or more toes.

Results

Experiment 1: Time of onset of transmammary transmission

One dam of each of three pairs of time-matched dams was inoculated 24 hr after parturition. Before returning the infected dam to her litter, one baby was transferred to an uninfected dam. After restoration of the infected dam to her litter, another baby was removed and transferred periodically up to 120 hr. At each transfer, one of the recipient's babies was removed to maintain litter size. Seven days after the inoculation of the dams, the baby rats were killed and their duodenums squashed between microscope slides and examined for worms.

No worms were found in any of the 17 nurslings killed at 17 different times up to 75 hr postinoculation whereas those killed at eight different times from 81 to 120 hr were all infected (Table 1). The three nurslings which had remained continuously with the infected dams and the six which had remained with the uninfected mothers served as controls.

Experiment 2: Duration of transmammary transmission

The offspring of an uninfected dam were transferred at intervals to an inoculated one. Eight days postinoculation, the transferred animals and the controls were examined.

Newborn rats were found infected at five intervals prior to but not at four intervals beginning 117.5 hr postinoculation of their foster mother (Table 2).

Experiment 3: Route of migration in the offspring

Litters were used which ranged in age from 10 hr to 9 days at the time of the inoculation of their dams. Suckling rats were killed at various times from 14 to 223 hr after the dam had been inoculated and their stomachs, duodenums, and lungs and, on occasion, liver, kidneys, spleen, and blood were examined by squashes on slides. These organs as well as mammary glands of dams were also minced and placed in a modified Baermann apparatus utilizing a pilsner glass. Some of the worms recovered were measured with an ocular micrometer after

Table 2. ***Strongyloides venezuelensis*** **recovered from nurslings transferred from an uninoculated to an inoculated dam at various times (hours) after inoculation and autopsied 8 days postinoculation of the foster mother.**

Time of transfer	Neg.	Pos.
24, 46, 72, 93, 103	0	5
117.5, 118.5, 142, 167	4	0
Not transferred, remained with		
infected dam	0	1
uninfected dam	2	0

being stained with iodine solution. Several larvae from mammary tissue were inoculated subcutaneously into an adult female rat and one larva was inoculated into a nursling. These animals were later examined by charcoal culture of feces and direct examination of feces and tissues.

None of the suckling rats examined at 12 intervals prior to 75 hr postinoculation of the dams had worms but after that 28 of 45 rats examined at 22 intervals had worms (Table 3). The larvae in the milk were swallowed, passed through the stomach and took up residence in the duodenum. Worms were not found in the lungs, kidneys, spleen, blood, or liver. Worms found in the stomach were always larvae. In the duodenum up to 94 hr postinoculation only larvae were observed; from 94 to 117 hr there were larvae and nonovigerous adults, 117 to 162 hr nonovigerous adults, and from 200 to 223 hr ovigerous adults.

Larvae recovered from the stomach and from mammary glands appeared basically third-stage-like. However, measurements indicated some growth had taken place; filariform larvae from cultures had a mean length of 568 ± 3.4 μm while those from mammary tissues and the stomach had mean lengths of 607 ± 7.1 and 603 ± 17.6 μm, respectively. Adults from the duodenum measured on the average 3.00 ± 0.075 mm.

Larvae were recovered from the mammary glands of two rats 4 days after inoculation but none were found in two others on the 7th day. These larvae failed to produce patent infections in either an adult or suckling rat.

Experiment 4: Need for extraintestinal migration

Using a feeding needle, an inoculum of 125 coproculture filariform larvae in 0.05 ml was placed in the pharyngeal region of each of 10 members of a suckling litter. The baby rats were kept separate from the mother for 10 min. At 15 min postinoculation and at various intervals thereafter, nurslings were killed and their lungs, stomachs, and duodenums examined by tissue squashes.

Table 3. ***Strongyloides venezuelensis*** **recovered from nurslings at various times (hours) after inoculation of their mothers.**

		No. with worms		
Time	No. examined	Stomach only	Duodenum only	Stomach and duodenum
14–74	29	0	0	0
75	1	1	0	0
76–109	35	1	12	5
117–223	10	0	10	0

Table 4. ***Strongyloides venezuelensis*** **recovered from nurslings at various times (hours) after their oral inoculation with larvae from cultures.**

Time	Location and stage of worms		
	Lungs	Stomach	Duodenum
0.25	—	Larvae	—
3.5–68.5	—	—	—
75.5	Larvae	—	Nonovigerous adults
92.0	—	—	Nonovigerous adults

Worms were found in the lungs and the duodenum 75.5 hr postinoculation but not at seven intervals prior to that (Table 4).

Experiment 5: Importance of the transmammary route

Two virgin females and four dams 3 to 6 days postpartum were inoculated with filariform larvae. Seven days postinoculation the adults and the nurslings were killed and the total number of worms in their duodenums was determined by direct count in tissue squashes.

Of the initial inoculum, 43.2% ended up in the litters while only 0.87% of the worms were found in the dams. The duodenums of the control rats yielded 3.16% of the inoculum. The male and female babies harbored the same numbers of worms.

Discussion and Conclusions

The experiments reported here show that transmammary transmission of *Strongyloides venezuelensis* can take place when the lactating dam is inoculated with filariform larvae 10 to 216 hr postpartum. The passage to the nurslings starts approximately 72 hr postinoculation and lasts only 24 to 48 hr.

Various factors may influence transmammary transmission (Wilson et al., 1978a, b). The fact that transmammary transmission starts later in *S. venezuelensis* than in *S. ratti* (Katz, 1969; Zamirdin and Wilson, 1974) is consistent with biological differences of these two species (Petriello and Katz, 1966; Wertheim, 1970).

The patent infections from the oral inoculations of filariform larvae probably resulted from larvae penetrating the buccal mucosa. Because larvae were found in the stomach 15 min after an oral inoculation but did not take up residence in the duodenum until 75 hr, it appears that the larvae which were swallowed before making a tissue migration could not establish themselves in the duodenum. This is not surprising since the literature (Michel, 1974) on prenatal and transmammary transmission indicates that while *Strongyloides* species in general require a tissue migration, larvae which have had a sojourn in tissue, as would those passed in milk, need not have another extraintestinal migration.

Under the conditions used in experiment 5, the transmammary route seems to be favored over the normal migratory pathway in the lactating female rat. Wilson et al. (1976a) reported for *S. ratti* that 24.4% of the initial inoculum ended up in the litter while only 2% was present in the dam. It appears that the controls in experiment 5 had a worm burden lower than what one might expect. However, the yield from the controls was still greater than that of the dams.

Wilson (1977) has proposed for *S. ratti* that a switch in the larvae's migration to the mammary gland, instead of the intestine, occurs in the lungs. This switch would take place at the time the larvae first enter the lungs; instead of going into the alveoli, the larvae would remain in the blood and eventually be carried to the mammary gland. Because *S. venezuelensis* larvae in the mammary gland are about the same size as those in the lungs, it would seem that Wilson's theory may also hold for *S. venezuelensis*.

Literature Cited

Brown, R. C., and M. H. F. Girardeau. 1977. Transmammary passage of *Strongyloides* sp. larvae in the human host. Am. J. Trop. Med. Hyg. 26:215–219.

Chauhan, P. P. S., R. D. Agrawal, and S. S. Ahluwalia. 1974. A note on the presence of *Strongyloides papillosus* and *Neoascaris vitulorum* larvae in the milk of buffaloes. Curr. Sci. 43:486–487.

Katz, F. F. 1969. *Strongyloides ratti* (Nematoda) in newborn offspring of inoculated rats. Proc. Pa. Acad. Sci. 43:221–225.

Lyons, E. T., J. H. Drudge, and S. C. Tolliver. 1970. Strongyloides larvae in milk of sheep and cattle. Mod. Vet. Pract. 51:65–68.

Lyons, E. T., J. H. Drudge, and S. C. Tolliver. 1973. On the life cycle of *Strongyloides westeri* in the equine. J. Parasitol. 59:780–787.

Lyons, E. T., J. H. Drudge, and S. C. Tolliver. 1977. Observations on development of *Strongyloides westeri* in foals nursing dams treated with Cambendazole or Thiabendazole. Am. J. Vet. Res. 38:889–892.

Michel, J. F. 1974. Arrested development of nematodes and some related phenomena. Pages 279–366 *in* B. Dawes, ed. Advances in Parasitology. Academic Press, New York.

Moncol, D. J., and E. G. Batte. 1966. Transcolostral infection of newborn pigs with *Strongyloides ransomi*. Vet. Med. Small Anim. Clin. 61:583–586.

Moncol, D. J., and M. J. Grice. 1974. Transmammary passage of *Strongyloides papillosus* in the goat and sheep. Proc. Helminthol. Soc. Wash. 41:1–4.

Petriello, R. P., and F. F. Katz. 1966. Observations on the life cycles of a homogenetic strain of *Strongyloides venezuelensis* and homo/heterogenetic strain of *S. ratti* (Nematoda, Rhabditoidea) in different host species. Bull. N.J. Acad. Sci. 11:40.

Stewart, T. B., W. N. Smith, and W. M. Stone. 1969. Strongyloidiasis: Perinatal infection of pigs with Strongyloides ransomi. Georgia Vet. 21:13–18.

Stewart, T. B., W. M. Stone, and O. G. Marti. 1976. *Strongyloides ransomi*: Prenatal and transmammary infection of pigs of sequential litters from dams experimentally exposed as weanlings. Am. J. Vet. Res. 37:541–544.

Stone, W., and F. W. Smith. 1973. Infection of mammalian hosts by milk-borne nematode larvae: A review. Exp. Parasitol. 34:306–312.

Wertheim, G. 1970. Growth and development of *Strongyloides venezuelensis* Brumpt, 1934 in the albino rat. Parasitology 61:381–388.

Wilson, P. A. G. 1977. The effect of the suckling stimulus on the migration of *Strongyloides ratti* in lactating rats. Parasitology 75:233–239.

Wilson, P. A. G., M. Cameron, and D. S. Scott. 1978a. Patterns of milk transmission of *Strongyloides ratti*. Parasitology 77:87–96.

Wilson, P. A. G., M. Cameron, and D. S. Scott. 1978b. *Strongyloides ratti* in virgin female rats: Studies of oestrous cycle effects and general variability. Parasitology 76:221–227.

Wilson, P. A. G., M. Gentle, and D. S. Scott. 1976a. Milk-borne infection of rats with *Strongyloides ratti* and *Nippostrongylus brasiliensis*. Parasitology 72:355–360.

Wilson, P. A. G., M. Gentle, and D. S. Scott. 1976b. Dynamic determinants of the route of larval *Strongyloides ratti* in lactating rats and the control of experimental error in quantitative studies of milk transmission of skin-penetrating roundworms. Parasitology 73:399–406.

Zamirdin, M., and P. A. G. Wilson. 1974. *Strongyloides ratti*: Relative importance of maternal sources of infection. Parasitology 69:445–453.

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Nematospiroides dubius Baylis, 1926 (Syn., *Heligmosomoides polygyrus* (Duj., 1845) Railliet et Henry, 1909) from the Uinta Ground Squirrel, *Spermophilus armatus* Kennicott, 1863 from Teton National Park, Wyoming[1]

ROBERT C. BERGSTROM AND BARBARA A. WERNER
Division of Microbiology and Veterinary Medicine,
University of Wyoming, Laramie, Wyoming 82071

ABSTRACT: Uinta and Richardson's ground squirrels are common in northwestern, western, and southern Wyoming. They range and feed in the same general habitat areas as voles which are infected by *Heligmosomoides polygyrus*. The nematode species was found in the small intestine of Uinta ground squirrels from Teton National Park, Wyoming, during the summer of 1979. This is a new host record and a new locality record for *H. polygyrus*. Six of six squirrels examined were infected by *H. polygyrus*, so it appears that prevalence of the nematode in ground squirrels is high in some localities during some seasons and/or years.

Uinta ground squirrels, *Spermophilus armatus*, are numerous in northwestern Wyoming, especially in the foothill and mountainous areas (Burt and Grossenheider, 1952).

Much of the early literature concerning *H. polygyrus* indicated the nematode's presence in mice (Baylis, 1926, 1927; Travassos and Darriba, 1929; Dikmans, 1940). After several years and additional fieldwork, *H. polygyrus* (then reported as *N. dubius*) was found in mice in many localities in the United States (Rausch and Tiner, 1948, north central states), in Wyoming (Kuns and Rausch, 1950), in Quebec and Labrador (Schad, 1954), and in many other areas.

More recent fieldwork with mammals from Utah (Grundmann and Warnock, 1964) and the biogeographic data of Durette-Desset (1971), Durette-Desset et al. (1972) are of interest because none of the field collections have shown natural infections of ground squirrels by *H. polygyrus*.

The present study revealed an interestingly high prevalence of *H. polygyrus* in a few Uinta ground squirrels from Teton National Park, Teton Co., Wyoming during the summer of 1979.

Materials and Methods

Carcasses of six Uinta ground squirrels were collected by Mr. Robert Wood, biologist, Teton National Park, Wyoming, who gave the specimens to Dr. Jack Konitz, a veterinarian of Jackson, Wyoming, who submitted the carcasses to the Wyoming State Veterinary Laboratory, Laramie, Wyoming. Dr. Konitz requested an examination for ectoparasites, expressing most concern as to possible plague-bearing fleas infesting the squirrels and the potential for plague transmission to visitors to Teton National Park. During the course of examination of the ground squirrels for ecto- and endoparasites, fecal samples from the squirrels were examined microscopically after concentration of helminth ova by centrifu-

[1] Published with the approval of the Director, University of Wyoming Agricultural Experiment Station, as J.A. 1083.

gation-concentrated sucrose flotation procedures. Subsequently, the intestinal tracts were opened and examined after screening the contents to detect the presence of immature and/or adult helminths. Total recovery of all helminths in the tracts was attempted. Nematodes were picked from screens and finger bowls, fixed in 70% ethyl alcohol with 5% glycerol and examined with an American Optical Spencer stereo dissecting microscope and an A. O. Spencer compound microscope at 45, 100 and 430×. Measurements were made of 10 male and 10 female worms. Photomicrographs were made of representative anterior and posterior portions of male and female worms. Eggs in the uterus of the female worms and eggs from the washings of the intestinal tract were compared and measured. Males and females were sectioned transversely in order to count longitudinal striations of the cuticle. Two male and three female nematodes, tentatively identified as *H. polygyrus* were submitted to Dr. J. Ralph Lichtenfels, Parasite Classification and Distribution Unit, Animal Parasitology Institute, U.S.D.A., Beltsville, Maryland, for species confirmation. Four males and four females were sent to the Manter Museum (Curator, Dr. Mary H. Pritchard).

Results

From two to 12 *H. polygyrus* males and females were recovered from the small intestine of each of 6 ground squirrels. Male worms were 7.4–8.5, mean 8.2 mm long. Mean length of esophagus was 625 μm in the males. Spicules measured 279–342, mean 298 μm. Bursal lobes were asymmetrical (Fig. 1). The externodorsal rays were slender with a slight lateral "elbow" near the proximal end. The small dorsal lobe was 78 μm long and was bifurcated at the distal end. All lateral rays were typical of *H. polygyrus* (syn., *N. dubius*) (Fig. 1). Thirty to 32 longitudinal striations were found at midbody. Female worms were 16–19, mean 17.8 mm long. The anterior end bore a cephalic inflation; a small spine much like that of *Nematodirus* spp. was noted on the truncated posterior ends. Eggs taken from the uterus had a mean length of 66 μm with a mean width of 32 μm. Eggs collected after intestinal washings had a mean length and width of 78 by 45 μm respectively. Thirty-four to 38 longitudinal striations were noted at midbody.

Dr. J. Ralph Lichtenfels and Mr. Alan Fusco, Beltsville, Maryland, confirmed our tentative identification of the nematodes as *H. polygyrus*. The occurrence of this species in *Spermophilus* (syn., *Citellus*) *armatus* is a new locality record and a new host record for the United States.

University of Nebraska State Museum, Manter Lab. No. 21173 has been assigned to *H. polygyrus* specimens submitted September 12, 1980.

Discussion

Heligmosomoides polygyrus was found in *Spermophilus* (syn., *Citellus*) *citellus* in Rumania (Roman-Chiriac and Hamar, 1966). With that exception, it appears that *Heligmosomoides* spp. have not been found in ground squirrels of the world. All of the six Wyoming ground squirrels examined were positive for the nematode species. The prevalence of the parasite in the ground squirrel populations in Teton Park may be high.

We have examined several dozen Richardson's ground squirrels (*S. richardsoni,* Kennicott, 1863) during the past 20 years in Albany Co., Wyoming, without finding nematodes, much less heligmosomoidid nematodes. Uinta ground squir-

Figure 1. Bursa of male *N. dubius* syn. *H. polygyrus* from small intestine of Uinta Ground squirrel.

rels are similar in general appearance, size, coloration, and habits to the Richardson's ground squirrel, both species feeding on low, succulent herbs and grasses, hibernating in August and breaking hibernation in March–April. The two ground squirrel species overlap in geographical distribution in western Wyoming. The Uinta ground squirrel has a darker-colored tail than that of the other species.

Administrators and biologists are concerned in the parks where the ground squirrels become very tame and approach human visitors to the parks. The ground squirrels are so abundant around campgrounds, visitor centers, and scenic observation points that feeding of the squirrels becomes routine. Children, especially, could be bitten and the movement of arthropod ectoparasites, especially fleas, from squirrels to man is a distinct possibility. Many ground squirrels of the two species noted above have been submitted to the Wyoming State Veterinary Laboratory for rabies examination, but most of those examined had no nematode parasites in any part of the gastrointestinal tract.

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tailed the nomenclature and identification procedures for ground squirrels while Mr. Antai opened squirrel intestinal tracts and helped recover nematodes. We thank Mr. John Austin, graduate student in zoology for additional specimens of *H. polygyrus* for comparison with the original material.

Literature Cited

Baylis, H. A. 1926. On a trichostrongylid nematode from a wood mouse (*Apodemus sylvaticus*). Ann. Mag. Nat. Hist. 9.5(108)18:661–665.

Baylis, H. A. 1927. A further note on *Nematospiroides dubius* Baylis, 1926. Ann. Mag. Nat. Hist. 9.5(115)20:102–105.

Burt, W. H., and R. P. Grossenheider. 1952. A Field Guide to the Mammals. Houghton-Mifflin Co., Boston, Massachusetts. 200 pp.

Dikmans, G. 1940. A note on the genera Nematospiroides Baylis, 1926 and Sincosta Roe, 1929 (Nematoda, Heligmosomidae) with descriptions of two new species of *Nematospiroides*. Proc. Helminthol. Soc. Wash. 7:79–82.

Durette-Desset, M. C. 1971. Essai de classification des nematodes héligmosomes. Corrélations avec la paléobiogéographie des hôtes. Mém. Mus. Nat. Hist. Nat. Paris, Sér. A. Zool. 69:1–26.

Durette-Desset, M. C., J. M. Kinsella, and D. J. Forrester. 1972. Arguments en faveur de la double origine des nematodes néartiques du genre Heligmosomoides Hall, 1916. Ann. Parasitol. Hum. Comp. 47:365–382.

Grundmann, A. L., and R. G. Warnock. 1964. A population study of eight parasitic nematode species from mammals of the Bonneville-Basin of Utah. Proc. Utah Acad. Sci. 41:228–233.

Kuns, M. L., and Rausch, R. 1950. An ecological study of helminths of some Wyoming voles (*Microtus* spp.) with a description of a new species of *Nematospiroides* (Heligmosomidae: Nematoda). Zoologica 35:181–188.

Rausch, R., and J. D. Tiner. 1948. Studies on the parasitic helminths of the north central states. I. Helminths of Sciuridae. Am. Midl. Nat. 39:728–747.

Roman-Chiriac, E., and M. Hamar. 1966. Contributions à la connaissance des helminthes des petits Mammifères (rongeurs, insectivores) de la Roumania. Acta Parasitol. Polon. 14:61–72.

Schad, G. A. 1954. Helminth parasites of mice in northeastern Quebec and the coast of Labrador. Can. J. Zool. 32:215–224.

Travassos, L., and A. Darriba. 1929. Notas sobre Heligmosominae. Sci. Med. 7:432–438.

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Eufilaria hibleri sp. n. (Nematoda: Filarioidea) from the Common Grackle (*Quiscalus quiscula versicolor*)

WILLARD O. GRANATH, JR.
Department of Biology, Wake Forest University, Winston-Salem, North Carolina 27109

ABSTRACT: *Eufilaria hibleri* sp. n. (Nematoda: Filarioidea) is described from the subcutaneous tissue of the common grackle (*Quiscalus quiscula versicolor* Vieillot). This parasite was discovered during a survey of wild birds from Illinois. Eighty-six of 203 grackles (42%) were infected with *E. hibleri* whereas none of the English sparrows, red-winged blackbirds, slate-colored juncos, brown-headed cowbirds, blue jays, starlings, barn swallows, robins, or horned larks examined were infected with this filariid. Distinguishing characters of *E. hibleri* are: short, slender esophagus, short, subequal spicules, anus of male flanked by two fleshy protuberances which may or may not have papillae, and sheathed microfilariae which occur in the blood. This is only the second species of *Eufilaria* to be fully described from a North American bird.

A new species of Onchocercidae was found in the subcutaneous tissue of the common grackle (*Quiscalus quiscula versicolor,* Vieillot) during a survey of wild birds from Illinois. Interestingly, of all the avian species examined (English sparrow (*Passer domesticus*), red-winged blackbird (*Agelaius phoeniceus*), slate-colored junco (*Junco hyemalis*), brown-headed cowbird (*Molothrus ater*), blue jay (*Cyanocitta cristata*), starling (*Sturnus vulgaris*), barn swallow (*Hirundo rustica*), robin (*Turdus migratorius*), horned lark (*Eremophila alpestris*)), only grackles were infected with this filariid.

Odetoyinbo (1960) reported that 64 of 112 grackles in Iowa had microfilariae of *Chandlerella quiscali* (Onchocercidae: Filarioidea) and 25 of 112 grackles had another, morphologically distinct sheathed microfilaria which he termed "microfilaria X." However, he was not able to locate or recover the adult worms which produced "microfilaria X." This report describes a new *Eufilaria* species found in the subcutaneous tissue of grackles from central Illinois. It is believed that this is the species of filariid that produced "microfilaria X" observed by Odetoyinbo (1960).

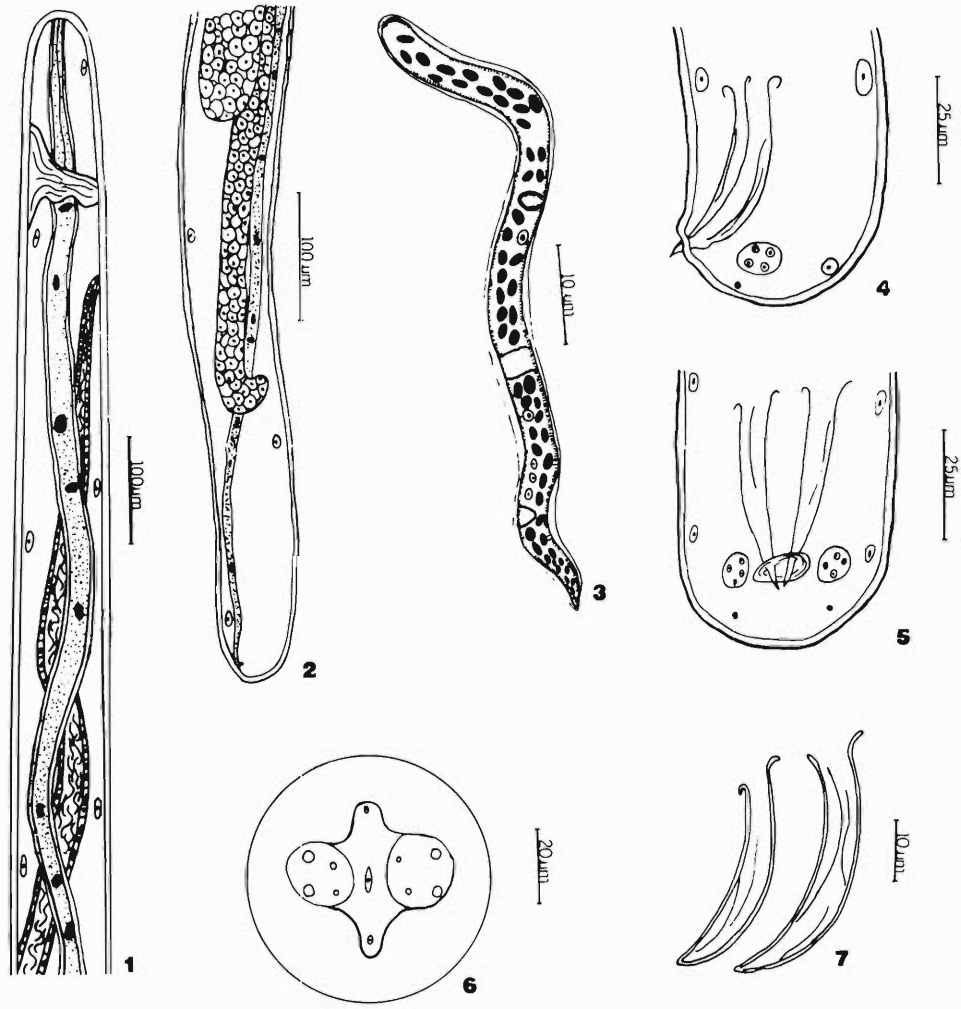
Materials and Methods

Adult nematodes were obtained during the examination of wild birds from central Illinois during the springs and summers of 1976, 1977, and 1978. Eighty-six of 203 (42%) common grackles were infected with the parasite and worms were dissected from the subcutaneous tissue of 19 of these birds. Measurements were based on 20 female and three male nematodes selected from specimens fixed in hot 70% ethyl alcohol and cleared in glycerin. Microfilariae were numerous in blood obtained from the lungs of infected birds. Measurements were based on 25 microfilariae selected at random from blood smears stained with Wright's or Giemsa's stain.

Results

Eufilaria hibleri sp. n. (Figs. 1–7)

Eighty-six of 203 (42%) common grackles examined were infected with *Eufilaria hibleri* whereas 0 of 56 English sparrows, 0 of 33 red-winged blackbirds, 0



Figures 1–7. ***Eufilaria hibleri*****. 1. Anterior end of adult female. 2. Posterior end of adult female. 3. Microfilaria. 4. Caudal end of male, lateral view. 5. Caudal end of male, ventral view. 6. En face view of female. 7. Spicules of male.**

of 27 slate-colored juncos, 0 of 24 brown-headed cowbirds, 0 of 16 blue jays, 0 of 11 starlings, 0 of 11 barn swallows, 0 of 7 robins, and 0 of 6 horned larks from the same area, were infected with *E. hibleri*.

DESCRIPTION: Filarioidea; Onchocercidae (Leiper, 1911) Chabaud and Anderson, 1959; Lemdaninae Lopez-Neyra, 1956; *Eufilaria* Seurat, 1921. Cuticle thin with fine transverse striations. Cephalic extremity with 8 submedian papillae. Buccal capsule absent. Excretory pore not observed. Esophagus short and slender, without observable muscular or glandular zones and imperfectly demarcated from the intestine. Intestine slender and usually containing refractive bodies. Phasmids minute, subterminal and situated ventrolaterally.

MALE: Length 7.5 (7.1–8.1) mm, width 100 (97–103) µm. Lateral width of cephalic end 59 (58–60) µm. Nerve ring 143 (141–144) µm from anterior end of

body, width at nerve ring 71 (69–73) μm. Esophagus 172 (170–174) μm long, 6 (5–7) μm wide. Anus 12 (12–13) μm from posterior end of body, width at anus 50 (48–51) μm. Posterior portion of body curved ventrally but not coiled. Anus subterminal and flanked by fleshy protuberances. One specimen had no papillae on these protuberances while 2 other specimens had 4 each. Spicules similar, subequal, terminating in a point; left 55 (53–56) μm, 4 (4–5) μm wide; right 54 (53–54) μm long, 5 (4–5) μm wide.

FEMALE: Length 20.9 (18.5–28.4) mm, width 173 (160–190) μm. Lateral width of cephalic end 75 (65–85) μm. Nerve ring 168 (130–190) μm from anterior end of body, width at nerve ring 92 (80–110) μm. Esophagus 239 (160–420) μm long, 12 (8–15) μm wide. Vulva 622 (540–760) μm from anterior end of body, width at vulva 99 (70–120) μm. Lips of vulva not prominent. Vagina 541 (350–750) μm long. Uteri didelphic. Anus 3 (0–13) μm from posterior end of body; width at anus 61 (45–90) μm. Ovaries end posteriorly at different levels. Posterior end bluntly rounded.

MICROFILARIA: Length 80 (71–95) μm, width 4 (3–6) μm. Anterior extremity truncated. Caudal extremity tapering to a fine point surrounded by a delicate hyaline sheath. Cuticle with fine transverse striations. Inner body 5 (3–6) μm long. Excretory pore and anal space prominent. The fixed points, expressed as percentages of total body length are: nerve ring 27 (22–30), excretory pore 39 (32–43), excretory cell 43 (42–50), start of inner body 59 (53–70), end of inner body 65 (57–78), length of inner body 6 (3–9), 1st rectal cell 71 (60–80), 2nd rectal cell 78 (64–87), 3rd rectal cell 81 (77–90), 4th rectal cell 83 (77–93), and midpoint anal space 87 (84–96).

HOST: *Quiscalus quiscula versicolor* Vieillot (Icteridae), common grackle.

SITE OF INFECTION: adults in subcutaneous tissue, microfilaria in blood.

LOCALITY: Brown County and McLean County, Illinois.

SPECIMENS DEPOSITED: holotype (male), USNM Helm. Coll. No. 75763; allotype (female), USNM Helm. Coll. No. 75764; microfilaria (1 slide), USNM Helm. Coll. No. 75765.

ETYMOLOGY: This species is named in honor of Dr. Charles P. Hibler, Wild Animal Disease Center, Colorado State University, Fort Collins, Colorado, in recognition of his contributions to the study of wildlife diseases.

Discussion

Eufilaria hibleri is only the second species of *Eufilaria* to be fully described from a North American bird. Hibler (1964) described *E. longicaudata* from the black-billed magpie in Colorado. Table 1 summarizes the hosts and localities of the known species of *Eufilaria*.

E. hibleri is distinguishable from the 12 known species of *Eufilaria* as classified by Anderson and Bain (1976, p. 64). A comparison of the morphological features of male and female adults of the 13 species of *Eufilaria* is summarized in Table 2 and Table 3, respectively.

E. hibleri is only the third species of *Eufilaria* described to have sheathed microfilariae. *E. cypseli* Annett, Dutton, and Elliot, 1901 and *E. mcintoshi* Anderson and Bennett, 1960 have sheathed microfilariae, whereas *E. utae* (Deshmuckh, 1968) Anderson and Bain, 1976, *E. coua* (Chabaud, Brygoo, and Richard, 1964) Anderson and Prestwood, 1969, *E. delicata* Supperer, 1958, *E. capsulata*

Table 1. Hosts and localities of the known species of *Eufilaria*.

Species	Host		Locality	Reference
	Scientific name	Common name		
E. alii	*Turnix tanki*	Button quail	Aurangabad, Maharashtra, India	Deshmukh (1968)
E. asiatica	*Corvus splendens*	Crow	Hyderabad-Deccan, India	Singh (1949)
E. buckleyi	*Caprimulgus asiaticus*	Nightjar	Hyderabad-Deccan, India	Rasheed (1960)
E. capsulata	*Pycnonotus barbatus*	Bulbul	West Africa	Annett et al. (1901)
E. coua	*Coua raynaudii*	Coua	Perinet, Malagasy region	Chabaud et al. (1964)
E. delicata	*Turdus viscivorus* *Turdus merula* *Garralus glandarius*	Mistle thrush European blackbird Eurasian jay	Nieder-Osterreich, Austria	Supperer (1958)
E. hibleri	*Quiscalus quiscula*	Common grackle	Illinois, USA	This report
E. longicaudata	*Pica pica hudsonia*	Black-billed magpie	Colorado, USA	Hibler (1964)
E. mcintoshi	*Padda oryzivora*	Java sparrow	Java; imported to Ontario, Canada	Anderson and Bennett (1960)
E. sergenti	*Passer hispanolensis*	Spanish sparrow	Algeria, Africa	
	Serinus serinus	Serin	Algeria, Africa	Seurat (1921)
	Garrulax leucolophus	White-crested jay-thrush	Himalayas	Yeh (1957)
E. singhi	*Lalage sykesii*	Black-headed cuckoo shrike	Hyderabad, India	Sultana (1961)
E. utae	*Perdicula asiatica*	Bush quail	Aurangabad, Maharashtra, India	Deshmukh (1968)

Table 2. Comparison of body measurements of male adults of the known species of *Eufilaria*.

	Length (mm)	Width (μm)	Anterior end to nerve ring (μm)	Length of esophagus (μm)	Distance anus from posterior of body (μm)	Length of spicules (μm)		Number of lateral swellings
						lf	rt	
Species								
E. alii	8–12	110–130	120–150	190–220	10–20	70–80	60–70	2
E. asiatica	9–10	115–120	100–120	233	ST*	113–114	119–124	4
E. buckleyi	12	281	200–300	986	110	200	200	0
E. capsulata	4	170	NG†	390	ST	NG	NG	NG
E. coua	10	105	130	260	T‡	65	70	4
E. cypseli	4	150	NG	320	ST	NG	NG	NG
E. delicata	11–13	100	150–160	230–277	ST	57–61	60–65	0
E. hibleri	7–8	97–103	141–144	170–174	12–13	53–56	53–54	2
E. longicaudata	7–11	41–59	138–170	325–410	ST	59–73	52–59	2
E. mcintoshi	6	71	92	186	14	64	52	2
E. sergenti	3	NG	NG	NG ("short")	ST	NG	NG ("short")	0
E. singhi	4	60	40	60	ST	64	39	0
E. utae	8–9	110–130	130–150	230–250	10	70–80	50–60	2

* Anus situated subterminally.
† Not given in original description.
‡ Anus situated terminally.

(Annett, Dutton, and Elliot, 1901) Seurat, 1921, *E. sergenti* Seurat, 1921, and *E. longicaudata* Hibler, 1964 do not have sheathed microfilariae. The descriptions of *E. alii* (Deshmuckh, 1960) Anderson and Bain, 1976, *E. singhi* (Sultana, 1961) Anderson and Prestwood, 1969, *E. asiatica* Singh, 1949, and *E. buckleyi* Rasheed, 1960 do not mention the presence or absence of sheathed microfilariae.

The male of *E. hibleri* has two lateral swellings, while the males of *E. asiatica*

Table 3. Comparison of body measurements of female adults of the known species of *Eufilaria*.

	Length (mm)	Width (μm)	Anterior end to nerve ring (μm)	Length of esophagus (μm)	Distance anus from posterior of body (μm)	Anterior end to vulva (μm)	Length of vagina (μm)
E. alii	18–37	190–280	130–170	210–280	80–160	500–950	88–97
*E. asiatica**	—	—	—	—	—	—	—
E. buckleyi	14	300	210–310	129	130	490	NG†
E. capsulata	41	440	NG	540	T‡	250	1600
E. coua	19	170	140	240	55	590	200
E. cypseli	24–27	220	NG	450	T	700	NG
E. delicata	20–24	147	220–265	260–298	ST§	769–782	848–857
E. hibleri	18–28	160–190	130–190	160–420	0–13	540–760	350–750
E. longicaudata	11–21	69–124	132–186	225–415	35–80	500–600	205–240
E. mcintoshi	16–18	143–150	162–170	185–232	14–15	450–460	900
E. sergenti	14	NG	NG	NG ("short")	NG	NG	NG
E. singhi	16	90	50	80	26	580	260
E. utae	25–34	190–220	180–220	260–280	ST	540–960	NG

* Original description based on male worms only.
† Not given in original description.
‡ Anus situated subterminally.
§ Anus situated terminally.

and *E. coua* have four lateral swellings and a terminal protuberance. The males of *E. sergenti, E. buckleyi,* and *E. delicata* have no lateral swellings on the caudal extremity. The vagina of *E. hibleri* is much shorter than the vagina of *E. mcintoshi* and *E. delicata* but is much longer than the vagina of *E. singhi* and *E. alii.* The systematic position of *E. capsulata* is uncertain because the female was reported to be over 40 mm long, which is almost twice the length of *E. hibleri* females.

E. longicaudata bears the closest resemblance to *E. hibleri.* However, the microfilariae of *E. longicaudata* are longer (98) than the microfilariae of *E. hibleri* (80), and do not have a sheath. Also, the microfilariae of *E. longicaudata* have a longer inner body (6–12) than *E. hibleri* (3–6). Females of *E. longicaudata* are shorter in length, have a shorter vagina, and the distance from the anterior end to the vulva is much less than in *E. hibleri.* The males of *E. hibleri* are greater in width, and have shorter spicules.

