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PROCEEDINGS OF THE
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Final Hosts of *Diphyllobothrium sebago* (Cestoda: Pseudophyllidea) in Nature[1]

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ABSTRACT: In an examination of 322 piscivorous birds (11 spp.) and 23 mammals (6 spp.), from the Rangeley Lakes region in western Maine, where *Diphyllobothrium sebago* (Ward) is enzootic, only the herring gull (*Larus argentatus* Pontoppidan) was found to be of epizootiological importance. The ecological relationship between the final avian host and the intermediate fish hosts is discussed.

Information on the intermediate and final hosts in nature of *Diphyllobothrium* tapeworms and other helminths is fragmentary and too often based only on experimental infections in life cycle laboratory studies, which may not reflect accurately the natural cycle. In numerous cases just a single or a few related final host species have been examined and the helminth fauna analyzed, qualitatively and quantitatively. But intensive surveys seeking a particular species of parasite in the possible host species in an appreciable number of fish-eating avian and mammalian hosts in search of the natural final host(s) of a species of *Diphyllobothrium* are few. Kuhlow's (1953) search for the final hosts of *D. dendriticum* Nitzsch in Germany has been supplemented by Hickey and Harris (1947) in Ireland and Vik (1957) in Norway.

Meyer and Robinson (1963) and Meyer and Vik (1961, 1963, 1968) have shown that the plerocercoid stage of *Diphyllobothrium sebago* (Ward, 1910) occurs in the landlocked salmon, *Salmo salar* Linn., brook trout, *Salvelinus fontinalis* (Mitchill), and landlocked rainbow smelt, *Osmerus mordax* (Mitchill). The final host becomes infected through eating fish or fish offal containing the viable larvae. This study was undertaken to assess the relative importance of the final host(s) in the circulation of *D. sebago* in an enzootic area.

Material and Methods

From 1959 through 1964, 322 fish-eating birds and 23 mammals, representing 17 species, in the Rangeley Lakes region in western Maine were examined for *D. sebago*. Two hundred and 46 gulls, comprising 218 herring gulls, *Larus argentatus* Pontoppidan (64 chicks, 107 immatures, 47 adults), 19 ring-billed gulls, *L. delawarensis* Ord, and nine Bonaparte's gulls, *L. philadelphia* (Ord), were necropsied. The herring gull chicks were taken from the nests and from the water near the small rookery-island in Mooselookmeguntic Lake; older gulls and other birds were shot. More herring gulls were examined because they were present in greater numbers throughout most of the year; there was no objection from local residents to our capturing them for examination; and early in the study they were found to harbor *D. sebago*.

Gulls were assigned to three age groups, based mainly upon plumage; *chick*, a bird before flight, most of which were without flight feathers; *immature*, a fully feathered bird, young-of-the-year through third year; and *adult*, a bird 4 years or older. In the case of *L. argentatus* and *L. philadelphia*, both of which harbored adults, no distinction was made between immature and mature tape-

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Table 1. Monthly distribution of aged gulls by species infected with *Diphyllobothrium sebago*.

Species	Month								Total
	A	M	J	J	A	S	O	N	
	Number infected/Number examined								
Larus argentatus									
Chick	—	—	53/64	—	—	—	—	—	53/64
Immature	0/3	4/48	12/19	2/7	3/5	0/10	2/3	2/12	25/107
Adult	0/2	2/12	1/3	2/7	1/5	0/14	—	0/4	6/47
Total	0/5	6/60	66/86	4/14	4/10	0/24	2/3	2/16	84/218
L. philadelphia									
Immature	—	1/3	—	—	—	—	—	—	1/3
Adult	—	—	—	—	—	—	3/6	—	3/6
L. delawarensis									
Immature	—	0/7	—	—	0/4	0/4	—	—	0/15
Adult	0/2	0/2	—	—	—	—	—	—	0/4

worms in an infection. The term "incidence of infection" is used to mean the ratio of the number of infected hosts to the total number of hosts examined: in some cases this ratio is expressed as a percentage. Most of the material on the biology of the gulls presented in this paper is taken from Vik (1964).

Seventy-six birds, in addition to the gulls, were examined during late spring and early summer; they include: 39 common merganser, *Mergus merganser*; 22 belted kingfisher, *Megaceryle alcyon*; 6 common loon, *Gavia immer*; 4 common crow, *Corvus brachyrhynchos*; 2 great blue heron, *Ardea herodias*; 1 horned grebe, *Podiceps auritus*; 1 American bittern, *Botaurus lentiginosus*; and 1 common goldeneye, *Bucephala clangula*. Birds were returned to the laboratory and necropsied as soon after capture as possible, usually within a few hours.

Also included in the survey were 23 mammals from the area: 9 mink, *Mustela vison*; 6 raccoon, *Procyon lotor*; 3 bobcat, *Lynx rufus*; 2 common striped skunk, *Mephitis mephitis*; 2 red fox, *Vulpes fulva*; and 1 black bear, *Ursus americanus*. No organized effort was made to collect mammals for examination; most carcasses were contributed during the open season (1960–61) by hunters and trappers.

Results

No *D. sebago* were found in the mammals. With the exception of the herring gulls and Bonaparte's gulls and one common goldeneye, no birds were infected. Two plerocercoids were recovered from the one common goldeneye. But the presence of larvae only in a host, unaccompanied by adults, does not unequivocally establish it as a final host. Since no *L. delawarensis* were infected, this species will not be considered further.

As Table 1 shows, from April through November the incidence of infection of all herring gulls by *D. sebago* was 84/218 (39%). The highest infection occurred during the summer months, 74/110 or 67%, with a maximum in June 66/86, or 77%. During June the incidence of infection in chicks was 53/64 (83%); in immatures it was 12/19 (63%); and one of the three adults examined was infected. Fewer birds were infected at other times in the year: none during April, 6/60 in May, and 4/43 during the three fall months.

There was no significant difference in the incidence of infection between aged male and female gulls captured from April through No-

Table 2. Monthly distribution of aged and sexed *Larus argentatus* infected with *Diphyllobothrium sebago*.

Month	Sex	Immature Infected/ Examined	Adult Number Infected/ Examined	Total Infected/ Examined
April	♂	0/2	0/2	0/4
	♀	0/1	—	0/1
May	♂	0/27	1/7	1/34
	♀	0/21	0/3	0/24
June	♂	—	1/1	1/1
	♀	0/2	0/1	0/3
July	♂	1/2	0/2	1/4
	♀	1/3	2/5	3/8
August	♂	1/1	1/2	2/3
	♀	2/4	0/3	2/7
September	♂	0/6	0/3	0/9
	♀	0/4	0/1	0/5
October	♂	1/2	—	1/2
	♀	1/1	—	1/1
November	♂	1/4	0/2	1/6
	♀	1/8	0/2	1/10
Total		9/88 (♂ 4/44; ♀ 5/44)	5/34 (♂ 3/19; ♀ 2/15)	14/122 (♂ 7/63; ♀ 7/59)

vember (Table 2). Thus, of the 122 immature and adult birds examined 7/63 or 9.0% of the males and 7/59 or 8.4% of the females were infected.

Discussion

The seasonal fluctuation is probably a result of several factors, chief among which is the availability of the plerocercoid-infected smelt and the relative short duration of the adult stage in the final host. Kuhlow (1953) reported that the longest stay of *D. dendriticum* in experimentally infected laughing gulls (*Larus ridibundus*) was 4 months. He also found that the infection in the gulls was seasonal, being synchronized with the return of the three-spined stickleback (*Gasterosteus aculeatus*), which harbor the plerocercoids, beginning in February and March. Gulls which he examined from November through January were negative.

The parasite–host relationship may be influenced by a number of factors; some, such as the age and sex of the host, may operate through the immunological response of the host to the presence of the parasite, while others, such as the seasonal and geographical distribution of both the hosts and the parasite, are dependent upon the ecological relationship between the final and intermediate hosts. Some of these factors, especially the ecological ones, will be discussed.

The Adult Cestode and the Final Host

Herring gull

Herring gulls are seasonal residents of the region, arriving early in April and remaining until the waters become ice-covered, about mid-November. The gull population in the area fluctuates; it totals nearly 100 in June and July, after which the number is reduced to about 50. The main nesting site is a small island south of Student's Island in Mooselookmeguntic Lake. The following breeding observations, which are believed typical, were made in 1961: egg-laying began 6–11 May, the first hatching occurred 8 June, and the community produced 50 eggs.

While the food varies from place to place, depending primarily upon what is available, herring gulls are mainly scavengers, feeding for the most part on fish, fish offal, and garbage discarded by man. In the area, smelt is the most important food during spring and early summer; in the absence of fish, in late summer and fall, they feed on garbage at the nearby town dump. During the first 5 days the food of herring gull chicks studied by Leonov (1960) consisted of regurgitated fish pulp, of the same sort of food eaten by the adults, and whole small fish during the second 5 days. Thereafter, the chicks start fending for themselves. Thus, the chicks are subject to infection with *Diphyllobothrium sebago* as soon as they start taking food.

Bonaparte's gull

Larus philadelphia is only a spring and fall migrant visitor to the Rangeley Lakes region. Ten immature birds were present late in October 1960. In 1961, five adult and five young birds were present in May. None was observed during the summer of either year. Nine of the 20 migrants were examined. Of these, four harbored tapeworms typical of *D. sebago*. The results in relation to season and age group are given in Table 1. But since Bonaparte's gulls were only occasional transients, whose brief presence may have been shorter than the 7-day prepatent period of the tapeworm, it is likely that these infections were acquired elsewhere. In view of these data, combined with the relatively few *L. philadelphia* frequenting the region, it is unlikely that it is of much, if any, epizootiologic importance.

The Plerocercoids and the Fish Hosts

In the area there are two sources of the *D. sebago* plerocercoids for the final hosts: (1) landlocked smelt, which is of greatest importance; and (2) two species of Salmonidae: landlocked salmon and brook trout. Salmon are the more important, because they form the greater part of the catch and, generally speaking, the incidence of infection and the mean intensity of infection is greater in salmon than in trout.

Smelt

The usual summer habitat of freshwater smelts consists of the clear, cool waters found in the deeper portions of the deep, stratified lakes. Spawning takes place in lake tributaries

at night during late April and early May. Large numbers of gravid fish ascend the stream a short distance and select a spawning area in brisk current. Following spawning the adults return to the lake, after which there is a heavy smelt mortality. It is these dead and dying smelt which are the chief source of the plerocercoids for the gulls. Based upon an examination of 4,500 smelt, Meyer and Vik (1968) found an infection rate of nearly 5%.

Salmonidae

Except for an occasional legal-size salmonid, dying from some undetermined natural cause, fishermen cooperate in making the plerocercoids available to the final hosts. The practice of anglers gutting their catch and discarding the viscera over the side of the boat into the water, where they are promptly taken by gulls, is a source of infection. Another practice involves the sub-legal size salmon; after they are unhooked and returned to the water they often remain on the surface temporarily and are taken by gulls prior to recovery and descent. But this is the least important of the sources of plerocercoids, because of the relatively light infection in these small fish, which are 35 cm or less in total length (Meyer and Vik, 1968).

Acknowledgments

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Orientation of *Tylenchorhynchus martini* Swarmers to Chemical Stimuli

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ABSTRACT: The use of swarming *Tylenchorhynchus martini* nematodes provided a rapid and efficient in vitro method for the study of nematode orientation mechanisms as affected by chemical stimuli. Orientation of the nematodes occurred in concentration gradients diffusing from agar discs containing 0.05, 0.1, 0.25 M of both $AlCl_3$ and $CdCl_2$, and 0.25 M of NH_4Cl. Attraction of the nematodes did not occur at the other concentrations tested: 0, 0.5, or 1.0 M, or with the other chemicals: $MgCl_2$, $MgSO_4$, $Na_2S_2O_4 \cdot 2H_2O$, or ascorbic acid. There was no repulsion in any of these tests to nematodes.

Swarming nematodes of *Tylenchorhynchus martini* (Fielding, 1956) have continual and rapid jerky movements which result apparently from attempts of individuals to break free from the sticky cuticle of swarmers (Hollis, 1958, 1960, 1962; McBride and Hollis, 1966). Hollis suggested that swarming may be conditioned by the state of polysaccharide, lipid, or protein substances of the cuticle and that it is characterized by innate morphological modifications of the cuticle. The use of the electron microscope revealed morphological modifications in the cuticle of swarming *T. martini* (Ibrahim, 1967). These modifications must be related to the sticky condition of the cuticle. Thus, the use of swarming *T. martini* provided a rapid and efficient in vitro method for the study of orientation mechanisms of nematodes.

Orientation is the process whereby nematode movement is influenced by external stimuli. Little information is available on the mechanisms of nematode orientation and almost nothing is known of stimuli reception by nematodes (Klinger, 1965; Van Gundy, 1965; Wallace, 1964; and others). Chemical stimuli are of importance because they play a role in nematode orientation to germinating seeds and host plant roots. In view of this, the present study was made to determine the effect of certain chemicals on the orientation of swarming *T. martini*. A preliminary report stressing the reliability of the method has been published (Ibrahim and Hollis, 1967).

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Materials and Methods

The following chemicals were tested for their attractiveness to swarming *T. martini*: aluminum chloride ($AlCl_3$), cadmium chloride ($CdCl_2$), ammonium chloride (NH_4Cl), magnesium chloride ($MgCl_2$), magnesium sulfate ($MgSO_4$), sodium thiosulfate ($Na_2S_2O_4 \cdot 2H_2O$), and ascorbic acid. Agar discs containing these chemicals of molarities 0, 0.05, 0.1, 0.25, 0.5, and 1.0 were substituted for 5-mm-diameter agar discs taken from the edges of 50-mm petri plates containing 6 ml of 2% water agar.

A swarming population of *T. martini* was reared on rice plants grown in a green house. Nematodes were collected from the infested soil by the sifting and gravity method. Nematode swarmers in water suspension were dispersed by agitation, then 5 ml of this suspension containing about 200 nematodes were spread evenly over the agar surface of each petri plate containing the tested chemicals. The plates were covered and incubated at room temperature for 6 hr.

Duplicate tests containing four replicates and six treatments of each tested chemical were set up in randomized blocks. Average distance from the agar disc to the resultant one to three swarms forming in each plate was then determined and the data evaluated by the analysis of variance.

Results and Discussion

The data obtained from the duplicate tests of each of the tested chemicals were significantly similar and Table 1 shows the results of one of these tests. Significant orientation of *T. martini* swarmers occurred in concentration

Table 1. Effect of seven chemicals on the orientation of *Tylenchorhynchus martini* swarmers.

Chemical concentration in moles	Average distance (mm) from discs to swarms[a]						Ascorbic acid
	$AlCl_3$	$CdCl_2$	$MgCl_2$	$MgSO_4$	$Na_2S_2O_4 \cdot 2H_2O$	NH_4Cl	
1.0	26.3	26.8	27.8	26.0	28.8	27.5	28.0
0.5	25.3	26.8	28.3	28.8	28.0	26.8	28.3
0.25	13.3**	21.0**	31.3	30.5	30.3	23.8**	31.3
0.1	5.8**	22.5**	29.8	26.3	26.5	27.5	30.0
0.05	16.8**	23.8**	28.8	25.5	25.3	26.5	29.3
Control	28.5	29.8	30.8	29.8	29.8	30.0	30.3
LSD (99:1)	4.6	4.5	3.5	4.7	4.6	3.8	3.7

[a] Mean of four replicates. ** Highly significant.

gradients diffusing from agar discs containing: 0.05, 0.1, and 0.25 M $AlCl_3$ or $CdCl_2$, and 0.25 M NH_4Cl. Attraction did not occur at any of the tested concentrations of the other four chemicals, $MgCl_2$, $MgSO_4$, $Na_2S_2O_4 \cdot 2H_2O$, and ascorbic acid. There was no repulsion effect of these chemicals on the nematode movements in any of the tested concentrations.

One-tenth molar $AlCl_3$, in particular, had a maximum attractive value whereas 0.25 M $AlCl_3$ attracted the nematodes more than 0.05 M. The three effective concentrations of $CdCl_2$ attracted the nematodes in the following descending order: 0.25 M > 0.1 M > 0.05 M and their attractive values were less than those of the similar concentrations of $AlCl_3$. The concentration 0.25 M of NH_4Cl was the only attractive one of this chemical, and it had the minimum attractive value.

The present results show that the nematodes responded to a diffusible factor operating at some distance from the agar disc containing the attractive concentration of the chemical. Orientation of nematodes resulted from direct or indirect migration of the swarmers toward the source of stimulation. It is probable that both the chemical properties and the concentration of tested chemicals were responsible for the orientation of the nematodes. In the cases of no attraction, the nematode migration and aggregation took place on a purely chance basis.

Similar work on the orientation of *Meloidogyne javanica* larvae by different amino acids was done by Oteifa and Elgindi (1961). They found that the amino acid tyrosine attracted this nematode more than 13 other amino acids tested, which were either neutral or repellent to the nematode larvae.

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Two New Species of *Scutellonema* from Cultivated Soils in Africa with a Description of *Hoplolaimus aorolaimoides* sp. n. from Portugal (Nematoda: Hoplolaiminae)

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ABSTRACT: *Scutellonema labiatum* sp. n. from tomato and banana soils from Malawi is bisexual and is characterized by having a projecting, 5 to 7 μ long epiptygma. *S. brevistyletum* sp. n. found in wheat soil from Tanzania has an offset lip region exhibiting three annules, 21 to 23 μ long spear, and inconspicuous epiptygma. *Hoplolaimus aorolaimoides* sp. n. from tomato soil from Portugal has the smallest spear and body size among the known species of *Hoplolaimus*.

The genus *Scutellonema* was proposed by Andrássy (1958) under the subfamily Hoplolaiminae and its generic diagnosis was emended by Sher (1961, 1964). While revising this genus, Sher (1964) described four known and seven new species and gave a differential key which he revised later (1965). The following species have since been described under the genus *Scutellonema*: *S. siamense* Timm, 1965; *S. mangiferae* Khan and Basir, 1965; *S. magna* Yeates, 1967; and *S. africanum* Smit, 1971. My examination of a male and a female paratype of *S. mangiferae* did not reveal any significant difference from Sher's (1964) description of *S. grande* Sher, 1964, and I regard these two species as subjective synonyms. Smit (1971) synonymized *S. magna* with *Morulaimus geniculatus* Sauer, 1966, and gave a key to 14 species of *Scutellonema*.

Sher (1964) stated that the greatest distribution and speciation of *Scutellonema* appeared to be in Africa. This seems to be true because all the nine *Scutellonema* species in the collection of the Commonwealth Institute of Helminthology have been received from African countries. These species are listed in Table 1 with their hosts and locality.

The two new species of *Scutellonema* and *Hoplolaimus aorolaimoides* sp. n. are described below. All the measurements were made on specimens relaxed and killed by gradually heating in water, fixed and stored in F.A. 4:10, processed through lactophenol, and mounted in dehydrated glycerin containing traces of picric acid. In most cases the measurement ranges are followed by their means shown within brackets. Figure "b′" is the ratio of the body length divided by the distance from the anterior end of the body to the base of the esophageal glands, "o" is the distance from the orifice of the dorsal esophageal gland to the base of the spear expressed as percentage of the spear length, and "m" is the length of the anterior tapering part of the spear expressed as percentage of the spear length.

Scutellonema labiatum sp. n.

(Fig. 1, A–K)

MEASUREMENTS: 30 females (paratypes): L = 0.57–0.77 (0.61) mm; a = 23–32 (26); b = 5.8–8.0 (7); b′ = 4.6–6.5 (5.7); c = 45–70 (55); V = 55–61 (58); spear = 20–25 (22.5) μ; m = 48–52 (50); o = 14–28 (19).

12 males (paratypes): L = 0.54–0.64 (0.58) mm; a = 26–32 (28.5); b = 6.0–7.6 (6.6); b′ = 4.7–5.4 (5.2); c = 49–81 (65); T = 40–56 (46); spear = 20–25 (21.5) μ; m = 49–51 (50); o = 18–28 (21); spicules = 27–32 (28.5) μ; gubernaculum = 12–14 (12.7) μ.

Holotype female: L = 0.63 mm; a = 26; b = 6.3; b′ = 5.1; c = 53; V = ^{26}-60-^{27}; spear = 22.5 μ; m = 49; o = 23.

Description

FEMALE: Body usually lying in a single spiral, sometimes C-shaped, marked by distinct annules averaging 1.5 μ wide near middle. Lateral fields with 4 smooth incisures forming 3 equally wide bands which are not areolated except in neck region, one-fourth to one-fifth as wide as body. Phasmids 3.0–4.5 μ in diameter, usually at anal latitude, but variable from 4 body annules anterior to 3 annules posterior to anus; aperture of phasmids porelike

Table 1. Distribution, hosts, and locality of *Scutellonema*.

Nematode	Host	Locality
Scutellonema africanum Smit	Banana	Masambanjati, Malawi
S. brachyurum (Steiner)	Hops	Georgetown, Republic of South Africa
	Tomato	Tzaneen, N. Transvaal, Republic of South Africa
	Rice	Malkens Research Station, Middle Veld, Swaziland
S. brevistyletum sp. n.	Wheat	West Kilimanjaro, Tanzania
S. cavenessi Sher	Sugar cane	Bacita Estate, Jebba, Nigeria
S. clathricaudatum Whitehead	Cotton	Gezira, Sudan
	Sweet potato	Abunaama, Fung Area, Sudan
	Tomato	Um Shoka, Fung Area, Sudan
S. labiatum sp. n.	Potato and tomato	Bvumbwe Experimental Station, Limbe, Malawi
	Banana	Mangwalala, Malawi
S. magniphasmum Sher	Maize	Chitedze, Malawi
	Potato, tomato, and avocado	Bvumbwe Experimental Station, Limbe, Malawi
	Banana	Masambanjati and Mangwalala, Malawi
	Ninde (*Aeolanthus myrianthus*)	Mbawa and Mzuzu regions, Malawi
	Sugar cane	Tentativa Estate, 30 miles north of Luanda, Angola
S. truncatum Sher	Tomato	Tzaneen, N. Transvaal, Republic of South Africa
S. unum Sher	Giant Rhodes grass (*Chloris gayana*)	Near Salisbury, Rhodesia
	Lucerne	South Kilimanjaro, Tanzania
	Banana	Thekerani, Malawi
	Sugar cane	Tentativa Estate, Angola; Hippo Valley, Rhodesia

(Fig. 1, H–J). Excretory pore opposite esophageal glands, 93–124 (105) μ (in holotype 98 μ) from anterior end. Hemizonid 2–3 annules long, 0–3 annules anterior to excretory pore; hemizonion indistinct, 8–12 annules behind hemizonid.

Lip region broadly rounded, slightly offset by a constriction, usually exhibiting 4 to 5 annules (occasionally 3 and 6), basal annule marked by 7 to 15 longitudinal striae (4 face views). Labial framework well sclerotized, with outer margins incurved and extending 1–2 body annules. Spear rather weakly developed (Fig. 1, A); basal knobs small, rounded, with flattened anterior surfaces, 3.2–4.3 μ (3.5 μ in holotype) across and about 2 μ high. Median esophageal bulb round to slightly oval, extending through 7 to 9 annules; distance from anterior end to its base 64–85 (71) μ, in holotype 72 μ. Epiptygma double, conspicuous, 5–7 μ long, projecting from the body surface (Fig. 1, G). Spermathecae oval, axial, with sperms in most specimens. Ovaries outstretched, mostly with a single row of oocytes; eggs not seen. Rectum partly overlapped by intestine. Tail rounded, often with a greater curvature dorsally, usually around 0.6 times anal body-width long, bearing 6–13 annules; outer layer of cuticle at terminus thicker and usually marked by coarser annules (Fig. 1, H–J).

MALE: Body open to a close C-shaped, with striae 1.4 μ apart near middle. Lip region hemispheroidal, offset by a constriction, with 4–5 annules. Basal knobs of spear 3.0–3.5 μ across. Distance from anterior end to base of esophageal bulb 63–74 (66) μ. Excretory pore 77–104 (92) μ from anterior end, posterior to esophagointestinal junction. Tail conoid with a subdigitate tip, 0.6–0.9 times anal body width long. Spicules slightly arcuate, with large distal flanges; gubernaculum carinate distally (Fig. 1, K). Bursa coarsely striated. Phasmids 3.2–4.0 μ in diameter, at cloacal latitude or just anterior.

TYPE MATERIAL: Holotype female isolated from soil sample sent by Dr. Moid A. Siddiqui, Limbe (Malawi), in December 1969, deposited with the Department of Nematology, Rothamsted Experimental Station, Harpenden, England. Paratypes distributed as follows: 8 ♀♀ and 4 ♂♂ at Rothamsted Experimental Station; 10 ♀♀ and 4 ♂♂ at Commonwealth Institute of Helminthology, St. Albans, En-

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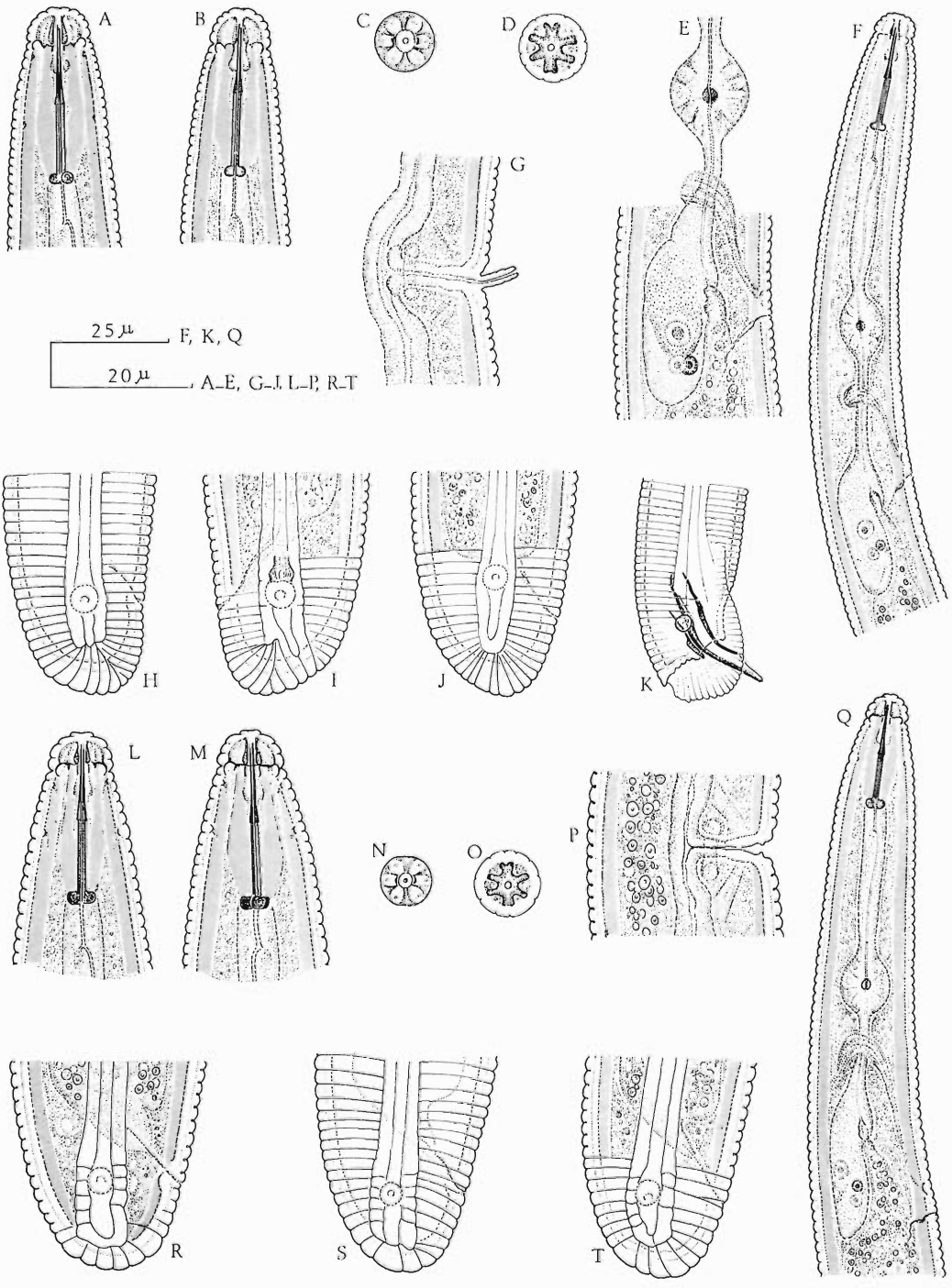
Figure 1. A–K. *Scutellonema labiatum* sp. n. L–T. *S. brevistyletum* sp. n. B and K. Male, rest female. A, B, L, M. Head ends. C and N. *En face* views. D and O. Cross sections through basal annule of lip region. E, F, and Q. Esophageal regions. G and P. Vulval regions. H–K and R–T. Tail ends.



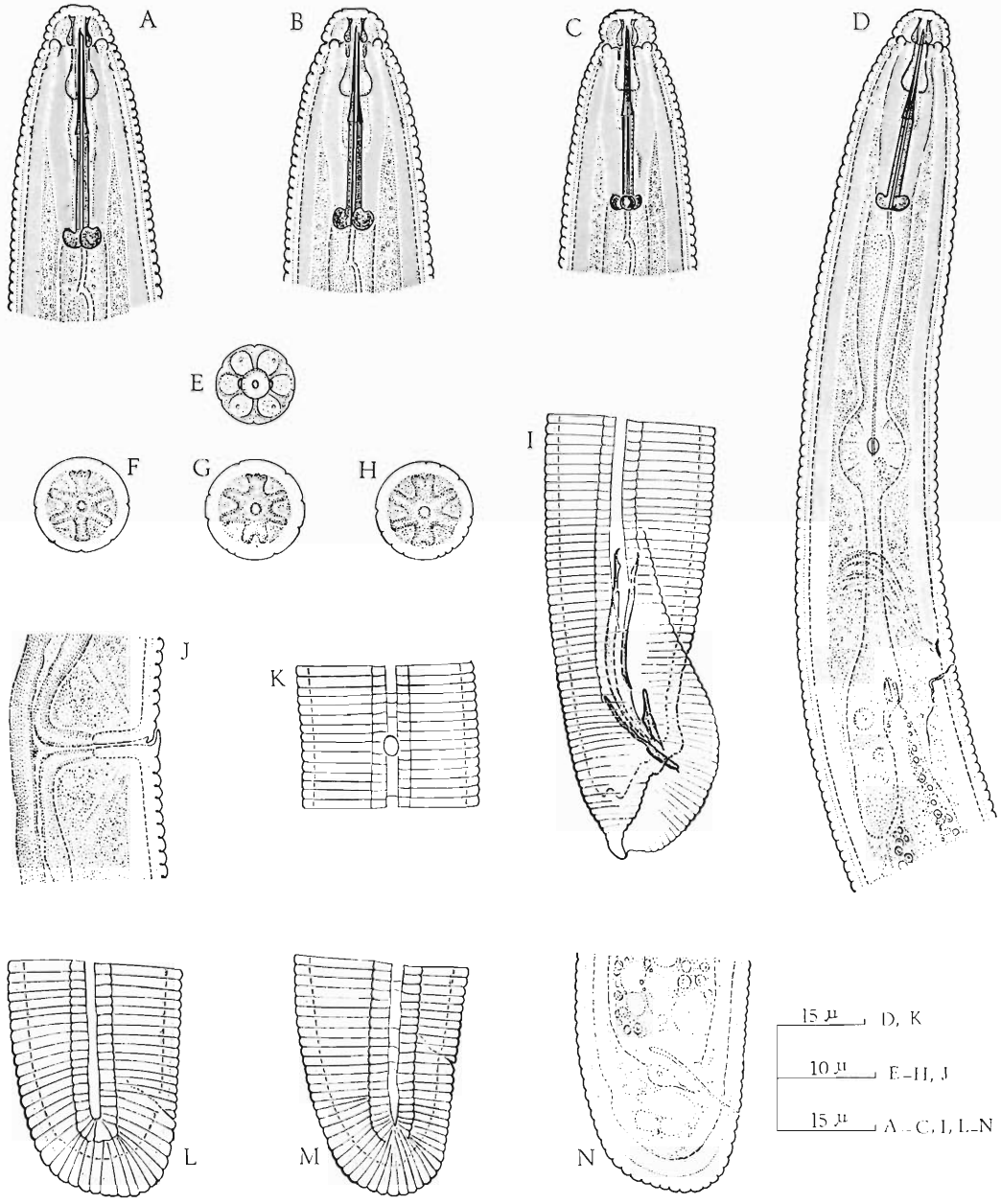


Figure 2. ***Hoplolaimus aorolaimoides*** **sp. n. C and I. Male, rest female. A–C. Head ends. D. Esophageal region. E.** ***En face*** **view. F–H. Cross sections through basal annule of lip region. J. Vulval region. K. Lateral field at phasmid. I, L–N. Tail ends.**

gland; 10 ♀♀ and 2 ♂♂ at Department of Nematology, University of California, Riverside, USA; 8 ♀♀ and 2 ♂♂ at Department of Nematology, Landbouwhogeschool, Wageningen, Holland; and 6 ♀♀ and 1 ♂ at Division of Nematology, Indian Agricultural Research Institute, New Delhi, India.

TYPE HABITAT AND LOCALITY: Soil after lifting a tomato crop in 1969 which was preceded by potatoes in 1968 and roses and apple root-stock in 1967, Experimental Plot, Bvumbwe Experimental Station, Limbe, Malawi. Male and female paratypes were also collected from soil around the roots of banana plants at Mangwalala, Cholo District, Malawi, sent in by Dr. L. K. Mughogho in April 1970.

RELATIONSHIP: *Scutellonema labiatum* sp. n. is recognized by its long projecting epiptygma and is close to *S. siamense* Timm, 1965, and *S. africanum* Smit, 1971. From *S. siamense* it can be differentiated by a larger number of labial annules, anterior part of the spear not conspicuously shorter than the posterior part, a well developed epiptygma, and the absence of the areolations of the lateral fields at the phasmids. From *S. africanum* it differs in having a crenated last labial annule, a projecting epiptygma, a weaker spear with smaller basal knobs, and the distal portion of the bursa in male not deeply indented. From *S. brachyurum* (Steiner, 1938) and *S. unum* Sher, 1964, this new species can easily be differentiated by its shorter spear, functional spermathecae, and conspicuous epiptygma.

Scutellonema brevistyletum sp. n.
(Fig. 1, L–T)

MEASUREMENTS: 10 females (paratypes): L = 0.59–0.69 (0.63) mm; a = 24–27 (25.5); b = 6.5–7.6 (7.1); b′ = 5.4–6.2 (5.7); c = 52–91 (61); V = 55–60 (57); spear = 21–23 (22.6) μ; m = 49–52 (50); o = 21–28 (24).

Holotype female: L = 0.69 mm; a = 25; b = 7.6; b′ = 6; c = 63; V = ^{29}-55-^{29}; spear = 22.5 μ; m = 49; o = 27.

Description

FEMALE: Body ventrally arcuate to C-shaped, marked by coarse striae, 1.6–1.9 (1.75) μ apart near middle. Lateral fields with four smooth, equidistant incisures, areolated in neck region up to a little behind esophageal glands and at phasmids, about one-fifth as wide as body. Anterior and posterior cephalids 1 and 5–6 body annules behind lip region, respectively. Phasmids, 3.5–4.0 μ in diameter, at latitude of anus or just behind it. Excretory pore usually at base of esophageal glands, 100–112 (106) μ (in holotype 112 μ) from anterior end. Hemizonid 2–3 body annules long, 0–3 annules anterior to excretory pore; hemizonion occupying a single body annule, 8–10 annules behind hemizonid.

Lip region hemispheroidal, offset by a constriction, bearing a distinct terminal disc and marked by two striae forming three annules (Fig. 1, L and M); in two paratypes there are 4 annules on one side of lip region. Last labial annule exhibiting 10 longitudinal striae (one face view, Fig. 1, O). Labial sclerotization strongly developed, outer margins sharply incurved extending one annule into body. Spear knobs rounded with flattened anterior surfaces, 4–5 μ across by 2.2–2.5 μ high. Median esophageal bulb extending through 5–6 annules; anterior end to base of median esophageal bulb 62–70 (66) μ, in holotype 67 μ. Epiptygma inconspicuous, double (Fig. 1, P). Spermathecae small-sized, rounded, axial, without sperms, not formed in some paratypes. Ovaries outstretched, oocytes in single row. Intestine slightly extending over rectum. Tail rounded, 7–12 μ or 0.4–0.7 times anal body width long, with 5–10 (7) annules; terminal annules usually coarser than body annules (Fig. 1, R–T).

MALE: Not found.

TYPE MATERIAL: Holotype female isolated from wheat soil sample sent by Mr. D. R. W. Watson, Plant Pathologist, Arusha, in July 1966; deposited with the Department of Nematology, Rothamsted Experimental Station; Harpenden, England. Paratypes with same data as holotype, distributed as follows: 3 ♀♀ at Rothamsted Experimental Station; 2 ♀♀ at Commonwealth Institute of Helminthology, St. Albans, England; 2 ♀♀ at Department of Nematology, University of California, Riverside, USA; 2 ♀♀ at Department of Nematology, Landbouwhogeschool, Wageningen, Holland; and 1 ♀ at Division of Nematology, Indian Agricultural Research Institute, New Delhi, India.

TYPE HABITAT AND LOCALITY: Isolated from

soil around roots of wheat plants from West Kilimanjaro, Tanzania.

RELATIONSHIP: *Scutellonema brevistyletum* sp. n. differs from *S. brachyurum* to which it is closely related, in having a shorter spear (spear = 26–31 μ in syntype and topotype females, after Sher, 1964), usually three annules in the lip region, and median esophageal bulb extending five to six body annules as compared to eight or more in *S. brachyurum*. It differs from *S. unum* in having a shorter spear, fewer annules in the lip region, and smaller phasmids. *S. siamense* has three annules in the lip region but the new species differs from it in having an offset, hemispherical lip region which is not truncate, a smaller spear with anterior part not distinctly shorter than the posterior one, and nonfunctional spermathecae.

Hoplolaimus aorolaimoides sp. n.
(Fig. 2, A–N)

MEASUREMENTS: 25 females (paratypes): L = 0.80–0.92 (0.85) mm; a = 24–30 (26.8); b = 7.3–8.2 (7.9); b′ = 6.0–7.1 (6.5); c = 53–140 (79); V = 54–60 (57); spear = 31–35 (32.8) μ; m = 48–52 (50); o = 10–17 (15); anterior phasmid = 31–40 (37)%; posterior phasmid = 64–81 (73)%.

6 males (paratypes): L = 0.70–0.83 (0.79) mm; a = 27–32 (29); b = 6.8–7.8 (7.2); b′ = 5.4–6.2 (5.9); c = 41–49 (45); T = 39–58 (49); spear = 30–34 (32) μ; m = 49–54 (51); o = 15–19 (17); spicules = 31–37 (34) μ; gubernaculum = 13–19 (16) μ; capitulum = 10–14 (12) μ; anterior phasmid = 32–45 (37)%; posterior phasmid = 71–84 (79)%.

Holotype female: L = 0.86 mm; a = 28; b = 7.5; b′ = 6.1; c = 86; V = ^{22}-58-^{25}; spear = 32 μ; m = 50; o = 18; right phasmid = 40%; left phasmid = 78%.

Description

FEMALE: Body ventrally arcuate to a closed C-shape; marked by transverse striae 1.4–1.7 μ apart. Lateral fields conspicuous, about 0.4 times as wide as body, with four incisures forming three bands behind neck region; outer bands wider than the inner and regularly areolated throughout; inner band exhibiting irregular areolation in some specimens. Anterior cephalids 2–3 annules behind lip region, posterior ones indistinct, 9–12 annules behind lip region. Hemizonid 2–3 annules long, usually just anterior to excretory pore but varying from 2 annules anterior to 1 annule posterior to it; hemizonion indistinct, 8–14 annules behind hemizonid. Excretory pore opposite esophagointestinal junction or a little posterior, 87–135 (112) μ from anterior end. Phasmids about 4 μ in diameter, left and right ones occurring anterior or posterior to vulva with equal frequency. Caudalid 7–8 annules anterior to anus.

Lip region hemispherical, set off from body by a constriction, with 4–5 (rarely 3 or 6) annules; basal annule exhibiting 6–13 longitudinal striae (Fig. 2, F–H). Labial sclerotization not as massive as in most other species; outer margins of framework incurved, extending one body annule; inner margins forming an inverted funnel-shaped spear guide and extending 5–6 annules into body cavity. Basal knobs of spear rounded, 5.0–6.5 μ across and 3 μ high, each with one or two blunt projections anteriorly (basal knobs are not compact and "tulip-shaped" as in most other species). Orifice of dorsal esophageal gland not distinct. Median esophageal bulb spheroidal to oval, about half as wide as adjacent body; distance from anterior end of body to its base 72–90 (83) μ. Esophageal glands offset, rather short, with three gland nuclei; dorsal gland nucleus larger and anterior to those of subventrals (Fig. 2, D). Esophagointestinal valve oval, distinct. Epiptygma double, inconspicuous. Spermathecae axial, spheroidal, distinct in impregnated females. Ovaries outstretched with oocytes usually in one row, imperfectly developed in a few specimens; eggs not seen. Intestine slightly overlapping rectum. Tail broadly rounded, 0.2–0.9 (0.54) times anal body width long, with 6–17 (10) annules.

MALE: Body ventrally arcuate. Lip region more prominently offset than in female, usually with 5 annules and a distinct labial disc. Spear knobs 5–6 μ across and 3.0–3.5 μ high. Excretory pore 100–124 (114) μ from anterior end, at, or just anterior to, hemizonid. Tail 1.0–1.3 times anal body width long with terminal 40–60 per cent nonprotoplasmic. Bursa large, crenate. Spicules not distinctly cephalated, with large ventral flanges distally;

gubernaculum with titillae at distal end (Fig. 2, I).

