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## Studies on the Seasonal Dynamics of *Crepidostomum cooperi* in the Burrowing Mayfly, *Hexagenia limbata*<sup>1</sup>

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**ABSTRACT:** Recruitment of metacercariae of *Crepidostomum cooperi* by nymphs of *Hexagenia limbata* in Gull Lake, Michigan, begins in June and continues through August. The seasonal dynamics of adults of *C. cooperi* is characterized by their disappearance from centrarchid definitive hosts during the fall months. More than 99% of the metacercariae in subimagos of *H. limbata* occur in the abdomen and >70% in IV, V, and VI abdominal segments; the distribution of metacercariae among the abdominal segments is virtually identical in the two sexes. During April and May and among the smallest nymphs during periods of recruitment, metacercariae distributions were best fit by the Poisson. With one exception, following the initiation of recruitment and continuing throughout each sampling period, the negative binomial model provided the best fit to observed frequency distributions of metacercariae in larger nymphs and subimagos. Based on these observations, it is suggested that the frequency distributions are generated by the compounding of Poisson variates which arise as a consequence of random waves of invasion by cercariae.

*Crepidostomum cooperi* Hopkins, 1931, is one of the most common and geographically widespread of the allocreadid trematodes. It has been reported from at least 20 genera of fishes throughout the United States and Canada (Hoffman, 1967). There are, however, only three studies which provide any data regarding seasonal dynamics or other ecological information for the parasite. Two of these (Cannon, 1972; McDaniel and Bailey, 1974) documented seasonal changes in the definitive host and the third (Hazen and Esch, 1977) examined seasonal dynamics in an intermediate host, *Hyaella azteca*.

For the past 12 years, the population biology of *C. cooperi* in the burrowing mayfly, *Hexagenia limbata*, has been studied in Gull Lake, Michigan, U.S.A. (Esch and Hazen, manuscript in preparation). As a part of this investigation, and because so little is known about the seasonal dynamics of the parasite, the present study was undertaken.

### Study Area

Gull Lake is spring-fed, mesotrophic tending toward eutrophic, and is located in southwestern, lower Michigan. The lake is 9.7 × 1.6 km, has a surface area of 822 ha, and reaches a maximum depth of 31 m. The lake is predominantly marl. Dominant aquatic macrophytes include *Chara* spp., *Najas flexilis*, *Myriophyllum heterophyllum*, *Potamogeton* spp., *Utricularia* sp., *Elodea canadensis*, and *Vallisneria americana* (Moss, 1972). The parasite fauna for Gull Lake centrarchids was described by Esch (1971).

### Methods

Mayfly nymphs were collected from several locations at approximately 4-m depths using an Ekman-type grab sampler. Mayflies were separated from the

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<sup>1</sup> Publication No. 454 from the W. K. Kellogg Biological Station, Hickory Corners, Michigan 49060.

**Table 1.** Distribution of *Crepidostomum cooperi* in the abdominal segments of male and female subimagoes.

| Segment | Percent metacercariae/segment |                   |
|---------|-------------------------------|-------------------|
|         | Males (N = 151)               | Females (N = 314) |
| Thorax  | <1.0                          | <1.0              |
| I       | 3.3                           | 5.1               |
| II      | 11.3                          | 8.9               |
| III     | 13.2                          | 11.1              |
| IV      | 19.2                          | 20.7              |
| V       | 29.1                          | 26.1              |
| VI      | 20.5                          | 26.4              |
| VII     | 4.0                           | 3.2               |
| VIII    | 0.0                           | <1.0              |

sediments and other debris by washing through a screen having a mesh size of 0.20 mm. Nymphs collected in this manner were immediately preserved in 70% ethanol and returned to the laboratory for microscopic examination. Metacercariae of *C. cooperi* were recorded and pertinent data regarding host sex and size were noted.

Beginning in late July, subimagoes of *H. limbata* were collected on the evening of their emergence from the lake. The positive phototaxis exhibited by the subimagoes facilitated their capture from window screens and light posts. Mayflies were immediately placed in 70% ethanol and returned to the laboratory for study. Metacercariae were counted with the aid of an ordinary 40× dissecting microscope. Host sex, total length and/or abdominal length were recorded.

The life cycle characteristics of *H. limbata* have been well documented by Hunt (1952); in Gull Lake, *H. limbata* has a 1-year cycle, with emergence beginning in late July and continuing into September. The life cycle pattern of *C. cooperi* was initially described by Hopkins (1934). In Gull Lake, the definitive hosts for *C. cooperi* include bluegill, *Lepomis macrochirus*, rock bass, *Ambloplites rupestris*, and smallmouth bass, *Micropterus dolomieu*. The primary second intermediate host in Gull Lake is *H. limbata*, although the amphipod, *Hyaella azteca*, also serves as an important second intermediate host.

Data were analyzed using an HP3000 computer. The negative binomial, log-normal, and Poisson models were all fitted to the observed distributions using the procedure of Bliss and Fisher (1953). An estimate of goodness of fit was obtained by the value of  $\chi^2$  derived from comparing observed and expected frequencies. The procedures used were similar to those of Lester (1977).

### Results

Metacercariae of *Crepidostomum cooperi* are restricted almost entirely to the abdominal segments of *H. limbata* subimagoes (Table 1), with less than 1% of the parasites encysted in the head and thoracic regions. The fourth, fifth, and sixth abdominal segments carry nearly 70% of all metacercariae present in the host. There is no apparent difference in the distribution of metacercariae in subimagoes of the two sexes.

Nymphs collected in April and May had few metacercariae; those which were present were randomly distributed (Table 2). Parasites which were present were

**Table 2. Seasonal changes in various population parameters of *C. cooperi* metacercariae in nymphs and subimagoes of *H. limbata*.**

| Date | Stage of host (sex) | Prevalence | $\bar{x}$ (N) | Variance | Dispersion pattern | $k$  | $P$    |
|------|---------------------|------------|---------------|----------|--------------------|------|--------|
| 4/2  | Nymph (not sexed)   | 6%         | 0.06 (33)     | 0.06     | Random             | —    | —      |
| 4/17 | Nymph (not sexed)   | 9%         | 0.15 (73)     | 0.29     | Random             | —    | —      |
| 5/2  | Nymph (not sexed)   | 8%         | 0.10 (70)     | 0.11     | Random             | —    | —      |
| 5/23 | Nymph (not sexed)   | 4%         | 0.05 (69)     | 0.08     | Random             | —    | —      |
| 7/7  | Nymph (male)        | 45%        | 0.97 (86)     | 3.83     | Contagious         | 0.54 | 0.067  |
| 7/29 | Nymph (male)        | 71%        | 1.90 (77)     | 3.61     | Contagious         | 1.15 | 0.366  |
| 7/30 | Subimago (male)     | 75%        | 1.96 (105)    | 4.04     | Contagious         | 1.95 | 0.875  |
| 7/30 | Subimago (female)   | 89%        | 6.01 (104)    | 37.82    | Contagious         | 1.38 | 0.030* |
| 8/28 | Subimago (male)     | 86%        | 3.26 (76)     | 14.89    | Contagious         | 1.37 | 0.425  |
| 8/28 | Subimago (female)   | 96%        | 7.00 (91)     | 39.74    | Contagious         | 1.65 | 0.971  |

\* While overdispersed, the negative binomial model did not provide a satisfactory fit to the observed frequency distribution.

most probably recruited during the previous fall. Recruitment of metacercariae by nymphs of *H. limbata* begins in June and continues at least through August. After the onset of parasite recruitment in June, and continuing through the August collections, metacercariae were contagiously distributed among both nymphs and newly emerged subimagoes. The negative binomial model provided an adequate fit for all samples after May 23 except among female subimagoes collected on July 30. A separate collection made approximately two weeks later revealed a mean density of 9.19 metacercariae/host and the negative binomial provided a satisfactory fit to the observed distribution ( $k = 1.32$ ;  $P = 0.738$ ). The value of  $k$ , which is an index of overdispersion, ranged from 0.54 (indicating a high degree of contagion) to 1.95 (indicating less overdispersion).

The prevalence and mean infrapopulation densities of *C. cooperi* metacercariae were measured in three, arbitrarily established, size classes of nymphs collected on July 7 and July 23 (Table 3). For each date, there was a positive relationship between total body length and the prevalence and density of the parasites. The parasites were randomly distributed in the smallest nymphs (<19 mm) collected on both dates in July. Metacercariae were overdispersed in all other size classes during each sampling period. In the two larger size classes collected in July, the

**Table 3. Various population parameters of *C. cooperi* metacercariae within male nymphs of different size classes during early and late July.**

| Size range (mm) | July 7     |               |          |                    |       |       |
|-----------------|------------|---------------|----------|--------------------|-------|-------|
|                 | Prevalence | $\bar{x}$ (N) | Variance | Dispersion pattern | $k$   | $P$   |
| ≅19             | 33%        | 0.37 (45)     | 0.28     | Random             | —     | 0.067 |
| 20–23           | 40%        | 1.04 (25)     | 4.12     | Contagious         | 0.388 | 0.237 |
| ≅24             | 69%        | 1.38 (16)     | 2.25     | Contagious         | 2.51  | 0.567 |
| July 29         |            |               |          |                    |       |       |
| ≅19             | 47%        | 0.77 (17)     | 1.07     | Random             | —     | 0.564 |
| 20–23           | 73%        | 1.67 (43)     | 3.99     | Contagious         | 1.59  | 0.245 |
| ≅24             | 88%        | 4.18 (16)     | 13.65    | Contagious         | 1.89  | 0.993 |

**Table 4.** Changes in various population parameters of *C. cooperi* metacercariae within subimagos of varying abdominal lengths.

| Abdomen length (sex) | Prevalence | $\bar{x}$ (N) | Variance | k    | P     |
|----------------------|------------|---------------|----------|------|-------|
| 13 mm (male)         | 60%        | 1.67 (15)     | 5.38     | 0.77 | 0.450 |
| 14 mm (male)         | 84%        | 3.02 (51)     | 8.70     | 1.89 | 0.793 |
| 15 mm (male)         | 85%        | 2.63 (80)     | 4.77     | 3.24 | 0.482 |
| 16 mm (male)         | 88%        | 3.28 (50)     | 11.68    | 1.79 | 0.360 |
| 17 mm (female)       | 91%        | 3.91 (23)     | 10.81    | 2.50 | 0.837 |
| 18 mm (female)       | 86%        | 3.69 (58)     | 10.01    | 2.04 | 0.385 |
| 19 mm (female)       | 89%        | 5.89 (92)     | 30.30    | 1.29 | 0.219 |
| 20 mm (female)       | 89%        | 5.39 (74)     | 20.33    | 1.47 | 0.573 |
| 21 mm (female)       | 100%       | 7.63 (18)     | 49.36    | 1.72 | 0.855 |

negative binomial model provided a satisfactory fit to the observed frequency distributions.

The mean density of *C. cooperi* metacercariae in subimagos was positively correlated ( $r = 0.922$ ) with length of the abdomen, increasing from 1.67 in males with abdomens 13–14 mm in length to 7.63 in females with abdominal lengths of 21–22 mm (Table 4). In all size classes, metacercariae were overdispersed; the negative binomial model provided a satisfactory fit to the observed frequency distribution of parasites in subimagos within each size class. The pattern of overdispersion is clear in all size classes, with the majority (>50%) of the metacercariae occurring in approximately 20% of the hosts.

The positive relationship between prevalence of *C. cooperi* metacercariae and size of *H. limbata* is also consistent for the other dominant intermediate host in Gull Lake. Thus, the prevalence of metacercariae in amphipods, *Hyalella azteca*, increased from <1% among individuals with <17 antennal segments to >25% among individuals with >25 antennal segments (Fig. 1).

### Discussion

The recruitment of *C. cooperi* by mayfly nymphs in Gull Lake is clearly a seasonal phenomenon, beginning in June and continuing at least through August. This pattern, not surprisingly, is similar to that which occurs among *Hyalella azteca* in the same system (Hazen and Esch, 1977). Unpublished observations by one of us (GWE) also indicate that the prevalence of *C. cooperi* adults in cetrarchid definitive hosts is also highly seasonal, with parasites present in each month from May through September, but absent during the winter. Collectively, the seasonal dynamics of *C. cooperi* in the second intermediate and definitive hosts suggest that the parasite overwinters as an egg, or in the first intermediate host (fingernail clams of the genus *Sphaerium*). While we have no direct observation to support the contention, indirect evidence from Gale (1973) suggests that *C. cooperi* occurs in *Sphaerium transversum* from Pool 19 in the Mississippi River during the winter months.

The seasonal pattern of *C. cooperi* metacercariae and adults in Gull Lake is similar to that reported by Cannon (1972) among yellow perch, *Perca flavescens*, in Lake Opeongo, Ontario, Canada, but not for *Lepomis* spp. in Little River, Oklahoma, U.S.A. (McDaniel and Bailey, 1974). Thus, the latter investigators

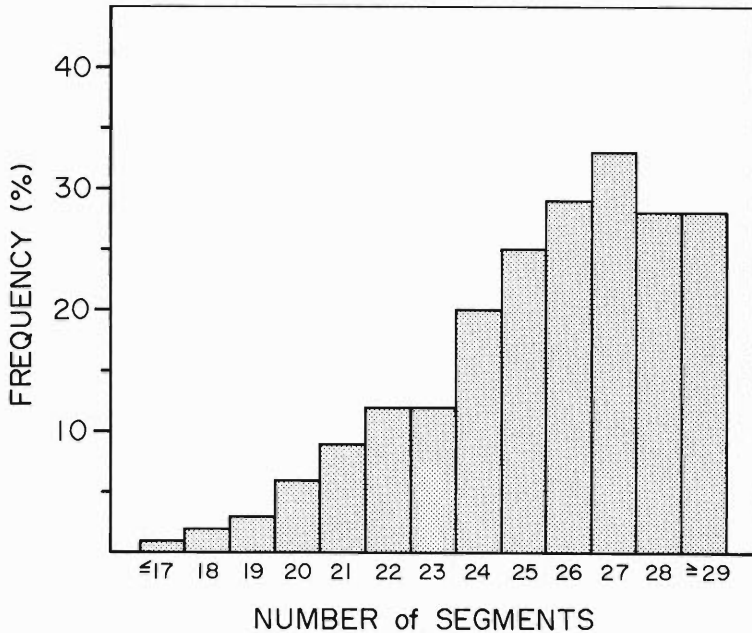


Figure 1. Relationship between prevalence of *C. cooperi* and numbers of antennal segments of *Hyalella azteca*; numbers of antennal segments are also a function of size and age for *H. azteca*.

reported peak prevalence during the winter months and then an almost linear decline until September when infection percentages reach zero. The similarity in seasonal dynamics of *C. cooperi* in Gull Lake and Lake Opeongo and its decline to zero during the summer months in Little River would be expected if, as proposed by Chubb (1979), water temperature is a major factor in regulating the population biology of *C. cooperi*. It is clear, however, that the seasonal periodicity of *C. cooperi* must also be synchronized with the seasonal dynamics of intermediate and definitive host populations. Thus, in Gull Lake, the burrowing mayfly, *Hexagenia limbata*, follows a 1-year cycle (rather than two years as in other lake systems), with peak emergence in August–September and maximum growth rates from May through July of each year. It would consequently be advantageous for the parasite to be recruited by the intermediate host during that time when probability of transmission to the definitive host is maximized. Since size selective predation is known to be a strategy of the bluegill (Werner and Hall, 1974) and probably of other centrarchid hosts in Gull Lake as well, then it would be best to have greater transmission during the months of June through August when nymphs of *H. limbata* grow to their greatest size.

Anderson (1978) proposed that three processes are most important in producing what he termed, "stable dynamical equilibrium," or regulation. These are: (1) density-dependent constraints on parasite population growth; (2) nonlinear, parasite-induced host mortality; and (3) overdispersion of parasite infrapopulations within their hosts. It was Crofton (1971), however, who first emphasized that "it is overdispersion and the relationship of parasite density to lethal factors which produce a disparity in parasite and host deaths." It is clear that *C. cooperi*

metacercariae are highly overdispersed and that the negative binomial model provided the best fit for the observed frequency distributions. The data presented herein do not suggest whether *C. cooperi* and *H. limbata* may be acting in a mutually regulatory fashion. The only feature which may be suggestive of regulation is that  $k$  values are low. According to Anderson and May (1978), a "high degree of overdispersion tends to confer stability"; this is, however, but one of several conditions which must be met in order to have effective regulation.

The mechanisms which may operate to generate overdispersion and, more precisely, a frequency distribution to which the negative binomial model can be fitted, are several. According to Crofton, these include: (1) compounding of Poisson variates through a series of random waves of infection; (2) clumped, or contagious distribution of infective agents; (3) increasing the probability of infection by a previous infection; (4) decreasing the chance of further parasite recruitment because of previous infection; (5) altering the probability of infection by changes in size or age of the host; (6) changing the probability of infection with time. The mechanism by which the overdispersion of *C. cooperi* metacercariae was generated has not been determined experimentally, but based on several life history features of the parasite and host, a reasonable hypothesis may be formulated. First, the parasite is randomly distributed among mayfly nymphs during the months of April and May, suggesting that recruitment by nymphs occurred during the previous fall and that it was probably a consequence of random exposure to cercariae shed from *Sphaerium* spp. during the tail-end of the seasonal cycle. Second, small nymphs collected on July 7 and July 29 had metacercariae which were dispersed in a random fashion. These nymphs, being smaller, were probably also younger and, as a consequence, were less likely to have been exposed to infection for as long a period of time as the larger individuals. The existence of randomly dispersed metacercariae in smaller nymphs, collected from the same location as larger nymphs having contagiously distributed metacercariae infrapopulations, tends to suggest that overdispersion was generated by the compounding of Poisson variates. Such a contention is further supported by the knowledge that nymphs do not move about in the substrate once construction of a burrow has been completed (Hunt, 1952); they are, therefore, sedentary and would unlikely be exposed to contagiously distributed masses of infective agents. The other five possibilities identified by Crofton (1971) as mechanisms for generating overdispersion which can be described by the negative binomial model seem less likely to have occurred than the first. The absence of any evidence indicating that probabilities of infection may be increased or decreased by changes in size, age, or as a function of differences in sample sizes, seem to support our assertion that *C. cooperi* overdispersion is generated by random waves of infection by cercariae shed from infected *Sphaerium*.

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### Literature Cited

- Anderson, R. M.** 1978. The regulation of host population growth by parasitic species. *Parasitology* 76:119–157.
- Anderson, R. M., and R. M. May.** 1978. Regulation and stability of host-parasite population interactions. I. Regulatory processes. *J. Anim. Ecol.* 47:219–247.
- Bliss, C. I., and R. A. Fisher.** 1953. Fitting the negative binomial distribution to biological data. *Biometrics* 9:176–200.
- Cannon, L. R. G.** 1972. Studies on the ecology of the papillose alloeocreadid trematodes of the yellow perch in Algonquin Park, Ontario. *Can. J. Zool.* 9:1231–1239.
- Chubb, J. C.** 1979. Seasonal occurrence of helminths in freshwater fish. Part II. Trematoda. *In* W. H. R. Lumsden, R. Muller, and J. R. Baker, eds. *Advances in Parasitology*. Vol. 17. Academic Press, New York.
- Crofton, H. D.** 1971. A quantitative approach to parasitism. *Parasitology* 62:179–193.
- Esch, G. W.** 1971. Impact of ecological succession on the parasite fauna in centrarchids from oligotrophic and eutrophic ecosystems. *Am. Midl. Nat.* 86:160–168.
- Gale, W. F.** 1973. Predation and parasitism as factors affecting *Sphaerium transversum* (Say) populations in Pool 19, Mississippi River. *Res. Popul. Ecol.* 14:169–187.
- Hazen, T. C., and G. W. Esch.** 1977. Studies on the population biology of two larval trematodes in the amphipod, *Hyaella azteca*. *Am. Midl. Nat.* 98:213–219.
- Hoffman, G. L.** 1967. *Parasites of North American Freshwater Fishes*. University of California Press, Berkeley and Los Angeles. 486 pp.
- Hopkins, S. A.** 1934. The papillose Alloeocreadidae. III. *Biol. Monogr.* 13:45–124.
- Hunt, B. P.** 1952. The life history and economic importance of the burrowing mayfly, *Hexagenia limbata*, in southern Michigan lakes. *Bull. Inst. Fish. Res.* 4:1–151.
- Lester, R. J. G.** 1977. An estimate of the mortality in a population of *Perca flavescens* owing to the trematode *Diplostomum adamsi*. *Can. J. Zool.* 55:288–292.
- McDaniel, J. S., and H. H. Bailey.** 1974. Seasonal population dynamics of some helminth parasites of centrarchid fishes. *Southwest. Nat.* 18:403–416.
- Moss, B.** 1972. Studies on Gull Lake, Michigan. II. Eutrophication evidence and prognosis. *Freshwater Biol.* 2:309–320.
- Werner, E. E., and D. H. Hall.** 1974. Optimal foraging and the size selection of prey by the bluegill sunfish (*Lepomis macrochirus*). *Ecology* 55:1042–1052.

## Attachment of *Cyclocoelum oculeum* Miracidia to Snails and Subsequent Penetration by Their Redial Stage<sup>1</sup>

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**ABSTRACT:** The miracidium of *Cyclocoelum oculeum* contains a fully formed redia. After attachment, the snail's epithelial cells are breached by the distal portion of the apical papilla. Retraction of its tip pulls a plug of snail tissue into the papillar cavity, thus forming a stable attachment between miracidium and snail. During this process secretions from the miracidium's apical and lateral glands are released, presumably causing lysis of snail tissues. Then, miracidial membranes near the apical papilla are digested by secretions from the redial esophageal glands, resulting in an opening through which the redia passes into the snail when the apical papilla of the miracidium retracts. Upon locating a hemolymph vessel the redia migrates to the hemocoel surrounding the buccal bulb.

Cyclocoelid miracidia are unusual, each containing a fully formed redia. When contact is made with a suitable snail, they attach and the redia penetrates, leaving the empty miracidia behind.

Even though a number of authors have observed this phenomenon, including Szidat (1932), Stunkard (1934), Ginetsinskaia (1949), Taft (1973, 1975) and Taft and Heard (1978), a detailed study of miracidial attachment and subsequent penetration by the redia has not been published.

This paper presents aspects of miracidial attachment, escape of the redia from the miracidium and early migration of the redial stage of *Cyclocoelum oculeum* through snail tissues.

### Materials and Methods

Gravid adults of *C. oculeum* were removed from the orbits of American coots, *Fulica americana*, and were dissected in previously boiled aquarium water. Miracidia thus obtained were exposed to snails (*Gyraulus hirsutus*) for 10 min to 1.5 hr. Exposed snails were fixed in Gendre's fluid, embedded in paraplast, and sectioned at 5-8  $\mu\text{m}$ . Sections stained with mercuric bromphenol blue (Mazia et al., 1953) a general protein stain, allowed observation of general redial morphology, and in particular, musculature of the miracidial apical papilla. The PAS technique (Pearse, 1960) for polysaccharides and polysaccharide complexes delineated the lateral and apical glands of the miracidium and, to a lesser extent, the redial esophageal glands. Aldehyde fuchsin (Cameron and Steele, 1959), considered a neurosecretory stain, proved good for general morphology and also stained contents of the apical and esophageal glands purple, indicating the time of release of their contents.

The examination of over 200 sectioned snails with miracidia and rediae in various stages of attachment and penetration provided clarification of many aspects of the attachment and penetration process.

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<sup>1</sup> Supported in part by research grant 8408 from University of Wisconsin Regents.

### Results and Discussion

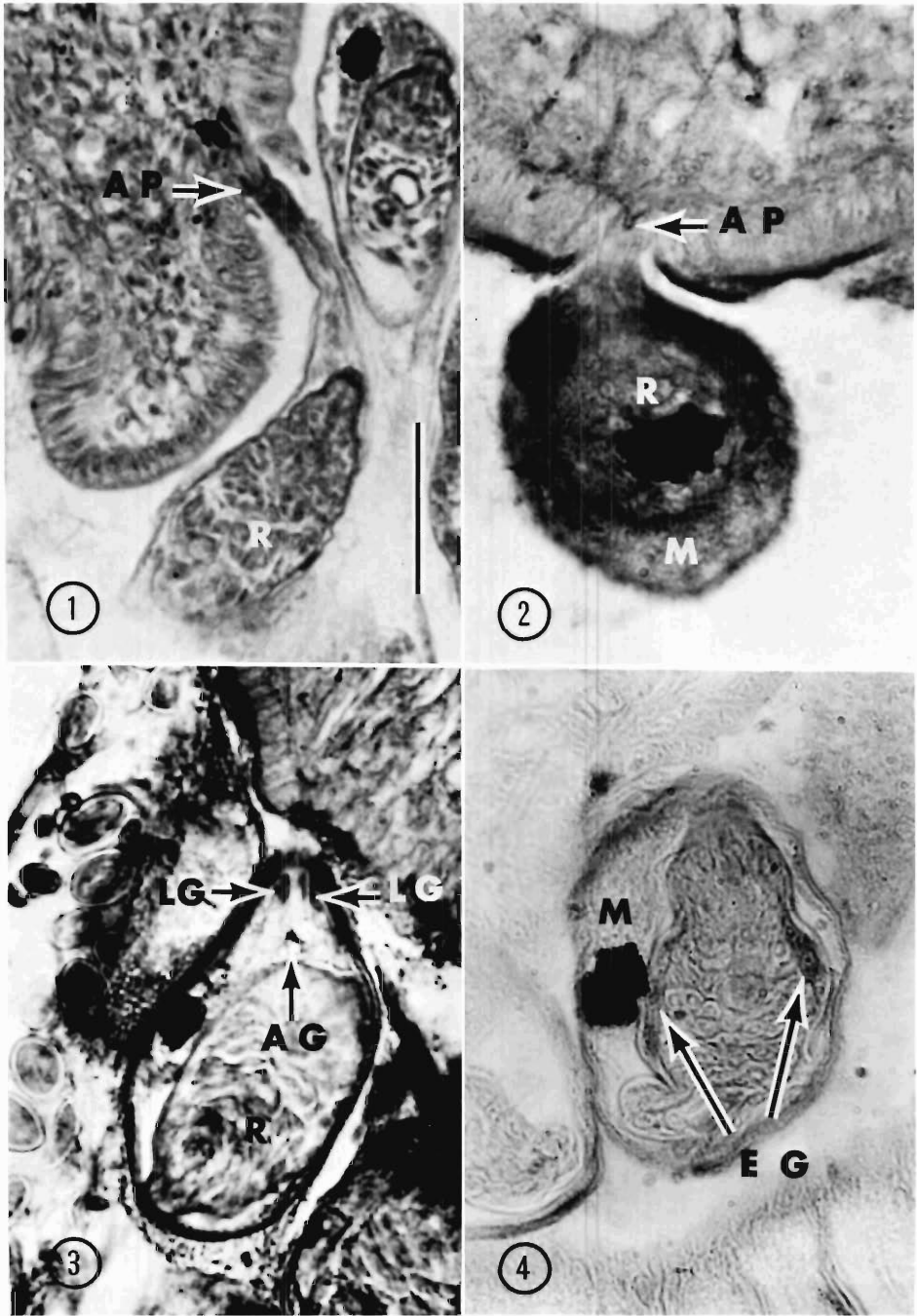
Initial attachment of miracidia to the molluscan host is difficult to observe because of their tendency to fall off at the stage when the snail is placed in fixative. However, several authors working at both light and EM levels have speculated on that process. Dawes (1960) suggested that the apical papilla of *Fasciola hepatica* miracidia retracts on contact with the snail, resulting in negative pressure which assures adhesion to the host at that point. Studying schistosomes, Wadji (1966) assumed that two mechanisms were involved: beating of the cilia to keep the miracidium pushed against the snail; and use of a mucoid substance secreted by the lateral (adhesive) glands of the miracidium causing it to adhere to the snail. Wilson et al. (1971) also postulated an adhesive role for the lateral glands of *F. hepatica* miracidia.

Wilson (1969), Kinoti (1971), LoVerde (1975), and Blankespoor and van der Schalie (1976) postulated that filaments or corrugations on the apical papilla of various miracidia aid in attachment. However, Kjøie and Frandsen (1976) theorized that folds on the apical papilla act as a "rasp," or play some role in the discharge of glandular secretions. Coil (1977), and Wilson et al. (1971) suggest that formation of a sucker caused by invagination of the tip of the apical papilla may also be responsible for initial miracidial attachment.

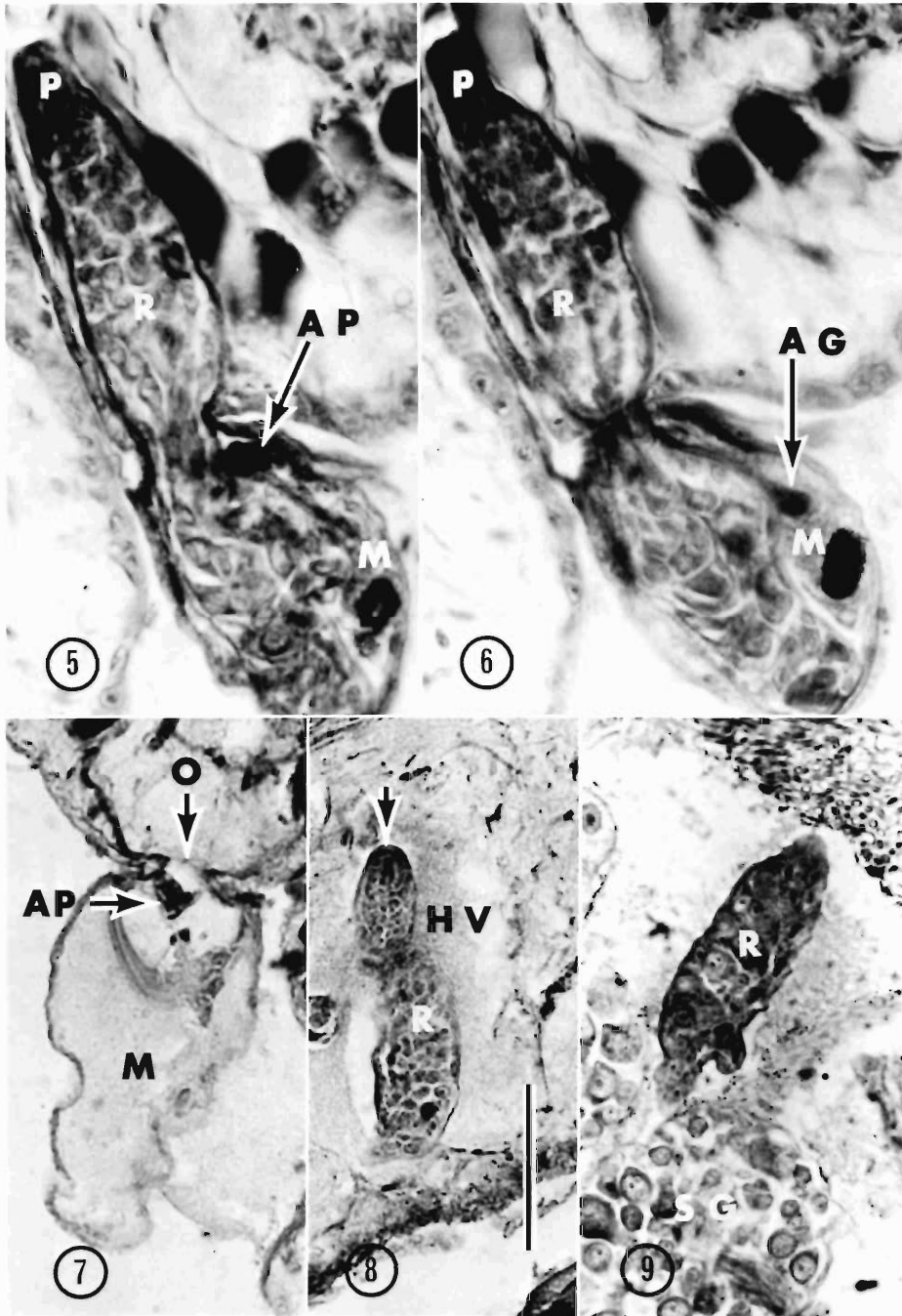
Living *C. oculeum* miracidia are approximately  $0.192 \times 0.069$  mm. They are covered externally with cilia, except for the apical papilla. Internally, an apical gland, flanked by lateral glands, is located immediately posterior to the apical papilla. During penetration, all three glands empty via ducts opening on the apical papilla. Behind these glands is a cavity containing a fully formed redia. Anteriorly, the redia bears two minute appendages; posteriorly, there are two larger appendages and a tail. Internally, the redia possesses a pharynx, with a so-called esophageal gland on either side, and a sacciform intestine.

Like other miracidia which have been studied, initial attachment of *C. oculeum* miracidia to the snail's surface is still unclear. However, once the surface is breached, attachment and penetration becomes easier to observe. Retraction of the apical papilla at the tip forms a cavity into which a plug of the snail's tissue is pulled (Figs. 1, 2). This connection is firm between miracidium and snail, as evidenced by numerous observations of it in tissue sections. By the time the miracidium has penetrated the snail's epithelium, a portion of the apical and lateral gland contents has been voided (Fig. 3). Whether secretions of these glands have more than one function is still debatable, but circumstantial evidence indicates that cytolysis is involved.

Concomitant with miracidial gland emptying is the release of redial esophageal gland contents (Fig. 4). If one studies these redial glands from the time the miracidium breaches the snail's epithelium until the redia enters the snail, a rapid decrease in the contents of the glands can be observed. Occasionally, a small amount of glandular material was seen near the anterior edge of the redial pharynx (Fig. 8). From these observations it is presumed the function of the redial glands is to lyse the wall of the miracidium between the first tier of epidermal plates and the apical papilla, thus allowing the redia to escape. Ginetsinskaia (1949) figured what appears to be esophageal glands in a redia of *Cyclocoelum microstomum* just after it had recently entered snail mantle tissue. From her figure it is difficult to tell whether these glands are empty or full.



Figures 1-4. *Cyclocoelum oculum* miracidia and rediae. Scale = 95  $\mu$ g. 1. Plug of snail tissue drawn into a suckerlike cup formed at the end of the greatly extended apical papilla (ap) of the miracidium containing a redia (r). 2. Plug of snail muscular and connective tissue drawn into invaginated distal end of apical papilla (ap) of a miracidium (m) with a redia (r). 3. Attached miracidium with contained redia (r), showing partially empty apical (ag) and lateral glands (lg). 4. Esophageal glands (eg) of the redia (r) within miracidium (m).



Figures 5-9. *Cyclocoelum oculum* miracidia and rediae. 5. Redia (r) passing from the miracidium (m) into snail tissue. Note the redial pharynx (p), and the retraction of the muscular apical papilla (ap) into the miracidium. Scale = 95  $\mu$ m. 6. Redia (r) passing from the miracidium (m) into snail tissue. Note the pharynx (p) of the redia (r) and apical gland (ag) of the miracidium. Scale = 95  $\mu$ m. 7. Empty miracidium (m), with retracted apical papilla (ap) and apical gland, still attached to snail. Observe the opening (o) through which the redia passed. 8. Redia (r) within hemolymph vessel (hv) of snail. Note residual material (arrow) from the esophageal glands at the anterior end of the redia. Scale = 200  $\mu$ m. 9. Redia (r) near snail's salivary glands (sg). Scale = 200  $\mu$ m.

Once the miracidial membranes are dissolved, the apical papilla retracts (Fig. 5) and the redial stage squeezes through the opening. At this time, the redia is two to two and one-half times as long as the miracidium and is constricted as it passes through the opening (Figs. 5, 6).

Once the redial stage has left, the remnant of the miracidium remains attached. The opening through which the redia passed is evident (Fig. 7). Eventually, the miracidium falls from the snail.

Immediately upon leaving the miracidium the redia enters a hemolymph vessel. At that time, the esophageal glands are empty, except for occasional residues of material near the anterior end of the pharynx (Fig. 8). The redia migrates via these vessels to a sinus surrounding the snail's buccal bulb and salivary glands (Fig. 9). Recently liberated rediae always migrate to this area regardless of the point of entry.

Sixteen-millimeter cinephotomicrography of living miracidia, as well as TEM and SEM studies are currently under way to further elucidate the miracidial attachment process and subsequent release and migration of the rediae.

### Acknowledgments

I would like to thank Dr. Lyle Nauman, Jerry Bartelt, and Cindy Swanberg for aid in collecting American coots, Drs. William LeGrande and Martin J. Ulmer for critically reading the manuscript, and Dr. Robert Price for translating a Russian article.

### Literature Cited

- Blankespoor, H. D., and H. van der Schalie. 1976. Attachment and penetration of miracidia observed by scanning electron microscopy. *Science* 191:291-293.
- Cameron, M. L., and J. E. Steele. 1959. Simplified aldehyde-fuchsin staining of neurosecretory cells. *Stain Technol.* 34:265-266.
- Coil, W. H. 1977. The penetration of *Fascioloides magna* miracidia into the snail host *Fossaria bulimoides*: a scanning electron microscope study. *Z. Parasitenkd.* 52:53-59.
- Dawes, B. 1960. A study of the miracidium of *Fasciola hepatica* and an account of the mode of penetration of the sporocyst into *Lymnaea truncatula*. Libro Homenaje al Dr. Eduardo Caballero y Caballero 95-111. Mexico: Escuela Nacional de Ciencias Biologicas.
- Ginetsinskaia, T. A. 1949. The life-cycle of the trematode *Cyclocoelum microstomum* (Creplin, 1829). *C.R. Acad. Sci. URSS* 58:509-512.
- Kinoti, G. K. 1971. The attachment and penetration apparatus of the miracidium of *Schistosoma*. *J. Helminthol.* 45:229-235.
- Kjøie, M., and F. Frandsen. 1976. Stereoscan observations of the miracidium and early sporocyst of *Schistosoma mansoni*. *Z. Parasitenkd.* 50:335-344.
- LoVerde, P. T. 1975. Scanning electron microscope observations of the miracidium of *Schistosoma*. *Int. J. Parasitol.* 5:95-97.
- Mazia, D., A. Philip, and M. Alfert. 1953. The cytochemical staining and measurement of protein with mercuric bromphenol blue. *Biol. Bull.* 104:57-67.
- Pearse, A. G. 1960. *Histochemistry, Theoretical and Applied*. Second edition. Little Brown and Company, Boston.
- Stunkard, H. W. 1934. The life history of *Typhlocoelum cymbium* (Diesing, 1850), Kossack, 1911 (Trematoda, Cyclocoelidae). A contribution to the phylogeny of the monostomes. *Bull. Soc. Zool. France* 59:447-466.
- Szidat, L. 1932. Zur Entwicklungsgeschichte der Cyclocoeliden. Der Lebenszyklus von *Tracheophilus sisowi*. *Skrj. Zool. Anz.* 100:205-213.
- Taft, S. J. 1973. Some aspects of the larval development of *Cyclocoelum obscurum* (Trematoda: Cyclocoelidae). *J. Parasitol.* 59:90-93.

- Taft, S. J.** 1975. Aspects of the life history of *Cyclocoelum brasilianum* Stossich 1902 (Trematoda: Cyclocoelidae). *J. Parasitol.* 61:1041-1043.
- Taft, S. J., and R. W. Heard.** 1978. Aspects of the larval development of *Ophthalmophagus* sp. (Trematoda: Cyclocoelidae). *J. Parasitol.* 64:597-600.
- Wadji, N.** 1966. Penetration by the miracidia of *Schistosoma mansoni* into the snail host. *J. Helminthol.* 40:235-244.
- Wilson, R. A.** 1969. Fine structure of the tegument of the miracidium of *Fasciola hepatica* L. *J. Parasitol.* 55:124-133.
- Wilson, R. A., R. Pullin, and J. Denison.** 1971. An investigation of the mechanism of infection by digenetic trematodes: the penetration of the miracidium of *Fasciola hepatica* into its snail host *Lymnaea truncatula*. *Parasitology* 63:491-506.

### Report on the Brayton H. Ransom Memorial Trust Fund

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| Receipts: Net interest received in 1981 .....   | <u>608.10</u> |
|   | \$6203.03     |
| Disbursements: Grant to The Helminthological Society of<br>Washington for 1980 (made on February 3, 1981) ..... | 50.00         |
| Grant to The Helminthological Society of<br>Washington for 1981 .....   | <u>50.00</u>  |
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## Prevalence of Avian Schistosomes in *Physa integra* from Southwestern Michigan<sup>1</sup>

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**ABSTRACT:** During this study, 26,775 *Physa integra* were collected in southwestern Michigan and examined for larval avian schistosomes. Approximately 0.19% of all snails isolated were found to have patent infections. Cercariae of *Trichobilharzia physellae* were obtained from 42 snails (0.16%), and 10 snails (0.04%) shed cercariae of *Gigantobilharzia huronensis*. In 1977-1979, collections were made during April-August; however, in 1979 the collection period was extended into November. Data obtained in 1979 suggest that infection rates for *Physa integra* show a bimodal curve. During the fall collections, 1,074 snails were collected; six (0.56%) were infected with *T. physellae*. This represents an increase in the infection rate compared to those snails obtained during July and August of the same year. Schistosome infections were more prevalent in larger *Physa integra*.

Gastropods representing three pulmonate families (Lymnaeidae, Physidae, and Planorbidae) are known to serve as intermediate hosts for nonhuman schistosomes in Michigan. In the southern part of the Lower Peninsula, *Physa integra* is the most important intermediate host for two genera of avian schistosomes (*Gigantobilharzia* and *Trichobilharzia*), causative agents of schistosome cercarial dermatitis.

Clampitt (1970) reported that *P. integra* is characteristically found in two general types of lake habitats, rocky shorelines and vegetated off-shore areas. Similar habitat preference by *P. integra* was observed in this study.

Strohm (1979) studied snails from several lakes in southeastern Michigan during 1976-1977, and recovered five cercarial types from *P. integra*, including *Trichobilharzia* sp. Clampitt (1972) lists *P. integra* as a host for both *Trichobilharzia physellae* and *Gigantobilharzia huronensis*. However, both investigators examined a relatively small sample of snails. The purpose of this study was to survey a large natural population of *P. integra* to determine the prevalence and seasonal abundance of patent avian schistosome infections.

### Materials and Methods

*Physa integra* snails examined in this study were collected from April through November of 1977-1979. All snails were obtained from two collection sites on the north shores of Gun Lake in Barry County, Michigan. They were collected either by hand or with a dip net in water ranging in depth from 2 cm to 1 m. Snails were segregated into groups of 25 per 7-cm finger bowl, which contained filtered lake water. The bowls were placed under heavy black cloth for at least 5 hr, and were then exposed to fluorescent light for 2 hr.

If a dish was observed to contain cercariae, the snails were removed, rinsed individually in filtered lake water, and then isolated in vials for an additional

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<sup>1</sup> This study was supported in part by the National Science Foundation (Undergraduate Research Participation) Grant SPI-7683612 AO2 and by the Michigan Department of Natural Resources.



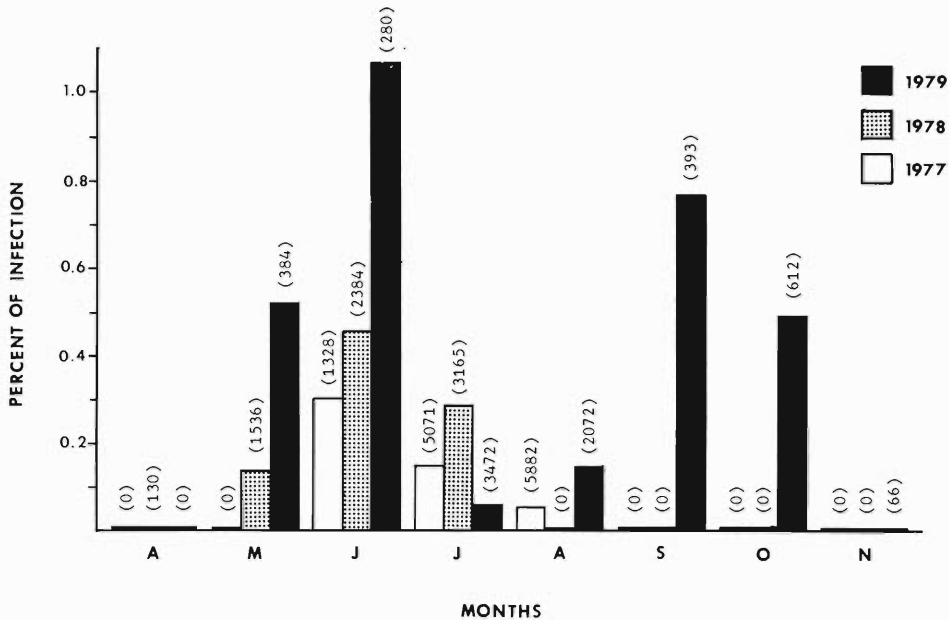


Figure 1. Percentage of *Physa integra* infected with avian schistosomes for each of the 3 yr (1977-1979). The number in parentheses represents the sample size.

shedding period. The 21,168 "mature" snails were each measured to determine the length (in mm) of the shell.

Cercariae were first examined alive; others were fixed in hot 3-5% buffered formalin, and dehydrated in increasing concentrations of ethanol. They were stained with Mayer's paracarmine, counterstained with fast green, cleared, and mounted on slides.

Identification of the schistosome cercariae was made on the basis of (1) size and morphology of mounted specimens, and (2) appearance and behavior of living specimens. *Gigantobilharzia huronensis* cercariae have tails approximately equal in length to their bodies, and rest at the surface of the water with their tails hanging down. *Trichobilharzia physellae* cercariae have tail lengths much greater than body lengths, and rest attached to the bottom or sides of the container.

## Results

Of 26,775 *Physa integra* examined for patent avian schistosomes, 52 (0.19%) were infected. Forty-two of the infections were *Trichobilharzia physellae*, and 10 were *Gigantobilharzia huronensis*. In 1979, no *P. integra* were found infected with *G. huronensis*.

Snails infected with avian schistosomes were first observed in May. In all 3 yr, infection rates increased in June and decreased in July (Fig. 1). In 1977 and 1978, snails were collected between April and August. In 1979, collections of *P. integra* were made from May to November. Six of 1,071 snails (0.56%) collected during September-November 1979 were infected with *T. physellae*. This represents a substantial increase in the percentage of infected snails when compared to those collected in July-August 1979 (0.09%).

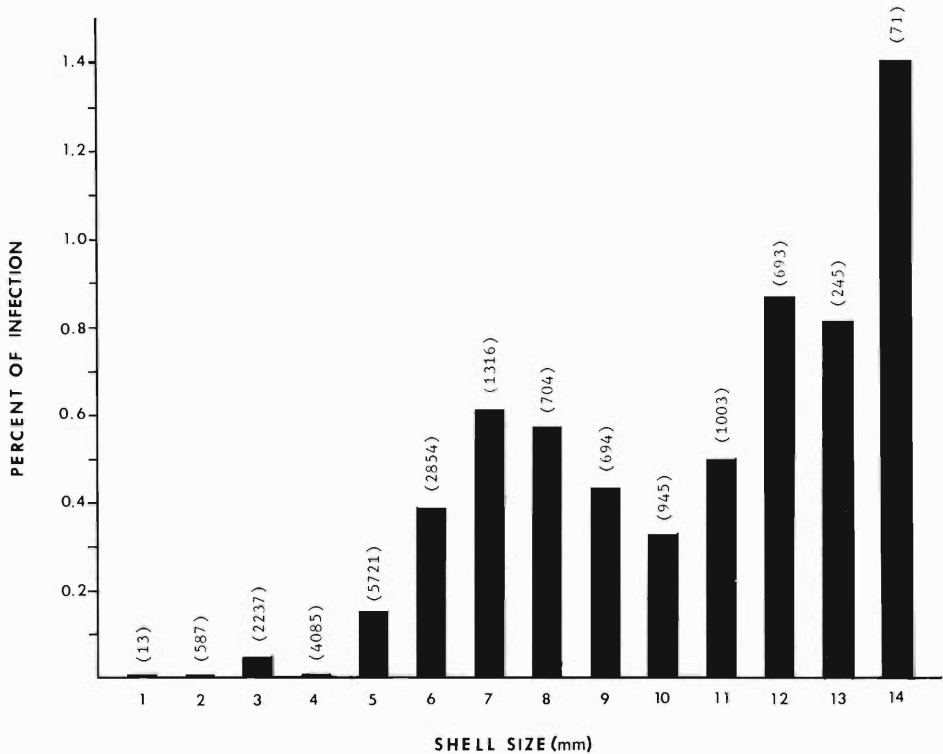


Figure 2. Prevalence of avian schistosomes in various size classes of *Physa integra* (1977-1979). The number in parentheses represents the sample size.

Data obtained during the 3-yr study indicate a general increase in infection rates for *P. integra* 1-7 mm in length (Fig. 2). Approximately 79% (16,813/21,168) of all measured snails fell into this size range. There was no regular trend in infection percentages in snails 8-14 mm in length.

In 1977 and 1978, the largest numbers of snails collected were 3,003 and 1,250, respectively, in the 5-mm size class. In 1979, the largest number (1,538) of any size was that of 4-mm snails.

### Discussion

Physid snails are important intermediate hosts for some species of avian schistosomes that cause swimmers' itch. Data from this study, involving a large sample of *Physa integra*, show that a very small percentage of snails bear patent infections with either *Gigantobilharzia huronensis* or *Trichobilharzia physellae*.

Clampitt (1970) indicated that egg production by *P. integra* is at a high level in May, and consequently, juvenile snails (1-2 mm) are present in great numbers in June. However, as the water temperature rises, mortality of adult snails increases dramatically, usually at the end of June or the beginning of July. This results in a physid population comprised primarily of juveniles. Data from the present study support Clampitt's (1970) findings. A decrease in the number of *P. integra* with patent infections in July and early August, may result because of the

mortality of adult snails. Strohm (1979) suggested this as a possible reason for declines in schistosome dermatitis outbreaks during the same months.

In all 3 yr, infection rates increased in May and June, and then decreased in July. These data support those of Strohm (1979), who examined a limited number of *P. integra* from southeastern Michigan. Unfortunately, most studies that involve prevalence of digenetic trematodes in snail intermediate hosts are conducted only during the summer months. Similarly, data gathered during the first 2 yr of this study followed such a pattern. However, in 1979, sampling of *P. integra* for schistosomes continued into November. Data from snails collected during this period indicate that infection levels increased sharply. This could result in large numbers of fall migratory birds becoming infected.

The average size of the six infected snails collected during the fall of 1979, was 7 mm; thus these snails were less than 6 mo old. This is contrary to speculation by Strohm (1979) who stated that physid snails can support patent infections after June, but that this is not commonly observed.

It is not known whether snails, shedding schistosome cercariae in early spring, overwinter with the infection or whether they become infected immediately after the ice melts. Work conducted by one of the present authors substantiates that physids can harbor infections throughout the winter, even under the ice. Blankespoor placed marked, laboratory-infected *Physa gyrina* in cages for overwintering in ponds. In the spring, the caged snails were found to still contain patent infections of *Gigantobilharzia huronensis*.

The alternate possibility is that snails become exposed early in the spring, when large numbers of definitive hosts accumulate. If this occurs, it would seem unlikely that snails with patent infections would be found in early May, because cold temperatures would delay development of the sporocysts.

There is little doubt that larger snails are more likely to carry patent infections. Of the seven infected *P. integra* isolated by Strohm (1979), the mean length was 11.3 mm. Similarly, the overall mean length of infected snails in the present study was 8.1 mm. Of 6,992 *P. integra* 4 mm in length or smaller, only one snail (3 mm) was found with a patent schistosome infection. Cort et al. (1955), studying the development of *T. physellae* in *Physa parkeri*, observed that young snails (less than 4 mm) do not usually harbor patent infections. They concluded that sporocyst development in small snails usually proceeds at such a rapid rate that the majority of the snails died prior to or just after reaching patent infections because of damage to the hepatopancreas.

Based on the data presented in this study, *Physa integra* is an effective intermediate host species for avian schistosomes in the Lower Peninsula of Michigan. Attempts to eradicate or control swimmers' itch with molluscicides should be undertaken with the understanding that huge volumes of lake water would have to be treated in order to kill a relatively small percentage of these infected snails.

#### Acknowledgments

This study was conducted at W. K. Kellogg Biological Station (Michigan State University), Hickory Corners, Michigan (Contribution 440). A special note of thanks is extended to Dr. David Daniell for the encouragement and assistance he offered during the project, and to Dr. Sam Loker for reviewing this manuscript.

### Literature Cited

- Clampitt, P. T. 1970. Comparative ecology of the snails *Physa gyrina* and *Physa integra* (*Basommatophora*: Physidae). *Malacologia* 10:113-151.
- Clampitt, P. T. 1972. The biology of swimmer's itch. *Explorer* 14:17-31.
- Cort, W. W., D. J. Ameel, and A. Van Den Woude. 1955. Germinal development in the sporocysts of a bird schistosome *Trichobilharzia physellae* (Talbot, 1936). *J. Parasitol.* 41:24-39.
- Strohm, B. 1979. The epizootiology and epidemiology of schistosome dermatitis in southeastern Michigan. *Dissertation Abstracts* 40:659-B.

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## A Rencolid Trematode Developing in the Limpet, *Collisella digitalis* (Rathke, 1833)

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**ABSTRACT:** A rencolid digenetic trematode uses the marine limpet, *Collisella digitalis*, as first and second intermediate host in southern California. Other limpets, *C. scabra*, *C. limatula*, *C. strigatella*, and *Lottia gigantea* from the same collecting area were negative. Sporocysts containing cercariae were found in the gonad and digestive gland; cercariae encyst in the same organs. The cercaria has a stylet, Y-shaped bladder, and simple tail. Because of its habit of feeding on limpets, the Black Oystercatcher, *Haematopus bachmani*, is suggested as a likely definitive host.

Cohn (1904) established the genus *Rencicola* for certain digenetic trematodes whose adults parasitize the kidneys of birds frequenting marine or brackish waters. Gastropods serve as first intermediate, and in some cases, second intermediate hosts. Pelecypods and fish may also serve as second intermediate hosts. Life cycles have been described by Stunkard (1964), Werding (1969), and Martin (1971).

In a recent survey of mollusks at Point Fermin, San Pedro, California, a rencolid cercaria was found in the limpet *Collisella digitalis* (Rathke, 1833) which also serves as the second intermediate host.

Limpets isolated in finger bowls and examined each day for 3 days did not shed cercariae but dissection revealed sporocysts and cercariae in some of them. The parasites were fixed in hot (near boiling) 5% formalin without pressure, stained in Mayer's paracarmine, and mounted in Canada balsam. Measurements are in micrometers averages in parentheses. Limpets were identified using McLean's "Key to the Marine Shells of Southern California."

### Observations

Of 1,252 *Collisella digitalis* examined by dissection, seven had sporocysts, cercariae, and metacercariae and one had only metacercariae. In no case did cercariae emerge from limpets over a 3-day period. When placed in seawater or physiological saline, cercariae did not swim but rocked back and forth with body and tail bent in a U-shape. Only daughter sporocysts were seen; they were yellow-brown and occupied much of the gonad and digestive gland. Two limpets appeared to be castrated by the parasites.

Other species of limpets from the same locality, examined in the same way, were negative. They included 1,204 *Collisella scabra* (Gould, 1846), 48 *C. limatula* (Carpenter, 1864), 61 *C. strigatella* (Carpenter, 1864), and 29 *Lottia gigantea* (Sowerby, 1834).

### Sporocysts

Mother sporocyst not seen. Young daughter sporocysts nearly cylindrical, with few germ balls; older ones partially or completely filled with germ balls and cercariae. Wall of sporocyst with granules 0.75-1.5 in diameter. Twenty stained and mounted sporocysts measure: length 389-833 (556); minimum width 33-89 (51); maximum width 144-322 (182).

## Cercaria

Distomate xiphidiocercaria with simple tail. Suckers subequal, well developed; acetabulum usually in posterior half of body. Tegument with transverse rows of spines decreasing in length toward acetabulum to disappear at that level. Three adhesive (?) glands on each side of hind body. Genital primordium immediately anterior to, or partially overlapping acetabulum. Mouth subterminal; pharynx immediately posterior to oral sucker; remainder of digestive tract incompletely developed. Numerous cystogenous glands and globules posterior to pharynx. Excretory bladder Y-shaped with arms embracing acetabulum. Wall of bladder cellular with bulges at nuclear locations. Main collecting ducts attached to stem of bladder; remainder of excretory system not determined. Tail can be extended to body length but always shorter in fixed specimens.

Measurements of 20 cercariae in whole mounts: body length 212–264 (232); body width 54–92 (71); stylet 2–2.5 wide and 13–14 long; oral sucker diameter 26–36 (30); pharynx 8–10 (9) long, 10–16 (11) wide; acetabulum 28–33 (31) long and 28–31 (29) wide; excretory bladder stem 72–90 (82) long and 13–33 (23) wide; bladder arms 26–44 (34) long and 10–23 (18) wide; tail 95–167 (137) long and 18–26 (21) wide.

Metacercariae in the digestive gland and gonad could be confused with the limpets eggs but are smaller and more translucent. Fixed and stained cysts are 110–113 in diameter. The cyst wall is about 6 thick and radially striated. Within it, the worm is bent upon itself, penetration glands are evident, and the detached stylet is partially dissolved. The conspicuous structure is the excretory bladder filled with concretions and its arms grown to reach the pharyngeal level.

## Discussion

The cercaria is of a well-known type represented by the larvae of *Renicola thaidus* (Stunkard, 1964); *R. roscovita* (Stunkard, 1932) Werding, 1969; *Cercaria parvicaudata* Stunkard and Shaw, 1931; *C. caribbea* XXXII Cable, 1956; *C. caribbea* XXXIII Cable, 1956; *C. opaca* Holliman, 1961; and the cercaria from *Buccinum* described by Kjøie (1969). She did not report metacercariae from *Buccinum* but Tsimbaliuk et al. (1968) found them in that host and suggested that they might be metacercariae of *Renicola lari*. In having five pairs of penetration glands, the subject of this paper differs from all of the above cercariae except that of *R. thaidus* and possibly the cercaria from *Buccinum* for which Kjøie did not determine the number of penetration glands. However, both of those cercariae emerge from their hosts whereas the present one apparently does not. Moreover, each of the three develops in a different molluscan species for which larval trematodes show considerable host specificity.

Several authors have had difficulty in infecting birds closely related to those found to have natural infections. Werding (1969) described the life cycle of *Renicola roscovita* (Stunkard, 1932) whose cercaria develops in *Littorina littorea* and *L. saxatilis* and suggested as possible synonyms: (1) *R. thaidus* (Stunkard, 1964) with cercariae developing in *Thais lapillus* and *T. lamellosa*; (2) *Cercaria parvicaudata* Stunkard and Shaw, 1931 that develops in *Littorina littorea*, *L. saxatilis*, and *L. obtusata*. Werding obtained adults of *R. roscovita* experimentally in the gull, *Larus argentatus*, but Stunkard (1971) fed thousands of *Cercaria parvicaudata*

*data* metacercariae to *L. argentatus* with negative results. From them, he concluded that *R. roscovita*, *C. parvicaudata*, and *R. thaidus* probably are different species. In the present work, *Collisella digitalis* was the only species of limpet from the collecting area that was infected.

The definitive host of this trematode has not been determined. If, as the results of this study indicate, the cercariae do not emerge from the limpet it must be a bird that feeds on that mollusk. In answer to my inquiry concerning such birds, Dr. David G. Ainley of the Point Reyes Bird Observatory, Bolina, California, wrote, "The only bird on this coast that eats appreciable numbers of limpets is the Black Oystercatcher. In fact a more proper name might be the Black Limpetcatcher." Hence, the definitive host of the species whose cercaria is the subject of the present study is likely to be *Haematopus bachmani*, the Black Oystercatcher.

#### Acknowledgments

I wish to thank associate directors of the Cabrillo Museum, Dr. S. Lawrenz-Miller and Mr. J. Olguin, for providing laboratory facilities.

#### Literature Cited

- Cohn, L. 1904. Helminthologische Mitteilungen II. Arch. Naturgesch. 70:229-252.
- Køie, M. 1969. On the endoparasites of *Buccinum undatum* L. with special reference to the trematodes. Ophelia 6:251-279.
- Martin, W. E. 1971. Larval stages of renicolid trematodes. Trans. Am. Microsc. Soc. 90:188-194.
- McLean, J. H. 1969. Marine shells of southern California. Los Angeles Co. Mus. Nat. Hist. Sci. Ser. 24, Zool. 11:1-104.
- Stunkard, H. W. 1950. Further observations on *Cercaria parvicaudata* Stunkard and Shaw, 1931. Biol. Bull. 99:136-142.
- Stunkard, H. W. 1964. Studies on the trematode genus *Renicola*: observations on the life-history, specificity, and systematic position. Biol. Bull. 126:467-489.
- Stunkard, H. W. 1971. Revue critique. Renicolid trematodes (Digenea) from the renal tubules of birds. Ann. Parasitol. Hum. Comp. 46:109-118.
- Stunkard, H. W., and C. R. Shaw. 1931. The effect of sea water on the activity and longevity of certain marine cercariae, with descriptions of two new species. Biol. Bull. 61:242-271.
- Tsimbaliuk, A. K., V. V. Kulikov, T. I. Baranova, and E. M. Tsimbaliuk. 1968. [Littoral invertebrates of Bering Island—intermediate and supplementary hosts of helminths of birds and mammals. Helminths of Animals of the Pacific Ocean.] Acad. Sci. SSSR 129-152. (In Russian.)
- Werdning, B. 1969. Morphologie, Entwicklung und Ökologie digener Trematoden-Larven der Strand-schnecke *Littorina littorea*. Mar. Biol. 3:306-333.

## Two New Species of *Lissorchis* Magath (Digenea: Lissorchiidae) from the Spotted Sucker, *Minytrema melanops* (Rafinesque), with a Key to Species

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**ABSTRACT:** *Lissorchis calentinei* sp. n. and *L. minytremi* sp. n. are described from the intestine of *Minytrema melanops* (Rafinesque, 1820) from Kentucky. These new species are most similar to *L. gullaris* Self and Campbell, 1957 (1956), *L. heterorchis* Krygier and Macy, 1969, and *L. polylobatum* (Haderlie, 1950), but differ from these and each other in body spination, egg size and shape, and the presence or absence of a prepharynx, vas deferens, metraterm, and cirrus spines. A key to the species of *Lissorchis* Magath, 1918 is provided.

Since Magath (1918) originally described the genus *Lissorchis*, 12 species have been described from freshwater fishes in North America. Although Simer (1929) proposed the genera *Triganodistomum* and *Alloplagiorchis* for species recovered from the Tallahatchie River, these were later considered to be synonyms of *Lissorchis* by Smith (1968) and Yamaguti (1958), respectively. Krygier and Macy (1969) described *L. heterorchis* from *Catostomus macrocheilus* Girard and in that work included a key to the 11 species then known. Recently *L. kritskyi* was described from *Carpionodes carpio* (Rafinesque) in Iowa (Barnhart and Powell, 1979).

In our studies of the ichthyoparasite fauna in Kentucky we recovered two unknown species of *Lissorchis* from *Minytrema melanops* (Rafinesque, 1820). This paper describes these new species and modifies the key to the known species of *Lissorchis* presented by Krygier and Macy (1969) to include *L. kritskyi* and the new species described herein.