There is also ecological evidence which indicates that *E. longicaudata* and *E. hibleri* are different species. It is well known that some species of avian filarial worms lack host specificity. For example, Granath (1980) found that birds in three different families (Icteridae, Corvidae, Sturnidae) were all infected with *Chandlerella quiscali.* However, in the present study, such lack of host specificity was not the case. Magpies and bluejays belong to the same family (Corvidae) whereas red-winged blackbirds, cowbirds, and grackles belong to the family Icteridae. If *E. longicaudata* showed lack of host specificity, it would be expected that other corvids (i.e., blue jays) would become infected, if the parasite were present in a given locality. However, blue jays were not infected with any *Eufilaria* species. Moreover, *E. hibleri* showed a marked host specificity within the Icteridae in that grackles were parasitized, but red-winged blackbirds and brown-headed cowbirds were not infected. It is also interesting to note that the range of the black-billed magpie (Bent, 1946), the host of *E. longicaudata,* does not overlap the range of the common grackle (Bent, 1958), which is the host of *E. hibleri.*

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Literature Cited

Anderson, R. C., and O. Bain. 1976. Keys to the order of Spirurida. Part 3. Diplotriaenoidea, Aproctoidea, and Filarioidea. In "CIH keys to the nematode parasites of vertebrates." Commonwealth Agricultural Bureaux, Farnham Royal, Bucks., England, p. 64.

Anderson, R. C., and G. F. Bennett. 1960. *Eufilaria mcintoshi* n. sp. from *Padda oryzivora* (C). Proc. Helminthol. Soc. Wash. 27:113–115.

Anderson, R. C., and A. K. Prestwood. 1969. *Singhfilaria hayesi* n. sp. from *Meleagris gallopavo* and a revision of Lemdaniinae (=Eufilariinae). Can. J. Zool. 47:1015–1019.

Annett, H. E., J. E. Dutton, and J. H. Elliott. 1901. Report of the malaria expedition to Nigeria of the Liverpool School of Tropical Medicine and Medical Parasitology. Part II. Filariasis. Thompson Yates Lab. Rep. 4:1–92.

Bent, A. C. 1946. Life histories of North American jays, crows, and titmice. U.S. Natl. Mus. Bull. 191:1–495.

Bent, A. C. 1958. Life histories of North American blackbirds, orioles, tanagers, and allies. U.S. Natl. Mus. Bull. 211:1–549.

Chabaud, A. G., E. R. Brygoo, and J. Richard. 1964. Filaires d'oiseaux malgaches (Deuxième note). Ann. Parasitol. Hum. Comp. 39:69–94.

Deshmukh, P. G. 1968. Two new species of a new genus *Neofilaria* from Indian quails. Riv. Parassitol. 29:123–128.

Granath, W. O. 1980. Fate of the wild avian filarial nematode *Chandlerella quiscali* (Onchocercidae: Filarioidea) in the domestic chicken. Poult. Sci. 59:996–1000.

Hibler, C. P. 1964. New species of Onchocercidae (Nematoda: Filarioidea) from *Pica pica hudsonia* (Sabine, 1923). J. Parasitol. 50:667–674.

Odetoyinbo, J. 1960. Biology of *Splendidofilaria quiscali* (Von Linstow, 1904) n. comb. (Nematoda: Onchocercidae). Ph.D. dissertation. Iowa State University. 142 pp.

Rasheed, S. 1960. The nematode parasites of the birds of Hyderabad (India). Biologia (Lahure) 6:1–116.

Seurat, L. G. 1921. La filaire du moineau. Bull. Soc. Hist. Nat. Afr. Nord 12:28–31.

Singh, S. N. 1949. Studies on the helminth parasites of birds in Hyderabad State. Nematoda IV. J. Helminthol. 23:39–56.

Sultana, A. 1961. Two new species of *Lemdana* Seurat, 1917, from Indian birds. J. Parasitol. 47:717–719.

Supperer, R. 1958. Zwei neue filarien (S.L.), *Eufilaria delicata* spec. nov. und *Ornithofilaria böhmi* spec. nov. aus der misteldrossel, *Turdus viscivorus* L. Z. Parasitenkd. 18:312–319.

Yeh, L. S. 1957. On *Chanderella braziliensis* n. sp. from a green-billed toucan and a discussion on some related genera. J. Helminthol. 31:33–38.

Proc. Helminthol. Soc. Wash.
48(1), 1981, pp. 24–37

Observations on the Prevalence and Intensity of *Capillaria* spp. (Nematoda: Trichuroidea) in Wild Carnivora from Ontario, Canada

ERIC W. BUTTERWORTH[1] AND MARY BEVERLEY-BURTON
Department of Zoology, College of Biological Science,
University of Guelph, Guelph, Ontario N1G 2W1, Canada

ABSTRACT: Four *Capillaria* spp. were found in eight species of wild Carnivora taken in Ontario, Canada, over a period of 18 months. *C. plica* was found in raccoons (*Procyon lotor*), red foxes (*Vulpes vulpes*), coyotes (*Canis latrans*), fishers (*Martes pennanti*), and striped skunks (*Mephitis mephitis*); *C. putorii* in short-tailed weasels (*Mustela erminea*), mink (*M. vison*), fishers, martens (*Martes americana*), striped skunks, and raccoons; *C. aerophila* in red foxes and martens (new host record), and *C. procyonis* in raccoons and striped skunks.

Data on location within each host species, prevalence, intensity, dispersion, and association are presented. The numerical host-parasite relationships are examined with regard to host species, season, age, and sex. An attempt was made to relate seasonal data with the biology (including feeding habits) of the hosts.

Capillaria aerophila (Creplin, 1839) Travassos, 1915, *C. plica* (Rudolphi, 1819) Travassos, 1915, *C. putorii* (Rudolphi, 1819) Travassos, 1915, and *C. procyonis* Pence, 1975 have been reported from a variety of Carnivora in North America (Law and Kennedy, 1932; Swales, 1933; Read, 1949; Pence, 1975). Unfortunately, most papers concerning *Capillaria* spp. only contain prevalence data and involve a single host species. Although both prevalence and intensity data are available for *C. aerophila* and *C. plica* in foxes (*Vulpes vulpes* L.) maintained on fur farms (Watkins and Harvey, 1942) little is known about prevalence, intensity, and transmission of *Capillaria* spp. in populations of wild Carnivora. The population dynamics of any parasite will be affected by the structure of the host community (Holmes, 1979). It is necessary to know the distribution of the parasite within the community of hosts before one can evaluate the population dynamics of any parasite species. Also, in a temperate climate, seasonal changes have the potential to affect the distribution patterns of a parasite within its host populations.

In the present study we examine the numerical host-parasite relationships of four *Capillaria* species found in wild Carnivora from central southern Ontario, Canada, with reference to species of host, host's age and sex, and season.

Materials and Methods

Carcasses of wild Carnivora (278) from Ontario, Canada, were obtained directly from trappers or indirectly through the Ministry of Natural Resources during the 1973–1974 and 1974–1975 trapping seasons (Table 1). Some of the animals collected during the spring, summer, and early fall were road kills, others were live trapped using baited No. 11 National (Tomahawk, Wisconsin) traps. Live trapped animals were transported to the laboratory, anesthetized with chloroform and

[1] Present address: Department of Zoology, University of Alberta, Edmonton, Alberta T6G 2E1, Canada.

killed by intracardiac injection of 1–2 ml of sodium pentobarbitol (Nembutal, Abbott Laboratories).

Species, date, sex, stomach contents and, where possible, age and weight of each animal were recorded. All of the red foxes and most of the raccoons (*Procyon lotor* L.) (n = 129 of 140) were aged as juveniles (<1 yr) or adults (>1 yr) by Ontario Ministry of Natural Resources personnel (I. Watt and D. E. Johnston). Red foxes were aged using the cementum annuli technique (Monson et al., 1973) while raccoons were aged using the presence or absence of open root canals and the size of pulp cavities (D. E. Johnston, pers. comm. 1975). Host species other than red fox and raccoon were not aged.

Necropsies were performed as soon after death as possible (48 hr maximum) or the carcasses were frozen (−20°C) until examination. Techniques used for finding and preparing worms for identification are described elsewhere (Butterworth and Beverley-Burton, 1980). Representative specimens have been deposited in the National Museum of Natural Science Collection of Invertebrates (Parasites) Ottawa, Ontario K1A 0M8, Canada (Nos. NMCIC (P) 1980-79 to 1980-111), the Commonwealth Institute of Helminthology, 103 St. Peter's Street, St. Albans, Herts., U.K. (Nos. 3372–3392) and the U.S. National Museum, Parasite Collection, Beltsville, Maryland (Nos. 75693–75702).

Chi-square analyses were used to compare prevalences of *Capillaria* spp. in relation to host, age, and sex. Index of affinity (Fager and McGowan, 1963), and Southwood's (1966) modification of Whittaker and Fairbanks' (1958) coefficient of association and the point correlation coefficient (Poole, 1974) were used to analyze association between *Capillaria* spp. Index of affinity was determined by:

$$I_{AB} = \frac{J}{(N_A N_B)^{\frac{1}{2}}} - \frac{1}{2(N_B)^{\frac{1}{2}}}$$

where J is the number of joint occurrences of species A and B, and N_A and N_B are the number of times each species occurred. The point correlation coefficient, also based on presence or absence, was determined by:

$$V = \frac{ad - bc}{[(a + b)(a + c)(b + d)(c + d)]^{\frac{1}{2}}}$$

where a, b, c, and d correspond to the standard notation of a 2 × 2 contingency table. The coefficient of association was determined by:

$$I_{ai} = 2\left[\frac{J_i}{A + B} - 0.5\right]$$

where J_i is the number of individuals of A and B in samples of joint occurrences, and A and B is the number of individuals of A and B in all samples.

The following procedures were applied to information collected from raccoons. Worm counts were grouped bimonthly based on the known biology of raccoons. In the present study young raccoons were first collected during July 1974 and the nine bimonthly periods used were as follows: Nov.–Dec. 1973; Jan.–Feb. 1974; Mar.–Apr. 1974; May–June 1974; July–Aug. 1974; Sep.–Oct. 1974; Nov.–Dec. 1974; Jan.–Feb. 1975; Mar.–Apr. 1975. Prevalence is defined as the number (expressed as a percentage) of infected animals in the sample while intensity is the mean number of parasites per infected host. A $\log_{10}$ transformation was applied

to the data to reduce heterogeneity of the variances. Reduction of the heterogeneity of variances was confirmed by Bartlett's test (Guenther, 1964). A one way analysis of variance and Scheffe's test were used to compare bimonthly intensity of infection.

Mann-Whitney U (two-tailed) and/or median test (one-tailed) were used to compare intensities of infections between untransformed data groups. $P < 0.05$ was used as the level of statistical significance.

Results

Location

Capillaria plica was located almost exclusively in the lumen of the urinary bladder of several host species (Table 1). However, one male from a raccoon was located in the submucosa of the bladder.

Capillaria putorii was located in the stomach of raccoons, mink (*Mustela vison* L.), martens (*Martes americana* Turton), fishers (*M. pennanti* Erxleben), striped skunks (*Mephitis mephitis* Schreber), and short-tailed weasels (*Mustela erminea* L.). It was found in the mucus lining the stomach wall and/or in the stomach contents of the host animals. In mink and martens, some worms were found in the lumen of the duodenum.

Capillaria aerophila was found within the mucosa of the trachea and bronchi of red foxes and martens. In red foxes most worms (66%) were found in the trachea.

Capillaria procyonis was found within the esophageal mucosa of raccoons and skunks.

Prevalence

Prevalences of all four *Capillaria* species are listed in Table 1. Red foxes were only obtainable in October, November, and December 1974 and prevalence of *C. plica* in this host (Table 1) was significantly lower than that in raccoons (84% of 33) collected during the same period. Similarly, mink were only available in winter and no significant difference was found in the prevalences of *C. putorii* in mink (Table 1) and raccoons (70% of 62) collected during the same time period (Nov.–Dec. 1973, 1974; Jan. 1975). Prevalences of *C. aerophila* and *C. procyonis* were not compared between species because host sample sizes were too small for statistical analyses. No significant differences were found between male and female hosts for *C. aerophila, C. plica,* and *C. putorii* (Table 1). Prevalences of *C. procyonis* in male and female raccoons were significantly different (Table 1). Prevalences of *C. plica, C. putorii,* and *C. procyonis* located in raccoons varied from 25% to 100% and were unrelated to season (Tables 1 and 2).

Prevalences of *C. aerophila* and *C. plica* in juvenile (56% of 27 and 62% of 29, respectively) and adult red foxes (29% of 21 and 42% of 19, respectively) were not significantly different. Prevalences of *C. putorii* and *C. procyonis* in juvenile and adult raccoons were not significantly different (Table 3). Prevalence of *C. plica* in adult raccoons was significantly higher than in juveniles (Table 3). However, considering prevalence only in bimonthly periods in which both juveniles and adults were collected the difference in prevalences was not significant, i.e., adults 62%, juveniles 40%.

Table 1. Occurrence of *Capillaria* spp. in wild Carnivora from Ontario, Canada.

Species	Host	No. examined (N)	Prevalence	Intensity*	No. of parasites	Male N	Male Prevalence	Male Intensity	Female N	Female Prevalence	Female Intensity
C. plica	Raccoon†	140‡	58	4.8 (±5.4)	1–25	74	60	4.9 (±5.8)	66	56	4.7 (±5.0)
	Red Fox§	48	54	3.7 (±3.3)	1–14	31	58	3.5 (±3.7)	17	47	4.1 (±2.6)
	Striped Skunk†	11	18	1.0	1	—	—	—	—	—	—
	Coyote§	6	33	3.0 (±1.4)	1–3	—	—	—	—	—	—
	Fisher‖	3	66	1.0	1	—	—	—	—	—	—
C. putorii	Raccoon	138‡	68	22.0 (±27.7)	1–117	74	72	19.6 (±25.1)	64	64	26.9 (±30.3)
	Mink†	45	60	42.0 (±44.9)	1–161	33	67	43.1 (±46.5)	12	50	31.5 (±39.9)
	Marten‖	13	54	9.8 (±6.3)	1–17	—	—	—	—	—	—
	Striped Skunk	11	18	5.0 (±5.7)	1–9	—	—	—	—	—	—
	Short-tailed Weasel†	10	40	6.3 (±3.8)	1–10	—	—	—	—	—	—
	Fisher	3	66	5.5 (±3.8)	3–8	—	—	—	—	—	—
C. aerophila	Red Fox	48	44	2.7 (±1.9)	1–8	31	42	3.4 (±2.1)¶	17	47	1.5 (±0.5)¶
	Marten	13	15	1.0	1	—	—	—	—	—	—
C. procyonis	Raccoon	129‡	59	4.3 (±3.2)	1–15	73	67¶	4.2 (±3.2)	56	48¶	4.5 (±3.6)
	Striped Skunk	11	18	5.5 (±3.5)	3–8	—	—	—	—	—	—

* Mean (±standard deviation).
† Central southern Ontario—including the counties of Huron, Perth, Wellington, and Waterloo.
‡ Total number of raccoons examined was 140: 2 stomachs were damaged and are excluded as were 11 oesophagi from raccoons examined prior to the initial finding of *C. procyonis*.
§ Central southern Ontario—including the counties of Huron, Middlesex, Perth, and southern Bruce.
‖ Central Ontario—District of Parry Sound.
¶ Significant difference at 95% level.

Table 2. Occurrence of *Capillaria plica*, *C. putorii*, and *C. procyonis* in raccoons (*Procyon lotor*) from central southern Ontario.

Months	*Capillaria plica*				*Capillaria putorii*				*Capillaria procyonis*			
	No. examined	Prevalence (%)	Intensity ($\bar{x}$ ± 1 SD)	Variance/mean	No. examined	Prevalence (%)	Intensity ($\bar{x}$ ± 1 SD)	Variance/mean	No. examined	Prevalence (%)	Intensity ($\bar{x}$ ± 1 SD)	Variance/mean
Nov.–Dec. 1973	30	40	1.7 ± 0.9	0.9:1	30	53	6.3 ± 7.1	8.1:1	23	48	2.6 ± 1.6	1.0:1
Jan.–Feb. 1974	21	57	1.8 ± 1.3	1.0:1	21	48	6.5 ± 6.8	7.0:1	21	48	2.1 ± 2.1	2.2:1
Mar.–Apr. 1974	5	100	1.4 ± 0.9	0.6:1	5	40	8.5 ± 10.6	13.2:1	5	60	4.3 ± 4.9	5.6:1
May–Jun. 1974	11	64	6.6 ± 6.7	6.8:1	10	80	35.4 ± 33.7	32.0:1	8	25	1.5 ± 0.7	0.3:1
Jul.–Aug. 1974	18	56	6.5 ± 5.9	5.4:1	18	72	25.9 ± 30.9	36.8:1	18	39	3.4 ± 2.0	1.2:1
Sep.–Oct. 1974	16	81	5.7 ± 5.3	4.8:1	16	88	26.4 ± 23.2	20.4:1	16	56	5.9 ± 4.2	3.0:1
Nov.–Dec. 1974	22	77	8.5 ± 7.0	5.9:1	21	90	41.6 ± 38.1	34.8:1	21	86	5.9 ± 3.8	2.5:1
Jan.–Feb. 1975	10	30	2.7 ± 2.1	1.6:1	10	90	10.6 ± 14.5	19.9:1	10	90	4.1 ± 2.5	1.6:1
Mar.–Apr. 1975	7	27	1.5 ± 0.7	0.3:1	7	57	13.5 ± 7.2	3.9:1	7	100	5.9 ± 3.3	1.9:1

* Solid lines indicate no significant difference between bimonthly period. Difference between groups (1 and 2) was significant at 95% level.

Table 3. Occurrence of *Capillaria plica*, *C. putorii*, and *C. procyonis* in juvenile and adult raccoons (*Procyon lotor*) from central southern Ontario.

Months	Juvenile			Adult		
	No. examined	Prevalence (%)	Intensity ($\bar{x}$ ± 1 SD)	No. examined	Prevalence (%)	Intensity ($\bar{x}$ ± 1 SD)
Capillaria plica						
Nov.–Dec. 1973	17	24	1.5 ± 0.6	9	56	2.0 ± 1.2
Jan.–Feb. 1974	10	40	1.0 ± —	9	67	1.6 ± 0.9
Mar.–Apr. 1974	1	—	1.0 ± —	3	100	1.7 ± 1.2
May–Jun. 1974	0	—	— —	11	64	6.6 ± 6.7
Jul.–Aug. 1974	5	0	— —	13	77	6.5 ± 5.9
Sep.–Oct. 1974	10	70	6.1 ± 6.2	5	100	6.0 ± 4.3
Nov.–Dec. 1974	6	50	4.0 ± 2.7	7	71	5.0 ± 3.7
Jan.–Feb. 1975	4	25	5.0 ± —	6	33	1.5 ± 0.7
Mar.–Apr. 1975	0	—	— —	7	29	1.5 ± 0.7
Total*	53	38†	3.6 ± 4.3	70	64†	4.6 ± 4.8
Capillaria putorii						
Nov.–Dec. 1973	17	53	4.1 ± 5.0	9	78	9.0 ± 8.8
Jan.–Feb. 1974	10	40	5.3 ± 6.6	9	67	7.3 ± 7.4
Mar.–Apr. 1974	1	—	1.0 ± —	3	0	— —
May–Jun. 1974	0	—	— —	10	80	39.6 ± 33.9
Jul.–Aug. 1974	5	40	1.5 ± 0.7†	13	85	30.4 ± 31.7†
Sep.–Oct. 1974	10	80	22.8 ± 29.2	5	100	30.0 ± 13.8
Nov.–Dec. 1974	5	80	35.3 ± 24.6	7	71	17.8 ± 12.6
Jan.–Feb. 1975	4	100	6.3 ± 5.9	6	83	14.0 ± 19.0
Mar.–Apr. 1975	0	—	— —	7	57	13.5 ± 7.2
Total	52	62	12.8 ± 20.1†	69	74	21.9 ± 27.7†
Capillaria procyonis						
Nov.–Dec. 1973	15	60	2.7 ± 1.7	6	17	4.0 —
Jan.–Feb. 1974	10	40	1.8 ± 0.5	9	44	3.0 ± 3.4
Mar.–Apr. 1974	1	100	2.0 ± —	3	33	1.0 —
May–Jun. 1974	0	—	— —	8	25	1.5 ± 0.7
Jul.–Aug. 1974	5	0	— —	13	62	3.5 ± 1.9
Sep.–Oct. 1974	9	44	7.5 ± 5.8	5	80	3.8 ± 1.7
Nov.–Dec. 1974	5	100	7.2 ± 4.9	7	57	5.0 ± 3.6
Jan.–Feb. 1975	4	100	4.3 ± 2.2	6	83	4.0 ± 3.0
Mar.–Apr. 1975	0	—	— —	7	100	5.9 ± 3.3
Total	49	53	4.4 ± 3.8	64	56	4.0 ± 2.8

* Ages available for 129 of 140 raccoons.
† Significant difference at 95% level.

Intensity

Intensities of all four species of *Capillaria* are listed in Table 1. Intensity of *C. plica* in raccoons (7.5 ± 6.5) (mean ± 1 SD) was significantly higher than intensity in red foxes (Table 1) collected during the same period (Oct.–Dec. 1974). Intensity of *C. putorii* in mink (Table 1) was significantly higher than in raccoons (21.9 ± 30.9) collected during the same time periods (Nov.–Dec. 1973, 1974; Jan. 1975).

Intensities of *C. aerophila* in male and female red foxes were significantly

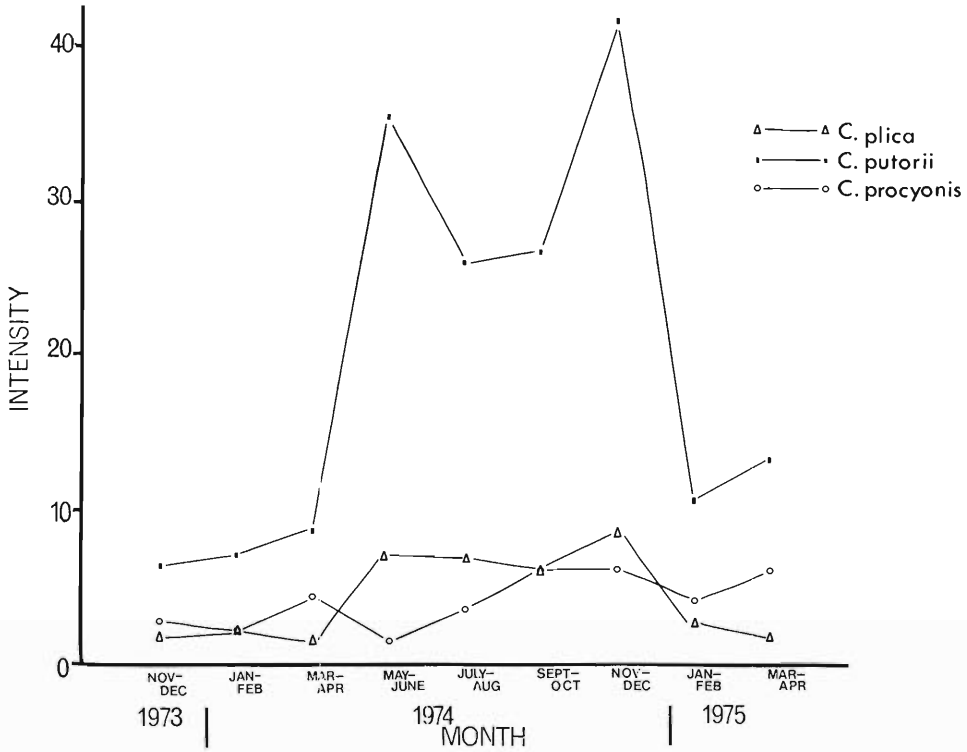

Figure 1. Changes in intensity of infection of *Capillaria plica*, *C. putorii*, and *C. procyonis* in raccoons (*Procyon lotor*) collected from central southern Ontario during the months November 1973 to April 1975.

different (Table 1). Comparison of intensities of other *Capillaria* spp. indicated no significant differences between male and female hosts (Table 1).

Intensities of *C. aerophila* and *C. plica* were not significantly different between juvenile (2.7 ± 2.1 and 4.1 ± 3.8, respectively) and adult (2.7 ± 1.6 and 2.9 ± 1.8, respectively) red foxes. Combined intensity from all bimonthly periods of *C. putorii* in adult raccoons was significantly higher than in juveniles (Table 3), although when juveniles born in 1974 were compared to adults during the same periods (Jul. 1974–Feb. 1975) the difference was not significant. Combined intensities from all bimonthly periods of *C. plica* and *C. procyonis* in juvenile and adult raccoons were not significantly different (Table 3).

Significant differences in intensity between bimonthly periods were found in both *C. plica* and *C. putorii* in raccoons. However, no significant differences were found between intensities of *C. procyonis* during the same periods (Table 2). Both *C. plica* and *C. putorii* had significantly higher intensities during spring, summer, and fall (May–Dec. 1974) than in winter (Jan.–Apr. 1974, 1975) (Table 2; Fig. 1). The decrease in intensity of *C. putorii* during the summer and early fall of 1974 was not significant (Table 2). Intensities of both *C. plica* and *C. putorii* were significantly higher in Nov.–Dec. 1974 than in the same period in 1973 (Table 2). Intensity of *C. putorii* in adult raccoons reached their highest value in spring (39.6) and remained high until early fall (30.0), decreasing in late fall (17.8), whereas intensity in juvenile raccoons increased from its lowest value in summer (1.5) to its highest value in late fall (35.3) (Table 3). Adult raccoons had a significantly higher intensity of *C. putorii* than juveniles during July–August

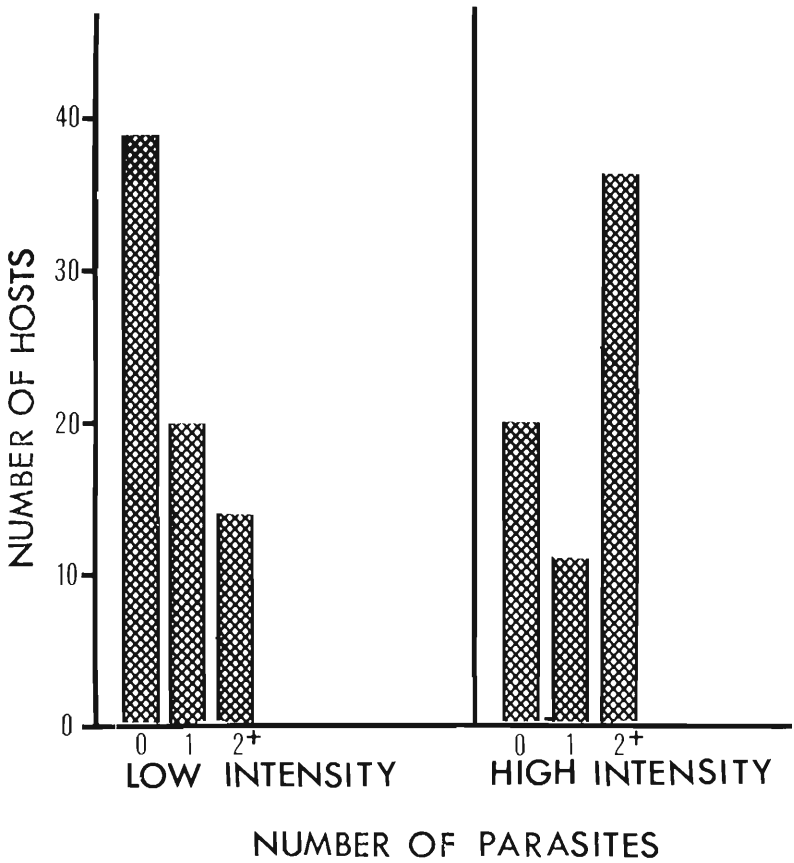

Figure 2. Frequency distribution of numbers of *Capillaria plica* in raccoons (*Procyon lotor*) collected from central southern Ontario during periods of low and high intensity.

1974. All other bimonthly comparisons of intensities of *Capillaria* spp. between juvenile and adult raccoons were not significant (Table 3).

Dispersion

Frequency distributions of *C. aerophila* and *C. plica* in red foxes and *C. plica*, *C. putorii*, and *C. procyonis* in raccoons were overdispersed. Generally, *C. plica*, *C. putorii*, and *C. procyonis* in raccoons had the largest variance to mean ratios (var.:mean > 1) during periods of high intensity (Table 2). Figures 2 and 3 indicate the change in the distribution of *C. plica* and *C. putorii* in the host population during periods of high and low intensity.

Association

Capillaria aerophila and/or *C. plica* occurred in 66% of the red foxes examined. The two species occurred concurrently in 13 of 46 (28%)[2] red foxes. The number of concurrent infections was not significant.

[2] A total of 50 red foxes was examined, two had damaged urinary bladders and two had damaged tracheae, all four were excluded from analyses.

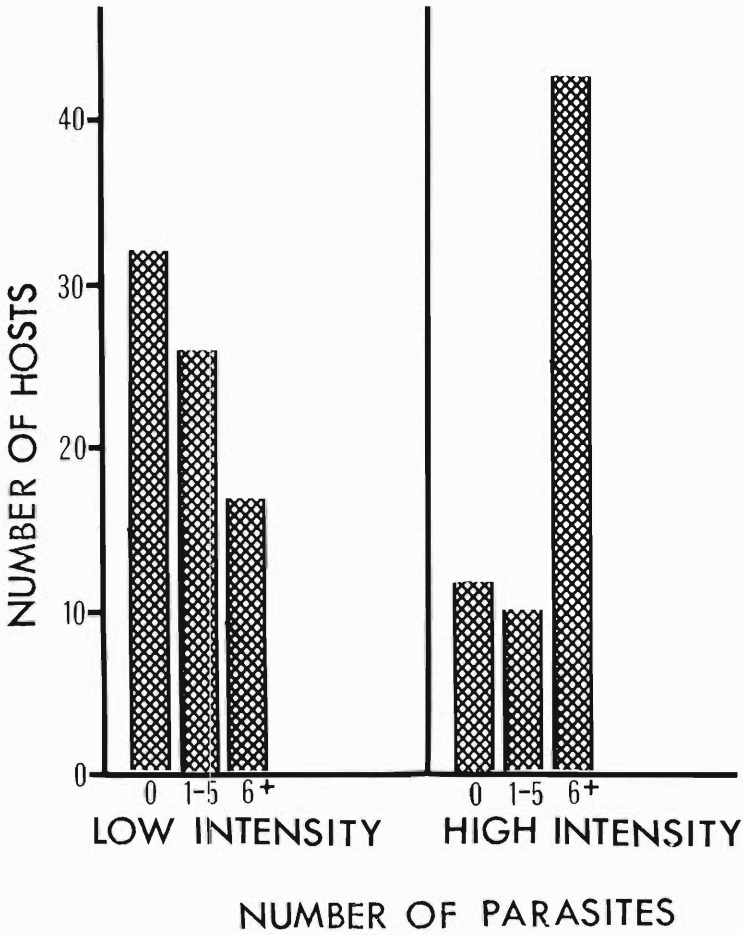

Figure 3. Frequency distributions of numbers of *Capillaria putorii* in raccoons (*Procyon lotor*) collected from central southern Ontario during periods of low and high intensity.

One or more of *C. procyonis*, *C. putorii*, and *C. plica* occurred separately or concurrently in 133 of 140 (95%) raccoons examined. *C. procyonis*, *C. putorii*, and *C. plica* occurred concurrently in 46 (36%) raccoons; *C. procyonis* and *C. putorii* in 61 (48%) raccoons; *C. procyonis* and *C. plica* in 51 (40%) raccoons; and *C. putorii* and *C. plica* in 63 (46%) raccoons. The number of concurrent infections of *C. procyonis* and *C. putorii* and concurrent infections of *C. putorii* and *C. plica* were significant while concurrent infections of *C. procyonis* and *C. plica* were not.

The numbers of concurrent infections of *C. procyonis*, *C. putorii*, and *C. plica* in raccoons were examined during the five bimonthly periods of low intensity (Nov. 1973–Apr. 1974; Jan.–Apr. 1975) and the four periods of high intensity (May–Dec. 1974). During periods of low intensity the number of concurrent infections involving all three species (15 [27%]) or even pairs of species was not significant (Table 4). In comparison, during periods of high intensity the number of concurrent infections involving all three species (31 [50%]) was significant as was the number of concurrent pairs of species (Table 4).

Table 4. Concurrent pairs, 'Index of Affinity (I_{AB})', 'Point Correlation Coefficient (V)', and Coefficient of Association (I_{ai}) as measures of association of concurrent infections between *C. plica, C. putorii,* and *C. procyonis* in raccoons (*Procyon lotor*) from central southern Ontario.

Species	Concurrent pairs		I_{AB}	Point correlation coefficient (V)	I_{ai}
	Low intensity	High intensity			
Capillaria procyonis *Capillaria putorii*	26 (39%)	35 (57%)*	0.74	0.43	0.67
Capillaria procyonis *Capillaria plica*	20 (30%)	31 (49%)*	0.69	0.36	0.76
Capillaria putorii *Capillaria plica*	21 (29%)	42 (65%)*	0.79	0.46	0.86

* Significant difference at 95% level.

Index of affinity (I_{AB}) and point correlation coefficient (V) indicated a positive association in concurrent pairs during periods of high intensity (Table 4). In addition, the coefficient of association (I_{ai}) indicated high numbers of individuals occur most often in concurrent infections of *C. putorii* and *C. plica* (Table 4).

Levels of intensity of individual species, during periods of high intensity, were not significantly different in the presence or absence of other species with the exception of *C. putorii*. Levels of intensity of *C. putorii* were significantly higher in the presence of *C. plica* as compared to levels of intensity when *C. plica* was absent. In addition, rank correlations (Kendall's tau) indicated, during periods of high intensity, only *C. putorii* and *C. plica* varied significantly in concert.

Discussion

Capillaria spp. in Carnivora are widely distributed over North America (Law and Kennedy, 1932; Swales, 1933; Harkema and Miller, 1964; Miller and Harkema, 1964, 1968; Dorney and Lauerman, 1968; Holmes and Podesta, 1968; Pence, 1975; Smith, 1978).

Of the species observed in the present study *C. aerophila, C. plica,* and *C. putorii* have been found in their respective hosts over most of the holarctic region but *C. procyonis* has been reported only from North America where raccoons appear to be the natural definitive host. It is interesting that no *Capillaria* spp. have been reported from raccoons introduced into the Soviet Union (Aliev and Sanderson, 1966). *C. aerophila* and *C. plica* both occur in red foxes throughout most of its distribution (Skrjabin et al., 1957). In our study area, *C. plica* was found with a high prevalence in both raccoons and red foxes. Intensity and prevalence were both significantly higher in raccoons than in red foxes leading to the conclusion that raccoons are the required definitive host (sensu Holmes, 1979) in southern Ontario. However, this must be interpreted with caution as we lack information regarding the relative host population densities needed to make this conclusion (Holmes et al., 1977). Also, little is known about the behavior of these two nocturnal animals (Ables, 1975; Fritzell, 1978) and it is impossible to determine if the populations of *C. plica* in raccoons and red foxes are of mixed or separate origin. In the present study *C. putorii* was found in raccoons, skunks, weasels, martens, and mink. Elsewhere it occurs extensively in a variety of mustelid hosts (Skrjabin et al., 1957) and the European hedgehog (*Erinaceus europaeus*) (Skrjabin et al., 1957; Fahmy, 1964). *C. putorii* in raccoons shows con-

sistent morphological differences from specimens collected from mustelid hosts (Butterworth and Beverley-Burton, 1980). The morphological differences could indicate a genetic drift between the two populations, one in raccoons and the other in mustelids.

Depending on the species of *Capillaria* involved the life cycle may be monoxenous or heteroxenous. Eggs are passed in the feces or urine of the definitive host and embryonate in the external environment. *C. aerophila* and *C. plica* have been shown to use "earthworms" as intermediate hosts (Borovkova, 1941 (in Rysavy, 1969); Petrov and Borovkova, 1942; Enigk, 1950). *C. putorii* may be transmitted directly by ingestion of larvated eggs or indirectly by ingestion of oligochaetes containing larvae (Skarbilovich, 1945).

Significant differences in prevalence and intensity of *C. plica* and *C. aerophila* and the age of red foxes were not found in our study. However, differences between juveniles and adults in intensity of *C. plica* and *C. aerophila* have been reported by Watkins and Harvey (1942) who found juvenile foxes with a higher intensity, of both *C. aerophila* and *C. plica,* than adult foxes. This difference may relate to the higher frequency of occurrence of earthworms in the diet of juvenile red foxes compared to adults (Burrows, 1968; Jefferies, 1974; Richards, 1977). The reasons for a lower intensity of *C. aerophila* in female foxes than in males is unknown. Any behavioral difference would also be expected to affect the intensity of *C. plica* as well, unless dispersion of the species, one in the urine and the other in the feces is a major factor.

The significant difference in prevalence of *C. procyonis* in male and female raccoons is difficult to explain because the life cycle is unknown. Female raccoons are reported to have a smaller home range than male raccoons (Cowan, 1973; Fritzell, 1978). However, if home range size was important in determining prevalence of *C. procyonis* one might expect it to be important for all three *Capillaria* species.

In an environment with a seasonal climatic variation there will often be fluctuations in resource availability. These fluctuations have the potential of altering transmission patterns between hosts and their parasites. Oligochaetes (lumbricids) are reported to show differences in activity and population numbers related to seasonal climatic patterns (Edwards and Lofty, 1972). Both activity and population numbers increase as soil temperature and moisture increase (Gerard, 1967; Edwards and Lofty, 1972; Nordström, 1975) and, according to Gerard (1967) most oligochaetes reach a peak in activity and numbers in summer and fall unless conditions are dry. In addition, the daily activity pattern of oligochaetes is affected by light, temperature, and moisture: during periods of low light intensity (night), rainfall or heavy dew and soil temperatures of greater than 5°C, surface activity increases (Svendsen, 1957; Gerard, 1967). The variations of intensity of *C. plica* and *C. putorii* in raccoons in bimonthly periods probably reflect seasonal changes in the activity of both oligochaetes and raccoons.