TYPE MATERIAL: Holotype female isolated from a tomato soil sample forwarded by Mr. P. B. Brudenell of the Shell International Chemical Co., London; deposited with the Department of Nematology, Rothamsted Experimental Station, Harpenden, England. Paratypes distributed as follows: 8 ♀♀ and 2 ♂♂ at Rothamsted Experimental Station; 5 ♀♀ and 1 ♂ at Commonwealth Institute of Helminthology, St. Albans, England; 5 ♀♀ and 2 ♂♂ at Department of Nematology, University of California, Riverside, USA; 4 ♀♀ and 1 ♂ at Department of Nematology, Landbouwhogeschool, Wageningen, Holland; and 3 ♀♀ at Division of Nematology, Indian Agricultural Research Institute, New Delhi, India.

TYPE HABITAT AND LOCALITY: Soil and roots of tomato plants (*Lycopersicon esculentum*) at field Pomar do Italiano in Lagoalva farm, near Alpiarça, Portugal. Paratype specimens were also collected from tomato nursery soil, Capel Tomato Industries, near Chança, Portugal.

RELATIONSHIP: *Hoplolaimus aorolaimoides* sp. n. is characterized by the smallest spear and body size of species of this genus. In having four incisures in the lateral fields, it is related to *H. tylenchiformis* Daday, 1905; *H. galeatus* (Cobb, 1913) Thorne, 1935; *H. stephanus* Sher, 1963; *H. californicus* Sher, 1963; and *H. concaudajuvencus* Golden and Minton, 1970 which have bodies measuring over 1 mm in length, spear over 40 μ long in female, and a larger number of longitudinal striations on the basal annule of the lip region.

Sher (1963) revised the genus *Hoplolaimus* and erected a new genus *Aorolaimus* which was differentiated from *Hoplolaimus* by the following characteristics:

1. Less massive cephalic sclerotization.
2. Less massive spear with knobs not bearing distinct anterior projections.
3. Lateral fields areolated only anteriorly and at phasmids.
4. Smaller body size, spear, spicule, gubernaculum, and capitulum (= telamon).

H. aorolaimoides has less massive cephalic sclerotization and spear and the anterior projections of the spear knobs are not as prominent and toothlike as in most species of *Hoplolaimus* where the spear knobs appear tulip-shaped. The body size and length of the spear, spicule, gubernaculum, and capitulum of *H. aorolaimoides* are similar to those of *Aorolaimus* spp. but, on the other hand, the size of the spicule, gubernaculum, and capitulum compare well with that of *H. stephanus*. The areolated lateral fields, however, are typical of the genus *Hoplolaimus*.

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Zonorchis chandleri sp. n. (Digenea: Dicrocoeliidae) from the Yellow-Breasted Chat, *Icteria virens*

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ABSTRACT: *Zonorchis chandleri* sp. n. is described from the gall bladder and large bile ducts of two *Icteria virens* from Reelfoot Lake, Tennessee, and Augusta, Georgia. It is most similar to the North American strain of *Z. petiolatum* (Railliet, 1900), differing from it in body shape and proportions, in having a larger and more anteriorly situated acetabulum, more posteriorly located testes, and smaller and more anteriorly situated cirrus pouch.

In June 1938, three specimens of a sexually mature dicrocoeliid trematode were removed from the gall bladder of a yellow-breasted chat, *Icteria virens* (L.), collected at Reelfoot Lake, Tennessee. Recently 22 additional specimens were recovered from one of two chats examined from the vicinity of Augusta, Georgia. This fluke appears to be a new species of the genus *Zonorchis* Travassos, 1944, for which the name *Z. chandleri* sp. n. is proposed in honor of the late Dr. Asa C. Chandler.

Specimens were fixed in 10% formalin solution under slight cover glass pressure and stained with either Bullard's hematoxylin or alum-carmine. The drawing was made with the aid of a camera lucida. All measurements are in micra unless otherwise stated. The description is based on 10 selected specimens including the types.

Zonorchis chandleri sp. n.

(Fig. 1)

DESCRIPTION: Body elongate, semitransparent, 2.52–3.86 mm long and 375–700 wide in region of acetabulum, tapering abruptly in preacetabular region but narrowing gradually posterior to gonadal zone. Tegument aspinose; with retractile sensory papillae on margins of preacetabular region of body. Oral sucker subterminal, muscular, 157–227 long by 170–230 wide; anterodorsal liplike projection not prominent. Acetabulum large, strongly muscular, with deep cup-shaped lumen, 308–586 in diameter, situated relatively close to oral sucker within anterior fifth of body. Ratio of diameter of oral sucker to acetabulum 1:1.7–2.2. Pharynx globular, 53–110 in diameter. Esophagus thick-walled, glandular, usually straight, 130–150 long, bifurcating at approximately ⅔ distance from oral sucker to acetabulum; ceca slender, sinuous, passing lateral to genital organs and terminating about ⅛ distance from vitellaria to posterior end of body. Excretory pore terminal. Genital pore median at posterior level of pharynx or immediately postpharyngeal. Testes symmetrical, oval, borders smooth to slightly lobed in somewhat shrunken specimens, 143–243 in greatest diameter, contiguous with acetabulum or slightly posterior to it. Cirrus pouch oblong, 100–255 long by 40–114 wide, in midline anterior to acetabulum. Ovary transversely oval, smooth to slightly irregular in outline, 66–140 long by 117–214 wide, on either right or left side of body relatively close behind respective testis. Seminal receptacle large, globular, immediately posterior to ovary. Vitellaria composed of numerous medium-sized follicles, mainly extracecal, beginning at caudal margin of testes or slightly posterior, extending posteriorly 0.672–1.229 mm to terminate at equator of body. Uterus greatly convoluted, filling most of postacetabular region of body, then following undulating course between testes and dorsal to one side of acetabulum to genital pore. Metraterm thin-walled and poorly differentiated. Ova numerous, dark brown when mature, 29–35 long by 18–24 wide. Miracidium possessing stylet and two large posteriorly situated vesicles containing highly refractile granules.

HOST: *Icteria virens* (Linnaeus).

HABITATS: Gall bladder and large bile ducts.

LOCALITIES: Reelfoot Lake, Tennessee (type) and Augusta, Georgia.

TYPE SPECMENS: USNM Helm. Coll. No. 71906, holotype; No. 71907, paratype.

Zonorchis chandleri closely resembles the species described from North American birds as *Z. petiolatum* (Railliet, 1900) by Denton and Byrd (1951) but differs from it in body shape and proportions, in having a relatively larger and more anteriorly situated acetabulum, more posteriorly located testes, and smaller and more anteriorly situated cirrus pouch. From *Z. (Dicrocoelioides) petiolatum* from European and African birds, redescribed by Dollfus (1954) and Timon-David (1960), *Z. chandleri* is easily distinguished by its more anterior and oppositely situated testes, smaller cirrus pouch, and smaller ova.

The yellow-breasted chat normally winters in Mexico and Central America. The possibility exists that the chat, while on its wintering grounds, might be exposed to infection with one of the six species of *Zonorchis* described from birds in South America by Travassos (1944). Of these *Z. japuhybae* Travassos, 1944, described from only three specimens, seems most closely related to *Z. chandleri*. The latter differs in being a smaller worm with different body proportions, in having a relatively larger acetabulum, shorter ceca, and smaller vitelline follicles which extend to the equator of the body.

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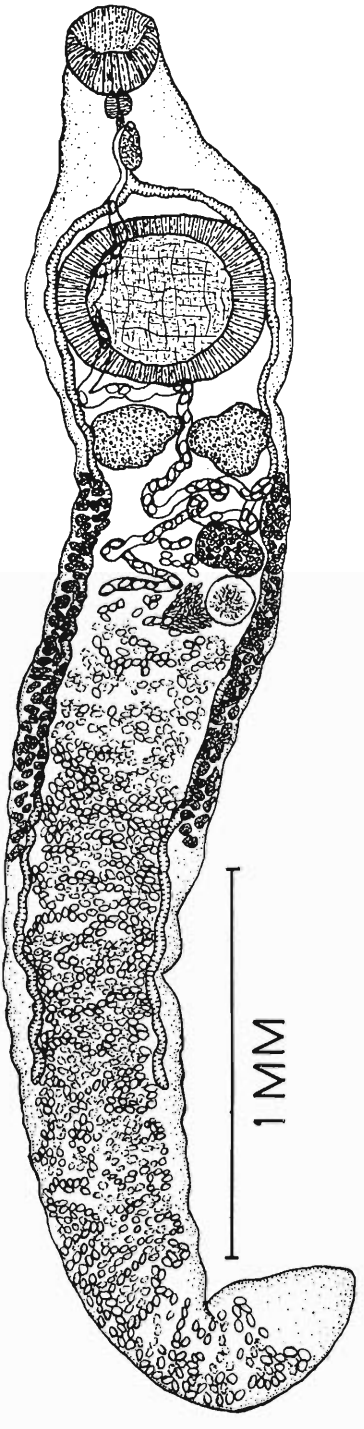

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Figure 1. ***Zonorchis chandleri*, holotype, ventral view.**

Anthelmintic Efficacy of Maretin in Cattle

H. CIORDIA[1]

ABSTRACT: Tests indicated that Maretin (naphthalophos), administered to calves as an oral drench at 50 mg/kg of body weight, had an average efficacy of 86.0% against adults of five genera of nematodes. The drug was highly efficacious (96–100%) against *Haemonchus placei*, *Cooperia punctata*, *C. oncophora*, and *Trichostrongylus colubriformis*, but less effective (86.9%) against *T. axei* and (78.1%) against *Ostertagia ostertagi*. The activity against *Oesophagostomum radiatum* was erratic. All the worms were eliminated during the first 2 days posttreatment. No signs of toxicity occurred in any of the calves.

Results of earlier investigations have indicated that Maretin* (N-Hydroxynaphthalimide diethyl phosphate) has a broad spectrum of activity against gastrointestinal nematodes of livestock. When given orally by drench at a dose level of 50 mg/kg of body weight, this compound is highly efficacious against *Haemonchus* spp., *Ostertagia* spp., *Cooperia* spp., and *Trichostrongylus* spp. in cattle, sheep, and goats (Federmann, 1964; Stober and Ende, 1964; Hebden and Hall, 1965; Guerrero-Ramírez and Chávez-G., 1966; Ciordia et al., 1966; Cox et al., 1967; Train et al., 1968; McDougald et al., 1968; Hansen and Zeakes, 1969; Partosoedjono, 1969; Mullee et al., 1970). The reportedly high efficacy and the relatively low toxicity (Nelson et al., 1970) makes Maretin a potentially useful anthelmintic. The purpose of the present investigation was to study its activity against the nematode cattle parasites enzootic in the area.

Materials and Methods

Four calves weighing an average of 264 kg each were obtained locally. All harbored naturally acquired parasitic infections and fecal egg counts showed an average of 557 nematode eggs per gram of feces. The calves were placed in individual stalls with a concrete floor 8 days before beginning the trial to get them accustomed to the environment, diet, and handling. Each calf was fed sorghum silage ad lib. and 1 kg of feed supplement daily.

All calves were given 50 mg of Maretin (80% Oral Drench Powder) per kg of body weight in the form of a drench. During the first 5 posttreatment days, the feces passed by each calf were collected from the concrete floor as soon as possible after being dropped and were stored under refrigeration until the end of each 24-hour period. The feces were then weighed, thoroughly mixed, and five 10-g samples were washed through a 100-mesh screen. The worms retained by the screen were counted and identified. The remainder of the feces was washed through a coarse mesh screen to recover larger worms. Worms remaining in the calves were recovered at necropsy 5 days after treatment and the numbers were used to estimate the efficacy of the anthelmintic.

The calves were observed frequently for signs of intoxication.

Results and Discussion

The numbers of worms by species passed with the feces after treatment, those recovered at necropsy from each calf, and the percentage of efficacy of Maretin against some species of nematodes are presented in Table 1. The worms eliminated during the first posttreatment day accounted for 93.6% of the worms passed. No worms were recovered from the feces after the second day. The drug, as used in this experiment, was 96–100% efficacious against *Haemonchus placei*, *Cooperia punctata*, *C. oncophora*, and *Trichostrongylus colubriformis*. It was also 86.9 and 78.1% effective against *T. axei* and *Ostertagia ostertagi*, respectively. The activity of the drug against *Oesophagostomum radiatum* was erratic (22–100%), although no definite conclusion can be drawn because of the relatively few specimens recovered. However, Maretin as a drench was found to be ineffective against *Oesophagosto-*

[1] From the Regional Parasite Research Laboratory Substation, Veterinary Sciences Division, Agricultural Research Services, U. S. Department of Agriculture.

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* Maretin® = Rametin® (naphthalophos), Reg. U. S. Pat. Off. by Farbenfabriken Bayer A. G., Chemagro Corporation licensee. This product is not presently registered in the United States as an anthelmintic for cattle or sheep.

Table 1. Anthelmintic efficacy in calves of Maretin oral drench at 50 mg/kg of body weight.

Calf No.	Numbers of worms by species*							
	O.o.	H.p.	T.a.	C.p.	C.o.	T.c.	O.r.	Total
	Worms eliminated during day 1 after treatment							
495	13,516	3,524	4,637	4,637	2,640	1,200	3	30,157
500	14,900	472	4,178	4,633	2,248	272	3	26,706
600	24,192	956	5,024	15,096	6,048	3,268	9	54,593
899	15,570	316	3,178	3,666	1,144	1,142	11	25,027
	Worms eliminated during day 2 after treatment							
495	144	807	807	0	0	0	3	1,761
500	0	0	151	661	0	0	0	812
600	1,200	56	700	2,400	1,300	350	0	6,006
889	373	0	0	0	374	0	0	747
	Adult worms recovered at necropsy							
495	3,665	0	0	0	0	0	19	3,684
500	3,198	0	1,998	0	0	0	3	5,199
600	7,720	0	0	1,120	80	0	32	8,952
889	5,005	0	825	0	0	0	0	5,830
	Percentage of efficacy							
495	78.8	100	100	100	100	100	24.0	89.6
500	82.3	100	68.4	100	100	100	50.0	88.1
600	76.7	100	100	94.0	98.9	100	22.0	87.1
889	76.1	100	79.4	100	100	100	100	81.6
	Average efficacy for the four calves							
	78.1	100	86.9	96.5	99.4	100	34.9	86.0

* O.o. = *Ostertagia ostertagi*; H.p. = *Haemonchus placei*; T.a. = *Trichostrongylus axei*; C.p. = *Cooperia punctata*; C.o. = *C. oncophora*; T.c. = *T. colubriformis*; B.p. = *Bunostomum phlebotomum*; O.r. = *Oesophagostomum radiatum*.

mum spp. in cattle (Train et al., 1968) and against *O. columbianum* in sheep (Federmann, 1964; Ciordia et al., 1966).

Bunostomum phlebotomum were recovered from two calves (31 and 6 specimens) at necropsy, but were never seen in the feces during the 5 posttreatment days. Because of the low numbers, the apparent inefficacy of the drug against this species is not conclusive.

Nematode larvae were not recovered from the feces. However, an average of 2,566 *O. ostertagi* larvae were counted from each abomasum at necropsy, of which 27.5 and 72.5% were third and fourth stages, respectively. It is not known if the absence of larvae in the feces was due to inefficiency of the drug or to the deficiency of the technique used.

There were no signs of toxicosis in any of the calves, nor were pathological changes attributable to the treatment observed at necropsy.

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Cryptobia cataractae sp. n. (Kinetoplastida: Cryptobiidae), a Hemoflagellate of some Cyprinid Fishes of West Virginia[1]

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ABSTRACT: *Cryptobia cataractae* sp. n. is described from the blood of some cyprinid fishes of West Virginia. The leech vector is *Cystobranchus virginicus*. It is compared with the *Cryptobia* species found in the blood of North American freshwater fishes.

Cryptobia (Leidy, 1846) (Synonym: Trypanoplasma) (Laveran and Mesnil, 1901) is a genus of biflagellated protozoan (Kinetoplastida: Cryptobiidae). It has been recorded in both marine and freshwater fishes of North America; from marine fishes as parasites of the blood (Strout, 1965) and digestive tract (Noble, 1968); from freshwater fishes as parasites of the skin (Swezy, 1919), gills (Wenrich, 1931), and blood.

The following are reports of *Cryptobia* found in North American freshwater fishes: *C. borelli* (Mavor, 1915), *C. gurneyorum* (Laird, 1961), and *C. salmositica* (Katz, 1951; Davison et al., 1954; Wales and Wolf, 1955; Becker and Katz, 1965a, b, c, 1966, 1969; Katz et al., 1966). Except for *C. salmositica*, the organisms have not been extensively studied.

Materials and Methods

The geographical area of natural study and specimen collection was the Opequon Creek at Dandridge Ford, Jefferson County, West Virginia, latitude 39°22′30″ N, longitude 77°57′30″ W.

Fishes from nature were collected by seining or electrofishing using a 115-volt alternating current system.

The leech vector of *Cryptobia cataractae*, *Cystobranchus virginicus* (Hoffman, 1964), was collected manually with small forceps from the host fishes, *Rhinichthys cataractae*, after anesthetization with MS-222 (tricaine methanesulfonate) at a concentration of 1 part to 15,000 parts water. *Piscicola salmositica* leeches used for comparative studies were collected and sent by Dr. Dale C. Becker, Biology Department, Battelle-Northwest, Richland, Washington 99352. Prior to examination, leeches were put in 500 ml of fresh spring water and placed into a 4 C refrigerator for 4 to 6 days. This permitted much of the blood within the leech to be digested.

After refrigeration, fasted leeches were placed on tissue paper to remove excess water, macerated in a mortar with Hanks' balanced salt solution, lightly centrifuged, let stand for 10 minutes, and the saline with contained *Cryptobia* decanted.

[1] A portion of a dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biological Sciences, Fordham University, The Bronx, New York 10458.

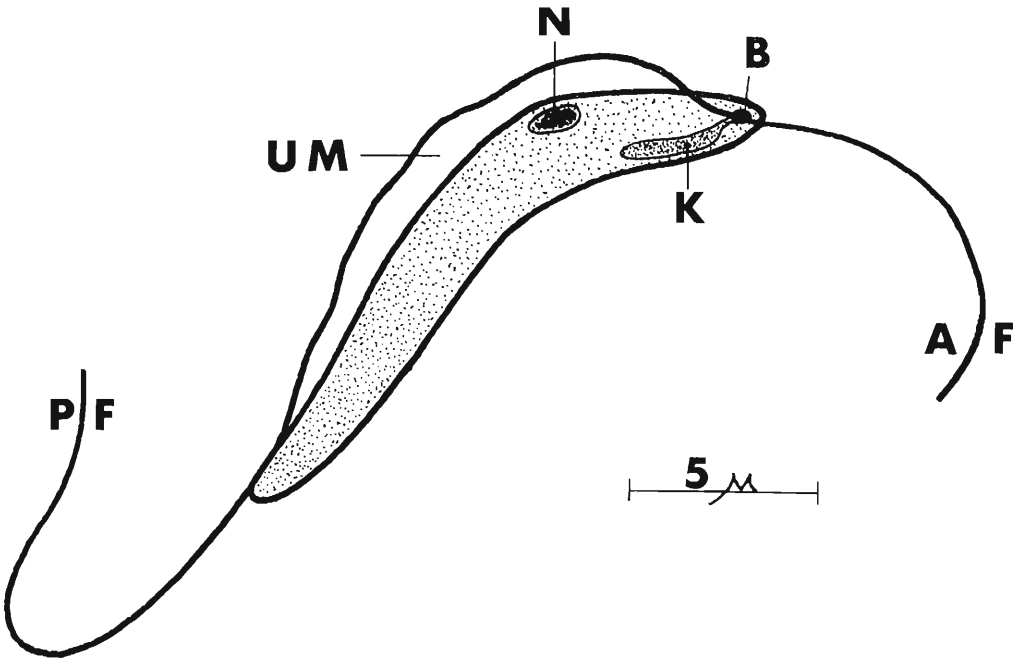

Figure 1. Freehand sketch of *Cryptobia cataractae* sp. n. showing organelles: Abbreviations: *AF*, anterior flagella; *B*, blepharoplast; *K*, kinetoplast; *N*, nucleus; *UM*, undulating membrane; *PF*, posterior flagella.

Fish used for transmission and host range studies were laboratory-reared or collected from streams. Those collected from streams were tested for the presence or absence of *Cryptobia* by withdrawal and microscopic examination of blood.

Blood containing *C. cataractae* was obtained from host fishes after anesthetization with MS-222 by cardiac puncture, puncture of vessels under the haemal arch in the caudal peduncle region, and severing of the tail at the posterior end of the caudal peduncle region.

To prevent coagulation of the blood, a drop of 2% heparinized saline was placed into containers receiving blood, including syringes and needles.

For transmission and host range studies, approximately 100 *Cryptobia* in 0.05 ml of Hank's balanced salt solution were injected into fishes intramuscularly near the base of the dorsal fin.

To determine transmission and host susceptibility, inoculated fishes were held for 10 days, anesthetized with MS-222, and examined. Blood was obtained by cardiac puncture using a heparinized 25-gauge needle and syringe, and diluted with equal part of BSS and examined microscopically.

Results

Cryptobia cataractae sp. n. (Fig. 1) is a biflagellated protozoan (Kinetoplastida: Cryptobiidae) which is in constant motion, with movements due to flagellar, undulating membrane, and entire body movements.

MORPHOMETRICS (in microns): From 50 formalin-fixed specimens measured with an ocular micrometer. Body length: 17 (14.7–18.9); body width: 2 (1.54–2.2); length of anterior flagellum: 11 (9.6–13.2); length of posterior flagellum (free part): 14 (11–16.4); nucleus diameter: 1–1.5; kinetoplast length: 2.6–3.1.

HOSTS, NATURAL: Longnose dace, *Rhinichthys cataractae* (primary host); blacknose dace, *Rhinichthys atratulus*; cutlips minnow, *Exo-*

Table 1. Comparison of *Cryptobia cataractae* with other North American *Cryptobia* from freshwater fishes. Measurements in microns.

Species	Vector	Body length	Body width	Length anterior flagella	Length posterior flagella	Nucleus	Kinetoplast length
Cryptobia cataractae[1]	*C. virginicus*	17 (14.7–18.9)	2 (1.54–2.2)	11 (9.6–13.2)	14 (11–16.4)	1–1.5	2.6–3.1
C. salmositica[2]	*P. salmositica*	14.94	2.46	16.05	8.96	1.5–3.5	4.58 × 0.78
C. gurneyorum[3]	None given	25.1 (19.2–28.2)	6.7 (3.1–7.1)	19	10	None given	None given
		Scattered giants					
		38.8	9.1				
C. borreli[4]	None given	20–25	3–4	None given	None given	None given	None given

Natural hosts:
[1] *R. cataractae, R. atratulus, E. maxillingua, C. anomalum.*
[2] *O. kisutch, C. rhotheus, C. aleuticus, S. gairdneri, O. tshawytscha, S. trutta, S. gairdneri gairdneri, C. snyderi, O. keta, O. gorbuscha, Prosopium williamsoni, C. bairdi, C. gubsus, C. beldingi, C. perplexus, C. asper, R. cataractae, Gasterosteus aculeatus.*
[3] *C. clupeaformis, S. namaycush, E. lucius.*
[4] *C. commersonii.*

glossum maxillingua; stoneroller, *Campostoma anomalum.*

HOSTS, EXPERIMENTAL: Creek chub, *Semotilus atromaculatus*; banded killifish, *Fundulus diaphanus.*

SITE OF INFECTION: Blood and body fluids.

VECTOR: *Cystobranchus virginicus* Hoffman, 1964.

TYPE LOCALITY: Opequon Creek at Dandridge Ford, Jefferson County, West Virginia, latitude 39°22′30″ N, longitude 77°57′30″ W.

TYPE SPECIMENS: USNM Helm. Coll. (No. 63198).

Discussion

Previous descriptions of *Cryptobia* species from North American freshwater teleosts have been based on morphometrics and hosts they were parasitizing. Experiments to elucidate the biology of the organism, i.e., life cycle, vector, experimental transmission, and possible physiological processes, were not used for determining species.

Mavor (1915), the first to report a *Cryptobia* from North America, found the organism in a moribund sucker, *Catostomus commersoni*, from Go Home Bay of Lake Huron. He states, "The trypanoplasm found in the sucker has all the morphological characters described and figured for *Trypanoplasma borreli* by Lavern and Mesnil (1902), size and shape of the body, position and shape of the nuclei, and length of the flagella. The writer therefore provisionally identifies it with this species."

Swezy (1919) discussed *T. carassii*, which he named in 1915, as an ectocommensal parasite of goldfish, *Carassius auratus*, in aquaria at the University of California. He reports, "A comparison of *T. carassii* with the blood inhabiting species of the genus shows no important differences." He does not indicate why he considered it a separate species.

Wenrich (1931) found a *Cryptobia* on the gills of the carp, *Cyprinus carpio*, from the Schuylkill River in Philadelphia. He stated, "The structure of this trypanoplasm is somewhat different from that attributed to *T. carassii* described by Swezy from the skin of goldfish and different from *T. cyprinus* from the blood of European carp." He doesn't say how it is different and does not elaborate as to its taxonomic status.

Katz (1951) described two new species of *Cryptobia*: *C. salmositica* from silver salmon, *Oncorhynchus kisutch*, and *C. lynchi* from two cottids, *Cottus rhotheus* and *C. aleuticus*, from the same geographical area in the state of Washington. He separated them on the basis of their morphometrics and host difference.

Wales and Wolf (1955) found *Cryptobia* from rainbow trout, *Salmo gairdneri*, king salmon, *O. tshawytscha*, steelhead rainbow trout, *Salmo gairdneri gairdneri*, brown trout, *S. trutta*, coho salmon, *O. kisutch*, Klamath

Table 2. Natural host range of *Cryptobia cataractae* over a 3-year period, 1967–69.

Fish family	Genera and species	Number examined	Number infected	Per cent infection
Cyprinidae	*Rhinichthys cataractae*	111	108	97
	Rhinichthys atratulus	84	25	30
	Exoglossum maxillingua	77	14	18
	Campostoma anomalum	69	13	19
	Semotilus corporalis	75	20	27
	Semotilus atromaculatus	57	0	0
	Notropis cornutus	72	0	0
Cyprinodontidae	*Fundulus diaphanus*	53	0	0
Cottidae	*Cottus* sp.	100	0	0
Centrarchidae	*Ambloplites rupestris*	65	0	0
	Micropterus dolomieui	48	0	0
Ictaluridae	*Noturus* sp.	61	0	0

large-scaled sucker, *Catostomus snyderi*, sculpin, *Cottus* sp., and the leech, *P. salmositica*, in the state of California. They designated the *Cryptobia* as *C. borreli* without giving any reasons for doing so.

Laird (1961) recorded a hemoflagellate from northern pike, *Esox lucius*, the salmonids *Coregonus clupeaformis* and *Salvelinus namaycush*, calling it *C. gurneyorum* (Minchin). He based speciation on morphometrics and the presence of cytoplasmic chromophilic granules.

The procedure of naming a new species of *Cryptobia* when infections are found in new hosts, and on minor morphometric differences alone, is questionable since the polymorphic diversity of *Cryptobia* species is well known (Table 1). This is further substantiated by the work of Becker and Katz (1965b). Becker (pers. comm.) believes *C. salmositica* from the Pacific Northwest of the United States to be a valid species primarily on the basis of the distribution and association with its common endemic leech vector, *P. salmositica*. *C. cataractae* is declared a new species after comparing its morphometrics with the other three species noted from North America (Table 1), natural and experimental host range with that of *C. salmositica* from the western United States (Tables 2, 3), and other biological parameters,[1] i.e., vectors, cryogenic preservation, histopathology, maintenance, and culture attempts. The specific name for this *Cryptobia* is taken from the primary host fish, *Rhinichthys cataractae*.

The natural host range of *C. cataractae* is within the family Cyprinidae. Natural infections were not observed in the families Cyprinidontidae, Cottidae, Centrarchidae, and Ictaluridae (Table 2). This natural host range was checked for 3 consecutive years to determine if any fluctuation occurred. It remained the same which indicated a similar constant host range for the vector, *C. virginicus*. Since the incidence of infection was the highest (97%) among the longnose dace, *R. cataractae*, and subsequent investigation showed this fish during its spawning season to be the only species in the endemic geographical area to harbor the vector, it is considered to be the reservoir host of *C. cataractae*.

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Table 3. Comparative experimental host range of *Cryptobia salmositica* from the Pacific northwestern U. S. and *Cryptobia cataractae* from the eastern U. S.

Fish	*C. salmositica* Western U. S.		*C. cataractae* Eastern U. S.	
	Number injected	Number infected	Number injected	Number infected
Salmonidae				
Salmo gairdneri	10	10	10	0
Salmo fontinalis	10	10	10	0
Salmo trutta	10	10	10	0
Oncorhynchus tschawytscha	10	10	10	0
Oncorhynchus kisutch	10	10	10	0
Cyprinidae				
Rhinichthys atratulus	10	10	10	10
Semotilus atromaculatus	5	5	10	7
Cyprinodontidae				
Fundulus diaphanus	0	0	10	10
Cottidae				
Cottus sp.	10	10	10	0
Centrarchidae				
Lepomis macrochirus	10	0	10	0
Lepomis cyanellus	10	0	10	0
Micropterus dolomieui	5	0	5	0
Ambloplites rupestris	10	0	0	0

C. cataractae showed a wider experimental host range (Table 3) than was found in nature (Table 2). An additional fish species of the Cyprinidae family, the creek chub, *S. atromaculatus*, when experimentally injected with *C. cataractae*, became infected as did the banded killifish, *F. diaphanus*, a member of another fish family, Cyprinodontidae.

When *C. salmositica* and *C. cataractae* were each injected into the same species of fishes, *C. salmositica* showed the widest experimental host range infecting eight species of fishes from three different families, while *C. cataractae* was infective to only three species of two families (Table 3).

Only two fish species, *R. atratulus* and *S. atromaculatus*, both of the family Cyprinidae, showed susceptibility to both *Cryptobia* species.

The leech vector, *Cystobranchus virginicus*, was found only on the longnose dace, *R. cataractae*. The probability of the natural host range of *Cryptobia cataractae* being a direct biological function of leech preference or specificity existed. This apparently is the case as shown by the natural and experimental host range of *C. cataractae*.

Acknowledgments

The author wishes to thank Mr. Charles P. Carlson, Hatchery Biologist, Experimental Fish Farming Station, Stuttgart, Arkansas, for noting the presence of *Cryptobia cataractae* sp. n. in the blood of longnose dace, *Rhinichthys cataractae*, while a trainee in fish diseases at this laboratory; Dr. John J. A. McLaughlin, chairman of the Department of Biological Sciences, Fordham University, and Director of The Louis Calder Conservation and Ecology Study Center for his mentorship during the study; Drs. S. F. Snieszko and G. L. Hoffman of the Eastern Fish Disease Laboratory for their assistance and critical review of the total dissertation.

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The Genus *Oxyspirura* (Nematoda: Thelaziidae) from Birds in Louisiana

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ABSTRACT: The eyeworms of the genus *Oxyspirura* of birds in southern Louisiana were studied. The number of known avian hosts for oxyspirurids in North America is increased to 28 species. *Oxyspirura petrowi* is recorded for the first time from *Agelaius phoeniceus, Cassidix mexicanus, Quiscalus quiscula, Molothrus ater, Tyrannus tyrannus, Myiarchus crinitus, Anthus spinoletta, Richmondena cardinalis, Vireo griseus, Iridoprocne bicolor, Butorides virescens,* and *Bubulcus ibis.* New host records for *Oxyspirura pusillae* are *Centurus carolinus, Melanerpes erythrocephalus, Thryothorus ludovicianus, Toxostoma rufum, Quiscalus quiscula, Myiarchus crinitus, Richmondena cardinalis, Sialia sialis,* and *Dendrocopos villosus. O. petrowi* is considered as a species of wide geographic range, which exhibits considerable metrical variation, and demonstrates little host specificity. *Oxyspirura lumsdeni* is rejected as a species distinct from *O. petrowi.* The natural history and systematics of these oxyspirurids are discussed.

Although 72 "valid" species of the genus *Oxyspirura* are described as parasites of the eyes of birds, only four have been reported from North America, north of Mexico (Addison and Anderson, 1969a). Ransom (1904) reported *Oxyspirura mansoni* from Louisiana, Puerto Rico, and adjacent islands. Saunders (1928) found *O. mansoni* in domestic fowl in Florida and was successful in experimentally infecting pigeons, bobolinks, shrikes, and blue jays. Cram (1937) described a species identified as *Oxyspirura petrowi* Skrjabin (1929) from ruffed grouse, the prairie chicken, sharp-tailed grouse, the eastern robin, and the meadow lark in Michigan. More recently, *Oxyspirura pusillae* was described from the brown-headed nuthatch, *Sitta pusilla,* in Georgia (Wehr and Hwang, 1957). Both *O. petrowi* and *O. pusillae* are reported from the yellow-throat, *Geothlypis trichas,* in South Carolina (Wells and Hunter, 1960). *Oxyspirura lumsdeni* was described from ruffed grouse, sharp-tailed grouse, lesser and greater prairie chickens, and pheasant in southern Canada and the United States by Addison and Anderson (1969b), who considered the specimens described by Cram (1937) from some of the same hosts as this species.

The present investigation was initiated in order to determine the occurrence of oxyspirurid infections in wild bird populations and to determine the species occurring in southern Louisiana. Birds were collected from several study areas, all within a radius of 150 miles of New Orleans, Louisiana. Eyeworms were removed from infected birds, fixed briefly in glacial acetic acid, and stored in a mixture of 5 parts glycerin and 95 parts of 70% ethyl alcohol. Specimens were studied in glycerin mounts after evaporation of the alcohol.

The morphological and metrical variations of specimens from different hosts were examined in order to determine the validity of certain characteristics used in oxyspirurid systematics. This information was compared with the original descriptions of certain described species. All measurements are in millimeters unless otherwise indicated. Specimens from the various hosts collected in Louisiana are deposited in the USNM Helm. Coll. Numbering for *O. petrowi* (No. 71766–71779) and *O. pusillae* (No. 71780–71789) follows sequences of hosts in Table 1.

Results

Eyeworms were recovered from 21 species of birds (Table 1). They were identified as *Oxyspirura petrowi* and *Oxyspirura pusillae.* Of the 14 species infected with *O. petrowi,* all but the meadow lark and robin, and of the 10 infected with *O. pusillae,* all but the nuthatch, are recorded as hosts of the respective species for the first time. Three species of birds, the purple grackle, crested flycatcher, and cardinal, were found infected on different occasions with both *O. petrowi* and *O. pusillae.* All the speci-

Table 1. The hosts of *Oxyspirura petrowi* and *O. pusillae* in Louisiana. *Denotes new host record.

Host	Incidence	Number of parasites collected
Oxyspirura petrowi		
1. Meadow lark (*Sturnella magna*)	20/24	51
2. Red-winged blackbird (*Agelaius phoeniceus*)*	3/20	10
3. Boat-tailed grackle (*Cassidix mexicanus*)*	4/12	22
4. Purple grackle (*Quiscalus quiscula*)*	1/11	12
5. Cowbird (*Molothrus ater*)*	1/19	1
6. Eastern kingbird (*Tyrannus tyrannus*)*	1/10	2
7. Crested flycatcher (*Myiarchus crinitus*)*	1/16	1
8. Water pipit (*Anthus spinoletta*)*	1/6	1
9. Cardinal (*Richmondena cardinalis*)*	1/20	2
10. White-eyed vireo (*Vireo griseus*)*	1/10	1
11. Tree swallow (*Iridoprocne bicolor*)*	1/69	1
12. Eastern robin (*Turdus migratorius*)	1/22	4
13. Green heron (*Butorides virescens*)*	1/15	1
14. Cattle egret (*Bubulcus ibis*)*	1/14	1
Oxyspirura pusillae		
1. Red-bellied woodpecker (*Centurus carolinus*)*	20/36	164
2. Red-headed woodpecker (*Melanerpes erythrocephalus*)*	5/12	29
3. Hairy woodpocker (*Dendrocopos villosus*)*	1/12	1
4. Carolina wren (*Thryothorus ludovicianus*)*	4/33	17
5. Brown thrasher (*Toxostoma rufum*)*	3/22	3
6. Brown-headed nuthatch (*Sitta pusilla*)*	2/15	16
7. Purple grackle (*Quiscalus quiscula*)*	1/11	4
8. Crested flycatcher (*Myiarchus crinitus*)*	1/16	4
9. Cardinal (*Richmondena cardinalis*)*	1/20	1
10. Blue bird (*Sialia sialis*)*	2/7	8

ments collected constitute new geographic records for these parasites.

Specimens from the water pipit, green heron, and cattle egret consisted of a single immature female and two mature female worms, respectively. These are regarded as *O. petrowi* since they are morphologically identical to the females of that species. Specimens from the remaining hosts conform in most respects to the descriptions of *O. petrowi* found in Skrjabin (1929), Cram (1937), Baruš (1965), and Ybarra (1948); however, metrical variations occur in certain key morphological characters in specimens from different hosts (Table 2). Specimens from the meadow lark and boat-tailed grackle represent the extremes of metric variability in certain characters. Especially notable is the considerable variation in the length of the right spicule, even in specimens from the same host.

Oxyspirura lumsdeni was proposed as a new species by Addison and Anderson (1969b) primarily because of the presence of deirids, which Skrjabin (1929) did not mention in his description of *O. petrowi*, and the greater length of the right spicule. However, according to Baruš (pers. comm.) deirids are present on all specimens identified as *O. petrowi* collected from several species of birds in eastern Europe, including some of the same species from which Skrjabin described this species. Comparison of specimens from Czechoslovakia identified as *O. petrowi* from *Saxicola rubetra* and specimens of *O. lumsdeni* from tetraonids from Canada with those collected in the present study revealed that eyeworms from all these sources are morphologically indistinguishable and the specimens from birds in Louisiana are intermediate in size between those from Europe and Canada.

Practically all of the size relationships authoritatively reported for *O. petrowi* fall within the ranges reported for the specimens collected in the present study (Table 3). In addition, most of the latter ranges fall within those reported for *O. lumsdeni*. Therefore, and in view of the observed lack of host specificity, *O. lumsdeni* is indistinguishable from *O. petrowi* and is rejected as a synonym.

Oxyspirura (*Oxyspirura*) *petrowi* Skrjabin 1929

GENERAL: (Based on 40 male and 40 female specimens.) Spiruroidea, Thelaziidae, Thelaziinae, *Oxyspirura* Drasche in Stossich, 1897, subgenus *Oxyspirura* Skrjabin, 1931, *Oxyspirura petrowi* Skrjabin, 1929. Body slender, yellow to cream color, bluntly rounded anteriorly, sharply attenuated posteriorly. Cervical alae present. Cuticle transversely striated. Mouth with four submedian pairs and three circumoral pairs of cephalic papillae. Amphids present. Cuticularized buccal capsule undivided. Esophagus not discernibly divided into muscular and glandular portions. Deirids lateral just anterior to nerve ring. Excretory pore posterior to nerve ring. Gravid uterus variable in position and sometimes extending into the esophageal region. Vulva and anus in posterior quarter of body. Spicules unequal and dissimilar. No gubernaculum or caudal

Table 2. Comparison of metric characteristics of *Oxyspirura petrowi* from different hosts in Louisiana.

Host Specimens	Meadow lark 20 ♂♂, 20 ♀♀	Boat-tailed grackle 10 ♂♂, 10 ♀♀	Red-winged blackbird 5 ♂♂, 5 ♀♀	Robin 2 ♂♂, 2 ♀♀	Purple grackle 1 ♂♂, 3 ♀♀	Cardinal 1 ♂♂, 1 ♀♀	Eastern kingbird 1 ♂♂, 1 ♀♀
Male							
Length	6.44–8.63 (7.75)	6.35–7.93 (6.89)	7.13–8.10 (7.61)	(8.31)	7.50	6.85	7.27
Esophagus length	0.53–0.65 (0.60)	0.53–0.60 (0.56)	0.55–0.63 (0.59)	(0.77)	0.58	0.64	0.60
Nerve ring—distance from anterior end	0.11–0.17 (0.14)	0.11–0.14 (0.13)	0.12–0.13 (0.13)	(0.15)	0.14	0.15	0.15
Excretory pore—distance to anterior end	0.22–0.33 (0.26)	0.22–0.26 (0.24)	0.22–0.28 (0.26)	(0.28)	0.25	0.26	0.25
Right spicule length	0.17–0.32 (0.21)	0.12–0.15 (0.14)	0.13–0.14 (0.14)	(0.20)	0.16	0.19	0.23
Left spicule length	0.33–0.51 (0.44)	0.26–0.33 (0.30)	0.29–0.32 (0.30)	(0.42)	0.34	0.36	0.41
Female							
Length	8.90–12.50 (10.80)	8.00–11.50 (9.20)	7.70–9.82 (8.76)	(11.70)	10.50	11.95	11.70
Esophagus length	0.55–0.73 (0.64)	0.57–0.65 (0.60)	0.58–0.60 (0.59)	(0.76)	(0.61)	0.66	0.68
Nerve ring—distance from anterior end	0.11–0.16 (0.13)	0.12–0.15 (0.14)	0.13–0.14 (0.13)	(0.17)	(0.15)	0.12	0.15
Excretory pore—distance from anterior end	0.14–0.34 (0.26)	0.19–0.28 (0.25)	0.23	(0.26)	(0.27)	0.28	0.28
Vulva—distance to posterior end	0.51–0.70 (0.61)	0.52–0.61 (0.57)	0.50–0.60 (0.55)	(0.59)	(0.60)	0.55	0.60
Egg length	(0.041)	(0.039)	(0.040)	(0.040)	(0.038)	0.035	0.044
Egg width	(0.028)	(0.024)	(0.028)	(0.027)	(0.027)	0.024	0.020

Table 3. Comparison of the metric characteristics of *Oxyspirura petrowi* and *O. lumsdeni*.