### Materials and Methods

From 1977 to 1980, 124 specimens of *M. melanops* were collected and necropsied for helminth parasites from three locations in Kentucky: 57 from Kentucky Lake, 49 from Lake Barkley, and 18 from the West Fork of Drakes Creek. For whole mounts, trematodes were fixed in AFA under light cover glass pressure and stained with Mayer's paracarmine. Several specimens were fixed in Bouin's fluid, sectioned at 8  $\mu$ m, and stained with Harris's hematoxylin and eosin for confirmation of some aspects of morphology, especially concerning the presence of a metraterm. Figures were drawn with the aid of a microprojector and ocular micrometer. Measurements are in micrometers unless otherwise stated, and given as range followed by the mean in parentheses; dimensions of organs are stated as length by width. Notations for deposited specimens are: USNM Helm. Coll. No. for United States National Museum Helminthological Collection, Beltsville, Maryland and HWML No. for Harold W. Manter Laboratory, Division of Parasitology, University of Nebraska State Museum, Lincoln, Nebraska.



*Lissorchis calentinei* sp. n.

(Fig. 1)

DESCRIPTION (based on five gravid specimens): Body elongate, tapering at both ends, 3.63–4.77 mm (4.29 mm) by 0.72–1.11 mm (0.87 mm), widest at posterior edge of acetabulum. Body spinose from anterior end to level of cecal bifurcation and from posterior ½ of acetabulum to level of anterior testis. Oral sucker subterminal, 396–464 (430) by 344–395 (368). Prepharynx shorter than pharynx, pharynx 159–198 (173) by 92–111 (102). Esophagus approximating length of pharynx. Cecal bifurcation anterior to acetabulum, extending to posterior end of body. Acetabulum 511–666 (600) by 522–696 (601), in anterior ⅓ of body. Sucker width ratio 1:1.41–1.85 (1:1.64). Forebody 17–20% of body length.

Testes tandem, in posterior ½ of body, anterior testis 467–696 (604) by 174–241 (208), posterior testis 586–726 (668) by 174–261 (214). Cirrus sac slightly post-acetabular, oblique, 583–783 (661) by 122–174 (139), opening at left body margin at level of posterior ½ of acetabulum. Internal seminal vesicle bipartite, cirrus protrusible, armed with minute spines. Prostatic gland cells numerous in distal ⅓ of cirrus sac. Vasa efferentia joining directly to seminal vesicle, vas deferens absent. Posttesticular body space 618–931 (778), 16–20% of body length.

Ovary with 12–16 (14) lobes, immediately pretesticular, 413–626 (481) by 319–444 (382). Seminal receptacle and Laurer's canal absent. Uterus arising at anterior edge of ovary, forming longitudinal loops on both sides of body between acetabulum and posterior end of body, opening immediately posterior to cirrus; metraterm absent. Vitelline glands convoluted, with 63–91 (77) interconnected follicles, extending from posterior margin of acetabulum to beyond posterior testis. Intrauterine eggs nearest genital pore amber, 12–18 (15) by 9–12 (11) ( $N = 24$ ).

Excretory pore terminal; excretory bladder I-shaped.

TYPE HOST: *Minytrema melanops* (Rafinesque, 1820).

TYPE LOCALITY: Kentucky Lake, Trigg County, Kentucky.

SITE: Intestine.

HOLOTYPE: USNM Helm. Coll. 76741.

PARATYPE: USNM Helm. Coll. 76742.

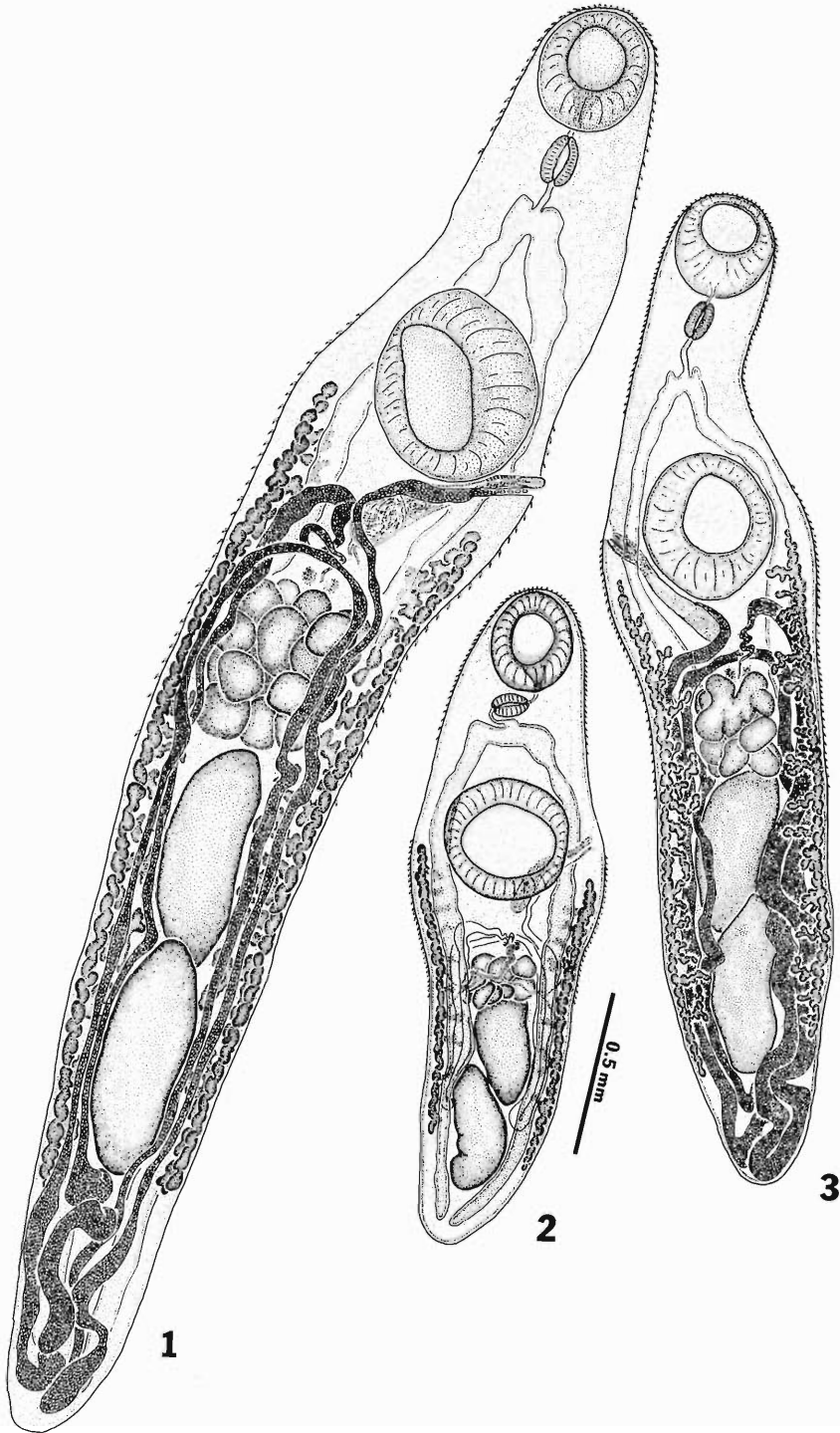
ETYMOLOGY: Named for Robert L. Calentine in recognition of his contributions to helminthology.

*Lissorchis minytremi* sp. n.

(Figs. 2, 3)

DESCRIPTION (based on 10 gravid specimens): Body elongate, tapering at both ends, 2.29–3.53 mm (2.95 mm) by 0.60–0.88 mm (0.73 mm), widest at level of acetabulum. Body spinose from anterior end almost to acetabular level and again from midacetabular level to that of anterior testis. Oral sucker subterminal, 277–348 (320) by 261–361 (317). Prepharynx shorter than pharynx, pharynx 110–150 (127) by 64–97 (80). Esophagus approximating length of pharynx. Ceca bifurcating anterior to acetabulum, extending to posterior end of body. Acetabulum in anterior ½ of body, 363–500 (442) by 396–491 (439). Sucker width ratio 1:1.29–1.51 (1:1.39). Forebody 22–27% of body length.

Testes tandem, in posterior ½ of body, anterior testis 309–624 (455) by 174–291 (205), posterior testis 452–755 (541) by 144–228 (185). Cirrus sac on left, obliquely



Figures 1–3. 1. *Lissorhis calentinei* sp. n., ventral view of holotype. 2, 3. *Lissorhis minytremi* sp. n. 2. Immature specimen, ventral view. 3. Dorsal view of holotype.

posteriolateral to acetabulum, 400–690 (503) by 62–96 (74), opening on body margin at midacetabular level. Internal seminal vesicle bipartite, cirrus protrusible, armed with minute spines. Prostatic gland cells numerous in distal  $\frac{1}{3}$  of cirrus sac. Vasa efferentia joining directly to seminal vesicle, vas deferens absent. Post-testicular body space 208–495 (348), 7–15% of body length.

Ovary with 9–12 (11) lobes, immediately pretesticular, 270–369 (320) by 216–361 (277). Seminal receptacle and Laurer's canal absent. Uterus beginning near anterior edge of ovary, forming longitudinal loops on both sides of body from posterior edge of acetabulum to posterior end of body, opening immediately posterior to cirrus; metraterm present. Vitelline glands convoluted, with 60–102 (83) interconnected follicles extending from posterior margin of acetabulum to beyond posterior testis. Intrauterine eggs nearest genital pore amber, 17–20 (18) by 7–9 (8) ( $N = 30$ ).

Excretory pore terminal; excretory bladder I-shaped.

TYPE HOST: *Minytrema melanops* (Rafinesque, 1820).

TYPE LOCALITY: West Fork of Drakes Creek, Simpson County, Kentucky.

SITE: Intestine.

HOLOTYPE: USNM Helm. Coll. 76743.

PARATYPES: USNM Helm. Coll. 76744; HWML 21341.

ETYMOLOGY: Named after its host.

### Discussion

*Lissorchis calentinei* and *L. minytremi* show the greatest similarity to each other and to *L. gullaris* Self and Campbell, 1957 (1956), *L. heterorchis* Krygier and Macy, 1969, and *L. polylobatum* (Haderlie, 1950). These trematodes differ from each other in body spination, egg size and shape, and the presence or absence of a prepharynx, vas deferens, metraterm, and cirrus spines. Body spination occurs in two defined areas in *L. calentinei* and *L. minytremi*, is entire in *L. gullaris* and is uninterrupted from the anterior end of the body to the testicular level in *L. heterorchis* and *L. polylobatum*. Eggs of *L. calentinei* are shorter and wider (15 by 11) than those of *L. minytremi* (18 by 8). Eggs are much larger in *L. gullaris* (23 by 11), *L. heterorchis* (26 by 16), and *L. polylobatum* (25 by 17). A prepharynx is present in *L. calentinei*, *L. minytremi*, and *L. gullaris*, but is not evident in *L. heterorchis* and *L. polylobatum*. Only *L. polylobatum* lacks cirrus spines. A vas deferens is absent in *L. calentinei* and *L. minytremi*, but is present in *L. gullaris*. A distinct metraterm present in *L. minytremi*, *L. gullaris*, and *L. heterorchis* is absent in *L. calentinei*. *Lissorchis calentinei* is also much larger than *L. minytremi*, a fact we consider significant considering both trematodes were recovered from the same host species.

The prevalence of *L. calentinei* was only 7% (4/57) in Kentucky Lake, the only site from which this species was recovered. Likewise, the parasite burden was light. One fish contained three specimens and each of three others contained a single worm. In two cases *L. calentinei* were found in fish that also contained *L. kritskyi*. The prevalence of *L. minytremi* in spotted suckers from the West Fork of Drakes Creek was 50% (9/18) and intensities exceeded 20 trematodes in several fish. Four of the fish infected with *L. minytremi* also harbored *L. kritskyi*.

Very likely, either *L. calentinei* or *L. minytremi* is the species that Aliff (1973, 1977) reported from *M. melanops* but did not name. However, its description is

so incomplete and the quality of the deposited specimens (USNM Helm. Coll. 72601 and 72602) so poor that valid comparisons were impossible.

We agree with Duncan (1972) and with Barnhart and Powell (1979) that taxonomy within the genus *Lissorthis* is controversial. Uncertainty as to the validity of certain species will likely remain until more data on their biology become available.

Following is a modified and updated version of the key to the species of *Lissorthis* given by Krygier and Macy (1969).

#### Key to the Adult Species of *Lissorthis* Magath, 1917

1. Vitelline follicles large, fewer than 16 on each side ..... 2  
    Vitelline follicles small, 16 or more on each side ..... 4
2. Posttesticular body length greater than 20% of total body length; eggs  
    average 24 by 12 ..... *L. simeri* (VanCleave and Mueller, 1932)  
    (from *Minytrema melanops*, *Catostomus commersoni*; KY, OH, NY,  
    L. Huron)  
    Posttesticular body length less than 20% of total body length ..... 3
3. Cirrus protrusible; vas deferens absent; eggs 14–18 by 8–11 .....  
    ..... *L. kritskyi* Barnhart and Powell, 1979  
    (from *Carpionodes carpio*, *Minytrema melanops*; IA, KY)  
    Cirrus nonprotrusible; vas deferens present; eggs 17–19 by 11–13 .....  
    ..... *L. mutabile* (Cort, 1918)  
    (from *Erimyzon sucetta*; MI, MN, TN)
4. Tegumental spines extending to posterior end of body ..... 5  
    Tegumental spines extending no farther than level of posterior testis ..... 6
5. Cirrus armed and protrusible; distinct esophagus present; eggs average  
    23 by 11 ..... *L. gullaris* Self and Campbell, 1956 (1956)  
    (from *Ictiobus cyprinella*, *I. bubalus*, *I. niger*, *Catostomus catostomus*;  
    OK, ND, SD, Labrador)  
    Cirrus not armed and nonprotrusible; esophagus absent; eggs average 20  
    by 10 ..... *L. fairporti* Magath, 1918  
    (from *Ictiobus cyprinella*, *I. bubalus*; IA, MS)
6. Ovary overlapping both testes; testes wider than long; body flask-  
    shaped; eggs average 16 by 11 ..... *L. garricki* (Simer, 1929)  
    (from *Carpionodes difformis*; MS)  
    Ovary not overlapping both testes; testes spherical or longer than wide;  
    body elongate ..... 7
7. Anterior limits of vitelline fields much posterior to acetabulum; eggs  
    19–20 by 12–13 ..... *L. hypentelii* (Fishthal, 1942)  
    (from *Hypentelium nigricans*, *Moxostoma aureolum*, *M. macrolepidotum*;  
    WI, MI, WV)  
    Vitelline fields reaching level of acetabulum ..... 8
8. Uterus consisting of single loop to posterior end of body; small trema-  
    tode averaging 1.0 mm in length; eggs 19–24 by 12–14 .....  
    ..... *L. translucens* (Simer, 1929)  
    (from *Ictiobus bubalus*; MS)
9. Tegumental spines confined to ventral body surface, absent on body  
    margins; combined width of ceca equal to  $\frac{1}{3}$  of body width; eggs

- average 21 by 18 ..... *L. crassicurum* (Haderlie, 1953)  
(from *Catostomus rimiculus*; CA)
- Tegumental spines on body margins, not confined to ventral surface;  
ceca narrower ..... 10
- 10. Prepharynx absent ..... 11
- Prepharynx present ..... 12
- 11. Cirrus spined; testes distinctly lobed; posterior testis about 1/3 longer  
than anterior testis; eggs average 26 by 16 .....  
..... *L. heterorchis* Krygier and Macy, 1969  
(from *Catostomus macrocheilus*; OR)
- Cirrus not spined; testes not distinctly lobed; testes of nearly equal size;  
eggs average 25 by 17 ..... *L. polylobatum* (Haderlie, 1950)  
(from *Catostomus occidentalis*; CA)
- 12. Tegumental spines uninterrupted from anterior end to level of posterior  
testis; vas deferens present; eggs average 20 by 12.....  
..... *L. attenuatum* (VanCleave and Mueller, 1932)  
(from *Catostomus commersoni*, *C. catostomus*, *C. macrocheilus*,  
*Moxostoma rubresques*, *Semotilus atromaculatus*, *Gila straria*; WI,  
WY, MI, KY, NY, OH, IA, NH, PA, ME, ND, Br. Columbia)
- Tegumental spines in two distinct zones, interrupted in area anterior to  
acetabulum; vas deferens absent ..... 13
- 13. Metraterm present; eggs 17–20 by 7–9 ..... *L. minytremi* sp. n.  
(from *Minytrema melanops*; KY)
- Metraterm absent; eggs 12–18 by 9–12 ..... *L. calentinei* sp. n.  
(from *Minytrema melanops*; KY)

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**Literature Cited**

Aliff, J. V. 1973. Digenetic trematodes from Kentucky fishes. Ph.D. Dissertation, University of Kentucky, Lexington, Kentucky, 159 pp.

Aliff, J. V. 1977. Digenetic trematodes from Kentucky fishes. *Trans. Ky. Acad. Sci.* 38:1–14.

Barnhart, M. C., and E. C. Powell. 1979. *Lissorchis kritskyi* sp. n. (Digenea: Lissorchiidae) from the river carpsucker, *Carpionodes carpio* (Rafinesque). *Proc. Helminthol. Soc. Wash.* 46:47–51.

Duncan, B. L. 1972. Three new lissorchiid cercariae of the *mutabile* group from *Laevapex fuscus* and *Ferrissia rivularis* with observations on their host-parasite relationships and ecology, and a review of the genus *Lissorchis*. Ph.D. Dissertation, Wayne State University, Detroit, Michigan, 185 pp.

Krygier, B. B., and R. W. Macy. 1969. *Lissorchis heterorchis* sp. n. (Trematoda: Lissorchiidae) from *Catostomus macrocheilus* Girard in Oregon. *Proc. Helminthol. Soc. Wash.* 36:136–139.

Magath, T. B. 1918. The morphology and life history of a new trematode parasite, *Lissorchis fairporti* nov. gen., et nov. spec. from the buffalo fish, *Ictiobus*. *J. Parasitol.* 4:58–69 (1917).

Simer, P. H. 1929. Fish trematodes from the lower Tallahatchie River. *Am. Midl. Nat.* 11:563–588.

Smith, R. J. 1968. Ancyliid snails as first intermediate hosts of *Lissorchis mutabile* comb. n. (Trematoda: Lissorchiidae). *J. Parasitol.* 54:283–285.

Yamaguti, S. 1958. *Systema Helminthum*. Vol. I. Interscience, New York. 1575 pp.

## ***Prohyptiasmus grusi* sp. n. (Trematoda: Cyclocoelidae) from Sandhill Cranes (*Grus canadensis*)**

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ABSTRACT: *Prohyptiasmus grusi* sp. n. is described from *Grus canadensis canadensis* and *G. canadensis rowani* from Texas, Oklahoma, Canada, Nebraska, and Alaska. *P. grusi* is similar to *P. robustus* except the uterine pore is postpharyngeal and the uterine coils do not extend beyond the posterior testis. Additionally, the new species also differs from other members of this genus in host range, geographic distribution and site of infection.

During 1979 and 1980, sandhill cranes (*Grus canadensis canadensis* and *G. canadensis rowani*) were examined for helminth parasites in conjunction with a study of their behavioral ecology in Texas, Oklahoma, Nebraska, Canada, and Alaska. Three hundred nineteen birds were examined. Of these, 32 (10.0%) were infected with an undescribed digenean that appeared to belong to the genus *Prohyptiasmus*. Specimens were fixed in formalin or alcohol and stained in Delafield's iron hematoxylin. In the following description measurements are in micrometers and the mean follows the range in parentheses.

### ***Prohyptiasmus grusi* sp. n. (Fig. 1)**

SPECIES DIAGNOSIS (based on 7 adult specimens): Body elongate with rounded extremities 15,600 (20,000-11,000) by 4,000 (2,500-6,000). Oral sucker nonmuscular and terminal 190 (120-320), pharynx 280 (180-320) and esophagus 160 (40-201) short, pharynx well developed; cecae without diverticula, fused at terminal end (continuous). Testes smooth, tandem in posterior 1/2 of body. Anterior testis 520 (160-250) long 250 (140-400) wide, posterior testis 610 (140-1,120) long, 310 (240) wide. Genital pore ventral, immediately postpharyngeal. Cirrus unarmed, enclosed by a unipartite seminal vesicle and cirrus pouch. Ovary ovoid, 290 (200-360) long, 220 (140-360) wide, between anterior and posterior testes. Ootype not observed. Uterus not extended beyond posterior testis. Vitelline follicles small, in lateral fields, not united posteriorly, extending from pharynx to posterior testis. Eggs small, operculate, 111 (90-140) long, 80 (70-85) wide.

HOLOTYPE: Adult; USNM Helm. Coll. 76305.

PARATYPES: USNM Helm. Coll. (3) 76449.

TYPE HOSTS: *Grus canadensis canadensis* and *G. canadensis rowani*.

INFECTION SITE: Body cavity.

TYPE LOCALITY: Texas, Oklahoma, Canada, Nebraska, Alaska.

### **Remarks**

*Prohyptiasmus grusi* sp. n. is similar to *P. robustus* (Stossich, 1902) in body shape and size. The new species differs from *P. robustus* in that the genital pore

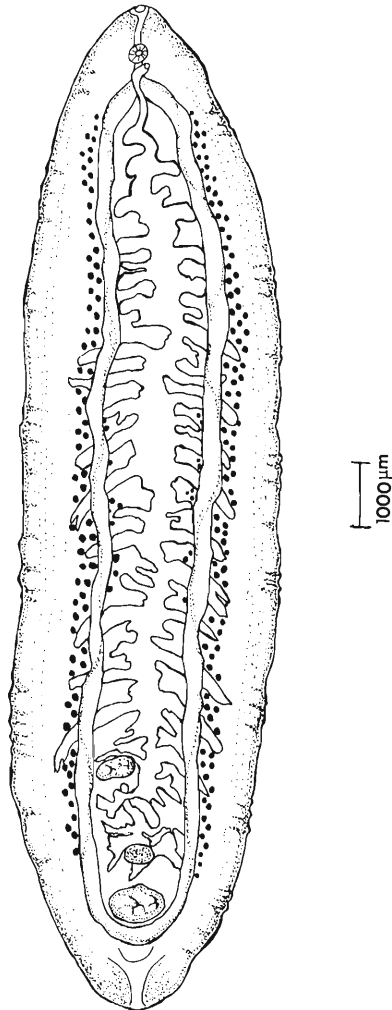


Figure 1. *Prohyptiasmus grusi* sp. n. adult, ventral view.

is immediately postpharyngeal and the uterine coils do not extend beyond the posterior testes. Previous reports of species in the genus *Prohyptiasmus* are from birds other than cranes in Europe, Asia, and Australia (Yamaguti, 1971). The site of infection is the nasal or intraorbital sinuses and pharynx. *Prohyptiasmus grusi* sp. n. was recovered from the body cavity of sandhill cranes that migrate from breeding areas in Alaska and Canada to winter in Texas and Oklahoma. The new species may be restricted to the midcontinental population of sandhill cranes since other parasite surveys of North American cranes have failed to observe this parasite (Burnham, 1972; Dubois and Rausch, 1964; Bush et al., 1973; Forrester et al., 1974, 1975).

The new species also bears some superficial likeness to the genus *Morishitium* (Witenberg, 1928) in the site of infection and the location of the genital pore. It

differs that the testes are tandem and not diagonal, the ovary is not contiguous or very close to the posterior testis and the ovary is not in a straight line with the testes. Additionally, the uterine coils extend laterally beyond the ceca and the overall size is much greater than has been reported for members of the genus *Morishitium*. For these reasons, the new species has been assigned to the genus *Prohaptiasmus* until life cycle studies can be completed to determine its exact relationship with other members of the family Cyclocoelidae.

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#### Literature Cited

- Burnham, G. L. 1972. Some helminth parasites of the sandhill crane in west Texas. *Southwest. Nat.* 17:200-201.
- Bush, A. O., D. B. Pence, and D. J. Forrester. 1973. *Tetrameres (Gynaecophila) williamsi* sp. n. (Nematode: Tetrameridae) from the white ibis, *Eudocimus albus*, with notes on *Tetrameres grusi* Shumakovich from the sandhill crane, *Grus canadensis*. *J. Parasitol.* 59:788-792.
- Dubois, G., and R. L. Rausch. 1964. Studies on the helminth fauna of Alaska. XL. *Strigia grusi* sp. n., a trematode parasite of *Grus canadensis* (L.). *J. Parasitol.* 50:445-447.
- Forrester, D. J., A. O. Bush, L. E. Williams, Jr., and D. J. Weiner. 1974. Parasites of greater sandhill cranes (*Grus canadensis tabita*) on their wintering grounds in Florida. *Proc. Helminthol. Soc. Wash.* 41:55-59.
- Forrester, D. J., A. O. Bush, L. E. Williams, Jr., and D. J. Weiner. 1975. Parasites of Florida sandhill cranes, *Grus canadensis protensis*. *J. Parasitol.* 63:547-548.
- Yamaguti, S. 1971. Synopsis of Digenetic Trematodes of Vertebrates. Keigaku Publ. Co., Tokyo. 1042 pp.



## *Styphlotrema artigasi* sp. n. (Plagiorchiidae), a New Trematode Parasite of Marine Fish<sup>1</sup>

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**ABSTRACT:** *Styphlotrema artigasi* sp. n. is described from *Guavina guavina* (Cuv. et Val.), a marine fish from Guanabara Bay, Rio de Janeiro State, Brazil. It differs from *S. solitaria* (Looss, 1899) Odhner, 1911, a parasite of marine turtles and the only other species in the genus, in having a saccate seminal vesicle, more extensive vitellaria with follicles almost meeting in the midline, and in having smaller eggs.

This paper reports a new species of *Styphlotrema*, a genus previously represented only by the type species, *S. solitaria* (Looss, 1899) Odhner, 1911, which has been reported from marine turtles in Egypt (Looss, 1899), Florida, U.S.A. (Luhman, 1935; Byrd et al., 1940), Puerto Rico (Fischthal and Acholonu, 1976), and Cuba (Groschaft et al., 1977). *Styphlotrema artigasi* sp. n. is described on the basis of a single mature specimen from a marine fish in Brazil.

The specimen was fixed under light coverglass pressure in Railliet and Henry's fluid, stained with alcoholic acid carmine, dehydrated in EtOH, cleared in Faya's creosote, and mounted in Canada balsam. The illustration was made with the aid of a drawing tube; measurements are in millimeters.

### *Styphlotrema artigasi* sp. n. (Fig. 1)

**DESCRIPTION:** Elongate distome with body tapering anteriorly, rounded posteriorly; 2.91 long, 0.59 wide. Suckers subequal, subspherical, 0.16 to 0.17 in diameter. Oral sucker subterminal; acetabulum pre-equatorial. Prepharynx short, pharynx 0.094 in diameter. Esophagus 0.16 long, bifurcating midway between suckers; ceca narrow, tubular, not entering posterior  $\frac{1}{4}$  of body length. Genital pore at anterodextral margin of acetabulum. Testes ovoid, symmetrical, post-equatorial; right testis 0.26 long, 0.18 wide; left testis 0.20 long, 0.14 wide. Cirrus sac straight, 0.50 long by 0.22 wide, reaching ovarian level; containing inflated saccate seminal vesicle. Cirrus evaginated, 0.11 long, 0.08 wide. Ovary smooth, 0.17 long, 0.12 wide; immediately anterior to right testis. Seminal receptacle 0.21 in diameter, just posterior to cirrus sac; Mehlis' gland and transverse vitelline ducts in that area. Laurer's canal not evident. Vitellaria consisting of relatively few round to oval follicles 0.023 to 0.075 by 0.030 to 0.056, extending from ovarian level past testes but not beyond ends of ceca; right and left vitelline fields lateral to, above, and between ceca but not confluent. Uterus fills body posterior to testes before extending anteriorly to join conspicuous metraterm 0.31 long, 0.11 wide. Eggs operculate, 0.019 to 0.028 by 0.015 to 0.019. Excretory system not observed.

**HOST:** *Guavina guavina* (Cuvier et Valenciennes) (Electridae).

<sup>1</sup> Supported in part by the National Research Council (CNPq)-Brazil.

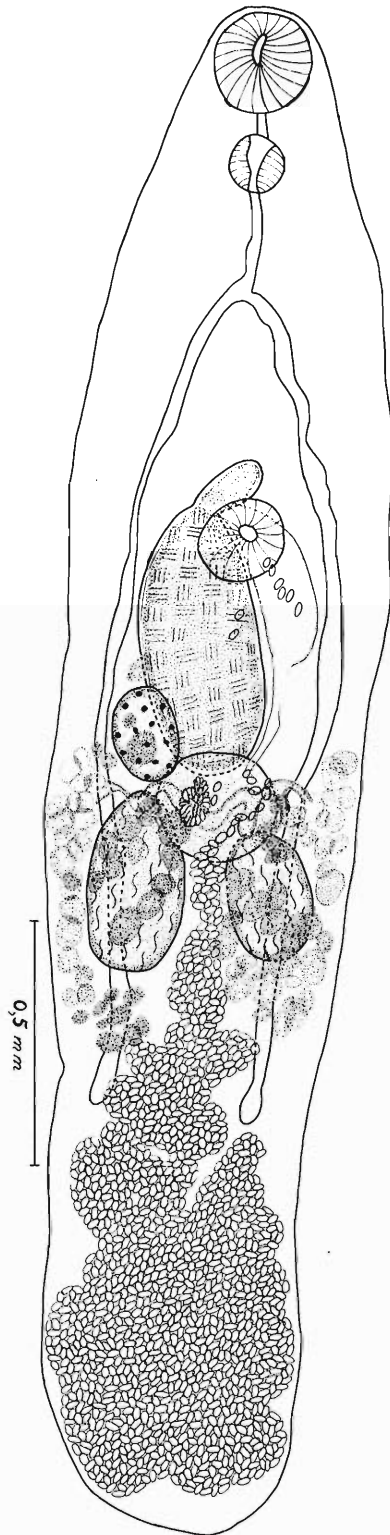


Figure 1. *Styphlotrema artigasi* sp. n. Ventral view of holotype.

HABITAT: Intestine.

LOCALITY: Guanabara Bay, Rio de Janeiro, Brazil.

DEPOSITED SPECIMEN: Holotype 31.819, Helm. Coll., Inst. Oswaldo Cruz.

ETYMOLOGY: The species is named in honor of Dr. Paulo de Toledo Artigas, São Paulo University, in recognition of his contributions to helminthological research in Brazil.

### Remarks

Besides its definitive host being a marine fish instead of a marine turtle, *Styphlotrema artigasi* sp. n. differs from *S. solitaria* in having smaller eggs, a sacciform seminal vesicle and vitellaria not extending into the intercecal region.

### Literature Cited

- Byrd, E. E., M. V. Parker, and R. J. Reiber. 1940. Taxonomic studies on the genus *Styphlodora* Looss, 1899 (Trematoda: Styphlodoridae), with descriptions of four new species. Trans. Am. Microsc. Soc. 59:294-326.
- Fischthal, J. H., and A. D. Acholonu. 1976. Some digenetic trematodes from the Atlantic hawksbill turtle, *Eretmochelys imbricata imbricata* (L.) from Puerto Rico. Proc. Helminthol. Soc. Wash. 43:174-185.
- Groschaft, J., O. A. Coy, and F. Tenora. 1977. Trematodes (Trematoda) from Cuban turtles, *Chelonia mydas mydas* (L.) and *Eretmochelys imbricata imbricata* (L.) (Testudinata-Cheloniidae). Acta Univ. Agric. 25:155-167.
- Looss, A. 1899. Weitere Beiträge zur Kenntniss der Trematoden-Fauna Aegyptens, zugleich Versuch einer natürlichen Gliederung des Genus *Distomum* Retzius. Zool. Jahrb. Abt. Syst. 12:521-784.
- Luhman, M. 1935. Two new trematodes from the loggerhead turtle (*Caretta caretta*). J. Parasitol. 21:274-276.

## Additional Records of Digenetic Trematodes of Marine Fishes from Israel's Mediterranean Coast

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**ABSTRACT:** In 1979, 375 fishes (50 species) from Israel's Mediterranean coastal waters were examined for digenetic trematodes; 115 (30.7%) harbored at least one species. One new species and one new larval form are described: *Lecithochirium jaffense* sp. n. (Hemiuridae) from *Trachinotus ovatus*, *Seriola dumerili*, *Epinephelus* sp., *Echeneis naucrates*, *Pomatomus saltatrix*, and *Gobius cobitus*; Immature Didymozoid I (Didymozoidae) from *Caranx dentex*. Twenty-four previously known species are reported from Israel, many representing new host records while seven are reported from Israel for the first time. Two species of Red Sea immigrant fishes each harbored one trematode species known only from the Mediterranean.

Fischthal (1980) reported 47 species (11 new) of digenetic trematodes of marine fishes from Israel's Mediterranean coast. The present study is a follow-up, especially in an attempt to examine additional Red Sea immigrant fishes. From 29 June to 21 August 1979 I examined 375 fishes comprising 50 species in 38 genera and 26 families. Three of these species are cosmopolitan, 34 Atlanto-Mediterranean, 1 Mediterranean, 11 Red Sea immigrants, and 1 fresh- and brackish-water (from Tel-Baruch). Only three species of Red Sea immigrants studied in 1979 were not taken in 1977 when 12 species were examined; therefore, a total of 15 species were examined for both years. At least one species of trematode was recovered from 115 (30.7%) of the 375 fishes examined. A total of 26 species were identified, including one new species and one new larval form; three others were identified to genus only. An asterisk (\*) preceding the host name indicates a new host record; one preceding the locality (for seven trematode species) indicates that it is reported from Israel's Mediterranean for the first time.

### Materials and Methods

Fresh fishes were obtained from local fishermen gill-netting or purse-seining in Israel's coastal waters, refrigerated, and examined within several hours for digenetic trematodes. All worms were alive when recovered. They were killed in hot water, fixed in AFA without coverglass pressure, and stored in 70% alcohol; subsequently, they were stained in Mayer's paracarmine and mounted in Permount. Specimens were deposited in the U.S. National Museum Helminthological Collection as noted. All measurements are in micrometers.

### Hemiuridae

#### *Lecithochirium jaffense* sp. n.

(Figs. 1, 2)

**HOSTS:** Type, *Trachinotus ovatus* (L.) (Carangidae); *Seriola dumerili* (Risso) (Carangidae); *Epinephelus* sp. (Serranidae); *Echeneis naucrates* (L.) (Echeneidae); *Pomatomus saltatrix* (L.) (Pomatomidae); *Gobius cobitus* Pallas (Gobiidae).

**SITE:** Stomach.

**LOCALITIES:** Tel-Baruch, Jaffa.

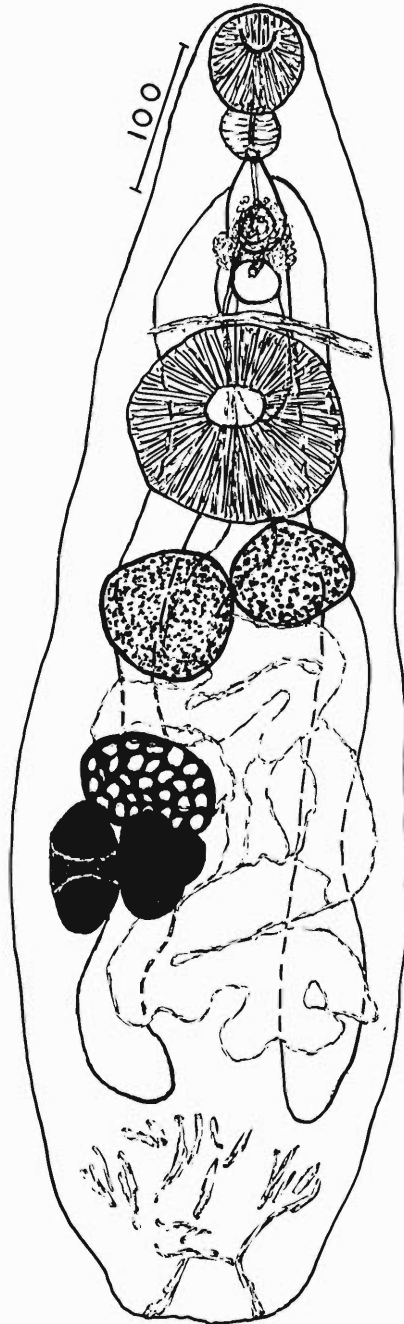


Figure 1. *Lecithochirium jaffense* sp. n. Adult, holotype, ventral view. Scale in micrometers.

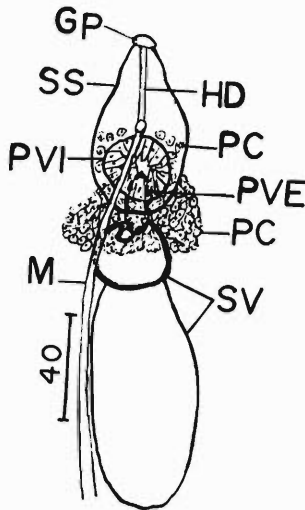


Figure 2. *Lecithochirium jaffense* sp. n. Terminal genitalia, holotype. Abbreviations: GP, genital pore; HD, hermaphroditic duct; M, metraterm; PC, prostate cells; PVE, external prostatic vesicle; PVI, internal prostatic vesicle; SS, sinus sac; SV, seminal vesicle. Scale in micrometers.

**SPECIMENS DEPOSITED:** No. 76389 (holotype, *T. ovatus*); No. 76390 (paratype, *T. ovatus*); No. 76391 (paratypes, *S. dumerili*); No. 76392 (paratype, *Epinephelus* sp.); No. 76393 (paratype, *E. naucrates*); No. 76394 (paratype, *P. saltatrix*); No. 76395 (paratype, *G. cobitus*).

**DESCRIPTION** (based on 17 worms, 8 measured): Body elongate, smooth, anterior extremity rounded, ecsoma short, completely retracted in all but two worms; body (without ecsoma) 511–843 long by 123–257 wide at vitellarian level. Forebody 158–230 long; hindbody (without ecsoma) 205–501 long; forebody-hindbody length ratio 1:1.34–2.43. Preacetabular pit absent. Oral sucker ventral, 48–71 by 53–77; preoral space 6–18 long. Acetabulum 94–140 in diameter, aperture round to transversely oval. Sucker length ratio 1:1.73–2.25, width ratio 1:1.173–2.12. Prepharynx absent; pharynx partly dorsal to oral sucker, 26–44 by 32–52; esophagus short, thick-walled, muscular; prececal sacs muscular, walls same thickness as esophagus or thinner, only posterior part with cellular lining which is continuous with that of ceca; cecal bifurcation 41–97 preacetabular; ceca narrow, undulating, possibly entering ecsoma. Excretory vesicle dilated posteriorly, arms uniting dorsal to pharynx or oral sucker.

Testes two, smooth, overlapping or contiguous, occasionally up to 16 apart, not separated by uterus, near or overlapping acetabulum, diagonal to symmetrical (in four worms); anterior testis dextral in two, sinistral in eight; right testis 35–88 by 35–92, left testis 32–77 by 33–85. Seminal vesicle overlapping acetabulum 32–61, bipartite; posterior chamber saccular, thin-walled, 55–81 by 34–63; anterior chamber muscular, thick-walled, subspherical, 14–32 in diameter, joined to external prostatic vesicle by thick-walled, muscular duct. That vesicle straight, 23–33 by 8–16, with epithelial lining, surrounded by large, compact mass of prostate cells. Sinus sac pyriform, with slightly thickened wall, 50–75 by 22–37, 23–54 preacetabular; containing hermaphroditic duct and a few prostate cells adjacent to spherical, internal prostatic vesicle 17–25 in diameter. Genital pore median to

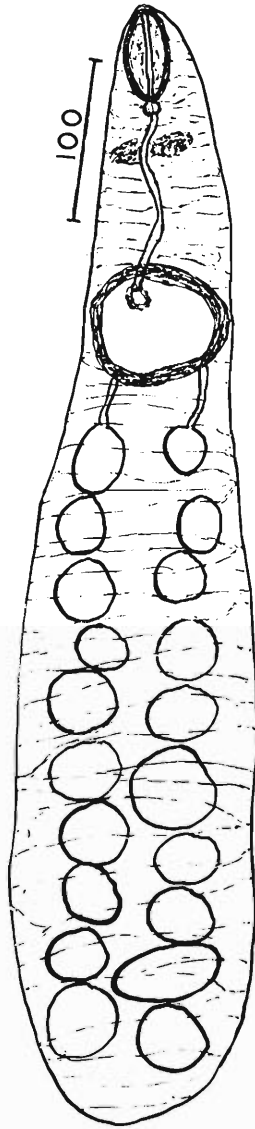


Figure 3. *Lecithochirium jaffense* sp. n. Larva. Immature Didymozoid I (new form). Scale in micrometers.

submedian, at pharyngeal level or occasionally slightly postpharyngeal. Ovary smooth, 45–75 by 58–99, dextral (nine worms) or sinistral (five), on same side of body as posterior testis with one exception, from a position overlapping posterior testis to one 28 posterior to that gonad. Vitellaria in two nearly compact masses overlapping ovary posteroventrally, surface indentations shallow to absent; right mass 42–87 by 42–55, left mass 38–90 by 35–90. Uterus not entering ecsoma, coiling from anterior to ends of ceca to acetabulum; sphincter absent at junction of uterus and metraterm. Metraterm slightly thick-walled, muscular, commencing dorsal to acetabulum, passing ventral to sinus sac, uniting slightly anterior to

internal prostatic vesicle to form hermaphroditic duct. Eggs yellow-brown, operculate, 20 measuring 17–27 (19.7) by 10–13 (11.2).

### Remarks

Seven adult worms were recovered from *T. ovatus*, four adults and one immature from *S. dumerili*, two adults from *P. saltatrix*, and one each from the other three host species. There are only three species in the genus possessing a bipartite seminal vesicle with the anterior chamber thick-walled and muscular: *L. goslinei* (Manter and Pritchard, 1960) Yamaguti, 1970, from a congrid fish from Hawaii; *L. thomasi* Fischthal, 1980 (homonym *Sterrhurus ghanensis* Fischthal and Thomas, 1972), from nine families of fishes from Ghana; *L. israelense* Fischthal, 1980, from trachinid and carangid fishes from Israel's Mediterranean. *L. goslinei* differs from the new species in having an unusually muscular forebody with its sides often curved ventrally, a preacetabular concavity with distinct muscle fibers, the two chambers of the seminal vesicle the same size, a thin-walled duct between the seminal vesicle and external prostatic vesicle, a cylindrical internal prostatic vesicle, a muscular sphincter between the uterus and metraterm, vitelline lobes considerably longer than wide, and slightly smaller eggs, and in lacking prececal sacs. *L. thomasi* differs in having a thin-walled duct between the seminal vesicle and the external prostatic vesicle, and vitelline lobes as long as wide, in the uterus descending into the ecsoma, and in lacking prostate cells in the sinus sac. *L. israelense* differs in having a preacetabular pit, a muscular sphincter between the uterus and metraterm, and vitelline lobes as long as wide, in the uterus descending into the ecsoma, and in lacking prostate cells in the sinus sac.

## Didymozoidae

### Immature Didymozoid I

(Fig. 3)

HOST: *Caranx dentex* (Schneider) (Carangidae).

SITE: Small intestine.

LOCALITY: Tel-Baruch.

SPECIMEN DEPOSITED: No. 76396.

DESCRIPTION (based on one worm): Enclosed in elongate muscular cyst. Body 668 by 157, extremities rounded. Oral sucker 58 by 25, composed of thin muscular layer enclosing vesicular parenchyma. Acetabulum absent. Pharynx 11 by 14, with outer thin muscular layer and inner nonmuscular part, contiguous with oral sucker; esophagus slender, sinuous, entering stomach 21 from its anterior margin; stomach at cecal bifurcation, 78 by 88, with muscular walls up to 10 thick, lying 157 from anterior extremity; ceca emerging from posterolateral part of stomach, narrow for short distance before forming a series of 10 symmetrically arranged chambers, terminating 23 from posterior extremity.

### Remarks

The new form belongs to the larval group *Monilicaecum* Yamaguti, 1942. Four larval didymozoids are known to have a stomach: *Monilicaecum ventricosum* Yamaguti, 1942, from Japan and Israel's Mediterranean; Didymozoidae (*Monil-*



*icaecum*) larvae I Nikolaeva, 1965, from Namibia, Ghana, and Israel's Mediterranean; Immature Didymozoid F Fischthal and Thomas, 1968, from Ghana; Didymozoid metacercaria Madhavi, 1968, from India. Lengy and Fishelson (1972) reported Immature Didymozoid H from Israel's Red Sea, noting that just before the cecal bifurcation the esophagus may or may not widen appreciably; in one instance, the junction of the esophagus to the cecal bifurcation appeared as a muscular bulb. All the above forms differ from the new form in possessing an acetabulum. The stomach is at the distal end of the esophagus in Didymozoidae (*Monilicaecum*) larvae I, Didymozoid metacercaria, and Immature Didymozoid H. The oral sucker is entirely muscular in Immature Didymozoid F and Didymozoid metacercaria. Most cecal chambers on each side alternate with one another in *Monilicaecum ventricosum*.

### Previously Known Species

1. *Bacciger israelensis* Fischthal, 1980 (Fellodistomidae) from ceca and small intestine of *Boops boops* (1, 2, and 3 adult worms in 3 of 16 fish) from Tel-Baruch and Jaffa; specimens deposited: No. 76397-8.

2. *Proctoeces lintoni* Siddiqi and Cable, 1960 (Fellodistomidae), small intestine, *Diplodus sargus* (1 immature, 1, 1, 2, 3 adult worms in 5 of 18 fish), *D. vulgaris* (1 immature worm in 1 of 5 fish), *D. trifasciatus* (1, 2 adult worms in 2 of 7 fish), *Sparus auratus* (1 adult and 3, 6 immature in 3 of 5 fish), \**Caranx rhonchus* (1 adult worm in 1 of 7 fish), \**Lichia amia* (1 adult worm in 1 of 11 fish), and *Mullus surmuletus* (2 immature worms in 1 fish), Tel-Baruch, Jaffa; specimens deposited: No. 76399 (*D. sargus*); No. 76400 (*D. vulgaris*); No. 76401 (*D. trifasciatus*); No. 76402 (*S. auratus*); No. 76403 (*C. rhonchus*); No. 76404 (*L. amia*); No. 76405 (*M. surmuletus*).

3. *Proctotrema bacilliovatum* Odhner, 1911 (Monorchiiidae), ceca, small intestine, *Mullus surmuletus* (33 adult worms in 1 fish), Jaffa; specimens deposited: No. 76406-8.

4. *Proparvipyrum israelense* Fischthal, 1980 (Zoogonidae), ceca, *Mullus surmuletus* (47 adult worms in 1 fish), Jaffa; specimens deposited: No. 76407-8.

5. *Lepocreadium album* (Stossich, 1890) Stossich, 1903 (Lepocreadiidae), ceca, *Diplodus vulgaris* (1 adult worm in 1 of 5 fish), Jaffa; specimen deposited: No. 76409.

6. *Lepocreadium pegorchis* (Stossich, 1901) Stossich, 1904, ceca, *Sparus auratus* (1, 1, 2 adult worms in 3 of 5 fish), \**Diplodus vulgaris* (1 adult worm in 1 of 5 fish), *Lithognathus mormyrus* (1 immature, and 1, 1, 1, 1, 2, 3, 4, 6 adult worms in 9 of 23 fish), \**Seriola dumerili* (1 adult worm in 1 of 9 fish), and \**Pomadasyus incisus* (1 adult worm in 1 of 10 fish), Tel-Baruch, Jaffa; specimens deposited: No. 76410 (*S. auratus*); No. 76411 (*D. vulgaris*); No. 76412 (*L. mormyrus*); No. 76413 (*S. dumerili*); No. 76414 (*P. incisus*).

7. *Echeneidocoelium indicum* Simha and Pershad, 1964 (Lepocreadiidae), small intestine, *Echeneis naucrates* (4 immature and 4 adult worms in 1 fish), \*Jaffa; specimens deposited: No. 76415; previously from India.

8. *Opecoeloides furcatus* (Bremser in Rudolphi, 1819) Odhner, 1928 (Opecoelidae), small intestine, *Mullus surmuletus* (77 immature and adult worms in 1 fish), Jaffa; specimens deposited: No. 76416.

9. *Cainocreadium labracis* (Dujardin, 1845) Nicoll, 1909 (Opecoelidae), small

intestine, \**Dicentrarchis punctatus* (3 immature and 1 adult worms in 1 fish), \*Jaffa; specimens deposited: No. 76417; previously from the Aegean and Adriatic Seas, and northern France.

10. *Podocotyle (Pedunculotrema) israelensis* Fischthal, 1980 (Opecoelidae), ceca, small intestine, *Pomadasyx incisus* (1, 1, 1, 3, 6, 7 [2 immature] 11 adult worm in 7 of 10 fish), Tel-Baruch, Jaffa; specimens deposited: No. 76418.

11. *Bucephalus gorgon* (Linton, 1905) Eckmann, 1932 (Bucephalidae), ceca, small intestine, *Seriola dumerili* (2, 5, 92, 151 immature and adult worms in 4 of 9 fish), \**Caranx dentex* (8 adult worms in 1 of 2 fish), \*Tel-Baruch, Jaffa; specimens deposited: No. 76419 (*S. dumerili*); No. 76420 (*C. dentex*); previously from Gulf of Mexico and U.S. Atlantic.

12. *Bucephalus labracis* Paggi and Orecchia, 1965, ceca, small intestine, \**Sphyræna chrysotaenia* (1 [in 4], 2, 2, 3, 4, 14, 17, 51, 66 immature and adult worms in 12 of 19 fish), \*Tel-Baruch, Jaffa; specimens deposited: No. 76421; previously from Tyrrhenian Sea.

13. *Bucephalus varicus* Manter, 1940, ceca, small intestine, *Trachinotus ovatus* (2 immature, 2 adult worms in 2 of 12 fish), \**Lichia amia* (9 [in 3], 18, 30, 32, 51+ [in 3] immature and adult worms in 9 of 11 fish), Tel-Baruch, Jaffa; specimens deposited: No. 76422 (*T. ovatus*); No. 76423 (*L. amia*).

14. Immature Didymozoid B Fischthal and Kuntz, 1964 (Didymozoidae), stomach, \**Euthynnus alletteratus* (21 immature worms in 1 of 2 fish), \*Tel-Baruch; specimens deposited: No. 76424; previously from Philippines and West Africa.

15. *Monilicaecum ventricosum* Yamaguti, 1942 (Didymozoidae), stomach, \**Euthynnus alletteratus* (1 immature worm in 1 of 2 fish), Jaffa; specimen deposited: No. 76425.

16. *Hemiurus appendiculatus* (Rudolphi, 1802) Looss, 1899 (Hemiuridae), stomach, *Boops boops* (1 adult worm in 1 of 16 fish), Tel-Baruch; specimen deposited: No. 76426.

17. *Parahemiurus merus* (Linton, 1910) Woolcock, 1935 (Hemiuridae), stomach, \**Caranx rhonchus* (1 adult worm in 1 of 7 fish), \**Lichia amia* (1 immature, 1 adult, 1 immature and 1 adult worms in 3 of 11 fish), \**Seriola dumerili* (1, 3 adult worms in 2 of 9 fish), \*Jaffa; specimens deposited: No. 76427 (*C. rhonchus*); No. 76428 (*L. amia*); No. 76429 (*S. dumerili*); previously from Namibia, West Africa, U.S. Atlantic, Bimini, Caribbean, Gulf of Mexico, Brazil, U.S. and Ecuador Pacific, and Bering, Okhotsk, and South China Seas.

18. *Ectenurus lepidus* Looss, 1907 (Hemiuridae), stomach, *Caranx rhonchus* (1, 2, 10 [2 immature], 11 adult worms in 4 of 7 fish), \**C. dentex* (1 adult worm in 1 of 2 fish), *Trachinotus ovatus* (1, 2, 4, 5 adult worms in 4 of 12 fish), *Lichia amia* (1 immature, 1, 2 adult worms in 3 of 11 fish), \**Seriola dumerili* (2 adult worms in 1 of 9 fish), Tel-Baruch, Jaffa; specimens deposited: No. 76430 (*C. rhonchus*); No. 76431 (*C. dentex*); No. 76432 (*T. ovatus*); No. 76433 (*L. amia*); No. 76434 (*S. dumerili*).

19. *Lecithocladium aegyptense* Fischthal and Kuntz, 1963 (Hemiuridae), stomach, *Epinephelus guaza* (2, 4 adult worms in 2 of 9 fish), Tel-Baruch; specimens deposited: No. 76435.

20. *Lecithocladium excisum* (Rudolphi, 1819) Lühe, 1901, stomach, *Caranx rhonchus* (1 immature worm in 1 of 7 fish), Jaffa; specimen deposited: No. 76436.

21. *Lecithocladium unibulbolabrum* Fischthal and Thomas, 1971, stomach,

*Trachinotus ovatus* (1, 1, 2, 3, 4, 6, 7, 8 immature and adult worms in 8 of 12 fish), \**Lichia amia* (1, 2 [in 3] immature and adult worms in 4 of 11 fish), \**Caranx dentex* (1 immature worm in 1 of 2 fish), *Cephalacanthus volitans* (3, 13 adult worms in 2 of 4 fish), Tel-Baruch, Jaffa; specimens deposited: No. 76437 (*T. ovatus*); No. 76438 (*L. amia*); No. 76439 (*C. dentex*); No. 76440 (*C. volitans*).

22. *Aponurus lagunculus* Looss, 1907 (Hemiuridae), stomach, *Trachinotus ovatus* (1 adult worm in 1 of 12 fish), Jaffa; specimen deposited: No. 76441.

23. *Aphanurus stossichi* (Monticelli, 1891) Looss, 1907 (Hemiuridae), stomach, *Boops boops* (1 [in 3], 2 [in 3], 4, 4, 5 [in 3], 6, 6, 9, 10, 13 immature and adult worms in 16 of 16 fish), \**Sparus auratus* (2 and 4 immature worms in 2 of 5 fish), \**Caranx rhonchus* (1 adult worm in 1 of 7 fish), *Trachinotus ovatus* (1 adult worm in 1 of 12 fish), Tel-Baruch, Jaffa; specimens deposited: No. 76442 (*B. boops*); No. 76443 (*S. auratus*); No. 76444 (*C. rhonchus*); No. 76441 (*T. ovatus*).

24. *Lecithochirium ghanense* Fischthal and Thomas, 1972 (Hemiuridae), stomach, \**Echeneis naucrates* (4 adult worms in 1 fish), \*Jaffa; specimens deposited: No. 76445; previously from West Africa.

#### Unidentified Immature Worms

1. *Lepocreadium* sp. (Lepocreadiidae), ceca, *Pomatomus saltatrix* (2 immature worms in 1 of 21 fish), Tel-Baruch; specimens deposited: No. 76446.

2. *Bucephalopsis* sp. (Bucephalidae), small intestine, *Pomatomus saltatrix* (many immature worms in 1 of 21 fish), Jaffa; specimens deposited: No. 76447.

3. *Lecithocladium* sp. (Hemiuridae), stomach, *Pomadasys incisus* (1 immature worm in 1 of 10 fish), Tel-Baruch; specimen deposited: No. 76448.

#### Zoogeographical Affinities

Only two of the 11 species of Red Sea immigrant fishes studied in 1979 had digenetic trematodes. *Epinephelus* sp. harbored one specimen of *Lecithochirium jaffense* sp. n. which, however, occurs in two Atlanto-Mediterranean and two cosmopolitan fish species; no doubt the trematode is Mediterranean in origin. *Sphyraena chrysotaenia* was infected with *Bucephalus labracis*, previously reported from the Tyrrhenian Sea; this trematode is Mediterranean.

The affinities of all but seven of the previously known species of trematodes reported in this study are already recorded by Fischthal (1980). The data for the seven are listed herein under each species.

#### Host-parasite List

The designations after the host's name is interpreted as follows: C—cosmopolitan species, A—Atlanto-Mediterranean species, M—Mediterranean species, R—Red Sea immigrant species, F—freshwater and brackish species. The first number in parentheses following the name of each fish indicates the number examined, while the second number indicates the number infected with at least one species of trematode.

*Anguilla anguilla* (L.), Anguillidae—A (3; 0)

*Atule djeddaba* (Forsk.) Carangidae—R (1; 0)

*Autisthes puta* (Cuvier), Theroponidae—R (3; 0)

*Blennius cristatus* L., Blenniidae—A (1; 0)

- Blennius pavo* Risso—A (12; 0)  
*Blennius sanguinolentus* Pallas—A (25; 0)  
*Boops boops* (L.), Sparidae—A (16; 16)  
     *Aphanurus stossichi* (Monticelli, 1891) Looss, 1907  
     *Bacciger israelensis* Fischthal, 1980  
     *Hemiurus appendiculatus* (Rudolphi, 1802) Looss, 1899  
*Caranx dentex* (Schneider), Carangidae—A (2; 2)  
     *Bucephalus gorgon* (Linton, 1905) Eckmann, 1932  
     *Ectenurus lepidus* Looss, 1907  
     Immature Didymozoid I (new larval form)  
     *Lecithocladium unibulbolabrum* Fischthal and Thomas, 1971  
*Caranx rhonchus* Geoffrey St. Hilaire—A (7; 7)  
     *Aphanurus stossichi* (Monticelli, 1891) Looss, 1907  
     *Ectenurus lepidus* Looss, 1907  
     *Lecithocladium excisum* (Rudolphi, 1819) Lühe, 1901  
     *Parahemiurus merus* (Linton, 1910) Woolcock, 1935  
     *Proctoeces lintoni* Siddiqi and Cable, 1960  
*Cephalacanthus volitans* (L.), Cephalacanthidae—A (4; 3)  
     *Lecithocladium unibulbolabrum* Fischthal and Thomas, 1971  
*Dasyatis violacea* (Bonaparte), Dasyatidae—M (1; 0)  
*Dicentrarchis punctatus* (Bloch), Serranidae—A (1; 1)  
     *Cainocreadium labracis* (Dujardin, 1845) Nicoll, 1909  
*Diplodus sargus* (L.), Sparidae—A (18; 6)  
     *Proctoeces lintoni* Siddiqi and Cable, 1960  
*Diplodus trifasciatus* (Rafinesque)—A (7; 2)  
     *Proctoeces lintoni* Siddiqi and Cable, 1960  
*Diplodus vulgaris* (Geoffrey St. Hilaire)—A (5; 3)  
     *Lepocreadium album* (Stossich, 1890) Stossich, 1903  
     *Lepocreadium pegorchis* (Stossich, 1901) Stossich, 1904  
     *Proctoeces lintoni* Siddiqi and Cable, 1960  
*Echeneis naucrates* (L.), Echeneidae—C (1; 1)  
     *Echeneido-coelium indicum* Simha and Pershad, 1964  
     *Lecithochirium ghanense* Fischthal and Thomas, 1972  
     *Lecithochirium jaffense* sp. n.  
*Epinephelus alexandrinus* (Valenciennes), Serranidae—A (5; 0)  
*Epinephelus guaza* L.—A (9; 2)  
     *Lecithocladium aegyptense* Fischthal and Kuntz, 1963  
*Epinephelus* sp.—R (1; 1)  
     *Lecithochirium jaffense* sp. n.  
*Euthynnus alletteratus* (Rafinesque), Scombridae—A (2; 2)  
     Immature Didymozoid B Fischthal and Kuntz, 1964  
     *Monilicaecum ventricosum* Yamaguti, 1942  
*Gobius cobitus* Pallas, Gobiidae—A (23; 1)  
     *Lecithochirium jaffense* sp. n.  
*Holocentrus ruber* (Forsk.) Holocentridae—R (3; 0)  
*Lichia amia* (L.), Carangidae—A (11; 10)  
     *Bucephalus varicus* Manter, 1940  
     *Ectenurus lepidus* Looss, 1907

- Lecithocladium unibulbolabrum* Fischthal and Thomas, 1971  
*Parahemiurus merus* (Linton, 1910) Woolcock, 1935  
*Proctoeces lintoni* Siddiqi and Cable, 1960  
*Lithognathus mormyrus* (L.), Sparidae—A (23; 10)  
*Lepocreadium pegorchis* (Stossich, 1901) Stossich, 1904  
*Liza aurata* (Risso), Mugilidae—A (8; 0)  
*Liza provensalis* (Risso)—A (7; 0)  
*Mullus surmuletus* L., Mullidae—A (1; 1)  
*Opecoeloides furcatus* (Bremser in Rudolphi, 1819) Odhner, 1928  
*Proctoeces lintoni* Siddiqi and Cable, 1960  
*Proctotrema bacilliovatum* Odhner, 1911  
*Proparvipyrum israelense* Fischthal, 1980  
*Pelates quadrilineatus* (Bloch), Theroponidae—R (11; 0)  
*Pempherus molucca* Cuvier, Pempheridae—R (3; 0)  
*Pomadasyus incisus* (Bowdich), Pomadasyidae—A (10; 9)  
*Lecithocladium* sp.  
*Lepocreadium pegorchis* (Stossich, 1901) Stossich, 1904  
*Podocotyle (Pedunculotrema) israelensis* Fischthal, 1980  
*Pomatomus saltatrix* (L.), Pomatomidae—C (21; 5)  
*Bucephalopsis* sp.  
*Lecithochirium jaffense* sp. n.  
*Lepocreadium* sp.  
*Pranesus pinguis* (Lacépède), Atherinidae—R (9; 0)  
*Sardinella aurita* Valenciennes, Clupeidae—C (5; 0)  
*Sardinella maderensis* Lowe—A (6; 0)  
*Sarpa salpa* (L.), Sparidae—A (4; 0)  
*Sciaena umbra* (L.), Sciaenidae—A (2; 0)  
*Scorpaena scrofa* (L.), Scorpaenidae—A (1; 0)  
*Scyris alexandrina* Geoffrey St. Hilaire, Carangidae—A (3; 0)  
*Seriola dumerili* (Risso), Carangidae—A (9; 5)  
*Bucephalus gorgon* (Linton, 1905) Eckmann, 1932  
*Ectenurus lepidus* Looss, 1907  
*Lecithochirium jaffense* sp. n.  
*Lepocreadium pegorchis* (Stossich, 1901) Stossich, 1904  
*Parahemiurus merus* (Linton, 1910) Woolcock, 1935  
*Siganus luridus* (Rüppell), Siganidae—R (11; 0)  
*Siganus rivulatus* (Forsk.)—R (17; 0)  
*Sillago sihama* (Forsk.), Sillaginidae—R (12; 0)  
*Solea lascaris* (Risso), Soleidae—A (5; 0)  
*Solea vulgaris aegyptica* Chabanaud—A (4; 0)  
*Sparus auratus* (L.), Sparidae—A (5; 5)  
*Aphanurus stossichi* (Monticelli, 1891) Looss, 1907  
*Lepocreadium pegorchis* (Stossich, 1901) Stossich, 1904  
*Proctoeces lintoni* Siddiqi and Cable, 1960  
*Sphyraena chrysotaenia* Klunzinger, Sphyraenidae—R (19; 13)  
*Bucephalus labracis* Paggi and Orecchia, 1965  
*Sphyraena sphyraena* (L.)—A (3; 0)  
*Tilapia zillii* (Gervais), Cichlidae—F (2; 0)

- Trachinotus ovatus* (L.), Carangidae—A (12; 10)  
*Aphanurus stossichi* (Monticelli, 1891) Looss, 1907  
*Aponurus lagunculus* Looss, 1907  
*Bucephalus varicus* Manter, 1940  
*Ectenurus lepidus* Looss, 1907  
*Lecithochirium jaffense* sp. n.  
*Lecithocladium unibulbolabrum* Fischthal and Thomas, 1971  
*Umbrina cirrosa* (L.), Sciaenidae—A (1; 0)

#### Acknowledgments

Appreciation is due Dr. Lev Fishelson, Department of Zoology, Tel-Aviv University, Ramat-Aviv, Israel, for providing laboratory space and necessary supplies, and to Dr. Adam Ben-Tuvia, Department of Zoology, The Hebrew University, Jerusalem, for identifying some of the fishes.

