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PROCEEDINGS OF THE
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Energetics of *Aphelenchus avenae* in monoxenic culture

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ABSTRACT: Energy budgets for *Aphelenchus avenae* feeding on *Botrytis cinerea* were constructed by calorific estimation of ingestion (using cinematographic techniques), respiration (using cartesian divers), growth (by calculating weights from measurement of volume throughout the growth cycle, and specific gravity), and reproduction (by observation of number of eggs produced). A daily energy budget and a cumulative energy budget were calculated. The efficiency of *A. avenae* as a converter of energy was calculated from the daily energy budget and found to be variable throughout the life cycle. The relevance of energetics to the study of host–parasite relationships is discussed as a means of quantifying pathogenicity, efficiency of parasitism, and the efficiency of a parasite.

Energy exchanges provide a unifying measure of biological processes and interactions. In an attempt to quantify the energy loss caused by nematodes to their hosts a model system of *Aphelenchus avenae* cultured on the fungus *Botrytis cinerea* was studied. A quantitative analysis was made by constructing a budget for *A. avenae*. There are two parts to the construction of energy budgets: firstly, the energy budget of each individual, and secondly, the productivity, i.e., the total number or biomass of individuals. This paper describes a laboratory study designed to obtain an energy budget for an individual. The productivity of *A. avenae* including additional population effects will be dealt with elsewhere.

An energy budget is based on calorific values of the following physiological parameters: ingestion, i.e., gross energy intake; respiration, i.e., energy cost of maintenance, work, and activity; growth and reproduction, i.e., energy contained in organic material synthesized by the organism; assimilation, i.e., metabolizable energy available for life processes; egestion, i.e., ingestion less assimilation. These definitions are modified after Weigert (1964). In the case of nematodes exuviae include organic and nitrogenous excretion, fecal material (egestion, if any), and molted cuticle. Estimates of these were not made. It was thought that this factor may have been appreciable, but its estimation would have been arbitrary, due, for example, to the possible breakdown and resorption of materials in molting. It was hoped that an estimate of egestion would be obtained by balancing the energy budget.

Materials and Methods

A population of *Aphelenchus avenae* originating from a single female was maintained on *Botrytis cinerea* on potato dextrose agar (PDA) at 26 C. One-week-old *B. cinerea* cultures derived from a 1-cm-diameter disc cut from a mycelial mat were used. Nematodes of known age were obtained by inoculating newly hatched larvae obtained by isolating eggs, incubating on the fungus, and extracting at given intervals to obtain the required age of *A. avenae*. The newly hatched larvae were

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Table 1. Ingestion.

Day	Volume of Valve (a) μ^3	Mean No. puls/sec. (b)	Volume of Food Ingest/s. in μ^3 (axb) = X	Feeding Time/15 m Period Secs. Y	Vol. Ingested/ 15 m period XY μ^3	Vol. Ingested for 24 hours XY × 4 × 24 μ^3	Vol. Ingested per 24 hrs. in ml.	Cals. Ingested Per Day
1	34.5496	8.14	281.2337	16.62	4674.1041	448713.9936	44871399^{-14}	0.0000396613
2	67.4838	7.47	504.1040	25.99	13101.6630	1257759.6480	125775965^{-14}	0.0001111721
3	84.3652	7.50	632.7390	15.66	9908.6927	951234.4992	95123450^{-14}	0.0000840786
4	147.8714	6.50	961.1641	10.11	9717.3691	932867.4336	93286743^{-14}	0.0000824552
5	200.6264	6.83	1370.2783	54.60	74817.1952	7182450.7392	718245074^{-14}	0.0006348496
6	200.6264	6.54	1312.0967	45.15	59241.1660	5687151.9360	568715194^{-14}	0.0005026816
7	227.7503	7.21	1642.0797	14.40	23645.9477	2270010.9792	227001098^{-14}	0.0002006440
8	227.7503	7.21	1642.0797	23.98	39377.0712	3780198.8352	378019884^{-14}	0.0003341279
9	227.7503	7.21	1642.0797	37.33	61298.8352	5884688.1792	588468818^{-14}	0.0005201417
10	227.7503	7.21	1642.0797	—	—	—	—	—
16	227.7503	7.21	1642.0797	30.80	50516.0548	4855301.2608	485530126^{-14}	0.0004291552
21	227.7503	7.21	1642.0797	17.82	29261.8603	2809138.5888	280913859^{-14}	0.0002482969
24	227.7503	7.21	1642.0797	36.47	59886.6467	5749118.0832	574911808^{-14}	0.0005081587

$\mu^3 = 10^{-12}$ ml
1 ml = 88.389 cals.

counted as 1 day old. A miniature bomb calorimeter was used in determining the calorific values of the materials (Phillipson, 1964). A minimum dry weight of 5 mg of material was required to obtain a reliable result.

Results

Ingestion

Aphelenchus avenae feeds by penetrating the host cell walls with its stylet. Then by the pulsation of the muscular esophagus and its bulb (metacorpus) and valve, the cell contents are sucked in, leaving the cell wall. Ingestion of *B. cinerea* cell contents could be assessed quantitatively because at each pulsation the valve fills with cell contents to the same extent as it opens and the fluid is pushed back into the gut when the valve closes. Observations of the valve action during feeding and cine film analysis suggested that this was the feeding mechanism. Pulsation of the medium bulb and the streaming of cell contents into the stylet was observed immediately after penetration of the cell wall. There is no time, therefore, for nematode secretions to be injected (Fisher and Evans, 1967) and affect the calorific value of ingested material. Only food entering through the stylet was considered as cuticular absorption of organic material is most unlikely (Lee, 1965).

Observations on feeding were made by placing one *A. avenae* of a known age on a 2-mm-thick disc of PDA with *B. cinerea* on a slide. Observations were made at 400× or 1,000× magnification.

Ingestion was studied through the considera tion of two factors: the volume of food ingested per day, and the calorific value per unit volume of food.

The volume of food ingested was estimated by measuring the volume of the open valve (*a*) and the rate of pulsation per second (*b*) of the valve. This was then related to the time spent feeding (*y*). The product of (*a*) × (*b*) × (*y*) expressed the volume of food ingested.

THE RATE OF PULSATION AND THE VOLUME OF THE MEDIAN VALVE: The pulsation of the median valve of *A. avenae* during feeding is too rapid to be accurately counted. Cine films were taken of the different ages at 24 frames per second using a Vinten 16mm camera. The films were projected at 8 frames per second at which speed the pulsation rate could be counted, and related back to normal speeds. Films were made of all stages from newly hatched larvae to adult females. An eyepiece scale was included in the microscope and micrometer slide was filmed, for reference, at the same magnification as the nematodes, at the end of each day. By viewing the film on a film editor, the internal diameter of the fully open valve was measured in microns. The valve opens to form a spherical cavity, the volume of which was calculated from the measurement of its diameter. The length and width of the median bulb of a few newly hatched (day 1) and 4-, 5-, 6-, 7-, 10-, 16-, and 24-day-old *A. avenae* were measured across the median valve to determine when growth stopped. It was apparent from these measurements that the bulb and the valve stopped growth after day 7. Therefore, films were made of a few *A. avenae* of each day up to day 6 and of an adult over 7 days old.

Table 1 presents the volume of the valve and the mean pulsation rate of aging *A. avenae*. Four decimal places are given where possible to facilitate greater accuracy of calculation. The volume of food ingested per second of the time spent feeding is calculated from these two measurements.

DURATION OF FEEDING: The duration of each feeding period was noted by observing *A. avenae* of different ages for 15 min each. Day 1 (L_2) and day 2 (L_3) *A. avenae* were observed during the night as well as during the day at approximately 22 and 26 C to test any possible periodicities or effects of temperature. Older *A. avenae* were observed only at approximately 22 C. Nematodes were incubated at 26 C in between all observations so that the rate of maturation would remain constant.

Table 2 presents the total time spent feeding and the number of feeds per 15-min periods of 1- and 2-day-old *A. avenae* at approximately 22 and 26 C during the day and approximately 22 C at night. It is apparent that the L_3 spend a longer time in feeding than the L_2. But there is no effect of the three given conditions on the time spent feeding of either age. The time spent feeding in a 15-min period of observation, and thereby the volume of food ingested per 24 hr by each age of *A. avenae*, is extrapolated and presented in Table 1.

CALORIFIC VALUE OF A UNIT VOLUME OF *B. CINEREA* CELL CONTENTS: *B. cinerea* was grown on 100 ml of standard potato dextrose broth in each of 12 250-ml conical flasks, for 1 week at 26 C. The mycelial mat was removed from the culture flasks, washed in distilled water, and the surface moisture removed by mopping with tissue. The mycelium was cooled to about 4 C and macerated in a vibro-mixer while the container was surrounded with ice. The macerated fungus was then introduced into 15-ml centrifuge tubes and centrifuged at 3,000 rpm for 15 min in a refrigerated centrifuge, maintained at 4 C. Drops of the supernatant were checked microscopically to verify the absence of cell walls. The dry weight of 1 ml of the supernatant was established by freeze-drying a known quantity in weighed ampules and reweighing. The rest of the supernatant was freeze-dried and the calorific value per gram ash free weight was established by using a Phillipson (1964) type bomb calorimeter.

Table 2. The total time spent feeding and the number of feeds per 15 min. period of 1 (L_2) and (L_3) day old *A. avenae* at 22 C at various times of day.

	Day at 22 C		Day at 26 C		Night at 22 C	
	Total feeding time	No. of feeds	Total feeding time	No. of feeds	Total feeding time	No. of feeds
L_2 number						
1	18.8	2	16.5	2	19.9	2
2	22.4	2	7.4	3	13.6	3
3	9.0	1	19.9	7	20.9	3
4	5.8	2	24.4	4	15.0	3
5	6.6	5	6.5	3	7.3	2
6	30.9	3	10.2	4	13.4	3
7	26.9	6	—	—	—	—
Mean	17.2		14.2		15.0	
L_3 number						
1	10.8	3	45.6	5	41.7	15
2	25.1	4	38.8	7	30.2	2
3	28.9	5	6.8	3	22.8	9
4	20.4	4	12.3	4	31.4	13
5	13.1	6	47.6	10	34.8	15
6	23.3	10	24.7	7	19.6	4
7	46.5	10	10.2	4	17.4	3
8	6.6	3	12.7	2	9.7	2
9	59.2	11	—	—	—	—
Mean	25.9		24.9		25.9	

Weight of 1 ml of *B. cinerea* cell content = 0.0252 g.

Calorific value of *B. cinerea* cell contents = 3,567.5 calories/g ash free weight.

1 ml of *B. cinerea* cell contents = 88.389 calories.

The calorific value of ingestion per day is calculated and presented in Table 1, knowing the volume of food ingested and the calorific value of a unit volume of food.

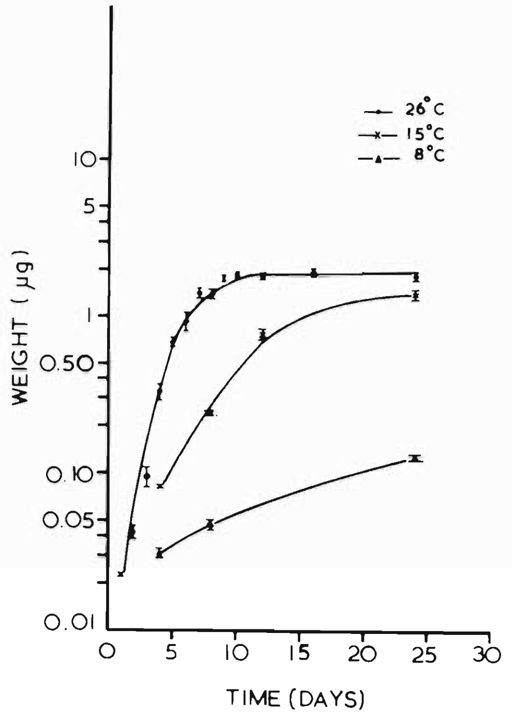

Figure 1. Growth rates of *A. avenae* at different temperatures.

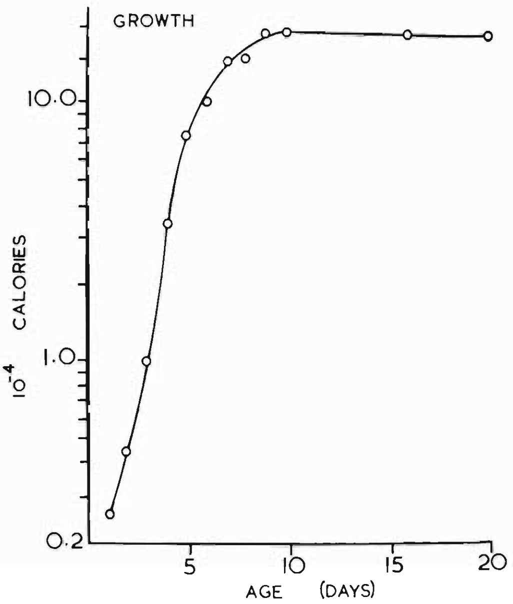

Figure 2. Cumulative calorific increase of *A. avenae*.

Growth

To obtain a calorific expression of growth it was first necessary to obtain the weights of each age group and then relate this to the calorific increase. The method described by Andrassy (1956) was used to obtain weights from linear measurements of lengths and widths. *A. avenae* were extracted from cultures at daily intervals. They were then straightened by gently heating, camera lucida drawings were made, and the lengths and the greatest widths were determined. A daily record of growth was thus made of newly hatched larvae to 10-day-old adults. From then on a less frequent record was kept until day 24. After maturation the nematodes were transferred every 3rd or 4th day into new cultures so that the original nematodes could be recognized from their progeny and to provide excess food. Andrassy's (1956) formula was used in obtaining wet weights.

$$G = \frac{a^2 \times b}{16 \times 100{,}000}$$

where G = body weight expressed in micrograms, a = greatest body width, and b = length of the body. The rounded tail end of *A. avenae* was extended to a point before the length was measured. Using a CDC 6600 computer linear measurements of each nematode were converted into wet weights. The cumulative growth curves of *A. avenae* at 26, 15, and 8 C were calculated in micrograms (Fig. 1).

The dry weight of all stages of *A. avenae* was determined as 19.78% of the wet weight using Myers' (1967) method. The calorific value of samples containing all stages of *A. avenae* was 5,453.2 cal/g dry ash free weight. Using these data, the wet weight of *A. avenae* at 26 C was converted into calories (Fig. 2), and daily calorific increase calculated (Fig. 3).

Egg production

A. avenae being parthenogenetic, egg production was studied by culturing a single fourth-stage larva, and counting the progeny at intervals. The number of eggs produced

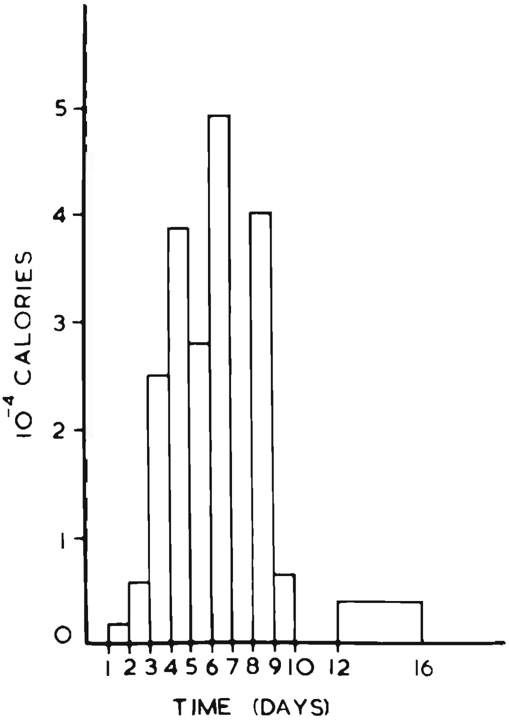

Figure 3. Daily calorific increase of *A. avenae* with age.

per day by progressively aging females was thus obtained. The dry weight was extrapolated by measuring the volume of newly laid eggs, their specific gravity, and water content.

Twenty 1.5-cm culture discs were incubated at 26 C each with a fourth-stage larva in covered solid watch glasses. After 4 days each disc was washed into a 2.5-cm-diameter nylon sieve (195-μ pore size), which was placed in a counting dish filled with water. The sieves were allowed to stand for 30 min. The agar was then squashed through the sieve so that any eggs or larvae remaining would pass through. The originally inoculated nematode was transferred onto a fresh culture, and extractions were subsequently done at 3- to 4-day intervals. Broken pieces of the female were seen in some extractions. More frequent counts of eggs laid could not be taken because of this loss. The procedure had to be repeated to obtain sufficient records of egg production for the longevity of the female. The results of two experiments are combined in Table 3.

Table 3. Reproduction of *A. avenae* at 26 C on *B. cinerea* on PDA.

Time: Days after inoculation	Time: Actual age of female (days)	No. of females observed	Total progeny: eggs	Total progeny: larvae	Mean progeny/female: eggs	Mean progeny/female: larvae
4	8	20	245	3	12.3	0.2
7	11	17	474	177	27.9	10.4
11	15	19	375	402	18.2	21.2
14	18	15	146	82	9.7	5.5
18	22	7	24	4	3.4	0.6
21	25	4	1	2	0.3	0.5

Daily egg production data were necessary to determine a daily energy budget for *A. avenae*. From the counts of eggs and larvae produced taken every 3 or 4 days, daily egg production was deduced. The eggs take 2 days to hatch at 26 C (Pillai and Taylor, 1967). Therefore, larvae counted on the 3rd or 4th day were taken to be eggs produced on the 1st and/or 2nd day. The eggs counted were produced on the 3rd and/or 4th day of each period. When total produce of 2 days was counted together, an average was taken for each day of egg production. All females were dead after 28 days at 26 C.

To convert the numerical data on egg production to calories of eggs produced/day, the calorific value of an average egg had to be established. The number of individuals contained in a pellet used for calorimetry could not be accurately determined. Therefore, the weight of an egg had to be established. The weights of eggs were obtained by measuring their volumes and the specific gravity.

To obtain the volume of newly laid eggs, *A. avenae* females were incubated in tap water for 1 hr, the eggs laid collected, and camera lucida drawings made of their outlines.

The eggs were elliptical in shape and on observation of the drawings it is clear that each egg could be described by (1) drawing a rectangle around it (Fig. 4) and (2) by taking the volume of the cube about the shorter side (b) of the rectangle less the sphere which fits within this cube (radius = ½b), the volume of the rectangular figure which is not occupied by the egg was found

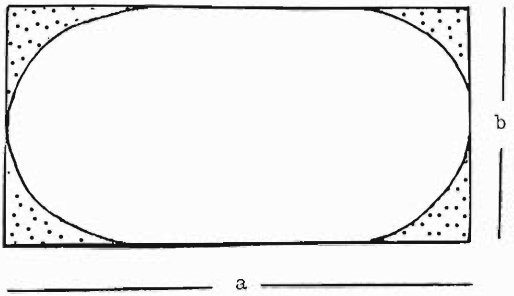

Figure 4. Diagram of egg.

(stippled in Fig. 4), and (3) by subtracting the value obtained from (2) (i.e., cube – sphere) from the volume of the rectangular figure, the volume of the egg was obtained.

Although there is a simpler method of calculating the volume of an oblong, the above method was considered more accurate as the egg is not always a perfect shape and the parameters required for the method used were very easily obtained from the drawings.

The density of *A. avenae* eggs, larvae, and adults was found by using a gradient of Ficoll[1] solutions. Ficoll was used in preference to other solutions because of its very low osmotic pressure at high concentrations.

From preliminary observations and from previous work (Andrassy, 1956; Ellenby, 1968) it was apparent that the density of *A. avenae* was more than 1.0 g/ml. Solutions of densities of 1.01 to 1.06 g/ml were prepared from a graph of the density per concentration (weight/volume) of Ficoll provided by Pharmacia Ltd.

Using a hypodermic syringe, 5 ml each of solutions of densities 1.03, 1.02, and 1.01 g/ml were layered on each other in a 25-ml centrifuge tube. When layering solutions, the hypodermic syringe was kept on the side of the tube at the level of the liquid and raised gradually as the level of liquid rose in the tube. Using a Pasteur pipette, 0.5 ml of a dense aqueous suspension of *A. avenae* (all stages) was pipetted on top of the Ficoll gradient. Another tube containing a gradient of solutions of densities 1.06, 1.05, and 1.04 g/ml was prepared as above. The tubes were centrifuged for 10 min at 3,000 rpm. On centrifugation the nematodes in the first tube dropped to the bottom indicating that they were of a higher density than 1.03 g/ml. In the second tube a few nematodes settled at each interface indicating that the density was 1.04 to 1.06 g/ml. All stages were found at each interface, therefore this does not show that the different stages have different densities.

Table 4. Distance dropped by *A. avenae* in 5 and 10 min. in solutions of Ficoll of different densities.

Density of ficoll solⁿ (g/ml.)	Max. distance dropped in 5 min.	Max. distance dropped in 10 min.	Position of concentration of *A. avenae* 10 min.
1.04	3.0 mm	11.0 mm	3.0 mm
1.05	2.0 mm	10.0 mm (few)	at interface
1.06	2.0 mm (very few)	10.0 mm (very few)	at interface

An aqueous suspension of *A. avenae* was carefully placed onto the surface of tubes containing Ficoll of 1.04, 1.05, and 1.06 g/ml. The distance from the surface that the nematodes dropped was observed at 5 and 10 min (Table 4). In solution of density 1.04 most nematodes sank in 10 min. In the solution of density 1.05 g/ml a few sank in 10 min but most were at the interface (water and Ficoll). The density of *A. avenae* was therefore taken as 1.05.

Although 10- to 14-day cultures contained large numbers of eggs, it was not possible to isolate enough for pellets. Therefore, the calorific value of 5.453 cal/g as obtained from the mixed samples of *A. avenae* was used to estimate the mean calorific value of an egg as 0.3718^{-4} cal/g ± SE 0.0734^{-4}.

Using the mean calorific value of *A. avenae* eggs, calories of eggs produced per day was determined (Fig. 5).

Respiration

A cartesian diver Zeuthen (1950) constructed and used in accordance with instructions given by Klekowski (1967) was used to obtain reliable values of oxygen consumption of all stages of *A. avenae* from egg to adult. The divers were of 1- to 3-μl gas volume, with fitted stoppers.

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[1] Pharmacia (Great Britain) Ltd.

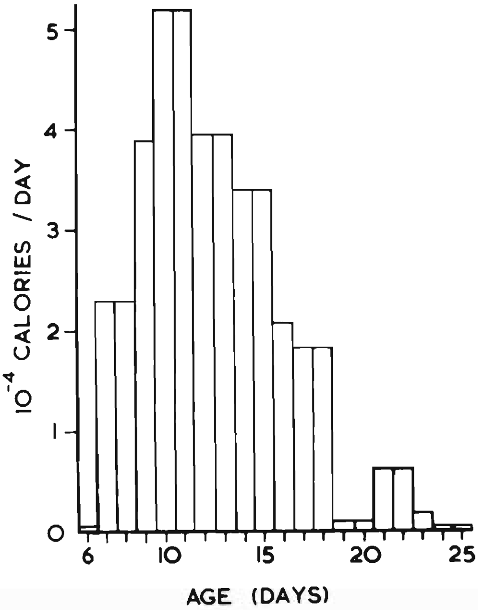

Figure 5. Calorific value of eggs produced per day by an average female.

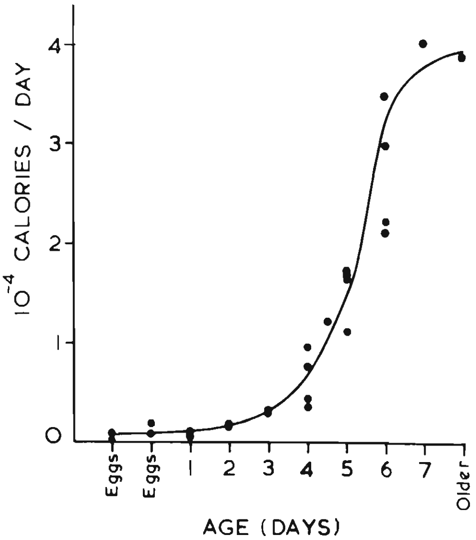

Figure 6. Calories respired by an individual per day by various ages of *A. avenae.*

Freshly laid eggs up to 18 hr and from 18 hr to hatching age (48 hr) were obtained by incubating adult females in water in watch glasses for 18 hr. The eggs laid were collected and either used immediately for respiratory studies or incubated at 26 C for a further 18 hr. Respiratory studies were made on these two arbitrarily aged groups of eggs, and on newly hatched larvae to adults at daily intervals. To obtain a reliable rate of oxygen consumption, it was necessary to use a minimum of 20 eggs or 20 1-, 2-, or 3-day-old larvae per diver. Divers usually held in excess of this number. For studies on older *A. avenae* between nine and 20 individuals were placed in each diver.

The calories utilized in respiration by the various stages of *A. avenae* are plotted in Figure 6. As explained by Brody (1945), and others, the range of calorific equivalents of oxygen consumption when varying substrates are used in respiration is 4.7 to 5.0 kcal per liter, which is within the limits of experimental error. Therefore, the respiratory quotient was not determined but a value of 4.825 kcal per liter of oxygen consumed was used in obtaining respiratory loss in calories.

Discussion

An energy budget may be constructed from the estimates of ingestion, growth, egg production, and respiration. Figure 7 and Table 5 present the calories ingested and used in growth, reproduction, and respiration on any particular day in the life of *A. avenae* at 26 C. Feeding commenced as soon as the larva hatched and reached a peak feeding level on about the 5th day. Growth was also very rapid during this period with the larva maturing into an adult on about the 5th day.

Of the three parameters used in estimating the volume of food ingested, the volume of the valve and the time spent feeding is dependent on the age, and has most bearing on the change in daily ingestion. The volume of the valve increases during the growth period, but levels off when the adult is produced. The pulsation rate does not depend on age, and is not widely variable throughout life. *A. avenae* stops feeding at various times in the life cycle. A few 3- and 4-day-old *A. avenae* from the sample were found to be in a molt,

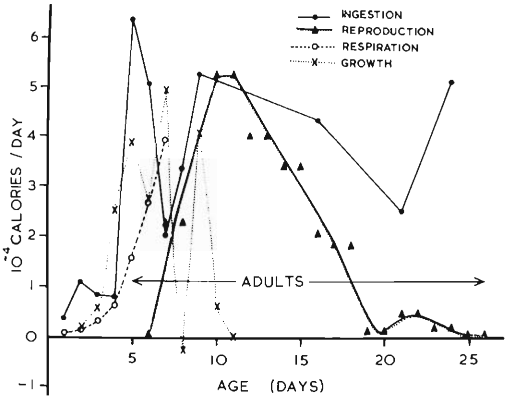

Figure 7. Mean individual energy budget of *A. avenae* per day.

Table 5. Mean individual energy budget per day of *A. avenae*.

Age in days	Ingestion in 10^{-4} calories	Growth in 10^{-4} calories	Egg-production in 10^{-4} calories	Respiration in 10^{-4} calories
1	0.3966	—	0.0000	0.0891
2	1.1117	0.1903	0.0000	0.1726
3	0.8408	0.5693	0.0000	0.3164
4	0.8245	2.5094	0.0000	0.6294
5	6.3484	3.8519	0.0000	1.5444
6	5.0268	2.7921	0.0558	2.6919
7	2.0064	4.9267	2.2791	3.9374*
8	3.3413	-0.2858	2.2791	3.9374
9	5.2014	4.0484	3.8667	3.9374
10	—	0.6355	5.2052	3.9374
11	—	0.0000	5.2052	—
12	—	0.0000	3.9411	—
13	—	0.0000	3.9411	—
14	—	0.0000	3.3834	—
15	—	0.0000	3.3834	—
16	4.2915	0.0000	2.0449	—
17	—	0.0000	1.8218	—
18	—	0.0000	1.8218	—
19	—	0.0000	0.1115	—
20	—	0.0000	0.1115	—
21	2.4829	0.0000	0.6321	—
22	—	0.0000	0.6321	—
23	—	0.0000	0.1859	—
24	5.0816	0.0000	0.0558	—
25	—	0.0000	0.0558	—

* Average respiration of 7 day and older adults.

and as such were not feeding during the time of observation. This depressed the mean value for the time spent feeding, and thereby ingestion per day. The time spent in feeding varies greatly between *A. avenae* of the same age. The standard error of the mean of the time spent feeding is thereby very large (Fig. 1). Both the adults and the larval stages show this variation. My estimate of ingestion is lower than is expected from values obtained for gross production (Table 5). This probably resulted from observations of feeding made with a cover slip placed on the nematode. This may have retarded feeding. The replacement of the cover slip with a thin sheet of polyethylene gives a better preparation.

Egg production started on the 6th day, and there was a tendency for the rate of feeding to be greatly reduced during this period. There was a slight loss in the average biomass of the adults between days 7 and 8, corresponding with the start of egg laying by the majority of adults. The calorific intake on day 7 was 2.0064^{-4} (Table 5) with a decrease in biomass of 0.2858^{-4} cal between days 7 and 8.

Growth ceased on the 10th day and egg production reached a peak level during this period. Ingestion varied from day to day during adult life. Egg production was reduced to 0.1115^{-4} cal by the 19th day, but ingestion continued at approximately the same rate as during the peak egg-laying period. Since net production (growth + egg production) was low after day 19, the energy ingested was either mostly not assimilated or the cost of maintenance (respiration) must have risen. The adult, therefore, became less efficient either in assimilation or in respiration as it aged. The cost to the host therefore persisted even though the nematode was not productive. From Figure 7 it appears that gross production (= respiration + net production = assimilation) may be in excess of ingestion on some days. Growth was initially measured as an increase in volume, and by determining an average calorific value (of all ages), it was converted to calories. Therefore, an increase in calorific value of the standing crop without an increase in volume may cause *an error*. The energy ingested on one day and used on succeeding days for processes such as egg production may not be appreciated in an individual daily budget as presented in Figure 7. Therefore, a cumulative energy budget was

Table 6. Cumulative energy budget.

Age (days)	Ingestion (I) 10^{-4} calories	Growth 10^{-4} calories	Egg Prodcn. 10^{-4} calories	Net Prodcn.*[1] 10^{-4} calories	Respiration 10^{-4} calories	Assimilation*[1] 10^{-4} calories
1	0.3966		0.0000	0.0000	0.0891	0.0891
2	1.5083	0.1903	0.0000	0.1903	0.2617	0.4520
3	2.3491	0.7596	0.0000	0.7596	0.5781	1.3377
4	3.1736	3.2690	0.0000	3.2690	1.2075	4.4765
5	9.5330	7.1209	0.0000	7.1209	2.7519	9.8728
6	14.5488	9.9130	0.0558	9.9688	5.4438	15.4126
7	16.5552	14.8397	2.3349	17.1746	9.3812	26.5558
8	19.8965	14.5539	4.6140	19.1679	13.3186	32.4865
9	25.0979	18.6023	8.4807	27.0830	17.2560	44.3390
10		19.2378	13.6859	32.9237	21.8748	54.7985
11		19.2378*	18.8911			
12		19.2378	22.8322			
13		19.2378	26.7733			
14		19.2378	30.1567			
15		19.2378	33.5401			
16		19.2378	35.5850			
17		19.2378	37.4068			
18		19.2378	39.2286			
19		19.2378	39.3401			
20		19.2378	39.4516			
21		19.2378	40.0837			
22		19.2378	40.7158			
23		19.2378	40.9017			
24		19.2378	40.9575			
25	105.00	19.2378*	41.0133	60.2511		

* No significant growth after the 10th day.
*[1] excluding exuviae.

constructed (Table 6) by summing the ingestion or production of one day to that of the preceding days, as outlined by Klekowski and Zyromska-Rudzka (1967). From this analysis, ingestion, growth, egg production, and cost of maintenance up to a given day may be obtained. There was no significant change in standing crop after day 10 and so the cumulative production of body tissue remained constant at 19.2378^{-4} cal. An average value of respiration was obtained from Table 5. From day 7 to day 10 the value was obtained from respiratory studies on day 7 and older, unaged but egg-laying females. No correction was made for the respiration of eggs produced within divers, since neither time of laying nor respiration of eggs *in utero* was known.

Ingestion was not measured continuously after day 9. When measured after day 9, daily ingestion varied between 2.4829^{-4} cal and 5.0816^{-4} cal (on days 21 and 24, respectively) and therefore it was not possible to obtain a reliable average cumulative figure. Therefore, cumulative values for ingestion are only available up to day 9. It is apparent that cumulative assimilation (= gross production) exceeded cumulative ingestion from day 4 onward. This error is probably caused by a low estimate of ingestion.

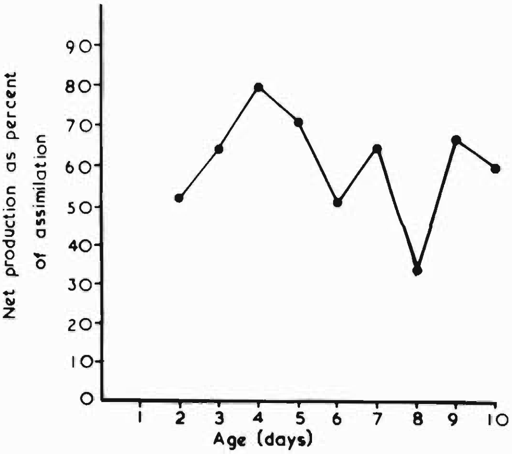

Figure 8. Net production efficiency of *A. avenae*.

The gross production efficiency, $k = P/I$ (Ivlev, 1939a, b), cannot be assessed without accurate values of ingestion. Net production efficiency, $k_2 = P/A$ (Ivlev, 1939a, b), is calculated (Fig. 8) from values given in Table 3. The exuviae fraction was not determined and therefore there is a small error in the net production quoted and assimilation quoted of the molting stages. The efficiency of *A. avenae* as a converter of energy (Fig. 8) increased in the larval stages from 52% in the youngest larvae to 80% in the fourth-stage larva. A minimum net production efficiency of 34% was obtained on day 8, caused by the loss in biomass between days 7 and 8.

From the energy budgets constructed the net loss to the host for 1 day by supporting a given aged nematode or the cumulative net loss of supporting that nematode up to any age of its life may be obtained. Since the estimate of ingestion for *A. avenae* is low the net loss assessed by assimilation of *A. avenae* is a minimum. For example, a 9-day-old adult assimilated 11.8525^{-4} cal (Table 5) on day 9 and 44.3390^{-4} cal (Table 6) throughout life. This is a minimum estimate of the net cost to the fungus of supporting one 9-day *A. avenae*. Gross loss to the fungus is probably even more than what is ingested.

The flow of energy through a host organism and the points at which a parasite may disturb this flow can be found and quantified by the construction of energy budgets for a host and its parasites. Although this may be an over-simplification of the host–parasite complex, energy flow studies provide a means of quantifying pathogenicity (by estimating energy budgets for parasitized and nonparasitized host), efficiency of parasitism (of any particular host–parasite system), and the efficiency of a parasite. In nature the system is greatly complicated by host reactions, tissue changes, secondary invasions, and so on. Nevertheless, the tools and notions of energetic studies may prove beneficial to the quantification of host–parasite relationships.

Acknowledgments

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Brachylaimid and Dicrocoeliid Trematodes of Birds from Palawan Island, Philippines[1]

JACOB H. FISCHTHAL AND ROBERT E. KUNTZ[2]

ABSTRACT: New species of brachylaimid trematodes of birds from Palawan Island, Philippines, are *Leucochloridium palawanense* and *L. philippinense*; new dicrocoeliids are *Brachylecithum palawanense*, *B. philippinense*, *Lyperosomum duculae*, *L. palawanense*, and *Zonorchis philippinensis*. *Leucochloridium* sp. and *Brachylecithum* spp. are reported but not allocated to species as the specimens are immature or incomplete. Previously reported species briefly redescribed are the brachylaimid *Brachylaima fuscata* (Rudolphi, 1819) and the dicrocoeliid *Conspicuum aenigma* (Gvozdev, 1956) comb. n. (syn. *Skrjabinus aenigma* Gvozdev, 1956). *Skrjabinus indicus* Jaiswal, 1957, from India is transferred to *Conspicuum* as *C. indicum* (Jaiswal, 1957) comb. n.

The trematodes of this report were part of a collection made by the junior author while a member of the U. S. Naval Medical Research Unit No. 2, Taipei, Taiwan, and serving as a guest investigator on the Silliman University–Bishop Museum Expedition to Palawan Island, Republic of the Philippines. Host names are those reported by Kuntz (1969). The trematodes were washed in saline, killed in hot water, and transferred immediately to FAA fixative. After 4 to 8 hr they were stored in 70% alcohol plus 2% glycerine; staining was in Harris' hematoxylin or carmalum and fast green; all were mounted in balsam. Specimens have been deposited in the U. S. National Museum Helminthological Collection as noted. All measurements are in microns. New host records are indicated by an asterisk (*).

Family Brachylaimidae

Brachylaima fuscata (Rudolphi, 1819) Joyeux, Baer, and Timon-David, 1932

HOSTS: **Anthracoceros marchei* Oustalet, Palawan hornbill (Coraciiformes: Bucerotidae); **Aplonis panayensis panayensis* (Scopoli), Philippine glossy starling (Passeriformes: Sturnidae).

HABITAT: Small intestine.

LOCALITY: Tarabanan Concepción, Palawan Island, Philippines.

DATE: 14 May 1962.

SPECIMEN DEPOSITED: No. 72164.

MEASUREMENTS AND SOME PERTINENT DATA BASED ON ONE ADULT WORM FROM *A. marchei*: Body 2,250 long by 600 wide at acetabular level; forebody 720 long; hindbody 1,275 long; forebody–hindbody length ratio 1:1.77; preoral space 24 long; oral sucker 245 by 215; acetabulum 255 by 250, center at anterior 38% of body length; sucker length ratio 1:1.04, width ratio 1:1.16; pharynx 125 by 145; esophagus 36 by 26; postcecal space 63 long; anterior testis 225 by 270, lying 650 postacetabular; posterior testis 185 by 220; posttesticular space 145 long; seminal vesicle 58 in maximum width, extending anteriorly and then looping posteriorly; pars prostatica with loop; cirrus sac 98 by 36; genital pore ventral to anterior testis, 100 from its anterior margin; ovary 175 by 225, overlapping both testes dorsally; vitellaria extending from posterior part of acetabulum to anterior testis; uterus probably not fully developed as few coils present, extending 162 preacetabular, 167 postbifurcal; metraterm C-shaped, dextral to cirrus sac, longer than latter; 10 eggs measuring 24–31 (27.5) by 15–19 (16.4); excretory bladder thick-walled, bifurcating between cecal ends.

DISCUSSION: From *Aplonis p. panayensis*

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were recovered one worm just beginning egg production (most abnormal) and one immature. In all probability Tubangui's (1928) immature *Harmostomum* sp. from the rough-crested cuckoo, *Dasylophus superciliosus* (Cuvier) (Cuculiformes: Cuculidae), from Luzon Island, Philippines, is *Brachylaima fuscata*.

Leucochloridium palawanense sp. n. (Figs. 1, 2)

HOST: *Pitta sordida sordida* (P. L. S. Müller), black-headed pitta (Passeriformes: Pittidae).

HABITAT: Small intestine.

LOCALITY: Puerto Princesa, Palawan Island, Philippines.

DATE: 22 May 1962.

SPECIMENS DEPOSITED: No. 72165 (holotype); No. 72166 (paratypes).

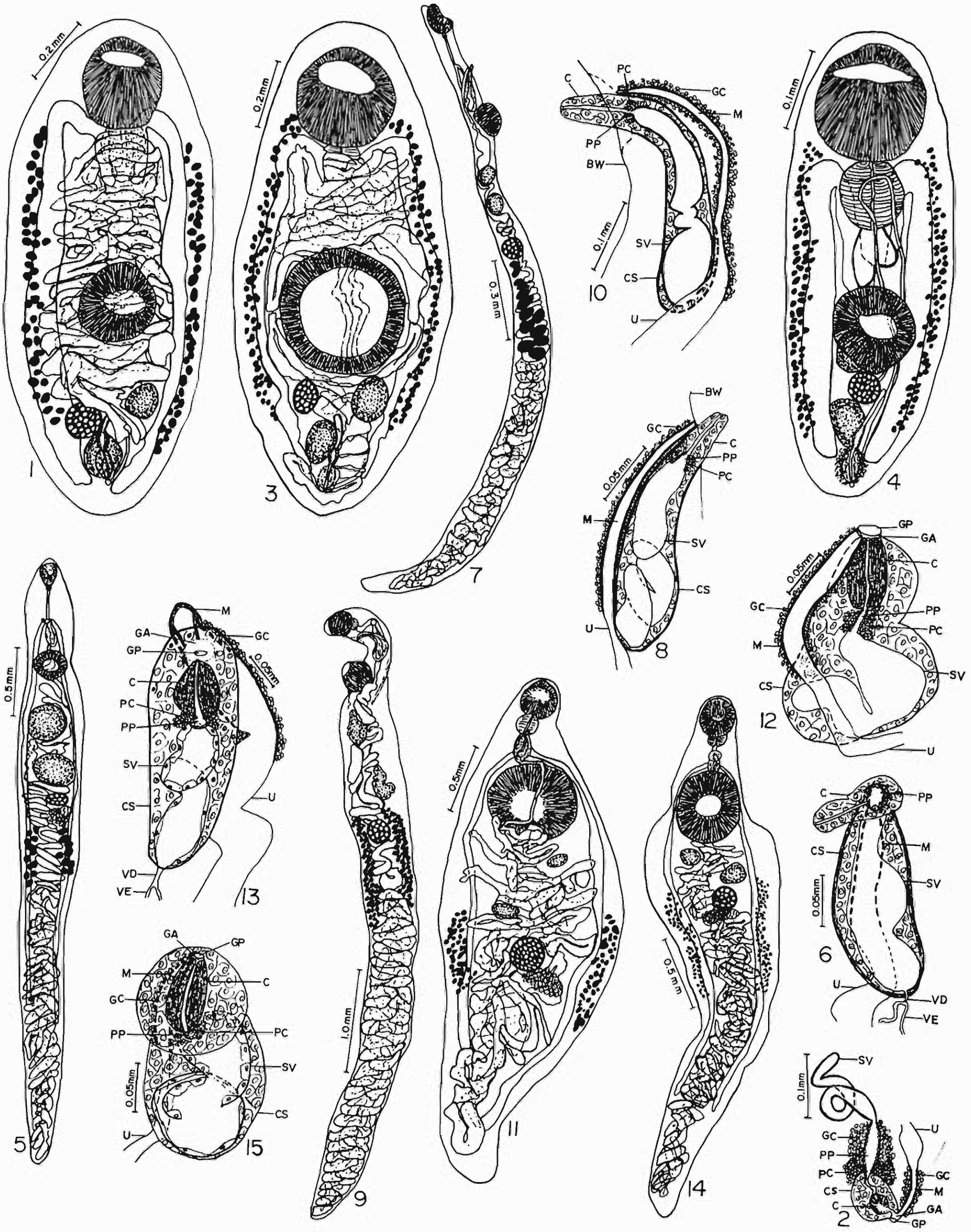
DIAGNOSIS (based on seven adult worms; six measured): Body elongate, relatively narrow, usually widest at acetabular level, extremities rounded, tegument entirely spined, 1,325–1,535 long by 415–600 wide. Forebody 630–795 long; hindbody 495–525 long; forebody–hindbody length ratio 1:0.65–0.79. Black pigment granules scattered throughout parenchyma. Oral sucker subterminal ventral, longer and slightly convex posteroventrally, somewhat shorter and slightly concave posterodorsally, rounded anteriorly, 235–270 by 190–235; preoral space 18–20 long in two slightly macerated specimens, 47–53 in normal specimens. Acetabulum round to longitudinally or transversely elongate, not filling intercecal space, 200–240 by 180–240, center at anterior four-sevenths of body length; sucker length ratio 1:0.79–0.91, width ratio 1:0.91–1.05. Prepharynx very short, not always apparent; pharynx usually contiguous with concave posterodorsal part of oral sucker, round to slightly longitudinally or transversely elongate, 131–148 by 118–153; esophagus thick-walled, muscular, 41–44 by 20–30, opening into expanded, thick-walled prececal sac (53–97 by 75–92); ceca surrounded by layer of cuboidal cells, lined internally with layer of columnar cells, constricted at point of emergence from prececal sac, passing dorsally and then anteriorly to sides of oral sucker (which it may overlap) before looping posteriorly, extending posttesticularly; postcecal space 36–77 long.

Gonads smooth, usually longitudinally elongate but one occasionally round, postacetabular, in triangular arrangement, testes usually contiguous with ovary but sometimes anterior testis separated by uterus. Anterior testis ventrosinistral, 104–130 by 90–109, lying 73–152 postacetabular; posterior testis dorsomedian, lying anterior to cecal ends by which it is more or less embraced, 114–130 by 94–125; posttesticular space 78–128 long. Seminal vesicle dextral, tubular, very thick-walled (5–13) with thin inner longitudinal muscle layer and thick outer circular muscle layer, winding, passing posteriorly ventral or just median to ovary. Pars prostatica straight, narrower and shorter than seminal vesicle, 60–110 by 26–28, walls thick (4–6) and muscular as for seminal vesicle, surrounded by large mass of gland cells, prostate cells numerous at junction with cirrus sac and smaller than gland cells. Cirrus sac thick-

→

Abbreviations: BW, body wall; C, cirrus; CS, cirrus sac; GA, genital atrium; GC, gland cells; GP, genital pore; M, metraterm; PC, prostate cells; PP, pars prostatica; SV, seminal vesicle; U, uterus; VD, vas deferens; VE, vas efferens.

Figures 1–15. *Leucochloridium palawanense* sp. n. 1. Whole mount, holotype, ventral view. 2. Terminal genitalia, paratype, ventral view. *Leucochloridium philippinense* sp. n. 3. Whole mount, holotype, ventral view. *Leucochloridium* sp. 4. Whole mount, ventral view. *Brachylecithum palawanense* sp. n. 5. Whole mount, holotype, ventral view. 6. Terminal genitalia, holotype. *Brachylecithum philippinense* sp. n. 7. Whole mount, holotype, dextrolateral view. 8. Terminal genitalia, holotype. *Lyperosomum duculae* sp. n. 9. Whole mount, holotype, body from just postacetabular anteriorly in sinistrolateral view, remainder of body in dorsal view. 10. Terminal genitalia, holotype. *Lyperosomum palawanense* sp. n. 11. Whole mount, holotype, dorsal view. 12. Terminal genitalia, holotype. *Conspicuum aenigma* (Gvozdev, 1956) comb. n. 13. Terminal genitalia, ventral view. *Zonorchis philippinensis* sp. n. 14. Whole mount, holotype, ventral view. 15. Terminal genitalia, holotype.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walled, muscular, longitudinally oval, 77–99 by 44–53, surrounded by gland cells. Cirrus muscular, sinuous posteriorly, anterior part inverted within cirrus sac, chamberlike, with long, slightly curved spines (8–19 by 2 at base), longest spines at bottom of chamber (at anterior end of cirrus when everted) and shortest anteriorly. Genital atrium shallow, small. Genital pore ventral, median to slightly submedian, 63–80 from posterior extremity, usually posttesticular but occasionally at posterior margin of posterior testis, at or just posterior to cecal ends.

Ovary dorsal, at same depth level as posterior testis, lying posterodextral to anterior testis and anterodextral to posterior testis, occasionally smaller or same size as either testis to larger than anterior testis, 106–123 by 92–111. Oviduct emerging from sinistrolateral margin of ovary. Ootype complex median to ovary and intertesticular. Vitellaria in lateral extracecal fields, extending from level of posterior margin of oral sucker or pharyngeal level to posterior testis level, terminating 153–225 from posterior extremity, well anterior to cecal ends, follicles ventral and lateral to ceca but not dorsal; transverse vitelline ducts emerging from near posterior end of each vitellarian field, uniting to form relatively large vitelline reservoir lying anterosinistral to posterior testis. Uterus with single ascending and descending limbs passing dorsal to acetabulum, ascending on right (rarely on left), descending on opposite side, coils many between posterior testis and oral sucker, without anterolateral loops extending prececally, transverse coils overlapping pharynx ventrally. Metraterm thick-walled, muscular, straight, longer than cirrus sac, lying sinistral to latter, opening into genital atrium. Eggs numerous, yellow near ovary, becoming slightly darker as they progress through uterus, operculate, 18 measuring 21–27 (23) by 12–14 (13).

Excretory bladder thick-walled, tubular, short, posttesticular; pore subterminal dorsal.

DISCUSSION: The distribution of the uterine coils separates our new species from all those that can be included in Kagan's (1952) concept of the genus *Urogonimus* Monticelli, 1888 (syn. of *Leucochloridium* Carus, 1835). Our form differs from Kagan's concept of *Leucochloridium* in lacking lateral prececal uterine loops. It differs from Kagan's new genus *Neoleucochloridium* (syn. of *Leucochloridium*) in lacking a pustulated cirrus and in having the uterine coils extending anterior to the cecal bifurcation. *L. palawanense* sp. n. differs from all known species in the genus in the shape of the oral sucker and presence of a thick-walled, muscular seminal vesicle, and from all but *L. cyanocittae* McIntosh, 1932 (syn. of *L. actitis* McIntosh, 1932) in having a spined cirrus. The latter species differs further in possessing prececal uterine loops on the sides of the oral sucker, the vitellaria extending postcecally or nearly so, and the anterior testis being near the posterior margin of the acetabulum.

Leucochloridium philippinense sp. n.
(Fig. 3)

HOST: *Pitta s. sordida.*

HABITAT: Small intestine.

LOCALITY: Puerto Princesa.

DATE: 22 May 1962.

SPECIMEN DEPOSITED: No. 72167 (holotype).

DIAGNOSIS (based on one worm): Body elongate, somewhat broad, widest at acetabular level, extremities rounded, tegument entirely spined, 1,452 long by 650 wide. Forebody 660 long; hindbody 410 long; forebody–hindbody length ratio 1:0.62. No black pigment granules in parenchyma. Oral sucker subterminal ventral, nearly round, 302 by 290; preoral space 70 long; acetabulum round, nearly filling intercecal space, separated from ceca by uterine coils, 382 by 385, center at anterior three-fifths of body length; sucker length ratio 1:1.26, width ratio 1:1.33. Prepharynx not apparent; pharynx nearly round, 100 by 110, overlapping oral sucker dorsally; esophagus very short; no prececal sac; ceca surrounded by layer of cuboidal cells, lined internally with layer of columnar cells, slightly ascending sides of oral sucker, extending to near posterior extremity; postcecal space 77 long.

Gonads smooth, longitudinally elongate, at same depth level; ovary and anterior testis lying just postacetabular, symmetrical; posterior testis mediodiagonal to other gonads; anterior testis sinistral, 155 by 116; posterior testis dextral, lying anterior to cecal ends, 142 by 107; posttesticular space 130 long. Seminal vesicle intertesticular, mediosinistral, tubular, very thick-walled (6–14) with thin inner longitudinal muscle layer and very thick outer circular muscle layer, slightly curved, 143 by 46, commencing at overy–anterior testis level. Pars prostatica elongate oval, 41 by 24, walls thick (5) and muscular as for seminal vesicle, narrowing for short distance before entering cirrus sac, surrounded by large mass of gland cells. Margins of cirrus sac obscured by eggs. Cirrus muscular, unspined, smooth. Genital atrium shallow, small. Genital pore ventral, median, postcecal, 60 from posterior extremity.

Ovary dextral, 109 by 82. Oviduct emerging from sinistrolateral margin of ovary. Ootype complex median to ovary, intertesticular. Vitellaria in lateral extracecal fields, extending from level of posterior part of oral sucker to level of anterior part of posterior testis, terminating 250 from posterior extremity, well anterior to cecal ends, follicles ventrolateral to ceca but not dorsolateral or dorsal; transverse vitelline ducts emerging from near posterior end of each vitelline field, uniting to form large vitelline reservoir lying intertesticular. Uterus with single dextral ascending and sinistral descending limbs passing dorsal to acetabulum, coils many between acetabulum and posterior margin of oral sucker, without anterolateral coils extending prececally, with few coils postacetabularly, ascending and descending between ovary and anterior testis, descending further intertesticularly, much coiled sinistral to posterior testis, with coils between latter, cecal ends, and posterior extremity. Metraterm thick-walled, muscular, partly posterior to left cecum, lying sinistral to cirrus sac, surrounded by gland cells. Eggs numerous, yellow-brown, operculate, 10 measuring 21–27 (24) by 12–14 (13).

Excretory bladder thick-walled, tubular, short, posttesticular; pore subterminal dorsal.

Discussion: This species differs from Kagan's concepts of the genera *Urogonimus*, *Neoleucochloridium*, and *Leucochloridium* as discussed for *L. palawanense*. In having a thick-walled, muscular seminal vesicle the present form differs from all species of the genus except *L. palawanense*. *L. philippinense* differs from the latter in the shape of the oral sucker, having the acetabulum larger than the oral sucker, lacking a prececal sac, the ovary and anterior testis being just postacetabular, and possessing an unspined cirrus and posttesticular uterine coils. Our form closely resembles *L. turdi* Yamaguti, 1939, which differs further in its more posteriorly placed genital pore and the anteriormost extent of the uterine coils being at the cecal bifurcation. In the posttesticular extension of the uterine coils and posterior extent of the vitellaria in relation to the cecal ends, our form also resembles *L. dasylophi* Tubangui, 1928, *L. hypotaenidiarum* Tubangui, 1927, and *L. nainitalensis* Baugh, 1962; these species differ further from our form in having the acetabulum equatorial and the gonads lying far posterior to the acetabulum. *L. dasylophi* also differs in having a dorsal genital pore and larger eggs; *L. hypotaenidiarum* in the posterior testis being far anterior to the cecal ends, the genital pore dorsal, and the vitellaria extending posttesticularly; and *L. nainitalensis* in the vitelline fields commencing postpharyngeally.

Leucochloridium sp. (Fig. 4)

Host: *Anthus gustavi gustavi* Swinhoe, wagtail (Passeriformes: Motacillidae).

Habitat: Small intestine.

Locality: Tarabanan Concepción.

Date: 14 May 1962.

Specimens deposited: No. 72168.

Description (based on 10 immature worms; four measured): Body elongate oval, widest just preacetabular, extremities rounded, tegument spined, 430–580 long by 182–285 wide. Forebody 222–295 long; hindbody 111–162 long; forebody–hindbody length ratio 1:0.49–0.56. Oral sucker subterminal ventral, slightly longitudinally elongate, 143–172 by 136–160; preoral space 10–24 long; acetabulum round to slightly longitudinally or transversely elongate, postequatorial, filling intercecal space, 97–123 by 92–121, center at anterior 61–64% of body length; sucker length ratio 1:0.68–0.76, width ratio 1:0.68–0.78. Prepharynx very short, often not apparent; pharynx not overlapping oral sucker, round to slightly longitudinally elongate, 75–92 by 65–90; esophagus thick-walled, very short, opening into thick-walled prececal sac; ceca thick-walled, passing dorsally and then anteriorly to sides of posterior part of oral sucker before looping posteriorly, extending posttesticularly to within 24–44 of posterior extremity.

Gonads smooth, usually longitudinally elongate but occasionally transversely elongate, contiguous to overlapping one another, tending to go from dorsal (anterior testis) to middepth (ovary) to ventral (posterior testis) position, usually with anterior testis sinistral and with ovary and posterior testis median to mediodextral and tandem to diagonal, sometimes all three tandem or with anterior testis dextral, in two worms ovary entirely posttesticular in position normally occupied by posterior testis. Anterior testis overlapping acetabulum dorsally, 52–66 by 41–75; posterior testis 53–69 by 44–69; posttesticular space 34–73 long. Seminal vesicle sinuous, walls appearing thick and cellular; pars prostatica elongate oval, narrowing to short duct before entering elongate oval, posttesticular cirrus sac, both structures surrounded by gland cells; cirrus smooth, unspined. Genital pore median to slightly submedian, ventral, very near posterior extremity. Ovary 39–48 by 34–53. Vitellaria in lateral fields, extending from posterior part of oral sucker to posterior testis level, terminating 76–97 from posterior extremity, well anterior to cecal ends, follicles only ventral and ventrolateral to ceca. Uterus only with single sinuous ascending and descending limbs extending anteriorly to pharynx, passing dorsal to acetabulum; metraterm thick-walled, longer than and sinistral to cirrus sac.

DISCUSSION: The present form differs from *L. palawanense* in having an unspined cirrus and the cirrus sac lying entirely posttesticular, and from *L. philippinense*, *L. dasylophi*, and *L. hypotaenidiarum* in having the acetabulum considerably smaller than the oral sucker. Because the coiling of the uterus and the body proportions are not fully developed, the worms cannot be identified.

Family Dicrocoeliidae

Brachylecithum palawanense sp. n.

(Figs. 5, 6)

HOSTS: Type, *Halcyon chloris collaris* (Scopoli), white-collared kingfisher; *Ceyx rufidorsus rufidorsus* Strickland, red-backed kingfisher (Coraciiformes: Alcedinidae).

HABITAT: Small intestine (?).

LOCALITY: Tarabanan Concepción.

DATES: 12, 14 May 1962.

SPECIMENS DEPOSITED: No. 72169 (holotype, from *Halcyon*); No. 72170 (paratypes, *Halcyon*); No. 72171 (paratypes, *Ceyx*).

DIAGNOSIS (based on seven adult worms in dorsal or ventral view from *Halcyon*, measurements of six given below; and five adults in lateral view from *Ceyx*, measurements not included below): Body elongate, narrow, extremities rounded, 3,305–4,535 long by 270–485 wide at testicular level. Forebody 535–695 long; hindbody 2,535–3,365 long; forebody-hindbody length ratio 1:4.3–6.2. Oral sucker subterminal ventral, longitudinally elongate, somewhat inverted pear-shaped, truncate or nearly so posteriorly, in ventral or dorsal view with compact layer of muscles just within posterior and posterolateral margins, in lateral view muscle layer posterior, posterodorsal and posteroventral, 148–177 by 114–153; preoral lip prominent, 31–61 long; acetabulum usually somewhat transversely elongate but occasionally round, 173–235 by 186–252; sucker length ratio 1:1.05–1.33, width ratio 1:1.47–2.41. Prepharynx absent; pharynx round or nearly so, sometimes with nipplelike projection at anterior end, 51–61 by 47–58; esophagus 121–230 long; cecal bifurcation 130–180 preacetabular; ceca extending short distance into posterior third of body.

Gonads smooth, transversely oval, separated from one another by uterine coils. Anterior testis slightly sinistral in four worms and slightly dextral in two, 90–270 by 120–285, lying 170–315 postacetabular in worms from *Halcyon*, lying closer to acetabulum in worms form *Ceyx*; posterior testis slightly dextral in four worms but slightly sinistral in two when anterior testis dextral, 110–242 by 118–310, lying 22–110 posterior to anterior testis. Cirrus sac elongate oval, straight, thick-walled, muscular, commencing dorsal to anteriormost part of acetabulum or entirely preacetabular, 157–196 by 65–78. Seminal vesicle winding, somewhat coiled, filling most of cirrus sac when cirrus protruded, 125–180 by 40–70. Pars prostatica roundish, small, surrounded by few prostate cells. Cirrus large, muscular, usually protruded, opening into posterior part of genital atrium. Genital pore median, at cecal bifurcation.

Ovary in tandem with posterior testis, slightly dextral in four worms but slightly sinistral in two when posterior testis sinistral, 97–120 by 127–170, lying 35–143 posterior to posterior testis. Seminal receptacle very large, 97–148 by 116–155, lying postovarian and slightly more lateral than latter. Mehlis' gland well developed, lying posteromedian to seminal receptacle at or near anterior limits of vitellaria. Latter follicular, in two short, subequal, lateral fields; field on ovarian side 335–430 long, with eight follicles in one worm, nine in five; opposite field 350–605 long, with 10 follicles in one worm, 12 in five; anteriormost follicle lying 93–190 postovarian; postvitellarian space 1,130–2,015 long. Uterus filling most of hindbody, extending to near posterior extremity, ascending median to ovary and posterior testis with single loop between them, crossing (with several loops) between testes to median side of anterior testis. Metraterm thick-walled, muscular, shorter than and lying dorsal to cirrus sac, opening into shallow genital atrium anterior to male opening. Eggs numerous, operculate, 25 measuring 29–37 (32.3) by 15–19 (17.9).

Excretory bladder tubular, sometimes dilated, where visible posteriorly; narrowing to short duct before opening through terminal pore.

DISCUSSION: This form keyed to the genus *Brachylecithum* Strom, 1940, in the key given by Odening (1964). While some of the five specimens from *Ceyx* were smaller than those from the other host, the morphology, measurements, and ratios of all were basically similar. It differs from all others in the genus in the shape of the oral sucker. It is closest to *B. halcyonis* (Yamaguti, 1941) Skrjabin and Evranova, 1953, *B. tetraogalli* Gvozdev, 1953, *B. bubo* Chibichenko, 1959, and *B. indicum* Singh, 1962. The latter species differs further in the shape of the gonads, the testes being tandem, very close to each other, and separated by only a single uterine coil, and the extent of the vitellaria never being more than twice the length of the ovary rather than three to four times its length. *B. halcyonis* differs further in the shape of the gonads, and in having tandem testes and larger eggs (42 by 21–22). *B. bubo* differs further in having tandem testes, an S-shaped cirrus sac, and somewhat larger eggs (36–40 by 18–20). *B. tetraogalli* differs further in having a postbifurcal genital pore and larger eggs (47 by 26–30).

***Brachylecithum philippinense* sp. n. (Figs. 7, 8)**

HOST: *Tersiphone cyanescens* (Sharpe), blue paradise flycatcher (Passeriformes: Musicapidae).

HABITAT: Small intestine (?).

LOCALITY: Tarabanan Concepción.

DATE: 14 May 1962.

SPECIMEN DEPOSITED: No. 72172 (holotype).

DIAGNOSIS (based on one worm in dextrolateral view; measurements are length by depth): Body elongate, narrow, extremities rounded, 2,563 by 143 at vitellarian level. Forebody 420 long; hindbody 2,005 long; forebody–hindbody length ratio 1:4.8. Oral sucker subterminal ventral, 141 by 100, with compact muscle layer inside posterior, posterodorsal, and posteroventral margins; preoral space 8 long; acetabulum 138 by 95; sucker length ratio 1:0.98, depth ratio 1:0.95. Prepharynx absent; pharynx very small, 29 by 32; esophagus 115 long; cecal bifurcation 120 preacetabular.

Testes two, smooth, round, tandem, 36 apart, both 75 in diameter, anterior testis lying 95 postacetabular. Cirrus sac thick-walled, muscular, somewhat oval with narrower part anteriorly, 172 by 52. Seminal vesicle filling most of cirrus sac, sinuous. Pars prostatica small, surrounded by few prostate cells. Cirrus large, protruded. Genital atrium shallow. Genital pore median, at cecal bifurcation.

Ovary smooth, in tandem with testes, 77 by 90, lying 97 posterior to posterior testis. Vitellaria follicular, in two short lateral fields 350 long, anteriormost limit at posterior margin of ovary. Uterus filling most of hindbody, with several loops between ovary and posterior testis and one loop between testes. Metraterm thick-walled, muscular, slightly shorter than cirrus sac, opening into genital atrium just anterior to male opening. Eggs numerous, operculate, 10 measuring 40–46 (43.1) by 20–26 (23.2).

DISCUSSION: This form appears closest to *B. baskokowi* (Ivanitsky, 1927) Strom, 1940, *B. filum* (Dujardin, 1845) Strom and Sondak, 1935, and *B. vanellicola* (Layman, 1922) Strom, 1940. *B. baskokowi* differs in having a larger sucker length ratio (1:1.43), and much larger pharynx (74 in diameter) and gonads even though the adult worm is smaller (2,059 long). *B. filum* differs in its much greater size (7 mm long) with all structures correspondingly much larger, and in having larger eggs (49–58 by 26–37). *B. vanellicola* differs in having longitudinally elongate testes, the ovary smaller than the testes, and eggs significantly smaller (22–31 by 18).

Brachylecithum spp.

HOSTS: *Streptopelia chinensis tigrina* (Temminck), spotted neck dove (Columbiformes: Columbidae); *Pitta erythrogaster thompsoni* Ripley and Rabor, red-breasted pitta (Passeriformes: Pittidae); *Pitta s. sordida*; *Pycnonotus plumosus cinereifrons* (Tweeddale), large olive bulbul (Passeriformes: Pycnonotidae).

HABITAT: Small intestine (?).

LOCALITY: Tarabanan Concepción.

DATES: 12, 14, 15 May 1962.

DISCUSSION: Two incomplete worms were recovered from one *P. sordida* and one incomplete worm from each of the other host species. More than one species of trematode appear to be present, but allocations are not possible.

Lyperosomum duculae sp. n.
(Figs. 9, 10)

HOST: *Ducula aenea palawanensis* (Blasius), green imperial pigeon (Columbiformes: Columbidae).

HABITAT: Small intestine (?).

LOCALITY: Tarabanan Concepción.

DATE: 15 May 1962.

SPECIMEN DEPOSITED: No. 72173 (holotype).

DIAGNOSIS (based on one complete adult worm with body from just postacetabular anteriorly in sinistrolateral view and remainder in dorsal view, and another with body missing anterior to middle of posterior testis; both measured): Body elongate, narrow, extremities rounded, 5,815 long by 565 wide. Forebody 875 long; hindbody 4,630 long; forebody–hindbody length ratio 1:5.3. Oral sucker subterminal ventral, with compact muscle layer just within posterior, posterodorsal, and posteroventral margins, 280 long by 265 deep; preoral space 17 long; acetabulum 310 long by 345 deep; sucker length ratio 1:1.11. Prepharynx absent; pharynx 84 in diameter; esophagus 255 long; cecal bifurcation 435 preacetabular; ceca conspicuously cell-lined, terminating about 1,175 from posterior extremity in partial worm with 3,010 long postvitellarian space.

Testes two, slightly lobed, pyriform, tandem–diagonal; anterior testis sinistromedian, 365 by 180, lying 180 postacetabular; posterior testis dextromedian, 365 by 225, lying 215 posterior to anterior testis. Cirrus sac elongate, thick-walled, muscular, 305 long by 73 deep, commencing 60 preacetabular. Seminal vesicle sinuous, thick-walled, muscular, 290 long by 65 deep. Pars prostatica very short, tubular, surrounded by few prostate cells. Cirrus muscular, protrusible, opening into posterior part of shallow genital atrium. Genital pore at level of posterior part of esophagus.

Ovary smooth to slightly lobed, median, diagonally oriented, 240–275 by 215–243, lying 150–210 posterior to posterior testis. Seminal receptacle posterolateral to ovary, 205 by 90. Mehlis' gland well developed, postovarian. Vitellaria follicular to dendritic, in lateral fields, anteriormost level of fields nearly equal and lying 120–125 preovarian, posterior level subequal with right field extending 620–825 postovarian and left field 840–1,100, right field 920–1,225 long, left 1,125–1,500 long; vitelline reservoir small; postvitellarian space 2,460–3,010 long. Uterus filling most of hindbody, separating gonads. Metraterm thick-walled, muscular, slightly shorter than cirrus sac, entering genital atrium anterior to male opening. Eggs numerous, operculate, 20 measuring 26–32 (29.8) by 19–21 (20.2).

DISCUSSION: Our form keyed to *Lyperosomum* Looss, 1899, in the keys given by Odening (1964) and Yamaguti (1971). It differs from all others in the genus in having pyriform testes, and from all but *L. turdia* (Ku, 1938) Travassos, 1944, and *L. anatis* Belogurov and Leonov, 1963, in having lobed testes. *L. turdia* differs further from our species in having a much wider body, vitellaria commencing at the testicular level, and a much shorter posttesticular space. *L. anatis* differs further from our species in having the vitellaria commencing at the testicular level and a much shorter posttesticular space.

Lyperosomum palawanense sp. n. (Figs. 11, 12)

HOST: *Dinopium javanense everetti* (Tweeddale), golden-backed three-toed woodpecker (Piciformes: Picidae).

HABITAT: Small intestine (?).

LOCALITY: Tarabanan Concepción.

DATE: 14 May 1962.

SPECIMEN DEPOSITED: No. 72174 (holotype).

DIAGNOSIS (based on one worm): Body elongate, lancet-shaped, extremities rounded, 3,740 long by 1,305 wide at level between ovary and posterior testis. Forebody 605 long; hindbody 2,540 long; forebody–hindbody length ratio 1:4.2. Oral sucker subterminal ventral, 290 by 295; preoral space 47 long; acetabulum 595 by 670, center at anterior one-fourth of body length, at posterior part of anterior third of body; sucker length ratio 1:2.05, width ratio 1:2.27. Prepharynx absent; pharynx 140 by 170; esophagus thick-walled, muscular; cecal bifurcation somewhat closer to acetabulum than pharynx; ceca thick-walled, cell-lined; postcecal space 710 long.

Testes two, diagonal, well separated by uterus, intercecal, smooth, transversely elongate; anterior testis dextral, 100 by 150, lying 75 postacetabular; posterior testis sinistral, 120 by 185, lying 410 postacetabular. Cirrus sac thin-walled, slightly muscular, elongate, somewhat pyriform, 205 by 150, commencing 8 preacetabular, terminating ventral to posterior part of pharynx, occupying 5.5% of body length. Seminal vesicle bipartite, sinuous, saccular, walls cellular internally; posterior chamber 85 by 97; anterior chamber 97 by 94. Pars prostatica very short, tubular. Cirrus elongate, muscular. Prostate cells few, surrounding pars prostatica, anterior part of seminal vesicle, and posterior part of cirrus. Genital atrium small. Genital pore median, ventral to posterior part of pharynx.

Ovary smooth, 236 by 245, lying 750 postacetabular and 225 posterior to posterior testis. Seminal receptacle posterior to ovary. Laurer's canal present. Mehlis' gland well developed, posteromedian to ovary. Vitellaria follicular, in extracecal fields up to 960 long, may overlap ceca, commencing 455 postacetabular at level of posterior testis, extending 285 postovarian, in middle third of body; postvitellarian space 1,225 long. Uterus filling most of hindbody intercecally but may overlap ceca, descending postcecally to near posterior extremity, ascending median to ovary and between testes. Metraterm thick-walled, muscular, shorter than cirrus sac, lying sinistral to latter, surrounded by gland cells. Eggs many, operculate, 10 measuring 32–39 (36.1) by 22–26 (23.5).

Excretory bladder tubular where visible posteriorly; pore terminal.

DISCUSSION: This species appears close to *L. turdia* (Ku, 1938) Travassos, 1944, and *L. indosinense* (Odening, 1964) Yamaguti, 1971. *L. turdia* differs by having testes larger than the ovary, the ovary located immediately or only slightly posterior to the posterior testis, and the cirrus sac relatively longer (occupying 9.5–14% of body length). *L. indosinense* differs in having the cecal bifurcation at the anterior margin of the acetabulum, the gonads of about equal size, the cirrus sac entirely prebifurcal, the genital pore lateral to the pharynx, the vitellaria commencing at the anterior testis level, and smaller eggs (28–32 by 16–21).

Conspicuum aenigma (Gvozdev, 1956) comb. n. (Fig. 13)

HOST: **Turnix susitator fasciata* (Temminck), barred button quail (Gruiformes: Turnicidae).

HABITAT: Gall bladder.

LOCALITY: Puerto Princesa.

DATE: 22 May 1962.

SPECIMENS DEPOSITED: No. 72175.

MEASUREMENTS AND SOME PERTINENT DATA (based on six adult worms; five measured): Body 3,705–7,275 long by 600–3,265 wide at gonadal level; forebody 625–1,385 long; hindbody 2,685–5,410 long; forebody–hindbody length ratio 1:3.8–4.5; oral sucker 344–595 by 285–600; preoral space 23–65 long; acetabulum 320–610 by 285–620, center at anterior 21–23% of body length; sucker length ratio 1:0.83–1.14, width ratio 1:1.00–1.23; pharynx 114–165 by 128–200; esophagus narrow but sometimes dilated, 133–390 by 48–130; cecal bifurcation 22–280 preacetabular; gland cells at posterior edge of pharynx, along esophagus, cecal bifurcation, and very beginning of ceca; latter narrow near bifurcation, becoming dilated short distance preacetabular; testes symmetrical to slightly oblique, right testis 105–405 by 125–495, left testis 123–425 by 111–455; vas efferens emerging from anterodorsal surface of each testis, usually entering cirrus sac side by side, sometimes joining to form short vas deferens up to 31 long before entering cirrus sac; latter 168–303 by 73–125, relatively thin-walled, slightly muscular, dextral or sinistral to esophagus, entirely prebifurcal, terminating at pharyngeal level or just postpharyngeal; seminal vesicle bipartite, posterior chamber elongate, usually straight, saccular, lined internally with long, flat cells, 47–162 by 26–67, anterior chamber elongate, thicker-walled, with cells larger and more cuboidal, 42–92 by 37–58; pars prostatica very short, tubular to slightly bulbous; cirrus very muscular, protrusible, 80–88 by 37–58 when within cirrus sac (in two largest worms) and 97–115 by 23–36 when protruded (in two smallest worms); prostate cells relatively few, surrounding anterior part of seminal vesicle, pars prostatica, and posterior part of cirrus; genital atrium large; genital pore submedian dextral or sinistral, at pharyngeal level or just postpharyngeal; ovary dextromedian in four worms, sinistromedian in two, 109–300 by 125–420, lying 463–1,120 postacetabular; seminal receptacle 80–150 by 83–245; Laurer's canal muscular, sinuous, median to seminal receptacle and ovary; Mehlis' gland well developed, posterior to posteromedian to ovary; vitellaria commencing at level of posterior part of acetabulum, terminating 960–1,895 from posterior extremity, fields may be subequal anteriorly and posteriorly, lying in middle third to half of body length; transverse vitelline ducts postovarian, uniting to form short common vitelline duct; uterus ascending sinistral to ovary if latter dextral and dextral if sinistral; metraterm thick-walled, slightly muscular, surrounded by gland cells, shorter than cirrus sac, lying dextral to latter if uterus ascending dextral to ovary and sinistral if ascending sinistral to ovary; eggs operculate, 30 measuring 27–35 (30.7) by 16–20 (17.7); excretory bladder tubular where visible posteriorly, narrowing to muscular duct surrounded by gland cells before opening through terminal pore.