Raccoons mate from January to April, with maximum mating activity from mid-January to mid-March. The gestation period lasts approximately 63 days and the young remain in the den for approximately 2 mo (Johnson, 1970; Sanderson and Nalbandov, 1973). Raccoons enter dens and movement is minimal during winter when temperatures fall below 0°C (Steuwer, 1943; Cowan, 1973). In the present study young raccoons were first collected during July 1974. The lowest

intensity of *C. putorii* was during the winter months (January to April) and the highest was during the spring (May–June) and fall (September–December). The increase in activity of raccoons in spring after winter denning appears to correspond with the spring increase of *C. putorii*. The slight decrease in intensity of *C. putorii* in summer (July–August) was associated with the presence of juvenile raccoons in the population with a significantly lower intensity. Most young raccoons leave the den and are weaned in July (Schneider et al., 1971; Cowan, 1973).

The intensity of *C. plica* did not decrease during the summer as did *C. putorii*. Juvenile raccoons examined in summer were uninfected with *C. plica* suggesting there was no transmission in the den. The presence of *C. putorii* in some juveniles during summer and not *C. plica* is possibly related to the shorter prepatent period (26–32 days) of *C. putorii* (Skarbilovich, 1945) than that of *C. plica* (58–63 days) reported by Enigk (1950).

Intensities of *C. plica* and *C. putorii* were higher in the fall of 1974 than those observed in the fall of 1973. One explanation for this difference is that conditions were less dry during the summer and early fall of 1974 compared to the same periods in 1973 (in July, August, and September, 1973 there were 17 days of precipitation >0.01 mm while in 1974 there were 30) (Canadian Department of Environment, Atmospheric Environment, Monthly Records, Meteorological Observations in Canada. 1973, 1974). The greater precipitation in 1974 may have provided increased opportunity for raccoons to feed on oligochaetes as seven raccoons, taken during the period July to September 1974, had oligochaete remains in their stomachs and two of these contained over 100 earthworms. In contrast, oligochaetes were not found in the stomach contents of raccoons during any other period.

Intensity of *C. procyonis* did not vary seasonally. Several explanations are possible: the host is being continually reinfected, the longevity of the worms is such that overlapping generations may mask seasonal fluctuations, the larval stages may reflect seasonal changes in transmission which were not detected, or a density-dependent mechanism may be operating to limit numbers.

Frequency distributions of all species were overdispersed (variance/mean > 1). An overdispersed distribution indicates an unequal chance of infection for all hosts, but whether this is a result of an aggregated distribution of intermediate stages or hosts, or variance in host susceptibility is unknown.

Caution must be exercised when methods or interpretations of association analysis are chosen. Good reviews of the methods and possible interpretations are presented by Fager (1957) and Goodall (1978). Association analyses (chi-square, index of affinity, point correlation) based on presence or absence only, indicated a positive association between all three species during periods of high intensity. The lumping of individuals in joint occurrences (Whittaker and Fairbanks' (1958) equation) also indicated a positive association. However, rank correlation indicated only *C. putorii* and *C. plica* varied in concert. Lumping of data over the entire period masked the difference in association between species in periods of high and low intensity. Analysis based on presence or absence of a species may simply reflect the utilization of the host involved, by the parasite, and not reflect any relationship between *Capillaria* species. The positive association found between *C. plica, C. putorii,* and *C. procyonis* during periods of high intensity may simply reflect the importance of this period in the transmission of the parasite to

the definitive host. The positive association between the number of worms of *C. plica* and *C. putorii* may reflect the utilization of oligochaetes by both species and a similar seasonal transmission pattern.

Acknowledgments

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Literature Cited

Ables, E. O. 1975. Ecology of the red fox in North America. Pages 216–236 *in* M. W. Fox, ed. The Wild Canids. Their Systematics, Behavioural Ecology and Evolution. Van Nostrand Reinhold Co., New York, Toronto. 508 pp.

Aliev, F. F., and G. C. Sanderson. 1966. Distribution and status of the raccoon in the Soviet Union. J. Wildl. Manage. 30:497–502.

Burrows, R. 1968. Wild Fox. Talinger Publ. Co., New York.

Butterworth, E. W., and M. Beverly-Burton. 1980. The taxonomy of *Capillaria* spp. (Nematoda: Trichuroidea) in carnivorous mammals from Ontario, Canada. Systematic Parasitology. In press.

Cowan, W. 1973. Ecology and life history of the raccoon (*Procyon lotor*) (Nelson and Goldman) in the northern part of its range. Ph.D. Thesis, University of North Dakota, Grand Forks, North Dakota. 161 pp.

Dorney, R. S., and L. H. Lauerman, Jr. 1968. A helminthological survey of wild mink in Wisconsin. Bull. Wildl. Dis. Assoc. 5:35–36.

Edwards, C. A., and J. R. Lofty. 1972. Biology of Earthworms. Chapman and Hall, London. 283 pp.

Enigk, K. 1950. Die Biologie von *Capillaria plica* (Trichuroidea, Nematodes). Z. Tropenmed. Parasitol. 1:560–571.

Fager, E. W. 1957. Determination and analysis of recurrent groups. Ecology 38:586–595.

Fager, E. W., and J. A. McGowan. 1963. Zooplankton species groups in the North Pacific. Science 140:453–460.

Fahmy, M. A. M. 1964. Some parasitic worms from insectivores. J. Vet. Sci. U.A.R. 1:75–97.

Fritzell, E. K. 1978. Aspects of raccoon (*Procyon lotor*) social organization. Can. J. Zool. 56:260–271.

Gerard, B. C. 1967. Factors affecting earthworms in pastures. J. Anim. Ecol. 36:235–252.

Goodall, D. W. 1978. Sample similarity and species correlation. Pages 99–149 *in* R. H. Whittaker, ed. Ordination of Plant Communities. Dr. W. Junk, The Hague. 388 pp.

Guenther, W. C. 1964. Analysis of Variance. Prentice-Hall Inc., Englewood Cliffs. 199 pp.

Harkema, R. and G. C. Miller. 1964. Helminth parasites of the raccoon, *Procyon lotor* in the Southeastern United States. J. Parasitol. 50:60–66.

Holmes, J. C. 1979. Parasite populations and host community structure. Pages 27–46 *in* B. B. Nickol, ed. *in* Host-Parasite Interfaces. Academic Press, New York.

Holmes, J. C., R. P. Hobbs, and T. S. Leong. 1977. Populations in perspective: Community organization and Regulation of Parasite Populations. Pages 209–245 *in* G. W. Esch, ed. Regulation of Parasite Populations. Academic Press, New York.

Holmes, J. C., and R. Podesta. 1968. The helminths of wolves and coyotes from the forested regions of Alberta. Can. J. Zool. 46:1193–1204.

Jefferies, D. J. 1974. Earthworms in the diet of red fox (*Vulpes vulpes*). J. Zool. London 173:251–252.

Johnson, A. S. 1970. Biology of the raccoon (*Procyon lotor varius*) Nelson and Goldman in Alabama. Agr. Exp. Stn. Auburn Univ. Bull. 402. 148 pp.

Law, R. G., and A. H. Kennedy. 1932. Parasites of fur-bearing animals. Ont. Dep. Game Fish Bull. 4. 30 pp.

Miller, G. C., and R. Harkema. 1964. Studies on helminths of North Carolina vertebrates. V. Parasites of the mink, *Mustela vison* Schreber. J. Parasitol. 50:717–720.

Miller, G. C., and R. Harkema. 1968. Helminths of some wild mammals in the Southeastern United States. Proc. Helminthol. Soc. Wash. 35:118–125.

Monson, R. A., W. B. Stone, and E. Parks. 1973. Aging red foxes (*Vulpes vulpes*) by counting annular cementum rings of their teeth. N.Y. Fish Game J. 20:54–61.

Nordström, S. 1975. Seasonal activity of lumbricids in Southern Sweden. Oikos 26:307–315.

Pence, D. B. 1975. *Capillaria procyonis* sp. nov. (Nematoda: Trichuroidea) from the esophagus of the raccoon, *Procyon lotor*. J. Parasitol. 61:815–818.

Petrov, A. M., and A. M. Borovkova. 1942. Contribution to the study of the cycle of development of *Capillaria plica* (Rudolphi, 1819), a nematode of the bladder of dog and fox. C.R. (Dokl.) Acad. Sci. USSR. 35:159–160.

Poole, R. W. 1974. An Introduction to Quantitative Ecology. McGraw-Hill, Inc., New York. 532 pp.

Read, C. P. 1949. Studies on North American helminths of the genus *Capillaria* Zeder, 1800 (Nematoda): I. Capillarids from mammals. II. Additional Capillarids from mammals with keys to the North American species. III. Capillarids from the lower digestive tract of North American birds. J. Parasitol. 35:223–249.

Richards, D. F. 1977. Observations on the diet of the Red Fox (*Vulpes vulpes*) in South Devon. J. Zool. London 183:495–504.

Rysavy, B. 1969. Lumbricidae—an important parasitological factor in helminthoses of domestic and wild animals. Pedobiologia 9:171–174.

Sanderson, G. C., and A. V. Nalbandov. 1973. The reproductive cycle of the raccoon in Illinois. Ill. Nat. Hist. Surv. Bull. 31:29–85.

Schneider, D. G., L. D. Mech, and J. R. Tester. 1971. Movements of female raccoons and their young as determined by radio-tracking. Anim. Behav. Monogr. 4:1–43.

Skarbilovich, T. S. 1945. Determination of two different types of development cycles in *Capillaria putorii* (Rud., 1819). Dokl. Akad. Nauk USSR 50:553–554. (In Russian.)

Skrjabin, K. I., N. P. Shikhobalova, and I. V. Orlov. 1957. Trichocephalidae and Capillariidae of animals and man and the diseases caused by them. *In* K. I. Skrjabin, ed. Essentials of Nematodology. Vol. VI. Akad. Nauk SSSR, Moscow. Israel Program for Scientific Translations Ltd., Jerusalem, 1970.

Smith, H. J. 1978. Parasites of red foxes in New Brunswick and Nova Scotia. J. Wildl. Dis. 14:366–370.

Southwood, T. R. E. 1966. Ecological Methods. Methuen & Co. Ltd., London, 391 pp.

Steuwer, F. W. 1943. Raccoons: Their habits and management in Michigan. Ecol. Monogr. 13:204–257.

Swales, W. E. 1933. A review of Canadian helminthology I and II. Can. J. Res. 8:468–482.

Svendsen, J. A. 1957. The behaviour of lumbricids under moorland conditions. J. Anim. Ecol. 26:423–439.

Watkins, C. V., and L. A. Harvey. 1942. On the parasites of silver foxes on some farms in the southwest. Parasitology 34:155–179.

Whittaker, R. H., and C. W. Fairbanks. 1958. A study of plankton copepod communities in the Columbia basin, southeastern Washington. Ecology 39:46–65.

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Two New Tetraphyllidean Cestodes from *Potamotrygon circularis* Garman (Chondrichthyes: Potamotrygonidae) in the Itacuaí River, Brazil[1,2]

MONTE A. MAYES,[3] DANIEL R. BROOKS,[4] AND THOMAS B. THORSON[5]

[3] Environmental Sciences Research, Dow Chemical USA, Midland, Michigan 48640

[4] Department of Zoology, University of British Columbia, Vancouver, British Columbia V6T 1W5, Canada, and

[5] School of Life Sciences, University of Nebraska–Lincoln, Lincoln, Nebraska 68588

ABSTRACT: The new genus *Rhinebothroides* is proposed to include three species of *Rhinebothrium*-like cestodes parasitizing freshwater stingrays in South America. They differ from *Rhinebothrium* species by having squared rather than elongate bothridia, by possessing internal seminal vesicles, and by exhibiting terminal genitalia at the ovarian level. *Rhinebothroides circularisi* sp. n. in *Potamotrygon circularis* from the Itacuaí River in northwestern Brazil differs from *R. moralarai* by having more testes and bothridial loculi and differs from *R. scorzai* by lacking vitelline follicles proximate to the genital pore, by having a straight rather than coiled vagina, and by possessing craspedote rather than acraspedote proglottids. *Potamotrygon circularis* from the Itacuai River also hosted *Potamotrygonocestus amazonensis* sp. n. which differs from the only other member of the genus, *P. magdalenensis*, by having bothridial hooks 58–78 μm long rather than 43–55 μm long, by possessing a shallow genital atrium rather than lacking one, and by having follicular rather than compact vitellaria.

Four reports (Lopez-Neyra and Diaz-Ungria, 1958; Brooks and Thorson, 1976; Rego and Dias, 1976; Mayes et al., 1978) list a total of seven species of tetraphyllidean cestodes parasitizing freshwater stingrays in South America. We collected specimens of two new species from the stingray *Potamotrygon circularis* Garman occurring in the Itacuaí River in northwestern Brazil. Worms were removed from their hosts' spiral valves and examined alive before fixing in hot AFA or were fixed in situ with 10% formalin; all were then stored in 70% ethanol. Some specimens were stained with Mayer's hematoxylin and mounted in Histoclad (commercial mounting medium produced by Clay Adams) or Canada balsam for study as whole mounts. Others were cut in serial cross sections at 8 μm and stained with hematoxylin-eosin for confirmation of some aspects of proglottid morphology. Measurements are in micrometers unless otherwise stated; figures were drawn with the aid of a drawing tube.

Three species of tetraphyllidean cestodes, including a new species described herein, resemble members of *Rhinebothrium*-like genera (*Rhinebothrium, Caulobothrium, Rhabdotobothrium*) but differ by possessing squared or quadrate rather than elongate bothridia and an internal seminal vesicle (see Brooks and Thorson, 1976, also Fig. 4 of this paper). They further differ by exhibiting reduced poral ovarian lobes and terminal genitalia at the ovarian level. Because of the above consistent morphological differences and the species' present restriction to freshwater stingrays, we propose the following new genus to accommodate them.

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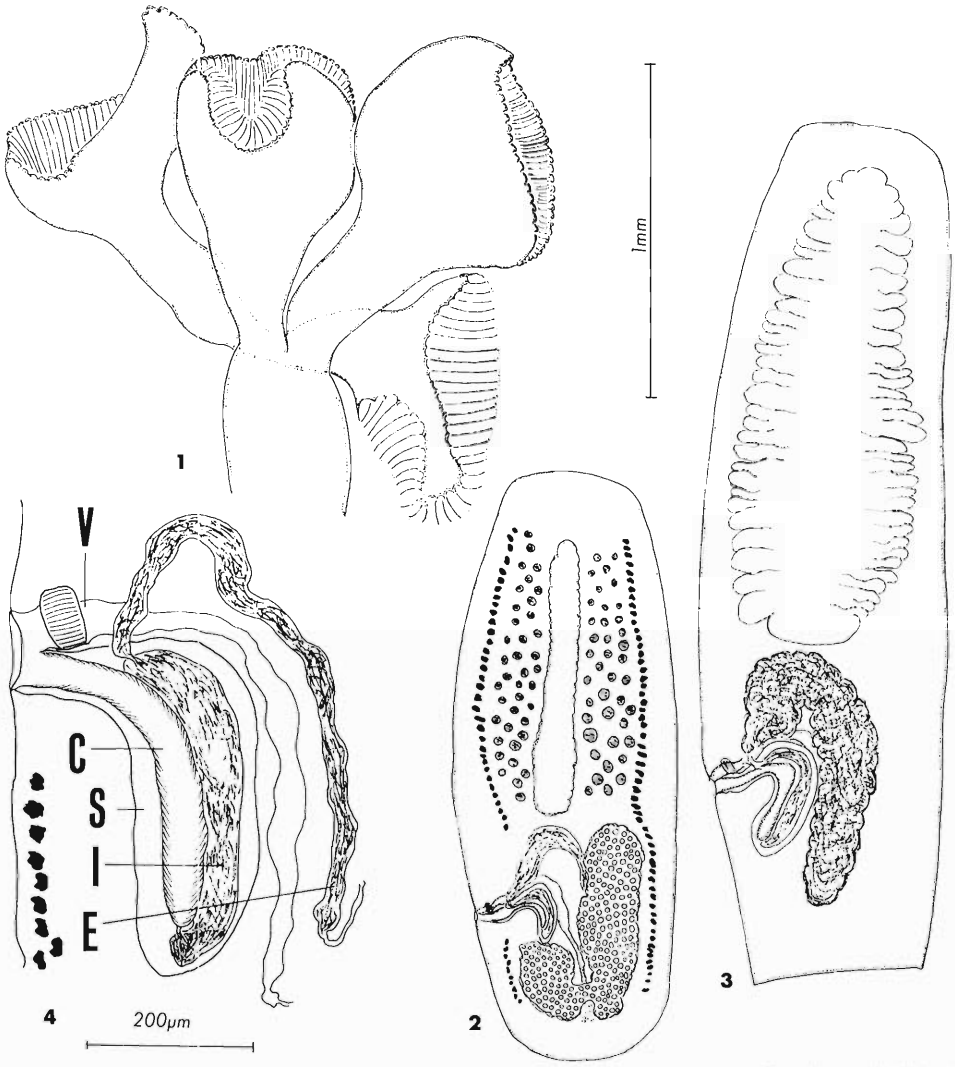
[2] Partial funding for the preparation of this manuscript was provided through NIH grant 5-T32-AI-07030-04 to DRB.



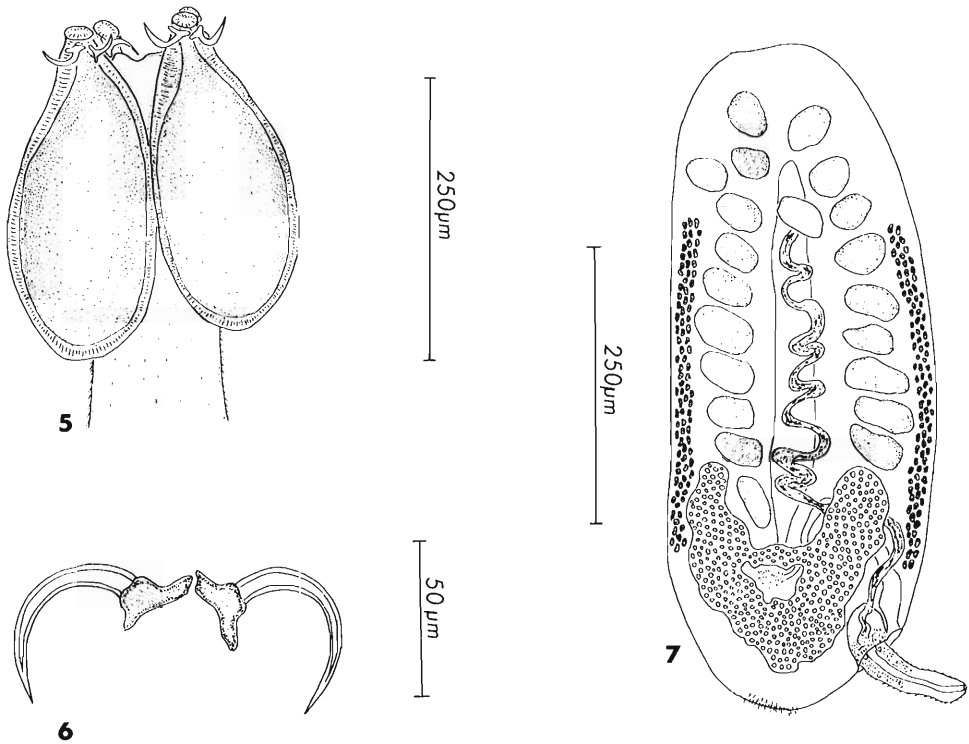
Figures 1–4. ***Rhinebothroides circularisi*****. 1. Scolex. 2. Mature proglottid. 3. Gravid proglottid. 4. Terminal genitalia. Abbreviations: V = vagina; C = cirrus; S = cirrus sac; I = internal seminal vesicle; E = external seminal vesicle.**

Rhinebothroides gen. n.

DIAGNOSIS: Tetraphyllidea, Phyllobothriidae. Scolex with 4 pedicellated quadrate bothridia; bothridia with shallow horizontal loculi. Ovary bilobed with greatly reduced poral lobe in frontal view; X-shaped in cross section. Testes pre-ovarian. Cirrus sac at ovarian level, containing spined eversible cirrus and internal seminal vesicle. Uterus with lateral diverticula. Parasites of freshwater stingrays (family Potamotrygonidae). South America.

TYPE SPECIES: *Rhinebothroides moralarai* (Brooks and Thorson, 1976) comb. n. (synonym *Rhinebothrium moralarai* Brooks and Thorson, 1976).

OTHER SPECIES: *R. scorzai* (Lopez-Neyra and Diaz-Ungria, 1958) comb. n.



Figures 5–7. ***Potamotrygonocestus amazonensis*****. 5. Scolex. 6. Bothridial hooks. 7. Mature proglottid. Note tegumental spines at posterior end.**

(synonym *Rhinebothrium scorzai* Lopez-Neyra and Diaz-Ungria, 1958), *R. circularisi* sp. n.

Rhinebothroides circularisi sp. n.
(Figs. 1–4)

DESCRIPTION (based on 10 specimens): Strobila acraspedote, apolytic, up to 27 mm long, composed of 18–24 proglottids (n = 10). Scolex 912–1,490 wide. Cephalic peduncle contractile, up to 360 long. Bothridia 675–1,000 long by 400–530 wide, divided longitudinally by indistinct median septum and transversely by 33–38 septa forming 2 parallel rows of 34–39 loculi plus terminal loculus at tip of each lobe; total number of loculi 70–80. Immature proglottids longer than wide; mature ones 1,000–1,440 long by 620–960 wide. Testes in 2 broad fields in anterior ⅔ of proglottid, 66–88 ($\bar{x}$ = 78, n = 25) in number, 46–74 long by 25–80 wide. External seminal vesicle extending entire length of cirrus sac, joining cirrus sac near poral end and vas deferens near posterior end of ovary. Genital atrium shallow; genital pores alternating irregularly, 28–39% of proglottid length from posterior end. Vagina anterior to cirrus sac; vaginal sphincter present; posterior portion dilated to form seminal receptacle. Ovary with aporal lobe 360–540 long, not extending into anterior ½ of proglottid; poral lobe shorter, extending to posterior margin of cirrus sac. Ovarian isthmus posteromedian to cirrus sac, 300–400 wide. Vitelline follicles lateral, extending from level of ovarian isthmus to near

anterior end of proglottid, interrupted near genital pore on poral side, 12–30 in diameter. Gravid proglottids 2,160–3,756 long by 786–1,320 wide, devoid of testes. Uterus saccate with 49–80 ($\bar{x}$ = 70, n = 15) lateral diverticula. Eggs 18–29 in diameter in utero, oncospheres 11–18 in diameter, unembryonated.

HOST: *Potamotrygon circularis* Garman.

SITE OF INFECTION: Middle ⅓ of spiral valve.

LOCALITY: Itacuaí River, 5 km south Atalaia do Norte, Brazil.

HOLOTYPE: USNM Helm. Coll. 76361.

PARATYPES: USNM Helm. Coll. No. 76362; University of Nebraska State Museum, Manter Laboratory No. 21020.

ETYMOLOGY: The species is named after its host species.

Rhinebothroides circularisi resembles *R. moralarai* by possessing a straight vagina and acraspedote proglottids, and by exhibiting vitelline follicles interrupted on the poral side of the proglottid in the area of the genital pore. The new species differs from *R. moralarai* by having more testes (an average of 78 rather than 63) and more bothridial loculi (70–80 rather than 46–48). *Rhinebothroides circularisi* resembles *R. scorzai* in number of testes and in bothridial loculi number, but examination of specimens of the latter species (Brooks, Mayes, and Thorson, unpublished) show that *R. scorzai* possesses craspedote proglottids and a coiled vagina, and exhibits no vitelline interruption.

Potamotrygonocestus amazonensis sp. n.
(Figs. 5–7)

DESCRIPTION (based on 5 specimens): Strobila acraspedote, hyperapolytic, 1,254–3,534 long, composed of 10–13 proglottids. Scolex 240–300 long by 240–300 wide, comprising 4 sessile nonseptate bothridia each with apical sucker and pair of simple hooks. Scolex and neck spinose, strobila sparsely spinose (Fig. 7), spines 5–15 long. Bothridia 330–408 long by 135–210 wide. Accessory suckers 38–55 in diameter. Hook prongs 58–78 long ($\bar{x}$ = 66, n = 12), bases 26–35 long ($\bar{x}$ = 30, n = 12). Neck 115–200 long. Immature proglottids initially wider than long, becoming longer than wide. Mature attached proglottids 420–690 long by 270–330 wide. Testes in 2 longitudinal rows in anterior ⅔ of proglottid, 21–24 ($\bar{x}$ = 23, n = 10) in number, 29–50 in diameter. Cirrus sac near posterior end of proglottid, 73–93 long by 44–50 wide, containing spined eversible cirrus. Everted cirrus in one proglottid 52 long by 20 wide. Genital atrium shallow; genital pore dextral or sinistral. Vagina anterior to cirrus sac with dorsal tegumental membrane. Ovary in posterior ⅓ of proglottid, 132–240 long by 102–162 wide at isthmus; ovarian lobes fused posteriorly. Vitellaria follicular; follicles in 2 lateral longitudinal rows extending from level of ovarian isthmus to within 32–38% of total proglottid length from anterior end; follicles 29–37 in diameter. Gravid proglottids not collected.

HOST: *Potamotrygon circularis* Garman.

SITE OF INFECTION: anteriormost chamber of spiral valve.

LOCALITY: Itacuaí River, 5 km south Atalaia do Norte, Brazil.

HOLOTYPE: USNM Helm. Coll. No. 76363.

PARATYPES: University of Nebraska State Museum, Manter Laboratory No. 21019.

ETYMOLOGY: The species is named for the major river system drainage in which it occurs.

Potamotrygonocestus amazonensis differs from *P. magdalenensis* Brooks and Thorson, 1976, the only other known species, by having a shallow genital atrium rather than lacking one, by exhibiting follicular rather than compact vitellaria, and by having bothridial hooks 58–78 μm long rather than 43–55 μm long.

Literature Cited

Brooks, D. R., and T. B. Thorson. 1976. Two tetraphyllidean cestodes from the freshwater stingray *Potamotrygon magdalenae* Dumeril, 1852 (Chrondrichthyes: Potamotrygonidae) from Colombia. J. Parasitol. 62:943–947.

Lopez-Neyra, C. R., and C. Diaz-Ungria. 1958. Cestodes de Venezuela. V. Cestodes de vertebrados Venezolanos (segunda nota). Nova Cienc. 23:1–41.

Mayes, M. A., D. R. Brooks, and T. B. Thorson. 1978. Two new species of *Acanthobothrium* Van Beneden, 1849 (Cestoidea: Tetraphyllidea) from freshwater stingrays in South America. J. Parasitol. 64:838–841.

Rego, A. A., and A. P. L. Dias. 1976. Estudos de cestoides de peixes do Brasil. 3a nota: cestoides de raias fluviais paratrygonidae. Rev. Bras. Biol. 36:941–956.

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The Editor

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Systematic Review of Cestodes Infecting Freshwater Stingrays (Chondrichthyes: Potamotrygonidae) Including Four New Species from Venezuela

DANIEL R. BROOKS,[1,2] MONTE A. MAYES,[3] AND THOMAS B. THORSON[4]

[2] Department of Pathology, National Zoological Park, Smithsonian Institution, Washington, D.C. 20008

[3] Environmental Sciences Research, The Dow Chemical Company, Midland, Michigan 48640, and

[4] School of Life Sciences, University of Nebraska–Lincoln, Lincoln, Nebraska 68588

ABSTRACT: Cestode parasites were collected in freshwater stingrays from localities in Colombia, Venezuela, Brazil, and Paraguay. Four new species are described from Venezuela. *Potamotrygonocestus orinocoensis* sp. n. from *Potamotrygon reticulatus* in the delta of the Orinoco River resembles *P. magdalenensis* by having compact rather than follicular vitellaria, but differs by having larger and differently shaped bothridial hooks. *Acanthobothrium regoi* sp. n. from *P. hystrix* in the delta of the Orinoco River most closely resembles *A. quinonesi*, but differs by having larger bothridial hooks. *Rhinebothroides glandularis* sp. n. from *Potamotrygon hystrix* in the delta of the Orinoco River most closely resembles *R. scorzai* by having a coiled vagina and poral ovarian lobes which extend anterior to the posterior margin of the cirrus sac, but differs in numbers of bothridial loculi and testes, and by exhibiting prominent parenchymal gland cells surrounding the terminal male genitalia. *Rhinebothroides venezuelensis* sp. n. from *Potamotrygon hystrix* in the delta of the Orinoco River and *P. yepezi* from the Río Cachirí, Zulia, Venezuela, resembles *R. moralarai* and *R. circularisi* by having a straight vagina and poral ovarian lobes not extending anteriorly beyond the posterior margin of the cirrus sac, but differs in testes number and number of bothridial loculi.

Eutetrarhynchus araya was collected in the new hosts *Potamotrygon reticulatus* and *P. falkneri* from the delta of the Orinoco River and the new locality of the Paraná River near Hohenau, Paraguay, respectively. *Eutetrarhynchus baeri* is a junior synonym of *E. araya*. Phylogenetic analysis suggests that *E. araya* is a member of a monophyletic group of species, including *E. thalassius, E. caribbensis, E. schmidti,* and *E. geraschmidti.*

Potamotrygonocestus represents a monophyletic group of three known species of uncertain affinities with other tetraphyllidean cestodes. *Potamotrygonocestus amazonensis* was collected in the new host *Potamotrygon yepezi* from the Río Cachirí, Zulia, Venezuela, a new locality. Phylogenetic analysis of the three species of *Potamotrygonocestus* suggests that *P. amazonensis* represents the plesiomorphic sister-species of the sister-species *P. magdalenensis* and *P. orinocoensis. Potomotrygonocestus travassosi* is considered a *species inquirenda.*

The four species of *Acanthobothrium* infecting freshwater stingrays form a monophyletic group most closely related to *A. holorhini, A. cartagenensis,* and *A. urolophi. Acanthobothrium quinonesi* is reported in the new host *Potamotrygon yepezi* from the new locality Río Cachirí, Zulia, Venezuela.

Rhinebothrium paratrygoni was collected in the new hosts *Potamotrygon hystrix* and *P. reticulatus* from the new locality of the delta of the Orinoco River and in *P. falkneri,* another new host, from the Paraná River, near Hohenau, Paraguay, a new locality. *Rhinebothrium paratrygoni* belongs to a monophyletic group containing *R. urobatidium, R. ditesticulum, R. spinicephalum, R. tetralobatum,* and a new species being described elsewhere.

Rhinebothroides represents a monophyletic group of six known species most closely related to *Phyllobothrium kingae* and its relatives. *Rhinebothroides moralarai, R. circularisi,* and *R. venezuelensis* form one monophyletic group within the genus and *R. glandularis, R. freitasi,* and *R. scorzai* another. *Rhinebothroides scorzai* is reported from the new hosts *Potamotrygon reticulatus* and *Elipesurus spinicauda* from various localities in the delta of the Orinoco River.

The cestode fauna of potamotrygonids comprises elements with closest relationships to species infecting marine elasmobranchs and not freshwater fishes.

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[1] Present address: Department of Zoology, University of British Columbia, 2075 Westbrook Mall, Vancouver, British Columbia V6T 1W5, Canada.

Figure 1. Map showing localities from which helminth parasites infecting potamotrygonid stingrays have been reported. Closed circles represent localities reported by the present authors, open circles localities reported by other authors.

Prior to 1976, two reports listed a total of three cestode species infecting freshwater stingrays (family Potamotrygonidae) (Woodland, 1934; López-Neyra and Diaz-Ungriá, 1958). Subsequently, four reports (Brooks and Thorson, 1976; Rego and Dias, 1976; Mayes et al., 1978; Rego, 1979; Mayes et al., 1981) have added an additional 10 species. This study presents descriptions of four new species collected in Venezuela and reviews the systematic status of the 14 presently known cestode species infecting potamotrygonids. Figure 1 shows localities from which potamotrygonid parasites have been reported.

Hosts were collected by seine, gig, or dipnet and dissected within 2 hr if taken alive, or immediately if collected dead. Worms were removed from host spiral valves, examined alive, then fixed with warm AFA and stored in 70% ethanol. Spiral valves were fixed with 10% formalin to preserve any overlooked helminths. Worms were stained with Delafield's or Mayer's hematoxylin and mounted in Canada balsam for study as whole mounts; serial cross sections of some speci-

mens cut at 8 μm and stained with hematoxylin-eosin were used to confirm some aspects of proglottid morphology. Measurements are in micrometers unless otherwise stated; figures were drawn with the aid of a drawing tube. The abbreviation USNM Helm. Coll. refers to the U.S. National Museum Helminthological Collection, Beltsville, Maryland; UNSM refers to the University of Nebraska State Museum, Lincoln, Nebraska.

Trypanorhyncha Diesing, 1863
Eutetrarhynchidae Guiart, 1927
Eutetrarhynchus Pintner, 1913
Eutetrarhynchus araya (Woodland, 1934) Rego and Dias, 1976

Tentacularia araya Woodland, 1934.
Eutetrarhynchus baeri López-Neyra and Diaz-Ungriá, 1958.
Eutetrarhynchus araya: Rego and Dias, 1976.

HOSTS: *Trygon* sp. (=*Potamotrygon* sp. or *Elipesurus* sp.); *Potamotrygon hystrix* (Müller and Troschel);[2] *P. motoro* (Müller and Henle); *P. reticulatus* (Gunther), new host;[2] *P. falkneri,* new host.

LOCALITIES: Itacuaí River, Brazil; delta of Orinoco River, Venezuela; Rio Salobra, Mato Grosso, Brazil; delta of Orinoco River, Venezuela, near El Toro and at km 82 of main channel, new localities; Paraná River, Paraguay, near Hohenau, new locality.

SITE OF INFECTION: Middle ⅓ of spiral valve.

SPECIMENS EXAMINED: UNSM No. 21011.

Rego and Dias (1976) described trypanorhynch specimens collected in *Potamotrygon motoro* from the Rio Salobra, Mato Grosso, Brazil. They concluded that their specimens, those described by Woodland (1934) from "*Trygon* sp." ("brown specimen speckled with black" 45 cm in disc length) in the Amazon River, and *Eutetrarhynchus baeri* López-Neyra and Diaz-Ungriá, 1958, from *P. hystrix* in the delta of the Orinoco River were conspecific. Based on our collections from the Orinoco and from the Paraná, we concur with Rego and Dias (1976). The tentacular armature of our specimens is identical to that reported by Rego and Dias and different from that reported by Woodland or López-Neyra and Diaz-Ungriá, who gave only cursory sketches. Further, our specimens possess 300–400 testes per proglottid but they lie in two layers in the parenchyma; Woodland (1934) noted that the "over 200" testes in his specimens also lay in two layers. López-Neyra and Diaz-Ungriá reported 150–200 testes per proglottid for *E. baeri,* but they may have interpreted their specimens as having cylindrical

[2] Our identifications of *P. hystrix* and *P. reticulatus* must be considered tentative pending a taxonomic revision of the potamotrygonids. According to Dr. Reeve Bailey, University of Michigan (personal communication), those specimens we identify as *P. hystrix* may actually be *P. humboldti,* if *P. hystrix* (*s.s.*) is found to be endemic to the Río de la Plata. Further, our specimens identified as *P. reticulatus* may represent a single species which has been called *P. reticulatus, P. brumi,* or *P. brachyurus*. Rego and Dias (1976) listed *Elipesurus* sp. as one host. *Elipesurus* has been used to refer to members of *Potamotrygon* as well as to *Elipesurus spinicauda*. The latter species, one specimen of which we examined in Venezuela, is apparently identical with *Disceus thayeri*. Specimens of hosts we examined have been deposited in the U.S. National Museum of Natural History, Washington, D.C., for future reference.

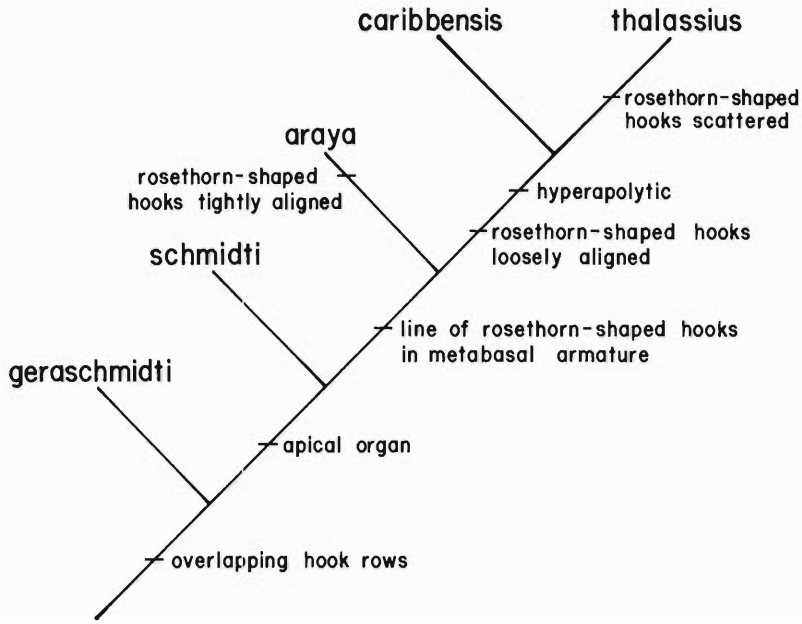


Figure 2. Cladogram depicting phylogenetic relationships of *Eutetrarhynchus araya* and its closest relatives. See text for explanation.