#### Literature Cited

- Fischthal, J. H. 1980. Some digenetic trematodes of marine fishes from Israel's Mediterranean coast and their zoogeography, especially those from Red Sea immigrant fishes. *Zool. Scr.* 9:11-23.  
 Lengy, J., and L. Fishelson. 1972. On an immature didymozoid larva (Trematoda: Didymozoidae) in the muscles and swim bladder of *Anthias squamipinnis* (Anthiidae) from the Gulf of Aqaba. *J. Parasitol.* 58:879-881.

#### 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.

## Five New Neotropical Species of Lecithodendriidae (Trematoda: Digenaea) Including Three New Genera, All from Panamanian and Colombian Mammals<sup>1</sup>

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**ABSTRACT:** Three new neotropical genera and species, of Lecithodendriidae (Trematoda: Digenaea) are described from Panamanian and Colombian mammals. *Proechimytrema phanerotermina* gen. et sp. n. from the spiny rat, *Proechimys* sp. and *Oryzomytrema tristoma* gen. et sp. n. from the rice rat, *Oryzomys* sp. both somewhat resemble the genus *Phaneropsolus* Looss, 1899, but both differ from the latter in having the genital pore lateral, and not anterior, to the acetabulum. Both of these new genera have elongate cirrus sacs which nearly encircle the acetabulum, but that of *Oryzomytrema* is the longer. *Proechimytrema* has a prominent metraterm, while *Oryzomytrema* lacks this structure. *Oryzomytrema*, on the other hand, has a large genital sucker, which the other new genus lacks. *Procyonophilus panamensis* gen. et sp. n. from the crab-eating raccoon, *Procyon cancrivorus*, has vitelline follicles lateral to the acetabulum, as in *Lecithodendrium* Looss, 1896. The new genus differs from the latter in having a genital pore lateral to, and not anterior to, the acetabulum and a massive, horizontally aligned cirrus sac. *Czosnowia noctilionis* sp. n. from the bat, *Noctilio labialis*, differs from the type species, *C. joannae* Zdzitowiecki, 1967, in that the genital pore is more lateral and the testes are opposite rather than diagonal. This new species also has smaller eggs and body. *Prosthodendrium calimaense* sp. n. from the woolly opossum, *Caluromys derbianus*, is placed in this genus because the ovary is entire, and not lobate. This new species resembles *P. buongerminii* Lent, Teixeira de Freitas and Proença, 1945, from a Paraguayan bat, but it differs from the latter in having a more anterior ovary and vitelline glands, as well as a smaller cirrus sac, larger testes and different host and geographic distributions. The problem of subfamilies in the Lecithodendriidae is discussed.

Lecithodendriidae is a family of digenetic trematodes occurring, as adults, in insectivorous vertebrates. The family presently contains more than 40 known genera, and numerous species have been described in some of these genera. The complexities and confusion existing in the systematics of the group is illustrated by the genus *Prosthodendrium* to which Yamaguti (1971) attributed 73 species, 6 subspecies, and 3 subgenera. The family has also been divided into subfamilies and tribes (Skarbilovich in Skrjabin, 1948). Many of these taxonomic divisions are ambiguously defined and undoubtedly will need to be revised or abandoned after more of the presently unknown tropical species have been described.

Since insects transmit Lecithodendriidae to the vertebrate host, it is to be expected that more different forms should occur in warm and tropical regions where insects are so numerous and varied. Skarbilovich (1948) listed 40 species from India and South Asia, as compared to only 17 from Africa and 16 from Central and South America. These figures suggest that many more species of Lecithodendriidae await discovery in the Neotropics, as well as in Africa. Travassos et al. (1969) listed 15 species of this family for Brazil, and Caballero and Grocott (1952, 1960) reported two species for the Republic of Panama. An ad-

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<sup>1</sup> Specimens used in this study were collected in the Republic of Panamá between 1962 and 1967 while the author was a staff member of the Gorgas Memorial Laboratory, and the Colombian material between 1968 and 1970 while he was associated with the Tulane-Universidad del Valle, International Center for Medical Research, Cali, Colombia.

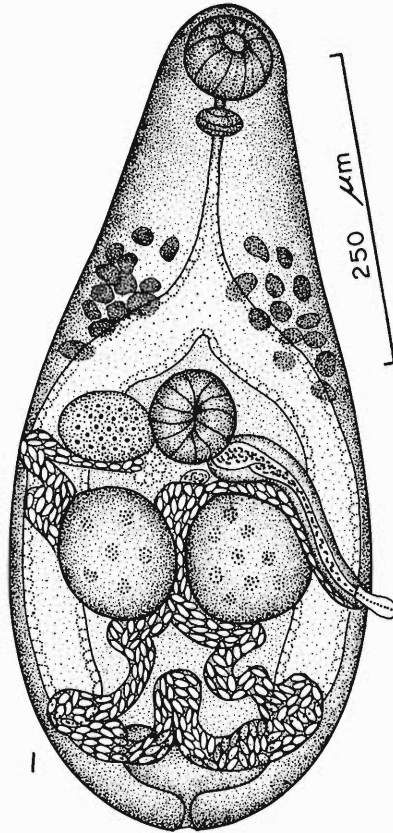


Figure 1. *Czosnowia noctilionis* sp. n., ventral view of holotype.

ditional species was reported from a Panamanian primate by Thatcher and Porter (1968). According to Yamaguti (1971) two representatives of this family have been reported from Colombia. The present paper adds two new species from Panama and three from Colombia, including a new Panamanian genus and two new Colombian genera. These worms were collected from a bat, two genera of rodents, a marsupial, and a raccoon.

#### Materials and Methods

The methods used in collecting and preparing the trematodes were those reported previously (Thatcher and Dossman, 1975). Drawings were made with the aid of a camera lucida and measurements with a measuring ocular. All measurements are given in micrometers, and the extremes are followed by the means, in parentheses.

#### *Czosnowia noctilionis* sp. n.

(Figs. 1, 2)

HOST: *Noctilio labialis labialis* (Kerr); "Little Bulldog Bat."

LOCATION: Small intestine.

LOCALITY: Panama, R.P.



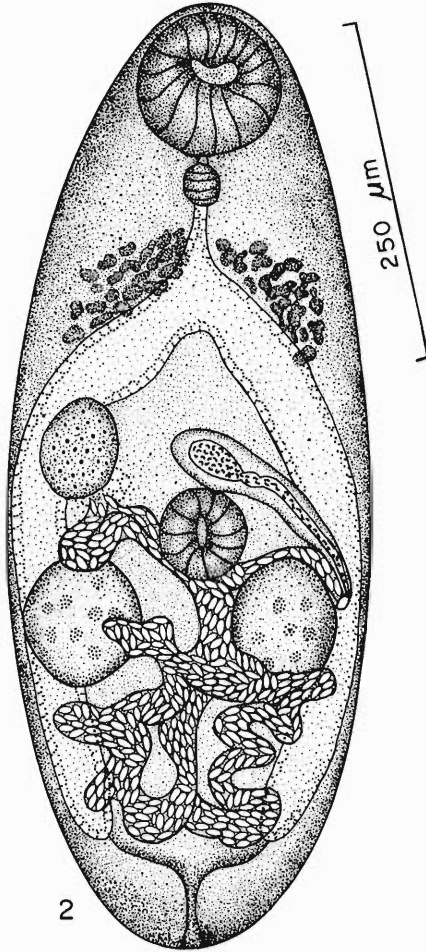


Figure 2. *Czosnowia noctilionis* sp. n., ventral view of a paratype showing variation in position of cirrus sac and ovary.

HOLOTYPE: U.S. Helm. Coll. 76162.

PARATYPES: Instituto Nacional de Pesquisas da Amazônia, Manaus, Brasil.

DESCRIPTION (17 specimens studied, 10 measured): Body 420–650 (498) long by 190–350 (275) wide, in equatorial region. Tegumental spines minute, extending posterior to testicular level. Oral sucker subterminal; 66–120 (85) long by 71–120 (89) wide; prepharynx short; pharynx spherical, 28–48 (35) in diameter; esophagus moderately long, 46–92 (69) in length; bifurcation between pharynx and acetabulum, position variable; ceca nearly full length, 50 in maximum diameter. Acetabulum equatorial or slightly postequatorial, 58–72 (67) in diameter. Testes spherical, opposite, posterior to acetabulum, 50–110 (82) in diameter. Cirrus sac elongate, slender, slightly curved, post- or preacetabular, sometimes overlapping testis; contains cirrus, seminal vesicle, and few prostatic cells; measures 120–190 (161) long by 35–43 (39) in maximum diameter. Genital pore ventromarginal, postacetabular, at level of testes. Ovary spherical, lateral or anterolateral to ac-

etabulum, on opposite side from genital pore, pretesticular; measures 48–74 (59) in diameter. Vitellaria composed of small follicles (about 10 in diameter) situated dorsolateral to intestinal bifurcation. Uterine coils mostly postacetabular; eggs small, numerous,  $18 \times 9$ . Excretory vesicle V-shaped; pore terminal.

### Remarks

*Czosnowia noctilionis* sp. n. resembles the type species of that genus, *C. joannae* Zdzitowiecki, 1967, in the general placement of the organs. The new species differs from the latter in the position of the genital pore which is ventral to the cecum and testis in *C. joannae* and lateral to these organs in *C. noctilionis*. The new species is also smaller (420–650 as compared to 502–723); the eggs are smaller ( $18 \times 9$  instead of  $22\text{--}24.5 \times 11\text{--}13$ ); the testes are opposite (not diagonal); the ovary is separated from the near testis by uterine loops (the same organs are contiguous in *C. joannae*); and the host is a Neotropical (not European) bat, of a different genus.

In the position of the genital pore, the new species shows some similarity to *Leyogonimus polyoon* (v. Linstow, 1887) Ginetsinskaia in Skarbilovich, 1948, which was described from Eurasian birds. The new species differs from the latter in having: a lateral nonlobate ovary; a much longer cirrus sac, of a different shape; and smaller vitelline follicles, of a more limited distribution.

Curiously, Yamaguti (1971) placed *Czosnowia* in the Subfamily Parabascinae Yamaguti, 1958, and *Leyogonimus* in Leyogoniminae Dollfus, 1951. The presently described form does not quite fit either of these subfamily diagnoses. After more of the Neotropical Lecithodendriidae are reported, it may be possible to perceive more meaningful subfamily groupings.

### *Prosthodendrium calimaense* sp. n.

(Fig. 3)

HOST: *Caluromys derbianus* (Waterhouse); "Woolly Opossum."

LOCATION: Small intestine.

LOCALITY: Calima Lake, Department of Valle, Colombia.

HOLOTYPE: U.S. Helm. Coll. 76163.

PARATYPES: Instituto Nacional de Pesquisas da Amazônia, Manaus, Brasil.

DESCRIPTION (6 specimens studied and measured): Body flattened, round, 640–770 (722) long by 650–780 (718) wide. Minute spines embedded in tegument; extend to near posterior extremity. Oral sucker subterminal, 100–140 (123) long by 100–160 (133) wide; prepharynx very short; pharynx spherical, 41–60 (47) in diameter, esophagus very short; ceca short, stout, horizontal; measure about 60 in maximum diameter. Testes opposite, preacetabular, of irregular outline; right testis 140–210 (175) long by 140–230 (180) wide; left testis 140–210 (171) long by 140–210 (180) wide. Cirrus sac ovoid, preacetabular; containing cirrus, sinuous seminal vesicle and prostatic cells; measures 120–145 (135) long by 82–110 (93) in maximum diameter. Genital pore submedial, between pharynx and acetabulum. Ovary irregular, but not lobate; situated medially, preacetabular; measures 90–150 (113) long by 85–170 (131) wide. Small seminal receptacle present, posterodorsal to acetabulum; ootype and Mehlis' gland dorsal to acetabulum. Vitellaria composed of few large follicles; situated anterior to ceca and lateral to oral sucker; measure about 45 in diameter. Uterine coils fill in postacetabular region;

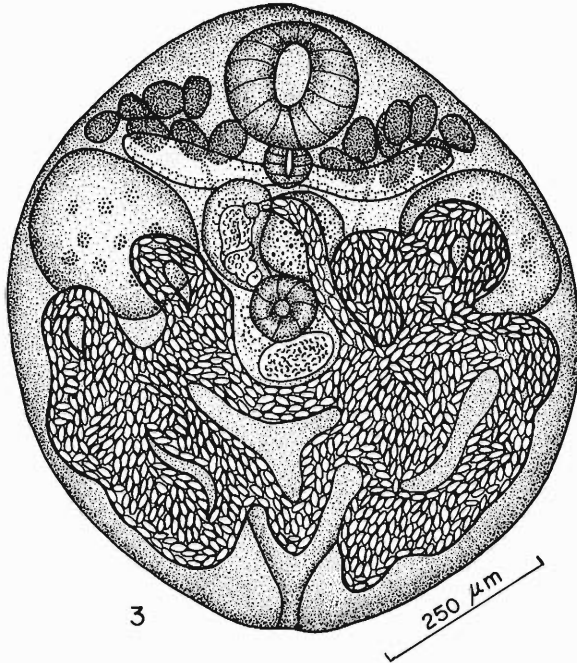


Figure 3. *Prosthodendrium calimaense* sp. n., ventral view of holotype.

eggs small, numerous; measure  $25 \times 12$ . Excretory vesicle V-shaped; pore terminal.

#### Remarks

*Prosthodendrium calimaense* sp. n. has an ovary which is entire and not lobate thereby placing it in the subgenus *Prosthodendrium* Dubois, 1960, and separating it from those species which have a lobate ovary and have been considered to constitute the subgenus *Paralecithodendrium* Odhner, 1910. The status of these subgeneric names is questionable, however. Yamaguti (1958) merged them, but in 1971, the same author recognized them as distinct. Including all of these disparate forms in one genus, divided into subgenera, is unhelpful and results in a generic diagnosis so over-extended as to be meaningless. *Prosthodendrium* should be retained as a generic name for those forms having a nonlobate ovary, and *Paralecithodendrium* for those with a lobate ovary. The use of these names in a subgeneric sense should be discontinued.

*Prosthodendrium calimaense* sp. n. shows some similarity to *P. buongerminii* Lent, Teixeira de Freitas and Proença, 1945, described from a Paraguayan bat. The new species differs from the latter in the following respects: (1) The ovary is anterior to the acetabulum and extends anterior to the cirrus sac (in *P. buongerminii* the ovary is near, and somewhat posterior, to the acetabulum); (2) the vitelline follicles are larger and more anterior in the new species; (3) the new form has a smaller cirrus; (4) *P. calimaense* has larger testes; and (5) the two species were found at opposite ends of the South American continent, and they infect marsupials and bats, respectively.

The species name refers to the type locality. Calima Lake is a large artificial lake and power complex near the city of Cali, Colombia.

*Proechimytrema* gen. n.

DIAGNOSIS: Lecithodendriidae. Body flattened, ovoid; tegument spinous. Oral sucker well developed; pharynx large; prepharynx short; esophagus moderately long; bifurcation between acetabulum and pharynx; ceca short, not extending posterior to testes. Acetabulum equatorial. Testes ovoid, opposite, lateral; acetabular or postacetabular. Cirrus sac elongate, hooked; largely anterodorsal to acetabulum and ovary; contains cirrus, elongate seminal vesicle, and prostatic cells. Genital pore ventrolateral, between acetabulum and testis. Ovary ovoid, between acetabulum and testis, on side opposite to genital pore. Metraterm large, swollen, pigmented. Vitellaria of few large follicles; prececal, at esophageal level. Uterine coils mostly postacetabular, but extending into prececal region. Eggs small, numerous. Excretory vesicle Y-shaped; pore terminal. Intestinal parasites of rodents.

TYPE SPECIES: *P. phanero termina* sp. n.

*Proechimytrema phanero termina* sp. n.

(Fig. 4)

HOST: *Proechimys* sp.; "Common Spiny Rat."

LOCATION: Small intestine.

LOCALITY: Leon River, Department of Antioquia, Colombia.

HOLOTYPE: U.S. Helm. Coll. 76164.

PARATYPES: Instituto Nacional de Pesquisas da Amazônia, Manaus, Brasil.

DESCRIPTION (9 specimens studied, 8 measured): Body 920–1,100 (1,000) long by 660–730 (725) wide. Oral sucker subterminal, 110–120 (116) long by 120–140 (131) wide; prepharynx short; pharynx large, spherical, 50–60 (57) in diameter; esophagus moderately long, 46–92 (69); ceca short, stout, nearly horizontal, measuring 69 in maximum diameter. Acetabulum equatorial, 110–135 (122) in diameter. Testes postacetabular, longer than wide; right testis 210–270 (232) long by 120–230 (172) wide; left testis 210–270 (230) long by 160–230 (180) wide. Cirrus sac large, hooked, horizontal; measures 390–500 (430) long by 90–130 (118) in maximum diameter. Genital pore midway between acetabulum and lateral body wall; surrounded by glandular cells. Ovary lateral to acetabulum, longer than wide; measures 160–180 (173) long by 110–160 (130) in diameter. Vitelline follicles large, few, prececal, about 55 in diameter. Metraterm large, elliptical; measures 200–270 (238) long by 72–110 (94) in maximum diameter. Ootype and Mehlis' gland posterolateral to acetabulum. Uterine coils extensive from region of esophagus to posterior extremity. Eggs small, numerous; measure 25 × 14. Excretory vesicle Y-shaped; pore terminal.

*Oryzomytrema* gen. n.

DIAGNOSIS: Lecithodendriidae. Body flattened, rounded; tegument spined. Oral sucker well developed, ventroterminal; prepharynx absent; pharynx small, spherical; esophagus absent; ceca short, preacetabular, horizontal. Acetabulum large, equatorial. Testes rounded, irregular, opposite, on either side of body at level of acetabulum. Cirrus sac very long, slender, fishhook-shaped; partially encircles

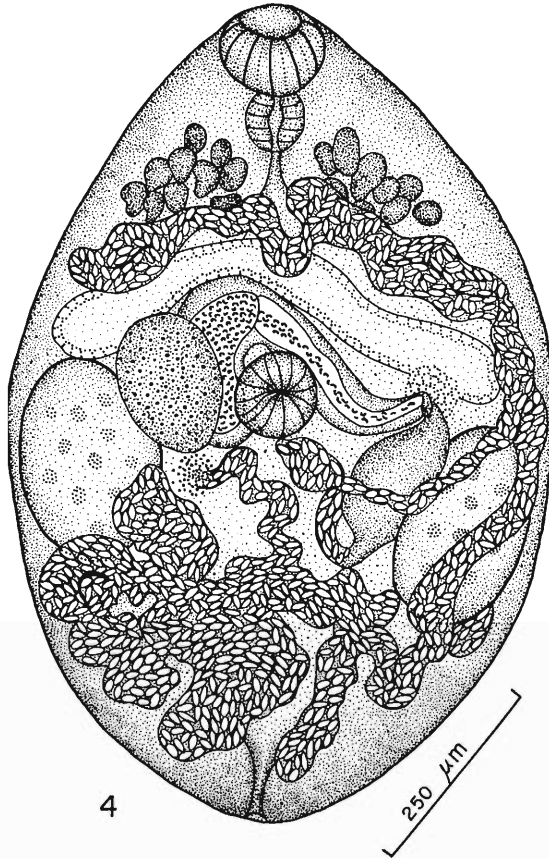


Figure 4. *Proechimytrema phanerotermis* gen. et sp. n., ventral view of holotype.

acetabulum; contains elongate seminal vesicle and prostatic cells. Genital pore between lateral body wall and acetabulum; provided with large genital sucker. Ovary irregularly rounded, between testis and acetabulum, opposite to genital pore. Vitellaria of numerous medium-sized follicles, largely prececal. Uterine coils mostly postacetabular, but extending into prececal region. Eggs small, numerous. Excretory vesicle Y-shaped; pore terminal. Intestinal parasites of rodents.

TYPE SPECIES: *O. tristoma* sp. n.

*Oryzomytrema tristoma* sp. n.

(Fig. 5)

HOST: *Oryzomys* sp.; "Rice Rat."

LOCATION: Small intestine.

LOCALITY: San Antonio River, near Buenaventura, Department of Valle, Colombia.

HOLOTYPE: U.S. Helm. Coll. 76165.

PARATYPES: Instituto Nacional de Pesquisas da Amazônia, Manaus, Brasil.

DESCRIPTION (7 specimens studied, 5 measured): Body 450–630 (564) long by

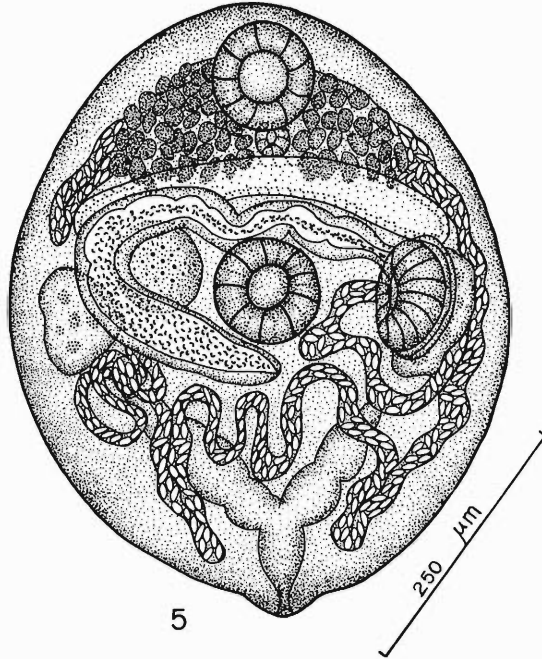


Figure 5. *Oryzomytrema tristoma* gen. et sp. n., ventral view of holotype.

360–470 (418) wide. Oral sucker ventroterminal, slightly wider than long, measures 74–100 (86) long by 77–110 (97) wide; prepharynx absent; pharynx spherical, measures 48–57 (53) in diameter; esophagus absent; ceca horizontal, measuring about 45 in maximum diameter. Testes irregularly rounded, at acetabular level; right testis 110 long by 72–90 (80) wide; left testis 96–120 (102) long by 72–90 (80) wide. Cirrus sac very long, fishhook-shaped; measures 360–500 (430) long by 48–50 (49) in maximum diameter. Ovary irregularly spherical, slightly longer than wide; measures 92–110 (99) long by 72–92 (83) wide. Genital pore between acetabulum and lateral body wall, provided with large sucker which measures 96–110 (103) long by 69–72 (70) wide. Vitellaria consisting of numerous small follicles of about 20 in diameter. Uterine coils extensive; eggs small, numerous,  $24 \times 9$ . Excretory vesicle Y-shaped; pore terminal.

#### Remarks

Both *Proechimytrema* gen. n. and *Oryzomytrema* gen. n. show some similarities to *Phaneropsolus* Looss, 1899, but they cannot be included in the subfamily Phaneropsolinae Mehra, 1935, as defined by Yamaguti (1971), because the genital pore is anterior to the acetabulum, in the latter, and lateral to the acetabulum in the two new forms. It is thought to be inadvisable to erect a new subfamily unit, or expand existing concepts, at this time. Such decisions should await more detailed and complete studies of the Neotropical lecitodendriids.

The two new forms can readily be distinguished from each other on the basis of the termination of the reproductive systems. *Proechimytrema* has a cirrus sac, which only partially encircles the acetabulum, and a prominent metraterm. *Ory-*

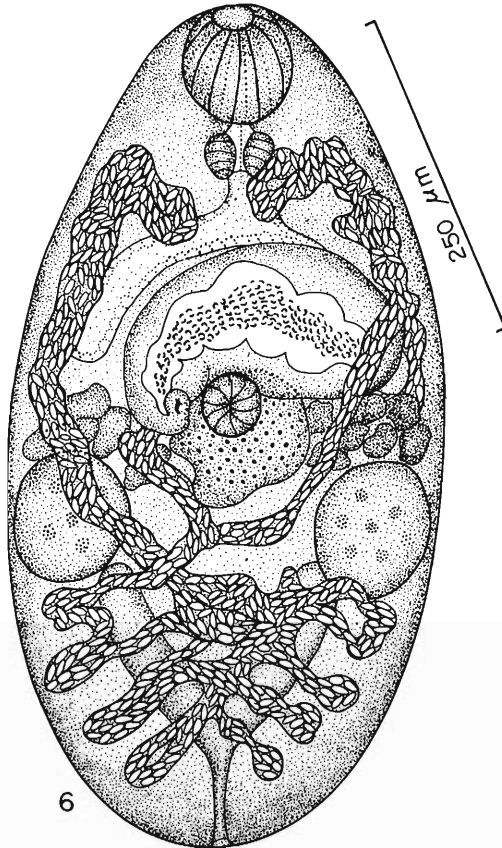


Figure 6. *Procyonophilus panamensis* gen. et sp. n., ventral view of holotype.

*zomytrema*, on the other hand, has a much longer cirrus sac, nearly encircling the acetabulum, a prominent genital sucker, and lacks a metraterm.

The new generic names were derived from those of the rodent hosts, and the specific designations refer, respectively, to the presence of a prominent metraterm (phaneros = visible + termina = termination) and to the genital sucker (tri = three + stoma = mouth).

#### *Procyonophilus* gen. n.

DIAGNOSIS: Lecithodendriidae. Body flattened, elliptical; tegumental spines present. Oral sucker large, subterminal; prepharynx short; pharynx well developed; esophagus short; ceca short, not surpassing acetabulum. Acetabulum small, equatorial. Testes spherical, opposite, postacetabular. Cirrus sac massive, horizontal, preacetabular; contains cirrus, sinuous seminal vesicle, and prostatic cells. Genital pore lateral to acetabulum. Vitellaria consisting of two groups of large follicles, dorsolateral to acetabulum. Uterine coils extensive. Eggs small, numerous. Excretory vesicle Y-shaped; pore terminal. Intestinal parasites of mammals.

TYPE SPECIES: *P. panamensis* sp. n.

*Procyonophilus panamensis* sp. n.

(Fig. 6)

HOST: *Procyon cancrivorus* Cuvier; "Crab-eating Raccoon."

LOCATION: Small intestine.

LOCALITY: Achiote, Colón Province, Panama.

HOLOTYPE: U.S. Helm. Coll. 76166.

PARATYPES: Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil.

DESCRIPTION (5 specimens studied, 5 measured): Body 480–640 (556) long by 330–380 (366) wide; tegument with minute spines to near posterior extremity. Oral sucker somewhat wider than long, measures 83–110 (97) long by 110–120 (112) wide; prepharynx short; pharynx wider than long, measures 38–48 (41) long by 37–58 (47) wide; esophagus short, about 20 long; ceca short, not surpassing acetabulum, measure about 45 in maximum diameter. Acetabulum small, equatorial, 53–55 (54) in diameter. Testes spherical, similar in size; measure 72–110 (93) long by 80–110 (93) wide. Cirrus sac massive, horizontal; measures 170–220 (204) long by 96–120 (110) in diameter. Ovary irregularly spherical, centrally situated; measures 93–96 (91) in diameter. Genital pore lateral to acetabulum. Vitellaria of few large follicles, lateral to acetabulum, measuring about 30 in diameter. Uterus extensive, from pharyngeal level to posterior of body. Eggs small, numerous; measure 18 × 8. Excretory vesicle Y-shaped, with arms reaching testes; pore terminal.

**Remarks**

The equatorial position of the reproductive organs, especially the vitelline glands, suggests that *Procyonophilus* gen. n. may be related to the subfamily Lecithodendriinae Looss, 1902. The new genus has a genital pore lateral to the acetabulum while in Lecithodendriinae the genital pore is immediately anterior to the acetabulum, according to Yamaguti (1971). This new form is another indication that more information is needed before meaningful subfamily designations can be formulated.

The massive cirrus sac serves to distinguish this new genus and species from all others known in the family.

The generic name was adapted from that of the raccoon host, and the specific designation refers to the country of origin.

**Acknowledgment**

I wish to express my gratitude to Miss Maria de Fátima Viera, staff artist, for her help in preparing the illustrations.

**Literature Cited**

- Caballero, E., and R. G. Grocott. 1952. Helminths from the Republic of Panama: II. A new trematode from the intestine of *Philander laniger pallidus* Thomas and a key to the species of the genus *Phaneropsolus* Looss, 1899 (Trematoda: Lecithodendriidae). J. Wash. Acad. Sci. 42:388–391.
- Caballero, E., and R. G. Grocott. 1960. Helmintos de la República de Panamá. XXIII. Estudio de los tremátodos de murciélagos, con descripción de una nueva especie. Ciencia (México) 19:244–248.
- Skarbilovich, T. S. 1948. Lecithodendriidae Odhner, 1911. Pages 337–590 in K. I. Skrjabin, ed. Trematodi Zhivotnikh i Cheloveka. Vol. II. Akad. Nauk SSSR, Moscow.
- Thatcher, V. E., and D. Dossman M. 1975. *Unicoelium prochilodorum* gen. et sp. n. (Trematoda:



- Haploporidae) from a freshwater fish (*Prochilodus reticulatus*) in Colombia. Proc. Helminthol. Soc. Wash. 42:28-30.
- Thatcher, V. E., and J. A. Porter, Jr.** 1968. Some helminth parasites of Panamanian primates. Trans. Am. Microsc. Soc. 87:186-196.
- Travassos, L., J. F. Teixeira de Freitas, and A. Kohn.** 1969. Trematódeos do Brasil. Mem. Inst. Oswaldo Cruz 67:1-886.
- Yamaguti, S.** 1958. Systema Helminthum, Vol. I. Pts. 1 and 2, The Digenetic Trematodes of Vertebrates. Interscience Publ. Inc., New York. 1575 pp.
- Yamaguti, S.** 1971. Synopsis of Digenetic Trematodes of Vertebrates, Vols. I and II. Keigaku Publ. Co., Tokyo. 1774 pp.

### Society Incorporation

The Helminthological Society of Washington became incorporated on November 4, 1981, in the state of Maryland. It was necessary to rearrange parts of the Constitution and to make some minor wording changes in preparation for incorporation. All these changes were voted on and passed unanimously by those present at the 541st meeting held on October 16, 1981. The entire Constitution as passed at that time, as well as the Articles of Incorporation, will be published in a later issue of the Proceedings.

Our special thanks go to incorporation committee members Drs. Harry Herlich and Harley G. Sheffield and especially to committee chairman, Dr. A. Morgan Golden, who spent numerous hours in meetings with lawyers and who will serve as resident agent for the Society.

NANCY D. PACHECO  
*Immediate Past President*

## A Simulations Approach to Discerning Possible Sister-groups of *Dioecotaenia* Schmidt, 1969 (Cestoda: Tetraphyllidea: Dioecotaeniidae)

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**ABSTRACT:** Apparent common architectural constraints in the structure of the bothridia of both species of *Dioecotaenia* permit testing of a number of models for the evolution of the scolex of *Dioecotaenia* from that found in other tetraphyllidean cestodes. Although presently known species of *Dioecotaenia* exhibit bothridial loculi in the shape of hexagons, derivation of those bothridia from an ancestor possessing a single central hexagonal bothridium with marginal loculi would not produce total bothridial loculi numbers consistent with those known. A more feasible derivation involves an ancestral form exhibiting three linear loculi. If the loculi are arranged vertically, as in species of *Rhinebothrium*, orientation of the bothridia would have undergone a 90° shift in orientation at some point to achieve the orientation seen today in *Dioecotaenia* spp. If the bothridial loculi were arranged horizontally, simple addition of rows of three loculi, with subsequent modification of their shape into hexagons permitting closer packing, would account for the observed bothridial morphology. *Tritaphros retzii*, a parasite of European rajiform elasmobranchs, exhibits three bothridial loculi arranged horizontally.

Schmidt (1969) redescribed *Rhinebothrium cancellatum* Linton and discovered that it possessed a number of features unique among tetraphyllidean cestodes. Those features included: (1) a dioecious nature, with separate female and male strobilae, (2) hypodermic impregnation, (3) compact vitellaria accreted to the ovary, (4) no vaginal pore or vagina, and (5) an internal seminal vesicle. The scolex of *Dioecotaenia cancellata*, as the species was renamed, comprises four sessile loculated bothridia exhibiting a locular architecture unknown among tetraphyllidean cestodes (Fig. 1). Because the species possessed a scolex formed of four bothridia, Schmidt placed it in the Tetraphyllidea; because of its unique traits, he placed it in its own family. To this date, no one has ventured an opinion about the relationships of *Dioecotaenia* to other cestodes.

I became interested in the problem of *Dioecotaenia* and its possible relatives while aiding description of the second member of the genus and family, *D. campbelli* Mayes and Brooks. I noticed that both species exhibited bothridial architecture marked by common structural constraints. I derived the simple algebraic formula describing those constraints and used it to simulate hypothetical bothridial morphotypes other than those known. The simulations produced three patterns of development, two of which were rejected because they satisfied only some of the architectural constraints. The accepted hypothetical line of development suggests an archetypal bothridial morphology which is found in a known tetraphyllidean, and demonstrates several possible scenarios deriving the scolices of *D. cancellata* and *D. campbelli* from the archetypal pattern. Because this study represents the first attempt to find the sister-group of *Dioecotaenia*, I make no claim that my analysis represents anything more than a "bold hypothesis" sensu Popper (1968). It does, however, make predictions which may be tested by new collections.

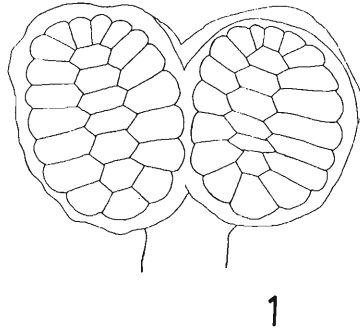


Figure 1. Bothridial architecture of *Dioecotaenia campbelli*.

### Architecture of *Dioecotaenia* Bothridia

Schmidt (1969) noted that the bothridial loculi of *Dioecotaenia cancellata* numbered 21, and that they could be viewed as arranged in three rows of seven loculi each or as one central row of five loculi surrounded by 16 marginal loculi. Mayes and Brooks (1980) noted for *D. campbelli* 24 loculi, in three rows of eight loculi each or one central row of six loculi surrounded by 18 marginal loculi. Algebraically, those observations may be expressed as follows,

$$3(X) = (X - 2) + (2X + 2)$$

where  $X = 7$  for *D. cancellata* and  $X = 8$  for *D. campbelli*. Additionally, central loculi in *Dioecotaenia* are in the form of hexagons, and marginal loculi are connecting modified hexagons. Thus, any scheme simulating development of the *Dioecotaenia* scolex must satisfy both sides of the equation and be consistent with the structural constraints of interlocking hexagons.

### Simulations

**SIMULATION 1:** This simulation begins with an assumption that the archetypal bothridium comprised a single central hexagonal loculus surrounded by marginal loculi. Expected values for marginal loculi and for total number of loculi predicted by the equation above for a single central loculus (Table 1) are eight and nine,

Table 1. Values for simulated bothridial loculi based on observed patterns in *Dioecotaenia cancellata* and *D. campbelli*.

| "X" | Central loculi no. | Marginal loculi no. | Total loculi no. |
|-----|--------------------|---------------------|------------------|
| 1   | (-1)               | (4)                 | 3                |
| 2   | (0)                | (6)                 | 6                |
| 3   | 1                  | 8                   | 9                |
| 4   | 2                  | 10                  | 12               |
| 5   | 3                  | 12                  | 15               |
| 6   | 4                  | 14                  | 18               |
| 7   | 5                  | 16                  | 21               |
| 8   | 6                  | 18                  | 24               |
| 9   | 7                  | 20                  | 27               |
| 10  | 8                  | 22                  | 30               |

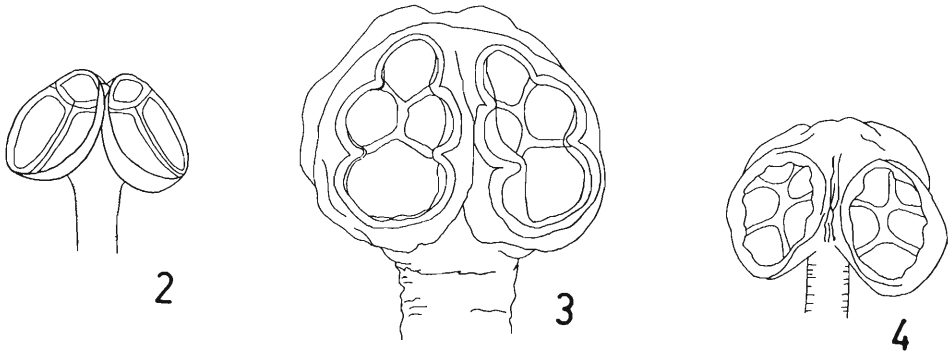
**Table 2.** Values for simulated bothridial loculi based on assumption that archetypal bothridial morphology comprised a single central hexagonal loculus and interlocking marginal loculi.

| "X" | Central loculi no. | Marginal loculi no. | Total loculi no. |
|-----|--------------------|---------------------|------------------|
| —   | 1                  | 6                   | 7                |
| —   | 2                  | 8                   | 10               |
| —   | 3                  | 10                  | 13               |
| —   | 4                  | 12                  | 16               |
| —   | 5                  | 14                  | 19               |
| —   | 6                  | 16                  | 22               |
| —   | 7                  | 18                  | 25               |
| —   | 8                  | 20                  | 28               |
| —   | 9                  | 22                  | 31               |
| —   | 10                 | 24                  | 34               |

respectively. However, a single hexagonal loculus can accommodate only six interlocking hexagonal marginal loculi, giving only seven total loculi. Table 2 presents predicted locular values under this simulation for a variety of values for the central loculi. In no case are the values required by the equation produced (compare with values in Table 1), so this model could not have produced the patterns seen in *D. cancellata* and *D. campbelli*. It is therefore rejected.

**SIMULATIONS 2 AND 3:** Table 1 lists as three the smallest number of total loculi expected. This means only that some archetypal plan involving three loculi represents the greatest extent of extrapolation under this model. In fact, I will show later that not until  $X = 3$  would bothridia characteristic of *Dioecotaenia* be recognizable unambiguously. Thus, any particular single loculus or simple sucker, or double loculus precursor would not be predicted from our observations. Two known tetraphyllidean genera each contain a single species characterized by triloculate bothridia. Simulations 2 and 3 investigate the implications of considering each as possessing the archetypal bothridial morphology.

**SIMULATION 2:** The scolex of *Trilocularia acanthiaevulgaris* as archetype. The bothridial architecture of *T. acanthiaevulgaris* is shown in Figure 2. It comprises three radially arranged interlocking loculi. Transformation of this plan to a structure characteristic of *Dioecotaenia* would require at least one doubling of loculi (to six) in order to produce a linear arrangement from a radial one, followed by progressive additions of three-loculi units. The transformation from radial to linear arrangement would have to be followed by some form of secondary radiality producing marginal loculi. I have reservations about this simulation for two reasons. The first is the large number of ad hoc assumptions which must be made to explain what we see today, suggesting a more parsimonious argument exists. Second, I believe we have evidence that the evolution of the bothridial morphology exhibited by *Trilocularia* proceeded in a manner different from that postulated in this simulation. Two additional tetraphyllideans, *Zyxibothrium kamienae* and *Pentaloculum symmetricum*, possess bothridia similar in shape and attachment to those of *Trilocularia*, but have four and five loculi per bothridium, respectively (Figs. 3, 4). Hayden and Campbell (1981) have discussed other similarities among these cestodes. It thus appears that bothridial evolution for this particular group of cestodes has proceeded by simple locular addition, from three to four to five.



Figures 2-4. 2. Scolex of *Trilocularia acanthiaevularis*. 3. Scolex of *Zyxibothrium kamienae*. 4. Scolex of *Pentaloculum symmetricum*.

SIMULATION 3: The scolex of *Tritaphros retzii* as archetype. *Tritaphros retzii* possesses linear trilobulate bothridia oriented horizontally (Fig. 5). In the latter respect they differ from bothridia of *Rhinebothrium* spp., of which they are reminiscent. Transformation of this bothridial structure into that characteristic of *Dioecotaenia* may be accomplished with a minimum of assumptions and without compromising the structural constraints postulated herein. Three different developmental scenarios leading to the structures exhibited by *Dioecotaenia* from that possessed by *T. retzii* are displayed diagrammatically in Figure 6. Of those three scenarios, two require two different kinds of mechanisms whereas the third requires only one. Thus, a progressive doubling of loculi from  $X = 1$  to  $X = 8$  could account for *D. campbelli* but not *D. cancellata* unless a secondary loss of one row was also postulated. Similarly, doubling from  $X = 1$  to  $X = 4$  followed by progressive addition of single rows of loculi would also require a change of mechanism. Because of our scanty knowledge, I prefer the third scenario, simple addition of three-loculi units, which requires only a single mechanism.

*Tritaphros* is pertinent from another standpoint. Because of the horizontal orientation of its loculi, *Dioecotaenia* bothridial patterns could be formed from a *Tritaphros* archetype directly at  $X = 3$ . If the archetypal bothridial plan included vertically oriented loculi as in *Rhinebothrium*, at  $X = 3$  a bothridial plan

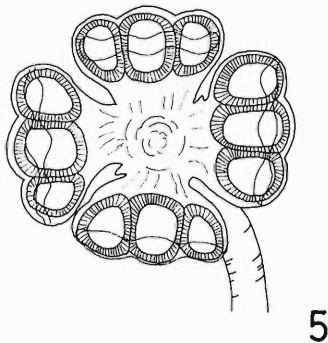


Figure 5. Scolex of *Tritaphros retzii*.

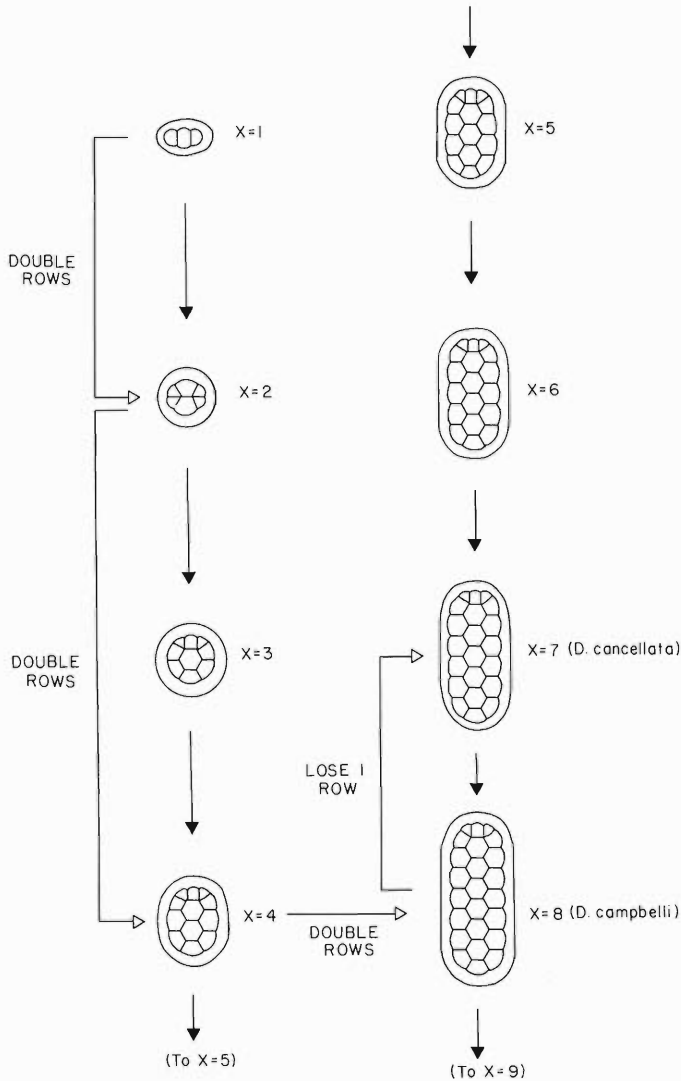


Figure 6. Results of simulation producing scolices of *Dioecotaenia* spp. from the scolex of *Tritaphros retzii* (upper left, X = 1). Arrows with solid head indicate sequence produced by simulation, requiring only a single mechanism of development, addition of rows of loculi. Arrows with open heads indicate possible alternative scenarios requiring fewer steps but invoking two developmental mechanisms, doubling of rows of loculi and loss of a single row.

similar to *Dioecotaenia* could be formed, but at X = 4 the orientation of the bothridial would be out of phase by 90°. In *Dioecotaenia* spp., the three rows of loculi occur vertically rather than horizontally. Thus, by the time X = 4 was achieved from a vertically oriented three-loculi pattern, a 90° rotation in locular orientation would be required (Fig. 6).

### Conclusions

By rejecting simulation 1 and accepting simulation 3, this study supports the notion that the hexagonal structure of *Dioecotaenia* loculi is a derived feature.

I have no data suggesting how a transformation from non-hexagonal to hexagonal loculi could have been achieved. I only note that closest packing of objects is achieved if the objects are hexagons. Discovery of species exhibiting six or nine loculi per bothridium might provide data bearing on this matter. This report makes a number of predictions about expected bothridial morphotypes for undescribed *Dioecotaenia* species. Departure from the locular numbers depicted in Table 1 in any new species would falsify the hypothesis presented in this paper.

This study does not make any predictions about the origins of the dioecious nature of *Dioecotaenia*. Because there is no sexual dimorphism in scolex structure, there does not appear to be any ontogenetic relation between the two characters beyond the fact that they are products of the same phylogenetic history. Thus, monoecious species exhibiting *Dioecotaenia* scolex morphology may exist. Alternatively, dioecious species possessing three or six bothridial loculi not immediately recognizable as *Dioecotaenia* might also exist.

#### Acknowledgments

Support for this study was provided by a grant from the Natural, Applied and Health Sciences Grant Committee of the University of British Columbia. Ms. Janine Caira prepared the illustrations.

#### Literature Cited

- Hayden, B. P., and R. A. Campbell. 1981. *Zyxibothrium* (Tetraphyllidea: Phyllobothriidae), a new genus of cestodes from skates, with suggestions for diagnoses, classification, and revision of the Rhinebothriinae Euzet, 1953. *J. Parasitol.* 67:262-267.
- Mayes, M. A., and D. R. Brooks. 1980. Cestode parasites of some Venezuelan stingrays. *Proc. Biol. Soc. Wash.* 93:1230-1238.
- Popper, K. R. 1968. *The Logic of Scientific Discovery*. Harper and Row, San Francisco. 479 pp.
- Schmidt, G. D. 1969. *Dioecotaenia cancellata* (Linton, 1890) gen. et comb. n., a dioecious cestode (Tetraphyllidea) from the Cow-Nosed Ray, *Rhinoptera bonasus* (Mitchill), in Chesapeake Bay, with the proposal of a new family, the Dioecotaeniidae. *J. Parasitol.* 55:271-275.

## *Proteocephalus micruricola* sp. n. (Cestoda: Proteocephalidae) from *Micrurus diastema affinis* in Oaxaca, Mexico

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**ABSTRACT:** *Proteocephalus micruricola* sp. n. is described from *Micrurus diastema affinis* (Jan) in Mexico. *P. micruricola* is characterized by possessing 121 to 169 testes in two lateral fields, an apical structure on the scolex, and equatorial genital pores. It differs from *P. jarara* (Fuhrmann, 1927) Brooks, 1978, from *Bothrops alternatus* in Brazil, in that it has a scolex diameter of 720 to 760  $\mu\text{m}$ , neck length of 11.75 mm, vaginal pores opening posterior to the cirrus pouch in the majority (85%) of proglottids, and onchosphere diameter of 15 to 18  $\mu\text{m}$ .

During an expedition into southern Mexico to examine snakes for mesocercariae of the trematode genus *Alaria* one of us (WLS) had the opportunity to examine a coral snake, *Micrurus diastema affinis* (Jan), for helminths. We found two gravid strobilae of an undescribed proteocephalid tapeworm in the small intestine. It is unusual to find proteocephalids in fossorial snakes as these worms usually infect aquatic vertebrates such as fish, amphibians, and semi-aquatic snakes. To our knowledge, no other parasites have been reported from this host.

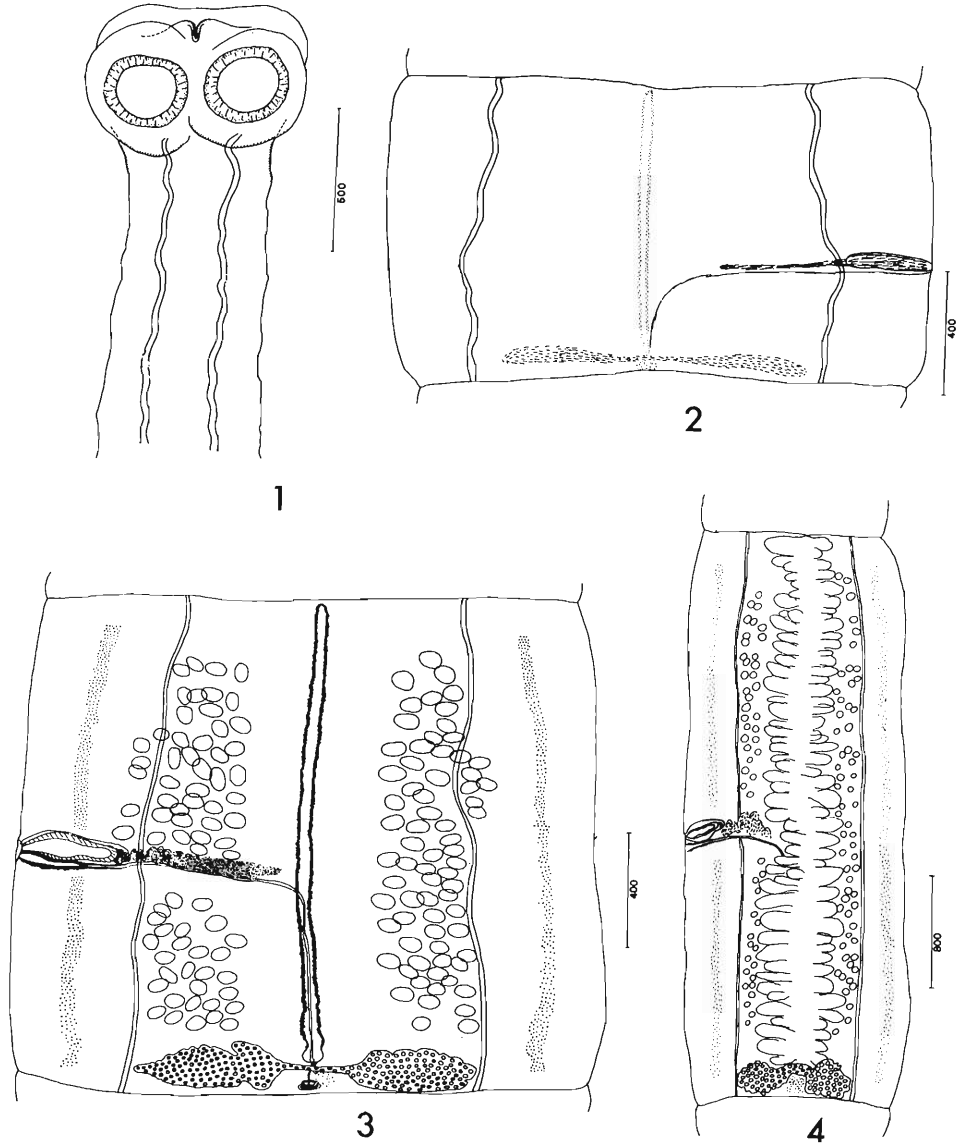
The worms were fixed in the field and stored in AFA. They were subsequently stained in Semichon's aceto-carmin, dehydrated in a series of alcohols, and mounted in Permount. Measurements are in micrometers unless otherwise stated; means are followed by the ranges in parentheses. After measurements were taken, one specimen was dismantled and prepared for scanning electron microscopy for additional observations on scolex morphology. Line drawings were prepared with the aid of a microprojector.

### *Proteocephalus micruricola* sp. n. (Figs. 1-4)

**DESCRIPTION** (based on two specimens): Proteocephalidea Mola, 1928; Proteocephalidae La Rue, 1911; *Proteocephalus* Mola, 1929. Length of strobila 275 (259-290) mm. Scolex globose, 475 (450-500) long by 740 (720-760) wide, with four muscular suckers; suckers 255 (250-260) long by 305 (300-310) wide. Apical structure consisting of a slight elevation with an inconspicuous, funnel-shaped depression. Neck 11.75 (11.5-12.0) mm long by 720 (510-930) wide. Scolex and neck spinous. Strobila acraspedote. Immature proglottids 795 (540-1,200) long by 1,780 (1,500-2,200) wide ( $N = 20$ ); mature proglottids 1,450 (1,150-1,800) long by 2,090 (1,850-2,400) wide ( $N = 20$ ); and gravid proglottids 3,200 (2,100-4,100) long by 1,750 (1,400-2,200) wide ( $N = 10$ ), with a width to length ratio of 1:1.9 (1.1-2.9). Calcareous corpuscles numerous in the cortical parenchyma. Excretory system composed of paired dorsolateral and ventrolateral longitudinal canals; ventrolateral canals displaced laterally and four times the diameter as the dorsolateral canals; no transverse commissures present.

**MALE GENITALIA:** Testes in two distinct lateral fields, 140 (121-169) total, 43 (29-56) preporally, 24 (17-29) postporally, 73 (60-90) antiporally; 75 (55-100) in diameter ( $N = 25$ ). Vas deferens describes a broad series of coils between the





Figures 1–4. *Proteocephalus micruricola* sp. n. 1. Scolex. 2. Immature proglottid. 3. Mature proglottid. 4. Gravid proglottid.

cirrus pouch and uterus. Cirrus pouch transverse, 333 (300–350) long by 97 (80–105) wide ( $N = 25$ ); ratio of cirrus pouch length to proglottid width 1:6.4 (5.1–6.9). Genital pores alternate irregularly in the anterior 51 (48–56) % of proglottids. Genital pore slightly elevated.

FEMALE GENITALIA: Ovary bilobate, 1,052 (820–1,250) wide ( $N = 20$ ), lobes joined by a narrow isthmus; ovary confined to the area between the dorsolateral excretory canals. Mehlis' gland complex conspicuous. Vagina opens posterior to the cirrus pouch in 85% of proglottids; distal part of the vagina thick walled,

without a sphincter. Uterus preformed as a simple tube in mature proglottids; when gravid the uterus expands laterally into 40 (35–53) horizontal, layered diverticula on each side ( $N = 10$ ). Vitellaria in two narrow bands just lateral to the ventrolateral excretory canal, and extends almost the entire length of the proglottid. Eggs 22 (20–23) in diameter ( $N = 10$ ) with onchospheres 17 (15–18) in diameter ( $N = 10$ ).

TYPE HOST: *Micrurus diastema affinis* (Jan).

SITE: Small intestine.

TYPE LOCALITY: Valle Nacional, Oaxaca, Mexico.

HOLOTYPE: USNM Helm. Coll. 76375.

### Discussion

Freze (1965) placed those proteocephalids with simple scolices from amphibians in *Batrachotaenia*, those from snakes in *Ophiotaenia*, and those from turtles in *Testudotaenia*. In addition, he placed similar proteocephalids from fish in a different genus, *Proteocephalus*, based on host specificity and absence of a preformed uterus in mature proglottids. Brooks (1978) evaluated the characters used by Freze and concluded that they were not significant in view of similar scolex morphology, arrangement of gonads, and available life history information. Consequently, Brooks considered the three genera, sensu Freze, as junior synonyms of *Proteocephalus*. We concur with Brooks until additional information becomes available.

*Proteocephalus micruricola* is most similar to *P. jarara* (Fuhrmann, 1927) Brooks, 1978, from *Bothrops alternatus* in Brazil, in possessing testes in two distinct fields, number of testes, presence of an apical structure, and equatorial genital pores. *P. micruricola* differs from *P. jarara* in the following characters: (1) *P. micruricola* has a scolex diameter of 720 to 760 whereas that of *P. jarara* measures 1,100 to 1,200; (2) the neck length of *P. micruricola* averages 11.75 mm while that of *P. jarara* is under 5 mm; (3) the vaginal pores of *P. micruricola* open posterior to the cirrus pouch in 85% of proglottids whereas *P. jarara* never open posterior to the cirrus pouch; and (4) the onchospheres of *P. micruricola* are 15 to 18 in diameter whereas those of *P. jarara* measure 10 in diameter.

### Acknowledgments

The authors express their appreciation to Randy H. Vaeth, Museum of Zoology, L.S.U., who kindly identified the coral snake used in this report.

### Literature Cited

- Brooks, D. R. 1978. Systematic status of proteocephalid cestodes from reptiles and amphibians in North America with descriptions of three new species. *Proc. Helminthol. Soc. Wash.* 45:1–28.
- Freze, V. I. 1965. *Essentials of Cestodology*. Vol. V. Proteocephalata in Fish, Amphibians, and Reptiles. Akad. Nauk, Moscow. (Israel Program for Scientific Translations, Jerusalem, 1969.) 597 pp.

## In Vitro Culture of *Anisakis* spp. Larvae from Fish and Squid in Newfoundland

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**ABSTRACT:** *Anisakis* spp. larvae obtained from herring, Atlantic salmon, mackerel and short-finned squid, taken in Newfoundland waters, were grown in vitro to determine the identity of the adult, and in the case of the latter host to use this information to determine migration patterns (east-west or north-south). *Anisakis simplex* pre-adults were obtained from all host species. The majority of the larvae from the squid, however, were significantly smaller ( $P < 0.001$ ) than those from fish, and would not grow in vitro. It is likely that they are a different species, but until this fact can be confirmed, no speculations can be made on the migratory movements of the host.

Adult *Anisakis* spp. are found in the stomach and intestine of a wide variety of marine cetaceans and pinnipeds (Davey, 1971; Oshima, 1972), and have a life cycle that involves one, or more, intermediate and paratenic hosts (Smith and Wootten, 1978). The species composition of the genus *Anisakis*, and the geographical distribution of valid species still require elucidation. Davey (1971) recognized only three valid species (*Anisakis simplex*, *A. typica*, *A. physeteris*), and retained four species as *species inquirendae*, while Smith and Wootten (1978) raised questions about several species Davey (1971) did not review in detail or omitted.

With our increasingly greater utilization of stocks of marine fish and other organisms, it behooves us to obtain as much information about the organisms we are exploiting as possible, so that we may use them rationally. This includes gathering data on stock sizes, areas where the animals reproduce, migration routes, and various other biological parameters.

To date scientists have relied to a large extent on mechanical tagging to determine the movement of fishes, and whether one, or more, stocks exist in a given area. This method is labor intensive, time consuming, expensive and gives relatively little data for the time and dollars invested. Parasites, including *Anisakis* sp. larvae (vide Smith and Wootten, 1978) have been used as biological tags, with Kabata (1963) noting the conditions which must be fulfilled before a parasite can be successfully used in this way.

It is well known that larval nematodes, including members of the genus *Anisakis*, are present in short-finned (or bait) squid (*Illex illecebrosus*) and a wide variety of marine fishes in Newfoundland waters. The identity of the adults of these larvae are, however, unknown. Beverly-Burton et al. (1977) suggested that all the *Anisakis* larvae type 1 found in the North Atlantic are those of *Anisakis simplex*, a cosmopolitan species in cold and temperate waters. The closely related *A. typica* is generally found in warmer water from 36°S to 40°N (Davey, 1971).

A study was, therefore, initiated in 1979, to determine the identity of the larval anisakines in squid, and to determine whether they were the same as those seen in herring (*Clupea harengus*) and Atlantic salmon (*Salmo salar*) in the Newfoundland region. If the larvae proved to be *A. simplex* it would not be unreasonable to suspect that the squids migrated relatively short distances, or west-east to a

**Table 1.** Measurements (mm) of *Anisakis* spp. larvae, from herring, short-finned squid and Atlantic salmon, which were grown in vitro.

|   | Days after culture commenced |            |           |      |            |           |
|---|------------------------------|------------|-----------|------|------------|-----------|
|   | 0                            |            |           | 1-10 |            |           |
|   | a*                           | b          | c         | a    | b          | c         |
| <b>Host: Herring</b>                      |                              |            |           |      |            |           |
| <i>(Clupea harengus)</i>                  |                              |            |           |      |            |           |
| Total length                              | 24                           | 26.2 ± 2.2 | 21.9-29.7 | 4    | 18.9 ± 4.6 | 13.9-25.0 |
| Maximum width                             | 24                           | 0.4 ± 0.1  | 0.3-0.6   | 4    | 0.4 ± 0.02 | 0.3-0.4   |
| Oesophagus length                         | 24                           | 2.1 ± 0.1  | 1.9-2.9   | 1    | 2.1        | —         |
| Ventricle length                          | 24                           | 1.1 ± 0.1  | 0.8-1.3   | 1    | 0.9        | —         |
| Anus to tip of tail<br>(excluding mucron) | 23                           | 0.1 ± 0.03 | 0.08-0.16 | 2    | 0.1 ± 0.01 | —         |
| <b>Host: Short-finned squid</b>           |                              |            |           |      |            |           |
| <i>(Illex illecebrosus)</i>               |                              |            |           |      |            |           |
| Total length                              | 3                            | 16.9 ± 2.3 | 15.0-19.1 | 33   | 12.6 ± 2.4 | 6.8-18.3  |
| Maximum width                             | 3                            | 0.4 ± 0.1  | 0.4-0.6   | 33   | 0.4 ± 0.1  | 0.3-0.5   |
| Oesophagus length                         | 3                            | 1.6 ± 0.2  | 1.4-1.8   | 31   | 1.2 ± 1.3  | 0.6-1.3   |
| Ventricle length                          | 3                            | 0.7 ± 0.1  | 0.6-0.8   | 31   | 0.6 ± 0.2  | 0.4-0.7   |
| Anus to tip of tail<br>(excluding mucron) | 2                            | 0.1 ± 0.04 | 0.08-0.1  | 23   | 0.1 ± 0.03 | 0.08-0.1  |
|   |                              | 1-10       |           |      | 21-30      |           |
| <b>Host: Atlantic salmon</b>              |                              |            |           |      |            |           |
| <i>(Salmo salar)</i>                      |                              |            |           |      |            |           |
| Total length                              | 5                            | 21.4 ± 2.1 | 17.7-23.3 | 5    | 28.5 ± 8.1 | 20.9-41.9 |
| Maximum width                             | 5                            | 0.6 ± 0.04 | 0.5-0.7   | 5    | 0.9 ± 0.3  | 0.8-1.4   |
| Oesophagus length                         | 4                            | 2.2 ± 0.3  | 1.8-2.4   | 4    | 2.5 ± 0.7  | 1.6-3.2   |
| Ventricle length                          | 3                            | 0.9 ± 0.1  | 0.8-1.0   | 4    | 0.7 ± 0.02 | 0.70-0.75 |
| Anus to tip of tail<br>(excluding mucron) | 5                            | 0.1 ± 0.03 | 0.05-0.11 | 3    | 0.2 ± 0.02 | 0.16-0.20 |

\* a. No. measured. b. Mean size ± SD. c. Range.

yet unknown spawning area, and not north-south. On the other hand if the adults were *A. typica* the squids must have migrated to a spawning ground south of latitude 40°N to acquire them.