Species Authority	*O. petrowi* Skrjabin, 1929	*O. petrowi* Baruš, 1965	*O. petrowi* Present author	*O. lumsdeni* Addison and Anderson, 1969
Male				
Length	5.5–6.4	4.8–6.0	6.3–8.6 (7.4)	6.9–16.4 (11.8)
Width	0.22–0.28	0.19–0.22	0.14–0.29 (0.21)	0.19–0.39 (0.29)
Esophagus length	0.74	0.57–0.62	0.53–0.68 (0.59)	0.61–1.02 (0.81)
Nerve ring—distance from anterior end	—	0.33	0.11–0.17 (0.13)	0.16–0.23 (0.19)
Excretory pore—distance from anterior end	—	0.18–0.19	0.22–0.33 (0.25)	0.25–0.44 (0.33)
Right spicule length	0.125	0.148–0.152	0.121–0.320 (0.183)	0.200–0.260 (0.220)
Left spicule length	—	0.243–0.340	0.264–0.517 (0.381)	0.440–0.600 (0.520)
Female				
Length	9.2	5.9–6.2	7.70–12.35 (10.17)	11.4–22.5 (16.4)
Width	0.42	0.25–0.32	0.20–0.46 (0.31)	0.27–0.60 (0.41)
Esophagus length	0.95	0.62	0.55–0.73 (0.62)	0.55–0.110 (0.86)
Nerve ring—distance from anterior end	0.16–0.17	0.16	0.11–0.16 (0.14)	0.16–0.24 (0.19)
Excretory pore—distance from anterior end	0.30	0.39	0.14–0.31 (0.26)	0.27–0.46 (0.33)
Egg length	0.039–0.041	0.038–0.040	0.035–0.044 (0.039)	0.029–0.048 (0.038)
Egg width	0.026–0.028	0.026–0.028	0.015–0.031 (0.026)	0.022–0.036 (0.027)
Vulva—distance from posterior end	0.41–0.43	0.29–0.36	0.50–0.70 (0.59)	0.48–0.83 (0.62)

alae. Ventral caudal papillae present in male. Phasmids present in both sexes.

MALE: Length 6.27–8.63 (7.40). Maximum width 0.185–0.330 (0.237). Buccal capsule: Length 14–29 μ (21); width 13–23 μ (18). Esophagus 0.530–0.680 (0.590) long. Nerve ring 0.110–0.165 (0.137) and excretory pore 0.219–0.330 (0.255) from anterior extremity. Right spicule 0.121–0.320 (0.183) long and boat-shaped. Left spicule slender with sharp tip, 0.264–0.517 (0.381) long. Tail sharp-pointed, 0.181–0.330 (0.255) long. Preanal papillae 4 to 6 in number, asymmetrically arranged. Postanal papillae 4 to 6 in number, asymmetrically arranged. Phasmids usually lateral, 0.055–0.154 (0.111) from posterior extremity.

FEMALE: Length 7.70–12.35 (10.17). Maximum width 0.200–0.455 (0.308). Buccal capsule 18–28 μ (22) long and 15–26 μ (21) wide. Esophagus 0.550–0.730 (0.620) long. Nerve ring 0.110–0.158 (0.137) and excretory pore 0.136–0.308 (0.260) from anterior extremity. Vulva 0.500–0.700 (0.591) from posterior extremity. Anus 0.242–0.400 (0.328) from posterior extremity. Phasmids usually lateral 0.100–0.176 (0.136) from posterior extremity. Eggs embryonated, 35–44 μ (39) long, 15–31 μ (26) wide.

Additional descriptions and good figures of *O. petrowi* are found in Skrjabin (1929), Cram (1937), Baruš (1965), and Addison and Anderson (1969b).

Oxyspirura pusillae was obtained from 10 species of birds. This species is differentiated from other similar species of the subgenus *Yorkeispirura* with a gubernaculum by the presence of six preanal, two adanal, and four postanal papillae and by the greater length of the spicules. It is very similar to *Oxyspirura tsingchengensis* Hsü, 1933, but differs in having two adanal papillae and a cuticularized thickening around the vulva. Although Hsü (1933) did not mention the presence of a gubernaculum in this species, figure 42 of his description indicates its possible presence. Also, *O. pusillae* is similar to *O. mansoni* except the latter species supposedly lacks a gubernaculum. However, some authors state that this structure is present in *O. mansoni*, while others contend that it is absent. On the basis of existing descriptions, *O. pusillae* can be differentiated from these two species only on the basis of the cuticularized thickening of the vulva and possibly by the presence of a gubernaculum.

Oxyspirura (Yorkeispirura) pusillae Wehr and Hwang, 1957

GENERAL: (Based on 40 male and 40 female specimens.) Spiruroidea, Thelaziidae, Thelaziinae, *Oxyspirura* Drasche in Stossich, 1897, subgenus *Yorkeispirura* Skrjabin, 1931, *Oxyspirura pusillae* Wehr and Hwang, 1957. Body slender, white in color, rounded anteriorly, sharply attenuated posteriorly. No cervical alae. Cuticle smooth. Mouth terminal, with four submedian pairs and three circumoral pairs of cephalic papillae. Amphids present. Well-cuticularized buccal capsule divided by a distinct constriction into two cylindrical parts. Esophagus not discernibly divided into a muscular and glandular portion. Deirids present in the region of the excretory pore. Excretory pore ventral and posterior to nerve ring. Vulva and anus in posterior quarter of body. Vulva with cuticularized thickening at its opening. Spicules unequal and dissimilar. Gubernaculum present. No caudal alae present. Ventral caudal papillae present in the male. Phasmids present in both sexes.

MALE: Length 6.65–9.85 (8.39). Maximum width 0.140–0.240 (0.191). Buccal capsule: Anterior part 11–18 μ (14) deep and 11–24 μ (20) wide; posterior part 14–28 μ (22) deep and 10–22 μ (14) wide. Esophagus 0.820–1.350 (1.080) long. Nerve ring 0.156–0.284 (0.198) and excretory pore 0.194–0.367 (0.296) from anterior extremity. Right spicule stout, 0.149–0.242 (0.209) long. Left spicule slender, often with undulations, sharp-pointed, 1.710–2.330 (2.032) long. Gubernaculum 39–88 μ (56) long. Ventral caudal papillae asymmetrically arranged, three pairs preanal, one pair adanal, and two pairs postanal. Phasmids lateral, 32–73 μ (55) from tip of tail.

FEMALE: Length 7.93–14.78 (11.72). Maximum width 0.140–0.330 (0.262). Buccal capsule: Anterior part 11–22 μ (17) deep and 14–26 μ (22) wide; posterior portion 15–20 μ (22) deep and 11–20 μ (15) wide. Esophagus 0.890–1.480 (1.160) long. Nerve ring 0.176–0.253 (0.204) and excretory pore 0.249–0.354 (0.311) from anterior extremity. Vulva 0.670–1.200 (0.852) and anus 0.220–0.350

(0.267) from posterior extremity. Phasmids 31–81 μ (56) from tip of tail. Eggs embryonated, 42–48 μ (45) long, 18–33 μ (27) wide.

Good figures of *O. pusillae* are found in Wehr and Hwang (1957).

Apparently, neither *O. petrowi* or *O. pusillae* is pathogenic. Even in heavy infections with as many as 30 worms per eye, no gross or histopathology was demonstrated.

The rates of infection ranged from one to as many as 68 worms in a single bird. Host species which were more commonly infected also tended to be more heavily infected.

Although meadow larks and blackbirds are more commonly infected with *O. petrowi*, sufficient specimens of hosts were not collected to enable the determination of the overall incidence in these avian populations. Also, *O. pusillae* appears to be a common parasite of the red-bellied woodpecker and red-headed woodpecker in southern Louisiana. It was found more often in these birds than in the type host, the brown-headed nuthatch.

Discussion

Ransom (1904) concluded that eyeworms were not reported more frequently because they were often overlooked. This is apparent since they were commonly found in the present study. The number of naturally infected hosts of oxyspirurids known in North America is now increased to 28 species. Undoubtedly, additional hosts will be recordcd.

The ecology and natural history of these infections present some interesting speculations. *Oxyspirura petrowi* is encountered primarily in birds found in open fields, submarginal grasslands, and marsh lands. Its presence in a number of species of avian hosts representing several families, but all occupying comparable ecological niches, indicates that host specificity is not dependent on definitive host physiology. Rather, it is probably dependent on the occurrence of the intermediate host(s) which is restricted to a particular habitat. Likewise, *O. pusillae* is found in a series of unrelated avian hosts which are most commonly found feeding on trees, removing insects from under bark, decaying wood, and on leaves. Other birds which appear to be more rarely infected probably only occasionally enter this niche. Although their life histories are unknown, it appears that these oxyspirurids have arthropod, probably insect, intermediate hosts which are found in particular ecological situations. Evidently their occurrence is directly dependent on the contact of the definitive host with the ecological niche within which the intermediate host is most often found.

The occurrence of both *O. pusillae* and *O. petrowi* in the same host species, reported here and by Wells and Hunter (1960), also indicates the lack of host specificity in these species. However, double infections in the same host individuals have not been reported.

Addison and Anderson (1969b) found that the following species cannot be distinguished from *O. petrowi* on the basis of existing descriptions: *O. dicruricola* Jairapuri and Siddiqi, 1967; *O. indica* Singh, 1948; *O. kaitingensis* Hsü, 1933; *O. malabarica* Jairapuri and Siddiqi, 1967; *O. rysavyi* Baruš, 1963; and *O. yehi* Ali, 1960. On the basis of the present study, the following species cannot be distinguished from *O. petrowi*: *O. cochlearispiculata* Caballero, 1951; *O. laharpurensis* Jairapuri and Siddiqi, 1967; *O. lalagea* Ali, 1960; *O. matogrosensis* Rodriques, 1963; *O. otocompsa* Rasheed, 1960; *O. peipingensis* Hsii, 1933; and *O. streparae* Johnson and Mawson, 1941. Most of these species are based on a few specimens, or a single specimen, from a single host individual and locality. Hence, nothing is known as to what extent intraspecific variations may occur in populations from the same host and locality or from different hosts and localities. The result is a series of described species in which the taxonomy is unworkable and cannot be conclusively resolved until more data based on additional specimens and comparison with the respective types are forthcoming.

In view of the present findings, it is concluded that the genus *Oxyspirura* probably consists of fewer valid species than are now described. It is suggested that future investigators consider more carefully the abovementioned factors before proposing new species in this genus.

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Activity of Cambendazole and Morantel Tartrate against Two Species of *Trichostrongylus* and Two Thiabendazole-resistant Isolates of *Haemonchus contortus* in Sheep

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ABSTRACT: The activity of cambendazole and morantel tartrate against *Trichostrongylus axei*, *T. colubriformis*, and two thiabendazole-resistant isolates of *Haemonchus contortus* was determined in controlled anthelmintic trials with experimentally infected lambs. Morantel tartrate at 8 mg/kg and cambendazole at 20 mg/kg were, respectively, 99 and 94% effective against the BPL-2 isolate of *H. contortus*; against the AH-2 isolate the efficacies of morantel tartrate and cambendazole were 97 and 81%, respectively. Cambendazole was more effective (99%) than morantel tartrate (88%) against *T. colubriformis*, but both drugs were highly effective, 100 and 99%, respectively, against *T. axei*. Only the AH-2 thiabendazole-resistant isolate of *H. contortus* showed any evidence of drug resistance in these trials.

The occurrence of drug-resistant strains of nematode parasites of sheep (Colglazier, Kates, and Enzie, 1970; Hotson, Campbell, and Smeal, 1970; Kates et al., 1971; Smeal et al., 1968; Theodorides et al., 1970) shows the need for alternative broad-spectrum anthelmintics. Recent reports indicate that cambendazole (Baker and Walters, 1971; Benz, 1971a, b; Ciordia and McCampbell, 1971; Egerton and Campbell, 1970; Egerton et al., 1970; Hoff et al., 1970) and morantel tartrate (Cornwell and Jones, 1970a, b, c; Howes, 1968; McFarland et al., 1969) are both effective against many nematode parasites of domestic rumi-

Table 1. Summary of trials comparing the efficacy of cambendazole and morantel tartrate against two species of *Trichostrongylus* and two isolates of *H. contortus* in lambs.

Parasites (lambs/group)	Larvae/lamb ± 95% CI[1]	Average worms at necropsy and calculated efficacy				
		Unmedicated controls	Cambendazole (20 mg/kg)		Morantel (8 mg/kg)	
		Worms	Worms	Efficacy (%)	Worms	Efficacy (%)
H. contortus						
BPL-2 Isolate (6)	5,000 ± 172	2,556 (21–3,654)[2]	144 (41–449)	94	2 (0–4)	99
AH-2 Isolate (6)	5,000 ± 176	3,881 (3,268–4,166)	746 (473–1,127)	81	127 (0–737)	97
T. axei (12)	20,000 ± 930	12,622 (10,540–14,220)	0	100	63 (0–680)	99
T. colubriformis (12)	20,000 ± 844	17,612 (14,720–19,680)	3 (0–40)	99	2,153 (380–7,380)	88

[1] Confidence Interval = SE × t (2.26 @ 9 degrees of freedom).
[2] Range.

nants. There are no published reports on the action of these newer anthelmintics against drug-resistant nematodes. Hence, trials were designed to compare the activity of cambendazole and morantel tartrate against two thiabendazole-resistant isolates of *H. contortus* (Colglazier et al., 1970), as well as against two species of *Trichostrongylus*, in experimentally infected lambs. Similar trials comparing the activities of levamisole, pyrantel tartrate, and rafoxanide against these parasites were reported previously (Colglazier, Kates, and Enzie, 1971a).

Materials and Methods

EXPERIMENTAL ANIMALS: The 36 Polled Dorset lambs used in these trials were raised parasite-free except for minimal infections of *Strongyloides papillosus* and coccidia. The lambs, 5 to 6 months of age, were randomly allotted to six groups of six lambs each; and each group was maintained during the 4-week experimental period in a separate concrete-floored pen that was cleaned daily. The lambs were fed alfalfa hay and grain sufficient to maintain a moderate rate of growth.

LARVAL INOCULATION AND TREATMENT PROCEDURES: Each of 18 lambs (three groups of six lambs) was given orally 5,000 infective larvae of the BPL-2 isolate of *H. contortus* and 20,000 infective larvae of both *T. axei* and *T. colubriformis*, a total of 45,000 larvae per lamb. The other 18 lambs were similarly infected with the AH-2 isolate of *H. contortus* and the two species of *Trichostrongylus* (Table 1). Before infecting the lambs, freshly harvested larvae were quantitated as previously described (Colglazier, Kates, and Enzie, 1969). Appropriate quantities of larval suspensions for each of the three nematode species were combined and given as a single dose to each lamb. The origin of the *H. contortus* isolates was previously reported by Colglazier et al. (1970). The *Trichostrongylus* larvae (bovine origin) were supplied by Dr. H. Herlich of this laboratory.

The lambs in two subgroups infected with each *H. contortus* isolate were dosed with one or the other of the test drugs; lambs in the third subgroup served as unmedicated controls. Because all lambs were given equivalent numbers of the two *Trichostrongylus* species, the control and medicated groups infected with these parasites consisted, in effect, of 12 lambs each, whereas groups involving the two *H. contortus* isolates contained six lambs each (Table 1).

Single therapeutic doses of cambendazole (20 mg/kg) and morantel tartrate (8 mg/kg) were given to the lambs 21 days postinfection. All lambs were necropsied for residual worm counts 6 to 8 days later, using standard techniques previously described (Colglazier et al., 1969). The data on anthelmintic efficacy were subjected to analysis of variance.

ANTHELMINTICS USED: [1]Cambendazole: 2-

[1] 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.

(4-thiazolyl)-5-isopropoxycarbonylaminobenzimidazole; 7.58% suspension (MK-905) for experimental use; Merck & Co., Rahway, New Jersey.

Morantel tartrate: trans-2[2-(3-methyl-2-thienyl) vinyl]-1-methyl-1,4,5,6-tetrahydropyrimidine tartrate; pure chemical for experimental use; Charles Pfizer & Co., Inc., Groton, Connecticut.

Results and Discussion

The results are given in Table 1. The data indicate certain differences in the anthelmintic effect of cambendazole and morantel tartrate against one of the two *Trichostrongylus* species as well as against the two isolates of *H. contortus*.

Necropsy worm counts of the unmedicated control lambs show that, with one exception, substantial numbers of all three parasite species were established in the test lambs. One control lamb in the BPL-2 group harbored only 21 mature *H. contortus* at necropsy, although the animal showed signs of anemia. It is possible, therefore, that the major portion of the *H. contortus* worm burden had been eliminated spontaneously shortly before necropsy.

In 1970, Colglazier et al. reported that two Maryland isolates of *H. contortus* were resistant to thiabendazole when the drug was given to sheep at the dose level of 50 mg/kg of body weight. A single dose of thiabendazole was only 39 and 67% effective against the two isolates, designated AH-2 and BPL-2, respectively. In trials reported here with the chemically related compound, cambendazole, the drug was 81% effective against the AH-2 isolate of *H. contortus* when given at a dose rate of 20 mg/kg of body weight. This moderate efficacy was noteworthy in view of the reports by other researchers that dosages of 22 mg/kg, or less, were 99 to 100% effective against *Haemonchus* infections in sheep (Egerton and Campbell, 1970) and in cattle (Benz, 1971a; Ciordia and McCampbell, 1971; Egerton et al., 1970). Benz (1971b) also reported similar high efficacy with single treatments of cambendazole (22 to 35.8 mg/kg) against *H. placei* in cattle. The BPL-2 isolate of *H. contortus*, which was less resistant than the AH-2 isolate to thiabendazole, was also less resistant to cambendazole; an efficacy of 94% was achieved against this isolate in sheep that were given the drug at the 20-mg/kg dose level. Excluding data from the aforementioned BPL-2 control lamb with the low *H. contortus* necropsy worm count (21), statistical analysis indicated that the AH-2 isolate was significantly ($P < 0.05$) more resistant to cambendazole than was the BPL-2 isolate. When the worm count data from this one control lamb are included in the calculations, however, the difference in efficacy between the two isolates was not significant at the 5% level. Nevertheless, our results with cambendazole, together with data previously reported for thiabendazole (Colglazier et al., 1970), indicated that cambendazole at 20 mg/kg was much more effective against both isolates of *H. contortus* than was thiabendazole at the 50-mg/kg dose level. Thus, our findings tend to support the observations of Hoff et al. (1970), who assayed drug activity with a laboratory animal model, that the anthelmintic potency of cambendazole was about 6 times that of thiabendazole.

Morantel tartrate, given at a dose rate of 8 mg/kg of body weight, was 99 and 97% effective against the BPL-2 and AH-2 isolates of AH-2 isolates of *H. contortus*, respectively. The efficacy data for the individual isolates were highly significant ($P < 0.01$); differences between isolates, however, were not significant ($P < 0.05$). The anthelmintic action of morantel tartrate, a 3-methyl analogue of pyrantel tartrate (Austin et al., 1966), compared favorably with that of levamisole, pyrantel tartrate, and rafoxanide (Colglazier et al., 1971a) against these two thiabendazole-resistant isolates of *H. contortus*. The results were comparable also to those of Cornwell and Jones (1970c), who reported complete removal of *H. contortus* from sheep with dosages ranging from 6 to 25 mg/kg of body weight.

The AH-2 and BPL-2 isolates of *H. contortus*, which were resistant to thiabendazole (Colglazier et al., 1970), were removed more effectively with morantel tartrate than with cambendazole ($P < 0.05$). However, the difference in efficacy between morantel tartrate and cambendazole at the dose levels used was greater for th AH-2 isolate (97 vs 81%) than for the BPL-2 isolate (94 vs 99%); both differences were significant at $P < 0.05$.

Evidence is accumulating that certain strains or isolates of parasitic nematodes are cross-resistant to various benzimidazole anthelmintics. Theodorides et al. (1970) reported that six thiabendazole-resistant strains of *H. contortus* (five from sheep and one from goats) were all cross-resistant to parbendazole at dose levels of 20 and 40 mg/kg. Moreover, Kates et al. (1971) also reported that a thiabendazole-resistant population of *H. contortus* in sheep was resistant to parbendazole. In Australia, Hotson et al. (1970) reported on two strains of *T. colubriformis* that were resistant to thiabendazole at the 50-mg/kg dose level and were effectively removed from sheep only after a threefold increase in dose rate. Subsequently, one of the strains was shown to be almost completely refractory to parbendazole at the 20-mg/kg dose level. This is the only instance, to our knowledge, in which drug resistance has been demonstrated in ruminant helminths other than *H. contortus* following previous exposure to an anthelmintic.

Data obtained in our trials with one of the local thiabendazole-resistant isolates of *H. contortus* (AH-2) suggest that the isolate may be resistant as well to cambendazole. Fortunately, the benzimidazole-resistant nematodes seem to be responsive to the nonbenzimidazole anthelmintics. Hotson et al. (1970) showed that their thiabendazole- and parbendazole-resistant strain of *T. colubriformis* was sensitive to therapeutic doses of *dl*-tetramisole and pyrantel tartrate. This is consonant with data presented here for morantel tartrate, as well as with our earlier findings (Colglazier et al., 1971a), showing that benzimidazole-resistant *H. contortus* in sheep were effectively removed with such other nonbenzimidazole anthelmintics as pyrantel, levamisole, and rafoxanide.

In the trials reported here, cambendazole given at 20 mg/kg was very effective (99 to 100%) against both *T. axei* and *T. colubriformis*. These results are consistent with those obtained by Egerton and Campbell (1970) against *Trichostrongylus* spp. in sheep, and by Baker and Walters (1971), Benz (1971a, b), Ciordia and McCampbell (1971), and Egerton et al. (1970) against *Trichostrongylus* spp. in cattle. We previously reported that the two related benzimidazole anthelmintics, thiabendazole (Colglazier et al., 1969b) and parbendazole (Colglazier et al., 1971b), also were highly effective against *T. axei* and *T. colubriformis*.

Morantel tartrate given at 8 mg/kg in the present trials was very effective against *T. axei* (99%), but significantly ($P < 0.05$) less effective (88%) against *T. colubriformis*. Cornwell and Jones (1970c) reported 99 to 100% efficacy against *T. axei* with dosages ranging from 6 to 25 mg/kg, and 94 per cent removal of 21-day-old *T. colubriformis* with a dose of 10 mg/kg. Against the latter species, therefore, the optimum dosage of morantel tartrate is probably somewhat larger than 8 to 10 mg/kg of body weight.

Acknowledgment

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Monogenea from Red Sea Fishes. I. Monogenea of Fish of the Genus *Siganus*

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ABSTRACT: Two new species of dactylogyrid monogenea are described from fish of the genus *Siganus*: *Pseudohaliotremotoides polymorphus* sp. n., with three subspecies: *P. polymorphurs elaticus* ssp. n. from S. *luridus* from the Gulf of Eilat (Aqaba), *P. polymorphus suezicus* ssp. n. from S. *rivulatus* from the Gulf of Suez, and *P. polymorphus indicus* ssp. n. from *Siganus* sp. off Mombasa. *Pseudohaliotrema plectocirra* sp. n. from S. *luridus* and S. *rivulatus* from Eilat and Suez gulfs. The infraspecific variability of these species as well as their taxonomical affinities are discussed.

A study of the monogenea infecting fish of the Red Sea, sponsored by "The Fauna Palaestina Committee" of the Israel Academy of Sciences and Humanities, has been carried out since 1969. This communication presents the first results of this study; it is the second report on monogenea from Red Sea fish. In the first report (Paperna, 1965) five new species were described from seven species of fish from the coral reef of Eilat. The genus *Siganus* is represented in the northern Red Sea by four species: S. *rivulatus* (Fôrskal), S. *luridus* (Rüppel), S. *rostratus* (Valenciennes), and S. *stellatus* (Fôrskal). The first two species entered the Mediterranean through the Suez Canal (Ben Tuvia, 1966) and are now common along the Mediterranean shores of Israel.

Sites of Collection and Methods

Fish were collected from the northern sandy shores of the Gulf of Eilat (Aqaba), from rocky shores and coral reefs in the northwest portion of the Gulf, in the Gulf of Suez near Ras abu-Rudeis (33°10′ east, 28°57′ north) (rocks and reefs), and in El-Blaim lagoon (33°16′ east, 28°33′ north) (sandy shores). The collected fish were killed in 2 to 4% formaldehyde, in separate jars for each species and site. Parasites were collected from the fixed gills and embedded either in glycerin gelatin or polyvinyl–lactophenol with added traces of cotton blue stain. Measurements were taken from the specimens embedded in glycerin gelatin. For the measurement method see Paperna (1959), as well as Bykhovskaya-Pavlovskaya et al. (1962). Measurements are given in microns.

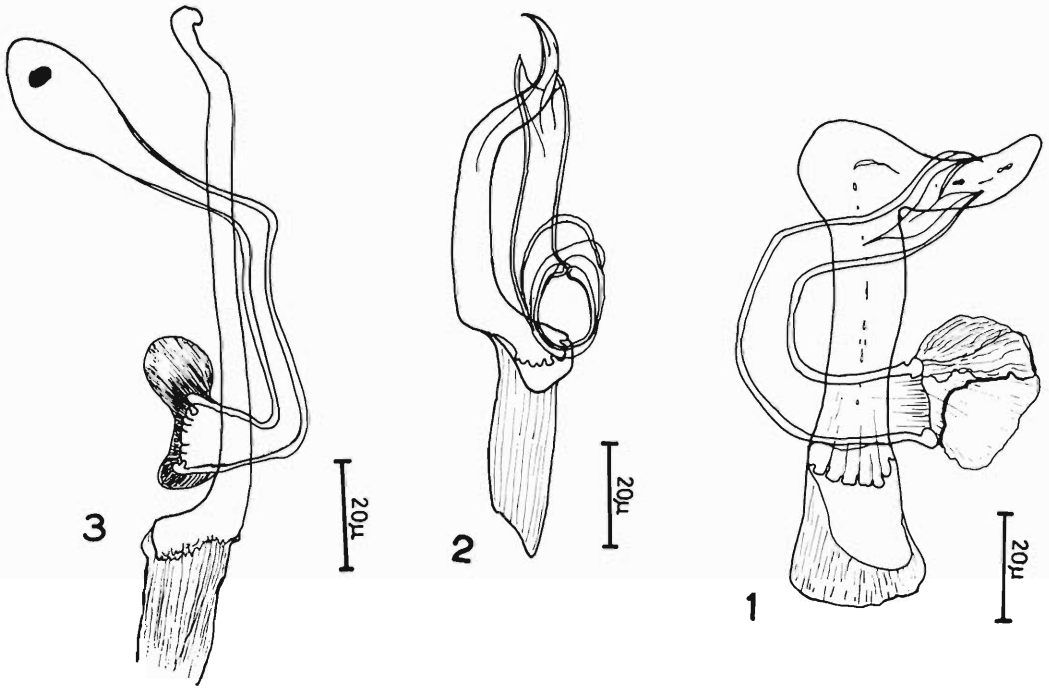
* The field work was carried out in the Marine Biological Laboratory, Eilat.

Results

Out of the three fish species studied in the Gulf of Eilat monogenea were found only in S. *luridus* (checked two) and S. *rivulatus* (checked 20). All the three checked S. *rostratus* were free from monogenea. In the Gulf of Suez only S. *rivulatus* were collected and were also found to be infected with monogenea. Additional material from the Mombasa Coast (from unidentified *Siganus*) was kindly provided by Dr. J. P. Thurston.

Pseudohaliotrematodides polymorphus sp. n.

DESCRIPTION: Medium-sized to large worms with a small opisthaptor embedded in the posterior end of the body. In the prohaptor two pairs of head organs and an additional pair of glands lie posterolateral to the oval pharynx. Eyes were observed in some specimens but not in others. The intestinal limbs originate from the pharynx without a distinct esophagus and join posteriorly. A single testis is located in a position posterior or latero-posterior to the ovary. The vas deferens winds anteriorly to the copulatory organ without looping around the intestine. A spindle-shaped seminal vesicle is located alongside the copulatory organ; prostate glands were seen in some specimens but were absent from the syntypes. The copulatory organ consists of a heavy tubiform cirrus and one solid accessory piece; attached to the piece is a basal "root." The rounded ear-shaped funnel is nonsclerotinoid. The copulatory organ sits in a muscular pouch.



Abbreviations to figures: AP—Accessory piece; C—Cirrus; CG—Cerebral ganglion; E—Intrauterine egg; Pg—Pharyngeal glands; P—Prostata; SV—Seminal vesicle; V—Vagina; G—glands, possibly prostata.

Figure 1. Copulatory organ of *Pseudohaliotrematoides polymorphus eilaticus* sp. n. ssp. n.
Figure 2. Copulatory organ of *P.p. suezicus* ssp. n.
Figure 3. Copulatory organ of *P.p. indicus* ssp. n.

The vagina opens on the right side of the body through a muscular sphincter. In the posterior end of the body large cement glands open into the opisthaptor. In the opisthaptor there are two pairs of anchors and two bars; hooklets are absent in mature specimens, but are present in very young ones. In one pair of anchors (the dorsal) the inner root is very short. The outer root and both roots in the second pair are long. The shafts of both anchors are short, and the tips are distinct in having their basal end thinner than the distal end of the shaft. The bars are thin and small. The species is very polymorphic; there is distinct infraspecific variability of populations collected from different regions. The different populations vary particularly in the shape of the copulatory organ, as well as in body size and the size of the anchors. Prostate glands present in some populations but absent in others.

Three subspecies can be differentiated morphologically:

P. polymorphus eilaticus ssp. n. (syntype of *P. polymorphus* sp. n.)

Medium size to large (610–1,200 long); cirrus robust, the accessory piece thick, with a short "root." Prostate glands not seen.

HOSTS AND LOCALITIES: *Siganus luridus* (150–200 mm long); northwestern Gulf of Eilat (Taba and Coral Beach).

P. polymorphus suezicus ssp. n.

Large worms (1,050–1,320 long). Cirrus short, accessory piece not robust and longer than the cirrus, accessory piece "root" long. Prostate glands present.

HOSTS AND LOCALITIES: *Siganus rivulatus* (90–100 mm long); Gulf of Suez near Ras abu-Rudeis.

Table 1. Measurements of the different populations and subspecies of *Pseudohaliotrematoides polymorphus* sp. n.

Subspecies	*P. p. elaticus*		*P. p. suezicus*		*P. p. indicus*
Host:	*Siganus luridus*				*Siganus* sp.
Population: (No. specimens examined)	Fish I (3)	Fish II (5)	Fish group I, II (5)	Fish group III (1)	Mombasa (2)
Total length	610–870	920–1,200	1,240–1,320	1,050	1,690
Width	280–410	390–450	340–380	180	550
Opisthaptor length	40–60	60–80	100–150	50	80
Opisthaptor width	70–120	100–190	100–190	60	100
Ventral anchors	50–55	67–80	67–87	75	95–120
Inner root	20–22	25–37	37–40	30	80
Outer root	20–22	20–35	30–30	20	64
Shaft	19–22	17–27	20–25	30	29
Tip	20–25	20–22	17–27	22	30
Bar	35–36	33–42	–	–	43
Dorsal anchor	60–60	65–75	70–75	67–75	85–120
Inner root	10–12	12–17	12–15	10–12	27
Outer root	22–25	35–35	35–37	22–27	74
Shaft	20–25	22–28	15–17	22–33	29
Tip	12–17	15–22	23–25	20–20	30
Bar	30	30–35	–	–	38
Cirrus	60–77	75–90	45–62		112–120
Width at funnel level	15–15	12–17	12–15		9–12
Width at midlevel	9–10	6–10	6–9		5–9
Accessory piece length	65–77	70–90	67–85		95–102
Accessory piece width	10–15	10–15	5–5		3–6
"–root–"	–	21–30	30–42		22–27
Ratio cirrus/Accessory piece	0.9–1.04	0.83–1.08	0.64–0.73		1.17–1.18
Mean	0.97	0.99	0.69		1.175

P. polymorphus indicus ssp. n.

Very large worms (1,690 long). Cirrus and accessory piece slender and long, cirrus terminating in a rounded widening. Prostate glands not seen.

HOST AND LOCALITY: *Siganus* sp.; Mombasa Coast, Indian Ocean (East Africa). Specimens were collected by Dr. J. P. Thurston.

MEASUREMENTS: Measurements of the specimens of the three subspecies are compared in Table 1.

REMARKS: *P. polymorphus* sp. n. differs from *P. fusiforme* (Yamaguti, 1953) mainly in the structure of the accessory piece of the copulatory organ.

P. chaetopteri (Caballero et Bravo-Hollis, 1960), as well as additional six new species assigned recently by Yamaguti (1968) to this genus and parasitizing fish of genera other than *Siganus*, are fundamentally different from *P. polymorphus* and *P. fusiforme* in the structure of the anchors and the bars, the pattern of the copulatory organ, and the position of the vaginal pore. The reduced inner root is characteristic of both *P. polymorphus* and *P. fusiforme* and is apparently of generic significance. A copulatory organ similar to that found in *P. polymorphus* and *P. fuisforme* is found in *Pseudohalitrema sigani* Yamaguti, 1953, which also parasitizes siganid fish. The latter, however, differs from these species in the structure of the anchors and the presence of hooklets, as well as other anatomical details characteristic of the genus.

Young (1967) has recently described two new species from siganid fish, which he assigned to the genus *Tetrancistrum* Goto and Kikuchi, 1917 (*T. nebulosi* and *T. oraminii*). Another species of similar morphology which was included in this genus (*T. nasonis*) was obtained from acanthurid host fish. From acanthurid fish (*Naso* spp.) Yamaguti (1968) described four more new species similar in morphology to the above-mentioned species but which he included in a new genus: *Pseudoancyrocephalus* Yamaguti, 1968. All these species, as well as the type species *T. sigani*, show close morphological affinities with *P. fusiforme* and *P. polymorphus*, all having a reduced inner root in the dorsal anchor. In fact, *P. fusiforme* was transferred by Young to the genus *Tetrancistrum*. On the other hand, the remaining species listed by Yamaguti (1963) in this genus are distinctly different from the type species as well as from the above-mentioned new species. *T. oraminii* is the

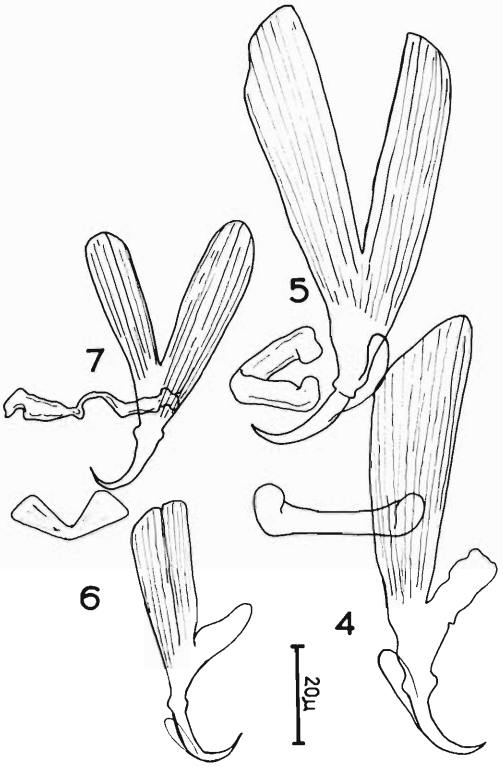


Figure 4. Dorsal anchor and bar of ***P.p. indicus.***
Figure 5. Ventral anchor and bar of ***P.p. indicus.***
Figure 6. Dorsal anchor and bar of ***P.p. eilaticus.***
Figure 7. Ventral anchor and bar of ***P.p. eilaticus.***

closest species to *P. polymorphus*, not only in the pattern of the anchors and other anatomical details, but also in the pattern of the accessory piece. However, mature specimens of *P. fusiforme*, *P. polymorphus*, and also *T. oraminii* lack hooklets, which occur in the type species of *Tetrancistrum* and in the remaining species. The type *T. sigani* as well as *T. nebulosi* are peculiar in having a diverticulate intestine. In view of these differences it seems most reasonable to retain *P. fusiforme* and *P. polymorphus* in the genus *Pseudohaliotrematoides* separate from those species included in *Tetrancistrum*. *T. oraminii*, which is allied to *P. polymorphus*, should be transferred to *Pseudohaliotrematoides*.

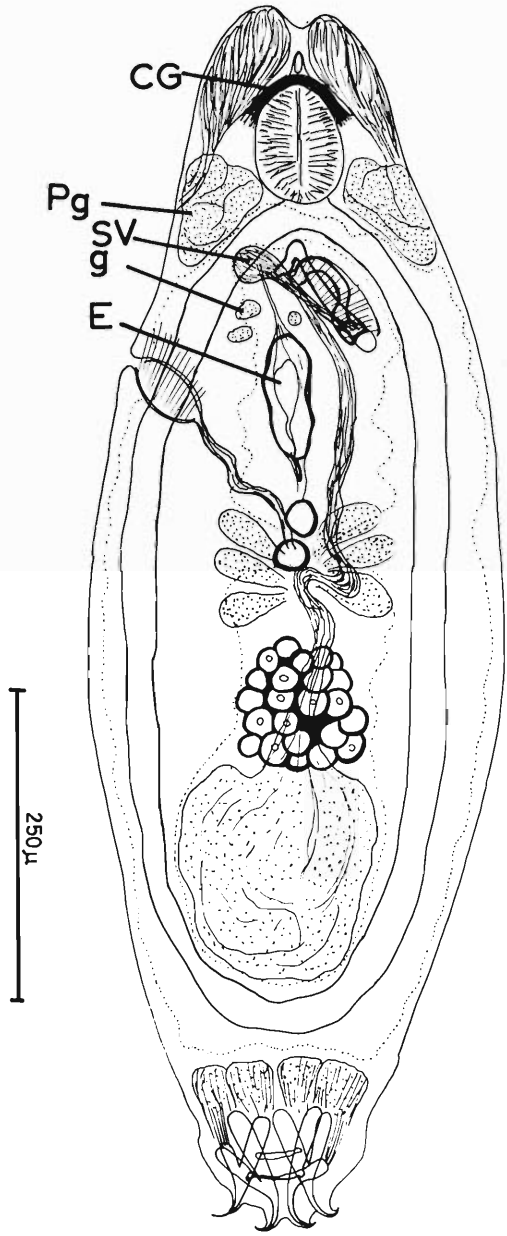


Figure 8. General view of ***P.p. eilaticus.***

Pseudohaliotrema plectocirra sp. n.

DESCRIPTION: Occurs in two forms, large elongated or small robust worms. Head organs are arranged in three or four pairs, eyes are

absent, the pharynx is oval. The alimentary canal originates from a distinct esophagus; the intestinal limbs join posteriorly. The vas deferens winds directly to the copulatory organ, without looping around the intestinal limbs. A seminal vesicle is present; prostatic glands are present in numerous follicles. The cirrus and the accessory piece are twisted and coiled around themselves as well as around each other, thus assuming extremely variable appearances in the different specimens. The vagina is highly coiled and opens on the right side of the body through a hard-walled pore. The opisthaptor is distinctly delineated from the entire body of the worm.

In the opisthaptors a dorsoventral constriction separates the disk into two lobes in each of which are located one anchor of each pair. The anchors are polymorphic, with the roots either widely separated or partly fused. The bars are narrow and delicate, the hooklets small, with relatively large blades and short handles.

The sclerotinoid elements (anchors, bars, and copulatory organ) were usually of similar size in both the small and the large specimens (Table 2).

MEASUREMENTS: Total body length 350–1,100, width 130–390, copulatory organ long axis 35–42, ventral anchors length 40–53, inner roots 12–22, outer roots 7–20, shaft 25–30, tip 15–17, ventral bar 22–25, dorsal anchor length 42–57, inner root 17–30, outer root 10–22, shaft 17–35, tip 12–20, dorsal bar 25–27, hooklets 10–17.

HOSTS AND LOCALITIES: *Siganus luridus*; northwestern Gulf of Eilat (Taba and Coral Beach). *S. rivulatus*; northwestern Gulf of Eilat (Taba and Coral Beach) and in the Gulf of Suez near Ras abu-Rudeis.

The larger specimens were found exclusively on *S. rivulatus* in both Eilat and Suez gulfs; the smaller specimens were found on *S. luridus* in the Gulf of Eilat, as well as on *S. rivulatus* in the Gulf of Suez.

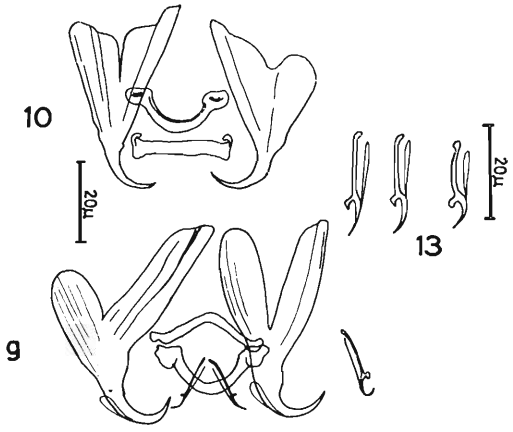


Figure 9. Anchors and bars of *Pseudohaliotrema plectocirra* sp. n. specimen with long roots.
Figure 10. Anchors and bar of *P. plectocirra*, specimen with joint roots.
Figure 13. Hooklets of *P. plectocirra*.