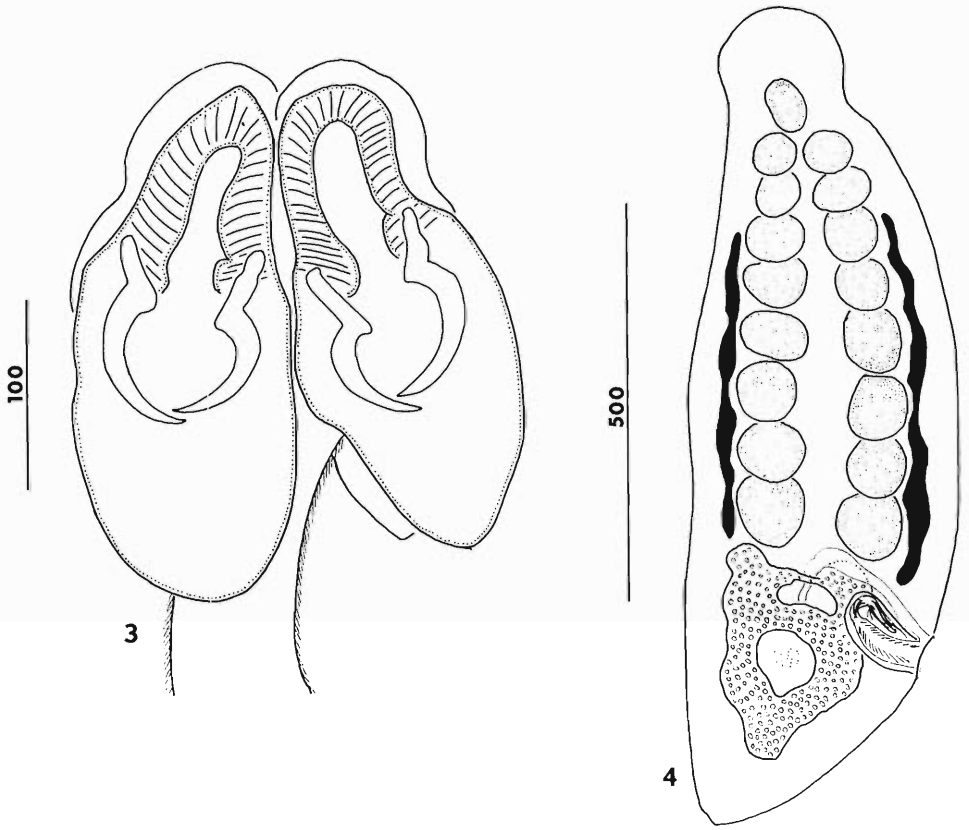
testes, as many trypanorhynchs do, and miscalculated the true number of testes present.

Eutetrarhynchus araya most closely resembles *E. thalassius* Kovacs and Schmidt, 1980, and *E. caribbensis* Kovacs and Schmidt, 1980, which infect *Urolophus jamaicensis* Cuvier in Jamaica. The above species possess rosethorn-shaped hooks in the metabasal armature, a trait no other species in *Eutetrarhynchus* exhibit. Those of *E. araya* are positioned in an overlapping longitudinal row of eight hooks, whereas in the other species the rosethorn-shaped hooks are either nonoverlapping (*E. caribbensis*) or scattered (*E. thalassius*).

Only two other members of *Eutetrarhynchus* exhibit overlapping hook rows and similar holdfast proportions—*E. schmidti* Heinz and Dailey, 1974, and *E. geraschmidti* Dollfus, 1974. The former species exhibits a glandular apical organ found in *E. araya, E. thalassius,* and *E. caribbensis* but lacking in *E. geraschmidti* or other members of the genus. Figure 2 depicts the distribution of the above special traits among the five species by means of a cladogram (cf. Hennig, 1966).

Tetraphyllidea Carus, 1863
Onchobothriidae Braun, 1900
Potamotrygonocestus Brooks and Thorson, 1976
Potamotrygonocestus orinocoensis sp. n.
(Figs. 3–4)

DESCRIPTION (based on 11 specimens): Strobila hyperapolytic, acraspedote, 1.3–1.6 mm long, composed of 5–10 proglottids. Scolex 153–230 long by 204–220 wide, composed of 4 simple bothridia each armed with pair of simple hooks and



Figures 3, 4. ***Potamotrygonocestus orinocoensis.*** **3. Scolex. 4. Mature detached proglottid.**

surmounted by apical portion of bothridium modified as accessory suckerlike structure 51–61 in diameter. Bothridia 240–265 long by 71–107 wide. Hooks dissimilar in shape (Fig. 3); outer hook base 30–50 ($\bar{x}$ = 42, n = 17) long, prong 73–108 ($\bar{x}$ = 92, n = 17) long; inner hook base 25–48 ($\bar{x}$ = 37, n = 17) long, prong 80–125 ($\bar{x}$ = 104, n = 17) long. Scolex and extremely short neck spinose; spines up to 8 long. Detached mature proglottids 714–1,020 long by 235–296 wide. Testes in 2 single file lines in anterior ¾ of proglottid, 8–14 ($\bar{x}$ = 10, n = 15) porally, 9–14 ($\bar{x}$ = 11, n = 15) aporally, 17–25 ($\bar{x}$ = 21, n = 15) in number; 41–66 in diameter. Cirrus sac lateral to ovary, 77–153 long by 31–46 wide, containing spined eversible cirrus up to 107 long. Ovary in posterior ¼ of proglottid, theta (θ) shaped, 214–311 long by 112–143 wide at isthmus. Vagina anterior to cirrus sac, vaginal sphincter lacking. Genital pore 68–77% of proglottid length from anterior end. Vitellaria composed of 2 lateral compact lines extending from 20–28% of proglottid length from anterior end to anterior margin of ovary, 32–41% of proglottid length from posterior end. Gravid proglottids not collected.

HOST: *Potamotrygon reticulatus.*

SITE OF INFECTION: Anteriormost chamber of spiral valve.

LOCALITIES: Delta of Orinoco River, Venezuela, near El Toro (type) and at km 82 of main channel.

HOLOTYPE: USNM Helm. Coll. No. 75713.

PARATYPES: USNM Helm. Coll. No. 75714; UNSM No. 21008, 21009.

ETYMOLOGY: The species is named for the Orinoco River, in which it was collected.

Potamotrygonocestus orinocoensis differs from the other two members of the genus by having hook prongs joining their bases at one end rather than in the middle. The new species' hooks are also larger than those of *P. amazonensis* Mayes, Brooks, and Thorson, 1981 (58–78 μm long) or *P. magdalenensis* Brooks and Thorson, 1976 (43–45 μm long). Its ovarian shape is unique. *Potamotrygonocestus orinocoensis* most closely resembles *P. magdalenensis* by having compact rather than follicular vitellaria and by lacking a genital atrium.

Potamotrygonocestus magdalenensis Brooks and Thorson, 1976

HOST: *Potamotrygon magdalenae* Dumeril.

SITE OF INFECTION: Anteriormost chamber of spiral valve.

LOCALITY: Magdalena River and associated cienagas near San Cristóbal, Bolívar, Colombia.

SPECIMENS EXAMINED: USNM Helm. Coll. No. 73542 (holotype) and 73543 (paratype); UNSM No. 20254 (paratypes).

DIAGNOSTIC FEATURES: Bothridial hooks 43–55 long, bases 19–29 long; prongs joining bases near middle. Testes 20–29 in number. Ovarian shape an inverted "A." Genital pore postovarian. Vitellaria compact. Genital atrium lacking.

Potamotrygonocestus amazonensis Mayes, Brooks, and Thorson, 1981

HOSTS: *Potamotrygon circularis* Garman; *P. yepezi,* Castex and Castello, 1970, new host; *P. reticulatus,* new host.

SITE OF INFECTION: Anteriormost chamber of spiral valve.

LOCALITIES: Itacuaí River, Brazil, 5 km south Atalaia do Norte, Brazil (type); Represa de Tulé, Río Cachirí, Zulia, Venezuela, new locality; Orinoco River delta, Venezuela, near km 82 of main channel, new locality.

SPECIMENS EXAMINED: USNM Helm. Coll. No. 76363 (holotype); UNSM No. 21019 (paratypes).

DIAGNOSTIC FEATURES: Bothridial hooks 58–78 long, bases 26–35 long; prongs join base near middle. Testes 21–24 in number. Ovarian shape an inverted "A." Genital pore posterolateral to ovary. Vitellaria follicular. Genital atrium shallow.

Potamotrygonocestus travassosi Rego, 1979, *species inquirenda*

HOST: *Potamotrygon hystrix.*

SITE OF INFECTION: Spiral valve.

LOCALITY: Amazon River, Maicuru, Para, Brazil.

SPECIMENS EXAMINED: None.

Rego (1979) described this species on the basis of ". . . dois proglotes livres e un plerocercoide . . . ." Examination of Rego's Figure 6 suggests that the free proglottids belong to *Eutetrarhynchus araya*; Figures 4 and 5 clearly depict the scolex of a species of *Potamotrygonocestus*. However, Figure 4 illustrates bothridial hook morphology similar to that exhibited by *P. amazonensis,* whereas Figure 5 depicts a single hook appearing more similar to those of *P. orinocoensis*.

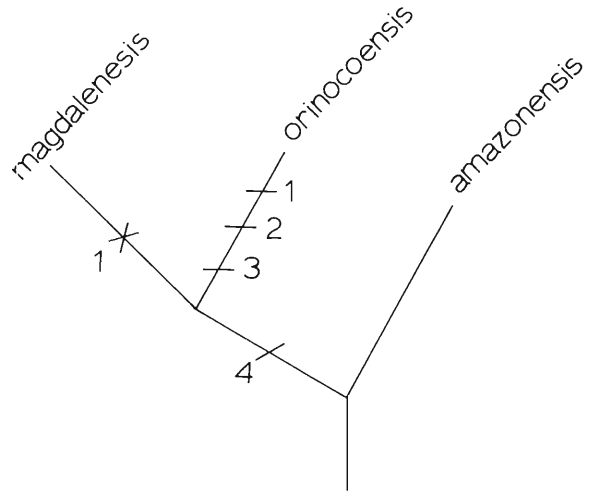


Figure 5. Cladogram depicting phylogenetic relationships of members of *Potamotrygonocestus*. For identities of characters denoted by numbers, see text.

We, therefore, consider *P. travassosi* a *species inquirenda* pending further collections of material from Maicuru.

Key to Species of *Potamotrygonocestus*

1a. Maximum bothridial hook length less than 80 μm, hook prongs join bases near middle 2
1b. Maximum bothridial hook length more than 100 μm, prongs join bases at end ..... *orinocoensis*
2a. Vitellaria compact, hooks 43–55 μm long ..... *magdalenensis*
2b. Vitellaria follicular, hooks 58–78 μm long ..... *amazonensis*

Phylogenetic Relationships

Potamotrygonocestus species exhibit perhaps the most simplified scolex morphology of any onchobothriid tetraphyllideans, possessing simple hooks and nonseptate bothridia. Members of *Potamotrygonocestus* are unusual because they exhibit posterolateral or postovarian genital pores and terminal genitalia. The three species of this monophyletic group may be related phylogenetically in one of four ways. The most parsimonious, based on the following four characters, is depicted in Figure 5.

1. Bothridial hook length. 0 = maximum bothridial hook length up to 78 μm; 1 = maximum hook length up to 125 μm; −1 = maximum hook length less than 60 μm.
2. Ovarian shape. 0 = inverted "A"; 1 = theta-shaped.
3. Hook prong attachment. 0 = prongs join bases near middle; 1 = prongs join bases at one end.
4. Vitelline configuration. 0 = vitellaria follicular; 1 = vitellaria compact.

The above characters support the phylogenetic hypothesis that *P. orinocoensis*

and *P. magdalenensis* are more closely related to each other than either is to any other species on the basis of relative recency of common ancestry.

Acanthobothrium Van Beneden, 1849
Acanthobothrium regoi sp. n.
(Figs. 6–8)

DESCRIPTION (based on 5 complete and 6 fragmented specimens): Strobila acraspedote, apolytic, up to 45 mm long, composed of 87–120 proglottids. Scolex 700–900 long by 800–1,100 wide, composed of 4 triloculate bothridia each armed with pair of bifid hooks and surmounted by apical sucker and pad. Posterior margins of bothridia attached to scolex; velum lacking. Bothridia 500–600 long by 300–350 wide; ratio of length to width 1:0.6–0.7 ($\bar{x}$ = 1:0.64, n = 20). Anterior loculus 200–220 long, middle loculus 80–100 long, posterior loculus 100–130 long; average ratio of locular lengths 1:0.45:0.55. Apical sucker 61–102 in diameter, pads 126–179 in diameter. Hook formula (modified from that of Euzet, 1956) for 30 hooks:

$$\frac{31\text{–}41\ (35)\quad 87\text{–}128\ (105)\quad 66\text{–}82\ (75)}{122\text{–}163\ (142)}$$

Cephalic peduncle up to 900 long, spinose; neck expanded at insertion to scolex. Immature proglottids wider than long. Mature proglottids 1,040–1,642 long by 337–510 wide. Testes in anterior ¾ of proglottid, 47–70 ($\bar{x}$ = 58, n = 41) in number, 5–11 (7) postporally, 10–28 (20) preporally, 23–40 (31) antiporally; up to 80 in diameter. Cirrus sac postequatorial, posterior end curved anteriorly, 153–255 long by 82–112 wide. Genital atrium shallow. Genital pore 51–59% of proglottid length from anterior end. Vagina anterior to cirrus sac, coiled. Vaginal sphincter weakly developed; seminal receptacle present. Ovary near posterior end of proglottid, H-shaped with equal-lengthed lobes not extending anteriorly to posterior margin of cirrus sac, 306–714 long by 153–179 wide at isthmus. Vitelline follicles extending from level of ovarian isthmus to near anterior end of proglottid; follicles single file, up to 40 in diameter. Mature detached proglottids 3,010–3,720 long by 714–832 wide. Cirrus sac 316–371 long by 255–337 wide. Genital pores 51–53% (52%) of proglottid length from anterior end. Ovary 918–1,020 long by 255–337 wide at isthmus. Gravid proglottids not collected.

HOST: *Potamotrygon hystrix*.

SITE OF INFECTION: Middle ⅓ of spiral valve.

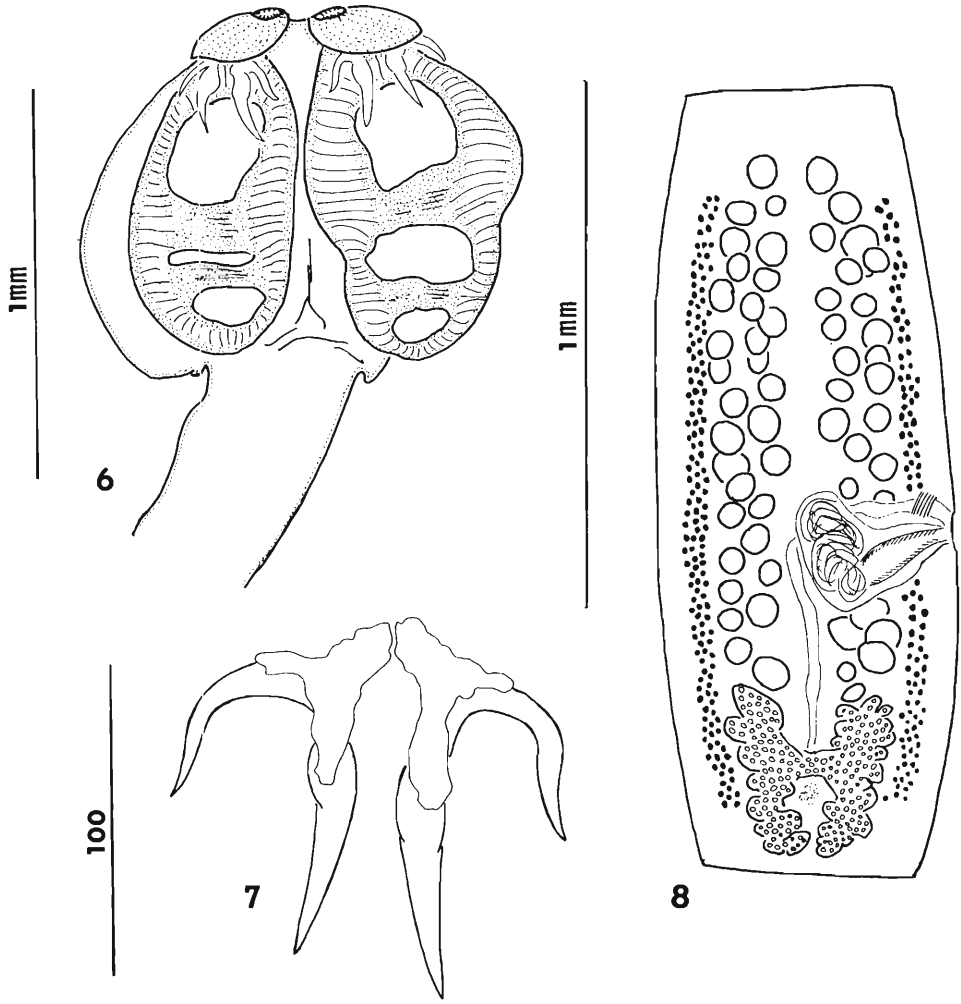
LOCALITIES: Orinoco River delta, Venezuela, near Curiapo (type); Orinoco River near Los Castillos, Venezuela.

HOLOTYPE: USNM Helm. Coll. No. 75709.

PARATYPES: USNM Helm. Coll. No. 75710; UNSM No. 21012, 21013.

ETYMOLOGY: This species is named in honor of Dr. A. Arandas Rego, Instituto Oswaldo Cruz, Rio de Janeiro, in recognition of his contributions to our knowledge of Neotropical cestodes.

By possessing recurved cirrus sacs, *Acanthobothrium regoi* most closely resembles *A. quinonesi* Mayes, Brooks, and Thorson, 1978, infecting *Potamotrygon magdalenae* in the Magdalena River of Colombia. The new species differs by having a larger scolex (800–1,100 μm wide vs. 460–620 μm wide), more pro-



Figures 6–8. ***Acanthobothrium regoi.*** **6. Scolex. 7. Bothridial hooks. 8. Mature proglottid.**

glottids (87–120 vs. 50–75), larger apical suckers ($\bar{x}$ = 102 vs. $\bar{x}$ = 66 μm), and larger bothridial hooks ($\bar{x}$ = 142 vs. $\bar{x}$ = 118–120 μm).

The four species of *Acanthobothrium* parasitizing freshwater stingrays form a monophyletic group characterized by a special (uniquely derived) bothridial hook dimorphism (Fig. 7), H-shaped ovaries, postequatorial genital pores, and scolices with sessile bothridia and expanded necks. *Acanthobothrium terezae* Rego and Dias, 1976, differs greatly from the other species in testes number, scolex size, and bothridial hook size. Characters distinguishing the other three species are slight but consistent. We consider them indicative of specific differences but if they are not, because of their consistency, the variations delimit morphotypes relating either to host-induced variation or to geographic (subspecific) variation. However, cestodes collected in three localities (Magdalena River, Lake Maracaibo area, and lower Orinoco River) from three different hosts (*Potamotrygon*

Table 1. Comparison of selected morphological characters among four species of *Acanthobothrium* infecting South American freshwater stingrays. The superscript [1] refers to specimens of *A. quinonesi* collected in the Magdalena River of Colombia whereas[2] refers to those collected in the Maracaibo area of Venezuela.

	terezae	*quinonesi*[1]	*quinonesi*[2]	*amazonensis*	*regoi*
Scolex width	2,000–3,000	508–620	460–620	612–790	800–1,100
Number of proglottids	200–260	55–75	50–60	75–100	87–120
Strobilar length (mm)	88–110	up to 25	up to 25	up to 35	up to 45
Total bothridial hook length					
range	180–326	100–142	100–140	145–184	122–163
mean ($\bar{x}$)	253	118	120	168	142
Apical sucker diameter	87	53–66	51–68	85–107	61–102
Number of testes					
range	120–140	43–60	50–55	50–72	47–70
mean ($\bar{x}$)	130	52	52	62	58
Position of genital pores	post-eq.	post-eq.	post-eq.	post-eq.	post-eq.
Cirrus sac length	450	137–237	170–220	243–293	153–255
Ovarian shape	H	H	H	H	H
Presence or absence of spines	absent	present	present	present	present

magdalenae, P. yepezi, and *P. hystrix*) all uniformly possess recurved cirrus sacs. Therefore, we consider *A. amazonensis* Mayes, Brooks, and Thorson, 1978, from *Potamotrygon circularis* in the upper Amazon River drainage, with straight cirrus sacs, distinct. Likewise, because specimens from *P. magdalenae* in Colombia and *P. yepezi* in the Lake Maracaibo area exhibit very similar scolex sizes, apical sucker diameters, bothridial hook sizes, and proglottid numbers, we consider them distinct from specimens parasitizing *P. hystrix* in the Orinoco. Table 1 presents a comparison for all species of *Acanthobothrium* infecting potamotrygonids showing pertinent characters.

Acanthobothrium terezae Rego and Dias, 1976

HOSTS: *Potamotrygon motoro* (as *Paratrygon m.*); *Elipesurus* sp.
SITE OF INFECTION: Spiral valve.
LOCALITY: Rio Salobra, Mato Grosso, Brazil.
SPECIMENS EXAMINED: None.
DIAGNOSTIC FEATURES: See Table 1.

Acanthobothrium quinonesi Mayes, Brooks and Thorson, 1978

HOSTS: *Potamotrygon magdalenae* (type); *P. yepezi,* new host.
SITE OF INFECTION: Middle ⅓ of spiral valve.
LOCALITIES: Magdalena River and associated cienagas near San Cristóbal, Bolívar, Colombia; Lake Maracaibo area near El Congo and Represa de Tulé, Río Cachirí, Zulia, Venezuela, new localities.
SPECIMENS EXAMINED: USNM Helm. Coll. No. 74804 (holotype) and 74805 (paratypes); UNSM No. 20563 (originally listed as 74806) (paratypes) and 21021, 21022, 21023 (voucher specimen from *P. yepezi*).

DIAGNOSTIC FEATURES: See Table 1. Mayes et al. (1978) reported that this species lacked tegumental spines, but small spines were discovered upon reexamination of the type material. Venezuelan specimens tend to have follicular ovaries, whereas Magdalenean ones have compact ovaries.

***Acanthobothrium amazonensis* Mayes, Brooks, and Thorson, 1978**

HOST: *Potamotrygon circularis* Garman.

SITE OF INFECTION: Middle ⅓ of spiral valve.

LOCALITY: Itacuaí River, 5 km south Atalaia do Norte, Brazil.

SPECIMENS EXAMINED: USNM Helm. Coll. No. 74806 (holotype) and 74807 (paratype); UNSM No. 20562 (paratype).

DIAGNOSTIC FEATURES: See Table 1.

Key to Species of *Acanthobothrium* Infecting Freshwater Stingrays

1a. Posterior end of cirrus sac recurved 2
1b. Posterior end of cirrus sac straight 3
2a. Bothridial hooks averaging 142 μm in total length, scolices 800–1,100 μm wide ..... *regoi*
2b. Bothridial hooks averaging 118–120 μm in total length, scolices 460–620 μm wide ..... *quinonesi*
3a. Testes averaging 62 in number, bothridial hooks averaging 168 μm in total length ..... *amazonensis*
3b. Testes averaging 130 in number, bothridial hooks averaging 253 μm in total length ..... *terezae*

Phylogenetic Relationships

We noted four possible cladograms representing the phylogenetic relationships of the three species of *Potamotrygonocestus*; for the four species of *Acanthobothrium* infecting potamotrygonids the number of possible cladograms is 26. However, we prefer the cladogram depicted in Figure 9, which is most consistent with the characters listed below.

1. Shape of cirrus sac. 0 = straight; 1 = recurved.
2. Strobilar spination. 0 = lacking; 1 = present.
3. Bothridial hook size. 0 = total hook length averaging 140–170 μm; 1 = total hook length averaging 118–120 μm; −1 = total hook length averaging 253 μm.
4. Number of proglottids. 0 = 75–120; 1 = 50–75; −1 = 200–260.

Phyllobothriidae Braun, 1900
***Rhinebothrium* Linton, 1889**
***Rhinebothrium paratrygoni* Rego and Dias, 1976**

HOSTS: *Elipesurus* sp. (type); *Potamotrygon hystrix*, *P. reticulatus*; *P. falkneri*, new hosts.

SITE OF INFECTION: Middle ⅓ of spiral valve.

LOCALITIES: Rio Salobra, Mato Grosso, Brazil (type); Orinoco River delta, near Curiapo and near km 82 of main channel, Venezuela, new localities; Paraná River, Paraguay, near Hohenau, new locality.

SPECIMENS EXAMINED: UNSM No. 21010 (voucher specimens from Orinoco) and 21016 (voucher specimens from Paraná).

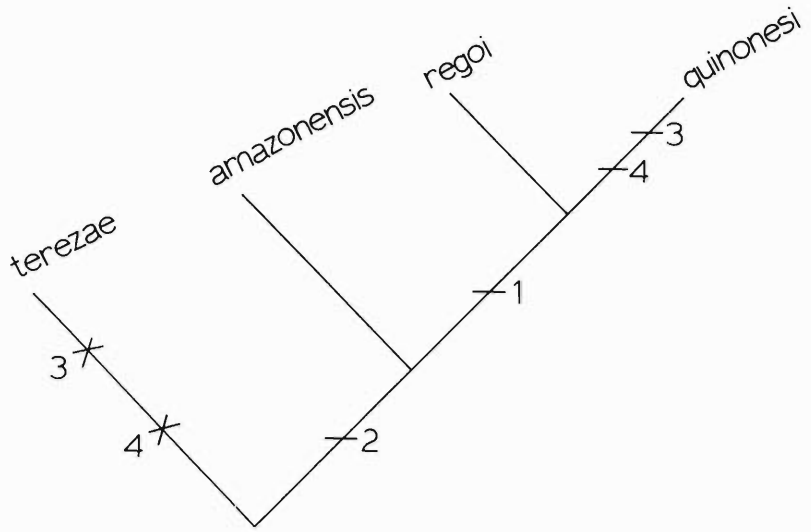


Figure 9. Cladogram depicting phylogenetic relationships of *Acanthobothrium* species infecting potamotrygonids.

Our specimens agree closely with those described by Rego and Dias (1976). They reported that proglottids generally contained five and occasionally contained six testes; we found four to eight testes with a mean of five (n = 50). Rego and Dias were not able to provide an accurate count of bothridial loculi, but our specimens exhibited 72–76 loculi arranged as follows: bothridia comprising two lobes separated by a horizontal hingelike constriction; bothridia with 36–38 horizontal septa divided by a median longitudinal septum forming 72–76 total loculi. Rego and Dias compared *R. paratrygoni* with *R. maccallumi* Campbell, 1970, *R. lintoni* Campbell, 1970, *R. walga* Euzet, 1956, and *R. minimum* Euzet, 1956. We do not believe that any of those species are closely related to *R. paratrygoni*. Rather, five other species and *R. paratrygoni* form a monophyletic group characterized by being small worms with relatively to markedly long cephalic peduncles, more than 25 proglottids per strobila, craspedote proglottids which are wider than long (except terminal proglottids), bilobed bothridia with a single median longitudinal septum and at least 32 loculi, and an average of fewer than 10 testes per proglottid. Those species include *R. urobatidium* (Young, 1955) Appy and Dailey, 1978, *R. spinicephalum* Campbell, 1969, *R. tetralobatum* Brooks, 1977, *R. ditesticulum* Appy and Dailey, 1978, and a new species being described elsewhere, in addition to *R. paratrygoni*. The phylogenetic relationships of those five species, depicted in Figure 10, were estimated using the following characters and their coded states.

1. Testes number. 0 = 6–12 ($\bar{x}$ = 8); 1 = 4–8 ($\bar{x}$ = 5); 2 = 3–6 ($\bar{x}$ = 4); 3 = 2.
2. Bothridial loculi number. 0 = 38–42; 1 = 48–55; 2 = 72–76; 2* = 32–34 (reversal).
3. Relative length of cephalic peduncle. 0 = short; 1 = long.
4. Ovarian morphology. 0 = compact; 1 = fragmented.

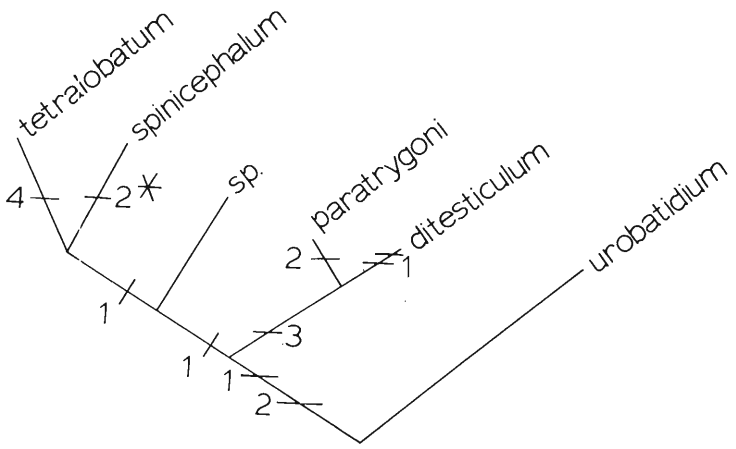


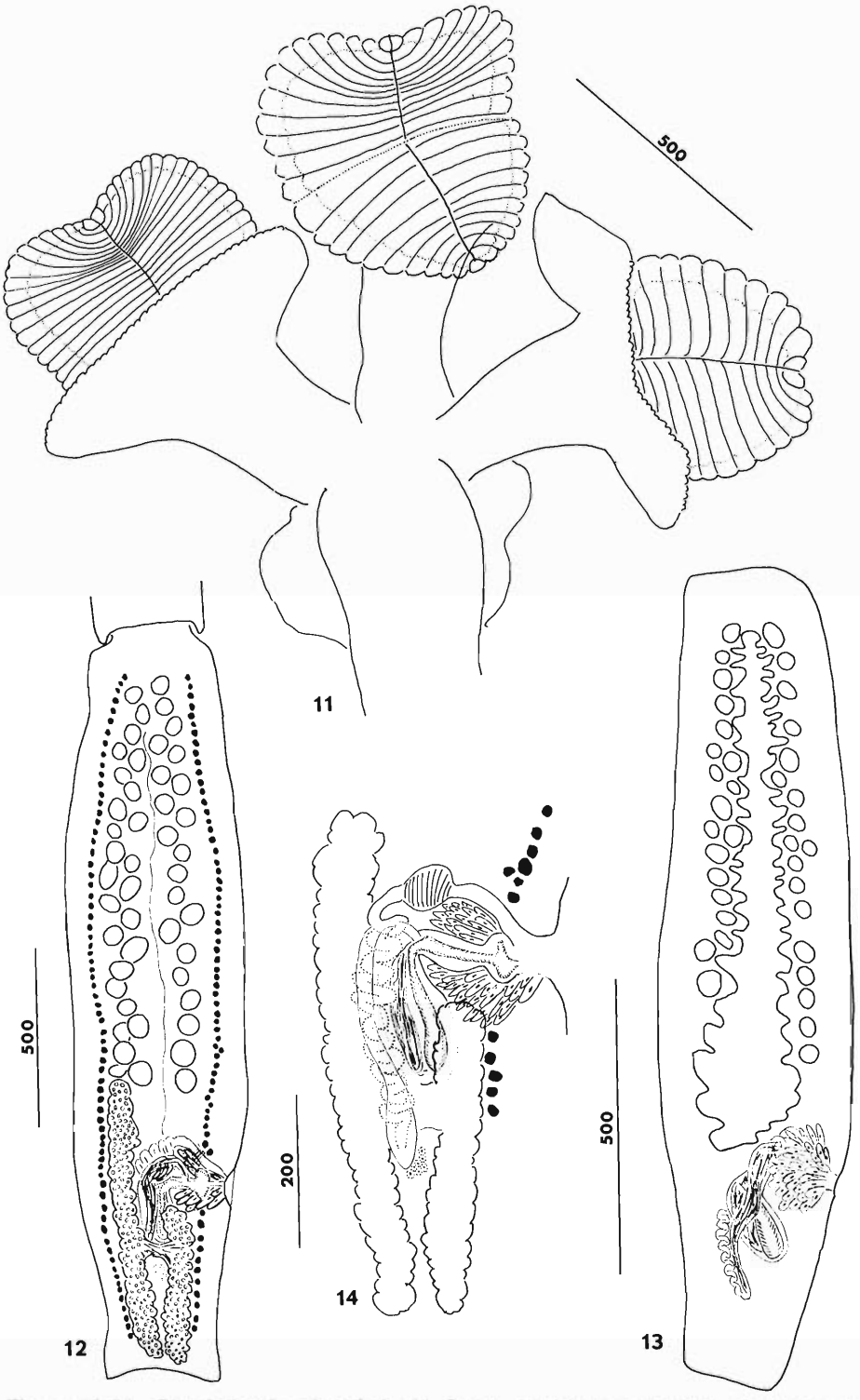
Figure 10. Cladogram depicting phylogenetic relationships of *Rhinebothrium paratrygoni* and its closest relatives.

The most parsimonious arrangement of those six species based on the above characters indicates a parallel reduction in testes number to a total of two (*R. ditesticulum* and *R. spinicephalum*/*R. tetralobatum*) and a reduction in bothridial loculi number following a trend of increasing loculi numbers, a form of evolutionary reversal (*R. spinicephalum*).

Rego and Dias (1976) noted the presence of a very long cephalic peduncle in *Rhinebothrium paratrygoni* and suggested that the trait invalidated the generic concept of *Caulobothrium* Baer, 1948. The critical characteristic separating *Caulobothrium* species from *Rhinebothrium* species is the presence of postvaginal testes in *Caulobothrium* and their absence in *Rhinebothrium*. In fact, three species of *Caulobothrium*—*C. anacolum* Brooks, 1977, from *Himantura schmardae* in Colombia, *C. myliobatidis* Carvajal, 1977, from *Myliobatis chilensis* in Chile, and *C. multorchidum* (Young, 1955) Appy and Dailey, 1978, from *Urolophus halleri* in California—all possess short cephalic peduncles. Appy and Dailey (1978) treated the generic status of *Rhinebothrium, Caulobothrium,* and *Rhabdotobothrium* Baer, 1948. They observed that *Rhinebothrium* and *Caulobothrium* appeared valid because they retained their membership intact without regard for relative peduncle length, but that *Rhabdotobothrium,* which differs from *Caulobothrium* by lacking any cephalic peduncles, might not be justifiably distinct. We concur with Appy and Dailey, recognizing *Rhinebothrium* for those species lacking postvaginal testes and *Caulobothrium* for those species possessing postvaginal testes. We consider the relative length of the cephalic peduncle a plastic trait exhibiting wide variation among the representatives of both genera.

Rhinebothroides Mayes, Brooks, and Thorson, 1981
Rhinebothroides glandularis sp. n.
(Figs. 11–14)

DESCRIPTION (based on 20 specimens): Strobila craspedote, apolytic, up to 50 mm long, composed of 25–30 proglottids. Scolex with 4 pedicellated, bilobed, squared bothridia; rostellum lacking. Pedicels contractile, up to 50 long. Bothridia 610–714 long by 460–714 wide; indistinct hingelike constriction between lobes;



Figures 11–14. ***Rhinebothroides glandularis*****. 11. Scolex. 12. Mature proglottid. 13. Gravid proglottid. 14. Close-up of terminal genitalia and ootype region.**

divided into marginal and medial portions by marginal septum; divided horizontally by 25–29 septa; medial loculi 51–59 in number; marginal loculi 51–59 in number. Immature proglottids wider than long; mature ones 1.6–1.9 mm long by 460–510 wide. Testes in 2 broad fields in anterior ⅔ of proglottid, 41–51 in number ($\bar{x}$ = 45, n = 50); 40–61 in diameter. Cirrus sac in posterior ⅓ of proglottid, 331–408 long by 57–102 wide, surrounded by darkly staining cells lying free in the parenchyma, containing spined eversible cirrus and internal seminal vesicle. External seminal vesicle extending length of cirrus sac, joining cirrus sac near poral end and vas deferens near posterior end of proglottid. Genital atrium shallow; genital pores alternating irregularly in posterior $^1/_5$ of proglottid. Vagina anterior to cirrus sac, coiled; vaginal sphincter and seminal receptacle present. Ovary bilobed in frontal view, X-shaped in cross section; aporal lobe 638–868 long, extending anteriorly to middle of proglottid; poral lobe extending anteriorly to middle of cirrus sac; 348–468 wide at isthmus. Vitelline follicles lateral, extending entire length of proglottid, not interrupted near genital pore; 20–32 in diameter. Gravid proglottids 1.90–2.35 mm long by 460–510 wide, devoid of gonads. Uterus saccate with 45–60 total lateral diverticula. Eggs 29–37 in diameter; oncospheres 27–31 in diameter, unembryonated in utero.

HOST: *Potamotrygon hystrix*.

SITE OF INFECTION: Middle ⅓ of spiral valve.