DISCUSSION: This species was described from *Coturnix coturnix* L. (Galliformes: Phasianidae) from Kazakh SSR as *Skrjabinus aenigma.* In the key to the genera of Eurytrematini from birds given by Yamaguti (1971) this species keyed to *Conspicuum* (Bhalerao, 1936) Strom, 1940, as the vitellaria commences at the acetabular level rather than in the preovarian zone. Therefore, we are transferring it to this genus as *Conspicuum aenigma* (Gvozdev, 1956) comb. n. (syn. *Skrjabinus aenigma* Gvozdev, 1956). Similarly, *Skrjabinus indicus* Jaiswal, 1957, should be transferred to this genus as *Conspicuum indicum* (Jaiswal, 1957) comb. n.

Zonorchis philippinensis sp. n.
(Figs. 14, 15)

HOST: *Gallus gallus gallus* L., red jungle fowl (Galliformes: Phasianidae).

HABITAT: Small intestine (?).

LOCALITY: Puerto Princesa.

DATA: 24 May 1962.

SPECIMENS DEPOSITED: No. 72176 (holotype); No. 72177 (paratype).

DIAGNOSIS (based on two adult worms): Body elongate, phylliform, with distinct shoulderlike expansions at acetabular level, extremities rounded, 2,680–4,090 long by 895–1,100 wide at testicular level. Forebody relatively narrow, 510 long; hindbody wide anteriorly, tapering gradually posteriorly, 1,850–2,690 long; forebody–hindbody length ratio 1:3.6–5.3. Suckers round to longitudinally elongate; oral sucker subterminal ventral, 245–250 by 230–245; preoral space 22–23 long; acetabulum 320–505 by 320–425, center at anterior 19–25% of body length; sucker length ratio 1:1.30–2.02, width ratio 1:1.39–1.73. Prepharynx absent; pharynx overlapping oral sucker dorsally, 107–130 by 114–115; esophagus thick-walled, muscular; cecal bifurcation 30–58 preacetabular, somewhat closer to acetabulum than oral sucker; ceca thick-walled, conspicuously cell-lined; postcecal space 500–745 long.

Testes two, diagonal, well separated by uterine coils, intercecal, smooth to very slightly lobed, transversely elongate; anterior testis dextral in holotype, sinistral in paratype, 125–126 by 148–162, lying 10–32 postacetabular; posterior testis on side opposite anterior testis, 120–158 by 165–180, lying 130–145 postacetabular. Cirrus sac thin-walled, slightly muscular, elongate, bending sharply ventral at level of genital atrium, longitudinal extent 155–205 by 110–121 wide, contiguous with acetabulum to overlapping latter 51, terminating 15–30 postpharyngeally. Seminal vesicle bipartite, sinuous, saccular; posterior chamber lined with flat, elongate cells, 82–97 by 90; anterior chamber thicker-walled, lined with larger, more cuboidal cells, 82–90 by 63–68. Pars prostatica very short, tubular. Cirrus elongate, muscular, longitudinal extent 70–90 by 29–39 wide. Prostate cells relatively few, surrounding pars prostatica, anterior part of seminal vesicle, and posterior part of cirrus. Genital atrium small. Genital pore median, lying 21–34 postpharyngeal, 128–133 preacetabular.

Ovary transversely elongate, smooth, posterior to and contiguous with or very slightly separated from posterior testis, 155–157 by 180–184, lying 235–290 postacetabular. Seminal receptacle posterior to and contiguous with ovary, 73–128 by 82–106. Laurer's canal present. Mehlis' gland well developed, posteromedian to ovary, median to seminal receptacle. Vitellaria follicular, in extracecal fields, occasionally overlapping ceca, commencing 120–250 postacetabular at testicular level, extending 810–975 postovarian, occupying middle third of body length; postvitellarian space 945–1,630 long. Uterus filling most of hindbody intercecally, occasionally overlapping ceca, descending postcecally to near posterior extremity, ascending median to ovary and between testes. Metraterm thick-walled, muscular, shorter than cirrus sac, lying dextrodorsal to latter if ovary sinistral or sinistrodorsal if ovary dextral, surrounded by gland cells. Eggs numerous, operculate, 10 measuring 36–45 (39.5) by 22–25 (23.8).

Excretory bladder tubular where visible posteriorly; pore terminal.

Discussion: Our form differs from all others in the genus *Zonorchis* Travassos, 1944, in its phylliform body resulting from the presence of distinct shoulderlike expansions of the body at the acetabular level. It is closest to *Z. panduriformis* (Railliet, 1900) Timon-David, 1953, and *Z. petiolatus* (Railliet, 1900) Denton and Byrd, 1951. The latter species differs further in having the cecal bifurcation closer to the oral sucker, the testes more nearly symmetrically placed, the ovary well separated from the posterior testis by the uterus, and the cirrus sac well anterior to the acetabulum and oval to pyriform. *Z. panduriformis* differs further in having a submedian, more anteriorly placed genital pore, and the cirrus sac pyriform.

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The Life Cycle and Notes on the Developmental Stages of *Microtetrameres corax* Schell, 1953 (Nematoda: Tetrameridae)[1]

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ABSTRACT: The life cycle and development of *Microtetrameres corax* Schell, 1953, are described. Experimentally, grasshoppers (*Melanoplus* spp.) served as intermediate hosts. Black-billed magpies (*Pica pica hudsonia*), which are natural hosts in northeastern Colorado, were raised from nestlings in the laboratory and used as definitive hosts. After ingestion of embryonated eggs by a grasshopper, first-stage larvae emerge from the shell within 6 hr and migrate to the hemocoel within 24 hr. Second-stage larvae appear in the hemocoel after 10 to 14 days. Third-stage larvae were recovered 27 to 56 days in the thoracic region of the hemocoel and among the fat bodies in the abdominal region. Grasshoppers containing third-stage larvae were fed to magpies; at necropsy, 48 days later, 110 females and 18 males were found in one bird and 74 females and 11 males were found in the other. Fourth-stage larvae were not recovered. The pathological effects of female *M. corax* are discussed.

Schell (1953) described *Microtetrameres corax* from the raven in Bovill, Idaho, and attempted to continue the life cycle by feeding eggs to mealworm larvae, *Tenebrio* spp., and German cockroaches, *Blattella germanica.* At necropsy, 32 to 38 days later, the mealworm larvae were negative, but in the cockroaches he found encysted larvae, assumed to be third-stage *M. corax*, which were then fed to chicks. The chicks, however, were not infected when examined 50 to 56 days later.

Cram's (1934) notes on larvae of *M. helix* Cram, 1927, and a more complete investigation by Ellis (1969a, b) on the life history and larval morphology of *M. centuri* Barus, 1966, are the only other existing reports of experimental life cycle studies on *Microtetrameres.*

Microtetrameres corax is a common helminth in black-billed magpies (*Pica pica hudsonia*) in northeastern Colorado (Wacha, 1966; Wacha and Schmidt, 1971). The abundance of material and the lack of experimental knowledge about this interesting genus prompted this study.

Materials and Methods

Adult *M. corax* males were obtained from the mucus and females from the proventricular glands of magpies. Seventy birds were collected from various sites in the vicinity of Greeley, Colorado, between April and November 1967. Magpie nestlings were raised in the laboratory for experimental use as definitive hosts. Grasshoppers (*Melanoplus* spp.) were collected at early nymphal stages from an area in which little or no magpie activity had been observed, and used experimentally as intermediate hosts. Preliminary necropsies of several of these revealed no natural infections of parasitic helminths.

Embryonated eggs from gravid females were

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[1] From a thesis submitted to the Graduate Division of Colorado State College (now the University of Northern Colorado) in partial fulfillment of the requirements for the degree of Master of Arts.

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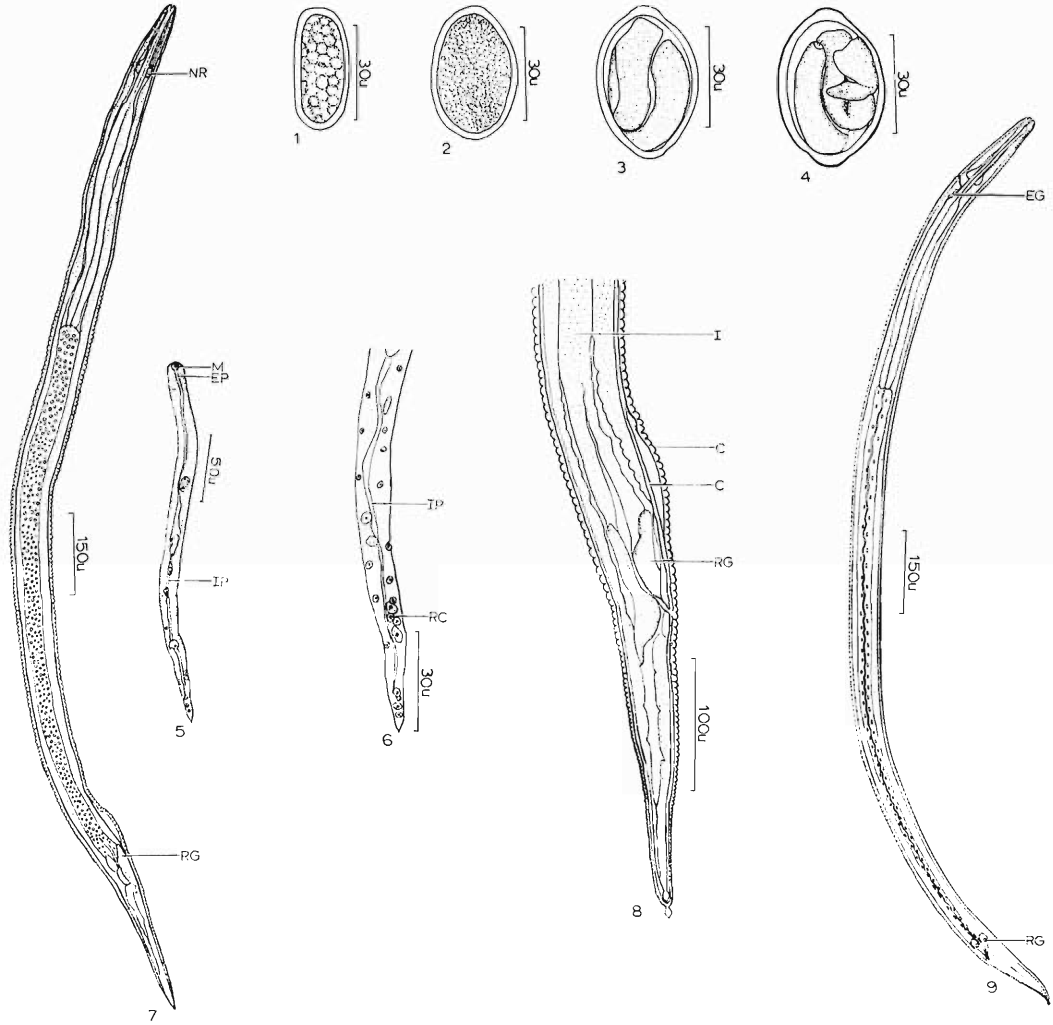

Abbreviations: C, cuticle; EG, excretory gland; EP, esophageal primordium; I, intestine; IP, intestinal primordium; M, mouth; NR, nerve ring; RC, rectal cells; RG, rectal gland.

Figures 1–9. Larval stages of *Microtetrameres corax*. 1. Egg in early morula stage. 2. Egg in late gastrula stage. 3. Egg in early embryo stage. 4. Egg containing first-stage larva. 5. First-stage larva. 6. Posterior end of first-stage larva. 7. Second-stage larva. 8. Posterior end of second-stage larva, lateral view. 9. Third-stage larva.

spread on leaves and fed to starved grasshoppers. The grasshoppers were then necropsied at intervals and examined for larval stages. Infected grasshoppers containing third-stage larvae were fed to the magpies. Both infected and control birds were necropsied after 48 days and examined for adult *M. corax*.

A 5% formalin solution proved the most suitable fixative for the extremely delicate first-stage larvae. The second and third stages were fixed in F.A.A., cleared by evaporation in glycerine–alcohol, and mounted in pure glycerine or glycerine jelly.

Drawings of eggs and larval stages were

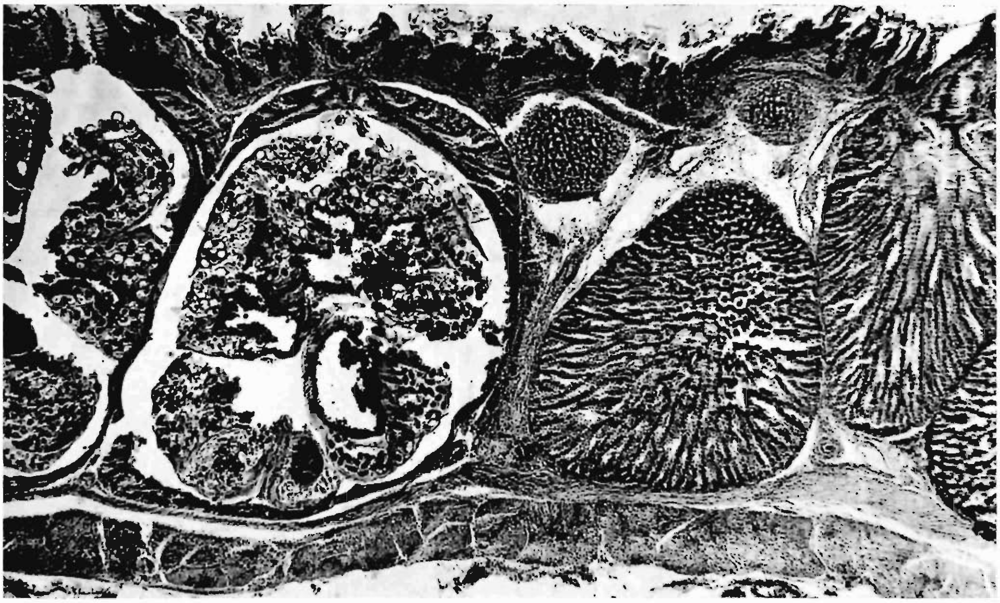

Figure 10. Cross section of proventriculus showing a gravid *M. corax* in gland. Normal glands can be seen on the right.

prepared with the aid of camera lucida. All measurements are in microns unless otherwise stated.

Results

Egg (Figs. 1–4)

Eggs in morula stage flattened on one side, 40 by 25. Eggs in late gastrula and early embryo oval, 40 by 28. Fully embryonated eggs oval with boss on each end, 45–47 by 31–32. Shells transparent, 2–3 thick.

First-stage larvae (Figs. 5, 6)

Eggs were found unhatched in the intestine 3 hr after ingestion. First-stage larvae emerged within 6 hr and were still found in the intestine after 12 hr. They were found in the thoracic region of the hemocoel at 24 and 36 hr. From the appearance of the empty egg shells, the larvae emerged from one of the poles of the eggs. After their emergence, motility is very rapid, eventually decreasing.

Description of first-stage larvae: Size 248–258 long by 13–15 wide. Cuticle thin with discontinuous striations anteriorly. Mouth blunt, terminal, communicates with esophageal primordium via short buccal cavity. Intestine in early stage of development, very irregular with sparsely scattered cells on each side. No excretory or anal openings; group of four large rectal cells present 32 from tip of tail. Other giant cells and groups of cells found in posterior tip, midsection, and anterior third of body, but fate of these cells undetermined.

Second-stage larvae (Figs. 7, 8)

Second-stage larvae appeared in the tissues of the thoracic region of the hemocoel 10 to 14 days after infection. Very rapid growth occurs during this stage. The dimensions range from those given for the largest first-stage larvae to the ones given below for stages in early ecdysis.

Description of second-stage larvae: Body 1.90 mm long by 0.070 mm wide. Cuticle shows signs of slippage toward posterior end; old cuticle as well as underlying new cuticle bears fine transverse striations. Mouth opens into narrow, V-shaped buccal capsule

10 long; muscular esophagus well developed, 196 long; glandular esophagus 422 long. Nerve ring 146 from anterior end. No excretory pore or gland cells. Esophagus and intestine sharply differentiated; intestine granular and incompletely developed, connecting posteriorly to indistinct anal canal. Rectal glands present 193 from tip of tail; anus not clearly visible but tail appears to be 180 long, ending in characteristic "ball-point" tip.

Third-stage larvae (Fig. 9)

Third-stage larvae were recovered 27 to 56 days postinfection, mainly in the thoracic region of the hemocoel and less frequently among the fat bodies of the abdominal region. At 56 days, the third-stage larvae were all encysted in a thin membrane which was loosely connected with the surrounding tissues. The cysts did not appear in the other larval stages or during the early development of the third stage. The encysted larvae were observed to push against the wall of the cyst in an uncoiling motion in an effort to break out of the membrane.

Description of third-stage larvae: Body narrower than in previous stage; tail appears contracted and somewhat shorter. Size of body 1.95–2.35 mm long by 55–72 wide. Buccal capsule narrow, 22 long. Muscular esophagus 198–237 long; glandular esophagus 412–505 long. Nerve ring 159 from anterior end. Excretory pore, gland and canal visible; pore 170 from anterior end. Intestine slenderer than in previous stage, connects posteriorly to cuticularized rectal canal. Tail 132 long, with "ball-point" tip. Size of third-stage cyst 950–990 by 560–575.

Fourth-stage larvae

Unfortunately, limited facilities and materials did not permit sequential examinations for fourth-stage larvae and migratory phases.

Several larvae were found in the proventricular mucosa of experimental (48 days) and naturally infected magpies. These are identical to the third-stage larvae except for an unidentified structure found in the posterior third of one experimental specimen. This structure is similar to one described in some third-stage larvae of *M. centuri* (Ellis, 1969a).

Adults

The adults obtained during the study are not described here, but were sent to Dr. Patricia M. Mawson who is currently examining the taxonomy of the genus.

Infection and pathology

Natural infection: Of the 70 magpies taken for examination, 35% were infected with *M. corax*. The intensity of infection with females ranged from 6 to 28. All birds appeared to be in good condition and well nourished. Stained sections (H & E) of proventriculi with females in the glands (Fig. 10) showed no connective tissue or cellular response, but pressure atrophy of the glandular tissue around the worms was evident. The females appear to replace most of the glandular tissue as they grow within the lumen, forcing the glandular epithelium against the surrounding connective tissue.

Experimental infection: Each experimental bird was fed two grasshoppers containing numerous third-stage larvae. The birds were necropsied 48 days later and found to be heavily infected with adult *M. corax*; 110 females and 18 males were recovered from one bird, 74 females and 11 males from the other. The pathology was identical to that in the natural infections. No *M. corax* were found in control birds; no other helminths were found in either experimental or controls.

There was a noticeable difference in activity and general appearance of experimental and control birds by the 35th day post-infection. The experimentals were less aggressive and less active than controls and appeared undernourished. They had numerous sores on their legs and appeared to have lost a considerable amount of feathers. The controls remained normal and sturdy throughout the study.

Discussion

The general morphology of the larvae of *M. corax* is very similar to that described for *M. centuri* by Ellis (1969a). The giant cells and groups of large cells formed three distinct bodies in the first stage, similar to the "refractile bodies" of *M. centuri*; the nuclei of these were easily visible under light-phase microscopy. Some structures, such as the cephalic projections and anal and excretory pores of first-stage and the excretory system of second-stage *M. centuri* were not apparent in *M. corax*.

The cyst membrane of third-stage larvae appeared only in the older larvae; its development, therefore, is quite different from that of the membrane surrounding the second- and third-stage larvae of *Physaloptera hispida*, which is formed by a cellular response of the colon wall of the intermediate host (Schell, 1952). The origin of the cyst membrane encountered in this study was not investigated.

The pathological effects of female *M. corax* are identical to those caused by *M. centuri* (Ellis, 1970): pressure atrophy which renders the proventricular gland totally nonfunctional, with no resulting inflammation or connective tissue encapsulation. The poor physical appearance and loss of weight in the experimentally infected birds may have been due to their inability to compete for food and/or digest it. The number of females recovered from their proventriculi far exceeds that of natural infections.

The few experimental studies on *Microtetrameres* spp. have not revealed any genital development in the larvae, or the existence of a fourth-stage larva. Ellis (1969a) recovered larvae of *M. centuri* from birds necropsied at various time intervals after the ingestion of infective third-stages, but considered them to be still in the third stage due to a lack of sexual differentiation. Although the onset of rapid sexual development does occur during the fourth stage of many nematodes, this should not be considered a "character" of this stage. The only criterion used for the identification of the larval stages during this study was the actual process of molting itself.

Seurat (1918) described genital primordia in the third-stage, and sexual differentiation in the fourth-stage larvae of *Tropidocerca (= Microtetrameres) spiralis* from naturally infected hosts. The validity of this early record might be questioned in light of the caution necessary when working with natural infections, particularly of the Tetrameridae. However, these larval features have been found in at least one of the experimental studies on the closely related genus *Tetrameres* (Zago and Barretto, 1962).

Acknowledgments

I would like to thank Dr. Gerald D. Schmidt for his guidance and assistance during the study, and Dr. John C. Holmes for reviewing the manuscript.

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New Host Records of *Hedruris siredonis, Falcaustra elongata,* and *F. mascula* from Mexican Salamanders[1]

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Abstract: Salamanders comprising five species of *Ambystoma* and three species of *Rhyacosiredon* collected in Mexico were found to harbor one or more of the nematode species *Hedruris siredonis, Falcaustra elongata,* and *F. mascula.* Parasites were recovered either directly by autopsy or indirectly as a result of their migration from the body or passage in the feces of recently collected hosts. *Ambystoma ordinarium, A.* sp., and *A. subsalsum* are new hosts for *H. siredonis*; *A. lacustris, A. subsalsum, A.* sp., and *Rhyacosiredon altamirani* are new hosts for *F. elongata. Falcaustra mascula* is reported for the first time from salamanders, in *Ambystoma tigrinum, R. altamirani, R. leorae,* and *R. rivularis,* and for the first time in Mexico.

Comparatively little information is available on helminths of salamanders in Mexico. This report is based on a collection of nematodes recovered from five species of *Ambystoma* and three of *Rhyacosiredon* from the Mexican states of México, Michoacán, and Puebla. Included are several new host records and range extensions.

Materials and Methods

Salamanders were obtained alive and transported to Illinois in May and December of 1970, and March of 1971. Some nematodes were recovered *in situ* by autopsy from the salamanders shortly after capture. Others were recovered as they spontaneously migrated from or were passed in feces from live salamanders a few days to a few weeks after the hosts were transferred from native water to charcoal-filtered tap water. Because individual salamanders were uniquely numbered in individual containers in a large laboratory colony, correct identification of host was assured.

Nematodes were killed and fixed in hot 70% ethanol, cleared in glycerine–alcohol, and mounted in either pure glycerine or glycerine jelly for microscopic study.

Results and Discussion

One or more of the nematode species *Hedruris siredonis, Falcaustra elongata,* and *F. mascula* were collected from each of the salamander hosts (Table 1).

Hedruris siredonis Baird, 1858

The geographical distribution of *Hedruris siredonis* Baird, 1858, has thus far been limited to Mexico, and previous to this investigation, this species has been known to occur in *Ambystoma mexicanum* only. Baird's description of *H. siredonis* proved inadequate as it was based on a single female specimen which was probably immature. Chandler (1919) found *Taricha torosa* in Oregon to be infected frequently with a nematode which he identified tentatively as *H. siredonis.* Because Baird's *H. siredonis* was so exiguously described, the identity of Chandler's specimens could only be inferred from circumstantial evidence. Later, Caballero and Hollis (1938) gave a detailed description of *H. siredonis* based on several mature males and females taken from *Ambystoma,* collected from Lake Xochimilco, Mexico, D. F., identified by them as *A. tigrinum.* In the absence of preserved host specimens, it is impossible to verify their actual identity. This difficulty arises because of inconsistency in older publications in applying the specific names *tigrinum* and *mexicanum* (e.g., Freitas and Lent, 1941a, b; see also discussion in Smith, 1969), and because both species may occur sympatrically in parts of Mexico. The names *A. tigrinum* and *A. mexicanum* have both been applied to the dark, paedogenetic, long-toed species occurring in the Lake of Mexico (obtained mainly at Lake Xochimilco),

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Table 1. Intestinal nematodes recovered from 40 Mexican salamanders.

Parasite	Host	Locality	No. of animals Infected	No. of animals Examined
Hedruris siredonis	†*Ambystoma ordinarium*	San Jose Lagunillas, Michoacán	2**	6
	†*A.* sp.	Zacapu, Michoacán	3**	3
	†*A. subsalsum*	Lake Alchichica, Puebla	1*	1
Falcaustra elongata	†*Ambystoma lacustris*	Lake Zumpango, México	6*	6
	†*A.* sp.	Zacapu, Michoacán	1* 2**	1 3
	†*A. subsalsum*	Lake Alchichica, Puebla	3**	3
	A. tigrinum	Nopaltepec, México	6*	6
		Xochimilco, México	4*	4
	†*Rhyacosiredon altamirani*	La Marquesa, México	1*	1
Falcaustra mascula	†*Ambystoma tigrinum*	North of Apizaco, Puebla	1*	1
	†*Rhyacosiredon altamirani*	La Marquesa, México	5*	5
	†*R. leorae*	Río Frío, México	1*	1
	†*R. rivularis*	Nevado de Toluca, México	2* 2**	2 5

* Parasites recovered from water.
** Parasites recovered on autopsy.
† New host record.

the axolotl which has been used extensively as an experimental laboratory animal during the past century. This species is now called *A. mexicanum.* It is now certain, however, that two different species of salamanders can be obtained at Xochimilco (Brandon, unpubl.), one of them *mexicanum* and the other probably what is called *tigrinum* farther north. Which animals Caballero and Hollis actually had one can only guess.

Freitas and Lent (1941a) reviewed the genus *Hedruris* and proposed *H. chandleri nom. nov.* for *H. siredonis,* Chandler, 1919 *nec* Baird, 1858. *H. chandleri* is distinct from the *H. siredonis* redescribed by Caballero and Hollis and can be differentiated by the relative positions of the excretory pore and nerve ring and the form of the spicules. Although Lehmann (1954, 1956) reported *H. siredonis* from *Taricha granulosa* and *Ambystoma gracile* in Oregon, those parasites are probably better identified as *H. chandleri.* Our 22 specimens from the stomach of six salamanders of three host species are not significantly different from *H. siredonis* as described by Caballero and Hollis (1938). Variation in the position of some of the caudal papillae as pointed out by these authors was also observed by us.

Chandler (1919) observed that *H. chandleri,* when present in large numbers, have an injurious effect on the host as heavily infected *T. torosus* were undersized and thin, and contained little or no food in the stomach. Infections seen by us were light (from one to 11 specimens per host) and no gross injurious effects on the hosts were noted except that in some cases the tissue lining the stomach was swollen where female worms were attached. The posterior portion of the female terminates in a retractile suckerlike invagination which is armed with a chitinized hook capable of being protruded or withdrawn within a pocketlike depression in the ventral wall of the sucker. Perrier (1871) demonstrated that the caudal hook of *H. armata* connects to a gland and suggested that the secretions of the gland probably function in irritating the host tissue sufficiently to cause a swelling thereby insuring a firm attachment.

H. siredonis has not heretofore been reported from any of the three hosts or localities listed in Table 1. Representative specimens are deposited in the USNM Helm. Coll., Nos. 72187–72189.

Falcaustra mascula (Rudolphi, 1819)

This species, first described by Rudolphi (1819) from specimens found in a Brazilian colubrid snake, has also been found in other Brazilian colubrid snakes as well as hylid and leptodactylid frogs, and in Paraguayan bufonid and leptodactylid anurans (Walton, 1964; Lent, Freitas, and Proenca, 1946). For a discussion of the synonymy of *F. mascula* the reader is referred to Freitas and Lent (1941b) and Gomes and Vicente (1966).

We found 39 specimens in 11 salamanders of four host species (Table 1). These agree with the description of *F. mascula* as given by Lent, Freitas, and Proenca (1946). This species has not previously been reported from salamander or from Mexico.

Representative specimens are deposited in the USNM Helm. Coll., Nos. 72195–72198.

Falcaustra elongata (Baird, 1858)

This nematode was first described from *Ambystoma mexicanum* by Baird (1858) and assigned to the genus *Leptodera* Dujardin, 1845. It was redescribed by Walton (1932) from the type material and transferred to the genus *Spironoura* Leidy, 1956. Although the original label documents Baird's identification of the type host as *A. mexicanum* ("*Leptodera elongata*, Baird, 1858, ex ints., *Siredonis mexicani*. Sir W. Jardine, Bart."; *fide* Walton, 1932), Walton followed the general opinion of earlier workers that *A. mexicanum* and *A. tigrinum* were the same species, and listed the type host as *A. tigrinum*. Caballero and Hollis (1938) did likewise in their redescription of *Spironoura* (= *Falcaustra*) *elongata* based on four male and five female worms taken from the large intestine of hosts from Lake Xochimilco. Freitas and Lent (1941b), in a revision of the subfamily Kathlaniinae, transferred *S. elongata* to the genus *Falcaustra* Lane, 1915.

Our observations were based on 53 females and 28 males from 23 salamanders of five host species (Table 1). Our specimens are larger than those studied by Walton (1932), but they agree very closely with the description of *F. elongata* given by Caballero and Hollis (1938) with some slight variability in the position of the male postanal papillae. Our record is apparently the first for this nematode in Mexican *A. tigrinum*, *A. lacustris*, *A. subsalsum*, *A.* sp., and *Rhyacosiredon altamirani*. To our knowledge, the report of this nematode in *A. tigrinum* from Utah by Frandsen and Grundmann (1960) is the only record of this parasite in a salamander host outside of Mexico.

Specimens are deposited in the USNM Helm. Coll., Nos. 72190–72194.

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The Ultrastructure of the Sperm of *Deontostoma californicum,* A Free-Living Marine Nematode[1]

K. A. Wright,[2] W. D. Hope,[3] and N. O. Jones[4]

ABSTRACT: Sperm in both male and female reproductive tracts of *Deontostoma californicum* are rod-shaped. Nuclear material is of an open fibrous type including granular bodies and perhaps remnants of centrioles. Cytoplasm surrounding the nucleus contains β glycogen particles. The peripherally located organelles include membrane specializations that open through the sperm surface, dense bodies that may be unopened membrane specializations, dense granules, and granular mitochondria. It is estimated that sperm in the female tract could have released as much as one-seventh their total volume into the tract through opening of the membrane specializations. The ectoplasmic zone of sperm adjacent the uterine epithelium is thickened and contains hemidesmosome attachments to the epithelium.

Nematode spermatozoa occur in many sizes and shapes (Walton, 1942). They are generally considered to be nonflagellate and to lack an acrosome (Foor, 1970). Sperm of several species of nematodes parasitic in animals and of two saprophytes have been described at the ultrastructure level (Favard, 1961; Jamuar, 1966; Beams and Sekhon, 1972; Lee and Anya, 1967; Foor, 1970; Pasternak and Samoiloff, 1972).

This report describes the mature sperm of the marine nematode *Deontostoma californicum* Steiner and Albin, 1933. It is the first fine structure study of sperm from nematodes of this group.

Materials and Methods

Specimens of *Deontostoma californicum* were the same as those used in earlier studies of this species (Hope, 1969). For light microscopy, 10% formalin-fixed worms were embedded in polyethylene glycol. Sections were stained by the Feulgen technique. For electron microscopy, worms were fixed in 1% Acrolein in 0.1 M phosphate buffer pH 7.2, postfixed in 1% osmium in veronal acetate buffer, oriented in agar, and embedded in Maraglas 655. Sections were stained with lead hydroxide or a sequence of potassium permanganate and lead citrate (Wright, 1972). Glycogen was stained cytochemically by the periodic acid–thiosemicarbazide–silver proteinate (PA–TSC–Ag proteinate) technique of Thiéry (1967).

Results

Sperm were studied by light microscopy in both the male and female reproductive tracts. The sperm appear to be bluntly rod-shaped, measuring about 8 by 18 μ. The central nuclear mass that stains by the Feulgen technique is about 3 by 15 μ (Fig. 1).

Sperm studied at the ultrastructure level occurred in the distal end of the uterus (close to the oviduct) in a female worm. The central Feulgen-positive region of the sperm contains

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a densely stained fibrous material oriented primarily in the longitudinal direction (Figs. 2–4). The interspaces of this material contain a less dense filamentous component (Fig. 5). In some areas these filaments are organized into parallel groups. Granular bodies also occur (Figs. 4, 5). In two sperm, patterns were seen that resemble cross-sectioned bundles of tubules. In one, the bundle consisted of five peripheral tubules around a single central tubule (Fig. 5 inset); in the other, there was a single central tubule, six peripheral, and one tubule outside this ring. The nuclear region is limited by an irregularly granular boundary that is not a membrane but appears to connect to the peripheral cytoplasm. In sections stained with permanganate and lead citrate, the cytoplasm around the nuclear region is unstained. However, in sections stained only with lead hydroxide, or those stained by the PA–TSC–Ag proteinate technique (Fig. 10), this region is found to contain numerous β particles of glycogen.

The periphery of the sperm contains several types of inclusions or organelles. The most prominent are membrane-bounded vesicles (average diameter: 0.55 μ, range: 0.70–0.44 μ) that contain microvilluslike internal projections of their membrane and are connected through a narrow neck (about 0.012 μ wide) to the peripheral plasmalemma of the sperm (Fig. 6). As these vesicles resemble those found in sperm of ascaridoid, spiruroid, filarioid, strongyloid, and rhabditoid nematodes (see Foor, 1970) the term introduced by Foor, i.e., membrane specializations, will be used here. Lee (1971) referred to similar structures in *Heterakis gallinarum* as alpha bodies while Pasternak and Samoiloff (1972) refer to V-bodies in *Panagrellus silusiae*. Most of the specializations are devoid of content, but in some a small amount of granular material can be found (Fig. 6). Similar material can be found in some intercellular spaces. The necklike connections of membrane specializations to the surface membrane are surrounded by dense collarlike rings about 85 Å thick and 720 Å wide. Further dense material occurs external to this (Figs. 6, 7). Oblique sections of sperm showing cross sections of the necks allow an estimate of the number of membrane specializations per unit area (i.e., 3.75 per μ^2) and in turn, as they appear to be uniformly distributed around the sperm, an estimate of

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Figure 1. Photomicrograph of part of a section of the vas deferens of a male *Deontostoma californicum* stained by the Feulgen technique. Sperm are seen in longitudinal, oblique, and cross sections. × 800.

Figure 2. Electron micrograph of an obliquely sectioned sperm in the distal uterus of a female *D. californicum*. The dense fibrous nuclear material occupies the central region of the sperm while organelles are more peripheral. ms = membrane specializations, db = dense bodies that may be unopened membrane specializations, g = dense granule, m = granular bodies presumed to be mitochondria. × 10,500.

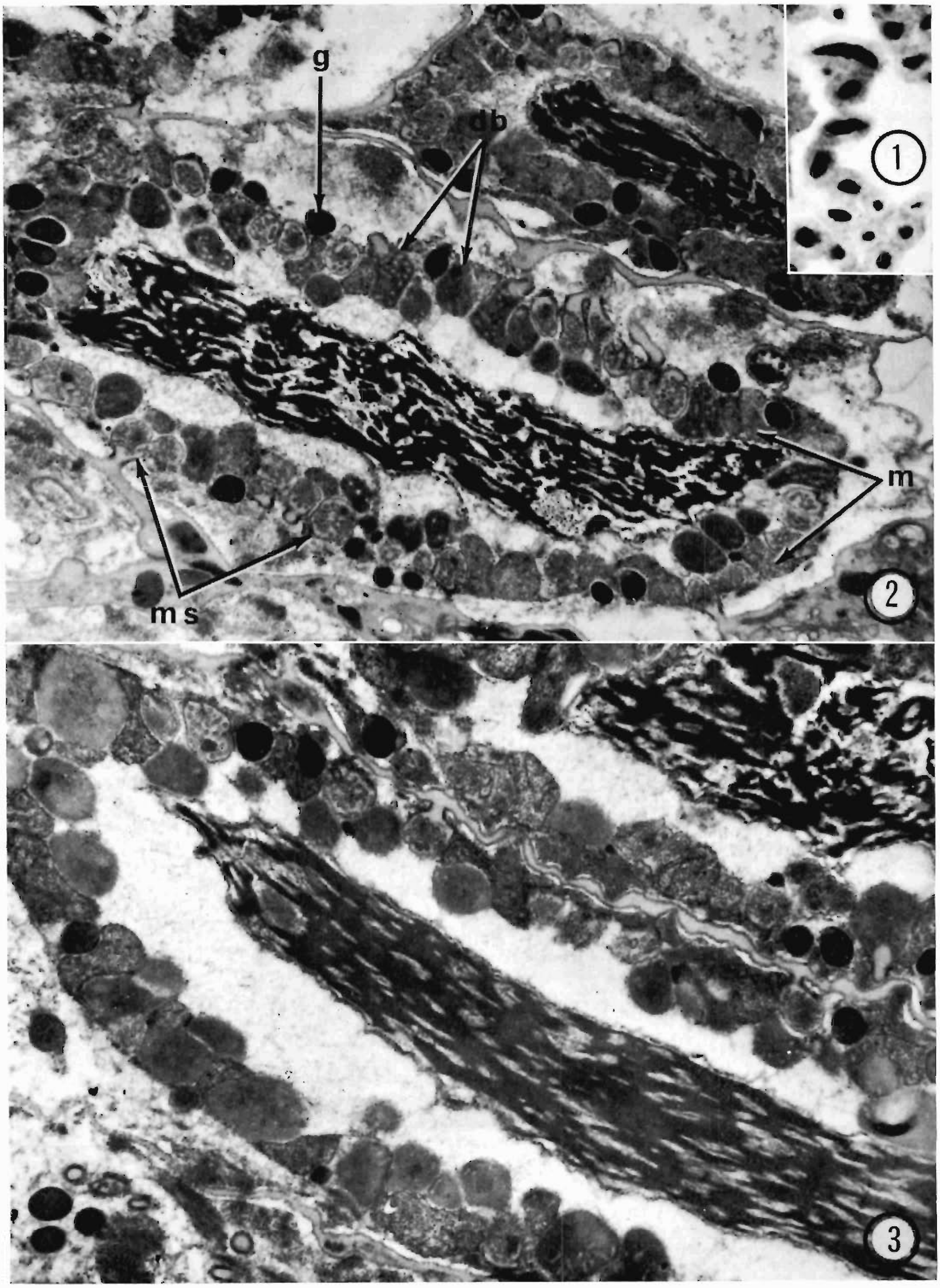
Figure 3. The dense fibrous nuclear material is oriented along the longitudinal axis of the sperm as seen in this longitudinal section. Note the lack of heavily stained material between the nucleus and peripheral organelles. × 14,700.

Figure 4. Cross section of a sperm including a granular body (arrow) within the nuclear material. ×9,300.

Figure 5. Higher magnification of the nuclear material showing less dense filaments and a granular body. × 55,000. Inset: a group of six cross-sectioned tubules within the nuclear material. × 67,000.

Figure 6. Two membrane specializations showing their internal microvilluslike structures and, in one, the necklike connection to the plasmalemma. The arrow indicates the ring of dense material just within the membrane of the neck. Asterisk indicates some content in one of the specializations. m = granular body that may be a mitochondrion. × 59,000.

Figure 7. Cross sections of necks of the membrane specializations show their dense collarlike ring (large arrow) and cell membrane (small arrow). × 81,000.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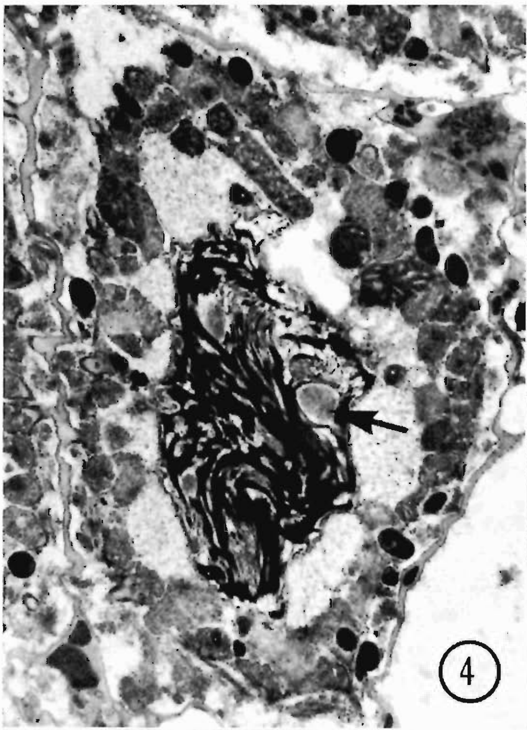

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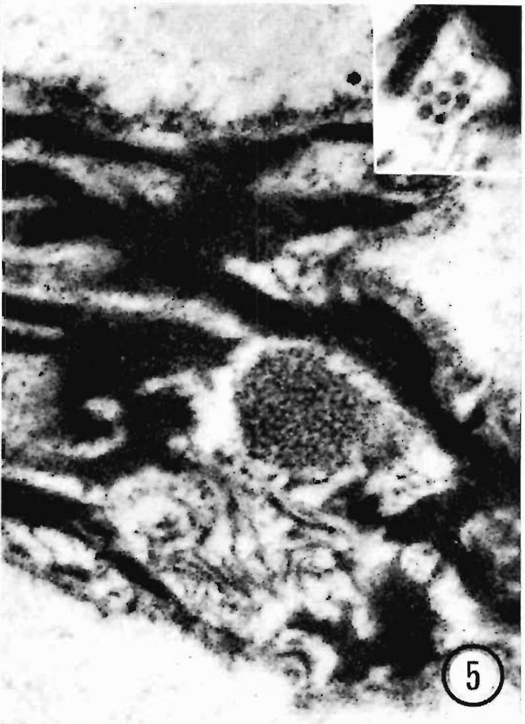

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their total number (about 1,700 per sperm if sperm are considered as cylinders with hemispherical ends, 18 μ long by 8 μ in diameter).

Interspersed between these membrane specializations, and almost equal in number, are dense bodies (average diameter: 0.64 μ, range: 1.01–0.48 μ) that are membrane-limited and also have tubular or microvilluslike processes projecting into them (Fig. 8). As well, they contain an indistinctly reticular center (Fig. 9). Some profiles of these bodies show a necklike projection resembling the necks that connect membrane specializations to the plasmalemma (Fig. 9). None of these dense bodies have been found open to the cell surface. A few dense spherical granules about 0.38 μ in diameter occur, as well as irregularly granular bodies that sometimes contain patterns resembling mitochondrial cristae. The latter bodies may be degenerate, or poorly preserved mitochondria.

Sperm lying adjacent the wall of the uterus are modified along their surface in contact with this epithelium. The ectoplasmic zone between inclusions and plasmalemma is increased in thickness and contains much dense, granular or filamentous material. Glycogen is not found in this region. At some points where the sperm membrane is closest to the seminal receptacle membrane, small dense hemidesmosomes occur along the inner surface of the sperm membrane (Fig. 9). Contrary to their distribution in sperm in the lumen of the uterus, membrane specializations were only rarely found to open to the surface through this modified ectoplasmic zone.

Discussion

The sperm of *Deontostoma* are relatively simple in structure. They show no gross differentiation and their organelles are uniformly distributed around their periphery. Nuclear material appears to be of an unusual form. Although most nematode sperm studied have uniformly dense nonmembrane-bound nuclei, the nuclear component of sperm of *Aspicularis tetraptera* consists of dense granular chromatin associated with microtubules and mitochondria (Lee and Anya, 1967). Fibrous nuclear material comparable to that found in sperm of *Deontostoma* is unknown in sperm of other animal groups as far as the authors are aware. Despite extensive searching clearly identifiable centrioles have not been found in *Deontostoma* sperm. The groups of cross-sectioned tubules found in only two sperm may be rudiments of centrioles. Although centrioles occur throughout sperm development in *Ascaris*, they have not been identified in the fully developed sperm (Foor, 1970). These findings may not be so disturbing as Pickett-Heaps (1971) has recently stressed that centrioles may be formed *de novo* in several cell types and may be of only secondary importance even in genesis of spindle fibers. Besides the absence of centrioles, a further similarity between *Ascaris* sperm and those of *Deontostoma* is the similar structure of their membrane specializations. These similarities are of interest as Sprent (1962) suggested that parasitic ascaridoid nematodes may have evolved from members of the marine order Enoplida to which *Deontostoma* belongs.

Foor (1970) has emphasized that final mat-

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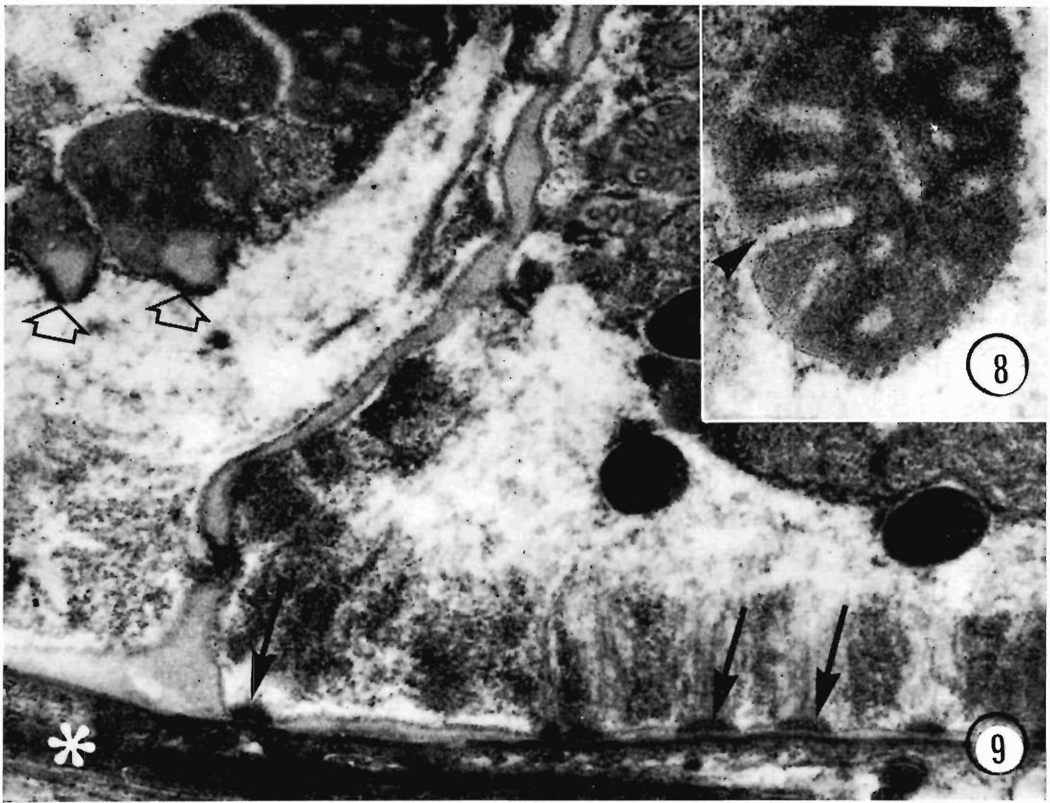
Figure 8. A dense body showing continuity of its peripheral membrane with the membrane profiles within it (arrow). × 65,600.

Figure 9. The ectoplasmic zone of this sperm adjacent the uterine epithelium (asterisk) is thick and contains hemidesmosomelike densities (arrows) on its membrane. Note two dense bodies (open arrows) with necklike structures similar to those of membrane specializations. The irregular center of another dense body is evident. × 30,000.

Figure 10. Beta glycogen (gly) stained with the PA–TSC–Ag proteinate technique in the cytoplasm between the nuclear material (N) and organelles. Note absence of glycogen from the ectoplasmic zone where this region is thickened in sperm attached to the uterine epithelium (asterisks). Density of nucleus and other structures is due to osmium fixation. × 27,600.



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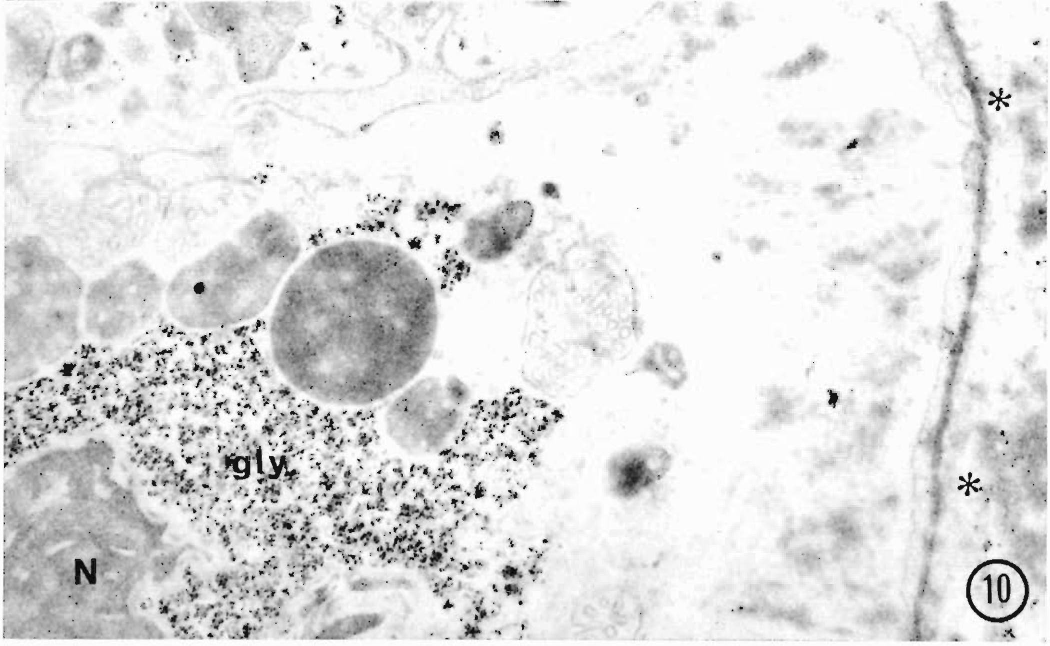

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uration of nematode sperm occurs in the female tract. Associated with this maturation in at least some species is the opening of membrane specializations to the cell surface and release of their contents. The membrane specializations of *Deontostoma* sperm are open in the female tract. Both membrane specializations and the dense bodies contain microvillus-like projections of their peripheral membrane, and some of the latter bodies have necks similar to those of membrane specializations. Perhaps the dense bodies are in fact membrane specializations that have not yet opened to the surface to eject their contents. The internally produced V-bodies of sperm of *Panagrellus silusiae* appear to attach to the plasmalemma by means of a preformed knob (Pasternak and Samoiloff, 1972) that is similar perhaps to the neck of the membrane specializations and dense bodies of *Deontostoma* sperm. As an estimate of size and number of membrane specializations in sperm of *Deontostoma* has been made, it is feasible to estimate the quantity of material that might have been released from them. Thus, 1,700 spherical bodies 0.5 μ in diameter could have contained 102 μ^3 of material. However, a considerable volume of the bodies is occupied by the microvilluslike projections—a reduction of volume by a factor of about one-third might be considered. However, if the dense microvillus-containing bodies are unopened membrane specializations, the original estimate may not be excessive as these bodies are larger in volume than the open membrane specializations. Thus each sperm as seen in our preparations may have released about 100 μ^3 (almost one-seventh its total volume) of material and may potentially contain almost again as much in membrane specializations that have not yet opened to the exterior. This estimate applies only to sperm in the lumen of the uterus in which the distribution of membrane specializations is uniform and an estimate of their number is feasible.

The morphology of the cortex of sperm adjacent to the uterine epithelium suggests that these sperm are attached to the epithelium of the female tract. Close associations between the female tract and sperm have been noted in *Heterakis* (see Lee, 1971) and in *Parascaris* (see Favard, 1961). In the latter species, Favard (1961) suggested that sperm were being resorbed. It seems more likely in *Deontostoma* that attachment of sperm to the female tract may be related to copulatory frequency and sperm longevity. An attachment mechanism might be required to prevent sperm from being expelled as the large oocytes pass by them into the more proximal uterus.

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Description and Observations on a Cuticular Infection of *Thelastoma pterygoton* sp. n. (Thelastomatidae: Nematoda) from *Oryctes* spp. (Scarabaeidae: Coleoptera)

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ABSTRACT: *Thelastoma pterygoton* sp. n. is described from larvae of *Oryctes monoceros* Ol. from Abidjan, Ivory Coast, West Africa. Distinguishing characters of *T. pterygoton* are the female lip cone distinctly set off from the rest of the body, the excretory pore located behind the basal bulb in both sexes, the presence of large lateral alae in the male, and the third pair of male anal papillae not fused as in other species in the genus.

T. pterygoton also occurs in *Oryctes boas* F., but in this host the adult nematodes often have a cuticular infection caused by bacterialike microorganisms closely appressed to the cuticle. Since all studied material was fixed, the microorganisms were not cultured, but resembled both bacteria and mycoplasmalike bodies. They appeared to dissolve the outer portion of the nematode's cuticle.

During a study on the nematode parasites and associates of members of the beetle genus *Oryctes* attacking coconut palms, thelastomid nematodes were recovered from the intestine of third-stage larvae of *Oryctes rhinoceros* L. in Western Samoa, Malaysia, and New Guinea and from *O. boas* F., *O. monoceros* Ol., and *O. owariensis* Beauv. from West Africa.

The larvae of *Oryctes* feed on decaying organic matter. The developmental biology of *O. rhinoceros* has been intensively studied and is probably more or less similar to that of other *Oryctes* species collected here. The larval period of *O. rhinoceros* lasts from 3 to 6 months and most of this time is spent in the third or last larval instar. The larvae occur in a variety of habitats, including decaying coconut trunks and other logs, sawdust, compost, and cattle dung. While the adult beetles cause considerable damage to palms, the larvae are innocuous except occasionally damaging timber posts and stumps set in the ground (Catley, 1969).

Because of the abundance of material, the nematodes found in the larvae of *Oryctes monoceros* and *O. boas* in West Africa were extensively studied and found to be undescribed. The present paper describes this species, discusses its morphology in comparison with other members of the genus, and describes a cuticular infection that occurs on this nematode.

Materials and Methods

Nematodes were removed from the intestine of third-stage field-collected larvae of *Oryctes monoceros* Ol. and *O. boas* F. from Port Bouet, Ivory Coast, West Africa. They were heat-killed, fixed in TAF (triethanolamine, formalin, and water), and processed to glycerin.

For electron micrograph studies of the cuticular region, specimens of *T. pterygoton* were prefixed in 1.5% glutaraldehyde for 2 hr, then washed in 0.05 M phosphate buffer and fixed in 1% osmium phosphate buffer (pH 7) for 1 hr. After dehydration in a graded ethanol series, the specimens were embedded in Araldite 6005 and sectioned with glass knives in a Porter–Blum microtome (MT-2). The sections were stained with a saturated aqueous solution of uranyl acetate and lead citrate (Reynolds, 1963) and examined with a Phillips EM-300 electron microscope.

Results and Discussion

Nematodes removed from the third-stage larvae of both *O. monoceros* and *O. boas* belong to the same species, which was undescribed. In the quantitative portion of the following description of this species, the first figure after the character represents the average value for that character, while the numbers in parentheses represent the range. All measurements are in millimeters.

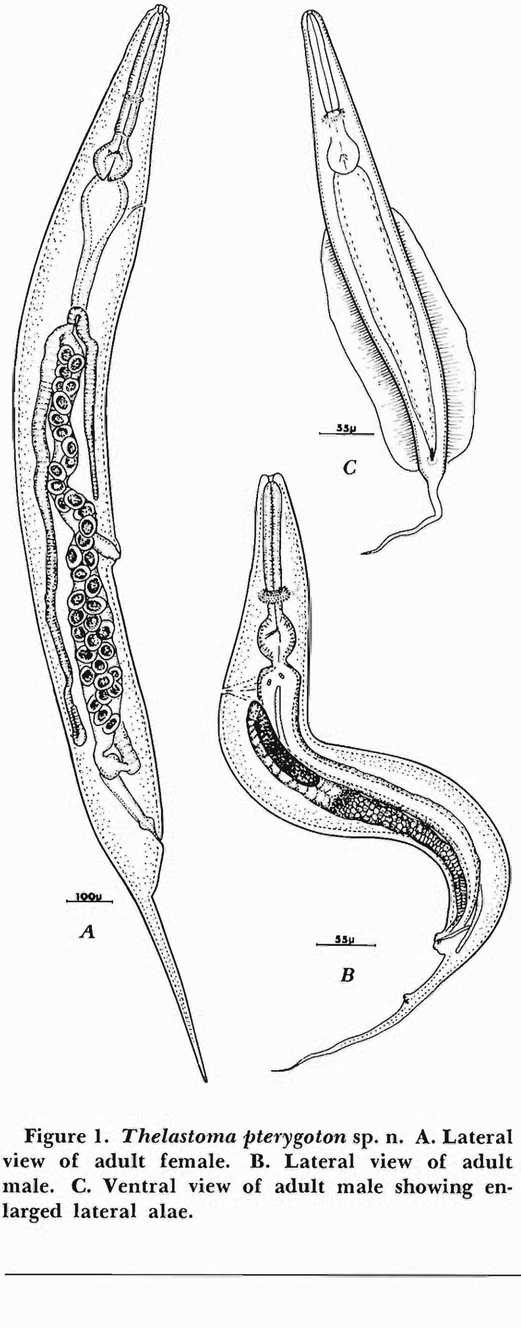

Figure 1. ***Thelastoma pterygoton*** **sp. n. A. Lateral view of adult female. B. Lateral view of adult male. C. Ventral view of adult male showing enlarged lateral alae.**

Thelastoma pterygoton sp. n. (Thelastomatidae: Oxyuroidea) (Figs. 1–2)

ADULTS: Head with eight distinct labial papillae and paired amphids; stoma simple, pharynx composed of a corpus, isthmus, and valvated bulb; tail of both sexes filiform, vulva near middle of body; ovaries paired; spicule present; male with four pairs of caudal papillae, well-developed lateral alae, and genital cone.

FEMALE ($n = 10$). Body covered with cuticular annulations approximately 8 μ apart; width of head at first annule 0.046 (0.037–0.054); distance of first annule from head 0.026 (0.024–0.031); total length 2.16 (1.41–2.95); width near vulva 0.18 (0.11–0.27); lip cone [width 0.025 (0.021–0.028); height 0.010 (0.009–0.012)] set off from remainder of body by a deep constriction; lip region slightly eight-lobed, bearing a row of eight terminal papillae and two emphids located laterally; an inner row of six papillae is also present. The sclerotized stomatal ring (= mouth cylinder = buccal cavity = vestibule wall) was 0.011 (0.010–0.012) long and 0.010 (0.009–0.011) wide. It is lined by an internal layer of pharyngeal tissue which is expanded at the base into three roughened areas. A faint ring of tissue just above the stomatal ring may represent the cheilorhabdions. Pharynx slender 0.39 (0.32–0.44) long with a constriction just above the basal bulb; nerve ring 0.20 (0.17–0.21) from head, located approximately in the center of the pharynx; body width at nerve ring 0.10 (0.08–0.14); excretory pore 0.44 (0.37–0.54) from head, approximately 0.04 behind the basal bulb; width of body at excretory pore 0.15 (0.10–0.24). Ventricular portion of intestine expanded and containing bacterial cells; % vulva 51 (48–54); length vagina 0.170 (0.125–0.229); spermatheca present at tip of uterine branches; ovary tips outstretched or reflexed; uterus containing eggs in various stages of division; tail length 0.49 (0.32–0.62); width

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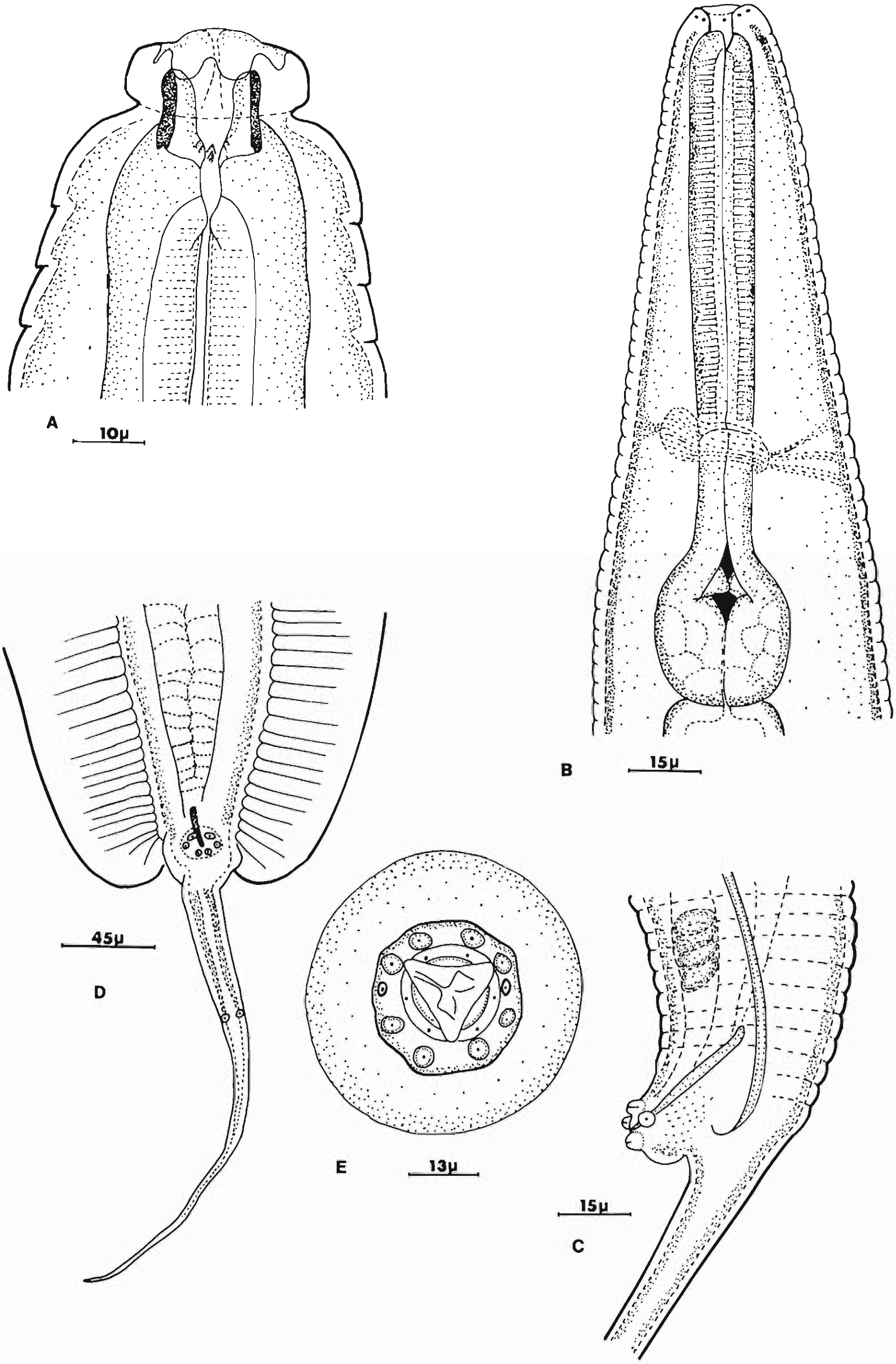
Figure 2. ***Thelastoma pterygoton*** **sp. n. A. Lateral view of female head. B. Lateral view of pharyngeal region of male. C. Lateral view of tail region of male. D. Ventral view of tail region of male. E. "En face" view of female.**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at tail 0.10 (0.08–0.13); tail extended into a narrow spine 0.39 (0.27–0.51) long.

MALE ($n = 8$): Body covered with cuticular annulations approximately 4–5 μ apart; total length 0.80 (0.69–0.98); greatest width 0.08 (0.07–0.11); lips fused but not set off from rest of body as a cone; eight terminal papillae—inner papillae not seen; length stoma 0.004 (0.003–0.005); width stoma 0.004 (0.003–0.006); pharynx slender, 0.15 (0.14–0.16) long, with constriction (isthmus) located farther above the basal bulb than in the female; nerve ring located in the posterior half of the pharynx, 0.09 (0.08–0.10) from the head; width of body at nerve ring 0.06 (0.05–0.07); excretory pore 0.20 (0.17–0.25) from head, approximately 0.03 behind the basal bulb; body width at excretory pore 0.08 (0.06–0.10); testis single, almost extending to excretory pore, reflexed at tip; spicule single, 0.034 (0.031–0.035) long, 0.002 (0.002–0.003) wide; anal area forming a genital cone [length 0.011 (0.010–0.014)] containing three pairs of papillae. The postanal pair are separate—not fused as in other thelastomids; the fourth pair of papillae are located at ⅓ of the length of the tail spine; distance from the base of the spine to the fourth pair of papillae 0.050 (0.041–0.058); distance from the fourth pair of papillae to the tip of the tail spine 0.141 (0.129–0.166); length total tail spine 0.20 (0.18–0.23); length tail 0.21 (0.19–0.24); body width at tail 0.04 (0.03–0.05); lateral alae from 0.028–0.050 wide, begin at the level of the ventricular portion of the intestine and extend to just above the base of the tail spine.

TYPE HOST: *Oryctes monoceros* (Ol.) (Coleoptera: Scarabaeidae).

TYPE LOCALITY: Abidjan, Ivory Coast, West Africa.

TYPE SPECIMEN: Deposited in the USDA Nematode collection, Beltsville, Maryland [Holotype (male), T-211t; Allotype (female), T-212t].

Diagnosis

The female of *Thelastoma pterygoton* possesses a distinct lip cone well set off from the body, the excretory pore behind the basal bulb, the nerve ring situated in the middle of the pharynx, and the tail less than one-quarter the total body length. The male contains an excretory pore also behind the basal bulb and possesses large lateral alae. The third pair of anal papillae just beneath the anus are separate. These latter two characters separate *T. pterygoton* from other males in the genus. Aside from being twice the size, *T. robustum* Christie (1938) possesses an eight-lobed lip cone that is not so distinctly separated from the rest of the body as in the present species. Also, the tail and pharynx of the male *T. pterygoton* are proportionally longer than in *T. robustum*.

The anteriorly placed excretory pore and nerve ring and the protruding vulva in the female of *T. alatum* Johnston (1914) separate it from the present species. The shape of the lateral alae and proportionally smaller tail in the male of *T. alatum* also separate it from *T. pterygoton*.

The larger size of the female of *T. toxi* van Waerebeke (1970) as well as its small lip cone and the presence of cuticular protuberances on the male distinguish it from *T. pterygoton*.

The excretory pore in the female of *T. macramphidum* Christie (1931) lies anterior to the basal bulb and the lip cone is not set off as in *T. pterygoton*. The head region of the male of *T. macramphidum* is set off by a constriction and contains narrower alae (4 μ) and a longer spicule (40–55) than *T. pterygoton*.

The female of the variety *gallica* Théodoridès (1955) of *T. macramphidum* possesses the lip cone less separated from the body than *T. pterygoton*. The male of variety *gallica* Théodoridès and three forms of this variety were described by Jarry (1964). The lateral alae of var. *gallica* extend to the nerve ring and the male tail is proportionally smaller than in *T. pterygoton*. The junction of the corpus and isthmus in the male *gallica* is further anterior than in *T. pterygoton*. However, the variety *gallica* itself differs from the typical *T. macramphidum* by having larger lateral alae (15–25 μ), a smaller spicule (28–30 μ), and the excretory pore in the female opening behind the basal pharyngeal bulb. Thus, *T. macramphidum*, as defined by Jarry (1964), is a variable species. Jarry and Jarry (1968)

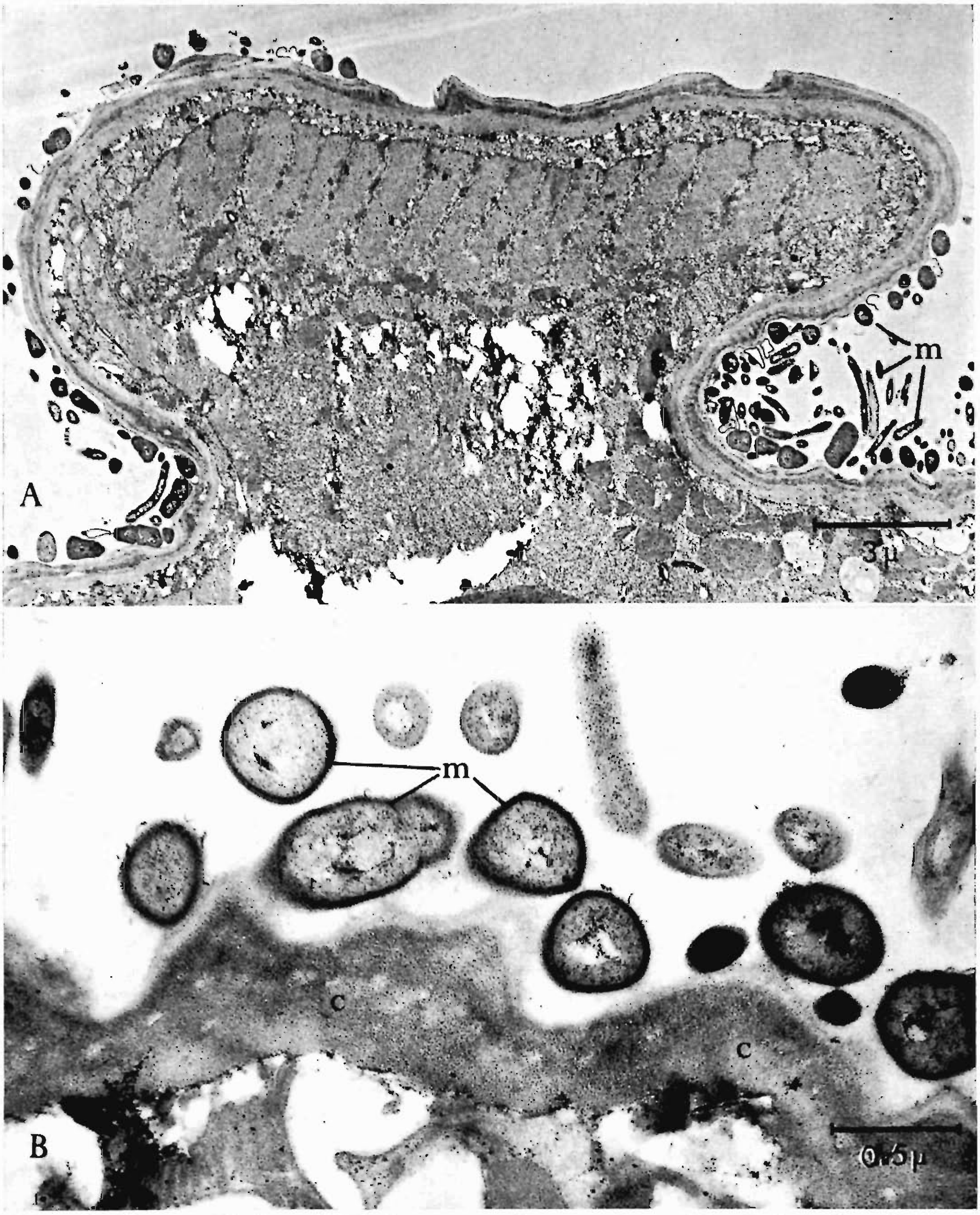

Figure 3. Cuticular infection of *Thelastoma pterygoton* sp. n. A. A portion of the body partially constricted from the remainder with associated microorganisms (m). B. Irregular grooves in the cuticle (c) and associated microorganisms (m).

feel that most of the described species in the genus *Thelastoma* are only varieties and have reduced all described species to three, *T. macramphidum*, *T. robustum*, and *T. attenuatum*, based on the position of the excretory pore in the female and the position of the genital papillae in the male. This can be determined with certainty only after additional studies have been conducted. Investigations on the morphological variation and interbreeding of thelastomatids is a challenging goal for the future. The separated pair of postanal papillae in the male of *T. pterygoton* is a diagnostic character and with the wide lateral alae and offset mouth cone, separates this species from previously described thelastomatids.