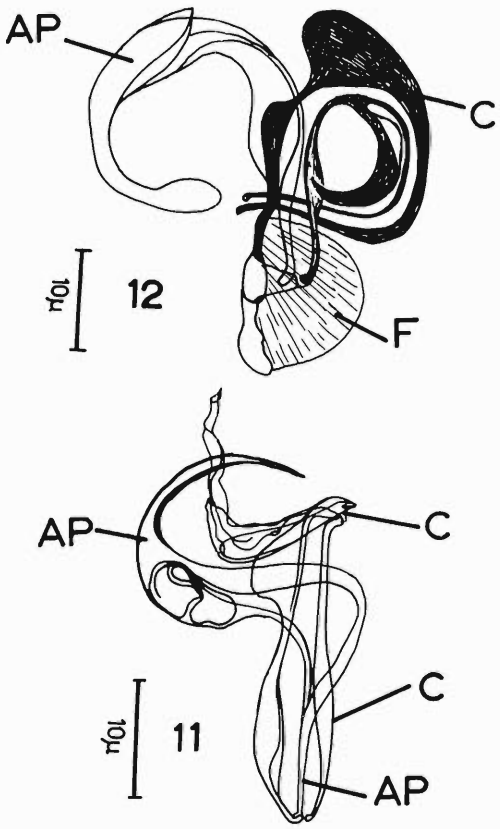
REMARKS: The described specimens differ from other species of the genus *Pseudohalitrema* in the structure of the copulatory organ.

Discussion

None of the species described earlier from fish of the genus *Siganus* (from Japan and Southeast Asia, Goto and Kikutchi, 1917; Yamaguti, 1953; Table 3) were found on the fish currently studied from the Red Sea zone; thus, fish of these two Indopacific zones have distinct populations of monogeneans. However, the parasites found in the two zones still belong to the same genera, i.e., *Pseudohaliotrema*, Yamaguti, 1953, and *Pseudohaliotrematoides* (Yamaguti, 1953), two closely related genera,

Table 2. Infraspecific variability in relation to host and locality.

Host Species:	*S. rivulatus*			*S. luridus*	
Size:	150–200 mm	200 mm	90–100 mm	200 mm	200 mm
Locality:	Taba (Eilat)	Suez	Suez	Taba (Eilat)	Coral Beach (Eilat)
Parasite:					
Length	700–1,100	570–670	780–830	420–500	350–500
Width	240–390	130–200	230–300	140–270	280–350
Ventral anchors	45–53			40–50	42–45
Dorsal anchors	42–57			42–45	45–47
No. measured	10	3	4	5	9



Figures 11 and 12. Copulatory organs of *P. plectocirra*.

which are apparently specific to siganid fish. Yamaguti (1963, 1968) also included in these genera species found on fish other than *Siganus*. However, these species differ fundamentally in their morphology from all the species included in these genera; therefore their present taxonomical status should be reviewed. Distinct infraspecific variability in *P. polymorphus* is associated with fish populations of different geographical zones, i.e., the Gulf of Eilat, the Gulf of Suez, and the East African coast of the Indian Ocean.

This monogenean could therefore be used as a "biological tag" to study the interrelation

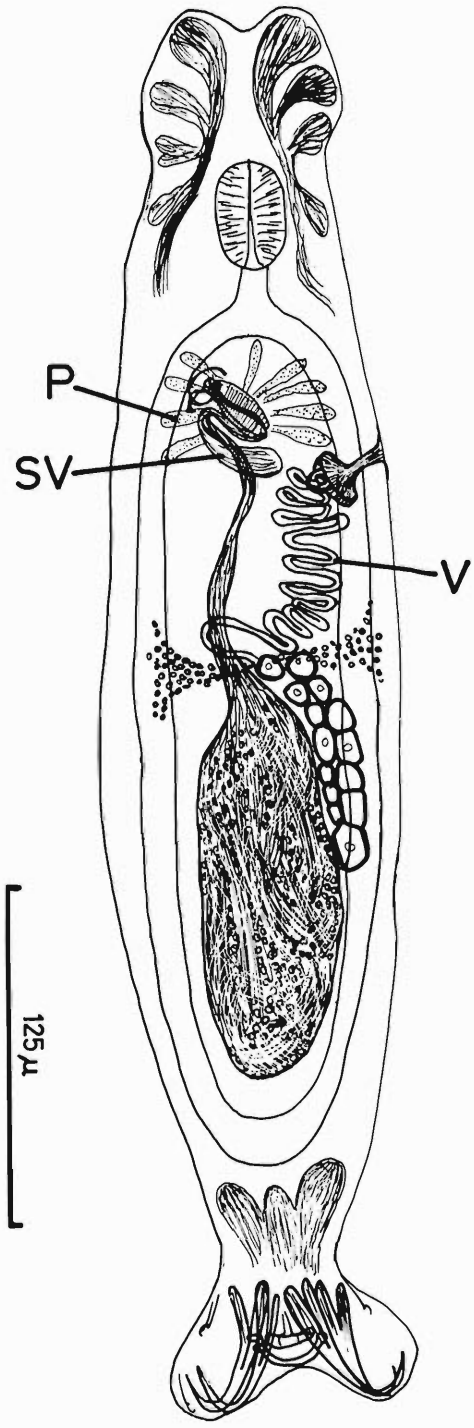


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Figure 14. *P. plectocirra*, general view.

Table 3. Host–parasite interrelations between *Siganus* spp. and Monogenea.

Fish species	Locality	No. studied	Monogenean spp.	No. specimens collected
S. *luridus*	NW Eilat Gulf	2	*P. polymorphus eilaticus*	25
S. *rivulatus*	NW Eilat Gulf		*P. plectocirra* (small)	107
S. *rivulatus*	Suez Gulf	12	*P. plectocirra* (large)	42
		5	*P. polymorphus suezicus*	6
			P. plectocirra (large)	20
			P. plectocirra (small)	7
S. *rivulatus* (juv.)	El Blaim lagoon (Suez)	3	—	0
S. *rostratus*	NW Eilat Gulf	3	—	0
S. sp.	Mombasa (Indian Ocean)	(coll. J. P. Thurston)		
			P. polymorphus indicus	2

between populations of *Siganus* in the different sectors of the Red Sea and possibly also the Indian Ocean. The variability found in the other species, *P. plectocirra*, is associated with the different species of *Siganus* rather than with geographical divisions.

The author wishes to thank Dr. George Kissel for his help in identification of the fish. Thanks are extended also to Miss M. McComb for her help in the preparation of the manuscript.

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Monogenea from Red Sea Fishes. II. Monogenea of Mullidae

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ABSTRACT: In the course of an investigation of ectoparasitic infection of fish of the family Mullidae (goat fish) from the Gulf of Eilat five species of monogenea were found: *Haliotrema alata* Yamaguti, 1942; *Haliotrema australe* Johnston and Tiegs, 1922; *Haliotrema curvipenis* sp. n.; *Haliotrema recurvata* Yamaguti, 1942; and *Gyrodactylus* sp. 1. Infection was heaviest in fish of the genus *Pseudupeneus*, while being mild or sporadic in *Upeneus* and *Mulloidichthys*. In *Upeneus* only *Gyrodactylus* were found.

Mullidae, or goat fish, are found in both the Indopacific zone and the Atlantic zone. In the Red Sea area representatives of the three genera—*Upeneus* Cuvier, *Pseudupeneus* Bleeker, and *Mulloidichtys* Whitley—are found and have been investigated in this study. Monogenea have been found on goat fish in Okinawa, Indonesia, Australia, and Hawaii

* The field work was carried out in the Marine Biological Station, Eilat.

(Johnston and Tiegs, 1922; Yamaguti, 1942, 1953, 1968). The present study of Red Sea fish ectoparasites was sponsored by the Fauna Palaestinae Committee of the Israel Academy of Sciences and Humanities.

Collection Sites and Methods

Goat fish were obtained only from the Gulf of Eilat (Aqaba). From the sandy littoral in the northern Gulf fish were obtained by seine net. From the reef areas in the northwest they were caught in traps. The methods of collecting, mounting, and measuring the monogeneans are described elsewhere (Paperna, 1965, 1972). Measurements are recorded in microns unless otherwise stated.

Taxonomical Account

GENUS: *Haliotrema* Johnston and Tiegs, 1922.

Haliotrema alata Yamaguti, 1942

MEASUREMENTS (from 6 specimens): Total length 520–630, width 150–160, ventral anchors 50–58, inner root 22–33, outer root 10–12, shaft 35–40, tip 10–11, bar 53–65, dorsal anchors 55–67, inner root 28–32, outer root 11–15, shaft 35–43, tip 8–15, bar 46–48, hooklets 10–14, copulatory organ 67–72.

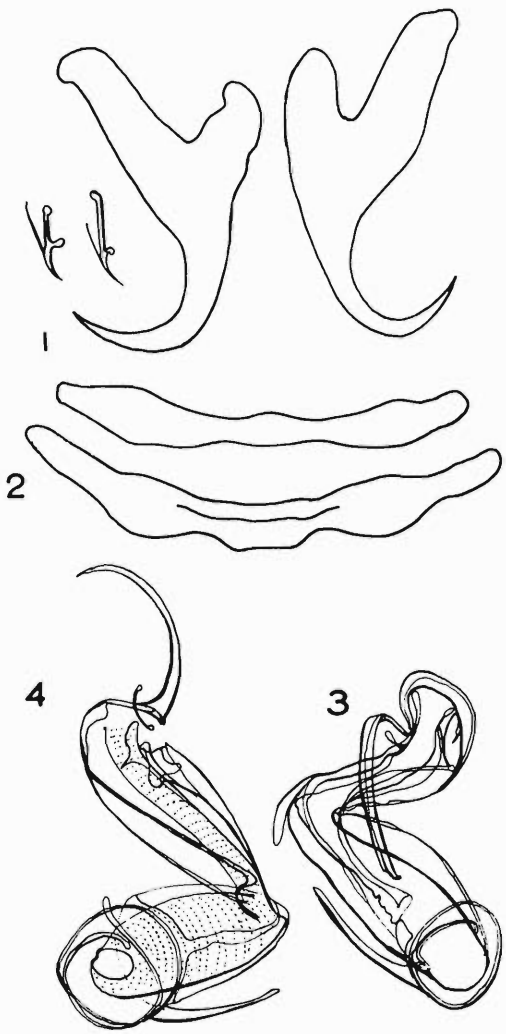
HOSTS AND LOCALITIES: *Pseudupeneus barberinus* (Lacepede); northern and southwestern Gulf of Eilat (in the vicinity of Taba).

REMARKS: Described earlier from Naha (Okinawa) from *Parupeneus chrysedros* and *P. multifasciata* (Yamaguti, 1942) and also from "some mullids" from Macassar (Yamaguti, 1953).

Haliotrema australe Johnston and Tiegs, 1922

MEASUREMENTS (from 14 specimens): Total length 312–624, width 52–208, ventral anchors 37–57, inner root 15–22, outer root 5–12, shaft 30–40, tip 10–15, bar 47–66, dorsal anchor 37–60, inner root 20–33, outer root 5–10, shaft 27–40, tip 10–15, bar 36–47, hooklets 7–11, copulatory organ 41–67.

MORPHOLOGICAL VARIABILITY: Morphological variability in the shape of the bars and the copulatory organ was observed among the examined specimens. This variability was observed even among specimens collected from



Figures 1–4. ***Haliotrema alata,*** **1. Anchors and hooklets. 2. Bars. 3–4. Copulatory organs of two specimens.**

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Figures 5–15. ***Haliotrema australe.*** **5. Whole worm, ventral view. 6–9. Copulatory organ of different specimens. 10–13, 15. Anchors and bars of different specimens. 14. Hooklets. CG: Cephalic glands. PG: Prostatic glands. PR: Prostatic reservoir. SV: Seminal vesicle.**

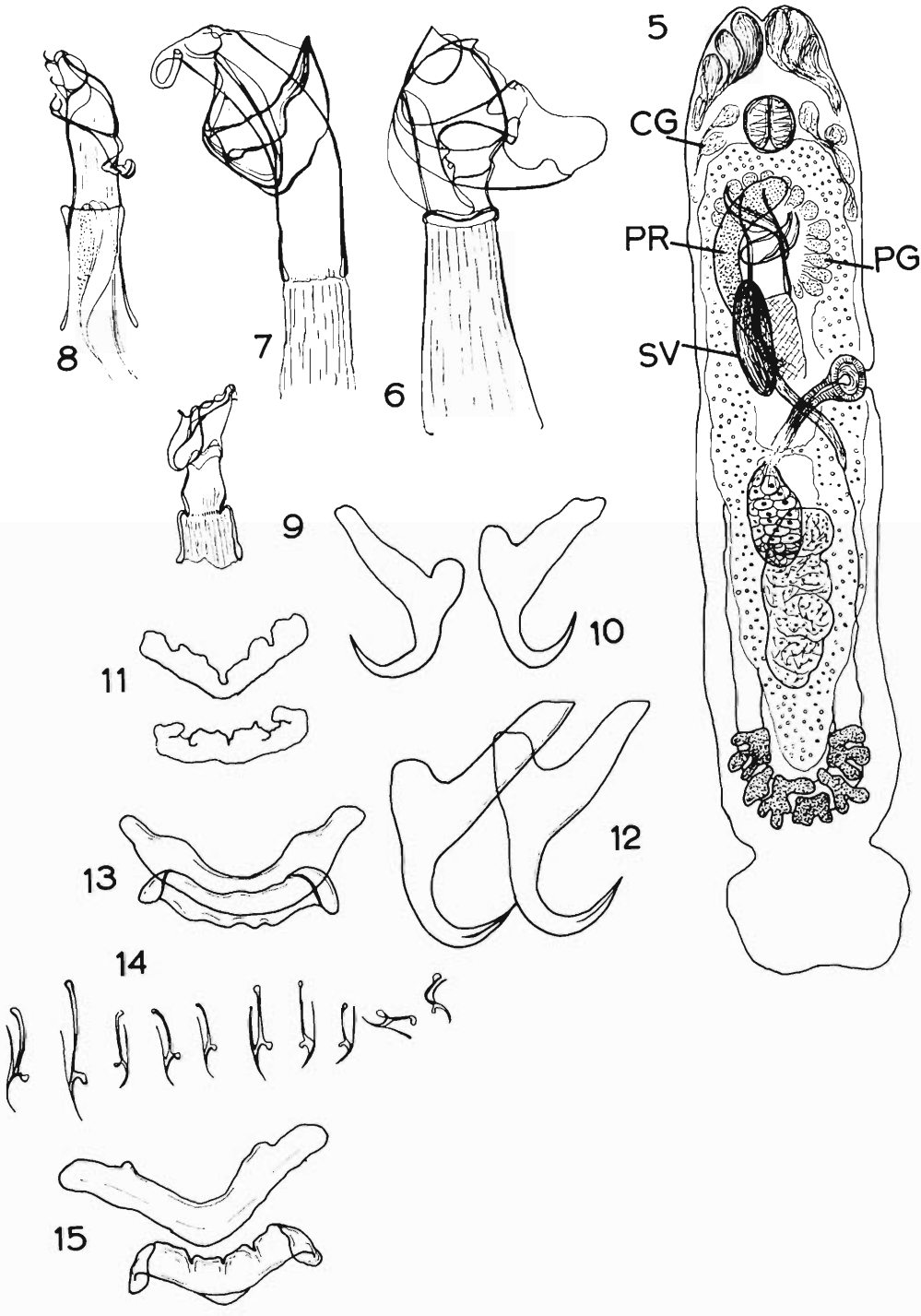


Table 1. Host–parasite relationships between goat fish and *Gerres* sp. and their specific monogeneans.

Fish species:	No. of parasites recovered						
	H. australe	*H. recurvata*	*H. alata*	*H. curvipenis*	*Gyrodactylus* sp. 1	No. of fish	Site
Pseudupeneus:							
P. barberinus	22	12	3	0	0	5	Eilat—North
"	34	10	1	0	0	6	Eilat—NW
P. pleurospilos	5	12	0	0	0	1	Eilat—North
P. cyclostomata	12	0	0	0	0	1	Eilat—North
"	4	0	0	1	0	1	Eilat—Aquarium
P. macronema	7	2	0	0	0	1	Eilat—Aquarium
Upeneus:							
U. tragula	0	0	0	0	4	10	Eilat—North
Mulloidychthys:							
M. auriflamma	0	0	0	20	0	10	Eilat—North
Gerres sp.	4	16	0	0	0	6	Eilat N—NW

the same host. To a certain extent this variability, particularly that of the copulatory organ, is due to an artificial distortion of the delicate sclerotinoid lamellae and vanes during the process of embedding.

HOSTS AND LOCALITIES: *Pseudupeneus barberinus*; *P. pleurospilos* (Bleerker); *P. cyclostomus* (Lacepede); *P. macronema* (Lacepede) and also *Gerres* sp. (Gerridae); northwestern Gulf of Eilat (in the Eilat port area).

REMARKS: Type was described from *Upeneus signatus* Günther, southeast Queensland, Australia.

Haliotrema curvipenis sp. n.

DESCRIPTION (from 7 specimens): Medium to large worms with the characters of the genus. In the prohaptor, posterior to the head organs and lateral to the pharynx, there are two large follicles of adhesive glands (cephalic glands). There are two eyes, but in some specimens they are absent. The copulatory organ consists of an elongated tubiform cirrus, originating from a funnel with a heavily sclerotized rim. The accessory piece is a flat plate partly enveloping the cirrus. A seminal vesicle is present; there is one large prostate reservoir and numerous smaller prostate glands. The vagina opens on the right side of the body through a muscular bulb. The opisthaptor is relatively small, the anchors have large roots and slender tips, and both bars are V-shaped.

MEASUREMENTS: Total length 470–832, width 260–312, ventral anchors 56–65, inner root 30–35, outer root 16–20, shaft 30–40, tip 10–17, bar 40–50, dorsal anchors 62–70, inner root 32–45, outer root 12–22, shaft 33–37, tip 10–15, bar 47–55, hooklets 15–19, cirrus 45–65, funnel length 12–23, funnel width 8–8, intrauterine egg 90 × 60.

HOST AND LOCALITIES: *Mulloidichthys auriflamma* (Fôrskal); northern Gulf of Eilat; *Pseudupeneus cyclostomus* (one specimen); northwestern Gulf of Eilat; this specimen was kept for a few weeks in the aquarium of the Marine Biological Station in Eilat.

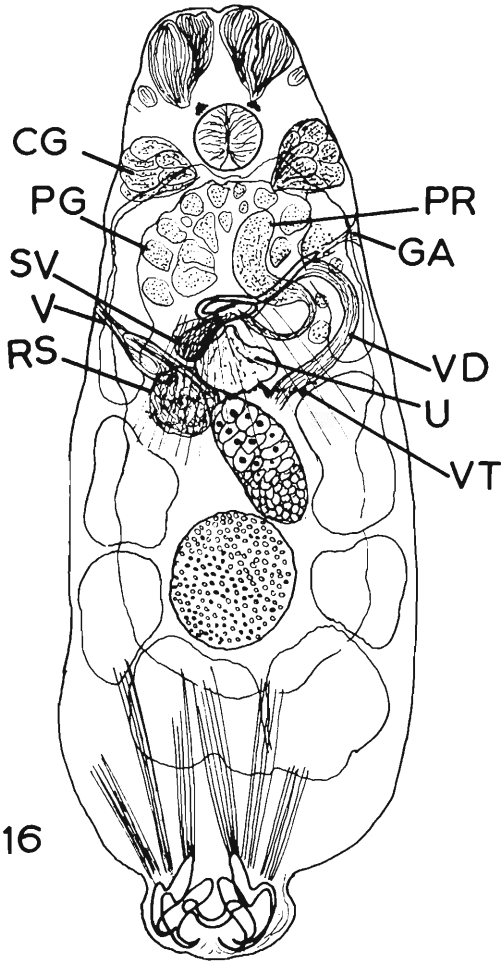
REMARKS: Differs from other species of *Haliotrema* in the structure of the copulatory organ, anchors, and bars. According to its morphological affinities it is allied to *H. recurvata* Yamaguti, 1942; *H. upenei* Yamaguti, 1953; and *H. spirophallus* Yamaguti, 1937 included by Young (1968) in *Haliotrema* species group no. 3.

Haliotrema recurvata Yamaguti, 1942

MEASUREMENTS (from 4 specimens): Total length 258–484, width 52–208, ventral anchors 37–50, inner root 15–17, outer root 5–7, shaft 30–33, tip 9–11, bar 60–65, dorsal anchors

→

Figures 16–18. *Haliotrema curvipenis*. 16. Whole worm, dorsal view. 17. Copulatory organ. 18. Anchors, bars, and hooklets. AP: Accessory piece. C: Cirrus. F: Funnel. GA: Genital atrium. RS: Seminal receptacle. U: Uterus. V: Vagina. VD: Vasdeferens. VT: Vitelline duct. For other abbreviations see Figures 5–13.



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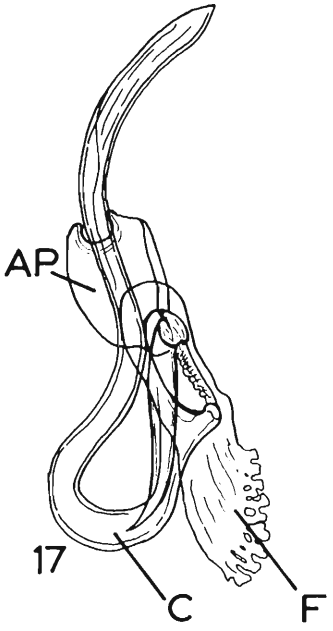

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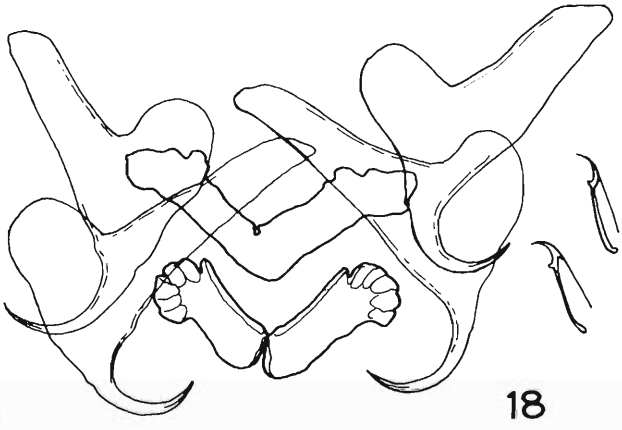

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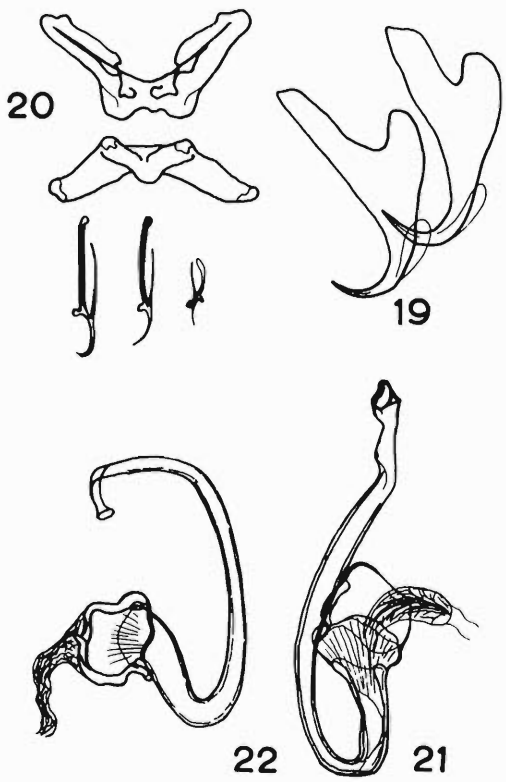


Figures 19–22. *Haliotrema recurvata*. 19. Anchors. 20. Bars and hooklets. 21, 22. Copulatory organ of two specimens.

40–45, inner root 17–20, outer root 2–7, shaft 30–33, tip 9–11, bar 48–52, hooklets 10–12.

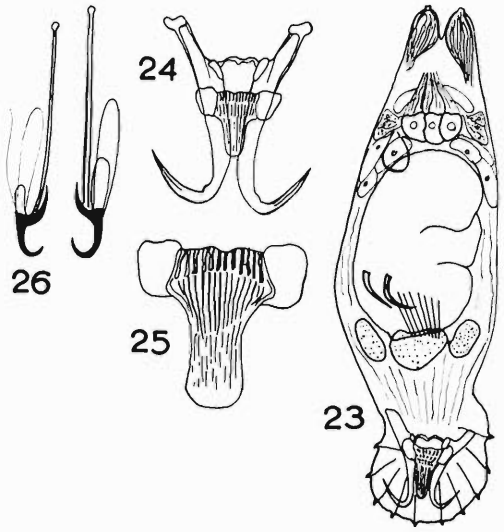
HOSTS AND LOCALITIES: *Pseudupeneus barberinus*; *P. pleurospilos*; and *P. macronema*, as well as *Gerres* sp. (Gerridae); northwestern Gulf of Eilat (Taba and Port of Eilat).

REMARKS: Type was described from *Parupeneus chrysedros* Naha, Okinawa. Specimens from Naha are much larger (800–1,230 long, 160–280 wide) than those presently described.

GENUS: *Gyrodactylus* Nordmann, 1832.

Gyrodactylus sp. 1

DESCRIPTION (from 4 specimens): Small-size worms with the characteristics of the genus. The two prohaptoral anterior lobes are prominent. The pharynx consists of large basal cells and elongated apical cells embedded in the apical membranous cone. A glandular area



Figures 23–26. *Gyrodactylus* sp. 1. 23. Whole worm. 24. Anchors and bars complex. 26. Hooklets.

can be observed lateral and posterior to the pharynx. The cirrus was not traceable, or else it is unarmed. The anchors have distinct, long roots with thickened rims. The ventral bar has a large membrane. In the marginal hook the sickle filament loop (terminology according to Malmberg, 1970) reaches half the length of the handle; the spike of the sickle base is prominent and pointed backwards.

MEASUREMENTS: Total length 260–312, width 80–90, opisthaptor 50–62 × 50–70, pharynx 13 × 14,* pharyngeal processes length 7–8,* anchors 40–44, root 10–12, shaft 30,* tip 18,* dorsal bar 16, ventral bar 19 × 5,* membrane 9 × 8,* hooklets 22–31, sickle 7,* sickle filament loop 10.*

HOSTS AND LOCALITIES: *Upeneus tragula* Richardson; northern shores of the Gulf of Eilat in three out of 10 fish studied.

REMARKS: Since only fixed specimens were available, the detailed structure of the excretory system could not be studied. Furthermore, the structure of the cirrus remained obscure. In the absence of information on these two important criteria (Malmberg, 1970) the taxonomic status of this *Gyrodactylus* species cannot be determined.

* Detailed measurements could be obtained only from one specimen.

Table 2. Distribution pattern of *Haliotrema* spp. associated with goat fish.

	North Red Sea	Indonesia	Japan	Australia	Hawaii
H. alata Y., 1942	+	+	+		
H. australe J. & T., 1922	+			+	
H. curvipenis sp. n.	+				
H. japonense Y., 1934			+		
H. recurvata Y., 1942	+		+		
H. spirophallus Y., 1937			+		
H. upenei Y., 1953		+			
H. spirale Y., 1968					+
H. minutospirale Y., 1968					+
H. curvicirrus Y., 1968					+
H. bifurcocirrus Y., 1968					+

Host–Parasite Relationship and Geographical Distribution

Young (1968) suggested that the species of *Haliotrema* described from goat fish be included in a separate infrageneric grouping within the genus *Haliotrema* (species group 4). Of the species found in the Red Sea, only one is unknown from elsewhere, while the remaining three have been found in goat fish from the Far East Pacific zone. Out of the four species found in goat fish in the Gulf of Eilat, three were found only in fish of the genus *Pseudupeneus*, while the fourth species was found mainly in *Mulloidichthys*. *Upeneus* was infected only with *Gyrodactylus*. On the other hand, the two common species of *Haliotrema* (*H. australe* and *H. recurvata*) were found also on nonmullid fish, on *Gerres* sp. (Gerridae). Out of the four species of *Haliotrema*, two were numerous and heavily infected their respective hosts; the other two occurred only in small numbers (one of these species was found mainly on *Mulloidthys auriflamma*). All the fish of the genus *Pseudupeneus* were found to be heavily infected, while infection in the other two genera appeared to be more sporadic.

ACKNOWLEDGMENTS: I wish to thank Dr. George Kissel of the Marine Biological Laboratory, Eilat, for his help in identifying the fish.

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Effect of Immunosuppressants on the Susceptibility of Hamsters to *Trichostrongylus axei* and *T. colubriformis*

LOUIS M. WIEST, JR.[1]

ABSTRACT: Neither *T. axei* nor *T. colubriformis* adapted readily to the hamster. The hamster was least susceptible to *T. colubriformis*. The administration of corticosteroids accelerated the rate of parasite development, increased daily egg production, and increased adult worm burdens. These effects were most pronounced with dexamethasone when administered for 3 weeks starting the day of infection. However, only cortisone effected translocation in the site of adult *T. axei* from the small intestine to the stomach. The immunosuppressant chlorambucil, which is not a steroid, had none of the corticosteroid effects on the hamster.

Several laboratory models utilizing species of *Trichostrongylus* in rodents have been used to study gastrointestinal helminths of ruminants. Only one instance involving trichostrongylids (*T. axei*) in hamsters has been reported (Drudge et al., 1955), hence the susceptibility of the hamster to *T. axei* was further investigated. In addition, its susceptibility to *T. colubriformis* was studied. Because the hamster was somewhat refractive to infection by these parasites, three immunosuppressant drugs, namely cortisone, dexamethasone, and chlorambucil, were employed to increase its susceptibility. Cortisone and related steroids have been found to increase worm burdens and suppress the inflammatory response with other nematodes (Ritterson, 1959; Parker, 1961; Miller, 1966; Campbell, 1968; and others). Chlorambucil, a nonsteroid anti-inflammatory drug, has been used to suppress the immune response in sheep against *Haemonchus contortus* (Soulsby and Owen, 1965; Owen et al., 1966). Data were obtained on the time required for development of the worms, egg production, and the percentage of worms developing.

Materials and Methods

One hundred thirteen male golden hamsters, 2 to 3 months old with an average weight of 105 grams, were given either *T. axei* or *T. colubriformis* larvae by gavage. These infective larvae from bovine isolates were obtained through the courtesy of Dr. Harry Herlich of the National Animal Parasite Laboratory, USDA, Beltsville, Maryland. As determined by Dr. Herlich, 4,000 *T. colubriformis* larvae produced death in guinea pigs within 3 days after infection. However, the infectivity of the bovine strain of *T. axei* in laboratory animals was not known.

Fecal egg counts, using a direct centrifugation egg flotation count method with saturated zinc sulfate solution, were made daily on 24-hr fecal collections from each animal approximately from patency to necropsy. Daily egg production per female was determined using the daily average egg production 3 days before euthanasia.

Eggs from feces of infected hamsters were cultured in a mixture of water-soaked feces and either animal charcoal or vermiculite at 25 C for 5 to 10 days.

At necropsy, the stomach, small intestine, and large intestine were separately cut into 1- to 2-cm lengths and digested in individual jars with an artificial pepsin solution as described by Kates and Thompson (1967). Counts of adult male and female worms were made from each organ. Immature developmental stages were also counted. Species identification of male worms was made, using procedures of Douvres (1957), to verify the purity of the larval inoculum.

Results

Pilot trials were conducted to develop baseline information on *Trichostrongylus* develop-

[1] This work was in partial fulfillment of the M.S. degree at the University of Maryland, Department of Zoology, College Park, Maryland, under the advisorship of Dr. Gilbert F. Otto. This work was also part of antiparasitic investigations at the National Animal Parasite Laboratory, USDA, Beltsville, Maryland. I thank Dr. A. O. Foster, Dr. K. C. Kates, Mr. M. L. Colglazier, and the staff at the National Animal Parasite Laboratory for the use of their facilities and resources for this work.

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ment and egg production in hamsters. Various doses from 500 to 10,000 were used in a total of 59 animals. The optimum development optimum occurred in the dose range from 3,000 to 4,500 larvae. Seven per cent of the infecting dose of *T. axei* was recovered as adult worms but less than 1% (0.2%) of *T. colubriformis*. *T. colubriformis* did, however, develop more rapidly than did *T. axei*. The former reached the adult stage in 11 to 13 days. As a result, a shorter prepatent period and earlier occurrence of maximum daily egg production were manifest with *T. colubriformis* than with *T. axei*. Many *T. axei* remained arrested in the fourth larval stage even after 35 days.

Accordingly, in the experiments on the effect of immunosuppressant agents (cortisone, dexamethasone, and chlorambucil), infecting doses of 3,000 or 4,500 larvae were used.

EXPERIMENT I: Twelve hamsters were given 4,500 *T. axei* larvae each and another 12 were given the same number of *T. colubriformis* larvae. Each group of 12 was divided in random block design into four treatment groups as follows: Group I, Saline controls, 0.5 cc daily from day 1 to day 24 postinfection; Group II, Chlorambucil$_1$ (4-p-bis (2-chloroethyl)-aminophenylbutyric acid, Mann Laboratories, New York), 2 mg/kg of body weight every third day from day 1 of the infection to day 24 (total dose 18 mg/kg); Group III, Chlorambucil$_2$, 3 mg/kg every third day from day 7 to day 15 (total dose 12 mg/kg); Group IV, Cortisone (Nutritional Biochemicals of Ohio), 5 mg/kg from day 1 to day 24 (total dose 120 mg/kg).

All drugs were administered subcutaneously. The animals were killed 30 days after infection. Of the immunosuppressants only cortisone rendered the hamster more susceptible to the trichostrongylid infections. In the 11 of 12 hamsters which became infected with *T. axei*, cortisone treatment permitted worms to produce 6 times as many eggs as in the untreated animals or those receiving chlorambucil. Maximum daily egg production was higher and occurred 3 days earlier in the cortisone-treated hamsters. Furthermore, more worms became established in the cortisone-treated animals; 13.2% of the inoculum was recovered as developing or adult worms from these animals in contrast to 8.1% recovery from the untreated and chlorambucil-treated animals. Cortisone not only increased the total number of *T. axei* but also doubled the number which developed into adult parasites. Even more conspicuous was the establishment of adults in the stomach of the cortisone-treated animals. Of the adults recovered, 76% were in the stomach; this is 45% of the total worm recovery. In contrast, in untreated and the chlorambucil-treated animals only 21% of the adults or 8% of the total worms were in the stomach.

With *T. colubriformis* cortisone also increased egg production and worm development, but the total number of worms was much less than with *T. axei*. In the six of 12 hamsters which became patent with *T. colubriformis*, 99% of the eggs produced in 10 days came from cortisone-treated animals. The day of maximum egg production was 6 days earlier in cortisone-treated hamsters than in the remaining animals (untreated and chlorambucil-treated).

EXPERIMENT II: Because of increased egg production and worm development with cortisone, a second corticosteroid (dexamethasone) was included in this experiment. Fifteen hamsters were divided into five treatment groups according to a random block design after they had been given 3,000 *T. axei* larvae. Another 15 were similarly divided after the same dose of *T. colubriformis*.

Group I, Saline controls, 0.5 cc daily from day 1 to 24; Group II, Chlorambucil, 3 mg/kg daily from day 1 to day 24 (total dose 72 mg/kg); Group III, Cortisone, 5 mg/kg daily from day 1 to day 24 (total dose 120 mg/kg); Group IV, Dexamethasone$_1$ (9-alpha-fluoro-16-alpha-methyl predisolone, Schering Corporation), 0.5 mg/kg; from day 1 to day 24 (total dose 12.0 mg/kg); Group V, Dexamethasone$_2$, 0.5 mg/kg daily from day 7 to day 20 (total dose 7.0 mg/kg).

The drugs were given subcutaneously. Hamsters in the corticosteroid-treated groups showed signs of stress after day 7. All animals were killed 30 days after infection.

Differences in egg production were demonstrated between species of worm, steroid and nonsteroid treatment, and type of steroid administered. As in previous experiments the daily egg production per female worm in the untreated animals and those treated with chlor-

Table 1. Daily egg production per female for 3 days preceding necropsy.

Treatment	*Trichstrongylus axei*	*T. colubriformis*
Untreated	7	1
Chlorambucil	5	1
Cortisone	3	17
Dexamethasone$_1$	22	24
Dexamethasone$_2$	1	24

ambucil was conspicuously greater by *T. axei* than by *T. colubriformis* (Table 1). However, the increased egg production per female worm as a result of corticosteroid treatment was more consistent and on the whole greater for *T. colubriformis* than for *T. axei*. However, with the increased number of adult *T. axei* (see below) the total daily egg production was far greater by this species, particularly in the animals receiving the longer treatment with dexamethasone (Dexamethasone$_1$, Figure 1). The egg production was essentially 100-fold greater than in the chlorambucil-treated animals. Again the day of maximum egg production occurred 3 to 4 days earlier in corticosteroid treated animals than in either the unmedicated controls or the chlorambucil-treated animals.

Again more *T. axei*, including large numbers of arrested fourth-stage larvae, than *T. colubriformis* were recovered (Table 2). Corticosteroids significantly increased the worm load of the trichostrongyles over that in noncorticosteroid-treated animals. Also, treatment with corticosteroid drugs enhanced the development of *T. axei*. More than three times as many developed into adults than in hamsters without steroid treatment. Moreover, the increased activity of dexamethasone over cortisone was reflected only in more adult *T. axei* but not more *T. colubriformis*. In contrast, however, only cortisone appeared to affect the location of the adult worms. Of the 196 adult worms recovered from these animals 168, i.e., 86%, were found in the stomach; this is 52% of the 323 total worms recovered. Whereas in the dexamethasone$_1$ animals only 38% of the adults were in the stomach, in the dexamethasone$_2$ 36% and in the untreated and chlorambucil-treated 48%.

EXPERIMENT III: Finally, an attempt was made to establish a second generation of trichostrongylids in hamsters. However, culturing eggs from the first hamster generation produced only a small harvest of larvae from either species. From 7,400 *T. axei* eggs collected for culture from the first hamster generation 4,500 larvae were recovered. One-

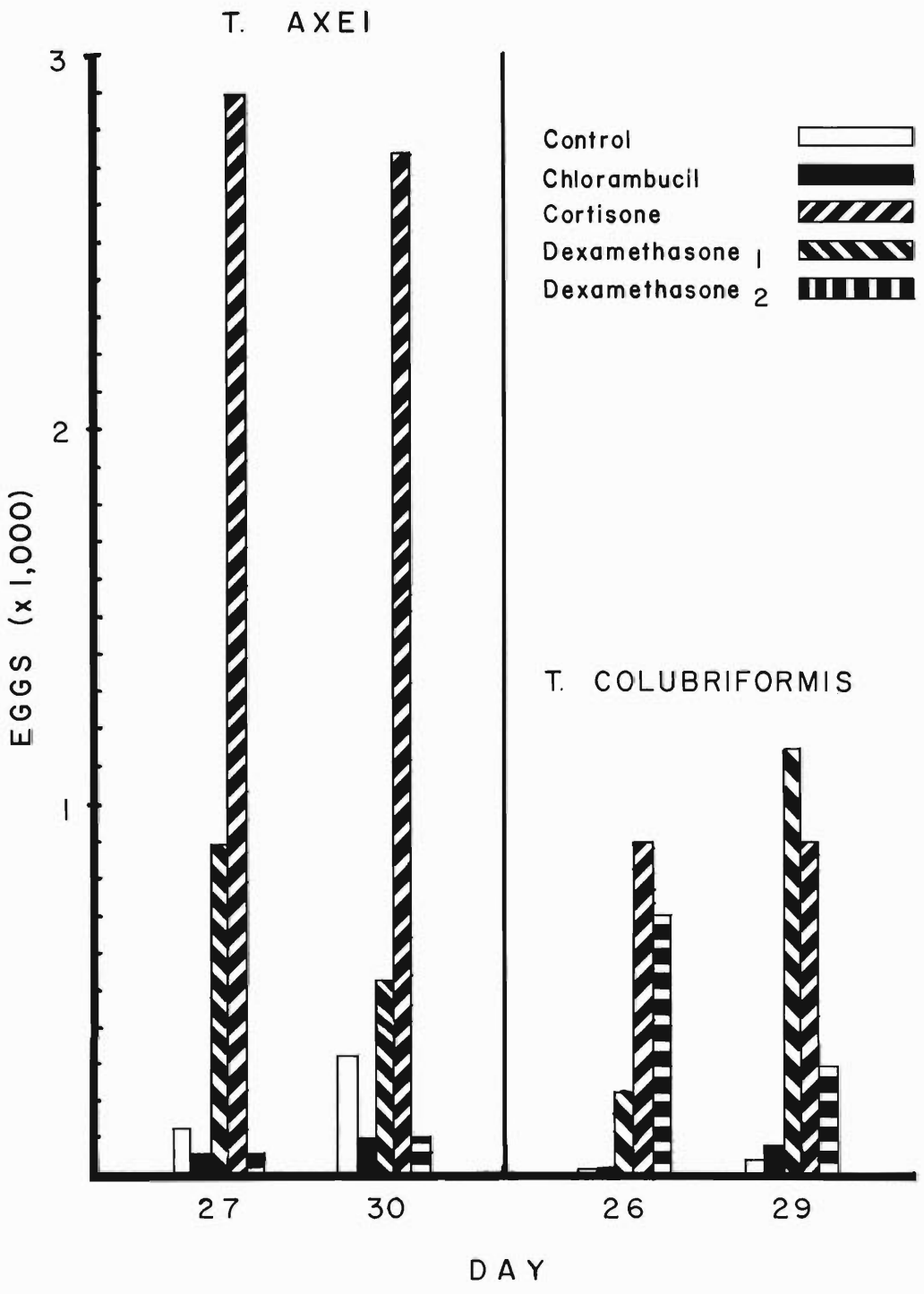
Table 2. Comparative effect of drugs on recovery of *Trichostrongylus axei* and *T. colubriformis* from the hamster 30 days after infection with 3,000 larvae (three animals per group).