LOCALITY: Orinoco River delta near Curiapo, Venezuela.

HOLOTYPE: USNM Helm. Coll. No. 75707.

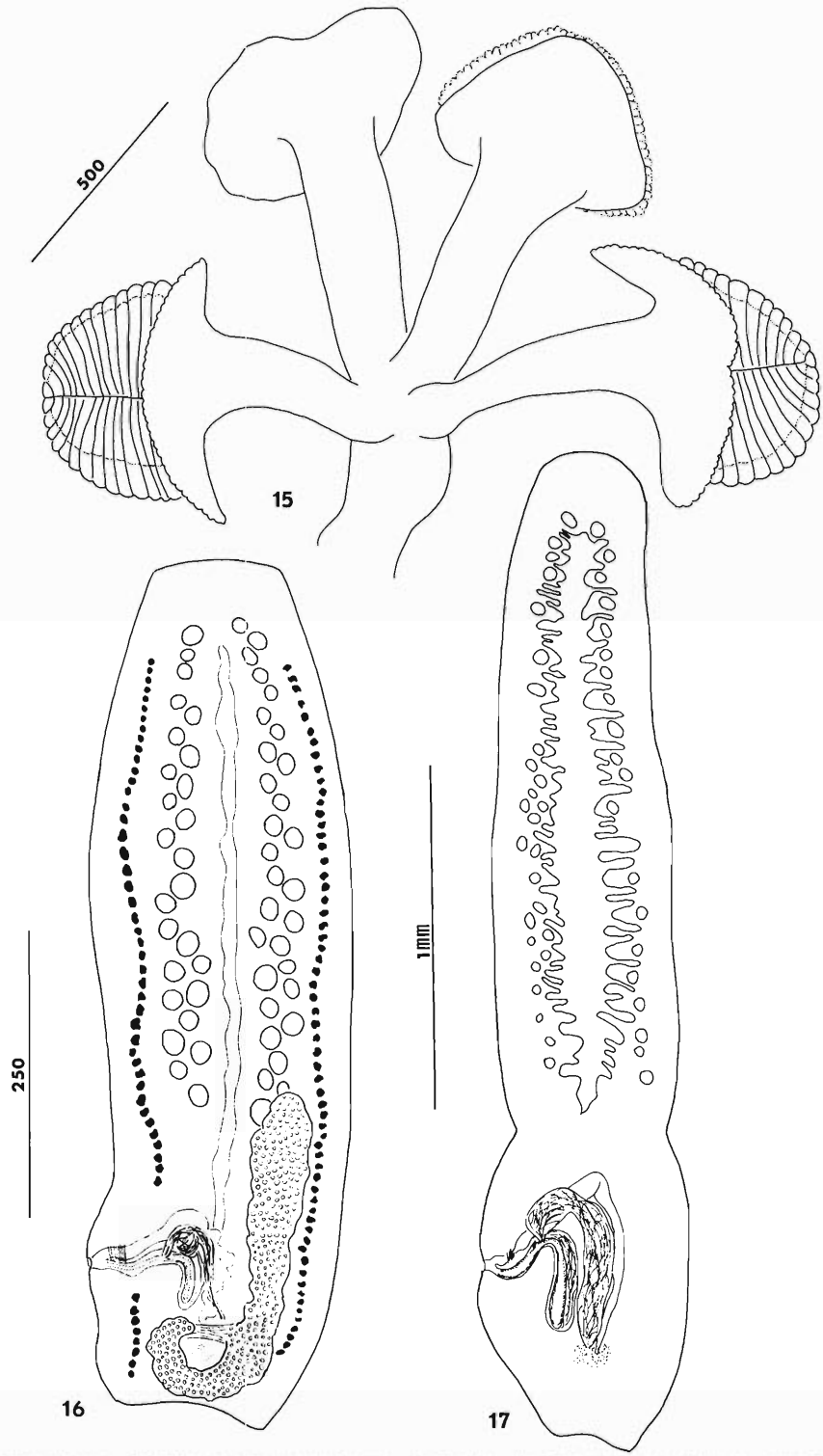
PARATYPES: USNM Helm. Coll. No. 75708; UNSM No. 21007.

ETYMOLOGY: This species is named after its diagnostic feature, the parenchymal gland cells surrounding the terminal genitalia.

Rhinebothroides glandularis differs from all other members of the genus by possessing prominent gland cells surrounding the terminal genitalia. It resembles *R. scorzai* more closely than any other species by having a coiled vagina, vitelline follicles uninterrupted near the genital pore, and poral ovarian lobes extending anterior to the posterior margin of the cirrus sac.

Rhinebothroides venezuelensis sp. n. (Figs. 15–17)

DESCRIPTION (based on 25 specimens): Strobila craspedote, apolytic, up to 60 mm long, composed of 20–30 proglottids. Scolex with four pedicellated, bilobed, squared bothridia; rostellum lacking. Pedicels contractile, up to 300 long. Bothridia 360–720 long by 260–690 wide; indistinct hingelike constriction between lobes; divided into marginal and medial portions by marginal septum; divided longitudinally by indistinct median septum; divided horizontally by 25–26 septa; medial loculi 51–53 in number; marginal loculi 51–53 in number. Immature proglottids wider than long; mature ones 714–1,160 long by 210–357 wide. Testes in 2 broad fields in anterior ⅔ of proglottid, 45–64 ($\bar{x}$ = 53, n = 50) in number, 40–80 in diameter. Cirrus sac in posterior $^1/_5$ of proglottid, 153–194 long by 31–51 wide, containing spined eversible cirrus and internal seminal vesicle. External seminal vesicle extending length of cirrus sac, joining cirrus sac near poral end, joining vas deferens near posterior end of proglottid. Genital atrium shallow, genital pores alternating irregularly in posterior $^1/_5$ of proglottid. Vagina anterior to cirrus sac, straight; vaginal sphincter and seminal receptacle present. Ovary



Figures 15–17. ***Rhinebothroides venezuelensis*****. 15. Scolex. 16. Mature proglottid. 17. Gravid proglottid.**

bilobed in frontal view, X-shaped in cross section. Aporal lobe 510–560 long, not extending into anterior ½ of proglottid; poral lobe extending anteriorly to posterior margin of cirrus sac; 123–160 wide at isthmus. Vitelline follicles lateral, extending entire length of proglottid, interrupted near genital pore; 20–40 in diameter. Gravid proglottids 1.53–3.05 mm long, devoid of gonads. Uterus saccate, with 80–100 total lateral diverticula. Eggs 19–24 in diameter; oncospheres 17–21 in diameter, unembryonated in utero.

HOSTS: *Potamotrygon hystrix* (type); *P. yepezi*.

SITE OF INFECTION: Middle ⅓ of spiral valve.

LOCALITIES: Orinoco River delta near Curiapo, Venezuela (type); Orinoco River near Los Castillos, Venezuela; Lake Maracaibo area, near El Congo and Represa de Tulé, Río Cachirí, Zulia, Venezuela.

HOLOTYPE: USNM Helm. Coll. No. 75705.

PARATYPES: USNM Helm. Coll. No. 75706; UNSM No. 21005, 21006.

ETYMOLOGY: This species is name for the Republic of Venezuela, the country in which it has been collected.

Rhinebothroides venezuelensis most closely resembles *R. moralarai* (Brooks and Thorson, 1976) Mayes, Brooks, and Thorson, 1981, and *R. circularisi* Mayes, Brooks, and Thorson, 1981, by having straight vaginae, vitelline follicles interrupted near the genital pore, and poral ovarian lobes extending anteriorly only to the posterior margin of the cirrus sac. The new species differs by having fewer testes (45–64) than *R. moralarai* (54–71) or *R. circularisi* (66–88) and by being craspedote rather than acraspedote. Specimens of *R. venezuelensis* from *Potamotrygon yepezi* in the Lake Maracaibo area differ from those collected in the Orinoco by having 45–64 ($\bar{x}$ = 54) rather than 45–55 ($\bar{x}$ = 51) testes per proglottid.

Rhinebothroides scorzai (López-Neyra and Diaz-Ungriá, 1958) Mayes, Brooks, and Thorson, 1981

Rhinebothrium scorzai López-Neyra and Diaz-Ungriá, 1958.
Rhinebothrium scorzai: Rego and Dias, 1976.
Rhinebothroides scorzai: Mayes, Brooks, and Thorson, 1981.

HOSTS: *Potamotrygon hystrix* (type); *P. motoro*; *P. reticulatus*, new host; *Elipesurus* sp.; *Elipesurus spinicauda*, new host.

SITE OF INFECTION: Middle of ⅓ of spiral valve.

LOCALITIES: Orinoco River delta, Venezuela (type); Rio Salobra, Mato Grosso, Brazil; Orinoco River delta, near El Toro, near km 82 of the main channel, and near Tucupita, Venezuela, new localities.

SPECIMENS EXAMINED: USNM Helm. Coll. No. 75704 (voucher specimen from *P. hystrix*); UNSM No. 21014 (voucher specimen from *E. spinicauda*), 21015 (voucher specimen from *P. reticulatus*).

DIAGNOSTIC FEATURES: Bothridial loculi 69–79 in number. Strobila craspedote, composed of 60–100 proglottids. Testes 60–99 in number. Cirrus sac up to 650 long. Poral ovarian lobes extending anterior to posterior margin of cirrus sac. Vitelline follicles not interrupted near genital pore. Vagina coiled. Uterine diverticula 54–80 in number.

Three reports (López-Neyra and Diaz-Ungría, 1958; Rego and Dias, 1976; present report), listing five hosts in five localities, exist for *R. scorzai*. Table 2 lists variability in testes number for *R. scorzai* based on those reports.

Rhinebothroides moralarai (Brooks and Thorson, 1976) Mayes, Brooks, and Thorson, 1981

Rhinebothrium moralarai Brooks and Thorson, 1976.
Rhinebothroides moralarai: Mayes, Brooks, and Thorson, 1981.

HOST: *Potamotrygon magdalenae*.
SITE OF INFECTION: Middle ⅓ of spiral valve.
LOCALITIES: Magdalena River, near San Cristóbal, Bolívar, and near La Dorada, Caldas, Colombia.
SPECIMENS EXAMINED: USNM Helm. Coll. No. 73544 (holotype) and 73545 (paratype); UNSM No. 20255 (incorrectly listed as 73546 in original description) (paratypes).
DIAGNOSTIC FEATURES: Bothridial loculi 69–79 in number. Strobila acraspedote, composed of 18–24 proglottids. Testes 66–88 in number. Cirrus sacs up to 650 long. Poral ovarian lobes not extending anteriorly beyond posterior margin of cirrus sac. Vagina not coiled. Vitelline follicles interrupted near genital pore. Uterine diverticula 49–80 in number.

Rhinebothroides circularisi Mayes, Brooks, and Thorson, 1981

HOST: *Potamotrygon circularis*.
SITE OF INFECTION: Middle ⅓ of spiral valve.
LOCALITY: Itacuaí River, Brazil, near Leticia, Colombia.
SPECIMENS EXAMINED: USNM Helm. Coll. No. 76361 (holotype) and 76362 (paratype); UNSM No. 21020 (paratype).
DIAGNOSTIC FEATURES: Bothridial loculi 69–79 in number. Strobila acraspedote, composed of 18–24 proglottids. Testes 66–88 in number. Cirrus sacs up to 650 long. Poral ovarian lobes not extending anteriorly beyond posterior margin of cirrus sac. Vagina not coiled. Vitelline follicles interrupted near genital pore. Uterine diverticula 49–80 in number.

Rhinebothroides freitasi (Rego, 1979) comb. n.

Rhinebothrium freitasi Rego, 1979.

HOST: *Potamotrygon hystrix*.
SITE OF INFECTION: Spiral valve.
LOCALITY: Amazon River, Maicuru, Para, Brazil.
SPECIMENS EXAMINED: None.
DIAGNOSTIC FEATURES: Bothridial loculi approximately 60 in number. Strobila acraspedote, composed of 12–15 proglottids. Testes 48–64 in number. Cirrus sacs up to 390 long. Poral ovarian lobes extending anterior to posterior margin of cirrus sac. Vagina coiled. Vitelline follicles terminating preovarially. Number of uterine diverticula not reported.

Key to Species of *Rhinebothroides*

1a. Vagina coiled, vitelline follicles not interrupted near genital pore, poral ovarian lobes extending anterior to posterior margin of cirrus sac 2
1b. Vagina straight, vitelline follicles interrupted near genital pore, poral ovarian lobes reaching anteriorly only to posterior margin of cirrus sac 4
2a. Strobila composed of 60–100 proglottids, testes 60–99 in number ..... *scorzai*

Table 2. Comparison of range and mean testes numbers for specimens of *Rhinebothroides scorzai* collected from various freshwater stingrays in Venezuela and Brazil. Superscript [1] refers to specimens reported by Lopez-Neyra and Diaz-Ungria (1958), [2] to specimens reported by Rego and Dias (1977), [3] to specimens collected in the present study.

Host	Range	Mean ($\bar{x}$)	Sample size (n)	Locality
Potamotrygon hystrix[1]	86–98	92 (est.)	—	Venezuela
Potamotrygon hystrix[3]	84–99	92	10	Venezuela
Potamotrygon reticulatus[3]	60–90	78	30	Venezuela
Potamotrygon motoro[2]	80–85	82.5 (est.)	—	Brazil
Elipesurus spinicauda[3]	78–90	82	10	Venezuela
Elipesurus sp.[2]	80–85	82.5 (est.)	—	Brazil

2b. Strobila composed of fewer than 35 proglottids, testes 41–64 in number 3
3a. Prominent gland cells in parenchyma surrounding terminal genitalia, strobila craspedote ..... *glandularis*
3b. Vitellaria terminating at level of anteriormost extent of ovary, strobila acraspedote ..... *freitasi*
4a. Strobila craspedote, bothridial loculi 51–53 in number, testes 45–64 in number ..... *venezuelensis*
4b. Strobila acraspedote 5
5a. Testes 54–71 in number, bothridial loculi 45–47 in number ..... *moralarai*
5b. Testes 66–68 in number, bothridial loculi 69–79 in number ..... *circularisi*

Phylogenetic Relationships

The six members of *Rhinebothroides* exhibit two traits possessed by no other tetraphyllideans presently known, their bothridial morphology and an internal seminal vesicle, which establishes them as a monophyletic group. The phylogenetic relationships of those six species (Fig. 18) were inferred using the following seven characters.

1. Vaginal morphology. 0 = vagina straight; 1 = vagina coiled.
2. Anterior extent of poral ovarian lobe. 0 = poral lobe extending anterior to posterior margin of cirrus sac; 1 = poral lobe reaching anteriorly only to posterior margin of cirrus sac.
3. Vitelline configuration. 0 = vitellaria not interrupted near genital pore; 1 = vitellaria interrupted near genital pore; −1 = vitellaria terminating preovarially.
4. Proglottid structure. 0 = acraspedote; 1 = craspedote.
5. Presence or absence of parenchymal gland cells surrounding terminal genitalia. 0 = lacking; 1 = present.
6. Testes number. 0 = 45–70; 1 = 60–100.
7. Bothridial loculi number. 0 = 69–79; 1 = 51–59; 2 = less than 50.

Study of numerous specimens of *Rhinebothroides*, including all five of the six known species, demonstrated that previous descriptions of bothridial morphology were incomplete. Bothridia are squared, bilobed, and hinged, with a median longitudinal septum and many horizontal septa forming numerous loculi as reported by Brooks and Thorson (1976) and Mayes et al. (1981). Two previously unreported

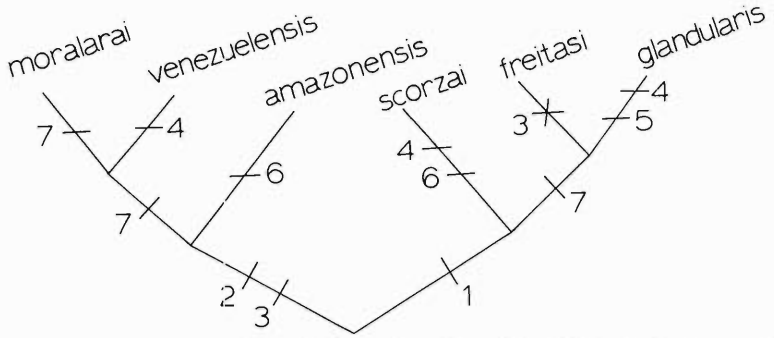


Figure 18. Cladogram depicting phylogenetic relationships of *Rhinebothroides* species.

features include (1) the presence of an indistinct circular septum (best seen in immature specimens) inside the margin of the bothridium creating a ring of marginal loculi equal in number to the medial loculi and (2) the asymmetrical nature of the terminal loculi, there being two at one end and one at the other end of each bothridium (Fig. 19).

Rhinebothroides species resemble *Phyllobothrium kingae* Schmidt, 1978, and *Phyllobothrium* cf. *kingae* Brooks and Mayes, 1980, by having squared bothridia

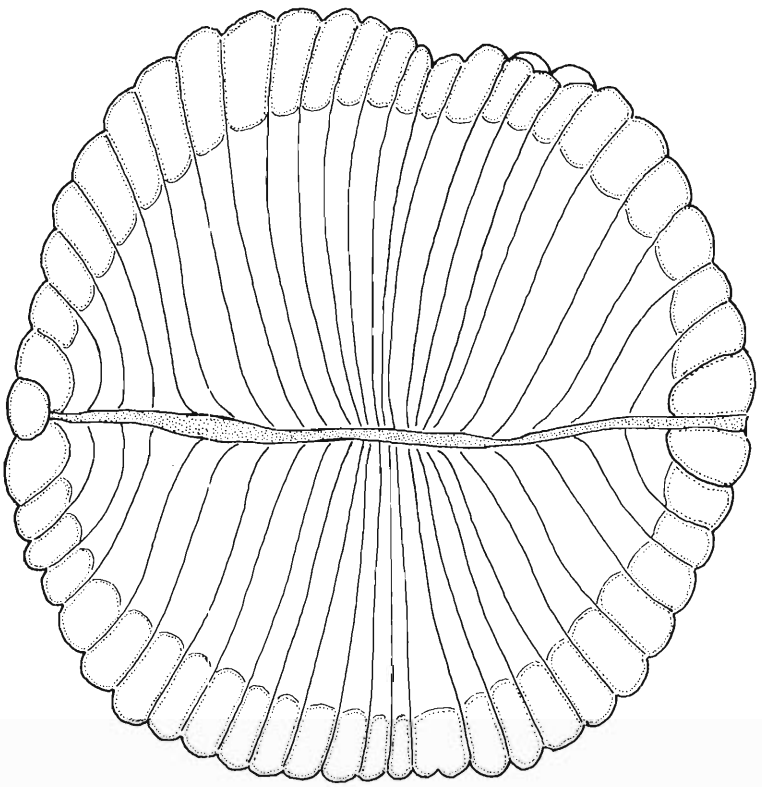


Figure 19. Generalized view of *Rhinebothroides* bothridial loculi architecture.

with indistinct horizontal septa and marginal loculi as well as proglottids with terminal genitalia situated near the ovary. The above-mentioned taxa differ from other phyllobothriid genera by virtue of those shared special traits. Members of *Rhinebothroides* have previously been classified with *Rhinebothrium* (López-Neyra and Diaz-Ungriá, 1958; Brooks and Thorson, 1976; Mayes et al., 1981), with which they share many generalized features, including septate bothridia. However, at least 12 separate tetraphyllidean genera are characterized by septate bothridia; thus, the presence of septate bothridia in *Rhinebothroides* does not necessarily imply close relationship with *Rhinebothrium*. Indeed, special traits exhibited by *Rhinebothroides* suggest other affinities.

Conclusions

Potamotrygonids host a diverse cestode fauna which reflects their phylogenetic relationships with other elasmobranchs more than their ecological affinities with freshwater fishes. Both the Trypanorhyncha and Tetraphyllidea comprise species exclusively parasitic as adults in elasmobranchs, and are represented in the cestode fauna of potamotrygonids. On the other hand, no potamotrygonid examined thus far has hosted any members of the Proteocephalidea, a group of cestodes displaying its greatest diversity in South American freshwater fishes (Brooks, 1978). Each of the cestode taxa occurring in potamotrygonids comprises a monophyletic group, either a species-group (*Acanthobothrium*) or a distinct genus (*Potamotrygonocestus* or *Rhinebothroides*). These findings suggest that potamotrygonid cestodes are derived from a common ancestral helminthfauna and support the hypothesis that potamotrygonids themselves comprise a monophyletic group. A more thorough analysis of the evolutionary history of potamotrygonid parasites is being prepared by the authors.

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Literature Cited

Appy, R., and M. D. Dailey. 1978. A new species of *Rhinebothrium* (Cestoda: Tetraphyllidea) and redescription of three rhinebothriate species from the Round Stingray, *Urolophus halleri* Cooper in southern California. Bull. South. Calif. Acad. Sci., 76:116–127.

Brooks, D. R. 1977. Six new species of tetraphyllidean cestodes, including a new genus, from a marine stingray *Himantura schmardae* (Werner, 1904) from Colombia. Proc. Helminthol. Soc. Wash. 44:51–59.

Brooks, D. R. 1978. Evolutionary history of the cestode order Proteocephalidea. Syst. Zool. 27:312–327.

Brooks, D. R., and M. A. Mayes. 1980. Cestodes in four species of euryhaline stingrays from Colombia. Proc. Helminthol. Soc. Wash. 47:22–29.

Brooks, D. R., and T. B. Thorson. 1976. Two tetraphyllidean cestodes from the freshwater stingray *Potamotrygon magdalenae* Dumeril, 1852 (Chondrichthyes: Potamotrygonidae) from Colombia. J. Parasitol. 62:943–947.

Euzet, L. 1959. Théses presentees à la Faculté des Sciences de Montepellier pour obtenir le grade de Docteur es Sciences Naturelles: 1. Recherches sur les cestodes Tetraphyllides de Selaciens des côtes de France. Causse, Graille, and Castelnau, Montpellier. 263 pp.

Hennig, W. 1966. Phylogenetic Systematics. University of Illinois Press, Urbana, Illinois. 263 pp.

Lopez-Neyra, C. R., and C. Diaz-Ungria. 1958. Cestodes de Venezuela. V. Cestodes de vertebrados Venezolanos (segunda nota). Nova Cient. 23:1–41.

Mayes, M. A., D. R. Brooks, and T. B. Thorson. 1978. Two new species of *Acanthobothrium* Van Beneden, 1849 (Cestoidea: Tetraphyllidea) from freshwater stingrays in South America. J. Parasitol. 64:838–841.

Mayes, M. A., D. R. Brooks, and T. B. Thorson. 1981. Two new tetraphyllidean cestodes from *Potamotrygon circularis* Garman (Chondrichthyes: Potamotrygonidae) in the Itacuaí River, Brazil. Proc. Helminthol. Soc. Wash. 48:38–42.

Rego, A. A. 1979. Contribuicao ao conhecimento dos helmintos de raias fluviais Paratrygonidae. Rev. Brasil. Biol. 39:879–890.

Rego, A. A. and A. P. L. Dias. 1976. Estudos de cestoídes de peixes do Brasil 3.a nota: cestóides de raias fluviais paratrygonidae. Rev. Brasil. Biol. 36:941–956.

Woodland, W. N. F. 1934. Six new cestodes from Amazon fish. Proc. Zool. Soc. London 1:33–44.

Survey or Taxonomic Papers

Authors submitting manuscripts of a survey or taxonomic nature for publication in the Proceedings of the Helminthological Society of Washington are urged to deposit representative specimens in a recognized depository such as the National Parasite Collection at Beltsville, Maryland and include the accession numbers in the manuscript.

Proc. Helminthol. Soc. Wash.
48(1), 1981, pp. 65–70

A Taxonomic Review of the Genus *Loxogenoides* (Digenea: Lecithodendriidae) with a Description of *Loxogenoides loborchis* sp. n. from *Rana catesbeiana* Shaw in Western Kentucky

BRUCE M. CHRISTENSEN
Department of Biological Sciences, Murray State University, Murray, Kentucky 42071

ABSTRACT: *Loxogenoides loborchis* sp. n. is described from the biliary system of *Rana catesbeiana* Shaw, 1802 from Calloway County, Kentucky. *Loxogenoides bicolor* (Krull, 1933) Kaw, 1945 is redescribed based on specimens recovered from *R. catesbeiana* and *Rana clamitans* Latreille, 1802. *Loxogenoides polyorchis* (Lautenschlager and Cheng, 1958) Yamaguti, 1971 is considered a junior synonym of *L. bicolor*. *Loxogenoides loborchis* differs from *L. bicolor* in egg size, sucker width ratio, body size and shape, pigmentation, and characteristics of the gut. The generic diagnosis of *Loxogenoides* is emended.

The genus *Loxogenoides* was created by Kaw (1945) when he reviewed the genus *Loxogenes* Stafford, 1905. After comparing *Loxogenes bicolor* with *Loxogenes arcanum*, the type species for the genus, he removed *L. arcanum* and placed it in the genus *Pleurogenoides* Travassos, 1921 and established the genus *Loxogenoides* to receive *L. bicolor*. Yamaguti (1971), however, considered *L. arcanum* to be the type species for the genus *Loxogenes* and separated it from species of the genus *Pleurogenoides* by the location of the uterine coils.

Lautenschlager and Cheng (1958) described a new heterophyid trematode, *Larelmintha polyorchis*, from the herring gull, *Larus argentatus* Pontoppidan, but Yamaguti (1971) transferred it from the Heterophyidae to the genus *Loxogenoides*, family Lecithodendriidae. He regarded this species as an accidental parasite of the herring gull probably resulting from the ingestion of an infected frog. Until the present study, *L. bicolor* and *L. polyorchis* were the only two species in the genus.

Outside of the excellent study by Byrd (1950) on the flame-cell pattern of *L. bicolor* (2[(3+3+3) + (3+3+3)]) and the work by Seitner (1945) that suggests *L. bicolor* develops virgulate distomatous xiphidiocercariae, in *Goniobasis depygis*, that encyst in Ephemeroptera and Odonata naiads, little work has been done on the genus.

Fourteen bullfrogs, *Rana catesbeiana* Shaw, 1802, were collected in western Kentucky and examined for helminth parasites during July, August, and September 1979. Three trematodes representing a new species in the genus *Loxogenoides* were found unencysted in the biliary system of one frog, and one *L. bicolor* was found encysted in the body cavity of another frog.

This paper describes the new species of *Loxogenoides*, redescribes *L. bicolor*, indicates that *L. polyorchis* is a junior synonym of *L. bicolor*, and emends the generic diagnoses of Kaw (1945) and Yamaguti (1971).

Materials and Methods

All trematodes were fixed in AFA under slight cover glass pressure and stained with Mayer's paracarmine. Figures were drawn with the aid of a microprojector

and ocular micrometer. All measurements are in micrometers unless otherwise stated. The mean is followed by the range in parentheses and dimensions of organs are stated as length by width. Specimens examined included the holotype (USNM Helm. Coll. 32876), two paratypes (USNM Helm. Coll. 32877), and one serial section of *L. bicolor*, three whole mounts of *L. bicolor* collected in Virginia (Campbell, 1968), and the holotype of *L. polyorchis* (USNM Helm. Coll. 38295). In addition, the one whole mount of *L. bicolor* collected during this study also was used for the redescription. Notations for deposited specimens are: USNM Helm. Coll. for United States National Museum Helminthological Collection, Beltsville, Maryland and HWML for Harold W. Manter Laboratory, Division of Parasitology, University of Nebraska State Museum, Lincoln, Nebraska.

Loxogenoides loborchis sp. n.
(Figs. 1, 2)

TYPE HOST: *Rana catesbeiana* Shaw, 1802.

TYPE LOCALITY: Calloway County, Kentucky.

SITE: Bile ducts of liver.

TYPE SPECIMENS: Holotype USNM Helm. Coll. 76097.

PARATYPES HWML 21193; USNM Helm. Coll. 76320.

DESCRIPTION (based on three mature specimens): Body elongate, 2.72 mm (2.43–2.98 mm) by 0.88 mm (0.74–0.99 mm), with rounded extremities, widest at midbody. Entire body spinose, spines 15 (13–17) long. Oral sucker subterminal, 318 (299–346) by 325 (306–346). Prepharynx absent; esophagus short; pharynx 212 (194–227) by 138 (119–151). Ceca extending to posterior of body, broad, with outpocketings. Acetabulum about ⅓ body length from anterior end, 245 (202–270) by 244 (216–270). Ratio of width of oral sucker to acetabulum 1:0.75 (1:0.71–1:0.78).

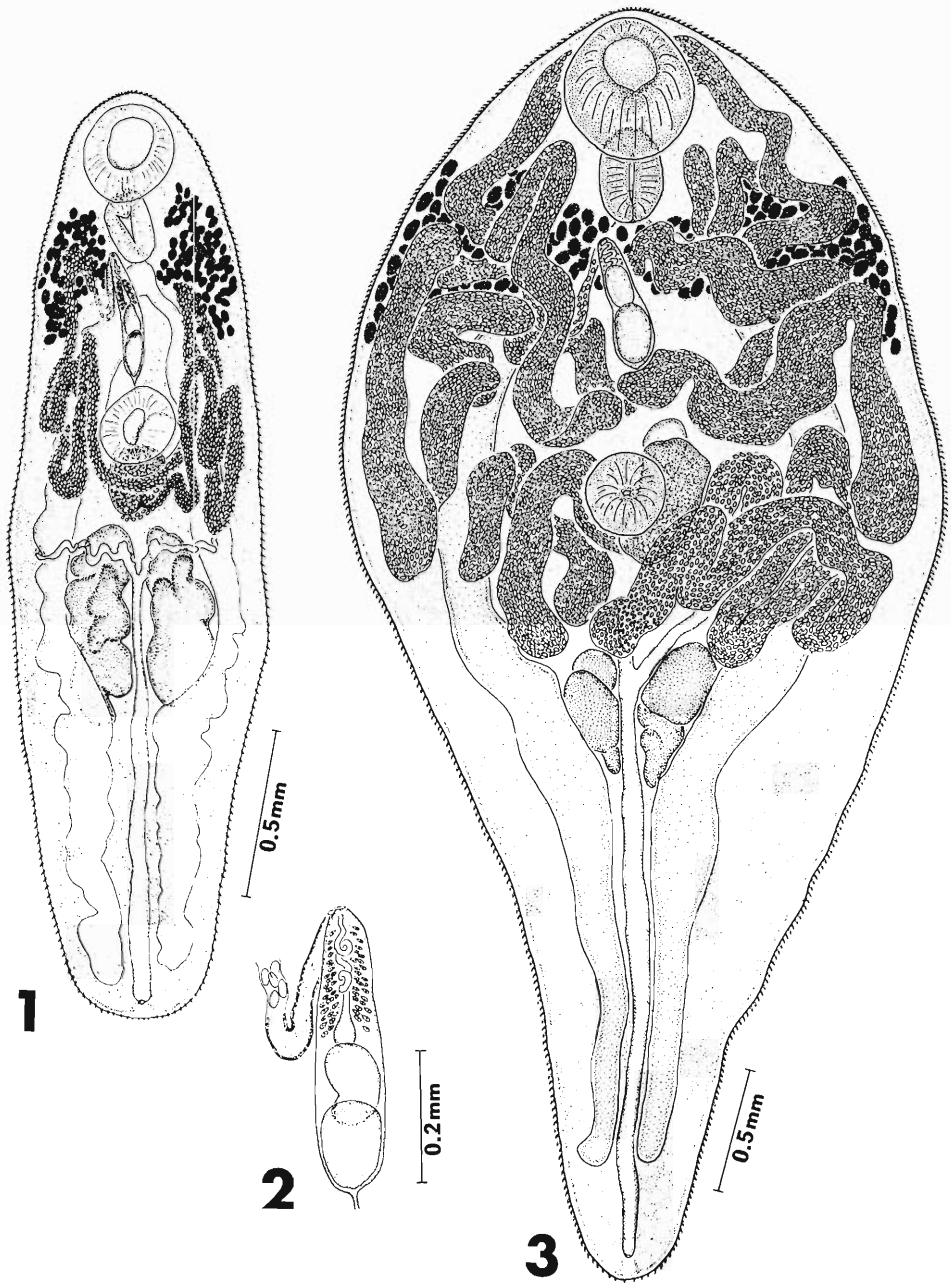
Testes slightly postequatorial, symmetrical, multilobed. Right testis 589 (504–735) by 251 (180–331), left testis 588 (486–706) by 214 (206–221). Cirrus sac preacetabular, elongate, 421 (360–461) by 101 (90–108). Seminal vesicle bipartite, proximal portion 155 (148–162) by 87 (74–94), distal portion 147 (139–157) by 75 (70–83). Narrow, coiled ejaculatory duct with numerous prostatic gland cells. Genital pore slightly dextral to posterior portion of pharynx.

Ovary overlapping posterior margin of acetabulum, slightly lobate, 253 (198–299) by 198 (187–209). Seminal receptacle anterosinistral to ovary, 218 (180–288) by 113 (97–126); ootype dorsal to ovary. Uterus arising at posterior edge of ovary, forming longitudinal loops on both sides of body between testes and pharyngeal level; metraterm shorter than cirrus sac, muscular, leading to genital pore. Vitelline follicles in forebody, lateral to pharynx and anterior portion of ceca; occasional follicles joining fields dorsally. Intrauterine eggs nearest genital pore amber, 23 (22–24) by 16 (15–19) (N = 19).

Excretory pore subterminal; excretory bladder T-shaped, bifurcating at level of anterior portion of testes, stem 1.20 mm (0.88–1.47 mm) long.

Remarks

Loxogenoides loborchis differs from *L. bicolor* in egg size, sucker width ratio, body size and shape, pigmentation, and characteristics of the gut. Eggs of *L. loborchis* are shorter and wider (23 by 16) than the more elongate eggs of *L.*



Figures 1, 2. ***Loxogenoides loborchis*** **sp. n. 1. Ventral view of holotype. 2. Terminal genitalia. Figure 3.** ***Loxogenoides bicolor*** **from** ***Rana clamitans*****. Ventral view.**

bicolor (30 by 13). Sucker width ratio of *L. loborchis* (1:0.75) is also different from *L. bicolor* (1:0.61). *Loxogenoides loborchis* is much smaller than *L. bicolor* and has a distinctly different shape. The characteristic broad anterior end and tapering posterior extremity seen in *L. bicolor* is not apparent in *L. loborchis*.

Loxogenoides loborchis lacks the yellow pigmented body and pink or red colored suckers that give *L. bicolor* its name. The presence or lack of pigmentation is evident in whole mount specimens. Prepared slides of *L. bicolor* retain a yellowish hue that reduces clarity of organs, but *L. loborchis* prepared in a similar manner become very clear. The ceca of all *L. bicolor* examined were straight, whereas those in *L. loborchis* have outpocketings.

Loxogenoides bicolor (Krull, 1933) Kaw, 1945
(Fig. 3)

Loxogenes bicolor Krull, 1933.
Larelmintha polyorchis Lautenschlager and Cheng, 1958.
Loxogenoides polyorchis (Lautenschlager and Cheng, 1958) Yamaguti, 1971.

HOSTS AND LOCALITIES: *Rana catesbeiana* Shaw, 1802, Georgia, Kentucky, North Carolina; *Rana clamitans* Latreille, 1802, Maryland, Virginia; *Rana sphenocephala* Cope, 1886 (=*Rana utricularia*), Maryland, Virginia.

SITES: Bile ducts of liver, pancreas, stomach, and body cavity.

REDESCRIPTION (based on seven mature specimens): Body elongate, 5.06 mm (3.66–6.00 mm) by 2.71 mm (2.25–3.29 mm), broader anteriorly, tapering to posterior extremity, widest at midbody. Living worms bright yellow to orange with reddish-colored suckers. Entire body spinose, spines 15 (13–16) long. Oral sucker subterminal, 683 (588–808) by 720 (551–956). Prepharynx absent; esophagus short; pharynx 373 (368–382) by 299 (294–309). Ceca broad, straight, extending to posterior of body. Acetabulum slightly preequatorial, 441 (368–551) by 435 (342–551). Ratio of width of oral sucker to acetabulum 1:0.61 (1:0.58–1:0.63).

Testes slightly postequatorial, symmetrical, multilobed. Right testis 636 (515–952) by 404 (221–732), left testis 719 (456–952) by 368 (221–588). Cirrus sac preacetabular, elongate, 570 (432–735) by 151 (126–180). Seminal vesicle bipartite, 289 (221–414) by 139 (126–167). Narrow, coiled ejaculatory duct with prostatic gland cells. Genital pore slightly dextral to posterior portion of pharynx.

Ovary overlapping acetabulum, irregular in shape, 520 (404–654) by 383 (331–478). Seminal receptacle overlapping anterior margin of ovary, 340 (221–463) by 274 (170–456); ootype dorsal to ovary. Uterus long, irregularly coiled, extending from extreme anterior end of body to level of, or slightly posterior to testes. Metraterm shorter than cirrus sac, muscular, leading to genital pore. Vitelline follicles in forebody, concentrated laterally; numerous follicles joining fields dorsally. Intrauterine eggs nearest genital pore amber, 30 (28–31) by 13 (11–14) (N = 21).

Excretory pore subterminal; excretory bladder Y- to T-shaped, bifurcating at level of anterior portion of testes, stem 2.25 mm (1.28–2.56 mm) long.