The presence of *T. pterygoton* in the larvae of *O. monoceros* and *O. boas* did not appear to have any effect on the hosts. However, adults of *Thelastoma pterygoton* removed from third-stage larvae of *O. boas* frequently exhibited rough areas on their cuticle that were visible under the light microscope. Portions of the body were sometimes partially constricted off from the remainder (Fig. 3A). Electron micrographs of the affected areas showed numerous microorganisms closely appressed to the cuticular surface. A close examination of the cuticle near these microorganisms showed the presence of irregular grooves and depressions often matching the shape of the associated cells (Fig. 3B). These results suggest that the microorganisms are capable of dissolving at least part of the nematode's cuticle and establishing colonies on the surface of the nematode.

Unfortunately, only fixed material was available; thus the cells were not cultured and their identification is not known. They exhibited a great irregularity in size and shape, many were empty, and the outer surface could not be definitely matched with any known organism. They may be some type of bacteria or mycoplasmalike body. Why these cuticular irregularities were never observed on *T. pterygoton* from the type host, *O. monoceros*, is not known.

Acknowledgments

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Special thanks are also extended to Roberta Hess for providing the electron micrographs and to Dr. M. Doudoroff of this university for his opinion on the associated microorganisms.

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Lindseyus costatus gen. n., sp. n., and Notes on the Roqueidae and Swangeriidae (Nematoda:Dorylaimida)[1]

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Abstract: *Lindseyus costatus* gen. n., sp. n., is described from Indiana as the second genus in the family Roqueidae (superfamily Belondiroidea). The diagnosis of Roqueidae is emended to accommodate the new genus. Similarities between the Roqueidae and the Swangeriidae are discussed.

A single large male belondiroid nematode resembling *Roqueus gracilis* Thorne, 1964, was recovered from soil obtained from southwestern Indiana in July 1967. The collecting site was an area of southern flora and fauna unusual for Indiana, at the edge of Hovey Lake in Posey County (Lindsey et al., 1969). Many large bald cypress trees (*Taxodium distichum*), swamp privet (*Forestiera acuminata*), and buttonbush (*Cephalanthus occidentalis*) border the lake. Broad-leaved arrowhead (*Sagittaria latifolia*) was the predominant plant in the immediate collecting site. Inasmuch as this lake is scheduled to be enlarged following completion of a dam on the adjacent Ohio River, and many features of the biological community despoiled, an attempt was made during October 1968 to secure more material. No specimens of this unusual nematode were found in soil processed in the laboratory on the Purdue University campus about 48 hr after collection. In June 1970, during another visit, soil processing and examination of residues from a No. 25 mesh sieve were carried out at the site. A total of six female specimens were found at this time, but no males.

Comparison with specimens of *R. gracilis* from Puerto Rico indicated that our specimens represent a new genus, which we name *Lindseyus* for Professor A. A. Lindsey, ecologist at Purdue University and diligent worker for the preservation of small natural areas in Indiana. *Lindseyus* is placed with *Roqueus* in the family Roqueidae, which is hereby emended to accommodate the new genus.

Family Roqueidae, Thorne, 1964, Emended

Belondiroidea: Body exceedingly slender, a = 67 – 117 in known representatives. Female tail long and filiform; male tail short, rounded. Cardia very elongated, with basal portion projecting into intestine. Supplements low, rounded, number 7–14 in addition to adanal pair. Ventro-submedian papillae present.

Type genus: *Roqueus* Thorne, 1964.

Genus *Lindseyus* gen. n.

Diagnosis: Roqueidae. Body long and slender (a = 67 – 86). Spear short and thin, with small aperture. Faint basketlike structure in lip region, guiding ring absent. Vulva transverse, simple. Ventromedian supplements seven, in addition to adanal pair. Lateral guiding pieces tapered and prominent.

Type species: *Lindseyus costatus* sp. n.

Lindseyus costatus is close to *Roqueus* from which it is distinguished by being less slender; by having a basketlike structure in the lip region and no guiding ring; by the sinistrally spiral twist of the musculature of the esophagus; by the simple transverse vulva; in the number and arrangement of supplements; and in the shape of the lateral guiding pieces. It has similarities also to members of the Swangeriidae in the basketlike structure in the lip region, elongate cardia, the sinistrally spiral musculature, and the long slender shape.

*Lindseyus costatus** sp. n.

(Fig. 1)

Females† (6): L = 5.20 mm ± 0.53 (4.3 – 5.67); a = 78.1 ± 6.8 (67.2 – 86.3); b = 14.7

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* The species name, *costatus*, is a Latin adjective meaning ribbed, and refers to the structure in the lip region.

† Figures given are for means, standard deviations, and ranges.

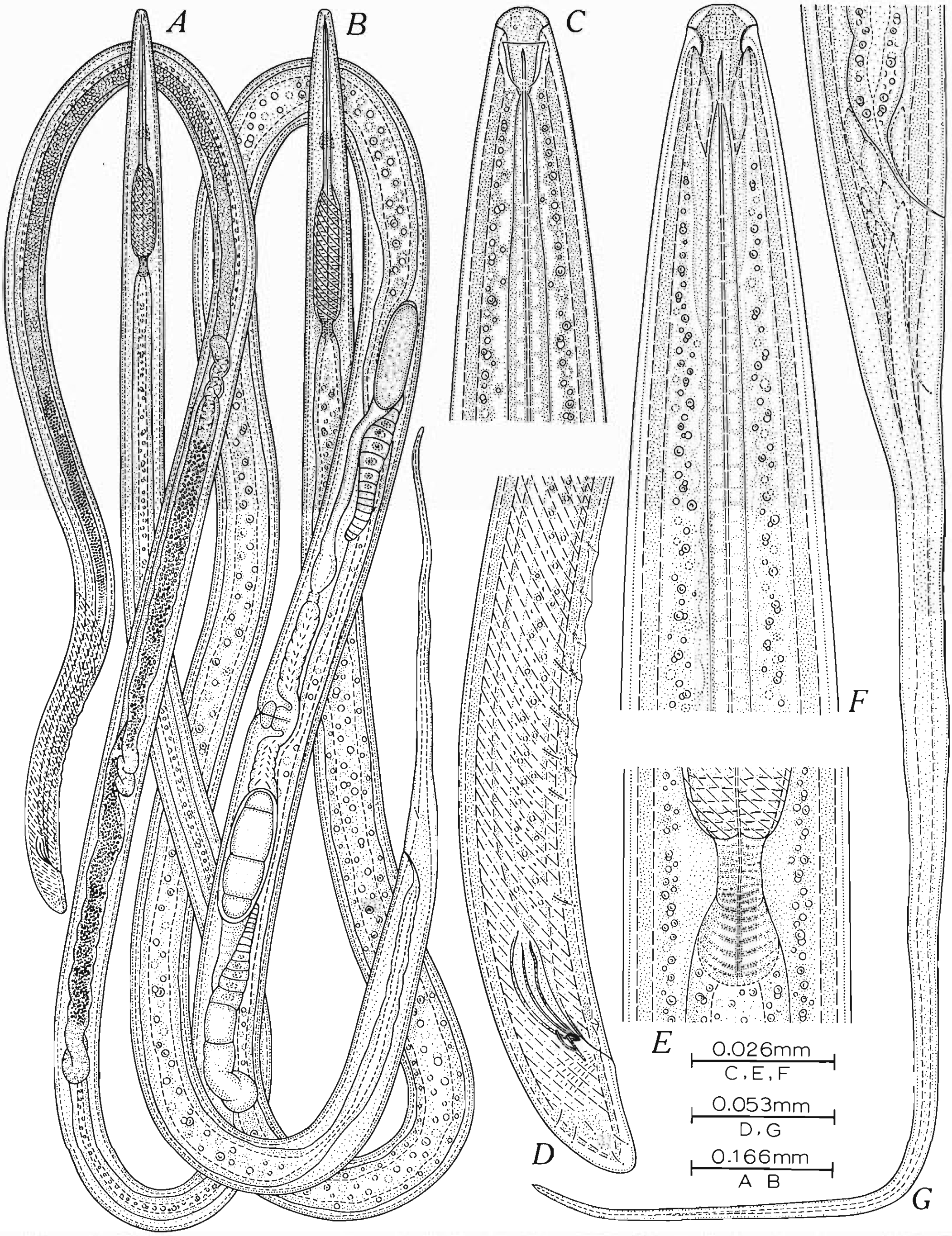

Figure 1. *Lindseyus costatus* sp. n. A, male; B, female; C, male head, lateral view showing amphid; D, male tail; E, cardia; F, female head, dorsoventral view showing basketlike structure in lip region; G, female tail.

Table 1. Known habitats of species of Swangeriidae and Roqueidae.

Species	Locality	Habitat	Authority
Swangeria fragilis	Sumatra	Soil around roots of rubber tree	Thorne, 1939
Swangeria bisexualis	Florida USA	Swampy soil	Hopper, 1961
Qudsiella gracilis	Middle Andamans, India	Soil around roots of papaya	Jairajpuri, 1967
Roqueus gracilis	Puerto Rico	Rain forest	Thorne, 1964
Roqueus africanus	Ivory Coast and Un. of S. Africa	Rice field and along Vaal River	Andrássy, 1970a, b
Lindseyus costatus	Hovey Lake, Indiana, USA	Wet soil around *Sagittaria* roots	Ferris and Ferris, this publ.

± 1.4 (12.6 – 16.5); c = 11.2 ± 1.4 (9.9 – 13.7); V = 37.9% ± 2.4 (33.9 – 41.2); g_1 = 10.7% ± 3.1 (8.7 – 16.9); g_2 = 10.4% ± 1.2 (9.1 – 11.7); body width = 0.07 mm ± 0.01 (0.06 – 0.08); esophagus length = 0.36 mm ± 0.04 (0.29 – 0.39); tail length = 0.47 mm ± 0.07 (0.35 – 0.57); prerectum length = 0.29 mm ± 0.06 (0.19 – 0.37).

FEMALE (holotype): L = 5.3 mm; a = 73.3; b = 13.7; c = 11; V = 37%; g_1 = 16.9%; g_2 = 9.5%; body width = 0.07 mm; esophagus length = 0.39 mm; tail length = 0.49 mm; prerectum length = 0.37 mm.

MALE: (paratype): L = 4.1 mm; a = 77.5; b = 13.9; c = 87; T = 59.5%; body width = 0.05 mm; esophagus length = 0.29 mm; tail length = 0.047 mm; prerectum length = 0.32 mm.

Body slender, cuticle marked by fine transverse striae. Neck tapering to narrow, rounded lip region. Amphid chambers about ⅔ width of adjacent body, the lower half filled with fine nerve fibrils as described by Thorne (1964) for *R. gracilis*. Sensillae pouches just posterior to base of spear extensions. Spear slender, 5–7 μ long, with small aperture. Spear extensions about 22 μ, exact length often difficult to determine. Lip region with suggestion of a basketlike structure of faintly sclerotized ribs as reported for *Swangeria* species (Thorne, 1939; Hopper, 1961). Enlargement of esophagus beginning at 58–70% of the esophageal length measured from anterior end, and surrounded by very prominent sinistrally spiral muscles. Prominent glands around anterior part of esophagus. Cardia long, basal portion attached to intestine as illustrated. "Stäbchensaum" present in intestinal lumen as reported by Thorne (1964) for *R. gracilis*. Prerectum four to six times as long as body width. Vulva transverse, appearing as simple open ellipse in ventral view. Vagina in lateral view composed of two sections. Cellular oviducts lead to what appear to be elongate–oval spermathecae, although these structures contained few, if any, sperms in our specimens. The two structures are separated by prominent sphincter. Ovaries reflexed ⅓ to ⅔ distance back to vulva.

Female tail filiform, attenuated portion shorter than in *R. gracilis*. Terminus a sharp sclerotized point. Male tail short, rounded, ventrally arcuate with adanal pair and seven ventromedian supplements. Ventro-submedian papillae present. Spicules slightly arcuate. Lateral guiding pieces tapered and quite prominent.

HOLOTYPE: Female in Purdue Nematode Collection (PNC) on slide designated 7/31/70B1.

PARATYPES: Male in PNC on slide designated 7/15/67A7. Five females in PNC on slides with the following designations: 7/31/70A1; 7/31/70A2; 7/31/70B2; 7/31/70B3; 7/31/70C1.

TYPE HABITAT: Swampy soil at edge of lake around roots of broad-leaved arrowhead (*Sagittaria latifolia*).

TYPE LOCALITY: Hovey Lake, Posey Co., Indiana, USA.

Discussion

The family Swangeriidae is comprised of two genera, *Swangeria* and *Qudsiella*, containing only three species, *S. fragilis* Thorne, 1939, *S. bisexualis* Hopper, 1961, and *Q. gracilis* Jairajpuri, 1967. Roqueidae now with two genera, *Roqueus* and *Lindseyus* also has three species, *R. gracilis*, *R. africanus* Andrássy, 1970, and *L. costatus* sp. n. The presence of the faint basketlike structure in the lip region of *L. costatus*, the elongate cardia, the sinistrally spiral musculature of the esophagus, and the long slender shape suggest a relationship between the Roqueidae and the Swangeriidae. *R. gracilis* has a guiding ring and no basketlike structure, but this structure is not a constant feature even of the Swangeriidae, as it is absent in *Qudsiella* (Jairajpuri, 1967). There are apparent similarities in the ecology of the six known species of the two families as well, as all have been found in southern areas and in moist to wet habitats (Table 1). The type habitat of *L. costatus*, although at 37°51′ N latitude, is in an area of southern flora and fauna considered unusual for Indiana.

Although genera in both families have the cardia greatly elongated, differences exist between the families in the nature of the posterior attachment. In *Swangeria* and *Qudsiella* the cardia is extremely elongate and attached to the intestine only at the posterior end. Relatively more of the cardia in both *Roqueus* and *Lindseyus* projects into the intestine. Detailed observations of the cardia in living specimens of *L. costatus* indicated no major structural differences between living and fixed specimens. The sexual dimorphism in tail shape serves also to separate the families Roqueidae and Swangeriidae, but we consider the two families to be closely related.

Acknowledgments

The authors are grateful to Mrs. Clara Wood and Dr. J. P. Tjepkema for technical assistance, to Mr. L. H. Wang for preparation of the illustrations, and to Prof. Gerald Thorne for critically reviewing the manuscript.

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Dunnifilaria ramachandrani gen. n., sp. n. (Nematoda: Filarioidea) from the Long-Tailed Giant Rat (*Rattus sabanus*) in Malaysia[1]

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ABSTRACT: *Dunnifilaria ramachandrani* gen. n., sp. n. (Nematoda: Filarioidea) is described from the long-tailed giant rat, *Rattus sabanus*, from West Malaysia. It belongs in the family Onchocercidae (Leiper, 1911) Chabaud and Anderson, 1959, subfamily Splendidofilariinae Chabaud and Choquet, 1953. Adult worms were found in the right ventricle and pulmonary arteries of 10% of over 100 rats examined. Microfilariae are small, sheathed, and found in the peripheral blood.

During a survey for blood parasites of Malaysian mammals trapped by the Medical Ecology Division of the Institute for Medical Research, sheathed microfilariae were found in about 10% of more than 100 long-tailed giant rats (*Rattus sabanus*) from the states of Pahang and Selangor. Adult worms were subsequently recovered from the right heart chambers and pulmonary arteries of the rats. Examination revealed that the worms belonged to a new genus in the subfamily Splendidofilariinae. Dunn and Ramachandran (1962) briefly described these worms and noted that the microfilariae were subperiodic, having a maximum peak in the late afternoon. They reported attempts to infect *Aedes aegypti* and *Mansonia uniformis*, but the larvae failed to develop in the mosquitoes. Sivanandam et al. (1965) provided additional measurements of the adults and microfilariae and recorded their presence in the pulmonary arteries.

The genus and species are named after Drs. F. L. Dunn and C. P. Ramachandran in recognition of their studies on filarial worms in Southeast Asia.

Materials and Methods

The animals were killed with chloroform, and thick films were made from heart blood in order to study the microfilariae. Films were allowed to dry overnight and were then stained with either dilute Giemsa (35 drops per 100 cc buffer) for 1 hr or hot hematoxylin after methanol fixation. Unstained microfilariae dissected from the uterus of adult worms after fixation and clearing were also examined.

Adult worms were dissected from the heart and pulmonary vessels and killed in glacial acetic acid. After 3 min they were transferred to a solution of 70% ethanol and 5% glycerin which was allowed to evaporate. The worms were studied in pure glycerin.

Measurements and drawings were made with the aid of a camera lucida and ocular micrometer. All measurements are in millimeters unless otherwise noted, with averages in parentheses.

Description

Dunnifilaria gen. n.

Filarioidea: Onchocercidae (Leiper, 1911) Chabaud and Anderson, 1959; Splendidofilariinae Chabaud and Choquet, 1953. Small slender worms of uniform diameter, tapering at the extremities. Head smooth and rounded. Oral opening small, simple, surrounded by four pairs of inconspicuous submedian papillae. Cuticle smooth. Esophagus moderately long, anterior muscular and posterior glandular portions not distinctly demarcated; sometimes taking a sinuous course, occasionally folding back on itself. Anus difficult to visualize, subterminal in position. Vulva in midesophageal region. Tail in male long, caudal papillae consisting of

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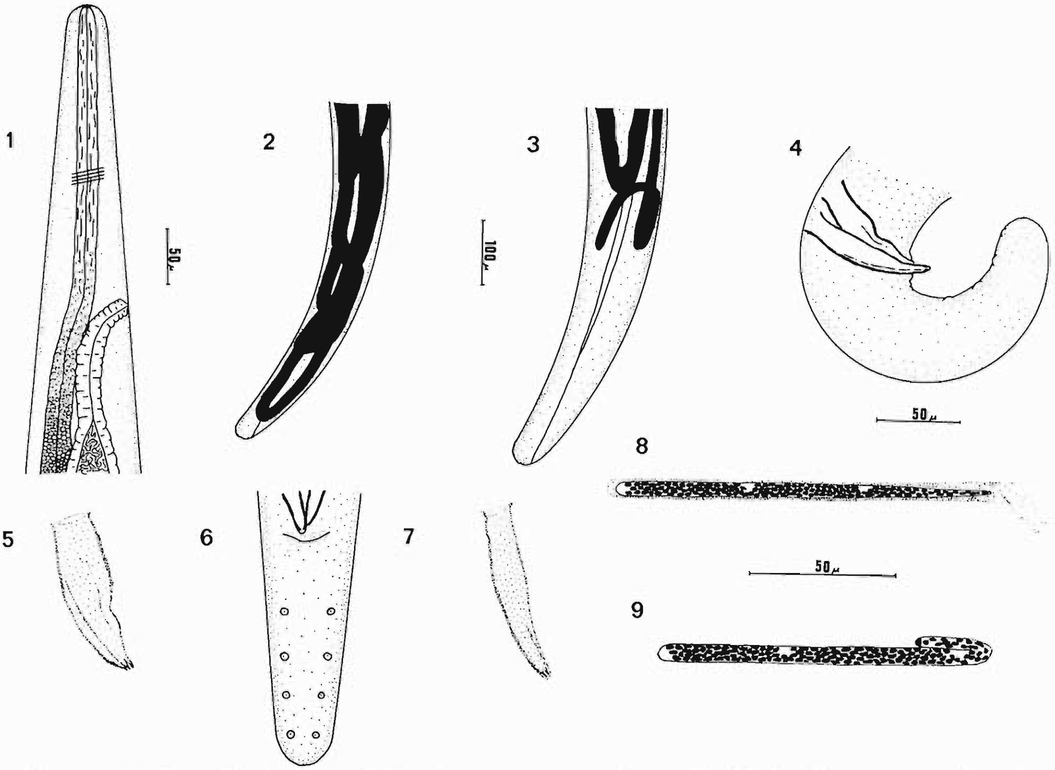

Figures 1–8. *Dunnifilaria ramachandrani* gen. n., sp. n.: Camera lucida drawings. 1. Anterior end of female showing midesophageal vulva. 2. Tail of immature female. 3. Tail of mature female. 4. Tail of male. 5. Left spicule. 6. Ventral view of male tail. 7. Right spicule. 8. Microfilaria stained with hematoxylin. 9. Microfilaria stained with dilute Giemsa.

four pairs, all postanal. Preanal papillae lacking. Spicules similar, nearly equal in length. Caudal alae absent. Microfilariae sheathed, found in blood. Parasites of rodents; type species *Dunnifilaria ramachandrani* sp. n.

Dunnifilaria ramachandrani sp. n.

With characters of the genus (description based on 10 males and 5 gravid females).

MALE: Body length 9.6–14.8 (13.6), width at midbody 0.09–0.13. Esophageal length 0.50–0.95 (0.65). Nerve ring approximately 60 μ from anterior end. Transition from muscular to glandular esophagus approximately 75–100 μ from anterior end. Caudal papillae only slightly elevated, inconspicuous, consisting of four pairs postanal; preanal and adanal papillae missing. Cloaca 0.11–0.16 (0.13) from posterior end of body. Spicules stout, subequal, dissimilar; length left 0.07–0.09 (0.077), right 0.065–0.08 (0.070). Left to right spicular ratio 1.1:1. Both spicules boat-shaped with spatulate tips, left broader than right. Tail tightly coiled, rounded, occasionally slightly knoblike.

FEMALE: Body length 18.5–30.3 (25.2), width at midbody 0.16–0.20 (0.18). Esophagus not measurable in gravid specimens; approximately 5% of total length in nongravid females. Nerve ring approximately 100 μ from anterior end. Transition from muscular to glandular esophagus approximately 150 μ from anterior end. Vulva in midesophageal region 0.32–0.45 (0.41) from anterior extremity. Vagina muscular, approximately 0.125 long, usually directed posteriorly without looping forward. Uterine coils longitudinal, filling body

Table 1. *Dunnifilaria ramachandrani* sp. n.: Measurements of microfilariae (10 in each staining category).

Part measured	Stain used		
	Hematoxylin	Giemsa	Unstained
Cephalic space L × W	2 × 4	2.2 × 6.3	—
Total length	112–136 (123)	122–144 (133)	110–126 (118)
Head to nerve ring	32–43 (38)	38–48 (42)	—
Head to excretory pore	68–77 (74)	—	—
Head to anal pore	—	107–122 (114)	—

cavity except in posterior end of body where they assume a spiral appearance; coils extending farther posteriorly in immature worms. Anus 0.025 from posterior end of body.

Microfilariae: Found circulating in blood. Each with sheath that stains poorly with Giemsa but well with hematoxylin. Cephalic space short. Head and tail blunt. Last tail nucleus prominent. Tail often folded back alongside body. Microfilariae on hematoxylin-stained films more compact and slightly shorter than on Giemsa-stained films (Table 1).

Type host: *Rattus sabanus*, No. 87776 Medical Ecology Museum, Institute for Medical Research, Kuala Lumpur, Malaysia.

Additional host: *R. sabanus*, No. 88205 Medical Ecology Museum, Institute for Medical Research, Kuala Lumpur, Malaysia.

Location: Right heart chambers and pulmonary arteries.

Type locality: Kuala Selangor, Selangor, West Malaysia.

Specimens: Holotype (male) USNM Helm. Coll. No. 72155, and paratypes No. 72156.

Discussion

Dunnifilaria gen. n. is placed in the family Onchocercidae (Leiper, 1911) Chabaud and Anderson, 1959, because it has a round, smooth head lacking chitinous peribuccal formations and an anterior vulva; produces microfilarioid embryos; and lacks caudal alae in the male. It is placed in the subfamily Splendidofilariinae, Chabaud and Choquet, 1953, because of its long digitiform tail, four pairs of cephalic papillae, lack of caudal alae, anterior vulva, and subequal similar spicules (Chabaud and Anderson, 1959).

Other genera listed by Yamaguti (1961) in the subfamily Splendidofilariinae that are parasitic in mammals include *Micipsella* Seurat, 1921; *Onchocercella* Yorke et Maplestone, 1931; *Johnstonema* Yeh, 1957; and *Protofilaria* Chandler, 1929. *Dunnifilaria* differs from each as follows: from *Micipsella* in lacking broad lateral lines of cuticular bosses, lacking a hemispherical cephalic prominence provided with a circle of small papillae, lacking preanal papillae, and having the vulva situated more anteriorly; from *Onchocercella* in lacking transverse striations and fusiform thickenings on the cuticle and lacking adanal papillae; from *Johnstonema* in lacking epauletlike structures on the head, lacking two large lateral cephalic papillae, lacking preanal and perianal papillae, and having a more anteriorly placed vulva; from *Protofilaria* in lacking a cleft dividing the tail into two lobes, and in having postanal papillae, a longer tail in the male, and a more anteriorly placed vulva.

Acknowledgments

We thank Dr. I. Muul and other members of the Medical Ecology Division of the Institute for Medical Research for providing the specimens and Professor P. C. Beaver, Dr. D. Little, and Dr. J. H. Esslinger, all of Tulane University, for their constructive comments on the manuscript.

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Raillietina (*R.*) *garciai* sp. n. (Cestoda: Davaineidae) from the Greater Antillean Grackle, *Quiscalus niger brachypterus* Cassin, in Puerto Rico

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ABSTRACT: *Raillietina* (*Raillietina*) *garciai* sp. n., a davaineid tapeworm from the Greater Antillean grackle, *Quiscalus niger brachypterus* Cassin, is described. The species most closely resembles *Raillietina* (*R.*) *weissi* (Joyeux, 1923) Fuhrmann, 1924, from which it differs in hosts and locality, size of strobila, cirrus pouch, testes, and egg capsules.

During a recent study of helminth parasites of some birds in Puerto Rico by Whittaker et al. (1970), 124 Greater Antillean grackles, *Quiscalus niger brachypterus* Cassin, were examined, of which three collected in a rural area near Isabela were found to be infected with 10 specimens of a heretofore undescribed cestode species of the genus *Raillietina* (*Raillietina*) Fuhrmann, 1920. Two of the birds harbored three worms each, and the third had four.

The worms were relaxed in tap water, fixed in AFA solution, stained in Harris' hematoxylin, and mounted in euparal vert. Drawings were made with the aid of a camera lucida. All measurements are in millimeters with average measurements in parentheses after the range. The following description is based on a study of the 10 specimens.

Raillietina (*R.*) *garciai* sp. n.
(Figs. 1–4)

DESCRIPTION: Davaineidae, Davaineinae. Length of strobila 97–120 (107); segmentation distinct and craspedote. Strobila widening posterior to scolex, gradually increasing in width throughout its length. Immature segments wider than long. Mature segments wider than long ranging in width from 0.651–0.741 (0.690) and in length from 0.167–0.176 (0.171). Gravid segments also wider than long, 0.814–1.040 (0.892) in width by 0.410–0.714 (0.520) in length. Scolex from 0.218–0.276 (0.252) in width; extended rostellum not observed; rostellum armed with double row of 132–140 (136) hooks 0.019–0.022 (0.020) in length in first row and 0.017–0.019 (0.018) in second row. Suckers subspherical, 0.054–0.074 (0.064) in diameter, each with approximately four circles of very minute hooks. Genital pores unilateral in middle third of segment. Cirrus pouch elongate, sometimes pyriform, 0.078–0.105 (0.094) in length by 0.023–0.035 (0.029) in width in mature segments, extending approximately one-half distance to longitudinal excretory canal. Cirrus unarmed. Testes spherical, 8–13 (10) in number per mature segment and measuring 0.035–0.054 (0.043) in diameter. Testes distributed in two groups, 1–3 (2) poral and 7–10 (8) aporal, between excretory canals. Vas deferens coiled from near midline to its entrance into cirrus pouch. Unconvoluted vagina parallel and posterior to vas deferens, entering genital atrium posterior to male system. Vagina in most specimens with distinct enlargement near genital atrium, at times similar in size to cirrus pouch. Vagina passing dorsal to ventral excretory canal. Ovary bilobed and situated medially in middle third of mature segment; lobes connected by short isthmus. Vitellarium compact, postovarian. Gravid segments containing 35–45 (39) egg capsules, each with 4–7 (5) eggs; capsules subspherical, 0.085–0.178 (0.133) in diameter, weakly arranged in 4–5 transverse rows in fully gravid segment. Diameter of eggs, 0.032–0.043 (0.037). The sample sizes on which the means are based are as follows: strobila, 10; mature segments, 40; gravid segments, 30; scolex, 9; hook number, on 9 scoleces; hook size, 85; suckers, 36; cirrus pouch, 50; testis number, in 50 mature segments; testis size, 76; egg capsule number, in 40 segments; egg capsule size, 83; egg number, in 87 capsules; egg size, 62.

HOST: *Quiscalus niger brachypterus* Cassin (Passeriformes: Icteridae).

HABITAT: Small intestine.

TYPE LOCALITY: Isabela, Puerto Rico.

TYPE SPECIMEN: USNM Helm. Coll. No. holotype 72283.

This species is named in honor of Dr. Julio Garciá Diaz of the University of Puerto Rico in recognition of his contributions to helminthology in Puerto Rico.

Discussion

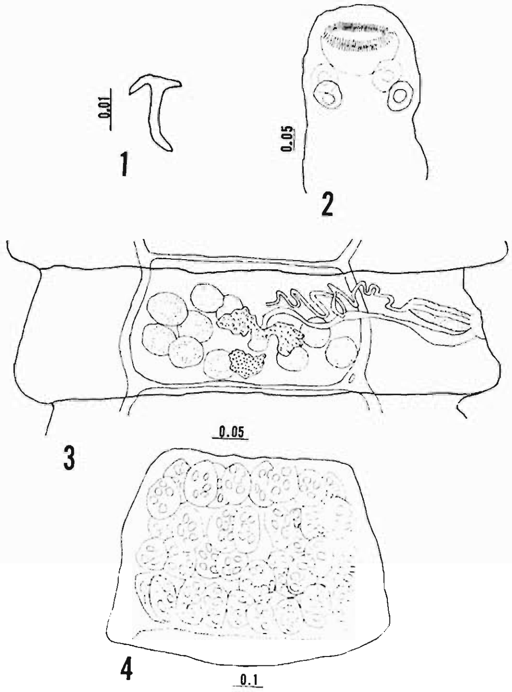
Artiukh (1966) has reviewed the literature concerning the genus *Raillietina*. Most of the 151 species of the subgenus *Raillietina* mentioned by Artiukh from birds and mammals are thoroughly described and figured. Species of the subgenus *Raillietina* described since Artiukh's work have also been considered in the description of *R. garciai.*

The size of the strobila, rostellar hooks, and cirrus pouch in combination with the number of rostellar hooks, testes, eggs per capsule, and capsules per segment serve to distinguish *R. garciai* from the other members of the subgenus.

Raillietina garciai most closely resembles *R. weissi* (Joyeux, 1923) Fuhrmann, 1924, from which it differs in hosts and locality (*R. weissi* was recovered from doves, *Columba livia* Gmelin and *Streptopelia turtur arenicola* Hartert, in Tunisia), size of strobila and cirrus pouch. The strobila of *R. weissi* is longer (142 mm) and proportionately wider (2 mm), and the cirrus pouch is longer (0.1–0.130 mm), extending nearer to the ventral excretory canal. *R. garciai* differs further from *R. weissi* in the size of the testes and egg capsules (0.060–0.080 and 0.105–0.135 mm in diameter, respectively, in *R. weissi*).

R. garciai may be differentiated from *R. korkei* Joyeux et Houdemer, 1927, and *R. columbiella* Ortlepp, 1938, by differences in the number of rostellar hooks and length of the strobilae. In the latter two species the hook number is 150–160 and 200, respectively. The length of the strobilae is 164 and 34–67 mm. Furthermore, *R. korkei* has 7–12 eggs per capsule, and *R. columbiella* has 50–80 egg capsules per segment. Also the cirrus pouch of the latter species is considerably longer (0.160–0.230 mm) than that of *R. garciai.*

One other species of cestode, *R. turaci* Baer, 1933, can be easily distinguished from *R. garciai* by the greater number of egg capsules (80) and rostellar hooks (150–160). Moreover, the hooks of *R. turaci* are considerably shorter (0.005–0.006 mm) than those of *R. garciai.*



Figures 1–4. *Raillietina garciai* sp. n. from the Greater Antillean grackle in Puerto Rico. 1. Rostellar hook, first row. 2. Scolex. 3. Mature proglottid. 4. Gravid proglottid.

These differences are considered adequate to justify designating the specimens from Puerto Rico as a new species.

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Investigations on the Trematode Fauna of Hyderabad, A. P., Part II. Parasites of Birds—(D). *Eumegacetes (Anterovitellum) centropius* sp. n. from a "Coucal," the Crow-pheasant, *Centropus sinensis.*

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ABSTRACT: *Eumegacetes (Anterovitellum) centropius* sp. n. is described from the "coucal," the crow-pheasant, *Centropus sinensis*, from Hyderabad, A. P., India, and compared with the other four previously described Indian forms and also with a Hyderabad species, *E. indicus* Jaiswal and Vasudev, 1960. It differs from all other species of the genus in size, shape, disposition of the gonads, location of the suckers as well as their ratio, and in the principal measurements of the body. The authors suggest the reduction of the genus *Posthovitellum* Khotenovskii, 1966 (along with its six species) to the rank of a subgenus under the genus *Eumegacetes* Looss, 1900 and all the 26 species described so far from different parts of the world have also been accommodated under a single genus *Eumegacetes*. A key based on the extent of vitellaria has also been given.

In 1960, Jaiswal and Vasudev, while discussing the systematic position of the controversial genus *Eumegacetes* Looss, 1900 objected to the erroneous attribution of this genus to the tribe Phaneropsolea of the subfamily Lecithodendriinae by Skrbilovich (1943) and opined that the above arrangement was not justifiable and needed revision. While describing five new forms of this genus from Hyderabad, they further suggested the retention of the genus *Eumegacetes* under a separate subfamily Eumegacetinae, Mehra, 1935, with several modifications and amendments. In 1966, Jaiswal and Reddy added one more new species to the genus and again in 1967 they described yet another form, retaining the classification of Jaiswal and Vasudev (1960). In 1966, Khotenovskii recognized the family Eumegacetidae Travassos, 1922 and placed *Eumegacetes* and other genera under this family.

The present work deals with one more new species of the genus *Eumegacetes*. The authors are in complete agreement with the Russian author, as far as the shifting of the genus *Eumegacetes* from the family Lecithodendriidae to Eumegacetidae is concerned, but they do not subscribe to the views of Khotenovskii (1966) in eliminating the following five species, namely (1) *Eumegacetes contribulans* Braun, 1901; (2) *E. brauni* Mehra, 1935; (3) *E. brevicoecus* Vigueras, 1940; (4) *E. Komarovi* Skrjabin, 1948; and (5) *E. longicirratus* Jaiswal and Vasudev, 1960, from the genus *Eumegacetes* and placing them under a new genus *Posthovitellum* created by Khotenovskii (1966).

As the above forms are established and recognized species of the genus *Eumegacetes*, it is not desirable at this stage to transfer them from their original position to the proposed new genus. On the contrary, it is suggested that the newly created genus *Posthovitellum* Khotenovskii, 1966 should be reduced to the rank of a subgenus under the genus *Eumegacetes* Looss, 1900 so that it may also be brought in line with the other already existing subgenus *Anterovitellum* created by Khotenovskii in 1966.

Thus, in consideration of the above facts, it is suggested that the genus *Eumegacetes* Looss, 1900 be divided into three subgenera, namely (1) *Eumegacetes* (Oshmarin, 1963) Khotenovskii, 1966—*sensu stricto*; (2) *Anterovitellum* Khotenovskii, 1966; and (3) *Posthovitellum* Khotenovskii 1966. All six species, including *Posthovitellum delichoni* from *Delichon urbica* described by the Russian author in 1966 and assigned to his new genus, should now be shifted to the proposed new subgenus *Posthovitellum* Khotenovskii, 1966. Thus all 26 forms, described so far as species of the genus *Eumegacetes*, may now be accommodated under a single genus *Eumegacetes* Looss, 1900. The above three subgenera can easily be differentiated from one another by the extent of their vitellaria, as per the following key:

1. The forward extent of vitellaria restricted only to the anterior level of the testes ..... subgenus *Eumegacetes* (Oshmarin, 1963) Khotenovskii, 1966
2. The forward extent of vitellaria reaches beyond the anterior level of the testes .... subgenus *Anterovitellum* Khotenovskii, 1966
3. The extent of vitellaria restricted only to the midlevel of the body and does not extend even to the anterior level of the acetabulum .... subgenus *Posthovitellum* (Khotenovskii, 1966)

The species belonging to the genus *Eumegacetes* Looss, 1900 are now assigned to the three subgenera as follows:

I. Subgenus *Eumegacetes* (Oshmarin, 1963) Khotenovskii, 1966 will now comprise the following 11 species:

Type species:

1)	*Eumegacetes*	(*Eumegacetes*)	*emendatus* Braun, 1901
2)	"	"	*medioximus* Braun, 1901
3)	"	"	*emendatus ibericus* Kurashvili, 1940
4)	"	"	*lanii* Yamaguti and Mitunaga, 1943
5)	"	"	*skrjabini* Tenora and Kopriva, 1958
6)	"	"	*hirundiosus* Jaiswal and Vasudev, 1960
7)	"	"	*hyderabadensis* Jaiswal and Vasudev, 1960
8)	"	"	*macroorchis* Brenes and Arroyo, 1962
9)	"	"	*lecithrosalus* Oshmarin and Oparin, 1963
10)	"	"	*sacculouterus* Oshmarin and Oparin, 1963
11)	"	"	*eudynamisius* Jaiswal and Reddy, 1966.

II. Subgenus *Anterovitellum* Khotenovskii, 1966 will now consist of the previously described nine species and the new species described herein:

1)	*Eumegacetes*	(*Anterovitellum*)	*perodiosus* Travassos, 1922
2)	"	"	*artamii* Mehra, 1935
3)	"	"	*microdiosus* Chauhan, 1940
4)	"	"	*mehrai* Jha, 1943
5)	"	"	*riparius* Gupta, 1957
6)	"	"	*singhi* Jaiswal, 1957
7)	"	"	*indicus* Jaiswal and Vasudev, 1960
8)	"	"	*megacetabulus* Jaiswal and Vasudev, 1960
9)	"	"	*acquillai* Jaiswal and Reddy, 1967
10)	"	"	*centropius* sp. n. (described herein).

III. Subgenus *Posthovitellum* (Khotenovskii, 1966) will comprise the following six species:

1)	*Eumegacetes*	(*Posthovitellum*)	*contribulans* Braun, 1901
2)	"	"	*brauni* Mehra, 1935
3)	"	"	*brevicoecus* Vigueras, 1940
4)	"	"	*komarovi* Skrjabin, 1948
5)	"	"	*longicirratus* Jaiswal and Vasudev, 1960
6)	"	"	*delichoni* Khotenovskii, 1966.

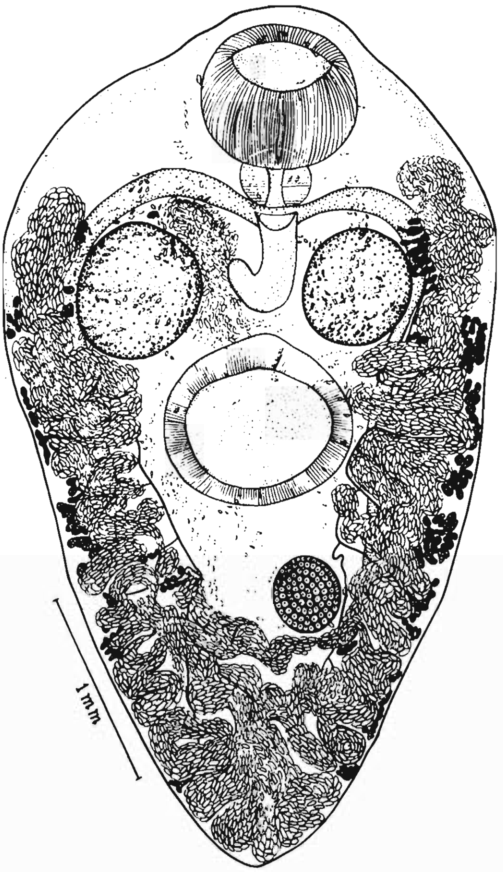

Figure 1. ***Eumegacetes*** **(*Anterovitellum*)** ***centropius*** **sp. n. Ventral view.**

Thus, in consequence of the above discussion, the family Eumegacetidae Travassos, 1922 will now comprise only two genera, namely (1) genus *Eumegacetes* Looss, 1900 (consisting of three subgenera) and (2) genus *Papillatrema* Oshmarin, 1965.

Eumegacetes (*Anterovitellum*) *centropius* sp. n. (Fig. 1)

In August 1961, the writers collected two specimens of this species from a "coucal," the crow-pheasant, *Centropus sinensis.* The worms were found crawling in the kidney tissue. The live flukes were examined in normal saline and were found oval-shaped, broader anteriorly and narrower posteriorly. The specimens were stained in hematoxylin for detailed study of their organs and body dimensions. The length of the body is 4.26–4.34 mm, and the maximum width is 2.28–2.53 mm. The acetabulum is slightly bigger than the oral sucker, it is strongly muscular and is placed in the middle of the body, measuring 0.84–0.88 by 0.91–0.96 mm. The oral sucker is subterminal and somewhat rounded in shape, measuring 0.73–0.76 by 0.71–0.79 mm. The distance between the suckers is 0.82–0.84 mm. The oral sucker opens into a muscular pharynx which is globular in shape, measuring 0.25–0.30 by 0.35–0.36 mm. The pharynx is followed by the intestinal ceca, which are completely overlapped on both sides of the body by the strongly convoluted ascending and descending loops of the uterus.

The excretory bladder is V-shaped, with long cornua, and opens to the exterior at the median excretory pore, located at the posterior end.

The male genitalia consist of two symmetrically placed oval testes, which extend prominently between the visible limbs of the ceca and the ventral sucker. The right testis is bigger than the left one and they measure 0.64–0.68 by 0.44–0.64 mm and 0.58–0.63 by 0.50–0.55 mm, respectively. The cirrus sac is curved at its base, somewhat broader at the anterior end, and opens immediately in front of the pharynx, above the ceca.

The female gonad consists of a rounded ovary, placed somewhat laterally at a distance from the acetabulum, and measures 0.38–0.44 by 0.34–0.35 mm. The uterus is highly convoluted and extends posteriorly covering the entire hind region of the body. It then proceeds anteriorly covering completely the lateral zones of the body, extending upward beyond the level of the testes, reaching up to the pharynx. The uterine coils are packed with eggs.

The vitellaria consist of small irregularly shaped bodies, lying laterally and mostly covered by the uterus; their anterior extent is slightly beyond the level of the testes and posteriorly they reach nearly up to the caudal region. The eggs are thin-shelled, measuring 18–23 μ long by 8–11 μ broad.

Discussion

The form under study differs from all the known species not only in its size and the shape and disposition of the gonads but also in the much smaller size of the eggs and the disposition of the vitellaria.

To some extent it resembles the following four forms, namely (1) *E. artamii* Mehra 1935; (2) *E. microdiosus* Chauhan, 1940; (3) *E. mehraii* Jha, 1943; and (4) *E. riparius* Gupta, 1957. However, it differs from all of them in the size, shape, disposition of the gonads, and the location of the suckers and their ratio. In the Hyderabad species the testes are very large and oval in shape; they occupy nearly the entire available space between the ceca and the acetabulum. Moreover, the ovary is rounded and is placed much below the acetabulum to the left side of the body. The genital pore, which is median in position, is placed on the intestinal bifurcation. In *E. artamii*, *E. microdiosus*, *E. mehraii*, and *E. riparius* the testes are comparatively small and are either irregular in shape or rounded. They are placed either in the middle of the space between the intestinal bifurcation and the acetabulum or they partly overlap the acetabulum. The ovary is rounded or elliptical in shape and mostly approximated with the acetabulum. The genital pore is either lateral or somewhat median but definitely below the level of the intestinal bifurcation. The new form differs from all the above-mentioned species also in the disposition and extent of its vitellaria which terminate in the posterior region slightly above the caudal end and extend anteriorly somewhat above the testes, terminating at about the level of the intestinal bifurcation. In all the above four species, the vitellaria extend posteriorly right up to the caudal end and terminate anteriorly below the level of the intestinal bifurcation.

The new form also differs from the other Hyderabad species, namely *E. indicus* Jaiswal and Vasudev, 1960, by the absence of epidydimoids at the outer margins of its testes and the lack of the body spines.

SPECIFIC DIAGNOSIS: Body oval, broader anteriorly and narrower posteriorly 4.26–4.34 mm in length and 2.28–2.53 mm in greatest width. Oral sucker 0.73–0.76 by 0.71–0.79 mm, acetabulum 0.84–0.88 by 0.91–0.96 mm, pharynx muscular, 0.25–0.30 by 0.35–0.36 mm, excretory bladder V-shaped with long cornua. Testes oval, between intestinal fork and acetabulum, the right and left testes measure 0.64–0.68 by 0.44–0.64 mm and 0.58–0.63 by 0.50–0.55 mm, respectively. Genital pore median and opens above the ceca. Ovary rounded, measuring 0.38–0.44 by 0.34–0.35 mm. Vitellaria irregularly, shaped mostly extracecal, extending anteriorly a little beyond the testicular level. Eggs 18–23 by 8–11 μ.

HOST: *Centropus sinensis.*

HABITAT: Kidney.

LOCALITY: Hyderabad, A. P.

The type specimens have been deposited in the Helminthological Section of the Zoological Museum of the Osmania University.

Acknowledgment

The authors take this opportunity to express their gratitude to Prof. S. N. Singh, D.Sc. (London), F.N.I., Department of Zoology, Osmania University, for providing all the facilities in the compilation of this work, without whose help this paper would not have been completed.

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The Sporulated Oocysts of *Eimeria tetartooimia* sp. n., and *E. duodenalis* and *E. pacifica* (Protozoa:Eimeriidae) Redescribed, from the Ring-necked Pheasant, *Phasianus colchicus*

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ABSTRACT: Oocysts of *Eimeria tetartooimia* sp. n., *E. duodenalis*, and *E. pacifica* were isolated from 30 ring-necked pheasants (*Phasianus colchicus*) in Montana. The sporulated oocysts of *E. tetartooimia* average 18.6μ (range 17.0 to 20.4) by 16.5μ (range 15.0 to 18.4). The sporulated oocysts of *E. duodenalis* and *E. pacifica* are redescribed. This appears to be the first record of *E. duodenalis* in North America.

During the fall of 1970, fecal samples from 30 ring-necked pheasants, 4 to 6 months of age, were examined for coccidian oocysts. As a result, one new and two previously reported species of *Eimeria* were found to be present, and are herein reported.

Materials and Methods

All pheasants were obtained from the Montana State Game Farm at Warm Springs, Montana, and transported for study to the Biology Department at Carroll College, Helena, Montana. In the laboratory, each bird was placed in a separate cage and samples of fecal material were collected at approximately 48-hr intervals. Fecal samples from each bird were combined into a common pool, sieved, washed with water, suspended in 2.5% $K_2Cr_2O_7$, poured into finger bowls to a depth of 5 to 10 mm, and allowed to sporulate at room temperature (22 C) for 5 to 7 days. After sporulation, the fecal material was refrigerated (5 C) until examined.

Sporulated oocysts, concentrated for study by centrifugation in Sheather's solution, were examined with a Zeiss microscope equipped with apochromatic objectives. All measurements were made with the aid of an ocular micrometer while using bright field illumination, and are expressed in microns. Range measurements are included in parentheses.

Results and Discussion

Eimeria tetartooimia sp. n.

DESCRIPTION: Oocysts (Fig. 1) subspherical. Oocyst wall smooth. Wall of intact oocyst 1.4 thick with brownish-yellow outer layer, reddish-brown inner layer. Micropyle absent. Polar granule present, usually as single spherical body, occasionally as two separate gran-

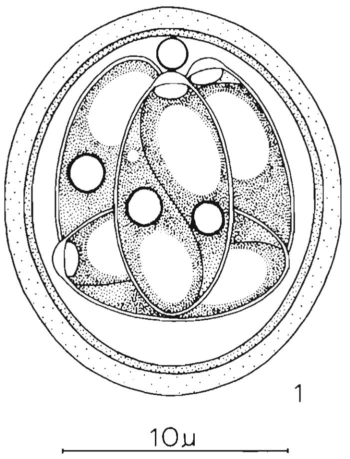
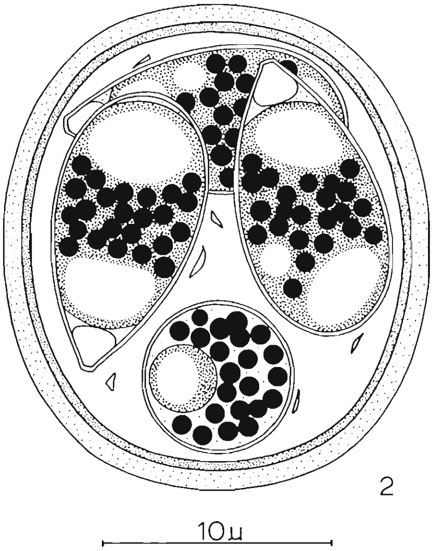


Figure 1. Sporulated oocyst of *Eimeria tetartooimia.*



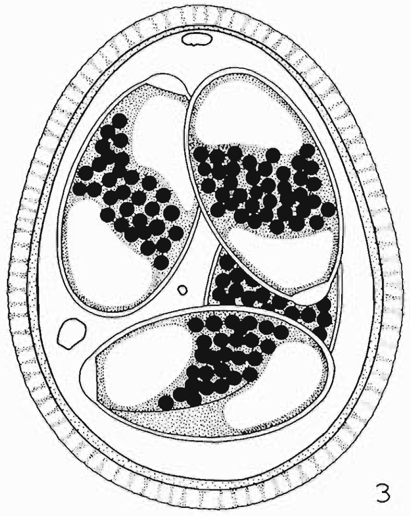


Figure 2. Sporulated oocyst of *Eimeria duodenalis.*

Figure 3. Sporulated oocyst of *Eimeria pacifica.*

ules. Thirty sporulated oocysts averaged 18.6 (17.0 to 20.4) by 16.5 (15.0 to 18.4); length/width ratio, 1.1 (1.0 to 1.2). Sporocysts narrowly ellipsoidal with Stieda body and substiedal body of about equal size. Sporocyst residuum absent. Thirty sporocysts averaged 10.8 (9.5 to 11.6) by 5.6 (4.8 to 6.1). Sporozoites with one large refractile body at broad end and occasionally one small refractile body at narrow end; nucleus usually visible in middle of sporozoite.

TYPE HOST: *Phasianus colchicus* Gmelin.

REMARKS: Seven recognized species of *Eimeria* have been reported from pheasants. These include *E. dispersa* Tyzzer, 1929; *E. phasiani* Tyzzer, 1929; *E. langeroni* Yakimoff and Matschoulsky, 1937; *E. megalostomata* Ormsbee, 1939; *E. pacifica* Ormsbee, 1939; *E. colchici* Norton, 1967; and *E. duodenalis* Norton, 1967. In addition to these, Ormsbee (1939) reported an *Eimeria* species, which he called "Type IV," from ring-necked pheasants in Washington state. The three oocysts which he assigned to this group averaged 18 by 15, had a mean length/width ratio of 1.2, and contained a polar granule. Of these species, the sporulated oocysts of *E. tetartooimia* bear the closest resemblance to those of "Type IV," hence the specific name *tetartooimia* meaning "like the fourth." The sporulated oocysts of *E. tetartooimia* may be distinguished from those of the other species of *Eimeria* reported from the pheasant by the following combination of characters: oocysts subspherical and comparatively small with a mean length/width ratio of 1.1 (1.0 to 1.2), oocyst wall smooth and without micropyle, polar granule present, and oocyst and sporocyst residua absent.

Eimeria duodenalis Norton, 1967

DESCRIPTION: Oocysts (Fig. 2) broadly ellipsoidal to subspherical. Oocyst wall smooth. Wall of intact oocyst 1.4 thick with brownish-yellow outer layer, reddish-brown to dark-brown inner layer. Micropyle absent. Three to 8 small, widely scattered refractile particles present in oocyst fluid. Fifty oocysts averaged 20.3 (18.4 to 21.8) by 18.1 (16.3 to 19.7); length/width ratio, 1.1 (1.0 to 1.2). Sporocysts pyriform with small inconspicuous Stieda body and relatively large substiedal body. Sporocyst residuum consists of densely packed, uniform, spherical granules. Fifty sporocysts averaged 12.6 (11.6 to 13.6) by 6.7 (6.1 to 6.8). Sporozoites largely obscured by sporocyst residuum; each has one large refractile body at one end with second, smaller refractile body sometimes visible at opposite end. Nucleus not discernible.

REMARKS: *Eimeria duodenalis* was originally described from pheasants in England by Norton (1967). As presently redescribed, *E. duodenalis* has a substiedal body in the sporocyst and refractile particles in the oocyst fluid, two morphological features previously unreported for this species. The substiedal body appeared as a relatively large, distinct, homogeneous body lying directly beneath the Stieda body, similar to that described for *E. utahensis* from kangaroo rats by Ernst, Hammond, and Chobotar (1968). The refractile particles in the oocyst fluid were located in most cases near or adjacent to the outer walls of the sporocysts. The significance of these particles is not known. The occurrence of *E. duodenalis* in North America appears to constitute a new geographical record for this parasite.

Eimeria pacifica Ormsbee, 1939

DESCRIPTION: Oocysts (Fig. 3) ovoid with slight thinning at polar end. Oocyst wall mammillated, with some oocysts appearing more heavily mammillated than others. Wall of intact oocyst appears 1.4 thick with yellow to greenish-yellow outer layer, reddish-brown to dark-brown inner layer. Outer layer appears striated in cross section, stippled in tangential section. Micropyle absent. One to four refractile granules usually present in oocyst fluid, with one granule usually present at polar end. Fifty oocysts averaged 21.1 (19.0 to 23.1) by 17.5 (15.6 to 19.0); length/width ratio, 1.2 (1.1 to 1.4). Sporocysts narrowly ellipsoid with Stieda body at one end. Substiedal body absent. Sporocyst residuum consists of densely packed, spherical granules of uniform size. Fifty sporocysts averaged 10.9 (10.2 to 12.2) by 6.1 (4.8 to 6.8). Sporozoites largely obscured by sporocyst residuum; each has one large refractile body at broad end. Nucleus not discernible.

REMARKS: The oocysts of *Eimeria pacifica* were originally described from four ring-necked pheasants in Washington state by Ormsbee (1939). However, Ormsbee's description included little detailed information regarding the morphological characteristics of the sporocysts. Since this information has been lacking, the sporulated oocysts of *E. pacifica* are herein redescribed. Ormsbee (1939) made no mention of the mammillated nature of the oocyst wall in this species. Since the degree of mammillation appeared to vary among the oocysts of the present study, and has been reported to vary in other species, viz., *E. auburnensis* from cattle as described by Christensen and Porter (1939), Ormsbee may have overlooked this character. In size, shape, and occurrence of polar granules the oocysts of *E. pacifica* found in the present study agree with those of the original description.

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Studies on Echinostomatidae (Trematoda) in Malaysia. XVI. The Life History of *Echinostoma ilocanum* (Garrison, 1908)*

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ABSTRACT: The life cycle and morphology of *Echinostoma ilocanum* (Garrison), a parasite of man and animals in Southeast Asia, are described. The first intermediate host in Malaysia is *Gyraulus convexiusculus* (Hutton). The miracidia penetrate through the exposed parts of the snail and develop into sporocysts in the ventricular cavity. Rediae migrate from the heart to the viscera and other organs, where they produce rediae but soon switch over to cercarial production. Metacercarial cysts are found in the pericardial sac and posterior part of the kidney of freshwater snails and in the kidney of tadpoles. The adult worms develop in the small intestines of rats, mice, and hamsters.

Echinostoma ilocanum was first described as a parasite of man in the Philippines (Garrison, 1908) and was later reported in man in Indonesia (Bonne et al., 1953). The parasite was also found in animals: *Rattus norvegicus* Berkenhout, 1769 in the Philippines (Tubangui, 1931); native dogs in Canton, China (Chen, 1934); and the rice field rats, *Rattus argentiventer* (Robinson and Kloss, 1916) in Java (Bonne et al., 1953).

We found *E. ilocanum* in West Malaysia in 1964 during a survey of echinostome infections in freshwater snails, and adult worms were maintained in laboratory bred white rats (Umathevy, 1965). Tubangui and Pasco (1933) described the life cycle of the parasite. The present paper reports the life history and morphology of the parasite, emphasizing those aspects not described or not fully detailed by previous authors.

Materials and Methods

Gyraulus convexiusculus (Hutton), collected early in 1971 from a pond in Rawang near Kuala Lumpur, shed cercariae having about 50 collar spines each. The cercariae encysted in various freshwater snails, and when the cysts were fed to white rats adult worms identical to *Echinostoma ilocanum* were obtained.

Eggs were obtained from the infected rats, and the life cycle was traced through the snail intermediate hosts to definitive hosts. Infected snails were kept in aquaria (at an average room temperature of 27 C). In the early stages of the work uninfected *G. convexiusculus* collected from a pond were used for the experiments; later on only laboratory raised snails were used. For the study of the development of sporocysts and rediae, snails were dissected at various intervals postexposure. Laboratory raised *Lymnaea rubiginosa* (Michelin) were used as second intermediate hosts. Techniques were the same as those described in previous papers on Malaysian echinostomes (Lie, 1963, 1965, 1966a, b). All measurements are in microns unless otherwise stated.

Results

Larval stages

Egg and miracidium (Figs. 1, 2): First eggs usually appeared in feces 14 days after infection, in uncleaved condition, yellowish-brown, with thickening at nonoperculated end of shell, 92–102 long by 61–66 wide. Eggs kept in distilled water in a petri dish at room temperature (average 27 C) hatched in 9 days or later.

Miracidia killed in hot 2% silver nitrate, 60–81 long by 30–36 wide. Retractile apical papilla 10 by 5 when protruded, with two pairs of hairs. Body covered with four rows

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of ciliated epidermal plates: first (anterior) row with six trianguloid plates, two ventral, two dorsal, and two lateral (one on each side), 19 long and 13 wide at base; second row with six squarish plates, three dorsal and three ventral, about 15 long and wide; third row with four squarish plates, two lateral, one dorsal, and one ventral, about 22 long and wide; and fourth row with two trianguloid plates, one ventral, and one dorsal, about 25 long and wide at base. Cilia 14 long. Two lateral processes, 3.5 long, each situated posterior to a lateral anterior epidermal plate, with short bristle immediately anterior to each process. Primitive gut filled with refractile granules with opening at tip of apical papilla. Penetration gland cells not visible. Eyespot shown in Figure 1. Two flame cells: anterior one with excretory pore dorsolateral and posterior one with excretory pore ventrolateral; both pores between third and fourth row of epidermal plates. Several germ cells and scattered pigment granules in body cavity.

Miracidia penetrated into the snail host in the mantle edge and along the edges of the foot, shedding the epidermal cells during penetration; they rarely also penetrated other exposed parts, such as the antennae and head region.

Sporocyst (Fig. 3): Sporocysts developed in the ventricular cavity of the heart (Umathevy, 1965) where they arrived about 2 days postexposure. Newly arrived, live sporocysts measured 80 by 36. They developed into contractile, elongated sacs, attached with the broader end to the heart muscle and with the narrow end free in the heart cavity, containing germ balls and one or two gray rediae. Sporocysts 3 days old measured 108 by 46, 6 days old 297 by 152, and mature sporocysts up to 600 by 147. Old sporocysts were dark gray, small, and empty. They died 30–55 days after exposure.

Redia (Fig. 4): First released from sporocyst 8–9 days postexposure, colorless, about 250 long and 74 wide; pharynx 35 wide, with conspicuous locomotor organs, continuous collar, and a gut reaching to locomotor organs; distance anterior end to locomotor organs 196; distance anterior end to collar 39. They all migrated to hemolymph space surrounding the viscera and albumen gland where they matured 18–22 days after exposure, producing rediae. Average mature redia at this stage 740 long by 139 wide; distance anterior end to locomotor organs 465; distance anterior end to collar 80; pharynx width variable 34–98; gut length variable, reaching halfway to locomotor organs or farther and reaching beyond locomotor organs in some; rediae with a large pharynx usually had a long gut; birth pore dorsal, immediately posterior to collar.

Redial production was soon replaced by production of cercariae, and 25–29 days postexposure all or almost all rediae produced cercariae only. At this stage, the average length of the mature rediae was 1.1 mm (maximum length 2.2 mm). Each redia contained up to 13 cercariae, and the total number of rediae, including immature small ones in each snail, ranged from 30–60.

Cercaria (Figs. 5–9): Cercaria released from snail 28 or more days after exposure. Measurements based on specimens killed in hot 2% silver nitrate: body 224–275 long by 71–86 wide; oral sucker subterminal, 37–41 by 31–34; prepharynx short; pharynx 15 wide; esophagus solid, consisting of six cells; ceca solid, reaching level of excretory bladder; protrusible acetabulum posterior to midbody, 41–50 by 42–48; collar 61–67 wide with minute collar spines 3–6 long (see adult for number and arrangement of spines). Body covered with scalelike spines, many posterior to collar but becoming scarce toward posterior end of body. Patterns of integumentary seta bearing papillae (see Lie, 1966a) shown in Figures 6–8. Cystogenous cells numerous, few

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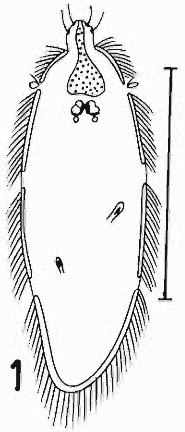
Figures 1–7. *Echinostoma ilocanum*. Camera lucida drawings unless otherwise stated. Projected scales are 50 μ. 1. Miracidium showing apical papilla, gut, eyespots, flame cells, and lateral processes. 2. Miracidium in silver nitrate solution, ventral view, showing epidermal cells and an excretory duct outlet. 3. Sporocyst containing redia. 4. First-generation redia containing redia. 5. Freehand drawing of cercaria showing arrangement of flame cells. 6. Pattern of seta-bearing papillae on ventral body surface of cercaria indicated with black dots. 7. Pattern of seta-bearing papillae on dorsal body surface of cercaria.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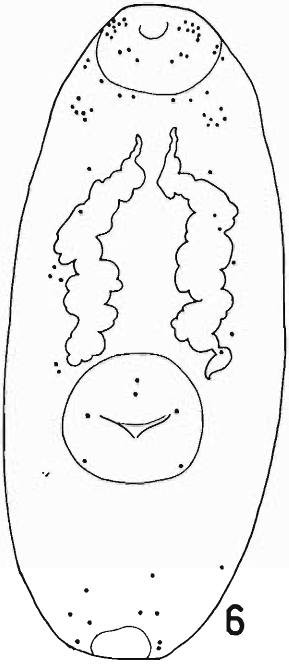
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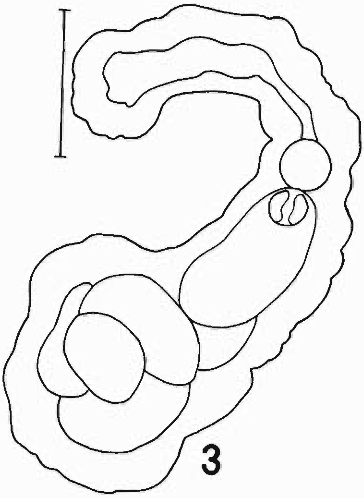
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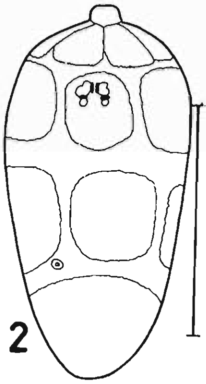
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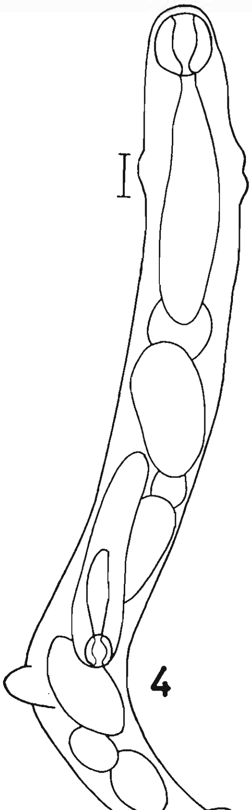
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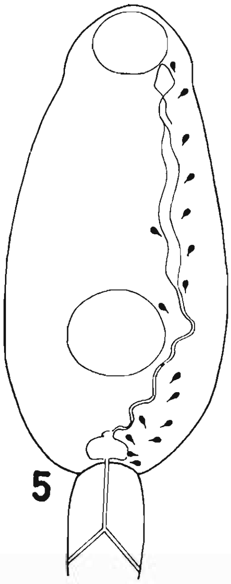
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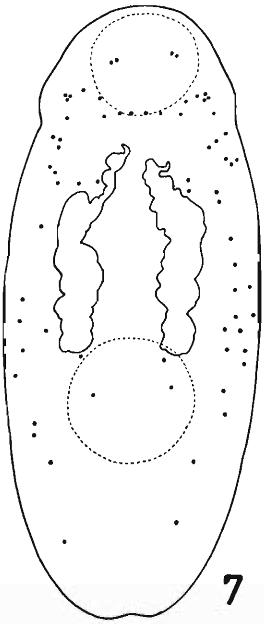
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near oral sucker and pharynx, contents granular. Paraesophageal gland cells (Lie, 1966b) and prepharyngeal body absent. Penetration gland cell ducts not visible. Genital primordia: two masses of cells, one at anterior margin of acetabulum, the other between acetabulum and base of tail, connected by string of cells, passing dorsal to acetabulum. Tail 377–387 by 40–51, without fins (Fig. 9). Excretory system stenostomate with main tubes extending from anterior parts of small bladder to sides of oral sucker, dilated between acetabulum and pharyngeal level, filled with 45–60 yellowish-brown refractile granules of different size, largest 70 in diameter. Flame cells difficult to see, probably 17 pairs arranged as in Figure 5; caudal branch of excretory system entering anterior fourth of tail where it bifurcates and terminates laterally at pair of primary pores.

Metacercarial cysts usually spherical, 93–110 in diameter; obtained experimentally in the pericardial sac and posterior part of kidney of *G. convexiusculus* and *L. rubiginosa* and in the kidney of tadpoles. Cyst wall 2–5 thick. Cysts remain infective after the tadpoles have metamorphosed into frogs.

Adult (Figs. 10–13)

Adult worms (Fig. 10) live in the duodenum and first part of the small intestine of white rats. Measurements are based on 24 specimens from a naturally infected *R. argentiventer* and six specimens from an experimentally infected white rat.

Body elongate, 5.460–9.204 mm by 0.620–1.560 mm, attaining maximum width in uterine region. Body scales posterior to collar, covering anterior two-thirds dorsally, anterior three-fourths laterally and ventrally. Collar 348–429 wide. Number of collar spines variable, 49–55, sometimes more. Size of spines 18–40 by 7.5–17. Spine arrangement (Figs. 12, 13): five corner spines on each side, three oral and two aboral; 16 laterals on each side arranged in a double row when complete; 11 dorsals or more, arranged in an irregular double row. Most dorsals small and inconspicuous, thin ones with longitudinal cracks, lateral aboral spine largest of the ventral group. Acetabulum within anterior fourth of body, 469–643 by 469–737. Oral sucker subterminal, 180–240 in diameter; prepharynx short; pharynx 180–228 by 135–210; esophagus 172–228 long, bifurcating anterior to acetabulum and genital pore; ceca extending almost to posterior end of body. Genital pore immediately preacetabular followed by shallow genital atrium. Testes intercecal, tandem, more or less oblong and constricted at their equator with shallow or moderately deep indentations; anterior testis at midbody, 469–1,179 by 241–804; posterior testis 523–1,206 by 228–697. Cirrus sac ovoid, extending almost to posterior margin of acetabulum and containing a seminal vesicle, pars prostatica, and cirrus. Ovary spherical or subspherical, pretesticular, 180–442 by 165–402. Mehlis' gland complex (Fig. 11) between ovary and anterior testis, similar to that of other echinostomes (Lie, 1965) with small ovicapt 50 by 33; ciliated oviduct about 300 long; Laurer's canal 270 long, only proximal third ciliated, remainder contractile, opening on dorsal surface, ootype about 140 long; no seminal vesicle. Uterine seminal receptacle between ovary and anterior testis, containing numerous spermatozoa. Uterus intercecal, pretesticular, with many eggs, and connecting with genital atrium through metraterm. Vitellaria extending in lateral fields from halfway between acetabulum and ovary to just posttesticular, overlapping posterior third of ceca, confluent dorsally distal to posterior testis and ventrally near posterior end of body. Excretory bladder extending from near hind testis to excretory pore at posterior tip of worm.

Adult worms also live for a long period in

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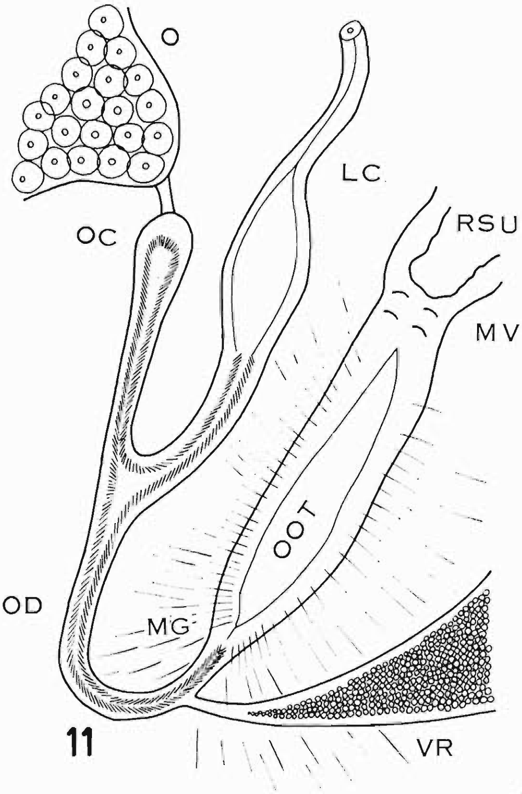
Figures 8–13. *Echinostoma ilocanum*. 8. Tail, lateral view showing pattern of seta-bearing papillae, primitive excretory pore indicated by a ring. 9. Freehand drawing of cercaria, lateral view, showing tail without fins. 10. Adult worm, collar spines not shown. 11. Diagram of Mehlis' gland complex: LC, Laurer's canal; MG, Mehlis' gland; MV, muscular valve; O, ovary; OC, ovicapt; OD, oviduct; OOT, ootype; RSU uterine seminal receptacle; VR vitelline reservoir. 12. Corner (three oral and two aboral) and lateral spines (consisting of 17 spines, eight pairs and one accessory spine between second and third pair). 13. Dorsal spines, consisting of 13 spines; note irregular arrangement.



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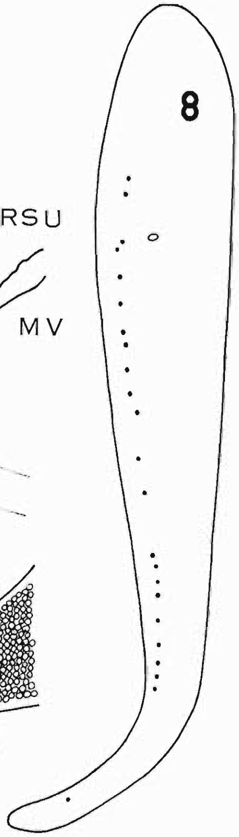

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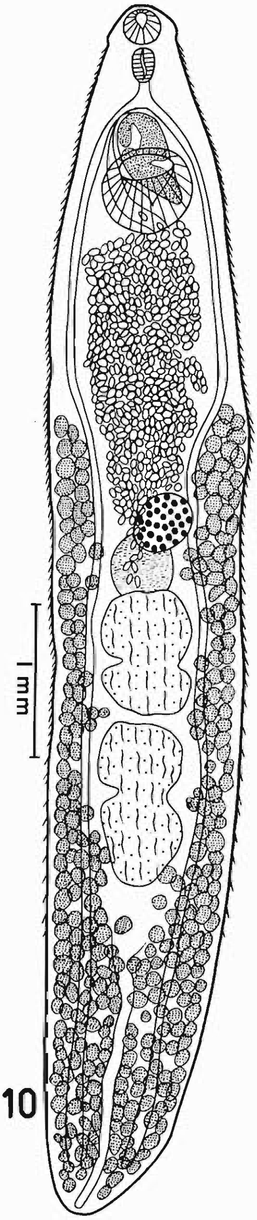

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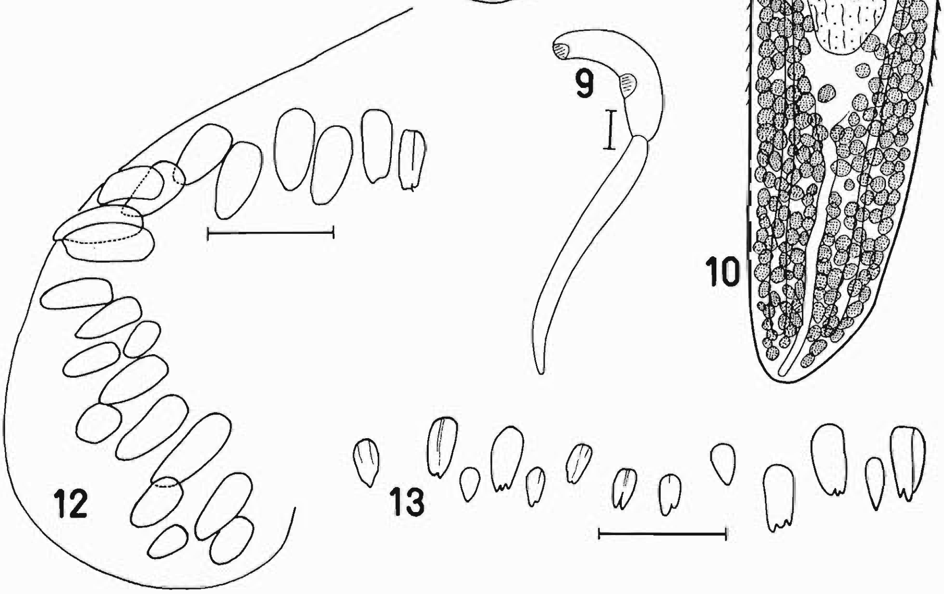

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white mice and hamsters. In white rats, they may live for more than 10 months.