Treatment	Stomach 5th	Duodenum 5th	Small intestine 5th	Large intestine 5th	Totals 4th	Totals 4th Molt	Totals 5th	Grand total	Recovery as % of infective dose	Male/ Female
T. axei										
Untreated	17	13	2	2	155	12	34	202	6.7	0.53
Chlorambucil	3	5	0	1	81	20	8	108	3.6	0.41
*Cortisone	168	21	6	5	107	17	196	323	10.8	0.61
Dexamethasone$_1$	135	114	15	88	78	25	352	455	15.1	0.76
Dexamethasone$_2$	42	42	3	24	117	23	116	257	8.6	0.95
T. colubriformis										
Untreated			7	1	0	0	8	8	0.3	0.33
Chlorambucil			4	1	0	0	5	5	0.2	1.50
*Cortisone			31	21	0	0	52	52	1.8	0.92
*Dexamethasone$_1$			28	16	0	0	44	44	1.5	0.50
Dexamethasone$_2$			22	8	0	0	30	30	1.0	0.71

* Two animals per group, other hamsters died of unknown causes.

→

Figure 1. Twenty-four-hour egg production of *Trichostrongylus axei* and *T. colubriformis* at day of maximum egg production and the day before sacrifice.



half (2,250 larvae) were given to each of two normal (i.e., unmedicated) hamsters. Eggs appeared in the feces of each during the fourth week after infection but were never numerous. The entire fecal output for a week from both hamsters yielded only 100 on culturing. No worms were found on necropsy. Similarly, one hamster was given 500 *T. colubriformis* larvae. Very few eggs appeared in the feces during the fourth week but no larvae were recovered from culture and no worms were recovered on necropsy.

Discussion

Susceptibility of the hamster to *Trichostrongylus axei* and *Trichostrongylus colubriformis* infection was less than that reported from the rabbit, guinea pig, gerbil, or mastomys. Even larval doses up to 10,000 *T. colubriformis* larvae did not produce death or discernible injury in hamsters, whereas 4,000 of these infective larvae were lethal to guinea pigs (Herlich, pers. comm.). Reduced susceptibility over other hosts was seen in terms of longer average prepatent period, delay in maximum egg production, and decreased total egg production. The number of worms developed after 30 days (*T. axei*—7.2% and *T. colubriformis*—0.2% worm recovery) was markedly lower than what has been reported, by the investigators cited below, from other laboratory hosts (range 11 to 46% recovery).

However, the administration of corticosteroid immunosuppressants increased the susceptibility of the hamster to *T. axei* and *T. colubriformis* to the point that worm development approached that in natural and other experimental hosts. Shorter prepatent periods, an earlier period of maximum daily egg passage, and an increased daily egg production per female were indicative of the effect of corticosteroids on hamster susceptibility. Furthermore, with dexamethasone, the *T. axei* daily egg production per female worm (22) even approached values in other hosts (calf—42, horse—22, rabbit—20, gerbil—28, and mastomys—51); (according to Leland, 1963, 1968; Leland and Drudge, 1957). Because host barriers were significantly lowered by corticosteroids, worm development also approached that recorded in other hosts even though many *T. axei* remained arrested in the fourth stage. The response of *T. colubriformis* was similar but apparently this species was less adaptable to the hamster and conspicuously fewer total worms developed. The effect of dexamethasone was less when administration of the drug was started 7 days after infection. These studies have given the first direct indication that corticosteroids can shift the establishment of *T. axei* in stomachs of small laboratory animals from the small intestine. This effect, however, was seen only with cortisone.

However, the nonsteroid antiinflammatory agent, chlorambucil, in the hamster evinced no increase in either worm development or egg production. This is contrary to the reported increase in number of eggs in sheep after treatment with chlorambucil (Soulsby and Owen, 1965).

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Spinitectus micracanthus sp. n. (Nematoda: Rhabdochonidae) from the Bluegill, *Lepomis macrochirus* Rafinesque, in Ohio

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ABSTRACT: *Spinitectus micracanthus* sp. n. is described from the intestine of *Lepomis macrochirus*. It differs from all other species of the genus in having a male with two parallel longitudinal rows of cuticular ridges anterior to the anus. The species most closely resembles *S. carolini* Holl, 1928, from which it differs in ratios of anatomical structures, number and type of cuticular spines, cuticular ridges; number of, and arrangement of postanal papillae; shape and size of long spicule; size of the females and eggs.

During 1966 and 1967, a survey of nematode parasites of some freshwater fishes of Ohio was undertaken. Spiruroid nematodes belonging to the genus *Spinitectus* Fourment, 1883, were taken from small intestine of bluegills, *Lepomis macrochirus macrochirus* Rafinesque, collected from a privately owned pond in Snortin Ridge Township, Fairfield County. These nematodes appear to represent a new species and are described such herein.

Specimens of both sexes of the nematodes were collected. Some of each sex were fixed in hot 75% alcohol or AFA, cleared in alcohol–glycerin, and temporarily mounted in glycerin under supported cover slips. Both living and fixed specimens were examined with ordinary light and phase contrast microscopes. The description is based on 20 males and 20 females. Unless otherwise indicated, the measurements are in microns. The drawings were made with the aid of a camera lucida.

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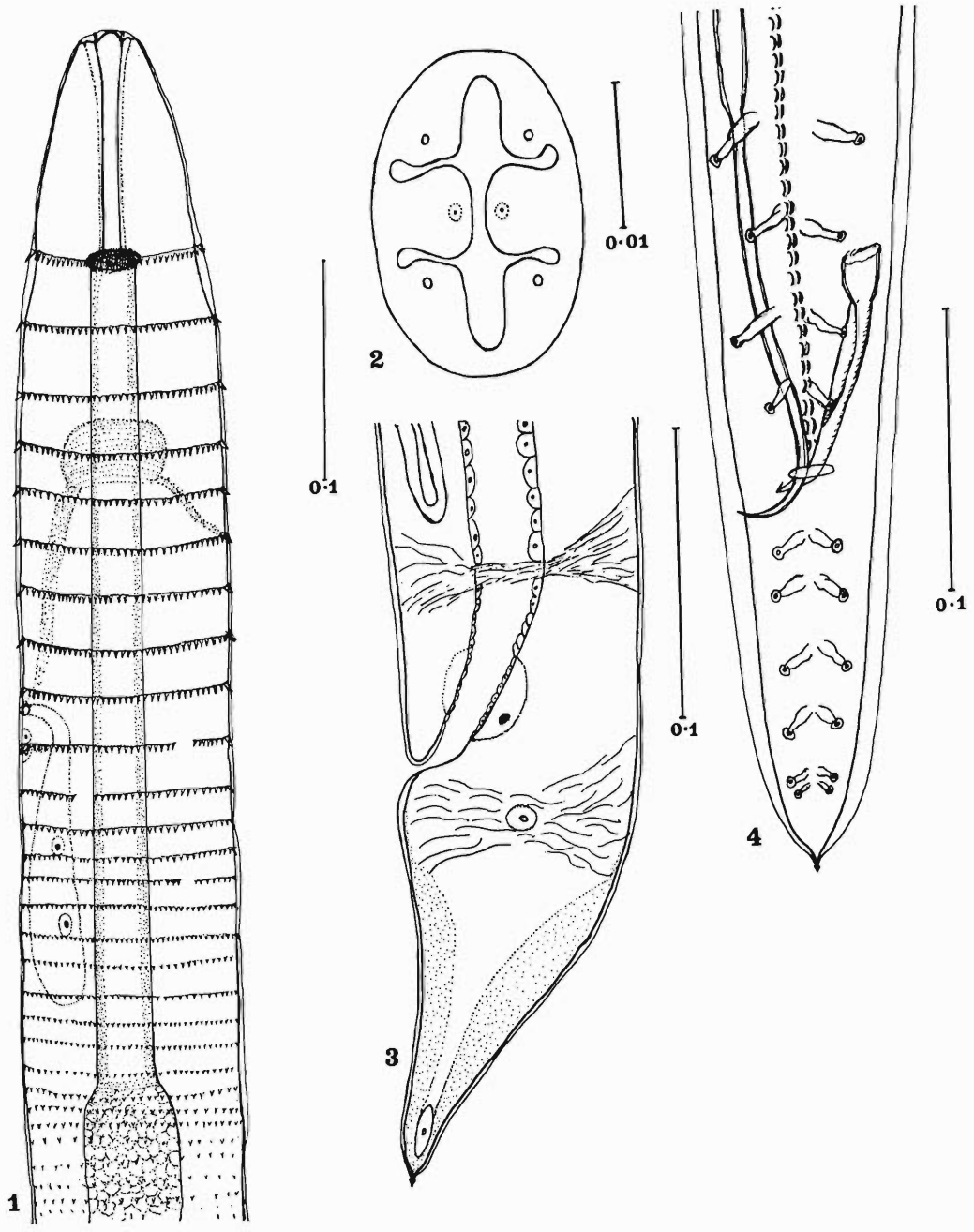
Spinitectus micracanthus sp. n. (Figs. 1–4)

DESCRIPTION: Body filiform, slightly attenuated at extremities; anterior end bluntly conical; posterior end pointed. Cuticular spines small, fine, in transverse circlets flush with body surface, each spinous circlet bearing 56–72 spines. Head region devoid of spines, demarcated posteriorly at level of first circlet of spines. Spinous circlets extending posteriorly to anterior portion of glandular esophagus. Mouth terminal surrounded by two pseudolabia, oral opening irregularly hexalobate. Head bearing two pairs of sublateral papillae and one pair of lateral amphids. Stoma cylindrical, terminating at level of first circlet of spines. Nerve ring surrounding muscular portion of esophagus at level of 4th spinous circlet. Distinct esophagointestinal valve present. Intestine straight, wider anteriorly than posteriorly; inconspicuous rectal–intestinal valve present. Tail slender, tapering posteriorly.

MALE: Length 7.6–8.2 mm, maximum width



Figures 1–4. *Spinitectus micracanthus* sp. n. (All scales are in millimeters.) 1. Anterior portion of female. 2. En face view. 3. Posterior portion of female. 4. Posterior portion of male showing spicules, genital papillae, caudal alae, and cuticular ridges.

Table 1. Comparison of ratio, measurements, and structural features of the *Spinitectus* species reported from freshwater fishes of North America.

Characteristic features	*S. gracilis**	*S. carolini**	*S. micracanthus*
1. Length of mature females	10–15 mm	7–8 mm	16–20 mm
2. Length of mature males	8–10 mm	slightly smaller than females	7.6–8.2 mm
3. Head length	not given	80	99–107
4. Stoma length	25	100	90–107
5. Direction vagina extends from vulva	anteriorly	posteriorly	posteriorly
6. Length of vagina	800	280	350–380
7. Ratio of esophagus to total body length	1:9 or 1:10	1:3 or 1:4	1:5 or 1:6
8. Ratio of muscular to glandular esophagus	1:3	1:5 or 1:6	1:6 male; 1:8 female
9. Length and shape of long spicule	600; cylindrical through its length and scoop-shaped	270; distal half compressed	305; anterior ⅓ thicker, distal ⅔ more slender and gutter-shaped
10. Length and shape of short spicule	150; bent twice and horn-shaped	70; arcuate, distally provided with a large ventral barb	86–94; boat-shaped, distally provided with a fine ventral barb
11. Size and number of spines per circlet	long; 35–50 per circlet	very long and sharp; 25–35 per circlet	small, short, very thin and fine; 56–72 per circlet
12. Number of rows of cuticular ridges	4–8 rows	4–5 rows	2 rows (54–56 ridges in each row)
13. Number of preanal papillae	4 pairs	4 pairs	4 pairs
14. Number of postanal papillae	6 pairs	5 pairs	6 pairs
15. Position of excretory pore	between 4th and 5th circlets	between 8th and 9th circlets	between 7th and 8th in males; between 9th and 10th circlets in females
16. Prevulva to postvulva ratio	3:1	4:3	1:1
17. Width of caudal alae	fairly wide	narrow	narrow
18. Size of egg	40 by 24	36 by 23	39 by 24

* According to Van Cleave and Mueller (1932).

90–100 at muscular esophageal level. Head region 94–99 long, 32–39 wide at level of first spinous circlet. Stoma 94–98 long, 5 wide. Muscular esophagus 297–312 long, 20–30 wide; glandular esophagus 1,914–2,030 long, 66–70 wide. Nerve ring 160–164 from anterior tip of body. Junction of muscular and glandular esophagus at level of 17th and 18th circlets, about 286–289 from anterior tip. Testis single, reflexed, near esophagointestinal valve. Spicules unequal, dissimilar. Short spicule thick, boat-shaped, 86–94 long, pointed distally, provided with fine ventrally curved barb. Long spicule 296–312 long, anterior third thicker, roughly cylindrical. No gubernaculum. Tail 130–140 long with somewhat rounded tip, with small, fine, spearlike knob, and always coiled. Caudal alae 20 wide, about 296–300 long, extending from 156 anterior to anus to tip of tail. Ten pairs of pedunculate caudal papillae present: 4 preanal pairs and 6 postanal pairs. Two parallel, longitudinal rows of cuticular ridges present, extending cephalad from about 39–40 anterior to anus to beyond anterior extremity of retractor muscle of long spicule.

FEMALE: Length 16–20 mm. Width at level of vulva 168–179. Stoma 90–107 long, 5 wide. Muscular esophagus 340–363 long, 31–34 wide; glandular esophagus 2,950–2,970 long, 98–109 wide. Nerve ring 168–172 from anterior tip. Vulva nonsalient, 8.5–10.2 mm from anterior tip, 8.2–9.7 mm from tip of tail. Ovejector present. Vagina strongly muscular, 350–380 long, directed posteriorly; uteri amphidelphic, extensively coiled filling almost entire width of nematode. Uteri and vagina usually full of eggs. Egg elliptical with thick, transparent shell, without polar filaments, containing coiled embryo at oviposition, and measur-

ing 39 long by 24 wide. Tail 158–165 long, slenderly tapering posteriorly and provided with a short, spikelike projection 5 long.

HOST: *Lepomis macrochirus* Rafinesque.

LOCALITY: Local pond, Snortin Ridge, Madison Township, Fairfield County, Ohio.

LOCATION: Intestine.

HOLOTYPE MALE: USNM Helm. Coll. No. 70749.

ALLOTYPE: USNM Helm. Coll. No. 70750.

PARATYPES: USNM Helm. Coll. No. 70751.

Discussion

The only two species of *Spinitectus* described from freshwater fishes of North America are *S. gracilis* Ward and Magath, 1917, and *S. carolini* Holl, 1928. Both species were redescribed and reported from other fish hosts by Van Cleave and Mueller (1932), who suggested that the shape, form, and size relationships of anatomical structures should be employed in separating species of this genus. Ali (1956) constructed a key in separating most of the species known at that time. Sahay and Prasad (1965) expanded on Ali's key but, unfortunately, some species were omitted.

Anthony (1963) reported four unidentified species of *Spinitectus* from freshwater fishes of Wisconsin, but did not describe or name them. The criteria suggested by Van Cleave and Mueller (1932) are here followed to differentiate the new species from *S. carolini* which it closely resembles superficially (Table 1). A table of comparative measurements and diagnoses of the three species of *Spinitectus* from the freshwater fishes in North America is presented.

Spinitectus micracanthus differs from all other known species of the genus in having the smallest size and largest number of spines per circlet. It is the only species known to have males with two parallel longitudinal rows of cuticular clefts anterior to the anus. In addition to size relationships and structural features in which *S. carolini* and *S. micracanthus* differ as shown in Table 1, the short spicule of the male of *S. carolini* has a large ventral barb whereas that of *S. micracanthus* has a fine ventral barb. Spines are borne on large cuticular plates in *S. carolini* but such plates are lacking in *S. micracanthus*. Because of the differences given above and in Table 1, *S. micracanthus* is considered a new species of *Spinitectus* and the third species to be described from freshwater fishes of North America.

Acknowledgments

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Prevalence and Distribution of Helminths of Swine in South Carolina[1]

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ABSTRACT: The prevalence of helminths of swine in South Carolina during 1968–69 was: *Oesophagostomum* spp., 34% (including *O. dentatum*, *O. quadrispinulatum*, and *O. brevicaudum*); *Ascaris suum*, 23%; *Strongyloides ransomi*, 7%; *Metastrongylus* spp., 6% (including *M. apri*, *M. pudendotectus*, and *M. salmi*); *Trichuris suis*, 5%; *Stephanurus dentatus*, 3%; *Ascarops strongylina*, 1%; *Physocephalus sexalatus*, <1%; and *Macracanthorhynchus hirudinaceus*, <1%. Neither *Trichinella spiralis* nor *Hyostrongylus rubidus* were encountered. The highest prevalence of infection by *Oesophagostomum* spp., *S. dentatus*, *P. sexalatus*, and *M. hirudinaceus* occurred in brood sows, whereas the highest *A. suum* infections occurred in market hogs. Pigs under 12 weeks of age had the highest prevalence of *T. suis* and *S. ransomi*. Little difference in prevalence was noted in purebred vs. crossbred swine except for *A. suum* and *S. ransomi*, which were more common in crossbreeds. No significant differences were found in the prevalence and distribution of the helminths geographically. Higher levels of infection were recorded during the summer months for *A. suum*, *T. suis*, and *S. ransomi*, whereas levels of infection by *Oesophagostomum* spp. were irregular over a 12-month period. Swine which had been treated with anthelmintics did not show reduced helminth populations. Feces of swine from farms which utilized central farrowing houses and concrete-floored parlors contained numbers of helminth eggs comparable to those from feces of swine from farms lacking these facilities. However, the prevalence of helminths appeared to be related directly to the overall quality of management, i.e., good management resulted in fewer parasites, although this trend was not statistically significant.

The United States Department of Agriculture has estimated the annual loss due to harmful effects caused by internal parasites of swine to be more than 275 million dollars (Stewart and Southwell, 1960). A large portion of this loss occurs in the southeastern states. In North Carolina, for example, internal parasites reduce the value of swine by at least one dollar for each pig marketed (Batte and Moncol, 1966). The control of swine parasites, therefore, is a major problem, particularly since intensification and expansion of the swine industry has not been accompanied by the reduction of worm populations (Batte et al., 1965). A knowledge of the parasites present in swine of different ages and breeds over the course of a year is essential to help formulate control programs that may involve management, sanitation, drugs, or combinations thereof.

With these facts in mind, an ecological study of the helminths of swine in South Carolina was designed in two phases: (1) a determination of the species present, their prevalence, and geographic distribution; (2) a determination of the seasonal dynamics of several gastrointestinal nematodes.

Materials and Methods

South Carolina can be divided into three broad geophysical areas, the Piedmont, Sandhill, and Coastal Plain regions (Fig. 1), which vary in soil texture, average annual precipitation, annual temperature ranges, and vegetation. The climate is described as temperate for the Piedmont and Sandhill regions and warm temperature to subtropical for the Coastal Plain region (Kish, 1968, 1969). Of the swine population of the state (432,000), 75% is located in the Coastal Plain region with the other 25% distributed about equally between the Sandhill and Piedmont regions (Lanham and Whitworth, 1967).

During May 1968, fresh feces were collected from swine in each of the 46 counties of the state. Table 1 shows the sample sizes

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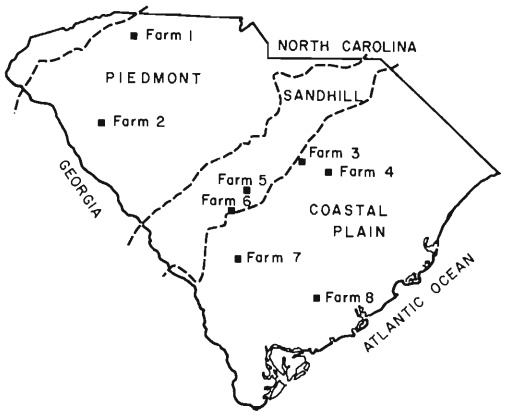

Figure 1. The Piedmont, Sandhill, and Coastal Plain regions of South Carolina with the locations of the eight swine farms sampled monthly from September 1968 to August 1969.

from swine populations in each of the three geophysical regions. For each sample information was obtained on type and breed of hog, quality of management ("above average," "average," "below average"), whether market hogs were maintained on ground or concrete, use of a central farrowing house, number of years swine had been raised on the farm, date of last anthelmintic application, and type of drug used. Farms classified as "above average" had balanced feeding programs, proper buildings and equipment, high overall levels of sanitation, and practical programs of prevention and treatment of diseases and parasites. "Average" farms had a moderate number of these practices in use, while "below average" farms utilized none of them. The number of samples collected was divided equally among three pig types: (1) brood sows, (2) pigs under 12 weeks of age, and (3) market hogs, which included some gilts. All of the samples were collected during the week of 23–30 May 1968.

A companion necropsy study was conducted in slaughterhouses, which handled animals from each region of the state. Lungs, diaphragms, kidneys and perirenal fat, and complete gastrointestinal tracts were collected. In some collections the samples were limited to lungs, diaphragms, and kidneys, or to kidneys only. The numbers of animals sampled are summarized in Table 1.

In order to study the seasonal dynamics of several nematode parasites, an additional 120 fecal samples were collected each month from September 1968 through August 1969 from swine on two farms in the Piedmont region, two farms in the Sandhill region, and four farms in the Coastal Plain region (Fig. 1). All of the eight farms were considered to be "average" management-level farms. Each month 15 fecal samples were taken from each farm, five samples coming from each of the three pig types (brood sows, pigs under 12 weeks of age, and market hogs).

Each of the 731 fecal samples collected during May 1968 from swine in each county was placed in 50 cc of 6% formalin and later was examined qualitatively for the presence of helminth eggs by a sodium dichromate flotation technique. The 1,440 fecal samples collected from swine on the eight farms over a 12-month period were analyzed quantitatively by a modified McMaster technique using a 5-cc liquified mixture of fecal matter and 6% formalin in a saturated salt solution. The formalin–

Table 1. Numbers of fecal samples and animals examined at necropsy in slaughterhouses in South Carolina during 1968–69.

Region	Swine population	No. fecal samples obtained	Numbers of animals sampled by necropsy						
			Complete*		Partial†		Totals		
			Brood sows	Mkt. hogs	Brood sows	Mkt. hogs	Brood sows	Mkt. hogs	All swine
Piedmont	53,100	196	2	3	0	20‡	2	23	25
Sandhill	50,900	119	2	0	2	20‡	4	20	24
Coastal Plain	328,000	416	4	3	0	40‡	4	43	47
Total	432,000	731	8	6	2	80‡	10	86	96

* Gastrointestinal tract, lungs, kidneys, and diaphragms.
† Lungs, kidneys, and diaphragms.
‡ In half of these samples, only kidneys were examined.

Table 2. Percentage prevalence of helminths of swine in South Carolina as determined by necropsies and fecal analyses during 1968–69.

Helminths	Per cent prevalence		
	Necropsies	Fecal analysis	Totals
Oesophagostomum spp.	57(14)*	34(731)	34(745)
Ascaris suum	0(14)	23(731)	23(745)
Strongyloides ransomi	—	7(731)	7(731)
Trichuris suis	15(14)	6(731)	6(754)
Metastrongylus spp.	23(48)	4(731)	5(779)
Stephanurus dentatus	3(96)	—	3(96)
Ascarops strongylina	43(14)	0(731)	1(745)
Physocephalus sexalatus	14(14)	0(731)	<1(745)
Trichinella spiralis	0(56)	—	0(56)
Macracanthorhynchus hirudinaceus	7(14)	0(731)	<1(745)
Totals	26(96)	74(731)	68(827)

* Numbers in parentheses indicate number of swine sampled.

feces mixture was obtained by adding 1 g of feces to 50 cc of 6% formalin.

Necropsies were performed following standard procedures. Nematodes and acanthocephalans were preserved in 70% glycerinated ethyl alcohol and later mounted in lactophenol and identified.

Results and Discussion

Prevalence of helminths

The prevalence of helminths is shown in Table 2. The prevalence of the several species of *Oesophagostomum*, determined from a sample of 149 specimens collected from eight pigs, was as follows: *O. dentatum* (60%), *O. quadrispinulatum* (27%), and *O. brevicaudum* (13%).

Table 4. Percentage prevalence of helminths in pigs,* brood sows, and market hogs in South Carolina as determined by fecal analysis during 1968–69.

Helminths	Pigs*	Brood sows	Mkt. hogs
Oesophagostomum spp.	14	61	27
Ascaris suum	18	20	33
Strongyloides ransomi	16	2	2
Trichuris suis	8	2	5
Metastrongylus spp.	2	6	3
Stephanurus dentatus	–	–	–
Ascarops strongylina	0	0	0
Macracanthorhynchus hirudinaceus	0	0	0
All helminths	12	18	14

* Pigs under 12 weeks of age.

In a sample of 31 lung nematodes from 11 pigs 64, 33, and 3% were *M. apri*, *M. pudendotectus*, and *M. salmi*, respectively. No cestodes or trematodes were encountered nor were *Trichinella spiralis* or *Hyostrongylus rubidus* recovered. The absence of *Hyostrongylus* could be attributed possibly to the small number of stomachs examined. Esophageal samples were not inspected for the presence of *Gongylonema pulchrum*.

In Table 3 the prevalence data obtained during the present study are compared with similar information from swine in northern Florida (Spindler, 1934), Georgia (Andrews and Connelly, 1945; Spindler, 1934), and North Carolina (Batte and Moncol, 1966). The results from these studies do not agree closely, but it does appear that *Oesophagostomum* spp., *A. suum*, and *S. ransomi* rank high in relative prevalence in swine in these southern states.

Table 3. Comparison of percentage prevalence of helminths of swine in northern Florida, Georgia, and North Carolina with the present study.

Helminth	Area (year of analysis)			
	South Carolina (1968–69)	Georgia and northern Florida* (1929–31)	Georgia† (1941–43)	North Carolina‡ (1965–66)
Oesophagostomum spp.	34	72	63	16
Ascaris suum	23	74	68	7
Strongyloides ransomi	7	26	55	20
Trichuris suis	6	23	31	1
Metastrongylus spp.	5	44	14	–
Stephanurus dentatus	3	51	0	–
Ascarops strongylina	1	53	71	–
Physocephalus sexalatus	<1	47	32	–
Macracanthorhynchus hirudinaceus	<1	25	16	–
Hyostrongylus rubidus	0	15	0	–

* From Spindler, 1934.
† From Andrews and Connelly, 1945.
‡ From Batte and Moncol, 1966.

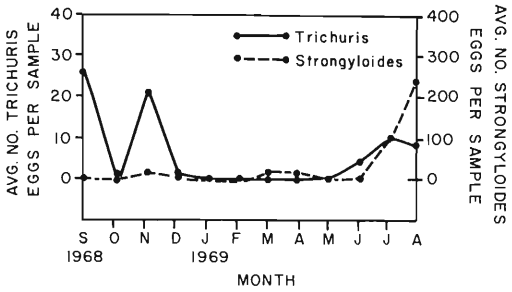

Figure 2. Average number of *Trichuris* and *Strongyloides* eggs in feces collected from all types of swine from September 1968 to August 1969 in South Carolina.

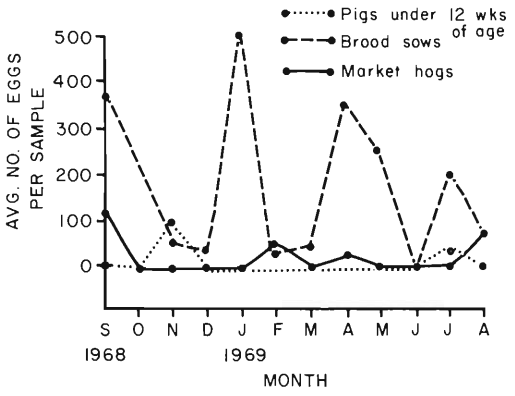

Figure 4. Average number of *Oesophagostomum* eggs in feces collected from pigs under 12 weeks of age, brood sows, and market hogs from September 1968 to August 1969 in South Carolina.

Influences on the prevalence of helminths

The results of the statewide fecal collection and the necropsy study (Table 4) showed that the brood sow was the major host of *Oesophagostomum* spp., *S. dentatus*, *P. sexalatus*, and *M. hirudinaceus*, that market hogs (both sexes) harbored the highest infections of *A. suum*, and that pigs under 12 weeks of age contained the highest prevalence of infection by *T. suis* and *S. ransomi*. *Metastrongylus* spp. and *A. strongylina* were distributed evenly among the three swine types.

No significant differences in the prevalence of all nematode eggs in swine feces were found when the results were studied with reference to breed of swine (pure vs. crossbred swine), geographic region in the state (Piedmont vs. Sandhill vs. Coastal Plain), recent anthelmintic experience (medicated vs. nonmedicated), usage of a central farrowing house (used vs. not used), and method of housing for market hogs (concrete vs. ground). However, certain differences in the prevalence of individual nematodes in various age classes of swine were apparent. For example, the prevalence of *Ascaris* and of *Strongyloides* eggs was significantly higher* in crossbred (21 and 17%) than in purebred (3 and 8%) pigs under 12 weeks of age. Eggs of *Oesophagostomum* spp. were significantly* more common in feces from market hogs raised on dirt-floored lots (32%) than in feces from market hogs raised on concrete-floored lots (20%).

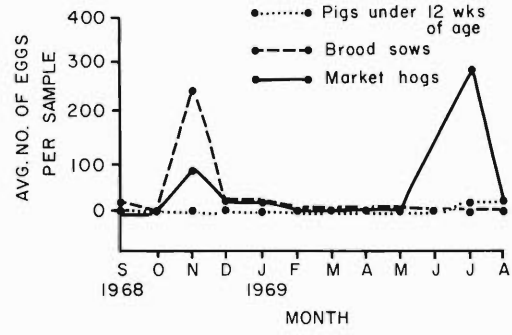

Figure 3. Average number of *Ascaris* eggs in feces collected from pigs under 12 weeks of age, brood sows, and market hogs from September 1968 to August 1969 in South Carolina.

In reference to the overall quality of husbandry, the degree of good management that existed on the farms appeared to be directly related to the incidence of nematodes although these differences were not statistically significant.

From September 1968 through August 1969 monthly egg counts of four nematodes, *A. suum*, *T. suis*, *Oesophagostomum* spp., and *S. ransomi*, were made on feces collected from swine on eight farms. Combined egg counts for all four species of nematodes varied irregularly between 20 and 160 eggs/sample throughout the 12-month period. A slight rise, although not significant, was seen in the counts for the month of July. Feces from brood sows con-

* 5% level of significance; chi-square test.

tained the highest combined egg counts for 8 of the 12 months. Market hogs and young pigs passed a higher number of eggs in their feces in July and August than at other times of the year. The number of *Trichuris* eggs in feces was highest during the summer and fall months (Fig. 2), although the incidence of this nematode was low throughout the study. Numbers of *Strongyloides* eggs were highest also during July and August in feces of pigs under 12 weeks of age (Fig. 2). The numbers of *Ascaris* eggs increased significantly in feces from brood sows during November, and in feces of market hogs during June and July (Fig. 3). Irregular peaks of egg production were characteristic of *Oesophagostomum* spp. throughout the 12-month period (Fig. 4).

Acknowledgments

The authors thank Dr. T. B. Stewart, Mr. C. W. Ackerman, Dr. W. E. Johnston, Dr. J. B. Kissam, and Mrs. Janice Riddle for advice and assistance in various aspects of this study.

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Transplantation of *Heterakis gallinarum* Larvae: Effects on Development of *H. gallinarum* and the Transmission of *Parahistomonas wenrichi*

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ABSTRACT: Following the rectal transfer of 10-day-old larvae of *Heterakis gallinarum* from young chickens to young turkeys, 45% of the worms were lost in the first 24 hr, and only 30% of those transferred survived 4 days or more. Most of these remained to maturity, but their development was retarded about 5 days. *Parahistomonas wenrichi* was detected in the cecal discharges of seven of the 12 turkeys only 7 days after they had received the 10-day larvae rectally, 3 days sooner than has ever been observed for infections acquired by natural means. These early infections must have been initiated by histomonads liberated during the first 4½ days following the transfer of the larvae, this being also the time of greatest loss of larvae in the recipient birds. Infections with *P. wenrichi* are probably initiated principally, if not exclusively, by organisms liberated by the death and dissolution of the larvae harboring them.

During a recent study of the acquisition of histomonads by the cecal worm, *Heterakis gallinarum* (Schrank, 1788) Madsen, 1949, worms were grown 10 days in one host and then transferred to the rectum of another to complete their development (Lund, 1971). Such

transfers appeared to have had certain influences on the development of the heterakids that could not be explored at that time because of the specific requirements of the experiment. The present study was designed to ascertain the effects of such transfer on both the heterakids and the nonpathogenic histomonad, *Parahistomonas wenrichi* (Lund, 1963) Honigberg and Kuldova, 1969, that these worms were carrying.

Materials and Methods

The eggs of *Heterakis gallinarum* were from a strain of cecal worms that had transmitted only *Parahistomonas wenrichi* during the past three generations. The chickens were first-generation crosses of New Hampshire males and Barred Plymouth Rock females, 5 weeks old when inoculated. The turkeys were Beltsville Small Whites, 6 weeks old when given the *Heterakis* larvae.

Two tests were run, the first to determine the proportion of larvae that were retained for certain intervals after rectal transfer, and the second to determine the effects of a single disturbance (the transfer) on the liberation of *P. wenrichi* and the ultimate maturation of the heterakids.

For the preliminary test, each of 15 chickens received 150 embryonated *Heterakis* eggs obtained and administered as described in an earlier report (Lund, 1967). Ten days later, each chicken was necropsied and its heterakid larvae were counted and quickly washed in six changes of physiologic saline at 37 C. The larvae were immediately rectally transferred to a turkey, which was suspended head down for 20 min so the larvae would be carried to the ceca (Browne, 1922; Lund, 1955). Five of these turkey foster hosts were necropsied after 1 day, two after 4 days, and the remainder after 13 days.

The second test was started in a similar manner except that each of 24 chickens was given 360 embryonated *Heterakis* eggs. Twelve of these chickens were necropsied 10 days later, each providing larvae for a turkey by the method previously described. These 12 turkeys were then caged individually and the cecal discharges of each were examined daily for *P. wenrichi*. The remaining chickens (now 10 because two had died from cage injuries) were held as controls in which the heterakids could develop undisturbed. These 10 control chickens and the 12 recipient poults were necropsied 40 days after the initial feeding of embryonated *Heterakis* eggs. Fresh smears of the cecal contents were examined microscopically for *P. wenrichi*, and all *Heterakis* were removed from each bird. These worms were then separated according to sex, counted, and all females placed in 1.0% formalin to permit embryonation of the eggs (Clapham, 1933). The embryonated eggs in each female heterakid were then counted so an estimate could be made of the degree of development and extent of fertility attained by each female in each bird.

Results

Ten days after having been fed 150 embryonated *Heterakis* eggs, the 15 chickens on the preliminary test yielded a total of 523 larvae. Because of losses during the several washings, only 451 were transferred to the 15 turkeys. Five turkeys necropsied the next day had a total of 83 larvae, or 55% of the number they had received. The two turkeys necropsied 4 days after the transfer retained only 28% of the larvae received. Retention apparently stabilized at about this level, as the eight turkeys necropsied on the 13th day retained 29% of the larvae given them. Inasmuch as worm counts could not be made at the above intervals for the recipient turkeys on the second test, the curve shown for retention of larvae by the latter birds during the interval between transfer and necropsy (Fig. 1) is based on the above findings.

Parahistomonas wenrichi was not detected in any of the 15 donor chickens or the first seven turkeys to be necropsied, but was present in three of seven turkeys in which it was sought at the 13-day necropsy. (Inadvertently, one turkey necropsied at 13 days was not examined for *P. wenrichi*.)

The results of the second test are summarized in Table 1. The 12 donor chickens, necropsied 10 days after having received 360 embryonated *Heterakis* eggs, yielded a total of 1,418 larvae, a recovery of 33%. Because of losses during the several washings, only 926 were transferred to the 12 turkeys, an average of 77 larvae per recipient, or (as depicted in Fig. 1) 21.4% of the original inoculum of 360 embryonated eggs.

Parahistomonas wenrichi was detected in

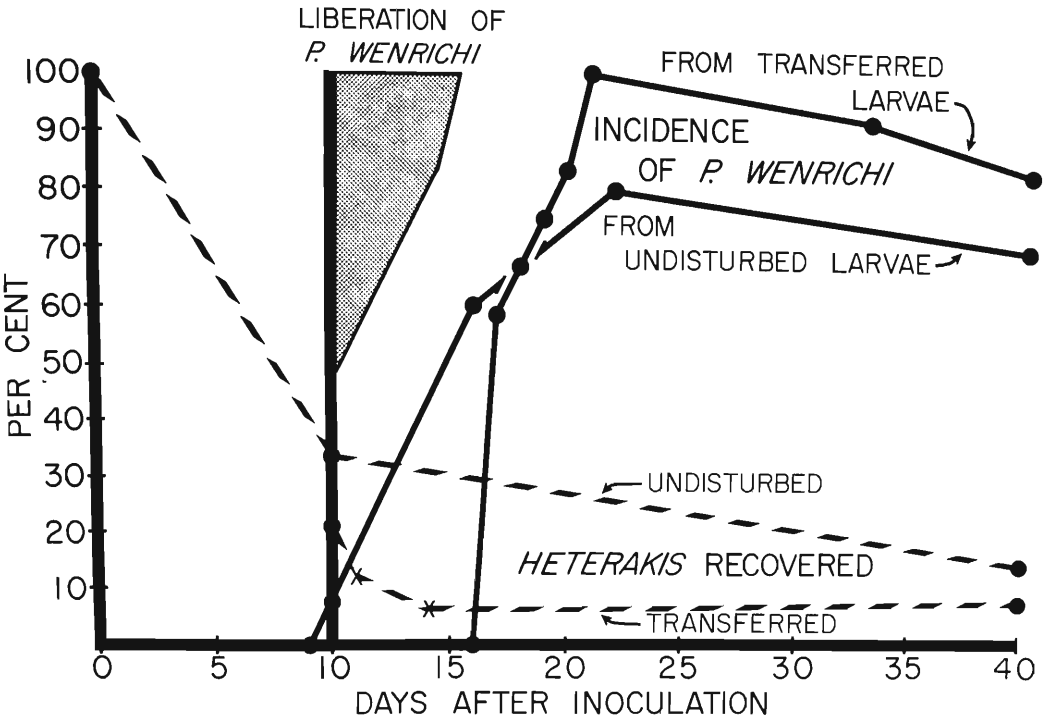

Figure 1. Comparison of the retention of undisturbed *Heterakis* with those transferred as 10-day larvae, and the cumulative incidence of *Parahistomonas wenrichi* accompanying each situation. The vertical line at 10 days represents the time of transfer of the larvae. On it, the incidence of infection with *P. wenrichi* from undisturbed larvae, the per cent of 10-day *Heterakis* larvae recovered and transferred, and the calculated frequency of effective liberations of *P. wenrichi* by transferred larvae (shaded area) are plotted. With regard to the latter circumstance, such liberations can be considered as having started soon after the larvae were transferred, to have attained 83% by day 4, and 100% by day 5 following transfer.

only one of the donor chickens. However, it appeared in the cecal discharges of seven of the 12 turkeys 7 days after the larvae were transferred. On each of the next 3 days, an additional turkey voided cecal discharges containing *P. wenrichi*, and by the 11th day, all birds were shedding numerous nonpathogenic histomonads. By the 23rd day after the transfer of the *Heterakis* larvae, *P. wenrichi* was no longer found in the cecal droppings of one of the turkeys, and at necropsy a week later still another had lost its histomonads. By this time these 12 turkeys harbored a total of only 307 heterakids, a third of the number transferred to them 30 days earlier. As shown in Figure 1, this amounts to 7% of the original 360 embryonated eggs given each donor chicken. Only 142 of the 307 heterakids were females, most of which appeared 4 or 5 days younger than their actual age of 40 days, as adjudged by the numbers of eggs they contained. Three weeks later, when embryonation should have been completed (Lund and Burtner, 1958), these worms contained an average of only 82 embryonated eggs.

As for the control birds, *P. wenrichi* first became numerous enough to be detected in the cecal discharges of one bird 10 days after the embryonated eggs had been given. Thereafter, the incidence rose gradually, so six of the 10 chickens had voided histomonads by the 16th day. The incidence culminated at 80% on the 23rd day. One of the eight infected birds had lost its histomonads by the 40th day, when

Table 1. Transplantation of 10-day-old *Heterakis gallinarum* larvae: Effects on development of heterakids and transmission of *Parahistomonas wenrichi*.

	Controls, in which larvae developed undisturbed	Donors, in which larvae developed 10 days
No. of *Heterakis* in original inoculum	360	360
No. of chickens	12	12
No. of chickens surviving to yield data	10*	12
Average no. of *Heterakis* 10 days after inoculation	–	118.2
Incidence of *Parahistomonas wenrichi* at 10 days, %	8	8
		Recipient turkeys, that received 10-day larvae
No. of turkeys given transplanted larvae	–	12
Average no. of larvae transplanted	–	77 (range 28–235)
Highest incidence of *P. wenrichi*	80% (at 22 days)	100% (at 21 days)
Incidence of *P. wenrichi* at necropsy (40 days),† %	70	75
Average no. *Heterakis* recovered at necropsy	49.9	25.6
Recovery of *Heterakis* larvae on basis of larvae transferred, %	–	33.2
Recovery of *Heterakis* on basis of 360 eggs, %	13.9	7.1
Average no. of mature female *Heterakis* per bird	25.3	11.8
Average no. of embryonated eggs per mature female	132	82

* Two chickens died early of causes unrelated to the experimental procedures.
† Days after the original use of the 360 embryonated eggs.

these control chickens were necropsied. At this time these 10 chickens yielded 499 heterakids, a recovery of 13.9%. All worms were mature; 253 were females containing an average of 132 eggs that embryonated.