Remarks

Krull (1933) based his description of *L. bicolor* on only the largest specimens and no information was available on variations present in this species. He stated that smaller specimens with the less extensive uterus tend to have large testes in proportion to the ovary, and therefore look quite different than the larger worms. These smaller specimens are no longer available for study (J. R. Lichtenfels, personal communication). Krull (1933) further noted the presence of a delicate Laurer's canal and a short, muscular cirrus, but these were not seen in any of

the specimens I examined. The holotype of *L. polyorchis* compares very closely with specimens of *L. bicolor,* and appears to be similar to what Krull (1933) described as the smaller specimens of *L. bicolor.* Body shape, egg size (30 (28–30) by 12 (11–14) (N = 18)), sucker width ratio (1:0.59), and the characteristics of the gut all compare favorably with *L. bicolor.* Although Lautenschlager and Cheng (1958) do not mention pigmentation, the type specimen appears pigmented. Because of these similarities, I consider *L. polyorchis* a junior synonym of *L. bicolor.*

Discussion

Krull (1933) recovered *L. bicolor* from *R. clamitans,* but *R. catesbeiana* and *Rana palustris* Le Conte, 1825 collected in the same location were negative. He therefore suggested the possibility of a marked host specificity. Campbell (1968) also recovered *L. bicolor* from *R. clamitans* but not from *R. catesbeiana* collected in the same location in Virginia. However, a marked host specificity does not exist because Brandt (1937) recovered *L. bicolor* from *R. catesbeiana* and *R. sphenocephala* in North Carolina and Byrd (1950) found two of 54 bullfrogs from Georgia and North Carolina infected with *L. bicolor.* In addition, one *R. catesbeiana* examined in the present study contained a single *L. bicolor.* In all of these studies the parasite burden did not exceed four *L. bicolor* in a single host. The prevalence rate also was low in these studies except for data presented by Brandt (1937). He reported prevalence rates as high as 44.7% for *L. bicolor* in *R. catesbeiana. Loxogenoides loborchis* presently seems to be similar in having low prevalence rates and a low intensity of infection. Studies are needed on life histories and host specificity of *L. bicolor* and *L. loborchis* to further clarify relationships between these species.

The generic diagnoses of Kaw (1945) and Yamaguti (1971) are emended in order to more reasonably delineate the characteristics of the genus.

Loxogenoides Kaw, 1945 Char. Emend.

Krullitrema Ogata, 1954.
Larelmintha Lautenschlager and Cheng, 1958.

DIAGNOSIS: Lecithodendriidae. Loxogenoidinae. Body elongate, flattened. Tegument spined. Cirrus sac elongate. Internal seminal vesicle bipartite. Uterus extensive or not, majority in anterior half of body. Metraterm present. Genital pore submedian, preacetabular. Excretory vesicle Y- to T-shaped with long stem. Excretory pore subterminal. Parasites of Amphibia.

TYPE SPECIES: *Loxogenoides bicolor* (Krull, 1933).

Acknowledgments

I sincerely appreciate the suggestions and help provided by M. H. Pritchard and M. J. Ulmer during the preparation of this manuscript. I also thank S. Nance and D. Strohlein for collecting the hosts, and R. A. Campbell and J. R. Lichtenfels for the loan of specimens. This study was supported in part by National Institutes of Health Grant AI 16472.

Literature Cited

Brandt, B. B. 1937. Parasites of certain North Carolina Salientia. Ecol. Monogr. 6:491–532.

Byrd, E. E. 1950. The excretory system in Trematoda. II. The excretory system of *Loxogenoides bicolor* (Krull, 1933). J. Parasitol. 36:139–144.

Campbell, R. A. 1968. A comparative study of the parasites of certain Salientia from Pocahontas State Park, Virginia. Va. J. Sci. 19:13–20.

Kaw, B. L. 1945. On the present status of the genus *Loxogenes*. Proc. Indian Acad. Sci. Sect. B 21:342–343.

Krull, W. H. 1933. *Loxogenes bicolor*, a new pigmented fluke from the frog, *Rana clamitans* Latr. Trans. Am. Microsc. Soc. 52:47–50.

Lautenschlager, E. W., and T. C. Cheng. 1958. *Larelmintha polyorchis* (Trematoda: Heterophyidae), a new fluke from the herring gull. Trans. Am. Microsc. Soc. 77:451–454.

Seitner, P. G. 1945. Studies on five new species of xiphidiocercariae of the Virgula type. J. Parasitol. 31:272–281.

Yamaguti, S. 1971. Synopsis of Digenetic Trematodes of Vertebrates. Vol. I. Keigaku, Tokyo. 1074 pp.

Fifth International Congress of Parasitology

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The 5th International Congress of Parasitology (ICOPA V) will be held at the Sheraton Centre, Toronto, Canada from 7–14 August 1982.

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Proc. Helminthol. Soc. Wash.
48(1), 1981, pp. 71–79

Additional Records of Digenetic Trematodes of Mammals from Taiwan[1]

JACOB H. FISCHTHAL AND ROBERT E. KUNTZ
Department of Biological Sciences, State University of New York at Binghamton, Binghamton, New York 13901, and
Department of Parasitology, Southwest Foundation for Research and Education, P.O. Box 28147, San Antonio, Texas 78284

ABSTRACT: Twenty-two digenetic trematodes of mammals are reported from Taiwan. Four new species are described: Lecithodendriidae, *Pseudocryptotropa taiwanense* from *Coelops frithii formosanus*, *Prosthodendrium* (*Prosthodendrium*) *taiwanense* and *Pycnoporus taiwanensis* from *Pipistrellus abramus*; Dicrocoeliidae, *Zonorchis taiwanensis* from *Callosciurus erythraeus centralis*. Previously known species reported are: Plagiorchiidae, *Plagiorchis* (*Plagiorchis*) *vespertilionis*, *P.* (*Multiglandularis*) *linkuoliangi*, *P.* (*M.*) *muris*, and *Encyclometra colubrimurorum*; Lecithodendriidae, *Lecithodendrium macrostomum*, *L. moedlingeri*, *Prosthodendrium* (*Prosthodendrium*) *cordiforme*, *P.* (*P.*) *parvouterus*, and *P.* (*P.*) *urna*; Echinostomatidae, *Echinostoma cinetorchis*, *E. gotoi*, *E. macrorchis*, and *Echinochasmus japonicus*; Dicrocoeliidae, *Platynosomoides muris*; Anchitrematidae, *Anchitrema sanguineum*; Heterophyidae, *Centrocestus caninus* and *Haplorchis pumilio*; Diplostomatidae, *Pharyngostomum cordatum*.

The digenetic trematodes of this report are part of the same collection from mammals reported by Fischthal and Kuntz (1975). Some of the species recorded in the 1975 paper are also mentioned herein since new hosts and/or localities are reported. Parasites were washed in saline, killed in hot water, and transferred immediately to FAA fixative; after 4–8 hr they were stored in 70% alcohol plus 2% glycerin. Host names preceded by an asterisk (*) represent new host records; prefectures followed by an asterisk are new locality records for Taiwan. Specimens of each trematode species have been deposited in the United States National Museum Helminthological Collection as noted. All measurements are in micrometers.

Lecithodendriidae

Pseudocryptotropa taiwanense sp. n. (Figs. 1, 2)

HOST: *Coelops frithii formosanus* Horikawa, tailless leaf-nosed bat (Chiroptera: Hipposideridae).

HABITAT: Small intestine.

LOCALITY: Yung Foh Lee, Yang Ming Shan, Taipei Prefecture.

DATE: 7 January 1958.

SPECIMENS DEPOSITED: No. 75780 (holotype and paratypes); No. 75781 (paratypes).

DESCRIPTION (measurements, except for eggs, are from holotype only): Body egg-shaped, spined, 585 long by 330 wide. Forebody 220 long; hindbody 288 long; forebody-hindbody length ratio 1:1.31. Oral sucker ventroterminal, 88 by 77.

[1] Contribution from the Department of Biological Sciences, State University of New York at Binghamton, Binghamton, New York 13901.

Acetabulum diameter 77. Sucker length ratio 1:0.88, width ratio 1:1. Prepharynx short, dorsal to oral sucker; pharynx diameter 39; esophagus narrow, 73 long; cecal bifurcation 12 preacetabular; ceca lined with large cuboidal to columnar cells, extending to testes level; postcecal space 200 long. Excretory vesicle Y-shaped; pore terminal.

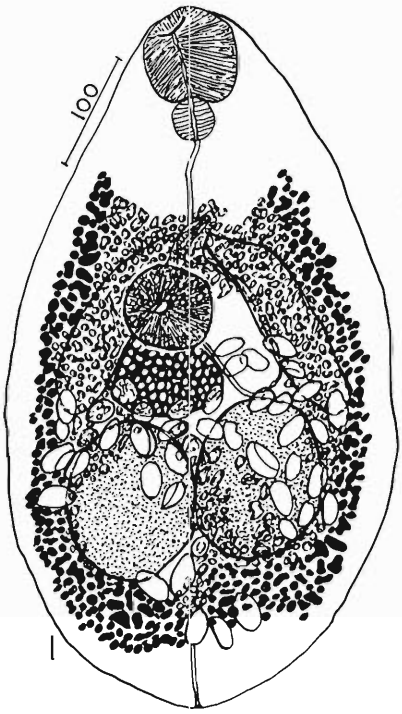
Testes 2, oval, smooth, subsymmetrical to symmetrical, contiguous to slightly separated, 133 by 110; posttesticular space 102 long. Cirrus sac 215 by 62, commencing 40 postacetabular near anterior margin of left testis, ascending diagonally toward median side of ovary and dorsal to sinistral part of acetabulum, turning sharply sinistral just preacetabular, ventral to cecal bifurcation, turning sharply dorsal and extending to dorsal surface, terminating 60 preacetabular. Seminal vesicle saccular, 152 by 60. Prostatic vesicle 60 by 39, commencing at sharp preacetabular turn of cirrus sac, lined with large cuboidal cells, surrounded by prostate cells. Cirrus extending dorsally. Genital pore dorsal, prebifurcal, sinistral and close to posterior part of esophagus. Ovary dextral, smooth, 80 by 95, posterodorsal to acetabulum, slightly separated from right testis. Seminal receptacle 50 by 105, overlapping ovary and testes dorsally. Vitellaria commencing at esophageal level, extending to posterior extremity, confluent dorsally between acetabular level and posterior part of esophagus as well as posttesticular, from latter space some follicles extending anteriorly essentially intertesticular, in lateral usually uninterrupted fields between acetabulum and testes, occasionally with very slight interruption at posterolateral part of one or both testes. Uterus with few coils between acetabulum and posterior extremity, ventral, lying mainly between gonads and proximal part of cirrus sac. Metraterm sinistral to and much shorter than cirrus sac. Eggs yellow-brown to brown, operculate, 15 measuring 34–39 (36.7) by 21–26 (23.7).

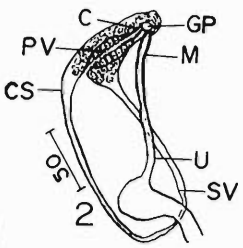
Discussion

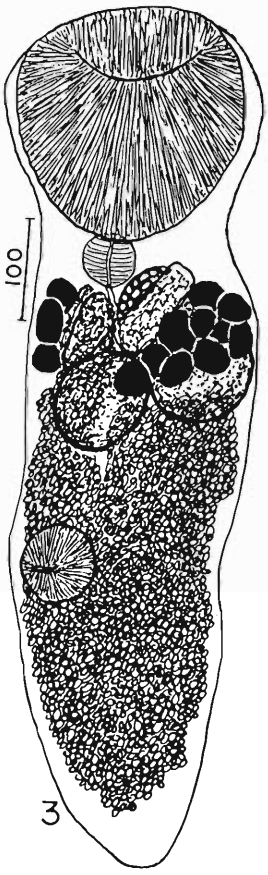
Our collection contains 79 worms from one host. All but the holotype were too macerated for measurements although pertinent observations could be made from some. Three species are known in the genus: *P. macrotestis* (Belopolskaja, 1954) Yamaguti, 1958, from a caprimulgid bird from the eastern Siberian maritime region; *P. nycticebi* (Rohde, 1962) Khotenovskii, 1965, from a lorisid primate from Malaya; *P. malaysiae* Fischthal and Kuntz, 1973, from a cuculid bird from North Borneo. They differ from the new species in having ceca extending only to the acetabular level, the shape (J or slightly curved) and posterior extent of the cirrus sac (preacetabular or dorsal to acetabulum), the genital pore lying at the level of the cecal bifurcation or postbifurcal, and the vitelline fields commencing at the pharyngeal level and always widely interrupted opposite the testes. *P. macrotestis* differs further in having a much shorter esophagus, the genital pore con-

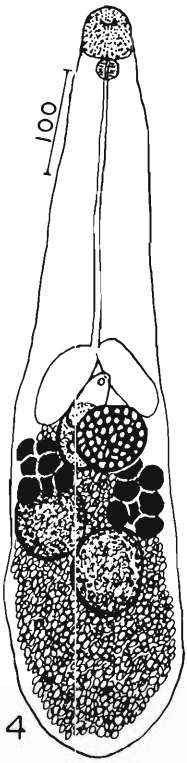
→

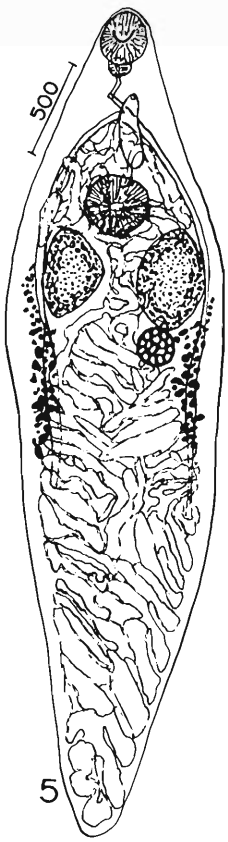
Figures 1–6. ***Pseudocryptotropa taiwanense*** **sp. n. 1. Whole mount, holotype, ventral view. 2. Terminal genitalia, holotype.** ***Prosthodendrium (Prosthodendrium) taiwanense*** **sp. n. 3. Whole mount, holotype, ventral view.** ***Pycnoporus taiwanensis*** **sp. n. 4. Whole mount, holotype, dorsal view.** ***Zonorchis taiwanensis*** **sp. n. 5. Whole mount, holotype, ventral view. 6. Terminal genitalia, holotype. Abbreviations: C, cirrus; CS, cirrus sac; GP, genital pore; M, metraterm; PV, prostatic vesicle; SV, seminal vesicle; U, uterus.**

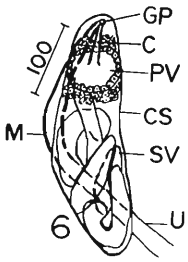












siderably lateral, and the vitellaria in separate fields anteriorly. *P. nycticebi* differs further in having the testes very widely separated from one another, the genital pore considerably lateral, and larger eggs with granular shells. *P. malaysiae* differs further in having the testes very widely separated from one another, and a bipartite seminal vesicle, the distal part of which is tubular, thick-walled, and muscular.

Prosthodendrium (Prosthodendrium) taiwanense sp. n.

HOST: *Pipistrellus abramus* Temminck, Japanese pipistrelle (Chiroptera: Vespertilionidae).

HABITAT: Small intestine.

LOCALITY: Taipei, Taipei Prefecture.

DATE: 20 August 1957.

SPECIMENS DEPOSITED: No. 75778 (holotype and 3 paratypes).

DESCRIPTION: Body elongate, narrowest just behind oral sucker where body constricted, widest at oral sucker level, extremities rounded, 760–840 long, width at oral sucker 203–242 and depth 235–275. Forebody 455–505 long; hindbody 215–250 long; forebody-hindbody length ratio 1:0.45–0.50. Oral sucker ventroterminal, very large, 215–235 long by 190–220 wide by 220–265 deep. Acetabulum median, 75–85 by 70–73 by 70–78. Sucker length ratio 1:0.32–0.40, width ratio 1:0.33–0.37, depth ratio 1:0.29–0.32. Prepharynx very short; pharynx 53–65 by 56–60 by 50–61; esophagus very short; ceca in holotype short, inflated, right cecum extending posterodextrally to lateral side of right testis, left cecum extending anterosinistrally along median side of left testis and lying ventral to ovary. Excretory vesicle obscured by uterus; pore terminal.

Testes 2, smooth, contiguous, subsymmetrical to diagonal, lying just anterior to acetabulum to 73 preacetabular at vitellarian level; right testis 75–120 by 94 by 75–100, left testis 60–92 by 65–99 by 60. Cirrus sac and genital pore not observed. Ovary smooth, median, pretesticular, contiguous with to slightly overlapping testes, anterior part may lie as far anteriorly as esophagus level, 90–105 by 87 by 95. Vitelline follicles large, in 2 anterolateral masses of 6–11 (usually 10) follicles each at cecal level, usually commencing at oral sucker level but at pharyngeal level in holotype. Uterus filling body from posterior part of vitellaria to just short of posterior extremity. Eggs yellow to yellow-brown, operculate, 20 measuring 19–24 (21.8) by 10–14 (12.4).

Discussion

The four worms were from one host. Two of the paratypes were mounted in lateral view. The new species is morphologically closest to *P. (P.) piriforme* Yamaguti, 1939, from a rhinolophid bat from Japan, and *P. (P.) chilostomum* (Mehlis, 1831) Macy, 1936, from a variety of rhinolophid and vespertilionid bats, including *Pipistrellus abramus,* from Europe and Vietnam. Both differ from the new species in body shape as well as in having a larger sucker ratio, the testes widely separated from one another, and larger eggs.

Pycnoporus taiwanensis sp. n.
(Fig. 4)

HOST: *Pipistrellus abramus* Temminck, Japanese pipistrelle (Chiroptera: Vespertilionidae).

HABITAT: Small intestine.

LOCALITY: Taipei, Taipei Prefecture.

DATE: 20 August 1957.

SPECIMENS DEPOSITED: No. 75779 (holotype and 2 paratypes).

DESCRIPTION: Body elongate, narrow, extremities rounded, 715–885 long by 150–169 wide by 185 deep. Forebody 370–440 long; hindbody 280–455 long; forebody-hindbody length ratio 1:0.76–0.88. Oral sucker ventroterminal, truncated posteriorly, 47–50 long by 48–50 wide. Acetabulum 60–75 by 58–60 by 95. Sucker length ratio 1:1.28–1.30, width ratio 1:1.20–1.42. Pharynx 17–20 by 20–26; esophagus 245–265 long; cecal bifurcation 40–102 preacetabular; ceca short, inflated, preacetabular to lying lateral to latter, ceca 80–105 by 35–46 by 45. Excretory vesicle obscured by uterus; pore terminal.

Testes 2, smooth, diagonal, contiguous and occasionally overlapping; anterior testis 75–110 by 70–82 by 95, lying 11–60 postacetabular, partly overlapping level of vitelline fields anteriorly; posterior testis 85–105 by 70–85 by 95, lying 50–125 postacetabular, occasionally just overlapping level of vitelline fields anteriorly; posttesticular space 145–210 long. Cirrus sac oval, diagonally oriented between ceca and overlapping acetabulum, 65–100 by 28–35 by 45, containing convoluted seminal vesicle. Genital pore submedian, preacetabular, postbifurcal. Ovary smooth, dorsolateral to acetabulum, 65–80 by 75–80 by 85. Vitelline follicles in 2 lateral masses of 6–7 follicles each, commencing at level of posterior half of acetabulum. Uterus filling hindbody. Eggs yellow to yellow-brown, operculate, 15 measuring 16–19 (17.6) by 9–10 (9.8).

Discussion

The three worms were from one host. One paratype mounted in lateral view has part of the body from the posterior part of the esophagus anteriorly missing. The new species is morphologically closest to *P. transversus* Ozaki, 1929, from the same host species from Japan, and *P. proattenuatus* Salem, 1971, from a molossid bat from India. *P. transversus* differs from the new species in having the posterior margin of the oral sucker rounded, a much greater sucker ratio (approximately 1:2.73–3.35), the testes widely separated from one other by the uterus, the cirrus sac transversely oriented, the ovary postacetabular, and the vitellaria a distance postacetabular. *P. proattenuatus* differs in having a pyriform body, the posterior margin of the oral sucker rounded, a greater sucker ratio (approximately 1:3.35–3.37, length; 1:1.79–1.93, width), and the testes subsymmetrical and widely separated from one another by the uterus.

Dicrocoeliidae

Zonorchis taiwanensis sp. n.

(Figs. 5, 6)

HOST: *Callosciurus erythraeus centralis* Bonhote, central Formosan red-bellied squirrel (Rodentia: Sciuridae).

HABITAT: Bile duct.

LOCALITY: Wu-sheh, Nan-tou Prefecture.

DATE: 16 April 1959.

SPECIMENS DEPOSITED: No. 75782 (holotype and 2 incomplete paratypes).

DESCRIPTION: Body elongate, tapering to blunt point at both extremities, widest at testes level, 4,255 long (holotype) by 1,040–1,090 wide. Forebody 810 long

(holotype); hindbody 3,030–3,120; forebody-hindbody length ratio (holotype) 1:3.85. Oral sucker 287 by 255 (holotype). Acetabulum 325–355 by 325–335. Sucker ratios (holotype), length 1:1.13, width 1:1.27. Pharynx diameter 110 (holotype); esophagus length 175 (holotype); cecal bifurcation 250–255 preacetabular; ceca narrow, extending postvitellarian but do not reach posterior extremity. Excretory vesicle obscured by uterus; pore terminal.

Testes 2, slightly lobed, symmetrical, intercecal, just postacetabular, separated by ascending uterus; right testis 490–555 by 355–360, left testis 535–600 by 335–375. Cirrus sac slightly curved, 315 by 102 (holotype), commencing 75 preacetabular; containing tubular coiled seminal vesicle, short prostatic vesicle (65 by 85) surrounded by prostate cells, and muscular cirrus. Genital pore submedian, sinistral at esophageal level, in holotype lying 138 posterior to oral sucker, 85 postpharyngeal, and 355 preacetabular. Ovary smooth, 205 by 210–240, dextral in 1 worm, sinistral in 2, contiguous to overlapping testis on its side; postovarian body length 2,335–2,425. Seminal receptacle dorsal to ovary, 155–175 by 185–195. Vitelline follicles in lateral extracecal fields but may overlap ceca, right field 935–1,000 long, left field 765–1,010 long, field on ovarian side shorter than other, commencing at level of midlength of testes to posterior part of latter; postvitellarian body 1,585–1,925 long. Uterus filling all of hindbody except parts occupied by gonads and vitellaria, coils present dorsal to acetabulum and preacetabularly up to cecal bifurcation. Metraterm muscular, shorter than cirrus sac. Eggs yellow-brown to brown, thick-shelled, operculate, 15 measuring 53–60 (57.1) by 36–40 (38.2).

Discussion

Only the holotype specimen was entire. Both paratypes had the prebifurcal part of the body missing, and in addition one of them had most of the posttesticular body gone. Three species of the genus are known from *Callosciurus* spp.: *Z. borneoensis* Fischthal and Kuntz, 1965, from *C. prevostii pluto* Gray and *C. notatus dilutus* Miller from North Borneo; *Z.* sp. Rohde, Lee, and Lim, 1968, from *C. notatus* (Boddaert) and *C. caniceps* (Gray) from Malaya; *Z. callosciuri* Nguyen Thi Le, 1968, from *C. erythraeus* and *C. macclelandi* from Vietnam. *Z. borneoensis* and *Z.* sp. agree in every respect except that in the former the testes are close together and the ovary is smooth while in the latter the testes are well separated by the uterus and the ovary is slightly lobed. Betterton and Lim (1977), in an analysis of *Zonorchis* from small mammals of Malaysia, noted the great similarity between these two forms and reported that rounded as well as deeply lobed ovaries were observed among otherwise exactly similar worms from a single host. They concluded that probably this character is unimportant taxonomically. The new species differs from *Z. borneoensis* and *Z.* sp. in having a smaller sucker ratio, much shorter vitelline fields which commence at the level of the posterior half of the testes, uterine coils dorsal and anterior to the acetabulum, and much larger eggs. *Z. callosciuri* differs from the new species in having a larger sucker ratio, the testes nearly lateral to the acetabulum, the ovary well separated from the testes, longer vitelline fields commencing at the acetabular level or preacetabular, and smaller eggs, and in lacking uterine coils dorsal and anterior to the acetabulum. In the genus only *Z. goliath* Travassos, 1945, from an opossum from Brazil has preacetabular uterine coils, but it differs from the new species in having

the sides of the body nearly parallel from the acetabulum to well posterior to the vitelline fields, a larger sucker ratio, longer vitelline fields commencing more anteriorly, the ovary well separated from the testes, and smaller eggs.

Previously Known Species

1. *Plagiorchis* (*Plagiorchis*) *vespertilionis* (Müller, 1784) Braun, 1900 (Plagiorchiidae) from the small intestine and liver of the long-winged bat, *Miniopterus schreibersi* Kuhl (Chiroptera: Vespertilionidae), *Pipistrellus abramus,* and **Coelops frithii formosanus* from Taipei and Yung Foh Lee, Yang Ming Shan, Taipei Prefecture* and Sun Moon Lake, Nan-tou Prefecture*; collected 5 March 1957, 7, 28 January 1958. Specimens deposited: No. 75783 (*M. schreibersi*); No. 75784 (*P. abramus*); No. 75781 (*C. frithii*).
2. *Plagiorchis* (*Multiglandularis*) *linkuoliangi* Tang, 1941, from the small intestine of the house shrew, *Suncus murinus* L. (Insectivora: Soricidae) from Hsin Yi Lu and Taipei, Taipei Prefecture*; collected 1957–1958. Specimens deposited: No. 75785.
3. *Plagiorchis* (*Multiglandularis*) *muris* (Tanabe, 1922) Shults and Skvortsov, 1931, from the small intestine of the Norway rat, *Rattus norvegicus* Berkenhout (Rodentia: Muridae), and the house rat, *Rattus rattus* (L.), from Taipei, Taipei Prefecture*; collected 5 August, 18 November, 12 December 1957, and 3 January 1958. Specimens deposited: No. 75786 (*R. norvegicus*); No. 75787 (*R. rattus*).
4. *Encyclometra colubrimurorum* (Rudolphi, 1819) Dollfus, 1929 (Plagiorchiidae) from the small intestine of **Rattus norvegicus, *R. rattus,* and the Indian bandicoot, **Bandicota indica nemorivaga* Hodgson (Rodentia: Muridae) from Shih-lin and Yung Foh Lee, Yang Ming Shan, Taipei Prefecture and Shan-sheng and Pu-yen, Chang-hua Prefecture; collected 24 April, 26, 30 July, 10 September 1958. Specimens deposited: No. 75788 (*R. norvegicus*); No. 75789 (*R. rattus*); No. 75790 (*B. indica*). This is the first record of the genus from mammals. Snakes, rarely lizards, serve as the usual definitive hosts, while amphibians harbor the metacercaria.
5. *Lecithodendrium macrostomum* (Ozaki, 1929) Macy, 1936 (Lecithodendriidae) from the small intestine of *Pipistrellus abramus* from Taipei, Taipei Prefecture*; collected 21 July 1958. Specimens deposited: No. 75791.
6. *Lecithodendrium moedlingeri* (Pande, 1935) Dollfus, 1937, from the small intestine of **Pipistrellus abramus* from Taipei, Taipei Prefecture*; collected 27 June 1958. Specimen deposited: No. 75792.
7. *Prosthodendrium* (*Prosthodendrium*) *cordiforme* (Braun, 1900) Macy, 1936 (Lecithodendriidae) from the small intestine of the large leaf-nosed bat, *Hipposideros armiger terasensis* Kishida (Chiroptera: Hipposideridae) from Yung Foh Lee, Yang Ming Shan, Taipei Prefecture*; collected 7 January 1959. Specimen deposited: No. 75793.
8. *Prosthodendrium* (*Prosthodendrium*) *parvouterus* (Bhalerao, 1936) Dubois, 1955, from the small intestine of *Miniopterus schreibersi* from Sun Moon Lake, Nan-tou Prefecture*; collected 28 January 1958. Specimens deposited: No. 75794.
9. *Prosthodendrium* (*Prosthodendrium*) *urna* (Looss, 1907) Macy, 1936, from the small intestine of *Pipistrellus abramus* and **Coelops frithii formosanus*

from Taipei and Keelung, Taipei Prefecture*; collected 5 March, 16 October 1957 and 27 June, 17, 21 July 1958. Specimens deposited: No. 75784, 75791, 75792 (*P. abramus*): No. 75780, 75781, 75803 (*C. frithii*).

10. *Echinostoma cinetorchis* Ando and Ozaki, 1923 (Echinostomatidae) from the small intestine of **Suncus murinus* and the house mouse, **Mus musculus* (L.) from Hsin Yi Lu, Taipei Prefecture and Shan-sheng, Chang-hua Prefecture; collected 6 September 1957, 24 April 1958. Specimens deposited: No. 75795 (*S. murinus*); No. 75796 (*M. musculus*).
11. *Echinostoma gotoi* Ando and Ozaki, 1923, from the small intestine of the small Formosan civet, **Viverricula indica pallida* Gray (Carnivora: Viverridae) from Wu-lai, Taipei Prefecture*; collected 11, 16 December 1958. Specimens deposited: No. 75797.
12. *Echinostoma macrorchis* Ando and Ozaki, 1923, from the small intestine of the Formosan brown country rat, **Rattus losea* Swinhoe (Rodentia: Muridae) and *R. norvegicus* from Yung Foh Lee, Yang Ming Shan, Taipei Prefecture, Ping-tung, Ping-tung Prefecture*, and Pu-yen, Chang-hua Prefecture*; collected 9, 18 December 1957, 7 February, 28, 29 July, 1 August, 4 September 1958. Specimens deposited: No. 75798 (*R. losea*); No. 75799 (*R. norvegicus*).
13. *Echinochasmus japonicus* Tanabe, 1926 (Echinostomatidae) from the small intestine of **Suncus murinus* from Shan-sheng, Chang-hua Prefecture*; collected 18 August 1958. Specimens deposited: No. 75800.
14. *Platynosomoides muris* (Shcherbakova, 1942) Yamaguti, 1971 (Dicrocoeliidae) from the small intestine of the spinous country rat, **Rattus coxinga coxinga* Swinhoe (Rodentia: Muridae) from Yung Foh Lee, Yang Ming Shan, Taipei Prefecture; collected 13 November 1957, 4 May 1958. Specimens deposited: No. 75801.
15. *Anchitrema sanguineum* (Sonsino, 1894) Looss, 1899 (Anchitrematidae) from the small intestine of **Pipistrellus abramus*, **Miniopterus schreibersi*, and **Coelops frithii formosanus* from Hsin Yi Lu and Keelung, Taipei Prefecture* and Sun Moon Lake, Nan-tou Prefecture*; collected 19 September, 16 October 1957, 28 January 1958. Specimens deposited: No. 75802 (*P. abramus*); No. 75783 (*M. schreibersi*); No. 75803 (*C. frithii*).
16. *Centrocestus caninus* (Leiper, 1913) Travassos, 1932 (Heterophyidae) from the liver of **Suncus murinus* from Shan-sheng, Chang-hua Prefecture*; collected 15 August 1958. Specimens deposited: No. 75804.
17. *Haplorchis pumilio* (Looss, 1896) Looss, 1899 (Heterophyidae) from the small intestine of **Suncus murinus*, **Pipistrellus abramus*, and **Rattus rattus* from Taipei, Taipei Prefecture and Shan-sheng, Chang-hua Prefecture*; collected 23 December 1957, 21 July, 15 August 1958. Specimens deposited: No. 75804 (*S. murinus*); No. 75806 (*P. abramus*); No. 75805 (*R. rattus*).
18. *Pharyngostomum cordatum* (Diesing, 1850) Ciurea, 1922 (Diplostomatidae): Adult from the small intestine of the domestic cat from Ping-tung, Ping-tung Prefecture*; collected 11 September 1958. Specimens deposited: No. 75807. Metacercaria from the small intestine, body cavity, and liver of **Suncus murinus* and **Rattus rattus* from Shan-sheng and Pu-yen, Chang-hua Prefecture; collected 21 April, 25, 26 July, 15, 18 August 1958. Specimens deposited: No. 75808 (*S. murinus*); No. 75809 (*R. rattus*).

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Literature Cited

Betterton, C., and B.-L. Lim. 1977. Patterns in the morphological variation of *Zonorchis* and *Skrjabinus* (Trematoda: Dicrocoelidae) from small mammals in Malaysia. Int. J. Parasitol. 7:73–82.

Fischthal, J. H., and R. E. Kuntz. 1975. Some digenetic trematodes of mammals from Taiwan. Proc. Helminthol. Soc. Wash. 42:149–157.

Obituary Notice

EVERETT ELMER WEHR
November 5, 1895–September 6, 1980

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Natural Infections of the Dermatitis-Producing Schistosome *Gigantobilharzia huronensis* Najim, 1950 in Passerines in Southeastern Michigan[1]

BRIAN C. STROHM,[2] HARVEY D. BLANKESPOOR,[3] AND PETER G. MEIER
Department of Environmental and Industrial Health, The University of Michigan, Ann Arbor, Michigan 48109

ABSTRACT: Three out of the 10 schistosome infections found in common grackles (*Quiscalas quiscala*) were identified as *Gigantobilharzia huronensis*. The same schistosome was found in four out of nine infected red-winged blackbirds (*Agelaius phoeniceus*) from 13 birds sampled. Since these are two new host records for *G. huronensis*, these findings suggest that a wider role is played by passerines in the epizootiology of avian schistosomiasis than previously indicated.

Cercariae of the genus *Trichobilharzia* have been identified as the etiologic agent of schistosome dermatitis in man; waterfowl are considered the primary definitive hosts (Cort, 1950). Recent evidence suggests that species of *Gigantobilharzia* may be underrated as a cause of schistosome dermatitis in North America. Brackett (1942) found *G. gyrauli* in yellow-headed (*Xanthocephalus xanthocephalus*) and red-winged blackbirds (*Agelaius phoeniceus*) in Wisconsin. Najim (1956) described *G. huronensis* from an American goldfinch (*Spinus tristus*) and cardinal (*Cardinalis cardinalis*) in southeastern Michigan. Recently, Guth et al. (1979) found red-winged blackbirds and common grackles (*Quiscalas quiscala*) to be infected with *Gigantobilharzia sp.* in Michigan. The present study is an effort to elucidate the epizootiology of *Gigantobilharzia huronensis*, for which limited field and laboratory information exists.

Materials and Methods

Birds were collected in southeastern Michigan during 1976 and 1977. They were shot, packed in ice, and transported to the laboratory. There, they were identified, sexed, and eviscerated within 2 to 3 hr. The intestines and liver were placed in avian Ringer's solution and stored in a cooler for examination within 2 days. A longer delay resulted in rapid deterioration of the schistosomes.

In examining the viscera, the intestine was cut into sections, split, rinsed, and teased apart in a Syracuse dish of avian Ringer's solution for examination with a dissecting microscope. If worms or eggs were not found, the fecal material was diluted in aerated tap water and examined for eggs and miracidia (McMullen and Beaver, 1945). The liver was pressed between glass plates and examined for worms and eggs. For identification, recovered schistosomes were fixed in warm 10% buffered formalin or AFA, and prepared as whole mounts, stained with Mayer's paracarmine, and counterstained with fast green. Mounted specimens

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[2] Present address: Duke University Medical Center, Office of Environmental Safety, Durham, North Carolina 27710.

[3] Present address: Biology Department, Hope College, Holland, Michigan 49423.

were identified using the taxonomic keys of Yamaguti (1971), Farley (1971), and Najim (1956).

Results

Thirteen red-winged blackbirds and 10 common grackles were examined. Schistosome infections were found in nine (69.2%) red-winged blackbirds. *G. huronensis* infections were confirmed in four of the red-winged blackbirds. Worm fragments obtained from the other five birds could not be positively identified. Three red-winged blackbirds were heavily infected with over 15 worms each. The others had fewer than 10 worms each.

All 10 common grackles harbored schistosome infections. *G. huronensis* infections were identified in three birds. Worm fragments extricated from the remaining birds were insufficient for species identification. The schistosome burden in five of the grackles exceeded 15 worms per bird. Three grackles harbored 10 to 14 worms and the remaining had less than 10 worms each. In one male, the infection was so heavy as to be evident to the unaided eye; the adult schistosomes appeared as faint black lines along the intestine.

In heavy infections, worms were in tangled masses in the mesenteric veins. Eggs were often present in the mucosa and villi of the posterior half of the intestine. In the villi, eggs were frequently observed stacked one on the other. No eggs or worms were found in the liver.

Discussion

A high percentage of red-winged blackbirds and common grackles collected in Washtenaw, Livingston, and Oakland counties were infected with schistosomes. The only species identified was *G. huronensis*. This is the first report of red-winged blackbirds and common grackles serving as definitive hosts for this organism. A large number of unidentifiable schistosome fragments were obtained from several birds. These may or may not have been *G. huronensis*.

The infection rates in this study were comparable to the *G. gyrauli* infection rates of 83% for yellow-headed blackbirds and 60% for red-winged blackbirds reported by Brackett (1942), but considerably higher than the *Gigantobilharzia sp.* infection rates of 14.8% in grackles and 11.0% in red-winged blackbirds observed by Guth et al. (1979). Their procedure was limited to fecal examination for eggs, whereas birds were sacrificed by Brackett (1942) and in the present study, revealing both patent and prepatent infections. The latter procedure gave a more accurate estimate of the schistosome burden.