Discussion

The adult worms closely resemble *Echinostoma ilocanum* (Garrison, 1908) as described by Tubangui and Pasco (1933) and Bonne et al. (1953). The larval stages show some differences from those described by Tubangui and Pasco, but these are not important and may not represent actual differences. The miracidium they described has three epidermal plates in the fourth row. Apparently, they studied the epidermal plates in the living miracidia. It is difficult to determine the number of epidermal plates without silver nitrate staining. They were unable to find the sporocyst stage and hypothesized that the miracidium develops into a mother redia after penetration into a snail. The cercaria they described and drew has 15 pairs of flame cells and 14 esophageal cells. However, flame cells in echinostome cercariae are difficult to see and almost impossible to count exactly. We counted 17 pairs but there may be more.

The miracidia penetrate through the exposed parts of the snail while shedding the epidermal plates as in other species of echinostomes (Lie, 1969b). After penetration the sporocysts migrate to the heart cavity. They do not grow during migration; growth takes place after they arrive in the heart cavity, as is also the case in *E. lindoense* Sandground and Bonne, 1940 (Lie, 1969b). Sporocysts of *Paryphostomum segregatum* Dietz, 1909 develop near the places of entry into the snail. Also in this species sporocysts do not develop in the first 2 days after penetration (Lie, 1969b). A period of adjustment to the new environment is probably required before development can start.

Sporocysts develop in the ventricular cavity in eight other echinostome species (Lie, 1969b). All rediae produced by the sporocysts migrate from the heart to the viscera and other organs, but in *E. audyi* Lie and Umathevy, 1965, *E. barbosai* Lie and Basch, 1966, and *E. paraensei* Lie and Basch, 1967 (see Lie and Basch, 1966, 1967) several rediae usually remain in the heart cavity where they become mature and produce rediae only. When all rediae outside the heart cavity have switched to cercarial production, those in the ventricular cavity keep producing rediae only. The heart cavity is apparently not a suitable milieu for rediae of *E. audyi*, *E. barbosai*, or *E. paraensei* to produce cercariae.

Various factors determine the type of larvae produced in rediae or sporocysts. In various species of echinostomes a switch from cercarial to redial production is observed in old, opaque, and shriveled rediae or in sick rediae, due to microsporidan infection or other causes (Lie, 1969b). Rediae of *Fasciola gigantica* Cobbold, 1855 switch to redial production when the temperature is 16 C or less (Dinnik and Dinnik, 1964). Daughter sporocysts of *Schistosoma mansoni* start producing sporocysts when they degenerate due to various causes such as trematode antagonism (Lie, 1969a). In *E. ilocanum* rediae, a rapid switch from redial to cercarial production is observed, resulting in a small redial population within the snail. This may be due to the small size of the *Gyraulus* snail host. Redial numbers in *E. revolutum* (Froelich, 1802) and in *P. segregatum* are related to the size of the infected snails (Zischke, 1967; Lim and Lie, 1969).

One characteristic of *E. ilocanum* is the variable number of collar spines (Bonne et al., 1953), usually 49–55, sometimes more. The group of corner spines is the most constant in number and an accessory spine is rare. The lateral group, when complete, consists of 16 spines arranged alternately in a double row. However, it is usually not complete, with the first aboral lateral spine missing either on one or both sides, and sometimes the second aboral spines are also missing. Occasionally an accessory spine is present in this group (Fig. 12). The number of lateral spines on each side may therefore be from 14 to 17. The dorsal group varies most in number, from 11 to 16 (Fig. 13). The same variations in number of the lateral and dorsal collar spines are also seen in cercariae. The cercarial collar spines can be seen under the oil immersion in specimens killed in hot water. The missing first and second lateral aboral spines often observed in adult worms are probably not due to loss of spines. They may never have been formed since they are often also missing in cercariae. Another characteristic of *E. ilocanum* is the irregular arrangement of the dorsal collar spines (Fig. 13). The dorsal collar spines are difficult to see since they are often small, thin, and inconspicuous. Moreover, they fall off easily.

Many species of echinostomes have a fairly constant number of collar spines. Among these are *E. revolutum*, *E. audyi*, *E. barbosai*, *E. lindoense*, *E. paraensei*, and *E. rodriguesi* Hsu, Lie, and Basch, 1968, all having 37 collar spines. *P. segregatum* and *E. hystricosum* Lie and Umathevy, 1966 also have a fairly constant number of spines, respectively 27 and 47. *E. malayanum* Leiper, 1911 has 43 spines, but about 20% of the worms have 45. *E. murinum* (Tubangui, 1931) has 45 and occasionally 43 spines. *Echinoparyphium dunni* Lie and Umathevy, 1965 has 43 and occasionally 45 spines. *Hypoderaeum dingeri* Lie, 1964 has a variable number, 49–54, with about 40% of the worms having 50. The variations are more frequent in *E. ilocanum* than in *H. dingeri*.

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Effect of Intramuscular Injections of Iron-Dextran on Ovine Haemonchosis

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ABSTRACT: The effect of intramuscular iron-dextran injections on the development of *Haemonchus contortus* and haemonchosis in 4- to 5-month-old lambs was determined in two experiments, using seven closely matched pairs of animals—three pairs in the first experiment and four pairs in the second experiment. In the second experiment, four injections, 28 and 9 days before inoculation, on the day of inoculation, and 14 days after inoculation, resulted in significantly different ($P < 0.05$) average hematocrit levels at necropsy among principals (21%) and controls (15%). However, in the first experiment injections of iron-dextran 14 days before inoculation and on the day of inoculation had no effect on hematocrit levels. The lambs were necropsied 27 to 34 days after infection. Although the average number of worms recovered from principals was less than from the controls, this difference was not statistically significant. The averages were 2,875 and 3,737, respectively, and all but one of the lambs given injections had fewer worms than their pair counterparts. Adult *H. contortus* recovered from principals were significantly ($P < 0.05$) shorter than those from controls. The male and female worms from the principals averaged 1.27 and 1.89 cm, respectively, while from controls they averaged 1.44 and 2.09 cm, respectively. No consistent difference was observed in average daily gain between the two groups.

The effect of minerals on infections with *Haemonchus contortus* in sheep has been recorded by many workers. Clunies Ross and Gordon (1933) found that acquired resistance was lost in sheep fed a protein- and mineral-deficient diet. However, mineral supplementation of the diet increased resistance to the effects of infection (Weir et al., 1948; Richard et al., 1954; Shumard et al., 1956). Similarly, lambs on a pelleted feed developed less severe clinical signs of disease than lambs fed alfalfa hay (Kates et al., 1962). Cane molasses, the binder used in this pelleted feed, contains 16.1 mg of iron per 100 gm of feed. Scott et al. (1971) showed that oral iron supplementation offset the clinical effects of infection with *H. contortus*. Iron-dextran has been used by many workers for the treatment of anemia of sheep (Holz et al., 1961; Carlson et al., 1961; Kiesel and Clark, 1964; Bezeau and Clark, 1965; Ricketts et al., 1965). The present experiments, pairing closely matched lambs, were conducted to determine if the intramuscular injection of iron-dextran would influence the levels of *H. contortus* infections established, the size of the worms, and their pathogenic effects.

Materials and Methods

Two experiments used 4- to 5-month-old purebred Dorset lambs. They were closely paired by pairs on the basis of sire, birth date, sex, weight, and whether they were born and raised as singles or twins. Three pairs were twins of the same sex. All lambs had been raised helminth-free to the time of infection.

In both experiments, one member of each pair was given intramuscular injections of iron-dextran at a dose rate of 400 mg of elemental iron.[1] The injections were placed intramuscularly in the rear leg, alternating between right and left.

Weights and hematocrit values (PCV) were determined every two weeks. At the end of the experiments, the lambs were necropsied and worms recovered and enumerated by standard methods. Measurements of 25 adult male and 25 adult female worms were recorded from worms chosen at random from the total collection from each lamb. The data were analyzed by least squares analysis of variance (Harvey, 1960).

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[1] Armidexan 100-iron dextran injection (Bradley Products Co., Bradley, Illinois) containing 100 mg of elemental iron per cc with 0.5% phenol as a preservative. (Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U. S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.)

Table 1. Effect of iron-dextran injections on weight gains hematocrit values, and worm loads of lambs.

Lamb pair	Avg. daily gain for period (kg)	Packed red blood cell volume at necropsy (%)*	Avg number *Haemonchus contortus*
Exp. 1—Duration-27 days—2 IM injections			
1 (NI)†	0.12	30	–
2 (I)	0.05	26	–
3 (NI)	0.07	32	2,613
4 (I)	0.16	27	1,933
5 (NI)	0.10	31	2,926
6 (I)	0.12	33	2,038
Avg (NI)	0.10	31	2,770
Avg (I)	0.11	29	1,986
Exp. 2—Duration-34 days—4 IM injections			
7 (NI)	0.06	16	4,240
8 (I)	0.07	18	2,740
9 (NI)	0.06	15	4,280
10 (I)	0.01	23	3,060
11 (NI)	0.08	14	4,160
12 (I)	0.05	22	5,200
13 (NI)	0.15	14	4,200
14 (I)	0.07	19	2,280
Avg (NI)	0.09	15	4,220
Avg (I)	0.05	21	3,320
Combined avg			
(NI)	0.09	22	3,737
(I)	0.08	24	2,875

* Between iron and no-iron in Experiment 2, significantly different ($P < 0.05$).
† NI = no iron.
I = iron.

Table 2. Effect of iron-dextran injections on the length of adult *Haemonchus contortus* from lambs.

	Length (cm)*			
	Male		Female	
Lamb pair	Avg	Range	Avg	Range
Exp. 1—Duration-27 days—2 IM injections				
3 (NI)†	1.42	1.2–1.7	1.94	1.7–2.2
4 (I)	1.27	1.0–1.5	1.74	1.6–2.0
5 (NI)	1.51	1.2–1.8	1.98	1.8–2.2
6 (I)	1.02	0.8–1.2	1.58	1.4–1.8
Avg (NI)	1.47	1.2–1.8	1.96	1.7–2.2
Avg (I)	1.14	0.8–1.5	1.66	1.4–2.0
Exp. 2—Duration-34 days—4 IM injections				
7 (NI)	1.56	1.0–2.1	2.39	2.0–2.8
8 (I)	1.47	1.3–1.6	2.12	1.4–2.5
9 (NI)	1.47	1.3–1.6	2.30	2.0–2.5
10 (I)	1.38	1.2–1.5	2.07	1.5–2.5
11 (NI)	1.42	1.2–1.6	2.30	2.0–2.7
12 (I)	1.51	1.4–1.6	2.38	2.0–2.6
13 (NI)	1.22	1.0–1.4	1.92	1.7–2.1
14 (I)	1.19	0.9–1.5	1.92	1.7–2.2
Avg (NI)	1.42	1.0–2.1	2.23	1.7–2.8
Avg (I)	1.39	0.9–1.6	2.12	1.4–2.6
Combined avg				
(NI)	1.44	1.0–2.1	2.09	1.7–2.8
(I)	1.27	0.8–1.6	1.89	1.4–2.6

* All worm lengths, NI vs. I, significantly different ($P < 0.05$).
† NI = no iron.
I = iron.

The *H. contortus* larvae used in the experiments originally came from the BPL strain maintained at this laboratory. The infective larvae were used the same day as isolated for the infection of the experimental animals in these tests.

Three pairs of 5-month-old lambs were used in Experiment 1. Two injections of iron were given: (1) two weeks before infection and (2) on the day of exposure to infective larvae. Each lamb received 15,000 infective larvae of *H. contortus.* Two pairs of lambs were necropsied 27 days after infection; one pair was not necropsied.

Four pairs of 4-month-old lambs were used in Experiment 2. Four injections of iron were given: (1) 28 days before infection; (2) 9 days before infection; (3) on the day of exposure to infective larvae; and (4) 14 days after infection. Each lamb was given 12,000 infective larvae of *H. contortus.* Experiment 2 ran for a longer period than Experiment 1; therefore, worm egg counts were determined twice in the last week before necropsy. All lambs were necropsied 34 days after larval inoculation.

Results and Discussion

The average number of *H. contortus* recovered at necropsy from all principals was 2,875 as compared with 3,737 from controls (Table 1). In Experiment 1, the average numbers of worms recovered were 2,770 and 1,986, respectively. In Experiment 2 they were 4,220 and 3,320, respectively. All but one lamb given injections had fewer worms than their counterparts, but the difference is not statistically significant. In general, principals had slightly less abomasitis than the controls.

The average and range of length of adult male and female worms recovered from each lamb are shown (Table 2). Male worms from principals averaged 1.27 cm long, whereas those from controls averaged 1.44 cm. Female worms averaged 1.89 and 2.09 cm, respectively. The differences in length of *H. contortus* from principals and controls, both male and female, were significant ($P < 0.05$). The only lamb pair (11, 12) in which the principal had longer worms (Table 2) was the exception to the general pattern in which there were more worms in the controls (Table 1).

Worm egg counts made in Experiment 2 showed, at 27 days after infection, an average of 21,550 eggs per gram of feces for controls and 12,350 for principals. One week later, at necropsy, the averages were 21,000 and 17,300, respectively. As is quite often true, the worm egg counts did not reflect precisely the true extent of differences in worm loads of the two groups of animals.

There were no consistent differences in average daily gain between principals and controls over the short experimental periods of the two experiments (Table 1). Also, there was no relationship between average daily gain and the number of *H. contortus* recovered at necropsy.

There was no difference in hematocrit levels between principals and controls in Experiment 1, whereas in Experiment 2 there was a significant difference ($P < 0.05$). In Experiment 1, only two injections of iron-dextran were given, but in Experiment 2 four injections were given. The last injection in the latter test, 14 days after larval inoculation, may have been the only one which was of direct benefit in helping the lambs to maintain the higher hematocrit levels. It is during the 20 days from this last injection to necropsy that the greatest blood loss probably occurred. Moreover, Ricketts et al. (1965) found that lambs treated at birth with iron-dextran and untreated lambs differed most in hematocrit levels when they were 21 days old.

The difference at necropsy in hematocrit levels between the first and second experiments was highly significant ($P < 0.01$), with an average of 29.8 for the lambs in the first experiment and 17.6 for those in the second experiment (Table 1). Evidently the nonheme iron reserves were sufficient for both principals and controls in Experiment 1, while in Experiment 2 the iron reserve pool of the lambs not given iron-dextran was considerably depleted. Necropsy of the lambs occurred 34 days after infection in the second experiment as compared with 27 days in the first experiment. Thus, in Experiment 2, the longer period of time resulted in more blood loss and consequent anemia. Also, in Experiment 2, one-third more worms were recovered than in Experiment 1.

The title of the work of Scott et al. (1971) indicates the subject to be the effect of oral iron supplement on active and passive immunity against *H. contortus* in sheep. However, their provocative results, except for that from 10 lambs, involved different preinoculation injections of lymph node cells, antigens, and globulins in addition to the oral iron supplementation. Furthermore, their conclusions were based only on worm egg counts. The present data certainly suggest that more work should be conducted in which the use of oral or injectable iron by itself is tested as a means of offsetting the effects of *H. contortus* infections in sheep.

Acknowledgments

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Freshwater Larval Trematodes. XXXI. Two New Species of Cercariae

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ABSTRACT: *Cercaria plieguicauda*, of gymnocephalic group, and *C. guanipensis*, of stylet cercariae, from the snail, *Pomacea glauca*, in Venezuela, are described. A detailed comparison is made with related species.

The freshwater snail *Pomacea glauca* (L.) has yielded the following cercariae from different regions in Venezuela: (Echinostomes) *Cercaria paraudoi* Nasir, Díaz, and Hamana, 1969, *Echinochasmus zubedakhaname* Nasir and Díaz, 1968, *Stephanoprora paradenticulata* Nasir and Rodriguez, 1969; (Furcocercariae) *Cercaria heteroglandula* Nasir and Díaz, 1968 (apharyngeal brevifurcate ocellate distomate), *C. cumanacoensis* Nasir, 1964 (pharyngeal longifurcate distomate), *C. monagasica* Nasir, Hamana, and Díaz, 1969 (Vivax), *C. neoorientalis* Nasir and Guevara, 1968 (Vivax); (Gymnocephalous) *C. barceloica* Nasir, 1971, *C. macarapanensis* Nasir and Acuña, 1966, *C. pomacea* Nasir and Díaz, 1968, *C. sanlorenzensis* Nasir and Acuña, 1964, *Guaicaipuria pseudoconcilia* (Nasir, Díaz, and Guevara, 1969) Nasir, Díaz, and Marcano, 1971 (syn. *Cercaria pseudoconcilia* Nasir et al., 1969); (Macrocercous) *C. latigazica* Nasir, Díaz, Hamana, and Guevara, 1969, *C. yacalicola* Nasir, Díaz, Hamana, and Guevara, 1969; and (Xiphidiocercariae) *C. arismendii* Nasir, Hamana, and Díaz, 1969 (Ubiquita type), *C. etgesi* Nasir, 1964 (Virgulate), *C. peculiaristylata* Nasir and Acuña, 1966, *C. pifanoi* Nasir and Díaz, 1967. The present paper adds two new species.

Cercariae were studied alive with or without the aid of intravital stains. Only freshly emerged cercariae were employed. The method for measurements has already been given (Nasir, 1971). Measurements are in mm and outlines of diagrams have been made with the aid of camera lucida.

Cercaria plieguicauda sp. n. (Fig. 1)

HOST: *Pomacea glauca* (L.)

LOCALITY: Caño Ajies, Sucre state, Venezuela.

DESCRIPTION: Gymnocephalic. Body aspi-

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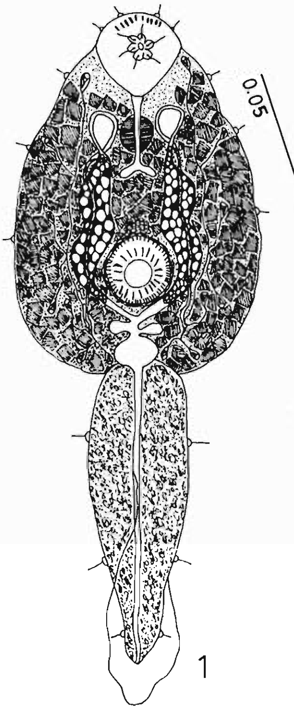

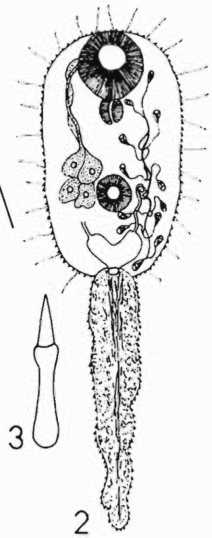

Figure 1. ***Cerceria plieguicauda*** **sp. n., ventral view, musculature of suckers not drawn.**

Figure 2. ***Cercaria guanipensis*** **sp. n., ventral view, flame cells and penetration glands shown on one side only.**

Figure 3. Stylet, note the absence of a basal bulb.

nose, with three five rows of setate papillae. Tail aspinose, with three rows of setate papillae. Caudal finfold only on about posterior half of tail. Oral orifice bordered with a ring of papillae, each bearing hairlike prolongation arising from a basal granule. Ten small, rough spines, in semicircle, in preoral region. Prepharynx, pharynx, esophagus, and intestinal ceca of about same length. Ventral sucker smaller than oral, in posterior half of body, with two sets of spines: small, delicate spines bordering acetabular periphery internally, and long, stout spines between acetabular aperture and peripheral spines. Penetration ducts not seen. Cystogenous glands with rhabditiform contents. Only anterior excretory loop present. Secondary excretory tubes ciliated internally, dividing postacetabularly. Flame cell formula $2[(2+2)+(1+2)]=14$. Measurements of 12 specimens: body 0.120–0.147 by 0.066–0.090; tail 0.105–0.138 by 0.030–0.039; oral sucker 0.027–0.039 in diam; ventral sucker 0.024–0.033 in diam. Encysting in gills of the fish, *Rivulus harti* (Boulenger). Rediae in hepatopancreas, with pharynx, saccate gut, undivided collar, and posterior locomotor appendages.

Cercaria guanipensis sp. n.
(Figs. 2–3)

Host: *Pomacea glauca.*

Locality: Laguna de Guanipe, Apure state, Venezuela.

Description: Xiphidiocercaria. Body with 13 rows of hairs, spinose. Tail spined, without finfold. Stylet as shown in Figure 3, without basal bulb. No virgula organ. Prepharynx, esophagus, and intestinal ceca absent. Pharynx present. Pentration glands in four pairs, acetabular, without differentiation into finely and coarsely granular contents, leading into two ducts on each side of body. Excretory vesicle V-shaped. Main excretory tubes dividing at equatorial level of ventral sucker. Flame cell formula $2[(2+2+2)+(2+2+2)]=24$. Measurements of 12 cercariae: body 0.072–0.084 by 0.027–0.050; tail 0.088–0.110 by 0.015–0.018; oral sucker 0.020–0.027 in diam; ventral sucker 0.010–0.014 in diam; pharynx 0.007–0.010 in diam; stylet 0.022 by 0.005. Development in saclike sporocysts.

Discussion

Gymnocephalic cercariae like *C. plieguicauda* sp. n., i.e., with a caudal finfold, rhabditiform contents of cystogenous glands, and about which adequate structural details are known, are: *Cercaria asaguensis* Nasir, Hamana, and Díaz, 1969, *C. helvetica* XVII Dubois, 1929 [= *Sphaeridiotrema globulus* (Rudolphi, 1819), after Szidat, 1937 (syn. *Cercaria obscura* Wesenberg-Lund, 1934, after Cable, 1938], *C. penthesilia* Faust, 1921, and *C. sucrensis* Nasir and Acuña, 1965. *Cercaria plieguicauda* differs from these in the pattern of its alimentary canal posterior to the pharynx and, in addition, from *C. asaguensis* (10 flame cells in all) and *C. sucrensis* (24) in the possession of 14 flame cells.

The identity of *Cercaria reflexae* Cort, 1914, which is also furnished with a caudal finfold, has already been discussed (Nasir, Hamana, and Díaz, 1969), and *C. plieguicauda* is distinguishable from it in the nature of the finfold, contents of cystogenous glands, and presence of an undivided collar in rediae.

Cercaria guanipensis sp. n. has been compared with only those freshwater xiphidiocercariae having four pairs of penetration glands, and lacking eyespots, virgula organ, and caudal finfold; these are: *Cercaria agglomerata* Porter, 1938, *C. aralica* Aristanov, 1967, *C. astrachanica* XI Ginetsinskaya and Dobrovolsky, 1968, *C. baakensis* Porter, 1938, *C. burrupiae* Faust, 1926, *C. burripioides* Porter, 1938, *C. cawstoni* Faust, 1919, after Porter, 1938, *C. dureni* Fain, 1953, *C. forskalia* Porter, 1938, *C. gunnisoni* Hurst, 1923, *C. helvetica* XI Dubois, 1929, *C. indicae* LI Sewell, 1922, *C. kansiensis* O'Roke, 1917, *C. mamagaliensis* Porter, 1938, *C. pachycystata* Miller, 1936 (for *C. tetradena* Miller, 1935), *C. pandora* Faust, 1921, *C. parvus* Khan, 1961, *C. pneumata* Fain, 1953, *C. pseudarmata* Brown, 1926, *C. pulicis* (von Linstow, 1892) Brown, 1926, *C. rumiensis* Pike, 1967, *C. stylata* Agrawal, 1956, *C. stylobuccalis* Faust, 1922, *C. tabitha* Faust, 1921, *C. tenuispina* Lühe, 1909 nec of Komiya, 1939 [= *Opisthoglyphe ranae* (Frölich, 1791) Looss, 1907], *C. vesiculosa* Diesing, 1855, after Wesenberg-Lund, 1934, *C. wentloogensis* Pike, 1967, *Cercaria* X3 Harper, 1929, *Xiphidiocercaria* sp. 4 Odening, 1962, *Xiphidiocercaria* gen. sp. Ginetsinskaya and Dobrovolsky, 1968, *Maritrema obstipum* (Van Cleave and Mueller, 1932) Mueller, 1934, after Etges, 1953, *Prosthogonimus* sp. Komiya, 1951, and *P. macrorchis* Macy, 1934. Of these *C. indicae* LI is readily separated from *C. guanipensis* in the arrangement of its penetration glands into two groups on each side of the body: three anterior to the ventral sucker, one posterior to it. The contents of the penetration glands are differentiated into fine and coarse granules in *C. astrachanica* XI, *C. baakensis*, *C. forskalia*, *C. rumiensis*, *C. tabitha*, *C. vesiculosa*, and *C. wentloogensis* and, thus, are distinctly set apart from *C. guanipensis* in which the contents are uniform. Of the remaining species, only *C. aralica*, *C. parvus*, and *C. stylata* have a total of 24 flame cells and, therefore, are closely related to *C. guanipensis*. However, the latter is easily distinguished in having a different stylet shape in addition to the differing specific characters discussed below.

Cercaria aralica has a prepharynx and gut posterior to the pharynx whereas these are absent in *C. guanipensis*.

Cercaria parvus is distinguishable from *C. guanipensis* in the possession of a well-developed gut beyond the pharynx.

In *C. stylata* the suckers are of subequal size while in *C. guanipensis* the oral sucker is twice as large as ventral.

Cercaria helvetica XI, originally described from *Bithynia tentaculata* (L.) in Switzerland, lacks a gut beyond the pharynx, bears nine flame cells on each side of the body, and the contents of its penetration glands are not differentiated. Probert (1965) described a "*C. helvetica* XI Dubois" from *B. tentaculata* in the Llangorse Lake, South Wales, in which "the two anteriormost cells contain coarsely granular contents. The posterior two pairs of cells have finely granular inclusions. The anteriormost cells have a lobulated outline," and there is a blind-ending esophagus. "*Cercaria helvetica* XI Dubois" of Ginetsinskaya and Dobrovolsky (1968) from *B. tentaculata* in the delta of the Volga River, has a flame cell formula $2[(2+2+2)+(2+2+2)]=24$, a long esophagus, rudimentary intestinal ceca, and the contents of its penetration glands are not differentiated. It is doubtful whether Probert as well as Ginetsinskaya and Dobrovolsky were dealing with the same species. In any case, *C. guanipensis* in contradistinction to *C. helvetica* XI of Dubois has a different number of flame cells; it differs from that of Probert in lacking a basal bulb in its stylet, undifferentiated contents of the penetration glands, and a different numbers of flame cells; from that of Ginetsinskaya and Dobrovolsky in the absence of a basal bulb in the stylet and digestive tract posterior to the pharynx.

Brown (1933) described an hypothetical cercaria of *Lecithodendrium chilostomum* (Mehling, 1831) Braun, 1900 "reconstructed from early postcercarial stage"; it was beset with four pairs of penetration glands of undifferentiated contents, well-developed intestinal ceca extending outward in the preacetabular region, the flame cells in the order of $2[(2+2+2)+(2+2+2)]=24$, and the stylet including a basal bulb. In *C. guanipensis* the basal bulb is absent and there is no gut posterior to the pharynx.

The other freshwater xiphidiocercaraiae lacking eyespots, virgula organ, and finfold like *Cercaria guanipensis* but equipped with a variable number of penetration glands are: *Cercaria lymnaea ovatae* von Linstow, 1884 [Honer (1963) showed four pairs of penetration glands in Fig. 10, but five pairs in accordance with the observations of Wesenberg-Lund (1934); Aristanov (1967) mentioned five pairs], *Alloglossidium corti* (Lamont, 1921) Van Cleave and Mueller, 1934 [life cycle worked out by McCoy (1928), McMullen (1935), and Crawford (1937), syn *Plagiorchis corti* Lamont, 1921 = *P. ameiurensis* McCoy, 1928 after McMullen, 1930, 1935] [penetration glands in four pairs (Crawford, 1937); four to five pairs (McCoy, 1928)], *Lissorchis fairporti* Magath, 1918 [four to six pairs], *Macroderoides spiniferus* Pearse, 1924, (Leigh, 1958) [four glands on one side, five on other], *Plagiorchis muris* Tanabe, 1922 [four pairs; seven or eight pairs (McMullen, 1937); seven pairs (Yamaguti, 1943)], *Telorchis ercolanii* (Monticelli, 1893) Braun, 1901 [four to five pairs (Ginetsinskaya and Dobrovolsky, 1968)], and *T. robustus* Goldberger, 1911 [four on one side, five on other (Krull, 1936)]. *Cercaria guanipensis* differs from all these species in the shape of its stylet, and certain other characters as discussed below.

In *Alloglossidium corti* the anterior two pairs of the penetration glands are coarsely granular while the posterior two finely granular, and this character alone suffices to separate it from *C. guanipensis.* The supposed larva of *A. corti* described by Brooks (1948) from helisome snails in Carrol Lake, Wisconsin, USA, bearing five pairs of glands, is of uncertain status.

Lissorchis fairporti is a distinct entity from *C. guanipensis* because of a gut posterior to the pharynx. *Macroderoides spiniferus* has a flame cell formula $2\ [(3+3+3)+(3+3+3)]=36$ in contrast to that of *C. guanipensis. Plagiorchis muris* also possesses 36 flame cells. In *Telorchis ercolanii* and *T. robustus* intestinal ceca extend to posterior end of body, and there are 36 flame cells in the former.

Linstow (1890) first worked out the life cycle of *Haplometra cylindracea* (Zeder, 1800) Looss, 1899, but the description of the cercaria is too poor to make a fair comparison. Combes (1968) in France and Grabda-Kazubska (1970) in Poland gave experimental demonstrations of the life cycle of *H. cylindracea.* The snail host for the French cercaria is *Galba truncatula* (Müller), and the penetration glands are in 11 pairs; the Polish cercaria involves *G. palustris* (Müller), and has 12 pairs. In both of these the flame cell formula is identical, i.e., $2\ [(3+3+3)+(3+3+3)]=36$, intestinal ceca extend to posterior end of body and, whereas the stylets are of the same shape, the Polish cercaria is provided with a basal bulb in its stylet. Moreover, the metacercaria referred to by Combes is twice as large.

Wesenberg-Lund (1934) came across a larva, from *Lymnaea stagnalis* in Denmark, furnished with four or five pairs of penetration glands, which was considered without experimental evidence as *Cercaria haplometrae cylindraceae.* It encysted in *Corethra* larvae in constrast to the aquatic beetle, *Ilybius fuliginosus,* as stated by Linstow (1890). Honer (1963) also described a cercaria of *Haplometra cylindracea,* from *Lymnaea ovata* in Holland, characterized by four pairs of penetration glands, but experimental proof is lacking.

It is clear from the foregoing observations that there are four independent species which are taken for the larva of the same adult, *Haplometra cylindracea.* In comparison with "cercaria" of *H. cylindracea,* the specific entity of *Cercaria guanipensis* is established when the constant number of penetration glands (four pairs) with undifferentiated contents, absence of the gut posterior to the pharynx, characteristic shape of the stylet, and the flame cells (24), are taken into account.

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Announcement: Initiation of Page Charges.

During 1971 and 1972 the number of papers submitted and subsequently accepted for publication in the Proceeedings increased considerably. Manuscripts in excess of those normally included in a given number were held over for the following issue sometimes resulting in delays of publication up to one year. Since it is the objective of the Helminthological Society of Washington and the Proceedings to provide prompt publication the Executive Committee of the Society authorized an increase in the size of the Proceedings and the assessment of page charges to help offset the increased costs. Members will be charged ten dollars ($10.00) per page for all pages of papers submitted after September 22, 1972. As before, nonmembers publishing in the Proceedings will be charged the full page cost of $29.80 per page.

HARLEY G. SHEFFIELD, Editor

The Aging of *Neoaplectana glaseri*

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ABSTRACT: Up to 60% of *Neoaplectana glaseri* survivalarvae (the ensheathed, third stage) that had been stored under 1–2 cm of water at 5C in the dark for more than 3 years became motile at room temperature. The majority of living nematodes in these populations exsheathed and molted twice to the adult stage when inoculated into a culture medium. However, the nematodes' ability to reproduce declined measurably with age.

One hundred per cent of cultures started with survivalarvae that had been stored for less than 1 year were able to reproduce. Newborn larvae first appeared in the medium after 6 days. Population increments were 40–100% after 2 weeks.

Of cultures started with survivalarvae more than 3 years old, only 41% were able to reproduce. More than half of these showed a delay in the first appearance of newborn larvae. Population increments were small.

Cultures started with survivalarvae stored for 1 to 3 years gave intermediate results.

Stages in the life cycle of *N. glaseri* other than the survivalarval do not persist for more than a few weeks under all reported conditions.

Information about the persistence of *Neoaplectana glaseri* is important for two purposes at least. As a parasite of insect pests (Glaser and Fox, 1930; Stoll, 1953; Jackson and Moore, 1969; Turco et al., 1970), this nematode species has been and is being used worldwide in biological control programs. As the first parasitic nematode to be cultured in species isolation, i.e., axenically (Glaser, 1940), *N. glaseri* has also been the subject of physiological and immunological studies in vitro. How long and how effectively the nematode survives between insect hosts or between cultures is, therefore, a practical question.

There has been no answer because usually the latest supply of nematodes is consumed rapidly for the next piece of work. Recently, however, availability of a certain number of survivalarval populations that had been stored under constant conditions for known periods prompted this test of the effects of larval age on subsequent maturation and reproduction. Survivalarvae (the ensheathed, third stage) are, of course, the only developmental segment of the *N. glaseri* life cycle that persists significantly. The other stages die and disintegrate after a few weeks under all reported conditions.

Materials and Methods

At various times during a 4-year period, axenic populations of 2 to 4 thousand *N. glaseri* survivalarvae were harvested from 3- to 6-week-old kidney cultures with a glass pipette containing 0.5 ml of doubly distilled water, then washed 3 times by sedimentation in tubes of water, and stored under 1–2 cm of water in dated Erlenmyer flasks with screw caps not fully tightened. These "stocks" for starting new cultures or infections were kept in a dark refrigerator at 5 C. Details of culturing, of harvesting, and of testing for contaminants have been described previously (Stoll, 1959; Jackson, 1962, 1969).

To test maturation and reproduction subsequent to storage, the survivalarval populatios of different ages were again washed 3 times in water and inoculated into flasks containing 10 ml of the liquid culture medium described in Table 1. The initial depth of the medium was 1 cm. To prevent crowding, never more than ca. one thousand nematodes were inoculated into a given flask. These flasks were incubated in the dark at 19 C on a shaker delivering 100 horizontal 1-cm strokes per minute. An inverted light microscope was used to observe the nematodes in their culture flasks at least every other day. Records of maturation, onset, and estimated amount of reproduction were kept.

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Table 1. The liquid test medium for *Neoaplectana glaseri*.

		% Final volume
"Pf" broth:	1,000 ml 50% wt/vol beef heart infusion 10 g Pfanstiehl peptone 5 g NaCl 20 ± ml 1 N NaOH (to pH 8.2) (heated to 100 C for 1 hr on 3 successive days)	50%
NCTC-109:	Microbiological Associates, Bethesda, Md.	20%
Defined medium for *Neoaplectana glaseri*:	(Jackson, 1962)	20%
Dextrose:	10 g/100 ml	10%

Results

After 3 years in water at 5 C, as many as 60% of survivalarvae are viable if judged by their motility in water at room temperature. Dead nematodes do not settle quickly in water and many were then removed by washing populations prior to their inoculation into the liquid culture medium.

Even with the oldest stock tested, the majority of living survivalarvae did exsheath and develop into adult nematodes. However, there were differences in the reproductive capacities of cultures depending on the age of the inoculum (Table 2).

Reproduction was judged by the initial day on which stage-1 larvae appear in the medium and by population size after 2 weeks in culture. It is with great regularity that newborn (free stage-1) larvae first appear after 6 days in these cultures started with survivalarvae less than 1 year old. Such cultures also produce the largest populations. With increasing age of the inoculum there are more nonreproducing populations; in reproducing cultures the number of offspring decreases and their first appearance tends to be delayed.

Discussion

Culture media other than that used in this work, or insect hosts, might provide more suitable environments for the maximum reproduction of *Neoaplectana glaseri* survivalarvae that are more than a year old. However, the medium described in Table 1, a composite of several adequate media, served well to stimulate the reproduction of survivalarvae that are less than 1 year old, hence the decline of reproductive potential with increasing age is thought to be a real phenomenon.

Since the reproductive potential of *N. glaseri* survivalarvae declines measurably after 1 year in refrigerated water, might other methods of storing the nematodes be preferable? Freezing methods, both slow and fast, have been tried repeatedly. Such methods that work well with *Turbatrix aceti* (J. Milhorat cited in Jackson, 1966) and other organisms have only killed *N. glaseri*.

These results with *N. glaseri* stored in water indicate that careful age records of stock survivalarvae ought to be kept for both experimental and field work. That some survivalarvae more than 3 years old are still able to reproduce, suggests that the species' continuity in nature might not depend entirely on a single year's generation. Of course, the persistence of survivalarvae in the field is probably variable in the extreme.

Acknowledgments

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Table 2. The aging of *Neoaplectana glaseri*.

Age of survivalarvae	Number of stocks tested	Stocks + for maturation	Stocks + for reproduction	Reproduction time delay	2-week population increments
>3 years	17	(100%)	(41%)	4 of 7 (57%)	0–5%
2–3 years	4	(100%)	(100%)	1 of 4 (25%)	1–10%
1–2 years	8	(100%)	(100%)	1 of 8 (12%)	5–50%
<1 year	20	(100%)	(100%)	0	40–100%

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The Parasitology of the Ground Squirrels of Western Utah

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Abstract: The parasitology of six species of ground squirrels (*Spermophilus* and *Ammospermophilus*) occupying the western half, or Bonneville Basin area, of Utah is reported. The study included intestinal protozoa (except Coccidia), endoparasitic helminths and dipteran larvae, and ectoparasites. A complete list is included. The probable results of host post-Pleistocene migration on parasitism are described. Parasite–host interrelationships are discussed.

A parasitological study of the ground squirrels (*Spermophilus* and *Ammospermophilus*) was conducted throughout the Bonneville Basin and surrounding mountains that make up the western half of Utah. Most of the six species included reach the limits of their geographical distribution in this region and represent the present state of the migration that has occurred since Pleistocene Lake Bonneville receded to form the present Great Salt Lake. The ranges of the species are, with several exceptions, allopatric, and provide a probable record of some host-restricted parasites carried in with host migrations as well as those acquired through interrelationships with other genera of mammals in the region. Included in the study were helminths, intestinal protozoa (excluding Coccidia), myiasis-producing Diptera larvae, and ectoparasites.

Two extensive systematic and distributional studies of the hosts in Utah are those by Durrant (1952) and Hansen (1954). In addition to systematics, Hansen also reported on the parasitic fauna, especially the ectoparasites. Helminths of the antelope ground squirrel were reported by Grundmann (1957, 1958), and Frandsen and Grundmann (1961) published on the helminths of several additional host species. Doran (1954, 1955) compiled parasite lists for rodent species.

Excellent publications on fleas that provide pertinent data are those of Stark (1958), Jellison (1945), Jellison et al. (1953), Hansen (1954), and Beck (1955). Sucking lice were reported by Bell (1950) and Hansen (1954). Edmunds (1951) compiled a check list of the

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ticks of Utah. Data contributed by these studies are acknowledged in Table 1.

Kirby and Honigberg (1949) and Evans (1955, 1956, 1957, 1959) studied the flagellates of ground squirrels. Becker (1926a, b) described *Entamoeba citelli*. Dajani (1962) studied amoeba from a number of host species.

Several studies similar to the present one have been conducted in North America. Most of these have been concerned with defined categories of parasites such as helminths or protozoa, and none has covered as broad a range as the present study. The most extensive previous study was that of McLeod (1933) in Manitoba, Canada, which covered helminths and ectoparasites of three species of squirrels. Rausch and Tiner (1948) surveyed 325 sciurids from the North Central States. Voge (1956) published a summary of California nematode parasites. A number of parasite species reported in these publications also were found in the Bonneville Basin.

Materials and Methods

A total of 490 specimens was live-trapped or shot from 60 localities distributed over the 14 western counties of Utah extending from Boxelder and Summit on the north to Washington on the south. Trapsites ranged in elevation from 4,200 feet adjacent to the Great Salt Lake to 4,800 at the southern end of the Bonneville Basin floor. In the higher mountain ranges in and surrounding the basin, the study extended to 11,000 feet elevation. The sample included 181 rock squirrels, *Spermophilus variegatus utah* Merriam; 100 golden mantled ground squirrels, S. *lateralis lateralis* (Say); 12 Belding squirrels, S. *beldingi crebus* Hall; 35 Uinta, S. *armatus* (Kennicott); 25 Townsend, S. *townsendi mollis* Kennicott; and 135 antelope ground squirrels, *Ammospermophilus leucurus leucurus* (Merriam).

Animals removed from traps were placed in muslin bags for transport to the laboratory. Animals shot were also bagged to gain maximum recovery of ectoparasites. Live animals were etherized while still in the bags, and upon removal were combed, washed in detergent, and beaten to remove ectoparasites before autopsy. Before discarding, the carcass was placed in a bag and refrigerated at 36 F for 24 hr. This procedure caused the ectoparasites remaining to move to the hair tips where they could be collected. In the case of animals examined for protozoa, specimens were taken from the ileum, cecum, and colon immediately after the abdominal cavity was opened. Slide material was fixed in Schaudinn's and stained with iron hematoxylin. Tapeworms were relaxed in chloretone-saline solution, flattened, fixed in AFA, stained in acid alum carmine, and mounted. Nematodes were fixed either in hot 70% alcohol or in Kahle's, washed to remove the formaldehyde, and stored in 70% alcohol. Specimens were cleared in chlorolactophenol d'Amann for study and returned to storage in 70% alcohol.

Examination for *Trichinella spiralis* was done by removing a sample of approximately 1.25 g of muscle tissue from the diaphragm, tongue, and leg muscles. The sample was cut into small pieces and subjected to digestion (Hoffman, 1956), at 37–39 C using a shaker.

Fleas and ticks were prepared for mounting by being placed in warm 10% KOH for 2 hr, neutralized in acid alcohol, dehydrated in alcohol, and mounted in Hoyer's solution. Mites and lice were prepared in a similar manner omitting the KOH step.

Host Distribution

The six ground squirrel species are currently divided among three subgenera of *Spermophilus* and one of *Ammospermophilus*. The subgenus *Spermophilus* is represented by S. *armatus*, S. *beldingi*, and S. *townsendi*. The former two species are thought to have entered the Bonneville Basin region from the north and the northwest while *townsendi* invaded from the west (Hansen, 1954). S. *beldingi* has the most limited distribution in the region and occurs only in the Raft River Mountains located in the extreme northwest corner of Utah where the animal also reaches the most southern and eastern limits of its range. The habitat of the Belding ground squirrel is primarily the moist upland meadow.

S. *armatus* inhabits the drier situations in mountains, foothills, and high valleys in the Wasatch and central mountain chain that forms the eastern rim of the Bonneville Basin. The range as it occurs marks both the western limit of migration in northern Utah with the southern limit being reached in Sevier County near the center of the state. These animals select cultivated fields and pastures around developed areas and are abundant in well-drained situations such as knolls and in man-constructed banks of canals, roads, and railroads. Where its habitat coincides with recreational areas, the species is a frequent inhabitant of garbage areas and campgrounds.

Spermophilus townsendi mollis inhabits the northern three-fourths of the Bonneville Basin and is a desert valley form located in discrete population groups about moist areas such as springs and seeps. *Townsendi* range is allopatric in relation to its two closest relatives described above although farther north in Idaho there are limited areas of overlap with *beldingi*. The Townsend squirrel is closely associated in the habitat with the antelope ground squirrel, *Ammospermophilus leucurus leucurus* Merriam, that also inhabits the desert conditions of the basin and with whom it shares most of its parasites.

The subgenus *Otospermophilus* is represented by *S. variegatus utah* Merriam. Rock squirrel distribution in Utah coincides with the distribution of the scrub oak, *Quercus gambellii* Nuttall, which the animal utilizes for its basic food resources. Rock squirrels have extended their distribution into some of the desert mountain ranges of the basin where it is usually the only squirrel present above the foothill habitat where one encounters the antelope ground squirrel. The rock squirrel, however, has extended its niche greatly by adapting to suburban and urban living in human communities. It has also moved into the niche afforded by forest service garbage dumps and campgrounds and by so doing has expanded both its population density and its parasite diversity. Rock squirrel range overlaps that of the golden mantled ground squirrel, *S. lateralis lateralis* Say, at elevations around 7,000 to 7,500 ft. *S. variegatus* reaches its northern limit near the Utah–Idaho border and is considered to have invaded the region from the south as ancient Lake Bonneville receded.

The subgenus *Callospermophilus* has one representative, *S. lateralis lateralis* Say, in the region. The golden mantled ground squirrel is an inhabitant of canyon bottoms and suitable forested areas in the aspen–fir and spruce–fir belts from about 7,000 to 10,500 feet elevation. Colorado is considered to be the center of dispersal of this species and its migration into Utah has been from the east and north down the central mountain chain. The golden mantled ground squirrel had the lowest incidence of parasitism in the study group (7%) and, to date, only two parasite species have been present that have not been recovered from other ground squirrels. These are a larval tapeworm, *Taenia mustelae* Gmelin, and an adult of *Cittotaenia*.

Ammospermophilus leucurus leucurus Merriam, the antelope ground squirrel, is the lone representative of its genus in the region. The species inhabits the dry desert of the basin floor and low foothills where the rainfall is less than 10 inches per year. Antelope ground squirrels favor rocky outcrops and vegetated dunes and do not extend above the pinyon pine–juniper region along hillsides. Parasitism was found to be more individualistic in this species than in other ground squirrels of the region, being approximated in this regard only by *S. variegatus*. The major species of helminth is *Citellina triradiata* Hall, present in 74.7% of specimens. Several additional species of nematodes, *Spirura infundibuliformis* McLeod, and a species of *Subulura* also appear to be restricted to this host.

Discussion and Results

It is interesting to note that *S. townsendi*, which occurs sympatrically with *A. leucurus*, also possesses *Citellina triradiata* as its major parasite rather than possessing the parasites of its two nearest relatives, *S. armatus* and *S. beldingi*. Townsend ground squirrels appear to be primarily vegetarian in diet and do not acquire the parasites requiring insect intermediate hosts found in *A. leucurus*. Townsend ground squirrels also have an activity period that varies greatly from that of *S. armatus*, *S. beldingi*, and *A. leucurus* in that these animals are active only from February to July after which they enter into a 7½-month period of inactivity. This unique activity pattern, vegetarian diet, and independent migration into the area from the west has produced an almost totally different parasitic fauna in the Townsend squirrel than occurs in its two nearest relatives in the subgenus. Additional evidence that the Townsend squirrel has ac-

Table 1. Parasite-host records for ground squirrels of Western Utah.*

	Subgenus *Otospermophilus* *Spermophilus variegatus utah* Merriam	Subgenus *Spermophilus* *Spermophilus armatus* (Kennicott)	*Spermophilus beldingi crebus* Merriam	*Spermophilus townsendi molis* (Kennicott)	Subgenus *Callospermophilus* *S. lateralis lateralis* (Say)	Genus *Ammospermophilus* *Ammospermophilus leucurus leucurus* Merriam	
Parasite species	1†	2	3	4	5	6	Previous reports in literature
PROTOZOA							
Rhizopoda:							
Entamoeba citelli Becker, 1926	18/50‡	9/16	3/5	8/20	15/38	3/10	Dajani, 1962 (1†, 5, 6)
Mastigophora:							
Chilomastix magna Becker, 1926	6/50	6/16			3/38	5/10	Evans, 1955 (6)
Hexamastix muris Wenrich, 1924	10/50	5/16	1/5		4/38	1/10	Kirby and Honigberg, 1949 (3, 5)
Hexamites teres Kirby and Honigberg, 1949		1/16	X		1/38	2/10	Kirby and Honigberg, 1949 (3, 5)
Monocercomonoides pilleata Kirby and Honigberg, 1949	28/50	15/16	4/5	4/20	20/38	10/10	Kirby and Honigberg, 1949 (3, 5) Evans, 1955 (6)
M. robustus Gabel, 1954	6/50	7/16	1/5		5/38	2/10	
Tritrichomonas muris Grassi, 1879	9/50	13/16	3/5	4/20	12/38	1/10	Kirby and Honigberg, 1949 (5) Evans, 1955 (6)
TREMATODA							
Brachylaimidae:							
Brachylaime microti Kruidenier and Gallecchio, 1959	4/154						
CESTODA (adult):							
Mesocestoididae:							
Mesocestoides corti Hoeppli, 1925	1/154						
Anoplocephalidae:							
Cittotaenia sp.					4/100		
Hymenolepididae:							
Hymenolepis citelli McLeod, 1933	4/154				2/100	8/135	Grundmann, 1958 (6) Hansen, 1954 (6)
Davaineidae:							
Raillietina retractilis Stiles, 1925	5/154						
CESTODA (larval)							
Taeniidae:							
Taenia hydatigena Pallas, 1866	2/154					2/135	Hansen, 1954 (1) Grundmann, 1958 (6)

Table 1 cont'd.

	Subgenus *Otospermophilus* *Spermophilus variegatus utah* Merriam	Subgenus *Spermophilus* *Spermophilus armatus* (Kennicott)	*Spermophilus beldingi crebus* Merriam	*Spermophilus townsendi mollis* (Kennicott)	Subgenus *Callospermophilus* *S. lateralis lateralis* (Say)	Genus *Ammospermophilus* *Ammospermophilus leucurus leucurus* Merriam	
Parasite species	1†	2	3	4	5	6	Previous reports in literature
T. mustelae Gmelin, 1790	5/154	1/35			2/100		
T. rileyi Loewen, 1929	2/154					1/135	Grundmann, 1958 (6)
T. taxidiensis Skinker, 1935	3/154						
Cladotaenia globifera Batsch, 1786	3/154						
NEMATODA							
Trichuridae:							
Trichuris citelli Chandler, 1945	18/154						
Trichuris sp. (possibly *T. perognathi*)	1/154						
Capillarinae:							
Capillaria hepatica Bancroft, 1843	3/154						
Trichostrongylidae:							
Citellinema bifurcatum Hall, 1916	7/154	6/35	1/12				
Ostertagia circumcincta Stradelmann, 1894		1/35	1/12				
Trichostronglus colubriformis Giles		1/35	1/12		1/100		
Oxyuridae:							
Citellina triradiata Hall, 1916		1/35		8/12		100/135	Grundmann, 1957 (6)
Syphacia citelli Tiner and Rausch, 1950	101/154	22/35	1/12				Tiner and Rausch, 1950 (2)
Spiruroidea:							
Rictulariidae:							
Ritcularia coloradensis Hall, 1916	1/54					3(135	Grundmann, 1957 (6)
Rictularia sp. (possibly *affinis* or *splendida*)					1/100		
Spiruridae:							
Spirura infundibuliformis McLeod, 1933						5/135	
Subuluridae:							
Subulura sp.						2/135	
Physalopteridae:							
Physaloptera massino Schultz, 1927				1/25		7/135	Grundmann, 1957 (6)
ACANTHOCEPHALA							
Moniliformidae:							
Moniliformis clarki Ward, 1917	1/154				1/100	2/135	

Table 1 cont'd.

Parasite species	Subgenus *Otospermophilus* *Spermophilus variegatus utah* Merriam 1†	Subgenus *Spermophilus* *Spermophilus armatus* (Kennicott) 2	*Spermophilus beldingi crebus* Merriam 3	*Spermophilus townsendi molis* (Kennicott) 4	Subgenus *Callospermophilus* *S. lateralis lateralis* (Say) 5	Genus *Ammospermophilus* *Ammospermophilus leucurus leucurus* Merriam 6	Previous reports in literature
ARTHROPODA†							
Siphonaptera:							
Fam. Hystrichopsyllidae (Tiraboschi)							
Subfam. Hystrichopsyllinae							
Hystrichopsylla dippei truncata Holland		X					Stark, 1958 (2)
Hystrichopsylla sp.		X			2/24		Stark, 1958 (2, 5)
Rhadinopsylla heiseri (McCoy)						X	Hansen, 1954 (6) Stark, 1954 (6)
Subfam. Neopsyllinae							
Neopsylla inopina Rothschild		X			1/24		Hansen, 1954 (2) Stark, 1958 (2)
Catallagia decipiens Rothschild	4/61	1/14					Stark, 1958 (1)
Meringis dipodomys Kohls						X	Stark, 1958 (6)
M. parkeri Jordan						X	Stark, 1958 (6)
Subfam. Anomiopsyllinae							
Conorhinopsylla stanfordi Stewart				X			Stark, 1958 (4)
Megarthroglossis smiti Mendez						1/14	Stark, 1958 (6)
Fam. Ceratophyllidae Dampf							
Thrassis acamatus utahensis (Wagner)	3/61	3/14		X	2/24		Hansen, 1954 (4) Stark, 1958 (1, 2)
T. francisi francisi (Fox)	X	X	X	2/12	1/24	X	Stark, 1958 (1, 2, 4, 6)
T. pandorae Jellison	1/61	3/14		X	2/24	X	Hansen, 1954 (2, 4) Stark, 1958 (1, 2, 4, 6)
T. petiolatus (Baker)		X					Stark, 1958 (2)
T. bacchi caducus (Jordan)					X	X	Stark, 1958 (5, 6)
T. b. gladiolus (Jordan)	X			1/12	X	X	Hansen, 1954 (4, 6) Stark, 1958 (1, 2, 4, 6)
T. b. consimilis Stark	X					X	Stark, 1958 (1, 6)
T. arizonensis arizonesis (Baker)		X					Stark, 1958 (2)
Diamanus montanus (Baker)	43/61	7/14		4/12	11/24	10/14	Hansen, 1954 (1, 2, 4, 5, 6) Stark, 1958 (1, 2, 5, 6)
Nosopsyllus fasciatus (Bosc)	X	X					Stark, 1958 (1, 2)
Oropsylla idahoensis (Baker)	1/61	4/14	X	3/12	4/24	2/14	Hansen, 1954 (2, 3, 4, 5)
O. rupestris		1/14					Stark, 1958 (1, 2, 5, 6)
Opisthocrostis tuberculatus (Baker)	8/61	3/14	X	2/12	3/24	6/14	Hansen, 1954 (2, 3, 4, 6) Stark, 1958 (1, 2)

Table 1 cont'd.

Parasite species	Subgenus *Otospermophilus* *Spermophilus variegatus utah* Merriam 1†	Subgenus *Spermophilus* *Spermophilus armatus* (Kennicott) 2	*Spermophilus beldingi crebus* Merriam 3	*Spermophilus townsendi molis* (Kennicott) 4	Subgenus *Callospermophilus* *S. lateralis lateralis* (Say) 5	Genus *Ammospermophilus* *Ammospermophilus leucurus leucurus* Merriam 6	Previous reports in literature
O. t. cynomuris Jellison	X	X					Stark, 1958 (1, 2)
O. hirsutus (Baker)		X			X	X	Hansen, 1954 (6) Stark, 1958 (2, 5)
O. labis (Jordan and Rothschild)		X		X		X	Stark, 1958 (2, 4, 6)
Dactylopsylla ignota utahensis (Wagner)	X					1/14	Stark, 1958 (6)
D. ignota apachina Fox	6/61						Stark, 1958 (1)
Orchopeas leucopus (Baker)					1/24		
O. sexdentatus (Baker)	X				5/24		Stark, 1958 (1, 5)
Opisodasys keeni keeni (Baker)	2/61						
Malareus bitterrootensis (Dunn)					X		Stark, 1958 (5)
M. sinomus Jordan						1/14	
Monopsyllus wagneri wagneri Baker	X	X		X	X	X	Hansen, 1954 (2, 5, 6) Stark, 1958 (1, 2, 5)
Monopsyllus vision (Baker)					X		Stark, 1958 (5)
M. eumolpi (Rothschild)	X	X			X		Hansen, 1954 (2, 5) Stark, 1958 (1, 2, 5)
M. e. cyrturus Jordan		X					Stark, 1958 (2)
Megabothris abantis (Rothschild)		X			X		Stark, 1958 (2, 5)
Fam. Pulicidae Stephens Subfam. Pulicinae *Pulex* sp. pos. *irritans*					1/24	1/24	
Subfam. Spilopsyllinae *Echidnophaga gallinacea* Westwood	X					X	Hansen, 1954 (1) Stark, 1954 (6)
Cediopsylla inaequalis inaequalis (Baker)	X				X		Hansen, 1954 (1) Stark, 1958 (5)
Hoplopsylla anomalus Baker	25/61	2/14		5/12	1/24	1/14	Stark, 1958
Phtheraptera: Fam. Hematopinidae							
Enderleinellus suturalis Osborn		1/14		X	1/24	X	Hansen, 1954 (2, 4, 5, 6) Bell, 1950 (4, 5)
Enderleinellus sp.		1/14					
Hoplopleura acanthopus Burmeister		X			X		Bell, 1950 (2, 5)
Neohaematopinus citellinus Ferris						X	Hansen, 1954 (6)
N. laeviusculus Grube	12/61	6/14	X	X	1/24	1/14	Hansen, 1954 (1, 2, 3, 4) Bell, 1950 (2, 4, 5)
ACARINA Suborder Mesostigmata Fam. Dermanyssidae Kolenati							

Table 1 cont'd.

Parasite species	Subgenus *Otospermophilus* *Spermophilus variegatus utah* Merriam 1†	Subgenus *Spermophilus* *Spermophilus armatus* (Kennicott) 2	*Spermophilus beldingi crebus* Merriam 3	*Spermophilus townsendi molis* (Kennicott) 4	Subgenus *Callospermophilus* *S. lateralis lateralis* (Say) 5	Genus *Ammospermophilus* *Ammospermophilus leucurus leucurus* Merriam 6	Previous reports in literature
Hirstionyssus carniflex Koch		X					Hansen, 1954 (2)
H. incomptus Eads and Hight						2/14	
H. tricanthus Koch		X				1/14	Hansen, 1954 (2)
Fam. Laelaptidae Berlese							
Hypoaspis leviculus Ewing					X		Hansen, 1954 (5)
Haemolaelaps glasgowi Ewing	3/61	2/14		X	4/24		Hansen, 1954 (2, 4, 5)
Haemolaelaps sp.		2/14					
Suborder Trombidiformes							
Fam. Trombiculidae Ewing							
Euschongastia sp.	2/61	1/14			4/24		Hansen, 1954 (5)
Trombicula sp.	1/61					X	Hansen, 1954 (6)
ACARINA (ticks)							
Dermacentor andersoni Stiles	23/61	9/14	X		21/24		Hansen, 1954 (1, 2, 3, 5)
D. parumapertus Neumann	2/61	2/14	1/5	X		X	Hansen, 1954 (4, 6)
Ixodes marmotae Cooley and Kohls		1/12			1/24		Edmunds, 1948 (2) Hansen, 1954 (2, 3, 5)
I. sculptus Neumann		X			1/24		Edmunds, 1948 (2)
I. kingi Bishopp				1/25		1/14	
INSECTA							
Order Diptera (larvae determined to family with the exception of one case)							
Anthomyidae	1/54				1/100		
Calliphoridae					1/100		
Cecidomyidae Sp. I					2/100		
Sp. II	1/154						
Sp. III						1/135	
Hylemya Sp. I					1/100		
Sp. II					1/100		
Sp. III					1/100		
Sp. IV					1/100		
Sp. V	1/154				1/100		
Sp. VI	1/154						
Sp. VII	1/154						
Sp. VIII		1/135			2/100		
Mycetophyllidae Sp. I					1/100		
Sp. II						1/135	
Muscidae		1/135					
Nitidylidae							
Chychramus adastris					2/100		
Phoridae	1/154						
Rhagoletis					2/100		
Sarcophagidae	1/154						

* Includes data from all sources. Data contributed by the present study is expressed by number of infected animals over number processed. Records from literature only are represented by X and followed by reference.

† Host species are numbered to correspond to previous reports in literature in right-hand column.

‡ Not all specimens were processed for protozoa and ectoparasites.

quired its parasites from its association with the antelope ground squirrel is that a second nematode, *Physaloptera massino* Shultz, is also present in both but not in other ground squirrels of the region.

Most of the helminths listed in Table 1 were restricted to one or two species of ground squirrels. Few are host-specific to the species listed except in this area, and the majority have a wide distribtuion in North America. Seven species were found to inhabit at least three of the six host species. Continued collection will probably increase this number. Parasites exhibiting broad host involvement in addition to residence in ground squirrels are: Trematodes: *Brachylaime microti* Kruidinier and Gallecchio (ground squirrels, deer mice, and voles). Cestodes: *Mesocestoides corti* Hoeppli (ground squirrels, house mice, and carnivores); *Hymenolepis citelli* McLeod (ground squirrels, deer mice, chipmunks, grasshopper mice, pocket gophers, and Ord kangaroo rats); *Raillietina retractilis* Stiles (ground squirrels, jack rabbits, and kangaroo rats); larval *Taenia mustelae* Gmelin (ground squirrels and deer mice). Nematodes: *Capillaria hepatica* Bancroft (ground squirrels, deer mice, pocket gophers, and voles); *Citellonema bifurcatum* Hall (ground squirrels and marmots); *Trichostrongylus colubriformis* Giles (ground squirrels, sheep, goats, and cattle); *Ostertagia circumcincta* Stradelman (ground squirrels, deer mice, chipmunks, tree squirrels, sheep, and goats); *Physaloptera massino* Shultz (ground squirrels, house mice); and *Rictularia coloradensis* Hall (ground squirrels, deer mice, chipmunks, pocket mice, and weasels). Acanthocephala: *Moniliformis clarki* Ward (ground squirrels, deer mice, pocket gophers, chipmunks, and voles).

Special mention should be made of *Trichostrongylus colubriformis* and *Ostertagia circumcincta*, both considered to be mainly parasites of sheep and goats. It is likely these species were introduced into the region along with sheep. These species probably were transferred from sheep to ground squirrels in mountain habitats capable of sustaining the life history of the worms but not in desert habitats used by sheep as winter range. What is important is that ground squirrels could possibly act as a reservoir of infection in suitable locations and complicate control measures designed for sheep.

Why S. *lateralis* and S. *variegatus* have but two helminth species of a total of 21 in common even though their habitats overlap can be explained only through physiological and behavioral differences resulting from speciation. However, both hosts share all of the protozoan species. It is easier to explain why *A. leucurus* and S. *lateralis* have two helminths in common because one is a desert rodent and the latter a high-mountain resident. Moreover, both common parasite species in this instance have intermediate hosts that are burrow-dwelling insects and are not restricted by altitudinal differences. S. *armatus* and S. *beldingi* have four helminth species in common, but these two hosts belong to the same subgenus and occupy adjacent ranges.

Parasites suggesting specificity to genus of host are the following: *Syphacia citelli* (S. *armatus*, S. *beldingi*, and S. *variegatus*). This oxyurid has been previously reported by Tiner and Rausch (1950) from S. *armatus* in Wyoming and from S. *variegatus* by Kruidinier and Peebles in Arizona. *Trichuris citelli* (S. *variegatus*) has been previously reported from S. *beecheyii* in California by Chandler (1945). Furthermore, in the total of 18 cases *T. citelli* was never found as a single infection but was always accompanied by *Syphacia citelli* suggesting dependence on either previous or concurrent infection with the latter species.

Infection rates were based on both the diversity and number of parasite species present in each host and the high incidence produced by principal species having highly developed patterns of dissemination. Most heavily parasitized were S. *variegatus* (70.5%), S. *townsendi* (72%), and *A. leucurus* (71.5%). These were followed by S. *armatus* (39.2%) and S. *beldingi* (42.8%). S. *lateralis* at 7.3% showed the lowest parasitism. S. *variegatus* would reasonably be expected to have the highest rate in that it (1) has the largest number of parasitic species, (2) has expanded its niche to include urban and suburban habitat as well as taking over the role of the Norway and brown rat in recreational areas, (3) normally inhabits the transitional habitat between desert and higher mountains where environmental conditions are more variable and the community consists of a greater number of species, and (4) is parasitized by an oxyurid species, *Syphacia citelli* Tiner and Rausch, that is extremely prevalent. In the case of *S. townsendi* and *A. leucurus leucurus*, both desert forms, the high percentage of parasitism is associated with the high incidence of *Citellina triradiata* Hall, mediated by the variety and succession of food in a desert habitat that ensures that fecal pellets will constitute a considerable part of the diet when other food is not abundant.

Males of most of the host species showed higher incidence of parasitism than females. Trapping success in *S. variegatus* yielded approximately 40% males and 60% females of which 35.6% of the former and 23.5% of the latter were parasitized with one or more species. A similar situation was present in *S. armatus* where 41.6% of males and 33.3% of females were infected. In *S. lateralis*, however, the majority of infections in males were cases of myiasis while females revealed *Rictularia coloradensis* and *Moniliformis clarki* cases not recovered from males, suggesting greater dependence of nesting females on burrow-inhabiting insects such as the camel cricket, *Ceuthophilus utahensis*, the natural host of these species. Lower infection rates in females also may be due to sex-hormone-induced resistance as established by Mathies (1959) in studies with *Aspiculuris tetraptera* in mice. Testosterone in males is also known to enhance susceptibility.

A trichinosis survey was conducted on 100 selected specimens from the six host species. The bulk of the specimens (*S. variegatus*, *S. armatus*, and *S. lateralis*) were collected about campgrounds and garbage disposal areas in the Wasatch Mountains where a potential for infection exists. Completely negative results were obtained but these results are not considered conclusive because the specimens came from too limited a number of localities. However, with the heavy visitor pressure now present in most recreational areas and with the present garbage disposal methods where Forest Service sanitary landfills are left exposed for long periods, it will be only a matter of time before *T. spiralis* is introduced. As far as is known, all ground squirrel species, with the possible exception of *S. townsendi*, are susceptible to infection.

Three protozoan species were present in all host species. These were *Entamoeba citelli* Becker which is specific to ground squirrels. The flagellates, *Monocercomoides pileata* Kirby and Honigberg, and *Tritrichomonas muris* Grassi, were also present in all host species. Additional study of the host species other than the antelope ground squirrel that was thoroughly studied by Evans (1955, 1956, 1959) might reveal that other protozoans listed may also occur universally in ground squirrels.

Little host selectivity could be demonstrated in the ectoparasites recovered. Most have been recovered from rodent species other than ground squirrels and it appears that considerable normal exchange occurs. Two species of flea, *Opisthocrostis tuberculatus* Baker and *Oropsylla idahoensis* Baker, were present on all six hosts, although individuals of these species were not as numerous nor did they have the incidence of parasitism that *Diamanus montanus* Baker and *Hoplopsyllus anomalus* Baker had on five of the six hosts. Among the lice, only *Neohematopinus laeviuscula* Grube was universal in distribution and quite numerous. Among the mites, the most frequently recovered was *Haemolaelaps glascowi* Ewing.

In western Utah, the Rocky Mountain spotted fever tick, *Dermacentor andersoni* Stiles, is generally present above 6,000 feet in elevation while *D. parumapertus* Neumann is present in the lower areas. These species lack host specificity and the absence of either species on a host is due to altitudinal restriction. The restriction of *Ixodes kingi* to *A. leucurus* could also be distributional.

The Diptera larvae recovered probably represent accidental ingestion of fly-infested food while the host was feeding in garbage cans and dumps about forest recreation areas. It was not possible to establish that true myiasis was present in these cases although a number of the genera represented are known to assume this role. Adult flies were not reared since most specimens were preserved at the time of collection.

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Controlled Trials with Levamisole, Cambendazole, and Morantel Tartrate against Naturally Acquired Helminth Infections in Sheep

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ABSTRACT: Forty-eight ram lambs, similarly exposed to helminth infections on a contaminated pasture, were assigned to six equal groups for use in controlled anthelmintic trials. The treated lambs were given single therapeutic doses of the following anthelmintics: Group 1, levamisole (a reference drug), 8 mg/kg; Group 2, cambendazole, 20 mg/kg; Group 3, cambendazole, 30 mg/kg; Group 4, morantel tartrate, 8 mg/kg; Group 5, morantel tartrate, 12.5 mg/kg; Group 6, unmedicated controls. Drug efficacies were assayed by standard methods. Both mature and immature parasites of some species were recovered from the controls at necropsy; the most numerous species were *Haemonchus contortus*, *Strongyloides papillosus*, *Trichostrongylus axei*, and *T. colubriformis*. For groups 1 to 5, respectively, the calculated efficacies against all stages of parasites were: *H. contortus*, 99, 38, 73, 99, and 99%; *Ostertagia* spp., 96, 96, 98, 95, and 96%; *T. axei*, all 99%; *T. colubriformis*, 99, 99, 100, 94, and 98%; *S. papillosus*, 71, 100, 99, 36, and 0%; *Nematodirus* spp., all 100%; *Cooperia* spp., all 100%; *Oesophagostomum venulosum*, all 100%; *Trichuris* spp., 50, 37, 75, 0, and 37%; *Moniezia expansa*, 25, 75, 100, 25, and 0%. Only cambendazole was highly effective against *S. papillosus* and *M. expansa*, but this drug was relatively ineffective, particularly at 20 mg/kg, against *H. contortus*. There were no significant differences in efficacy among the three drugs against fourth- and fifth-stage *H. contortus* and *Nematodirus* spp.

Levamisole (Bullock, Hand, and Waletsky, 1968), the levo isomer of *dl*-tetramisole (Thienpont et al., 1966) has proved to be a highly effective livestock anthelmintic. The pertinent literature on this drug was recently summarized by Kates et al. (1971). Cambendazole, first described by Hoff et al. (1970), has been tested on a limited scale in ruminants by Baker and Walters (1971), Benz (1971a, b), Ciordia and McCampbell (1971), Egerton and Campbell (1970), Egerton et al. (1970), Restani (1971), and Restani and Borrelli (1971). The anthelmintic activity of morantel tartrate, a methyl analogue of pyrantel tartrate, was first reported by Howes (1968); details of its chemistry and preliminary studies of its anthelmintic activity were reported by McFarland et al. (1969). This drug has been tested alone on a limited scale in sheep by Cornwell and Jones (1970a, b, c), and in combination with diethylcarbamazine by Cornwell, Jones, and Pott (1971).

Earlier, we reported on the comparative activity of levamisole, thiabendazole, and parbendazole (Kates et al., 1971), as well as levamisole, parbendazole, and pyrantel tartrate (Colglazier et al., 1971b), against naturally acquired infections of sheep helminths. In both instances, the same pasture in successive years was contaminated with helminth eggs by grazing it with the same flock of infected breeding ewes. Thereafter, lightly infected lambs were confined on the pasture until they acquired heavy helminth infections for use in controlled anthelmintic trials. These studies contributed to our knowledge of the broad spectrum activity of thiabendazole, parbendazole, levamisole, and pyrantel tartrate against sheep helminths. They also indicated that the populations of *Haemonchus contortus* in these lambs were somewhat resistant to the two benzimidazoles (thiabendazole and parbendazole) but not to the two non-benzimidazoles (levamisole and pyrantel tartrate). The present trials were designed to compare the activity of two of the newer anthelmintics, cambendazole and morantel tartrate, against similar naturally acquired infections in lambs, using levamisole as a reference drug. In earlier trials (Colglazier, Kates, and Enzie, 1972) we reported on the activity of these drugs against

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experimental infections of *H. contortus* and *Trichostrongylus* spp. in lambs.