Van Banning (1971) and Pippy and van Banning (1975) have successfully reared *Anisakis* larvae, to the adult stage, from a variety of fish species taken in the North Sea, and showed that the species of larvae normally involved in the infections were those of *A. simplex*. Grabda (1976) also cultured *A. simplex* from herring caught in Pomeranian Bay (Baltic Sea). To date, no similar work has been performed on *Anisakis* larvae from fish, and squid, taken in the northwestern North Atlantic.

### Materials and Methods

Fish and squid were obtained from commercial fishermen from several locations along the east coast of insular Newfoundland, during the period May-November 1979 and 1980, and examined for anisakine larvae. One thousand and seventy larvae were cultured (herring, *Clupea harengus*, 246 in 1979; Atlantic salmon, *Salmo salar*, 206, 73 in 1979, 133 in 1980; mackerel, *Scomber scombrus*, 144 in 1979; short-finned squid, *Illex illecebrosus*, 287, 174 in 1979, 113 in 1980).

Table 1. Continued.

| Days after culture commenced |            |           |       |             |            |       |            |            |
|------------------------------|------------|-----------|-------|-------------|------------|-------|------------|------------|
| 11-20                        |            |           | 21-30 |             |            | 31-40 |            |            |
| a                            | b          | c         | a     | b           | c          | a     | b          | c          |
| 6                            | 23.8 ± 9.1 | 15.6-35.3 | 17    | 24.2 ± 7.9  | 14.0-42.3  | 4     | 31.0 ± 5.9 | 24.3-37.3† |
| 6                            | 0.6 ± 0.1  | 0.3-0.7   | 15    | 0.7 ± 0.2   | 0.4-1.0    | 4     | 1.0 ± 0.2  | 0.8-1.3    |
| 3                            | 2.4 ± 0.4  | 1.9-2.8   | 3     | 1.9 ± 0.3   | 1.8-2.3    | —     | —          | —          |
| 1                            | 0.7        | —         | 3     | 0.6 ± 0.1   | 0.5-0.7    | —     | —          | —          |
| 2                            | 0.2 ± 0.02 | —         | 4     | 0.2 ± 0.02  | —          | —     | —          | —          |
| 2                            | 15.3 ± 2.6 | 13.4-17.1 | 5     | 20.1 ± 6.2  | 13.7-28.5  | 3     | 22.5 ± 1.7 | 21.3-24.5  |
| 2                            | 0.3 ± 0.2  | 0.1-0.4   | 5     | 0.6 ± 0.2   | 0.4-0.9    | —     | —          | —          |
| 1                            | 1.1        | —         | 1     | 2.3         | —          | —     | —          | —          |
| 1                            | 0.6        | —         | 1     | 0.7         | —          | —     | —          | —          |
| —                            | —          | —         | 4     | 0.1 ± 0.02  | 0.1-0.2    | 1     | 0.2        | —          |
|                              | 31-40      |           |       | 41-50       |            |       |            |            |
| 4                            | 36.0 ± 9.5 | 21.9-41.8 | 9     | 46.7 ± 14.9 | 26.7-67.9† |       |            |            |
| 4                            | 1.1 ± 0.2  | 0.8-1.2   | 9     | 1.5 ± 0.3   | 1.0-1.9    |       |            |            |
| 1                            | 3.5        | —         | —     | —           | —          |       |            |            |
| 1                            | 1.2        | —         | —     | —           | —          |       |            |            |
| 4                            | 0.2 ± 0.04 | 0.2-0.3   | 1     | 0.4         | —          |       |            |            |

† Pre-adult *A. simplex*.

Nematodes were placed directly into a culture medium, after teasing them out of the cysts they occupied, which were usually in the mesenteries of the hosts. Other workers, e.g., van Banning (1971) and Grabda (1976) digested their larvae from the cysts, with the latter worker stating that sterile conditions were necessary for proper culture. Van Banning (1971) and the present worker did not find that absolutely sterile conditions were necessary for good cultures to be obtained. The culture medium and method used was a modification of the van Banning (1971) method.

The culture medium used in the present study was prepared as follows. Fresh hog liver was liquefied in a 0.9% NaCl solution in a blender (100 g liver/500 ml NaCl solution). Better results were obtained with liver that had been chilled for 24 hr than with liver that was only 2-3 hr old. After blending all large pieces of material (e.g., blood vessels) were strained from the solution. Pepsin was then added to the above solution, the pH lowered to 1.0-1.5 using concentrated hydrochloric acid, and the resultant mixture left to digest, at 38°C, overnight (periods of longer than 16 hr made no difference to the final medium). Grabda (1976) used 1.25 g of pepsin in the aforementioned amount of solution. In the present study amounts of up to 1.5 g were used, with the optimum being 0.5 g. In this

study hog pepsin and liver were used rather than beef products, due to their availability.

After digestion the solution was once again strained and any undigested particles removed. The pH was raised to 2.0 using concentrated sodium hydroxide, and the whole mixture centrifuged at 5,000 rpm for 30 min. The resultant clear yellow supernatant fluid was removed, and 0.015 g of Nystatin/100 ml was added. The foregoing method, if followed exactly, will yield a superior medium that may be made in large batches and deep frozen for later use.

Medium was placed in 10-, 20-, or 40-ml vials, with one to three worms (almost invariably one). The yellow supernatant fluid is the activating medium for the larvae, but it is necessary to add fresh whole blood daily if maturation is to occur. Fresh hog's blood was obtained (sodium-citrated) every 3 days, and 1–2 drops added per vial per day. It served no useful purpose to add the blood until the larvae molted for the first time in the medium (mean 5 days; range 4–6 days) as was noted by Yasuraoka et al. (1967) and Koyama et al. (1967). The optimal temperature for culture was 36–37°C, with development ceasing and mortality increasing at temperatures of 38°C or higher.

### Results and Discussion

The prevalence and intensity of infection with larval ansakines was always high in salmon, but was found to vary on a seasonal, and geographical, basis in the case of herring and squid. The viscera of approximately 50 salmon taken in June 1979 yielded 111 larvae, while only 251 were taken from several hundred (approximately 750) pounds of herrings caught in May. Further, of the 251 larvae from herrings 186 (74%) were recovered from a 250 pound sample of fish from Bonavista Bay, while the other 65 (26%) were located in two samples of 250 pounds each from Trinity Bay and Conception Bay. When squid first arrived in inshore waters in both years, relatively few larval nematodes were present in them. From early June to mid-August 1979, 2,300 squid were necropsied and yielded 12 *Anisakis* larvae (only three in the first 1,000 squid). During the period mid-September to mid-October 1979, 230 squid were examined and 100 larvae located.

When placed in the culture medium the worms moved actively for the first few days, and molted after 4–6 days. The worms were not measured before culturing began to minimize handling, and thus mortality. Despite all efforts a heavy mortality still occurred (over 75%; 98% in the case of squid nematodes), it often being hard to tell live larvae from dead ones as they approached maturity and became quiescent. Very rapid breakdown of the worms took place in the medium after death, resulting in few animals being available for measuring, and consequent loss of data sets. Soon after culturing began there was an initial loss of length in the worms, followed by an increase in size (Table 1). A further molt followed 12–14 days after the first observed molt, resulting in pre-adults (resemble adults in most characters, but not sexually mature). As maturation occurred the worms became thicker (greater diameter or width, as is well seen in the case of the larvae from salmon (Table 1)). Details of the growth of larvae from different hosts are given in Table 1. Day 0 larvae are those that were removed from the host and immediately fixed and preserved.

It can be seen from Table 1 that the salmon nematodes grew most rapidly and

achieved the greatest size. Two pre-adult males and several females were obtained from the salmon nematode cultures. Using spicule size in the males, and lip-conformation in both sexes (see Davey 1971) the pre-adults were designated *Anisakis simplex*. The larvae from herring, and also a small number from mackerel, that matured were also identified as *A. simplex*. In the case of the latter host, however, small numbers of a recognizably different *Anisakis* sp. larva (smaller) was found (3–4 specimens per 100 *A. simplex* larvae). This smaller larva was reminiscent of that taken from squid, and bore large numbers of teeth on its lips. As with the small larvae from squid it proved difficult to culture these larvae. This observation is of interest inasmuch as the mackerel is a migratory species that is found only in Newfoundland waters at certain times of the year. In no case did the worms from herring and mackerel attain the size of those from salmon (maximum size reached by larvae from salmon 67.9 mm, herring 37.3, mackerel 29.0), even though they lived as long. The larvae from herring reached a pre-adult stage, stopped moving and, while living for as long as 54 days, did not mature. A characteristic of the maturation process is thickening of the body, as noted earlier.

The larvae from squid (Table 1) were significantly smaller ( $P < 0.001$ ) than those taken from any of the other hosts, and normally lived for only 1 or 2 days in the culture medium, then died. Initially all the larvae from this host (squid) were small, but in the latter part of the squid season, in both years, a small number of larger nematodes were found. The latter resembled in size and morphology those from the fish hosts. Ten were kept alive for periods of up to 37 days in 1979, with growth being evident in some, but no maturation. These larvae attained a mean diameter of 0.6 mm which is less than that of pre-adults cultured from larvae from salmon (pre-adult mean width 1.5 mm) or herring (1.0). Two specimens still possessed a boring tooth and mucron (tail spine) after 25 days in the culture medium, indicating that they had never molted. In 1980, however, six larvae did grow (one specimen  $30 \times 0.4$  mm, esophagus, 2.2 mm, ventricle 1.1 mm) and were identified as *A. simplex*. It would appear that squid contain small larvae, probably of a different species, when they arrive inshore, and secondarily acquire the larvae of *A. simplex* after spending the summer in warm shallow water. Until the identity of the small larva is established these organisms may not be used to speculate on the migratory habits of the host.

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#### Literature Cited

- Beverly-Burton, M., O. L. Nyman, and J. H. C. Pippy. 1977. The morphology, and some observations on the population genetics of *Anisakis simplex* larvae (Nematoda: Ascaridata) from fishes of the North Atlantic. *J. Fish. Res. Board Can.* 34:105–112.
- Davey, J. T. 1971. A revision of the genus *Anisakis* Dujardin, 1845 (Nematoda: Ascaridata). *J. Helminthol.* 45:51–72.
- Grabda, J. 1976. Studies on the life cycle and morphogenesis of *Anisakis simplex* (Rudolphi, 1809) (Nematoda: Anisakidae) cultured *in vitro*. *Acta Ichthyol. Piscatoria* 6:119–141.

- Kabata, Z.** 1963. Parasites as biological tags. ICNAF Spec. Publ. 4:31–37.
- Koyama, T., K. Yasuraoka, and M. Machida.** 1967. (Studies on the *in vitro* axenic development of *Anisakis* larvae. (11).) Jpn. J. Parasitol. 16:291. (In Japanese)
- Oshima, T.** 1972. *Anisakis* and Anisakiasis in Japan and adjacent area. Pages 301–393 in K. Morishita, Y. Komiya, and H. Matsubayashi, eds. Progress of Medical Parasitology in Japan. Vol. IV. Meguro Parasitological Museum, Tokyo.
- Pippy, J. H. C., and P. van Banning.** 1975. Identification of *Anisakis* larva (1) as *Anisakis simplex* (Rudolph, 1809 det. Krabbe 1878) (Nematoda: Ascaridata). J. Fish. Res. Board Can. 32:29–32.
- Smith, J. W., and R. Wootten.** 1978. *Anisakis* and Anisakiasis. Adv. Parasitol. 16:93–163.
- van Banning, P.** 1971. Some notes on a successful rearing of the herring-worm *Anisakis marina* L. (Nematoda: Heterocheilidae). J. Cons. Cons. Int. Explor. Mer 34:84–88.
- Yasuraoka, K., T. Koyama, and K. Kato.** 1967. (Studies on the *in vitro* axenic development of *Anisakis* larvae. (1).) Jpn. J. Parasitol. 16:290–291. (In Japanese)

## CALL FOR PAPERS

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The Editor



## Vaccine Trials with Ultraviolet Attenuated *Ostertagia ostertagi* in Calves

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**ABSTRACT:** Infective larvae of *Ostertagia ostertagi* attenuated by ultraviolet irradiation to prevent significant development beyond the fourth larval stage were used to vaccinate a total of 17 calves, 2 to 6.5 mo old, in three experiments using single, double, or triple immunizations. When challenged with normal infective larvae, there was no indication from either worm recovery at necropsy, clinical signs of infection, or rate of gain that vaccinated calves developed any resistance.

Attempts to stimulate protective immunity against the medium stomach worm, *Ostertagia ostertagi*, in cattle have generally resulted in failure or low levels of protection (Anderson et al., 1967; Michel et al., 1973; Ross, 1963; Ross and Dow, 1964; Herlich, 1976). Those attempts entailed the use of normal infective larvae (L<sub>3</sub>) administered orally at various dosage levels over a variety of intervals, and, in some instances, repeated, chemically terminated, immunizing infections. The results suggest that calves may develop immunity to reinfection very slowly, requiring up to 9 mo of daily exposure to L<sub>3</sub>. Other efforts to produce protection in calves by parenteral injection with the in vitro grown parasitic stages of *O. ostertagi* and exoantigens obtained from culture media were also unsuccessful (Herlich and Douvres, 1979). Finally, in attempts to exploit irradiated L<sub>3</sub>, in the fashion of the irradiated lungworm vaccine, Armour (1967) found that onset of diarrhea was delayed in immunized calves, mean fecal egg count was only 20% of that in the controls, and less than 50% of the total number of worms at necropsy were mature adults, whereas over 90% were mature in the controls. However, there was no significant difference between the total numbers of *O. ostertagi* between immunized and control calves. Similar results were reported by Burger et al. (1968).

We have shown that ultraviolet irradiation can attenuate the development of *O. ostertagi* L<sub>3</sub> (Herlich and Tromba, 1980). Indications that irradiated L<sub>3</sub> of other nematode species are more efficient than normal larvae in eliciting resistance (Jarrett et al., 1959; Dow et al., 1959), and Tromba's (1978a) successful immunization of swine using UV-attenuated *Ascaris suum* embryonated eggs, led us to undertake a series of experiments to determine whether UV-attenuated *O. ostertagi* L<sub>3</sub> might stimulate protection against reinfection in calves.

### Materials and Methods

Holstein-Friesian neutered male calves were raised helminth-free until used. The strain of *O. ostertagi* used was originally isolated from a calf in Auburn, Alabama and has been maintained by continuous passage through calves for over 20 years. Larvae were attenuated by exposure to UV radiation as described (Tromba, 1978b) except that a radiometer with digital readout was used to measure the dose (J-260 Radiometer, Ultra-Violet Products Inc). All calf inoculations were made by oral administration of L<sub>3</sub> suspended in tap water. Fecal egg counts

**Table 1. Protocol and results of vaccinating calves with single and double exposure to ultraviolet-attenuated *Ostertagia ostertagi* infective larvae (experiment 1).**

| Day  | Protocol<br>Groups (number of calves)          |                         |                         |               |
|------|--|-------------------------|-------------------------|---------------|
|      | I (4)  | II (4)                  | III (2)                 | IV (3)        |
| 0    | 50,000 L <sub>3</sub> *                        | 50,000 L <sub>3</sub> * | 50,000 L <sub>3</sub> † | Nil           |
| 14   | Nil  | 50,000 L <sub>3</sub> * | 50,000 L <sub>3</sub> † | Nil           |
| 35   | Challenge inoculation 300,000 L <sub>3</sub> † |                         |                         |               |
| 70   | Necropsy                                       |                         |                         |               |
|      | Results<br>Number worms recovered at necropsy  |                         |                         |               |
|      | 36,700   | 50,500                  |                         | 39,600        |
|      | 24,400   | 29,900                  | 42,800                  | 50,800        |
|      | <u>33,100</u>                                  | <u>45,200</u>           | <u>58,800</u>           | <u>34,700</u> |
| Ave. | 31,400   | 41,867                  | 50,800                  | 41,700        |
|      | [1,130]‡                                       | [760]‡                  |                         |               |

\* L<sub>3</sub> = infective larvae exposed to 500 ET ( $\mu\text{W}\cdot\text{min}/\text{cm}^2$ ) of ultraviolet radiation.

† L<sub>3</sub> = normal ensheathed infective larvae.

‡ Number of residual immunizing worms in a nonchallenged calf necropsied on day other calves were challenged.

were made by direct centrifugal flotation with zinc sulfate as the flotation fluid. Calves were observed daily for signs of anorexia, abnormal stool discharge, and other evidence of parasitic disease. In experiment 3, calves were weighed weekly. All calves were necropsied and numbers of *O. ostertagi* determined (Herlich, 1976).

EXPERIMENT 1 (single and double vaccination, Table 1): Infective larvae were exposed to a (estimated) total radiation dose (ET) of 500  $\mu\text{W}\cdot\text{min}/\text{cm}^2$ , a dose that permits less than 1% of the inoculum to achieve sexual maturity (Herlich and Tromba, 1980) while allowing extensive early development and involvement in the gastric pits (unpublished data). Thirteen 2-mo-old calves were allotted to four groups as follows: I—each of four calves inoculated once with 50,000 irradiated L<sub>3</sub>; II—each of four calves inoculated twice at a 2-week interval with 50,000 irradiated L<sub>3</sub>; III—two calves inoculated twice at a 2-week interval with 50,000 normal L<sub>3</sub>; and IV—three calves as challenge controls. Thirty-five days after the initial immunizing inoculation, three calves in each of groups I and II and both calves of group III were given a single challenge inoculation of 100,000 normal L<sub>3</sub>. One calf each in groups I and II were not challenged to serve as indicators of the level of infection produced by the irradiated L<sub>3</sub>. Simultaneously, three previously uninfected calves (group IV) were given the challenge inoculation. All calves were necropsied 35 days after challenge (DAC).

EXPERIMENT 2 (single vaccination, Table 2): Infective larvae were irradiated at an ET of 1,000, a dosage that prevents any adult development, but does allow early development and tissue involvement including production of typical early stage *O. ostertagi* lesions (unpublished data). Ten 6.5-mo-old calves were allotted to two equal groups. Five calves were given a single inoculation with a massive number, 750,000, of irradiated L<sub>3</sub>. These calves and five previously uninfected control calves were each given a single challenge inoculation of 120,000 L<sub>3</sub> 34 days after the immunizing inoculation. All calves were necropsied 35 DAC.

**Table 2. Protocol and results of vaccinating calves with a single massive dose of ultraviolet-attenuated *Ostertagia ostertagi* infective larvae (experiment 2).**

| Day  | Protocol<br>Groups (number of calves)         |                 |
|------|---|-----------------|
|      | I—Vaccinates (3)                              | II—Controls (3) |
| 0    | 750,000 L <sub>3</sub> *                      | Nil             |
| 34   | 120,000 L <sub>3</sub> †                      | Challenge       |
| 69   |   | Necropsy        |
|      | Results<br>Number worms recovered at necropsy |                 |
|      | 28,360  | 44,200          |
|      | 33,710  | 28,900          |
|      | <u>25,400</u>                                 | <u>48,000</u>   |
| Ave. | 29,157  | 40,367          |

\* Infective larvae exposed to 1,000 ET dose of ultraviolet radiation.

† Normal infective larvae.

EXPERIMENT 3 (triple vaccination, Table 3): Triple vaccination using *Ascaris suum* embryonated eggs irradiated at descending ET dosages appeared to provide enhanced protection against challenge (Tromba, 1978a). Therefore, we inoculated each of six 3-mo-old calves with doses of 100,000 *O. ostertagi* L<sub>3</sub> at 1-week intervals with L<sub>3</sub> irradiated at ET's of 600, 550, and 500 in successive weeks. A single challenge inoculum of 300,000 normal L<sub>3</sub> was given to each of the vaccinated calves 33 days after the last immunizing inoculation; six previously uninfected calves were similarly inoculated to serve as controls. All calves were necropsied 35 DAC.

Differences in worm burdens among groups were subjected to analysis of variance.

**Table 3. Protocol and results of vaccinating calves with three exposures to ultraviolet-attenuated *Ostertagia ostertagi* infective larvae (experiment 3).**

| Day  | Protocol<br>Groups (number of calves)          |                 |
|------|--|-----------------|
|      | I—Vaccinates (6)                               | II—Controls (5) |
| 0    | 100,000 L <sub>3</sub> *                       | Nil             |
| 7    | 100,000 L <sub>3</sub> *                       | Nil             |
| 14   | 100,000 L <sub>3</sub> *                       | Nil             |
| 37   | Challenge with 300,000 L <sub>3</sub>          |                 |
| 72   | Necropsy                                       |                 |
|      | Results<br>Number worms discovered at necropsy |                 |
|      | 48,740   | 45,470          |
|      | 47,000   | 78,930          |
|      | 42,640   | 54,620          |
|      | 60,000   | 53,370          |
|      | <u>58,160</u>                                  | <u>56,860</u>   |
| Ave. | 51,308   | 57,850          |
|      | [760]†   |                 |

\* UV dose at day 0 was 600 ET; at day 7—500 ET; at day 14—500 ET.

† One vaccinate not challenged but necropsied to serve as control on worm development of irradiated L<sub>3</sub>; number worms in brackets.

## Results

EXPERIMENT 1: All calves given the immunizing inoculation of *L*<sub>3</sub>, whether irradiated or not, developed patent infections. The fecal egg count rose sharply in all groups by 25 DAC, and at necropsy the average epg (eggs per gram of feces) per group ranged from 724 to 1,015, the differences not being significant. Before administration of the challenge inoculation, all calves appeared to be unaffected by the immunizing doses of irradiated and normal *L*<sub>3</sub>. At 25 DAC there was an onset of what proved to be a transitory mild anorexia and passage of abnormally soft to fluid feces in some calves of all groups. The nonchallenged vaccinated calves of groups I and II had 1,130 and 760 adult *O. ostertagi* when necropsied (Table 1) at the time challenge inoculations were given the other calves. Average number of *O. ostertagi* per group at necropsy ranged from 31,400 to 50,800 in the vaccinated groups (I to III) and 41,700 in the group IV controls (Table 1). The differences among groups are not significant. Of the worms recovered, from 1 to 2% were immature.

EXPERIMENT 2: No patent infections resulted from inoculation of calves with the irradiated *L*<sub>3</sub>, and all calves, vaccinated and controls, had patent infections 17 DAC. No calves showed any signs of parasitic disease. On day of necropsy the average epg was 222 for the vaccinates and 300 for the controls, and the average number of worms per group was 29,157 and 40,367, respectively (Table 2). The differences in epg and worm counts between groups are not significant.

EXPERIMENT 3: All calves developed patent infections as a result of inoculation with irradiated *L*<sub>3</sub>, but epg was low ranging from 14 to 62. The one vaccinated nonchallenged calf killed when the remaining calves were given challenge inoculations still had 200 adult and 560 *L*<sub>4</sub> *O. ostertagi* 23 days after the last immunizing dose of irradiated *L*<sub>3</sub> was given (Table 3). Peak average epg of 336 in vaccinates and 548 in controls occurred on day of necropsy; the difference between averages is not significant. All calves were anorectic from 25 DAC until necropsy and there was intermittent diarrhea from day 21 to necropsy. Vaccinated calves gained an average of 16.4 kg and controls gained an average 15 kg over the 10 weeks of the experiment. However, during the last 2 weeks the groups lost 13.6 and 13.1 kg, respectively.

At necropsy, the abomasa of some calves in both groups were highly congested and edematous and there was a mesenteric lymphadenitis characterized by a purulent exudate. The average number of worms was 51,308 in vaccinates and 57,850 in the controls (Table 3); the difference is not significant. Less than 0.1% of the worms recovered were immature.

## Discussion

These experiments confirm that the early parasitic development of UV-attenuated *O. ostertagi* proceeds with concomitant invasion of the gastric pits, but that few or none of the larvae mature if the ET is between 500 and 600. The results are in basic agreement with the work cited in the introduction, namely, that little or no protection can be stimulated in calves by short-term exposure to normal or irradiated *L*<sub>3</sub>. There was no evidence of reduced epg, delayed onset of diarrhea, or a shift in the challenge worm populations to a greater percentage of immatures as reported by Armour (1967) for x-irradiated *L*<sub>3</sub> immunization. The almost consistent failure to produce protective immunity by any technic other

than by long term repeated daily exposure to L<sub>3</sub> suggests that vaccination as a practical method for prevention and control of ostertagiasis in calves does not hold promise as a viable alternative to control by anthelmintics and grazing management.

### Literature Cited

- Anderson, N., J. Armour, W. F. H. Jarrett, F. W. Jennings, J. S. D. Ritchie, and G. M. Urquhart. 1967. Experimental infections of *Ostertagia ostertagi* in calves: results of two regimens of multiple inoculations. *Am. J. Vet. Res.* 28:1073-1077.
- Armour, J. 1967. *Ostertagia ostertagi* infections in the bovine: field and experiments studies. Unpublished thesis, Glasgow University.
- Burger H.-J., J. Eckert, H.-J. Chevalier, M. S. A. Rahman, and G. Konigsmann. 1968. Zur parasitaren Gastroenteritis des Rindes II. Mitteilung: Versuche zur kunstlichen Immunisierung von Kalbern mit vontgenbestrahlten Ostertagi- und Cooperia-Larven. *Vet. Med. Nachrichten* 4:312-332.
- Dow, C., W. F. H. Jarrett, F. W. Jennings, W. S. M. McIntyre, and W. Mulligan. 1959. The production of active immunity against the canine hookworm *Uncinaria stenocephala*. *J. Am. Vet. Med. Assoc.* 135:407-411.
- Herlich, H. 1976. Attempts to produce protection against *Ostertagia ostertagi* in cattle. *Am. J. Vet. Res.* 37:61-64.
- Herlich, H., and F. W. Douvres. 1979. Gastrointestinal nematode immunization trials in cattle. *Am. J. Vet. Res.* 40:1781-1782.
- Herlich, H., and F. G. Tromba. 1980. Effect of ultraviolet radiation on some gastrointestinal parasites of cattle. *J. Parasitol.* 66:692-694.
- Jarrett, W. F. H., F. W. Jennings, W. S. M. McIntyre, W. Mulligan, M. C. C. Sharp, and G. M. Urquhart. 1959. Immunological studies on *Dictyocaulus viviparus* infections in calves—Double vaccination with irradiated larvae. *Am. J. Vet. Res.* 20:522-526.
- Michel, J. F., M. B. Lancaster, and C. Hong. 1973. *Ostertagia ostertagi*: Protective immunity in calves. The development in calves of a protective immunity to infection with *Ostertagia ostertagi*. *Exp. Parasitol.* 33:179-186.
- Ross, J. G. 1963. Experimental infection of calves with the nematode parasite *Ostertagia ostertagi*. *Vet. Record.* 75:129-132.
- Ross, J. G., and C. Dow. 1964. Further experimental infections of calves with the nematode *Ostertagia ostertagi*. *Br. Vet. J.* 120:279-285.
- Tromba, F. G. 1978a. Immunization of pigs against experimental *Ascaris suum* infection by feeding ultraviolet-attenuated eggs. *J. Parasitol.* 64:651-656.
- Tromba, F. G. 1978b. 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.

## Pinworms and Primates: A Case Study in Coevolution

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**ABSTRACT:** Cladistic analysis of 13 species of *Enterobius* based on 31 morphological characters supports the notion that pinworms and primates have co-speciated. A possible exception is the relationship between *Enterobius vermicularis* and *Homo*. *Enterobius vermicularis* is postulated to be the sister-species of *E. buckleyi* + *E. lerouxi* + *E. anthropopithecii*. Thus, if co-speciation has occurred, *Homo* is the sister-group of *Pongo* + *Gorilla* + *Pan*. Examination of the possibility that *E. vermicularis* in *Homo* is the result of host-switching or that *Homo* has been misclassified demonstrates that the latter possibility is the more parsimonious one.

Oxyurid nematodes representing the genera *Enterobius* Leach, 1853 and *Trypanoxyuris* Vevers, 1923 parasitize a variety of primate hosts. Their occurrence in primate hosts and their pronounced host specificity in natural conditions has promoted speculation that these pinworms have coevolved with their hosts and thus could serve as markers of primate phylogeny. Cameron (1929) first proposed this notion, stating,

“The examination of forms described in this paper suggests that one species restricts itself to one genus of host rather than to one species; in other words the evolution of the parasite is slower than that of the primate. It would seem legitimate to assume, to some extent at least, that the parasite has evolved with the host . . . One would expect to find forms most closely related to the human parasites in apes, while those in Old World monkeys would be closer [to] *E. vermicularis* than those in the new world monkeys and the lorises but not so close as in apes. This actually does seem to be the case . . .” (pp. 180–181)

Sandosham (1950) reported several cases of host transfers occurring in zoo settings such that some species of *Enterobius* occurred in distantly related hosts. He considered those observations to be evidence refuting Cameron’s assertion of host specificity and evolutionary rates. Sandosham further stated, “. . . none of the characters of the parasites show a gradation in correspondence with the evolutionary position of the host.” (p. 197)

Inglis (1961) reexamined the problem based on study of available type material and concluded that Cameron had been correct in his assessment. Inglis responded to Sandosham’s assertions thus,

“It should be noted that all the atypical records are from hosts in captivity . . . It is reasonable to conclude that “Cameron’s Hypothesis”—one species of parasite: one genus of host—is a good general guide to the conditions which are likely to be found in the wild.” (p. 115)

Further, Inglis asserted,

“The second suggestion put forward also seems to be, at least in part, correct since, as pointed out above, in the genus *Enterobius* there is

**Table 1. Characters and their coded states for nine of 10 complex characters used to reconstruct phylogenetic relationships of *Enterobius* spp. Plesiomorphic state is given as 0 with 1, 1\*, and 1\*\* derived equally from 0 and 2 and 2\*\* derived from 1 and 1\*\*, respectively.**

| Name of character   | Names of states (numerical code for states)             |       |
|---|---|-------|
| Total body length of female worms                             | 5-9 mm  | (0)   |
|   | more than 12 mm   | (1)   |
| Relative length of esophagus (as % of total body length)      | 20  | (0)   |
|   | 13-16   | (1)   |
| Ratio of vulvar position to total body length of female worms | 1:2   | (0)   |
|   | 1:3   | (1)   |
| Ratio of female tail length to total body length              | 1:5   | (0)   |
|   | 1:4   | (1)   |
|   | 1:3   | (2)   |
|   | 1:6-8   | (1*)  |
|   | 1:10  | (1**) |
| Length of esophageal bulb                                     | 70-100 $\mu\text{m}$ ( $\bar{x}$ = 85 $\mu\text{m}$ )   | (0)   |
|   | 100-130 $\mu\text{m}$ ( $\bar{x}$ = 115 $\mu\text{m}$ ) | (1)   |
|   | 67-80 $\mu\text{m}$ ( $\bar{x}$ = 73 $\mu\text{m}$ )    | (2)   |
|   | 150-165 $\mu\text{m}$ ( $\bar{x}$ = 157 $\mu\text{m}$ ) | (1*)  |
| Spicule length  | 100-130 $\mu\text{m}$                                   | (0)   |
|   | 66 $\mu\text{m}$  | (1)   |
|   | 52-56 $\mu\text{m}$                                     | (2)   |
|   | 77-81 $\mu\text{m}$                                     | (1*)  |
|   | 200-240 $\mu\text{m}$                                   | (1**) |
|   | 300-350 $\mu\text{m}$                                   | (2**) |
| Vaginal direction   | posterior   | (0)   |
|   | anterior  | (1)   |
| Number of caudal papillae in males                            | 5   | (0)   |
|   | 6   | (1)   |
|   | 7   | (2)   |
|   | 4   | (1*)  |
| Buccal ornamentation  | lacking   | (0)   |
|   | present   | (1)   |

some tendency for the species with the more "advanced"—i.e. more fused—spicules to occur in the most advanced primates." (p. 115)

All the above authors cautioned against drawing precise conclusions from known data. Notably, although they differed in their coevolutionary conclusions, all three workers agreed to a great extent about the morphological characters of the pinworms being discussed. Inglis (1961) and Quentin et al. (1980) corrected many of the discrepancies noted previously (see also Materials and Methods).

Since Inglis' study, five new species of *Enterobius* have been described inhabiting cercopithecoid monkeys of the subfamily Colobinae (Wahid, 1961; Vuylsteke, 1964; Yen, 1973; Quentin et al., 1980). Quentin et al. (1980) placed all six species inhabiting colobine monkeys (*E. colobis* Vuylsteke, 1964; *E. inglisi* Wahid, 1961; *E. longispiculum* Quentin, Betterton, and Krishnashamy, 1980; *E. pesteri* Wahid, 1961; *E. presbytis* Yen, 1973; and *E. zakiri* Siddiqi and Mirza, 1954) in a separate subgenus, *Colobenterobius*. They noted that *E. buckleyi* Sandosham, 1950, inhabiting orangutans, possessed some traits similar to those listed above but did

**Table 2.** Data matrix for 30 binary and one 3-state character used in reconstructing the phylogenetic relationships of 13 species of *Enterobius*.

| Species name            | Characters 1-30 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|-------------------------|-----------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| <i>lemuris</i>          | 0               | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |   |
| <i>zakiri</i>           | 0               | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 |
| <i>inglisi</i>          | 0               | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 |   |   |   |   |   |
| <i>pesteri</i>          | 0               | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 |   |   |   |   |   |
| <i>presbytis</i>        | 0               | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 |   |   |   |   |   |
| <i>colobis</i>          | 0               | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 |   |   |   |   |   |
| <i>longispiculum</i>    | 0               | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 |   |
| <i>brevicauda</i>       | 0               | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 |   |   |
| <i>bipapillata</i>      | 0               | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |   |
| <i>vermicularis</i>     | 1               | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| <i>buckleyi</i>         | 0               | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| <i>lerouxi</i>          | 0               | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| <i>anthropopithecii</i> | 0               | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |

not place the latter species in *Colobenterobius*, thus basing their classification scheme initially on host identity and secondarily on morphological traits of the parasites.

We believe previous authors were correct in counseling caution in accepting coevolutionary conclusions when dealing with *Enterobius* spp. The approach used by Cameron, Sandosham and Inglis comprised (1) assessing degree of host specificity, (2) fitting parasite morphological trends to presumed host phylogeny, and (3) accepting or rejecting the coevolution on the basis of an intuitive assessment of the goodness of fit of the parasite data to the host phylogeny. There are two possible sources of error in such methods of analysis which severely limit their effectiveness. First, degree of co-accommodation (host specificity) may not be tied necessarily to degree of co-speciation (parallel host and parasite phylogenesis) (Brooks, 1979) and may be completely decoupled from co-speciation if host-switching accounts for parasite speciation. Second, the presumed host phylogeny may be incorrect, so no valid assessment of co-speciation may be made.

A more robust approach to testing hypotheses of co-speciation begins with formulation of parasite phylogenies based on data not including host identity or host phylogeny. Such parasite phylogenies, in the form of branching diagrams, or cladograms, may then be compared with cladograms of host relationships and some assessment of concordance can be made. Such a study has not been attempted previously for any parasites of primates.

### Materials and Methods

As mentioned earlier, there has been little disagreement about the morphological characters exhibited by most pinworm species. Inglis (1961) reported that the type specimens for most of the species of *Enterobius* were in very poor condition. He did reallocate some species to *Trypanoxyuris* because they possessed labial teeth and he corrected some information on numbers of caudal papillae in males for some species. Quentin et al. (1980) corrected some mistaken measurements and added additional characters for some species. Because of the recent analyses and because of the status of the type material, our analysis is based on published



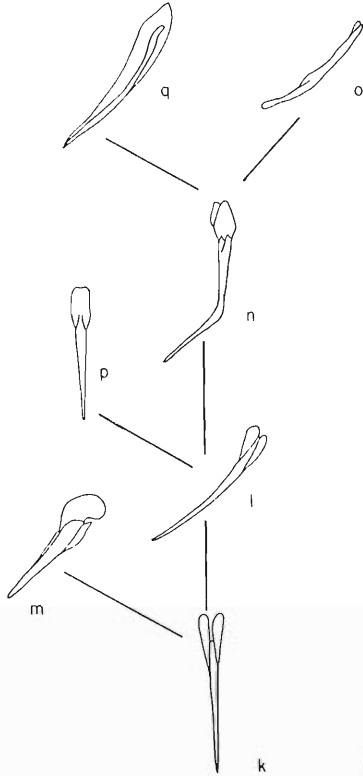


Figure 1. Pictorial representation of character-state tree for spicule morphology of *Enterobius* spp. (modified from that of Inglis, 1961). k = *E. lemurus* morphotype, m = *E. brevicauda*, l = *E. bipapillata*, p = *E. vermicularis*, n = *E. buckleyi*, o = *E. anthropopitheci*, q = *E. lerouxi*.

descriptive literature concerning *Trypanoxyuris* and *Enterobius*. Species analyzed were those members of *Enterobius* for which both males and females had been described and for which all the characters used had been noted. We utilized a total of 13 species (see Table 2 and Fig. 2). Species not used included: *E. foecundus* (Linstow, 1908); *E. parallela* (Linstow, 1908); *E. simiae* MacCallum, 1925; *E. pitheci* Cameron, 1929; and *E. macaci* Yen, 1973.

Traits for each character were arranged in transformation-series (*sensu* Hennig, 1966) polarized by out-group comparisons using *Trypanoxyuris* as the out-group. Table 1 depicts the transformation-series for nine of the 10 characters used. The transformation-series for the 10th character, spicule morphology, is shown pictorially in Figure 1. All the transformation-series except character 2 were standardized using Additive Binary Coding. The resulting data matrix (Table 2) was analyzed using the Wagner algorithm for phylogenetic inference (Farris, 1970) implemented by the Wagner-78 computer program developed by James S. Farris, State University of New York, Stony Brook. Additive Binary Coding was not necessary for character 2 because the plesiomorphic state (0) occurred only in one taxon and the secondarily derivative state (2) also occurs in only one taxon. *Enterobius pesteri* and *E. inglisi* were given the same female traits because both

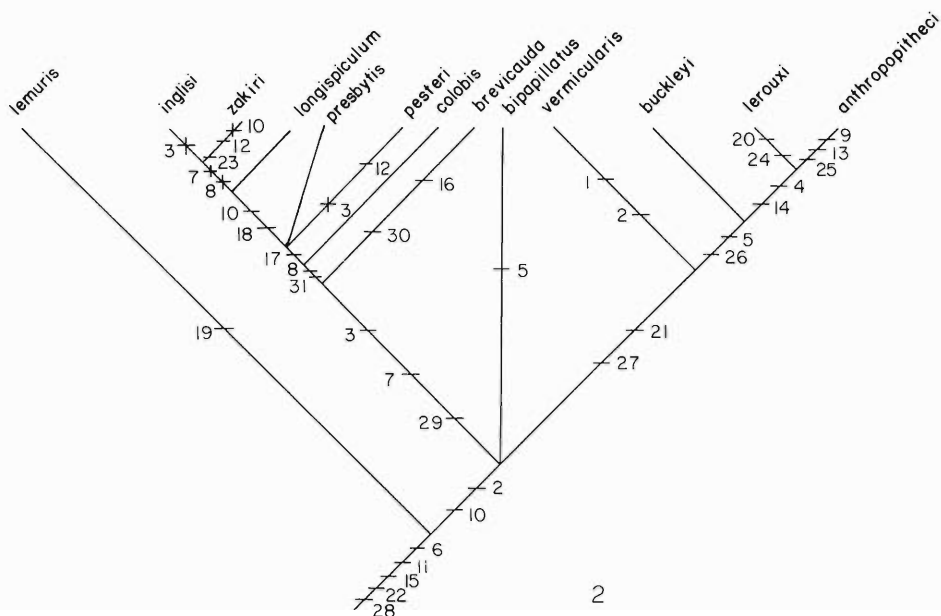


Figure 2. Cladogram summarizing data from Table 2. Slash marks indicate synapomorphies (shared derived traits) for characters from Table 2 indicated by accompanying number. Numbers accompanying an asterisk indicate a postulated reversal for the character. *Enterobius bipapillata* may also be shown as the sister-group of *E. vermicularis* + *E. buckleyi* + *E. lerouxi* + *E. anthropopitheci* if apomorphic trait for character 5 has a single origin with a reversal in *E. vermicularis*. *Enterobius bipapillata* is shown in its ambiguous position because of the ambiguity of character 5. No other ambiguities exist in the data set.

were described from a mixed infection containing males of each species and monomorphic females.

The original data matrix did not distinguish character-states 2 for character 5 and 1\* for character 6, and considered character 10, spicule morphology, in the same configuration as given by Inglis (1961). As a result of optimizing the resulting cladogram, the two character-states listed above as well as the transformation-series given in Figure 1 were found to provide a better fit of all data to the cladogram. The data matrix in Table 2 reflects the recoding of those characters.

## Results

The most parsimonious arrangement of the data is shown in Figure 2. The cladogram is rooted so that *E. lemuris* Baer, 1935 is the sister-species of all others in this analysis. The goodness of fit statistic (deviation ratio) for this type of analysis ranges from 0 to 1, with lower values indicating better fit. The deviation ratio for this analysis is 0.15. Consistency ratios are indicators of the fidelity of characters to the cladogram and the coding regime. Their values also range from 0 to 1, with higher values indicating better fit. For the 31 variables used in this analysis, 25 exhibited perfect consistency ratios of 1.0, four of the other six exhibited ratios of 0.5, and the remaining two exhibited ratios of 0.33. This indicates a very good fit to the cladogram by all data as coded. Thus, because we used the data presented by Cameron, Sandosham, Inglis, and Quentin et al., and

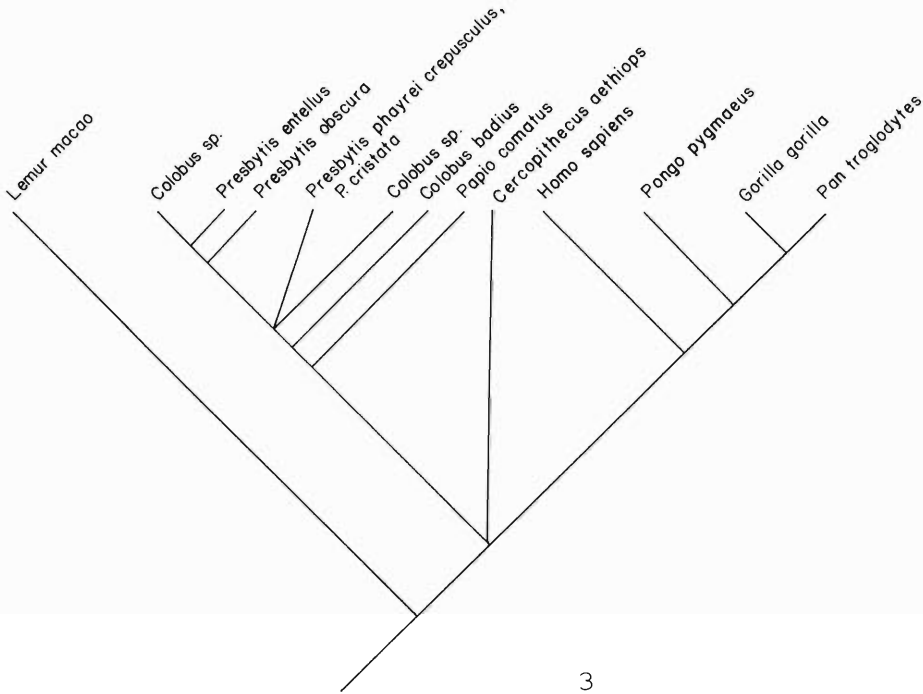


Figure 3. Host relationships predicted by phylogenetic relationships of *Enterobius* spp. *Lemur macaco* belongs in the Lemuridae, *Papio comatus*, *Presbytis entellus*, *Colobus* sp., and *Cercopithecus aethiops* belong in the Cercopithecidae, *Homo sapiens* represents the Hominidae, and *Pongo pygmaeus*, *Gorilla gorilla*, and *Pan troglodytes* belong in the Pongidae.

because the fit of data values are very high, we consider Figure 2 an accurate summation of the data upon which discussions of pinworm and primate coevolution have been based.

According to our analysis, the six species comprising the subgenus *Colobenterobius* do form a monophyletic group, corroborating the classification proposed by Quentin et al. (1980) as natural. Our cladogram also supports the exclusion of *E. buckleyi* from *Colobenterobius*. *Enterobius colobis* and *E. pesteri* from *Colobis* spp. and *E. presbytis*, *E. longispiculum*, and *E. zakiri* from *Presbytis* spp. appear in a sequence congruent with their hosts' phylogenetic relationships, but *E. inglisi* from *Colobis* sp. is not in sequence. Because *E. inglisi* and *E. pesteri* occurred in the same host in a mixed infection, one species is likely an invader of *Colobis*. Thus, *E. inglisi* may well be a parasite of *Presbytis* in Africa occasionally occurring in *Colobis*. Such an interpretation would be consistent with observed host relationships and with the biogeographic relationships of *Enterobius* spp. occurring in *Presbytis* spp. (*E. presbytis* in China and Malaysia, *E. longispiculum* in Malaysia, and *E. zakiri* in India). We would include *E. brevicauda* Sandosham, 1950 and *E. bipapillata* Gedoelst, 1916, which also inhabit cercopithecoid monkeys and which are the sister-species of *Colobenterobius*, in the above group as well rather than assigning separate subgeneric names to each of the latter two species.

Figure 3 presents the branching diagram from Figure 2 with generic names of natural hosts listed rather than parasite species names. With the exception of

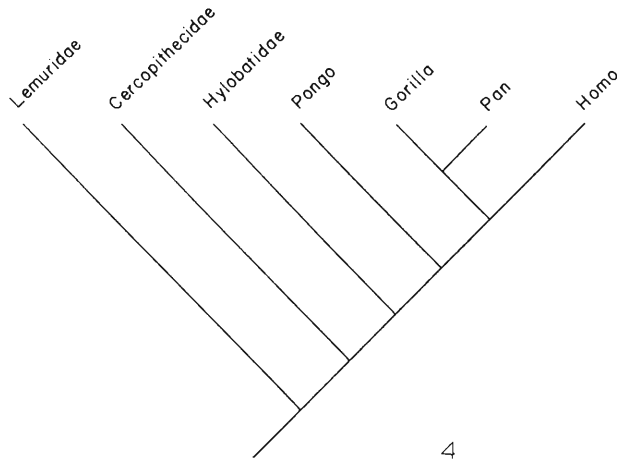


Figure 4. Commonly accepted phylogenetic hypothesis of the great apes plus Old World monkeys plus lemurs. Hylobatidae plus *Pongo*, *Gorilla*, and *Pan* are usually classified in an artificial grouping excluding *Homo*.

*Homo*, the diagram depicts primate relationships consistent with the most commonly accepted classifications (see Wiley, 1981).

We removed both sources of potential error, degree of co-accommodation and presumed host phylogeny, from the analysis. Thus, the general congruence of parasite and host phylogenies corroborates the ideas of Cameron and Inglis independently. The placement of *E. vermicularis* (Linnaeus, 1758) is not predicted by the work of those two authors and must therefore be the result of error in (1) assessing the significance of co-accommodation or (2) classifying *Homo*. We will consider each possibility.

## Discussion

### Host-switching and the Evolution of *Enterobius vermicularis*

If it is assumed that currently accepted notions of primate phylogeny are correct, *E. vermicularis* occurs in *Homo* as a result of host-switching, or parasite phylogenesis incongruent with host phylogenesis. Because the rest of *Enterobius* spp. exhibit co-speciation, one would have to explain why *Homo* does not host a species of *Enterobius* most closely related to *E. anthropopithecii* Gedoelst, 1916, the species inhabiting chimpanzees, or *E. lerouxi* Sandosham, 1950, the species inhabiting gorillas. If it were suggested that the evolution of *Homo* involved the loss of its co-speciating pinworm lineage, despite the fact that *Enterobius* spp. have direct life cycles, it must then be explained how *Homo* could then at a later time acquire pinworms from a parasite stock not closely related to pinworms inhabiting chimpanzees or gorillas. Additionally, it must be explained why, under natural conditions, no other primate besides *Homo* hosts *E. vermicularis* or a sister-species to *E. vermicularis*, because there must have been some ancestral pinworm population inhabiting some relatively primitive anthropoid host from which *E. vermicularis* was derived. Figure 2 suggests that the ancestral population from which *E. vermicularis* was derived occurred in the common ancestor of *Pongo* + *Gorilla* + *Pan*. If the hominoid lineage evolved concomitantly with

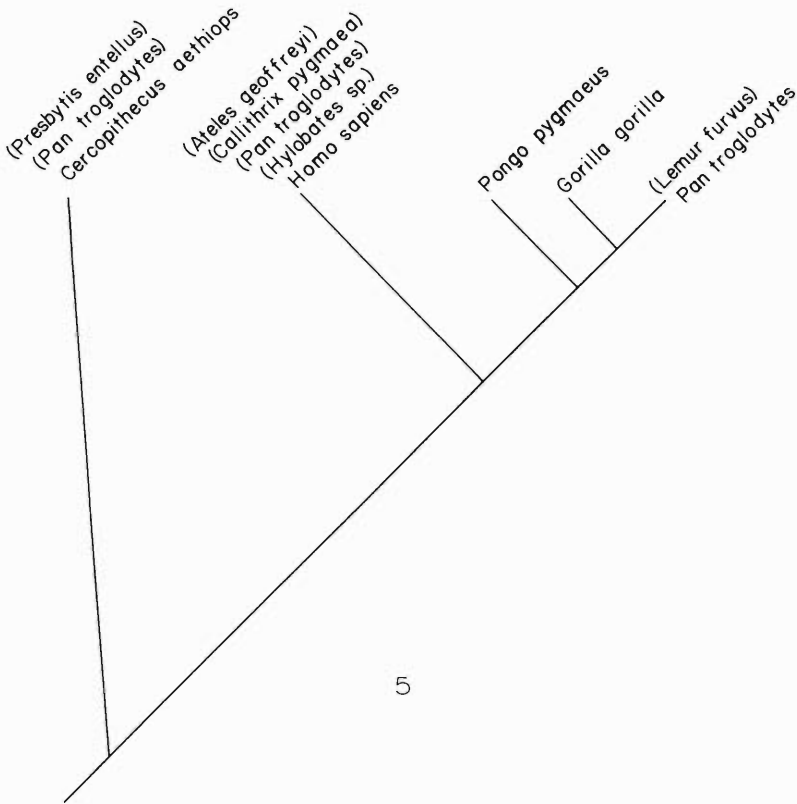


Figure 5. Natural hosts for *Enterobius bipapillatus*, *E. vermicularis*, *E. buckleyi*, *E. lerouxi*, and *E. anthropopitheci* (from left to right) with unusual hosts encountered in zoo settings in parentheses. Note that *Pan troglodytes* (chimpanzee) hosts three different species of *Enterobius* in zoos, two of which are not closely related to the species with which *Pan* has co-specified (*E. anthropopitheci*). Note also that the only species of pinworm hosts by *Hylobates* spp. is *E. vermicularis*.

*Pan*, or with *Pan* + *Gorilla*, as our current classifications suggest, the ancestral host and therefore the ancestral parasite from which *E. vermicularis* would purportedly be derived were not longer in existence when *Homo* came onto the scene. The occurrence of *E. vermicularis* in chimpanzees under zoo conditions cannot be used as evidence of close relationships between *Pan* and *Homo* because *Pan* has its own pinworm species and also hosts *E. bipapillata* Gedoelst, 1916 in zoo settings (Yamashita, 1963; see Fig. 5).

Clearly, invoking host-switching for the phylogenetic and host relationships of *E. vermicularis* is not a very parsimonious explanation. However, so long as *Enterobius* spp. retain the ability to infect unusual hosts under unusual conditions (Fig. 5), and so long as new pinworm species are still being described, the above scenario cannot be considered completely refuted.

#### Incorrect Host Phylogeny and the Evolution of *Enterobius vermicularis*

If one assumes that *Enterobius* spp. exhibit uniform co-speciation, i.e., that they possess evolutionary histories concordant with those of higher primates, the existing classification and phylogenetic hypothesis for higher primates must be

changed. That new classification would conform to the cladogram in Figure 3, wherein *Homo* would be considered the sister-group of *Pongo* + *Gorilla* + *Pan*. No other special, ad hoc, explanations of past evolutionary events of an unusual nature would be required. This is a more parsimonious explanation than host-switching.

### Conclusions

The most parsimonious explanation of the phylogeny of *Enterobius* is that *Enterobius* and the great apes, including *Homo*, have co-speciated and that our current placement of *Homo* in primate classifications is unjustified. This does not alter any notions about the "advancement" of humans, because such judgements are statements about degree of specialization along a lineage, or anagenetic conclusions, whereas classificatory or phylogenetic relationships are hierarchical, or cladogenetic, conclusions.

Secondarily, it could be argued that all *Enterobius* spp. except *E. vermicularis* have co-speciated with their hosts, that *Homo* lost its co-speciating pinworm lineage and later reacquired pinworms from a relatively primitive primate host which either no longer exists, no longer hosts *E. vermicularis* or its closest relative, or has not yet had its pinworm species described. In either event, "Cameron's Hypothesis" is corroborated, either totally or in all respects except one.

There are a number of ways in which these findings may be tested. First, reexamination of the bases for present classification of higher primates might produce evidence that *Homo* has indeed been misclassified. Second, discovery of new species of *Enterobius* and collection of new material representing known species could add evidence corroborating or refuting the findings of this study. Of particular interest would be finding pinworms in hylobatids, or gibbons. Hylobatids are thought to comprise the sister-group of all other great apes, and presently no pinworms are known from collections in hylobatids under natural conditions. If our hypothesis is correct, a species of *Enterobius* endemic to hylobatids would be most closely related to *E. vermicularis*, occurring one branch below on the cladogram in Figure 2. Only one species of *Enterobius* has ever been reported in zoo conditions for hylobatids, and that was *E. vermicularis* (Sandosham, 1950; Yamashita, 1963). As mentioned earlier, *E. vermicularis* also occurs in chimpanzees and has been reported in two species of New World monkeys in zoos (Fig. 5), but for all those hosts at least one other, endemic species of *Enterobius* or *Trypanoxyuris* also occurs. Thus, the finding of *only E. vermicularis* in hylobatids is suggestive of close relationships of hosts.

The final method of testing our hypothesis would involve performing cladistic analysis on all helminth groups occurring in primate hosts and examining predicted host phylogenies. If *Homo* is predicted consistently in the position shown in Figure 3, we must abandon the notion of random host-switching as an explanation. Recently, a technique has been formulated for analyzing more than one group of parasites simultaneously and assessing the predicted host phylogenetic relationships (Brooks, 1981). Thus, this latter test is feasible and would represent a test independent of any host characteristics gathered by primatologists.

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### Literature Cited

- Brooks, D. R.** 1979. Testing the context and extent of host-parasite coevolution. *Syst. Zool.* 28:299–307.
- Brooks, D. R.** 1981. Hennig's parasitological method: a proposed solution. *Syst. Zool.* 30:229–249.
- Cameron, T. W. M.** 1929. The species of *Enterobius* Leach, in primates. *J. Helminthol.* 7:161–182.
- Farris, J. S.** 1970. Methods for computing Wagner trees. *Syst. Zool.* 19:83–92.
- Hennig, W.** 1966. *Phylogenetic Systematics*. Univ. Illinois Press, Urbana. 263 pp.
- Inglis, W. G.** 1961. The oxyurid parasites (Nematoda) of primates. *Proc. Zool. Soc. London* 136:103–122.
- Quentin, J.-C., C. Betterton, and M. Krishnashamy.** 1980. Oxyures nouveaux ou peu connus, parasites de primates, de rongeurs et de dermoptères en Malaisie. Création du sous-genre *Colobenterobius* n. subgen. *Bull. Mus. Natl. Hist. Nat. Paris, 4e. ser., 1, 1979, section A, no. 4:1031–1050.*
- Sandosham, A. A.** 1950. On *Enterobius vermicularis* (Linnaeus, 1758) and some related species from primates and rodents. *J. Helminthol.* 24:171–204.
- Vuylsteke, C.** 1964. Mission de zoologie médicale au Maniema (Congo Leopoldville). (P. L. G. Benoit, 1959). 3. Vermes. Nematoda. *Ann. Mus. R. Afr. Cent., Ser. 8°, Zool.* 132:41–66.
- Wahid, S.** 1961. On two new species of the genus *Enterobius* Leach, 1853 from a colobus monkey. *J. Helminthol.* 35:345–352.
- Wiley, E. O.** 1981. *Phylogenetics, The Theory and Practice of Phylogenetic Systematics*. John Wiley and Sons, New York.
- Yamashita, J.** 1963. Ecological relationships between parasites and primates. *Primates* 4:1–96.
- Yen, W. C.** 1973. Helminths of birds and wild animals from Lin tsan prefecture, Yunnan province, China. II. Parasitic nematodes of mammals. *Acta Zool. Sinica* 19:354–364.

***Capillaria xenopodis* sp. n. (Nematoda: Trichuroidea) from the Epidermis of the South African Clawed Frog (*Xenopus laevis* Daudin)**

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**ABSTRACT:** *Capillaria xenopodis* sp. n. from the epidermis of wild caught South African clawed frogs (*Xenopus laevis*) is distinguished from all other amphibian capillariids by its location in the epidermis. The male has two lateral tail papillae located dorsally to the anus, one short spicule, and a long spicular sheath with rows of nonspinous cuticular projections; the female produces embryonated eggs in utero. Worm burrows are found in the epidermis of the frog.

The existence of a cutaneous capillariid in South African clawed frogs was first reported by Cosgrove and Jared (1974). These authors found one-third of *Xenopus laevis* they examined infected and suffering from extensive skin damage due to the presence of worms and ova in epidermal tunnels. The skin was roughened and thickened and the frogs were emaciated. Other signs of infection observed in the present study include excessive sloughing of small pieces of skin, emaciation with loss of muscle mass, atrophy of the ovaries and fat bodies, and, shortly before death, complete anorexia.

The nematodes described herein were recovered from *X. laevis* maintained in the laboratory of Dr. A. W. Blackler of Cornell University. These frogs had been received from South Africa at least 1½ years previously. The worms were found in epidermal tunnels or lying on the surface of the epidermis under a layer of mucus.

**Materials and Methods**

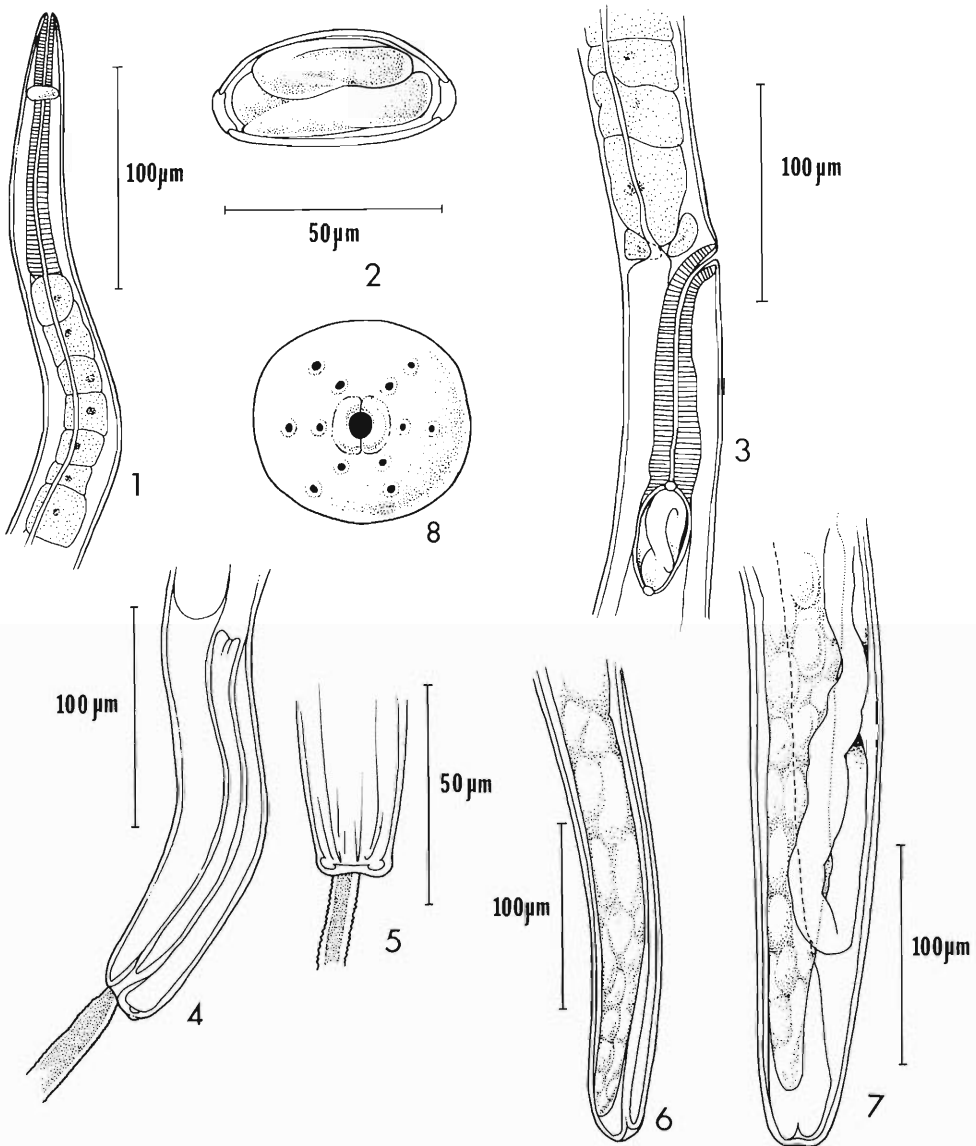
All nematodes were recovered from the epidermis during necropsy of *X. laevis*. Specimens were removed either from fresh skin or from skin that had been treated with pepsin-acid and placed in 4.5% NaCl solution.

Specimens were fixed in hot 70% ethyl alcohol, stored in 70% ethyl alcohol, and studied in alcohol mounts covered by coverslips ringed with petroleum jelly. Drawings were made with the aid of a Zeiss drawing tube (camera lucida). All measurements are in micrometers unless otherwise indicated.