The habits of red-winged blackbirds and grackles predispose them to avian schistosomiasis. They migrate into southeastern Michigan in the early spring. Males usually arrive first and establish breeding territories. Red-winged blackbirds prefer marsh nesting and feeding areas, but are adaptable to other sites (Albers, 1975). Grackles have been observed nesting in a variety of habitats ranging from marshes to holes in tree stumps and have the habit of lining their nests with mud (Bent, 1958). Habitat preference and behavior patterns bring the birds into frequent contact with water during the spring when the *Gigantobilharzia sp.* infection rate is highest in the gastropod intermediate host (Strohm, 1979). Repeated contact with the water, at this time, provides successive exposure to infection.

Heretofore, waterfowl have been implicated as a primary source of schistosome dermatitis infection. This study indicates that passerine birds should be given more attention, and that the role of red-winged blackbirds and common grackles in the epizootiology of avian schistosomiasis and schistosome dermatitis has been underrated.

Literature Cited

Albers, P. H. 1975. Avian habitat selection in a region of intensive agriculture: The red-winged blackbird. Ph.D. Dissertation. The University of Michigan, Ann Arbor. 189 pp.

Bent, A. C. 1958. Life histories of North American black-birds, orioles, tangers and allies. U.S. Natl. Mus. Bull. 211. 549 pp.

Brackett, S. 1942. Five new species of avian schistosomes from Wisconsin and Michigan with the life cycle of *Gigantobilharzia gyrauli* (Brackett, 1940). J. Parasitol. 28:25–42.

Cort, W. W. 1950. Studies on schistosome dermatitis XI. Status of knowledge after more than twenty years. Am. J. Hyg. 52:251–307.

Farley, J. 1971. A review of the family Schistosomatidae: Excluding the genus *Schistosoma* from mammals. J. Helminthol. 65:289–320.

Guth, B. D., H. D. Blankespoor, R. L. Reimink, and W. C. Johnson. 1979. Prevalence of dermatitis-producing schistosomes in natural bird populations of lower Michigan. Proc. Helminthol. Soc. Wash. 46:58–63.

McMullen, D. B., and P. C. Beaver. 1945. Studies on schistosome dermatitis IX. The life cycles of three dermatitis-producing schistosomes from birds and a discussion of the subfamily Bilharziellinae (Trematoda: Schistosomatidae). Am. J. Hyg. 42:128–155.

Najim, A. T. 1956. Life history of *Gigantobilharzia huronensis* Najim, 1950. A dermatitis-producing bird blood fluke. (Trematoda—Schistosomatidae). Parasitology 46:443–469.

Strohm, B. C. 1979. The epizootiology and epidemiology of schistosome dermatitis in southeastern Michigan. Ph.D. Dissertation. The University of Michigan. University Microfilms, Ann Arbor, Michigan. 170 pp.

Yamaguti, S. 1971. Synopsis of Digonetic Trematodes of Vertebrates. Keigaku Publ. Co., Tokyo, Japan. 1974 pp.

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Infectivity of *Amblosoma suwaense* (Trematoda: Brachylaimidae) in the Domestic Chick

BERNARD FRIED AND MARTIN S. SCHNIER
Department of Biology, Lafayette College, Easton, Pennsylvania 18042

ABSTRACT: Anesthetized domestic chicks were infected per cloaca with free metacercariae of *Amblosoma suwaense* removed directly from *Campeloma decisum* snails (fresh metacercariae) or metacercariae maintained for 24 hr in sterile Locke's at 38 ± 1°C (conditioned metacercariae). Worms from fresh metacercariae established only in the cloaca and were not recovered after 24 hr postinoculation. Worms from conditioned metacercariae established mainly in the bursa of Fabricius and were recovered up to 4 days postinoculation. Ovigerous worms were not obtained. Worms from both sites fed on host mucosa and blood, caused hemorraghic spots where suckers contacted host tissue, and showed a tendency to pair. The mean body area of worms from chicks was markedly greater than that of metacercariae. However, the area of the ovary of worms from chicks was markedly less than that of metacercariae.

Shimazu (1974) described the free (unencysted) brachylaimid metacercaria of *Amblosoma suwaense* from the viviparid snail *Sinotaia quadrata* in Japan, and Font (1980) recently found this metacercaria in *Campeloma decisum* snails in Wisconsin, USA. Adults of this parasite have not been described from natural or experimental hosts, although Shimazu (1974) and Font (1980) grew metacercariae to ovigerous adults in chick embryos. Schnier and Fried (1980) obtained ovigerous adults in vitro within 4 days in NCTC 135 plus 20% hens' egg yolk. Shimazu (1974) and Font (1980) were unable to infect domestic chicks with metacercariae and recent unpublished studies in our laboratory using unanesthetized chicks and per cloacal exposure were also unsuccessful. Fried and Harris (1971) used the cloacal drop procedure on anesthetized chicks to obtain infection with *Leucochloridiomorpha constantiae* and Herman and Bacha (1978) used this procedure to infect chicks with *Himasthla quissetensis* cercariae. The purpose of this study was to infect anesthetized chicks per cloaca with *A. suwaense* metacercariae.

Materials and Methods

Amblosoma suwaense metacercariae were removed from the visceral epithelium of *C. decisum* snails (Schnier and Fried, 1980), rinsed in sterile Locke's solution and used immediately for infection studies (fresh metacercariae), or were used following maintenance for 24 hr at 37 ± 1°C in Locke's (conditioned metacercariae). Conditioned metacercariae were live and active at 24 hr, but showed no postmetacercarial growth or development.

Chicks, 1 and 2 days old, were anesthetized with Equi-Thesin (Fried and Berry, 1961) and infected individually per cloaca with either five fresh or five conditioned metacercariae. A petri dish containing Locke's was placed under each chick to collect worms released in the stool, and chicks that defecated worms within 15 min were reinfected with an equal number of worms. Chicks remained anesthetized for 2–3 hr and were observed for the loss of worms in the stool up to 5 hr postinoculation.

Chicks were necropsied 1 to 5 days postinoculation and the lower ileum, caeca, rectum, bursa of Fabricius, and cloaca were examined. Metacercariae and worms

Table 1. Summary of *Amblosoma suwaense* infectivity data in chicks.

No. of chicks exposed to fresh (F) or conditioned (C) metacercariae		Age of chicks (days) at necropsy	No. of chicks infected with fresh (F) or conditioned (C) metacercariae		Total no. of worms in cloaca (Cl) or bursa of Fabricius (B)	
F	C		F	C	Cl	B
6	–	1	3	–	4	0
–	5	1	–	3	1	2
3	–	2	0	–	0	0
–	3	2	–	0	0	0
3	–	3	0	–	0	0
–	3	3	–	2	0	2
2	–	4	0	–	0	0
–	6	4	–	1	0	1
–	2	5	–	0	0	0
Total 14	19	–	3	6	5	5

from chicks were studied live, then prepared as stained whole mounts, and measured following the model of Berntzen and Macy (1969). Measurements were made of relative body area (product of length times midacetabular width) and area of the gonads and vitellaria.

To determine if conditioning of a brachylaimid metacercaria had subsequent effects on growth and development, additional chicks were each inoculated per cloaca with five metacercariae of *L. constantiae* maintained for 24 hr at 37 ± 1°C in Locke's. *L. constantiae* metacercariae were obtained from the uterus of *Campeloma decisum* snails as described previously (Harris et al., 1972). Chicks were necropsied 4 days postinoculation and worms from the bursa of Fabricius were examined as described above.

Results

Of 14 chicks inoculated with fresh metacercariae, seven (50%) expelled one to three worms in the stool, whereas seven (37%) of 19 chicks inoculated with conditioned metacercariae expelled one to two worms in the stool within 5 hr postinoculation. Results of infection in the chick with *A. suwaense* metacercariae are summarized in Table 1. Worms were recovered only in the cloaca or bursa of Fabricius. Of five worms recovered in the cloaca, four were from fresh metacercariae and all worms from the bursa were from conditioned metacercariae. In one chick inoculated with fresh metacercariae, two worms were recovered 1 day later, 2 mm from each other in the urodaeum of the cloaca. Another chick inoculated with conditioned metacercariae and necropsied 3 days later contained two worms within 2 mm of each other on adjacent folds of the bursa. Only single worms were recovered in the remaining infected chicks. In all infections worms attached tenaciously to the mucosal surface with the oral sucker and the acetabulum. Hemorrhagic areas were observed where the suckers attached to the mucosa. Worms intestinal contents were cream-colored suggesting that they fed on the mucosal surface. However, microscopic examination of worms also showed the presence of host erythrocytes in the intestinal caeca. Worms from chicks never contained black pigment seen in the intestinal caeca of metacercariae (Schnier and Fried, 1980).

Day-old *A. suwaense* from the cloaca increased their mean body area by 51%, their testicular area by 37%, and their vitelline area by 79%. No change in ovarian area was noted. Conditioned metacercariae showed no change in mean body or testicular areas, but showed a 14% decline in vitelline area, and a 60% decline in area of the ovary. As compared with fresh metacercariae, *A. suwaense,* 3 to 4 days old, from the bursa showed an increase in mean body area of 45%, in testicular area of 82%, and vitelline area of 7%, but showed a 67% decline in ovarian area.

Conditioned metacercariae of *L. constantiae* developed into ovigerous adults in the bursa of Fabricius in 4 days and were identical in size and development to adults of the same age from fresh metacercariae (Harris et al., 1972).

Discussion

Anesthetized chicks became infected following per cloacal administration of *A. suwaense* metacercariae. The use of anesthesia probably reduced chick defecation and helped in the establishment of worms as described by Herman and Bacha (1978) for *H. quissetensis*. Our study is the first to provide infection data on *A. suwaense* in an experimental definitive host, although Pojmanksi (1972) found young adults of *Amblosoma exile* in the cloaca of naturally infected ducks, *Aythya fuligula*.

A. suwaense became ovigerous within 4 to 5 days in chick embryos (Shimazu, 1974; Font, 1980) and in 4 days in vitro in NCTC 135-20Y (Schnier and Fried, 1980). However, the chick is apparently a poor host for *A. suwaense* since infections did not last beyond 4 days and ovigerous worms were not obtained. These observations are similar to those of Fried and Foley (1970) on *Clinostomum marginatum,* in which worms showed some postmetacercarial development, but did not survive in the mouth of the chick beyond 4 days.

Conditioned metacercariae established mainly in the bursa of Fabricius and survived longer in the chick than fresh metacercariae. Fresh metacercariae established only in the cloaca. Reasons for these findings are unclear. *L. constantiae* metacercariae establish only in the bursa of Fabricius of the avian host (Allison, 1943; Harris et al., 1972), whereas *Leucochloridium variae* metacercariae establish in both the cloaca and bursa (Fried and Guy, 1974). Cercariae of *H. quissetensis* establish in the lower ileum and bursa of the chick, but worms from the latter site often show gonadal atrophy (Herman and Bacha, 1978). As discussed for *H. quissetensis* by Herman and Bacha (1978), immunologic factors associated with the bursa may inhibit ovarian development in *A. suwaense*.

The body area of *A. suwaense* grown in vitro decreased (Schnier and Fried, 1980), whereas that of worms grown in chicks increased. The area of the ovary of worms grown in vitro increased (Schnier and Fried, 1980), whereas that of worms grown in chicks decreased. These findings are unusual since most trematodes show more marked somatic and reproductive growth in vivo than in vitro (Fried, 1978).

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Literature Cited

Allison, L. N. 1943. *Leucochloridiomorpha constantiae* (Mueller) (Brachylaemidae), its life cycle and taxonomic relationships among digenetic trematodes. Trans. Am. Microsc. Soc. 62:127–168.

Berntzen, A. K., and R. W. Macy. 1969. *In vitro* cultivation of *Sphaeridiotrema globulus* (Rudolphi) from the metacercarial stage to egg production. J. Parasitol. 55:136–139.

Font, W. F. 1980. *Amblosoma suwaense* (Trematoda: Brachylaimidae: Leucochloridiomorphinae) from *Campeloma decisum* in Wisconsin. J. Parasitol. 66. In press.

Fried, B. 1978. Trematoda. *In* A. E. R. Taylor and J. R. Baker, eds. Methods of Cultivating Parasites in Vitro. Academic Press, London.

Fried, B., and J. E. Berry. 1961. The use of Equi-Thesin as an anesthetic in avian helminth transplant studies. J. Parasitol. 47:660.

Fried, B., and D. A. Foley. 1970. Development of *Clinostomum marginatum* (Trematoda) from frogs in the chick and on the chorioallantois. J. Parasitol. 56:332–335.

Fried, B., and W. J. Guy. 1974. The use of a computer program to analyze growth of *Leucochloridium variae* McIntosh 1932 (Trematoda) in the domestic chick. Int. J. Parasitol. 4:73–77.

Fried, B., and K. R. Harris. 1971. Reproduction in single- and double-worm infections of *Leucochloridiomorpha constantiae* (Mueller, 1935) (Trematoda) in the chick. J. Parasitol. 57:866–868.

Harris, K. R., B. Fried, and D. A. Mayer. 1972. Infectivity, growth and development of *Leucochloridiomorpha constantiae* (Trematoda) in the chick and on the chorioallantois. J. Parasitol. 58:213–216.

Herman, S. M., and W. J. Bacha, Jr. 1978. Growth, development, and site location of the trematode *Himasthla quissetensis* in the chick following cloacal drop infections with cercariae. J. Parasitol. 64:827–830.

Pojmanska, T. 1972. *Amblosoma exile* g. n. sp. n. (Trematoda, Brachylaimidae, Leucochloridiomorphinae)—morphology of the adult and metacercaria. Acta Parasitol. Pol. 20:35–44.

Schnier, M. S., and B. Fried. 1980. *In vitro* cultivation of *Amblosoma suwaense* (Trematoda: Brachylaimidae) from the metacercaria to the ovigerous adult. Int. J. Parasitol. 10. In press.

Shimazu, T. 1974. A new digenetic trematode, *Amblosoma suwaense* sp. nov., the morphology of its adult and metacercaria (Trematoda, Brachylaimidae). Jpn. J. Parasitol. 23:100–105.

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Research Note

Eimeria canadensis: Intracellular Motility of a Sporozoite-Shaped Schizont in Vitro[1]

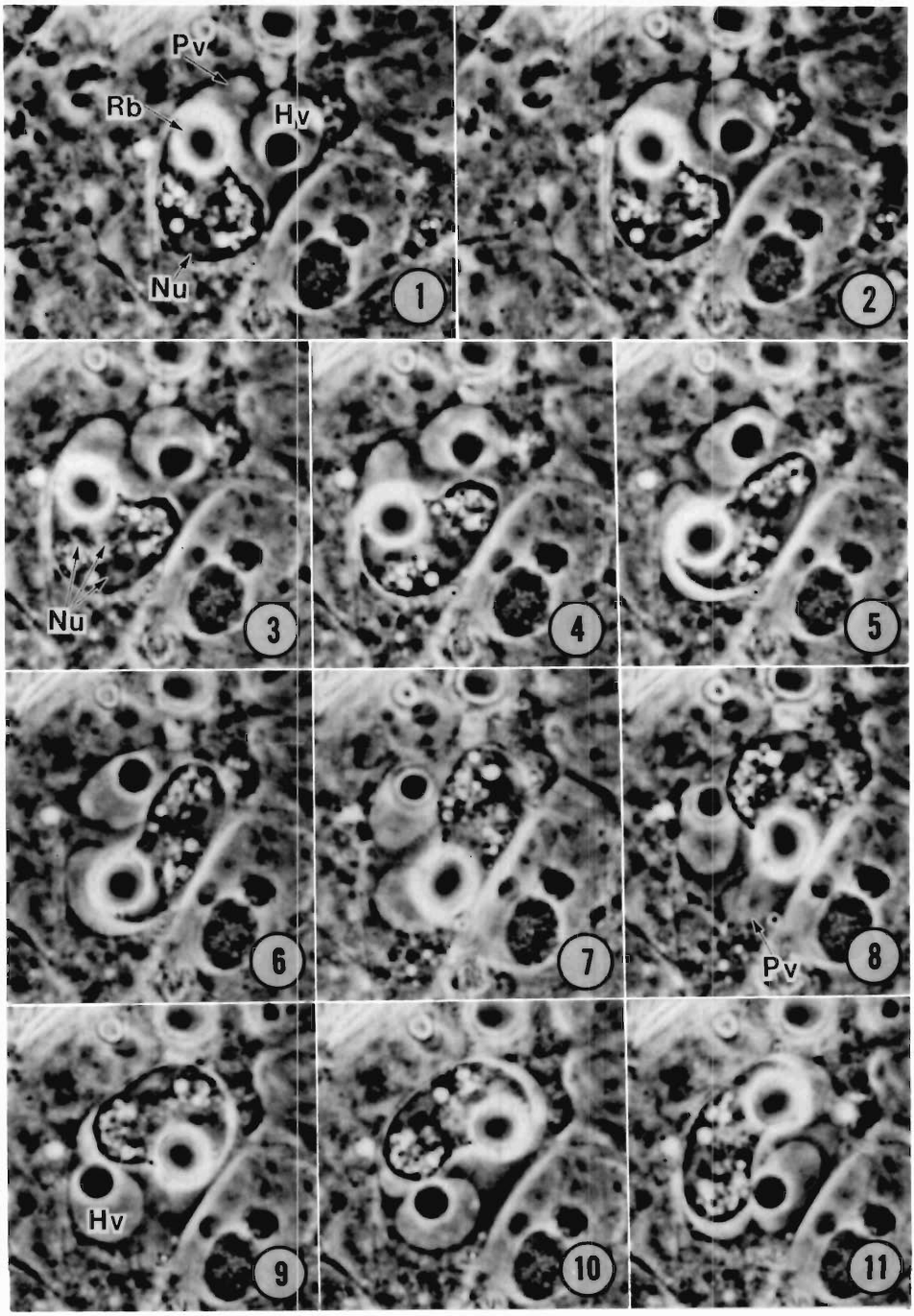
Motility is characteristically present in various stages of the coccidia and related Apicomplexa including ookinetes, sporozoites, merozoites, and microgametes. Motility has also been reported in sporozoite-shaped schizonts and multinucleate merozoites (Speer et al., 1973, J. Parasitol. 59:613–623; Speer and Hammond, 1970a, Z. Parasitenkd. 35:105–118; Speer et al., 1970, J. Protozool. 17:274–284) and macrogamonts (Fayer, 1970, Science 168:1104–1105; Speer and Hammond, 1972, Science 178:763–765).

Sporozoites of *Eimeria canadensis* Bruce, 1921 (Protozoa, Apicomplexa) undergo development to first-generation schizonts in several cell lines and primary cell aggregates (Mueller et al., 1973, J. Protozool. 20:293–297). Recently, we observed a gliding amoeboid-like motility in a sporozoite-shaped schizont at 12 days after inoculation of primary embryonic bovine kidney cell cultures with sporozoites of *E. canadensis*. At the beginning of the observation, the multinucleate organism was already moving within its host cell (Fig. 1). The parasite moved in a circular path within the host-cell cytoplasm changing considerably from elongate to ovoid and back again (Figs. 1–11, taken at intervals of 10 sec). As the parasite moved about it appeared to remain within its parasitophorous vacuole and to pull the vacuole along with it. It also appeared to push a separate host cell vacuole ahead itself as it moved about within the cell. The parasite evidently moved in an anterior direction since a large refractile body, which is characteristically located at the posterior end of sporozoites and sporozoite-shaped schizonts (Mueller et al., 1973, loc. cit.) was present at the trailing end of the organism. Movement of the parasite gradually slowed and eventually ceased after about 2 min, shortly after Figure 11 was photographed.

Coccidian microgametes move by flagella and macrogamonts of *E. magna* move by pseudopodia (Speer and Hammond, 1972, loc. cit.). Macrogamonts of a *Sarcocystis* sp. in cell culture rotated rapidly clockwise and counterclockwise on their longitudinal axis while within the parasitophorous vacuole, the mechanism of which is still unknown (Fayer, 1970, loc. cit.). Apicomplexan ookinetes and zoites have no apparent specialized organelles which might provide locomotion. They have a somewhat similar shape, undergo a similar type of swimming or helical gliding movement, and also have an apical complex, multilayered pellicle, and subpellicular microtubules (Aikawa, 1971, Exp. Parasitol. 30:284–320; Roberts et al., 1970, J. Parasitol. 56:907–917; Speer et al., 1975, J. Invertebr. Pathol. 25:73–78). Although the mechanism of motility in these stages is still unknown, it is generally considered to be caused by the multilayered pellicle and/or subpellicular microtubules.

After a certain number of nuclear divisions, transformations of elongate sporozoite-shaped schizonts of *E. canadensis* and certain other *Eimeria* species to spheroid stages occurs by a lateral outpocketing or an increase in width of the

[1] Research project 2054.



Figures 1–11. Phase-contrast photomicrographs of a moving sporozoite-shaped schizont of *Eimeria canadensis*, 12 days after sporozoite inoculation of primary bovine embryonic kidney cells; photomicrographs taken at 10-sec intervals; ×1,400; Hv, host cell vacuole; Nu, parasite nucleus; Pv, parasitophorous vacuole; Rb, refractile body.

parasite (Mueller et al., loc. cit.; Speer and Hammond, 1970a, b, loc. cit.; Speer et al., 1970, loc. cit.). Ultrastructural studies of such transforming organisms have shown that the inner membrane complex of the pellicle and the subpellicular microtubules gradually disappear (Roberts et al., 1970, J. Protozool. 17:584–592; Kelley and Hammond, 1972, Z. Parasitenkd. 38:271–284). The sporozoite-shaped schizont seen in the present observation appeared to be in an intermediate stage of transformation to a spheroid organism, indicating that perhaps many of the organelles believed to provide motility had already disappeared. Even so, the parasite was still capable of moving about within its host cell by a gliding amoeboid-like motion.

BODO E. G. MUELLER
Department of Biology
Bielefeld University
D-4800 Bielefeld 1, F.R.G.

CLARENCE A. SPEER
Department of Microbiology
University of Montana
Missoula, Montana 59812

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Research Note

Helminth Parasites of an American Flamingo from Newfoundland, Canada

On 7 November 1977 an American Flamingo (*Phoenicopterus ruber*) was shot at Woodstock, White Bay, Newfoundland. The plumage of the bird was in excellent condition, with no evidence of the bird having been pinioned. The colors of the bird varied from white, to deep pink through rose, with the underparts of the wing being most deeply colored. The bird was a female, weighing 2,163 g, and had the following meristic characters: wing length, flattened, 410 mm, chord of culmen 113 mm, tarsus 287 mm, tail 137 mm.

It is felt that this bird was blown into the Newfoundland region from southern regions, probably Florida, where American Flamingos are kept in large numbers in tourist attractions e.g., The Parrot Jungle and Hialeah Race Track, or where they occasionally occur in the wild on mud flats. Wind and weather patterns were such, just prior to the arrival of the bird in Newfoundland, as to account for its passage northwards. On 28 October, strong southerly winds (20–35 knots) began from Miami (25°45′N lat.) and continued until 30 October. On the evening of the 30th, the wind direction changed to south southwesterly, and to northwesterlies on 31 October. Between 1 and 4 November, southeasterlies (25 knots) blew as far north as New York, and southwesterly from New York north.

To date, few papers have been written on the parasites of flamingos in the

Western Hemisphere, one of the most extensive being that of Vigueras (1941, Mem. Soc. Cub. Hist. Nat. 15:327–336). The present specimen was, therefore, examined for both ectoparasites (none found), and endoparasites, using conventional techniques (see Eveleigh and Threlfall, 1976, Can. J. Zool. 54:1694–1711; Andrews and Threlfall, 1975, Proc. Helminthol. Soc. Wash. 42:24–28). All gut contents were strained through a fine wire mesh screen (149 μm) to trap any small parasites present, and any debris remaining in the screen was examined, in a petri dish, under a dissecting microscope (×40 magnification) to ensure that all parasites present were collected. The anterior region of a trematode was recovered from the trachea, and was identified as *Orchipedum* sp. (*jolliei* Schell, 1967?), this constituting a new host record. Fifty-three female *Tetrameres coccinea* (Seurat, 1914) were located in the bird's proventriculus. This species was described from *Phoenicopterus roseus*, taken in Algeria, by Seurat (1914, C. R. Soc. Biol. 76:814–817). The most abundant parasite was *Polymorphus obtusus* Van Cleave, 1918, with 191 specimens being taken from the posterior two-thirds of the small intestine, large intestine, and caeca (118, 34, 29, 10 specimens respectively). This constitutes a new host record. The large intestine and caeca were also the site of infection by not more than five *Leptotaenia ischnorhyncha* (Lühe, 1898) and approximately 52 hymenolepids. The latter were mostly immature and had lost the hooks from their scolices. However, small numbers of *Hymenosphenacanthus cirrostylifera* (Vigueras, 1941) and *Flamingolepis megalorchis* (Lühe, 1898) were identified. One unidentified, immature specimen bore eight hooks, approximately 27 μm long, on its scolex. The hooks had a long handle and short blade. Vigueras (op. cit.) and Rysavy and Macko (1973 (1971) An. Inst. Biol., Univ. Nac. Auton. Mex. Ser. Zool. 42:1–28) recovered *L. ischnorhyncha* and *F. megalorchis* from this host in Cuba. Five *Echinostoma revolutum* (Froelich, 1802) were taken from the large intestine, and two from the cloaca. This digenean is a very common parasite of waterfowl, other aquatic birds and mammals (McDonald, 1969, Catalogue of Helminths of Waterfowl (Anatidae). Bur. Sport Fish. and Wildl. Spec. Sci. Rep. Wildl. 126:692 pp.).

Specimens have been deposited in the USNM Natl. Parasite Coll. (Nos. 75893–75897).

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WILLIAM THRELFALL
Department of Biology
Memorial University
St. John's, Newfoundland A1B 3X9
Canada

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Research Note

Parasites of the Isopod, *Caecidotea communis*, and Amphipod, *Hyalella azteca*, in New Hampshire

The ecology and host-parasite relationships of several endohelminths infecting the fallfish, *Semotilus corporalis*, and the white sucker, *Catostomus commersoni*, collected from the Oyster River, Durham, New Hampshire, were reported by Muzzall and Bullock (1978, J. Parasitol. 64:860–865) and Muzzall (1980, J. Parasitol. 66:127–133). To elucidate better the host-parasite interactions that occur at this locality, 303 freshwater isopods, *Caecidotea communis* (Say), and 1,474 amphipods, *Hyalella azteca* (Saussure), were examined during the periods May 1975 through August 1977 and April 1977 through August 1979, respectively. Also 2,996 isopods, *C. communis*, were examined for parasites from the "Old Reservoir," Durham, New Hampshire, during March 1975 through August 1976. Crustaceans collected were brought to the laboratory alive and examined within 24 hr, or were fixed in 10% formalin in the field, and examined later.

Table 1 presents infection data for the parasite species found in this study. Five percent of the isopods examined from the Oyster River were infected with cystacanths of *Acanthocephalus jacksoni* Bullock, 1962 (Acanthocephala: Echinorhynchidae). Cystacanths were not encysted in the 15 isopods which were collected in May and June 1975 and June 1979. The ovaries of female cystacanths were fragmented, forming masses of large ovarian balls. Testes, seminal vesicles, and cement glands of male cystacanths stained dark, indicating possible presence of semen. Infected isopods were pigmented; these observations contrast with those of Muzzall and Rabalais (1975, Proc. Helminthol. Soc. Wash. 42:31–34), who found that isopods infected with *A. jacksoni* were nonpigmented. In the Oyster River, *A. jacksoni* was a common parasite of brook trout, *Salvelinus fontinalis*, and fallfish, *Semotilus corporalis*.

Seven of the 1,474 amphipods examined from the Oyster River were infected with *Pomphorhynchus bulbocolli* Linkins in Van Cleave, 1919 (Acanthocephala: Pomphorhynchidae). Infected amphipods were collected in September 1977, and June and August 1979. Cystacanths found were not encysted and were light green in color. It is not known if this color is natural or whether it occurred after the amphipods were fixed. This low infection rate of *P. bulbocolli* in amphipods is not surprising, since Esch et al. (1976, Trans. Am. Fish. Soc. 105:486–490) examined more than 9,000 amphipods for *P. bulbocolli* and none was infected.

Cystacanths, which I identified as *P. bulbocolli*, were also found in estuarine amphipods of the genus *Gammarus*. Seven of the 214 amphipods examined from Beards Creek, Durham, New Hampshire, during September through November 1975 were found infected; mean intensity was 1.0. Beards Creek is an estuarine system with tidal fluctuations; there is a freshwater pond above Beards Creek. Immature *P. bulbocolli* were found in the following fish species at Beards Creek: American eel, *Anguilla rostrata* (Anguillidae); nine-spine stickleback, *Pungitius pungitius* (Gasterosteidae); and tomcod, *Microgadus tomcod* (Gadidae). Similarly, Johnson and Harkema (1971, ASB Bull. 18:40) reported that *G. tigrinus* served as an intermediate host for *P. rocci* in an estuarine system.

Eleven and 14 isopods of those examined from the Oyster River and Old Res-

Table 1. Prevalence and mean intensity of *A. jacksoni*, *P. bulbocolli*, and *Hedruris* sp. in *C. communis* and *H. azteca* from the Oyster River and Old Reservoir.

	No. examined	No. infected (%)	No. parasites recovered (♂, ♀)	Mean intensity ±SD	Mean length (mm) ±SD of infected crustaceans
Oyster River					
A. jacksoni	303*	15 (5.0)	15 (8, 7)	1.0	9.6 ± 1.7
P. bulbocolli	1,474†	7 (0.5)	7 (3, 4)	1.0	5.1 ± 0.7
Hedruris sp.	303*	11 (3.6)	12 (5, 7)	1.1 ± 0.3	8.2 ± 1.4
Old Reservoir					
Hedruris sp.	2,996*	14 (0.5)	16 (6, 10)	1.1 ± 0.5	10.9 ± 0.6

* *C. communis*.
† *H. azteca*.

ervoir, respectively, were infected with larval stages of *Hedruris* sp. Nitzsch, 1821 (Nematoda: Hedruridae). Larvae were found in the anterior portion of the isopod hemocoel. Adult *Hedruris* sp. occurred in the stomachs of green frogs, *Rana clamitans*, and bullfrogs, *R. catesbeiana*, collected from the Oyster River. Gravid female *Hedruris* sp. were attached to the mucosa of the stomach by means of their clawlike hook on their posterior end; males found were coiled around the body of the female.

Hall (1929, Smithson. Misc. Collect. 81:1–77) noted that *Hedruris androphora* (parasite of amphibians) and *H. orestiae* (parasite of fish) utilize the aquatic isopod, *Asellus aquaticus*, and amphipod, *Allorchestes* sp., as intermediate hosts, respectively. However, the actual authors who reported this were not cited. The results of the present study, therefore, verify those of Hall, at least for the utilization of isopods in the life cycle of some hedrurid nematodes.

Only one of the 303 isopods examined from the Oyster River was infected with *Allocreadium lobatum* Wallin, 1909 (Trematoda: Allocreadiidae). Four gravid individuals were found in an isopod collected in May 1975. Similarly, DeGiusti (1962, J. Parasitol. 48[2, Sec. 2]:22) reported the occurrence of progenetic *A. lobatum* in amphipods (*Gammarus pseudolimnaeus* and *Crangonyx gracilis*).

PATRICK M. MUZZALL[1]
Department of Zoology
University of New Hampshire
Durham, New Hampshire 03824

[1] Present address: Department of Natural Science, Kedzie Laboratory, Michigan State University, East Lansing, Michigan 48824.

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Research Note

Intestinal Helminths of the Bat, *Myotis keenii* (Merriam), from Southeastern Wisconsin

During a 1979 study of helminths of Wisconsin chiropterans, two specimens of *Myotis keenii* (Merriam) were obtained. Both animals were captured by mist net from a hibernaculum in Dodge County, southeastern Wisconsin. *Myotis keenii* is rare in Wisconsin and attempts to obtain additional specimens were unsuccessful.

Four species of platyhelminths are known to parasitize *M. keenii* (Table 1); the cestodes *Hymenolepis christensoni* (Macy, 1931) Rausch, 1975 (Macy and Rausch, 1946, Trans. Am. Microsc. Soc. 65:173–175) and *H. roudabushi* (Macy and Rausch, 1946) Rausch, 1975 (Nickel and Hansen, 1967, Am. Midl. Nat. 78:481–486) and the digeneans *Prosthodendrium volaticum* Blankespoor, 1972 and *Plagiorchis vespertilionis* Braun, 1900 (Blankespoor and Szymusiak, 1974, J. Parasitol. 60:934). In the present study five species of digeneans are reported as parasites of *M. keenii*. The most abundant parasites were *Plagiorchis vespertilionis* (25/1) and *Prosthodendrium volaticum* (110/3). Numbers in parenthesis refer to number of parasites in each of the two hosts examined. One specimen of *Ototrema schildti* Font, 1978 and five of *Urotrema scabridum* Braun, 1900 were collected from the intestine, and two of *Limatulum gastroides* Macy, 1935 from the stomach of one male *M. keenii* trapped in July. *Myotis keenii* represents a new host for the latter three species of trematodes.

Ototrema schildti was described from *M. lucifugus* in Eau Claire Co., Wisconsin (Font, 1978, J. Parasitol. 64:391–392) and was noted to be absent from *Eptesicus fuscus* in the same locality. The present paper represents the second report of *O. schildti*. *Limatulum gastroides*, originally described by Macy (1935, Proc. Helminthol. Soc. Wash. 2:74–75) from Wisconsin in *M. lucifugus*, has previously been reported from *M. lucifugus* in Iowa and *M. californicus caurinus* in Oregon (Table 1). Martin (1969, Proc. Helminthol. Soc. Wash. 36:250–260) reported *L. gastroides* in the anterior ⅓ of the intestine from the bat *Peropteryx kappleri* from Colombia, South America. All other reports indicate the stomach as a location. *Limatulum istmicus* Caballero, 1964 (An. Esc. Nac. Cienc. Biol. 13:73–82) and *Ochoterenatrema costarricensis* Caballero and Brenes, 1957, recovered from *M. nigricans nigricans* in Costa Rica, are considered synonyms of *L. gastroides* (see Dubois, 1964, Rev. Suisse Zool. 71:371–381; Martin, 1969, loc. cit.). *Prosthodendrium volaticum* Blankespoor, 1972 was described from *E. fuscus* and *Lasiurus borealis*, Iowa (Blankespoor and Ulmer, 1972, Proc. Helminthol. Soc. Wash. 39:224–226) and also was reported from *M. keenii* from Iowa (Blankespoor and Szymusiak, 1974, loc. cit.). Wisconsin represents a new locality for *P. volaticum*. *Plagiorchis vespertilionis* Braun, 1900 has previously been found in *M. keenii* and *L. cinereus* from Iowa, and in *E. fuscus*, and *M. lucifugus* from Nebraska (Table 1). This digenean has also been reported from Europe, Mexico, Canada, and Egypt (see Yamaguti, 1958, Systema Helminthum, vol. 1. Interscience Publ.). The larval stage of this parasite is reported to occur in *Lymnaea auricularia* in Colorado (Acholonu, 1964, Diss. Abstr., 25, 3752).

Table 1. Host and locality records for platyhelminths of *Myotis keenii* (Merriam).

Parasite	Host	Locality	Reference
TREMATODA			
Lecithodendriidae			
Limatulum gastroides Macy, 1935	*Myotis lucifugus*	Wisconsin	Macy, 1935
Syn. *Limatulum istmicus*	*M. lucifugus*	Iowa	Blankespoor and Ulmer, 1970
Caballero, 1964	*M. californicus caurinus*	Oregon	Macy, 1947
Ochoterenatrema costarricensis	*M. nigricans nigricans*	Costa Rica	Caballero, 1964
Caballero and Barnes, 1957	*Peropteryx kappleri*	Colombia	Martin, 1969
	M. keenii	Wisconsin	Present report
Ototrema schildti Font, 1978	*Myotis lucifugus*	Wisconsin	Font, 1978
	M. keenii	Wisconsin	Present report
Prosthodendrium volaticum	*Eptesicus fuscus*	Iowa	Blankespoor and Ulmer, 1972
Blankespoor, 1972	*Lasiurus borealis*		
	Myotis keenii	Iowa	Blankespoor and Szymusiak, 1974
	M. keenii	Wisconsin	Present report
Plagiorchiidae			
Plagiorchis vespertilionis	*Myotis keenii*	Iowa	Blankespoor and Szymusiak, 1974
Braun, 1900	*Eptesicus fuscus*	Iowa	Blankespoor and Ulmer, 1970
	Lasiurus cinereus		
	M. lucifugus		
	M. lucifugus	Nebraska	Nickel and Hansen, 1967
	Eptesicus fuscus		
	M. keenii	Wisconsin	Present report
Urotrematidae			
Urotrema scabridum	*Myotis grisescens*	Kansas	Ubelaker, 1966
Braun, 1900	*M. grisescens*	Kansas	Nickel and Hansen, 1967
	M. lucifugus	Iowa	Blankespoor and Ulmer, 1970
	M. keenii	Wisconsin	Present report

Table 1. Continued.