Materials and Methods

Origin and infection of experimental lambs

These aspects were similar to those previously described (Kates et al., 1971; Colglazier et al., 1971b), except that the lambs were purchased in West Texas and shipped to Beltsville, Md., arriving 27 May 1970. Upon arrival, the lambs averaged 32 kg in weight, and fecal examinations indicated that they were lightly infected with nematodes of the genera *Haemonchus, Ostertagia, Trichostrongylus, Cooperia, Oesophagostomum,* and *Nematodirus.* On 1 June 55 lambs were confined on the 4.05-hectare helminth-contaminated pasture used in previous trials (loc. cit.). The lambs were kept under parasitological surveillance until they had acquired substantial helminth infections.

Test procedures and anthelmintic treatment*

On 20 July the lambs were removed from pasture to a clean concrete-floored pen. Forty-eight lambs were randomly separated into six groups of eight lambs each, and anthelmintic treatment was given as follows on 22 July:

Group 1: Levamisole (levo-tetramisole) [*l*-2,3,5,6-tetrahydro-6-phenylimidazo (2,1-*b*) thiazole hydrochloride]: 8 mg/kg (pure chemical for experimental use; American Cyanamid Co., Princeton, New Jersey).
Group 2: Cambendazole [2-(4-thiazolyl)-5-isopropoxycarbonylaminobenzimidazole]: 20 mg/kg (7.58% suspension for experimental use; Merck & Co., Rahway, New Jersey).
Group 3: Cambendazole: 30 mg/kg.
Group 4: Morantel tartrate [trans-2[2-(3-methyl-2-thienyl) vinyl]-1-methyl-1,4,5,6-tetrahydropyrimidine tartrate]: 8 mg/kg (pure chemical for experimental use; Charles Pfizer & Co., Inc., Groton, Conn.).
Group 5: Morantel tartrate: 12.5 mg/kg.
Group 6: Unmedicated controls.

Levamisole was used only at one dose level as a reference drug. The two newer anthelmintics were used at two dose levels within the range likely to be used under normal field conditions. Each dose was given as an aqueous drench with a Whitlock syringe. At 5 to 7 days posttreatment, all lambs were necropsied for residual worm counts. Drug efficacy calculations were done by the standard method and statistical analysis was done as previously described (Kates et al., 1971).

Results and Discussion

This trial is the third in a series to compare the efficacy of modern, broad spectrum anthelmintics against naturally acquired gastrointestinal helminths of sheep under similar conditions. The two earlier trials involved levamisole, thiabendazole, parbendazole, and pyrantel tartrate (Kates et al., 1971; Colglazier et al., 1971b). Generally, these four anthelmintics showed the broad-spectrum activity expected. It was noted, however, that the populations of *H. contortus* were somewhat resistant to the benzimidazole drugs, thiabendazole and parbendazole, but not to the other two anthelmintics, levamisole and pyrantel tartrate. The present trials extend these studies to the newer anthelmintics, cambendazole and morantel tartrate, using levamisole primarily as a reference drug. Because the test lambs acquired most of their helminth infections from the same pasture used in previous trials, their *H. contortus* populations were considered to be resistant to at least some benzimidazole anthelmintics, a group to which cambendazole belongs but morantel tartrate does not.

The data and the statistical analysis are summarized in Table 1. In these trials, the most numerous parasites in the control lambs were *H. contortus, Trichostrongylus* spp., and *S. papillosus,* but lesser numbers of *Ostertagia* spp., *Nematodirus* spp., *Oesophagostomum venulosum, Cooperia* spp., *Trichuris* spp., and *Moniezia expansa* were present also. Furthermore, substantial numbers of larvae (fourth stage) of *H. contortus* and *Nematodirus* spp. were recovered from the controls. When animals are removed from a contaminated pasture, as in these trials, and then treated with an

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* Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U. S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

Table 1. Average number of helminths recovered from unmedicated control and treated lamb groups, drug efficacies, and summary of statistical analysis of differences between groups (eight lambs per group).

Helminth species	Unmedicated controls (avg worms)	Levamisole 8 mg/kg		Cambendazole				Morantel tartrate			
				20 mg/kg		30 mg/kg		8 mg/kg		12.5 mg/kg	
		Avg worms	% Eff.	Avg worms	% Eff.	Avg worms	% Eff.	Avg worms	% Eff.	Avg worms	% Eff.
H. contortus											
4th-stage	$5,015^{a}$*	33^{c}	99	$3,380^{ab}$	33	$1,427^{b}$	72	15^{c}	99	2^{c}	99
5th-stage	$10,103^{a}$	65^{bc}	99	$5,980^{a}$	41	$2,660^{a}$	74	155^{b}	98	33^{c}	99
Total	$15,118^{a}$	98^{bc}	99	$9,360^{a}$	38	$4,087^{a}$	73	170^{b}	99	35^{c}	99
Ostertagia spp.											
4th-stage	25^{a}	2^{b}	92	0^{b}	100	0^{b}	100	0^{b}	100	0^{b}	100
5th-stage	260^{a}	8^{b}	97	10^{b}	96	5^{b}	98	15^{b}	94	10^{b}	96
Total	285^{a}	10^{b}	96	10^{b}	96	5^{b}	98	15^{b}	95	10^{b}	96
T. axei											
4th-stage	40^{a}	0^{b}	100	0^{b}	100	0^{b}	100	0^{b}	100	0^{b}	100
5th-stage	$1,730^{a}$	18^{b}	99	15^{b}	99	2^{b}	99	32^{b}	98	5^{b}	99
Total	$1,770^{a}$	18^{b}	99	15^{b}	99	2^{b}	99	32^{b}	99	5^{b}	99
T. colubriformis											
4th-stage	5^{a}	0^{b}	100	0^{b}	100	0^{b}	100	0^{b}	100	0^{b}	100
5th-stage	$3,065^{a}$	2^{b}	99	7^{b}	99	0^{b}	100	187^{c}	94	67^{c}	98
Total	$3,070^{a}$	2^{b}	99	7^{b}	99	0^{b}	100	187^{c}	94	67^{c}	98
Nematodirus spp.											
4th-stage	207^{a}										
5th-stage	168^{a} } all	0^{b}	100	0^{b}	100	0^{b}	100	0^{b}	100	0^{b}	100
Total	375^{a}										
S. papillosus†	$3,218^{a}$	943^{b}	71	0^{c}	100	2^{c}	99	$2,072^{a}$	36	$3,912^{a}$	0
Cooperia spp.†	30^{a}	0^{b}	100	0^{b}	100	0^{b}	100	0^{b}	100	0^{b}	100
O. venulosum†	21^{a}	0^{b}	100	0^{a}	100	0^{b}	100	0^{b}	100	0^{b}	100
Trichuris spp.†	8^{a}	4^{bc}	50	5^{bc}	37	2^{c}	75	11^{a}	0	5^{b}	37
M. expansa											
Scolices	4^{a}	3^{a}	25	1^{b}	75	0^{b}	100	3^{a}	25	4^{a}	0
Strobilar vol. ml	85^{a}	63^{a}	26	17^{bc}	80	0^{c}	100	35^{ab}	59	101^{a}	0

* Any means not followed by same superscript letter (read horizontally) are significantly different ($P < 0.05$).
† Only 5th-stage parasites recovered.

anthelmintic shortly thereafter, significant but unknown proportions of the worm populations at the time of treatment are usually immature. Therefore, when high efficacies ensue under these circumstances against immature and mature worm populations combined, they are more meaningful than high efficacies obtained only against mature worms when applied to helminth control in animals on the farm.

Levamisole, given at the recommended dosage of 8 mg/kg of body weight, was highly effective against all nematode species except *S. papillosus* (71%) and *Trichuris* spp. (50%). Interestingly, it had better activity against all stages of *Ostertagia* spp. (96%) than it had in the earlier tests, 76 and 82% (loc. cit.). Morantel tartrate at both dose levels was highly effective against many of the same nematode species as was levamisole, but it was less effective against *S. papillosus* (36 and 0%). The efficacy of cambendazole at both dose levels against the various nematode species generally paralleled that of the other two drugs, except that the drug was significantly less effective against *H. contortus* (38 and 73%) and significantly more effective against *S. papillosus* (100 and 99%). Also, only cambendazole showed marked action against *M. expansa*; the data, however, were limited.

There appeared to be no marked differences in efficacy among the three drugs with regard to the developmental status of the worms at the time of treatment.

In the only other published studies on the anthelmintic activity of cambendazole in sheep, Hoff et al. (1970) and Egerton and Campbell (1970) reported that dosages of 5 to 20 mg/kg were highly effective against *H. contortus*, and that the 20-mg dose removed more than 99% of these worms. Similar results against *H. placei* in cattle were reported by Benz (1971a, b), Ciordia and McCampbell (1971), Egerton et al. (1970), and Restani (1971). In our trials, however, the efficacy of cambendazole at 20 and 30 mg/kg was only 38 and 73%, respectively, against all stages of *H. contortus*, indicating that the population was somewhat resistant to this drug. Previously, we reported

(Kates et al., 1971; Colglazier et al., 1971b) that populations of *H. contortus* acquired by lambs on the same pasture were not removed effectively by doses of 50 and 100 mg/kg of thiabendazole and 15 and 20 mg/kg of parbendazole. It appears, therefore, that these populations of *H. contortus* were cross-resistant to the three benzimidazole anthelmintics. However, these benzimidazole-resistant populations of *H. contortus* were highly susceptible to therapeutic doses of such non-benzimidazole anthelmintics as levamisole, pyrantel tartrate, and morantel tartrate. Rafoxanide, another non-benzimidazole anthelmintic, has also proved effective in controlling some benzimidazole-resistant strains of *H. contortus* (Colglazier et al., 1971a).

The only data previously available on the activity of cambendazole against *S. papillosus* in ruminants are those reported by Egerton and Campbell (1970), a dosage of 5 mg/kg was 99% effective in a few sheep, and by Restani and Borrelli (1971) who reported high efficacy with somewhat higher doses in calves. In our present trials, substantial infections of this nematode were almost completely removed with dosages of 20 and 30 mg/kg. It appears, therefore, that cambendazole, in addition to thiabendazole and parbendazole, is another drug that may prove useful for the control of this parasite. Levamisole at 8 mg/kg was only partially effective (71%) against *S. papillosus*, and morantel tartrate was ineffective at both dose levels used.

Although the data for tapeworms were limited, cambendazole at the 30-mg/kg level showed promise of effective action against this parasite.

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Acanthobothrium urolophi sp. n., a Tetraphyllidean Cestode (Oncobothriidae) from an Australian Stingaree

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ABSTRACT: *Acanthobothrium urolophi* sp. n. is described from a common stingaree, *Urolophus testaceus*, from South Australia. It differs from all other species in being apolytic and acraspedote, in having hooks 105–115 μ long, one accessory sucker 80–90 wide on each bothridium, and 40–72 testes in two longitudinal rows.

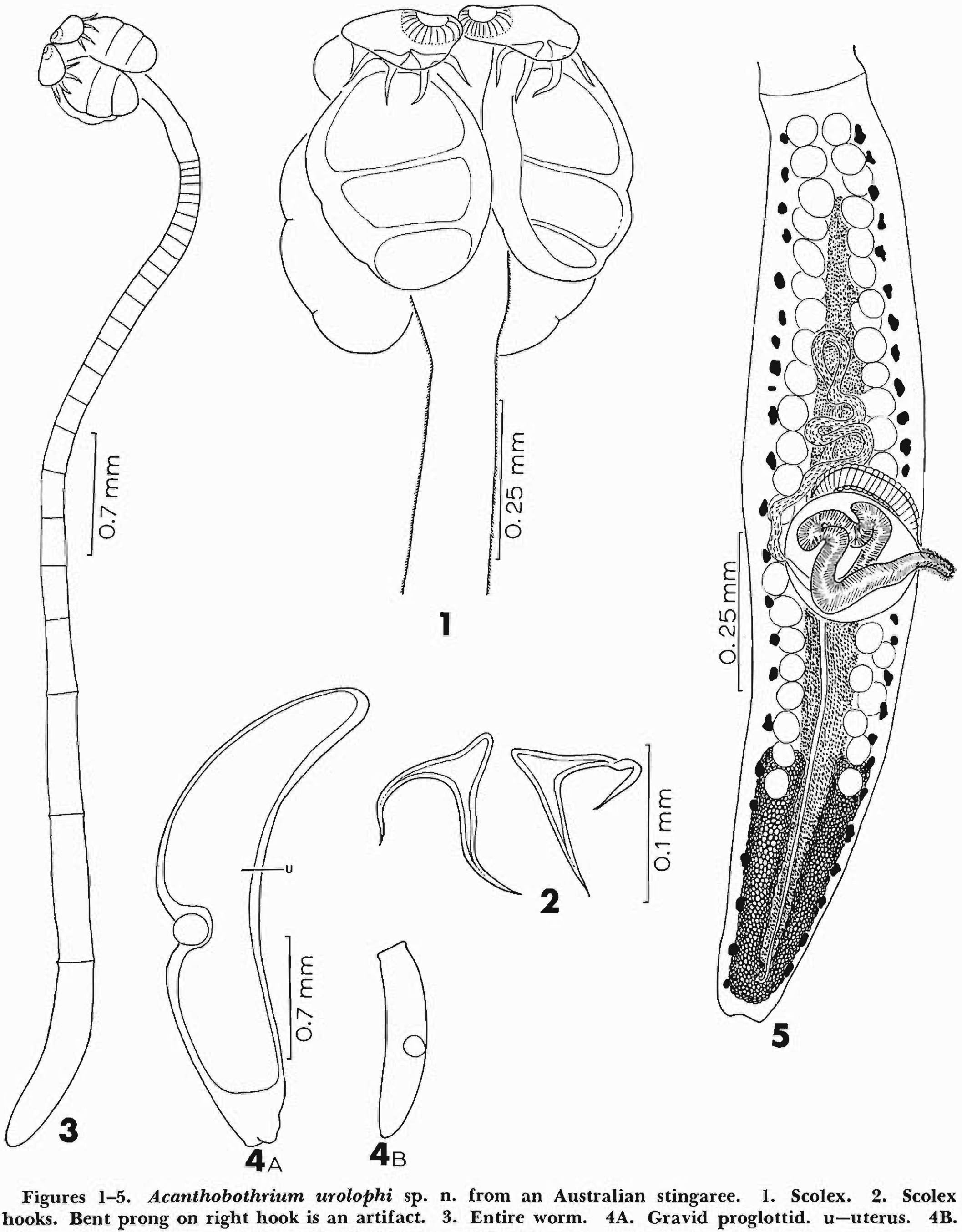
This report is based upon four specimens recovered from the spiral valve of a common stingaree, *Urolophus testaceus*, which I speared at Glenelg Beach near Adelaide, South Australia, in January 1970. They represent a new species and are the basis of the following measurements and description. All measurements are in microns unless otherwise stated.

Acanthobothrium urolophi sp. n.
(Figs. 1–5)

Description

Scolex (Fig. 1) broad, clearly set off from peduncle, 360 to 440 long, 440 to 640 greatest width. Four bothridia present, each 400 to 410 long, 280 to 320 greatest width. Each bothridium with three loculi of the following lengths: anterior 150 to 200, middle 100 to 130, posterior 80 to 90. Muscular pad present on anterior end of each bothridium, each with single accessory sucker 80 to 90 wide. Two bifurcated hooks (Fig. 2) present at base of each muscular pad. Handle of hook 25 to 30 long, inner prong 75 to 80 long, outer prong 75 to 90 long. Total hook length (from tip of outer prong to tip of handle) 105 to 115. Tips of prongs very delicate. Peduncle 560 to 720 long (measured from base of scolex to first discernible segmentation), covered with minute spines.

Strobila (Fig. 3) thin, delicate, apolytic, acraspedote, 8.0 to 9.5 mm long, with 23 to 26 proglottids. Last three proglottids mature, 1.3 to 1.5 mm long, 275 to 300 greatest width. Proglottids continue growth after detaching (Figs. 4A, 4B); when gravid they measure 2.7 to 3.8 mm long, 400 to 560 greatest width. Reproductive systems (Fig. 5) protandrous. Genital pores about equatorial in mature seg-



Figures 1–5. ***Acanthobothrium urolophi*** **sp. n. from an Australian stingaree. 1. Scolex. 2. Scolex hooks. Bent prong on right hook is an artifact. 3. Entire worm. 4A. Gravid proglottid. u—uterus. 4B. Mature proglottid, same scale as 4A. 5. Mature proglottid.**

ments, at about posterior third in gravid segments, irregularly alternating. Genital atrium absent.

MALE GENITALIA: Forty to 72 testes, arranged in two longitudinal rows. Each testis 40 to 100 wide in mature segment. When first discernible, about 40 testes are present, increasing to about 70 as proglottid matures. Twenty-one to 40 testes on aporal side, 13 to 31 prevaginal on poral side, 6 to 11 postvaginal on poral side. Cirrus pouch spheroid, thin-walled, 100 to 200 wide. Vas deferens anterior to cirrus pouch when filled with sperms. Cirrus long, slender, covered with small spines of similar size and shape throughout its length. Cirrus present only in mature proglottids, apparently being lost at copulation, although not seen in the vaginas of gravid proglottids.

FEMALE GENITALIA: Ovary at posterior end of segment, U-shaped with two anteriorly directed lobes that do not reach level of cirrus pouch, 280 to 320 total length. Vitelline follicles small, few, lateral. Distal end of vagina ventral or anterior to cirrus pouch, with thick cuticular lining and muscular walls. Proximal portion of vagina thin-walled. Seminal receptacle not seen. Uterus a median, longitudinal tube, becoming a thin-walled sac when gravid. Eggs spherical, about 46 wide (only very young eggs were not collapsed during preparation for slides).

TYPE HOST: Common stingaree, *Urolophus testaceus* (Muller et Henle) (Myliobatiformes: Urolophidae).

LOCATION: Spiral valve.

TYPE LOCALITY: Glenelg, South Australia (Gulf of St. Vincent).

TYPE SPECIMENS: USNM Helm. Coll. holotype No. 72284, 3 paratypes No. 72285.

REMARKS: Much attention has been centered on this genus in recent years. Goldstein (1967, 1969) and Williams (1969) have offered extensive reviews of the more than 70 species within it.

Four species have been reported from rays of the family Urolophidae. *Acanthobothrium dasybati* Yamaguti, 1934 was reported from *Urolophus fuscus* Garmen, from Japan (Yamaguti, 1952; and *A. crassicolle* Wedl, 1855, *A. dujardini* Beneden, 1849, and *A. parviuncinatum* Young, 1954, from *Urolophus halleri* (Cooper), all from California (Young, 1954).

Acanthobothrium urolophi sp. n. differs from *A. dasybati* in having an accessory sucker on the anterior part of each bothridium, which *A. dasybati* lacks, and in being apolytic with few segments while *A. dasybati* has a strobila of up to 80 segments.

Acanthobothrium urolophi has hooks 105 to 115 long and 40 to 72 testes, while *A. crassicolle* has hooks 180 to 230 long and 100 to 153 testes; *A. dujardini* has hooks 180 to 210 long and 20 to 38 testes; and *A. parviuncinatum* has hooks 87 long and 12 to 14 testes per segment. *Acanthobothrium urolophi* thus appears to differ significantly from other species reported from Urolophidae.

Williams (1969) discussed host specificity of this genus in considerable detail, concluding that it is nearly absolute. Although it is doubtful that *A. urolophi* would occur in other families of rays, I further compared it with published descriptions of all other species from elasmobranchs and found none with the combination of characters typical of *A. urolophi*: (1) apolytic; (2) 40 to 72 testes in two longitudinal rows; (3) hooks 105 to 115 in total length; (4) postvaginal testes present; and (5) one accessory sucker present on each bothridium.

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Anthelmintics for Nematode Parasites of Fish: I. Control of *Sterliadochona pedispicula* in *Salmo gairdnerii* by Diethyl 2-Chlorovinyl Phosphate and 2,2 Dichlorovinyl Dimethyl Phosphate Pellets

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ABSTRACT: Tests indicate that the oral administration of diethyl 2-chlorovinyl phosphate (SD1836) and 2,2 dichlorovinyl dimethyl phosphate (DDVP) plastic-incorporated pellets significantly reduces the intestinal nematode parasite *Sterliadochona pedispicula* in wild rainbow trout. No evidence of fish toxicity was noted with the treatments and dosages tested.

Nematodes are ubiquitous as parasites of fish, yet little is known about their effects upon fish biology or physiology. Even less is known about prophylaxis and treatment of the nematode diseases of fish. Only the Russians appear to recognize nematodes as serious parasites of fish and have made attempts to control them with anthelmintics, pond treatments, or feed incorporation (Engashev, 1965; Konovalov and Zavyalov, 1968; Marazas and Vasilkov, 1968; Vasilkov, 1969). This report concerns a wide-spectrum anthelmintic and an analog used against one of the most common nematode parasites, *Sterliadochona pedispicula* Maggenti and Paxman, 1971 found in rainbow trout, *Salmo gairdnerii* Richardson of California. The purpose of the experiment is to establish that it is feasible to reduce or eliminate nematodes from the intestinal tract of a freshwater fish.

Materials and Methods

Rainbow trout were collected from Jawbone Creek, Tuolumne County, California, with electric shocking equipment. At the time of collection (October) the mean intensity of infection was 44 nematodes per fish. Fish to be treated were selected, within limits of practicality, for uniformity of length and weight. In each treatment trout were identified by either fin clipping or lateroventral tattooing with freezing markers.

A wide-spectrum anthelmintic (Hass, 1971) 2, 2 dichlorovinyl dimethyl phosphate (DDVP, Vapona, Dichlorvos) and an analog (Lichtenthaler, 1961) diethyl 2-chlorovinyl phosphate (SD1836) incorporated in rodlike plastic pellets were tested. Pellet formulations and sizes are: DDVP, 26% (80 mg, 4 × 6 mm); DDVP, 21% (3.5 mg, 2 × 2 mm); DDVP, 5% (1.3 mg, 1 × 1.5 mm); SD1836, 15% (31.5 mg, 3.5 × 3.5 mm); SD1836, 30% (31.3 mg, 3 × 3 mm).

Pellets for all experiments were injected by either a modified syringe (DDVP, 26% and all SD1836 pellets) or a number one cork bore and plunger (DDVP, 5 and 21% pellets). The needle of the syringe was replaced with plastic tubing of a diameter to accommodate one pellet. The syringe and tubing were water-filled in order to supply pressure for pellet release; 0.1 ml of water was ejected with each pellet. The tubing was inserted down the gullet and the pellet was ejected in the region of the esophagus and stomach juncture. Because of the number and smaller size of the 5 and 21% DDVP pellets they were administered with a number one cork bore and plunger. The bore was inserted in a similar manner to the syringe tubing; when in place the plunger was dropped thus forcing all pellets out in a single application.

Studies with these chemicals were conducted under two sets of conditions: one initiated under natural stream conditions and transferred to the laboratory and the other conducted entirely under laboratory conditions.

STREAMSIDE TREATMENTS: Rates of DDVP, 26% pellet: ½ plus ½ pellet, 24-hr elapse between adminstration of each ½ pellet; and 4 pellets per fish. Rate of DDVP, 5% pellet: 30 pellets per fish. Rates of SD1836, 15% pellet: 1, 2, and 4 pellets per fish. Each treatment was replicated with four fish. Treated fish were held in the stream in nylon net-covered cylindrical cages 10 inches in diameter and 14 inches long. The nylon net was fine enough to allow pellet recovery and excluded pellet loss into the stream (verified by pellet recovery after passage and at necropsy). After 48 hr fish were transported to the laboratory and maintained in 10-gallon aerated milk cans; stream temperature (40 F) was maintained in transport and in the laboratory. After 96 hr of observation the trout were sacrificed and examined for parasitic nematodes.

LABORATORY TREATMENTS: 5% DDVP pellets, 45 per fish; 21% pellets, 15 and 30 pellets per fish; SD1836, 15% pellets, 8 per fish. Each treatment was replicated with four fish. Treated trout were maintained in 10-gallon temperature-controlled (40 F) aerated milk cans for 96 hr, after which they were sacrificed and examined.

Nematode evacuation and pellet passage were monitored while testing the anthelmintic efficacy of SD1836, 30% pellets. Water temperature within the treatment can was maintained at 58 F. Four pellets were administered to each of four fish. Pellets and nematodes were collected every 24 hr until all pellets were recovered from the treated trout. The fish were then transferred to a 100-gallon temperature-controlled (58 F), aerated, filtered stock tank and maintained for 4 weeks, then sacrificed and examined.

Dissection and isolation of parasitic nematodes from the alimentary canal of host trout were carried out in cold-blooded Ringer's solution.

Fish were maintained in stream water (pH 7.6, conductivity 0.05 millimhos/cm) or well water (pH 7.6, conductivity 0.90 millimhos/cm) at temperatures to which wild fish were naturally accustomed.

Results

No deleterious effects were observed with any of the handling procedures used in these experiments.

Among the SD1836, 15% pellet treatments, six pellets, administered as a single application, showed a significant reduction in the parasitic nematode burden (Table 1). Lack of significant control with eight pellets was due to the inability of test trout to retain so many pellets for a sufficient exposure period. Several pellets were regurgitated within a matter of hours. With a lesser dosage the pellets were retained and passed through the alimentary canal. Regurgitation was not influential in the 1-, 2-, and 4-pellet treatments; these treatments were clearly sublevel dosages (Table 1).

Table 1. Control of *Sterliadochona pedispicula* with diethyl 2-chlorovinyl phosphate (SD1836) and 2,2 dichlorovinyl dimethyl phosphate (DDVP) pellets injected into the esophagus of *Salmo gairdnerii*. Fish sacrificed 96 hr after treatment. Four fish in each treatment; temperature 40 F.

Treatment	No. pellets	Avg fish length (mm)	Avg fish weight (g)	Nematodes necropsy (mean)
Control	0	188	75.2	33–49 (41)
SD1836-15%	1	189	76.0	27–72 (50)
SD1836-15%	2	174	59.3	23–57 (35)
SD1836-15%	4	207	99.8	49–70 (56)
SD1836-15%	6	137	29.1	1–22 (9)*
SD1836-15%	8	179	66.2	17–52 (28)
[1]SD1836-30%	4	143	33.0	0
[2]DDVP-26%	½+½	180	66.0	1–17 (8)*
DDVP-26%	4	166	51.8	0– 8 (3)*
DDVP-21%	15	133	27.0	0–13 (5)*
DDVP-21%	30	137	28.8	0– 7 (4)*
DDVP-5%	30	155	42.6	0– 5 (2)*
DDVP-5%	45	149	38.8	0– 2 (1)*

* Significant reduction at the 0.01 level.

[1] Nematodes were collected as evacuated over a 72-hr period; the fish were then maintained for 4 weeks prior to necropsy. Treatment temperature 58 F.

[2] Repeated application 24-hr interval.

The single administration of four 30% pellets of SD1836 substantially increased anthelmintic efficacy (Table 1). Trout with a length of 120 mm or more can easily accommodate four pellets of this size. Within 48 hr 60% of the pellets had passed through the alimentary canal; at 72 hr 75% had passed. Passage of all pellets required 144 hr. After total pellet recovery treated trout were observed for 4 weeks; during this time no deleterious effects were detected upon either fish feeding or behavior. Thorough examination of the alimentary canal at necropsy established that all nematodes had passed (Table 1).

The DDVP 26, 21, and 5% pellets all significantly reduced the parasitic worm burden in the treated trout (1% level of confidence). Of these treatments the smaller 21 and 5% pellets were most easily accommodated by the fish. However, there were no significant differences among the DDVP treatments tested.

Discussion

The study of parasitic nematodes of fish and the need for controls must cease to be looked upon merely as an academic exercise. It is apparent that because of the ubiquity of nematode parasites of fish they are too often equated to commensalism rather than parasitism because of a lack of knowledge or techniques. This fallacy persists even in the face of the reports of severe infections which have had devastating results. Dogiel et al. (1958) describe an infection of Lake Sudochye fish with *Rhaphidascaris acus*. The effect of the worm burden was such that all fish perished during the winter as a result of extreme emaciation. Petrushevski and Shulman (1955) as well as others have reported on the severe liver damage and loss of weight (fats reduced by 47% in Baltic cod infected with *Contracoecum aduncum*). Hiscox (1972) reports the effects of *Bulbodacnitis ampullastoma* on food consumption, growth, and mortality of rainbow trout. The effects were observable with as few as five nematodes per fish. This nematode was originally collected and described from a hatchery brood trout that harbored several hundred nematodes in the ceca and cecal region of the gut (Maggenti, 1971).

Among the nematodes parasitizing fish the most likely to be of importance in pond fisheries are those with copepods as intermediate hosts such as the camallanids and cucullanids. However, nematodes such as S. *pedispicula* which require aquatic insects in the life cycle could under favorable circumstances become extremely important. The latter seem to be the prevalent parasites in streams and rivers.

Sterliadochona pedispicula has a wide distribution, high incidence, and a high intensity in rainbow trout throughout California. This parasite also occurs in *Salvelinus fontinalis* (brook trout) and *Salmo trutta* (brown trout).

The Jawbone Creek population of rainbow trout has an unusually high incidence of infection; previous surveys showed that all rainbow trout over 4 inches were infected. Three years of survey from May to November indicated that the highest intensity occurs in the fall. Among the trout collected for the present study the lowest intensity of S. *pedispicula* per fish was 22 and the highest 88. Moravec (1971) reports that a remarkably similar situation prevails with the parasite *Sterliadochona* (= *Cystidicoloides*) *tenuissima* in S. *trutta* in the river Bystřice in Czechoslovakia.

The success obtained with the anthelmintics tested in these experiments indicates it is just as feasible to control the nematode parasites of fish as it is to control their other helminths and diseases. The manual insertion of pellets is impractical for wide-scale control under natural conditions; however, it could be very useful for removal of parasitic nematodes from special fish such as brood stock. This type of treatment minimizes chemical contamination of fish environment and eliminates the problems in aquarial filtration of chemicals.

Anthelmintics incorporated directly or as pellets within pellets into feed could be used for treatments in hatcheries, pond fisheries, and holding ponds. Control of parasitic nematodes deserves consideration by any group involved in or responsible for the release of excess or older brood fish into natural streams as is done, for example, in trophy fish programs for sportsmen. Without prior anthelmintic treatment these fish present a real danger as carriers of high numbers of parasitic nematodes into previously uninfested areas. The danger also exists in the maintenance of untreated wild trout once they are selected for brood stock.

Perhaps also important at this time is the potential use of anthelmintics as a tool to elucidate the physiological effects of parasitic nematodes on fish. The use of individually administered pellets would permit one to study the effects of parasitism on the same fish with and without a nematode burden.

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Anthelmintics for Nematode Parasites of Fish: II. Aqueous Anthelmintic Bath Treatments using Diethyl 2-Chlorovinyl Phosphate and 2,2 Dichlorovinyl Dimethyl Phosphate for Control of *Sterliadochona pedispicula* in *Salmo gairdnerii*

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ABSTRACT: The toxicity of diethyl 2-chlorovinyl phosphate (SD1836) and 2,2 dichlorovinyl dimethyl phosphate (DDVP) was tested as an "in-water" treatment of rainbow trout and in vitro against the internal nematode parasite of fish, *Sterliadochona pedispicula*. As an "in-water" anthelmintic treatment SD1836 was 100% effective at concentrations of 25 μ liters/liter for 24 hr and 5 μ liters/liter for 72 hr; no toxic effects to rainbow trout were noted with these treatments.

The increasing importance of fish culture for purposes of food and recreation demands that more research be directed to the treatment of nematode parasitized fish. Most commonly efforts are directed toward those helminths potentially dangerous to man. The Russians recognize nematodes as serious parasites of fish which can cause severe economic losses and have made efforts to control them (Maggenti, 1972). This present report investigates the efficacy of a wide-spectrum anthelmintic and an analog applied in water against the widely distributed internal nematode parasite, *Sterliadochona pedispicula* Maggenti and Paxman, 1971, in rainbow trout, *Salmo gairdnerii* Richardson, of California.

Materials and Methods

The rainbow trout infected with S. *pedispicula* used in these experiments were collected from Jawbone Creek, Tuolumne County, California. Trout were collected in the early fall (October) when water temperature was 40 F and intensity of infection in trout over 4 inches averaged 44 nematodes per fish. Trout were collected with electric shocking equipment.

Uninfected hatchery trout, for anthelmintic toxicity studies, were obtained from the American River Hatchery of the California Department of Fish and Game, Sacramento, California.

Experimentation was divided into three phases: (1) toxicity of test chemicals to the nematodes; (2) toxicity to uninfected fish; and (3) anthelmintic efficacy as in-water treatments. Two anthelmintics were tested: a wide-spectrum anthelmintic (Hass, 1971), 2,2 dichlorovinyl dimethyl phosphate (DDVP, Vapona, dichlorvos), emulsifiable concentrate 2#/gallon formulation and diethyl 2-chlorovinyl phosphate (SD1836), technical consisting of a mixture of components: α 62%, β 28%, and γ 10% (Gatterdam et al., 1959). Concentrations all refer to active ingredient.

IN VITRO ANTHELMINTIC TOXICITY TO S. *PEDISPICULA*: Nematodes used in this investigation were dissected from infected wild rainbow trout and held in Ringer's Solution A for cold-blooded animals and maintained at two temperatures, 40 and 58 F. All anthelmintic concentrations were made with Ringer's Solution A. At 40 and 58 F, respectively, 10 nematodes were placed in petri dishes in six concentrations each of DDVP and SD1836 (Table 1). Solutions were changed at each observation period. At both temperatures 10 control animals were maintained in Ringer's Solution A. Observations were maintained for 96 hr.

TOXICITY TO UNINFECTED TROUT: All chemical toxicity tests of uninfected hatchery trout were carried out at 58 F, the hatchery rearing temperature. Treatments were carried out in 10-gallon temperature-controlled aerated milk cans. Each treatment was replicated with four fish. Control fish were maintained under the same conditions as treated fish. Anthelmintic concentrations were made with 10 liters of well water: pH 7.6, conductivity 0.90 millimhos/cm. Four concentrations of DDVP and two concentrations of SD1836 were tested (Table 2). Observations were made for a 48-hr period.

ANTHELMINTIC EFFICACY: Three concentrations of SD1836 in 20 liters of well water were tested at 58 F for anthelmintic activity with wild infested trout (Table 3). Treatment and control solutions were changed daily; evacuated nematodes were collected and counted. Surviving fish were transferred after treatment to 100-gallon temperature-controlled filtered tanks and maintained for 4 weeks of observation. The fish were then sacrificed and necropsied in Ringer's Solution A.

In each treatment trout were identified by lateroventral tattooing with freezing markers.

Results

The in vitro experiments run at 40 F showed that S. *pedispicula* was very susceptible to DDVP at a concentration of 50 μ liters/liter and exposure time of 12 hr (Table 1). At the concentration of 25 μ liters/liter one-half of the test nematodes were killed with a 12-hr exposure and all were dead after an exposure time of 36 hr. Complete kill was not obtained with 96 hr of exposure with any of the lower concentrations used. The table reports the data up to 48 hr because no change was noted after this period of time.

In no instance was complete kill obtained with any of the concentrations of SD1836 tested. Reductions on the order of one-half were obtained with 50 μ liters/liter at 24 hr, 25 μ liters/liter at 36 hr, and 5 μ liters/liter, 1 μ liters/liter, and 0.5 μ liters/liter at 48 hr (Table 1).

The second in vitro experiment (Table 1) was conducted at 58 F in order to test possible higher activity with increased temperature. Because of the generally poor results obtained with the in vitro studies with SD1836, higher concentrations were also included. Not unexpectedly the activity of DDVP did increase with increased temperature. Complete kill was obtained with 50 μ liters/liter, 25 μ liters/liter, and 5 μ liters/liter at 12, 24, and 36 hr, respectively (Table 1). Below these concentrations

Table 1. *In vitro* survival of *Sterliadochona pedispicula* exposed to 2,2 dichlorovinyl dimethyl phosphate (DDVP) and diethyl 2-chlorovinyl phosphate (SD1836).

Treatment	Rate μl/l a.i.	Numbers of nematodes surviving							
		40 F				58 F			
		12 hr	24 hr	36 hr	48 hr	12 hr	24 hr	36 hr	48 hr
Control	–	10	10	10	10	10	10	10	10
DDVP	50	1	0	0	0	0	0	0	0
DDVP	25	5	2	0	0	2	0	0	0
DDVP	5	5	5	3	3	5	5	1	0
DDVP	1	10	4	3	3	5	5	5	3
DDVP	0.5	10	6	6	6	5	5	3	3
DDVP	0.25	10	6	5	4	7	5	5	4
SD1836	100	–	–	–	–	2	0	0	0
SD1836	75	–	–	–	–	4	4	2	2
SD1836	50	8	4	1	1	4	4	3	3
SD1836	25	9	7	3	2	4	2	2	1
SD1836	5	10	9	9	5	8	7	7	5
SD1836	1	10	10	8	6	8	7	5	5
SD1836	0.5	10	10	8	5	–	–	–	–
SD1836	0.25	10	10	9	8	–	–	–	–

kill was on the order of one-half but did not change with longer exposure. Higher activity with SD1836 appeared to be more related to higher concentration rather than higher temperature (Table 1). Complete kill was obtained only with a 24-hr exposure to a concentration of 100 μ liters/liter. A comparison of toxicity at a concentration of 50 μ liters/liter does show increased activity with temperature in that one-half of the nematodes were dead in 12 hr at 58 F; at the same concentration at 40 F the same level of kill took 24 hr. A similar relationship exists for the other concentrations tested (Table 1).

The results of the in vitro tests determined the concentrations that were used to test fish toxicity. Because of its higher activity four concentrations of DDVP were selected and two concentrations of SD1836 (the highest concentration which showed best in vitro results with nematodes and the lowest concentration that had shown reasonable activity). DDVP at the concentrations tested was extremely toxic to rainbow trout (Table 2). Test fish did not live 1 hr at 50 μ liters/liter and survived only a little more than 12 hr at 0.25 μ liters/liter. It was surprising to find the toxicity of SD1836 so much lower than DDVP. At the 100-μ liters/liter concentration of SD1836 all test rainbow trout survived longer (17 hr) than they did in 0.25 μ liters/liter of DDVP (12 hr).

Because of the extreme toxicity to fish and the high levels indicative of nematicidal activity, investigation of DDVP was not included in an investigation of nematode evacuation by fish without nematicidal action. An exposure of 22 hr to the 100-μ liters/liter concentration of SD1836 was lethal (Table 3). However, three fish were alive at 21 hr. During this period as confirmed by necropsy all nematodes were passed. At the 25-μ liters/liter treatment one fish survived the 36-hr exposure. This fish was removed to an untreated tank where it

Table 2. Survival of uninfested rainbow trout exposed to 2,2 dichlorovinyl dimethyl phosphate (DDVP) and diethyl 2-chlorovinyl phosphate (SD1836) in-water at 58 F. Four fish in each treatment.

Treatment	Rate μl/l a.i.	Fish		Fish survival hours							
		Avg length (mm)	Avg weight (g)	1	2.5	5	12	17	24	36	48
Control	–	146	34.9	4	4	4	4	4	4	4	4
DDVP	50	142	32.2	0	–	–	–	–	–	–	–
DDVP	5	144	33.5	4	3	0	–	–	–	–	–
DDVP	1	145	34.2	4	4	0	–	–	–	–	–
DDVP	0.25	141	31.5	4	4	4	4	0	–	–	–
SD1836	100	138	29.5	4	4	4	4	4	0	–	–
SD1836	25	143	32.8	4	4	4	4	4	4	3	1

Table 3. In-water anthelmintic efficacy of diethyl 2-chlorovinyl phosphate (SD1836) against *Sterliadochona pedispicula* in infested wild rainbow trout at 58 F. Four fish in each treatment.

Treatment	Rate μl/l a.i.	Fish: Avg length (mm)	Fish: Avg weight (g)	Exposure hours	Fish survival	Nematode recovery: 24 hr	Nematode recovery: 48 hr	Nematode recovery: 72 hr	Nematode recovery: 144 hr	Necropsy 4 weeks
Control	–	136.5	29.1	–	4	–	–	–	–	61
SD1836	100	146.7	36.8	22	0	30	–	–	–	0*
SD1836	25	133.7	27.6	36	1	51	5	–	–	0*
SD1836	25	143.7	33.3	24	4	24	13	4	1	0
SD1836	5	123.5	21.1	72	4	2	25	9	2	0

* Examined immediately after death.

was maintained for 4 weeks without any signs of deleterious effects due to the treatment. In the 25-μ liters/liter treatment with exposure reduced to 24 hr and at the 5-μ liters/liter treatment with a 72-hr exposure all trout survived and were maintained for 4 weeks with no deleterious effects from the treatments. When these fish were sacrificed for necropsy the anthelmintic treatments were found to be 100% effective. Regardless of survival all treatments were 100% effective for evacuation of *S. pedispicula* from the intestinal tract. Because of the greater safety factor the 5-μ liters/liter treatment appears most promising.

Discussion

The in vitro studies for nematicidal activity contributed little to the establishment of parameters of chemical concentrations to be tested for anthelmintic activity. The most efficacious anthelmintic treatments were at concentrations far below concentrations required for nematicidal activity. For example, DDVP is approximately 100 times more toxic to immersed rainbow trout than it is to nematodes tested in vitro under the same conditions of temperature, concentration, and exposure period. A similar comparison with SD1836 indicates that the toxicological reaction of trout and nematodes is very similar; however, anthelmintic activity was well below the levels toxic to trout or the nematode. Anthelmintic efficacy, in these experiments, was measured by nematode release and passage rather than kill. No difference in anthelmintic activity was noted for different ages or stages of nematodes.

Because of the extreme toxicity of DDVP only SD1836 was tested as an in-water anthelmintic treatment of naturally infested wild trout. The results obtained with these in-water anthelmintic treatments are unlike those obtained with plastic-incorporated anthelmintic pellets (Maggenti, 1972). In the latter the administration of DDVP pellets directly into the gullet produced no direct toxic effects or indirect secondary effects over the range of rates tested. In addition, DDVP had a good anthelmintic potential over the range tested.

In-water treatments, as opposed to direct pellet treatments, provide the researcher with an opportunity to test anthelmintic efficacy with large populations of fish. Since these treatments can be accurately monitored they provide specific information as to the amount of chemical used and exact time of fish exposure. This study also indicates that a great latitude in size of fish treated is possible since anthelmintic activity is well below fish toxicity levels. The smallest fish in these tests was 11.7 cm and the largest 22 cm; no differences were noted in anthelmintic efficacy with size.

The primary concern in a study of nematodes as parasites of fish must be directed to the effects upon the fish. These parasites should not be ignored because they rarely utilize humans as alternate or definitive hosts. The expanding culture of fish in the United States, not just for recreational purposes but as a source of food, requires that a thorough understanding of all helminths and diseases be encouraged. Among the least appreciated parasites of fish are the nematodes. Treatments such as described here point to the feasibility of controlling at least the intestinal nematodes of freshwater fish. The use of anthelmintics to control nematodes of fish would act to prevent the introduction of dangerous parasites into hatcheries, lakes, and streams. That nematodes can severely debilitate fish and cause serious damage to their tissues and organs is well documented by Dogiel et al. (1958). In addition, the use of anthelmintics would prevent the contamination of eggs with the eggs of the nematode at the time of spawning. That contamination of eggs in the spawning nest can occur is documented by the work of Platzer (1967).

The release of nematodes prior to the time of spawning would serve to aid the fish during a time of stress. This may be especially true with steelhead trout which often must be maintained in holding ponds for prolonged periods of time prior to egg and sperm maturation. During this time when the fish are not being fed it would be far better for the fish to utilize its stored energies to support itself rather than to have these stores drained by parasites at the expense of gonadal maturation and fish survival.

Acknowledgments

I am grateful for the assistance of my wife, Mary Ann, and Mr. A. W. Johnson in the collection and transportation of the wild trout utilized in this research. Appreciation is due to Mr. G. A. Paxman for assistance in experimentation.

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Two New Gizzard Worms from Louisiana Birds, *Viguiera coccyzae* sp. n. from *Coccyzus americanus* and *Viguiera pari* sp. n. from *Parus carolinensis* and *Parus bicolor* (Nematoda: Spiruridae)

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ABSTRACT: Two new species of gizzard worms of the genus *Viguiera* are described from three species of North American birds. *Viguiera coccyzae* sp. n. from *Coccyzus americanus* differs from other species of the genus by the size and structure of the cephalic elaborations in the form of cuticular shields on the pseudolabia and its much greater spicule ratio of 1:30 or more. *Viguiera pari* sp. n. from *Parus carolinensis* and *P. bicolor* is most similar to *Viguiera osmanhilli* Yeh, 1955, but differs in the structure and size of the cephalic shields on the head, absence of a cephalic collar, and greater spicule ratio of 1:25 or more as compared to 1:20 in the latter. These are the first records of this genus from North America and from the host families Cuculidae and Paridae.

Nematodes of the genus *Viguiera* were recovered from under the gizzard lining of three species of Louisiana birds. These represent species distinct from others of the genus and are described herein as *Viguiera coccyzae* sp. n. from *Coccyzus americanus* and *V. pari* sp. n. from *Parus carolinensis* and *P. bicolor*, respectively.

Worms were fixed briefly in glacial acetic acid, stored in a mixture of 95 parts of 70% ethyl alcohol and 5 parts glycerin, and studied in glycerin mounts after the evaporation of the alcohol. All measurements are in millimeters unless otherwise indicated and the range of measurements and their means of the paratypes follow in parentheses the values for the holotype and allotype. Drawings were made with the aid of a camera lucida.

Viguiera coccyzae sp. n.
(Figs. 1–8)

DESCRIPTION: Spiruridae Oerley, 1885, Habronematinae Chitwood and Wehr, 1932, *Viguiera* Seurat, 1913. With characters of the genus. Long slender brownish-red nematodes. Cuticle thick, transversely striated, transparent, slightly inflated to form an indistinct collar in the cervical region. Oral opening dorsoventrally elongate, two pairs of teeth on anterior border of each pseudolabium. Anteriorly each pseudolabium bears one large dorsally and one large ventrally directed cuticular elaboration in the form of a winglike shield which extends some distance beyond the body margins and laterally a pair of smaller bifurcate shields which extend slightly beyond the pseudolabial margin. These processes form a rooflike covering over the head. Three pairs of small papillae in the inner circle, two pairs of large papillae in the outer circle represented by a single pair on each labium, one pair of amphids located between bifurcate shields on each pseudolabium. Pharynx cuticularized, short. Esophagus with short muscular and very long glandular portions. Excretory pore at level of, or slightly above, nerve ring. Cervical papillae lateral, slightly posterior to nerve ring. Vulva in extreme posterior of body, just anterior to anus. Posterior extremity of male twisted and spirally rolled, caudal alae asymmetrical, six pairs of large sessile preanal papillae, two pairs of sessile postanal papillae. Spicules very un-

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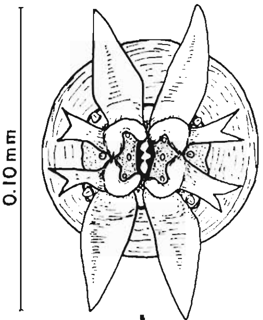
Figures 1–8. *Viguiera coccyzae* sp. n. 1. *En face* view of head. 2. *En face* optical section at level where labia and pseudolabia separate (pseudolabia stippled). 3. Lateral view of head. 4. Ventral view of head. 5. Anterior extremity of female. 6. Egg. 7. Posterior extremity of female. 8. Posterior extremity of male.



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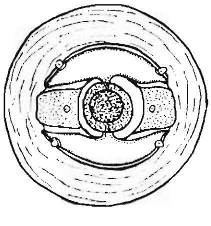

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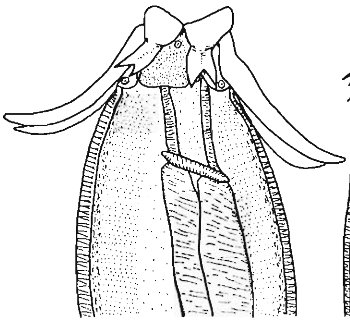

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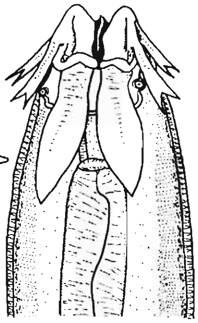

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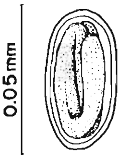

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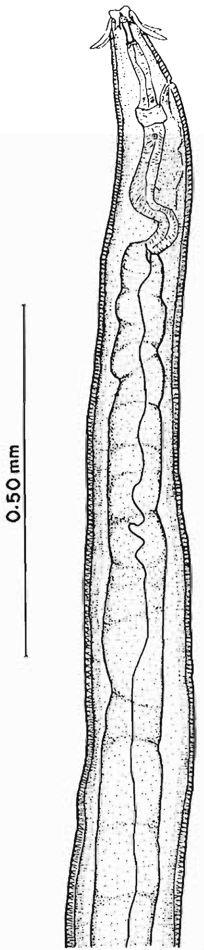

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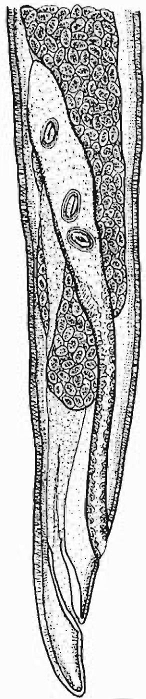

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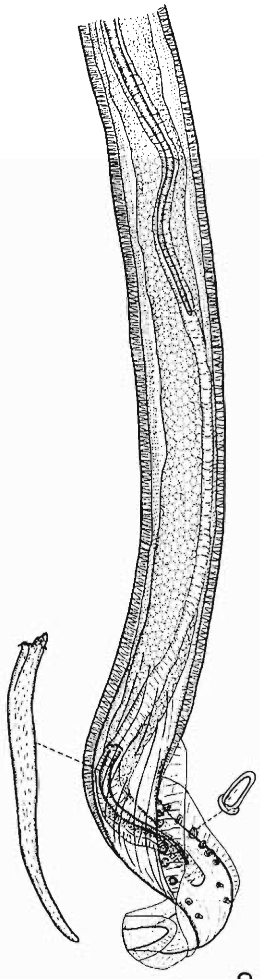

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equal and dissimilar, right spicule short and stout, left spicule long and slender about 30 times length of right spicule sometimes reaching level of posterior of glandular esophagus. Eggs thick-shelled, with well-develop larvae when laid.

Male (based on holotype and five paratypes): 10.1 (10.0 to 11.5, 10.2) long, 0.150 (0.125 to 0.150, 0.140) wide (maximum). Excretory pore, nerve ring, and cephalic papillae 0.136 (0.120 to 0.145, 0.137), 0.136 (0.132 to 0.143, 0.136), and 0.165 (0.143 to 0.200, 0.176) from anterior extremity, respectively. Pharynx 46 μ (33 to 46, 39) long. Muscular and glandular portions of esophagus 0.280 (0.240 to 0.320, 0.290) and 2.30 (1.80 to 2.50, 2.20) long, respectively. Right and left spicules 0.200 (0.175 to 0.215, 0.200) and 6.85 (5.60 to 7.70, 6.85) long, respectively. Ratio of right and left spicules 1:34 (1:28 to 1:38, 1:35). Anus 0.200 (0.155 to 0.210, 0.190) from posterior extremity.

Female (based on allotype and 14 paratypes): 17.7 (12.9 to 20.0, 15.3) long, 0.190 (0.145 to 0.200, 0.170) wide (maximum). Excretory pore, nerve ring, and cervical papillae 0.136 (0.115 to 0.160, 0.140), 0.135 (0.125 to 0.150, 0.135), and 0.165 (0.135 to 0.205, 0.165) from anterior extremity, respectively. Pharynx 44 μ (36 to 50, 41) long. Muscular and glandular portions of esophagus 0.300 (0.205 to 0.400, 0.295) and 2.80 (2.15 to 2.60, 2.35) long, respectively. Vulva and anus 0.215 (0.210 to 0.300, 0.225) and 0.090 (0.090 to 0.120, 0.105) from posterior extremity, respectively. Eggs 44 μ (37 to 44, 41) long, 26 μ (20 to 26, 23) wide.

Host: *Coccyzus americanus*, the yellow-billed cuckoo.

Location: Under lining of gizzard.

Locality: Mandeville, Louisiana, USA (2 ♀♀, 1 ♂ from a single host collected 12 May 1970 and 10 ♀♀, 3 ♂♂ and 3 ♀♀, 3 ♂♂ collected from two hosts, respectively, 6 June 1970 by D. B. Pence).

Holotype: ♂, USNM Helm. Coll. No. 72246.

Allotype: ♀, USNM Helm. Coll. No. 72247.

Paratypes: 2 ♂♂, 2♀♀, USNM Helm. Coll. No. 72248, remaining specimens in collection of author.

Pathology: These worms form small tracts in the mucosa under the lining of the gizzard as they migrate, but these produce little observable effect on the host.

Viguiera pari sp. n.
(Figs. 9–16)

Description: Spiruridae Oerley, 1885, Habronematinae Chitwood and Wehr, 1932, *Viguiera* Seurat, 1913. With characters of the genus. Long slender white nematodes. Cuticle thick, transparent, transversely striated. Oral opening dorsoventrally elongate, teeth not observed. Anteriorly each pseudolabium bears cuticular extensions in the form of one dorsally and one ventrally directed short process which does not extend beyond the body margins but is directed posteriorly lying flat against the cuticle and a pair of larger and wider laterally directed winglike shields which extend a short distance beyond the body margins. These processes form a rooflike covering over the head. Three pairs of small papillae in the inner circle, two pairs of large papillae in the outer circle represented by one pair on each labium, amphids located on lateral winglike expansions on pseudolabia. Pharynx cuticularized, long. Excretory pore at level of, or slightly anterior to, nerve ring. Muscular esophagus short, glandular esophagus very long. Vulva and anus very close to posterior extremity, vulva slightly inflated and cuticularized at opening. Posterior extremity of male twisted and spirally coiled, caudal alae asymmetrical, with six to eight pairs of preanal papillae, two pairs of postanal papillae. Spicules very unequal and dissimilar, right spicule short and stout, left spicule long and slender

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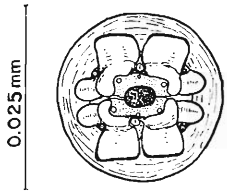
Figures 9–16. ***Viguiera pari*** **sp. n. 9.** ***En face*** **view of head. 10.** ***En face*** **view at optical section where labia and pseudolabia separate (Pseudolabia stippled). 11. Lateral view of head. 12. Ventral view of head. 13. Anterior extremity of female showing pharyngoesophageal junction in detail. 14. Egg. 15. Posterior extremity of female. 16. Posterior extremity of male.**



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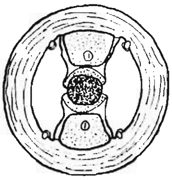

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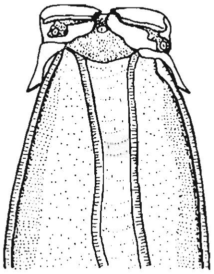

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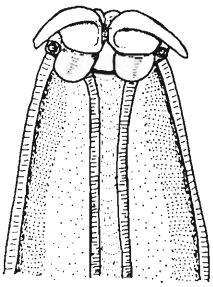

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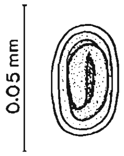

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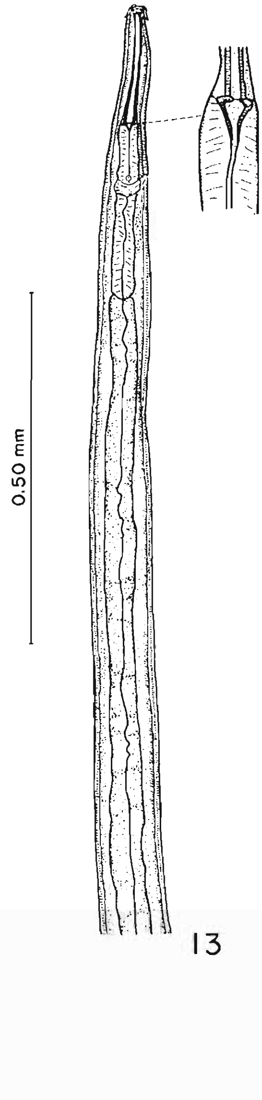

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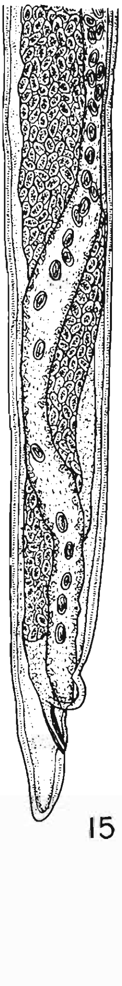

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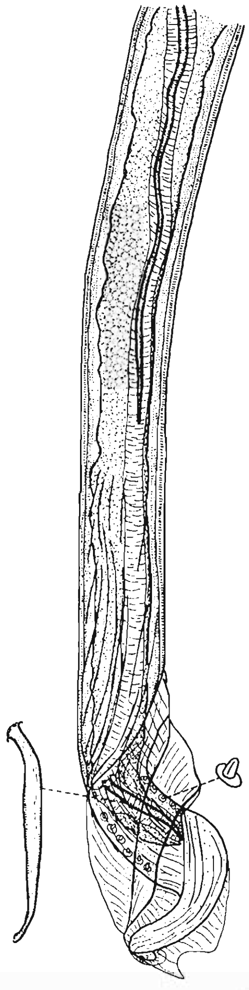

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about 25 times longer than right spicule and sometimes reaching to level of posterior of glandular esophagus. Eggs thick-shelled, with well-developed larvae when laid.

MALE (based on holotype and five paratypes): 7.85 (7.35 to 8.70, 7.95) long, 0.140 (0.125 to 0.145, 0.135) wide (maximum). Excretory pore, nerve ring, and cephalic papillae 0.190 (0.170 to 0.215, 0.200), 0.195 (0.175 to 0.205, 0.190), and 0.185 (0.160 to 0.200, 0.185) from anterior extremity, respectively. Pharynx 0.130 (0.110 to 0.150, 0.130) long. Muscular and glandular esophagus 0.220 (0.165 to 0.240, 0.220) and 1.75 (1.45 to 1.85, 1.70) long, respectively. Right and left spicules 0.165 (0.150 to 0.175, 0.160) and 4.10 (4.20 to 5.00, 4.50) long, respectively. Ratio of length of right and left spicules 1:25 (1:25 to 1:31, 1:28).

FEMALE (based on a single specimen, the allotype): 17.3 long, 0.165 wide (maximum). Excretory pore, nerve ring, and cervical papillae 0.180, 0.185, and 0.190 from anterior extremity, respectively. Pharynx 0.130 long. Muscular and glandular portions of esophagus 0.230 and 2.40 long, respectively. Vulva and anus 0.165 and 0.105 from posterior extremity, respectively. Egg (last egg in uterus) 34 long, 22 wide.

HOSTS: *Parus carolinensis*, the Carolina chickadee (type host), and *Parus bicolor*, the tufted titmouse.

LOCATION: Under lining of gizzard.

LOCALITY: Covington, Louisiana, USA (5 ♂♂ from *Parus carolinensis* collected 17 May 1969 and 1 ♂, 1 ♀ from *Parus bicolor* collected 15 June 1969 by D. B. Pence).

HOLOTYPE: ♂, USNM Helm. Coll. No. 72249.

ALLOTYPE: ♀, USNM Helm. Coll. No. 72250.

PARATYPES: 2 ♂♂, USNM Helm. Coll. No. 72251, remaining specimens in collection of author.

PATHOLOGY: There was no observed gross pathology.

The two new species reported herein are the first records for the genus *Viguiera* from North America. Eleven other species are known from birds in South America, Africa, Madagascar, Europe, India, Pakistan, and Australia. Mawson (1968) has provided a key for these species.

The systematic position of *Viguiera* and allied genera which were originally placed in the Schistorophinae (Acuariidae) by most authors has long been the subject of considerable confusion. However, Chabaud (1960) demonstrated that the head of *Viguiera* and certain other genera is typical of the Habronematinae (Spiruridae). More recently, Inglis (1965) studied the comparative morphology of the nematodes parasitic in the gizzards of birds. Many of the conclusions of Chaubaud (1958, 1960) and Dollfus and Chabaud (1957) concerning the systematics of these genera were confirmed and a convincing case for morphological convergence in the head structures in the species of the Spiruridae (Habronematinae), Acuariidae, and Amidostomidae which occur under the gizzard lining of avian hosts was presented.

Viguiera coccyzae is the first record of this genus from the host order Cuculiformes. The new species differs from all others by the shape and large size of the cuticular shields in the form of a pair of very long dorsoventrally directed processes and a smaller pair of lateral bifurcate shields on each pseudolabium. It differs from all other species except *V. chabaudi* Mawson, 1967 and *V. pari* in having a spicule ratio of 1:30 or more which is 1:20 or less in the remaining species of the genus. It is decidedly different from these two species in the structure of the head shields.

The new species, *V. pari*, appears most similar to *V. osmanhilli* described from *Cyanerpes cyaneus* from Brazil by Yeh (1954), but differs in its larger spicule ratio of 1:25 or greater as compared to 1:20 or less, absence of a head collar, and principally in the structure of the cephalic extensions on the head which are more pronounced and with a different structure in the lateral winglike processes. This is the first record of this genus from the host family Paridae.

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Capillaria maseri sp. n. (Nematoda) from Insectivores (Soricidae and Talpidae) in Oregon

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ABSTRACT: *Capillaria maseri* sp. n. is described from the urinary bladder of shrews, *Sorex* spp. (type host: *S. yaquinae* Jackson) in Oregon, and is recorded also from the shrew-mole, *Neürotrichus gibbsii* (Baird). *C. maseri* is the first capillariid to be reported from the urinary bladder of North American insectivores.

In 1970–72, nematodes of the genus *Capillaria* Zeder, 1800, were found in the urinary bladder of insectivores collected in Oregon by Chris and Rita Maser, Oregon Coast Ecological Survey, Puget Sound Museum of Natural History, who kindly sent the specimens to us for study. The species involved differs from any recorded from insectivores in Eurasia and North America and is described herein.

Materials and Methods

Most of the specimens had been fixed *in situ* in 10% formalin solution. Fifty-six were studied in detail, including some that were broken in dissecting the anterior ends from tissue of the urinary bladder. They were cleared by evaporation of a mixture of 2% glycerin in 70% ethanol, after which selected specimens were stained in light green SF dissolved in glycerin. Two bladders with nematodes *in situ* were embedded in paraffin, sectioned at 0.005 or 0.010 mm, and stained in hematoxylin–eosin, in Mallory's aniline blue collagen stain, or by the periodic acid-Schiff reaction. For comparison, sections were similarly prepared from the normal urinary bladder of a shrew collected in Alaska. The hosts were identified by Mr. Maser. The nomenclature of shrews is in accordance with the recommendations of Johnson and Ostenson (1959).

Results

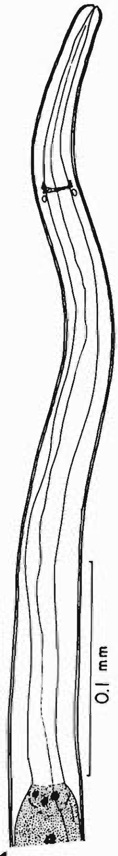
Description

Numbers of specimens from which the respective measurements were derived are shown in parentheses. All measurements are in millimeters.

Capillaria maseri sp. n.
(Figs. 1–8)

Slender nematodes; white in color when preserved. Bacillary lines present. Mouth simple. Cuticle smooth.

MALE (23 specimens; 8 intact): Length (8) 7.3 to 20.5 (avg 17.1). Maximum width (22) 0.099 to 0.155 (avg 0.123), attained in posterior third of body. Width at cephalic extremity (8) 0.008 to 0.011 (avg 0.010); width at posterior extremity (23) 0.031 to



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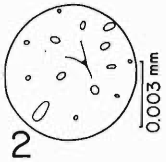

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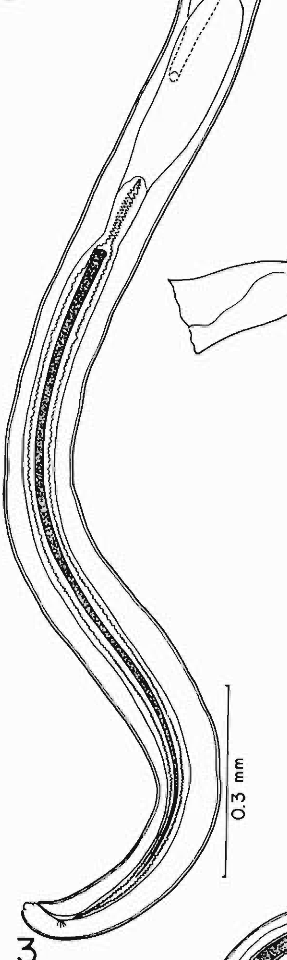

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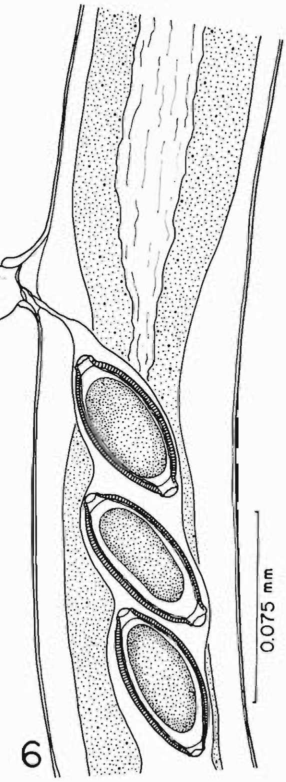

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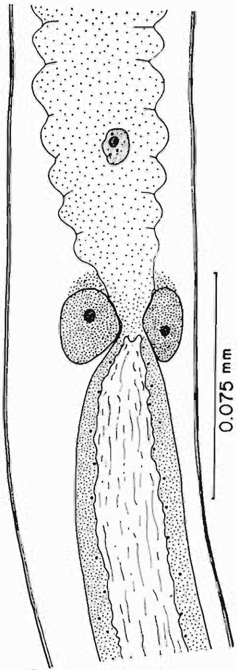

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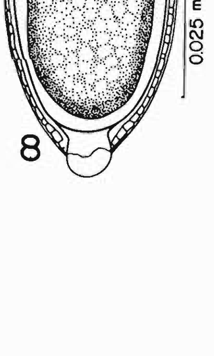

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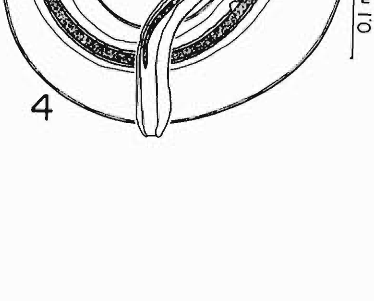

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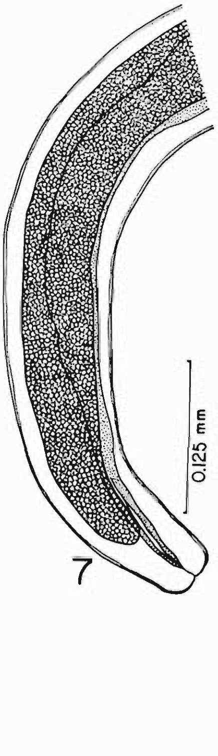

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0.036 (avg 0.032). Esophagus (8) 3.7 to 7.9 long (avg 6.5); width at base (19) 0.049 to 0.065 (avg 0.056). Length of muscular esophagus (4) 0.356 to 0.389 (avg 0.375). Ratio of length of esophagus to length of body (8) 1:1.9 to 1:3.1 (avg 1:2.6). Twenty-six to 48 esophageal cells observed. Distance from posterior end of esophagus to testis (19) 0.356 to 1.178 (avg 0.645). Length of spicule (23) 0.710 to 1.134 (avg 0.970). Spicule sheath aspinose, with minute folds. Caudal alae not present.

FEMALE (33 specimens; 10 intact): Length (10) 7.7 to 26.8 (avg 18.4). Maximum width (32) 0.073 to 0.168 (avg 0.145), attained in posterior third of body (containing uterus). Width at cephalic extremity (7) 0.008 to 0.011 (avg 0.010). Width at posterior extremity (33) 0.032 to 0.057 (avg 0.044). Width at level of vulva (30) 0.057 to 0.121 (avg 0.092). Esophagus (10) 2.93 to 10.4 (avg 7.5) long; width at base (28) 0.049 to 0.070 (avg 0.062). Length of muscular esophagus (5) 0.378 to 0.470 (avg. 0.434). Thirty-nine to 41 esophageal cells observed. Ratio of length of esophagus to length of body (10) 1:1.6 to 1:2.9 (avg 1:2.5). Nerve ring (5) ca. 0.090–0.115 from anterior end. Distance from posterior end of esophagus to vulva (28) 0.518 to 1.871 (avg 1.145). Distance from cephalic extremity to vulva (9) 3.5 to 11.4 (avg 9.0). Anus terminal. Egg (178) 0.060 to 0.073 by 0.024 to 0.032 (avg 0.065 by 0.028); shell smooth; polar plugs well defined.

TYPE HOST: *Sorex yaquinae* Jackson. Other hosts *S. vagrans* Baird, *S. bendirii* (Merriam), *S. trowbridgii* Baird, *S. pacificus* Coues, and *Neürotrichus gibbsii* (Baird).

TYPE LOCALITY: Cascade Head Experimental Forest, Lincoln County, Oregon.

HABITAT: Urinary bladder.

TYPES: USNM Helm. Coll. No. 72256 holotype (male) and No. 72257 allotype; Nos. 72258 and 72259 (paratypes).

Discussion

With the exception of *C. hepatica* (Bancroft, 1893), which has been reported from mammals of diverse groups, species of *Capillaria* from mammals are usually host-specific. That certain species have been found in hosts representing two or more related families indicates that their occurrence may be influenced by ecologic, as well as phylogenetic, factors. Such is the case with *C. maseri*, occurring in both shrews (Soricidae) and moles (Talpidae). However, in any host infected, the respective species appear to be organ-specific. In Eurasia, nematodes of the genus *Capillaria* have been found in the urinary bladder of moles and shrews, but none has been reported from this organ in North American insectivores. The two species known from insectivores in North America, *C. rauschi* Read, 1949, and *C. blarinae* Ogren, 1953, were described from the small intestine of *Sorex cinereus* Kerr and the esophagus of *Blarina brevicauda* (Say), respectively. Wakelin (1968) suggested that *C. rauschi* is possibly identical with *C. kutori* Rukhliadeva, 1946, described from the stomach of water shrews, *Neomys fodiens* (Pennant), from the Khopersk Reserve, European Russia. His hypothesis, if valid, would add another species of the genus *Capillaria* from mammals to those few known to be holarctic, of which none is from insectivores. It seems improbable, on zoogeographic grounds, that the two are conspecific; unfortunately, a conclusion cannot be made from the descriptions of *C. kutori* (Rukhliadeva, 1946; Soltys, 1954). *C. kutori* appears to be a common parasite of shrews in eastern Europe (Prokopič, 1959; Arzamasov et al., 1969), and it was identified by Wakelin (1968) from the intestine of *Sorex araneus* L. in England.

Comparisons

A distinctive morphological characteristic of *C. maseri* is the relatively great distance between the end of the esophagus and the vulva

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←

Figures 1–8. *Capillaria maseri* sp. n. 1. Anterior end of female. 2. *En face* view of cephalic end of male, slightly oblique. 3. Posterior end of male. 4. Caudal extremity of male, with partially everted spicule. 5. Terminal portion of esophagus, female. 6. Details of vulva and associated structures. 7. Caudal extremity of female. 8. Structure of egg.

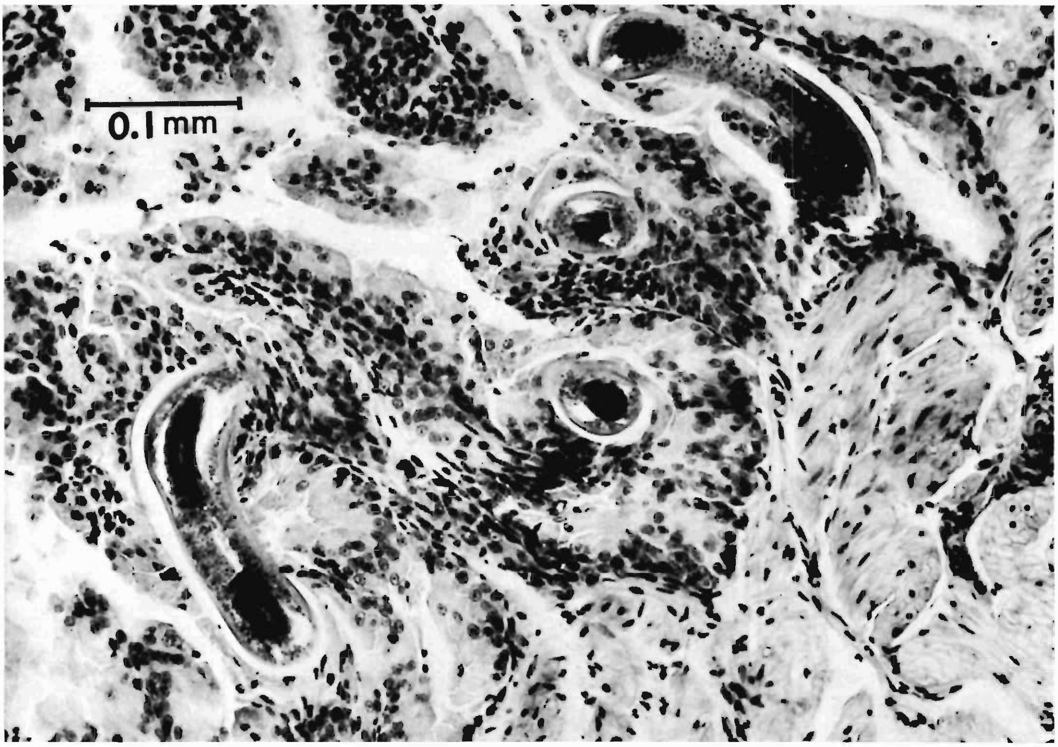

Figure 9. **Section of wall of bladder (*Sorex yaquinae*) showing anterior ends of *C. maseri* embedded in the mucosa. PAS stain, 0.010-mm section.**

(avg 1.145 mm). *C. maseri* differs from the two species previously described from North American shrews in other characters as well as in habitat. From *C. rauschi*, it is distinguished by the presence of bacillary lines, smooth cuticle, different position of the vulva, and smooth-shelled eggs of larger size; from *C. blarinae* by the larger size, longer esophagus, longer spicule, absence of spines in spicule sheath, and, in females, by the presence of an external vulvar process and a terminal anal opening.