***Capillaria xenopodis* sp. n.  
(Figs. 1-8)**

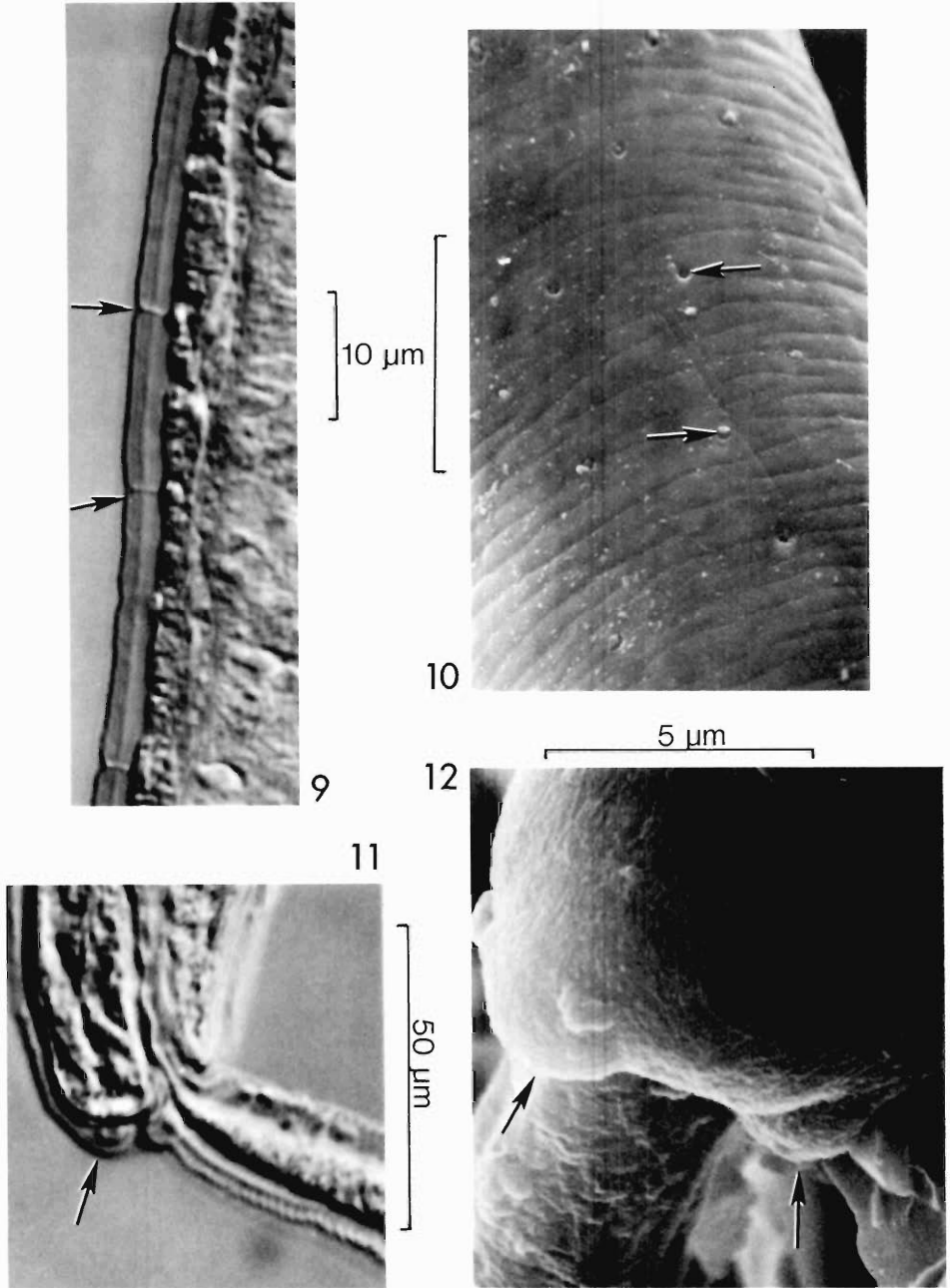
**DESCRIPTION:** Trichuridae Railliet 1915; Capillariinae Railliet 1915; *Capillaria* Zeder 1800. Small filiform worms. Diameter increased from the anterior end to about midbody. Body of male divided almost equally into anterior body containing muscular and stichosome esophagus, and posterior body containing intestine and reproductive system. In the female the posterior body is longer than the anterior. Cuticle has fine transverse striations. Bilateral bacillary bands extend from region just posterior to nerve ring to the tail. Mouth simple, surrounded by two liplike projections. Cephalic papillae arranged in two circles around oral opening (Fig. 8).





Figures 1–8. *Capillaria xenopodis* sp. n. from epidermis of *Xenopus laevis*. 1. Male, anterior extremity, lateral view. 2. Egg with first-stage larva. 3. Female, esophageal-intestinal junction, lateral view. 4. Male, caudal extremity, lateral view. 5. Male, caudal extremity, dorsal view. 6. Female, caudal extremity, lateral view. 7. Female, caudal extremity, ventral view. 8. Female, en face view.

MALE (based on 20 specimens): 1.4–2.2 mm (1.8 mm) long, width at head 8–12 (10), at base of muscular esophagus (beginning of stichosome) 20–31 (24), at base of stichosome 26–39 (31). Esophagus 0.73–0.96 mm (0.83 mm), muscular esophagus 0.11–0.15 mm (0.13 mm), nerve ring 25–68 (49) from anterior end to middle of ring, posterior body 0.66–1.2 mm (0.97 mm). Number of stichocytes 16–49 (31). Spicule 115–224 (183) long, with flared irregular proximal end 6–20 (12) wide and rounded distal end; sheath long, transversely striated with rows of cuticular



Figures 9–12. Lateral view of female, showing pores of bacillary band (arrows) in area of stichosome esophagus, Nomarsky differential interference contrast. 10. Surface view of female, showing pores of bacillary band (arrows), scanning electron microscope. 11. Lateral view of male, showing caudal extremity with lateral tail papilla (arrow) dorsal to anus, Nomarsky differential interference contrast. 12. Dorsal view of male, showing caudal extremity with lateral tail papillae (arrows) dorsal to anus, scanning electron microscope.

projections or folds extending the entire length. Testis anterior to spicule. Tail with two lateral papillae dorsal to anus (Fig. 12), anus subterminal (Fig. 11). Ratio of length of esophageal region to length of the posterior body 1:0.85–1:1.51 mm (1:1.17 mm). Each bacillary band contains double row of pores in region of stichosome and single row in posterior portion of body; pores widely spaced especially in midbody.

FEMALE (based on 25 specimens): 3.3–6.4 mm (4.4 mm) long, width at head 7–14 (11), at base of muscular esophagus (beginning of stichosome) 19–40 (31), at base of stichosome 45–77 (52), postvulvar region 38–50 (44), preanal region 42–50 (46). Esophagus 0.8–1.5 mm (1.2 mm), muscular esophagus 140–210 (170), nerve ring 54–66 (63) from anterior end to middle of ring, posterior body 2.2–4.9 mm (3.2 mm), Number of stichocytes 26–37 (32). Vulva immediately behind esophagus, muscular vagina 60–104 (84) long. Ovary just anterior to tail, oviduct joining ovary at anterior end, reflexing twice before joining anteriorly directed uterus. Egg 52–58 (55) by 23–29 (25), with polar plugs and containing larva at oviposition. Anus subterminal. Ratio of length of esophageal region to posterior body 1:1.79–1:3.79 (1:2.77). Each bacillary band contains two rows of pores in region of muscular esophagus, 3–4 rows in region of stichosome esophagus, and two rows in posterior part of body; pores occurring at irregular intervals (Figs. 9, 10).

HOLOTYPE MALE: USNM 75379.

PARATYPES: USNM 75380.

HOST: South African clawed frog (*Xenopus laevis*).

LOCATION: Epidermis.

LOCALITY: South Africa.

### Discussion

This nematode is placed in the genus *Capillaria* according to the key in Skrjabin et al. (1957) because, although the spicular sheath has cuticular projections, it lacks true spines. *Capillaria xenopodis* is not placed in either of two related genera (*Anatrichosoma* Smith and Chitwood, 1954 and *Paratrichosoma* Ashford and Miller, 1978) that include forms parasitic in epidermal tunnels because in both genera, the male worm lacks a spicule. The male worm is also larger than the female worm in *Anatrichosoma* (Ashford and Miller, 1978; Smith and Chitwood, 1954). In *C. xenopodis* the male worm has a spicule, and is much smaller than the female.

*Capillaria philippinensis* was the first recorded capillariid in which eggs develop in utero to the first larval stage (Chitwood et al., 1968). *Capillaria xenopodis* eggs also develop to the first larval stage in utero, but unlike *C. philippinensis*, these never hatch until laid. The larvated eggs have been observed in the epidermal tunnels made by the worms.

Data pertaining to *Capillaria* previously reported from amphibians are summarized in Table 1. Note that all of these worms are located in the intestine and liver except for *C. buccalis* which is found in the mucosa and submucosa of the pharynx, tongue and palate of *Bufo vulgaris japonicus*. Thus, *C. buccalis* somewhat resembles *C. xenopodis* with respect to habitat, but *C. xenopodis* is the first species of this group to be reported from the epidermis of an amphibian. *Capillaria buccalis* is also closest in number of stichocytes (male 36–42, female 23–38)

Table 1. *Capillaria* spp. Reported from Amphibia.

| Measurements in mm   | Total length               | Length of muscular esophagus | Length of stichosome esophagus | Number of stichocytes | Spicule<br>Spicular sheath | Ova               | Anus position              | Host and geographical location  | Location in host                                   |
|--|----------------------------|------------------------------|--------------------------------|-----------------------|----------------------------|-------------------|----------------------------|---|--|
| <i>Capillaria bombinatoris</i> (Linstow, 1892) Travassos, 1915 (4,5) | (Larva)<br>1.2             | 0.98                         |                                |                       |                            |                   | Terminal                   | <i>Bombina bombina</i><br>Europe  | Intestine  |
| <i>Capillaria brachyau-<br/>chenta</i> Walton, 1935 (5)              | ♂ 4.0-5.5<br>♀ 6.3-6.6     | .22-.33<br>.49-.52           | 1.6-2.3<br>2.0-2.3             | 40-45<br>48-52        | .20-.26<br>Spinose         |                   | Subterminal<br>Terminal    | <i>Notophthalmus viridescens</i><br>North America   | Intestine  |
| <i>Capillaria buccalis</i> Yamaguti, 1943 (4,6)                      | ♂ 6.8-8.8<br>♀ 8.1-12.0    | 2.7-3.9<br>2.9-4.1           |                                | 36-42<br>23-38        | .57-.61<br>Smooth          | .07 × .02-.04     | Terminal                   | Japan<br><i>Bufo vulgaris japonicus</i>   | Mucosa and submucosa of tongue, palate and pharynx |
| <i>Capillaria bufonis</i> Morishita, 1926 (3,5)                      | ♂ 8.5-9.0<br>♀ 10.0-12.0   | .40-.41<br>.39-.43           | 3.6-3.8<br>3.6-3.9             | 60-63<br>65-68        | .43-.65<br>Smooth          | .07 × .03         | Subterminal<br>Terminal    | <i>Bufo vulgaris japonicus</i> ,<br><i>Cynops pyrrhogaster</i><br>Japan   | Small intestine                                    |
| <i>Capillaria costaruzi</i> Travassos, 1932 (4)                      | ♂ 7.6<br>♀ 4.2-8.0         | .21<br>.22-.24               | 1.5<br>1.6-2.7                 | —                     | 1.2-1.3<br>Spinose         | .04-.06 × .02-.03 | Subterminal<br>Terminal    | <i>Rana</i> sp.<br>Brazil, Portugal   | Intestine  |
| <i>Capillaria fagei</i> Arvy, 1951 (4)                               | ♂ 15.0-20.0<br>♀ 40.0-60.0 | Shorter than female<br>.25   |                                | —<br>100              | .07<br>Smooth              | .06-.07 × .02-.03 | Subterminal<br>Subterminal | <i>Triturus helveticus</i> ,<br><i>Triturus vulgaris</i><br>Europe  | Liver  |
| <i>Capillaria filiformis</i> Linstow, 1885 (4,5)                     | ♂ 7.9<br>♀ 12.5            | .70-.73<br>.50-.53           | 4.8-4.9<br>5.1-5.2             | 55-58<br>75-78        | .30<br>Spinose             | .06 × .03         | Subterminal<br>Terminal    | <i>Triturus alpestris</i> ,<br><i>T. vulgaris</i> ,<br><i>Cynops pyrrhogaster</i><br><i>Onychodactylus japonicus</i> ,<br><i>Hynobius stejnegeri</i><br>Europe, Japan | Intestine  |
| <i>Capillaria hepatophila</i> Babos, 1954 (1)                        | ♂ 1.9-2.1<br>♀ 2.5-2.7     | —<br>—                       | —<br>—                         | —<br>—                | .14<br>Smooth              | .05-.06 × .02     | Terminal<br>Subterminal    | <i>Triturus cristatus</i><br>Hungary  | Liver  |

Table 1. Continued.

| Measurements in mm  | Total length           | Length of muscular esophagus   | Length of stichosome esophagus | Number of stichocytes    | Spicule/Spicular sheath | Ova       | Anus position              | Host and geographical location  | Location in host          |
|---|------------------------|--------------------------------|--------------------------------|--------------------------|-------------------------|-----------|----------------------------|---|---------------------------|
| <i>Capillaria inequalis</i> Walton, 1935 (4,5)                            | ♂ 6.8<br>♀ 11.4-13.2   | .31<br>.50-.60                 | 2.9<br>3.3-4.4                 | 52-54<br>70-75           | .24<br>Spinose          | .06 × .03 | Subterminal<br>Terminal    | <i>Notophthalmus viridescens</i> ,<br><i>Amblystoma opacum</i><br><i>Desmognathus</i> sp.,<br><i>Plethodon glutinosus</i><br>U.S.A. | Intestine                 |
| <i>Capillaria recondita</i> Freitas and Lent, 1942 (2,4)                  | ♂ 3.7-4.3<br>♀ 3.7-6.4 | —<br>—                         | —<br>—                         | —<br>—                   | .14-.15<br>Spinose      | —         | Subterminal<br>Subterminal | <i>Crossodactylus gandichaudii</i><br>Brazil, South America   | Intestine                 |
| <i>Capillaria tenua</i> Mueller, 1932 (4,5)                               | ♂ 7.5<br>♀ 10.0        | .40<br>.40                     | 3.4<br>3.1                     | 90<br>110                | .32<br>Spinose          | .05 × .02 | Subterminal<br>Terminal    | <i>Notophthalmus viridescens</i> ,<br><i>Rana clamitans</i><br><i>Rana palustris</i><br>U.S.A.                                      | Intestine                 |
| <i>Capillaria tritonis-cristati</i> (Diesing, 1861) Travassos, 1915 (4,5) | ♂ U<br>♀               | N<br>NOT DESCRIBED             | K                              | N                        | O                       | W         | N                          | <i>Triton cristatus</i><br>Europe   | Liver, muscles of abdomen |
| <i>Capillaria tritonis-punctati</i> (Diesing, 1851) Travassos, 1915 (4,5) | ♂ U<br>♀ 16.0-17.0     | N<br>—                         | K                              | N                        | O                       | .07 × .03 | N                          | <i>Lissonotriton punctatus</i><br>Europe  | Intestine                 |
| <i>Capillaria xenopodis</i> sp. n.  | ♂ 1.4-2.2<br>♀ 3.3-6.4 | .11-.15 (.13)<br>.14-.21 (.17) | .62-.81 (.70)<br>.70-1.3 (.98) | 16-49 (31)<br>26-37 (32) | .12-.22 (.18)<br>Smooth | Smooth    | Subterminal<br>Subterminal | <i>Xenopus laevis</i><br>South Africa   | Epidermis                 |

(1) Babos, 1954; (2) de Freitas and Lent, 1942; (3) Morishita, 1926; (4) Skjabin et al., 1957; (5) Walton, 1935; (6) Yamaguti, 1943.

to that of *C. xenopodis* (male 16–49, female 26–37); the other amphibian capillariids have more stichocytes. The length of the male *C. hepatophila* (1.9–2.1) corresponds most closely to that of the male *C. xenopodis* (1.44–2.2), while the female of *C. recondita* (3.73–6.39) corresponds most closely with the female of *C. xenopodis* (3.31–6.36) in total length. The spicules of *C. brachyauchenia*, *C. hepatophila*, and *C. recondita* are of similar length to that of *C. xenopodis*; all other species have longer spicules.

*Capillaria xenopodis* is the first amphibian capillariid to be described from Africa, the others being from Europe, Japan, and North and South America. Thurston (1970), in an addendum reported that Dr. Puylaert had recovered a *Capillaria* sp. from the intestine of *Xenopus* sp. from Ogbomosho, Nigeria but no further information appears to be available.

The combination of location in the epidermis, small size, few stichocytes, and short spicule readily distinguishes *C. xenopodis* from other described members of the genus.

#### Acknowledgments

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#### Literature Cited

- Ashford, R. W., and R. Muller. 1978. *Paratrichosoma crocodilus* n. gen. n. sp. (Nematoda: Tricho-  
somoidea) from the skin of the New Guinea crocodile. *J. Helminthol.* 52:215–220.
- Babos, A. 1954. Über eine neue *Capillaria*-art aus der Leber von *Triturus cristatus*. *Acta Vet. Buda.*  
4:141–146.
- Chitwood, M. B., C. Valesquez, and N. G. Salazar. 1968. *Capillaria philippinensis* sp. n. (Nematoda:  
Trichinellida), from the intestine of man in the Philippines. *J. Parasitol.* 54:368–371.
- Cosgrove, G. E., and D. W. Jared. 1974. Diseases and parasites of *Xenopus*, the clawed toad. Pages  
225–242 in R. L. Amborski, M. A. Hood, and R. R. Miller, eds. *Proceedings of Gulf Coast  
Regional Symposium on Diseases of Aquatic Animals*. Center for Wetlands Research, Louisiana  
State University, Baton Rouge.
- de Freitas, J. F. T., and H. Lent. 1942. Primeira espécie de "Capillaria" parasita de batráquio Sul-  
Americano (Nematoda, Trichuroidea). *Rev. Brasil. Biol.* 2:325–330.
- Morishita, K. 1926. Studies on some nematode parasites of frogs and toads in Japan, with notes on  
their distribution and frequency. *J. Fac. Sci. Univ. Tokyo, Zool. sect. IV.* 1:1–37.
- Skrjabin, K. I., N. P. Shihobalova, and I. N. Orlov. 1957. Trichocephalidae and Capillariidae of  
animals and man and the diseases caused by them. In K. I. Skrjabin, ed. *Essentials of Nema-  
tology*. Vol. VI.
- Smith, W. N., and M. B. Chitwood. 1954. *Anatrichosoma cynamolgi*, a new trichurid nematode from  
monkeys. *J. Parasitol.* 40(suppl.):12.
- Thurston, J. P. 1970. Studies on some protozoa and helminth parasites of *Xenopus*, the African  
clawed toad. *Rev. Zool. Bot. Afr.* 82:349–369.
- Walton, A. C. 1935. The nematoda as parasites of amphibia. II. *J. Parasitol.* 21:27–50.
- Yamaguti, S. 1943. *Capillaria buccalis* n. sp. (Nematoda) from *Bufo vulgaris japonicus*. *Annot. Zool.*  
*Jpn.* 22:5–7.

## Metazoan Parasites of Fish from the Red Cedar River, Ingham County, Michigan

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**ABSTRACT:** From October 1979 through May 1981, 328 fish representing five orders, eight families and 32 species were collected from the Red Cedar River, Ingham Co., Michigan and examined for intestinal helminths. Of these fish, 191 (58%) were infected. Twenty-five species in 17 genera were identified. These included seven species of digenetic trematodes, seven cestodes, five nematodes, and six acanthocephalans. Eight species are reported from new hosts and 17 for the first time from Michigan. The trematode, *Plagioporus sinitsini*, was found in the gall bladder of fish in the family Cyprinidae. The anchor worms, *Lernaea cruciata* and *L. cyprinacea* (Copepoda) infected fish in the family Centrarchidae. There was no evidence of host mortality caused by the parasites.

Several studies on new species, life cycles, host-parasite relationships, and the histopathology of fishes caused by parasites have been reported from inland waters of Michigan (Van Cleave, 1919; Weller, 1938; Dobrovolny, 1939a, b; Larsh, 1941; Fischthal, 1942a, b, 1943; Strandine, 1943; Garoian, 1960; Esch and Huffines, 1973; Allison et al., 1977; and Fallon and Wallace, 1977). The present investigation was undertaken in an effort to gain additional information on the parasite fauna of fish in south-central Michigan.

### Materials and Methods

Fish were collected from the Red Cedar River, Ingham Co., Michigan from October 1979 through May 1981. Scientific and common names utilized for fish are those recognized by the American Fisheries Society (1970). The Red Cedar River is a slow moving, warm-water stream located in the south-central portion of the lower peninsula of Michigan; it flows through farm and suburban land. Fish were collected by angling, electrofishing and seining (1/4-in mesh minnow seine) in that part of the Red Cedar River from Meridian Park (Meridian Rd. and Grand River Ave.) downstream approximately 11 kilometers to the Administration Building on the Michigan State University campus. This section of river bottom varies from silt-detritus and mud substrate to sand gravel and boulder.

Fish were brought to the laboratory alive and examined within 36 hr of collection. Total length in centimeters and sex were determined at necropsy. The entire digestive tract, and in some instances the gall bladder, were placed in saline solution and examined. Normal techniques for identifying the parasites were employed. Ecotypes of most specimens have been deposited in the United States National Museum Helminthological Collection. Accession numbers are listed in Table 1. Remaining specimens have been retained in the collection of the author.

Fish in the families Catostomidae and Cyprinidae have, to a certain extent, undifferentiated digestive tracts, and because of this, parasites found were recorded to be in the anterior, middle, or posterior intestine. Centrarchidae, Esocidae, Gasterosteidae, Ictaluridae, Percidae, and Umbridae, have digestive tracts that consist of some combination of stomach, pyloric ceca, intestine, and rectum; parasites found in these organs are so designated. Prevalence is the percent of

**Table 1. Endohelminths, anatomic location, and parasite prevalence in piscine hosts from the Red Cedar River.**

| Parasite                            | USNM no. | Host and location   | Prevalence  |
|-------------------------------------|----------|---|---|
| <b>Digenea</b>                      |          |   |   |
| * <i>Allocreadium lobatum</i>       | 76691    | † <i>Lepomis cyanellus</i> ‡<br><i>Notropis cornutus</i><br><i>Semotilus atromaculatus</i>  | 10 (10:1)§<br>28 (47:13)<br>26 (19:5)   |
| * <i>Crepidostomum cooperi</i>      | 76692    | <i>Micropterus dolomieu</i> (pc, ai)  | 40 (10:4)   |
| * <i>Crepidostomum cornutum</i>     | 76693    | <i>Ambloplites rupestris</i> (pc, ai)<br><i>Micropterus dolomieu</i> (pc, ai)   | 7 (15:1)<br>30 (10:3)   |
| * <i>Crepidostomum ictaluri</i>     | 76694    | <i>Ictalurus natalis</i> (ai)   | 60 (5:3)  |
| * <i>Crepidostomum isostomum</i>    | 76695    | <i>Etheostoma nigrum</i> (pc)   | 4 (24:1)  |
| * <i>Cryptogonimus chyli</i>        | 76696    | <i>Ambloplites rupestris</i> (pc, ai)   | 67 (15:10)  |
| <i>Microphallus opacus</i>          | 76697    | <i>Ictalurus nebulosus</i> (ai)<br><i>Micropterus dolomieu</i><br>† <i>Notropis rubellus</i>  | 50 (2:1)<br>10 (10:1)<br>10 (10:1)  |
| <i>Podocotyle</i> sp.               |          | <i>Etheostoma nigrum</i> (pc, ai)<br><i>Percina maculata</i> (pc, ai)<br>‡ <i>Pimephales notatus</i> (ai)   | 17 (24:4)<br>57 (7:4)<br>6 (31:2)   |
| <b>Cestoda</b>                      |          |   |   |
| <i>Bothriocephalus formosus</i>     | 76699    | <i>Etheostoma nigrum</i> (pc)   | 42 (24:10)  |
| * <i>Corallobothrium fimbriatum</i> | 76700    | <i>Ictalurus natalis</i> (ai)<br><i>Ictalurus nebulosus</i> (ai)  | 20 (5:1)<br>50 (2:1)  |
| * <i>Corallobothrium giganteum</i>  | 76701    | <i>Ictalurus melas</i> (ai)   | 50 (2:1)  |
| <i>Glaridacris catostomi</i>        | 76702    | <i>Catostomus commersoni</i> (ai)   | 40 (10:4)   |
| * <i>Isoglaridacris folius</i>      | 76703    | † <i>Hypentelium nigricans</i> (ai)   | 22 (9:2)  |
| <i>Isoglaridacris</i> sp.           |          | <i>Catostomus commersoni</i> (ai)   | 20 (10:2)   |
| * <i>Proteocephalus buplanensis</i> | 76704    | <i>Semotilus atromaculatus</i>  | 5 (19:1)  |
| <i>Proteocephalus pinguis</i>       | 76705    | † <i>Esox a. vermiculatus</i><br><i>Lepomis cyanellus</i>   | 50 (2:1)<br>10 (10:1)   |
| <i>Proteocephalus</i> spp.          |          | ‡ <i>Ambloplites rupestris</i> (pi, r)<br>‡ <i>Campostoma anomalum</i><br>‡ <i>Ictalurus natalis</i> (pi)<br>‡ <i>Lepomis cyanellus</i><br>‡ <i>Micropterus dolomieu</i> (pi, r)<br>‡ <i>Micropterus salmoides</i><br>‡ <i>Nocomis biguttatus</i> (pi, r)<br>‡ <i>Notropis cornutus</i> (pi, r)<br>‡ <i>Notropis rubellus</i> (pi, r)<br>‡ <i>Notropis stramineus</i> (r)<br>‡ <i>Perca flavescens</i> (r)<br>‡ <i>Pimephales notatus</i> (r)<br>‡ <i>Pomoxis nigromaculatus</i> (pi) | 40 (15:6)<br>50 (2:1)<br>20 (5:1)<br>10 (10:1)<br>50 (10:5)<br>50 (4:2)<br>23 (31:7)<br>17 (47:8)<br>18 (11:2)<br>20 (15:3)<br>75 (4:3)<br>3 (31:1)<br>25 (4:1) |
| <b>Nematoda</b>                     |          |   |   |
| * <i>Camallanus oxycephalus</i>     | 76706    | † ‡ <i>Nocomis biguttatus</i><br><i>Pomoxis nigromaculatus</i> (r)<br>‡ <i>Semotilus atromaculatus</i>  | 3 (31:1)<br>75 (4:3)<br>5 (19:1)  |
| <i>Contraecaecum brachyurum</i>     | 76707    | <i>Ambloplites rupestris</i> (s, ai)  | 20 (15:3)   |
| * <i>Rhabdochona cascadilla</i>     | 76708    | <i>Nocomis biguttatus</i><br><i>Notropis cornutus</i>   | 3 (31:1)<br>64 (47:30)  |



Table 1. Continued.

| Parasite                             | USNM no. | Host and location                                | Prevalence |
|--------------------------------------|----------|--|------------|
| <i>Rhabdochona</i> sp.               |          | <sup>1</sup> <i>Campostoma anomalum</i>          | 50 (2:1)   |
|                                      |          | <sup>1</sup> <i>Moxostoma anisurum</i>           | 25 (4:1)   |
|                                      |          | <sup>1</sup> <i>Notropis rubellus</i>            | 18 (11:2)  |
|                                      |          | <sup>1</sup> <i>Notropis stramineus</i>          | 7 (15:1)   |
|                                      |          | <sup>1</sup> <i>Pimephales notatus</i>           | 3 (31:1)   |
|                                      |          | <sup>1</sup> <i>Rhinichthys atratulus</i>        | 14 (7:1)   |
| * <i>Spinitectus carolini</i>        | 76709    | <i>Ambloplites rupestris</i>                     | 33 (15:5)  |
|                                      |          | <i>Micropterus dolomieu</i>                      | 10 (10:1)  |
| * <i>Spinitectus micracanthus</i>    | 76710    | † <i>Lepomis cyanellus</i>                       | 18 (11:2)  |
|                                      |          | † <i>Lepomis gulosus</i>                         | 100 (1:1)  |
|                                      |          | <i>Lepomis macrochirus</i>                       | 50 (2:1)   |
|                                      |          | † <i>Micropterus dolomieu</i>                    | 80 (10:8)  |
|                                      |          | † <i>Micropterus salmoides</i>                   | 50 (4:2)   |
| Acanthocephala                       |          |  |            |
| <i>Leptorhynchoides thecatus</i>     | 76711    | <i>Ambloplites rupestris</i> (pc)                | 20 (15:3)  |
|                                      |          | <sup>1</sup> <i>Etheostoma nigrum</i> (pc)       | 8 (24:2)   |
|                                      |          | <i>Lepomis cyanellus</i> (pc)                    | 20 (10:2)  |
|                                      |          | <sup>1</sup> <i>Lepomis gulosus</i> (pc)         | 100 (1:1)  |
|                                      |          | <i>Micropterus dolomieu</i> (pc)                 | 10 (10:1)  |
| * <i>Neoechinorhynchus cristatus</i> | 76712    | <i>Catostomus commersoni</i><br>(mi, pi)         | 50 (10:5)  |
| <i>Neoechinorhynchus cylindricus</i> | 76713    | <i>Micropterus salmoides</i>                     | 50 (4:2)   |
|                                      |          | † <sup>1</sup> <i>Pimephales notatus</i>         | 3 (31:1)   |
| * <i>Neoechinorhynchus saginatus</i> | 76714    | <i>Semotilus atromaculatus</i> (mi)              | 3 (31:1)   |
| * <i>Paulisentis missouriensis</i>   | 76715    | <i>Semotilus atromaculatus</i> (mi)              | 37 (19:7)  |
| <i>Pomphorhynchus bulbocollis</i>    | 76716    | <i>Ambloplites rupestris</i>                     | 33 (15:5)  |
|                                      |          | <i>Catostomus commersoni</i><br>(mi, pi)         | 50 (10:5)  |
|                                      |          | <sup>1</sup> <i>Etheostoma nigrum</i> (pi)       | 4 (24:1)   |
|                                      |          | <i>Hypentelium nigricans</i> (pi)                | 11 (9:1)   |
|                                      |          | <sup>1</sup> <i>Ictalurus melas</i> (mi, pi)     | 50 (2:1)   |
|                                      |          | <sup>1</sup> <i>Ictalurus natalis</i> (pi)       | 20 (5:1)   |
|                                      |          | <sup>1</sup> <i>Lepomis gulosus</i>              | 100 (1:1)  |
|                                      |          | <sup>1</sup> <i>Micropterus dolomieu</i>         | 40 (10:4)  |
|                                      |          | <sup>1</sup> <i>Moxostoma anisurum</i> (pi)      | 25 (4:1)   |
|                                      |          | <i>Nocomis biguttatus</i> (mi, pi)               | 10 (31:3)  |
|                                      |          | <sup>1</sup> <i>Notropis cornutus</i> (mi, pi)   | 15 (47:7)  |
|                                      |          | <sup>1</sup> <i>Notropis rubellus</i> (pi)       | 9 (11:1)   |
|                                      |          | <sup>1</sup> <i>Notropis stramineus</i> (pi)     | 7 (15:1)   |
|                                      |          | <sup>1</sup> <i>Percina maculata</i> (pi)        | 14 (7:1)   |
|                                      |          | † <sup>1</sup> <i>Pimephales notatus</i> (pi)    | 6 (31:2)   |
|                                      |          | <sup>1</sup> <i>Semotilus atromaculatus</i> (mi) | 16 (19:3)  |
|                                      |          | † <sup>1</sup> <i>Umbra limi</i> (pi)            | 33 (3:1)   |

\* New state record.

† New host record.

‡ No postscript indicates intestine as location. (ai) anterior intestine, (mi) middle intestine, (pi) posterior intestine, (s) stomach, (pc) pyloric caeca, (r) rectum.

§ First number denotes number of fish examined; second denotes number infected.

¶ Immature parasites in fish host. No prescript in host and location column indicates adult parasites in fish host.

infected hosts in a given sample. Density is the number of worms per infected host.

### Results and Discussion

Three hundred and twenty-eight fish representing five orders, eight families, and 32 species were collected from the Red Cedar River and examined for intestinal helminths. Of these fish, 191 (58%) harbored intestinal helminths. Five species of parasites was the most recovered from a single fish, a smallmouth bass (*Micropterus dolomieu*). Twenty-five species in 17 genera were identified. Four parasitic forms were identified only to genus. These parasites are systematically arranged showing hosts, location within hosts, and prevalence in Table 1. Eight parasite species are reported from new hosts and 17 for the first time from Michigan.

Seven species of digenetic trematodes were found in the present study, six representing new species records in Michigan. *Allocreadium lobatum*, *Microphallus opacus*, and *Podocotyle* sp. each occurred in three different fish species. *M. opacus* had the highest density of the trematodes with over 60 worms occurring in each of the three infected fish. Although *Crepidostomum* spp. and *Cryptogonimus chyli* were almost all limited in their occurrence to one host species, they have been reported from several fish species (Hoffman, 1967). The prevalence (66%) of *C. chyli* in rock bass (*Ambloplites rupestris*) was the highest of the trematode species encountered.

The present report of the cestode species (*Corallobothrium fimbriatum*, *C. giganteum*, *Isoglaridacris folius*, and *Proteocephalus buplanensis*) represents new species records in Michigan. The northern hog sucker (*Hypentelium nigricans*) and grass pickerel (*Esox americanus vermiculatus*) are new hosts for *I. folius* and *Proteocephalus pinguis*, respectively. Over 100 scoleces of *P. pinguis* were found in the intestine of the infected grass pickerel. *Bothriocephalus formosus* infected 42% of the johnny darters (*Etheostoma nigrum*) and was always found attached in the pyloric ceca. Fish of 13 species were infected with small, immature *Proteocephalus* spp. *Proteocephalus* sp. plerocercoids occurred in the livers of the black crappie (*Pomoxis nigromaculatus*), largemouth bass (*Micropterus salmoides*), and smallmouth bass.

Four of the five nematode species (*Camallanus oxycephalus*, *Rhabdochona cascadilla*, *Spinitectus carolini*, and *S. micracanthus*) found are new species records for Michigan. *C. oxycephalus* and *S. micracanthus* are reported from new hosts. *C. oxycephalus* occurred in three species of fish, but gravid females were found only in the black crappie. *S. micracanthus* infected the most fish species, and had the highest prevalence, 80% in smallmouth bass, of all nematodes found. *R. cascadilla* infected 64% of the common shiner (*Notropis cornutus*) and had a maximum density of 17 worms in that species.

The recovery of three acanthocephalan species (*Neoechinorhynchus cristatus*, *N. saginatus*, and *Paulisentis missouriensis*) represent new state records, and considerably extends their known distribution. *Pomphorhynchus bulbocolli* was the most widespread endohelminth found, occurring in 17 fish species; however, gravid females were found in only four species. Gravid females of the three

species of *Neoechinorhynchus* and *P. missouriensis* were host specific. Densities of *N. cristatus* and *P. missouriensis* ranged from one to 39 worms in white suckers (*Catostomus commersoni*), and one to 12 worms in creek chubs (*Semotilus atromaculatus*), respectively.

The total prevalence of infection by intestinal helminths in fish, organized by fish family (number fish examined—number infected (%)) are: Catostomidae, 27—15 (56%); Centrarchidae, 50—38 (78%); Cyprinidae, 164—89 (54%); Esocidae, 2—1 (50%); Gasterosteidae, 15—0 (0%); Ictaluridae, 9—9 (100%); Percidae, 49—38 (78%); Umbridae, 12—1 (8%). Fish species examined but not infected with intestinal helminths (number examined in parentheses) were: Catostomidae—*Moxostoma erythrurum* (2), Centrarchidae—*Lepomis gibbosus* (3), Cyprinidae—*Cyprinus carpio* (2), Gasterosteidae—*Culaea inconstans* (15), Percidae—*Etheostoma caeruleum* (14).

The trematode *Plagioporus sinitsini* was first reported from Michigan as a parasite of the gall bladder of several cyprinids and one catostomid from the Huron River, by Dobrovoly (1939a). In the present study, *P. sinitsini* was found in the gall bladder of four species of fish in the family Cyprinidae; it infected three of 14 hornyhead chubs (*Nocomis biguttatus*), seven of 12 common shiners, one of 15 sand shiners (*Notropis stramineus*), and one of 19 creek chubs (*Semotilus atromaculatus*). The maximum density of *P. sinitsini* was 24 worms in a common shiner. Specimens of *P. sinitsini* have been deposited at the U.S. National Museum (USNM) (USNM Helm. Coll. 76698). Species whose gall bladders were negative for parasites (number examined in parentheses) were: Catostomidae—*Catostomus commersoni* (5), *Hypentelium nigricans* (3); Centrarchidae—*Ambloplites rupestris* (8), *Lepomis cyanellus* (8), *L. gibbosus* (1), *Micropterus dolomieu* (5); Cyprinidae—*Campostoma anomalum* (2), *Cyprinus carpio* (1); *Pimephales notatus* (11), *Rhinichthys atratulus* (4); Gasterosteidae—*Culaea inconstans* (6); Percidae—*Etheostoma caeruleum* (8), *E. nigrum* (11); Umbridae—*Umbra limi* (8). One trematode identified as *Phyllodistomum* sp. was found in the urinary bladder of one brown bullhead (*Ictalurus nebulosus*).

The gills, fins, and external surfaces of fish in the family Centrarchidae collected throughout the sampling period were examined for *Lernaea* sp. (Lernaeidae: Copepoda). The anchor worm, *L. cruciata*, occurred on two of 15 rock bass, one of 10 green sunfish (*Lepomis cyanellus*), and one of two bluegills (*L. macrochirus*). This species was not found on three *Lepomis gibbosus*, one *L. gulosus*, 10 *Micropterus dolomieu*, four *M. salmoides*, and four *Pomoxis nigromaculatus*. *L. cyprinacea* infected two of the 10 green sunfishes; all other centrarchids were negative. Specimens of *L. cruciata* and *L. cyprinacea* have been deposited at the U.S. National Museum (USNM Helm. Coll. 76717, USNM Helm. Coll. 76718, respectively).

Although several endohelminth species were found in fish in the present study, there was no evidence of host mortality caused by the parasites. Generally, the low prevalence and density of the parasites found did not result in noticeable effects on fish. However, in heavy infections (over 400 worms) of the acanthocephalan *Pomphorhynchus bulbocollis* in rock bass and white suckers, the infected intestinal areas had large amounts of fibrous connective tissue and several encapsulated presomas, presumably those of this species.

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### Literature Cited

- Allison, L. N., J. G. Hnath, and W. G. Yoder. 1977. Manual of common diseases, parasites, and anomalies of Michigan fishes. Mich. Dep. Nat. Res., Fish. Manage. Rep. 8:1-132.
- American Fisheries Society. 1970. A list of common and scientific names of fishes from the United States and Canada. Third edition. Spec. Publ. 6:150 pp.
- Dobrovoly, C. G. 1939a. Life history of *Plagioporus sinitsini* Mueller and embryology of new co-cylocercous cercariae (Trematoda). Trans. Am. Microsc. Soc. 58:121-155.
- Dobrovoly, C. G. 1939b. The life history of *Plagioporus leponis*, a new trematode from fishes. J. Parasitol. 25:461-470.
- Esch, G. W., and W. J. Huffines. 1973. Histopathology associated with endoparasitic helminths in bass. J. Parasitol. 59:306-313.
- Fallon, M. E., and D. C. Wallace. 1977. The occurrence of *Phyllodistomum undulans* in the urinary bladder of the mottled sculpin, *Cottus bairdi*. Trans. Am. Fish. Soc. 106:189-191.
- Fischthal, J. H. 1942a. Three new species of *Phyllodistomum* (Trematoda: Gorgoderidae) from Michigan fishes. J. Parasitol. 28:269-275.
- Fischthal, J. H. 1942b. *Triganodistomum hypentelium* n. sp. (Trematoda: Lissorchiidae) from the hog sucker, *Hypentelium nigricans* (Le Sueur). J. Parasitol. 28:389-393.
- Fischthal, J. H. 1943. A description of *Phyllodistomum etheostomae* Fischthal, 1942 (Trematoda: Gorgoderidae), from percid fishes. J. Parasitol. 29:7-9.
- Garioian, G. S. 1960. *Schistocephalus thomasi*, n. sp. (Cestoda: Diphylobothriidae) from fish-eating birds. Proc. Helminthol. Soc. Wash. 27:199-202.
- Hoffman, G. L. 1967. Parasites of North American freshwater fishes. Univ. Calif. Press, Berkeley. 486 pp.
- Larsh, J. E., Jr. 1941. *Corallobothrium parvum* n. sp., a cestode from the common bullhead, *Ameiurus nebulosus* Le Sueur. J. Parasitol. 27:221-227.
- Strandine, E. J. 1943. Variations in *Microphallus*, a genus of trematodes, from fishes of Lake Lelanau, Michigan. Trans. Am. Microsc. Soc. 62:293-300.
- Van Cleave, H. J. 1919. Acanthocephala from fishes of Douglas Lake, Michigan. Occas. Pap. Mus. Zool. Univ. Mich. 72:1-12.
- Weller, T. H. 1938. Description of *Rhabdochona ovifilamenta* n. sp. (Nematoda: Thelaziidae) with a note on the life history. J. Parasitol. 24:403-408.

## A Survey of Helminth Parasites in the Salamanders and Certain Anurans from Wisconsin

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**ABSTRACT:** During 1979 and 1981, 117 salamanders and 18 anurans representing 14 host species were collected from seven localities in Wisconsin and examined for helminth parasites. Forty-two percent of salamanders and 89% of anurans were infected with one or more species of parasite. Four nematode species, seven digenean species, one species of cestode, and one monogenean were recovered. *Cosmocercoides dukae* was the most commonly occurring parasite, found in 11 host species. This is the first report of salamander parasites in Wisconsin.

Although there is an extensive literature on parasites of amphibians of the United States, most reports have dealt primarily with anurans. Much of this work has been reviewed by Yamaguti (1958), Walton (1964), and Ulmer (1970). However, excluding new species descriptions, there have been few investigations of the helminth parasites of salamanders (Harwood, 1932; Rankin, 1937, 1945; Fischthal, 1955). In the midwest, Dyer and Brandon (1973) investigated helminths of cave-dwelling salamanders of Illinois, Price and St. John (1980) and Price and Buttner (in press) studied the parasites of *Ambystoma texanum* and *Notophthalmus v. louisianensis* in Illinois, and Ulmer (1970) and Ulmer and James (1976) included *A. tigrinum* in their studies of the helminth fauna of Iowa amphibians. Brooks (1976) also collected *A. tigrinum* in his study of amphibian platyhelminths in Nebraska. Published accounts of the helminth fauna of Wisconsin amphibians are sparse. Williams and Taft (1980) studied the parasites of northwestern Wisconsin anurans. The present report is apparently the first survey of parasites from Wisconsin salamanders. At present there are eight salamander species endemic to Wisconsin; at least two specimens representing each species were collected during this investigation.

### Materials and Methods

Amphibians were collected during March–October 1979 and May 1980, from Fond du Lac, Oneida, Portage, Rock, Sheboygan, and Waukesha counties, Wisconsin. Although most specimens were taken in spring, scattered collections continued during spring and summer. Animals were anesthetized in a solution of chlorotone or pithed, and examined for helminth parasites. Trematodes and cestodes were fixed in AFA, stained in borax carmine, and mounted in Canada balsam. Nematodes were killed in 70% ethanol, cleared in glycerol, and examined as temporary mounts. Further information concerning collection localities may be obtained from the authors. Voucher specimens of all parasites recovered have been deposited in the UNSM, Manter Helm. Coll. 20278–20281, 21343–21352.

### Results and Discussion

One hundred-seventeen salamanders, representing eight species, were collected during this study. Forty-nine, 42%, were infected with one or more helminth parasite. Sixteen of 18 anurans (89%) were also found to be infected (Table 1).

Table 1. Helminth parasites collected from Wisconsin amphibians.

| Parasite   | No. infected hosts                    |                                       |                                      |                                     |                                     |  |   |                                      |                                   |                                 |                                  | Total<br>(135,48) |                                  |                                |
|--|---------------------------------------|---------------------------------------|--------------------------------------|-------------------------------------|-------------------------------------|--|---|--------------------------------------|-----------------------------------|---------------------------------|----------------------------------|-------------------|----------------------------------|--------------------------------|
|  | <i>Ambystoma laterale</i><br>(26,19)* | <i>Ambystoma maculatum</i><br>(20,35) | <i>Ambystoma tigrinum</i><br>(29,41) | <i>Ambystoma tobiacum</i><br>(2,50) | <i>Necturus maculosus</i><br>(6,67) | <i>Hemithysalis diactylum</i><br>(12,67) | <i>Natophthalmus v. louisi-<br/>anensis</i><br>(8,38) | <i>Plethodon cinereus</i><br>(14,64) | <i>Bufo americanus</i><br>(10,90) | <i>Hyla crucifer</i><br>(2,100) | <i>Rana clamitans</i><br>(1,100) |                   | <i>Rana palustris</i><br>(2,100) | <i>Rana pipiens</i><br>(2,100) |
| <b>MONOGENEA</b>   |                                       |                                       |                                      |                                     |                                     |  |   |                                      |                                   |                                 |                                  |                   |                                  |                                |
| <i>Sphyranura osteri</i><br>Wright, 1879                           | —                                     | —                                     | —                                    | —                                   | 3 <sub>μ</sub> †                    | —  | —   | —                                    | —                                 | —                               | —                                | —                 | —                                | —                              |
| <b>DIGENEA</b>   |                                       |                                       |                                      |                                     |                                     |  |   |                                      |                                   |                                 |                                  |                   |                                  |                                |
| <i>Brachycoelium salamandrae</i><br>Dujardin, 1845                 | —                                     | 6 <sub>cr</sub>                       | —                                    | —                                   | —                                   | —  | —   | 8 <sub>dr</sub>                      | —                                 | —                               | 1 <sub>e</sub>                   | —                 | —                                | —                              |
| <i>Cephalogonimus salaman-<br/>drinus</i> Dronen and<br>Lang, 1974 | —                                     | —                                     | 1 <sub>μ</sub>                       | —                                   | —                                   | —  | —   | —                                    | —                                 | 1 <sub>e</sub>                  | —                                | —                 | —                                | —                              |
| <i>Glypthelminis pennsylvani-<br/>ensis</i> Cheng, 1961            | —                                     | —                                     | —                                    | —                                   | —                                   | —  | —   | —                                    | —                                 | —                               | —                                | —                 | —                                | —                              |
| <i>Gorgoderina bilobata</i><br>Rankin, 1937                        | —                                     | —                                     | —                                    | —                                   | —                                   | —  | —   | —                                    | 6 <sub>mk</sub>                   | —                               | —                                | —                 | —                                | —                              |
| <i>Gorgoderina attenuata</i><br>Stafford, 1902                     | —                                     | —                                     | —                                    | —                                   | —                                   | —  | —   | —                                    | —                                 | —                               | —                                | —                 | —                                | —                              |
| <i>Phyllodistomum americanum</i><br>Osborn, 1903                   | —                                     | —                                     | —                                    | —                                   | —                                   | —  | —   | —                                    | —                                 | —                               | —                                | —                 | —                                | —                              |
| Unidentified metacercariae   | —                                     | —                                     | —                                    | —                                   | —                                   | —  | —   | —                                    | —                                 | —                               | —                                | —                 | —                                | —                              |
| <b>CESTODA</b>   |                                       |                                       |                                      |                                     |                                     |  |   |                                      |                                   |                                 |                                  |                   |                                  |                                |
| <i>Proteocephalus loenbergti</i><br>Fuhrmann, 1895                 | —                                     | —                                     | —                                    | —                                   | —                                   | —  | —   | —                                    | —                                 | —                               | —                                | —                 | —                                | —                              |
| <b>NEMATODA</b>  |                                       |                                       |                                      |                                     |                                     |  |   |                                      |                                   |                                 |                                  |                   |                                  |                                |
| <i>Cosmoeceroideis dukae</i><br>Holl, 1928                         | —                                     | —                                     | —                                    | —                                   | —                                   | —  | —   | —                                    | —                                 | —                               | —                                | —                 | —                                | —                              |
| <i>Oswaldoerizia pipiens</i><br>Walton, 1929                       | —                                     | —                                     | —                                    | —                                   | —                                   | —  | —   | —                                    | —                                 | —                               | —                                | —                 | —                                | —                              |
| <i>Rhabdias ranae</i><br>Walton, 1929                              | —                                     | —                                     | —                                    | —                                   | —                                   | —  | —   | —                                    | —                                 | —                               | —                                | —                 | —                                | —                              |
| Unidentified spirurid cysts  | —                                     | —                                     | —                                    | —                                   | —                                   | —  | —   | —                                    | —                                 | —                               | —                                | —                 | —                                | —                              |

\* Numbers in parentheses refer to number of hosts collected and percentage infected.  
† a = Fond du Lac Co. b = Milwaukee Co. c = Oneida Co. d = Portage Co. e = Rock Co. f = Sheboygan Co. g = Waukesha Co.

Nematodes comprised the bulk of the worm burden among both salamanders and anurans (Table 1). The most common nematode was *Cosmocercoides dukae* Holl, 1928, collected from the intestine in 11 of the 14 host species. However, the density of this nematode within each infected host remained low throughout the collection. Other nematodes collected included *Oswaldocruzia pipiens* Walton, 1929, found in the intestine of two *Bufo americanus* and *Rhabdias ranae* Walton, 1929, recovered from both *Plethodon cinereus* and *B. americanus*. Adults of two *R. ranae* were found in the lungs; juveniles inhabited the body cavity. Cysts containing an unidentified spirurid were found in the stomach wall of three *A. tigrinum* and one *A. laterale*.

Few digeneans were collected during this survey. *Brachycoelium salamandrae* Dujardin, 1845, was the most commonly recovered digenean and was found in both salamanders (*A. maculatum*, *P. cinereus*) and in *Rana palustris*. *Cephalogonimus salamandrus* Dronen and Lang, 1974, was found in four host species while *Glythelmins pennsylvaniensis* Cheng, 1961, occurred only in *Hyla crucifer*. The difficulty in identification of adult *C. salamandrus* has been discussed by Dronen and Lang (1974). In the present study, identification of this species was made from the adult and was confirmed by Brooks (personal communication).

The bladder fluke *Phyllodistomum americanum* Osborn, 1903, was commonly collected from *A. tigrinum* but was absent from all other hosts. One specimen of *Gorgoderina bilobata* Rankin, 1937, was found in the urinary bladder of *Hemidactylium scutatum*. The authors believe this to represent the first report of *Gorgoderina* sp. in a plethodontid salamander. *Gorgoderina attenuata* Stafford, 1902, and *G. bilobata* were recovered from the urinary bladder of *R. clamitans* and *B. americanus*, respectively. *Ambystoma tigrinum* and *H. scutatum* harbored unidentified metacercariae in the liver parenchyma.

*Necturus maculosus* is the only permanently aquatic salamander in Wisconsin. The monogenean *Sphyrnura osleri* Wright, 1879, was recovered from the gills of three hosts; the cestode *Proteocephalus loennbergii* Fuhrmann, 1895, occurred in the intestine of two *N. maculosus*.

The present study is the first survey of the helminth parasite fauna of Wisconsin salamanders. Lack of data on salamander parasites may indicate the difficulty of collecting these animals. In the current study most animals were collected during spring following melting of the normally heavy snow cover or during spring rains. The relatively sparse helminth parasite fauna of these salamanders may be a reflection of the time of collection. Animals were probably collected during their spring migration and before heavy feeding had begun. However, most anurans taken during the same period were more heavily infected. Results of the present study are consistent with previous reports of salamander parasites (Rankin, 1937; Dyer and Brandon, 1973; Price and St. John, 1980) although *A. tigrinum* was less heavily parasitized than reported by Ulmer (1970).

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### Literature Cited

- Brooks, D. R.** 1976. Parasites of amphibians of the Great Plains. 2. Platyhelminths of amphibians in Nebraska. *Bull. Univ. Nebr. State Mus.* 10:63-92.
- Dronen, N. O., and B. Z. Lang.** 1974. The life cycle of *Cephalogonimus salamandrus* sp. n. (Digenea: Cephalogonimidae) from *Ambystoma tigrinum* (Green) from eastern Washington. *J. Parasitol.* 60:75-79.
- Dyer, W. G., and R. A. Brandon.** 1973. Helminths of three sympatric species of cave-dwelling salamanders in southern Illinois. *Trans. Ill. State Acad. Sci.* 66:23-29.
- Fischthal, J. H.** 1955. Ecology of worm parasites in south-central New York salamanders. *Am. Midl. Nat.* 53:176-183.
- Harwood, P. D.** 1932. The helminths parasitic in the Amphibia and Reptilia of Houston, Texas, and vicinity. *Proc. U.S. Natl. Mus.* 81:1-71.
- Price, R. L., and T. St. John.** 1980. Helminth parasites of the small-mouthed salamander, *Ambystoma texanum* Matthes, 1855 from Williamson County, Illinois. *Proc. Helminthol. Soc. Wash.* 47:273-274.
- Price, R. L., and J. K. Buttner.** In press. Gastrointestinal helminths of the central newt, *Notophthalmus viridescens louisianensis* Wolterstorff, from southern Illinois, with a checklist of helminths from the red-spotted newt *N. v. viridescens* Rafinesque. *Proc. Helminthol. Soc. Wash.*
- Rankin, J. S.** 1937. An ecological study of parasites of some North Carolina salamanders. *Ecol. Monogr.* 7:171-269.
- Rankin, J. S.** 1945. An ecological study of the helminth parasites of amphibians and reptiles of western Massachusetts and vicinity. *J. Parasitol.* 31:142-150.
- Ulmer, M. J.** 1970. Studies on the helminth fauna of Iowa I. Trematodes of amphibians. *Am. Midl. Nat.* 83:38-64.
- Ulmer, M. J., and H. A. James.** 1976. Studies on the helminth fauna of Iowa II. Cestodes of amphibians. *Proc. Helminthol. Soc. Wash.* 43:191-200.
- Walton, A. C.** 1964. The parasites of Amphibia. *Wildl. Dis.* 39(Wd 63-4), 40(Wd 64-5-Wd 64-14).
- Williams, D. D., and S. J. Taft.** 1980. Helminths of anurans from NW Wisconsin. *Proc. Helminthol. Soc. Wash.* 47:278.
- Yamaguti, S.** 1958. *Systema Helminthum*, Vol. 1. The Digenetic Trematodes of Vertebrates. Vol. III. The Nematodes of Vertebrates. Interscience Publishers, Inc., New York.



## Endoparasites of the Double-crested Cormorant (*Phalacrocorax auritus*) in Florida

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**ABSTRACT:** Seventy-six double-crested cormorants, from two localities in Florida, were examined for endoparasites in 1973–1977. Quantitative data are given on the 19 species of worms that were recovered (nine new host records; eight species of Digenea, one of Cestoda, seven of Nematoda, three of Acanthocephala). Birds from the west coast of the Peninsula were the most frequently and heavily infected and carried the greatest number of species. Nestlings had the least number of species of parasites (five, mean per infected bird  $2 \pm 0.6$ ). The taxonomic status of *Mesostephanus cubäensis* Alegret, 1941 is discussed, with the species being relegated to synonymy with *M. appendiculatoides* (Price, 1934).

The cormorants (Phalacrocoridae) are a widely distributed family of birds, with the double-crested cormorant (*Phalacrocorax auritus*) being the most abundant and widespread species in North America (Palmer, 1962). The parasites of this group are well known (Yamaguti, 1959, 1961, 1963, 1971), but the majority of the literature is taxonomic/qualitative, rather than quantitative in nature. Few workers have surveyed the endoparasites of the double-crested cormorant. Hutton and Sogandares-Bernal (1960) and Hutton (1964) listed parasites from this host in Florida, while Alegret (1941) reported five species from the same bird host in Cuba. A study was, therefore, initiated to determine the prevalence and intensity of infection with helminths in the double-crested cormorant in Florida. Data on age and geographical differences in helminth burden were also to be collected.

### Materials and Methods

Forty-two fledged birds were obtained from Citrus Country south to Lee County on the west coast of Florida, in October 1973–February 1976, with a further 34 birds being taken in June–July 1977 in Brevard and Indian River counties, on the east coast. Procedures for recovering, killing, and fixing and staining the helminths were similar to those described by Kinsella and Forrester (1972) and Andrews and Threlfall (1975). The former sample contained two subspecies, namely *P. auritus* and *P. a. floridanus*, while the latter was composed only of *P. a. floridanus*. The two subspecies differ in meristic characters and in the fact that *auritus* is migratory, *floridanus* a permanent resident.

Data obtained were analyzed utilizing chi-square evaluations,  $2 \times k$  contingency tables, *t* statistic for two means and a one-way analysis of variance. Representative specimens have been deposited in the U.S. National Parasite Collection, Beltsville, Maryland (USNM Helm. Coll. 76588 to 76602).

### Results and Discussion

Nineteen species of helminths were recovered from the 76 cormorants examined at necropsy (Table 1). Nine are new host records. Birds from the west coast contained significantly more species ( $P < 0.05$ ) than those from the east coast (18 species; 12 species respectively), with individual west coast birds also having

**Table 1. Endoparasites recovered from 76 double-crested cormorants from Florida.**

| Helminth  | Age      | Origin of bird      |   |         |                     |   |            |
|---|----------|---------------------|---|---------|---------------------|---|------------|
|   |          | East coast          |   |         | West coast          |   |            |
|   |          | No. (%)<br>infected | Mean no. $\pm$ SD<br>(range)<br>worms per infected bird |         | No. (%)<br>infected | Mean no. $\pm$ SD<br>(range)<br>worms per infected bird |            |
| <i>Ornithobilharzia</i> sp.<br>(7)*                                   | Adult    | —                   | —   | —       | 2 (5)               | 1 $\pm$ 1   | (1–2)      |
| <i>Parorchis acanthus</i><br>(6)*                                     | Adult    | —                   | —   | —       | 1 (2)               | 1   | (1)        |
| <i>Drepanocephalus spathans</i> (3a, b, c)*                           | Adult    | 1 (6)               | 1   | (1)     | 6 (14)              | 263 $\pm$ 398   | (6–956)    |
| <i>Renicola</i> sp. ( <i>thapari</i> ?)<br>(10)*                      | Adult    | —                   | —   | —       | 1 (2)               | many  | (many)     |
| <i>Phagicola longus</i><br>(3a, b)                                    | Nestling | 9 (53)              | 138 $\pm$ 154   | (1–400) |                     |   |            |
|   | Adult    | 10 (59)             | 77 $\pm$ 74   | (3–197) | 16 (38)             | 54 $\pm$ 120  | (1–488)    |
|   | Total    | 19 (56)             | 106 $\pm$ 119   | (1–400) |                     |   |            |
| <i>Clinostomum marginatum</i> (8, 9)                                  | Adult    | —                   | —   | —       | 4 (10)              | 3 $\pm$ 3   | (1–7)      |
| <i>Mesostephanus appendiculatoides</i> (3a, b)*                       | Nestling | 14 (82)             | 97 $\pm$ 159  | (1–624) |                     |   |            |
|   | Adult    | 8 (47)              | 20 $\pm$ 38   | (1–113) | 7 (17)              | 50 $\pm$ 89   | (1–249)    |
|   | Total    | 22 (65)             | 69 $\pm$ 133  | (1–624) |                     |   |            |
| <i>Hysteromorpha triloba</i><br>(3a, b, c)                            | Adult    | —                   | —   | —       | 7 (17)              | 4,169 $\pm$ 7,193                                       | (1–19,727) |
| <i>Parvitaenia</i> sp. (immature, <i>eudocimi</i> ?)<br>(3a, b, c, d) | Adult    | 4 (24)              | 27 $\pm$ 49   | (1–100) | 9 (21)              | 48 $\pm$ 108  | (1–334)    |
| <i>Capillaria carbonis</i><br>(3d, 4, 5)*                             | Adult    | 7 (41)              | 13 $\pm$ 17   | (1–50)  | 11 (26)             | 44 $\pm$ 77   | (1–227)    |
| <i>Capillaria contorta</i><br>(1)*                                    | Adult    | 2 (12)              | 5 $\pm$ 4   | (2–8)   | 6 (14)              | 7 $\pm$ 10  | (1–26)     |
| <i>Contraecum</i> spp.<br>(1, 2)*                                     | Nestling | 15 (88)             | 92 $\pm$ 90   | (3–302) |                     |   |            |
|   | Adult    | 17 (100)            | 163 $\pm$ 141   | (9–550) | 41 (98)             | 240 $\pm$ 271   | (2–1,178)  |
|   | Total    | 32 (94)             | 129 $\pm$ 124   | (3–550) |                     |   |            |
| <i>Desmidocercella incognita</i> (8, 9, 11)                           | Nestling | 1 (6)               | 15  | (15)    |                     |   |            |
|   | Adult    | 6 (35)              | 5 $\pm$ 6   | (1–15)  | 17 (40)             | 49 $\pm$ 76   | (1–100)    |
|   | Total    | 7 (26)              | 7 $\pm$ 6   | (1–15)  |                     |   |            |
| <i>Skrjabinocara squamatum</i> (1, 2)                                 | Adult    | 1 (6)               | 1   | (1)     | 14 (33)             | 2 $\pm$ 1   | (1–4)      |
| <i>Tetrameres microspinosus</i> (1, 2)                                | Adult    | 6 (35)              | 23 $\pm$ 17   | (4–54)  | 30 (71)             | 58 $\pm$ 97   | (1–445)    |
| <i>Polymorphus obtusus</i><br>(5)†                                    | Adult    | —                   | —   | —       | 13 (31)             | 2 $\pm$ 1   | (1–2)      |
| <i>Andracantha</i> spp.‡<br>(3c, d, 4, 5)                             | Nestling | 1 (6)               | 1   | (1)     |                     |   |            |
|   | Adult    | 10 (59)             | 5 $\pm$ 4   | (1–13)  | 31 (74)             | 24 $\pm$ 34   | (1–129)    |
|   | Total    | 11 (34)             | 5 $\pm$ 4   | (1–13)  |                     |   |            |

Numbers in parentheses below parasite name indicate most frequent site in host: (1) esophagus; (2) proventriculus/gizzard; (3) small intestine: (a) duodenum, (b) anterior third, (c) mid-third, (d) posterior third; (4) ceca; (5) large intestine; (6) cloaca; (7) blood vessels; (8) trachea; (9) lungs; (10) kidneys; (11) air sacs.

\* New host record; † a complex of 2 species (ratio 10:1) *C. spiculigerum* and *C. multipapillatum*\*; ‡ a complex of 2 species (see text).

**Table 2. Prevalence and intensity of infection with major groups of endoparasites of two populations of double-crested cormorants from Florida.**

|                | East coast     |           |        |             |           |        | West coast  |               |           |
|----------------|----------------|-----------|--------|-------------|-----------|--------|-------------|---------------|-----------|
|                | Nestlings (17) |           |        | Adults (17) |           |        | Adults (42) |               |           |
|                | a*             | b*        | c*     | a           | b         | c      | a           | b             | c         |
| Digenea        | 15 (88)        | 171 ± 184 | 1-626  | 11 (65)     | 78 ± 78   | 2-204  | 23 (55)     | 1,391 ± 4,404 | 1-20,336  |
| Cestoda        | —              | —         | —      | 4 (24)      | 27 ± 49   | 1-100  | 9 (35)      | 48 ± 108      | 1-334     |
| Nematoda       | 16 (94)        | 102 ± 96  | 3-302  | 17 (100)    | 184 ± 141 | 13-573 | 42 (100)    | 311 ± 345     | 3-1,465   |
| Acanthocephala | 1 (6)          | 1         | 1      | 10 (59)     | 5 ± 4     | 1-13   | 32 (76)     | 24 ± 34       | 1-115     |
| Total          | 17 (100)       | 247 ± 187 | 10-661 | 17 (100)    | 243 ± 193 | 40-711 | 42 (100)    | 1,109 ± 3,389 | 26-21,189 |

\* a: no. (%) infected; b: mean intensity ± SD; c: range numbers.

a greater intensity of infection (Table 2). Five species of digeneans and one species of acanthocephalan were found only in birds from the west coast. The number of species of parasites per infected bird was also significantly higher in the west than the east ( $6 \pm 2$ ;  $3 \pm 2$  respectively). *Contracaecum* spp. were found at both sample sites in similar prevalences. However, the intensity of infection was significantly heavier in the west coast birds, a similar relationship being noted in brown pelicans (*Pelecanus occidentalis*) by Courtney and Forrester (1974). The prevalence of *Capillaria carbonis* was greatest in east coast birds, but the intensity was highest in hosts from the west coast. *Skryabinocara squamatum* showed the greatest prevalence in Gulf coast birds, while *Desmidocercella incognita*, *Tetrameres microspinosa*, *Polymorphus obtusus*, and *Andracantha* spp. were most frequent and located in greatest numbers in the latter group of birds. The differences noted in the worm burdens of the two groups may be due to a variety of factors, such as slight differences in host diet in the two sampling areas, or the actual time (year/month) of sampling.