For direct comparison of the performance of undisturbed heterakids and those transferred at 10 days, the above results and certain other data are shown in Table 1. The times at which *P. wenrichi* attained various incidences in the control chickens and the recipient turkeys, and the relationship between such incidences and the retention of *Heterakis* as determined at necropsy of the birds in both tests are depicted graphically in Figure 1.

Discussion

The loss in 24 hr of 45% of the larvae transferred on the preliminary test was no doubt occasioned partly by the ordeal of washing, but also by the relatively unfavorable distribution of the larvae as they arrived in the ceca of the recipients. When they were removed from the donors, 54% of all of the larvae were in the distal quarters of the ceca, regions of relative quiet to which all vigorous larvae migrate shortly after their emergence from the cecal mucosa or its glands or deep crypts. Efficient as the system of rectal perfusion is in delivering water promptly to the ceca (Browne, 1922), and accepting the probability that the larvae were also carried in, it is highly improbable that such a large portion of their numbers could be as favorably placed as they were before being disturbed. These relatively large (3.0–4.5 mm) larvae are not known to reenter the mucosa. Neither were larvae found in sufficient numbers in the cecal discharges to suggest that the heavy losses were due largely to their being voided intact. Apparently, half or more of the transferred larvae die and undergo dissolution during the first 4 days in their foster host, with the majority being lost during the first day. The extent of such losses have been confirmed various times since this test was completed. Larvae that survived this period had once more migrated to the quiet distal end of the cecum, where the vast majority were found 13 and 30 days after transfer. At this site, losses prior to maturity were infrequent, but the struggle had apparently cost the survivors about 5 days of development: these 40-day females yielding only about as many eggs capable of embryonation as indicated are usual for females 35 days old (Lund, unpublished observations).

The most conspicuous effect of transplanting the larvae was the early and precipitous rise

in the incidence of *P. wenrichi*. When transmitted through embryonated *Heterakis* eggs or by the larvae in earthworms, *P. wenrichi* has never been detected in the cecal discharges until 10 days after inoculation. Allowing for a multiplication period of 5½ days, these early detections must have resulted from liberations at 4½ days (Lund, 1968). There is no basis whatsoever for assuming that the transfer of the *Heterakis* larvae could shorten the multiplication period of the histomonad. Consequently, detections of *P. wenrichi* 7 to 11 days after the *Heterakis* larvae were transferred must indicate that the histomonads initiating such infections were liberated by 5½ days after such transfers. Moreover, 60% of the infections became evident on the 7th day, indicating that liberations during the first 36 hr were frequent. These periods of liberation, designated by the shaded area on Figure 1, correspond precisely with those of greatest losses of *Heterakis*, indicating that liberations following the death and accompanying the disintegration of larvae may be the usual means by which *P. wenrichi* infections are initiated. These nonpathogenic histomonads are not known to invade the tissues of the definitive host (Lund, 1963). One may now question whether they have the means of escaping from the intact tissues of the intermediate host.

As can be observed in Figure 1, 21.4% of the potential 360 *Heterakis* larvae were transferred to the recipient turkeys, and only 6% were retained by the 4th day after transfer. Thus, the calculated average heterakid loss during the first 4 days (the period of liberation of *P. wenrichi*) was 21.4% minus 6% = 15.4%, or about 55 larvae. The incidence of *P. wenrichi* infections resulting from liberations during this time was 100%. On the preliminary test in which 150 embryonated eggs were given each donor chicken, three of seven turkeys necropsied 13 days after transfer of the heterakids had *P. wenrichi*, an incidence of 43%. During the period of liberation of the histomonads initiating these three infections, the birds lost an average of 14% of their initial potential of 150 larvae, or 21 larvae. It is noteworthy that the ratio of larvae lost to incidence of *P. wenrichi* infections was similar: $\frac{55}{100}$ and $\frac{21}{43}$ on the second and preliminary tests, respectively. This similarity may or may not be entirely coincidental.

Hitherto there have been only two ways in which the prevalence of *P. wenrichi* in *Heterakis* could be expressed: (1) by the ratio of female heterakids that carried histomonas-bearing eggs to those that did not, and (2) by the number of embryonated eggs fed to test birds for each *P. wenrichi* infection (Lund and Burtner, 1957; Lund, 1971). These relationships may still be useful, but it is now rather obvious that many heterakid larvae must be carrying *P. wenrichi* without liberating the organism under usual conditions. Moreover, it appears necessary to relate liberations to heterakids lost, rather than merely to those that can be counted at necropsy.

Among the implications of these findings are at least the following:

1. If the same situation prevails with *Histomonas meleagridis*, anyone who might attempt to control histomoniasis by destroying heterakids might induce liberation of more histomonads than would otherwise be freed.

2. If some or many heterakis larvae carry histomonads without liberating them, could such histomonads survive to be carried in the eggs of the new generation, with another opportunity to be liberated?

3. Is it not in keeping with the economy of nature that heterakid larvae with the stamina to develop to maturity help perpetuate their own kind, but that at least some of those larvae that cannot do this, may help perpetuate the histomonad?

Acknowledgment

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Redescription of *Heterodera rostochiensis* (Nematoda: Heteroderidae) with a Key and Notes on Closely Related Species

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ABSTRACT: *Heterodera rostochiensis* is redescribed on the basis of topotype specimens from Rostock, and a neotype is designated. Morphology of the stylet guide and vulval bodies are briefly discussed, and "race 1" is the suggested designation for this nematode in the United States rather than "pathotype A" as in England. New data on the original cysts of *H. leptonepia* are presented and types established for the species. *Heterodera pseudorostochiensis* is placed as a new synonym under *H. tabacum*. The nomenclatural status of the "Mexican cyst nematode" is discussed, and it is considered to be conspecific with *H. virginiae*. A key to the six *Heterodera* species of the *rostochiensis* group (round cysts) is presented.

The golden nematode, *Heterodera rostochiensis* Wollenweber, 1923, is a major pest of potatoes, and recently was reported by Spears (1968) as occurring in 40 countries throughout the world. Since then its occurrence has been established in two additional countries, Tunisia[2] and Venezuela.[3] Within the United States, this potato parasite is known only in Steuben County and Long Island in New York state, but still poses a potential threat to potato production in other states. It was found in Delaware (Spears, 1969) on a single farm, but was subsequently reduced below detection levels by vigorous regulatory procedures.

This cyst nematode was first reported on potatoes in Germany by Kuhn (1881) at which time it was though to be the sugar beet nematode, *H. schachtii* Schmidt, 1871. [See Franklin (1951) for historical details to about end of 1948.] Wollenweber (1923) first recognized morphological differences between the sugar beet nematode and the cyst nematode on potato, and at the same time he briefly described the latter as *H. rostochiensis*. For the next few years the specific status of this form was not generally recognized or accepted, and most workers considered it a "strain," or at most a subspecies of *H. schachtii*. Franklin (1940) presented a more complete description of *H. rostochiensis*, and gave it full recognition as a valid species with authorship credited to Wollenweber as we do today.

During the last 20 years the description of additional species in the "*rostochiensis* group" has increased greatly the need for a more complete description of the golden nematode. This paper presents a redescription of *H. rostochiensis* based on specimens obtained from potatoes in Rostock, German Democratic

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[2] Personal communication from Mr. J. F. Spears, Associate Director, Plant Protection Division, Agricultural Research Service, U. S. Department of Agriculture, Hyattsville, Maryland.
[3] Specimens submitted for identification to senior author through Dr. W. F. Mai, Cornell University, Ithaca, New York, by Dr. F. Dao D. of Aragua, Venezuela.

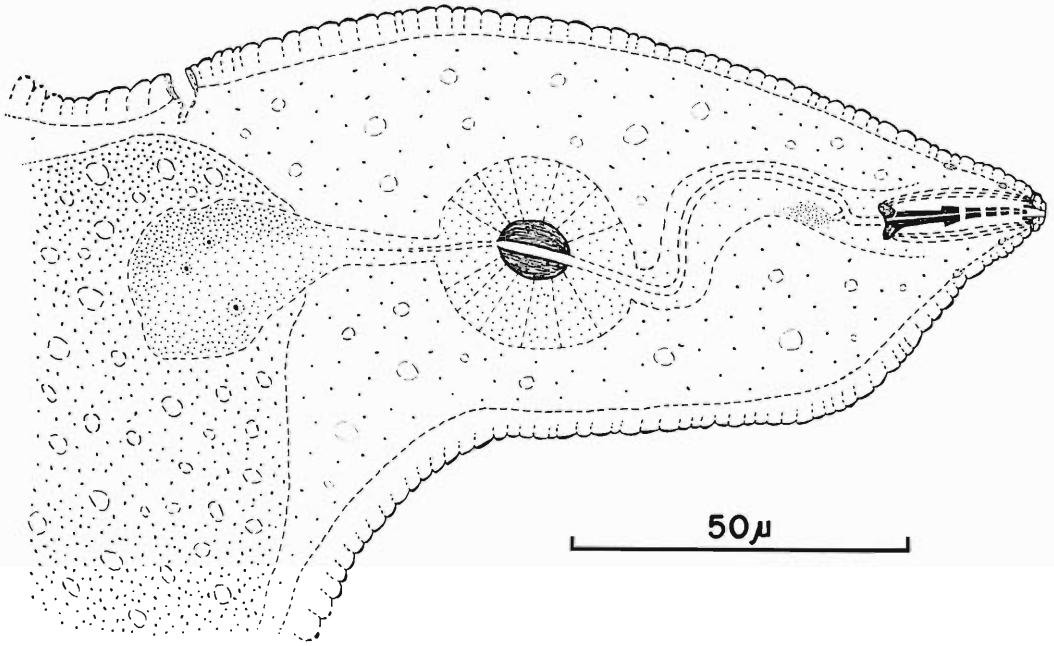

Figure 1. Drawing of anterior portion of female of *H. rostochiensis*.

Republic,[4] with designation of a neotype; new details and lectotype designation for *H. leptonepia* Cobb and Taylor, 1953; comments on the relationship of the Mexican cyst nematode and *H. virginiae* Miller and Gray, 1968; and a key to the described species of the "*rostochiensis* group."

Heterodera rostochiensis Wollenweber, 1923

Syn.: *H. schachtii* f. *solani* Zimmermann, 1927
H. schachtii rostochiensis Kemner, 1929
H. schachtii O'Brien and Prentice, 1930
H. (*Globodera*) *rostochiensis* Wollenweber, 1923 (Skarbilovich, 1959)

MEASUREMENTS: 50 females (Figs. 1, 3, 5, 6, 7, 8, and 9)—Length (including neck) 0.52 mm (0.42–0.64); width 0.34 mm (0.27–0.43); L/W ratio 1.5 (1.2–2.0); stylet 23 μ (22–24); outlet of dorsal esophageal gland 6.2 μ (5.8–7.0).

Data on neotype (female): Length 0.47 mm; width 0.32 mm; L/W ratio 1.4; stylet 23.8 μ; outlet of dorsal esophageal gland 6.4 μ; excretory pore at base of neck and 130 μ from anterior end; vulva slit 10 μ in length; anus 41 μ from nearest edge of the hyaline vulval membrane, the latter measuring 13 μ in length (on the longer axis) and 7 μ in width.

DESCRIPTION OF FEMALES: Body pearly white, ovate to subspherical in shape, with elongate, protruding neck, rounded posteriorly. As maturity continues toward the cyst stage, body undergoes color changes through yellow to light golden. Cuticle thick, outer layer rugose, and punctations near or just beneath the surface. Head slightly set off, bearing two annules, and commonly appearing about as illustrated. Cephalic framework weakly developed. Stylet fairly strong, with slight curvature, and well-developed basal knobs sloping posteriorly. Anterior and posterior cephalids generally located as illustrated. Median bulb large, nearly spherical, with well-developed

[4] For kindly providing this material, sincere appreciation is extended to Dr. H. Stelter and Dr. Ulrich, Amt. Institutsdirektor, Deutsche Akademie der Landwirtschaftswissenschaften zu Berlin, Institut fur Pflanzenzuchtung, 2551 Gross-Lusewitz, Kreis Rostock, Deutsche Demokratische Republik.

valve. Esophageal glands often obscured but appearing custered near base of neck. Excretory pore prominent, located 131 μ (105–175) from anterior end and always at or near base of neck. Vulva ellipsoid in shape, quite small, and measuring 12 μ (7–14) in length and 7 μ (5–11) in width. Vulva slit 9 μ (6–11) in length. Often underneath the vulva and generally in a cluster (see Fig. 9) are vulval bodies, being highly variable in size and shape. Anus much smaller than vulva and is located 47 μ (39–80) from the nearest edge of vulva and generally opposite the long axis of the latter.

MEASUREMENTS: 50 males (Figs. 2C, D, E, F, G, and H)—Length 1.08 mm (0.89–1.27); a = 27 (22–36); b = 5.9 (4.9–7.3); c = 267 (161–664); stylet 26 μ (25–27); outlet of dorsal esophageal gland 6.4 μ (5.3–7.0); spicules 35 μ (32–39); gubernaculum 12 μ (10–14); tail 4.4 μ (1.7–6.7).

DESCRIPTION OF MALES: Body slender, vermiform, tapering slightly at both extremities. Cuticle with prominent annulation; subcuticular annulation less distinct and occurring twice as often as on cuticle. Lateral field measuring 7.0 μ (6.7–8.4) in width at midbody, with 4 equally spaced lines except at its beginning in anterior portion. About midway, body measures 39 μ (31–46) in width. Head slightly set off, hemispherical, with six annules. Cephalic framework heavily sclerotized. Stylet very stong, with prominent knobs appearing in lateral view generally as illustrated. Stylet guide seen anteriorly as the usual lyre-shaped structure with a ring at its base encircling the stylet; attached to the base of this lyre-shaped guide is a membranous, sleevelike extension of the guide reaching about half the length of the basal stylet shaft, ending in another ring encircling the stylet at that point (see Fig. 2, D). Anterior and posterior cephalids present, located about as illustrated (Fig. 2, D). Median bulb ellipsoidal with its center located 99 μ (85–112) from anterior end. Excretory pore about two annules posterior to commonly distinct hemizonid. One testis. Spicules slightly arcuate, with tips rounded, unnotched. Tail short, variable in both length and shape (see Figs. 2 C, E, F, G, and H).

MEASUREMENTS: 50 second-stage larvae (Figs. 2A and B)—Length 0.43 mm (0.37–0.47); a = 19 (16–23); b = 2.3 (2.2–2.5); c = 8 (7–9); stylet 22 μ (21–23); outlet of dorsal esophageal gland 5.5 μ (5.0–6.7); tail 51 μ (44–57); hyaline tail terminal 24 μ (18–30); caudal ratio A = 3.4 (2.8–4.4); caudal ratio B = 10.8 (5.5–17.0).

DESCRIPTION OF SECOND-STAGE LARVAE: Body tapering at both extremities but much more so posteriorly. Subcuticular annulation twice as frequent as on cuticle. Lateral field with four lines for most of body length, the outer two crenate but without aerolation. Body measures 23 μ (19–26) at widest part. Head slightly set off, bearing five annules, and considerably wider at its base than in height, presenting a rounded though rather anteriorly flattened appearance as in Fig. 2 A. Cephalic framework heavily sclerotized. Stylet well developed, with prominent knobs appearing in lateral view as illustrated. Stylet guide as described above for males. Anterior and posterior cephalids present, located about as shown. Valvated median bulb prominent, ellipsoidal, with its center located 68 μ (64–76) from anterior end. Isthmus and esophageal glands typical for the genus. Excretory pore posterior and almost adjacent to hemizonid. Genital primordium located slightly posterior to midbody and commonly consists of four cells. Tail tapering to small, rounded terminus. Phasmids generally difficult to see, located about halfway on tail.

MEASUREMENTS: 50 cysts (Figs. 4, 10, 11, 12, 13, 14, 15, and 16)—Length (including neck) 0.68 mm (0.45–0.99); width 0.54 mm (0.25–0.81); L/W ratio = 1.27 (1.0–1.8); diameter, or longest axis of fenestra (A) 15 μ (8–20); distance from anus to nearest edge of fenestra (B) = 68 μ (29–116); B/A ratio (Granek's ratio) = 4.5 (2.0–7.0).

DESCRIPTION OF CYSTS: Cysts brown in color, ovate to spherical in shape, with protruding

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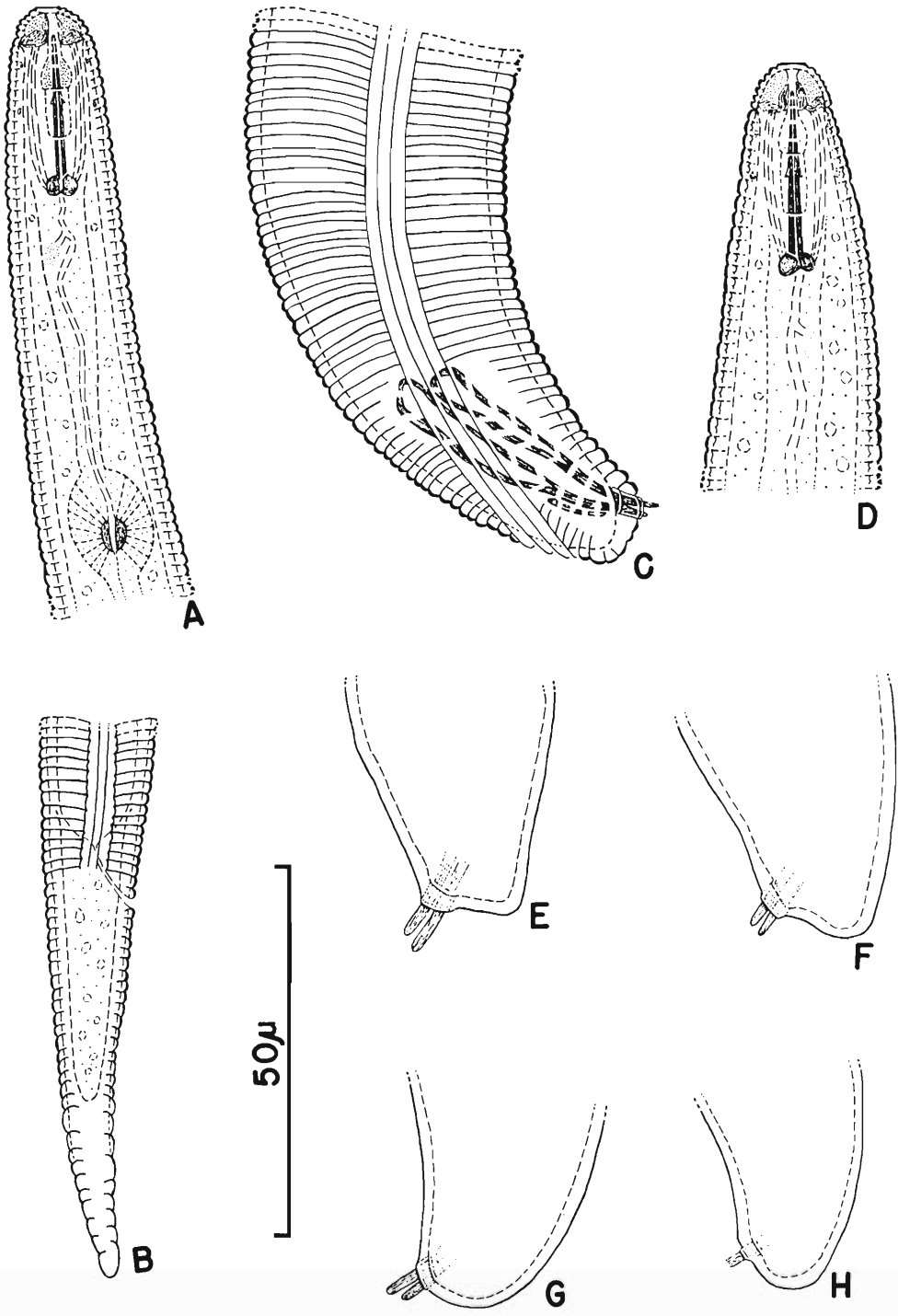
Figure 2. Drawings of *H. rostochiensis*. Second-stage larva: A—Anterior; B—Posterior. Male: C—Posterior; D—Anterior; E–H—Outline of posterior portion showing variations in tail shape.



neck; circumfenestrate, abullate, and without the distinct "vulval bodies" commonly seen in white females. Fenestra much larger than the small but distinct, V-shaped anus. Cyst wall pattern basically as in female but often more prominent, and especially near midbody, tends to form wavy lines going latitudinally around body (see Fig. 16). Punctation generally present but variable in intensity and arrangement.

MEASUREMENTS: 50 eggs—Length 105 μ (95–115); width 45 μ (42–48); L/W ratio = 2.3 (2.0–2.6). Egg shell hyaline, without visible markings. (See key for the distinguishing specific characteristics.)

NEOTYPE: Female: Collected by Dr. H. Stelter in May 1970. Slide T-203t, United States Department of Agriculture Nematode Collection, Beltsville, Maryland.

TYPE HABITAT, HOST, AND LOCALITY: Roots of potato (*Solanum tuberosum*) in Rostock, German Democratic Republic (East Germany).

Communication from Dr. Stelter indicated this nematode material to be "Rasse A," which is not known to break the *andigena* source of resistance.

Discussion

1. The golden nematode is perhaps the single most important species of plant nematode, and is subject to strict regulatory actions in the United States and other countries. Accurate identification of *H. rostochiensis* is, therefore, particularly critical, but has become increasingly difficult because of descriptions of closely related forms in recent years. The prospect of another species being split from *H. rostochiensis* (Jones et al., 1970) further intensifies the need for clear identity of the golden nematode. The designation of a neotype from topotype material in the present description is especially important in firmly establishing this species.

2. As background information for the present study, we collected and examined populations of *H. rostochiensis* from most countries of occurrence during the past several years. As have other workers [ex. Evans and Webley (1970), Webley (1970), and Jones et al. (1970)], we noted that certain populations, including those from Peru, were in many respects not morphologically identical. However, specimens examined in great detail from Belgium and from the two areas of occurrence in the United States (Long Island and Steuben County, New York) proved to be morphologically similar to those from Rostock in all essential points. In view of its morphology and failure to attack potato varieties having the *andigena* source of resistance (Peconic and Wauseon), the nematode in the United States is clearly equivalent to "pathotype A" in England (Jones et al., 1970; Webley, 1970). We therefore suggest the use of the term, race 1 (or race A), for the population in this country rather than "pathotype A." This would be consistent with terminology in use for such infraspecific forms in *H. glycines* (Golden et al., 1970); in other nematodes, as races of *H. avenae* Wollenweber, 1924, and *Ditylenchus dipsaci* (Kuhn, 1857) Filipjev, 1936; and in other disciplines, as parasitology, zoology, and plant pathology.

3. The stylet guide, as illustrated for larvae in Figure 2A and for males in Figure 2D, is accurate for this species as far as could be determined with fixed material (in formalin or glycerin). However, in certain other *Heterodera* species where shown, the sleevelike extension from the anteriorly placed lyrelike structure is different than depicted herein for *H. rostochiensis*. Hirschmann (1959) showed in *H. glycines* Ichinohe, 1952, a short sleevelike extension ending in a ring encircling the anterior portion of the basal shaft of the stylet (in *H. rostochiensis* the ring is located at about one-half the basal shaft). In *H. betulae*, Hirschmann and Riggs (1969) showed the sleevelike extension reaching almost to the stylet knobs and with no indication of its having a ring at its terminus. Miller and Gray (1968) illustrated the guide ring in a similar manner in their description of *H. virginiae*. It appears now that the stylet guide, when clearly and

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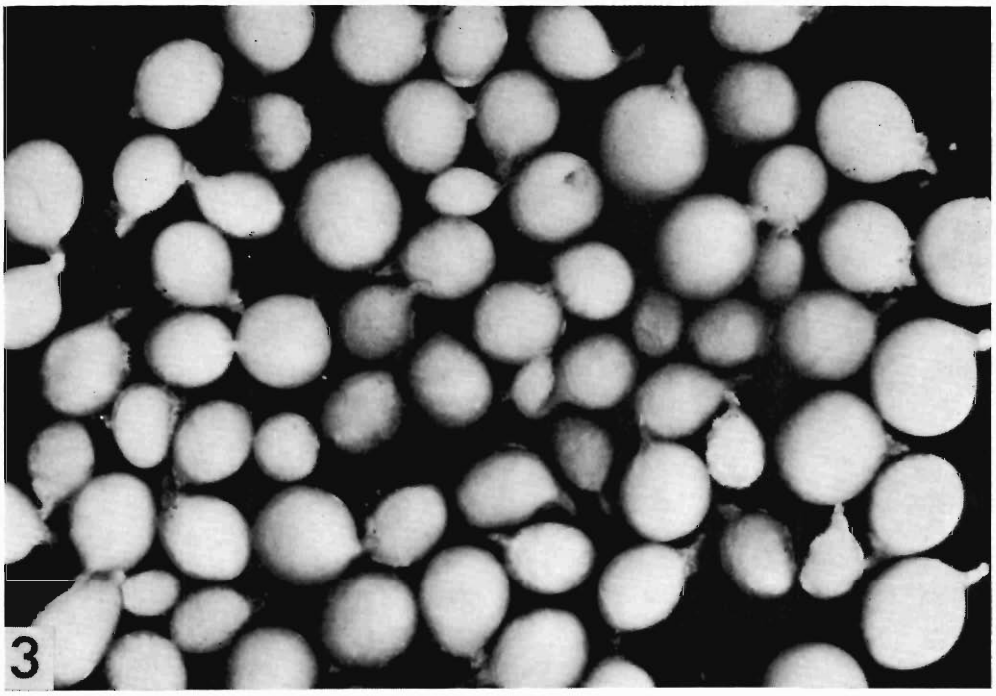
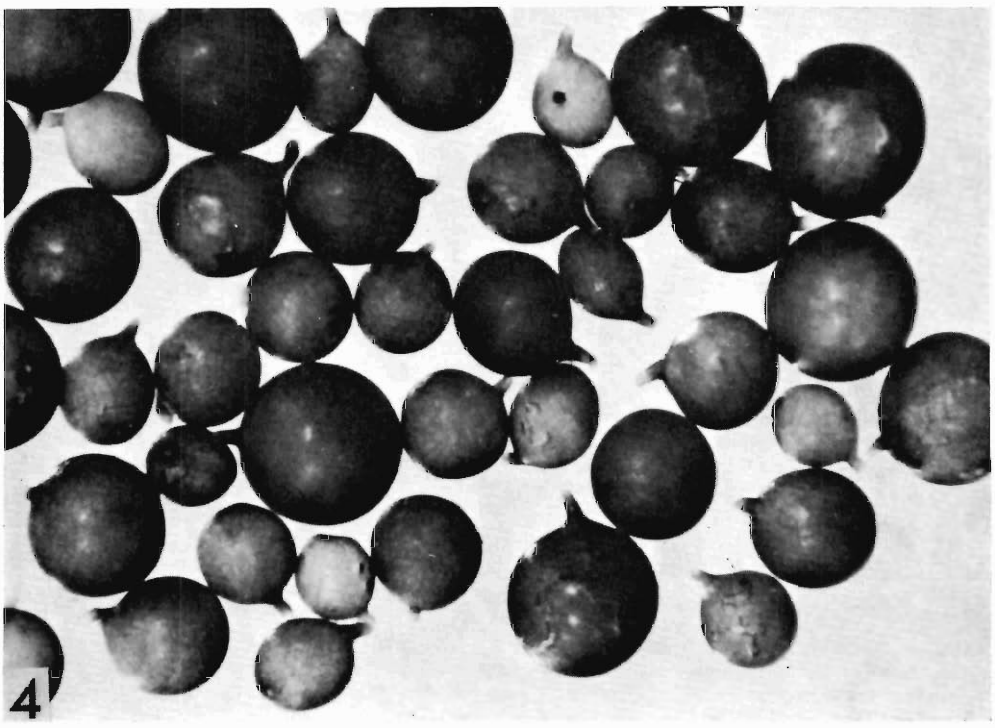
Photomicrographs of whole specimens of *H. rostochiensis*. Fig. 3—White females; Fig. 4—Cysts. (Both at same magnification.)





completely described for all of the various *Heterodera* species, might prove to be of taxonomic value.

4. In describing *H. millefolii*, Kirjanova and Krall (1965) referred to "bullae" in both that species and *H. rostochiensis*. They also pointed out that in the latter species the "bullae" formed compact circles around the "fenestra" while in *H. millefolii* the "bullae" were not numerous and occurred singly or in small groups a short distance from the "fenestra." Wilson (1968), without reference to the work by Kirjanova and Krall, reported on similar structures in *H. rostochiensis*, *H. tabacum*[5] Lownsbery and Lownsbery, 1954, and *H. schachtii*, calling them "vulval bodies." He stated they were embedded in the hypodermis and in close association with the "fenestra" in the species examined. Our findings support both of the above observations relative to *H. rostochiensis*, and we accept, pending a more appropriate choice, Wilson's name of "vulval bodies." As indicated in Figure 9 the vulval bodies in the golden nematode are deep in the hypodermis, and are compact and clustered in the immediate vulval area of the white female. Sometimes there are few, sometimes many, clustered in the area. The individual vulval bodies seem to be variable in size and shape, often appearing as irregular, deflated balloons. These vulval bodies also might prove in time to have some taxonomic value in the cyst nematodes.

Heterodera leptonepia Cobb and Taylor, 1953

This very interesting species was described almost 20 years ago by Cobb and Taylor (1953) on the basis of three cysts collected from soil with potatoes taken on as ship's stores at Callao, Peru. Presumably the cysts and potatoes came from Peru although the ship was known to travel the Pacific west coast almost entirely on the nitrate run from Peru and Chile to the United States. Two of these cysts contained eggs with larvae while the third was evidently empty. Since all of this material was retained, we remounted it in glycerin and examined the specimens again.

A striking feature emphasized in the excellent original description was the extreme slenderness of the larvae (a = 39). Other distinctive larval characters included an average length of 0.56 mm; a short stylet of 18 μ; the outlet of the dorsal esophageal gland being 12 μ (or ⅔ of stylet length) from the base of stylet; and no annules on the head. Our observations confirmed these and other points reported in the original description.

Through the years the opinion apparently developed among many workers that the cysts of *H. leptonepia* are essentially similar to those of *H. rostochiensis*. However, such is not the case, except for shape. In *H. leptonepia* the cyst wall pattern and B/A ratio are different from *H. rostochiensis* as shown in photomicrographs herein and described below.

LECTOTYPE: Cyst (Fig. 1D in original description and Figs. 17, 20, and 21 in present paper)—Circumfenestrate, abullate. Fenestra about 29 μ at greatest diameter; small anus 12 μ from nearest edge of fenestra, giving a B/A ratio (Granek's ratio) of 0.4.

PARALECTOTYPES: Two cysts, larvae, and eggs—cysts circumfenestrate, abullate. One cyst with fenestra about 24 μ at greatest diameter; small anus 23 μ from nearest edge of fenestra; B/A ratio = about 1. Second cyst (Figs. 18 and 19) with fenestra measuring 18 μ at greatest diameter; anus not located. Excretory pore in one cyst seen located at base of neck.

From original description—"Cysts: Light brown, more or less ovate, about 0.5 by 0.3

[5] Without explanation Kirjanova (1963) proposed the name "*H. pseudorostochiensis*, sp. nov." and placed *H. tabacum* in synonymy under it. Since no possible justification for this action is known to us, we propose the continued recognition of *H. tabacum* as a valid name and species, and the placement of *H. pseudorostochiensis* Kirjanova, 1963, as a new synonym under *H. tabacum*.

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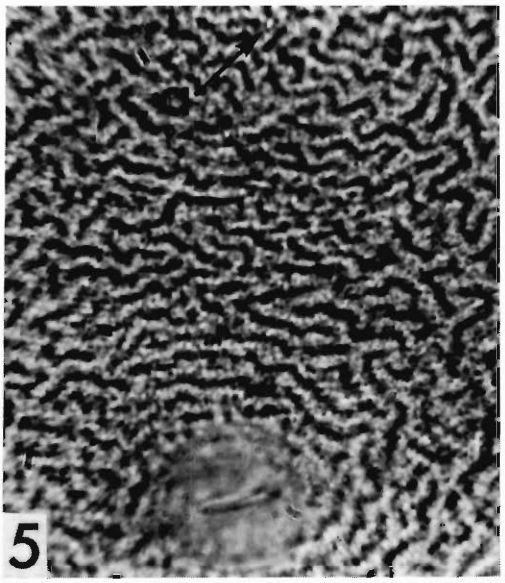
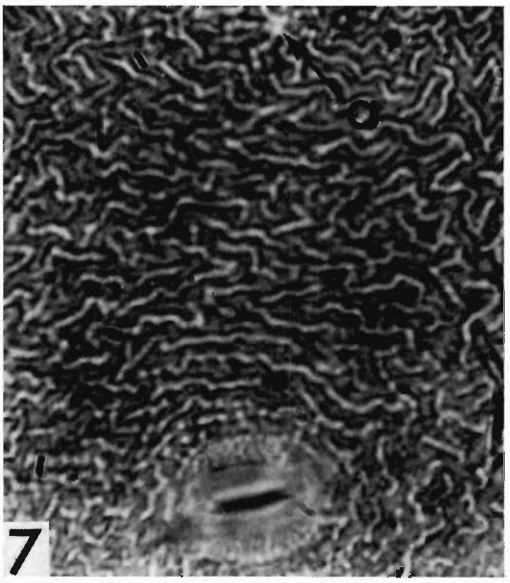
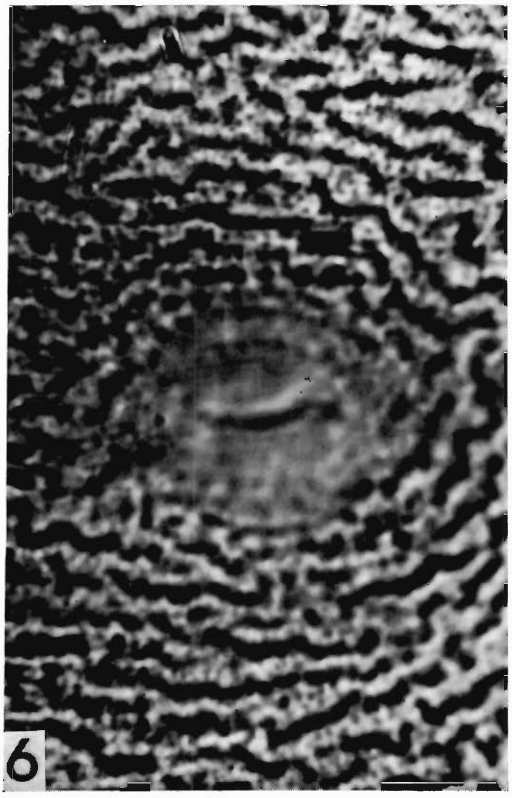
Photomicrographs of posterior portion of a single female of *H. rostochiensis*. Fig. 5—Vulval–anal area at outer surface. Fig. 6—Enlarged vulval area on surface. Fig. 7—Same as Fig. 5, but at deeper focus to under surface (note difference in appearance of pattern). Fig. 8—Same as Fig. 6, but at deeper focus to under surface. "a" with arrow indicates anus.







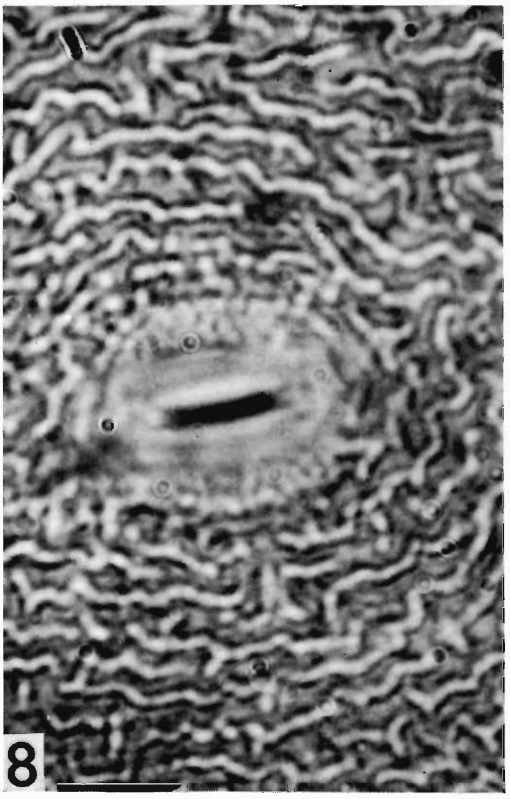


Figure 9. Photomicrograph of vulval bodies in white female of *H. rostochiensis*. "a" with arrows indicate vulval bodies. "b" with arrow indicates inner area of vulva.

mm, with distinct neck; smoothly rounded posteriorly as in *H. rostochiensis* and *H. punctata* Thorne, 1928. Vulvar opening round and much larger than the minute, pore-like anus, as is the case with *H. rostochiensis*; different from *H. punctata* which has anus located at a thin spot of cyst wall so that vulvar and anal openings appear about same size (Franklin 1940). Outer layer of cyst wall with rugose pattern of striae extending from neck to near vulva; immediately around vulva, striae interrupted, forming an irregular pattern as shown in Figure 1D. A lower layer of the cyst wall distinctly punctate, with minute dots arranged in closely spaced parallel rows at right angles to axis of cyst; dots irregularly spaced in rows."

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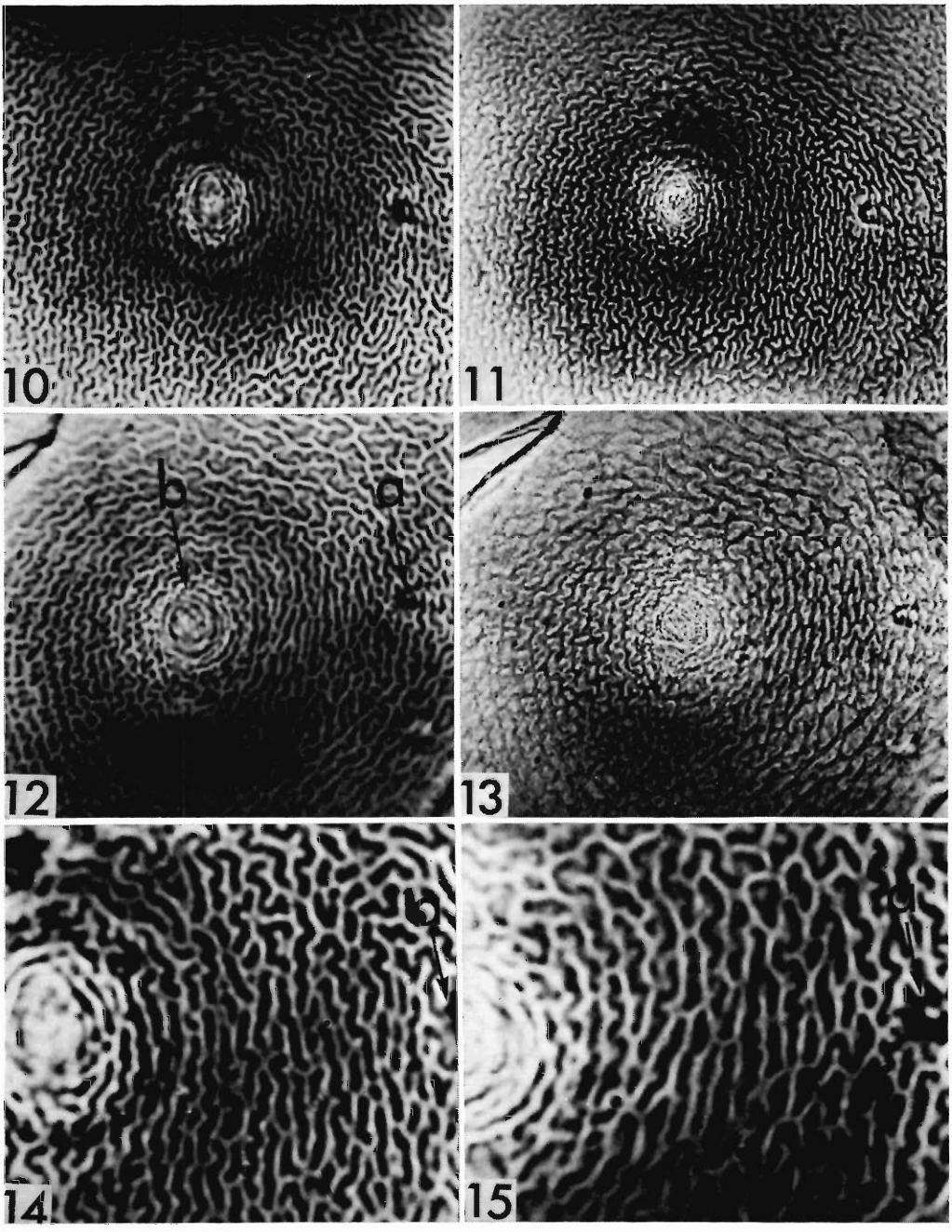
Photomicrographs of posterior portion of two cysts of *H. rostochiensis*. Fig. 10—Fenestral–anal area of one cyst at outer surface. Fig. 11—Same area and cyst as Fig. 10 but at deeper focus to under surface. Fig. 12—Fenestral–anal area of the second cyst at outer surface. Fig. 13—Same area and cyst as Fig. 12 but at deeper focus to under surface. (Note differences in pattern between the two cysts and at different



focus.) Fig. 14—Cyst wall pattern of Fig. 10 between fenestra and anus at higher magnification. Fig. 15—Cyst wall pattern of Fig. 12 between fenestra and anus at higher magnification. "a" with arrow indicates anus. "b" with arrow indicates fenestra.