Several species of *Capillaria* occurring in the urinary bladder of insectivores have been described in Eurasia. *C. capillaris* (von Linstow, 1882) and *C. incrassata* (Diesing, 1851) have been recorded from both moles and shrews. Wakelin (1968) concluded that *C. capillaris* could be synonymized with *C. incrassata*, a determination that was not accepted by Prokopič and Mahnert (1970) or Nadtochii and Rasskazova (1971). Both species have been found in western Eurasia as well as in Kamchatka, in northeastern Siberia (Nadtochii and Rasskazova, 1971).

C. urinicola Soltys, 1952, described from shrews in Poland, was later considered by Soltys (1954) to be identical with *C. capillaris*. However, comparisons by Furmaga (1959) led to the conclusion that *C. urinicola* is distinct.

The similarity between *C. sunci* Chen, 1937, described from *Suncus caeruleus* (= *S. murinus* L.) (Soricidae) in China, and *C. incrassata* was recognized by López-Neyra (1947, p. 140). Wakelin (1968, p. 392) stated that this species ". . . may well be considered a synonym of *C. incrassata*."

C. reni Shaldybin, 1968, described from water shrews from the Mordovsk Reserve in European Russia, might also be considered here, although it was found in the renal pelvis rather than in the urinary bladder.

C. maseri may be distinguished as follows from these other species inhabiting the urinary system of insectivores: from *C. capillaris* by its larger size, longer esophagus, vulva situated farther posteriorly, and smooth-shelled egg; from *C. incrassata* by its larger size, more slender anterior end, smooth cuticle, and longer spicule; from *C. urinicola* by its larger size, longer esophagus, presence of vulvar process, and a much larger egg; from *C. reni*, of similar large size, by its longer spicule and, in the female, by its terminal anal opening and smaller egg. The description of *C. reni* does not permit comparison of other anatomical details.

Parasite–host relationships

C. maseri occurred in 43 (31%) of 138 shrews collected during November 1970–April 1972. It was found also in one of seven shrew-moles, *Neürotrichus gibbsii*, but in none of 16 Townsend's moles, *Scapanus townsendii* (Bachman), or in 17 coast moles, *S. orarius* True. In 32 infected shrews, not segregated by species, numbers of nematodes ranged from 1 to 29, with an average of 4. The ratio of males to females in this series was 1:1.7. Five specimens were found in the bladder of the shrew-mole. Findings in mammals of the respective species are summarized in Table 1. Mammals of both sexes were infected.

The nematodes formed localized aggregations on the floor of the urinary bladder in the vicinity of the neck, with the anterior portion of their bodies deeply embedded in the mucosa. Males penetrated to the full length of the esophagus, females to the level of the vulva, permitting discharge of eggs directly into the lumen of the bladder. In sections of incised, contracted bladders, in which the mucosa was thrown into deep, irregular folds, the anterior portions of the nematodes extended erratically through the thick epithelial layer, but not penetrating the connective tissue of the lamina propria, nor did they pass through the mucosa into the underlying muscularis (Fig. 9). No observations were made on relationships in the distended bladder, in which the respective layers of tissue would be much flattened. Findings in tissue sections indicate that *C. maseri* is not pathogenic in shrews. The embedded portions of the nematodes were immediately surrounded by normal-appearing epithelial cells; leukocytic infiltration, fibrosis, or other evidence of an inflammatory response were not observed. No eggs were found within the tissue of the host. The tissue reaction, if any, evoked by other species of *Capillaria* inhabiting the urinary bladder of insectivores apparently has not been described. A slide with sections of the nematode *in situ* has been deposited in the USNM Helm. Coll., No. 72260. No early-stage infections involving newly established nematodes were detected, although a few immature specimens were found. In the shrew-mole, one of the nematodes was within the ureter; however, migration from the bladder might have taken place after the death of the host.

Table 1. Occurrence of *C. maseri* in shrews and moles.

Host	No. examined	No. infected
Soricidae	138	43
Sorex vagrans	16	10
S. pacificus	27	5
S. yaquinae	23	9
S. bendirii	13	3
S. trowbridgii	59	16
Talpidae	40	1
Neürotrichus gibbsii	7	1
Scapanus orarius	17	–
S. townsendii	16	–

Most of the insectivores were captured along the coast of Oregon in the vegetational zone characterized by Sitka spruce, a region of low elevation, mild climate, and high annual precipitation (Franklin and Dyrness, 1969). Of the five species of shrews involved, *S. trowbridgii*, *S. pacificus*, and *S. yaquinae* inhabit forest and are nocturnal. *S. bendirii* is found near water, while *S. vagrans* inhabits meadows as well as grassy areas along streams; both are active at any time of day. During the drier months (summer and autumn), all but *S. vagrans* were usually trapped within 25 m of streams. The shrew-mole has shrewlike habits; it usually occurs in forest and is active at any time of day. The infected insectivores were trapped in moist habitat in seral vegetation. *C. maseri* was found also in a single specimen of *S. bendirii* from the western foothills of the Cascade Range (Lane County), at an elevation of about 1,000 m. (The foregoing data were provided by C. Maser.)

Seasonal differences in rates of infection in shrews could not be discerned. Infected animals were obtained during all months but September and October. The lowest annual rates would be expected in autumn, when the populations of shrews consist predominantly of young animals.

Acknowledgments

We express thanks to Mr. and Mrs. Chris Maser, who provided the material studied as well as a record of their field observations, and to Dr. B. Hörning, Vet.-Bakteriologisches und Parasitologisches Institut, Universität Bern, Switzerland, who provided the otherwise unavailable description of *C. reni*.

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A New Subfamily, Two New Genera, and Three New Species of Haploporid Trematodes[1]

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ABSTRACT: A new subfamily, Unisaccinae, of the family Haploporidae is established to include the new genus *Unisaccus* and the new species, *U. brisbanensis* and *U. spinosus*; and the new genus and species *Unisaccoides vitellosus* found in the intestines of mullet in the Brisbane River, Queensland, Australia. These worms are unique among haploporids in having a single cecum.

During sabbatical leave (1970–71) spent in the Parasitology Department, University of Queensland, Brisbane, Australia, Brisbane River mullet were found to harbor in their small intestines some trematodes that are unique among haploporids in the possession of an undivided cecum. They are described as two new genera and three new species of a new subfamily.

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[1] Supported by NSF G6962.

The worms were fixed without pressure by injecting them into hot 5% formalin. They were stained with Mayer's iron alum or Mayer's paracarmine. Living specimens were also studied. All measurements are in microns unless designated otherwise; averages are in parentheses.

Unisaccinae n. subfamily

DIAGNOSIS: Haploporidae. Body small, tegument spinose. Eyespot remnants present. Oral sucker subterminal, acetabulum in anterior half of body. Cecum undivided. Hermaphroditic duct with or without spirally arranged pads bearing spines. Internal and external seminal vesicles present, the former enclosed along with prostate cells and prostatic bulb in hermaphroditic sac. Testis single in posterior half of body. Vitellaria follicular. Ovary immediately anterior to testis. Laurer's canal and seminal receptacle uterinum present. Embryonated egg with eyespotted miracidium (as far as known). Excretory bladder Y-shaped with sphincter near exit. Type genus *Unisaccus*.

Unisaccus gen. n.

DIAGNOSIS: Unisaccinae. Body fusiform, oral sucker subterminal, acetabulum in anterior half of body. Prepharynx, pharynx, and esophagus well developed. Cecal sac lobed or nonlobed. Vitelline follicles few, lateral between levels of testis and acetabulum. Ovary small, simple, near anterior margin of testis. Laurer's canal and receptaculum seminis uterinum present. Uterus extends to near posterior end of body. Eggs relatively large and numerous, each mature one with eyespotted miracidium containing the outline of a redia. Testis in posterior half of body. Hermaphroditic duct with spined pads. Genital pore midventral between acetabulum and pharynx. Type species *Unisaccus brisbanensis*.

Unisaccus brisbanensis sp. n.
(Figs. 1–3, 6)

DESCRIPTION (measurements based on 24 mounted specimens): With characters of the genus. Tegument spined to near posterior end of body. Body length 700–840 (780), width 260–400 (320). Oral sucker 73–90 (75) long, 64–100 (90) wide; acetabulum 80–84 (82) in diam; prepharynx and esophagus subequal, 170–280 (230) long; pharynx 22–44 (35) long, 30–68 (55) wide; cecum may bear shallow pouches at corners. Hermaphroditic sac 106–166 (129) long, 90–190 (150) wide; hermaphroditic duct with spirally arranged pads about 20 long, each bearing two spines about 7 long and supported by a delicate sclerotized basal lattice about 7 long. Internal seminal vesicle tubular; external vesicle saccular and filled with sperm in mature specimens. Sperm about 142 long. Testis oval to spherical, 97–147 (122) long, 56–101 (81) wide; ovary median, oval to spherical, 13–18 (16) long, 12–22 (17) wide: Laurer's canal opens dorsally posterior to ovary, with proximal bulb containing cilia which beat toward exit. Vitelline follicles 4 to 6. Uterus from near posterior end of body to acetabular level. Eggs operculate, with thin yellow shells 46–64 (58) long, 24–29 (27) wide. Arms of excretory bladder longer than stem, bladder often containing a small concretion.

Holotype deposited as No. 715 in the Hancock Parasitology Collection, University of Southern California.

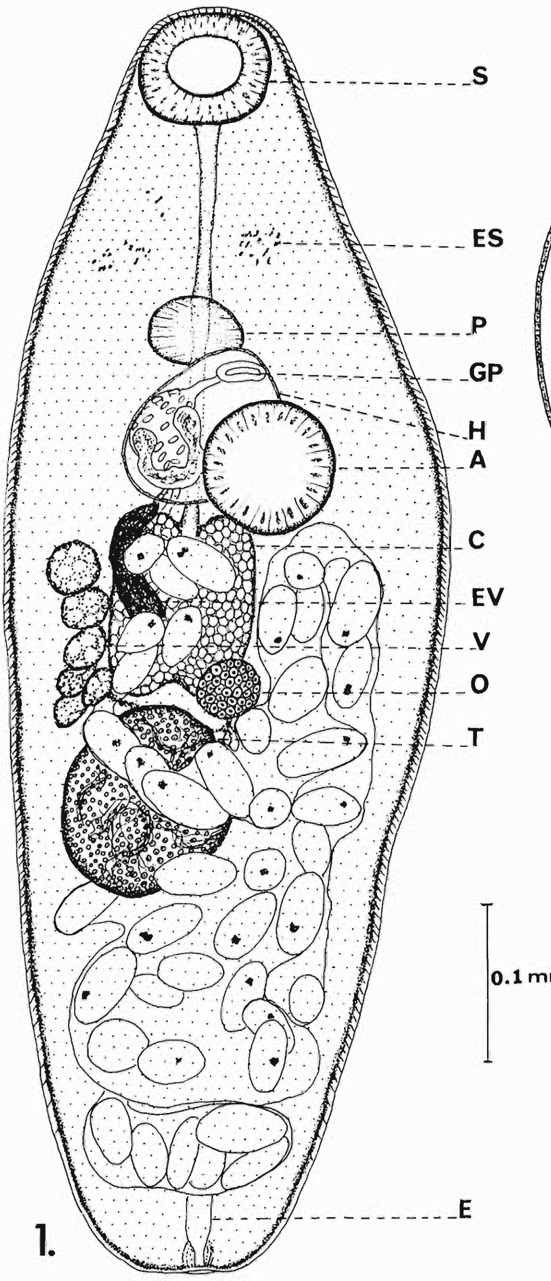
HOST: *Mugil cephalus* L.

SITE: Small intestine.

LOCALITY: Brisbane River, Brisbane, Queensland, Australia.

Unisaccus spinosus sp. n.
(Figs. 4, 5, 8)

DESCRIPTION (measurements based on 55 mounted specimens): With characters of genus. Tegument spined, body 500–774 (700) long, 146–399 (274) wide. Mouth subterminal; oral sucker 112–188 (159) long, 130–217 (192) wide; acetabulum spined, 97–160 (131) in diameter. Prepharynx 195–290 (248) long; pharynx 72–116 (91) long, 130–203 (174) wide; esophagus about as long as pharynx; cecum with shallow pockets at corners. Testis nearly spherical to oblong, 73–206 (171) long, 80–210 (168) wide. External seminal vesicle elongate and saccular. Hermaphroditic duct lining bears spiraling rows of pads, each pad with two or rarely three spines on a reticular sclerotized base. Ovary nearly spherical, 43–73 (61) in diam. Laurer's canal as in preceding species. Eggs with thin,



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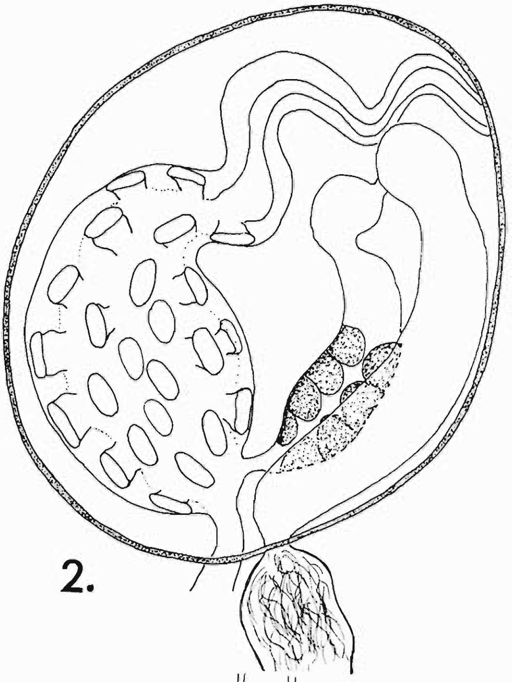

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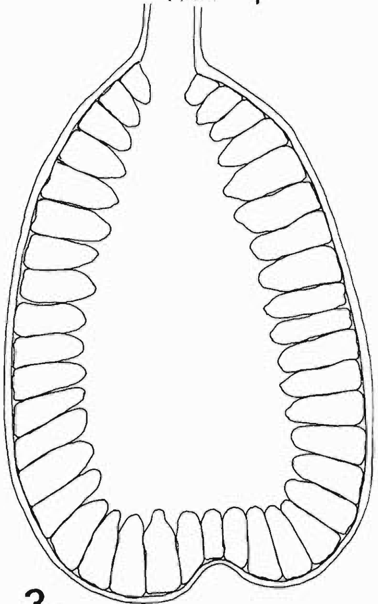

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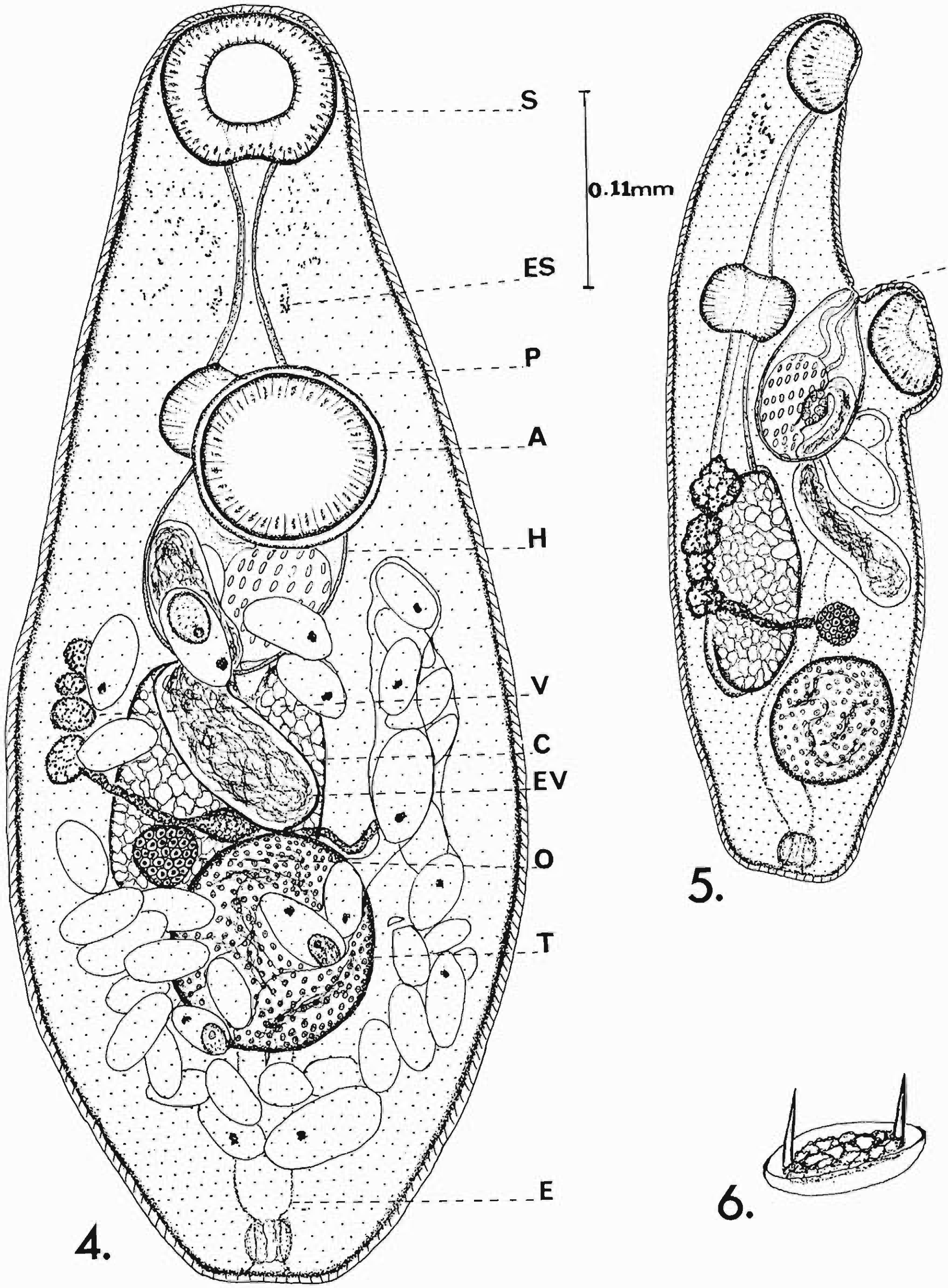
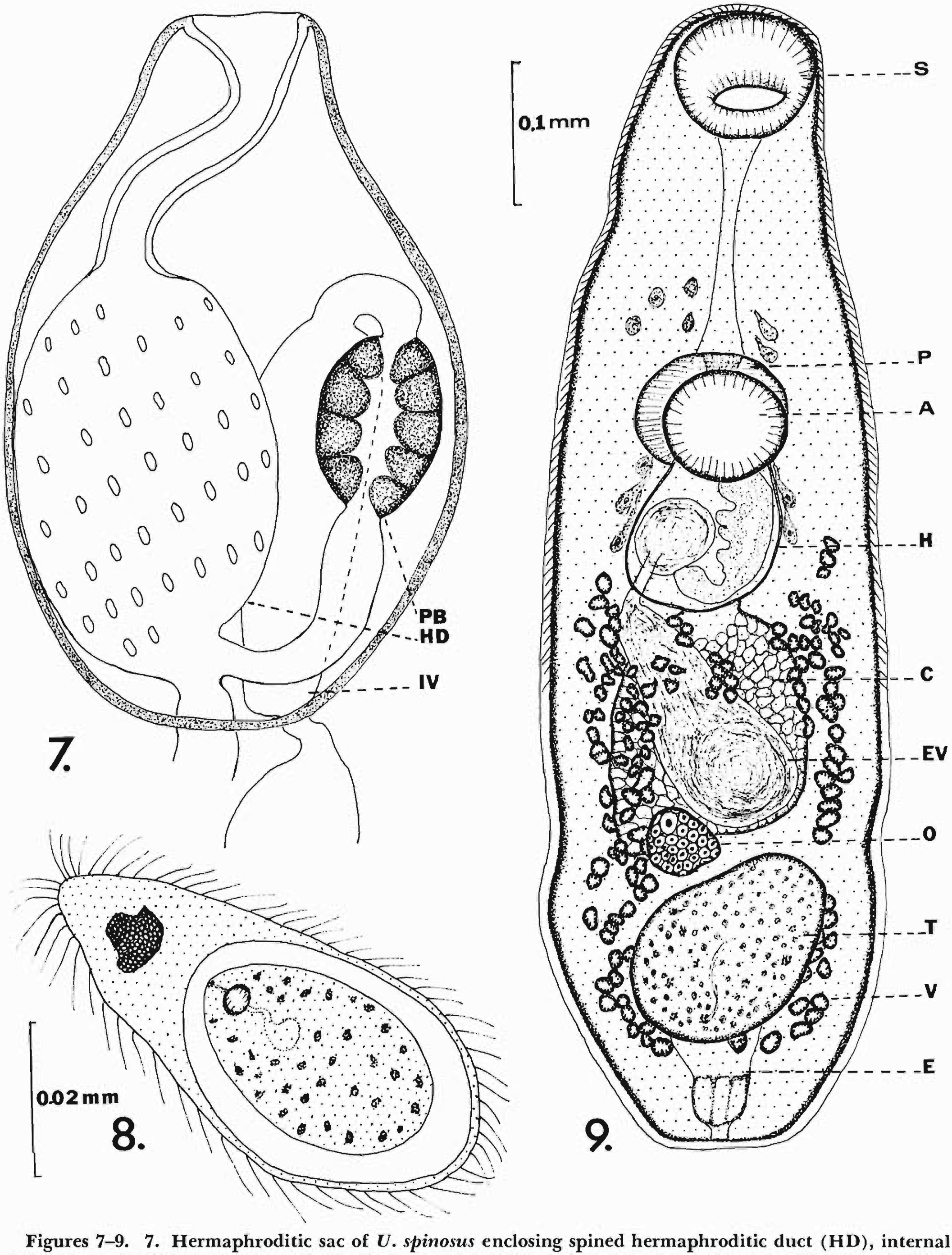
Figures 1–3. 1. Ventral view of *Unisaccus brisbanensis*. A, acetabulum; C, cecum; E, excretory bladder; ES, eyespot remnants; EV, external seminal vesicle; GP, genital pore; H, hermaphroditic sac; O, ovary; P, pharynx; S, oral sucker; T, testis; V, vitellaria. 2. Hermaphroditic sac enclosing internal seminal vesicle, prostate bulb, and spined hermaphroditic duct. 3. Optical section of cecum.



Figures 4–6. 4. Ventral view of *Unisaccus spinosus*. Symbols same as Figure 1. 5. Lateral view of *U. spinosus*. 6. Spine pad, spines, and sclerotized support of *U. brisbanensis*. Spine pads of *U. spinosus* similar but smaller and more numerous.



Figures 7–9. 7. Hermaphroditic sac of *U. spinosus* enclosing spined hermaphroditic duct (HD), internal seminal vesicle (IV), and prostate bulb (PB). 8. Miracidium of *U. spinosus* with enclosed redia. 9. *Unisaccoides vitellosus*, ventral view.

yellow, operculated shells, 90–106 (102) long and 40–66 (53) wide; embryonated egg with ocellate miracidium containing a well-developed redia about 37 long and 25 wide, with pharynx about 10 long and 12 wide. Hermaphroditic sac 203–300 (260) long, 130–260 (210) wide. Genital pore immediately anterior to acetabulum. Excretory bladder with fairly long stem, arms reaching to level of vitellaria.

Holotype deposited as No. 717 in the Hancock Parasitology Collection.

HOST: *Lisa argentea* (Quoy and Gaimard).

SITE: Small intestine.

LOCALITY: Brisbane River, Brisbane, Queensland, Australia.

Unisaccoides gen. n.

DIAGNOSIS: As in *Unisaccus* except vitelline follicles numerous, mainly lateral, from hermaphroditic sac to near posterior end of body where they may become confluent; uterus from testis to hermaphroditic sac. Type species *Unisaccoides vitellosus*.

Unisaccoides vitellosus sp. n.
(Fig. 9)

DESCRIPTION (measurements based on four specimens, only one ovigerous): Body 510–790 (650) long, 140–300 (217) wide. Oral sucker 50–80 (67) long, 59–90 (77) wide. Prepharynx 90–109 (99) long; pharynx 44–80 (60) long, 59–110 (85) wide; esophagus about 60 long; cecum 100–134 (115) long, about 112 wide. Acetabular diameter 56–74 (66). Hermaphroditic sac about 90 long, 50 wide. Ovary 31–62 (47) in diam; testis 75–165 (120) long, 50–90 (76) wide. Eggs with thin, yellow operculated shells, 59–65 (62) long and about 42 wide; none showed miracidia but perhaps would if older. Stem of excretory bladder shorter than arms which reach to near the acetabular level.

Holotype deposited as No. 716 in the Hancock Parasitology Collection.

HOSTS: *Mugil cephalus* and *Lisa argentea.*

SITE: Small intestine.

LOCALITY: Brisbane River, Brisbane, Queensland, Australia.

Discussion

Attempts to determine the life cycles were made by exposing laboratory-reared snails, *Posticobia brazieri* (Smith), to the miracidia of *Unisaccus brisbanensis* and *U. spinosus* but none became infected. *Posticobia brazieri* was chosen because it serves as intermediate host for some other haploporids (Martin, 1973).

The description of the digestive tract in the family Haploporidae must be emended to include those with an undivided cecum. In young adults of the species described in this paper the cecum lacks the lobes of older specimens. Evidently the cercaria has a single cecum. Also the cercaria must bear eyespots since all adults showed remnants of these structures. The single saccular cecum might indicate a fairly close relationship to the Haplosplanchnidae in which the cecum is tubular and usually longer. Certain species of Haploporidae and Haplosplanchnidae develop in the same species of mullet.

While alive, specimens of the trematodes described above were seen to evert the hermaphroditic duct through the genital pore in a manner suggesting its functioning as a male copulatory organ. When not everted, the hermaphroditic duct may serve as a vagina.

After the specimen shown in Figure 9 was drawn, it was accidentally crushed while removing immersion oil. Additional fish were examined and another specimen was found that contained eggs. This was deposited as the type specimen although it does not show some structures as clearly as the original specimen because of the eggs. Unfortunately the eggs collapsed during fixation so their measurements are as near approximations to actual size as could be obtained.

Acknowledgments

I am greatly indebted to Professor J. F. A. Sprent, Head of the Parasitology Department, University of Queensland, for the use of laboratory facilities and encouragement in many ways; to Dr. John Pearson, Reader in Parasitology, for furnishing laboratory-reared snails and help in many other ways; to Miss Marilyn Gadisckie for assistance in making slides; and to Mr. Jim Davie for help in collecting fish.

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Scanning Electron Microscope Study of Invasion of Host Cells by *Eimeria larimerensis* Sporozoites*

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Department of Zoology, Utah State University, Logan

ABSTRACT: Monolayers of MDBK cells on cover slips in petri dishes were inoculated with oocysts, sporocysts, and sporozoites of *Eimeria larimerensis*. Oocysts had an irregular surface, with numerous granular particles. The surface of sporocysts was smooth, and the Stieda body appeared as an elevated area at one end. Excysting sporozoites were constricted at the site of exit from the sporocyst and had adhering granular material. Sporozoites had a smooth surface, a bluntly rounded posterior end, and the anterior portion of the body was tapered. At the anterior end was a protrusion, probably consisting of the anterior annuli and anterior portion of the conoid (conoidal complex). This was protruded farther in some specimens than in others. Sporozoites fixed in the process of flexion had folds along the concave side of the flexed region. In sporozoites in an early stage of invasion of host cells, a slender anterior portion of the body (anterior protuberance) was seen within the host cell, lying immediately beneath the surface. In later stages of invasion, the sporozoite was constricted at the point of entrance and the internal portion of the body was as large in diameter as the external portion. In a sporozoite fixed in the process of leaving a host cell, an anterior protuberance with a protruding conoidal complex was observed. These findings confirm those of earlier studies of penetration of host cells by sporozoites of this species with the light and electron microscopes.

The morphology of the oocyst wall and excysting sporozoites of *Eimeria larimerensis* was studied with the transmission electron microscope (TEM) (Roberts, Speer, and Hammond, 1970). Penetration of sporozoites of this species into host cells was investigated with the light microscope (LM) and TEM (Roberts, Speer, and Hammond, 1971). Vetterling, Madden, and Dittemore (1971) described the surface appearance of the oocysts, sporocysts, and sporozoites of *E. tenella* and *E. adenoeides* as seen with the scanning electron microscope (SEM); a stage in penetration of a cultured cell by a sporozoite was also depicted. In our study, we used the SEM to observe the surface characteristics of the oocysts, sporocysts, and sporozoites of *E. larimerensis*. Also, entrance of sporozoites into cells in cultures was observed.

Materials and Methods

Oocysts of *E. larimerensis* were collected, cleaned, and sporulated as previously described (Speer and Hammond, 1970). The oocysts were crushed with a teflon tissue grinder to release the sporocysts, which were excysted as described by Roberts, Speer, and Hammond (1971). The free sporozoites were washed and resuspended in Eagle's minimum essential medium (MEM). Monolayers of Madin–Darby bovine kidney (MDBK) cells (passage No. 288) were grown on round, 12-mm glass cover slips by placing the cover slips in petri dishes and inoculating the dish with cells and

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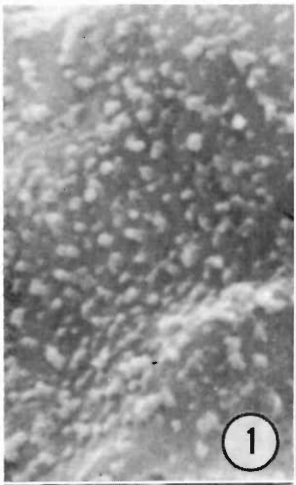
Scanning electron micrographs of oocysts and sporocysts of *Eimeria larimerensis*, as well as sporozoites free or in the process of entering or leaving cells. Abbreviations (all figures): C, conoid; PR, polar ring; SB, Stieda body; SP, sporozoite.

Figures 1–8. Oocyst, sporocysts, and sporozoites. 1. Surface of an oocyst; note granules. × 10,000. 2. Sporocyst. × 10,000. 3. Sporocyst with excysting sporozoite. × 5,000. 4. Sporozoite. × 5,000. 5. Anterior end of sporozoite; note polar ring and partially protruded conoidal complex. × 10,000. 6. Flexing sporozoite; note the folds on the concave side (arrow). × 5,000. 7. Sporozoite leaving host cell; note anterior narrow protuberance, with protruded conoidal complex. × 5,000. 8. Sporozoite penetrating host cell; note the anterior protuberance which has entered the cell and lies immediately beneath its plasmalemma (between arrows). × 10,000.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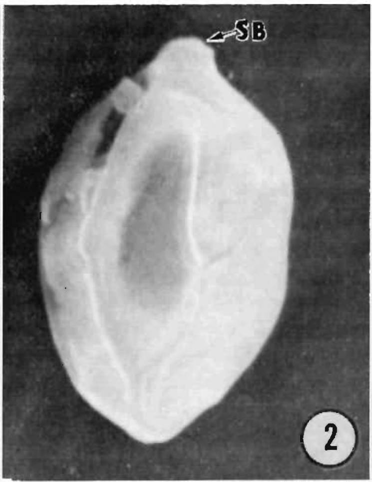
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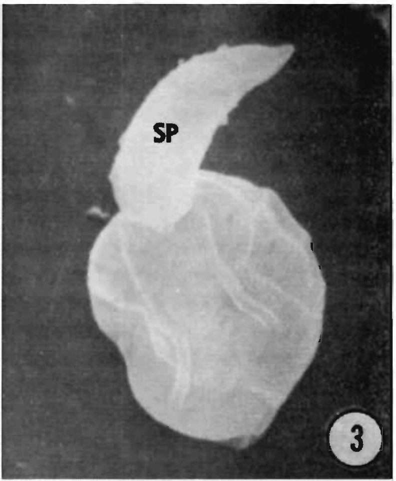
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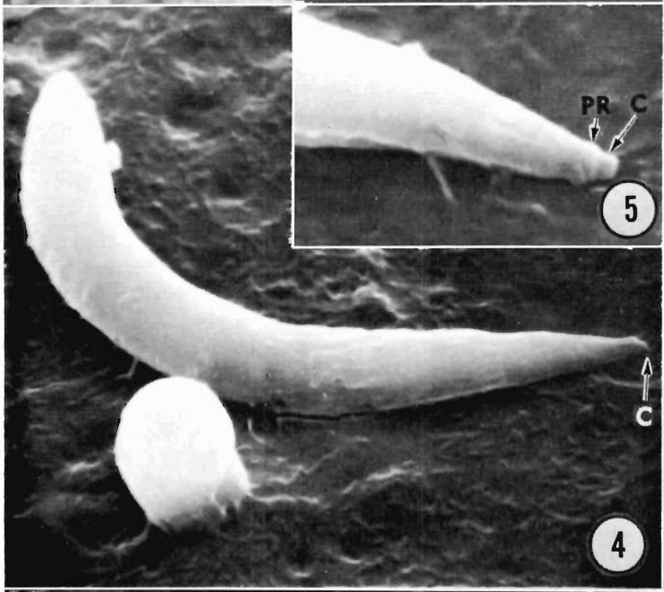
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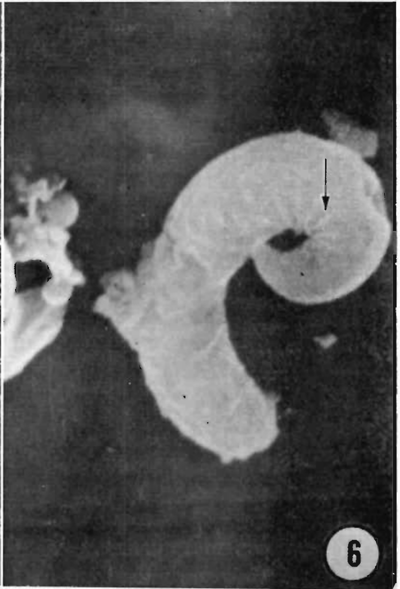
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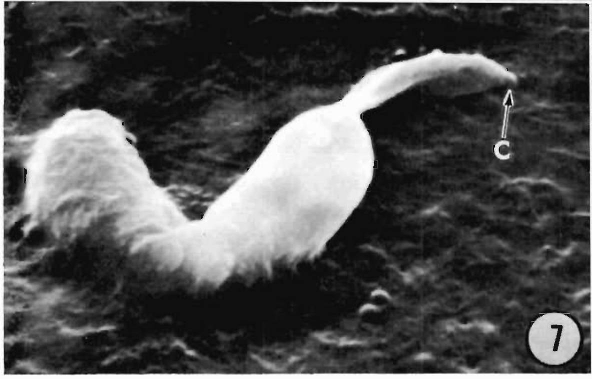
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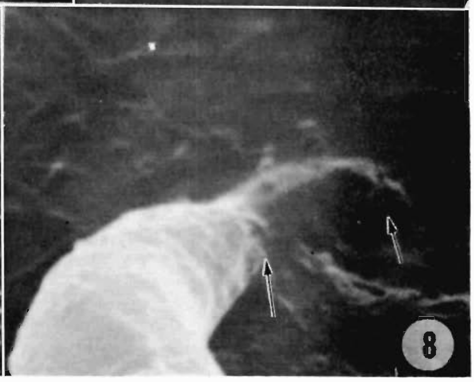
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MEM. After 48 hr, the cover slips were taken out of the MEM and placed, monolayer side up, on a glass slide. A few drops of the suspension, containing 0.5 to 1.5 million sporozoites per ml of MEM, were placed on the monolayer. The preparation was observed with a microscope at 100 and 400×. After about 2 min, when a sufficient number of sporozoites were in the process of penetration, excess suspension was removed with filter paper and several drops of Karnovsky's (1965) fixative were added. After 5 to 10 min, the fixative was removed by filter paper. Cover slips with fixed cells, sporozoites, and penetrating sporozoites were placed in Saline A for ½ hr, then transferred to 2.0% osmium tetroxide in cacodylate buffer for 1 hr. Cover slips were washed in distilled water for 15 min, run through a series of acetone solutions (25, 50, 75%, 3 min each) and two changes of absolute acetone (1½ hr each). They were then air dried and stored in a desiccator jar containing phosphorous pentoxide. Drops of MEM containing oocysts and excysting sporocysts were placed on other cover slips with monolayers and prepared as described for the sporozoites. Even though many of them were washed off during fixation, sufficient numbers adhered to make the study possible. The cover slips were mounted on specimen pegs with conductive silver paint. The mounted specimens were placed in a vacuum evaporator and coated with about 10 to 15 nm of gold while revolving 360 degrees through an arc of 85 degrees. Specimens were examined in a Cambridge Scientific Instruments Mark II A "Stereoscan" Scanning Electron Microscope, with micrographs recorded on Polaroid PN-55 film.

Results and Discussion

The surface of the oocyst wall (Fig. 1) appeared to be covered with small particles, probably corresponding to the electron-dense granules seen with the TEM by Roberts et al. (1971) and considered to be artifacts associated with the manipulation of the oocysts. The oocysts in that study were broken to release the sporocysts, whereas intact oocysts were photographed in the present study. The finding of the granules in the present study indicates that they are a characteristic component of the oocyst wall in *E. larimerensis*. Vetterling et al. (1971) and Nyberg and Knapp (1970) described the oocyst walls of *E. tenella* as having a textured surface or rough appearance; no granular particles similar to those found in *E. larimerensis* were reported.

The sporocysts (Figs. 2, 3) were somewhat distorted as a result of the techniques used. The surface appeared smooth. The Stieda body region appeared as a nipple-shaped protrusion at one end. An excysting sporozoite (Fig. 3) appeared to be constricted at the point of passage through the opening in the sporocyst wall. Several particles were observed on the surface of the sporozoite. This specimen resembled one seen with the LM in a previous study (Roberts et al., 1970).

Sporozoites (Figs. 4–7) were vermiform, tapering at the anterior end, and had a relatively smooth surface. The conoidal complex (conoid and anterior annuli) apparently protruded in some specimens (Figs. 4, 5, 7). Comparison with Figs. 18 and 21 of Roberts et al. (1971) indicates that the conoidal protrusion includes the annuli and anterior portion of the conoid, covered by a single membrane, and the swelling immediately posterior to the protrusion represents the anterior polar ring. Vetterling et al. (1971) observed a protruded complex, which they termed "rostrum," in the sporozoites of *E. tenella*. The rostrum had a truncated appearance.

Sporozoites fixed in the process of flexion showed distinct folds of the pellicle on the

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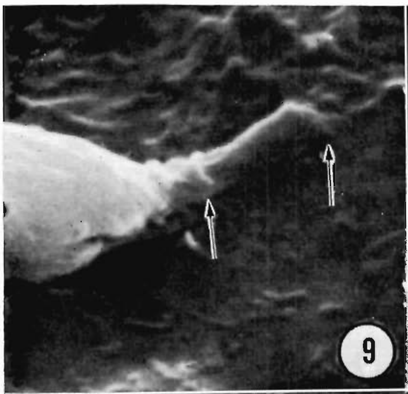
Figures 9–13. Sporozoites entering or leaving cells. 9. Sporozoite penetrating host cell; note anterior protuberance (between arrows). × 10,000. 10. Sporozoite leaving host cell; note site of exit of the anterior protuberance (arrow). × 12,000. 11. Sporozoite with about 1/3 of body within host cell; note constriction at site of entrance of host cell (arrow) and depression at both sides of internal portion of sporozoite. × 10,000. 12. Sporozoite with anterior 2/3 of body within host cell; note constriction at site of entrance (arrow). × 5,000. 13. Sporozoite with about half of body within host cell; note that sporozoite is apparently less constricted at the site of entrance (arrow) than the specimen in Figure 12. × 10,000.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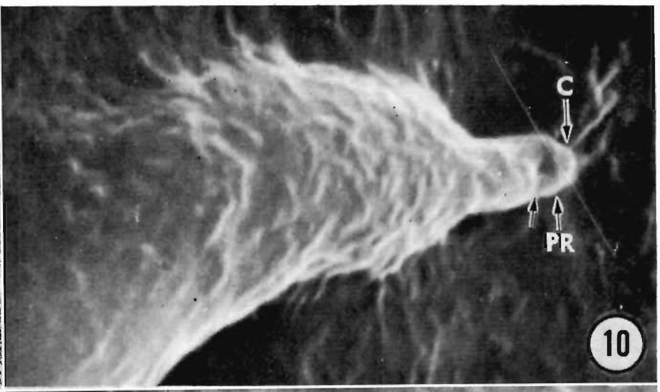

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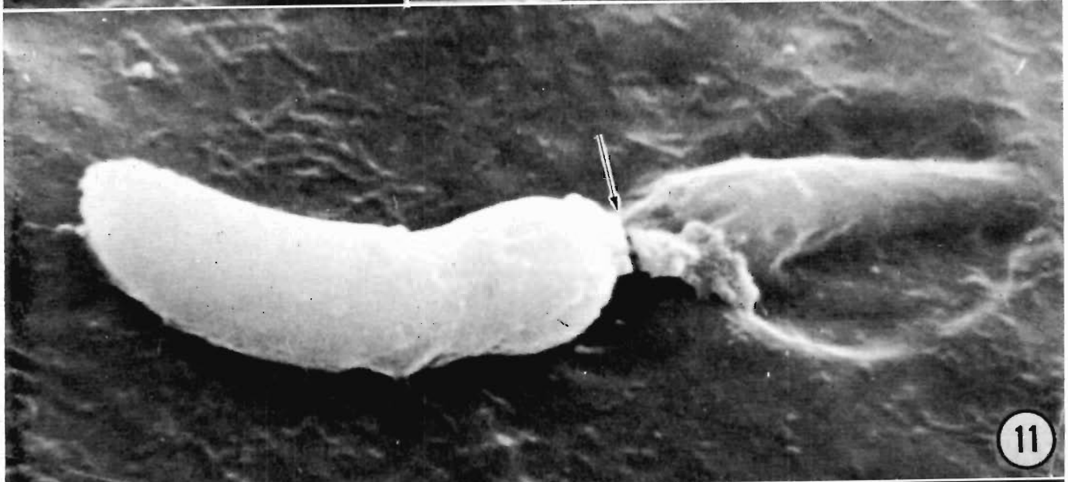
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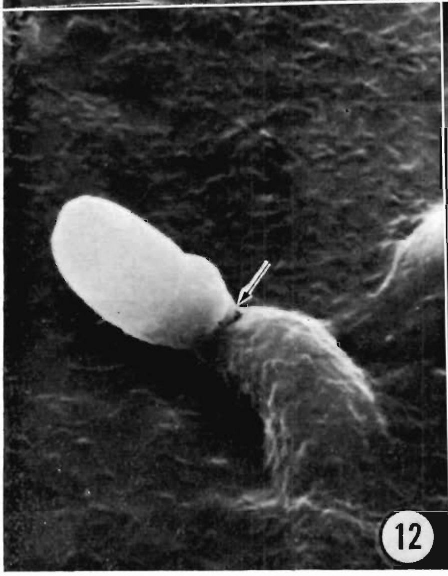
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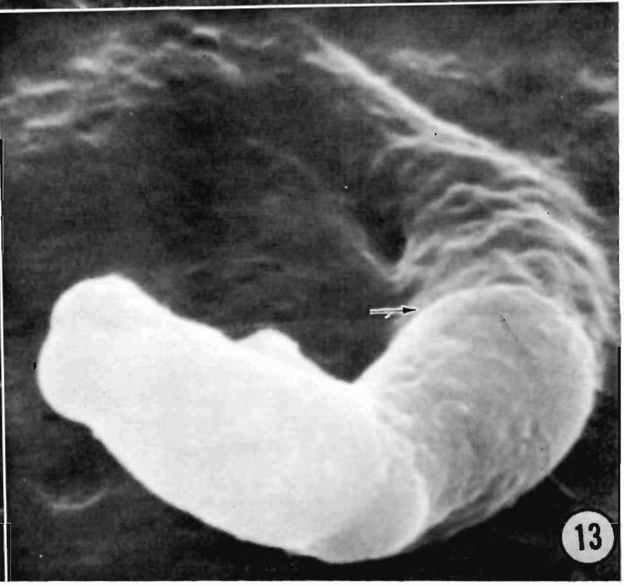
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concave side (Fig. 6). In several sporozoites, the anterior portion of the body appeared to be extended and the protruded conoidal complex was visible at the anterior end (Fig. 7). This extended anterior region may correspond with the protuberance seen in LM studies and described by Roberts et al. (1971) and by Speer, Davis, and Hammond (1971) in sporozoites just before and during the early stages of cell penetration. With high-speed cinemicrography, Fayer (1972) observed a similar structure, which he termed an anterior spinous projection, in sporozoites of *E. tenella* and *E. meleagrimitis* as they were penetrating host cells.

Sporozoites were observed in the process of penetrating or leaving host cells (Figs. 7–13). Many were fixed with the slender anterior protuberance inside the host cell plasmalemma (Figs. 8, 9). The surface of the host cell was not as smooth as that of the sporozoite so that the part of the sporozoite that was within the host cell could readily be distinguished from the part that was outside. In an LM study, Roberts et al. (1971) found that the protuberance of sporozoites was sometimes repeatedly retracted and inserted into cells. They described the protuberance as moving laterally for 1 to 2 sec in the host cell, and then swelling, increasing in width and decreasing in length, after which the sporozoite entered the cell. The penetrating sporozoite in Figure 11 may have moved its protuberance laterally just before fixation, and caused the shallow depression around the anterior portion of the sporozoite in the host cell. Fayer (1972) found that the anterior spinous projection of *E. tenella* and *E. meleagrimitis* sporozoites became swollen soon after it had entered a host cell. In later stages of penetration, the body of *E. larimerensis* sporozoites was constricted at the point of entrance (Figs. 11, 12). Refractile bodies of the sporozoite could not be identified in *E. larimerensis* as they were in *E. tenella* by Vetterling et al. (1971).

The results of a TEM and LM study by Roberts et al. (1970) indicated that the host cell membrane was interrupted during penetration. In the present study, the surface of the host cell was not depressed at the site of entrance of sporozoites and no gap between the entering sporozoite and the edge of the host cell membane was observed (Figs. 12, 13). This indicates that the host cell membrane was interrupted during penetration instead of being pushed in as it is when merozoites of *Plasmodium* species invade erythrocytes (Ladda, 1969).

One sporozoite appeared to have been fixed just as it was beginning to leave a cell (Fig. 10). The tip of the slender anterior protuberance apparently had just broken through the plasmalemma. Roberts et al. (1971) reported that as sporozoites begin to leave host cells, a slender anterior protuberance is extended beyond the margin of the host cell. Other sporozoites were about halfway out of host cells (Fig. 7).

With the SEM, the three-dimensional appearance of oocysts, sporocysts, and sporozoites, as well as the mode of penetration of the latter, is demonstrable. The SEM micrographs are of greater significance if they are compared to TEM and LM micrographs of similar stages, as in the present study, the findings of which confirm those in LM and TEM studies by Roberts et al. (1970).

Acknowledgment

We hereby express appreciation to Mrs. Yoko Elsner for preparing the cell cultures and sporozoites used in the study.

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A Synopsis of the Genera and Species in the Tylenchorhynchinae (Tylenchoidea, Nematoda)[1]

A. C. TARJAN[2]

ABSTRACT: The genera *Uliginotylenchus* Siddiqi, 1971, *Quinisulcius* Siddiqi, 1971, *Merlinius* Siddiqi, 1970, *Tylenchorhynchus* Cobb, 1913, *Tetylenchus* Filipjev, 1936, *Nagelus* Thorne and Malek, 1968, and *Geocenamus* Thorne and Malek, 1968 are discussed. Keys and diagnostic data are presented. The following new combinations are made: *Tetylenchus aduncus* (de Guiran, 1967), *Merlinius alboranensis* (Tobar-Jiménez, 1970), *Geocenamus arcticus* (Mulvey, 1969), *Merlinius brachycephalus* (Litvinova, 1946), *Merlinius gaudialis* (Izatullaeva, 1967), *Geocenamus longus* (Wu, 1969), *Merlinius parobscurus* (Mulvey, 1969), *Merlinius polonicus* (Szczygiel, 1970), *Merlinius sobolevi* (Mukhina, 1970), and *Merlinius tatrensis* (Sabová, 1967). *Tylenchorhynchus galeatus* Litvinova, 1946 is withdrawn from the genus *Merlinius*. The following synonymies are made: *Merlinius berberidis* (Sethi and Swarup, 1968) is synonymized to *M. hexagrammus* (Sturhan, 1966); *Tylenchorhynchus chonai* Sethi and Swarup, 1968 is synonymized to *T. triglyphus* Seinhorst, 1963; *Quinisulcius nilgiriensis* (Seshadri et al., 1967) is synonymized to *Q. acti* (Hopper, 1959); and *Tylenchorhynchus tener* Erzhanova, 1964 is regarded a synonym of *T. clarus* Allen, 1955. The following are regarded in *species inquirendae*: *Tetylenchus dimidius* Kirjanova, 1951; *Aphelenchus dubius* Steiner, 1914; and *Pratylenchoides gadeai* Arias-D, Jiménez-M. and Lopez-P., 1965.

Since my first compendium on *Tylenchorhynchus* (Tarjan, 1964), there have been a number of new species proposed, two additional keys prepared (Baqri and Jairajpuri, 1970 and de Guiran, 1967), and new genera proposed which are closely related to *Tylenchorhynchus* (Siddiqi, 1970, 1971; Thorne and Malek, 1968). In his first paper Siddiqi (1970) erected the new subfamily Tetylenchinae to include the genus *Tetylenchus* and proposed a new genus *Merlinius* to accommodate species of *Tylenchorhynchus* having six incisures in the lateral field, a small trough-shaped nonprotrusible gubernaculum, and stout spicules with distal ends notched and without large ventral flanges. Siddiqi's second paper proposed the new subfamily Merliniinae to accommodate *Merlinius* and formed two new genera within the Tylenchorhynchinae made up of certain species formerly in *Tylenchorhynchus*. The first of these is *Uliginotylenchus* with three incisures comprising an areolated lateral field, female tail clavate to cylindroid and with over 25 annules, and proximal end of gubernaculum bent dorsally. The second genus, *Quinisulcius*, has a nonareolated lateral field with five incisures, distal flanges of spicula are small-sized, and the proximal end of gubernaculum directed dorsally.

It is the object of the present study to evaluate the position of *Tylenchorhynchus* and closely related genera, to determine membership in such genera, and to present keys and a table of diagnostic data on species. The work presented was compiled only from publica-

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tions and not from actual examination of specimens.

Subfamily considerations

The Tylenchorhynchinae are, and have been, a relatively homogenous group readily recognized by their cuticular markings, labial structure, stylet characteristics, esophagus structure, sexual system arrangement, and tail shape. I see no practical value in assigning such closely related genera as *Tetylenchus*, *Tylenchorhynchus*, and *Merlinius* to three different subfamilies, e.g., Tetylenchinae, Tylenchorhynchinae, and Merliniinae, respectively (Siddiqi, 1971). Accordingly, I prefer to follow the arrangement suggested by Golden (1971) in recognizing one subfamily, the Tylenchorhynchinae, in which the new genus *Merlinius* (Siddiqi, 1970) can also be conveniently accommodated.

Previous departures

In addition to the accurate list given by Baker (1962), Table 2 cites departures from *Tylenchorhynchus* as given by various authors during the past two decades.

The genus *Uliginotylenchus* Siddiqi, 1971

This genus was characterized essentially as having an areolated lateral field with three incisures, moderately sclerotized cephalic framework, labial region not offset, spermatheca offset, spicules with large-sized distal flanges, gubernaculum with proximal portion directed dorsally, female tail with 25 or more annules and broadly rounded, and occurring in wet soil. The characters above appear to be valid except for the cephalic framework which is invariably lightly, not moderately, sclerotized for the five species included and for the offset nature of the spermatheca which is unknown for four of the five species. Of the species included, *U. bifasciatus* (Andrássy, 1961) appears to deviate most from the generic criteria in that: (a) its lip region is weakly offset, not continuous; (b) its males do not show spicules with large-sized distal flanges; and (c) its type locality was in garden soil, not marshy or wet soil.

Uliginotylenchus cannot be evaluated without referring to the current controversy on the present status of *Trichotylenchus* Whitehead, 1959 and *Telotylenchus* Siddiqi, 1960. Jairajpuri (1969) stated that *Trichotylenchus* differed from *Telotylenchus* only by a slightly narrower head, delicate spear, and three-incisured lateral field. In a later paper (1971), he presented a more detailed account corroborating and illustrating these differences. Jairajpuri regarded *Telotylenchus* a junior synonym of *Trichotylenchus*. Seinhorst (1971) did not refer to the Jairajpuri references and regarded the two genera as distinct, separated by stylet appearance, shape of female tail, lateral field, and shape of gubernaculum. He included *Trichotylenchus falciformis* Whitehead, 1959; *T. rectangularis* Netscher and Germani, 1969; *T. rhopalocercus* (Seinhorst, 1963); *T. palustris* (Merny and Germani, 1968); *T. uliginosus* (Siddiqi, 1970); and *T. papyrus* (Siddiqi, 1970) in the genus. He justified inclusion of some of these species, which in their original descriptions do not all have the same type of basal portion of the esophagus, by suggesting that the original authors misinterpreted the true structure of the esophagus. Seinhorst's placement of these species thus results in a genus in which: (a) two species (*falciformis* and *rectangularis*) have pronounced esophageal overlaps, while four species do not; (b) five species have delicate or slender stylets while one (*palustris*) does not; (c) five species have three-incisured lateral fields while one (*rectangularis*) has four incisures; and (d) four species have somewhat clavate tails while two species (*rectangularis* and *palustris*) have cylindrical tails. Then, too, no mention is made of Whitehead's (1959) original characterization of *Trichotylenchus* as having a head divided into four lobes and a distinctly forked stylet. Because of these discrepancies and because I feel that the distinct and pronounced overlap of the esophagus on the intestine is of cardinal importance in identifying species of *Trichotylenchus*, I regard Siddiqi's (1971) assignment of species as the most logical.

It would be convenient to include the four remaining three-incisured "*Tylenchorhynchus*" species in *Uliginotylenchus*, i.e., *T. chonai*, *T. divittatus*, *T. sculptus*, and *T. triglyphus*. This is not feasible principally because: (a) none of the species have areolated lateral fields, broadly rounded female tails with more than 25 annules, or occur in wet soils, and (b) most of these species do not have gubernacula with proximal parts directed dorsally, spicules with large-sized distal flange, or lightly sclerotized cephalic frameworks.

Key to Females of *Uliginotylenchus**

1. Tail with 24–35 annules, lip region with 4–5 annules, tail terminus smooth .... *palustris* (Merny and Germani, 1968) Siddiqi, 1971

   Tail with 42–56 annules, lip region with 5–8 annules, tail terminus annulated 2
2. T/ABW = 2.6–2.7, lip region 5–6 annules, lip region weakly offset .... *bifasciatus* (Andrássy, 1961) Siddiqi, 1971

   T/ABW = 3.7–5.6, lip region with 6–8 annules, lip region continuous 3
3. Tail shape subcylindrical to slightly clavate, T/ABW = 5.6, tail with 42 annules .. *rhopalocercus* (Seinhorst, 1963) Siddiqi, 1971

   Tail shape clavate, T/ABW < 5.6, tail with 52–56 annules 4
4. Stylet 14–16 μ long, T/ABW = 4.6–5.0, body 0.40–0.64 mm long .... *uliginosus* (Siddiqi, 1970) Siddiqi, 1971

   Stylet 23–24 μ long, T/ABW = 3.7–4.2, body 0.80–0.94 mm long .... *papyrus* (Siddiqi, 1970) Siddiqi, 1971

The genus *Tylenchorhynchus* Cobb, 1913

Because of the appearance of new closely related and more exactly defined genera, *Tylenchorhynchus* becomes the depository for species with four incisures in the lateral field, as well as the four species with three incisures without areolation. The generic diagnosis given by Siddiqi (1970) is inadequate because the generic criteria listed apply only to some species, *not all*. For example, males are unknown for nine of the 46 species included; accordingly it is impractical to rely on precise male characters as characterizing the genus. The generic diagnosis given by Golden (1971), patterned after previous diagnoses, is more inclusive. However, one of the principal characters of the genus, "cephalic framework present," becomes suspect when diagnostic data on *Tylenchorhynchus* species is inspected (Table 1). Under the heading of "framework sclerotization" it can be seen that among 45 species, 13 have inconspicuous cephalic frameworks, 6 have frameworks which were not mentioned or depicted, while 15 have lightly sclerotized frameworks.

Siddiqi (1970) transferred *Tylenchorynchus galeatus* Litvinova, 1946 to *Merlinius*. He later agreed (in litt.) that the species best be taken out of *Merlinius* because it is reported to have only four incisures in the lateral field. Accordingly, it is returned to the genus *Tylenchorhynchus*.

The only difference existing between the description and figures for *T. clarus* Allen, 1955 and *T. tener* Erzhanova, 1964 is the b ratio which is 4.0–5.0 and 5.3–9.8, respectively. In almost all other important respects, e.g., stylet length, number of tail annules, tail–anal body width ratio, head and tail shape, etc., differences are not evident. Therefore, *T. tener* Erzhanova, 1964 is regarded a junior synonym of *T. clarus* Allen, 1955.

Tylenchorhynchus gadeai (Arias-D., Jiménez-M., and López-P., 1965) was originally described as a *Pratylenchoides*. Braun and Loof (1966) examined two type specimens and concluded that the species belonged to *Tylenchorhynchus* but stated that the original description may have involved more than one species. Neither one of the accounts give the number of incisures in the lateral field. Since the description is lacking essential details and since reasonable doubt exists as to its true identity, the species is regarded as *species inquirenda*.

Pertinent data and figures for *T. triglyphus* Seinhorst, 1963 and *T. chonai* Sethi and Swarup, 1968 are almost identical. The only differences are claimed to be in head shape and stylet length (25–28 μ for *chonai*: 20–23 μ for *triglyphus*). Inspection of the figures for each species fails to show any essential differences in head shape and a 2-μ difference in stylet length ranges is not considered sufficient difference for recognizing separate spe-

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* The keys presented are based primarily on female characteristics except that male characters are also used in some cases. Any abbreviations or symbols used are explained at the end of Table 1.

cies. Accordingly, *T. chonai* is regarded a junior synonym of *T. triglyphus* Seinhorst, 1963.

Golden (1971) recently proposed the binomen *Tylenchorhynchus annulatus* (Cassidy, 1930) Golden, 1971 for *Chitinotylenchus annulatus* after studying Cassidy's original specimens. He stated, "This nematode is clearly a *Tylenchorhynchus* species closely related to *T. martini* Fielding, 1956." Since Golden did not further describe the species nor add it to its original description and since, under Tylenchorhynchinae standards, the description of the species is questionable, it is best to regard it as *species inquirenda* as Sher (1970) suggested.

Key to Females of *Tylenchorhynchus*

1\. Lateral field with 3 incisures 2
Lateral field with 4 incisures 4
2\. Cuticle of lateral fields extended on female tail, stylet 16–17 μ long, lip region offset .... *divittatus* Siddiqi, 1961
Cuticle of lateral fields normal on female tail, stylet 20–28 μ long, lip region continuous 3
3\. Tail with 9 annules, cephalic framework heavily sclerotized, anterior surface of stylet knobs inclined anteriorly .... *sculptus* Seinhorst, 1963
Tail with 13–15 annules, cephalic framework lightly sclerotized, anterior surface of stylet knobs inclined laterally .... *triglyphus* Seinhorst, 1963 (syn. *T. chonai* Sethi and Swarup, 1968)
4\. Body with longitudinal striae 5
Body without longitudinal striae 12
5\. Body with 29 longitudinal striae at midbody, tail with 10 annules .... *claytoni* Steiner, 1937
Body with 20 or less longitudinal striae, tail with 13 or more annules 6
6\. Lip region with 1–2 annules, tail with 13 annules, anterior surface of stylet knobs inclined anteriorly .... *pachys* Thorne and Malek, 1968
Lip region with 5–7 annules, tail with 22 or more annules, anterior surface of stylet knobs inclined posteriorly or laterally 7
7\. Tail terminus annulated 8
Tail terminus smooth 9
8\. Stylet 24–28 μ long, T/ABW = 1.9, V% = 46–53 .... *lamelliferus* (de Man, 1880) Filipjev, 1936
Stylet 21 μ long, T/ABW = 2.2–2.6, V% = 54–56 .. *judithae* Andrássy, 1962
9\. Stylet 16 μ long, tail shape subcylindrical, tail terminus hemispherical .... *brevilineatus* Williams, 1960
Stylet 19–27 μ long, tail shape conoid, tail terminus bluntly pointed 10
10\. Body with 16–20 longitudinal striae, tail with 53–55 annules, body 0.77–0.94 mm long .... *microphasmis* Loof, 1960
Body with 12 longitudinal striae, tail with 22–50 annules, body 0.54–0.77 mm long 11
11\. Bursa distally recurved, female tail with 22 annules, spicules 24 μ long .... *phaseoli* Sethi and Swarup, 1968
Bursa continuing to end of tail, female tail with 30–50 annules, spicules 27 μ long .... *sulcatus* de Guiran, 1967
12\. Tail terminus annulated 13
Tail terminus smooth 23
13\. Stylet 27–38 μ long 14
Stylet 17–24 μ long 15
14\. Tail with 13–14 annules, T/ABW = 1.1, stylet 37–38 μ long .... *brevicaudatus* Hopper, 1959
Tail with 31–33 annules, T/ABW = 2.5, stylet 27–30 μ long .... *magnicauda* (Thorne, 1935) Filipjev, 1936
15\. Tail with 20–29 annules, lip region with 4 annules 16
Tail with 31–66 annules, lip region with 3 or 5–7 annules 17
16\. Cuticular annulation irregular, lip region offset, anterior surface of stylet knobs inclined posteriorly .... *irregularis* Wu, 1969
Cuticular annulation coarse, lip region continuous, anterior surface of stylet knobs inclined anteriorly .... *eremicolus* Allen, 1955
17\. Tail with 66 annules, anterior surface of stylet knobs inclined anteriorly .... *canalis* Thorne and Malek, 1968

Tail with 31–48 annules, anterior surface of stylet knobs inclined posteriorly or laterally 18

18. Stylet 21–24 μ long 19

Stylet 17–19 μ long 20

19. Tail subcylindrical, tail terminus subhemispherical, body 0.76–0.86 mm long .... *bryobius* Sturhan, 1966

Tail cylindrical, tail terminus hemispherical, body 0.98–1.40 mm long .... *maximus* Allen, 1955

20. Tail shape clavate, lip region with 3 annules, T/ABW = 3.8–4.0 .... *clavicauda* Seinhorst, 1968 .. (syn. *clavicaudatus* Seinhorst, 1963)

Tail shape cylindrical to subcylindrical, lip region with 5–7 annules, T/ABW = 2.0–3.2 21

21. T/ABW = 2.0, spicule 30 μ long, lip region with 5 annules .... *huesingi* Paetzold, 1958

T/ABW = 3.0–3.2, spicule 12–24 μ long, lip region with 7 annules 22

22. Tail subcylindrical, tail terminus subhemispherical, tail with 46–48 annules .... *dubius* (Bütschli, 1873) Filipjev, 1936

Tail cylindrical, tail terminus hemispherical, tail with 35–43 annules .... *parvus* Allen, 1955

23. Tail with 40–58 annules 24

Tail with 8–40 annules 26

24. Tail with 40–45 annules, stylet 23 μ long, tail cylindrical, tail terminus hemispherical .... *robustus* Thorne and Malek, 1968

Tail with 45–58 annules, stylet 28–39 μ long, tail subcylindrical or conoid, tail terminus subhemispherical 25

25. Tail with 45–47 annules, stylet 31–39 μ long, T/ABW = 2.2 .... *galeatus* Litvinova, 1946

Tail with 51–58 annules, stylet 28–31 μ long, T/ABW = 3.3–4.8 .... *kegenicus* Litvinova, 1946

26. Stylet 23–27 μ long 27

Stylet 11–23 μ long 29

27. Tail with 17–23 annules, lip region continuous, tail cylindrical, tail terminus hemispherical .... *silvaticus* Ferris, 1963

Tail with 14–16 annules, lip region offset, tail subcylindrical to conoid, tail terminus subhemispherical to bluntly pointed 28

28. Tail conoid, tail terminus bluntly pointed, body 0.65–0.99 mm long, V% = 54–65 .... *cylindricus* Cobb, 1913

Tail subcylindrical, tail terminus subhemispherical, body 0.50–0.62 mm long, V% = 52–54 .... *tarjani* Andrássy 1969

29. Lip region with only 2 annules 30

Lip region with 3 or more annules 31

30. Tail conoid, tail terminus hemispherical, T/ABW = 2.8, tail with 18–20 annules .... *nudus* Allen, 1955

Tail subcylindrical, tail terminus subhemispherical, T/ABW = 4.0, tail with 29 annules .... *delhiensis* Chawla et al., 1968

31. Tail with 8–15 annules 32

Tail with 15–40 annules 34

32. Tail cylindrical, tail terminus hemispherical, tail with 8–10 annules .... *georgiensis* Eliashvili, 1971

Tail conical, tail terminus bluntly pointed, tail with 10–15 annules .. 33

33. Lip region offset, T/ABW = 2.2 .... *latus* Allen, 1955

Lip region continuous, T/ABW = 2.6 .... *clarus* Allen, 1955 (syn. *T. tener* Erzhanova, 1964)

34. Lip region with 3–4 annules 35

Lip region with 5–7 annules 40

35. Stylet 11–14 μ long, tail with 28–32 annules .. *ventrosignatus* Tobar-J., 1969

Stylet 16–23 μ long, tail with 15–33 annules 36

36. Lip region well set off, tail conoid, tail terminus bluntly pointed .... *brassicae* Siddiqi, 1961

Lip region continuous or only weakly set off, tail cylindrical to subcylindrical, tail terminus hemispherical or subhemispherical 37

37. Spermatheca and males not observed .... *martini* Fielding, 1956

Spermathecae and males present 38

38. Stylet 20–23 μ long, spicule 22–25 μ long, anterior surface of stylet knobs inclined laterally .... *agri* Ferris, 1963

Stylet 16–20 μ long, spicule 18–22 μ

long, anterior surface of stylet knobs inclined posteriorly 39

39. Stylet 16–19 μ long, tail terminus subhemispherical to hemispherical ...... *mashhoodi* Siddiqi and Basir, 1959

Stylet 18–20 μ long, tail terminus subhemispherical .... *ewingi* Hopper, 1959

40. Lip region well offset, stylet 13–15 μ long .... *goffarti* Sturhan, 1966

Lip region continuous or weakly set off, stylet 15–22 μ long 41

41. Tail with 35–38 annules, T/ABW = 3.3 .... *manubriatus* Litvinova, 1946

Tail with 16–27 annules, T/ABW = 2.0–2.8 42

42. Stylet 21–22 μ long, cephalic framework heavily sclerotized .... *ebriensis* Seinhorst, 1963

Stylet 15–18 μ long, cephalic framework lightly to moderately sclerotized 43

43. Lateral field areolated, lip region set off, cephalic framework moderately sclerotized .... *aerolatus* Tobar-J., 1970

Lateral field not areolated, lip region continuous, cephalic framework lightly sclerotized 44

44. Body 0.58–0.72 mm long, proximal end of gubernaculum curved anteriad almost 90° .. *striatus* Allen, 1955

Body 0.42–0.63 mm long, proximal end of gubernaculum only slightly curved anteriad .. *contractus* Loof, 1964

The genus *Quinisulcius* Siddiqi, 1971

The salient characteristic of this genus was given as five incisures in a nonareolated lateral field. An offset lip region, moderately sclerotized framework, ventrally arcuate female tail, well-developed bursa, spicules with small distal flanges, and protrusible gubernaculum with proximal end directed dorsally were given as additional characters. Of these characters, only a lateral field with five incisures is distinctive to all included species. The lateral field *generally* is nonareolated although *Quinisulcius cacti* has an areolated lateral field anterior to the metacorpus, but not posterior to it. The lip region is generally offset (exception: *Q. goodeyi* Marinari, 1962) and the well-developed bursa presumably is a sound character since it so appears in three species. Males for the remaining four species in the genus have not yet been discovered. The cephalic framework is only moderately sclerotized in one species, lightly sclerotized in three, inconspicuous in two, and not referred to in one species. I regard spicules in only one species as bearing small-sized distal flanges; the illustrations of two species do not show flanged spicules while males have not been found in four species.

The diagnostic data for *Quinisulcius nilgiriensis* (Seshadri et al., 1967) and *Q. acti* (Hopper, 1959) closely parallel each other. There are no apparent major differences. The former species was said to differ from the latter by the slope of the stylet, a slight esophageal overlap, and shape of the tail terminus. None of these differences are deemed of sufficient weight to justify acceptance of a separate species. Accordingly, *Q. nilgiriensis* (Seshadri et al., 1967) is considered a synonym of *Q. acti* (Hopper, 1959).

The description for *Tylenchorhynchus acutoides* Thorne and Malek, 1968 was accompanied by illustrations incorrectly labeled. Figure 16, C–F on page 43 of Thorne and Malek (1968) was identified as "*T. paracutus*." This obviously was a *lapsus calami*; the *nomen* should be "*T. acutoides*."

Key to Females of *Quinisulcius*

1. Stylet 20–24 μ long, lip region continuous, tail with 49–56 annules .... *goodeyi* (Marinari, 1962) Siddiqi, 1971

Stylet 15–19 μ long, lip region offset, tail with 15–42 annules 2

2. Lip region with 8 annules, tail with 26–42 annules 3

Lip region with 4–6 annules, tail with 15–23 annules 4

3. Tail with 42 annules, tail terminus hemispherical, anterior surface of stylet knobs inclined laterally .... *acti* (Hopper, 1959) Siddiqi, 1971 (syn. *nilgiriensis* Seshadri et al., 1967)

Tail with 26 annules, tail terminus bluntly pointed, anterior surface of stylet knobs inclined posteriorly .... *capitatus* (Allen, 1955) Siddiqi, 1971

4\. Spermatheca and males present, body 0.80 mm long .... *acutoides* (Thorne and Malek, 1968) Siddiqi, 1971

Spermatheca and males absent, body 0.49–0.70 mm long 5

5\. T/ABW = 2.1, anterior surface of stylet knobs inclined anteriorly .... *acutus* (Allen, 1955) Siddiqi, 1971

T/ABW = 2.7–3.0, anterior surface of stylet knobs inclined laterally or posteriorly 6

6\. Tail with 23 annules, lip region with 6 annules, body 0.60–0.70 mm long .... *cacti* (Chawla et al., 1968) Siddiqi, 1971

Tail with 15–22 annules, lip region with 4–5 annules, body 0.49–0.63 mm long .... *curvus* (Williams, 1960) Siddiqi, 1971

The genus *Merlinius* Siddiqi, 1970

Perhaps the most important alteration occurring within the Tylenchorhynchinae has been the establishment of the genus *Merlinius* to accommodate those forms, previously in *Tylenchorhynchus*, that have six incisures in the lateral field. In the generic diagnosis presented by Siddiqi (1970), two additional characters in males are claimed to be definitive. These are: (a) distal end of spicules broadly rounded and notched, and (b) a nonprotrusible gubernaculum. In personal correspondence with Siddiqi, I pointed out that there are a number of species reported without males which were included in the genus and that males were illustrated for some species with pointed spicules and/or protruding gubernacula. His response was logical in pointing out that the salient and consistent generic character is the six-incisured lateral field and that some illustrations by authors may not be wholly accurate. Although there can be reluctance to accept a new genus based on only one character, viz. six incisures, the character *is* consistent and easily recognizable. Since the objective of such action is to make the unwieldly genus *Tylenchorhynchus* less cumbersome, erection of the genus *Merlinius* is justifiable.