Prevalence and intensity of infection with helminths in nestlings and adults from the east coast are given in Tables 1 and 2. No difference was seen in the total prevalence and intensity of infection in the two groups, but a highly significant difference ( $P < 0.001$ ) was detected in the mean number of species in infected birds in the two groups ( $2 \pm 0.6$  in nestlings;  $4 \pm 1.3$  in adults). Nestlings contained only five species as opposed to the 13 in east coast adults, and 18 in west coast birds. These differences are undoubtedly related to differences in feeding habits of the various age groups. *Phagicola longus* and *Mesostephanus appendiculatoides* were the only helminths which showed a greater intensity of infection in nestlings than in adults. In the case of the latter species the prevalence was also greater. *Contracaecum* spp. occurred in lower numbers in nestlings than in adults.

All the specimens of the *Renicola* species were gravid but resembled most closely *R. thapari*. *Drepanocephalus spathans* is reported from this host for the first time, Dietz (1909) having described it from *Phalacrocorax brasiliensis* (Dietz, 1910; Travassos et al., 1969).

Price (1934) described *Prohemistomum appendiculatoides* (= *Mesostephanus appendiculatoides*) from a brown pelican (*Pelecanus occidentalis*) taken in the Dominican Republic, while Alegret (1941) described *Mesostephanus cubäensis*

**Table 3.** Measurements of *Mesostephanus appendiculatoides* (Price, 1934), *M. cubäensis* Alegret, 1941, and *Mesostephanus* sp. specimens obtained during the present study.

|                        | <i>Mesostephanus appendiculatoides</i><br>(Price, 1934) | <i>Mesostephanus cubäensis</i><br>Alegret, 1941  | <i>M. appendiculatoides</i><br>Present study     |           |
|------------------------|---|--|--|-----------|
|                        |   |  | Mean $\pm$ SD                                    | Range     |
| Body length            | 680-970   | —  | 700 $\pm$ 159                                    | 548-1,177 |
| width                  | 240-369   | —  | 252 $\pm$ 54                                     | 193-416   |
| Oral sucker (diam.)    | 40-48   | 67 by 55-60                                      | 58 $\pm$ 12                                      | 42-90     |
| Pharynx length         | 28-32   | 60   | 50 $\pm$ 10                                      | 33-67     |
| width                  | 20-32   | 47   | 50 $\pm$ 10                                      | 33-67     |
| Ventral sucker (diam.) | 20-28   | 85   | 38 $\pm$ 11                                      | 25-71     |
| Esophagus length       | 40-120  | 100  | 51 $\pm$ 19                                      | 33-87     |
| Ovary (diam.)          | 40  | 100  | 54 $\pm$ 4                                       | 50-58     |
| Testes                 |   |  |  |           |
| Anterior length        | 80-100  | 230  | 105 $\pm$ 62                                     | 71-269    |
| width                  | 60-80   | 260  | 108 $\pm$ 64                                     | 54-269    |
| Posterior length       | 100-120   | 220  | 121 $\pm$ 55                                     | 62-232    |
| width                  | 70-80   | 270  | 138 $\pm$ 54                                     | 62-232    |
| Cirrus pouch length    | 280-360   | 500-520  | 225 $\pm$ 63                                     | 83-399    |
| width                  | 50-85   | 80-90  | 61 $\pm$ 16                                      | 33-87     |
| Eggs no. in uterus     | 2-4   | 4-6  |  | 0-7       |
| length                 | 80-112  | 94-107   | 90 $\pm$ 6                                       | 79-108    |
| width                  | 60-80   | 73-76  | 62 $\pm$ 6                                       | 50-71     |
| Host                   | <i>Pelecanus occidentalis</i><br>(Pelecaniformes)       | <i>Phalacrocorax auritus</i><br>(Pelecaniformes) | <i>Phalacrocorax auritus</i><br>(Pelecaniformes) |           |

from double-crested cormorants taken in Cuba. Neither author noted, in their descriptions, the number of specimens they measured, and Alegret (1941) failed to give any differential diagnosis or rationale for the erection of a new species. During this study specimens of the *Mesostephanus* recovered were morphologically identical to *M. appendiculatoides*, which in turn is identical to *M. cubäensis* except for its size (smaller). Table 3 gives the measurements of *M. appendiculatoides* (Price, 1934), *M. cubäensis* Alegret, 1941, and 35 specimens of *Mesostephanus* obtained during the present study. From the data presented it is quite clear that *M. appendiculatoides* is a highly variable species with respect to linear measurements. Most measurements presented by Price (1934) and Alegret (1941) fit within, or closely approximate, the mean  $\pm$  standard deviation, or the range, obtained during the present study. Therefore, all three samples are considered conspecific and *M. cubäensis* is declared a synonym of *M. appendiculatoides*. The size range of the latter digenean is greatly extended, with the differences noted perhaps reflecting host influence on development. The size variations may also be a function of how the specimens were treated (fixed, stained, coverslip pressure, etc.), the age of individual helminths, or the number of specimens measured by the original workers.

While large numbers of cestodes were found, all proved to be immature. The specimens best fitted the description of *Parvitaenia eudocimi*, which was described from white ibises (*Eudocimus albus*) taken in Cuba, by Rysavy and

Macko (1971). These authors also recovered specimens of this cestode from double-crested cormorants. Adult female birds from both sample areas were more heavily infected with this helminth than the males.

Schmidt (1975) erected the genus *Andracantha* to contain three species of acanthocephalans which possessed genital spines and two fields of trunk spines. He included in this genus *Corynosoma gravida* Alegret, 1941 (= *Andracantha gravida* (Alegret 1941)) which had been described from double-crested cormorants taken in Cuba (Alegret, 1941). During the present study large numbers of this species were recovered, together with almost equal numbers of a previously undescribed species of this genus (Threlfall and Nickol, unpublished data).

Further studies on this host would prove worthwhile. A comparison of the helminth fauna of the migratory subspecies, with that of permanent residents, would establish whether a variety of helminth communities are found in double-crested cormorants depending on sampling sites. It seems likely that northern and/or freshwater breeders would bear a different worm burden than those examined in the present study, as indicated by necropsies on three specimens of the closely related great cormorant (*Phalacrocorax carbo*) from Brunette Island, Newfoundland (47°18'N, 55°54'W). The latter birds contained *C. spiculigerum* (all birds infected, mean no. worms 251, range 193–331), *Cryptocotyle lingua* (2 infected, 650, 121–1,180), *Desmidocerca nudicauda* and *Desmidocercella incognita* (3 infected, 176, 123–219, ratio, of 10:1 respectively).

It seems likely that birds which are living in the same area and which have relatively similar food habits will have closer helminth profiles than closely related species which are geographically widely separated. This is well illustrated by the brown pelican which, in Florida, shares with the double-crested cormorant at least eight species of parasites. The differences noted between the two species can be related to slight differences in their diets.

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#### Literature Cited

- Alegret, M. J. 1941. Contribution al conocimiento de los vermes parásitos de *Phalacrocorax Auritus Floridanus*. D.Sc. Thesis, Havana Univ., Cuba. 32 pp.
- Andrews, S. E., and W. Threlfall. 1975. Parasites of the common crow (*Corvus brachyrhynchos* Brehm, 1822) in insular Newfoundland. Proc. Helminthol. Soc. Wash. 42:24–28.
- Courtney, C. H., and D. J. Forrester. 1974. Helminth parasites of the brown pelican in Florida and Louisiana. Proc. Helminthol. Soc. Wash. 41:89–93.
- Dietz, E. 1909. Die Echinostomiden der Vogel. Zool. Anz. 34:180–192.
- Dietz, E. 1910. Die Echinostomiden der Vogel. Zool. Jahrb., Suppl. 12:265–512.
- Hutton, R. F. 1964. A second list of parasites from marine and coastal animals of Florida. Trans. Am. Microsc. Soc. 83:439–447.

- Hutton, R. F., and F. Sogandares-Bernal.** 1960. A list of parasites from marine and coastal animals of Florida. *Trans. Am. Microsc. Soc.* 79:287-292.
- Kinsella, J. M., and D. J. Forrester.** 1972. Helminths of the Florida duck, *Anas platyrhynchos fulvigula*. *Proc. Helminthol. Soc. Wash.* 39:173-176.
- Palmer, R. S. (Ed.).** 1962. *Handbook of North American Birds. Vol. 1.* Yale Univ. Press, New Haven, Connecticut. 567 pp.
- Price, E. W.** 1934. New trematode parasites of birds. *Smithson. Misc. Coll.* 91:1-6.
- Rysavy, B., and J. K. Macko.** 1971. Bird cestodes of Cuba 1. Cestodes of birds of the Orders Podicipediformes, Pelecaniformes and Ciconiiformes. *An. Inst. Biol. Univ. Nal. Autón. México* 42, Ser. Zoología (1):1-28.
- Schmidt, G. D.** 1975. *Andracantha*, a new genus of Acanthocephala (Polymorphidae) from fish-eating birds, with descriptions of three species. *J. Parasitol.* 61:615-620.
- Travassos, L., J. F. Teixeira de Freitas, and A. Kohn.** 1969. Trematódeos de Brasil. *Mem. Inst. Oswaldo Cruz* 67:1-886.
- Yamaguti, S.** 1959. *Systema Helminthum. Vol. II. The Cestodes of Vertebrates.* Interscience Publ., New York. 860 pp.
- Yamaguti, S.** 1961. *Systema Helminthum. Vol. III. The Nematodes of Vertebrates. Pt. 1 and Pt. 2.* Interscience Publ., New York. 1261 pp.
- Yamaguti, S.** 1963. *Systema Helminthum. Vol. V. Acanthocephala.* Interscience Publ., New York. 423 pp.
- Yamaguti, S.** 1971. *Synopsis of Digenetic Trematodes of Vertebrates.* Keigaku Publ. Co., Tokyo. Vol. I, 1074 pp. Vol. II, 349 plates.

## Helminths of Bushy-tailed Wood Rats, *Neotoma cinerea* subsp. from Colorado, Idaho, and Wyoming<sup>1</sup>

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**ABSTRACT:** Forty-four *Neotoma cinerea rupicola*, one *N. c. orolestes*, and three *N. cinerea* subsp. were examined for endoparasites. *Aspicularis ackerti* Kruidenier and Mehra, 1959, is redescribed and a key to the species of the genus *Aspicularis* Schultz, 1924, is provided. *Nematodirus neotoma* Hall, 1916, is redescribed and *N. tortuosus* Tucker, 1942, is placed in synonymy with it. Cysticercoids of *Paruterina candelabraria* (Goeze, 1782) Fuhrmann, 1906, and immature *Monoecocestus* sp. constitute new host records.

During 1974-1977 44 bushy-tailed woodrats, *Neotoma cinerea*, were collected from northern Weld County, Colorado and southeastern Laramie County, Wyoming. Thirty-seven contained one or more species of helminths (Table 1). A single *N. c. orolestes* was collected near Red Feather Lakes, Larimer County, Colorado, and three *N. cinerea* were collected by Floyd M. Seese at Saint Baldy Mountain, Bonewah County, Idaho. The helminths were fixed and studied by conventional techniques. Ectoparasites were not collected. All measurements are in micrometers unless otherwise stated.

### *Aspicularis ackerti* Kruidenier et Mehra, 1959

We compared our specimens and the type specimens (USNM Helm. Coll. 56163) with the original description by Kruidenier and Mehra (1959). Discrepancies in the original description indicate a need for the following redescription of the species, based on 55 males and 54 females from northern Weld County, Colorado and southeastern Laramie County, Wyoming, U.S.A.

Body small, cylindrical, males shorter than females. Gubernaculum and spicules absent. Cuticle transversely striated, cephalic vesicle present. Three undivided lips, one pair of lateral epaulettes with amphids, six labial papillae, and four large submedian cephalic papillae (Fig. 2). Anterior esophagus clubshaped, followed by well-developed bulb. Cervical alae narrow smoothly, continued posteriorly as narrow lateral alae (Fig. 3).

**MALE:** Length 3.01-6.35 (5.03) mm, greatest width 137-293 (236) at middle of body. Anterior esophagus 260-480 (343) long and 33-60 (51) wide. The bulb measures 113-183 (157) long by 65-120 (90) wide. Total length of esophagus 373-590 (501). Distance from anterior end to nerve ring is 150-213 (186). Cuticular vesicle 83-138 (111) long by 98-130 (111) wide. Excretory pore posterior to junction of esophageal bulb and intestine, 0.69-1.21 (0.96) mm from anterior end. Distance from anterior end to beginning of cervical alae is 33-55 (45); the cervical alae are 43-60 (51) thick and 499-636 (581) long, narrowing smoothly and continuing posteriorly. Length of the combined cervical and lateral alae is 1.18-1.96 (1.72) mm. Caudal alae do not reach tip of tail. The tail spike is 49-117 (92) long. Length of caudal alae is 489-772 (620). The anus is 205-401 (315) from tip of tail. There are

<sup>1</sup> Published with the support of the Brayton H. Ransom Memorial Trust Fund.

**Table 1.** Helminths found in *Neotoma cinerea*.

| Parasite  | No. infected hosts |
|---|--------------------|
| Cestoda   |                    |
| Anoplocephalidae  |                    |
| <i>Paranoplocephala neotomae</i> (Voge, 1946) Rausch, 1976 (small intestine) (*75512)                       | 6                  |
| <i>Monoecocestus</i> sp. (small intestine) (75511)  | 1                  |
| Davaineidae   |                    |
| <i>Railletina</i> sp. (small intestine) (75510)   | 2                  |
| Dilepididae   |                    |
| <i>Paruterina candelabraria</i> (Goeze, 1782) Fuhrmann, 1906 (liver, mesenteric lymph nodes) (75508, 75509) | 3                  |
| Taeniidae   |                    |
| <i>Echinococcus multilocularis</i> Leuckart, 1863 (liver)   | 1                  |
| Nematoda  |                    |
| Oxyuridae   |                    |
| <i>Aspicularis ackerti</i> Kruidenier and Mehra, 1959 (cecum) (75506)                                       | 22                 |
| Trichostrongylidae  |                    |
| <i>Nematodirus neotoma</i> Hall, 1916 (small intestine) (75507)   | 35                 |

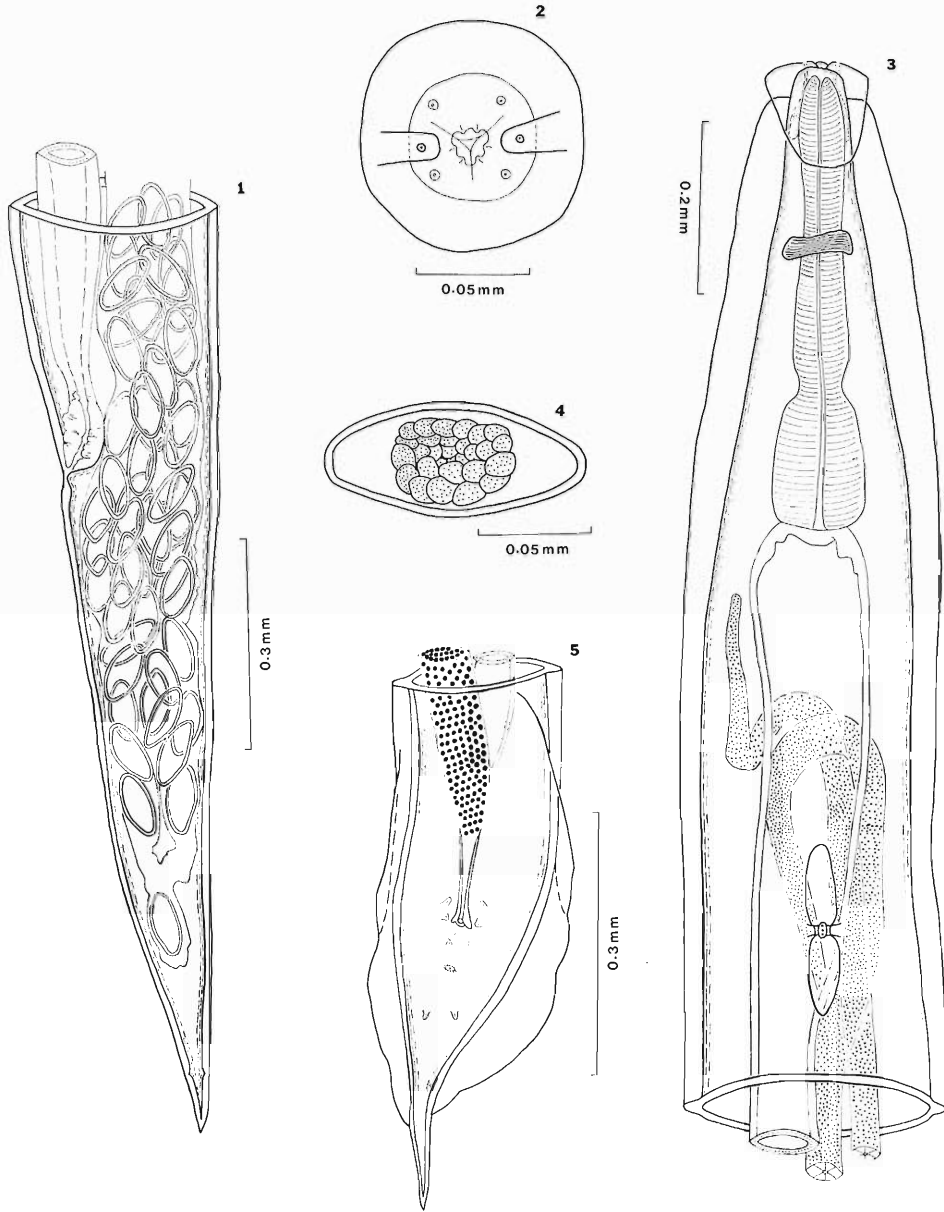
\* Voucher specimens deposited in USNM Helm. Coll.

ten anal papillae as follows (Fig. 5): one pair praeanal, one pair adanal, a single median postanal closely associated with anus, one posterior pair followed by second single median and one more posterior pair. Distance from the anus to most posterior single median papilla is 43–75 (61); distance from single posterior median papilla to most posterior pair is 38–73 (58); distance from the posterior pair to tip of the tail spike is 175–250 (210).

**MALE HOLOTYPE:** 4.46 mm long with greatest width being 196. Length of anterior esophagus is 263, its width is 43. Bulb length 145, width 70. Total esophagus length is 408. From anterior end to nerve ring is 170. The cuticular head vesicle is 105 long and 100 wide. Distance from anterior end to beginning of cervical alae is 40. All caudal papillae were not clearly seen. Distance between most posterior single median and posterior pair is 58. The tail spike is 78 long.

**FEMALE:** 5.46–8.97 (7.41) mm long with greatest width at vulva 225–391 (330). Anterior esophagus length is 305–465 (387), width is 45–80 (61). The bulb is 150–225 (177) long, 80–150 (107) wide. Total esophagus length is 476–655 (565). From anterior end to nerve ring is 162–238 (202). The cuticular vesicle is 100–178 (132) long by 95–148 (126) wide. Location of excretory pore similar to that of the male, 0.82–1.67 (1.10) mm from anterior end; it is well defined by a cuticular marking 200–288 (238) long (Fig. 3). Distance from anterior end to beginning of cervical alae is 30–63 (48). Thickness of cervical alae is 35–50 (38), while their length is 538–743 (656). Combined length of cervical and lateral alae is 0.97–2.60 (1.85) mm. Vulva is 1.52–2.89 (2.49) mm from anterior end, and 3.68–6.10 (4.89) mm from posterior end. The anus is 0.66–1.20 (0.96) mm from tip of tail (Fig. 1). Uterus loops posterior to anus; the egg in the loop in the tail measures 88–110





Figures 1-5. *Aspicularis ackerti* Kruidenier et Mehra, 1959. 1. Lateral view of female posterior end. 2. En face view. 3. Ventral view of female showing cervical alae and excretory pore. 4. Egg. 5. Caudal view of male showing caudal alae and papillae.

(101) long, by 38-50 (41) wide; terminal egg nearest vulva is 93-110 (102) long by 38-55 (45) (Fig. 4).

FEMALE ALLOTYPE: 5.68-6.31 (6.00) mm long; greatest width at vulva 177-215 (196). Anterior esophagus length 313-350 (332) by 40-43 (42) wide. Length of bulb 158-170 (164), width of bulb 73-75 (74). The total esophagus length is

483–508 (496). Distance from anterior end to nerve ring is 175–180 (178). The cuticular vesicle is 113 long by 105–108 (107) wide. The cuticular marking of the excretory pore is 230 long. It is posterior to the junction of the bulb and intestine and is 1.03 mm from anterior end. Anterior end to beginning of cervical alae is 35–50 (43). The cervical alae continue as lateral alae. Vulva 2.18–2.38 (2.28) mm from anterior end and 3.50–3.95 (3.73) mm from posterior end. Anus is 704–714 (709) from tip of tail. Uterus loops into tail posterior to anus. Most posterior egg in uterine loop 100 long by 40–45 (43) wide. Voucher specimens deposited in USNM Helm. Coll. 75506.

### Discussion

Kruidenier and Mehra (1959) described the caudal alae of the male “with two deep notches each” indicating that these “could be interpreted as three bilateral pairs of alae.” We did not observe this condition on the holotype male or ours, but did observe one notch, giving the alae the superficial appearance of being paired.

They also described the anal papillae as follows: “Eleven caudal papillae, two preanal, two adanal, and two postanal pairs closely associated with the anus, a single median, and two postanal pairs.” We found ten papillae: one pair preanal, one pair adanal, a single median postanal closely associated with the anus, one posterior pair followed by a second single median, and one more posterior pair. We did not observe the two most posterior pairs on the holotype but did observe a single pair as on our specimens.

Kruidenier and Mehra (1959) described the en face views of the female and male as being different. We found no difference in the en face views. Added to their description of the en face view is the presence of epaulettes bearing the amphids and four large, submedian cephalic papillae.

Akhtar (1955) subdivided the genus *Aspicularis* into five subgenera on the basis of presence or absence of a cephalic vesicle, cervical alae terminations, and presence or absence of a gap between cervical and lateral alae.

Erhardova-Kotrla and Daniel (1970), in describing *A. arianica*, used Akhtar’s key in part stating, “Akhtar divided the genus into five subgenera on the grounds of the shape of the cervical alae and the presence or absence of the cephalic cuticular vesicle,” omitting reference to the presence or absence of lateral alae. They described the cervical alae of *A. arianica* as being “entirely different from those of all species described,” being “short, paired, and not sickle-shaped.”

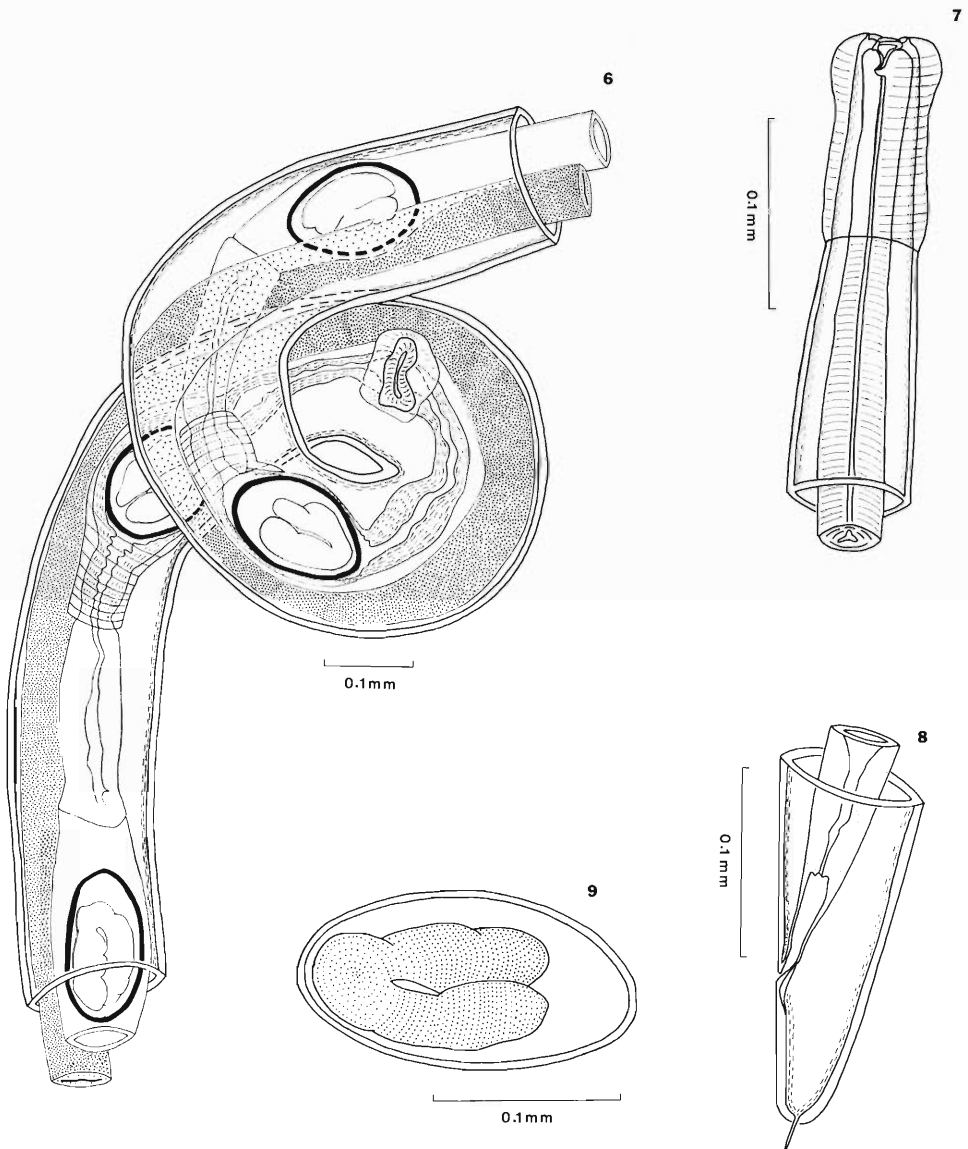
Quentin (1975) considered that Akhtar’s division into subgenera had complicated the systematics; he assigned *A. caviellae* Freitas, Lent, and Almeida, 1937, to the genus *Heteroxyxynema* Hall, 1916, as it is a caviomorph parasite. *A. arianica* was identified from the sciurid *Marmota caudata* and according to Vaughn (1978) the affinity of the family Sciuridae to other rodents is uncertain. Aside from host, *A. arianica* is otherwise atypical of the genus. Due to lack of measurements and immaturity of the described female, we feel this is an inadequately described species and place it incertae sedis until additional data are available.

Quentin (1975) divided the species into two large groups according to the form of their cervical alae. In the first group the cervical alae are abruptly truncated at the level of the bulb, giving the cephalic extremity the aspect of an arrowhead.

In the second group, the cervical alae progressively diminish in width; their edges fit together at the wall of the body or extend out of lateral wings when these exist. In describing *A. witenbergi*, Quentin noted that the edge of the cervical ala does not end abruptly in an acute angle; nor is it extended gradually into a lateral wing. This places his species as an intermediate between the two groups.

It becomes increasingly apparent that identification of species within the genus by the use of alae terminations is insufficient. We propose a key to the species of *Aspiculuris* as follows:

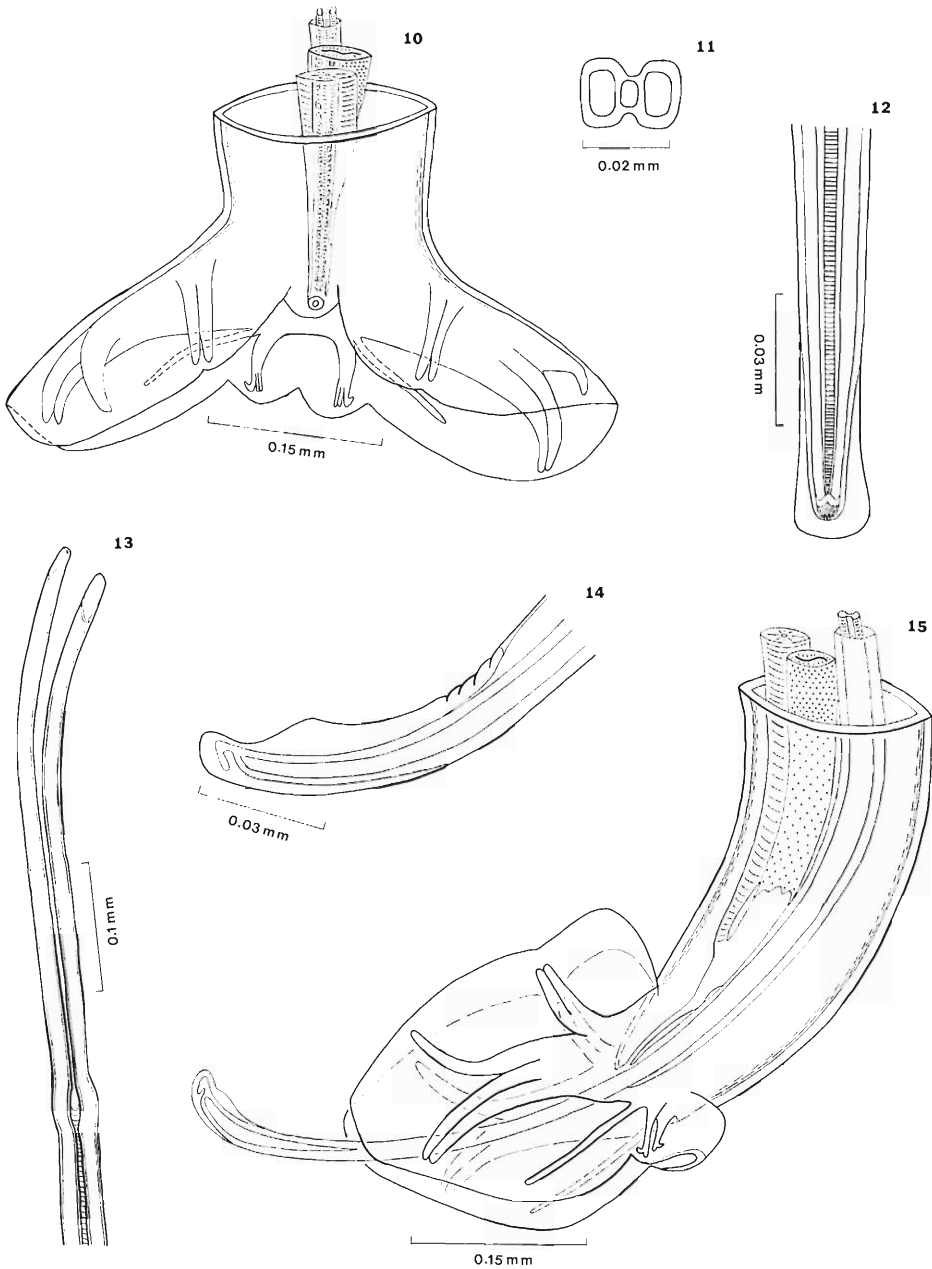
1. Cervical alae ending in a definite concave margin ..... 5  
Cervical alae ending otherwise ..... 2
2. Caudal alae of male enclosing tip of tail ..... 3  
Caudal alae of male not enclosing tip of tail ..... 4
3. Tail of male bearing 11 papillae, eggs  $81 \times 37$  ..... *A. asiatica* Schulz, 1927  
Tail of male bearing 16 papillae, eggs  $85-89 \times 34-38$  .....  
..... *A. tschertkovi* Tarzhimanova, 1969
4. Tail of male bearing 10 papillae, eggs  $88-110 \times 38-50$  .....  
..... *A. ackerti* Kruidenier and Mehra, 1959  
Tail of male bearing 11 papillae, eggs  $87-90 \times 42-46$  .....  
..... *A. africana* Quentin, 1966  
Tail of male bearing 12 papillae, eggs  $72 \times 40-42$  .....  
..... *A. witenbergi* Quentin, 1975
5. Termination of cervical alae at level of junction of anterior esophagus  
and esophageal bulb ..... *A. schulzi* Popov and Nasarova, 1930  
Termination of cervical alae near middle of esophageal bulb ..... 6  
Termination of cervical alae near posterior end of bulb or below ..... 7
6. Tail of male bearing 10 papillae, eggs  $75-78 \times 33-36$  .....  
..... *A. lahorica* Akhtar, 1955  
Tail of male bearing 10 papillae, eggs  $84-90 \times 36-39$  .....  
..... *A. artigasi* Araujo, 1965  
Tail of male bearing 14 papillae, eggs  $81-90 \times 42-51$  .....  
..... *A. tetraptera* (Nitzsch, 1821) Schulz, 1924
7. Male of species unknown ..... 8  
Male known ..... 9
8. Female length 3.1-3.25 mm, eggs  $85-90 \times 33-38$  ..... *A. ratti* Johnson, 1969  
Female length 4.3-4.62 mm, eggs  $95-100 \times 43-47$  .....  
..... *A. aserbaidjanica* Tarzhimanova, 1969
9. Length of female less than 6 mm ..... 11  
Length of female greater than 6 mm ..... 10
10. Caudal alae of male not enclosing tip of tail ..... *A. dinniki* Schulz, 1927  
Caudal alae of male enclosing tip of tail .....  
..... *A. rysavyi* Erhardova-Kotrla and Daniel, 1971
11. Caudal alae of male not enclosing tip of tail .. *A. pakistanica* Akhtar, 1955  
Caudal alae of male insufficiently described regarding enclosure of tail .... 12
12. Male with 4 pair caudal papillae, eggs  $76-83 \times 27-35$  .....  
..... *A. americana* Erickson, 1938  
Male with at least 9 papillae, eggs  $93 \times 43$  .....  
..... *A. kazakstanica* Nasarova and Sweshnikova, 1930



Figures 6–9. *Nematodirus neotoma* Hall, 1916, female. 6. Vulva and ovijectors. 7. Dorsal view anterior end female. 8. Posterior view anus and tail spike of female. 9. Egg.

#### *Nematodirus neotoma* Hall, 1916

*Nematodirus neotoma* was first described by Hall (1916) from four woodrats from Colorado: *Neotoma desertorum* Cary, *N. mexicana fallax* Merriam, *N. floridana baileyi* Merriam, and *N. cinerea rupicola* Allen. In the original description many important measurements were omitted, so we feel a description of the species, based on our material, is necessary. The following is based on 125 males and 99 females from northern Weld County, Colorado and southeastern Laramie County, Wyoming, U.S.A.



Figures 10–15. *Nematodirus neotoma* Hall, 1916, male. 10. Ventral view of bursa. 11. Cross section of united spicules. 12. Dorsal view distal end of spicules. 13. Proximal free portion and fusion of spicules. 14. Lateral view distal end of spicules. 15. Lateral view of bursa.

Body filiform, with longitudinal ridges. Cephalic vesicle (Fig. 7) transversely striated. Cervical papillae absent. Buccal cavity with a single dorsal tooth.

MALE: 10.29–20.24 (14.57) mm long, greatest width 93–192 (134) at midbody. Cephalic dilation length 95–140 (118), width 45–73 (54). Head width exclusive of

cephalic dilation 25–43 (29). There are 22–48 (34) longitudinal cuticular ridges, 5–12 (8) apart. Esophagus claviform, 391–577 (492) long, 28–68 (43) wide. Excretory pore 411–675 (526) from anterior end.

Prebursal papillae absent. Bursa (Fig. 10) symmetrical, with two large lateral lobes. Dorsal lobe with deep apical notch. Posterolateral and mediolateral rays bend posteriorly, anterolateral rays bend anteriorly (Fig. 15). Lateroventral and ventroventral rays arise from ventral surface of anterolateral ray and curve anteriorly. Externodorsal ray arises from base of dorsal ray, which is bifurcated; dorsal raylet appears again bifurcated. Ventral bursal surface covered with numerous cuticular bosses. Lateral lobes are 220–340 (267) long, 158–352 (220) wide, 490–510 (500) outspread width. Externodorsal ray 100–173 (135) long. Dorsal ray length (to bifurcation) 43–75 (53), lateral raylet 15–28 (19) long, dorsal raylet 10–23 (15).

Spicules filiform and united by a membrane for about  $\frac{7}{8}$  their length. They are yellow-brown in color, covered with transverse striations and are equal or nearly equal in length (Fig. 13). The distal tips are fingerlike, and are enclosed in a membranous bulb (Figs. 12, 14). Spicules are 2.50–4.17 (3.39) mm long. The fused portion is 205–577 (461) long.

FEMALE: 15.26–31.90 (24.23) mm long; width at the vulva 103–274 (200). The cephalic dilation is 103–148 (122) long and 53–78 (59) wide. There are 26–68 (50) longitudinal ridges, 5–10 (8) apart, extending past vulva. Esophagus claviform, 440–655 (533) long, 35–73 (50) wide. Excretory pore 440–646 (524) from anterior end. Vulva 10.36–22.00 (17.07) mm from anterior end, 3.50–10.93 (7.35) mm from posterior end. Ovijectors equal in length; including sphincters they are 0.69–1.58 (1.12) long (Fig. 6). Anus 58–120 (89) from end of body, excluding tail spike; spike 10–28 (19) long (Fig. 8). Eggs are oval, smooth shelled, 133–220 (171) long, 68–123 (94) wide (Fig. 9). Voucher specimens are deposited in USNM Helm. Coll. 75507.

### Discussion

Tucker (1942) described *N. tortuosus* as a new species from *Neotoma lepida* and *Neotoma fuscipes*. As his measurements of his specimens fall within the ranges of our specimens and the description of *Nematodirus neotoma* by Hall (1916), we consider *N. tortuosus* to be a junior synonym of *N. neotoma*.

### Acknowledgments

We wish to thank Dr. J. Ralph Lichtenfels, Animal Parasitology Institute, Beltsville, Maryland, for loan of the type specimens of *Aspicularis ackerti*.

### Literature Cited

- Akhtar, S. A. 1955. On nematode parasites of rats and mice of Lahore with some remarks on the genus *Aspicularis* Schulz, 1924, and two new species of the genus. Pak. J. Sci. Res. 7:104–111.
- Erhardova-Kotrla, B., and M. Daniel. 1970. Parasitic worms of small mammals from the mountain regions of the Eastern Hindu Kush. Folia Parasitol. 17:201–216.
- Hall, M. D. 1916. Nematode parasites of mammals of the orders Rodentia, Lagomorpha, and Hyracoidea. Proc. U.S. Natl. Mus. 50:1–258.
- Kruidenier, F. J., and K. N. Mehra. 1959. *Aspicularis ackerti* n. sp. (Nematoda: Oxyuridae) from the wood rats of Arizona. Proc. Helminthol. Soc. Wash. 26:147–150.

- Quentin, J. C.** 1975. Essai de classification des Oxyures Heteroxynematidae. Mém. Mus. Natl. Hist. Nat. Nouvelle Série A, Zool. 94:51-96.
- Tucker, H.** 1942. *Nematodirus tortuosus* n. sp., from the rat, *Neotoma*. J. Parasitol. 28:159-163.
- Vaughn, T. A.** 1978. Mammalogy. Second edition. W. B. Saunders Company, Philadelphia. 522 pp.

### **Guide to Parasite Collections**

“A Guide to the Parasite Collections of the World” edited by J. R. Lichtenfels and M. H. Pritchard describing 74 major collections in 25 countries has been published by the American Society of Parasitologists, 1041 New Hampshire Street, Lawrence, Kansas 66044, USA. Send \$3.00 to cover costs of handling and mailing.

## Endogenous Development of *Isospora arctopitheci* Rodhain, 1933 in the Marmoset *Saguinus geoffroyi*<sup>1</sup>

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**ABSTRACT:** Thirteen marmosets (*Saguinus geoffroyi*) were experimentally infected with sporulated oocysts of *Isospora arctopitheci*. Tissue specimens were prepared and examined by light and scanning electron microscopy. Parasites were found at 1–7 days postinoculation (DPI) in epithelial cells of the villi of the small intestine, primarily in the jejunum. No parasites were found in any of the extraintestinal tissues examined. Sporozoites invaded host cells and divided several times by endodyogeny to form as many as 16 zoites ( $3.7 \times 1.5 \mu\text{m}$ ) within a single parasitophorous vacuole. Usually, zoites escaped from host cells and entered other cells to undergo gametogony. Occasionally, zoites remained within the same parasitophorous vacuole in which they had developed and underwent gametogony to form micro- or macrogamonts. Microgamonts were  $12.1 \mu\text{m}$  in diameter and contained about 50–100 microgametes. Macrogamonts were  $11.7 \times 14.9 \mu\text{m}$ . An eosinophilic body (Eb) was associated with the surface of micro- and macrogamonts. In mature microgamonts, the Eb usually remained with the centrally located residual body, whereas it appeared to be incorporated into the oocyst wall in zygotes. Oocysts were present at 7 DPI, measured  $14.2 \times 18.1 \mu\text{m}$ , and were passed into the intestinal lumen unsporulated. Sloughing of the intestinal epithelium occurred at 7 DPI. Four of the 13 marmosets died during the infection, one at 3 and 5 DPI, and 2 at 7 DPI.

Hendricks (1974) redescribed *Isospora arctopitheci* Rodhain, 1933 which he found in naturally infected marmosets and a capuchin. Subsequently, Hendricks and Walton (1974) and Hendricks (1977) reported an unusually large host range for *I. arctopitheci* which included four families of carnivores, six genera of primates, and one marsupial species. We report herein the endogenous development of *I. arctopitheci* in one of its hosts, the marmoset *Saguinus geoffroyi*.

### Materials and Methods

Thirteen titi marmosets, *Saguinus geoffroyi*,<sup>3</sup> were purchased from animal vendors who had trapped the animals in the Republic of Panama. Animals were housed in separate cages which were cleaned daily and were fed commercial canned animal foods and fresh fruits from the United States. Feces of each animal, which were collected for a 10–14 day period before parasite inoculation and examined for parasites isolated by the formalin-ether technique (Ritchie, 1948) or by flotation in Sheather's sugar solution, were found to be negative for Coccidia.

Oocysts of *I. arctopitheci* were collected from the feces of naturally infected marmosets, suspended in a 2.5% (w/v)  $\text{K}_2\text{Cr}_2\text{O}_7$  solution and allowed to sporulate at 24°C for 5–7 days. After removing the  $\text{K}_2\text{Cr}_2\text{O}_7$ , each of the 13 marmosets was

<sup>1</sup> Based on a thesis submitted by A. T. Olcott to the Graduate School of the University of Montana in partial fulfillment of the requirements for the Master of Science degree.

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<sup>3</sup> In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council.



inoculated by gavage with a 2-ml aqueous suspension containing  $1-2 \times 10^5$  sporulated oocysts of *I. arctopitheci*. Animals died or were killed and necropsied at 1, 2, 3, 5, 6, 7, and 10 days postinoculation (DPI). Tissues, obtained from the duodenum, jejunum, ileum, cecum, colon, liver, lungs, kidneys, pancreas, spleen, diaphragm, and mesenteric lymph nodes, were fixed in Bouin's fluid, embedded in paraffin, sectioned and stained with hematoxylin-eosin. Specimens were examined and photographed by a model 18 Zeiss photomicroscope with planapochromatic lens. Size ranges are expressed in micrometers ( $\mu\text{m}$ ) followed by the mean in parentheses.

Specimens for scanning electron microscopy (SEM) were obtained from intestinal tissues which had been embedded previously in paraffin and sectioned for light microscopy. After the paraffin was removed by toluene, the tissues were placed in absolute ethanol, critical-point dried in a Tousimis Samdri Critical-point dryer, mounted on metal studs with the sectioned surface upward, coated with 25 nm gold:palladium (60:40) in a SPI sputter, and examined by a Zeiss Nova-scan 30 scanning electron microscope.

## Results

### Location and incidence

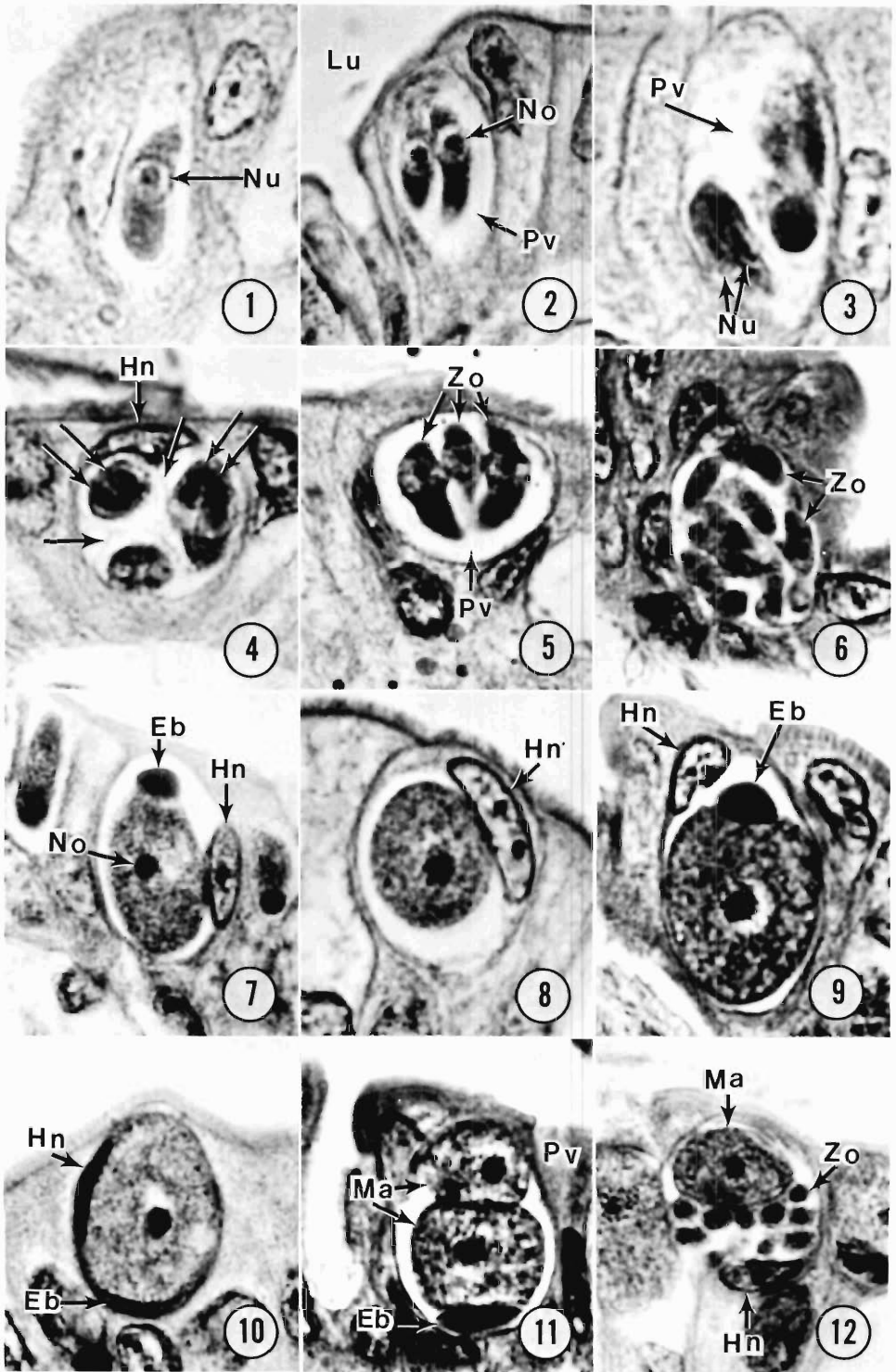
Endogenous stages of *I. arctopitheci* were seen in epithelial cells of the distal  $\frac{2}{3}$  of villi in the duodenum, jejunum, and upper ileum. The jejunum contained the greatest density of parasites. Parasites were usually situated beneath or alongside the host cell nucleus within a parasitophorous vacuole (Figs. 2-15, 19). As the parasites developed and increased in size, the host cell nucleus became flattened against the margin of the cell (Figs. 4, 7-10, 12, 15). Some epithelial cells were multiply infected (Fig. 4). Parasites were first seen at 1 DPI, were most numerous at 7 DPI, and were not seen at 10 DPI. At 7 DPI, a single young macrogamont was seen within the lumen of a blood vessel in the lamina propria of the jejunum. Parasites were not seen in any of the other tissues examined.

### Asexual development

Intracellular sporozoites, which were seen only at 1 and 3 DPI (Fig. 1), were crescent-shaped with a blunt posterior and a pointed anterior end, measured  $8.5-10.5 \times 2.5-3.5$  ( $9.4 \times 3.3$ ;  $N = 4$ ), and contained a spheroidal nucleus and nucleolus which were  $3.2 \mu\text{m}$  and  $2 \mu\text{m}$  in diameter, respectively. Asexual stages which were present at 3-7 DPI, appeared to multiply by endodyogeny (Figs. 3, 4) which resulted in two daughter zoites (Fig. 2). Zoites in pairs each measured  $6.5-10 \times 2.5-5.5$  ( $7.6 \times 2.3$ ;  $N = 24$ ) and had a nucleus and nucleolus which were 2.3 and 1.5 in diameter, respectively. Usually, zoites remained within their original parasitophorous vacuole and underwent one to three additional generations of endodyogeny to form as many as 16 zoites (Figs. 5, 6). Zoites became progressively smaller with each generation of endodyogeny. Zoites in a single parasitophorous vacuole containing 12-16 zoites measured  $3-4 \times 1.5$  ( $3.7 \times 1.5$ ;  $N = 50$ ).

### Sexual development

Immature gamonts were present at 5-7 DPI; mature gamonts at 6 and 7 DPI. Immature macrogamonts were recognized easily by their prominent nucleus and



nucleolus (Figs. 7, 8, 11). Immature micro- and macrogamonts could also be differentiated from asexual stages by an eosinophilic body (Eb) which was present at the surface of each gamont (Figs. 7, 9, 11, 13). In intermediate gamonts, the Eb was hemispherical, measured  $3.5\text{--}9.6 \times 0.9\text{--}3.5$  ( $5.7 \times 2.3$ ;  $N = 15$ ) and was located at one end of the parasite (Figs. 7, 13). The Eb increased in size coincidentally with an increase in the size of the gamont. In zygotes, the Eb became flattened and spread over the surface of the organism (Figs. 10, 23, 24) to form a uniform eosinophilic layer,  $0.5\text{--}1 \mu\text{m}$  thick, which eventually appeared to become part of the outer layer of the oocyst wall. By SEM, the surface of the Eb was continuous with the surface of the macrogamont (Figs. 23, 24). Fingerlike processes were present on the surfaces of relatively small Eb's and some processes appeared to contact the inner surface of the parasitophorous vacuole (Fig. 24). Such fingerlike processes were smaller or absent on the surfaces of larger Eb's (Fig. 23).

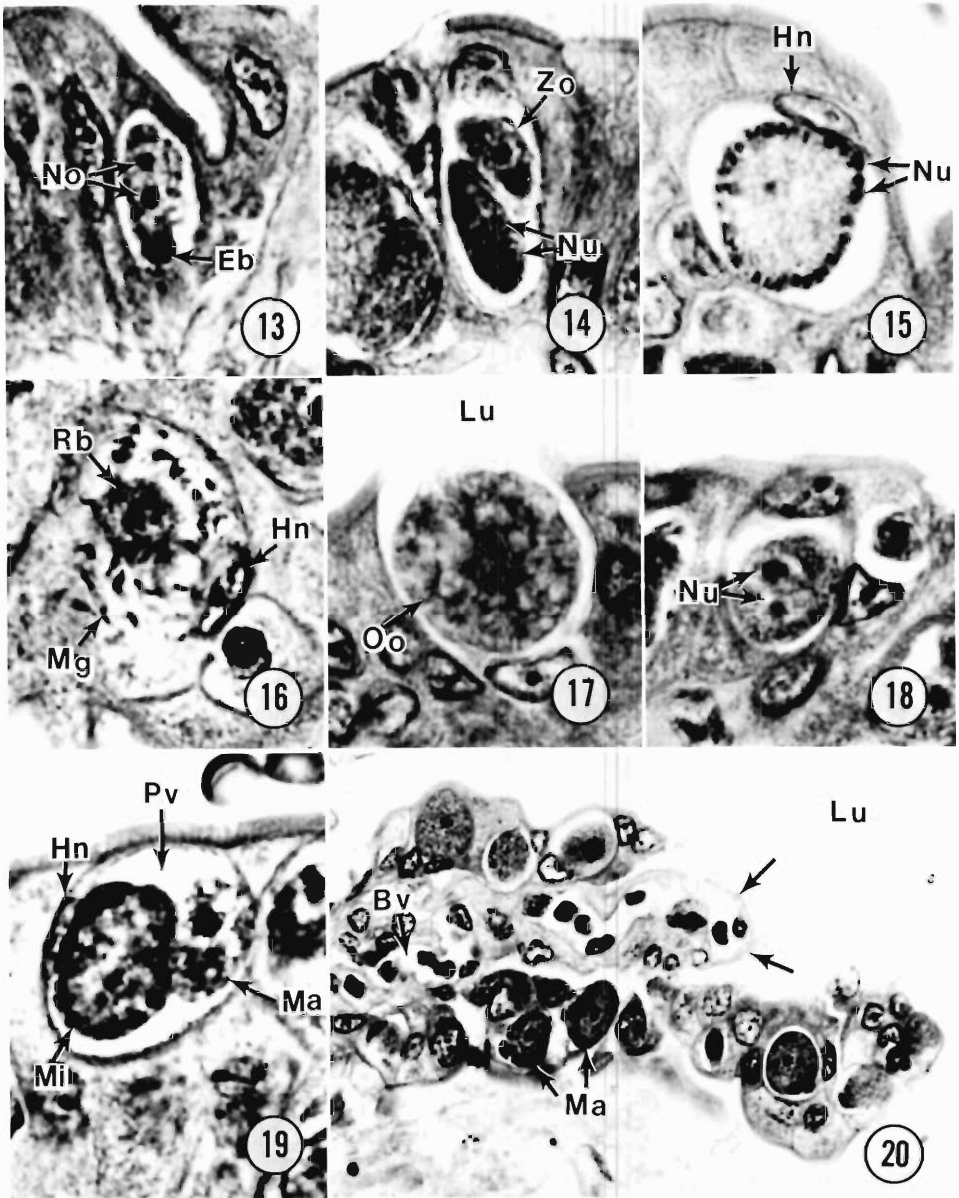
Intermediate macrogamonts were elongate and measured  $10\text{--}17.5 \times 6\text{--}12$  ( $13.2 \times 8.1$ ;  $N = 20$ ) (Fig. 7), whereas mature macrogamonts were ovoid and measured  $12\text{--}18.5 \times 9\text{--}15$  ( $14.9 \times 11.7$ ;  $N = 26$ ).

Several nuclear divisions occurred in young microgamonts while the organism was still elongate (Figs. 13, 14). As nuclear division progressed, the nuclei became smaller and indistinct (compare Figs. 13 and 14). The nucleolus was prominent in young microgamonts (Figs. 13, 14), but was not visible in the nuclei of intermediate and nearly mature microgamonts (Fig. 15). Intermediate microgamonts had numerous nuclei distributed randomly throughout their cytoplasm, whereas the nuclei were arranged at the periphery of nearly mature gamonts (Fig. 15). Folding or segmenting of the microgamont was not seen. The Eb was usually located within the central residual body or occasionally at the margin of the gamont. Mature microgamonts measured  $9.5\text{--}14$  ( $12.1$ ;  $N = 14$ ) in diameter and contained about 50–100 microgametes which were arranged peripherally about a central residual body (Figs. 16, 21). By SEM, microgametes were found to be biflagellate and measured  $2\text{--}2.7 \times 0.5\text{--}0.7$  ( $2.3 \times 0.6$ ;  $N = 20$ ).

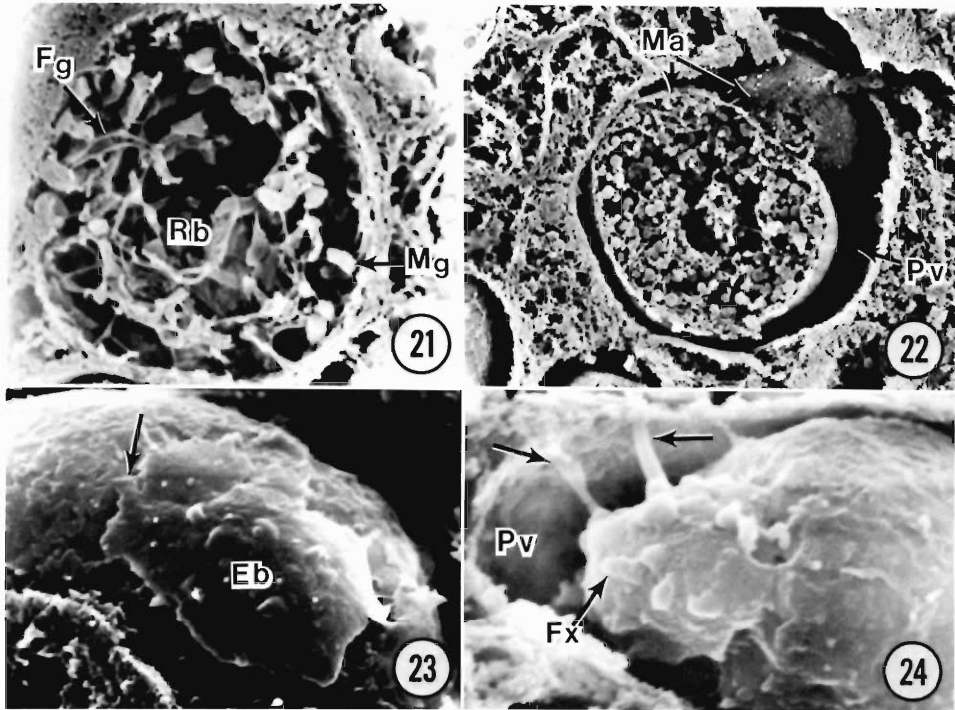
In some specimens, macro- or microgamonts were present within a host cell

←

**Figures 1–12.** Photomicrographs of the endogenous stages of *Isospora arctopitheci* in the small intestine of marmosets. Abbreviations for figures: Bv, blood vessel; Eb, eosinophilic body; Fg, flagellum of microgamete; Fx, fingerlike extension of eosinophilic body; Hn, host cell nucleus; Lu, lumen of intestinal tract; Ma, macrogamont; Mi, microgamont; No, nucleolus of parasite nucleus; Nu, parasite nucleus; Oo, oocyst; Pv, parasitophorous vacuole; Rb, residual body; Zo, zoite. 1. Sporozoite in intestinal epithelial cell;  $\times 2,000$ . 2. Two zoites in the same parasitophorous vacuole (Pv);  $\times 2,200$ . 3. Portions of three organisms in the same parasitophorous vacuole (Pv); note the two nuclei (Nu) in one organism which is undergoing endodyogeny;  $\times 1,700$ . 4. Three organisms in the same host cell; note that two organisms are undergoing endodyogeny (double arrows) and that a membrane (single arrows) separates each of the three organisms;  $\times 2,200$ . 5. Three zoites in the same parasitophorous vacuole;  $\times 2,200$ . 6. Group of about 10 relatively small zoites (Zo) in the same parasitophorous vacuole;  $\times 2,400$ . 7. Intermediate macrogamont with prominent nucleolus (No) and eosinophilic body (Eb);  $\times 2,000$ . 8. Intermediate macrogamont;  $\times 2,100$ . 9. Mature macrogamont with centrally located nucleus and nucleolus, and eosinophilic body (Eb);  $\times 2,000$ . 10. Zygote; note eosinophilic body (Eb) and flattened host cell nucleus (Hn);  $\times 2,200$ . 11. Two macrogamonts (Ma) in the same parasitophorous vacuole (Pv);  $\times 2,100$ . 12. Several zoites (Zo) and a macrogamont (Ma) within the same parasitophorous vacuole;  $\times 2,200$ .



Figures 13–20. Photomicrographs of the endogenous stages of *Isospora arctopitheci*. 13. Young microgamont with two nuclei, each with a prominent nucleolus (No), and an eosinophilic body (Eb);  $\times 2,200$ . 14. Zoite (Zo) and microgamont with several nuclei (Nu) in the same parasitophorous vacuole;  $\times 2,200$ . 15. Nearly mature microgamont with peripheral nuclei (Nu);  $\times 2,200$ . 16. Mature microgamont with numerous microgametes (Mg) and residual body (Rb);  $\times 2,200$ . 17. Oocyst (Oo); host cell appears to have lysed;  $\times 2,200$ . 18. Macrogamont with two nuclei (Nu);  $\times 2,200$ . 19. Macrogamont (Ma) and microgamont (Mi) in the same parasitophorous vacuole (Pv);  $\times 2,100$ . 20. Tip of villus showing sloughed epithelium (arrows); note numerous parasites in the epithelium, and blood vessel (Bv) in lamina propria;  $\times 650$ . Abbreviations as in Figures 1–12.



Figures 21–24. Scanning electron micrographs of the sexual stages of *I. arctopiheci*. 21. Mature macrogamont containing several microgametes (Mg) with two flagella (Fg), and a central residual body (Rb);  $\times 3,300$ . 22. Two macrogamonts (Ma) within the same parasitophorous vacuole (Pv);  $\times 2,400$ . 23. Eosinophilic body (Eb) which appears to have spread over the surface of the macrogamont; note small fingerlike extensions of the Eb and that the Eb is continuous (single arrow) with the thin outer layer of the organism;  $\times 12,800$ . 24. Eosinophilic body (Eb) on the surface of a young gamont; note fingerlike extensions (Fx) of the Eb and that two Fx contact the inner surface of the parasitophorous vacuolar (Pv) membrane (single arrow);  $\times 9,500$ . Abbreviations as in Figures 1–12.

parasitophorous vacuole which also contained several merozoites or 1–3 other micro- or macrogamonts (Figs. 11, 12, 19, 22). In a few specimens, a micro- and macrogamont were seen within the same parasitophorous vacuole (Fig. 19). Occasionally, young and intermediate macrogamonts each with 2–4 prominent nuclei were seen (Fig. 18).

### Oocysts

Oocysts, which were present only at 7 DPI, measured  $15.7\text{--}19.1 \times 10.4\text{--}18.3$  ( $18.1 \times 14.2$ ;  $N = 20$ ), had an indistinct oocyst wall and a granular, eosinophilic sporont (Fig. 17).