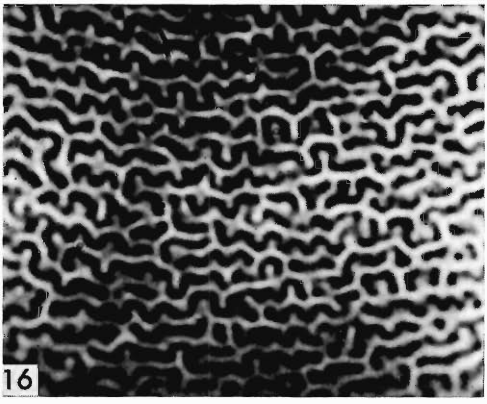


Figure 16. Enlarged photomicrograph of *H. rostochiensis* cyst near midbody.

For details on larvae, eggs, and diagnosis, see the original description, and the above comments on the larvae. Also, note certain characters in key to some *Heterodera* species in this paper.

LECTOTYPE: Cyst: Collected April 26 1952 by Inspector C. H. Oatridge of the Bureau of Entomology and Plant Quarantine, USDA, at the Oakland, California, port of entry, from soil (in ship's stores) with potatoes taken aboard at Callao, Peru. Slide T-202t, United States Department of Agriculture Nematode Collection, Beltsville, Maryland.

PARALECTOTYPES: Two cysts, larvae, and eggs: Same data and collection as lectotype. Slides T-989p–T-992p.

TYPE HABITAT AND HOST: Unknown. Thought possibly to be the roots of some solanaceous plant.

TYPE LOCALITY: Unknown. Thought to be some area in Peru.

As far as known, this nematode has never been found since the initial collection was made although it is obviously a very distinctive species. Perhaps more specimens and a host can be found in the future by sampling various plants in and around potato fields in Peru.

Mexican cyst nematode

As his doctorate thesis at the University of Wisconsin, Campos Vela (1967) made a very thorough study on the taxonomy, life cycle, and host range of a cyst nematode from Mexico which he named "*Heterodera mexicana* n. sp." in his thesis. As far as we know, there has not yet been a regular publication describing this proposed species. Consequently, in accordance with the present International Code of Zoological Nomenclature (especially Articles 7–9), this name is still unavailable (not properly published) as a specific name. When used as a specific name under these circumstances, as by Jones et al. (1970) and by Franklin (1971), it becomes a *nomen nudum* since it has never been published in the sense of the Code. Mayr (1969) discusses such a point clearly in his recent book (p. 347), and we agree that an unavailable name has no standing in zoological nomenclature and is best never recorded.

In describing *H. virginiae*, Miller and Gray (1968) correctly did not refer to the above nematode name as used in Campos Vela's thesis research. With both specimens and description of *H. virginiae* on hand, we soon had an opportunity also to examine material of the Mexican cyst nematode and Campos Vela's thesis. We were unable to find any consistent morphological differences of a specific nature between *H. virginiae* and the Mexican cyst specimens. Also, the measurements on most characters of specific value were identical, or nearly so, in both the thesis description of the Mexican cyst nematode and the published description of *H. virginiae*. As a matter of fact, Campos Vela pointed out (p. 43) that the morphological difference between his specimens and *H. virginiae* was the shape of the dorsal knob of the white female stylet. This character seemed to us to vary considerably between individual females and in the position of the specimen when viewed. This variable character, and the host differences indicated

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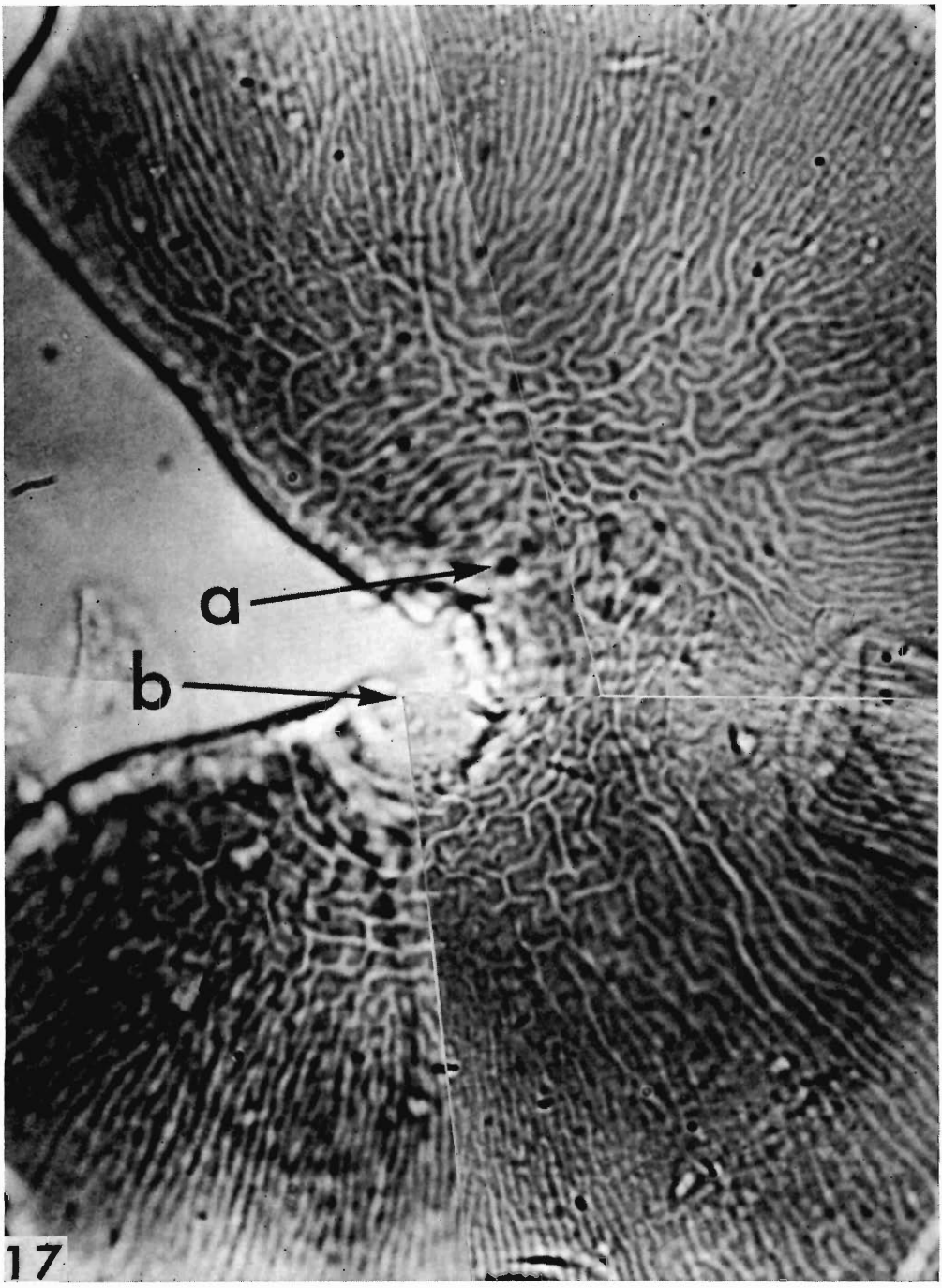
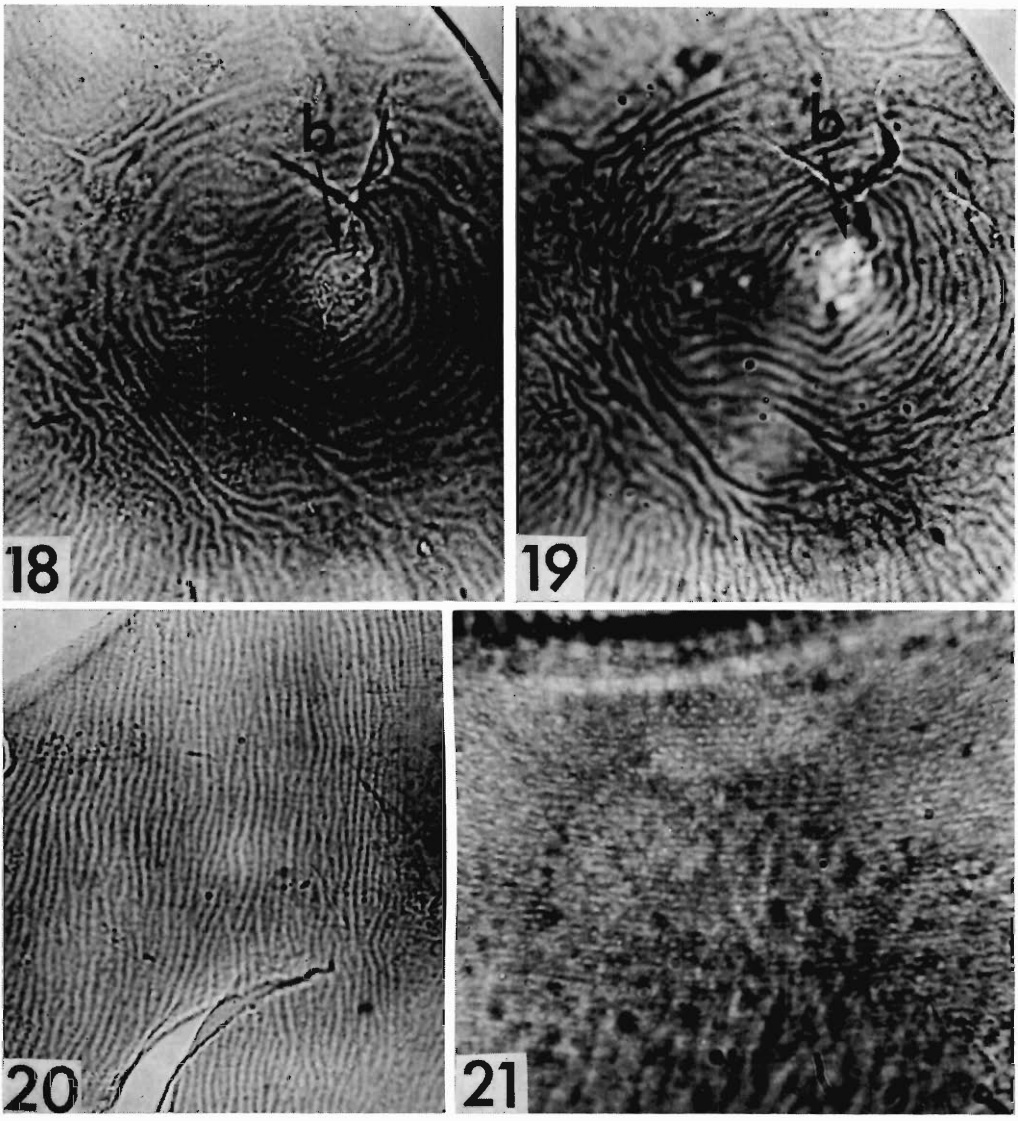
Fig. 17. Composite photomicrograph of the posterior portion of a cyst of *H. leptonepia* (note pattern around fenestra and heavy striae leading anteriorly). "a" with arrow indicates anus. "b" with arrow indicates fenestra.





Photomicrographs of cysts of *H. leptonepia*. Fig. 18—Posterior portion with focus at outer surface. Fig. 19—Same as Fig. 18 but with deeper focus to under surface. Fig. 20—Portion of cyst near midbody showing heavy longitudinal striae. Fig. 21—Latitudinal rows of punctation shown at high magnification. "b" with arrow indicates fenestra.

by Campos Vela, do not seem to justify specific status for this nematode. In the absence of a full published description giving clearer and more reliable characters, we prefer to consider the Mexican cyst nematode as conspecific with *H. virginiae*. In the meantime, should some identity of the population of the cyst nematode from Mexico be needed, perhaps the term, Mexican race (or race 1) of *H. virginiae* would be appropriate.

Key to the *Heterodera* species of the *rostochiensis* group (round cysts)

1. Cysts with large fenestra and very small anus 2
   Cysts with conspicuous fenestra and anus, both about equal in size ..... *H. punctata*
2. Cysts with excretory pore near base of neck; vulva slit* generally straight, less than 25 μ in length 3
   Cysts with excretory pore at mid-neck; vulva slit bow-shaped, about 35 μ in length ..... *H. millefolii***
3. Cyst wall pattern with rugose striae at midbody primarily extending latitudinally; larvae with "a" = about 18–25; stylet 20 or more μ in length; outlet of dorsal esophageal gland about ⅓ or less of stylet length 4
   Cyst wall pattern with prominent striae extending longitudinally from fenestral area to near base of neck; larvae with "a" = 39; stylet 18 μ long; and outlet of dorsal esophageal gland about ⅔ of stylet length ..... *H. leptonepia*
4. Cysts with B/A ratio (Granek's ratio) averaging 2.8 or less; outlet of dorsal esophageal gland of males averaging about 3.5 μ from base of stylet 5
   Cysts with B/A ratio averaging 4.5; outlet of dorsal esophageal gland of males approximately 6.4 μ from base of stylet ..... *H. rostochiensis*
5. Cysts with B/A ratio averaging 2.8; cyst wall pattern in fenestral area mazelike ..... *H. virginiae*
   Cysts with B/A ratio averaging 1.5; cyst wall pattern in fenestral area appearing as wavy but rather continuous lines encircling the fenestra ..... *H. tabacum*

As with other keys, this one is presented to aid in identification, perhaps eliminating some species from further consideration while pointing to one or more others for more careful examination. In any case, it is always advisable to refer to a particular species description before making a final decision on the identity of the nematode.

* Exact nature of vulva and slit in *H. leptonepia* is not known but statement probably applies.

** *Heterodera millefolii* was described by Kirjanova and Krall on the basis of one female only. A more complete description involving the various other stages is needed in order to know better the characters of this species.

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Enzyme Histochemistry of the Holdfast Organ and Forebody Gland Cells of *Alaria marcianae* (La Rue, 1917) (Trematoda: Diplostomatidae)[1]

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ABSTRACT: Histochemical staining tests for hydrolytic enzymes in adult *Alaria marcianae* (La Rue, 1917) revealed B-glucuronidase, aryl sulfatase B, and leucine aminopeptidase in the holdfast organ gland cells, chymotrypsinlike enzymes in the tegumental surface layer of the holdfast organ, and a cathepsinlike indoxyl C-esterase in the anterior mass of holdfast organ gland cells, forebody gland cells, and certain anterior forebody tegumentary cells; gamma glutamyl transpeptidase and trypsinlike enzymes were not present. Strong protease and hyaluronidase activity was indicated by substrate film methods in areas corresponding to the holdfast organ, the forebody gland cells and ducts, and the anterior forebody tegumentary cells. The results support the view that the gland cells associated with the holdfast organ and lappets secrete hydrolases for extracorporeal digestion and indicate a similar function for certain forebody tegumentary cells. Lysosomal enzymes apparently are not involved in extracorporeal digestion.

Recent studies on adult strigeoids indicate that at least one function of the holdfast organ and forebody gland cells is the synthesis and release of hydrolytic enzymes for extracorporeal digestion (Lee, 1962; Erasmus and Öhman, 1963, 1965; Öhman, 1965, 1966a, b; Bogitsh, 1966a, b; Erasmus, 1968, 1969a, b, c, 1970). In strigeoids which lack lappets and/or forebody gland cells other specialized cells (lappet cells, "subcuticular cells") apparently serve a similar function (Öhman, 1966a; Bogitsh, 1966a; Bogitsh and Aldridge, 1967; Erasmus, 1969a). Information on these enzymes has been derived from histochemical staining techniques and in vitro methods involving enzyme secretion.

The subject of the present study is the diplostomatid strigeoid, *Alaria marcianae* (La Rue, 1917). In a preliminary study of this parasite, Johnson, Bhatti, and Kanemoto (1971) reported acid phosphatase and nonspecific esterases in the tegumental surface layer and gland cells of the holdfast organ and nonspecific esterases in the forebody gland cells and ducts. The purpose of this study was to investigate further the nature of the hydrolases in these cells.

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Materials and Methods

Mesocercariae of *Alaria marcianae* were collected from naturally infected leopard frogs, *Rana pipiens* (Steinhilber and Co., Oshkosh, Wis.). Two dogs were injected intraperitoneally with 2,000 and 2,500 larvae, and killed 34 and 30 days, respectively, after infection.

ENZYME HISTOCHEMISTRY: Free and attached adults for esterases and B-glucuronidase were fixed in cold (4 C) formol–calcium for 24 hr, rinsed in cold 0.1 M cacodylate buffer (pH 7.4) containing 2.2 M sucrose for 24 hr, and stored at 4 C in this solution until used. Material for esterases, aryl sulfatase, gamma glutamyl transpeptidase, B-glucuronidase, leucine aminopeptidase, chymotrypsin- and trypsinlike enzymes, and for the substrate film methods were quenched in liquid nitrogen (–180 C), and then stored in stoppered tubes in a desiccator at –20 C. Sections cut at 8 μ on an International Harris Cryostat (–20 C) for enzyme staining or at 18 μ for the substrate film methods were mounted on albuminized slides.

In all enzyme staining methods mammalian tissues served as a positive control and deletion of the substrate served as a negative control. Control sections with inhibitors were preincubated for 60 min at room temperature in the appropriate buffer containing the inhibitor which was then also incorporated in the substrate medium.

The following enzyme staining techniques were used: esterases, a-naphthyl acetate method; cholinesterases, acetylthiocholin iodide method; leucine aminopeptidase, L-leucyl B-naphthylamide HCl method (Barka and Anderson, 1963); esterases, indoxyl method (Pearse, 1960); B-glucuronidase, naphthol AS-BI glucuronide method (Hayashi, Nakajima, and Fishman, 1964); aryl sulfatase, barium capture method (Hugon and Borger, 1967); gamma glutamyl transpeptidase, a-L-glutamyl B-naphthylamide method (Glenner, Folk, and McMillan, 1962); trypsinlike enzymes, DL-BANA method (Glenner, Hopsu, and Cohen, 1964); chymotrypsinlike enzymes, benzosalicylanilide B-phenylpropionate method (Lagunoff and Benditt, 1964).

Esterase inhibitors employed with the indoxyl method were: sodium fluoride (2×10^{-3} M), nonspecific esterases (Gomori, 1955); eserine sulfate (10^{-5} M), cholinesterases (Pearse, 1960); sodium fluoride (7.5×10^{-2} M) and eserine sulfate (2×10^{-4} M), both nonspecific esterases and cholinesterases (Nachlas and Seligman, 1949).

Inhibitors used for types of nonspecific esterases with the indoxyl method were (Pearse, 1960): silver nitrate (10^{-2} M), types A and B—100%, type C—50%; copper sulfate (10^{-3} M), types A and C—100%; lead nitrate (10^{-3} M), type A—50%. The inhibitor employed for the cathepsinlike indoxyl C-esterase (Hess and Pearse, 1958) was lead nitrate (10^{-3} M)—100%; activators used were ascorbic acid (2×10^{-3} M) and cysteine (10^{-3} M).

The inhibitor for B-glucuronidase was potassium hydrogen saccharate (1.25×10^{-4} M); for gamma glutamyl transpeptidase, lead nitrate (10^{-3} M), and for leucine aminopeptidase, potassium cyanide (10^{-2} M).

The following substrate film methods were employed: Fratello's (1968) method and a modification of Shear's method (1969) for proteases, and the Szemplinska, Sierakowska, and Shugar method (1962) for hyaluronidase. Alternate sections, briefly fixed in Bouin's fluid, were mounted on albuminized slides and stained with hematoxylin and eosin for comparison with substrate film sections.

In Shear's method, sections were mounted directly on exposed and developed photographic plates (Kodak, Tri-X Panchromatic plates, Type B) previously softened in phosphate buffer (pH 4, 6, and 8) and dampdried. Sections were incubated for 2 hr at 39 C in a humid atmosphere, subsequently air-dried, dehydrated in ethanol, cleared in xylene, and mounted in Permount. Control sections were softened in 4% formalin. The procedure was the same for Fratello's method except that the sections were mounted on Kodacolor film (ASA 80).

In the Szemplinska method for hyaluronidase, a 0.24% sodium hyaluronate solution (pH 8) was mixed with an equal volume of 5% gelatin. The mixture was warmed to 40 C and films prepared on microscope slides (Cunningham, 1967). Films were dried at 15 C in a stream of air, fixed for 12 hr in 10% formalin, washed for several minutes in 1% sodium chloride, and dried again. Tissue sections placed directly on the films were incubated

Table 1. Lysosomal enzymes in holdfast organ and forebody gland cells of *Alaria marcianae*.

Enzyme	B-glucuronidase (present study)	Aryl sulfatase B (present study)	Acid phosphatase (after Johnson et al., 1971)
Tegumental surface layer of holdfast organ	+	0	++
Holdfast organ gland cells	+	+	++
Forebody gland cells	+[a]	0	0
Forebody gland cell ducts	0	0	0

++, moderate activity; +, slight activity; 0, no activity.
[a] Considered here to be a nonlysosomal B-glucuronidase.

for 2 hr at room temperature, stained with 1% toluidine blue (pH 6) for 15 min, washed briefly in water and mounted in glycerin jelly.

Results

The holdfast organ, lappets, and associated gland cells in *Alaria marcianae* have been described (Johnson et al., 1971), so only a brief description will be given here.

The elongate, acetabular type of holdfast organ is situated on the ventral surface of the forebody posterior to the ventral sucker. The ventral surface of the holdfast organ lacks spines present on other areas and small bands of muscles are present under the tegumental surface layer ("cuticle"). A series of excretory spaces lie under the muscle bands. The gland cells (tegumentary cells) are located under these spaces throughout the entire length of the holdfast organ but form a more dense mass anteriorly. This anterior mass of cells and the associated tegumental surface layer will be referred to here as the anterior mass of holdfast organ gland cells. The remaining holdfast organ gland cells and associated tegumental surface layer will be called the posterior holdfast organ tegument.

The two earlike lappets are located at the anterior end of the body, one on each side of the oral sucker. The forebody gland cells are situated in the forebody between the levels of the pharynx and the holdfast organ. The lateral margin of each lappet is perforated by ducts from these cells.

Enzyme staining (Tables 1, 2): An intense staining for esterases was observed in the anterior mass of holdfast organ gland cells (Figs. 1, 2) and in the forebody gland cells and ducts (Figs. 3, 4) with both the a-naphthyl acetate and indoxyl methods. Only a moderate reaction was present in the posterior holdfast organ tegument (Fig. 2). No difference in activity was noted between fresh-frozen and fixed tissues.

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Abbreviations (all figures): AH, anterior portion of holdfast organ; C, cecum; E, enzyme reaction product; ES, excretory space; F, forebody; FD, forebody gland cell ducts; FG, forebody gland cells; H, hindbody; HO, holdfast organ; HG, holdfast organ gland cells; L, lappet; M, host mucosal tissue; PH, posterior portion of holdfast organ; S, spermary (testis); T, tegumental surface layer; TC, tegumentary cells; V, vitellaria.

Figures 1–9. Enzyme histochemistry of adult *Alaria marcianae*. Figs. 1–4, 6–8 from formol–calcium fixed sections, Figs. 5, 9 from fresh-frozen sections. 1. Longitudinal section of forebody, showing esterase activity in anterior mass of holdfast organ gland cells and forebody gland cells and ducts. × 50. 2. Longitudinal section of holdfast organ, showing intense esterase activity in anterior mass of gland cells. × 200. 3. Section of anterior forebody, showing esterase activity in forebody gland cells. × 500. 4. Section of lappets, showing intense esterase activity in forebody gland cell ducts. × 500. 5. Longitudinal section of forebody, showing strong cholinesterase activity in posterior region of holdfast organ and light activity in anterior region of holdfast organ and general forebody tegument. × 20. 6. Section of anterior forebody, showing esterase activity in tegumental surface layer and tegumentary cells. × 500. 7. Section of anterior region of holdfast organ, showing B-glucuronidase activity in tegumental surface layer and gland cells of holdfast organ. × 500. 8. Section of anterior forebody, showing B-glucuronidase activity in forebody gland cells. × 500. 9. Section of anterior region of holdfast organ, showing aryl sulfatase activity in gland cells. Note absence of activity in tegumental surface layer. × 500.

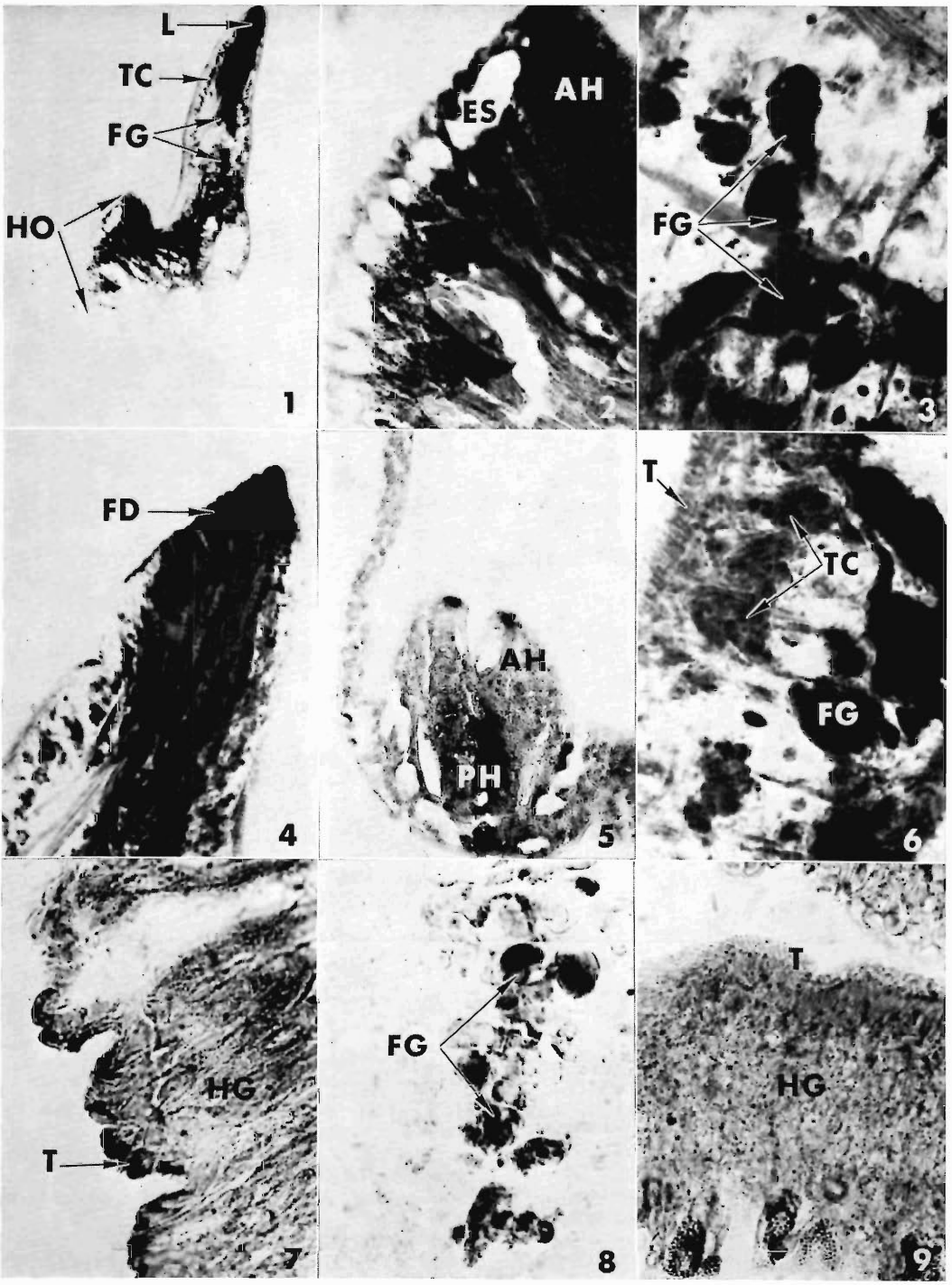

Table 2. Effect of additives on esterase activity in *Alaria marcianae*.

Substrate	Additives	Tissue[a] 1	2	3	4
5-bromoindoxyl acetate	None	++++	+	++	++++
	Eserine (10^{-5} M)	+++	0	++	++++
	Eserine (2×10^{-4} M)	0	0	0	0
	NaF (2×10^{-3} M)	+	+	+	+
	NaF (7.5×10^{-2} M)	0	0	0	0
	$AgNO_3$ (10^{-2} M)	++	+	0	++
	$Pb(NO_3)_2$ (10^{-3} M)	0	0	0	0
	$CuSO_4$ (10^{-3} M)	++	+	+	++
	Ascorbic acid (2×10^{-3} M)	+++++	+	+++	+++++
	Cysteine (10^{-3} M)	+++++	+	+++	+++++
Acetyl thiocholin iodide	None	+	++	+	0
	Eserine (10^{-5} M)	0	0	0	0

[a] 1 = anterior mass of holdfast organ gland cells; 2 = posterior holdfast organ tegument; 3 = anterior forebody tegumentary cells; 4 = forebody gland cells and ducts.
+++++, very strong activity; ++++, strong activity; +++, moderate activity; ++, weak activity; +, very weak activity; 0, no activity.

A slight decrease in esterase activity occurred in the anterior mass of holdfast organ gland cells and in the forebody gland cells and ducts with 10^{-5} M eserine, with complete inhibition in the posterior holdfast organ tegument. Complete inhibition was observed in both cell types with 2×10^{-4} M eserine. Sodium (2×10^{-3} M) produced a marked reduction in activity in the anterior mass of holdfast organ cells and in the forebody gland cells and ducts, but only a slight reduction in the posterior holdfast organ tegument. Complete inhibition occurred in all sites with 7.5×10^{-2} M sodium fluoride. Therefore, most of the activity in the anterior mass of holdfast organ gland cells and the forebody gland cells and ducts is due to nonspecific esterases. The activity in the posterior holdfast organ tegument is due to cholinesterases.

The staining pattern with the acetyl thiocholin iodide method was similar to that with the indoxyl method. However, except for a moderately strong reaction in the posterior holdfast organ tegument (Fig. 5), the reaction was weak, again indicating cholinesterase localization as viewed above with inhibitors. The staining was completely inhibited with 10^{-5} M eserine.

The effects of the additives used here to characterize the nonspecific esterases are given in Table 2. Partial inhibition by silver nitrate and copper sulfate and complete inhibition with lead nitrate indicated the presence of a type C-esterase. Complete inhibition

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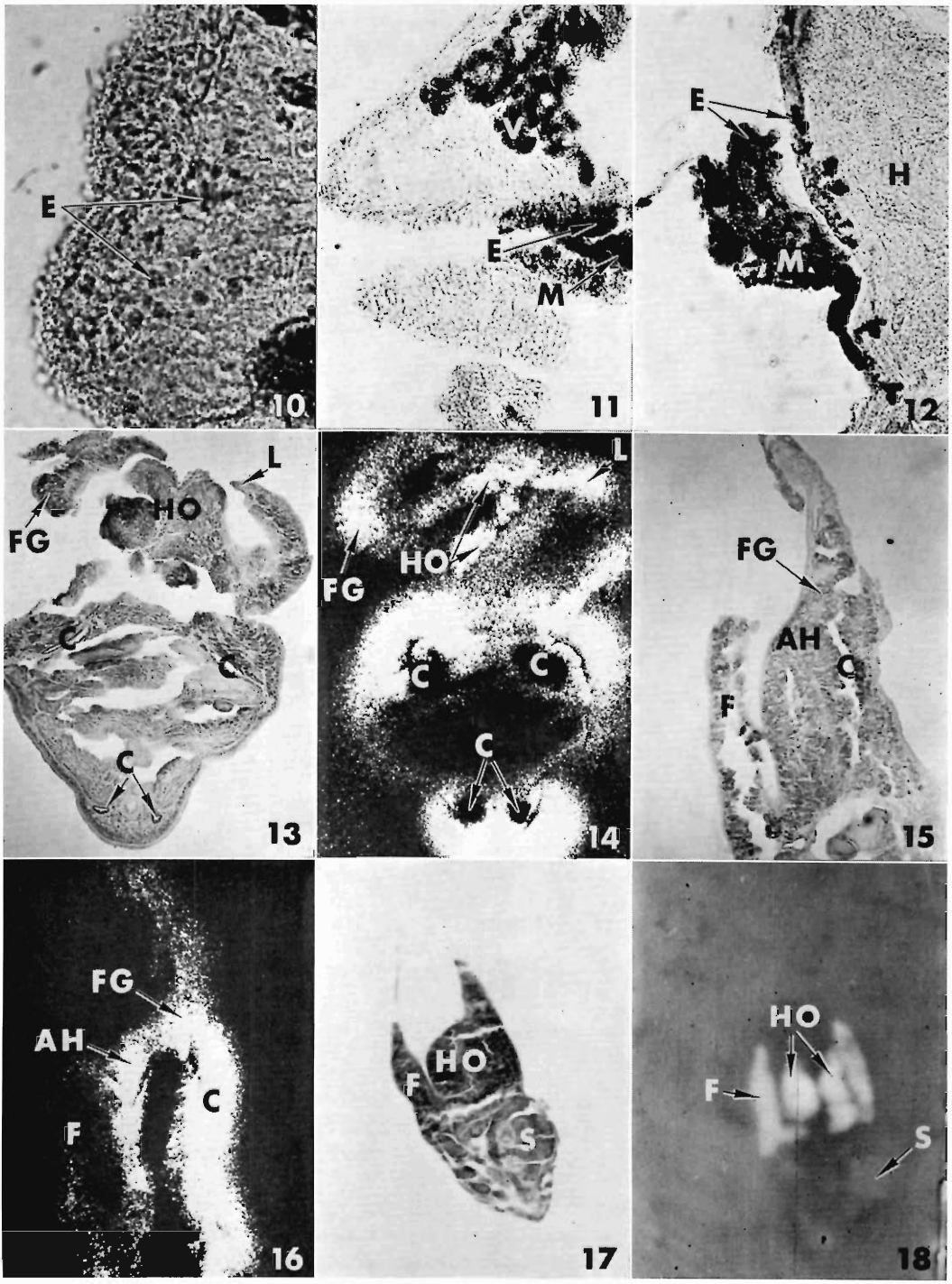
Figures 10–18. Enzyme histochemistry of adult *Alaria marcianae*. From fresh-frozen sections. 10. Section of holdfast organ, showing leucine aminopeptidase activity in gland cells. × 500. 11. Section of holdfast organ with host tissue adhering. Note intense leucine aminopeptidase activity in host tissue, in holdfast organ tegument, and in the vitellaria. × 200. 12. Section of hindbody with host tissue adhering. Note strong leucine aminopeptidase activity in host tissue and hindbody tegument. × 200. 13. Section of adult stained with hematoxylin and eosin. × 50. 14. Panchromatic film plate exposed to section of adult adjacent to that in Fig. 13. Note strong proteolytic activity in areas corresponding to holdfast organ, forebody gland cells, and ceca. × 50. 15. Section of adult stained with hematoxylin and eosin. × 50. 16. Panchromatic film plate exposed to section of adult adjacent to that in Fig. 15. Note strong proteolytic activity in areas corresponding to anterior region of holdfast organ, forebody gland cells, and cecum. × 50. 17. Section of adult stained with hematoxylin and eosin. × 20. 18. Gelatin–hyaluronic acid film exposed to section of adult adjacent to that in Fig. 17. Note high hyaluronidase activity in areas corresponding to holdfast organ and forebody. Some activity is also evident in the area corresponding to a testis. × 20.



of activity with lead nitrate showed that this type C-esterase is similar to the cathepsinlike indoxyl C-esterase reported by Hess and Pearse (1958). This view was further supported by an increase in activity with ascorbic acid and cysteine.

Esterase activity was also found in the tegumental surface layer and tegumentary cells in the anterior region of the forebody (Figs. 1, 6). Studies with additives showed that this activity was due to both cholinesterases and a cathepsinlike indoxyl C-esterase (Table 2). It should be emphasized that these are tegumentary cells and not specialized gland cells ("subcuticular cells") such as those found in the forebody of certain strigeoids (Öhman, 1965, 1966a; Bogitsh, 1966a; Bogitsh and Aldridge, 1967).

A weak reaction for B-glucuronidase (inhibited by potassium hydrogen saccharate) at pH 6 was found in the tegumental surface layer and gland cells of the holdfast organ and in the forebody gland cells (Fig. 7, 8).

Aryl sulfatase activity was observed in the gland cells of the holdfast organ at pH 5.5, which is optimum for aryl sulfatase B (Hayashi et al., 1964) (Fig. 9). Only a light staining reaction was noted.

A weak reaction for leucine aminopeptidase (inhibited by 10^{-2} M potassium cyanide) at pH 6.5 was detected in the holdfast organ gland cells of both free and attached adults (Fig. 10). However, in a few instances with free adults where host tissue adhered to the holdfast organ and general forebody tegument, a strong reaction was evident in the tegument (Fig. 11). In a few worms this was also true for the hindbody tegument (Fig. 12). The reason for this difference in staining intensity is not known.

A light staining reaction for chymotrypsinlike enzymes was observed in the tegumental surface layer of the holdfast organ. No activity for trypsinlike enzymes or gamma glutamyl transpeptidase was found in the parasites.

SUBSTRATE FILM METHODS: Protease activity was evident in the areas corresponding to the holdfast organ and the anterior region of the forebody at pH 6 and pH 8 using panchromatic plates (Figs. 13–16), but none was seen at pH 4. In the holdfast organ this activity appeared to be more intense in the anterior region (anterior mass of holdfast organ gland cells) than in the posterior region (Figs. 15, 16). The activity in the forebody corresponded to the areas of the lappets, forebody gland cells, and the anterior tegumentary cells (Figs. 13, 14). The region of the forebody posterior to the level of the forebody gland cells showed little or no activity (Figs. 15, 16). The area around the ceca displayed high protease activity (Figs. 13–16).

The activity pattern obtained with color film was similar to that with panchromatic plates, but was more sensitive with regard to intensity. Strong protease activity is indicated by clear blue areas, while red and yellow clear areas represent areas of high and low activity, respectively (Fratello, 1968). Thus, using color film, more activity was apparent in the cecal and anterior forebody regions than in the holdfast organ area.

Hyaluronidase activity was present in the areas corresponding to the entire holdfast organ and the anterior part of the forebody (Figs. 17, 18). This latter area would include the lappets, forebody gland cells, and anterior tegumentary cells. It was not possible to determine any regional difference in intensity with hyaluronidase. As might be expected, some hyaluronidase activity was also seen in the area corresponding to the testes in the hindbody (Figs. 17, 18).

Discussion

Acid phosphatase has been reported in the tegumental surface layer and gland cells of the holdfast organ of *Alaria marcianae* and certain other strigeoids (Erasmus and Öhman, 1963; Öhman, 1965, 1966a, b; Erasmus, 1968; Johnson et al., 1971). Since this enzyme is a marker for lysosomes in mammalian tissue (Novikoff, 1963; de Duve and Wattiaux, 1966), its presence might indicate that lysosomal enzymes are secreted (exocytosis) for extracorporeal digestion.

In this study two additional lysosomal enzymes, B-glucuronidase and aryl sulfatase B, were found in the tegumental surface layer and gland cells of the holdfast organ of *A. marcianae*. The distribution in the holdfast organ was similar to that reported for acid phosphatase, although the intensity was less (Table 1). B-glucuronidase was also found in

the forebody gland cells. However, considering its absence from the forebody gland cell ducts and the absence of other lysosomal enzymes (Table 1), we believe it is not of lysosomal origin. This B-glucuronidase may be involved in the metabolism of mucopolysaccharides (Conchie, Findlay, and Levvy, 1959; Hayashi, 1964), which are known to be present in these cells (Johnson et al., 1971).

In *A. marcianae* lysosomal enzymes are then apparently absent from the forebody gland cells, which secrete material exhibiting nonspecific esterase activity (Johnson et al., 1971; present study). Furthermore, an increased enzyme activity was not present in the anterior mass of holdfast organ gland cells which stain intensely for nonspecific esterases. Thus, it appears that lysosomal enzymes are not secreted for extracorporeal digestion in this strigeoid.

Although proteases would presumably be released by gland cells involved with the breakdown of host tissue, little information has been derived from histochemical staining techniques. In this study tests for gamma glutamyl transpeptidase and trypsinlike enzymes were negative. Only a weak reaction was noted in the tegumental surface layer and in the gland cells of the holdfast organ for chymotrypsinlike enzymes and for leucine aminopeptidase, respectively. Tests for the latter enzyme in other strigeoids have either been negative or a weak reaction found in the holdfast organ and forebody gland cells (see Johnson et al., 1971, for review).

A further characterization of the nonspecific esterases reported by Johnson et al. (1971) in the anterior mass of holdfast organ and forebody gland cells of *A. marcianae* was made in this study. The results indicate that it is a cathepsinlike indoxyl C-esterase such as that reported by Hess and Pearse (1958). The C-esterase was also found in certain anterior forebody tegumentary cells and associated tegumental surface layer. This enzyme may then function physiologically as a proteolytic enzyme.

Nonspecific esterases have been reported in the holdfast organ gland cells and in gland cells associated with the lappets in all strigeoids studied. They were considered by Öhman (1966a, b) to represent B-type esterases in *Apatemon gracilis minor* and *Holostephanus luehei*, while types A and B were reported by Lee (1962) in *Diplostomum phoxini*. Erasmus and Öhman (1963) in *Cyathocotyle bushiensis* and Öhman (1965) in *Diplostomum spathaceum* described a type B and a second type of esterase which was partially inhibited by silver nitrate. They referred to this type as an "unclassified resistant esterase." This esterase may be similar to cathepsinlike indoxyl C-esterase reported here in *A. marcianae*. Why several different types of nonspecific esterases occur in strigeoids is not known, although they presumably serve different functions. Nonspecific esterases secreted to the outside are probably concerned with extracorporeal digestion.

Erasmus and Öhman (1963) and Öhman (1965, 1966a, b) used an in vitro method to demonstrate the release of material exhibiting proteolytic activity by the holdfast organ gland cells and gland cells associated with the lappets. This method of incubating living isolated adults on solid gelatin, agar, or coagulated albumen was successful with two cyathocotylids (*Cyathocotyle bushiensis* and *Holostephanus luehei*), but was less suitable with a diplostomatid (*Diplostomum spathaceum*) and a strigeid (*Apatemon gracilis minor*).