The following species are transferred into *Merlinius*:

- *M. alboranensis* (Tobar-Jiménez, 1970) comb. n.
- *M. brachycephalus* (Litvinova, 1946) comb. n.
- *M. gaudialis* (Izatullaeva, 1967) comb. n.
- *M. parobscurus* (Mulvey, 1969) comb n.
- *M. polonicus* (Szczygiel, 1970) comb. n.
- *M. sobolevi* (Mukhina, 1970) comb. n.
- *M. tatrensis* (Sabová, 1967) comb. n.

Siddiqi (1970) questioned the transfer of *brachycephalus* to *Merlinius*. Litvinova (1946) depicted two tails for this species, one of which certainly is not at variance with tails of some other species in the genus. Also, the head of the species, although unusually flat, bears affinities to other species in the genus. Transfer of the species to *Merlinius* is logical.

The nomen *Merlinius nanus* (Allen, 1955) Siddiqi, 1970 is not included in the list of species given by Siddiqi (1970). However, he makes this combination in his illustrations and in a discussion of deirids within the same paper.

Siddiqi (1970) listed "*M. dubius* (Steiner, 1914) comb. n." The history of this species begins with Steiner (1914) describing a population of females from Switzerland as *Aphelenchus dubius*, having a wide lateral field with longitudinal striations, a "Tylench"-type head, and a typical *Aphelenchus*-type tail. Goodey (1932) arbitrarily decided that a bisexual population which he found was conspecific with Steiner's species. Since Goodey regarded these as species of *Anguillulina*, and *Anguillulina dubius* (Bütschli, 1873) preempted use of Steiner's specific epithet, Goodey gave the new name of *Anguillulina macrura* to the species. Allen (1955) examined topotype specimens and found that the species was actually composed of a large form and a small form. Wallace and Greet (1964) concurred and designated the small form as *Tylenchorhynchus macrurus* (Goodey, 1932) while the large form became *T. icarus* Wallace and Greet, 1964. Finally, Siddiqi (1970) transferred both species to *Merlinius*. Since, however, *Tylenchorhynchus dubius* (Bütschli, 1873) remained in another genus, Steiner's original specific name once again became valid and the species became *Merlinius dubius* (Steiner, 1914) Siddiqi, 1970.

In thus reviewing the history of this species, I fail to find any justification for Goodey (1932) to assume that his species from England was the same as Steiner's Swiss population. Even Allen (1955, p. 130) pointedly *assumed* that Goodey's identification was correct. Steiner's account lacks much essential information and it is questionable whether his species again could be recognized. It is interesting that Steiner (1920), Micoletzky (1922), and Goffart (1930) transferred this species into genera that now are *Helicotylenchus*, *Tylenchorhynchus*, and *Rotylenchus*, respectively. Accordingly, *Aphelenchus dubius* Steiner, 1914 [= *Merlinius dubius* (Steiner, 1914) Siddiqi, 1970] is regarded as *species inquirenda*, while *Anguillulina macrura* Goodey, 1932, which is adequately described and illustrated, becomes *Merlinius macrurus* (Goodey, 1932) Siddiqi, 1970 because of its citation in synonymy with *M. dubius*.

Merlinius sobolevi (Mukhina, 1970) comb. n. was originally described as having 10–12 longitudinal striations in the lateral field and illustrated with 12 striations. It is sometimes possible to detect faint lines between the striations in a lateral field. Assuming that this was the case, *M. sobolevi* would then have the usual six longitudinal striations. This thesis is supported by the observation of Geraert (1966) concerning *M. microdorus* (Geraert, 1966). He wrote, "The lateral field bears six incisures but in quite a number of animals I had the impression that additional incisures were present between the original six. Closer examination revealed rows of punctations between the incisures. . . ." Accordingly, *M. sobolevi* is judged to have six longitudinal lines in the lateral field.

A comparison of the critical diagnostic data between *Merlinius hexagrammus* (Sturhan, 1966) and *M. berberidis* (Sethi and Swarup, 1968) shows no differences, with the sole exception of body length (1.04–1.28 mm for *hexagrammus*; 0.72–0.95 for *berberidis*). Sethi and Swarup apparently were unaware of the existence of Sturhan's species at the time they published. It has been amply shown in the literature that temperature and host can affect body length of populations. With the absence of concrete criteria separating the species, *M. berberidis* (Sethi and Swarup, 1968) is regarded as a junior synonym of *M. hexagrammus* (Sturhan, 1966).

Key to Females of *Merlinius*

1. Body with longitudinal striae 2
   Body without longitudinal striae 11
2. Tail with 43–60 annules 3
   Tail with 21–40 annules 4
3. Tail with 58–60 annules, stylet 17–19 μ long, tail subcylindrical, tail terminus subhemispherical .... *hexincisus* (Jairajpuri and Baqri, 1968) Siddiqi, 1970
   Tail with 43–46 annules, stylet 23–25 μ long, tail conical, tail terminus bluntly pointed .... *koreanus* Choi and Geraert, 1971
4. Body with 30–48 longitudinal striae at midbody 5
   Body with 20–28 longitudinal striae at midbody 8
5. Body with 48 longitudinal striae, tail terminus annulated, stylet 18–20 μ long .... *tesselatus* (Goodey, 1952) Siddiqi, 1970
   Body with 30–36 longitudinal striae, tail terminus smooth, stylet 20–24 μ long 6
6. Lip region continuous, tail with 26–27 annules, T/ABW = 3.7 .... *cylindricaudatus* (Ivanova, 1968) Siddiqi, 1970
   Lip region offset, tail with 22–24 annules, T/ABW = 2.7–3.0 7
7. Tail terminus hemispherical, lip region with 5 annules, body 0.64–0.73 mm long .... *quadrifer* (Andrássy, 1954) Siddiqi, 1970
   Tail terminus bluntly pointed, lip region with 6–7 annules, body 0.80–0.90 mm long .. *rugosus* (Siddiqi, 1963) Siddiqi, 1970
8. Body with 20 longitudinal striae, body 0.55–0.58 mm long .... *sobolevi* (Mukhina, 1970) comb. n.
   Body with 24–28 longitudinal striae, body 0.63–1.04 mm long 9

9\. T/ABW = 3.7, stylet 18–20 μ long .... *lenorus* (Brown, 1956) Siddiqi, 1970

T/ABW = 1.9–2.3, stylet 20–24 μ long 10

10\. T/ABW = 1.9, stylet 24 μ long, tail with 30–37 annules .... *tartuensis* (Krall, 1959) Siddiqi, 1970

T/ABW = 2.3, stylet 20–22 μ long, tail with 21–22 annules .... *stegus* (Thorne and Malek, 1968) Siddiqi, 1970

11\. Tail terminus annulated 12

Tail terminus smooth 24

12\. Tail terminus hemispherical, T/ABW = 2.0–2.8 13

Tail terminus bluntly pointed or subhemispherical, T/ABW = 3.0–4.5 17

13\. Tail with 20–21 annules, lip region with 4–5 annules .... *gaudialis* (Izatullaeva, 1967) comb. n.

Tail with 35–59 annules, lip region with 5–10 annules 14

14\. Lip region offset, stylet 21–23 μ long, body 0.58–0.70 mm long .... *bogdanovikatjkovi* (Kirjanova, 1941) Siddiqi, 1970

Lip region continuous, stylet 24–42 μ long, body 0.83–1.96 mm long 15

15\. V% = 59–60, lip region with 5–6 annules, spicule 28–29 μ long .... *socialis* (Andrássy, 1962) Siddiqi, 1970

V% = 50–59, lip region with 8–10 annules, spicule 39–40 μ long 16

16\. Stylet 25–34 μ long, tail with 39–47 annules, body 0.83–1.19 mm long .... *macrurus* (Goodey, 1932) comb. n.

Stylet 34–42 μ long, tail with 50–59 annules, body 1.45–1.96 mm long .... *icarus* (Wallace and Greet, 1964) Siddiqi, 1970

17\. Stylet 12–18 μ long 18

Stylet 20–27 μ long 20

18\. Lip region offset, cephalic framework heavily sclerotized .... *undyferrus* (Haque, 1967) Siddiqi, 1970

Lip region continuous, cephalic framework lightly sclerotized 19

19\. Stylet 12–15 μ long, lip region with 7 annules, T/ABW = 3.8 .... *nanus* (Allen, 1955) Siddiqi, 1970

Stylet 16–18 μ long, lip region with 6 annules, T/ABW = 3.0 .... *nothus* (Allen, 1955) Siddiqi, 1970

20\. Tail with 65 annules, T/ABW = 4.5, body 1.25–1.50 mm long .... *parobscurus* (Mulvey, 1969) comb. n.

Tail with 40–60 annules, T/ABW = 3.0–4.0, body 0.63–1.20 mm long 21

21\. Lateral fields areolated in posterior part of body, lip region with 5 annules, spicules 19 μ long .... *polonicus* (Szczygiel, 1970) comb. n.

Lateral fields not areolated, lip region with 6–9 annules, spicules 27–30 μ long 22

22\. Lip region well offset, stylet 20–21 μ long .... *laminatus* (Wu, 1969) Siddiqi, 1970

Lip region slightly offset or continuous, stylet 23–27 μ long 23

23\. Spermatheca present, lip region with 7 annules, lip region continuous .... *obscurus* (Allen, 1955) Siddiqi, 1970

Spermatheca not observed, lip region with 8–9 annules, lip region slightly offset .... *leptus* (Allen, 1955) Siddiqi, 1970

24\. Stylet 11–24 μ long 25

Stylet 25–67 μ long 31

25\. Stylet 11–16 μ long, body 0.43–0.70 mm long 26

Stylet 20–24 μ long, body 0.70–0.90 mm long 28

26\. Stylet 11 μ long, tail with 26–33 annules, body 0.43–0.47 mm long .... *alboranensis* (Tobar-Jiménez, 1970) comb. n.

Stylet 13–16 μ long, tail with 42–54 annules, body 0.54–0.70 mm long 27

27\. Lip region with 6 annules, spicule 22 μ long, cephalic framework moderately sclerotized .... *brevidens* (Allen, 1955) Siddiqi, 1970

Lip region with 4 to 6 annules, spicule 20–22 μ long, cephalic framework lightly sclerotized .... *microdorus* (Geraert, 1966) Siddiqi, 1970

28\. Tail with 27–49 annules, T/ABW = 2.9–3.8, tail terminus bluntly pointed 29

Tail with 20–23 annules, T/ABW = 2.3–2.6, tail terminus hemispherical to subhemispherical 30

29\. Lip region with 4 annules, lip region continuous, T/ABW = 2.9 .... *bavaricus* (Sturhan, 1966) Siddiqi, 1970

Lip region with 6 annules, lip region offset, T/ABW = 3.8 .... *obscurisulcatus* (Andrássy, 1959) Siddiqi, 1970

30\. V% = 58–59, stylet 23–24 μ long, anterior surface of stylet knobs inclined posteriorly .... *tatrensis* (Sabová, 1967) comb. n.

V% = 55, stylet 20–22 μ long, anterior surface of stylet knobs inclined laterally .... *varians* (Thorne and Malek, 1968) Siddiqi, 1970

31\. Stylet 67 μ long ... *superbus* (Allen, 1955) Siddiqi, 1970

Stylet 25–48 μ long 32

32\. Tail with 10–12 annules, T/ABW = 1.1–1.5 .... *brachycephalus* (Litvinova, 1946) comb. n.

Tail with 28–58 annules, T/ABW = 2.3–3.6 33

33\. Lip region with 9 annules, T/ABW = 3.4–3.6 34

Lip region with 6–8 annules, T/ABW = 2.0–3.0 35

34\. Tail terminus hemispherical, tail with 54–58 annules, cephalic framework heavily sclerotized .... *alpinus* (Allen, 1955) Siddiqi, 1970

Tail terminus bluntly pointed, tail with 49–55 annules, cephalic framework sclerotization inconspicuous .... *conicus* (Allen, 1955) Siddiqi, 1970

35\. Stylet 43–48 μ long, male spicule 37 μ long .... *macrodens* (Allen, 1955) Siddiqi, 1970

Stylet 25–38 μ long, male spicule 26–34 μ long 36

36\. Tail with 28–33 annules, lip region with 8 annules .... *affinis* (Allen, 1955) Siddiqi, 1970

Tail with 36–52 annules, lip region with 6–7 annules 37

37\. Lip region continuous .... *hexagrammus* (Sturhan, 1966) Siddiqi, 1970 (syn.: *berberidis* Sethi and Swarup, 1968)

Lip region well offset 38

38\. Cephalic framework heavily sclerotized, tail with 36–42 annules .... *grandis* (Allen, 1955) Siddiqi, 1970

Cephalic framework inconspicuous, tail with 42–44 annules .... *lineatus* (Allen, 1955) Siddiqi, 1970

The genus *Geocenamus* Thorne and Malek, 1968

Thorne and Malek (1968) referred to the perioral disc, from which a slender stylet guide extends back almost ⅓ the length of a very slender stylet. They referred to an offset lip region and weakly developed cephalic framework as distinguishing the genus. One species was included, *G. tenuidens* Thorne and Malek, 1968, from which the generic characters were obtained.

Dr. M. R. Sauer kindly let me examine what appears to be a new species of *Geocenamus* from Australia and which clearly shows the generic characters listed above. He further influenced me to reexamine the characters of *Tylenchorhynchus arcticus* Mulvey, 1969 which he and R. H. Mulvey conclude belongs to *Geocenamus*. I concur and regard the taxon as *Geocenamus arcticus* (Mulvey, 1969) comb. n.

The original description and illustrations for *Tylenchorhynchus longus* Wu, 1969 fulfill all of the generic criteria listed above, except for reference to a slender stylet guide. Knobloch (1971) emended the description of the species principally to include longitudinal striations on the labial region and body, and enlarged phasmids. However, she pointed out similarities of the species to *Tylenchorhynchus mamillatus* Tobar-Jiménez, 1966, which has become *Scutylenchus mamillatus* (Tobar-Jiménez, 1966) Jairajpuri, 1971. *Scutylenchus* Jairajpuri, 1971 was based on characters of *S. mamillatus* which included: (a) a subdigitate tail shape, (b) enlarged scutella-like phasmids, (c) areolated lateral field, and (d) sloping stylet knobs. Admittedly, similarities between *S. mamillatus* and *T. longus* are noteworthy, viz.: (a) double-layered cuticle, (b) enlarged phasmids, and (c) tesselated nature of cuticle due to longitudinal striae. However, the dissimilarities between the two species are even more important, i.e., (a) occurrence of perioral disc in *T. longus*, (b) stylet long and slender for *T. longus*, short and stout for *S. mamillatus*, and (c) cephalic sclerotization weak for *T. longus* and moderately strong for *S. mamillatus*. The presence of a prominent perioral disc and perhaps also the slender stylet in *T. longus* relate the species more cogently to *Geocenamus*. Siddiqi (*in litt.*) stated that *T. longus* could not be accommodated in *Merlinius* because of the conspicuous labial disc which he used to separate *Geocenamus* from *Merlinius*. Accordingly, the species is designated *Geocenamus longus* (Wu, 1969) comb. n.

Key to Females of *Geocenamus*

1. Stylet 53–65 μ long, T/ABW = 2.0–2.2 .... *longus* (Wu, 1969) comb. n.
Stylet 27–38 μ long, T/ABW = 2.9–3.0 2
2. Stylet 27 μ long, body 0.83 mm long .... *tenuidens* Thorne and Malek, 1968
Stylet 34–38 μ long, body 1.05–1.27 mm long .... *arcticus* (Mulvey, 1969) comb. n.

The genus *Nagelus* Thorne and Malek, 1968

The asymmetrical fine striation of the lip region, inconspicuous cephalic framework, angular stylet knobs, attachment of stylet protractor muscles and presence of epiptygma at the vulva characterizes this genus. It has only one species, *N. aberrans* Thorne and Malek, 1968.

The genus *Tetylenchus* Filipjev, 1936

Filipjev (1936) characterized this genus as "Head without chitinization, cuticle finely striated (with some exceptions), esophagus tylenchoid, ovaries double, spear of moderate size, males unknown." He made *T. tenuis* (Micoletzky, 1922) the type and included *T. clavicaudatus* (Micoletzky, 1922) and *T. granulosus* (Cobb, 1893). *Tetylenchus clavicaudatus* (Micoletzky, 1921) was transferred to *Psilenchus* in 1949 by Thorne (1949) and later regarded as *species inquirenda* by Jairajpuri (1966). *Tetylenchus granulosus* (Cobb, 1893) was either overlooked or ignored by authors until Sher (1968) placed it in synonymy with *Radopholus similis* (Cobb, 1893) Thorne, 1949. Thus, the only remaining representative to the genus is the type *T. tenuis* (Micoletzky, 1922). Micoletzky described *T. tenuis* from one female. He felt that the long esophagus (b = 3.8), clear annulation of the cuticle, delicate weakly knobbed stylet, amphidelphic sexual system, and conical tail with minutely rounded terminus characterized the species. He depicted a head with delicate stylet showing what one could interpret as a moderately sclerotized framework. Hence, it is seen that Filipjev's definition of *Tetylenchus* based on Micoletzky's one female specimen deviates in regard to head sclerotization, cuticular annulation, and stylet size. Thorne (1949) emended the generic diagnosis. He diagnosed the genus as *without* sclerotized cephalic framework, stylet with *or without* basal knobs, tails tapering to acute or subacuate terminus, deirids and phasmids present, bursa subcaudal extending almost to terminus, etc. He placed four species in the genus, *T. tenuis*, and three new species.

In view of the foregoing historical analysis, it appears that the genus, as it is now recognized, differs considerably from the characters Micoletzky (1922) ascribed to *T. tenuis*. It is unfortunate that the genus is based on a type species described from only one female. Yet it also becomes clear that *Tetylenchus* is defined by some characters which are not valid, e.g., the absence of cephalic sclerotization. An examination of illustrations and specimens of *T. joctus* reveals the presence of a cephalic framework, admittedly very lightly sclerotized in some cases, but nonetheless there. This is similar to the rest of the Tylenchorhynchinae for which the intensity of cephalic sclerotization will vary within genera. Hence, the most important single characterization of *Tetylenchus* would be the presence of an acute or subacute female tail with finely rounded terminus. This character is unique among the genera in the subfamily. In addition, the cephalic framework can be regarded as inconspicuous to lightly sclerotized and never heavily sclerotized. In all other respects, *Tetylenchus* appears to be close to *Tylenchorhynchus* and *Merlinius*.

Tylenchorhynchus aduncus de Guiran, 1967, because of the tail shape of both sexes, tail terminus shape, and light cephalic sclerotization shown in the figures, fulfills the criteria stated above and becomes *Tetylenchus aduncus* (de Guiran) comb. n.

Tetylenchus dimidius Kirjanova, 1951 was thought to be a *Tylenchorhynchus* by Loof (1959). Merny (1964) also did not consider the species a true *Tetylenchus*. Both authors felt that the cylindrical tail and subhemispherical terminus precluded its placement in *Tetylenchus*; I concur. Accordingly *Tetylenchus dimidius* Kirjanova, 1951 is regarded as *species inquirenda*.

Key to Females of *Tetylenchus*

1. Stylet without knobs .... *abulbosus* Thorne, 1949
   Stylet with knobs 2
2. T/ABW = 5.7, 'a' ratio 42 .... *tenuis* (Micoletzky, 1922)
   T/ABW = 2.5–3.8, 'a' ratio 18–33.. 3
3. Lateral field with 3–4 incisures 4
   Lateral field with 6 incisures 5
4. Lip region offset, tail with 19–27 annules, lateral field with 4 incisures .... *aduncus* (de Guiran, 1967) comb. n.
   Lip region continuous, tail with 17 annules, lateral field with 3 incisures .... *annulatus* Merny, 1964
5. Stylet 29 μ long, tail with 35 annules, spicule 34 μ long .... *curiosus* Wilski, 1964
   Stylet 12–15 μ long, tail with 42–67 annules, spicule 19–21 μ long 6
6. Stylet 12 μ long, tail with 67 annules, lip region with 4–5 annules .... *productus* Thorne, 1949
   Stylet 15 μ long, tail with 42 annules, lip region with 7 annules .... *joctus* Thorne, 1949

Based on the foregoing data, the Tylenchorhynchinae can be regarded as made up of eight genera.

Key to Tylenchorhynchinae Eliava, 1964

1. Female tail subdigitate, phasmids enlarged .... *Scutylenchus* Jairajpuri, 1971
   Female tail not subdigitate, phasmids normal 2
2. Female tail acute or subacute, terminus finely rounded .... *Tetylenchus* Filipjev, 1936
   Female tail bluntly conical to cylindrical, terminus bluntly pointed to hemispherical 3
3. Stylet knobs characteristically angular, protractor muscles of stylet attached to inner wall of labial cavity, epiptygma present at vulva .... *Nagelus* Thorne and Malek, 1968
   Stylet knobs rounded, protractor muscles of stylet attached to base of cephalic framework, vulva seldom with epiptygma 4
4. Labial region with prominent perioral disc, stylet characteristically slender .... *Geocenamus* Thorne and Malek, 1968
   Labial region without perioral disc, stylet moderately robust to heavy 5
5. Lateral field with 3–4 incisures 6
   Lateral field with 5–6 incisures 7
6. Lateral field areolated, with 3 incisures .... *Uliginotylenchus* Siddiqi, 1971
   Lateral field usually with 4 incisures, never areolated when with 3 incisures .... *Tylenchorhynchus* Cobb, 1913
7. Lateral field with 5 incisures .... *Quinisulcius* Siddiqi, 1971
   Lateral field with 6 incisures .... *Merlinius* Siddiqi, 1970

Table 1 is a compendium of morphological measurements and other information of diag-

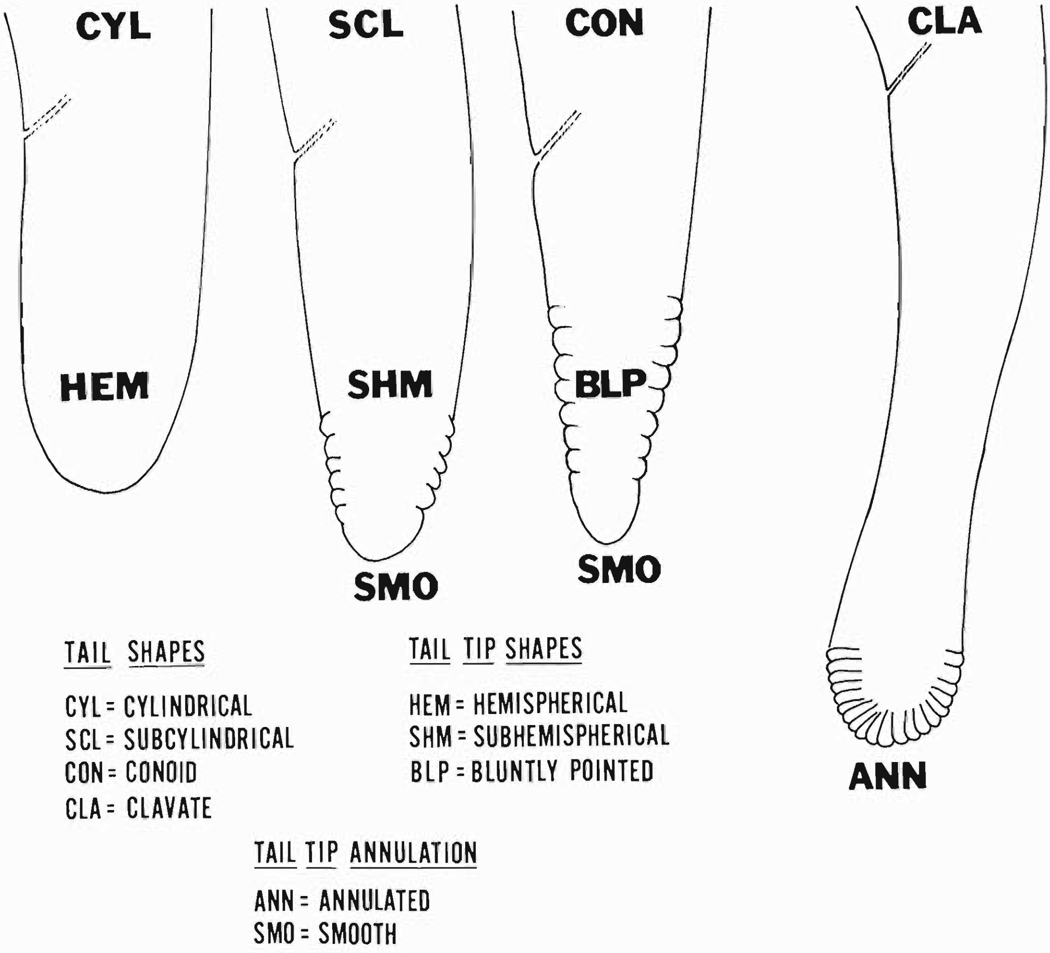

Figure 1. Tylenchorhynchinae tail shapes and code designations used in Table 1.

nostic value on the species within each genus, as covered in the preceding keys. The table is based on data concerning females, except for the last two columns which refer to male characters. When specific data were not presented in the text of the original publication, these were obtained from the accompanying drawings, if possible, e.g., spicule length, T/ABW (tail divided by body width at the anus), number of tail or head annules, etc. If certain information on a species was unknown, a dash (—) was inserted in the table.

An explanation of the abbreviations and code symbols used appears at the end of Table 1. Figure 1 is a diagrammatic explanation of the tail codes used in Table 1.

Acknowledgments

Miss Janell Kay Haglund, laboratory aide, contributed significantly to this work in preparing tables of diagnostic data and by conscientiously reviewing the text for mistakes. Dr. E. Krall amiably supplied photocopies of Russian references which were unavailable to me. Drs. M. R. Siddiqi, A. M. Golden, S. A. Sher, and R. P. Esser kindly reviewed the manuscript.

Table 1. Diagnostic data on species in genera within the subfamily Tylenchorhynchinae.

Uliginotylenchus Siddiqi, 1971	Length in mm	a	b	c	V%	Labial region	Labial annules	Frm. scl.	Stylet length in μ	Sty. cli.	Lateral inci-sures	No. long. striae	No. tail annules	Tail shape	Tail terminus	T/ABW	Spicule length in μ	Gubern. length in μ
bifasciatus (Andrássy, 1961)	0.65–0.73	26–31	5.6–5.9	13–17	51–52	OFF	5–6	LSC	19–20	POS	3	–	42–46	SCL	HEM-ANN	2.6–2.7	25	12
palustris (Merny and Germani, 1968)	0.46–0.62	27–34	4.1–5.8	12–16	52–59	CNT	4–5	LSC	14–17	LAT	3	–	24–35	SCL	HEM-SMO	2.7–3.9	18–22	9–11
papyrus (Siddiqi, 1970)	0.80–0.94	37–43	5.5–6.6	13–16	52–55	CNT	7–8	LSC	23–24	POS	3	–	56	CLA	HEM-ANN	3.7–4.2	26–29	13
rhopalocercus (Seinhorst, 1963)	0.62–0.81	38–46	6.5	11–13	49–55	CNT	7–8	LSC	17–19	POS	3	–	42	SCL	HEM-ANN	5.6	21	8
uliginosus (Siddiqi, 1970)	0.40–0.64	30–37	4.4–5.6	11–13	52–58	CNT	6–7	LSC	14–16	LAT	3	–	52	CLA	SHM-ANN	4.6–5.0	19–21	10–11
Tylenchorhynchus Cobb, 1913																		
aerolatus Tobar-Jiménez, 1970	0.54–0.70	26–31	4.7–6.0	15–18	54–59	OFF	5–6	MSC	15–17	POS	4	–	16–22	SCL	BLP-SMO	2.0–3.0	19–22	10–12
agri Ferris, 1963	0.66–0.77	28–33	4.7–5.5	15–21	55–58	OFF	4	INC	20–23	LAT	4	–	18–26	SCL	HEM-SMO	2.6	22–25	13–14
brassicae Siddiqi, 1961	0.58–0.72	26–35	5.0–6.0	14–17	52–58	OFF	4	LSC	16–17	LAT	4	–	18–33	CON	BLP-SMO	2.5–3.0	18–21	9–11
brevicaudatus Hopper, 1959	1.28–1.37	34–36	6.7–6.9	34–44	57–59	CNT	8	HSC	37–38	LAT	4	–	13–14	SCL	HEM-ANN	1.1	32	13
brevilineatus Williams, 1960	0.56–0.65	34	5.6	14	53–56	OFF	5–6	LSC	16	POS	4	8–12? CER	40–41	SCL	HEM-SMO	2.9	23	12
bryobius Sturhan, 1966	0.76–0.86	31–37	5.8–6.6	14	52	CNT	5–6	MSC	21–24	POS	4	–	35–45	SCL	SHM-ANN	2.5–3.5	23–26	13
canalis Thorne and Malek, 1968	1.00	39	7.1	13	53	OFF	5–6	MSC	20	ANT	4	–	66	SCL	HEM-ANN	2.8	25	13
clarus Allen, 1955	0.49–0.61	28–33	4.0–5.0	16–20	57–61	CNT	5	LSC	15–17	ANT	4	–	10–15	CON	BLP-SMO	2.6	?	?
clavicauda Seinhorst, 1968	0.54–0.72	31–43	4.5–5.9	12–18	53–59	CNT	3	LSC	18–19	POS	4	–	31	CLA	HEM-ANN	3.8–4.0	19	12
claytoni Steiner, 1937	0.64–0.73	24–25	5.0–6.2	18–19	55–57	OFF	3	?	20	POS	4	29 MID	10	CON	BLP-SMO	1.7	25	15
contractus Loof, 1964	0.42–0.63	22–30	3.8–5.0	13–15	56–60	CNT	5–6	LSC	16–18	LAT	4	–	20–23	CON	SHM-SMO	2.5	17–18	8–9
cylindricus Cobb, 1913	0.65–0.99	28–35	4.2–6.0	13–20	54–65	OFF	5	MSC	24–27	ANT	4	–	15–16	CON	BLP-SMO	2.5	25	16
delhiensis Chawla et al., 1968	0.60–0.70	24–33	5.0–6.0	13–18	47–48	CNT	2	INC	14–16	LAT	4	–	29	SCL	SHM-SMO	4.0	?	?
divittatus Siddiqi, 1961	0.55–0.72	32–38	5.4–6.4	16–19	53–55	OFF	5	INC	16–17	LAT	3	–	21	SCL	'HEM'-SMO	2.8	17	8
dubius (Bütschli, 1873)	0.62–0.78	30–35	5.0–6.0	13–16	54–57	OFF	7	LSC	18–19	POS	4	–	46–48	SCL	SHM-ANN	3.2	24	12
ebriensis Seinhorst, 1963	0.52–0.59	23–29	4.9–5.4	14–16	54–56	CNT	5	HSC	21–22	POS	4	–	25	CON	BLP-SMO	2.6	21	10

Table 1 cont'd.

	Length in mm	a	b	c	V%	Labial region	Labial annules	Frm. scl.	Stylet length in μ	Sty. cli.	Lateral inci- sures	No. long. striae	No. tail annules	Tail shape	Tail terminus	T/ABW	Spicule length in μ	Gubern. length in μ
eremicolus Allen, 1955	0.70–0.76	28–30	4.5–5.8	14–16	55	CNT	4	MSC	19–20	ANT	4	–	27–29	CON	BLP-ANN	2.7	22	9
ewingi Hopper, 1959	0.55–0.75	30–35	4.3–5.5	13–16	53–59	CNT	3	INC	18–20	POS	4	–	15–19	SCL	SHM-SMO	2.5	22	12
galeatus Litvinova, 1946	1.68	31	7.3	19	54	CNT	10–13	?	31–39	POS	4	–	45–47	CON	SHM-SMO	2.2	51–53?	13–18
georgiensis Eliashvili, 1971	0.57–0.69	29–35	4.3–5.0	19–23	57–61	OFF	5–6	?	19–21	POS	4	–	8–10	CYL	HEM-SMO	2.4	?	?
goffarti Sturhan, 1966	0.54–0.69	29–37	4.9–6.0	13–20	52–57	OFF	6–7	LSC	13–15	POS	4	–	23–40	SCL	SHM-SMO	2.5–3.5	21–23	11–13
huesingi Paetzold, 1958	0.78–0.92	26–31	5.0–5.9	15–21	53–55	CNT	5	?	18–19	POS	4	–	32–36	CYL	HEM-ANN	2.0	30	14–15
irregularis Wu, 1969	0.69–0.83	26–33	4.8–5.8	15–19	55–59	OFF	4	LSC	19–21	POS	4	–	20–26	CON	BLP-ANN	2.0–2.5	26–29	16–18
judithae Andrássy, 1962	0.88–0.97	33	6.7–7.2	17–23	54–56	OFF	6–7	LSC	21	LAT	4	14	36–40	CON	SHM-ANN	2.2–2.6	?	?
kegenicus Litvinova, 1946	0.79–1.17	25–35	4.3–6.8	10–14	42–55	CNT	7–10	?	28–31	POS	4	–	51–58	SCL	SHM-SMO	3.3–4.8	26–31	9
lameliferus (de Man, 1880)	0.86–1.10	25–31	4.3–6.8	16–24	46–53	CNT	6	INC	24–28	LAT	4	14 CER 16 MID	41–45	CON	BLP-ANN	1.9	31	16
latus Allen, 1955	0.58–0.70	30–40	4.9–5.4	18–22	56–61	OFF	6	LSC	16–17	ANT	4	–	14–15	CON	BLP-SMO	2.2	?	?
magnicauda (Thorne, 1935)	0.79–1.00	23–32	4.4–6.0	13–19	56–62	CNT	6	HSC	27–30	ANT	4	–	31–33	CYL	HEM-ANN	2.5	?	?
manubriatus Litvinova, 1946	0.75	29	5.4	15	55	CNT	6	?	18	POS	4	–	35–38	SCL	HEM-SMO	3.3	?	?
martini Fielding, 1956	0.75	31	5.0	14	54	OFF	3	?	19	LAT	4	–	22–27	SCL	HEM-SMO	3.7	?	?
mashhoodi Siddiqi and Basir, 1959	0.49–0.76	26–37	4.4–5.8	13–19	50–59	CNT	3–4	LSC	16–19	POS	4	–	15–30	CYL	SHM-SMO	2.5–4.0	18–22	10–15
maximus Allen, 1955	0.98–1.40	37–47	5.4–8.1	16–20	47–54	CNT	7	LSC	21–24	POS	4	–	38–41	CYL	HEM-ANN	2.6	?	?
microphasmis Loof, 1960	0.77–0.94	31–39	5.2–6.1	15–18	49–55	OFF	6–7	INC	24–27	POS	4	16–20	53–55	CON	BLP-SMO	3.0	29	14
nudus Allen, 1955	0.71	30	4.1–4.8	14–15	54–56	CNT	2	INC	19–23	ANT	4	–	18–20	CON	HEM-SMO	2.8	23	13
pachys Thorne and Malek, 1968	0.63	24	5.0	16	57	CNT	1–2	LSC	13–15	ANT	4	16 MID	13	CON	BLP-SMO	2.4	23	15
parvus Allen, 1955	0.65–0.72	25–30	4.8–5.6	13–16	52–57	CNT	7	INC	17–18	LAT	4	–	35–43	CYL	HEM-ANN	3.0	12	5
phaseoli Sethi and Swarup, 1968	0.61–0.77	28–35	4.0–5.4	17–20	54–58	OFF	6	LSC	19–26	POS	4	12	22	CON	BLP-SMO	2.3	24	12
robustus Thorne and Malek, 1968	1.00	31	7	15	52	CNT	?	MSC	23	ANT	4	–	40–45	CYL	HEM-SMO	3.5	27	15

Table 1 cont'd.

	Length in mm	a	b	c	V%	Labial region	Labial annules	Frm. scl.	Stylet length in µ	Sty. cli.	Lateral inci- sures	No. long. striae	No. tail annules	Tail shape	Tail terminus	T/ABW	Spicule length in µ	Gubern. length in µ
sculptus Seinhorst, 1963	0.54–0.59	23–25	4.3–4.7	18–19	56–57	CNT	2–3	HSC	22	ANT	3	–	9	SCL	SHM-SMO	2.3	22	12
silvaticus Ferris, 1963	0.80–1.00	24–33	5.5–6.3	18–23	53–58	CNT	4	INC	23–26	LAT	4	–	17–23	CYL	HEM-SMO	2.5	29	18
striatus Allen, 1955	0.58–0.72	29–34	5.0–5.3	13–16	55–57	CNT	5	LSC	16–17	ANT	4	–	20–27	SCL	SHM-SMO	2.8	20	12
sulcatus de Guiran, 1967	0.54–0.71	27–37	5.1–5.7	14–17	52–56	OFF	6–7	INC	19–22	POS	4	12	30–50	CON	BLP-SMO	2.5–3.3	27	15
tarjani Andrássy, 1969	0.50–0.62	24–27	4.7–5.5	16–18	52–54	OFF	4–5	INC	24–25	POS	4	–	14–15	SCL	SHM-SMO	2.7–2.8	26	15
triglyphus Seinhorst, 1963	0.58–0.68	24–30	4.6–5.6	16–19	55–58	CNT	4	LSC	20–23	LAT	3	–	13–15	CON	BLP-SMO	2.5	22	10
ventrosignatus Tobar-Jiménez, 1969	0.45–0.62	28–36	3.9–6.0	13–16	53–57	OFF	4	INC	11–14	POS	4	–	28–32	SCL	BLP-SMO	2.6–3.2	20–21	11
Quinisulcius Siddiqi, 1971																		
acti (Hopper, 1959)	0.65–0.71	30–36	4.6–4.9	15–16	55–57	OFF	8	INC	17	LAT	5	–	42	CON	HEM-SMO	3.0	?	?
acutoides Thorne and Malek, 1968)	0.80	35	5.4	20	57	OFF	4–5	MSC	18	ANT	5	–	18	CON	BLP-SMO	2.8	20	10
acutus (Allen, 1955)	0.63–0.70	31–38	4.8–5.3	17–22	56–57	OFF	6	LSC	16–17	ANT	5	–	17	CON	BLP-SMO	2.1	?	?
cacti (Chawla et al., 1968)	0.60–0.70	25–33	4.0–6.0	14–19	54–59	OFF	6	?	15–19	LAT	5	–	23	SCL	SHM-SMO	3.0	?	?
capitatus (Allen, 1955)	0.63–0.85	30–38	5.0–5.8	12–17	51–58	OFF	8	LSC	16–18	POS	5	–	26	CON	BLP-SMO	2.8	19	7
curvus (Williams, 1960)	0.49–0.63	29–37	4.6–5.5	15–18	52–57	OFF	4–5	INC	17	POS	5	–	15–23	CON	BLP-SMO	2.7	?	?
goodeyi (Marinari, 1962)	0.51–0.81	24–29	4.4–5.4	14–16	52–56	CNT	6–7	LSC	20–24	POS	5	–	49–56	CON	HEM-ANN	2.8–3.2	25–29	8–10
Merlinius Siddiqi, 1970																		
affinis (Allen, 1955)	0.80–0.90	30–33	4.2–5.0	14–15	55–58	CNT	8	HSC	26–27	POS	6	–	28–33	SCL	SHM-SMO	2.3	27	9
alboranensis (Tobar-Jiménez, 1970) n.c.	0.43–0.47	25–30	4.5–5.3	12–15	58–61	OFF	6	LSC	11	POS	6	–	26–33	SCL	SHM-SMO	2.4–2.8	17–20	5–7
alpinus (Allen, 1955)	0.83–0.95	26–27	5.0–5.3	11–13	52–54	OFF	9	HSC	39–42	POS	6	–	54–58	SCL	HEM-SMO	3.6	?	?
bavaricus (Sturhan, 1966)	0.75	28	5.2	13	57	CNT	4	LSC	21	POS	6	–	49	SCL	BLP-SMO	2.9	?	?
bogdanovikatjkovi (Kirjanova, 1941)	0.58–0.70	23–27	4.0–5.0	13–14	54–58	OFF	7–9?	MSC	21–23	POS	6	–	47?	CON	HEM-ANN	2.8	?	?

Table 1 cont'd.

	Length in mm	a	b	c	V%	Labial region	Labial annules	Frm. scl.	Stylet length in µ	Sty. cli.	Lateral incisures	No. long. striae	No. tail annules	Tail shape	Tail terminus	T/ABW	Spicule length in µ	Gubern. length in µ
brachycephalus (Litvinova, 1946) n.c.	0.66–0.77	26–28	4.4–5.4	35–42	52–69	CNT	5–7	HSC	28–30	POS	6	–	10–12	CON	BLP-SMO	1.1–1.5	37	11
brevidens (Allen, 1955)	0.54–0.69	23–27	4.2–5.2	11–13	52–58	CNT	6	MSC	14–16	POS	6	–	42–49	SCL	HEM-SMO	2.8	15	10
conicus (Allen, 1955)	0.78–1.10	31–39	4.4–5.8	11–14	52–56	OFF	9	INC	40–45	POS	6	–	49–55	CON	BLP-SMO	3.4	32	9
cylindricaudatus (Ivanova, 1968)	0.77–0.90	28–32	5.0–5.8	14–16	57–60	CNT	7	LSC	22–24	POS	6	30–34 MID	26–27	SCL	SHM-SMO	3.7	?	?
gaudialis (Izatullaeva, 1957) n.c.	0.66–0.70	23–26	4.7–6.0	17–22	55–60	CNT	4–5	MSC	22–25	POS	6	–	20–21	SCL	HEM-ANN	2.0–2.4	34–36	11–14
grandis (Allen, 1955)	0.96–1.11	34–41	5.0–6.4	13–16	50–54	OFF	6	HSC	26–30	POS	6	–	36–42	SCL	SHM-SMO	2.8	30	9
hexagrammus Sturhan, 1966	1.04–1.28	27–34	5.2–6.4	15–20	51–56	CNT	6–7	HSC	33–36	POS	6	–	39–52	CON	BLP-SMO	2.0–3.0	32–34	11–12
hexincisis (Jairajpuri and Baqri, 1968)	0.85–1.08	32–39	6.0–8.2	18–23	52–55	OFF	7	INC	17–19	POS	6	16 MID	58–60	SCL	SHM-ANN	3.0–4.0	28–31	8–9
icarus (Wallace and Greet, 1964)	1.45–1.96	29–34	5.9–6.9	19–25	50–57	CNT	8	HSC	34–42	POS	6	–	50–59	CYL	HEM-ANN	2.2	40	12
koreanus Choi and Geraert, 1971	0.75–0.92	30–44	5.1–6.1	12–16	53–60	OFF	6	LSC	23–25	ANT	6	15–17	43–46	CON	BLP-ANN	3.4–3.8	?	?
laminatus (Wu, 1969)	0.80–1.20	31–39	6.0–7.3	13–16	52–55	OFF	6–8	LSC	20–21	POS	6	–	50	CON	BLP-ANN	3.0–3.9	27–30	9–11
lenorus (Brown, 1956)	0.63–0.78	22–29	5.2–6.7	12–15	52–58	OFF	6	LSC	18–20	POS	6	24 MID	28–30	CON	SHM-SMO	3.7	27	7
leptus (Allen, 1955)	0.64–0.96	26–34	4.5–5.7	11–12	51–56	OFF	8–9	LSC	23–27	POS	6	–	50–58	CON	BLP-ANN	3.0–4.0	?	?
lineatus (Allen, 1955)	0.85–0.86	30–33	4.8–5.0	12–13	51–53	OFF	6	INC	25–28	POS	6	–	42–44	SCL	HEM-SMO	3.0	29	11
macrodens (Allen, 1955)	1.02–1.12	30–32	5.1–6.0	14–15	50–55	OFF	7	HSC	43–48	POS	6	–	34–37	SCL	SHM-SMO	2.6	37	11
macrurus (Goodey, 1932)	0.83–1.19	19–25	4.8–6.3	14–20	54–59	CNT	8–10?	HSC	25–34	POS	6	–	39–47	CYL	HEM-ANN	2.4	39	11
microdorus (Geraert, 1966)	0.58–0.70	24–29	4.8–5.9	11–13	55–59	OFF	4–6	LSC	13–15	POS	6	–	53–54	SCL	BLP-SMO	2.5–3.0	20–22	7–8
nanus (Allen, 1955)	0.52–0.64	27–31	4.5–5.3	10–12	52–57	CNT	7	LSC	12–15	POS	6	–	55–60	CON	BLP-ANN	3.8	23	8
nothus (Allen, 1955)	0.55–0.70	24–30	4.0–5.1	10–11	53–57	CNT	6	LSC	16–18	POS	6	–	39–48	CON	SHM-ANN	3.0	?	?
obscurisulcatus (Andrássy, 1959)	0.79	50	5.8	17	55	OFF	6	LSC	22	POS	6	–	27–32	CON	BLP-SMO	3.8	?	?
obscurus (Allen, 1955)	0.63–0.79	26–37	4.6–5.3	13–16	53–58	CNT	7	LSC	24–27	POS	6	–	53–58	CON	BLP-ANN	3.2	29	8

Table 1 cont'd.

	Length in mm	a	b	c	V%	Labial region	Labial annules	Frm. scl.	Stylet length in μ	Sty. cli.	Lateral inci-sures	No. long. striae	No. tail annules	Tail shape	Tail terminus	T/ABW	Spicule length in μ	Gubern. length in μ
parobscurus (Mulvey, 1969) n.c.	1.25–1.50	41–46	5.7–6.8	13–16	50–53	CNT	6–7	INC	22	POS	6	–	65	CON	BLP-ANN	4.5	30	?
polonicus (Szczygiel, 1970) n.c.	0.76–0.81	34–41	5.6–6.2	15–18	53–56	OFF	5	INC	21–25	POS	6	–	40–60	SCL	SHM-ANN	3.1–3.6	19	5–6
quadrifer (Andrássy, 1954)	0.64–0.73	26–28	5.0–5.9	17	57–59	OFF	5	INC	20–22	POS	6	30 MID	22	CYL	HEM-SMO	2.7	?	?
rugosus (Siddiqi, 1963)	0.80–0.90	30–32	4.4–5.1	16–17	55–56	OFF	6–7	INC	23	POS	6	32–36 MID	24	CON	BLP-SMO	3.0	?	?
sobolevi (Mukhina, 1970) n.c.	0.55–0.58	23	5.1	12–13	54–55	OFF	5	?	18	LAT	6	20 MID	38–40	CON	BLP-SMO	2.6	?	?
socialis (Andrássy, 1962)	0.89–0.92	29	5.2–5.6	14–20	59–60	CNT	5–6	LSC	24–26	LAT	6	–	35–39	SCL	HEM-ANN	2.0–2.8	28–29	8
stegus (Thorne and Malek, 1968)	0.70	26	5.1	17	55	OFF	4–5	MSC	20–22	LAT	6	24	21–22	CON	BLP-SMO	2.3	28	9
superbus (Allen, 1955)	1.12	28	5.6	22	56	OFF	8	INC	67	POS	6	–	26–29	SCL	HEM-SMO	1.4	33	12
tartuensis (Krall, 1959)	0.76–1.04	31–35	4.6–5.1	17–19	55–56	OFF	6	HSC	24	POS	6	24–28	30–37	CON	BLP-SMO	1.9	?	?
tatrensis (Sabová, 1967) n.c.	0.71–0.85	31–35	4.3–4.4	13–18	58–59	OFF	5–6	HSC	23–24	POS	6	–	20–23	CYL	HEM-SMO	2.3–2.6	?	?
tessellatus (Goodey, 1952)	0.83–0.94	28–31	5.7–6.5	12–14	50–55	OFF	7	LSC	18–20	POS	6	48	36	SCL	SHM-ANN	2.4	34	11–12
undyferrus (Haque, 1967)	0.54–0.61	21–29	4.5–5.4	9–14	52–58	OFF	7	HSC	16	POS	6	–	45	SCL	SHM-ANN	3.5	23	8
varians (Thorne and Malek, 1968)	0.90	29	5.7	17	55	CNT	5	HSC	20–22	LAT	6	–	21	SCL	SHM-SMO	2.3	?	?
Geocenamus Thorne and Malek, 1968																		
arcticus (Mulvey, 1969) n.c.	1.05–1.27	33–41	5.8–6.5	15–19	52–58	OFF	6–7	LSC	34–38	POS	6	–	46	SCL	SHM-SMO	3.0	26–28	7.8
longus (Wu, 1969) n.c.	0.98–1.07	29–31	5.3–5.9	17	55	OFF	6–7	INC	53–65	POS	6	50 MID	32	CON	BLP-ANN	2.0–2.2	33–34	8
tenuidens Thorne and Malek, 1968	0.83	30	6.7	12	50	OFF	5	LSC	27	POS	6	–	?	CON	BLP-?	2.9	22	7

Table 1 cont'd.

	Lenth in mm	a	b	c	V%	Labial region	Labial annules	Frm. scl.	Stylet length in µ	Sty. cli.	Lateral inci-sures	No. long. striae	No. tail annules	Tail shape	Tail terminus	T/ABW	Spicule length in µ	Gubern. length in µ
Nagelus Thorne and Malek, 1968																		
aberrans Thorne and Malek, 1968	0.90	31	5.8	15	62	OFF	8	INC	27	POS	6	–	55	CON	BLP-ANN	3.4	?	?
Tetylenchus Filipjev, 1936																		
abulbosus Thorne, 1949	1.00–1.40	39	6.6–7.4	10	52	CNT	7	INC	17	–	4	–	57			5.1	?	?
aduncus (de Guiran, 1967) n.c.	0.68–0.75	18–33	5.2–6.0	14–17	57–59	OFF	4–5	LSC	18–20	POS	4	–	19–27	Acute or subacute	Finely rounded	2.5–3.0	22–25	13
annulatus Merny, 1964	0.62–0.76	22–29	4.3–6.1	12–17	52–58	CNT	4–5	LSC	19–22	LAT	3	–	17			2.7–3.5	21–27	11–13
curiosus Wilski, 1964	1.01	24	5.7	11	52	CNT	5–6	LSC	29	POS	6	–	35			3.7	34	9
joctus Thorne, 1949	0.70	30	4.5	9	55	OFF?	7	INC	15	POS	6	–	42			3.8	19	6
productus Thorne, 1949	1.20	25	7.1	17	53	CNT	4–5	INC	12	POS	6	–	67			3.2	21	9
tenuis Micoletzky, 1922)	0.69	42	3.8	11?	52	OFF	?	LSC	20	?	?	–	38			5.7	?	?

CODE

Labial region:
CNT = Continuous
OFF = Offset or constricted

Frm. scl. = Cephalic framework sclerotization:
HSC = Heavily sclerotized
MSC = Moderately sclerotized
LSC = Lightly sclerotized
INC = Inconspicuous

Sty. cli. = Inclination of anterior surface of stylet knobs:
ANT = Anterior
LAT = Lateral
POS = Posterior

No. long striae = Number (and position) of longitudinal striations:
CER = At cervical region
MID = At midbody

No. tail annules = Range of annules cited in the description or counted in the accompanying drawing.

Tail shape and tail terminus:
See Figure 1.

T/ABW = Tail length divided by width of body at anus.

Table 2. Departures from *Tylenchorhynchus*.

Tylenchorhynchus	To	By
alatus (Cobb, 1930)	*species inquirendae*	Tarjan (1964)
browni (Kreis, 1929)	*species inquirendae*	Allen (1955)
bucharicus (Tulaganov, 1949)	*species inquirendae*	Tarjan (1964)
bursifer Loof, 1960	*Paratrophurus*	Siddiqi (1971)
caromatus (Tulaganov, 1949)	*species inquirendae*	Tarjan (1964)
clavicaudatus Seinhorst, 1963	became *clavicauda*	Seinhorst (1968)
coffeae Siddiqi and Basir, 1959	*species inquirendae*	Tarjan (1964)
crassicaudatus Williams, 1960	syn. of *mashhoodi*	Baqri and Jairajpuri (1970)
dactylurus Das, 1960	syn. of *mashhoodi*	Baqri and Jairajpuri (1970)
digitatus Das, 1960	syn. of *mashhoodi*	Baqri and Jairajpuri (1970)
dissitus Colbran, 1969	*Paratrophurus*	Siddiqi (1971)
elegans Siddiqi, 1961	syn. of *mashhoodi*	Baqri and Jairajpuri (1970)
graminicolus Kirjanova, 1951	*species inquirendae*	Tarjan (1964)
indicus Siddiqi, 1961	syn. of *brevilineatus*	Siddiqi (1963)
mamillatus Tobar-Jiménez, 1966	*Scutylenchus*	Jairajpuri (1971)
ornatus Allen, 1955	syn. of *quadrifer*	Andrássy in Tarjan (1964)
paucus Kirjanova, 1951	*species inquirendae*	Meyl (1961)
rhopalocercus Seinhorst, 1963	*Trichotylenchus*	Seinhorst (1968)
sexamammillatus (Kirjanova, 1938)	*species inquirendae*	Tarjan (1964)
spinicaudatus Sch. Stekhoven, 1944	*Hirschmanniella*	Luc and Goodey (1963)
styriacus (Micoletzky, 1922)	*species inquirendae*	Allen (1955)
symmetricus (Cobb, 1914)	*species inquirendae*	Allen (1955)
trilineatus Timm, 1963	syn. of *triglyphus*	Andrássy in Tarjan (1964)
zeae Sethi and Swarup, 1968	syn. of *mashhoodi*	Baqri and Jairajpuri (1970)

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Plagioporus hypentelii sp. n. (Trematoda: Opecoelidae) from the Hogsucker, *Hypentelium nigricans* (LeSueur) (Osteichthys: Catostomidae)[1]

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ABSTRACT: *Plagioporus hypentelii* sp. n. (Trematoda: Opecoelidae) is described from the intestine of the hogsucker, *Hypentelium nigricans* (LeSueur), in streams of Adams County, Pennsylvania. It most closely resembles *P. serotinus* Stafford, 1904, but differs from it chiefly in its less elongate shape, smaller size, smaller testes, ovary, and eggs, straight cirrus sac, in hosts and geographic location of the hosts.

During the course of examination of freshwater fishes for endohelminths, an undescribed trematode of the genus *Plagioporus* Stafford, 1904, was recovered from the intestine of the northern hogsucker, *Hypentelium nigricans* (LeSueur).

Fish hosts were collected with nets or by electrofishing and usually autopsied within a few hours of capture. The worms were studied alive and then fixed in hot AFA with slight coverslip pressure. Whole mounts were stained with Semichon's acetocarmine and mounted in permount. Sections were stained with Harris' hematoxylin and eosin.

Unless stated otherwise, the following description is based upon 25 stained whole mounts and five sectioned specimens. All measurements are in micrometers. Means are given first followed by the range in parentheses and are from the whole mounts.

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[1] From a dissertation submitted to the Graduate School, University of Maryland, in partial fulfillment of the requirements for the Ph.D. degree in Zoology.

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Plagioporus hypentelii sp. n.
(Fig. 1)

Description: Body lanceolate, aspinous, length 837 (520–1162); width at acetabulum 251 (152–300). Acetabulum in anterior part of middle third of body, length 191 (152–232), width 202 (174–240). Oral sucker subterminal, length 100 (72–120), width 106 (84–129); prepharynx very short; pharynx length 50 (42–57), width 46 (38–56); esophagus length 64 (42–90), width 21 (14–27). Intestinal bifurcation preacetabular; intestinal crura extending to near posterior end of worm, width 27 (20–35). Excretory bladder short, reaching the posterior testis, length 121 (75–187), width 45 (40–53) (six bladders measured); flame cell formula $2[(2+2)+(2+2)] = 16$. Testes postacetabular, tandem, foretestis length 104 (68–144), width 148 (120–188); hind testis length 117 (81–180), width 142 (101–196). Cirrus sac elongate, partially anterior to acetabulum, length 199 (120–255), width 56 (45–72); containing seminal vesicle, length 125 (58–195), width 56 (42–72). Prostate complex leading to genital pore which is on left of midline just anterior to cecal bifurcation. Cirrus short, eversible, unarmed. Ovary just postacetabular and dextral, length 66 (38–104), width 88 (68–115). Seminal receptacle, Laurer's canal, ootype, and Mehlis' gland present. Vitelline reservoir triangular in shape, length 71 (53–106), width 43 (24–63). Uterus extending from ootype anteriorly; metraterm short, joining ejaculatory duct just prior to genital pore. Vitelline follicles numerous, mainly in two lateral bands from region of esophagus to near posterior extremity, length 37 (17–78), width 24 (9–38) (125 follicles measured). Eggs usually fewer than 20 in number, uterine egg length 58 (45–71), width 33 (26–41) (62 eggs measured).

Host: *Hypentelium nigricans* (LeSueur).

Location in host: Middle and posterior thirds of intestine.

Incidence: Of 34 *H. nigricans* examined 31 were infected with from one to 460 (mean 72.5) specimens of *P. hypentelii.*

Localities: Marsh Creek (US 30) and Middle Creek (US 15), Adams County, Pa.

Holotype: USNM Helm. Coll. No. 72458.

Paratype: USNM Helm. Coll. No. 72459.

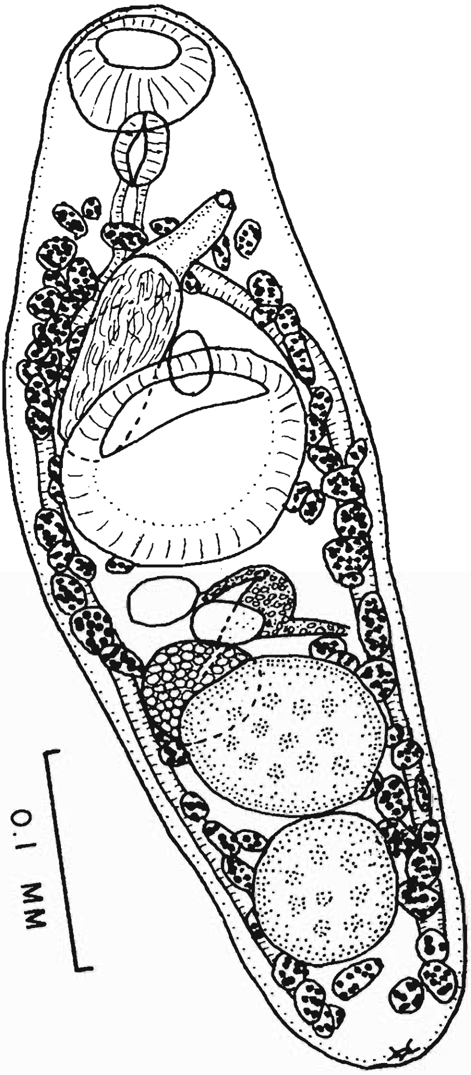

Figure 1. ***Plagioporus hypentelii*** **sp. n., ventral view of holotype.**

Discussion

There are currently seven species of *Plagioporus* from North American freshwater fishes. They include the following: *P. angusticolis* (Hausmann, 1896) Dobrovolny, 1939, in *Salmo*; *P. cooperi* (Hunter and Bangham, 1932) Price, 1934, in *Amocrypta, Cottus, Etheostoma, Gila, Notropis, Rheocrypta,* and

Richardsonius; *P. macrouterinus* Haderlie, 1953, in *Ptychocheilus*; *P. serotinus* Stafford, 1904, in *Moxostoma* and *Archoplites*; *P. serratus* Miller, 1940, in *Hiodon*; *P. siliculus* Sinitsin, 1931, in *Salmo*; *P. sinitsini* Mueller, 1934, in *Campostoma*, *Catostomus*, *Hyborhynchus*, *Hypentelium*, *Nocomis*, and *Notropis*; also *Plagioporus* sp. in *Salmo* and *Ictalurus* (Hoffman, 1967).

Plagioporus hypentelii differs from *P. angusticolis*, *P. macrouterinus*, and *P. siliculus* in its smaller size, smaller oral sucker, pharynx, ovary, testes, and cirrus sac. It differs further from *P. macrouterinus* in having smaller eggs and a uterus that does not extend posterior to the anterior margin of the foretestis.

P. hypentelii differs from *P. sinitsini* and *P. serratus* in location within the fish host. The latter two are found in the gall bladder while *P. hypentelii* is found only in the intestine. *P. hypentelii* differs further from *P. sinitsini* in its smaller acetabulum, oral sucker, pharynx, and eggs. *P. hypentelii* differs from *P. serratus* in being aspinous, having a larger body, acetabulum, oral sucker, and pharynx, in the position and larger size of the testes, and in host.

P. hypentelii differs from *P. cooperi* in body shape, shorter pharynx, smaller and fewer eggs, longer intestinal crura, and in the host and geographic location.

In the key for the subgenus *Plagioporus* (Stafford, 1904) in Arai and Dooley (1964), my specimens keyed to *P. serotinus*. This is also true for the key to the North American species of *Plagioporus* given by Miller (1940). Although *P. hypentelii* is found in the intestine of the common white sucker, *Catostomus commersoni* (Lacépède), sampled at different seasons, it does not attain sexual maturity in this host, while *P. serotinus* does. *P. hypentelii* differs further from *P. serotinus* in its less elongate shape, smaller body, testes, ovary, and eggs, and in having a straight rather than S-shaped cirrus sac. In addition to the above, *P. hypentelii* has a different host and host locality.

Based upon morphological criteria, two other species of North American *Plagioporus* have been removed from the genus by Pritchard (1966). These are now *Allopodocotyle lepomis* (Dobrovolny, 1939) and *A. virens* (Sinitsin, 1931). I agree that these two species do not belong in the genus *Plagioporus*.

Acknowledgments

I would like to thank Dr. Chu-Fa Tsai, Natural Resources Institute, University of Maryland, for his aid in collecting the fish, and Dr. J. Ralph Lichtenfels, Animal Disease and Parasite Research Division, USDA, Beltsville, Md., for the loan of slides of *P. cooperi* and *P. sinitsini* from the U. S. National Museum Helminthological Collection. Special thanks go to Dr. Leo A. Jachowski for his aid and encouragement in this work.

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Development of Second-generation Schizonts and Immature Gamonts of *Eimeria larimerensis* in Cultured Cells[1]

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ABSTRACT: The in vitro development of *Eimeria larimerensis* was studied by inoculation of sporozoites into Leighton tubes with monolayers of Madin–Darby bovine kidney (MDBK) and embryonic lamb thyroid (LETh) cells. Mature first-generation schizonts were seen in MDBK as well as LETh cells. First-generation merozoites left these cells, invaded others, and developed into second-generation trophozoites and schizonts. Some merozoites transformed into second-generation trophozoites without leaving the original host cell, but further development was not seen. Mature second-generation schizonts with two or four merozoites occurred only in MDBK cells. *Eimeria larimerensis* merozoites of the second generation from squirrels were inoculated into monolayers of MDBK and primary cell aggregates of embryonic rabbit kidney (REK). Merozoites did not develop in REK cells, whereas many merozoites in MDBK cultures transformed into spherical trophozoites and then developed into gamonts. Macrogamonts with a relatively large nucleus and prominent nucleolus as well as a few small plastic granules in the cytoplasm were seen at 24–120 hr; most observed after 72 hr appeared degenerate. Immature microgamonts with 10–15 nuclei were seen at 36–48 hr, whereas more advanced stages with more numerous and smaller nuclei as well as invaginations at the surface of the gamont were observed at 48–72 hr. Further development was not observed during a period of 120 hr from inoculation of the cultures.

Speer and Hammond (1970) reported the development of first-generation schizonts and immature second-generation schizonts of *Eimeria larimerensis* in cell cultures. The development of sporozoites to mature second-generation schizonts of a mammalian *Eimeria* species in cell culture has been described previously in only *E. magna* from rabbits (Speer and Hammond, 1971). The development of sporozoites to mature second-generation schizonts and of merozoites to immature gamonts of *E. larimerensis* in cultured cells is reported herein.

Materials and Methods

Established cell-line cultures of Madin-Darby bovine kidney (MDBK; 112, 113, and 114 serial passage) and cell-line cultures of embryonic lamb thyroid (LETh; 12 and 13 serial passage) were used. The methods of obtaining and maintaining the cell cultures as well as for handling the oocysts were similar to those described by Fayer and Hammond (1967). One ml of suspension containing 150,000 sporozoites in serum-free culture medium was inoculated into each Leighton tube containing a monolayer of one of the two cell types mentioned above. At 6-hr intervals for 120 hr, the monolayers were examined in double-coverslip preparations (Parker, 1961) with Zeiss–Nomarski interference-contrast or phase-contrast microscopy, and then fixed in Schaudinn's fluid and stained with Harris' hematoxylin and eosin (H & E).

A method similar to that described by Speer and Hammond (1972) was used to obtain further development of *E. larimerensis* in cell cultures. Monolayers of MDBK (261 and 263 serial passage) and primary cell aggregates of embryonic rabbit kidney (REK) were each inoculated with merozoites obtained by scraping the mucosa of the lower ⅔ of the small intestine of ground-squirrels inoculated 3½ and 4 days earlier with approximately 200,000 oocysts of *E. larimerensis.* The mucosal scrapings were gently stirred with a glass rod, and the mixture was then rinsed in saline containing 5,000 μg dihydrostreptomycin and 5,000 units penicillin G/ml. The suspension was centrifuged at 200 g for 1 min and the supernatant fluid, containing the merozoites, was removed. The merozoites, resuspended in Minimal Essential Medium (MEM) in

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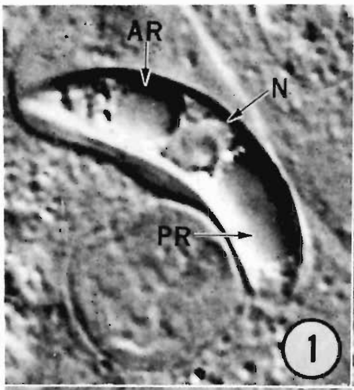

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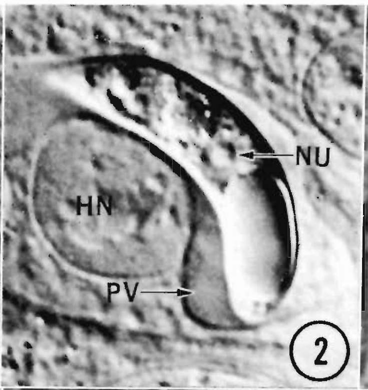

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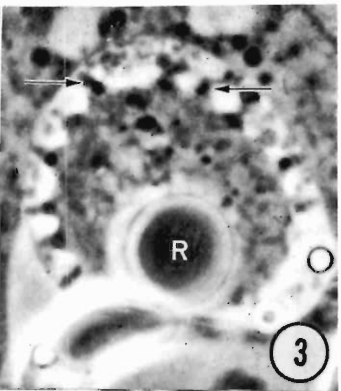

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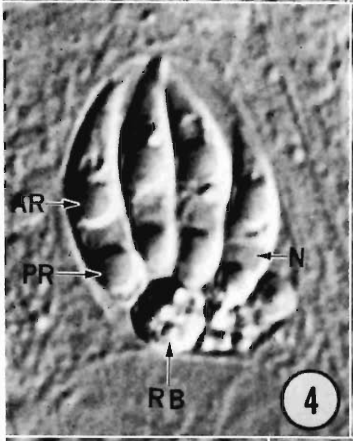

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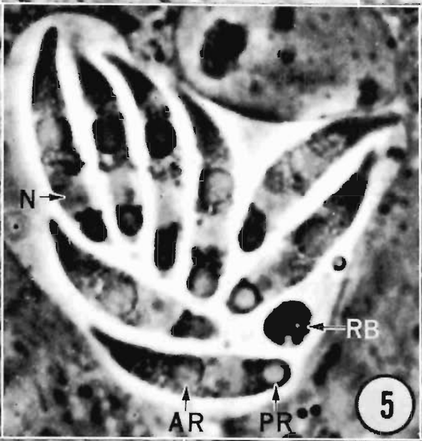

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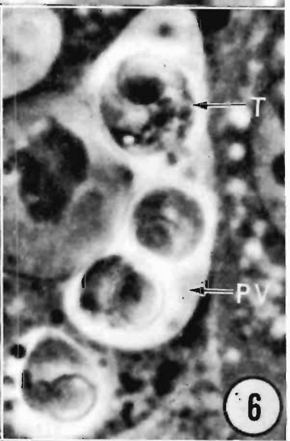

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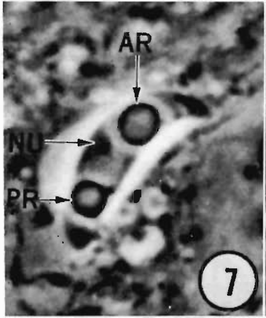

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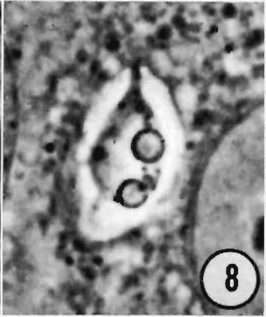
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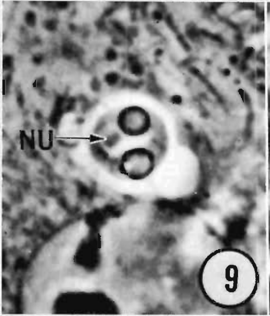

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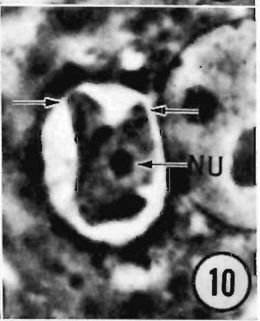

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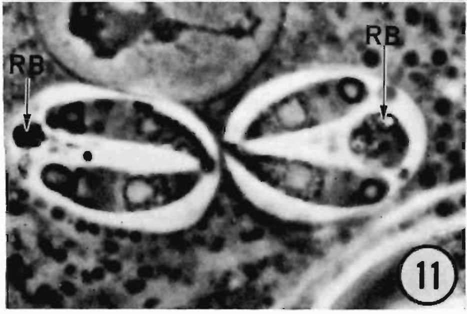

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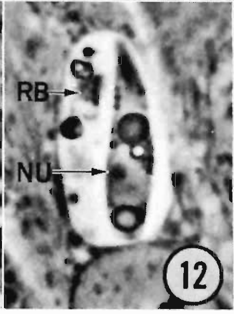

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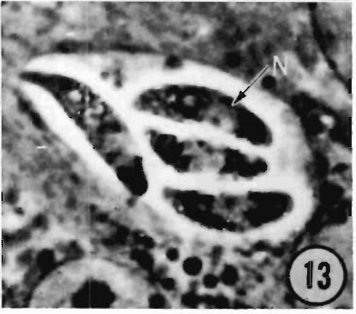

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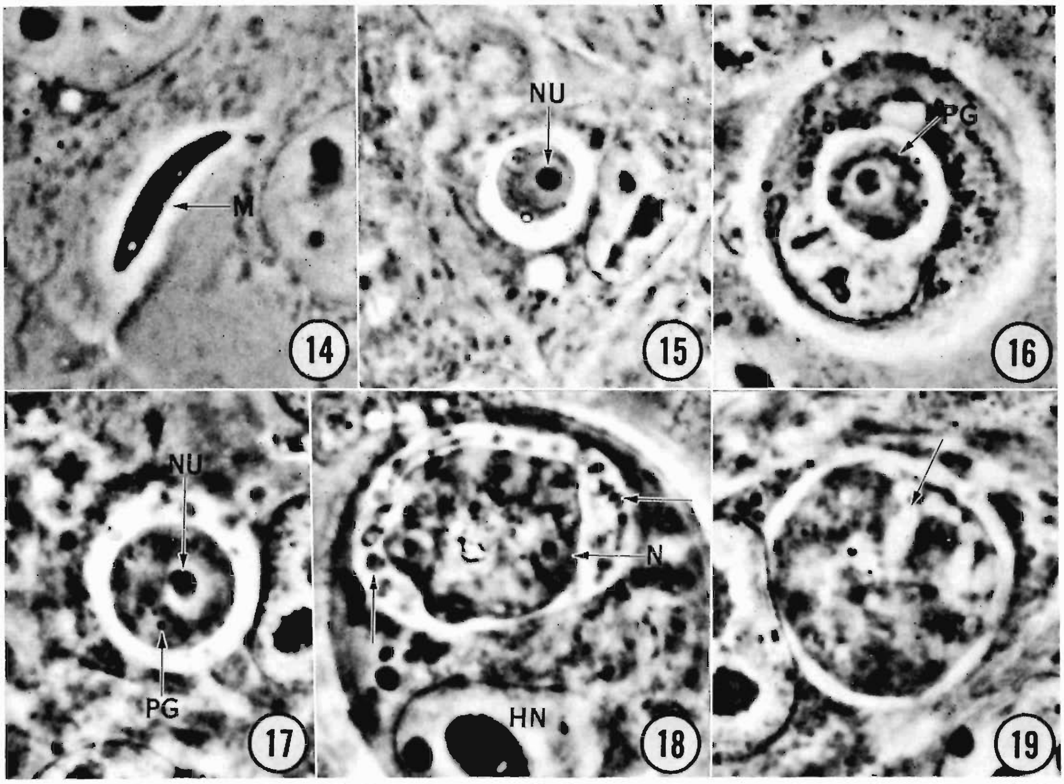

Figures 1–19. Photomicrographs of development of schizonts and gamonts of *Eimeria larimerensis* in Madin–Darby bovine kidney cells. Figs. 1–5 development of first-generation schizonts; 113-passage MDBK cells. Figs. 6–13 development of second-generation schizonts; 113-passage MDBK cells. Figs. 14–19 development of immature gamonts; 263-passage MDBK cells. All are of living specimens photographed with Zeiss–Nomarski interference-contrast (Figs. 1, 2, 4) or phase-contrast (Figs. 3, 5–19) microscopy; × 2,000. Hours after inoculation of sporozoites or merozoites into cultures listed in parentheses. Abbreviations: AR = anterior refractile body; HN = host cell nucleus; M = merozoite; N = nucleus of parasite; NU = nucleolus of parasite; PG = plastic granule; PR = posterior refractile body; PV = parasitophorous vacuole; R = refractile body; RB = residual body; RG = refractile granule; T = trophozoite. Fig. 1. Sporozoite (6). Fig. 2. Binucleate sporozoite-shaped schizont (18). Fig. 3. Schizont with initial stage of merozoite formation (arrows) (24). Fig. 4. Schizont with budding merozoites; merozoites are attached to a small residual body (24). Fig. 5. Mature first-generation schizont with eight merozoites (36). Fig. 6. Merozoites which have transformed to trophozoites without leaving the host cell (48). Fig. 7. Intracellular first-generation merozoite (36). Fig. 8. Stage in transformation of first-generation merozoite to second-generation trophozoite; note increased width of the middle region of the merozoite (36). Fig. 9. Second-generation trophozoite (36). Fig. 10. Early stage in formation of two second-generation merozoites (arrows) (42). Fig. 11. Two second-generation schizonts, each with two merozoites and a residual body (48). Fig. 12. Second-generation merozoite in original parasitophorous vacuole; note presence of residual body material (48). Fig. 13. Mature second-generation schizont with four merozoites (60). Fig. 14. Merozoite (12). Fig. 15. Young macrogamete (24). Fig. 16. Immature macrogamete; host cell appears spheroidal in contrast to the flat appearance of uninfected cells (36). Fig. 17. Immature macrogamete. Fig. 18. Intermediate microgamont with 10 to 15 nuclei, six of which are visible; note presence of spherical masses (arrows) in parasitophorous vacuole (36). Fig. 19. Intermediate microgamont; note invaginations at the margin of the gamont (arrow) (72).