### Pathology

Four of the 13 marmosets died during the infection; one at each 3 and 5 DPI, and 2 at 7 DPI. The two marmosets that died at 7 DPI had bloody diarrhea for 2 days before death, whereas the animals that died at 3 and 5 DPI showed no noticeable signs of intestinal disorder.

Host cells containing oocysts showed various degenerative changes such as loss of the brush border and lysis (Fig. 17). Destruction of several contiguous epithelial cells caused sloughing of the intestinal epithelium, especially at the tips of the villi, which exposed the lamina propria to the intestinal lumen (Fig. 20).

### Discussion

The endogenous development of *I. arctopitheci* in the small intestine of titi marmosets was similar to that described for other isosporids (Dubey, 1978a, 1979; Dubey and Frenkel, 1972; Dubey and Mahrt, 1978; Lepp and Todd, 1974; Lindsay et al., 1980; Mahrt, 1967; Shah, 1971) except that asexual multiplication occurred exclusively by endodyogeny. Merogony in addition to endodyogeny has been found to occur in *I. neorivolta* (Dubey and Mahrt, 1978) and *I. ohioensis* (Dubey, 1978a) in dogs, *I. rivolta* (Dubey, 1979) and *Toxoplasma gondii* (Dubey and Frenkel, 1972) in cats, and *I. suis* (Lindsay et al., 1980) in pigs. In cell cultures, sporozoites of *I. canis* (Fayer and Mahrt, 1972), *I. felis* (Fayer and Thompson, 1974), and *I. rivolta* (Fayer, 1972) multiply by endodyogeny only. As far as we know, *I. arctopitheci* is the first coccidian found to undergo asexual multiplication exclusively by endodyogeny.

Zoites of *I. arctopitheci* usually remained within the same parasitophorous vacuole in which they had developed and underwent several generations of endodyogeny to form as many as 16 zoites. Such development in which several generations of merogony or endodyogeny occur within the same parasitophorous vacuole is known to occur in vivo or in vitro in other *Coccidia* such as various *Eimeria* species (see review by Speer, 1981), *I. canis* (Lepp and Todd, 1974), *I. felis* (Shah, 1971), *I. ohioensis* (Dubey, 1978a), *I. neorivolta* (Dubey and Mahrt, 1978), *I. rivolta* (Dubey, 1979), *T. gondii* (Dubey and Frenkel, 1972), and *Hammondia hammondi* (Sheffield et al., 1976).

Certain merozoites of *E. magna* (Speer and Danforth, 1976), *E. nieschulzi* (Sibert and Speer, 1980), *I. suis* (Lindsay et al., 1980), and zoites of *I. arctopitheci* (present study) developed into macro- or microgamonts without leaving their original parasitophorous vacuole. In the present study of *I. arctopitheci*, usually macro- or microgamonts only occurred in the same parasitophorous vacuole. However, in *I. suis* (Lindsay et al., 1980) as well as *I. arctopitheci*, a macro- and microgamont were occasionally seen in the same parasitophorous vacuole. Such observations indicate that a single zoite is capable of giving rise to both macro- and microgamonts or that two merozoites penetrated the same host cell and were somehow incorporated within the same parasitophorous vacuole.

In the present study, we occasionally observed multinucleate macrogamonts of *I. arctopitheci*. Multinucleate macrogamonts have been reported for *Eimeria magna* (Speer, 1979, 1982) and *E. tenella* (Doran and Augustine, 1973) which were considered to be oddities of in vitro cultivation. Speer (1979) found that multinucleate macrogamonts of *E. magna* were formed by the fusion of several organisms which were in direct surface contact within the same parasitophorous vacuole of the host cell. Multinucleate macrogamonts of *I. arctopitheci* probably form by a similar mechanism since in the present study several macrogamonts were often found to occupy the same parasitophorous vacuole. Some multinucleate macrogamonts of *E. magna* were found to form unusually large multinu-

cleate oocysts in cultured cells (Speer, 1982). Such multinucleate oocysts were not observed in *I. arctopitheci*.

The eosinophilic body seen in gamonts of *I. arctopitheci* resembles the crescent body associated with intracellular sporozoites, meronts and/or gamonts of *E. auburnensis* (Chobotar et al., 1969), *E. bovis* (Fayer and Hammond, 1967; Hammond et al., 1946), *E. leuckarti* (Barker and Remmler, 1972), *E. nieschulzi* (Sibert and Speer, 1981), *E. ninakohlyakimovae* (Kelley and Hammond, 1972; Wacha et al., 1971), and *E. zuernii* (Speer et al., 1973). Both bodies are eosinophilic. However, in contrast to the homogeneous eosinophilic body of *I. arctopitheci*, crescent bodies are granular (Kelley and Hammond, 1972; Sibert and Speer, 1981) and are seldom associated directly with the surface of the parasite. Also, crescent bodies do not appear to be incorporated into the developing parasite, whereas the eosinophilic body appeared to become part of the outer layer of the oocyst wall in *I. arctopitheci*.

The histologic lesions caused by *I. arctopitheci* are similar to those described for *I. neorivolta* (Dubey and Mahrt, 1978) and *I. ohioensis* (Dubey, 1978b) in dogs and *I. rivolta* in cats (Dubey, 1979), in which necrosis and desquamation of the tips of villi occurred in those areas of the intestine occupied by the parasite. *Isospora ohioensis* (Dubey, 1978b) and *I. rivolta* (Dubey, 1979; Dubey and Streitl, 1976) are pathogenic to newborn pups and kittens, respectively, but are non-pathogenic to weaned hosts. In contrast, *I. arctopitheci* is pathogenic to adult marmosets.

### Literature Cited

- Barker, I. K., and O. Remmler. 1972. The endogenous development of *Eimeria leuckarti* in ponies. *J. Parasitol.* 58:112-122.
- Chobotar, B., D. M. Hammond, and M. L. Miner. 1969. Development of the first-generation schizonts of *Eimeria auburnensis*. *J. Parasitol.* 55:385-397.
- Doran, D. J., and P. L. Augustine. 1973. Comparative development of *Eimeria tenella* from sporozoites to oocysts in primary kidney cell cultures from gallinaceous birds. *J. Protozool.* 20:658-661.
- Dubey, J. P. 1978a. Life-cycle of *Isospora ohioensis* in dogs. *Parasitology* 77:1-11.
- Dubey, J. P. 1978b. Pathogenicity of *Isospora ohioensis* in dogs. *J. Am. Vet. Med. Assoc.* 173:192-197.
- Dubey, J. P. 1979. Life cycle of *Isospora rivolta* (Grassi, 1879) in cats and mice. *J. Protozool.* 26:433-443.
- Dubey, J. P., and J. K. Frenkel. 1972. Cyst-induced toxoplasmosis in cats. *J. Protozool.* 19:155-177.
- Dubey, J. P., and J. L. Mahrt. 1978. *Isospora neorivolta* sp. n. from the domestic dog. *J. Parasitol.* 64:1067-1973.
- Dubey, J. P., and R. H. Streitl. 1976. *Isospora felis* and *I. rivolta* infections in cats induced by mice or oocysts. *Br. Vet. J.* 132:649-651.
- Fayer, R. 1972. Cultivation of feline *Isospora rivolta* in mammalian cells. *J. Parasitol.* 58:1207-1208.
- Fayer, R., and D. M. Hammond. 1967. Development of first-generation schizonts of *Eimeria bovis* in cultured bovine cells. *J. Protozool.* 14:764-772.
- Fayer, R., and J. L. Mahrt. 1972. Development of *Isospora canis* (Protozoa; Sporozoa) in cell culture. *Z. Parasitenkd.* 38:313-318.
- Fayer, R., and D. E. Thompson. 1974. *Isospora felis*: Development in cultured cells with some cytological observations. *J. Parasitol.* 60:160-168.
- Hammond, D. M., G. W. Bowman, L. R. Davis, and R. T. Simms. 1946. The endogenous phase of the life cycle of *Eimeria bovis*. *J. Parasitol.* 32:409-427.
- Hendricks, L. D. 1974. A redescription of *Isospora arctopitheci* Rodhain, 1933 (Protozoa: Eimeriidae) from primates of Panama. *Proc. Helminthol. Soc. Wash.* 41:229-233.

- Hendricks, L. D.** 1977. Host range characteristics of the primate coccidian, *Isospora arctopitheci* Rodhain, 1933 (Protozoa: Eimeriidae). *J. Parasitol.* 63:32-35.
- Hendricks, L. D., and B. C. Walton.** 1974. Vertebrate intermediate hosts in the life cycle of an isosporan from a non-human primate. *Proc. Int. Congr. Parasitol.* 1:96-97.
- Kelley, G. L., and D. M. Hammond.** 1972. Fine structural aspects of early development of *Eimeria ninakohlyakimovae* in cultured cells. *Z. Parasitenkd.* 38:271-284.
- Lepp, D. L., and K. S. Todd, Jr.** 1974. Life cycle of *Isospora canis* Nemeseri, 1959 in the dog. *J. Protozool.* 21:199-206.
- Lindsay, D. S., B. P. Stuart, B. E. Wheat, and J. V. Ernst.** 1980. Endogenous development of the swine coccidium, *Isospora suis* Biester 1934. *J. Parasitol.* 66:771-779.
- Mahrt, J. L.** 1967. Endogenous stages of the life cycle of *Isospora rivolta* in the dog. *J. Protozool.* 14:754-759.
- Ritchie, L. L.** 1948. An ether sedimentation technique for routine stool examinations. *Bull. U.S. Army Med. Dep.* 8:326.
- Shah, H. L.** 1971. The life cycle of *Isospora felis* Wenyon, 1923, a coccidian of the cat. *J. Protozool.* 18:3-17.
- Sheffield, H. G., M. L. Melton, and F. A. Neva.** 1976. Development of *Hammondia hammondi* in cell cultures. *Proc. Helminthol. Soc. Wash.* 43:217-225.
- Sibert, G. J., and C. A. Speer.** 1980. Fine structure of zygotes and oocysts of *Eimeria nieschulzi*. *J. Protozool.* 27:374-379.
- Sibert, G. J., and C. A. Speer.** 1981. Fine structure of nuclear division and microgametogony of *Eimeria nieschulzi* Dieben, 1924. *Z. Parasitenkd.* 66:179-190.
- Speer, C. A.** 1979. Further studies on the development of gamonts and oocysts of *Eimeria magna* in cultured cells. *J. Parasitol.* 65:591-598.
- Speer, C. A.** 1982. The coccidia, in J. B. Jensen (ed.). *In Vitro Cultivation of Protozoan Parasites of Man and Domestic Animals*, CRC Press, Inc. (In press.)
- Speer, C. A., and H. D. Danforth.** 1976. Fine-structural aspects of microgametogenesis of *Eimeria magna* in rabbits and kidney cell cultures. *J. Protozool.* 23:109-115.
- Speer, C. A., A. J. deVos, and D. M. Hammond.** 1973. Development of *Eimeria zuernii* in cell cultures. *Proc. Helminthol. Soc. Wash.* 40:160-163.
- Wacha, R. S., D. M. Hammond, and M. L. Miner.** 1971. The development of the endogenous stages of *Eimeria ninakohlyakimovae* (Yakimoff and Rastegaieff, 1930) in domestic sheep. *Proc. Helminthol. Soc. Wash.* 38:167-180.



## Varying the Serum Component in Axenic Cultures of *Naegleria fowleri*

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**ABSTRACT:** Sera from representatives of five classes of vertebrates varied greatly in their ability to support growth of *Naegleria fowleri* in axenic cultures. Of the 17 sera tested, calf serum produced the highest cell yields while fetal calf serum produced the lowest cell yields. Serum proteins, including  $\alpha$ -,  $\beta$ -, and  $\gamma$ -globulins and albumin, which comprise the various Cohn fractions, were used in place of serum. The globulin-containing fractions yielded equivalent growth which, however, was somewhat less than that obtained with calf serum. Cell yields were lower with albumin than with the serum globulin fractions. Several lipids (phosphatidylcholine, sphingomyelin, phosphatidylethanolamine, and cholesterol), present in brain and serum, when added to Nelson medium base, enhanced growth of *N. fowleri*.

The media used for axenic cultivation of *Naegleria fowleri* contain various kinds and concentrations of serum. Liquid media developed specifically for growing *N. fowleri* by Červa (1969), Nelson (unpublished, see Weik and John, 1977), and Chang (1978) contain horse serum, calf serum, and fetal calf serum, respectively. A medium originally developed for axenic cultivation of *N. gruberi* (Balamuth, 1964) but used as a growth medium for *N. fowleri* also contains calf serum.

For good growth, i.e., high cell yield and rapid generation time, *N. fowleri* requires serum in the liquid media presently being used. Therefore, in this study the concentration of calf serum, various kinds of serum, serum fractions, and possible serum substitutes were evaluated for their ability to support growth of *N. fowleri* in liquid axenic cultures.

### Materials and Methods

**AMEBA STRAIN AND MAINTENANCE:** The LEE strain (ATCC #30894) of *N. fowleri* was used in all experiments. It was isolated from cerebrospinal fluid of a fatal human case of primary amebic meningoencephalitis at the Medical College of Virginia (MCV) in 1968 by E. C. Nelson. Two sub-lines of the LEE strain were used and should be identified. The two lines differed only in the way they had been maintained since isolation. The one line, used in all experiments, has been maintained in our laboratory in Nelson medium (Weik and John, 1977) consisting of Page ameba saline (Page, 1967) supplemented with 0.1% (w/v) Panmede liver digest (Harrison & Crosfield, Inc., Bronxville, New York), 0.1% (w/v) glucose, and 2% (v/v) calf serum (Gibco). For these experiments, amebae were maintained unagitated in 100 ml of Nelson medium in 75-cm<sup>2</sup> tissue culture flasks (Falcon Plastics) at 30°C and transferred weekly.

The other line of LEE strain, used only in one experiment, was obtained through the courtesy of R. J. Duma (Division of Infectious Diseases, MCV). This

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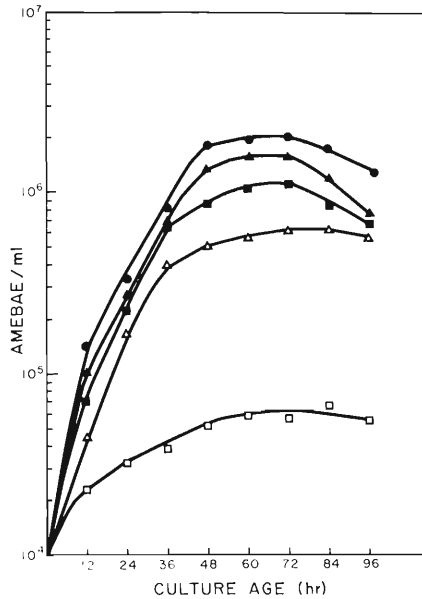


Figure 1. Growth of *N. fowleri* in Nelson medium base with various concentrations of calf serum. (□) 0%; (△) 1%; (■) 2%; (▲) 4%; (●) 8%.

stock of LEE had been maintained unagitated at 37°C in Nelson medium with 10% fetal calf serum.

**MEDIA AND CULTIVATION:** For all growth studies, 100 ml of each medium was prepared, adjusted to pH 5.0–5.5 (Weik and John, 1977), inoculated with 10<sup>4</sup> amebae/ml from a 72-hr stock culture, and then distributed in 25-ml amounts into four sterile, siliconized, screw-capped, 125-ml Erlenmeyer flasks. All cultures were incubated at 37°C at 100 rpm in a gyrotory shaker (New Brunswick).

Amebae were counted in a Coulter counter (model Z<sub>31</sub>) using procedures and settings described elsewhere (Weik and John, 1977). Cell counts were made at 12-hr intervals for 96 hr. Each point on a growth curve represents the average count of two samples from each of four replicate cultures.

These investigations gave rise to several changes and substitutions in the serum component of Nelson medium. Hence, Nelson medium base will refer to Page saline with 0.1% glucose and 0.1% Panmede liver digest (unchanged from Nelson medium); the serum component only will be varied. The percentage of calf serum was varied to give concentrations of 0, 1, 2, 4, and 8%. Various sera were substituted at 2%: fetal calf, dialyzed calf, gamma globulin-free calf, newborn calf, pig, lamb, chicken, rabbit, horse, and human (all from Gibco), *Rhesus* monkey (Flow Laboratories, Rockville, Maryland), dog (MCV Central Animal Facility), turtle and frog (animals supplied by Carolina Biological Supply Co., Burlington, North Carolina), mouse (animals supplied by Flow Research Animals, Inc., Dublin, Virginia), and fish (obtained from the James River, Richmond, Virginia). All sera were tested for mycoplasma (by respective suppliers) except dog, turtle, frog, mouse, and fish sera.

Blood for serum collection was obtained by cardiac puncture from frogs (*Rana*

**Table 1. Growth of *N. fowleri* in Nelson medium base with various sera or serum substitutes.**

| Type of serum (2%)           | Maximum cell yield (amebae/ml) | Time of maximum cell yield (hr) |
|------------------------------|--------------------------------|---------------------------------|
| Calf                         | $1.48 \times 10^6$             | 60                              |
| Pig                          | $1.36 \times 10^6$             | 84                              |
| Dialyzed calf                | $1.32 \times 10^6$             | 60                              |
| Monkey                       | $1.19 \times 10^6$             | 60                              |
| Newborn calf                 | $1.17 \times 10^6$             | 60                              |
| Lamb                         | $1.17 \times 10^6$             | 72                              |
| Turtle                       | $1.13 \times 10^6$             | 48                              |
| Dog                          | $9.99 \times 10^5$             | 60                              |
| Chicken                      | $9.69 \times 10^5$             | 60                              |
| Mouse                        | $8.74 \times 10^5$             | 96                              |
| Rabbit                       | $8.36 \times 10^5$             | 96                              |
| Frog                         | $8.28 \times 10^5$             | 48                              |
| Horse                        | $5.53 \times 10^5$             | 96                              |
| $\gamma$ -globulin-free calf | $5.01 \times 10^5$             | 96                              |
| Fish                         | $3.41 \times 10^5$             | 96                              |
| Human                        | $3.05 \times 10^5$             | 48                              |
| Fetal calf                   | $2.09 \times 10^5$             | 60                              |
| Cerebrospinal fluid*         | $1.04 \times 10^5$             | 60                              |
| Hemin†                       | $4.69 \times 10^4$             | 60                              |

\* 2% pooled normal CSF in place of serum.

† 5  $\mu$ g/ml hemin in place of serum.

*pipiens*), mice (DUB/ICR), and turtles (*Pseudemys scripta*). Blood from catfish (*Ictalurus nebulosus*) was collected in test tubes following decapitation. Blood was placed in serological test tubes at room temperature for an hour, ringed with a sterile applicator stick, and stored at 4°C overnight. Serum was separated from other blood components by centrifugation and stored at -20°C until needed.

Serum substitutes included pooled normal cerebrospinal fluid (from MCV patients) at 2%, hemin at 5  $\mu$ g/ml, Cohn serum fractions II, III, and IV, and bovine serum albumin, each at 0.1% (Sigma).

Brain and serum lipids were investigated for their ability to enhance the growth of *N. fowleri*. Using aseptic technique, L- $\alpha$ -phosphatidylcholine, sphingomyelin, and cerebrosides (Sigma) were solubilized in 95% ethanol and 0.1 ml was added to 100 ml of Nelson medium to give a concentration of 100  $\mu$ g/ml. L- $\alpha$ -phosphatidylethanolamine (Sigma), solubilized in ether, and cholesterol (Sigma), not previously solubilized, were added to Nelson medium at 100  $\mu$ g/ml. All cultures contained 1% calf serum and penicillin (200 U/ml) and streptomycin (200  $\mu$ g/ml). Control flasks contained 0.1% ethanol or 0.1% ether, with 1% calf serum and antibiotics. Antibiotics were used only in lipid-containing medium.

In order to ensure that growth differences of *N. fowleri* in Nelson medium were not merely a result of strain adaptation to maintenance with 2% calf serum, two lines of the LEE strain (maintained in different laboratories) were compared under each other's maintenance conditions and also under their own.

## Results

The percentage of calf serum in Nelson medium base was varied from 0 to 8% (Fig. 1). Even medium containing no serum supported some growth (about 2.5

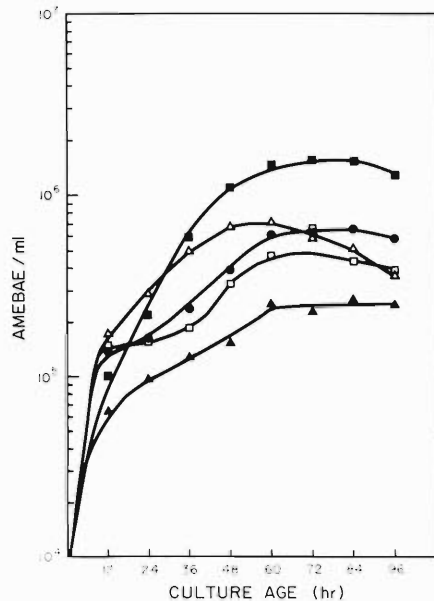


Figure 2. Growth of *N. fowleri* in Nelson medium base with various Cohn fractions, at a concentration of 0.1%, replacing calf serum. (●) Cohn fraction II ( $\gamma$ -globulins); ( $\Delta$ ) Cohn fraction III ( $\beta$ -globulins); ( $\square$ ) Cohn fraction IV ( $\alpha$ -globulins); ( $\blacktriangle$ ) bovine serum albumin (Cohn fraction V); ( $\blacksquare$ ) calf serum control (2%).

doublings) of *N. fowleri*. As the percentage of calf serum was increased from 1 to 8%, the amount of growth increased accordingly, with the growth curves nearly parallel. At 72 hr, the cell yield ( $1.21 \times 10^6$  amebae/ml) in the cultures containing 2% serum was about double that in cultures containing 1% serum. However, this relationship did not exist for 4 and 8% serum. Although the cell yield increased with the increased percentage of serum, the increased cell yield was not proportional to the amount of serum added.

Other forms of calf serum, various mammalian sera, and sera from representatives of other classes of vertebrates, as well as cerebrospinal fluid and hemin, were substituted for 2% calf serum in Nelson medium base (Table 1). Dialyzed calf and newborn calf sera supported good growth although cell yields were not quite as great as for calf serum, which supported the highest cell yields. Removing the gamma globulin component from calf serum resulted in substantially reduced growth. Although commonly used in media for growing *Naegleria*, fetal calf serum did not support good growth. In fact, of the 17 sera tested, it supported the lowest cell yields.

Pig serum proved to be the best of the other mammalian sera tested. Monkey, lamb, dog, mouse, rabbit, and horse sera supported growth ranging between about  $5 \times 10^5$  and  $10^6$  amebae/ml. Human serum produced markedly reduced cell yields.

Sera from representatives of other vertebrate classes including turtle, chicken, frog, and fish supported cell yields from about  $3 \times 10^5$  to  $10^6$  amebae/ml. Spinal fluid and hemin did not support much growth; cell yields were less than for any of the sera tested.

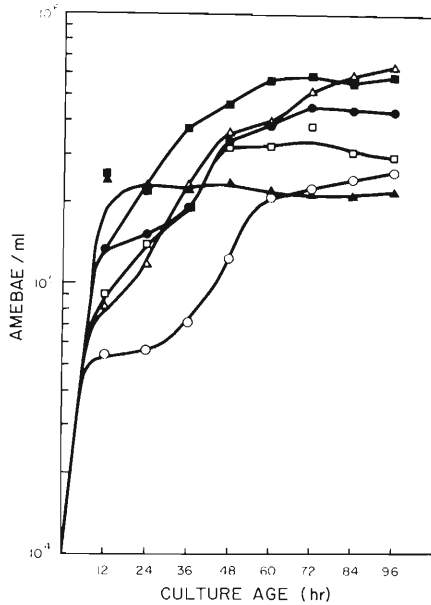


Figure 3. Growth of *N. fowleri* in Nelson medium base with 1% calf serum supplemented with various lipids (100  $\mu\text{g/ml}$ ). ( $\square$ ) cholesterol; ( $\Delta$ ) L- $\alpha$ -phosphatidylcholine; ( $\blacksquare$ ) sphingomyelin; ( $\bullet$ ) L- $\alpha$ -phosphatidylethanolamine; ( $\blacktriangle$ ) cerebrosides; ( $\circ$ ) calf serum control (1%).

Various Cohn fractions, containing primarily  $\alpha$ -,  $\beta$ -,  $\gamma$ -globulins or albumin, were used at a concentration of 0.1% in place of serum (Fig. 2). The globulin-containing Cohn fractions were intermediate between the calf serum control (best growth) and albumin (poorest growth) in their ability to support growth of *N. fowleri*.

The results for growth of *N. fowleri* in the presence of various lipids in Nelson medium base are illustrated in Figure 3. Cholesterol, sphingomyelin, L- $\alpha$ -phosphatidylcholine, and L- $\alpha$ -phosphatidylethanolamine all substantially increased growth as compared to the control which contained 1% serum alone. Growth in flasks containing cerebrosides did not change much after 12 hr, remaining rather constant at about  $2 \times 10^5$  amebae/ml.

Figure 4 represents the growth for the two lines of LEE strain maintained in Nelson medium base with either 2% calf serum or 10% fetal calf serum, and tested in Nelson medium base with both 2% calf serum and 10% fetal calf serum. The line of LEE normally maintained in Nelson medium with 2% calf serum grew to nearly the same level with 10% fetal calf serum as it did with 2% calf serum. LEE adapted to growing in Nelson medium base with 10% fetal calf serum grew far better with 2% calf serum than it did with 10% fetal calf serum.

### Discussion

Increasing the concentration of calf serum in Nelson medium resulted in higher cell yields. However, above 2% the increased growth was not proportional to the amount of serum added, indicating a limit to the amount of growth that can be achieved. This may be due to exhaustion of an essential growth factor or simply to the carrying capacity of the medium (Koch-Weik et al., 1980).

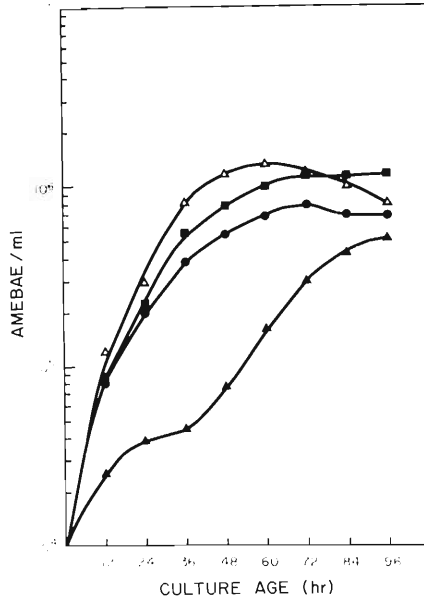


Figure 4. Growth of *N. fowleri*, maintained in Nelson medium with 2% calf serum; (△) in Nelson medium with 2% calf serum, (■) in Nelson medium base with 10% fetal calf serum. Growth of *N. fowleri*, maintained in Nelson medium base with 10% fetal calf serum; (●) in Nelson medium with 2% calf serum, (▲) in Nelson medium base with 10% fetal calf serum. Both isolates were LEE strain.

We did not examine the effect upon growth of calf serum concentrations greater than 8%. It has been shown, though, that calf serum concentrations greater than 10% inhibit the growth of *N. fowleri* (Pringle et al., 1979). The inhibition of growth was attributed to the toxicity of inorganic salts.

Of the 17 sera tested in Nelson medium base, calf serum supported the highest cell yields while fetal calf serum produced the lowest cell yields (7-fold less). To achieve equivalent growth, over five times as much fetal calf serum as calf serum was needed in Nelson medium (Fig. 4). Perhaps fetal calf serum lacks or has reduced amounts of growth factors such as serum globulins which when removed from serum resulted in substantially reduced growth (Table 1). Conversely, fetal calf serum contains certain maternal hormones such as prolactin and parturition hormones (Honn et al., 1975) which may have inhibited the growth of *N. fowleri*.

Some adaptation to a given medium (or serum) obviously does occur with prolonged maintenance. Nonetheless, the maintenance conditions may not be optimal for the amebae. This relationship is illustrated in Figure 4. When amebae which had been maintained for years with 10% fetal calf serum were placed in medium with 2% calf serum, cell yields were greater for cultures containing calf serum, even though 5-fold less serum was used. That cell yields for the LEE line normally maintained with fetal calf serum were less than for LEE amebae maintained with calf serum may be attributed to strain differences which arise through transfer of large populations of amebae in stock cultures. Also, individual investigators' techniques for ameba transfer and maintenance vary considerably. For example, we maintain our stock cultures at 30°C without antibiotics whereas

R. J. Duma (from whom we obtained the other LEE line) maintains his *N. fowleri* stocks at 37°C with penicillin and streptomycin. Undoubtedly, these seemingly insignificant differences probably affect the growth of the amebae.

All the sera in Table 1 increased the growth of *N. fowleri* over that achieved for Nelson medium base without serum (Fig. 1). Next to fetal calf serum, human serum supported the poorest growth. This may have been due to an amebicidal factor which reportedly is present in normal human serum (Carter, 1970).

Although *N. fowleri* amebae may be abundant in the cerebrospinal fluid of human cases, this substance does not appear to be an adequate growth substrate when used in place of serum in culture.

Band and Balamuth (1974) found hemin to be a suitable replacement for serum as a growth requirement for *N. gruberi*. However, replacing serum with hemin in Nelson medium base did not produce good growth of *N. fowleri*. In addition to the liver, glucose, and serum components of Nelson medium, the medium of Band and Balamuth also contains proteose peptone and yeast extract. The two organisms clearly have different nutritional requirements and, in general, a liquid medium which supports good growth of one species yields poor growth with the other. *Naegleria fowleri* grows better in simpler organic media whereas *N. gruberi* requires a more enriched medium (Haight and John, 1980).

Cohn fraction IV ( $\alpha$ -globulins) has been used successfully as a replacement for serum in growing *N. gruberi* (Balamuth, 1964; Fulton, 1970). The use of various Cohn fractions in Nelson medium base in place of serum indicated that whole serum was better than any of the individual fractions. Perhaps combinations of Cohn fractions would have supported growth comparable to that produced by serum; this was not tested.

A unique feature of the brain and myelin sheath is the large amount of specific lipids which they contain. Phospholipids, cerebrosides, and cholesterol collectively make up from 15 to 60% of the various parts of the nervous system (Volker and Woods, 1964). Demyelination has been described in human primary amebic meningoencephalitis (Chang, 1979; Duma et al., 1971) and has been attributed by Chang (1979) to be the result of the action of a specific phospholipolytic enzyme produced by *N. fowleri* amebae.

Perhaps lipids are nutritional substrates for *N. fowleri*; or they may act as binding or receptor sites for toxins or enzymes produced by the amebae. All of the lipids tested in this study enhanced the growth of *N. fowleri* in Nelson medium base containing 1% serum. The growth in this experiment (Fig. 3), however, was less than it was for the other experiments. It may be that growth was affected by the low (1%) serum level used in the cultures or by the presence in the medium of ethanol or ether, used to solubilize the lipids.

Since amebae grew better with than without lipids present in the medium, it is reasonable to assume that *N. fowleri* was able to utilize the lipids. Perhaps the high levels of lipids in the brain serve as an attractant and source of nutrition for *N. fowleri* amebae. Mice that have been inoculated intravenously or intraperitoneally with *N. fowleri* die from meningoencephalitis in the same way as mice inoculated intranasally (John et al., 1977). Considering the evidence, it would seem that *N. fowleri* amebae probably are neurotropic. Clearly, a potential area for research would be to examine host susceptibility to *N. fowleri* using animal models with lipid metabolism disorders.

### Acknowledgments

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### Literature Cited

- Balamuth, W.** 1964. Nutritional studies on axenic cultures of *Naegleria gruberi*. J. Protozool. 11 (Suppl.):19-20.
- Band, R. N., and W. Balamuth.** 1974. Hemin replaces serum as a growth requirement for *Naegleria*. Appl. Microbiol. 28:64-65.
- Carter, R. F.** 1970. Description of a *Naegleria* sp. isolated from two cases of primary amoebic meningoencephalitis, and of the experimental pathological changes induced by it. J. Pathol. 100:217-244.
- Červa, L.** 1969. Amoebic meningoencephalitis: axenic culture of *Naegleria*. Science 163:576.
- Chang, S. L.** 1978. Resistance of pathogenic *Naegleria* to some common physical and chemical agents. Appl. Environ. Microbiol. 35:368-375.
- Chang, S. L.** 1979. Pathogenesis of pathogenic *Naegleria* amoeba. Folia Parasitol. (Prague) 26:195-200.
- Duma, R. J., W. I. Rosenblum, R. F. McGehee, M. M. Jones, and E. C. Nelson.** 1971. Primary amoebic meningoencephalitis caused by *Naegleria*. Ann. Intern. Med. 74:923-931.
- Fulton, C.** 1970. Amebo-flagellates as research partners: the laboratory biology of *Naegleria* and *Tetramitus*. Methods Cell Physiol. 4:341-476.
- Haight, J. B., and D. T. John.** 1980. Growth of *Naegleria fowleri* in several axenic media. Folia Parasitol. (Prague) 27:207-212.
- Honn, K. V., J. A. Singley, and W. Chavin.** 1975. Fetal bovine serum: a multivariate standard (38804). Proc. Soc. Exp. Biol. Med. 149:344-347.
- John, D. T., R. R. Weik, and A. C. Adams.** 1977. Immunization of mice against *Naegleria fowleri* infection. Infect. Immun. 16:817-820.
- Koch-Weik, S. M., R. R. Weik, and D. T. John.** 1980. Inoculum size and pH affecting growth of *Naegleria*. Proc. Helminthol. Soc. Wash. 47:270-272.
- Page, F. C.** 1967. Taxonomic criteria for limax amoebae, with descriptions of 3 new species of *Hartmannella* and 3 of *Vahlkampfia*. J. Protozool. 14:499-521.
- Pringle, H. L., S. G. Bradley, and L. S. Harris.** 1979. Susceptibility of *Naegleria fowleri* to  $\Delta^9$ -tetrahydrocannabinol. Antimicrob. Agents Chemother. 16:674-679.
- Volker, J. F., and J. W. Woods.** 1964. Lipid metabolism. Pages 301-327 in J. C. Muhler, ed. Textbook of Biochemistry. The C. V. Mosby Co., St. Louis.
- Weik, R. R., and D. T. John.** 1977. Agitated mass cultivation of *Naegleria fowleri*. J. Parasitol. 63:868-871.



**Research Note**

**Metazoan Parasites of Three Species of Fish from Florida**

Bluegill sunfish (*Lepomis macrochirus* Rafinesque) are widespread in eastern North America, as are chain pickerel (*Esox niger* LeSueur) and redbfin pickerel (*Esox americanus* Gmelin) (Blair et al., 1968, Vertebrates of the United States. McGraw-Hill Book Co., New York. 616 pp.). Parasites of the former are well known, while those of the latter, piscivorous, organisms have received far less attention (Hoffman, 1967, Parasites of North American Freshwater Fishes. Univ. of Calif. Press, Berkeley. 486 pp.). Previous work on the parasites of these organisms has been qualitative, rather than quantitative. A study was, therefore, undertaken in June-July 1979 to obtain data on the metazoan parasites of bluegills, and the two species of pickerel.

Sixty fish (40 bluegill sunfish, 10 chain pickerel, 10 redbfin pickerel) were collected using a 640 volt alternating DC electrofisher in three Florida lakes. The three lakes were Orange Lake (~4,921 surface hectares, in Alachua and Marion counties), Lake Pearl (~23.5 surface hectares, Orange County), and Lake Wales (~140 surface hectares, Polk County). All specimens were placed on ice immediately after capture, and then measured (mm) and weighed (g), before being deep frozen for later examination. The fish were necropsied, and parasites found treated by conventional methods (Fernando et al., 1972, Univ. Waterloo Biol. Ser. 12). Table 1 details the meristic characters of the fish examined, as it is well known that different ages/sizes of fish, and those from different geographical locations, often have different parasite faunas. Bluegills from Lake Pearl were significantly lighter ( $P < 0.001$ ) than those from the other two lakes, despite the fact that the mean total lengths of fish samples from the three lakes were similar.

Orange Lake bluegills had a significantly higher prevalence and intensity of acanthocephalans and parasitic copepods than did bluegills from the other two lakes (Table 2). The pickerel examined also showed a high prevalence of Acanthocephala. The low prevalence of *Leptorhynchoides thecatus* in bluegills from Lake Pearl is unexpected, particularly as 50% of the redbfin pickerel caught therein were infected. This observation suggests differences in the diet of the bluegills

**Table 1.** Meristic characters of bluegill sunfish, chain and redbfin pickerel examined for metazoan parasites.

| Origin (N)       | Total length (mm)<br>mean $\pm$ SD (range) | Weight (g)<br>mean $\pm$ SD (range) |
|------------------|--|-------------------------------------|
| Bluegill sunfish |  |                                     |
| Orange Lake (15) | 148 $\pm$ 33 (111-228)                     | 77 $\pm$ 70 (25-274)                |
| Lake Pearl (15)  | 159 $\pm$ 28 (128-210)                     | 45 $\pm$ 32 (18-110)                |
| Lake Wales (10)  | 132 $\pm$ 28 (103-178)                     | 82 $\pm$ 53 (39-186)                |
| Chain pickerel   |  |                                     |
| Orange Lake (10) | 534 $\pm$ 90 (400-645)                     | 459 $\pm$ 143 (271-681)             |
| Redfin pickerel  |  |                                     |
| Lake Pearl (10)  | 228 $\pm$ 72 (135-386)                     | 84 $\pm$ 52 (39-202)                |

**Table 2. Metazoan parasites from bluegill sunfish, chain and redbin pickerel from three Florida Lakes.**

| Host                    | Total parasites*            | Parasite                        |  |                                  |                                 |                           |
|-------------------------|-----------------------------|---------------------------------|--|----------------------------------|---------------------------------|---------------------------|
|                         |                             | <i>Diplostomulum scheuringi</i> | <i>Posthodiplostomum minimum metacercariae</i> | <i>Contracaecum</i> sp. (larvae) | <i>Leptorhynchoides thecaus</i> | <i>Ergasilus</i> spp.†    |
| <b>Bluegill sunfish</b> |                             |                                 |  |                                  |                                 |                           |
| Orange Lake             | 15 (100) 79 ± 27<br>40-137  | 2 (13) 3 ± 2<br>1-5             | 15 (100) 27 ± 22<br>4-74                       | 6 (40) 4 ± 3<br>2-10             | 14 (93) 40 ± 16<br>19-77        | 10 (67) 19 ± 19<br>1-61   |
| Lake Pearl              | 15 (100) 39 ± 31<br>7-91    | 1 (7) 1<br>—                    | 14 (93) 36 ± 29<br>4-91                        | 4 (27) 1<br>—                    | 1 (7) 1<br>—                    | 7 (47) 1 ± 1<br>1-3       |
| Lake Wales              | 10 (100) 112 ± 70<br>19-227 | 1 (10) 1<br>—                   | 10 (100) 109 ± 71<br>19-227                    | — —<br>—                         | 6 (60) 4 ± 2<br>2-8             | — —<br>—                  |
|                         |                             |                                 |  |                                  |                                 | <i>Argulus versicolor</i> |
| <b>Chain pickerel</b>   |                             |                                 |  |                                  |                                 |                           |
| Orange Lake             | 10 (100) 6 ± 4<br>2-13      | 2 (20) 4 ± 3<br>2-5             | 2 (20) 3 ± 2<br>2-5                            | 3 (30) 2 ± 1<br>1-3              | 9 (90) 4 ± 3<br>1-12            | 2 (20) 4 ± 3<br>1-6       |
| <b>Redfin pickerel</b>  |                             |                                 |  |                                  |                                 |                           |
| Lake Pearl              | 7 (70) 4 ± 3<br>1-8         | 3 (30) 1 ± 1<br>1-2             | 3 (30) 1 ± 1<br>1-2                            | 1 (10) 1<br>—                    | 5 (50) 4 ± 2<br>1-6             | — —<br>—                  |

\* Values represent number of fish infected, percentage given in parentheses. Mean ± SD parasites/infected fish. Range of parasites/infected fish.

† Mixture of *E. caeruleus* and *E. centrarchidarum*.

in the three lakes. Digenea were most intense in Lake Wales bluegills, while overall intensity of infection with parasites in Lake Pearl fishes was significantly lower than in fish from other sample sites.

All the parasites recovered from the redbin pickerel, and the *Posthodiplostomum minimum* and *Contracaecum* sp. larvae from the chain pickerel are new host records. The *Contracaecum* larvae are almost certainly those of *C. spiculigerum* and/or *C. multipapillatum*, widespread and common nematodes in fish-eating birds in Florida (Courtney and Forrester, 1974, Proc. Helminthol. Soc. Wash. 41:89-93; Threlfall, 1982 Proc. Helminthol. Soc. Wash. 49:103-108).

*Diplostomulum scheuringi* was located in the vitreous humor of the eyes of the fishes, while *P. minimum* was recovered from transparent or opaque cysts in the stomach and intestinal walls, liver, kidneys, and swim bladder. Two fishes from

**Table 3. Intensity, and location, of infection with *Posthodiplostomum minimum* in bluegill sunfish from Florida.**

| Origin of host | Site of infection   |                        |                          |                           |                     |
|----------------|---------------------|------------------------|--------------------------|---------------------------|---------------------|
|                | Stomach             | Intestine              | Liver                    | Kidney                    | Swim bladder        |
| Orange Lake    | —                   | 6 (40) 6 ± 4*<br>1-12  | 15 (100) 16 ± 17<br>1-58 | 11 (73) 11 ± 10<br>2-35   | 2 (13) 2 ± 1<br>1-3 |
| Lake Pearl     | 5 (33) 3 ± 2<br>1-4 | 7 (47) 8 ± 13<br>1-37  | 14 (93) 22 ± 18<br>1-58  | 8 (53) 24 ± 22<br>2-60    | —                   |
| Lake Wales     | 1 (10) 1<br>—       | 6 (60) 22 ± 26<br>3-72 | 9 (90) 45 ± 28<br>15-115 | 10 (100) 52 ± 48<br>4-175 | —                   |

\* Values represent number of fish infected, percentage given in parentheses. Mean ± SD parasites/infected fish. Range of parasites/infected fish.

Lake Wales had cysts containing these organisms in their eyes. The intensity of infection in the various organs, and in fish from the different sample sites, varied greatly (Table 3). The liver and kidneys contained significantly ( $P < 0.001$ ) more parasites than other organs, while Lake Wales fishes were the most heavily infected. No differences were noted that could be related to length, weight, or sex of the host animals.

*Contracaecum* larvae were normally found associated with the stomach, intestinal wall, or swim bladder, and to a lesser extent with the liver and kidneys. Immature, and maturing, specimens of *Leptorhynchoides thecatus* were found in the intestine, and occasionally the stomach. Cystacanths of *L. thecatus* were found in three bluegills (one fish with three in the mesenteries and one in the liver, two other fish with one parasite in the liver) and one redbfin pickerel (five in the mesenteries). *Ergasilus* spp. were always attached to the gills.

The lack of adult helminths in these fish is somewhat surprising and worthy of further investigation, as is the fact that all three species carried essentially the same parasite fauna, suggesting an overlap in feeding habits. The larval nematodes may have been acquired by the pickerel at an early stage in their life before they became truly predatory. Some of the *L. thecatus* in the pickerel were undoubtedly acquired through predation on bluegills which can act as paratenic hosts for this acanthocephalan. Small differences were noted in the water chemistry (e.g., levels of the calcium, magnesium, organic nitrogen, ammonia nitrogen, nitrate nitrogen, total phosphate, and orthophosphate; pH; conductivity; chlorophyll a; turbidity) of the three lakes but none could explain the anomalies in prevalence and intensity of infection which were detected.

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*Research Note*

**Ectoparasites of the Rainbow Darter, *Etheostoma caeruleum* Storer, from Harrods Creek, Oldham County, Kentucky**

During the collection of several hundred rainbow darters, *Etheostoma caeruleum* Storer, for the purpose of initiating laboratory infections with the ciliate *Ichthyophthirius multifiliis*, 58 of these fish were selected at random and examined for their ectoparasite fauna. This brilliantly colored riffle-dwelling carnivore, which ranges up to approximately 70 mm (SL), is not of obvious economic importance, but may serve as a reservoir host for various parasites of fishes.

Fish were obtained with the aid of a 3-m minnow seine of 6.25-mm<sup>2</sup> mesh from 23 September 1973 through 5 October 1974 at three collection stations on Harrods Creek, Oldham Co., Kentucky. Specimens were transported to the laboratory live in styrofoam coolers containing creek water. They were necropsied by pithing and immediately examined under a dissecting microscope (40×) for the presence of ectoparasites. During the examination, fish were kept moist with creek water filtered through a 0.22 μm Millipore filter. First, the intact fish was examined and then each fin and gill arch was excised and reexamined separately. When protozoans were observed, the appropriate body or fin surface was gently scraped with a scalpel and the scrapings placed together with a drop of filtered creek water on a slide for viewing under higher magnification (100×). Gill filaments were macerated before further examination. Standard techniques were used in the preservation, staining, mounting, and identification of the parasites.

Ectoparasites were observed on 46 of 58 (79%) of the *E. caeruleum* examined. Twenty-nine fish harbored one species of parasite, 12 had two, and 5 had three. Table 1 shows the percent prevalence, intensity, and location of the parasites on the host.

Of the seven ectoparasite species recovered, *E. caeruleum* constitutes a new host record for five: *Trichodina globosa* Wellborn; *Epistylis* sp.; *Myxosoma* sp.; *Gyrodactylus etheostomae* Wellborn and Rogers; *Urocleidus moorei* Mizelle. *Trichodina globosa* has previously been found on the fins and body of the orangebelly darter, *E. radiosum* (Hubbs and Black) (Wellborn, Jr., 1967, J. Protozool. 14:412-414) in Fulton Co., Arkansas. *Myxosoma neurophila* Guilford was seen in the brain of the Johnny darter, *E. nigrum* Rafinesque, in Wisconsin (Guilford, 1963, J. Parasitol. 49:474-478). *Gyrodactylus etheostomae* has been reported previously on the fins and body of *E. radiosum* (Wellborn, Jr. and Rogers, 1967, J. Parasitol. 53:10-14) from Fulton Co., Arkansas, and *G. bretinae* Wellborn on the fins and body of the speckled darter, *E. stigmaeum* (Jordan) from Clay Co., Arkansas (Wellborn, Jr., 1967, Proc. Helminthol. Soc. Wash. 34:55-59). Mizelle (1940, Trans. Am. Microsc. Soc. 59:285-289) noticed *U. moorei* on fantail darters, *E. flabellare* Rafinesque, from Tennessee.

Harrises and Vickery (1970, Am. Midl. Nat. 84:437-443) identified a number of species of *Urocleidus* from various darters in Mississippi. Six species of *Etheostoma*, including *E. caeruleum* from the Rockcastle River at the Laurel-Pulaski Co. line in Kentucky, have been found to harbor *Pisciolaria reducta* Meyer (Bauer and Branson, 1975, Trans. Ky. Acad. Sci. 36:18-19). Glochidia larva are

**Table 1. Percent prevalence, intensity, and location of ectoparasites recovered from 58 rainbow darters, *Etheostoma caeruleum* Storer, from Oldham County, Kentucky.**

| Parasite                        | Percent prevalence | Intensity (mean and range) | Location               |
|---------------------------------|--------------------|----------------------------|------------------------|
| Protozoa                        |                    |                            |                        |
| <i>Trichodina globosa</i>       | 2                  | 13                         | Anal, caudal fins      |
| <i>Epistylis</i> sp.            | 7                  | 12 (1-38)                  | Pectoral, caudal fins  |
| <i>Myxosoma</i> sp.             | 5                  | 75 (50-100)                | Gills                  |
| Trematoda                       |                    |                            |                        |
| <i>Gyrodactylus etheostomae</i> | 55                 | 4 (1-29)                   | All fins, body surface |
| <i>Urocleidus moorei</i>        | 29                 | 5 (1-20)                   | Gills                  |
| Hirudinea                       |                    |                            |                        |
| <i>Pisciolaria reducta</i>      | 9                  | 1 (1)                      | Pectoral, caudal fins  |
| Pelecypoda                      |                    |                            |                        |
| Glochidia                       | 7                  | 9 (5-17)                   | Gills                  |

common on many species of freshwater fishes (Hoffman, 1967, *Parasites of North American Freshwater Fishes*, Univ. of Calif. Press, Berkeley. 486 pp.).

*Trichodina globosa*, *Epistylis* sp., and *G. etheostomae* were observed most frequently on the caudal fin, and *P. reducta* was found three times on the caudal fin and twice on the pectoral fins. Body surfaces were parasitized only by *G. etheostomae*.

Although hosts were examined during each month of the year, data were not treated statistically because of the small sample size. The following general trends regarding seasonal incidence of infection were noted however. *Gyrodactylus etheostomae* was observed on hosts during every month, but was most prevalent during January and February. *Urocleidus moorei* was most prevalent during June, July, and August and again during January and February. *Myxosoma* sp. was seen only during November, December, and January. Rawson and Rogers (1972, *Proc. Helminthol. Soc. Wash.* 39:159-162) found that numbers of *U. principalis* (Mizelle) and *U. furcatus* (Mueller) peaked in June, mid-September, and again in December on largemouth bass, *Micropterus salmoides* (Lacépède) in a Russell Co., Alabama reservoir. Rawson and Rogers (1972, *J. Wildl. Dis.* 8:255-260) also found five other species of ancycrocephalinaen trematodes abundant in the summer and again in December and January on bluegill, *Lepomis macrochirus* Rafinesque, in the same reservoir.

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*Research Note*

**New Host Records for Acanthocephala of  
Some Alabama Freshwater Fishes**

Williams (1974, Diss. Abstr. Int. 35:1461B) examined in excess of 5,000 marine, brackish, and freshwater fishes representing 252 species from Alabama and adjacent areas. A new genus and species (Williams, 1976, J. Parasitol. 62:102-104) and four new host records (Williams and Gaines, 1974, J. Mar. Sci. Ala. 2:135-148) have been reported from this material. Eleven new host records are reported here (Table 1) and a host-parasite list of freshwater fishes parasitized by Acanthocephala in the study is presented (Table 2). Locations are in Alabama unless otherwise noted.

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Table 1. New host records for Acanthocephala of some Alabama freshwater fishes.

| Parasite   | Host   | Locality   | Date                                 | USNM Coll. No. |
|--|--|--|--------------------------------------|----------------|
| <i>Neoechinorhynchus cylindratum</i><br>(Van Cleave, 1913) | <i>Carpiodes velifer</i> (Rafinesque)<br>highfin carpsucker    | Cahaba River, north of Highway 80, NW of Selma                                       | January 1973                         | 76526          |
|  | <i>Esox americanus</i> Gmelin<br>redfin pickerel               | Uphapee Creek, NE of Tuskegee, Macon County, and Line Creek, Macon-Montgomery County | 2 June 1969                          | 76527          |
|  | <i>Moxostoma lachneri</i> Robins and Raney<br>greater jumprock | Miller Creek, north of Valley, Lee County  | 12 February 1971<br>10 March 1972    | 76528          |
|  | <i>M. poecilurum</i> (Jordan)<br>blacktail redbhorse           | Miller Creek east of Valley, Hallawakee Creek, covered bridge, Lee County            | 12 February 1971<br>19 February 1969 |                |
|  | <i>Moxostoma</i> n. sp. cf. <i>poecilurum</i>                  | Lake Martin, west of Dadeville   | 27 January 1971                      | 76529          |
|  | <i>Ictalurus nebulosus</i> (LeSueur)<br>brown bullhead         | Miller Creek north of Valley, Lee County   | 10 March 1972                        | 76530          |
| <i>N. cylindratum</i> (encysted larva)                     | <i>Ictalurus nebulosus</i> (LeSueur)<br>brown bullhead         | Unnamed tributary of Loblockee Creek, NNE of Loachapoka, Lee County                  | 16 February 1972                     | 76531          |
|  | <i>Lepomis microlophus</i> (Gunther)<br>redecor sunfish        | Line Creek, Macon-Montgomery County  | 10 March 1972                        | 76532          |
|  | <i>L. punctatus</i> (Valenciennes)<br>spotted sunfish          | Uphapee Creek, NE of Tuskegee, Macon County  | 16 March 1970                        | 76533          |
|  | <i>Micropterus notius</i> Bailey and Hubbs<br>Savannah bass    | Ichetuckee River, east of Hildrith, Florida, Columbia County                         | 29 March 1969                        | 76534          |
| <i>Leptorhynchoides thecatus</i><br>(Linton, 1891)         | <i>Minytrema melanops</i> (Rafinesque)<br>spotted sucker       | Mouth of Santa Fe River, Gilchrist County, Florida                                   | 12 December 1972                     | 76535          |
|  | <i>Percina nigrofasciata</i> (Agassiz)<br>blackbanded darter   | Hallawakee Creek, NE of Opelika, Lee County  | 18 February 1971                     | 76536          |

**Table 2. Acanthocephala collected from freshwater fishes of Alabama and adjacent areas.**

| Host  | Parasite  |
|---|---|
| <i>Amia calva</i> Linnaeus, bowfin                                    | <i>Neoechinorhynchus cylindricus</i> (Van Cleave, 1913) |
| <i>Aphredoderus sayanus</i> (Gilliams), pirate perch                  | <i>Pilum pilum</i> Williams, 1976                       |
| <i>Carpionotus velifer</i> (Rafinesque), highfin carpsucker           | <i>Neoechinorhynchus cylindricus</i>                    |
| <i>Cyprinus carpio</i> Linnaeus, carp                                 | <i>Acanthocephalus</i> sp.                              |
| <i>Cyprinodon variegatus</i> Lacépède, sheepshead minnow              | <i>Atactorhynchus verecundum</i> Chandler, 1935         |
| <i>Dorosoma cepedianum</i> (LeSueur), gizzard shad                    | <i>Gracilisentis gracilisentis</i> (Van Cleave, 1913)   |
|   | <i>Tanaorhamphus longirostris</i> (Van Cleave, 1913)    |
| <i>Dorosoma petenense</i> (Gunther), threadfin shad                   | <i>Gracilisentis gracilisentis</i>                      |
| <i>Erimyzon sucetta</i> (Lacépède), lake chubsucker                   | <i>Neoechinorhynchus cylindricus</i>                    |
| <i>Esox americanus</i> Gmelin, redbreast pickerel                     | <i>Neoechinorhynchus cylindricus</i>                    |
|   | <i>Pilum pilum</i>                                      |
| <i>Fundulus grandis</i> Baird and Girard, gulf killifish              | <i>Leptorhynchoides thecatus</i> (Linton, 1891)         |
| <i>Fundulus jenkinsi</i> (Evermann), saltmarsh topminnow              | <i>Leptorhynchoides thecatus</i>                        |
| <i>Hypentelium etowanum</i> (Jordan), Alabama hogsucker               | <i>Acanthocephalus</i> sp.                              |
| <i>Ictalurus natalis</i> (LeSueur), yellow bullhead                   | <i>Pilum pilum</i>                                      |
| <i>Ictalurus nebulosus</i> (LeSueur), brown bullhead                  | <i>Neoechinorhynchus cylindricus</i> *                  |
|   | <i>Pilum pilum</i>                                      |
| <i>Ictalurus punctatus</i> (Rafinesque), channel catfish              | <i>Neoechinorhynchus cylindricus</i>                    |
| <i>Ictiobus bubalus</i> (Rafinesque), smallmouth buffalo              | <i>Neoechinorhynchus strigosum</i> Van Cleave, 1949     |
| <i>Lepomis auritus</i> (Linnaeus), redbreast sunfish                  | <i>Pilum pilum</i>                                      |
| <i>Lepomis cyanellus</i> Rafinesque, green sunfish                    | <i>Neoechinorhynchus cylindricus</i>                    |
|   | <i>Neoechinorhynchus cylindricus</i> *                  |
| <i>Lepomis gulosus</i> (Cuvier), warmouth                             | <i>Neoechinorhynchus cylindricus</i>                    |
|   | <i>Pilum pilum</i>                                      |
| <i>Lepomis macrochirus</i> Rafinesque, bluegill                       | <i>Eocollis arcanus</i> Van Cleave, 1947                |
|   | <i>Neoechinorhynchus cylindricus</i>                    |
|   | <i>Pilum pilum</i>                                      |
| <i>Lepomis megalotis</i> (Rafinesque), longear sunfish                | <i>Neoechinorhynchus cylindricus</i>                    |
| <i>Lepomis microlophus</i> (Gunther), redbreast sunfish               | <i>Leptorhynchoides thecatus</i>                        |
|   | <i>Neoechinorhynchus cylindricus</i> *                  |
| <i>Lepomis punctatus</i> (Valenciennes), spotted sunfish              | <i>Neoechinorhynchus cylindricus</i> *                  |
|   | <i>Pilum pilum</i>                                      |
| <i>Lucania parva</i> (Baird), rainwater killifish                     | <i>Leptorhynchoides thecatus</i>                        |
| <i>Micropterus coosae</i> Hubbs and Bailey, redeye bass               | <i>Acanthocephalus</i> sp.                              |
| <i>Micropterus notius</i> Bailey and Hubbs, Suwannee bass             | <i>Leptorhynchoides thecatus</i>                        |
| <i>Micropterus salmoides</i> (Lacépède), largemouth bass              | <i>Neoechinorhynchus cylindricus</i>                    |
|   | <i>Neoechinorhynchus cylindricus</i> *                  |
| <i>Minytrema melanops</i> (Rafinesque), spotted sucker                | <i>Leptorhynchoides thecatus</i>                        |
| <i>Moxostoma lachneri</i> Robins and Raney, greater jumprock          | <i>Neoechinorhynchus cylindricus</i>                    |
| <i>Moxostoma poecilurum</i> (Jordan), blacktail redbreast             | <i>Acanthocephalus</i> sp.                              |
|   | <i>Neoechinorhynchus cylindricus</i>                    |
| <i>Moxostoma</i> sp. n. cf. <i>poecilurum</i>                         | <i>Neoechinorhynchus cylindricus</i>                    |
| <i>Mugil curema</i> Valenciennes, white mullet                        | <i>Floridosentis elongatus</i> Ward, 1953               |
| <i>Paralichthys lethostigma</i> Jordan and Gilbert, southern flounder | <i>Leptorhynchoides thecatus</i>                        |
| <i>Percina nigrofasciata</i> (Agassiz), blackbanded darter            | <i>Leptorhynchoides thecatus</i>                        |

\* Encysted larval forms.



*Research Note*

**Ancyloid Snails as Hosts for *Posthodiplostomum minimum*  
(MacCallum, 1921) (Digenea: Diplostomatidae)**

Miller (1954, J. Parasitol. 40:255–270) described the cercaria of *Posthodiplostomum minimum* (MacCallum, 1921) from naturally infected *Physa heterostropha* Say in New York. Bedinger and Meade (1967, J. Parasitol. 53:985–988), working with natural infections among *Physa halei* Lea in East Texas, described another cercaria, distinguishable from that of Miller (1954, loc. cit.) by its lack of caudal bodies, arrangement of flagellets on the tail stem, and by having 13 rather than 10 pairs of flame cells in the body and tail.

Turner and Corkum (1979, Tulane Stud. Zool. Bot. 21:67–89) described a strigeid cercaria (designated *Cercaria* type III), which developed in southeastern Louisiana populations of freshwater limpet (ancyloid) snails. Three ancyloid species—*Ferrissia fragilis* (Tyron), *Hebetancyclus excentricus* (Morelet), and *Laevapex fuscus* (Adams)—hosted infections with this cercaria, which closely resembled that of Bedinger and Meade (1967, loc. cit.).

Our 8-mo survey of over 2,000 *L. fuscus*, from a freshwater marsh near Vinton in southwestern Louisiana, indicated a monthly prevalence of infection with *Cercaria* type III ranging from 0 to 7%.

Twenty-five hatchery-reared, fingerling sunfish, *Lepomis macrochirus* Rafinesque, were exposed for 24 hr to undetermined numbers of cercaria (type III) shed by *Laevapex fuscus*. Necropsy of fish at 43 days postexposure revealed each harboring from one to five larvae, identical in appearance to the description of *Posthodiplostomum minimum* metacercariae given by Hunter and Hunter (1940, Trans. Am. Microsc. Soc. 59:52–63). Ten controls were uninfected.

Twenty metacercariae were force-fed to each of three 1-day-old, unfed chicks, which were necropsied at 36, 60, and 84 hr after inoculation. Fifteen adult worms, morphologically identical to specimens of *P. minimum* deposited by Bedinger and Meade (USNM Helm. Coll. 62931), were recovered from the upper small intestine of the 36-hr chick. Upon necropsy the 60- and 84-hr chicks hosted three and zero worms, respectively.

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*Research Note*

**Helminths of the Scaled Quail, *Callipepla squamata*,  
from Northwest Texas**

The Scaled Quail, *Callipepla squamata*, is an endemic game bird of arid and semiarid regions of the southwestern United States and northern Mexico. Its present range extends northward to include southwestern Kansas and southeastern Colorado, eastward into the Oklahoma and Texas panhandles, west to include most of New Mexico and southeastern Arizona, and south through the Texas Trans Pecos through central and eastern Mexico to the Yucatan (Johnsgard, 1973, Grouse and Quails of North America, Univ. Nebr. Press, Lincoln, Nebraska. 553 pp.). Because there are few studies on the helminth fauna of this species (Chandler, 1935, Trans. Am. Microsc. Soc. 54:33-35; Rollins, 1980, M.S. Thesis, Okla. St. Univ., Stillwater, Oklahoma. 59 pp.; Wallmo, 1956a, Ecology of Scaled Quail in West Texas, Texas Game and Fish. Comm., Austin, Texas. 134 pp.), the present study was initiated to determine the prevalence, density, and ramifications of helminth parasitism in conjunction with an ongoing study by the USDA Forest Service, Rocky Mountain Forest and Range Experiment Station, on the food habits, reproductive ecology, and habitat use of Scaled Quail in the Texas Panhandle.

One hundred four Scaled Quail were collected by shooting from February 1980 through January 1981 with most birds collected during summer 1980. Hosts were collected from Oldham (63 birds), Bailey (23), Dallam (13), Andrews (three), and Lubbock (two) counties, Texas. Entire carcasses were frozen for later examination and necropsy. Sex and age (as juvenile or adult) (Wallmo, 1956b, J. Wildl. Manage. 20:154-158) were determined for all birds examined. Nematodes were briefly fixed in glacial acetic acid, preserved in a mixture of 75% ethyl alcohol with 5% glycerine, and examined in glycerine wet mounts after evaporation of the alcohol. Cestodes were stained in Celestine blue B and mounted in Canada balsam.