Parasite	Host	Locality	Reference
Syn. *Urotrema lasiurensis* Alicata, 1932	*Nycteris borealis*	Washington, D.C.	Alicata, 1932
	Nycticeius humeralis	Maryland	Penner, 1941
Syn. *Urotrema minuta* May, 1933	*Lasionycteris noctivagans*	Minnesota	Macy, 1933
Urotrema shillingeri Price, 1931	*Ondatra zibethica*	Maryland	Price, 1931
	O. zibethica	Maryland	Penner, 1941
	N. humeralis	Texas	Chandler, 1938
CESTODA			
Hymenolepididae			
Hymenolepis christensoni (Macy, 1931) Rausch, 1975	*Myotis grisescens*	Kansas	Ubelaker, 1966
	M. lucifugus	Oregon	Rausch, 1975
	M. lucifugus	Alaska	Rausch, 1975
	M. lucifugus	Wisconsin	Rausch, 1975
	M. keenii	Minnesota	Macy and Rausch, 1946
	M. evotis	Oregon	Rausch, 1975
	M. californicus	Oregon	Rausch, 1975
	M. yumanensis	Oregon	Rausch, 1975
Hymenolepis roudabushi (Macy and Rausch, 1946) Rausch, 1975	*Eptesicus fuscus*	Iowa	Blankespoor and Ulmer, 1970
	E. fuscus	Ohio	Rausch, 1975
	Myotis lucifugus	Iowa	Blankespoor and Ulmer, 1970
	M. keenii	Kansas	Nickel and Hansen, 1967
	Nycticeius humeralis	Iowa	Ubelaker and Kuntz, 1971
	M. humeralis	Ohio	Rausch, 1975
	Lasionycteris noctivagans	Ohio	Rausch, 1975

Previous to the present report, *Urotrema scabridum* Braun, 1900 has not been reported from any chiropteran host in Wisconsin. Thus, both host and locality reported in this study are new. *Urotrema scabridum* Braun, 1900 has been synonymized variously with *U. shillingeri* Price, 1931, *U. lasiurensis* Alicata, 1932, and *U. minuta* Macy, 1933 (see Yamaguti, 1958, loc. cit.). *Urotrema scabridum* has been reported from various species of bats in the United States (Table 1).

Voucher specimens of the five species of digeneans currently reported from *M. keenii* have been deposited in the H. W. Manter Laboratory, University of Nebraska State Museum (UNSM No. 20991–20995). Dr. Daniel R. Brooks kindly confirmed all identifications.

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JAMES R. COGGINS, JOHN L. TEDESCO, AND CHARLES RUPPRECHT
Department of Zoology and Center for Great Lakes Studies
University of Wisconsin–Milwaukee
Milwaukee, Wisconsin 53201

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Research Note

Survival of Metacercariae of *Zygocotyle lunata* (Trematoda) in Half-Strength Locke's Solution under Refrigeration

Willey (1941, Zoologica 26:65–88) found that the oldest *Zygocotyle lunata* metacercariae which successfully infected rats had been maintained in water for 138 days. Griffiths and Christensen (1974, J. Parasitol. 60:335) found that *Fascioloides magna* metacercariae remained infective to guinea pigs after storage in tap-water at 3–5°C for 393 days, but not for 407 or 421 days. The present study examined the survival of metacercariae of *Z. lunata* in half-strength Locke's solution under refrigeration.

Cercariae of *Z. lunata* emitted from naturally infected *Helisoma trivolvis* snails (Fried, 1970, J. Parasitol. 56:44–47) encysted on Saran wrap placed in aquaria. Within 1 week postencystment strips of Saran wrap containing cysts were placed in jars containing 200–300 ml half-strength Locke's (Paul, 1975, Cell and Tissue Culture, 5th Ed. Churchill and Livingstone, Edinburgh) solution. The jars were loosely capped and maintained in a refrigerator at 3–5°C. To determine the infectivity, 11 day-old chicks were fed 25 to 45 cysts from 237 to 444 days after storage. After storage, cysts were pretreated and fed in 3% $NaHCO_3$ as described by Fried (1970, loc. cit.). Chicks were necropsied within 13 days postexposure and the number of worms in the ceca was determined. The results of the experiment are presented in Table 1 and show that cysts stored as described for 444 days were infective.

Table 1. Effects of storage of *Zygocotyle lunata* cysts in half-strength Locke's solution at 3–5°C on infectivity in the domestic chick.

Chick no.	No. of days of cyst storage	No. of cysts fed	Days postfeeding until necropsy	No. of worms Recovered
1	237	45	13	10
2	237	45	13	1
3	237	20	13	7
4	237	20	13	12
5	237	20	13	0
6	237	20	13	1
7	358	45	9	10
8	358	45	9	13
9	444	25	6	10
10	444	25	12	0
11	444	25	12	0

BERNARD FRIED AND BRIAN D. WILSON
Department of Biology
Lafayette College
Easton, Pennsylvania 18042

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Research Note

Decrease in the Body Weight of Domestic Chicks Infected with *Echinostoma revolutum* (Trematoda) or *Zygocotyle lunata* (Trematoda)

Fried and Nelson (1978, Parasitology 77:49–53) reported that infection with *Zygocotyle lunata* decreased the weight and area of the chick cecum. The present study was designed to determine if infection with *Z. lunata* or *Echinostoma revolutum* decreased the body weight of the domestic chick.

Day-old chicks were exposed orally to either 50 to 75 *E. revolutum* cysts (Fried and Butler, 1978, J. Parasitol. 64:175–177) or 30 to 40 *Z. lunata* cysts (Fried, 1979, J. Parasitol. 56:44–47) or left unexposed (controls). Equal numbers of chicks from each group were placed in the same electric brooder cage (12 or 15 chicks per cage) and given food and water ad lib. from 18 hr postexposure to 1 day prior to necropsy. All chicks had an equal opportunity to feed and drink, although it was not determined if infected chicks ate or drank less than controls. Chicks were weighed and necropsied 14 days postexposure and the number of worms per chick was determined.

Chicks infected with *E. revolutum* had one to 50 (avg. 24) adults mainly in the lower ileum, whereas those infected with *Z. lunata* contained one to 25 (avg. 7) adults in the ceca. Results of the experiment are presented in Table 1. Student's *t*-test showed that the mean weight of chicks infected with *E. revolutum* at 14 days was significantly less ($P < 0.01$) (Exposure A vs. C) and that the weight of

Table 1. Decrease in the body weight of chicks infected with *Echinostoma revolutum* or *Zygocotyle lunata*.*

Exposure	Species vs. controls	No. of infected and control chicks	Mean number of worms at necropsy	Mean weight of chicks (grams) ± SD at necropsy	*t*	*P*
A	*E. revolutum*	27†	24	191.4 ± 21.9	2.56	<0.01
B	*Z. lunata*	54‡	7	195.3 ± 26.9	2.00	<0.025
C	Controls	47	—	205.5 ± 23.4	—	—

* All chicks necropsied 14 days postexposure.
† Each chick became infected with 1–50 worms.
‡ Each chick became infected with 1–25 worms.

chicks infected with *Z. lunata* was also significantly less ($P < 0.025$) (Exposure B vs. C). We propose no mechanism for the decrease in chick body weight at 14 days postinfection. Worms probably compete for host ingesta, although other factors such as diminished absorption or interference with digestion (von Brand, 1973, Biochemistry of Parasites, 2nd Ed., Academic Press, New York) may be involved.

We acknowledge the assistance of Dr. Randy Stonesifer, Department of Mathematics, Lafayette College, Easton, Pennsylvania for aid with statistical analysis.

BERNARD FRIED AND BRIAN D. WILSON
Department of Biology
Lafayette College
Easton, Pennsylvania 18042

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Research Note

Observations on *Eustrongylides* sp. Infection of Brown and Rainbow Trout in the Firehole River, Yellowstone National Park

Infection by large red larvae of the nematode *Eustrongylides* (Dioctophymatida) can render fish aesthetically displeasing to anglers and diminish the commercial value of the stock (Cooper et al., 1978, J. Parasitol. 64(1):102–107). Adult worms utilize primarily piscivorous birds as definitive hosts wherein they concentrate in the proventriculus and can result in host mortality (Wiese et al., 1977, J. Wildl. Dis. 13(4):376–382). Although Karmanova (1968, cited by Cooper et al., 1978) has speculated that freshwater oligochaetes are the first intermediate hosts for *Eustrongylides*, conclusive evidence of that relationship has yet to be reported.

We encountered a conspicuous *Eustrongylides* infection of trout while investigating the diets of fish from the Firehole River of Yellowstone National Park, Wyoming (Kaeding and Kaya, 1978, Trans. Am. Fish. Soc. 107(3):432–438). These worms, tentatively identified as larvae of *Eustrongylides tubifex* (Nitzsch

1819) Jägerskiold 1909, were encysted intraperitoneal in fibrous capsules similar to those described by Paperna (1974, J. Fish. Biol. 6:67–76). Representative specimens have been deposited in the National Parasite Collection as USNM Helm. Collection numbers 76253 and 76254. The prevalence of this infection differed greatly between trout from the two sampling stations and provides the basis for this report.

The Firehole River originates as a cool mountain stream but beginning about midway along its length it passes through three of the major geyser basins of the park where its thermal and chemical regimens are significantly altered by the addition of appreciable amounts of geothermally heated waters. Trout were collected from two stream reaches, the first upstream from the major geothermal areas of the drainage and consisting of two physicochemically similar stream locations separated by a natural upstream-movement barrier (unaltered station), and the other 17 km downstream in the warmest part of the river (altered station). Mean water temperatures at the altered station were about 10.5°C greater throughout the year than at the unaltered station. Annual temperature regimens for both stations were described by Kaeding and Kaya (1978, loc. cit.).

Collections were made between August 1974 and May 1975 at about monthly intervals except during winter when sampling frequency was approximately bimonthly at the altered station and somewhat longer at the unaltered station. Brown trout (*Salmo trutta*) were taken from the unaltered station while brown and rainbow trout (*Salmo gairdneri*) were taken from the altered station. Descriptions of collection methods, the sampling stations and their fish populations were provided by Kaya (1977, Trans. Am. Fish. Soc. 106(4):354–361), Kaeding and Kaya (1978, loc. cit.), and Kaeding (1980, Prog. Fish-Cult. 42(3):174–176).

Collected fish were subjected to customary field measurements, individually marked, and transported on ice to the laboratory. Refrigerated specimens were dissected, general locations of *Eustrongylides* cysts were recorded, and digestive tracts were preserved in formalin. Digestive tracts and cysts were later opened under a dissecting microscope and the contents searched for parasites. Nematodes were measured and preserved in glycerine-alcohol. Trout age was determined by the scale method (Kaeding and Kaya, 1978, loc. cit.).

The overall prevalence of larval *Eustrongylides* infection was 1.2% for brown trout from the unaltered station and 17.5 and 17.6% for brown and rainbow trout from the altered station, respectively. However, while *Eustrongylides* was found in three trout from the unaltered station, there was no evidence of the infection in resident fish. Kaeding (1980, loc. cit.) presented data indicating infected trout collected from the unaltered station, all large mature fish from below the upstream-movement barrier, were not residents but were members of downstream populations engaged in a seasonal spawning migration. Similarity in prevalence of infection between brown and rainbow trout at the altered station is perhaps not unexpected in view of the similar diets of these fish (Kaeding and Kaya, 1978, loc. cit.). No seasonal trend in prevalence was observed.

Prevalence and intensity of infection increased with age of rainbow trout but the sympatric brown trout did not exhibit a similar relationship (Table 1). Cooper et al. (1978, loc. cit.) attributed a positive relationship between prevalence of *E. tubifex* infection and size (age) of Lake Erie fishes to an accumulation of worms through time and a modification in host diet to include small, infected fish as

Table 1. Prevalence and intensity of larval *Eustrongylides* sp. infection of trout of various ages from the unaltered and altered stations on the Firehole River, Yellowstone National Park.

	Station, host		
	Unaltered	Altered	
	Brown trout	Brown trout	Rainbow trout
Age 0			
Examined/infected (%)	0	4/0 (0)	17/1 (5.9)
Intensity $\bar{x}$ ± SD			1.00
Age I			
Examined/infected (%)	48/0 (0)	40/9 (22.5)	71/7 (9.9)
Intensity $\bar{x}$ ± SD		1.89 ± 2.32	1.14 ± 0.38
Age II			
Examined/infected (%)	80/0 (0)	45/6 (13.3)	92/22 (23.9)
Intensity $\bar{x}$ ± SD		1.67 ± 1.63	1.36 ± 0.73
>Age II			
Examined/infected (%)	115/3 (2.6)	14/3 (21.4)	13/4 (30.8)
Intensity $\bar{x}$ ± SD	1.0	2.67 ± 1.53	2.25 ± 2.50

prey. Predation on fish by trout was only rarely encountered in the Firehole River (Kaeding and Kaya, 1978, loc. cit.). The overall mean intensity of infection was 1.56 ± 1.37 SD. Seventy-eight percent of the infected fish contained only one worm; the maximum worm burden was eight.

The majority of 82 cysts was found attached to the mesentaries of digestive tract organs, although some cysts were found attached to, or within the walls of, the organs themselves; 44% of the cysts were associated with the stomach, 22% with the esophagus, 12% with the intestine, and 10% with the pyloric caeca. The remaining 12% was associated with the other viscera with one cyst in epaxial muscle. Length of 71 worms averaged 57.2 mm ± 10.5 SD and ranged between 30 and 75 mm. No seasonal trend in minimum or average worm length was apparent (cf. Kennedy and Lie, 1976, J. Fish Biol. 8:293–302).

Because avian and piscine vectors of *Eustrongylides* infection have access to the unaltered station, absence of infection in trout resident there suggests an inability of the parasite to persist in that environment. One possible explanation is lack of an obligate first intermediate host, presumably an invertebrate. Although detailed analyses of the macroinvertebrate communities of the Firehole River have yet to be performed, significant differences have been reported between the benthic insect faunas (Armitage, 1961, Hydrobiology 17(1):152–174) and between the diets of trout (Kaeding and Kaya, 1978, loc. cit.) from the altered and unaltered stations. Kaeding and Kaya found that snails (*Physa* sp.) constituted a very significant part of the diets of trout at the altered station but were virtually absent from trout at the unaltered station.

Kennedy and Lie (1976, loc. cit.), in summarizing the accounts of *Eustrongylides* infection of fish in Britain, reported that infections occurred only in fish from lentic environments, and that reports on American and African infections almost exclusively involved similar environments. Perhaps the first intermediate host of *Eustrongylides* is an invertebrate generally occurring in lentic environments and also found at the altered station of the Firehole River. The altered

station contains areas of organic sediments frequently seen in lentic environments and generally considered the habitat utilized by many oligochaete forms (Brinkhurst and Jamieson, 1971, Aquatic Oligochaeta of the World. Oliver & Boyd, Edinburgh. 860 pp.).

Although no quantitative data are available, an additional nematode parasite, *Metabronema salvelini* (Fujita 1922), was commonly seen during the study. This infection was almost entirely confined to trout from the unaltered station.

These observations were made as part of a study funded by a grant from the former Energy Research and Development Administration to my major advisor, C. M. Kaya of the Biology Department, Montana State University. His assistance, and that of D. E. Burkhalter, is greatfully acknowledged. Beth Kaeding kindly provided technical assistance in the laboratory. I thank John L. Crites for his identification of the specimens.

LYNN R. KAEDING[1]
Department of Biology
Montana State University
Bozeman, Montana 59717

[1] Present address: Colorado River Fishery Project, U.S. Fish and Wildlife Service, 2708 N. 4th Street, Flagstaff, Arizona 86001.

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Research Note

Comparative Evaluation of Collection and Fixation Techniques for *Echinococcus granulosus*[1]

During the past several years we have frequently prepared *Echinococcus granulosus* adults by standard techniques for microscopic examination only to find that most, if not all, of the hooks were missing from the scolices. Since hooks are essential to species identification in the genus *Echinococcus,* we devised a study to determine which of several collection and fixation methods were best for leaving hooks intact and specimens relaxed.

Two dogs were infected experimentally, each per os with approximately 50,000 *E. granulosus* protoscolices of ovine origin. Fifty days following infection the dogs were euthanized and their small intestines removed and longitudinally sectioned. Both dogs were fasted for 24 hr prior to sacrifice in order to facilitate collection of tapeworms. One intestine was placed in 0.85% NaCl (PSS) and the other in tap water. Only worms which floated free from the intestines were used

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Table 1. Comparative evaluation of collection and fixation techniques for *Echinococcus granulosus*.

Collection		Fixation		Hook retention (%)			Specimen quality (%)		
Solution	Time	Solution	Time	Complete	Partial	Absent	Bad	Poor	Good
PSS	30 min	Cold formol-alcohol	24 hr	100	0	0	75	25	0
		10% formalin		100	0	0	14	64	22
		70% ethanol		100	0	0	43	57	0
		Hot formol-alcohol		100	0	0	29	42	29
		10% formalin		100	0	0	0	60	40
		70% ethanol		100	0	0	70	30	0
PSS	12 hr	Cold formol-alcohol	24 hr	100	0	0	18	76	6
		10% formalin		96	4	0	71	29	0
		70% ethanol		79	16	5	79	21	0
		Hot formol-alcohol		97	0	3	61	39	0
		10% formalin		96	0	4	50	39	11
		70% ethanol		97	3	0	86	14	0
Tap water	30 min	Cold formol-alcohol	24 hr	88	12	0	12	12	76
		10% formalin		70	20	10	0	40	60
		70% ethanol		83	17	0	33	67	0
		Hot formol-alcohol		31	15	54	15	0	85
		10% formalin		56	33	11	0	4	96
		70% ethanol		91	9	0	9	55	36
Tap water	12 hr	Cold formol-alcohol	24 hr	7	7	86	0	4	96
		10% formalin		0	0	100	20	53	27
		70% ethanol		0	0	100	8	12	80
		Hot formol-alcohol		0	0	100	46	18	36
		10% formalin		0	0	100	12	12	76
		70% ethanol		0	0	100	14	0	86

in the experiment in order to prevent unnecessary hook loss in extracting attached worms. All worms were collected at room temperature, since *E. granulosus* is usually collected in the field (37°C is generally accepted as optimum for collecting specimens for preservation). Tapeworms from either PSS or tap water were immediately fixed in each of three fixatives (10% formalin, 70% ethanol, and formol-alcohol) at room temperature, and in the same three fixatives heated to approximately 50°C. Each intestine was then refrigerated for 12 hr at 4°C, and free-floating tapeworms were again fixed as previously described. Treatment groups contained an average of 17 tapeworms. Specimens varied in number between treatment groups because of the difficulty in rapidly identifying intact worms. Although extra specimens were included in each group, there were still a few groups with lower than desired numbers of intact worms. Due to the danger of working with adult *E. granulosus,* we feel that the number of tapeworms in these groups is sufficient to indicate adequately whether their respective collection and fixation methods are suitable for this parasite. All tapeworms were stained with Semichon's acetic-carmine and mounted in Permount®.

Table 1 lists the collection and fixation techniques used and the percentages of hook retention and overall specimen quality. In general, collection in PSS gave excellent hook retention, but the overall appearance of these specimens was not good. In most cases these worms were contracted, and frequently marked swelling (particularly of the scolex) was evident. Tapeworms collected and maintained in tap water for 30 min showed fairly complete hook retention, whereas those collected and left in tap water for 12 hr showed nearly total hook loss. All tap water collected groups exhibited superior specimen quality when compared to those corresponding groups collected in PSS.

Fixation with formol-alcohol and 10% formalin generally gave better specimen quality and hook retention than 70% ethanol, and no consistent preference for either hot or cold fixative was noted. Our study suggests that for best results, specimens of *Echinococcus* should be collected in tap water and then within 30 min fixed for 24 hr in either hot or cold solutions of formol-alcohol or 10% formalin. If worms cannot be transferred to a fixative within a reasonably short period of time, they should be collected in PSS and then transferred later to either formol-alcohol or 10% formalin.

GEORGE A. CONDER
Department of Microbiology and Public Health
Michigan State University
East Lansing, Michigan 48824

JOHN R. CRELLIN AND FERRON L. ANDERSEN
Department of Zoology
Brigham Young University
Provo, Utah 84602

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Research Note

A Note on an Abnormal Cysticercoid of *Raillietina echinobothrida* (Megnin, 1881)

While collecting the infective larvae of the fowl cestode, *Raillietina echinobothrida* from naturally infected ant vector, *Pheidole* sp. for an experimental study in White Leghorn chickens we came across an abnormal cysticercoid. This was the only cysticercoid harbored by a particular ant host. Recovered from the ant, the scolex of this viable larva became everted almost immediately on placing on the slide. The specimen was fixed in 10% formalin and processed for taking various measurements and general observation.

The striking feature of this cysticercoid was the presence of six suckers arranged around the scolex in the normal manner (Fig. 1). In general the various measurements of this larva are in agreement with those of the normal cysticercoids of *R. echinobothrida* given by the earlier workers (Sawada, 1952, Zool. Mag. 61:311–313; Nadakal et al., 1973, Trans. Am. Microsc. Soc. 92:273–276). The present cysticercoid with its six, instead of the normal four suckers, may be considered as a 'terata' formed as a result of disturbance in genetical or developmental processes.

M. Rajendran, K. Vijayakumaran Nair, and A. M. Nadakal
Department of Zoology
Mar Ivanios College
Trivandrum-695 015, Kerala, India

Figure 1.

PRESENTATION

1980 Anniversary Award of the Helminthological Society of Washington

This year the Helminthological Society of Washington, in presenting its 1980 Anniversary Award to Dr. O. Wilford Olsen, honors a distinguished parasitologist, biologist, educator, and author.

Dr. Olsen was born in Brigham City, Utah, in 1901. After spending 4 years in the Tongan Islands of the South Pacific, as a Mormon missionary, he returned to Utah determined to make education a part of his life's work. He received a Bachelor of Science degree from Brigham Young University in 1929, and a Master of Science degree from the University of Minnesota in 1931. In 1932, he accepted an assistant professorship in the Zoology Department at the University of Hawaii. The following year he attended Harvard University for advanced graduate study, and then returned to the University of Minnesota, completing his Ph.D. in 1936 under the guidance of Dr. William A. Riley. After graduation Dr. Olsen remained at the University of Minnesota as the curator of the parasitology collection.

In 1939, Dr. Olsen joined the Zoological Division, Bureau of Animal Industry, U.S. Department of Agriculture, and was stationed at the Texas Experimental Station, Substation Number 3, in Angleton, Texas. There he worked on the problem of liver fluke infections of cattle. Realizing that biological control of liver flukes was not practical, he focused his investigations on finding a new anthelminthic for controlling the infection. His development of hexachloroethane as a vermifuge in the treatment of liver fluke infections earned him a U.S. Department of Agriculture Meritorious Citation in 1945. In time, hexachloroethane received worldwide acceptance for the control of liver fluke infections of both cattle and sheep.

Dr. Olsen's lifelong interest in the biology of parasites and in education prompted him to accept the position of Chairman of the Zoology Department at Colorado A&M in 1948 (now Colorado State University). He remained active in research, and spent the next 11 summers in Alaska on the Pribilof Islands working on the life cycle of the fur seal hookworm, *Uncinaria lucasi*. Utilizing his knowledge of parasite biology he made a major breakthrough with the discovery that the infective hookworm larvae were transmitted in the mother's colostrum to the nursing fur seal pup. This discovery of trans-mammary infection by nematode parasites initiated further investigations which demonstrated the existence of this method of parasite transmission in other animals. In 1964, Dr. Olsen presented his paper on the life cycle of *Uncinaria lucasi* at the first International Congress of Parasitology held in Rome.

Dr. Olsen was instrumental in organizing and promoting the graduate program in the Zoology Department at Colorado State University. Under his tutelage, fifteen candidates received the Doctor of Philosophy degree and twenty-four candidates the Master's degree. Many of these former students have made significant contributions to parasitology.

Although Dr. Olsen retired in 1967, he maintains an office and laboratory as Professor Emeritus in the Department of Zoology-Entomology at Colorado State University, where he continues to conduct research in parasitology.

Dr. Olsen became a member of the Helminthological Society of Washington in 1949. In addition he is a member of the American Society of Parasitologists, the Rocky Mountain Conference of Parasitologists, the American Microscopical Society, Sigma Xi, Phi Kappa Phi, and the Colorado-Wyoming Academy of Science. Other professional activities have included serving as a consultant for television wildlife programs, and research consultant for the Welder Foundation in Texas to develop programs for control of liver flukes in cattle.

Dr. Olsen's first scientific articles concerned the grasshoppers of Utah and were published during his senior year at Brigham Young University. Since then he has published over 150 scientific articles, plus numerous popular nature articles for both adult and juvenile readers. He is the author and illustrator of "Animal Parasites: Their Life Cycles and Ecology" (Third Edition) and "Essentials of Parasitology" (Third Edition). Recently his text "Animal Parasites" has been published in Spanish.

In recognition of his many and diverse contributions to the field of parasitology and as an outstanding educator, the Committee proudly presents the 1980 Anniversary Award of the Helminthological Society of Washington to Dr. O. Wilford Olsen.—LOUIS S. DIAMOND, Chairman, Award Committee (October 8, 1980)

Acceptance of the 1980 Anniversary Award

The honor extended to me this evening by you, fellow members of the internationally renowned Helminthological Society of Washington, in recognition of my efforts in parasitology far exceeds my expectations. That honor is enhanced by John's participation in the presentation. Whatever the achievements, my effort in parasitology has been sincere, dedicated, and most of all enjoyed.

Although my work in parasitology has been done alone, I have been inspired immensely by the great contemporaries who dominated the American scene. Chief among them was William A. Riley at Minnesota. He introduced me to parasitology. I went to him a dedicated and fairly well-trained entomologist but came away a parasitologist. I have remained one. I was soon convinced that entomology would provide excellent support for studies in life cycles, morphology, and taxonomy, phases of parasitology that appealed to me. Others who inspired me were Henry B. Ward and that galaxy of brilliant stars trained in his laboratory. They included LaRue, Cort, Faust, Stunkard, Van Cleave, and others. Soon after becoming a practicing parasitologist, I met that stimulating group of professional government parasitologists at Beltsville. They were engaged in advanced studies in morphology (the Chitwoods), taxonomy (Price, Cram, McIntosh, Becklund, M. Chitwood), life cycles (Krull, Wehr, Kates), control of parasites by medication (Harwood, Foster), and one who knew all about what these people were doing—Benjamin Schwartz. These people were the giants who occupied the parasitology stage at that time. I am deeply indebted to each of them.

I began working in parasitology in 1936 as an instructor at the University of Minnesota, in a laboratory devoted to a survey of parasites in wildlife. Thousands

Dr. O. Wilford Olsen was presented the 1980 Anniversary Award of the Helminthological Society of Washington by his son Dr. John L. Olsen. The citation was made by Dr. Louis S. Diamond, Chairman of the Awards Committee.

of animals were examined. One of my duties was to curate the collection. This proved a valuable experience in learning the parasitological literature, about the people who produced it, and the parasites themselves.

In 1939 I joined the Zoological Division, now Animal Parasite Institute of the U.S. Department of Agriculture. The assignment was to find means of controlling liver flukes (*Fasciola hepatica*) in cattle. The Division dealt in broad assignments in those days! I was located in Angleton at a substation of the Texas Experiment Station 42 miles south of Houston, in the very heart of liver fluke and cattle country.

The challenge of this assignment in applied parasitology was so stimulating that great efforts were made to reach a solution to the problem.

Clarification of the life cycle, ecology, and distribution of the snail intermediary (*Stagnicola bulimoides techella*) early in the study revealed the immensity of the problem. It clearly demonstrated the futility of attempts to eradicate snails in the gulf coast terrain as a means of controlling liver flukes.

Spectacular success of phenothiazine as a nematocide, introduced by Harwood,

prompted a search for a successful fasciolicide. Preliminary trials conducted in Germany and Hawaii with hexachloroethane as a fasciolicide showed promise. It was tested on a grand scale at Angleton.

Requirements for a successful anthelmintic must include the following attributes: low cost, ease of administration, broad spectrum of safety, and high efficacy. The water-bentonite-hexachloroethane suspension developed at Angleton met all these requirements as a fasciolicide.

Hexachloroethane was tested on a massive scale. Major cooperators included the managers of three large farms of the Texas Prison System who furnished around 5,000 cattle for experimental use and an abundance of free help for carrying out the project. In addition there were numerous cooperative ranchers who provided cattle, help, and pastures for experiments. Dow Chemical Company furnished gratis two tons of hexachloroethane in crystalline form, all of which was used. In addition private packing companies and the Texas Prison System packing plant freely permitted examination of livers of medicated and unmedicated cattle. Thousands of cattle were treated and examined. Results showed excellent fasciolicidal efficacy of hexachloroethane in suspension, often with dramatic recovery of sick cattle following removal of flukes. The general health of treated herds showed marked improvement.

Any plan to develop a regime of systematic treatment to control flukes on a regional basis must be founded on seasonal fluctuation of metacercariae on pastures. Treatment needs to be at the time when flukes in the cattle are in the adult stage and susceptible to the drug. By placing groups of five uninfected sheep on an infested pasture at the beginning of each month for a period of two years and examining each lot 90 days later, it was possible to ascertain by the presence of adult flukes when metacercariae were present. Fall and spring were determined to be favorable seasons for maximum benefits from treatment of herds.

An attempt was made to eradicate flukes from an experimental pasture where a herd of 250 cattle was confined for three years. While it was possible to reduce greatly the fluke population by semi-annual treatments of the cattle, the flukes reappeared in numbers greater than seemed likely from the repeated medication. In looking for the source of infestation, it was found that numerous rabbits and hares served as wild reservoir hosts that kept the pastures heavily infested with fluke eggs.

Hexachloroethane was acknowledged worldwide as an efficient, safe, and inexpensive fasciolicide for over 30 years. The Food and Drug Administration recently withdrew it from use in the United States because of claimed carcinogenic action in experimental animals.

In 1948 a long-dreamed-of opportunity came to pursue an academic career in the west. I succeeded the late Wendell Krull, formerly of the Zoological Division, as head of the three-year-old Department of Zoology at Colorado State University, remaining in that position until retirement. This situation provided a base for developing a strong program in parasitology. From it the gospel of parasitology was spread to a group of excellent and enthusiastic graduate students who in turn are magnifying the calling. In addition it furnished an opportunity to pursue for 10 summers a unique field project in parasitology on the Pribilof Islands.

The project with the U.S. Fish and Wildlife Service was to develop means of reducing or preventing the awesome mortality of Alaskan fur seal pups caused

by the deadly hookworm *Uncinaria lucasi*. Twenty percent of around 500,000 pups born annually died from uncinariasis within a few weeks after birth.

Up to 90% of the pups of the year harbor adult hookworms in the gut. Yearling and old seals are totally free of them. Third-stage larvae are present in the rookery sand in great numbers each spring at the time seals return from sea. Thus it was presumed that pups acquired infection from the soil, as is the case with other species of hookworms. Experiments to determine the life cycle of the hookworm by exposing pups to third-stage larvae collected from rookeries or hatched from eggs failed. Adult worms never appeared in the gut, as expected.

With third-stage larvae present in great numbers in the rookery sand each spring, destruction of them before arrival of the seals and birth of the pups seemed like a practical and effective means of preventing, or, at least, lessening the degree of infection.

When it was demonstrated experimentally that an aqueous emulsion of cresylic acid sprayed on the rookeries killed all third-stage larvae, the stage was set for a practical field trial.

A small rookery heavily infested with third-stage larvae and known to have a history of high annual pup mortality was sprayed prior to the spring arrival of the seals and birth of the pups. Numerous soil samples taken after spraying showed the area free of third-stage larvae. Pup mortality that summer from hookworm disease was just as high as in years prior to spraying. Results from spraying the same area the following year were similar to the first. Obviously infection was not coming directly from the soil, as suspected.

Examination of 39 caesarean pups the following summer showed all to be negative for larvae, which demonstrated that infection was not prenatal. Moreover pups dead from hookworm disease harbored only adult worms. Hence it was concluded that infection must be associated with the mother and occurred early in the life of the pup and at no other time.

Pups born in captivity under controlled conditions in the absence of third-stage larvae became infected shortly after birth. It was discovered from them that the source of infection was third-stage larvae in the colostrum. Milk stripped from pregnant cows captured on the rookeries contained numerous third-stage larvae. These larvae readily infected caesarean pups when administered by mouth. Larvae soon disappeared from the milk, a condition that explained the presence of only adult worms in pups dead of uncinariasis. That the larvae are carried in the body for more than a year was demonstrated by finding them in the mammary glands of pregnant cows taken at sea during their return from the Pacific Ocean to the Pribilof Islands.

This new, heretofore unsuspected, type of nematode life cycle was demonstrated for the first time. It consists of three parts: (1) a gut phase in which adult worms occur only in young pups; (2) a soil phase consisting of eggs, first-, second-, and third-stage larvae; and (3) a tissue phase in which infective third-stage larvae occur in the mammary glands of pregnant seals. Third-stage larvae in the sand enter all seals through the flippers and locate in the belly blubber in a noninfective state. They go to the mammary glands of the pregnant cows where infectivity to the pups is attained. On the basis of this life cycle, hope for controlling hookworms in fur seals vanished. Thus ended my last major project in parasitology.

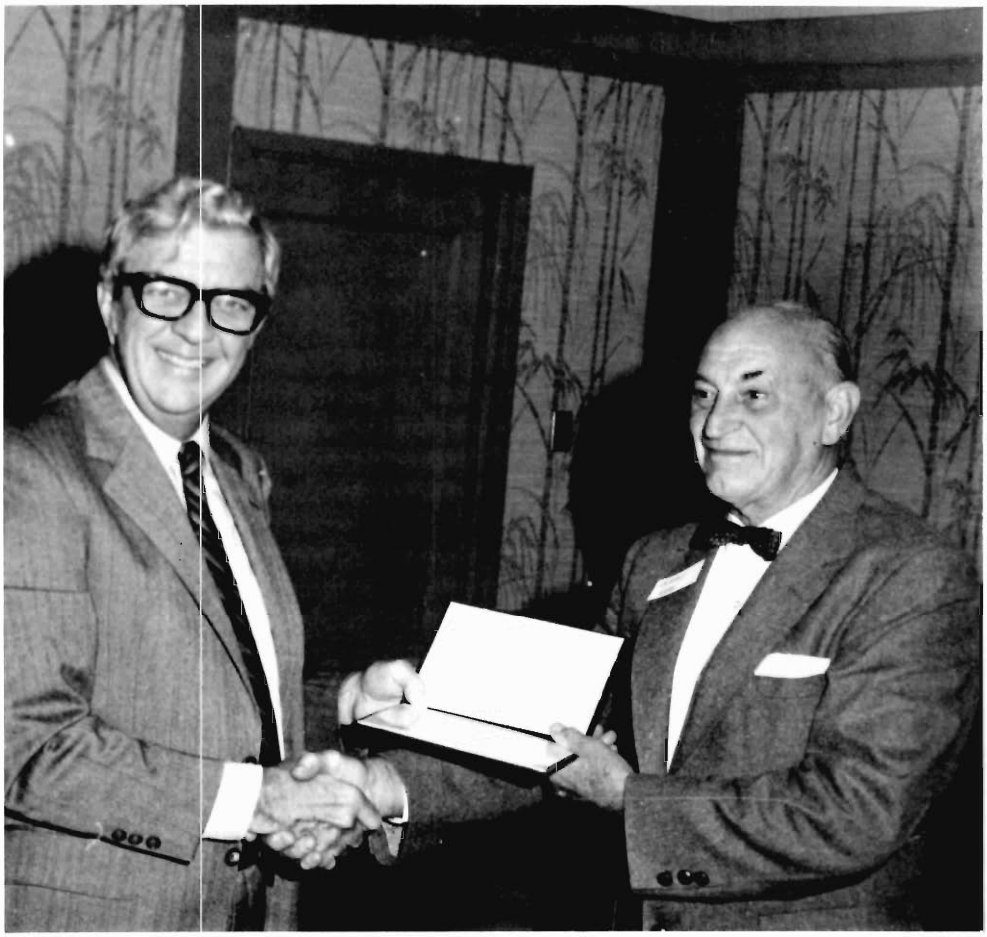
In conclusion, I repeat it is heartwarming to be honored in this manner by you and pleasing to share this brief sketch of a career in parasitology that has been fascinating, challenging, and rewarding. Were I to begin my career again at some future time and in different surroundings, I would choose to be a parasitologist and become a member of HelmSoc. Thank you.—O. WILFORD OLSEN (October 8, 1980)

70th Anniversary

The Helminthological Society of Washington

The first meeting of the Helminthological Society of Washington was held on October 8, 1910, in the Zoological Division of the Hygienic Laboratory, Public Health and Marine Hospital Service (the predecessor of the National Institutes of Health). In commemoration of that event, the Society held a 70th Anniversary Dinner on October 8, 1980. The program highlighted the Society's history and the presentation of awards to several members. A Special Service Award was presented to Edna M. Buhrer and to Gilbert F. Otto for their long record of outstanding contributions to HelmSoc. John S. Andrews received Life Membership in the Society, and the 1980 Anniversary Award was presented to O. Wilford Olsen.

Edna M. Buhrer was the recipient of a Special Service Award at the Society's 70th Anniversary Dinner on October 8, 1980. The presentation was made by Aurel O. Foster.



Gilbert F. Otto received a Special Service Award at the 70th Anniversary Dinner. The presentation was made by A. James Haley, Editor of the Proceedings of the Helminthological Society of Washington.

Life Members in attendance at the 70th Anniversary Dinner were (L to R) David R. Lincicome, Margaret A. Stirewalt, Lloyd E. Rozeboom, Gilbert F. Otto, Marion M. Farr, Carlton M. Herman, Mildred A. Doss, Aurel O. Foster, May Belle Chitwood, and John S. Andrews.

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