Earle's balanced salt solution without serum or antibiotics, were counted with the aid of a hemacytometer. One ml of MEM, containing approximately 400,000 merozoites, was then inoculated onto the monolayer grown on a 10- by 35-mm coverslip. After 6 hr of incubation at 37 C, 2 ml of MEM containing 3% fetal calf serum as well as 50 units/ml penicillin G and 5 μg/ml dihydrostreptomycin were added to each tube. At intervals of 12 hr for 120 hr, coverslips were removed from the tubes and examined with phase-contrast microscopy. After examination, coverslips were fixed in methanol or Schaudinn's fluid and stained with Giemsa's or H & E. Measurements of 20 or more living specimens were made with an ocular micrometer at a magnification of 1,000× to determine each mean value. Measurements are given as the mean in microns, with the range in parentheses.

Results

Asexual stage development

One to 5 days after inoculation, some intracellular sporozoites (Fig. 1) had developed to mature first-generation schizonts (Fig. 5) by a process similar to that described previously for this species by Speer and Hammond (1970). Sporozoite-shaped schizonts (Fig. 2) and schizonts with developing merozoites (Figs. 3, 4) were seen at 18 to 108 hr after inoculation. At 36 hr, first-generation merozoites were 10.5 by 2.2 (6–13.5 by 1.5–3) and each had spherical anterior and posterior refractile bodies 1.5 (1–3) and 1.2 (0.5–2) in diameter, respectively. Merozoites were seen leaving MDBK and LETh cells as early as 36 hr after inoculation. Extracellular merozoites were observed flexing and gliding; some entered cells. Merozoites did not appear to change in body size or in appearance of refractile bodies after penetration of cells (Fig. 7). Transformation of the first-generation merozoite to a trophozoite usually occurred by an increase in width of the middle region of the merozoites (Fig. 8). Some merozoites which transformed into trophozoites while still within the parasitophorous vacuole in which they had developed (Fig. 6) were not observed to undergo any further development. Second-generation trophozoites (Figs. 6, 9), which were 4.1 (3.3–5.5) in diameter, were first seen at 42 and 60 hr in MDBK and LETh cells, respectively. Trophozoites had a nucleus, 2.2 (1.5–3), with a prominent nucleolus, 1 (0.5–1.5), as well as one or two refractile bodies. Occasionally, fusion of the two refractile bodies in a trophozoite was seen. Development beyond the second-generation trophozoite stage was not observed in LETh cells, whereas mature second-generation schizonts with two or four merozoites occurred in MDBK cells.

Two conical elevations representing an early stage in the formation of two merozoites appeared at the margin of some trophozoites (Fig. 10). At this time the single refractile body usually fragmented into several small refractile granules, some becoming incorporated into the anterior region of the developing merozoite. Division of the nucleus and nucleolus then occurred, and a daughter nucleus with its nucleolus and additional refractile granules were incorporated into each forming merozoite. At first, the merozoites were attached to a residual body, whereas in mature schizonts the separate residual body was usually located posterior to or between the two parallel merozoites (Fig. 11). Some specimens had anterior and posterior refractile granules, but others had only a single large refractile granule. At 60 hr, these merozoites were 8.3 by 2.2 (6–10 by 1.5–3) and had nuclei and nucleoli 2.3 by 2 (1.5–3 by 1.5–2.5) and 1 (0.5–1.5), respectively.

In second-generation schizonts which formed four merozoites, two nuclear divisions occurred before the appearance of the conical elevations at the periphery of the schizont. A single nucleus was incorporated into each forming merozoite, with several refractile granules anterior and posterior to the nucleus (Fig. 13). Usually, mature second-generation schizonts with four merozoites had no residual bodies (Fig. 13). Such schizonts were 12 by 8.5 (8–14 by 6–10); merozoites were 7.2 by 2.2 (6–9.5 by 1.5–3) and had nuclei which were 2.2 by 2 (1.5–2.5 by 1.5–2.5) with small eccentric nucleoli. Schizonts with two merozoites were seen at 48 to 120 hr, whereas those with four were seen at 60 to 120 hr. Of 100 schizonts observed, 72 had two merozoites and 28 had four. Apparently, some second-generation merozoites had left the schizont and host cell as indicated by the occurrence within a parasitophorous vacuole of a single second-generation merozoite as well as a residual body (Fig. 12).

Sexual stage development

Intracellular merozoites (Fig. 14), 8 by 2 (6–9 by 1.5–3), were first seen 12 hr after inoculation in MDBK and REK cells. At 24 to 26 hr, most merozoites in MDBK cells had transformed into spherical trophozoites, whereas development beyond the merozoite stage was not observed in REK cells. At 24 hr, young macrogamonts (diameter, 4.1) were usually distinguishable from microgamonts by the relatively large nucleus (2.5) and prominent nucleolus (1.5) (Fig. 15). Macrogamonts seen at 36 hr had corresponding values of 6.1, 3, and 2 (Fig. 16); at 48 hr these were 7.5, 3, and 2 (Fig. 17). A few small plastic granules were present in the cytoplasm of gamonts observed at 36 and 48 hr (Figs. 16, 17). At 72 to 120 hr, most specimens appeared to be degenerate and further development was not observed.

Young microgamonts observed at 24 hr were 4 (3.5–5) in diameter and had a nucleus and nucleolus, 2 and 1 in diameter, respectively. At 36 and 48 hr, stages which had approximately 10 to 15 nuclei were 11.5 (8.5–13) in diameter (Fig. 18). Usually, at this time the parasitophorous vacuole was partially filled with small spheroidal bodies, 1.5 in diameter (Fig. 18). In more advanced stages, seen at 48 to 72 hr, the nuclei were relatively small and indistinct (Fig. 19) and invaginations of the surface extended into the central region of the gamont. At 72 hr, microgamonts were 13.2 (10–15.5) in diameter. Further development was not observed, as in the macrogamonts.

At 36 to 72 hr, most host cells which harbored immature macro- and microgamonts were spheroidal (Figs. 16, 18), whereas non-infected cells had a flat appearance.

Discussion

The formation of second-generation schizonts of *E. larimerensis* with two or four merozoites was similar to that described for second-generation schizonts of *E. magna* in cell cultures (Speer and Hammond, 1971). In schizonts of both species in which a pair of merozoites was formed, a single nuclear division occurred simultaneously with merozoite formation. Such a process appears similar to endodyogeny in *Toxoplasma gondii* (Sheffield and Melton, 1968), *Besnoitia jellisoni* (Sheffield, 1966), *Frenkelia* sp. (Kepka and Scholtyseck, 1970), and *Sarcocystis* (Sénaud, 1967) as well as to the early stages of schizogony in *E. callospermophili* (Roberts et al., 1970) and *E. magna* (Danforth and Hammond, 1972). In endodyogeny, as well as in schizogony resulting in formation of two merozoites in *E. magna* (Speer and Hammond, 1971) and *E. larimerensis*, the developing daughter organisms appear in association with a single, dividing nucleus. However, in *E. callospermophili* four to 10 nuclei are present in each schizont before merozoites begin forming and, with the additional nuclear divisions occurring during merozoite formation, eight to 20 merozoites result (Roberts et al., 1970). This process resembles endopolygeny, which was first described in *Toxoplasma gondii* in ascitic fluid or peritoneal cells from mice (Vivier, 1970). The asexual stages of *T. gondii* occurring in the intestine of the cat reproduce by such a process (Sheffield, 1970; Piekarski, Pelster, and Witte, 1971). In endodyogeny and in the endopolygeny-like process occurring in *E. callospermophili* (Roberts et al., 1970), *E. magna* (Danforth and Hammond, 1972), and *E. stiedai* (Heller, 1971), merozoites are initially formed internally within the parent organism. The site of origin of the merozoites cannot be determined without electron microscopy, which was not used in the present study, so that we were unable to decide how closely the merogony process in *E. larimerensis* resembles endodyogeny or endopolygeny. Schizonts with only two merozoites have been described in the first-generation of *E. media* and *E. magna* (Rutherford, 1943; Pérard, 1925; Speer and Hammond, 1971) and in the second-generation of *E. perforans*, *E. media* (Rutherford, 1943), and *E. magna* (Speer and Hammond, 1971).

Todd and Hammond (1968) found that second-generation *E. larimerensis* schizonts in ground-squirrels had 22 to 46 merozoites which were larger than those of the first generation. In the present study of in vitro development, we found in second-generation schizonts two or four merozoites which were smaller than those of the first-generation. Rutherford (1943) found eight to 75 merozoites in second-generation schizonts of *E. magna* in rabbits, whereas Speer and Hammond (1971) found two or four merozoites in cell cultures.

In our first study of *E. larimerensis* in cell cultures (Speer and Hammond, 1970), sporozoites underwent development to bi- and trinucleate second-generation schizonts in MDBK cells which had been serially passed 180 times (passage number) from one culture vessel to another. In the present study, sporozoites developed to mature second-generation schizonts in MDBK cells with passage numbers of 112, 113, and 114. Such a finding suggests that the lower passage numbers of MDBK cells were more suitable for supporting growth of the parasite than higher ones.

Development of gamonts of a mammalian eimerian species in cell culture has been previously reported only in *E. magna*. By inoculating MDBK cell cultures with merozoites taken from a rabbit inoculated 5¼ days earlier, Speer and Hammond (1972) observed the development of gamonts and oocysts as well as a few schizonts in vitro. Their method was similar to that used previously by Bedrnik (1967) in which second-generation merozoites of *E. tenella* from chickens developed to gamonts and oocysts in cell culture. Strout and Ouellette (1969) reported gametogony in primary kidney cell cultures of embryonic chickens inoculated with *E. tenella* sporozoites. Doran (1970) also obtained development of *E. tenella* sporozoites to mature oocysts by using kidney cell aggregates from 2- to 3-week-old chicks. He (Doran, 1971) found that this method was successful for development of asexual stages in certain additional avian *Eimeria* species. In the present study, merozoites did not develop in rabbit kidney cell aggregates. In attempting to obtain development beyond the first-generation schizont and by using a method similar to that of Bedrnik (1967), Hammond et al. (1969) inoculated various cell cultures with first-generation merozoites of *E. bovis* obtained from infected calves. Although small numbers of intracellular merozoites were seen, no further development occurred. Speer and Hammond (1972) suggested that since sporozoites of *E. magna* developed only to mature second-generation merozoites, whereas merozoites from rabbits developed to oocysts in cell cultures, certain of the intermediate schizont stages must require substances or conditions for further development which are found only in the natural host. The findings of the present study indicate that this suggestion also holds true for *E. larimerensis*, although complete development of the sexual stages of this species in cell cultures was not observed.

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Reciprocal Transfer of *Heterakis gallinarum* Larvae between Chickens and Chukar Partridges: Effects on *H. gallinarum*, *Histomonas meleagridis*, and *Parahistomonas wenrichi*

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ABSTRACT: Ten-day-old larvae of *Heterakis gallinarum* were transferred rectally from chickens to chukar partridges, chickens to chickens, chukars to chickens, and chukars to chukars. In control chickens and chukars, the worms were allowed to develop undisturbed. Recovery of mature heterakids was higher, and the worms were larger, in recipient birds than in control birds. The incidences of infection with *Histomonas meleagridis* and *Parahistomonas wenrichi* were much higher in chickens than in chukars. Both parasites were detected in recipient chickens as early as 3 days after transfer of heterakid larvae. The transfer did not affect the ability of the heterakids to transmit *H. meleagridis* to test poults. We attribute the better survival and growth of *Heterakis gallinarum* in recipient birds to two main factors: (1) the larvae that die during or just after transfer are presumably weaker than those that survive, and (2) the transferred larvae are in a host that has not been immunized against them by tissue penetration of larvae less than 10 days old.

In six previous tests (Lund and Chute, 1971; Lund and Chute, 1972c) the cecal worm, *Heterakis gallinarum* (Schrank, 1788) Madsen, 1949, usually developed well in young chukar partridges, *Alectoris graeca* (Meisner, 1804) Kaup, 1829, for only the first 10 to 14 days. As growth slowed, worm numbers dwindled so that recovery of mature heterakids ranged from 0 to 4% of the number of embryonated eggs given, even if the incidence of histomoniasis was as low as 2%. Only 8 to 20% of the young chukars retained any heterakids to maturity. In contrast, *H. gallinarum* has almost always thrived in young chickens with *Histomonas meleagridis* (Smith, 1895) Tyzzer, 1920, if cecal involvement has not been severe (Lund and Burtner, 1958; Lund, 1958, 1967a, b; Lund and Chute, 1970; Lund et al., 1970). These findings raised several questions, including:

1) Did the heterakid larvae arouse such effective immune responses in most chukars, probably as a result of invasion of the cecal tissues, that after 2 to 3 weeks the worms were unable to develop normally or even survive?

2) If so, would transfer of young larvae to another chukar or to a more compatible host (a chicken) permit continued development?

3) Would transfer of the young larvae from a compatible host (a chicken) to a chukar after the larvae were exclusively lumen dwellers still arouse immunity in the chukar, or result in any adverse host–parasite response?

4) Is the apparent incompatibility of the chukar partridge and *Heterakis gallinarum* present with all strains of hosts and parasites?

It was possible to design a rather simple study that should help resolve Questions 1 to 3, but this required minimizing the variables suggested by Question 4. Thus, we used the same strain of host as in the previous studies (Lund and Chute, 1971; Lund and Chute, in press) but a different strain of *H. gallinarum*. Because we had no histomonad-free strain, we chose heterakids carrying the least virulent histomonads available.

Earlier studies (Lund, 1972; Lund and Chute, 1972b) had indicated that transfer of heterakid larvae affects the liberation of *H. meleagridis* and *Parahistomonas wenrichi* (Lund, 1963) Honigberg and Kuldova, 1969. Since these protozoa were carried by heterakids used in the present study, we continued to investigate their liberation and transmission by heterakids transferred to a new host.

Materials and Methods

The eggs of *Heterakis gallinarum* were from worms that had been maintained in chickens for several years. Histomoniasis was rare and mild. More than a year after the last passage in chickens, a single passage in turkeys was accompanied by only 4% incidence of infection with *Histomonas meleagridis*. Worms recovered from these turkeys were kept in 0.5% formalin solution for 3 weeks at room temperature to allow their eggs to embryonate before storage for 6½ months at 4 C. The eggs were then pooled and suspended in physiologic saline so that 1 ml contained about 133 embryonated eggs; this dose was believed to be well suited for these studies in view of the previous performance of this strain.

The chukar partridges and New Hampshire chickens used for the major part of this study, and the Beltsville Small White turkeys used to test for transmission of *H. meleagridis*, were from strains maintained at the Animal Parasitology Institute (formerly the National Animal Parasite Laboratory) for several years. All birds were incubator-hatched, brooded on wire, and maintained in wire-floored cages suspended over dropping pans with removable plastic-coated liners, to assure access to fresh cecal discharges at all times. Throughout the tests, each bird was caged individually. The chickens and turkeys were 6 weeks old when given heterakid eggs; the chukar partridges, not being in production throughout the year, were 11 weeks old.

Each of 30 chickens and 30 chukars was given about 133 embryonated eggs of *Heterakis gallinarum* by pipette to the crop. In 10 chickens and 10 chukars, designated as controls, the heterakids were permitted to develop undisturbed for 37 days. The cecal discharges of these 20 control birds were examined microscopically for protozoan parasites each day from 5 to 10 days after inoculation, and on alternate days thereafter through the 22nd day. The other 20 birds of each species were killed as donors of 10-day heterakid larvae to be transferred to recipient hosts by a process previously reported (Lund, 1972; Lund and Chute, 1972b). In each instance, half of the donors contributed larvae to recipient hosts of the same species (i.e., chickens to chickens or chukars to chukars) and the remaining donors provided larvae for recipients of the other species (chickens to chukars, chukars to chickens). Cecal discharges of all recipient birds were examined microscopically each day from 3 to 10 days after transferring the heterakid larvae, and on alternate days thereafter through the 22nd day. Five days later, when the heterakids were 37 days old, all birds were killed and

Table 1. Reciprocal transfer of 10-day-old *Heterakis gallinarum* larvae between chickens and chukar partridges: effects on the heterakids and on the transmission of *Histomonas meleagridis* and *Parahistomonas wenrichi*. Each control and donor bird was given 133 embryonated heterakid eggs.

	Chicken controls	Donors				Chukar controls
		Chickens	Chickens	Chukars	Chukars	
No. of birds	10	10	10	10	10	10
Recovery of 10-day *Heterakis*, %	–	75	69	94	54	–
Incidence of *Histomonas* at 10 days, %	20	30	50	0	0	0
		Recipients				
		Chickens	Chukars	Chickens	Chukars	
No. of birds	(10)	10	9	10	8	(10)
Heterakis larvae transferred, %	–	73	65	88	51	–
Recovery of 37-day *Heterakis*, % of:						
133 eggs fed to donors	16	21	17	18	16	18
Larvae transferred	–	29	26	21	34	–
Avg length of 37-day *Heterakis*, mm:						
Females	10.4	11.4	10.7	11.1	11.3	10.1
Males	8.8	9.4	9.0	9.3	9.1	8.4
Incidence by 37 days, %:						
Histomonas	50	50	0	70	12	0
Parahistomonas	100	90	10	90	12	0
Embryonated *Heterakis* eggs fed per *Histomonas* infection in test poults	180	165	125	160	215	225

examined for signs of histomoniasis. Fresh smears were made from the contents of each cecum for microscopic examination. All heterakids were washed from the ceca, counted, sexed, and measured; all mature females were kept in 0.5% formalin solution at room temperature for 3 weeks to permit the eggs to embryonate. The ability of these eggs to transmit *Histomonas meleagridis* to young turkeys was then determined by feeding entire female worms with known numbers of embryonated eggs, and by feeding comparable numbers of such eggs pooled from worms from the same sources. This method has been employed in studies already described (Lund and Burtner, 1957; Lund, 1968; Lund and Chute, 1972c).

Results

The results are summarized in Table 1. The recovery of 10-day heterakids averaged 72% for the 20 chickens killed as donors, and 74% for the 20 chukars. The recoveries of mature heterakids from recipient birds of each species 27 days after transfer, based on the number of embryonated eggs given, were very similar to each other and to the recoveries of undisturbed worms in the two control groups. An average of about 4% of the larvae were lost during the transfer procedure. Adjusting the data for the recipient groups according to this loss, the recovery of mature worms was somewhat better in recipient birds than in birds of either control group. Moreover, the heterakids recovered from recipient birds were, on the average, longer than those recovered from control birds. Differences in worm size were found to be statistically significant ($P < 0.05$) using Student's *t* test, as shown in Table 2.

Histomonas meleagridis was first detected in cecal discharges 7 days after feeding the heterakid eggs. By the 10th day, more than 30% of the 30 chickens were infected. The incidence of infection in the control chickens eventually reached 50%. Histomonads were not seen in any of the 30 chukars given heterakid eggs. However, the protozoa were found in 70% of the chickens that received 10-day heterakid larvae from chukars and in 50% of those that received larvae from chickens. In these recipients, *H. meleagridis* was first detected only 3 days after transferring the larvae. Histomonads were found in only one chukar that received larvae, and this infection required 24 days to become detectable.

The incidence of infection with *Parahistomonas wenrichi* was also high in chickens and low in chukars. Because this organism characteristically appears late when introduced by embryonated heterakid eggs (Lund, 1963), it was never found in donors of 10-day larvae. *P. wenrichi* was first detected in control chickens 14 days after inoculation, and the

Table 2. Statistically significant differences in length of mature worms recovered from control and recipient birds.

Group with shorter worms	Group with longer worms: Chicken-to-chicken recipients	Chukar-to-chicken recipients	Chukar-to-chukar recipients
Chicken controls	+*	+	+
Chicken-to-chukar recipients	+	+	+
Chukar-to-chicken recipients	+	—	—
Chukar-to-chukar recipients	+	—	—
Chukar controls	+	+	+

* Student's t test, $P < 0.05$.

incidence reached 100% by the 23rd day. This organism was never found in the control chukars. In recipients of both chicken-to-chicken and chukar-to-chicken transfers, the incidence of infection with *P. wenrichi* reached 90% with the first detection occurring 3 days after transfer of heterakid larvae. Only one chukar in each recipient group became infected, and both infections were light.

The values in the final horizontal column of Table 1 indicate the relative ability of heterakid eggs from the six groups to effectively transmit *H. meleagridis* to young turkeys. For reasons presented in the discussion, the differences in these values are not considered important.

Discussion

In planning this study, we reasoned that if exposure of the host to the tissue stage of *Heterakis gallinarum* could be avoided, immunity might not be stimulated, or it might be delayed, less intense, or altered in some way. Tentatively, we attribute the apparently greater success of *H. gallinarum* in both chukars and chickens following transfer of larvae to the heterakids completing their development in a nonimmunized host. The larvae pass their tissue stage in the donor host and if not transferred to another bird, they must continue developing in the same bird with whatever immune responses they have aroused in it. The recipient of 10-day larvae does not experience tissue penetration by the heterakids. To be sure, many larvae die as a result of the transfer procedure (Lund, 1972; Lund and Chute, 1972b). But presumably it is the weaker larvae that die and the more viable ones that survive. Thus, those surviving the ordeals of transfer constitute a select group, as well as being in a more hospitable host from the immunological standpoint. We believe that these circumstances account for the higher percentage of transferred larvae surviving to 37 days, and their greater average size, compared in both respects to the unmolested larvae in the control birds.

We have no explanation of the means by which the strain of *Histomonas meleagridis* became so poorly adapted to the chukar partridge as to appear in only one bird, and then only as a transitory lumen dweller. It may have possessed this characteristic throughout. We knew the performance of these histomonads in only turkeys and chickens. Even in turkeys the incidence of infection was low, and clinical histomoniasis was rarely observed; such circumstances have often been accompanied by poor invasive powers in other species of birds.

Parahistomonas wenrichi has not been reported from chukar partridges, nor had we encountered it in this host until we found very transitory infections in two recipient chukars in this study. Obviously, there are strains of *P. wenrichi* that can survive in the chukar, but we know of none that thrives.

The transmission of *H. meleagridis* by eggs of *Heterakis gallinarum* from control and recipient chukars and chickens was similar to transmission by other strains of heterakids tested previously; in those, one infection with *Histomonas* was produced for each 125 to 230 embryonated eggs fed to young turkeys (Lund, 1968; Lund and Chute, 1970, 1972a) or young chickens (Lund and Chute, 1971). *However*, we attribute most liberations of histomonads to heterakids that die in the bird (Lund, 1972; Lund and Chute, 1972b). The proportion of larvae that carry viable histomonads varies, as does the proportion of larvae that die at times and in sites favorable to the survival of the histomonads. Hence, the ratio of embryonated eggs given to *Histomonas* infections initiated must likewise vary considerably. Additionally, individual test poults vary somewhat in their responses to both *Heterakis* and *Histomonas.* Consequently, the range of 125 to 225 embryonated eggs given per detectable infection with *H. meleagridis* is considered normal, attributable only to variations beyond the control of this type of study. If this strain of heterakids had become physiologically adapted to a single species of galliform bird by long passage in only that species, the results of the initial introduction into birds of another species might have been quite different (Lund et al., 1970).

Because of circumstances such as those mentioned above, we caution against concluding that transfers of *Heterakis gallinarum* larvae can be made with comparable success using any two species of galliform birds. If one were to assemble considerable data using various combinations of hosts, a pattern might emerge suggesting degrees of physiological similarity among the various hosts. However, one might also consider the degrees of similarity of the bacterial constituents of the biotic community of the hosts' ceca (Lund, 1969).

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BENJAMIN G. CHITWOOD

1907 - 1972

Benjamin G. Chitwood

I have been feeling sad about Ben Chitwood for a long time. His death, on November 19, 1972, of a heart attack, accentuates this sadness concerning a brilliant man and vigorous investigator whose personal life became marred by adversity and whose professional career suffered thereby.

My fortune was in meeting Ben at the height of his professional career, in 1936. He taught me nematology and encouraged me in research. His style of life, which consisted principally of work, resulted in enormous productivity and encyclopedic knowledge of nemas and related organisms. He had come to Washington in 1928 with a fresh baccalaureate from Rice Institute. From then until 1931 he had worked as a junior nematologist under Dr. N. A. Cobb in the Bureau of Plant Industry, USDA. During those same years he earned his doctorate at George Washington University, by night courses and study. That is really a remarkable record, to complete a doctorate in three years while maintaining a full-time job, and it speaks for his considerable intellect, genius, and his enormous energy and zeal. He was busy on the beginnings of his "Introduction to Nematology," which was to be published piecemeal over the next several years, and he was developing an interest in the chemistry and physiology of nemas, at a time when only a few people were turning their attention in that direction.

In 1937, he returned to the Bureau of Plant Industry and went to work at Babylon, Long Island, on nemas of ornamental plants. He returned to Beltsville in 1947, but stayed only three years. In 1950, he went to Catholic University as Associate Professor of Parasitology, where he remained for two years. He left the Washington scene, and most of us here lost contact with him. There was a severe disruption of his domestic life and of his own emotional life. I believe that these should remain his secrets.

One of his few recreational pursuits was long distance running. In his professional career, he was more of a sprinter. He turned out almost two hundred papers on a wide range of subjects, and there is his book, which will stand as a memorial to him. This prodigious effort was mostly in his early years, before his departure from Washington. Since that time I have seen him only a few times, at national meetings, and then only briefly. I am told by others who have had more recent contact with him, in the 1960's, that he sometimes displayed the brilliance and depth of knowledge that had earlier characterized him, but this was not constant. There were periods when he was not affiliated with any research organization, and he retired early, at the age of 57.

Rest well, Ben! You earned that entitlement early. And your troubles are over, although your friends will continue to regret them.

LEON JACOBS

Research Note

Capillaria hepatica (Bancroft, 1893): in vitro Hatching

Studies on the in vitro hatching of parasitic nematodes have been directed toward both an understanding of the mechanism of the hatching process as well as obtaining various developmental stages for experimental purposes. While the infective larvae of several phasmid nematodes have been obtained in such a manner, few comparable procedures have been developed for the aphasmids (Rogers and Sommerville, 1962, Adv. in Parasit. 1: 109–177). Chapman and Undeen (1968, Exp. Parasit. 22: 213–218) have reported on the conditions necessary to hatch *Trichosomoides crassicauda.* This communication reports a method to obtain viable first-stage larvae of *Capillaria hepatica* in quantity.

The general procedures for maintenance and continuous passage of *C. hepatica* were similar to those of Wright (1961, Can. J. Zool. 38: 167–182). Stock infections were maintained routinely with eggs prepared in the following manner. Adult male mice were killed 35 to 40 days after oral infection with 750 to 900 embryonated eggs. The livers were removed, homogenized in a Waring blender, and further ground in a glass tissue grinder. Following repeated centrifugation with distilled water to remove larger debris, eggs were washed three times in 0.6% formalin and incubated in screw-capped vials at 27 C for 7 to 8 weeks, which were aerated three times a week. Following embryonation and development of first-stage larvae within the eggs, vials were stored at room temperature. Standard dilution and counting procedures were used to prepare eggs for infection. Anesthetized mice were infected by gavage.

To obtain embryonated eggs free of liver tissue and debris, eggs were passed through two 400-mesh sieves, centrifuged, and washed. Egg suspensions then were layered on the surface of a gradient of 10 and 60% sucrose in 50-ml conical tubes and centrifuged at 2,800 rpm for 5 min. Clean eggs, at the interface between the sucrose solutions, were removed, washed five times in distilled water, and reconcentrated. The sucrose gradient separation was repeated when debris remained.

Washed eggs were incubated in equal parts of hypochlorite (Chlorox) solution and saline at room temperature for 45 min, washed three times by centrifugation in warm saline, and the number of embryonated eggs determined by dilution counts. Eggs were then placed in a 30-ml screw-cap test tube with the prehatching medium (PHM) consisting of 10 ml 0.1 M sodium bicarbonate containing 52.2 mg sodium dithionite (0.03 M), and 3 mg magnesium chloride and incubated in a shaker water bath at 37 C. The test tube mouth was covered with Parafilm® and a gas mixture of 90% nitrogen and 10% carbon dioxide was bubbled through at a slow and continuous rate. After incubation in PHM, eggs and larvae were transferred to minimum essential medium (MEM) for 2 hr further incubation. The number of hatched first-stage larvae was determined by dilution methods.

The present study demonstrated that, under in vitro conditions, a two-step process was involved in hatching: (1) pretreatment of eggs in a reducing medium and (2) transfer to a suitable medium for hatching. Studies using different times of incubation in PHM and MEM indicated that optimal hatching occurred with a 2-hr incubation in PHM followed by 2 hr in MEM, producing a maximum hatch of 68% (Fig. 1). During the period of exposure to PHM, a small percentage of hatched larvae were seen. However, 15 min after transfer to MEM there was a surge of hatching, amounting to approximately 50%. Following this, hatching continued at a lower level for the remainder of the 2-hr period in MEM. Upon hatching, first-stage larvae were extremely active and moved rapidly; however, mobility gradually decreased and the larvae became relatively quiescent after 2 hr in MEM.

Prior treatment of eggs with hypochlorite was necessary to produce good hatching. Indeed, such treatment alone followed by incubation in MEM led to hatching, but not of the order of that seen when PHM was used. Eggs not treated with hypochlorite failed to hatch or hatched in very low percentages in phosphate-buffered saline, 5% carbon dioxide

+ 5% oxygen, or in media lacking sodium dithionite or magnesium chloride, or in the presence of MEM alone. Preliminary studies indicated that a greater percentage hatch and viability occurred in MEM than in isotonic saline. Thus MEM was selected as the medium of choice. However, other tissue culture media may provide these conditions, but they have not been tested.

Under the above conditions, with a 2-hr incubation in the PHM and 2 hr in MEM, embryonated eggs 1 year of age or older did not hatch; however, embryonated eggs of 6 months of age produced a 50% hatch. It is possible that prolonged maintenance of the eggs in formalin may have been detrimental since Freeman and Wright (1960, J. Parasit. 46: 373–382) noted that infectivity of *C. hepatica* eggs decreased with prolonged storage in formalin.

Structurally, larvae hatched by the above method resembled those described by Wright (1961), who obtained first-stage larvae from eggs of *C. hepatica* by a squash technique. First-stage larvae hatched by the method described above were sieved through a 15-μM Nitex® nylon mesh filter to free them of shells and unhatched eggs and were then injected into the portal circulation of mice following laparotomy. Mature infections comparable to oral infections with embryonated eggs were produced. Eggs obtained from infections created by the intravenous injection of larvae developed in a normal manner and were infective for mice.

The present studies, while not directed toward an understanding of the hatching mechanism of *C. hepatica*, would seem to provide a point of departure for such a physiologically oriented investigation.

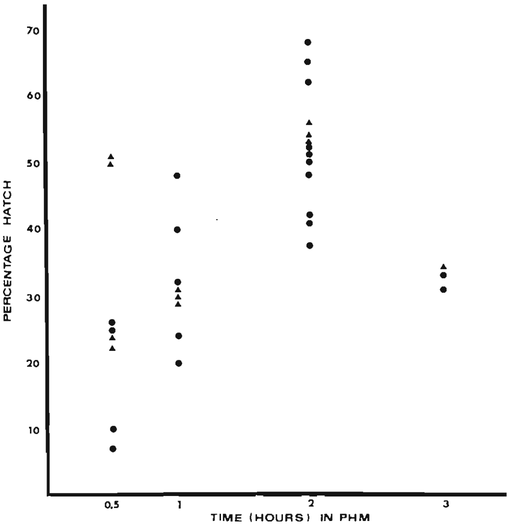

Figure 1. Relationship between hatching and time of incubation in prehatching medium followed by 2 hr in minimum essential medium. ▲—Hatched less than 5 weeks after embryonation. ●—Hatched 8 or more weeks after embryonation. Each figure represents an individual test.

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Research Note

Development of *Eimeria zuernii* in Cell Cultures

Eimeria zuernii is considered to be one of the most pathogenic species of *Eimeria* occurring in cattle. The endogenous stages of *E. zuernii* in calves were described by Davis and Bowman (1957, Am. J. Vet. Res. 18: 569–574). The development of large first-generation schizonts of *E. zuernii* in cultured bovine and ovine cells is reported herein.

Cell-line cultures of embryonic bovine synovial (BESy), tracheal (EBTr), and kidney (BEK-5), primary cultures of embryonic bovine kidney (BEK), and embryonic ovine

Table 1. Extent of development of *Eimeria zuernii* in various cell types.

Cell type*	Serial passage	No. of sporozoites or merozoites inoculated/tube	Extent of development‡
MDBK	123	120,000 Sp	MS
MDBK	244	130,000 Sp	ImS
BESy	16	100,000 Sp	Sp
EBTr	34	150,000 Sp	MS
BEK	1†	200,000 Sp	MS
BEK	5	130,000 Sp	MS
LEK	1	100,000 Mer	NP
EBTr	37	100,000 Mer	NP

* MDBK = Madin–Darby bovine kidney; BESy = embryonic bovine synovial; EBTr = embryonic bovine tracheal; BEK = embryonic bovine kidney; LEK = embryonic lamb kidney.

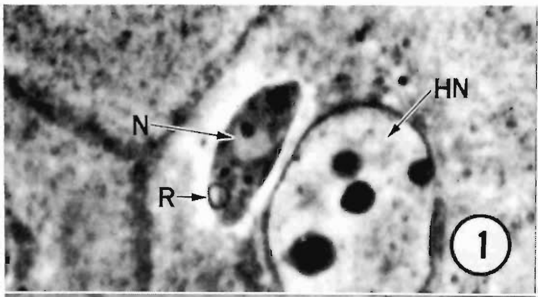
† 1 = Primary cell culture.

‡ Extent of development represents the most advanced stage of the parasite found throughout the experiment, days 1 to 20. MS = mature schizont; ImS = immature schizont; Sp = intracellular sporozoite; NP = no penetration of the host cell by the parasite.

kidney (LEK) as well as established cell-line cultures of Madin–Darby bovine kidney (MDBK) were used (Table 1). The methods of obtaining and maintaining the cell cultures and for handling the oocysts were similar to those described by Fayer and Hammond (1967, J. Prot. 14: 764–772). Each Leighton tube, containing a monolayer of one of the cell types mentioned above except for LEK, was inoculated with a 1-ml suspension of 100,000 to 200,000 sporozoites in serum-free culture medium [minimal essential medium (MEM) in Earle's balanced salt solution]. After 6 hr and at 3- to 4-day intervals for 20 days, the medium was removed and replaced with 2 ml of fresh MEM, containing 1 to 3% fetal calf serum. At 1- to 2-day intervals for 1 to 20 days, cover slips from Leighton tubes were removed and examined in double-coverslip preparations (Parker, 1961, Methods of Tissue Culture. 3rd ed. Paul B. Hoeber, Inc., New York, p. 145–151) with phase-contrast or Zeiss–Nomarski interference-contrast microscopy. These monolayers were then fixed in Schaudinn's fluid, stained with iron hematoxylin, and examined with bright field microscopy. For each of the stages, 10 to 20 live specimens were measured with an ocular micrometer at 1,000×. All measurements are in microns; ranges are in parentheses.

At 30 min after inoculation, intracellular sporozoites were located in close proximity to the host cell nucleus. These sporozoites were 8.3 by 2.4 (7 to 9 by 2 to 3) and each had a centrally located nucleus, 1 to 2 in diameter, as well as 0 to 6 small refractile granules randomly located in the cytoplasm. At 1 day, most sporozoites had 1 to 2 small refractile bodies and a nucleus with a prominent nucleolus (Fig. 1). At 1 to 8 days, there was little change in the appearance of the sporozoites except that a few had become shorter and wider. Trophozoites were first seen at 9 days (Fig. 2); these were 9.5 by 8.2 (8 to 13 by 6 to 10) with a nucleus and nucleolus 3.5 by 3 (3 to 5 by 2.5 to 3.5) and 1.5 in diameter, respectively. Most trophozoites had several small refractile granules. Usually, a dense-appearing crescent body (with phase-contrast microscopy), 7 by 1.5, was present in the parasitophorous vacuole (Fig. 2).

At 9 to 20 days in MDBK, EBTr, BEK, and BEK-5 cells, immature schizonts with 10 to several hundred nuclei, unusually large and prominent, were observed (Figs. 3, 4). In schizonts with 10 to 50 nuclei these were 2.7 (1.5 to 4) in diameter. Some refractile granular material was located in the cytoplasm. At this stage the crescent body (Fig. 4), 7 by 1.5 (1 to 8 by 1 to 2), appeared more dense than those seen in association with trophozoites; occasionally it was located in a separate vacuole in the host cell cytoplasm in close proximity to the parasite. At 18 days, infoldings of a peripheral layer of nuclei were observed. Merozoites were formed from the surface of such infoldings, blastophores, or from the surface of small schizonts by a budding process similar to that described for *E. bovis* (Hammond, Ernst, and Miner, 1966, J. Prot. 13: 559–564). Mature schizonts (Fig. 5), 61 by 53 (55 to 77 by 45 to 60), with 500 to 1,000 merozoites, were first seen in BEK cells at 18 days and in EBTr, MDBK (123rd passage), and BEK-5 cells at 19 days. Fully formed, rod-shaped merozoites (Fig. 6) were 6.5 by 1.5 (5.5 to 7 by 1 to 2) and had a centrally located nucleus. Extracellular merozoites were observed to undergo flexing (Fig. 6) and gliding movements. In an attempt to obtain further development, first-generation merozoites grown in EBTr cells were harvested at 20 days after inoculation of sporozoites, resuspended in fresh serum-free MEM, and inoculated into EBTr and LEK cell cultures. Five tubes of each cell type were each inoculated with 1 ml of MEM containing 100,000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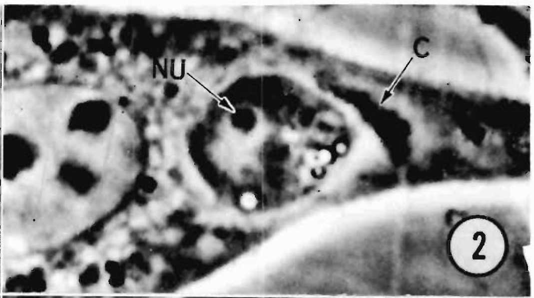
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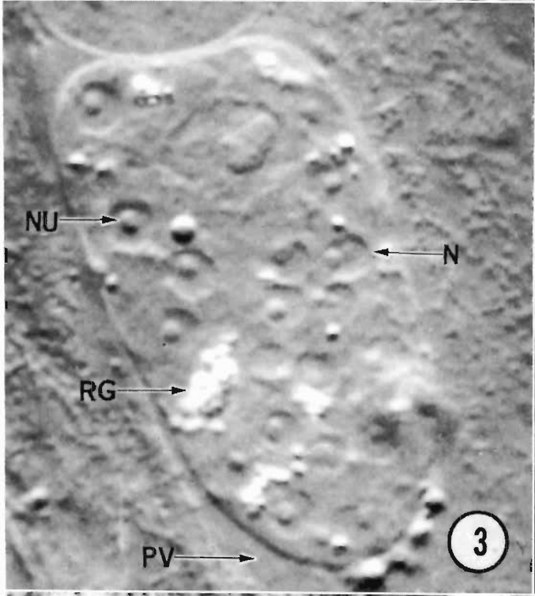
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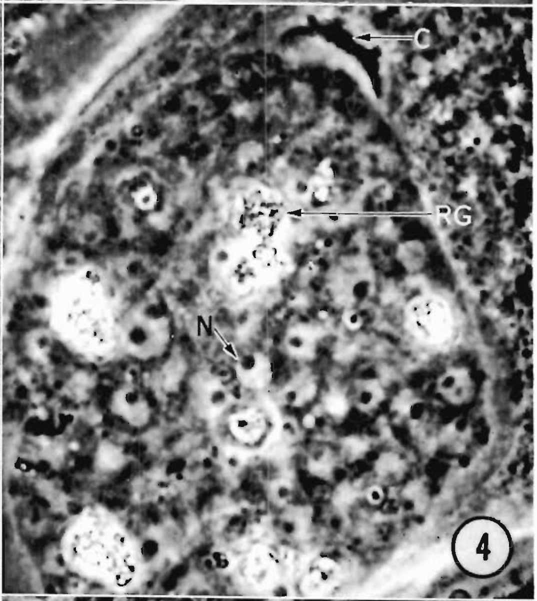
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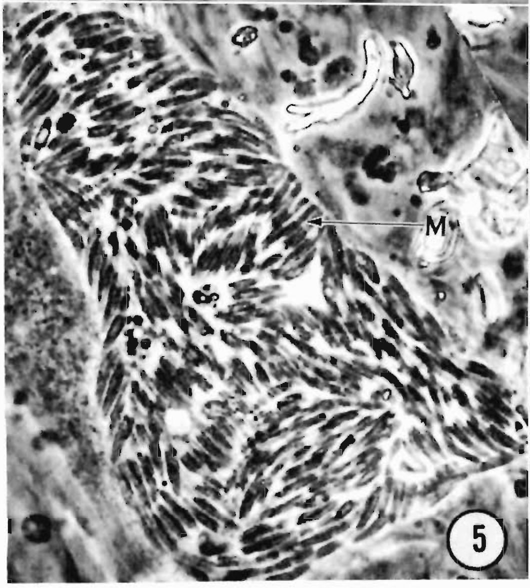
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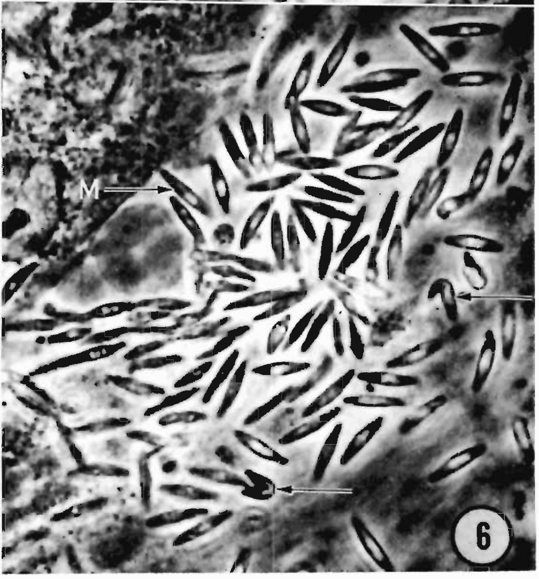
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merozoites. No intracellular merozoites or other evidence of development was seen.

Davis and Bowman (loc. cit.) found mature or nearly mature schizonts in the intestine of calves inoculated 6 to 19 days earlier with *E. zuernii* oocysts. These schizonts were from 7.0 by 9.8 to 10.5 by 16.1 and had 24 to 36 merozoites. Davis and Bowman (loc. cit.) found merozoites ranging in length from 7.0 to 16.1 at 7 to 19 days after inoculation. Stained merozoites had two refractile globules and a nucleus near the tapered end. Davis and Bowman (loc. cit.) found some indications of the occurrence of more than one generation of schizonts. In the present study, we found that *E. zuernii* sporozoites developed into first-generation schizonts several times larger than those seen by Davis and Bowman (loc. cit.). Each schizont had 500 to 1,000 merozoites, which were rod-shaped, had a centrally located nucleus, lacked refractile globules, and averaged 6.5 in length.

Eimeria species of ruminants which have previously been found to have large schizonts in cell cultures also develop such schizonts in the host (*E. bovis*, Fayer and Hammond, loc. cit.; Hammond, Ernst, and Miner, loc. cit.; *E. auburnensis*, Clark and Hammond, 1969, J. Prot. 16: 646–654; Chobotar, Hammond, and Miner, 1969, J. Parasit. 55: 385–397; *E. ninakohlyakimovae*, Kelley and Hammond, 1970, J. Prot. 17: 340–349; Wacha, Hammond, and Miner, 1971, Proc. Helm. Soc. Wash. 38: 167–180). Therefore, it seems likely that *E. zuernii* may have in its life cycle a large first-generation schizont, which was not observed by Davis and Bowman (loc. cit.).

The authors hereby express their appreciation to Mrs. Yoko Y. Elsner for assistance in maintenance of cell cultures and to Dr. L. Niilo of the Animal Diseases Research Institute, Lethbridge, Alberta, for supplying the majority of the oocysts used in this study.

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Figures 1–6. Photomicrographs of live developmental stages of *Eimeria zuernii* in cell cultures. Figures 1, 2, 4–6, were photographed with phase-contrast microscopy, Figure 3 with interference-contrast microscopy. Type of cell culture, passage number, interval between inoculation of sporozoites into culture and photographing, and magnification are listed in parentheses. Abbreviations: C, crescent body; HN, host cell nucleus; M, merozoite; N, nucleus of parasite; NU, nucleolus of parasite; PV, parasitophorous vacuole; R, refractile body; RG, refractile granular material. Fig. 1. Intracellular sporozoite (MDBK, 244, 0.5 hr, 2,200 ×). Fig. 2. Trophozoite (EBTr, 34, 24 hr, 2,200 ×). Fig. 3. Intermediate schizont with several nuclei (BEK, 5, 13 days, 2,000 ×). Fig. 4. Intermediate schizont (EBTr, 34, 14 days, 2,000 ×). Fig. 5. Mature schizont (BEK, 5, 20 days, 1,100 ×). Fig. 6. Extracellular merozoites; note that some merozoites (arrows) are in the process of flexing (BEK, 1, 18 days, 1,400 ×).

Research Note

Reighardia sternae (Cephalobaenida: Reighardiidae), a Pentastome from Gulls and Terns in Louisiana

While surveying the internal helminths and acarids of aquatic and semiaquatic birds in southern Louisiana, several specimens of *Reighardia sternae*, the only known pentastome from avian hosts, were recovered from the lungs and air sacs of *Larus delewarensis*, the ring-billed gull (in 2 of 14), *Larus atricilla*, the laughing gull (4 of 39), and *Sterna hirundo*, the common tern (1 of 12). These are reported herein as new host records for *R. sternae* from the two species of gulls and this is the first report of this parasite from the southern United States. The ring-billed gulls were collected about 50 miles south of New Orleans at Buras and the latter two host species were collected at Rockefeller Wild Life Refuge in Cameron Parish about 150 miles southwest of New Orleans.

Apparently, *R. sternae* is host-specific for birds of the family Laridae (gulls and terns). This species was first reported by Filippi (1862, Arch. Zool. Anat. Fisiol. 1: 52–76) in Europe and it was later recorded by Diesing (1864, Sitz.-Ber. Math.-Naturw. Classe Kais. Wiss. Akad. Wien 48, 49: 200–245) as "*Pentastomum Sternae Hirundinis.*" It was subsequently reported from North America by Ward (1899, Proc. Amer. Assoc. Adv. Sci. 1899: 254 Abstr.) who first used the generic name *Reighardia*. Adults were found in *Larus philadelphia* and *Sterna hirundo* from the north central United States. Faust (1927, Am. J. Trop. Med. 7: 311–325) reported nymphal stages of *R. sternae* from the hepatic portal blood and lungs of *Sterna fluviatilis* in China. Heymons (1935, Pentastomida. In Bronns, Klassen und Ordnungen das Tierreichs. Vol. 5, Sec. 4, Book 1, Leipzig, 268 p.) was the first to provide an adequate description of the adults from specimens collected in Europe and South America. More recently, this parasite has been reported from *Larus argentatus* and other gulls in Russia by Dubinina and Smogorzhevskaia (1956, Akad. Nauk SSSR Zool. Inst. Parazit. Sborn. 16: 213–216), Smogorzhevskaia (1956, Trudy II. Nauc. Kon. Parazit. USSR, Kiev 111–112), Michelson (1959, Lutvijas Podomju Soc. Rep. Zinatnu Akad. Trudy Biol. Inst. 12: 335–336), in Bulgaria by Buchvarov (1963, Bulg. Akad. Naukite. Investiia Tsentralnata Khelm. Lab. 8: 145–146), in Czechoslovakia by Macho [1964, Biologia (Bratislava) 19: 118–122], and in England by Pemberton (1963, J. Helm. 37: 57–88).

Specimens collected in the present study are in agreement with the description of the adults of *R. sternae* by Heymons (1935, loc. cit.). Although Osche (1963, Z. Morph. Okol. Tiere 52: 487–596) studied in detail the egg and embryo, he was unable to find larval stages or eggs with developing larvae. In the present study, larval stages were present in eggs from some of the larger females and larval development in eggs could be induced after a few days by incubation in buffered saline at pH 7 at room temperature. These stages are in agreement with the descriptions by Haffner and Rack (1965, Zool. Jb. Anat. 72: 419–444).

Although the life history of this species is unknown, Stiles and Hassel (1927, U. S. Pub. Health Serv. Hyg. Lab. Bull. 148: 197–289) and Heymons (1935, loc. cit.) suggested the possibility of fish as intermediate hosts. However, this has not been proven and, as Haffner and Rack (1965, loc. cit.) have suggested, this species may have a direct life cycle as found in certain other species of the Cephalobaenida.

Apparently, these parasites have little effect as adults on the host since no gross pathology was observed. The rates of infection ranged from one to eight pentastomes per host in birds examined in the present study.

This appears to be only the second report of *R. sternae* from North America. Although only a relatively small number of hosts were examined, it does not appear to be an uncommon parasite of these birds in Louisiana. Several other species of gulls and terns were examined, but these were negative for pentastome infections. However, it is probable that these parasites have a greater host and geographic range than is supposed. The birds examined in Louisiana are common over large areas of North America and they are migratory.

Specimens of *R. sternae* from the above hosts are deposited in the USNM Helm. Coll. (Nos. 63083 to 63085 from *Larus delewarensis*, *L. atricilla*, and *Sterna hirundo*, respectively), in the collection of Dr. J. Teague Self of the University of Oklahoma at Norman, and in the collection of the author.

Appreciation is expressed to Mr. Allen Ensminger, Refuge Director, and Messrs. Ted Joanen and Larry McNease of the Louisiana Wild Life and Fisheries Commission for assistance and permission to collect many of the birds examined in this study from the Rockefeller Wild Life Refuge.

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Research Note

Effect of Thiabendazole on a Drug-resistant Ovine Isolate of *Haemonchus contortus* in Cattle

Larvae of *Haemonchus contortus*, of bovine origin, readily infect both cattle and sheep, but those of ovine origin have a low order of infectivity for cattle (Roberts, 1942, Proc. Roy. Soc. Queensland 53: 97–100; Porter, 1953, Am. J. Vet. Res. 14: 550–554; Herlich, Porter, and Knight, 1958, Am. J. Vet. Res. 19: 866–872). Studies by Roberts, Turner, and McKevett (1954, Austral. J. Zool. 2: 275–295) confirmed by Bremner (1955, Austral. J. Zool. 3: 312–323; 1956, Austral. J. Zool. 4: 146–151), established two distinct species, *H. contortus* in sheep and *H. placei* in cattle. Certain ovine isolates of *H. contortus* are resistant to phenothiazine, thiabendazole, parbendazole, and cambendazole (Colglazier, Enzie, and Lehmann, 1967, Am. J. Vet. Res. 28: 1711–1722; Colglazier, Kates, and Enzie, 1970, J. Parasit. 56: 768–772; Theodorides, Scott, and Laderman, 1970, Am. J. Vet. Res. 31: 859–863; Colglazier, Kates, and Enzie, 1972, Proc. Helm. Soc. Wash. 39: 28–32). Surprisingly, however, there is no documentation of resistant bovine isolates, although Drudge (1970, J. Parasit. 56: 84–85) has noted resistance to phenothiazine and thiabendazole in equine strongyles. This study was designed to test the effect of thiabendazole on a drug-resistant ovine isolate of *H. contortus* in cattle.

Infective larvae of the AH-2 isolate of *H. contortus*, resistant to phenothiazine, thiabendazole, parbendazole, and cambendazole, were used (Colglazier et al., 1967, 1970, 1972, loc. cit.; Colglazier et al., 1971, J. Parasit. 57: 356–362). Each of 10 parasite-free Holstein calves, 8 to 9 months old, was given 15,000 ± 347 larvae. Each of five calves was drenched with 55 mg/kg thiabendazole 21 days after inoculation of larvae; the other five were not medicated. All calves were killed 7 days after medication. Counts of worms in duplicate 10% aliquots of the abomasal contents served to estimate the number of worms that remained.

An average of 1,432 (SD 348; SE 156) fifth-stage and 128 (SD 16; SE 8) fourth-stage worms were recovered at necropsy from the medicated calves. Nonmedicated calves had an average of 1,032 (SD 451; SE 202) fifth-stage and 104 (SD 23; SE 13) fourth-stage larvae. No more than eight eggs per gram were found in feces taken at necropsy and only a few adult females contained fertilized eggs.

It is clear that thiabendazole was ineffective against the AH-2 isolate of *H. contortus* in calves. By contrast, Colglazier et al. (1970, loc. cit.) reported an efficacy of 39% against the same isolate in sheep with a lower dosage of the same drug (50 mg/kg) and Herlich (1963, Vet. Med. 58: 874, 881–882) found a dosage of 55 mg/kg 95% effective against *H. placei* in calves.

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Research Note

Three Digenetic Trematodes of *Clarias mossambicus* Peters (Clariidae) from Ethiopia

The catfish, identified by Dr. Keith E. Banister, British Museum (Natural History), were collected in Wallo Province from the Awash River at Dubti on 5 February 1969 and Lake Haik near Dessie on 21 February and 19 April 1969. The worms were killed in hot water, transferred immediately to FAA fixative for 24 hr, and then stored in 70% alcohol plus 2% glycerin; they were stained in Mayer's carmalum and mounted in permount. From the small intestine of two of eight Awash River fish examined one and two adult *Eumasenia ghanensis* Fischthal and Thomas, 1968 (Maseniidae) were recovered; from two other fish one and three adult *Orientocreadium indicum* Pande, 1934 (Orientocreadiidae) were taken. The only African record for these two species is from Ghana in *Heterobranchus longifilis* Cuvier and Valenciennes (Clariidae) by Fischthal and Thomas (1968, Proc. Helm. Soc. Wash. 35: 126–140). From three of four Lake Haik fish examined hundreds of adult and immature *Eumasenia ghanensis* and *Glossidium pedatum* Looss, 1899 (Macroderoididae) were recovered; the former species occurred mainly in the upper small intestine with a few worms in the middle intestine, while the latter was found mainly in the large intestine with a few in the lower small intestine. *G. pedatum* has been reported only from *Bagrus bayad* Forskal and *B. docmac* (Forskal) (Bagridae) from the River Nile at Cairo by Looss (1899, Zool. Jahrb., Syst. 12: 521–784).

Inasmuch as my specimens of *G. pedatum* are smaller than those reported by Looss (3.3–3.5 mm long by 0.68 mm wide) and he gave very few measurements, a redescription, with additional data and measurements in microns of 10 adults, is presented: Body entirely spined, slightly rounded anteriorly, bifid and expanded laterally at posterior extremity, 1,175–1,920 long by 205–310 wide at acetabular level; forebody 450–715 long, hindbody 595–1,050 long, forebody–hindbody length ratio 1:1.29–1.71; oral sucker slightly rounded to truncate or slightly concave anteriorly, wider than long, 112–157 by 126–172, with compact muscle layer inside as well as outside posterior and posterolateral margins; ducts of glands at anterior margin of oral sucker but glands not visible; preoral space 15–30 long; acetabulum round to slightly longitudinally or transversely elongate, 110–155 by 110–155; sucker length ratio 1:0.95–1.12, width ratio 1:0.85–0.95; prepharynx 58–102 long, more thick-walled and muscular posteriorly, folded inwards just prepharyngeal to form sphincterlike structure; pharynx 63–90 by 65–105, four-lobed anteriorly with dorsal lobe longer and ventral lobe shorter than two lateral ones, gland cells lying anterolaterally and posteriorly; esophagus distinct, thick-walled, muscular, 35–77 long; cecal bifurcation 150–222 preacetabular, posterior tip between ceca devoid of cells; ceca conspicuously lined with tall columnar cells, terminating subequally 97–250 beyond testes and 102–235 from posterior extremity; testes longitudinally elongate, smooth, diagonal or sometimes nearly tandem; anterior testis dextral, 105–136 by 82–102, lying 153–310 postacetabular and 53–162 postovarian; posterior testis sinistral, 124–174 by 87–114, usually separated (12–39) from anterior testis in older worms, occasionally contiguous and with levels overlapping in young adults; posttesticular space 228–475 long (19–27% of body length); cirrus sac inverted J-shaped in ventral view, C-shaped in dextrolateral view, thick-walled, muscular, longitudinal extent 185–316 by 53–100 wide at level of seminal vesicle, commencing 39–123 postacetabular and dextral to ovary, dorsal to acetabulum and 29–53 anterior to it; seminal vesicle bipartite, posterior chamber longitudinally elongate, 56–122 by 48–94, anterior chamber transversely elongate, 22–44 by 36–60; pars prostatica longitudinally elongate, 55–85 by 39–58; prostate cells few; cirrus long; genital atrium small; genital pore anterodextral to acetabulum; ovary sinistral, smooth, longitudinally elongate but sometimes nearly round, 78–114 by 63–94, lying 0–59 postacetabular; ootype complex just postovarian; Laurer's canal sinuous, muscular, not reaching dorsal surface; seminal receptacle usually contiguous with ovary, round to longitudinally or transversely elongate, 27–49 by 29–53; vitelline follicles in lateral fields overlapping ceca, extending from posterior part of acetabulum or just postacetabular to posttesticular space, anterior limits usually at same level but sometimes subequal, posterior limits always subequal with shorter field sometimes ending at posterior testis level; postvitellarian space 128–375 long (11–22% of body length); uterus in mature adults filling most of hindbody, filling posttesticular space, lying ventral to gonads and proximal part of cirrus sac, occasionally overlapping ceca and vitellaria laterally, passing between testes; uterus in young adults filling most of posttesticular space with few coils anteriorly; metraterm thick-walled, muscular, slightly sinuous, usually commencing postacetabular, shorter than cirrus sac, ascending dextral to latter; eggs yellow to yellow-brown, operculate, 30 measuring 30–38 (34.1) by 18–23 (20.8); excretory bladder long, tubular, somewhat sigmoid-shaped in dorsal view, commencing medianly or nearly so at ovarian or seminal receptacle level, passing dorsally over sinistrolateral part of anterior testis, intertesticularly, dorsally over dextrolateral part of posterior testis, then medianly to excretory pore, latter subterminal ventral at median notch.

Specimens deposited in the U. S. National Museum Helminthological Collection are: *Eumasenia ghanensis*, No. 72224; *Orientocreadium indicum*, No. 72225; *Glossidium pedatum*, No. 72226.

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Research Note

A Simple Technique for Isolation of *Pelodera chitwoodi*, a Bacteriophagous Nematode, from Soil

Separation of nematodes from soil using Cobb's sifting and gravity method (Thorne, 1961, Principles of Nematology, McGraw-Hill, p. 1–553) involves much time and labor. Moreover, further separation by visual methods is necessary because nematodes extracted by this procedure comprise of a mixture of both bacteriophagous and nonbacteriophagous species. The attraction of some bacterial-feeding nematodes toward bacteria which serve as sole source of food for these nematodes has been demonstrated in our laboratory (Wilt and Smith, 1970, Water Resources Res. Inst. Bull. 701, Auburn Univ., p. 1–67; Joshi et al., 1971, Southern Soc. Parasitologists, Sec. Ann. Meet.; Joshi, 1972, M.S. thesis, Auburn Univ., p. 1–92). This report describes a technique which employs bacteria for the isolation of *Pelodera chitwoodi* from soil.

An *Agarbacterium* sp. and a *Vibrio* sp., which are excellent food for *P. chitwoodi* and some other free-living bacteriophagous nematodes, were grown separately in nutrient broth for 24 to 48 hr and the cells were harvested by centrifugation at 4,000 rpm. The bacterial pellet formed at the bottom of the tube was broken up with the aid of a Vortex Jr. mixer. Two drops of this thick bacterial suspension were placed on 2% water agar in a petri dish, each being 2 to 3 cm from the center of the dish. Ten grams of freshly collected mud, which was infested with species of *Tylenchus*, *Dorylaimus*, *Rotylenchus*, *Tylenchorhynchus*, *Aphelenchoides*, *Wilsonema*, *Criconemoides*, and *Pelodera*, were placed in the center of the dish and the cultures were incubated at ambient temperature (25 C). The dishes were examined under a dissecting microscope 3 to 4 hr after placing the mud sample. Ninety-nine per cent of the nematodes which accumulated in and around the bacterial colonies were identified as *P. chitwoodi*. These nematodes utilized washed cells of above bacteria as a sole food source. This technique was repeated several times with essentially the same results.

In order to obtain sterile larvae for monoxenic culture of the nematode, a gravid female was picked from a bacterial colony and transferred to a petri dish containing water agar where it was allowed to lay eggs. As soon as the larvae were observed, they were washed from the agar plate using sterile distilled water and suspended in 1:1,000 aqueous merthiolate (sodium ethyl mercuri thiosalicylate) for 30 min. After two more rinses in sterile distilled water, larvae were transferred to nutrient agar dishes containing one of the food bacteria. Active monoxenic cultures were obtained within 3 to 5 days.

We wish to thank Dr. John P. Hollis for his helpful suggestions. This work was supported by the office of the Water Resources Research of the Department of Interior and the Water Resources Research Institute, Auburn University, Alabama.

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Research Note

Helminths of Some Northern Arizona Lizards

During April and May of 1970 a total of 56 lizards representing eight species were collected in Yavapai and Coconino counties of northern Arizona and examined for helminths. Each lizard was necropsied immediately after death. Cestodes were fixed in alcohol–formalin–acetic acid solution (AFA), stained with Semichon's carmine, and mounted in balsam; nematodes were fixed in AFA and cleared in lactophenol.

Helminths were not found in three *Sceloporus undulatus*, one *Cnemidophorus velox*, or one *Crotophytus collaris*. The number of lizards

Table 1. Helminths of some northern Arizona lizards.

			New record of parasite for	
Host	Number examined	Parasite	Host	Arizona
Holbrookia texana	3	Cestoda *Oochoristica* sp.		
		Nematoda *Crytosomum heynemani* Gambino, 1958		
Holbrookia maculata	40	Cestoda *Oochoristica* sp.		
		Nematoda *Cyrtosomum heynemani* Gambino, 1958		
Phrynosoma douglasii	2	Cestoda *Oochoristica parvovaria* Steelman, 1939	*	*
Urosaurus ornatus	3	Nematoda *Pharyngodon warneri* Harwood, 1932	*	
Sceloporus magister	3	Cestoda *Oochoristica scelopori* Voge and Fox, 1950	*	*
		Nematoda *Physaloptera retusa* Rudolphi, 1819	*	*
		Crytosomum penneri Gambino, 1957		

of each of the other five species examined, the helminths found, as well as new host and locality records are reported in Table 1.

Although the *Oochoristica* spp. from *Holbrookia maculata* and *H. texana* appear to be new, additional material is necessary to provide an adequate description.

We are indebted to Mr. Grant Delph and Mr. Gene Olsen for providing host specimens.

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Research Note

Interaction of *Trichostrongylus axei* and *Haemonchus contortus* Administered Simultaneously to Calves

A few investigators have tested the interaction of simultaneously administered species of abomasal nematodes of ruminants. On the average, Herlich (1959, Proc. Helm. Soc. Wash. 26: 97–102) recovered similar numbers of *Ostertagia ostertagi* and *Trichostrongylus axei* from calves inoculated with either species as from calves inoculated with an equal number of larvae of both simultaneously. However, the average gain of singly infected calves in about 1 month was 4.54 kg whereas the dually infected calves lost 3.19 kg. Turner, Kates, and Wilson (1962, Proc. Helm. Soc. Wash. 29: 210–216) found that *T. axei* and *Ostertagia circumcincta* interfered with the establishment of *Haemonchus contortus* administered simultaneously to the same lambs. Establishment of *O. circumcincta* may have been adversely affected by the other species but neither establishment nor development of *T. axei* were adversely affected. In calves, Ross et al. (1968, Br. Vet. J. 124: 299–305) found no evidence to suggest that *T. axei* or *O. ostertagi* retarded the development of the other species administered 0 to 3 weeks after the first. The average percentage recovery of *T. axei* was similar whether administered alone or with *O. ostertagi*. The average recovery of *O. ostertagi* was 46% when given first and followed by *T. axei*, 23% when *T. axei* was given first, and 18% when given alone.

In the present study, interaction of *T. axei* and *H. contortus* was tested in calves. Six Holstein steers, averaging 8.7 (8.3 to 8.8) months old, raised worm-free and maintained under conditions preventing extraneous infec-

Table 1. Single and simultaneous dual inoculation with *Trichostrongylus axei* and *Haemonchus contortus:* worm recovery from six calves at 1 month postinoculation.

Larvae administered						
Species	*T. axei*		*H. contortus*		*T. axei* + *H. contortus*	
Number						
T. axei	150,000	150,000	0	0	150,000	150,000
H. contortus	0	0	10,000	10,000	10,000	10,000
T. axei						
Mature adults	50,091	62,290	—	—	56,530	56,200
Immature fifth stage	4,728	920	—	—	1,511	650
Late fourth stage	40	40	—	—	10	0
Total	54,859	63,250	—	—	58,051	56,850
H. contortus						
Mature adults	—	—	356	150	600	194
Immature fifth stage	—	—	4	0	64	2
Inhibited fourth stage	—	—	0	50	0	0
Total	—	—	360	200	664	196

tion, were inoculated with larvae obtained from sphagnum-cultured feces from lambs artificially infected with either species. Two calves were inoculated with *T. axei* alone, two with *H. contortus* alone, and two with both species, all simultaneously. The doses of *T. axei* and *H. contortus* were 150,000 and 10,000 larvae, respectively, from a single batch of each species. The calves were necropsied 29 or 30 days later. Depending upon the number of worms present, duplicate 0.25 to 1% portions from the combined contents and washings of the abomasum were examined for worms. In addition, 25 to 50% of this material from the calves given *H. contortus* was screened (23.6 meshes/cm) and examined for that species. The abomasum was digested after thorough washing, and duplicate 5% portions were examined for worms. Because large numbers of *T. axei* were present, the smaller portions (to 1%) were considered adequate, but since fewer *H. contortus* were present, the numbers determined from the larger portions (to 50%), considered more accurate, were used for that species. In both cases, the numbers from the digests were included in the total.

Nongravid fifth-stage females, not obviously senescent, and fifth-stage males, before darkening of the spicules, were counted as immature fifth-stage worms. Fourth-stage larvae which had not grown since attaining that stage, despite an available period, were counted as inhibited fourth-stage larvae.

The numbers and stages of the worms of each species recovered from the calves with the dual infection were similar to those from the calves with the single infection (Table 1). This finding indicated that neither species interfered with establishment or development of the other.

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PRESENTATION

1972 Anniversary Award of the Helminthological Society of Washington

470th Meeting, 17 November 1972

Mrs. MayBelle Hutson Chitwood

At the request of the Awards Committee and in behalf of the membership of the Society, I have the singular honor and great pleasure of presenting the 1972 Anniversary Award of the Helminthological Society of Washington to an outstanding parasitologist and long-time member of this Society, Mrs. MayBelle Hutson Chitwood.

The Award, first presented on the occasion of the fiftieth anniversary of the Society, 8 October 1960, is given not oftener than annually to a member in recognition of unusually meritorious service to the Society, or outstanding contributions to science, or possibly some other activity that advances the stature and influence of the Society. Previous recipients have been Miss Edna Buhrer, Miss Mildred Doss, Drs. McIntosh, Christie, Otto, LaRue, Cort, Dikmans, Schwartz, Wright, Foster, and Herman. Mrs. Chitwood thus becomes the honored and lucky thirteenth recipient.

Mrs. Chitwood, whom most of us have long known as "MayBelle", was born in Lubbock, Texas, 17 September 1908. She received formal education from a half dozen institutions, beginning with Texas Technical College in Lubbock and later including Rice Institute; George Washington, Maryland, and Colorado Universities, not to mention the Marine Biological Laboratory at Woods Hole and the Graduate School of the U. S. Department of Agriculture. Her one formal degree is a Bachelor of Arts from the University of Maryland, 1958. These exposures, even the degree, were probably important to MayBelle even if seemingly noncontributory, so far as her scientific work is concerned. My personal opinion is that she generically can be called a "self-made man", and that her years of greatest educational and scientific development were those from 1927 to 1952—the 25 years that she worked indefatigably with her former husband, Dr. B. G. Chitwood, whose reputation as a nematode morphologist probably stems as much from the untiring researches of a devoted wife as from his own great effort and notable scientific acumen. Whatever may be the full story most parasitologists today recognize that MayBelle is without a peer in the areas of nematode structure and classification!

Employment-wise, MayBelle entered the erstwhile Zoological Division of the late and lamented Bureau of Animal Industry, USDA, as a GS–4 Biological Aid in 1948. In fact, she entered the USDA in 1930 and served without pay, so far as I know, in one capacity and another, for 17 years. She survived her own trials and those of the organization that she served for 24 years when in 1971 she retired from the USDA and became Parasitologist in Charge, Primate Parasite Registry, University of California, Primate Research Center, Davis, California, 95616. Regarding the work that she has done, with apparently the greatest of freedom and joy, in less than a year with that organization, I can only say that I wish that the Laboratory she served so well and so long could take credit for it.

Mrs. Chitwood is an author of some 53 books, papers, and notes. Her contributions, as we all know so well, are in nematode systematics. Most of us are familiar with the 7 classical volumes entitled "Introduction to Nematology", published from 1937 to 1950. These are probably collector's items. Most of us, however, may be less familiar with a few aspects of MayBelle's work that have seemed to me to be important. How do you evaluate identification of parasites in tissue sections? And, if anyone seeks practical spin-offs from basic research, how about the intriguing stories of human angiostrongylosis and capillariasis? Her contribution to diagnosis and understanding of these two diseases are enough of them-

Mrs. MayBelle H. Chitwood receiving the 1972 Anniversary Award of the Helminthological Society of Washington (Presented by Dr. Aurel O. Foster)

selves to justify all the support of whatever kind she has ever had.

And what of the work on parasites as contaminants of foods? This problem is larger than any of us and its significance wholly underestimated.

There is one more item, namely, MayBelle's readiness to help anyone at any time in any way that she can. She has been a silent partner in publications which in number far exceed her own. In my own humble book on people, this is not unimportant.

Mrs. Chitwood was probably elected to membership in this Society in the middle thirties. I have found only the record that she served on innumerable committees and in several special assignments and that she was Vice President in 1966 and President in 1967. She also served on important committees of the American Society of Parasitologists and of the First and Second Congresses of Parasitology, and is currently a member of many other societies and committees.

I am personally gratified to be privileged to participate in presenting this award. I am happy, too, that the Awards Committee, consisting of Ralph Lichtenfels, Chairman; Lloyd Rozeboom, and Guillermo Pacheco, chose so worthy a recipient.

A. O. Foster

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