Herein, prevalence refers to the ratio of the number of hosts infected to the total number of hosts examined. Density refers to the number of individuals of a particular helminth species, or of all helminths, occurring in an infected bird. Mean density is the mean of the total individuals recovered from all infected birds. Abundance refers to prevalence times mean density for a particular helminth species. The effects of host age and sex on helminth prevalence and mean density were determined by a Kruskal-Wallis test and Mann-Whitney U-test, respectively. Sample sizes were not sufficiently large for determining the influence of different habitats on helminth prevalence and density. The female : male ratio (FMR) of *Aulonocephalus lindquisti* and a coefficient of correlation ( $r$ ) were calculated for densities versus the FMR (Sokal and Rohlf, 1969, Biometry, W. H. Freeman, San Francisco, California. 776 pp.). Simpson's index (Holmes and Podesta, 1969, Can. J. Zool. 46:1193-1204) and Sorenson's index of similarity (Greg-Smith, 1964, Quantitative Plant Ecology, Butterworth and Co., London. 256 pp.) were used to measure the concentration of dominance and similarity of helminth faunas between the Texas Panhandle and Texas Trans Pecos (data from Wallmo,

Table 1. Helminths of Scaled Quail from the Texas Panhandle.

| Helminth species                 | Prevalence                    |    | Densities |      |
|----------------------------------|-------------------------------|----|-----------|------|
|                                  | No. infected/<br>no. examined | %  | Range     | Mean |
| <i>Oxyuris petrowi</i>           | 2/104                         | 2  | 1         | 1    |
| <i>Rhabdometra odiosa</i>        | 12/104                        | 12 | —         | —    |
| <i>Aulonocephalus lindquisti</i> | 77/104                        | 74 | 1-263     | 40   |

1956a, loc. cit.), respectively. Critical values in tests for significance were established at the 0.05 level.

Representative specimens of helminth species recovered in this study are deposited in the Medical Zoology Collection, The Museum of Texas Tech University, TTU-MZ 16,760-16,915, and in the USNM Helm. Coll., 76,543.

Two nematode and one cestode species were recovered (Table 1). Eighty-one of 104 (78%) Scaled Quail were infected; 71 of 81 (88%) and 10 of 81 (12%) harbored one and two helminths species, respectively.

The eyeworm, *Oxyuris petrowi* Skriabin, 1929, is a widespread nematode of ground-dwelling and/or ground-feeding avian species in North America (Pence, 1975, Proc. Helminthol. Soc. Wash. 42:181-183). It is previously reported from the Scaled Quail in the Trans Pecos of Texas (Pence, 1975, loc. cit.) and the Ring-necked Pheasant, *Phasianus colchicus* (Pence et al., 1980, Proc. Helminthol. Soc. Wash. 47:144-147), and Lesser Prairie Chicken, *Tympanuchus pallidicinctus* (Pence and Sell, 1979, Proc. Helminthol. Soc. Wash. 46:146-149), from the Texas Panhandle. Only two of 104 (2%) Scaled Quail from this area were each infected with a single eyeworm.

The most abundant helminth of Scaled Quail in northwest Texas was the cecal worm, *Aulonocephalus lindquisti* Chandler, 1935. This helminth is previously reported from this host by Chandler (1935, loc. cit.) in south Texas, Wallmo (1956a, loc. cit.) in the Trans Pecos, and Rollins (1980, loc. cit.) in Texas, Oklahoma, and New Mexico. Seventy-four percent of the birds examined were infected with densities from one to 263 ( $\bar{x} = 40$ ) nematodes per host.

There were no significant differences in prevalence or mean density of *A. lindquisti* between sexes (67 male and 37 female) or ages (73 juvenile and 31 adult) of this host. The FMR of *A. lindquisti* was 1.4:1. The correlation coefficient was 0.94 and the FMR was not significantly increased with an increase in helminth densities.

The cestode, *Rhabdometra odiosa* (Leidy, 1887) Jones, 1929, is previously reported from the Bobwhite (Kellog and Calpin, 1971, Avian Dis. 15:704-715), Plain Chachalaca (*Ortalis vetula*) (Christensen and Pence, 1977, J. Parasitol. 63:830), and Prairie Chicken (Pence and Sell, 1979, loc. cit.) in Texas. It is reported from the Scaled Quail by Wallmo (1956a, loc. cit.) from the Trans Pecos, Texas. The species occurs in relatively low prevalence (12%) and densities in Scaled Quail from northwest Texas. Incomplete recoveries and fragmentation of specimens precluded quantification of densities of this species, but only one to a few cestodes were recovered from infected hosts.

Simpson's index, which is a measure of concentration of dominance of particular helminth species, was relatively high (0.56) reflecting the common occurrence

of *A. lindquisti* and low prevalences of tapeworms and eyeworms. Sorenson's index comparing the helminth faunas of the Texas Trans Pecos (Wallmo, 1956a, loc. cit.) and northwest Texas was 86, indicating little difference in the helminth fauna between the two regions.

In previous studies, Chandler (1935, loc. cit.) described *A. lindquisti* from a single Scaled Quail in south Texas. Wallmo (1956a, loc. cit.) found *R. odiosa* and *Raillietina* sp. (25% infected) as well as *A. lindquisti* (97%) and *Oxyspirura* sp. (33%) in 48 Scaled Quail examined from the Trans Pecos, Texas. Rollins (1980) reported on *A. lindquisti* and *Subulura brumpti* as cecal worms from Scaled Quail in the Texas and Oklahoma panhandles and in New Mexico. The above and present studies are indicative of the limited diversity of the helminth fauna of this host. The absence of pathology associated with the above helminth species in the birds examined herein and the relatively low prevalences and densities of most species indicate that helminth parasitism is of limited importance under present conditions in the management of this species on the High Plains of Texas.

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### *Research Note*

## **Helminths from Stejneger's Beaked Whale *Mesoplodon stejnegeri* and Risso's Dolphin *Grampus griseus* in Alaska**

Two Stejneger's beaked whales, a male from Moffet Point (55°27'N, 162°35'W) in the southeastern Bering Sea that beached on 18 June 1977 and another male from Mud Bay, near Homer (59°38'N, 151°33'W) in Cook Inlet that stranded on 13 November 1977, were necropsied by Fay. A male Risso's dolphin was examined by Hall on Middleton Island (59°26'N, 146°20'W) in the central Gulf of Alaska. Helminths from these specimens were prepared and studied by Shults.

Due to intensive scavenging by brown bears, *Ursus arctos*, the only visceral

organs remaining in the Moffet Point beaked whale were the kidneys, which were found heavily infected with nematodes of the genus *Crassicauda* Leiper and Atkinson, 1914. Specific determination of these was not feasible because of their advanced state of decomposition. Representative specimens have been deposited in the USNM Helminthological Collection (USNM 75814).

The Mud Bay beaked whale was intact and well preserved by cool weather. Its small intestine was found to contain nine scolices and a few strobilar fragments of the cestode *Tetrabothrius forsteri* (Kreffft, 1871) (USNM 75813). These cestodes compared well with the description of *T. forsteri* given by Delyamure (after Fuhrmann, 1899) (1955, Akad. Nauk SSSR, Moscow. 522 pp.); there were 22 to 24 testes. The scolices were much larger than described (0.77 rather than 0.28 mm wide), but this probably was due to their partial decomposition before fixation. Helminths were not found in any of the other organs.

The small intestine of the Risso's dolphin contained about 100 cestodes of the genus *Tetrabothrius* Rudolphi, 1819, specific identification of which was not feasible because of advanced decomposition (USNM 75812).

These are the first records of helminths from Stejneger's beaked whale and of *Tetrabothrius* sp. from Risso's dolphin. Cestodes of the genus *Tetrabothrius* have been recorded previously in the North Pacific region from five other species of cetaceans: the gray whale, *Eschrichtius robustus*, fin whale, *Balaenoptera physalus*, sei whale, *B. borealis*, striped dolphin, *Stenella coeruleoalba*, sperm whale, *Physeter macrocephalus* (Dailey and Stroud, 1978, J. Wildl. Dis. 14:503-511; Delyamure, 1955, loc. cit.; Margolis and Dailey, 1972, NOAA Tech. Rept. NMFS SSRF-647. 23 pp.). In the North Pacific, nematodes of the genus *Crassicauda* have been found previously in fin and sei whales, the humpback whale, *Megaptera novaeangliae*, bottlenose whale, *Berardius bairdii*, harbor porpoise, *Phocoena phocoena*, Risso's dolphin, and the belukha, *Delphinapterus leucas* (Dailey and Stroud, 1978, loc. cit.; Delyamure, 1955, loc. cit.; Margolis and Dailey, 1972, loc. cit.).

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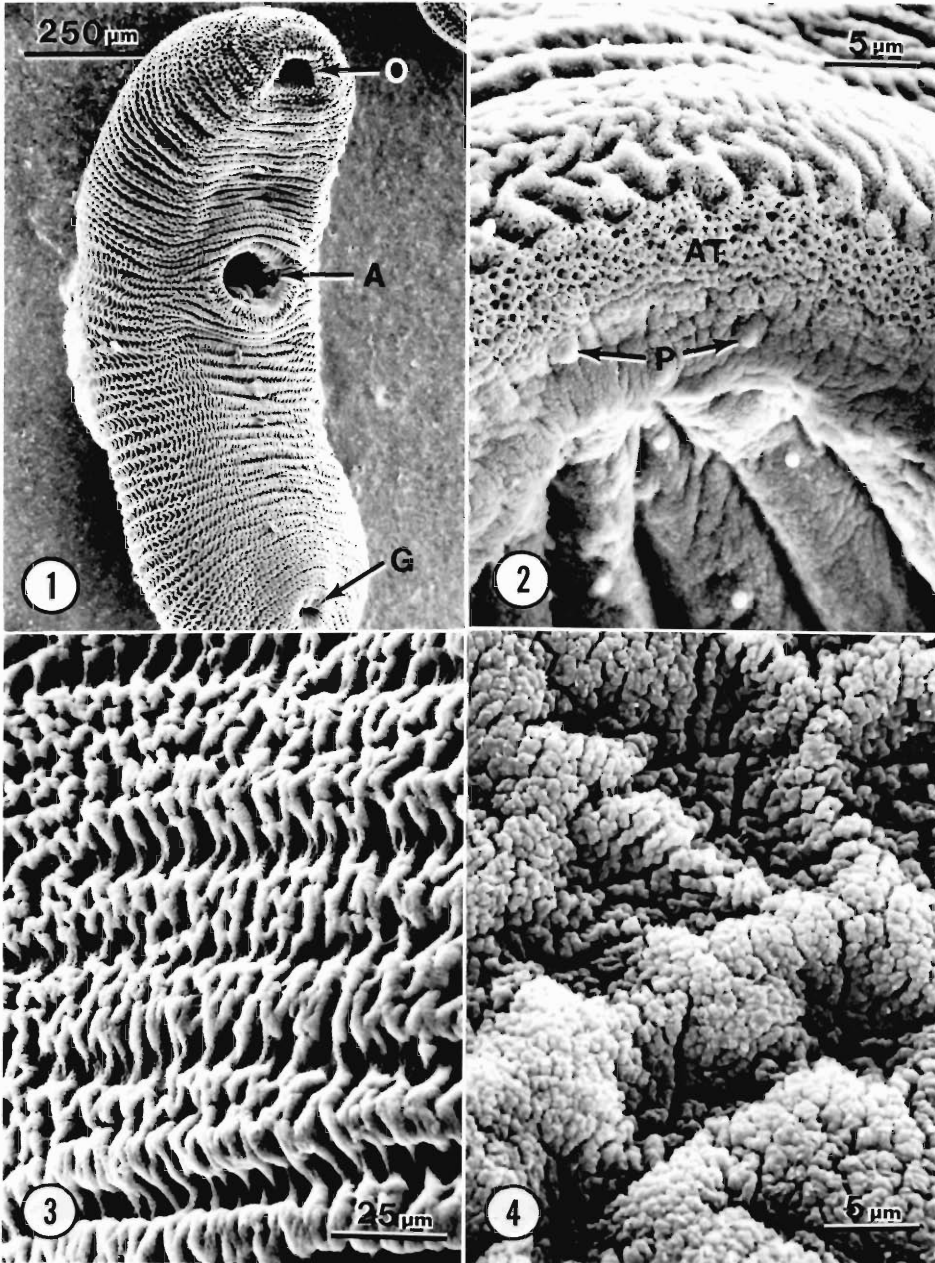
**Ultrastructure of the Tegument of  
*Amblosoma suwaense* Metacercariae**

Metacercariae of the Leucochloridiomorphae are unusual in that they do not encyst in their second intermediate hosts. *Leucochloridiomorpha constantiae* metacercariae occur in the uterus of the freshwater snail *Campeloma decisum* (Allison, 1943, Trans. Am. Microsc. Soc. 62:127-138), and *Amblosoma* spp. metacercariae attach to the external surface of the visceral epithelium of freshwater snails, *A. exile* in *Viviparus viviparus* (Pojmanska, 1972, Acta Parasitol. Pol. 20:35-44), *A. pojmanske* in *Viviparus georgianus* (Fischthal, 1974, Acta Parasitol. Pol. 22:165-169), and *A. suwaense* in *Sinotaia quadrata* (Shimazu, 1974, Jpn. J. Parasitol. 23:100-105) and *Campeloma decisum* (Font, 1980, J. Parasitol. 66:861-862).

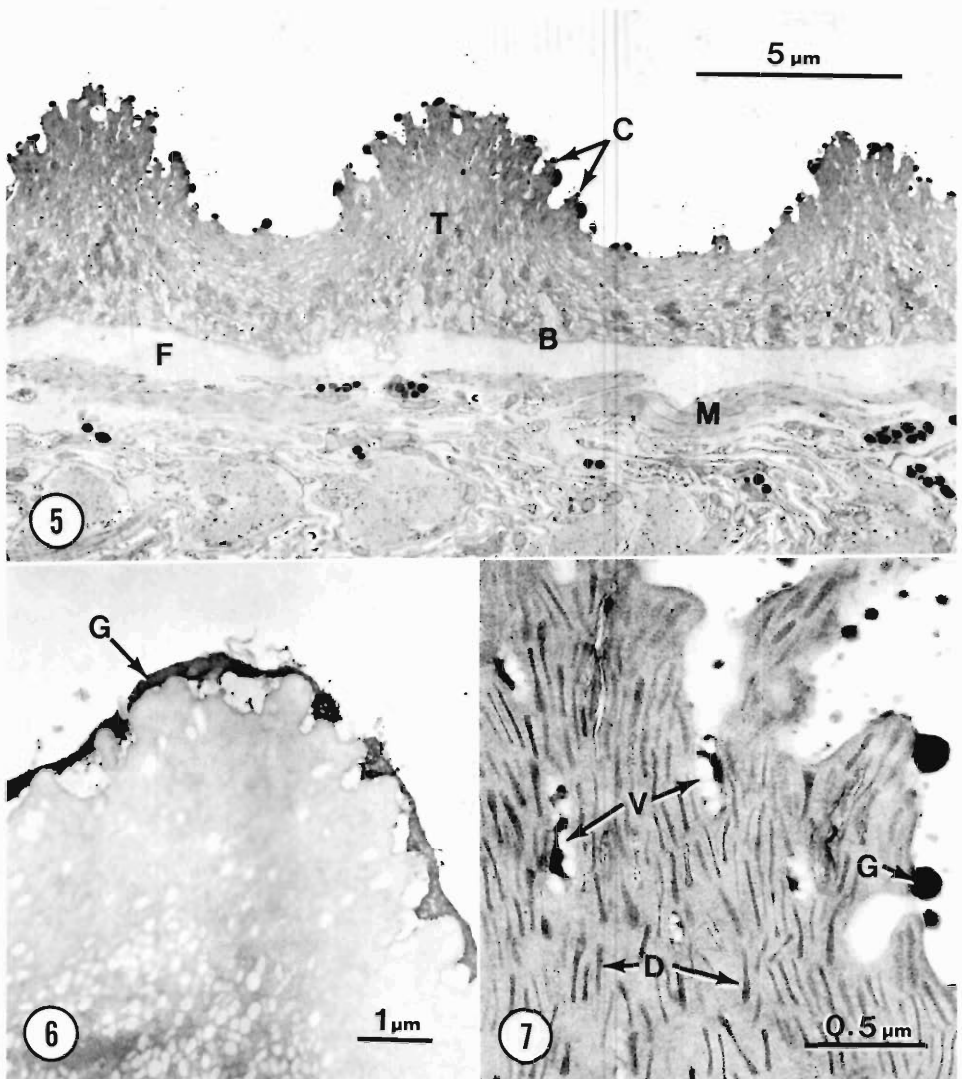
The tegument of unencysted metacercariae is of interest because it may perform protective functions analogous to the cyst wall which surrounds most metacercariae. The tegument of *L. constantiae* metacercariae and adults has been studied with transmission electron microscopy (Harris et al., 1974, Parasitology 68:57-67) and scanning electron microscopy (Font and Wittrock, 1980, J. Parasitol. 66:955-964). The present note describes the ultrastructure of the tegument of *A. suwaense* metacercariae and compares its structure with that of *L. constantiae*.

*Amblosoma suwaense* metacercariae were obtained from *Campeloma decisum* collected in Moose Ear Creek, Barron County, Wisconsin. Metacercariae, found between the visceral epithelium and shell, were placed in non-nutrient Locke's solution. Worms for light microscopy were fixed in 10% buffered formalin or AFA, embedded in paraffin, sectioned, and stained with PAS or alcian blue pH 2.5 (Humason, 1979, Animal Tissue Techniques, 4th ed.). Specimens for transmission electron microscopy were processed according to the protocol of Lumsden (1970, in Experiments and Techniques in Parasitology, MacInnis and Voge, eds.). Thin sections, stained with uranyl acetate and lead citrate, were examined with a Hitachi HS-9 electron microscope. Additionally, some specimens were processed in fixatives containing ruthenium red (Luft, 1971, Anat. Rec. 171:369-416) and examined with transmission electron microscopy with no further staining. Metacercariae for scanning electron microscopy were prepared and examined as described by Font and Wittrock (1980, loc. cit.).

Three structures were located on the ventral surface of *Amblosoma suwaense* metacercariae: a subterminal oral sucker, an acetabulum slightly anterior to the midbody, and a genital atrium near the posterior extremity (Fig. 1). The excretory pore was posterodorsal. The rugose surface was apparent both in living worms examined with light microscopy and in fixed worms viewed with SEM. The appearance of the surface was due to corrugation of the tegument into regular rows of ridges arranged in transverse bands (Fig. 3). Bands were continuous around the worm, giving the surface a similar appearance dorsally, ventrally, and laterally. The distance between bands and between individual ridges and depressions within bands was modified by muscular activity of living specimens and the degree of contraction of fixed specimens. At higher magnifications, extensive convolu-



Figures 1-4. Electron micrographs of *Amblosoma suwaense* metacercariae. 1. Ventral surface showing oral sucker (O), acetabulum (A), and genital atrium (G). 2. Acetabulum. Note modified tegument of acetabular margin (AT) and papillae (P). 3. Tegument posterior to acetabulum showing rows of ridges arranged in transverse bands. 4. High magnification of ridges formed by many fine convolutions of tegumental surface.



Figures 5-7. Electron micrographs of *Amblosoma suwaense* metacercariae. 5. Low magnification of tegument (T) showing three ridges. The surface of each ridge is composed of knoblike convolutions (C). A fibrous layer of connective tissue (F) containing muscle bands (M) lies beneath the basal lamina (B) of the tegument. 6. Ruthenium red staining of glycocalyx (G) adhering to tegumental surface. 7. High magnification of disk-shaped secretory vesicles (D) and ovoid vesicles containing opaque secretion (V). Note contact of ovoid vesicles with tegumental surface and apparent release of contents to form glycocalyx (G.)

tions of the tegumental surface of the ridges and depressions were evident (Fig. 4).

Corrugations of the orifices were somewhat modified from those of the body surface. Ridges surrounding the oral sucker were taller and not arranged into discernable rows. The inner margin of the acetabulum was composed of finely dissected ridges and occasional knob-shaped papillae (Fig. 2).



TEM was employed to examine the nature of both the large surface ridges and the finer tegumental convolutions. In areas where the tegument was not contracted by the underlying musculature, the appearance of convolutions on the surface of the larger ridges was most apparent (Fig. 5). The surface of the metacercariae consisted of a syncytial tegument similar to that of most digenetic trematodes. Two types of vesicles were present in the outer tegumentary zone. The first, biconcave disks similar to those described by Harris et al. (1974, loc. cit.) in *L. constantiae*, were numerous and tended to be oriented with their long axis parallel to the outer surface of the tegument (Fig. 7). The second type of vesicle was ovoid and contained electron-opaque material. Its contents were apparently released at the outer surface of the tegument (Fig. 7). This material, which stained intensely with alcian blue at the light level and ruthenium red at the EM level indicating the presence of acid mucopolysaccharides, appears to form a tightly adherent glycocalyx (Fig. 6).

The syntegument was limited internally by a thin basal lamina and an irregular layer of fibrous connective tissue containing bands of circular and longitudinal muscle (Fig. 5). A nucleated cytotelement containing both types of secretory vesicles occurred beneath the muscle layers and was connected to the tegument by thin cytoplasmic bridges.

Results of this study indicate a similarity in the teguments of *L. constantiae* and *A. suwaense* metacercariae. Because both metacercariae lack a cyst, the tegument must provide a protective function analogous to a cyst wall. The tightly adherent glycocalyx may well serve this important function.

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#### *Research Note*

### **Thin Layer Chromatographic Analysis of Neutral Lipids in Chick Intestinal Mucosa and Media Used for the in Vitro Cultivation of Trematodes**

Little information is available on the physical and chemical characteristics of various media used for the in vitro cultivation of trematodes (Fried, 1978, *in Methods of Cultivating Parasites in Vitro*, Academic Press, London). Information on the neutral lipid profile of the mucosa of the intestine of the domestic chick, an experimental host for trematodes, is not available except for an analysis of the upper intestinal mucosa (Fried and Butler, 1977, *J. Parasitol.* 63:831-834). The purpose of this study was to analyze by thin layer chromatography (TLC) the neutral lipid composition of various chick intestinal mucosa regions and media used for the in vitro cultivation of trematodes.

Intestinal mucosa from the cloaca, bursa of Fabricius, rectum, cecum, lower and upper ileum of 7-day-old domestic chicks were obtained as described by Basch et al. (1973, *J. Parasitol.* 59:319–322), and 1 mg wet weight of mucosa from each region was added to 100  $\mu$ l of the defined medium NCTC 135 (Grand Island Biological Co., Grand Island, New York). Each mucosal-NCTC 135 mixture was extracted in 2 ml chloroform/methanol (2/1) (Fried and Shapiro, 1975, *J. Parasitol.* 61:906–909). Three common *in vitro* cultivation media were analyzed for neutral lipids: NCTC 135, NCTC 135 + 20% hens' egg yolk (NCTC 135-20Y) and NCTC 135 + 20% hens' egg albumin (NCTC 135-20A). Additionally, hens' egg yolk (100Y) and hens' egg albumin (100A) were also analyzed for neutral lipids. NCTC 135 was purchased from Grand Island Biological Co. and the NCTC 135-20Y and NCTC 135-20A were prepared as described by Fried and Contos (1973, *J. Parasitol.* 59:936–937). For the analysis of cultivation media and hens' egg fractions, 100  $\mu$ l of each sample was extracted in 2 ml chloroform/methanol (2/1) as described above. Just prior to TLC each sample was reconstituted with 50  $\mu$ l of chloroform/methanol (2/1) and 10  $\mu$ l of each sample was spotted on silica gel sheets. Two neutral lipid standards were used: 18-1A (Nu-Chek Prep, Inc., Elysian, Minnesota) containing equal amounts of monoolein, diolein, triolein, and methyl oleate; and 18-4A (Nu-Chek Prep, Inc.) containing equal amounts of cholesterol, cholesterol oleate, triolein, oleic acid, and methyl oleate. TLC analysis was performed on 20  $\times$  20 cm silica gel sheets (Baker-flex IB2, J. T. Baker Chemical Co., Phillipsburg, New Jersey) (Fried and Shapiro, 1975, *loc. cit.*), and chromatograms were developed in the double solvent system of Skipski et al. (1965, *Biochem. Biophys. Acta* 106:386–396), which consists of a first development in isopropyl ether/acetic acid (96/4) followed by a second development in the same direction using petroleum ether/diethyl ether/acetic acid (90/10/1). Neutral lipids were detected by spraying chromatograms with 5% phosphomolybdic acid (PMA) in ethanol.

The cloacal mucosa contained only trace amounts of free sterol, whereas the major neutral lipid fractions of the bursal mucosa were free sterols and triglycerides. The rectal mucosa showed less free sterols than the bursal mucosa and only trace amounts of other neutral lipids. The major neutral lipid fraction of the cecum, lower ileum, and upper ileum mucosa was free sterol. The lower ileum also contained a large triglyceride fraction, whereas the cecum and upper ileum contained lesser amounts of triglycerides. The cecum also contained a fraction which showed a chromatographic mobility greater than cholesterol but less than diolein and is presumably 1,2-diglycerides (Skipski et al., 1965, *loc. cit.*). NCTC 135-20Y and 100Y showed mainly triglyceride and free sterol along with lesser amounts of diglycerides and sterol esters. Neutral lipids were not detected in NCTC 135, NCTC 135-20A, and 100A.

Extracts of NCTC 135 + mucosa from the various regions of the chick intestine showed different neutral lipid patterns. Since trematodes are site specific these chemical differences may be involved in habitat selection of flukes. The mucosa of the bursa of Fabricius had considerably more free sterol than that of the rectum or cloaca. *Leucochloridiomorpha constantiae* metacercariae placed on the cloacal lips of the domestic chick localize in the bursa within 5 min and remain there (Harris et al., 1972, *J. Parasitol.* 58:213–216). Fried and Gioscia (1976, *J. Parasitol.* 62:326–327) provided thin layer chromatographic evidence indicating that free

sterol is involved in the in vitro pairing of *L. constantiae*. Perhaps free sterol from the bursal mucosa serves as a chemoattractant for site selection of *L. constantiae*.

In this study in which equal volumes of culture media were analyzed, the only medium that contained neutral lipid was NCTC 135-20Y. Postmetacercarial growth and development in vitro of *Sphaeriodotrema globulus* by Berntzen and Macy (1969, J. Parasitol. 55:136-139), of *Leucochloridiomorpha constantiae* by Fried and Contos (1973, loc. cit.), of *Echinostoma revolutum* by Butler and Fried (1977, J. Parasitol. 63:1041-1045), and of *Amblosoma suwaense* by Schnier and Fried (1980, Int. J. Parasitol. 10:391-395) in NCTC 135-20Y have been reported. Neutral lipids in NCTC 135-20Y probably contribute to the successful in vitro cultivation of the the above-mentioned organisms.

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#### Research Note

### Survival of Metacercariae of *Echinostoma revolutum* (Trematoda) in Half-Strength Locke's Solution Under Refrigeration

Fried and Wilson (1981, Proc. Helminthol. Soc. Wash. 48:96-97) reported that encysted metacercariae of *Zygoxotyle lunata* stored in half-strength Locke's solution at 3-5°C for 444 days were infective to domestic chicks. The present study examined the survival of encysted metacercariae of *Echinostoma revolutum* in half-strength Locke's solution under refrigeration.

The methods of Fried and Weaver (1969, Proc. Helminthol. Soc. Wash. 36:153-155) were used to expose laboratory-reared *Physa* sp. snails to cercariae of *E. revolutum* emitted from naturally infected *Helisoma trivolvis* snails, and to recover encysted metacercariae of the parasite from the kidney of *Physa* snails. Within 2 to 5 days postencystment, *Physa* snails were dissected, and cysts were removed from the kidneys. Fifty to 100 cysts in 25 to 50 ml of half-strength Locke's solution in loosely capped shell vials were maintained in a refrigerator at 3 to 5°C for up to 300 days. To determine cyst viability, about 200 encysted metacercariae were used to study chemical excystation following 240 days of storage. Excystation studies were made in an alkaline trypsin-bile salt medium maintained at 39°C (Fried and Butler, 1978, J. Parasitol. 64:175-177). More than 50% of the encysted metacercariae excysted within 90 min. The excysted metacercariae were live, active, and appeared identical to those obtained from fresh cysts. To determine if stored cysts were infective to chicks, five 1-day-old chicks were each fed 75 cysts that had been stored for 300 days. Each chick was necropsied 15 days postexposure (Fried and Butler, 1978, loc. cit.). Two of the five chicks were infected, one with three worms and the other with four. All worms

were live, active, sexually mature, and appeared identical to those obtained following infection of chicks with fresh cysts. The results of this experiment show that *E. revolutum* cysts stored for 240 to 300 days as described could be excysted chemically and are infective to domestic chicks.

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### *Research Note*

## *Anatrichosoma* from the Eye of a *Cynomolgus* Monkey

An adult male cynomolgus monkey (*Macaca fascicularis*) was observed with acute iritis of the left eye. The problem persisted and 10 days later the animal was examined by an ophthalmologist at which time a nematode was noticed swimming free in the aqueous humor. Severe iris degeneration was also noted at this time. Three days later the ophthalmologist removed the parasite from the eye.

Although somewhat damaged and in two pieces, the morphological features of the recovered parasite indicated that the worm was a male *Anatrichosoma* sp. The nematode measured 15.75 mm long and had a maximum diameter of 70  $\mu\text{m}$ . The esophagus measured 6.55 mm in length, with a ratio of esophagus to body length of 1:2.4. The stichocytes were not distinct and could not be counted accurately. Prominent bacillary bands were evident throughout most of the length of the worm. A spicule and spicule sheath were absent. The cuticle was thin, averaging about 2  $\mu\text{m}$  in thickness, and lacked cephalic expansions. The posterior extremity had two pairs of small, lateral papillae.

*Anatrichosoma* has been reported frequently from the nasal mucosa and infrequently from skin lesions on the extremities of Asian monkeys. Those from the extremities were described originally as *A. cutaneum* (Swift et al., 1922, *J. Exp. Med.* 35:599–620) while those reported from the nares are considered to be *A. cynamolgi* (Smith and Chitwood, 1954, *J. Parasitol.* 40 (Suppl.):12; Long et al., 1976, *J. Parasitol.* 62:111–115). A third species from primates, *Anatrichosoma ocularis*, has been described from the eye of tree shrews (File, 1974, *J. Parasitol.* 60:985–988). Although *A. ocularis* normally inhabits the eye, the male of the species is less than half the size of the parasite recovered from the eye in the present case. Morphologically, the features of the male specimen in this report closely match those of *A. cynamolgi* and it is therefore identified as such.

Although *Anatrichosoma* is frequently encountered in primates, including cynomolgus monkeys, the report of the parasite in the eye is unique. It does serve to point out our lack of knowledge about the parasite in general, including migratory habits of the worm in tissue. Why and how the worm entered the eye in this instance is unknown. The rapid onset of clinical symptoms may indicate that

the worm migrated to the eye just prior to being observed there. It may be noteworthy that nasal swabs taken at the time the worm was removed were negative for *Anatrichosoma* eggs. However, three of five other cynomolgus monkeys in the same group were positive for eggs by nasal swab. The parasite in question may have undertaken an aberrant migration in search of other worms.

The specimen was deposited in the National Parasite Collection as USNM Helm. Coll. 76603.

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#### *Research Note*

### **Contaminative Ability of *Baylisascaris procyonis* Infected Raccoons in an Outbreak of Cerebrospinal Nematodiasis**

The ascarids, *Baylisascaris procyonis* and *B. columnaris*, of the raccoon (*Procyon lotor*) and skunk (*Mephitis mephitis*), respectively, are important causes of cerebrospinal nematodiasis and visceral larva migrans in various mammals and birds (Nettles et al., 1975, J. Am. Vet. Med. Assoc. 167:600-602; Sass and Gorgacz, 1978, J. Am. Vet. Med. Assoc. 173:1248-1249; Richardson et al., 1980, Avian Dis. 24:498-503). In naturally occurring cases of fatal central nervous system (CNS) disease due to these parasites few larvae were seen or recovered from the brain, despite the large number which were encysted elsewhere in the body. This further demonstrated the pathogenetic capabilities of individual larvae when they enter the CNS, as first determined by Tiner (1953, J. Infect. Dis. 92:105-113) and Sprent (1955, Parasitology 45:41-55). There is no information available, however, on the infective dose or length of exposure necessary to produce the clinical manifestations which were seen. Also, data on the fecundity of these parasites are lacking. This and the contaminative ability of infected animals are important from an animal and public health standpoint. In a recent extensive outbreak of fatal CNS disease in bobwhites (*Colinus virginianus*) (Reed et al., Avian Dis., in press), we had the unique opportunity to examine these parameters as they related to naturally occurring cerebrospinal nematodiasis. These considerations are the subject of this report.

In that outbreak 85 bobwhites had access to a 12 × 24 ft dirt-bottomed run.

Three young pet raccoons had been previously housed in the run for 3 mo. The birds were placed in the enclosure 6 weeks after the raccoons had been removed. The birds had access to the run for 2–3 mo, and all died of severe CNS disease beginning 3–4 weeks after introduction to the area. *Baylisascaris* larval migration was diagnosed histologically as the cause of death. Larvae were recovered from brains by Baermannization. The three raccoons were positive for *B. procyonis* eggs. Over 10,000 larvated *B. procyonis* eggs were recovered from 1,500 g of soil in the run (Reed et al., *ibid.*). These eggs caused 100% mortality by cerebrospinal nematodiasis in experimentally inoculated hamsters and mice (Kazacos, unpublished data).

To examine *B. procyonis* egg production in the raccoons, quantitative fecal flotations were performed (McMaster method). Individual fecal samples were collected and weighed. The raccoons were shedding *B. procyonis* eggs at rates of 5,400, 1,700, and 1,300 eggs per gram (epg) of feces, respectively (means of four determinations per animal). Eleven individual fecal samples were collected; the 5,400 epg animal averaged 106.4 g of feces per defecation (four samples), the 1,700 epg animal averaged 99.0 g (four samples), and the 1,300 epg animal averaged 93.5 g (three samples). The animals were fed a diet of dry dog food *ad libitum*. The owner stated that they defecated at least twice a day, which was similar to my experience with wild raccoons kept in the laboratory on a similar diet.

Using these rates and a twice daily defecation as a basis, the animals were shedding 1,149,120 eggs, 336,600 eggs, and 243,100 eggs per day, respectively (total 1,728,820 eggs per day). Over the 3-mo period, they would thus have contaminated the run with a total of 155,593,800 eggs. All of the eggs would have been potentially infective to the birds, since infectivity (second stage larva) is reached in 30 days (Lindquist, 1978, *Am. J. Vet. Res.* 39:1868–1869; Kazacos et al., 1981, *J. Am. Vet. Med. Assoc.* 179, in press). Based on this exposure, and the pathogenicity of the parasite, it was essentially inevitable that the birds would develop clinical disease in this situation. Following anthelmintic treatment using piperazine monohydrochloride at a dosage of 170 mg/6 lb body weight, 15 female and 10 male *B. procyonis* were recovered from the raccoons. Based on the total egg production, average fecundity of one *B. procyonis* female in this case approximated 115,000 eggs per day. These estimates are based on the assumption that egg production was constant, which is probably not true. However, egg production per animal was probably higher than this prior to the time they were examined, since some loss of worms took place over the 3-mo period (Reed et al., *loc. cit.*).

This report indicates that infected raccoons can shed very large numbers of *B. procyonis* eggs. Other animals may thus be exposed to extremely high numbers due to accumulation. The potential for accidental human infection must also be considered when large numbers of eggs are present. Although no human cases with *B. procyonis* have as yet been identified, human infection has been predicted by others (Sprent, 1965, *Trans. R. Soc. Trop. Med. Hyg.* 59:365–366; Beaver, 1969, *J. Parasitol.* 55:3–12). Kazacos et al. (1981, *loc. cit.*) recently reported 100% mortality in squirrel monkeys experimentally inoculated with *B. procyonis* eggs. Extensive visceral and ocular larva migrans and cerebrospinal nematodiasis oc-

curred in the subhuman primates in that study, indicating probable human susceptibility to infection by *B. procyonis*.

The present outbreak involved artificial concentration of raccoons and their feces, which is potentially a very serious situation for both animals and man. In the natural situation with wild raccoons, however, similar levels of contamination undoubtedly occur in certain areas, especially in areas with high population densities of infected raccoons. In an outbreak of CNS disease in commercial poultry linked to wild raccoons, Richardson et al. (1980, loc. cit.) documented the death of 622 birds following a single exposure. Jacobson et al. (1976, J. Wildl. Dis. 12:357-360), investigating an outbreak of CNS disease in rabbits and woodchucks, found a massive number of larvae in the viscera of one affected woodchuck, and a heavily infected wild raccoon shedding 25,750 epg. The interaction of these parasites and their hosts with wild and domestic animal populations and man deserves further attention.

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#### *Research Note*

### ***Polydelphis* (Nematoda: Ascarididae) Larvae Encysted in a Feral African Green Monkey (*Cercopithecus aethiops*)**

An adult feral African green monkey (*Cercopithecus aethiops*) from east Africa, that had been housed at the Delta Primate Center for 1 year, presented with an apparent intestinal obstruction. The animal was subjected to an exploratory laparotomy for removal of the obstruction. Incidental to this, numerous (25-50) small nodules scattered diffusely throughout the mesentery and omentum were observed. They were irregularly round, 1-2 mm in size, white and firm. A small piece of mesentery containing nodules was removed and fixed in 10% formalin.

Microscopically, the nodules consisted of granulomas which were composed of a thin fibrous capsule, a band of lymphocytes, and an inner zone of large foamy macrophages. Occasional multinucleate giant cells and neutrophils were present. In the center of each granuloma was a single nematode larva (Fig. 1A). In tissue section the worms were observed to have a muscular esophagus, an intestine

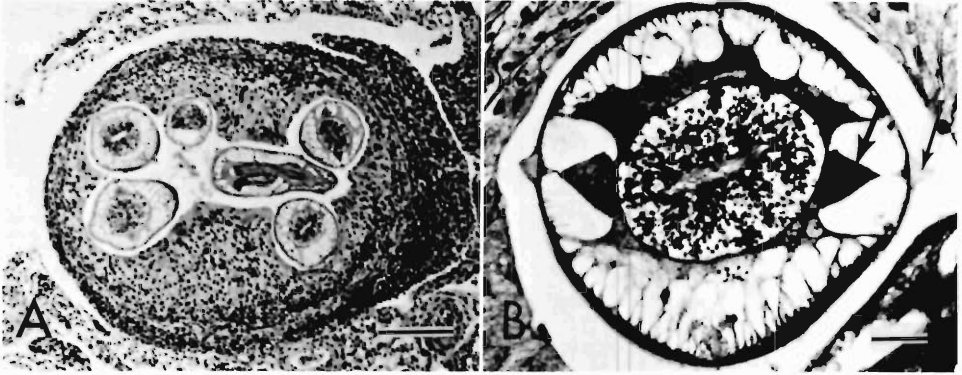


Figure 1. A. Mesenteric nodule containing coiled larvae. Hematoxylin and eosin stain. Line = 200  $\mu\text{m}$ . B. Cross section of larva at level of intestine showing lateral alae (small arrow) and excretory gland cells (large arrow). Heidenhain's aniline blue stain. Line = 25  $\mu\text{m}$ .

composed of a small number of cells, paired excretory gland cells, polymyarian-coelomyarian musculature, and prominent lateral alae along most of body length (Fig. 1B). They were identified as larval ascarids.

In an effort to further identify the larvae, specimens were dissected from nodules in the remaining portion of the fixed tissue and cleared in glycerin for morphological study. Larvae dissected from nodules were found to be morphologically indistinguishable from third-stage larvae of *Polydelphis anoura* recovered from experimentally infected mice (Sprent, 1970, *Parasitology* 60:375–397). Briefly, larvae in the present case were characterized as follows (measurements in mm): 5.5–6.5 in length by 0.17–0.20 in maximum diameter, a muscular esophagus which averaged 0.8 in length, a sharply pointed tail which averaged 0.1 in length, a small cecum at the esophageal intestinal junction, and an H-shaped excretory system. For a more extensive description of the larvae, including illustrations, the reader is referred to Sprent (1970, *ibid.*). Representative specimens have been deposited as USNM Helm. Coll. 76577.

The larvae recovered in this report are tentatively identified as those of *Polydelphis*. There are several factors that support this conclusion. Both *Travassosascaris* and *Hexametra* are similar morphologically to *Polydelphis*, but were excluded for various reasons. *Travassosascaris* appears to be restricted to rattlesnakes in Central and South America. Also, Araujo (1972, *Ann. Parasitol.* 47:91–120) found that in experimentally infected mice, the larvae of *Travassosascaris* remained in the liver, unlike those of *Polydelphis* which ultimately migrate to the mesentery. On the other hand, in those species of *Hexametra* that have lateral alae, the alae do not extend beyond the level of the esophageal-intestinal junction (Sprent, 1978, *J. Helminthol.* 52:355–384).

Sprent (1970, *ibid.*) recognized only a single species, *Polydelphis anoura*, in pythons from Africa, Australia, Ceylon, India, and Thailand. Kagei et al. (1974, *Snake* 6:44–46) described *Polydelphis elaphis* as a new species from *Elaphe quadrivirgata* in Japan. Although Sprent had not considered *P. elaphis* in his review, the worms conform to his diagnostic characters of the ascarid genus *Hexametra*: without interlabia and six uterine branches. In addition, taking all features into



consideration, the species *elaphis* could not be distinguished from *Hexametra quadricornis* as defined by Sprent (1970, *ibid.*). *Polydelphis elaphis* should be considered synonymous with *Hexametra quadricornis*. Therefore, the larvae recovered in the present report are considered to be those of *Polydelphis anoura*.

Whether the monkey in the present case became infected by ingesting snake feces containing eggs or by eating a small rodent infected with larvae is unknown. Regardless, the observation is unusual in that larval ascarids are rarely encountered in primates. There is a single published report of larval ascarids in a South American monkey (Chitwood and Lichtenfels, 1972, *Exp. Parasitol.* 32:407–519). This is the first known report of a larval ascarid infection in an African primate and the first known report of larval *Polydelphis* in a vertebrate host other than rodents.

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### *Research Note*

## **Exposure of Rodents to *Babesia microti* or *Entopolypoides macaci***

Although *Babesia microti* was described from *Microtus* in Europe in 1910 by Franca (*Arch. R. Inst. Bacteriol. Camera Pestana* 3:11–18), records for distribution of natural infections in rodents are somewhat limited and less attention than might be expected has been given to experimental introduction into wild or domestic mammals. Healy et al. (1970, *Science* 192:479–480), however, have demonstrated a high percentage of infection of *Microtus pennsylvanicus* and *Peromyscus leucopus* on Nantucket Island, Massachusetts. Babesiosis of rodent origin has become a zoonotic disease of medical concern since infections have been reported in a number of human populations in the northeastern United States, including Nantucket Island (Ruebush, 1980, *Trans. R. Soc. Trop. Med. Hyg.* 74:149–152). This situation has stimulated studies of the basic biology of the organism in its definitive hosts and efforts to determine patterns of susceptibility. Similar studies have involved *Entopolypoides macaci*, a babesiid of lesser medical significance which occurs naturally in Old World cercopithecoid primates but has recently been incriminated as a cause for disease in man (Wolf et al., 1978, *Ann. Int. Med.* 88:769–773).

The present report is concerned with a comparative study of *B. microti* and *E. macaci* in several species of mammalian hosts. A similar study has been made in nonhuman primates (Moore and Kuntz, 1981, *J. Parasitol.* 67:454–456). For these investigations *B. microti* (Gray strain) maintained in golden hamsters was obtained from Dr. George R. Healy, Center for Disease Control, Atlanta, Georgia. Blood with *E. macaci* was drawn from a naturally infected African baboon (*Papio cynocephalus*) captured in Kenya and subsequently held at the Foundation for 12 years.

Five spleen intact and five splenectomized golden hamsters (*Mesocricetus auratus*), white rats, hooded rats, jirds (*Meriones unguiculatus*), and pack rats (*Neotoma micropus*) were inoculated with blood containing *B. microti* or *E. macaci*. The former consisted of 0.1 ml heparinized heart blood drawn from a group of stock hamsters demonstrating parasitemias of 6 to 8% (postpeak infections) while the latter was 0.1 ml heparinized peripheral blood drawn from the naturally infected African baboon showing parasitemias of 3 to 5%. Recipients were examined at least once per week for 8 weeks beginning at 5 to 7 days postinoculation. Dried blood smears were fixed in absolute methanol and stained for 45 min with Giemsa with 1% Triton-X at pH 7.4.

The hamster has been generally regarded as a suitable maintenance and experimental host for *B. microti* and Ruebush et al. (1980, *J. Parasitol.* 66:107–110) have used it for isolation of the parasite from wild rodents. In the present study, intact and splenectomized hamsters demonstrated parasitemias of approximately 20% at 2 to 4 weeks postinoculation. All intact white rats and pack rats showed parasitemias of 1 to 5% at 2 to 4 weeks after inoculation while parasitemias in those splenectomized were recorded at 5 to 15%. All hooded rats were refractive. All jirds became infected, with parasitemias of 7 to 15% for intact as well as splenectomized individuals. None of the intact or splenectomized rodents, representing a total of 50 mammals, examined by the same postinoculation schedule employed for *B. microti* was receptive to *E. macaci*. Earlier cursory attempts (Moore and Kuntz, 1975, *J. Med. Primatol.* 41:1–7) to infect two albino mice (one intact, one splenectomized), albino rats (one intact, one splenectomized), jirds (one intact, one splenectomized), pack rats (one intact, one splenectomized), and ground squirrels (*Citellus mexicana*) (one intact, one splenectomized) or intact opossums (*Didelphis marsupialis*) with this parasite also were unsuccessful. In contrast to our observations, Wolf et al. (1970, *Ann. Intern. Med.* 88:769–773) reported transient, low-level parasitemias in splenectomized laboratory rats and hamsters inoculated intraperitoneally with blood from a human infection diagnosed as entoplopyoidiasis.

Although *B. microti* and *E. macaci* are closely related taxonomically, our results indicate that the parasites vary considerably in their preferences for definitive hosts.

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*Research Note*

**Experimental and Natural Transplacental Transmission of *Trypanosoma theileri* and its Possible Effects on the Bovine Fetus<sup>1</sup>**

*Trypanosoma theileri* Laveran, 1902 is an ubiquitous and cosmopolitan blood parasite of cattle and, in general, is considered nonpathogenic to its host (Hoare, 1972, *The Trypanosomes of Mammals*. Blackwell, Oxford). The parasite is typically cryptic in chronic infections but occasional reports of infection have been found often associated with clinical disease due to some other organism (Dikmans et al., 1957, *Cornell Vet.* 47:344-353; Levine et al., 1956, *J. Parasitol.* 42:553). In some cases *T. theileri* may appear in considerable numbers apparently due to inhibition of the immune response to trypanosomes in the presence of concurrent disease (Carmichael, 1939, *Parasitology* 31:498-500; Van Der Maaten et al., 1973, *Am. J. Vet. Res.* 34:341-343).

No reports of experimental transplacental transmission of *Trypanosoma theileri* have been found in the literature but Lundholm et al. (1959, *Virology* 8:394-396) reported natural fetal infections with trypanosomes contaminating fetal bovine kidney cell cultures, and Woo and Limebeer (1971, *Acta Trop. (Basel)* 23:61-63) found trypanosomes in fetal spleen cultures and fetal blood cultures. Abortion in the bovine, associated with fetal infection where trypanosomes were found in the aborted fetal stomach contents, has been reported (Dikmans, op. cit.).

This study was undertaken to explore experimental transplacental transmission of *Trypanosoma theileri* in the pregnant bovine and to study the effect of natural infection with this parasite on bovine pregnancy.

Cross-bred cows were pregnancy tested by rectal palpation 55 and 120 days after breeding. Trypanosome-positive donor cows were bled from the jugular vein (50 ml blood) into partial vacuum bottles containing 50 ml Alsever's solution and this mixture, or fractions of it, was then transfused into seven pregnant trypanosome-negative recipient bovines approximately 10-15 days prior to the midpoint of each gestational trimester. Inoculated recipients were bled at 2-day intervals thereafter and the blood checked by direct examination (DE) in heparinized microhematocrit tubes centrifuged at 12,500 g (Bennett, 1962, *Can J. Zool.* 40:124-125). Trypanosomes, when present, were seen moving in the plasma above the buffy coat when viewed under the scanning lens (2.5×) of a compound microscope using phase contrast illumination. Infection was followed in each dam until its fetus was delivered by caesarean section (CS). Fetuses and placentae were removed from infected dams by laparotomy at approximately the midpoint of the first, second, and third trimester of pregnancy which coincided with the peak of trypanosome infection in the dams. Fetuses were bled from the umbilical cord or from the jugular vein and the blood was examined DE or by culture methods using veal infusion medium (VIM) or Novy, MacNeal, Nicoll medium

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**Table 1.** Experimental vertical transmission of *Trypanosoma theileri* from dam to fetus.

| Period of infection | Number dams | Number days pregnant | Infection dams pos./neg. | Infection fetuses pos./neg. |
|---------------------|-------------|----------------------|--------------------------|-----------------------------|
| 1st trimester       | 3           | 64-72                | 2/1*                     | 0/2                         |
| 2nd trimester       | 2           | 100-120              | 2/0                      | 0/2                         |
| 3rd trimester       | 2           | 227-310              | 2/0                      | 1†‡/1                       |

\* Lack of infection perhaps due to previous undetected infection.

† Blood positive; organ cultures from all fetuses uniformly negative.

‡ Delivered by CS, 310 days, fetal crown-rump length 29 inches.

(NNN) (Matthews et al., 1979, Am. J. Vet. Res. 40:623-629). Fetal organs—spleen, liver, kidney, lung, placenta, heart, brain, and spinal cord—were cultured in VIM (with blood from an uninfected bovine added) and organ imprints were made, fixed in methyl alcohol, and stained with Giemsa. Blood and organ cultures were examined weekly for up to 5 weeks after collection and culturing by examining drops of culture material using phase contrast microscopy.

The results of the experimental exposure of seven pregnant bovines to infection with *Trypanosoma theileri* and the effects on their fetuses are given in Table 1. Only one fetus from one cow was infected and this was a third trimester fetus (Table 1). The culture was not seen as positive until 34 days after initial culture. Ordinarily this culture would have been scored as negative since, routinely, cultures were only read for 21 days after inoculation of the culture medium. The culture from the fetus was kept beyond this period and was examined yet again before being discarded. It is probable that the slow growth of organisms in this culture was due to a low level of infection with small numbers of parasites being present in the fetal blood inoculum. Trypanosomal stages seen in culture were epimastigotes which are typical of culture forms (Matthews et al., 1979, op. cit.).

Calves from other bovines, experimentally and naturally infected (i.e., field cases), were examined at birth, usually before nursing, using DE and VIM and NNN cultures to determine infections in newborn animals. Two calves, one from a naturally infected cow and one from an experimentally infected cow which had been used in a chronic study, were positive at birth. Calves from seven other cows were negative by DE and blood culture. The calf from the naturally infected cow was born dead (as the result of a dystocia); trypanosomes were seen in two of four microhematocrit tubes on DE; the blood was negative on culture. The trypanosome-positive calf from the experimental chronic cow was born approximately 2 years after the cow had been used in a chronic study on bovine trypanosomiasis. The infection in the dam had been followed for 13 mo after infection but not thereafter (Matthews et al., 1979, op. cit.). This cow was refractory to reinfection when challenged.

Blood (10 ml) containing 400 trypanosomes/ml from a trypanosome-infected bovine was fed to a 6-mo-old calf. Milk from the trypanosome-positive bovine was fed to the twin of this calf. The milk was cultured, alone, and with trypanosome-negative blood, with negative results. Blood from these recipients when examined DE and cultured in VIM was negative.

A group of 92 yearling heifers, born in 1973 and destined to serve as replacement heifers in the University of Wyoming Beef Herd, were bled periodically during their second year (1974) following exposure to two fly seasons. The blood

**Table 2. Calving success in a trypanosome-infected beef herd. *N* = 92; born 1973; bred 1974.**

|                   | Trypanosome positive (1974)<br><i>N</i> = 22 |                 |             | Trypanosome negative<br><i>N</i> = 70 |                 |             |
|-------------------|--|-----------------|-------------|---------------------------------------|-----------------|-------------|
|                   | Aborted                                      | Pregnant : open | Calved      | Aborted                               | Pregnant : open | Calved      |
| 1975              | 1 (6.7%)                                     | 3 (20%)         | 11 (73.3%)* | 1 (2.2%)                              | 0               | 46 (97.8%)* |
| 1976              | 1 (11.1%)                                    | 1 (11.1%)       | 7 (77.8%)   | 0                                     | 1 (5%)          | 19 (95%)    |
| 1977              | 1 (12.5%)                                    | 0               | 7 (87.5%)   | 0                                     | 0               | 14 (100%)   |
| Total calf losses | 7 (21.87%)                                   |                 |             | 2 (2.47%)                             |                 |             |
| Total calved      | 25 (78.13%)                                  |                 |             | 79 (97.53%)                           |                 |             |

\* Seven (31.8%) of the trypanosome-infected cohort, and 23 (32.9%) of the trypanosome negative cohort, were open at the first pregnancy check; these and other open animals detected in 1976 and 1977 are not included in the above tabulations.

was cultured and the incidence of infection with *T. theileri* determined. Twenty-two (23.91%) were positive for trypanosomes. These animals were bred, pregnancy tested, and calving results the following year were noted. Blood from the previously negative animals was cultured the following year. Calving results for all dams were obtained for a period of 3 years (Table 2). The trypanosome-infected 1973 heifers which calved in 1975–1977 showed what appeared to be differences in calving success when compared with their trypanosome-free cohorts. Animals infected with trypanosomes prior to bearing their first calves lost four calves in 1975 either by abortion (one) or were known pregnant and then open (three) as compared with uninfected dams where one dam may have aborted (calf born dead). Those dams (12) infected after calving successfully once showed no losses in subsequent years. Further losses in the early infected group occurred in 1976 where two calves were lost and in 1977 with one calf loss as compared with one loss in the uninfected group (Table 2). In the Beef Unit Herd, in general, calving was also more successful than in the trypanosome-infected group discussed above. Approximately 3–6% of heifers were pregnant and then open in 1975–1977; 1–2% delivered calves dead at birth.

Transplacental transmission of *Trypanosoma theileri* to the bovine fetus from the experimentally and acutely infected dam has not been demonstrated previously though natural congenital transmission has been shown to occur (Dikmans, 1957, op. cit.; Lundholm et al., 1959, op. cit.; Van Der Maaten et al., 1973, op. cit.; Woo and Limebeer, 1971, op. cit.). Hoare (1972, op. cit.) mentions one case with a heavy infection with trypanosomes in the blood of an aborted bovine fetus with congenital infection. Further, no previous systematic examination has been made of the effects of *T. theileri* on naturally infected, pregnant cows. The results reported here demonstrate that transplacental transmission can occur from dam to fetus during experimental acute infection of the dam during the third trimester of pregnancy. Also, bovines naturally infected prior to dropping their first calf showed a disproportionate number of calf losses during that (four losses) and subsequent pregnancies (three losses). Fetuses were aborted by such trypanosome-infected dams during the middle or latter part of the third trimester of pregnancy one dam aborted ca. 251–270 days; this cow had successfully calved between these abortions). Another cow produced a viable calf which died of scours; this dam then aborted in the subsequent calving at about 276–280 days,

producing a dead calf which had degenerated and was considered to have died 2 weeks earlier.

Calf losses in the pregnant-open trypanosome-infected groups occurred sometime between the time of the first pregnancy check (i.e., about 55 days post AI) and the second pregnancy testing (at about 115 days). These fetuses may have been resorbed or aborted with the aborted fetal remains not being detected. Natural infections of dams which aborted or resorbed fetuses must have occurred in the summer early in pregnancy (presuming horse-fly transmission; Hoare, 1972, *op. cit.*). Effects on the dam and fetus were not revealed until abortion some months later and with the dam in a chronic state of infection (Matthews et al., 1979, *op. cit.*). Early calf losses would have occurred shortly after infection of the dam (July to September; blood cultured 10 September 1974), possibly during the acute phase of infection. Our finding of experimental transplacental transmission of *T. theileri* in the third-trimester fetus supports the above contention though the experimental protocol was somewhat different with transmission to the fetus occurring during the acute period of infection in the dam. Our failure to demonstrate transplacental transmission in the late first- or early second-trimester fetuses possibly may be ascribed to too small an experimental sample to reliably detect such transmission. Further, we lack proof that aborted fetuses from naturally infected dams were positive for trypanosome infection (noted by Hoare, 1972, *op. cit.*). The fact remains, however, that a group of 15 such naturally infected dams lost seven calves over a 3-year period while a group of negative animals nearly three times the size of the infected group lost but two calves (Table 2). Twelve bovines from the uninfected cohort became naturally infected with *T. theileri* following the first calving (1975): these dams showed no calf losses in subsequent calvings and fewer than uninfected bovine cohorts (one loss). It would appear, then, that if *Trypanosoma theileri* plays a role in bovine abortions, its major effect is expressed in heifers infected before, or during the primary pregnancy.

We wish to thank Drs. Colin Kaltenbach and Tom Dunn and Messrs. Steve Mann and Roger Coles for technical assistance during some of this work.

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Note added in proof: Since preparation of this ms., a report of bovine fetal death due to *Trypanosoma theileri* infection (Mitchell and Long, 1980, *Am. Assn. Veterinary Laboratory Diagnosticians*, 23rd Ann. Proc. 62-72) has come to our attention. Pathology was seen in kidney, heart, and lung associated with trypanosomes. Numerous viable trypanosomes were recovered from thoracic fluid.

## PRESENTATION

### 1981 Anniversary Award of the Helminthological Society of Washington

Madam President, guests, and members of the Helminthological Society of Washington:

This evening, we honor Dr. Frank Dorr Enzie with the 1981 Anniversary Award of the Helminthological Society of Washington.

Dr. Enzie was born in 1917 on a farm in Dansville, New York. Shortly thereafter, the family moved to Long Beach, California. Upon graduation from high school, Dr. Enzie spent several months in the Civilian Conservation Corps before attending veterinary school at Ohio State University, where he received his DVM degree in 1940. After graduation, he accepted a position in North Carolina with the Tuberculosis Eradication Division, Bureau of Animal Industry (BAI), U.S. Department of Agriculture. While in North Carolina, he met Helen, and they were married in 1941 on Christmas day. The following year, Dr. Enzie transferred to the Zoological Division, BAI, in Beltsville, Maryland. This was the beginning of a distinguished career in parasitology that spanned the next 37 years. He served for 10 years as Leader of Antiparasitic Investigations, Beltsville Parasitological Laboratory, ARS, and in 1971 he became Director of the National Animal Parasite Laboratory, ARS, USDA. In 1972, he was appointed Chairman of the newly created Animal Parasitology Institute (API) in the Science and Education Administration (SEA), USDA, a position in which he provided capable leadership until his retirement in 1979.

During his professional career, Dr. Enzie authored or coauthored more than 80 scientific publications and review articles. Most of these contributions dealt with the chemotherapy of parasitic diseases in livestock and poultry. At one time or another, he evaluated, primarily from standpoints of safety, efficacy, and ease of administration, many of the major drugs that were available for the treatment of parasitic infections of veterinary importance. In reviewing these studies, it was apparent that Dr. Enzie always endeavored to improve the methods by which anthelmintics were evaluated and to standardize testing procedures.

His considerable expertise in parasite chemotherapy is well recognized by the scientific community. Dr. Enzie had a major role in the development of sodium fluoride for the removal of large roundworms from swine and of toluene as an antiparasitic for companion animals. He and his co-workers were the first to develop effective agents against the fringed tapeworm in sheep. They were among the first to report the occurrence of phenothiazine-resistant *Haemonchus contortus*, and the first to demonstrate that certain nonbenzimidazole anthelmintics were effective against benzimidazole-resistant strains of the parasite. Although researchers had tried unsuccessfully for many years to produce drug-resistant strains of worm parasites experimentally, Dr. Enzie and his co-workers achieved this goal with cambendazole and *H. contortus* of sheep in 1973. He prepared and contributed to a number of government publications on the chemical control of parasitic diseases of domestic animals, and was consulted frequently by researchers in the Food and Drug Administration (FDA) and other federal agencies, vet-



Dr. Frank D. Enzie, recipient of the 1981 Anniversary Award of the Helminthological Society of Washington.

erinary practitioners, and representatives from industry in this country and abroad on various matters pertaining to the treatment and control of livestock and poultry parasites. In 1958, he was selected to represent parasitology as a member of the first six-man U.S. Veterinary Exchange Delegation to review veterinary education and research in the Soviet Union. From 1966 to 1968 he served as Chairman of the Panel on Anthelmintics established by the National Research Council/National Academy of Sciences Committee on Veterinary Drug Efficacy. This committee was appointed in response to legislation requiring efficacy to be demonstrated for marketed drugs. They evaluated some 3,000 drug applications to determine whether these drugs met the legal requirements of efficacy. Much of this work serves as the basis for the efficacy evaluation of anthelmintics submitted to the FDA for market approval. Recently, Dr. Enzie served on FDA expert committees to prepare guidelines for assessing the efficacy of veterinary anthelmintics.

Some of the most important contributions Dr. Enzie made to the field of parasitology involved his leadership, research management, and development of guidelines for personnel evaluation in his various administrative capacities at Beltsville. High priority was given to the upgrading and augmentation of facilities and equipment to provide the best possible working environment for staff scientists. During his tenure as institute chairman, he recruited several outstanding scientists for API, reassigned staff personnel to the mutual advantage of both scientist and project requirements, and provided encouragement, guidance, and appropriate fiscal support for the several institute programs. This approach contributed to the development and recognition of award-winning scientists and to



the establishment of an exceptional research environment at the Animal Parasitology Institute.

Dr. Enzie has been a long-time supporter of the Helminthological Society of Washington. He joined the Society in 1942 on his arrival at Beltsville. During his research career, he published 18 papers in the Proceedings of the Society. He gave 8 presentations at Society meetings and, as administrative head, supported fully the annual November meeting at the Animal Parasitology Institute for many years. He served the Society as Recording Secretary in 1948, Vice-President in 1954, and President in 1956. When the need for format standardization between the Proceedings and other parasitological journals was brought to his attention, he organized, supported, and cochaired the Conference of Parasitology Editors at API in 1978. Not the least of his contributions to the Helminthological Society was the continuing encouragement he provided for young scientists and other staff researchers to take an active role in Society affairs. His success in these efforts is evident from the contributions that have been made over the years by many members of the Beltsville parasitology group.

On behalf of the Helminthological Society of Washington and members of the Awards Committee (Harley G. Sheffield and Micheal D. Ruff), I am pleased and honored to present the 1981 Anniversary Award to Dr. Frank D. Enzie.—KEN-DALL G. POWERS, Chairman, Awards Committee (November 13, 1981)

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#### Acceptance of the 1981 Anniversary Award

Madam President, Dr. Powers and other members of the Awards Committee, members of the Helminthological Society:

It is with considerable humility and profound gratitude that I accept the 1981 Anniversary Award of the Helminthological Society of Washington. To say that it came as a complete surprise would be to deny the obvious and to stretch credibility beyond the breaking point. Nevertheless, I am very pleased indeed to be counted among my many illustrious predecessors who have been similarly honored.

I have always believed that noteworthy performance or service should be recognized, appropriately rewarded, and announced to colleagues, friends, and associates. Indeed, during my tenure as Laboratory Director and Institute Chairman, I encouraged all personnel in leadership roles to take this action whenever warranted. Admittedly, there were some who disagreed with this policy, but to my mind their arguments were not persuasive. From a purely personal standpoint, however, I can truthfully say that it is not only more blessed, but more comfortable, to give than to receive.

In closing, I wish to take this opportunity to thank Dr. Powers, other members of the Awards Committee, and members of the Society for the fine honor that this award represents. I regret that circumstances precluded my presence at the meeting to accept the award in person.

Best wishes and warm regards to one and all.

Sincerely,  
FRANK D. ENZIE

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