In this study substrate film methods for proteases and hyaluronidase were used with the diplostomatid *A. marcianae* instead of an in vitro method. These methods demonstrated strong protease and hyaluronidase activity in areas corresponding to the holdfast organ and anterior forebody. The latter area would contain the forebody gland cells and ducts and the anterior forebody tegumentary cells.

Although determination of enzyme intensity is not as good as with histochemical staining methods, more protease activity was evident in the anterior than in the posterior region of the holdfast organ. This regional difference then is similar to that found for the cathepsinlike indoxyl C-esterases. We believe that these results indicate that the anterior region of the holdfast organ is more concerned with extracorporeal digestion than the posterior region. This regional difference in enzyme activity, which probably reflects a difference in function, is apparently not unique to *A. marcianae*. Reid and Harkema (1970) reported a similar type of difference with esterase activity in

the holdfast organ of *Procyotrema marsupiformis*.

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ABSTRACT: *Parapronocephalum* Belopolskaia, 1952, originally linked with the trematode family Pronocephalidae by placement in the subfamily Pronocephalinae as the only member of this subfamily found in birds, is assigned to the family Notocotylidae based on relative positions of ovary and Mehlis' gland. Because of major differences displayed by this genus, a new subfamily, Parapronocephalinae, has been erected in the Notocotylidae. A new species, *Parapronocephalum reversum*, is described from four species of shore birds of the Woods Hole area of Massachusetts and Sapelo Island, Georgia. This species appears to be seasonally regimented, infections occurring from midfall to early spring, and has the dunlin (*Erolia alpina*) as its principal definitive host.

Parapronocephalum symmetricum Belopolskaia, 1952, a monostomous trematode possessing a head collar, was collected during a survey of shore bird parasites in the years 1941–42 in eastern Murman from the intestinal ceca of the purple sandpiper (*Calidris maritima*). From a later survey in the same area in 1948, Belopolskaia (1953) recorded its hosts as purple sandpiper, turnstone, ruff, and oyster catcher. Belopolskaia (1952) further referred to the finding of this species in the ruddy turnstone (*Arenaria interpres*) by D. A. Naumov in 1947; Ginetsinskaia and Naumov (1959) recorded this finding in the literature. Larval development of this species was stated as occurring in *Littorina saxatilis*, and we are given an accounting of that development by Chubrik (1955). As far as can be determined, these are the only indications of findings of this organism. Skrjabin (1955) recorded it, as does Yamaguti (1958), in their compilations of trematode literature. However, if Yamaguti (1958) is used as the sole source for identification, one would be led astray, as he stated that this species occurs in the liver of *Calidris maritima*.

Placing this species in the subfamily Pronocephalinae by Belopolskaia (1952), an error in itself, has been made more awkward by removal of the subfamily from the Notocotylidae

and the giving of familial rank to the Pronocephalidae by Skrjabin (1955), and the similar inference by Yamaguti (1958). In earlier literature, pronocephalids were also accorded familial status: Price (1931); Harwood (1939); etc., but Szidat (1939) reduced them to subfamilial rank in the Notocotylidae, Belopolskaia (1952) then following Szidat's classification. What impressions were held by Belopolskaia in the 1953 publication are unknown as species name and hosts are merely logged under Notocotylidae, and none of the later Russian publications referring to the genus *Parapronocephalum* list this reference nor does Yamaguti (1958).

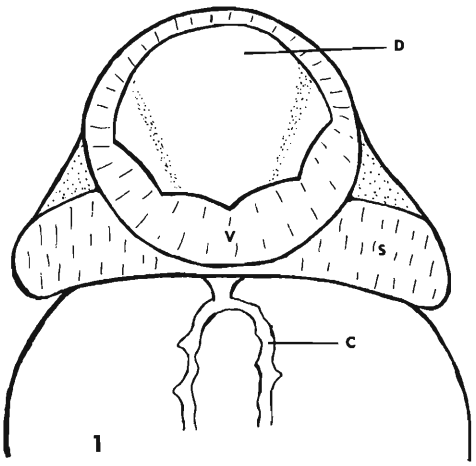
Referring to Chubrik (1955), at first, seemingly, would cause no difficulties as *Parapronocephalum symmetricum* was stated as belonging to the family Notocotylidae in the opening sentence, with no mention of subfamilial affiliations. However, the concluding sentence of this article refers to the species as a pronocephalid and goes on to compare its life cycle, schematically, with two other pronocephalids. Literature of this species is further complicated by the translators of Skrjabin's monumental work, "The Trematodes of Animals and Men—Trematody Zhivotnykh i Cheloveka" (Arai and Dooley, 1964). *Parapronocephalum* is allotted one notation by these authors, the genus being listed under Supplementary Illustrations of the family Pronocephalidae (p. 91). Workers not having access to the Skrjabin volumes would thus assume concurrence with Belopolskaia by Skrjabin concerning this genus, and who, by implication, had allotted it little interest. Such is not the case, as Skrjabin (Vol. X, 1955) devoted 10 pages (pp. 201–210) to this organism, heading it, a supplement to the family Notocotylidae. This discussion is appended to the Pronocephalidae, hence the probable cause for misinterpretation by Arai and Dooley (1964), as the Notocotylidae had been covered in a previous volume (VIII).

Yamaguti (1958) also seemed to have mixed impressions with regard to this organism. Curiously, Yamaguti, in his key to genera of Notocotylinae from birds, lists *Parapronocephalum*, but does not diagnose it with this group but does so under the Pronocephalidae in the following section. His dilemma, perhaps, further indicated by inclusion of two identical figures of *P. symmetricum* (save for size), Figures 1233 and 1286, on two separate plates of his tome, neither of which is placed in his section of figures of Digenea of Birds but are labeled as miscellaneous. Further, only the first of these figures is referred to in his generic analysis.

Pronocephalinae are monostomous trematodes parasitic in reptiles and fish, primarily marine turtles. *Parapronocephalum symmetricum* was listed by its describer (Belopolskaia, 1952) as being the only member of this subfamily parasitic in birds, an inference carried on by Yamaguti (1958) et al., a possibility quite allowable in itself, but certainly subject to conjecture. Belopolskaia (1952) believed that the species, because of its characteristic head collar, had a strong resemblance to pronocephalids, particularly to the genus *Adenogaster*. *Adenogaster*, unlike other pronocephalids, has dermal glands like the Notocotylidae and similar to those possessed by *Parapronocephalum*, which are squarish in outline and netlike in structure, and are thus quite unlike the typically round dermal glands of the Notocotylidae. Skrjabin (1955) argued, and rightly so, that dermal glands in *Adenogaster*, while similar to the Notocotylidae, should hold little weight systematically and similarity to those possessed by *Parapronocephalum* was shaky grounds on which to link this genus with the Pronocephalidae. The head collar of *Parapronocephalum* then, according to Skrjabin (1955), is left as the only tie with this group, as notocotylids were not indicated as having head collars. However, a subfamily of the Notocotylidae, the Hippocrepinae Mehra, 1932 (parasites of rodents), do have head collars of a sort.

Head collars of Pronocephalidae are emphasized as having a midventral split and usually described as of shield or triangular shape, much like those found in the philophthalmids and echinostomes save for the spination: *Renogonius* and *Criocephalus* like *Parorchis*; *Adenogaster* and *Pronocephalus* like *Echinochasmus*; *Glyphicephalus* like *Stephanoprora*. Further, the genera *Desmogonius* and *Diaschistorchis* of the family Pronocephalidae are lacking head collars making it not a universal character of this family.

Parapronocephalum, in the unique structure of its oral region, is quite unlike the typical



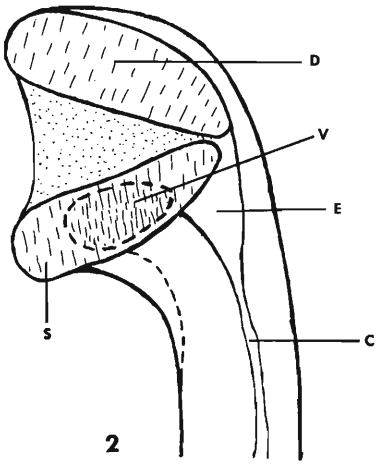


Figure 1. Ventral view of oral region of *Parapronocephalum reversum* sp. n.
Figure 2. Lateral view of oral region of *Parapronocephalum reversum* sp. n. c—intestinal ceca, d—dorsal muscle band, e—esophagus, s—shoulder collar, v—ventral muscle band.

pronocephalid (Fig. 1). The collar is composed of a pair of heavily muscled shoulders extending laterally and dorsally from the ventral portion of the sucker. Thinner cup-shaped depressions reach from these shoulders to the rear of the oral sucker. These depressions, so noticeable in live specimens, are drawn back against sucker musculature and behind the shoulders in fixed material. The large oral sucker, while superficially appearing as a single circular structure, is composed of two major and essentially separate groups of muscle (Fig. 2). The prominent dorsal musculature is a lens-shaped band with edges narrowing laterally. A scalloped ventral portion having four rounded protuberances projecting into the orifice also thins laterally. Laterally viewed, this ventral musculature is masked by the prominent shoulder collar. Thin sidewalls plus the cuplike depressions extending from the shoulders form the lateral aspects of the oral sucker, the whole complex appearing to be a very efficient attachment and sucking mechanism.

Skrjabin (1955), in associating the genus *Parapronocephalum* with the Notocotylidae, argued his case primarily on an ecological basis by stating that it should not be placed with the Pronocephalidae due to differences in host species as, heretofore, the families had been nicely divided on host affiliations, pronocephalids being found in turtles and notocotylids in birds and mammals. Strongly relying on this point and ignoring other hosts of pronocephalids (lizards and fish), Skrjabin (1955) reiterated those differences already pointed out by Belopolskaia (1952) in the original description of *Parapronocephalum* in relation to *Adenogaster*, namely, the presence of four rows of dermal glands in that genus and only three in *Parapronocephalum* and the median position of the genital pore in *Parapronocephalum* as opposed to the off-center position in the Pronocephalidae. Without really adding anything to our knowledge of the genus *Parapronocephalum*, Skrjabin (1955) designated the genus as belonging to the Notocotylinae despite presence of its head collar. In counterargument, having three rows of dermal glands is not a universal character of the Notocotylidae: the genus *Quinqueserialis* possesses five rows, the genus *Hippocrepis* more than five, and the genus *Paramonostomum* of this family is lacking dermal glands as all but the genus *Adenogaster* of the Pronocephalidae. Position of the genital pore also is a variable character in each of the families and should not be overstressed for differentiation.

Parapronocephalum symmetricum was described and depicted as having intestinal ceca laterally placed with regard to testes and

vitellaria, another factor differentiating it from the Notocotylidae, but not indicated by Skrjabin (1955). Testes do occur intercecally in the Pronocephalidae, for example, in the genera *Pronocephalus* and *Charaxicephalus*, but in *Adenogaster*, the genus heavily relied upon by Belopolskaia for comparison with *P. symmetricum*, testes are extracecal as in the notocotylids, a situation seemingly ignored by that author and Skrjabin. So it would seem that *Adenogaster* is as much an enigma in its own right with regard to the Pronocephalidae.

While I concur with Skrjabin's removal of *Parapronocephalum symmetricum* from the Pronocephalidae, his reason for doing so was slim and overstressed, and overlooked were a number of points that would equally as well associate this species with the Pronocephalidae.

Controversy concerning placement of this organism should never have arisen. Belopolskaia, the original describer, seems to have been unaware of the oft-quoted distinction between the families Pronocephalidae and Notocotylidae, the relative positions of ovary, testes, and Mehlis' gland. The two groups are indeed closely related, but Price (1931) and Harwood (1939) have pointed out that these two families can be easily separated by position of the Mehlis' gland which is cephalic to the ovary in the Notocotylidae but never cephalic to that structure in the Pronocephalidae, a discriminating factor true for all members of these two families. In the figure furnished by Belopolskaia (1952), as well as in written description, the Mehlis' gland of the species in question is forward of the ovary, thus eliminating it from the Pronocephalidae altogether. Skrjabin, too, seems to have overlooked this most important of features and argued solely on grounds of less specific and unsupportable characters, that each, in itself, is found to vary. Position of the ovary is also a discriminating character. This structure in the Pronocephalidae is dextrally displaced with regard to the midline. In *Parapronocephalum* and the Notocotylidae, the ovary is positioned medially (Figs. 3–5).

Disturbingly, the oral sucker of *Parapronocephalum* is much larger in proportion and actual size than that of other notocotylids, which, with addition of head collar and differences in shape and structure of dermal glands, does cause some awkwardness systematically (Fig. 3). Adding the species herein to be described to the already notably different aspects of the genus *Parapronocephalum*, it would seem reasonable that a new subfamily, the Parapronocephalinae, be erected.

Parapronocephalum reversum sp. n.

A new and easily distinguished species that can be assigned to the genus *Parapronocephalum* has been recovered from the intestinal ceca of shore birds of the Woods Hole region of Massachusetts and at Sapelo Island, Georgia. This new species, while having overall morphometry quite similar to *P. symmetricum*, can be distinguished from it primarily by positioning of intestinal ceca medial to testes and vitellaria, the typical notocotylid pattern. Because of this opposing position of intestinal ceca in this species, I have chosen to name it *Parapronocephalum reversum* (Fig. 3). Other less easily detectable differences will also be noted.

Parapronocephalum reversum occurs in the intestinal ceca of several species of shore birds of the Woods Hole region: ruddy turnstone (*Arenaria interpres*), purple sandpiper (*Erolia maritima*), knot (*Calidris canutus*), dunlin (*Erolia alpina*). This last host, the dunlin, seems to be the principal definitive host of this new species, the greatest incidence of infection and highest populations of this parasite being found in this host. As well, it is the only carrier of *P. reversum* at Sapelo Island, Georgia, even though two other known hosts are present there indicating a more northern source for this parasite with worms retained in travel during southward migration of their host. A further indication of a northern source of infection is the absence of immature specimens in dunlins found at Sapelo Island and the reduced incidence of infection. Table 1 presents recovery data for these two collecting areas.

Host records indicate that *P. reversum* is seasonally regimented, infections occurring between late October and early April, and primarily between mid-November and mid-January. Infection is apparently picked up in the Woods Hole area in addition to or exclusion of northern breeding grounds of bird hosts as migratory flocks of the various bird hosts are well established in that area by the time first instances of *P. reversum* are noted, and these

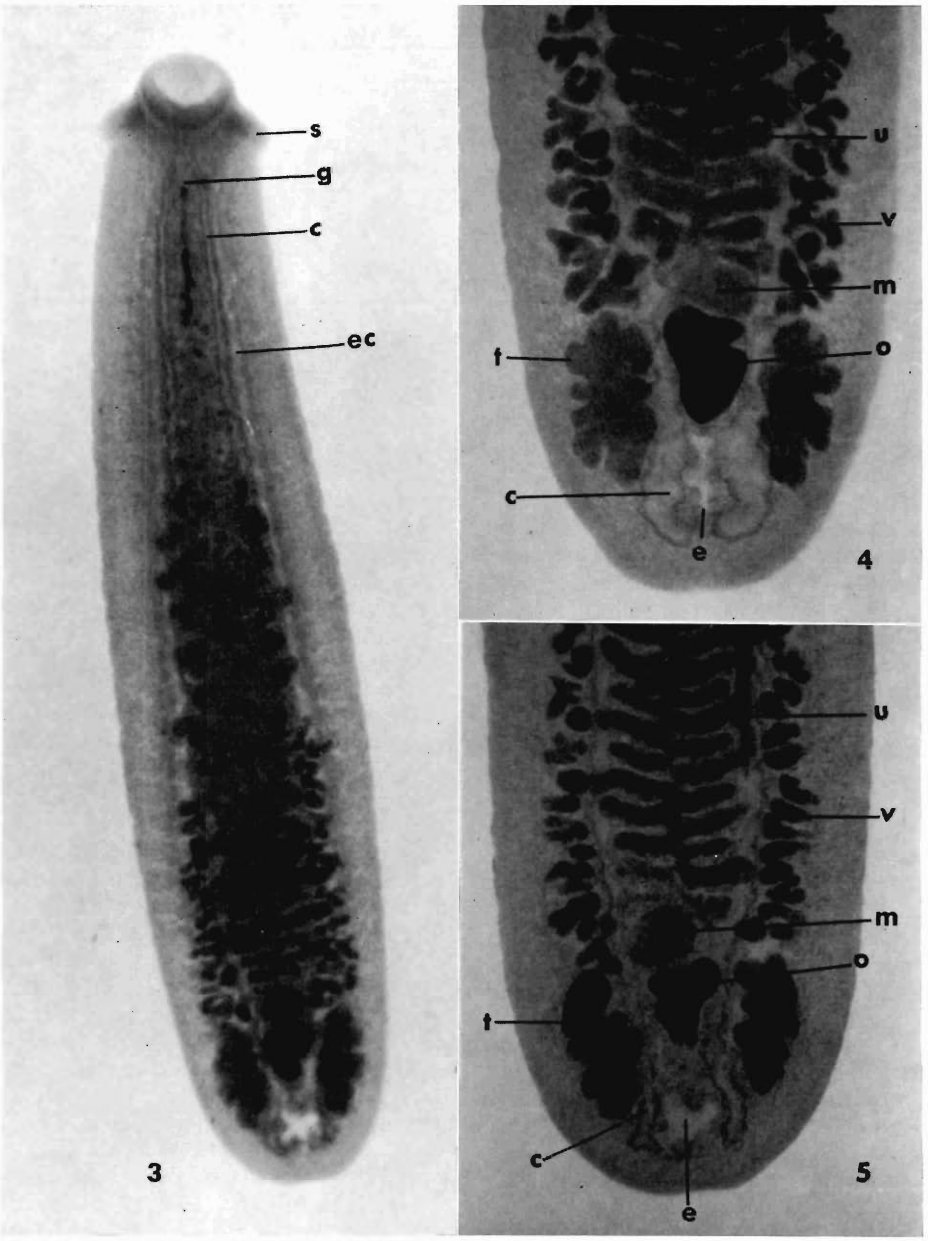


Figure 3. *Parapronocephalum reversum* sp. n. from intestinal ceca of dunlin (*Erolia alpina*), Sapelo Island, Georgia.

Figure 4. Posterior extremity of *Parapronocephalum reversum* sp. n.

Figure 5. Posterior extremity of *Parapronocephalum reversum* sp. n. c—intestinal ceca, e—excretory bladder, ec—excretory canal, g—genital pore, m—Mehlis' gland, o—ovary, s—shoulder collar, t—testis, u—uterus, v—vitellaria.

Table 1. Incidence of infection in hosts of *Parapronocephalum reversum* sp. n.

	Woods Hole, Massachusetts			Sapelo Island, Georgia		
	Sample size	No. infected	No. parasites	Sample size	No. infected	No. parasites
Purple sandpiper (*Erolia maritima*)	30	3	1, 12 + 21 (mostly immature)			
Ruddy turnstone (*Arenaria interpres*)	12	2	4 + 38 (all mature)	10	0	
Knot (*Calidris canutus*)	2	1	19 (all immature)	13	0	
Dunlin (*Erolia alpina*)	13	7	2–62 (all stages)	61	6	1–10 (all mature)

are mainly quite immature worms. These specimens often completely lack sexual organs and are similar in size and structure to metacercariae and newly infecting specimens of *P. symmetricum* (Chubrik, 1955). A search for the snail intermediate host of *P. reversum* has been unfruitful to date. *Littorina saxatilis* (the host of *P. symmetricum*) is found in the Woods Hole area and does harbor monostomous cercariae, but as yet no metacercariae have been obtained for experimental infections to link it with the new species. While it is hazardous to ascribe causation at this point, *L. saxatilis* does appear to be the likely candidate.

Dunlins taken during spring northward migration apparently have not retained infections of *P. reversum* during their southern sojourn. Ruddy turnstones have been collected in May, August, and September without infection, the only known instances of infection occurring in mid-November, one bird carrying only very mature worms and the other only immature specimens. Only two knots were collected in the Woods Hole area, one in August uninfected, the other in mid-November had a moderate infestation of immature *P. reversum*. Purple sandpipers (the principal definitive host of *P. symmetricum*) winter in the Woods Hole area on the Weepecket and Elizabeth islands and show infections from mid-November to early April. The only adult *P. reversum* found in this bird species were recovered in April. However, most worms in this particular specimen were immature indicating a local source of infection. It is entirely possible, but not indicated by present information, that although infection seems restricted to colder months, worms could be retained for a considerable time in light of size of some recovered specimens.

Parapronocephalum reversum is primarily a blood-feeder, digestive ceca in most specimens are noticeably engorged. It is assumed derivatives of this food source cause the notable change in pigmentation with maturation of this parasite. Immature specimens of *P. reversum* have the typical transparency of juvenile trematodes. An opaque cream-colored cast, taken on with maturity, changes through a bright yellow, orange, to reddish orange in the largest of specimens. No mention was made of coloration in the description of *P. symmetricum* (Belopolskaia, 1952) so it is not known whether this is of specific importance.

As stated, the most noteworthy differentiation point of the two known species of *Parapronocephalum* is placement of intestinal ceca. In conjunction with this, meanderings of the uterus in mature worms often cross the intestinal ceca in *P. reversum*, a point emphasized as not occurring in *P. symmetricum* (Figs. 3–5). Structure of the digestive ceca is different from that in *P. symmetricum* also as it has numerous outpocketings along its length as in the typical pronocephalid (Figs. 4, 5). *P. symmetricum* was noted in description as lacking these structures.

The ovary is depicted as round in Belopolskaia's figure of *P. symmetricum*, while in *P. reversum* this organ is decidedly lobed, sometimes elongate or quadripointed, but most often somewhat heart-shaped in outline (Figs. 3–5).

Dermal glands number 27 in lateral rows with 25 centrally placed, size of glands in all rows decreasing in forebody. Glands of the central row are displaced slightly forward of their counterparts in outer rows forming thus a triangular arrangement point foremost in any given transverse rank of glands. Position of the missing two glands of the central row is taken up by this displacement and by the

centrally placed genital pore in the forebody. In the description of *P. symmetricum*, rows were stated as being comprised of 29 glands centrally and up to 30 in outer rows without notation of a decrease in size. Depiction showed them all to be of similar dimensions, but having a displacement forward of the central row as in *P. reversum*. In the original figure of *P. symmetricum* there appear 30 glands in each of the three rows, which have thus been carried forward in copies of this figure in subsequent literature.

No mention was made of the excretory system of *Parapronocephalum symmetricum* by Belopolskaia (1952). Chubrik (1955), however, described the excretory vesicle of *P. symmetricum* as round with wide lateral branches running parallel to the intestinal ceca. In review, Yamaguti (1958) afforded it, "Excretory vesicle?" In *P. reversum* there is a large, very noticeable excretory vesicle (Fig. 3) lobed in outline with two very noticeable main connecting tubules having many side branches leading forward to the head collar region; finer details of this system have not been recorded. Excretory vesicles in the Notocotylidae are short, often not easily distinguishable, those of the Pronocephalidae V- or Y-shaped. Thus is added still another discriminating factor for the subfamily Parapronocephalinae.

Belopolskaia's original account of maturation in *P. symmetricum* proposed, by implication, a long developmental period as specimens of 3 mm in length were not mature and mature specimens were 6.5 mm in length; worms of intermediate length were not recovered. Chubrik (1955) alters this information slightly, by stating that eggs are present in the uterus of worms reaching not less than 6.5 mm in length. The gap in information provided by Belopolskaia (1952) for *P. symmetricum* may perhaps be comparable to maturation size observed in *P. reversum* where eggs begin to form when worms are 2.7 to 3.1 mm in length and large specimens of this species attain a length of 7.2 mm.

Specimen preparation

Worms extracted from host ceca were transferred live to 0.85% saline, then placed in hot water for relaxation. Relaxed specimens were fixed in alcohol–formaldehyde–acetic acid solution (AFA) and stained with Van Cleave's combination hematoxylin.

Family Notocotylidae

Family diagnosis sensu Yamaguti (1958) but amended as follows: Head collar usually absent. Oral sucker usually small. Ceca simple or with outpocketings. Vitellaria usually lateral to ceca. Excretory vesicle short or long.

Parapronocephalinae subfam. n.

DIAGNOSIS: Notocotylidae: Head collar present. Oral sucker proportionally large when compared to other notocotylids. Ventral surface provided with longitudinal series of glands squarish in outline and netlike in structure. Genital pore median and postintestinal bifurcation. Ovary intertesticular, round or lobed, medially positioned behind Mehlis' gland. Intestinal ceca with or without outpocketings, inter- or extratesticular in position. Vitellaria follicular and anterior to testes. Excretory vesicle small or large, round or lobed, two long arms extending forward to head collar region. Parasites of shore birds.

Parapronocephalum reversum sp. n. (Figs. 1–5)

DESCRIPTION: Notocotylidae, Parapronocephalinae: small to medium elongate monostomes 2.7–7.2 mm long by 0.6–1.6 mm in width. Sides parallel through most of body length. Head collar ½ to ⅔ body width (0.454–1.082 mm). Oral sucker terminal (0.216–0.475 mm × 0.302–0.605 mm). Pharynx absent. Esophagus short (0.124–0.194 mm). Ceca having outpocketing and extending to near posterior extremity. Testes elongate (0.227–0.572 mm × 0.097–0.313 mm), lobed, lateral to termination of ceca. Genital pore postbifurcal. Ovary lobed (0.259–0.432 mm × 0.097–0.270 mm), median between anterior parts of testes, immediately posterior to Mehlis' gland. Vitellaria follicular (14–20 each field) in pretesticular lateral fields, often having two or three lobes or arms per follicle. Uterus coiled transversely in median field often crossing ceca. Metraterm alongside cirrus pouch. Eggs small (0.019–0.025 mm × 0.009–0.013 mm), filamented at each pole. Excretory vesicle large, lobed, between posterior parts of testes and ceca.

HOSTS: *Erolia alpina, E. maritima, Arenaria interpres, Calidris canutus.*

LOCALITY: Woods Hole, Massachusetts, and Sapelo Island, Georgia.

SITE OF INFECTION: Intestinal ceca.

Holotype (No. 72000) and paratypes (Nos. 72001 and 72002) in USNM Helm. Coll., Beltsville, Maryland.

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Life Cycle of *Phyllodistomum bufonis* (Digenea: Gorgoderidae) from the Boreal Toad, *Bufo boreas*

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ABSTRACT: The life cycle of *Phyllodistomum bufonis* is described and illustrated. Hosts include fingernail clams (*Pisidium adamsi*), naiads of dragonflies (*Libidula* sp.), and toads (*Bufo b. boreas*). Experimental infections performed by feeding progenetic metacercariae to various amphibians and fish indicated that only *B. boreas* could serve as the definitive host. The formation of testes from nine primordia appears to characterize the genus *Gorgoderina* whereas the development of the testes from a single primordium is distinctive of *Phyllodistomum.*

The validity of the trematode genera *Gorgoderina* Looss, 1902, and *Phyllodistomum* Braun, 1899, has been questioned by various authors since Osborn (1903) described a transitional species, *P. americanum*, from *Ambystoma tigrinum* in North America and noted its similarity to *G. translucida* Stafford, 1902, from *Bufo lentiginosus* and *Rana virescens* (probably *B. americanus* and *R. pipiens*). Goodchild (1943) listed the various authors who have commented on the identity of these trematode genera.

Crawford (1939, 1940) published brief accounts of the life cycle of *P. americanum* based on specimens collected in Colorado from the boreal toad, *B. boreas* Baird and Girard, 1852, and the tiger salamander, *A. tigrinum* (Green, 1825). Adult flukes passed eggs containing

* From a dissertation submitted in partial fulfillment of the requirements for the Ph.D. degree, Colorado State University.

miracidia which upon hatching penetrated *Pisidium* sp. Cystocercous cercariae, shed by the bivalve, encysted in the esophagus when eaten by trichopteran larvae, diving beetles, or by naiads of damselflies. Juvenile flukes in *Bufo* migrated first to the kidneys where they remained for 2 weeks before proceeding to the urinary bladder where they matured in approximately 5 weeks.

Frandsen (1957) described *P. bufonis* from *B. boreas* in Utah and distinguished this species from *P. americanus* "by its different sucker-size ratio, the significantly smaller size of its capsules, and by the fact that *P. americanum* has a slight posterior notch." Tonn (1950, 1961) found morphological variation in *P. bufonis* from *B. boreas* in Colorado sufficiently great to include all species described for the genus.

Since Crawford (loc. cit.) did not publish detailed observations on the material that he studied and may have confused two species, the present study was undertaken to determine the morphology of the larval states and the specific identity of the *Phyllodistomum* from *B. boreas* in Colorado.

Materials and Methods

Mature *Phyllodistomum* were obtained from *Bufo boreas* collected at Trapp Lake, Larimer Co., Colorado. Fingernail clams, *Pisidium adamsi* Prime, collected at the same locality, shed cercariae of the *Phyllodistomum* sp. in the toad.

Parasite-free clams were raised in stender dishes at room temperature for several weeks before being exposed to miracidia. Monomiracidial races of *Phyllodistomum* were established and development of the larval stages studied. Naiads of dragonflies, *Libellula* sp., were collected from small pools lacking fish or amphibia and allowed to eat cercariae from experimentally infected clams. Metacercariae obtained by dissection of the naiads were fed by stomach tube to numerous hosts: *B. boreas* from Trapp Lake; *B. woodhousei woodhousei* Girard, 1854, from gravel pits 4 miles east of Windsor Reservoir; *Rana pipiens brachycephala* Cope, 1889,* from tree dump, north edge of Fort Collins; *Ambystoma tigrinum mavortium* Baird, 1850, from tree dump, north edge of Fort Collins; *B. woodhousei woodhousei* Girard, 1854, from gravel pits 4 miles east of Windsor Reservoir; and a topminnow, *Fundulus sciadicus* Cope, 1865, from the tree dump. *B. woodhousei*, *Bufo boreas*, and *F. sciadicus* were reared from eggs or fry and maintained free of trematodes. Other amphibians were examined in the laboratory for helminths before being used in experiments.

Adult trematodes were killed in warm AFA, stained in Grenacher's alum carmine, cleared in beechwood creosote, and mounted in Piccolyte before measuring. Measurements are based on five specimens from each of 10 toads. All measurements are in millimeters unless otherwise indicated. Drawings were made with the aid of a camera lucida, microprojector, and by tracing projected photomicrographs.

* Post and Pettus (1966) report that *Rana pipiens* of Fort Collins may differ from those in southern Colorado.

Results

Egg and miracidia (Figs. 1, 2)

Eggs freshly deposited, 24.0 to 35.3 μ long by 18.4 to 23.1 μ wide, ovoid, nonoperculated, increasing in size while *in utero* to accommodate developing miracidia; eggs in metraterm often with dark shell and resistant to immediate hatching in water.

Miracidia fully developed in eggs when laid. Hatching occurs within minutes after eggs are placed in water. Body mucrocuneate in shape, often assumes pyriform shape when swimming, 0.053–0.067 long by 0.030–0.042 wide. Fifteen ciliated epidermal plates present, arranged in three transverse rows with six plates in each of the first two rows and three in the last row. Apical gland in anterior region of body, 0.012 to 0.010 long by 0.029 to 0.021 wide, opens in a small apical pore. Large asymmetrical gland of Goodchild (1943) opens in space at anterior end of body; smaller homologue gland of Goodchild present in miracidia younger than 12 hr, opens similar to asymmetrical gland. Two flame cells present near middle of body, ducts open on lateral margins just anterior to last row of epidermal plates. Four germ cells present in encapsulated miracidia.

Wootton and Peters (1957) listed 15 epidermal plates for miracidia of *Gorgoderina attenuata*, *P. superbum*, *P. staffordi*, and *P. undulans*, all parasites of fishes as adults except *G. attenuata*. Goodchild (1943) reported that 16 plates were present on miracidia of *P. solidum*,

and Schell (1967) found 18 plates on *P. staffordi*. The morphology of miracidia of *P. bufonis* are similar to those of other gorgoderids having a 6, 6, 3 pattern of epidermal plates.

Mother sporocyst (Fig. 3)

Development of the mother sporocyst occurs within the gill lamella of *Pisidium adamsi* as described by Goodchild (1943) for *P. solidum* and Schell (1957) for *P. staffordi*. After 36 hr, the central cavity can be differentiated from the wall of the sporocyst and by 70 hr the cells with granular cytoplasm appear (Fig. 3). Embryos of daughter sporocysts are present as early as 80 hr after infection. Sixteen days after infection, the sporocyst moves to the inner surfaces of the gill lamellae. Twenty-one days after infection, mother sporocysts 0.45 long split, releasing the daughter sporocysts.

Daughter sporocysts (Fig. 4)

Daughter sporocysts are located in the gills anchored by the end with the birth pore with the opposite end extending into the interlamellar gill space. Sporocyst body tubular, 1.00 to 1.96 long by 0.55 to 0.70 wide; sporocyst wall generally wrinkled, thinner than wall of mother sporocyst, and contains similar granules. Birth pore subterminal on anterior end. Body cavity with 10 to 12 developing cercariae. Cercariae shed 38 to 48 days after initial exposure to miracidia.

Cercariae (Figs. 5–7)

Cercariae of *P. bufonis* are macrocercous, tail 1.46 to 1.93 by 0.14 to 0.19; cercarial chamber 0.14 to 0.16 by 0.11 to 0.13, neck 0.01 to 0.04 in length; anterior portion of tail as wide as chamber, diminishes gradually in width posteriorly, ending bluntly.

Body of cercaria fusiform, emerges readily from chamber under slight pressure, 0.21 to 0.25 by 0.09 to 0.11. Cuticle with fine striations and grooves. Stylet robust, 0.018 to 0.025 by 0.003 to 0.004. Oral sucker 0.040 to 0.055 by 0.054 to 0.060; acetabulum 0.041 to 0.070 by 0.062 to 0.082. Twelve unicellular penetration glands present, six per side, located dorsolateral to acetabulum, open in two pores on each side of stylet. Esophagus bifurcates just anterior to acetabulum into ceca which extend to posterior margin of the body. Excretory pore terminal; bladder extends to posterior margin of acetabulum, receives two main collecting ducts anteriad (subterminally), ducts extend to near posterior border of oral sucker before dividing. Flame cell pattern 2 $[(4 + 4) + (4 + 4 + 4 + 4)] = 48$; bladder surrounded by cystogenous cells. Genital primordium immediately posterior to acetabulum and ventral to the anterior end of excretory bladder, 0.008 to 0.012 by 0.03 to 0.04.

Remarks

Cercariae of *P. bufonis* leave via the birth pore after rupturing the thin host membrane surrounding the sporocyst are free in the interlamellar space and epibranchial cavity eventually to emerge through the excurrent siphon during day or night. The cercariae and sporocysts appear to do little physical damage to the host. Sporocysts, however, inhibit reproduction. Infected clams from experimental or natural infections never contained young clams.

The cercaria resembles other gorgoderid cercariae that are macrocercous, possess stylets, and have 12 penetration glands. The wide anterior portion of the tail is similar to *Cercaria conica* Goodchild, 1939; however, the remainder of the tail is not set off as distinctly and the stylet shape is different.

Metacercaria (Fig. 8)

Macrocercous cercariae liberated from the clam adhere to the substratum where they contract and extend vigorously for several hours. Naiads of dragonflies, *Libellula* spp., readily ate the cercariae which penetrated the intestinal wall and encysted in the hemocoel by means of secretions from the cystogenous glands as described by Sinitsin (1905), Goodchild (1943), and Thomas (1958). Metacercariae were usually found in the hemocoel near the posterior end of the body. Two-day-old meta-

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Figures 1–6. Larval stages of *Phyllodistomum bufonis* Frandsen, 1957. 1. Miracidium. 2. Miracidium plates. 3. Mother sporocyst, 5 days old. 4. Daughter sporocyst, 31 days old. 5. Macrocercous cercaria, anterior region of tail. 6. Body of cercaria.

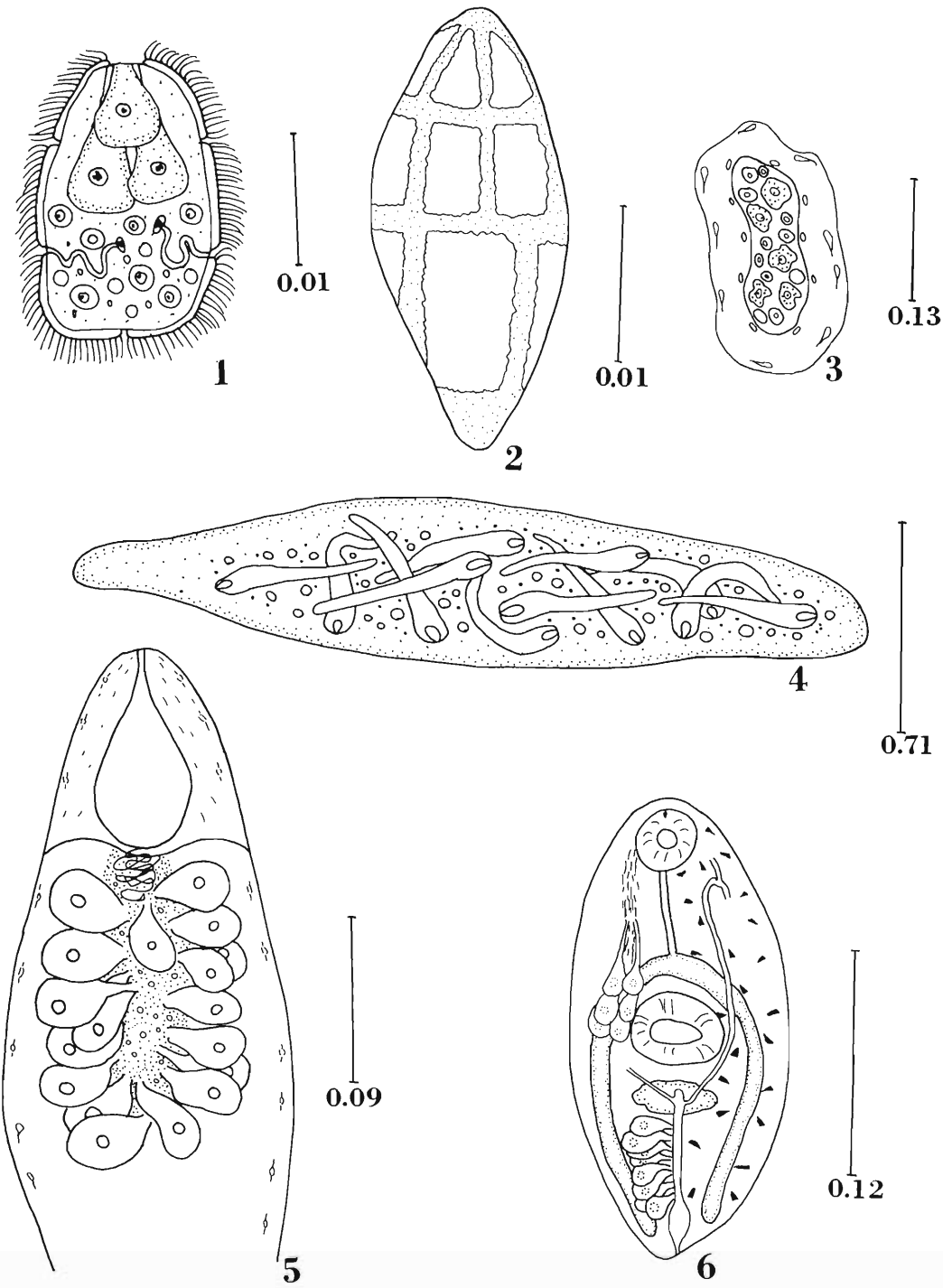


Table 1. Results of feeding amphibians and fish with 10 metacercariae of *Phyllodistomum bufonis*.

Hosts	No. specimens	Percentage infected after 48 hr
Ambystoma tigrinum uthaense	5	0
Ambystoma tigrinum mavortium	11	0
Rana pipiens (northern variety)	6	0
Bufo woodhousei	18	0
Bufo boreas	34	97
Fundulus sciaticus	6	0

cercariae measure 0.16 to 0.20 in diameter. Older ones are slightly larger. Genital organs are differentiated. In the oldest metacercariae eggs are already present in the uterus.

This is the first report of a progenetic *Phyllodistomum* from the United States. Rai (1964) reported progenetic metacercariae of *P. srivastiava* occurred in *Macrobrachium dayanus*, a freshwater shrimp in India.

Adult trematode (Figs. 9, 10)

The adult trematode used in this study resembles *P. americanum* and *P. bufonis* with minor exceptions. Measurements given by Osborn (1903) and by Frandsen (1957) reveal that *P. americanum* is smaller in size, the acetabulum is located more anteriorly, and the excretory bladder extends only to the posterior testis. Flame cell formula 2 $[(4 + 4) + (4 + 4 + 4 + 4)] = 48$ in *P. bufonis*.

Crawford (1940) reported that both *Bufo* and *Ambystoma* were infected with *P. americanum*. Tonn (1950) noted that *Rana pipiens*, *Ambystoma* spp., and *Pseudacris triseriata* collected with *B. boreas* were never infected with *Phyllodistomum*. To determine if various amphibians and fish could serve as experimental hosts, 10 metacercariae were fed to each of several hosts (Table 1). Only *B. boreas* became infected, whereas *Ambystoma* spp., *R. pipiens*, *B. woodhousei*, and *F. sciaticus* did not.

Goodchild (1943) was unable to infect *Triturus viridesans*, *R. pipiens*, *R. palustris*, *R. catesbeiana*, *R. clamitans*, *Micropterus dolomieu*, *Eupomotis gibbosus*, *Carassius auratus*, and *Cyprinus* sp. with metacercariae of *Phyllodistomum solidum* from *Desmognathus fuscus*.

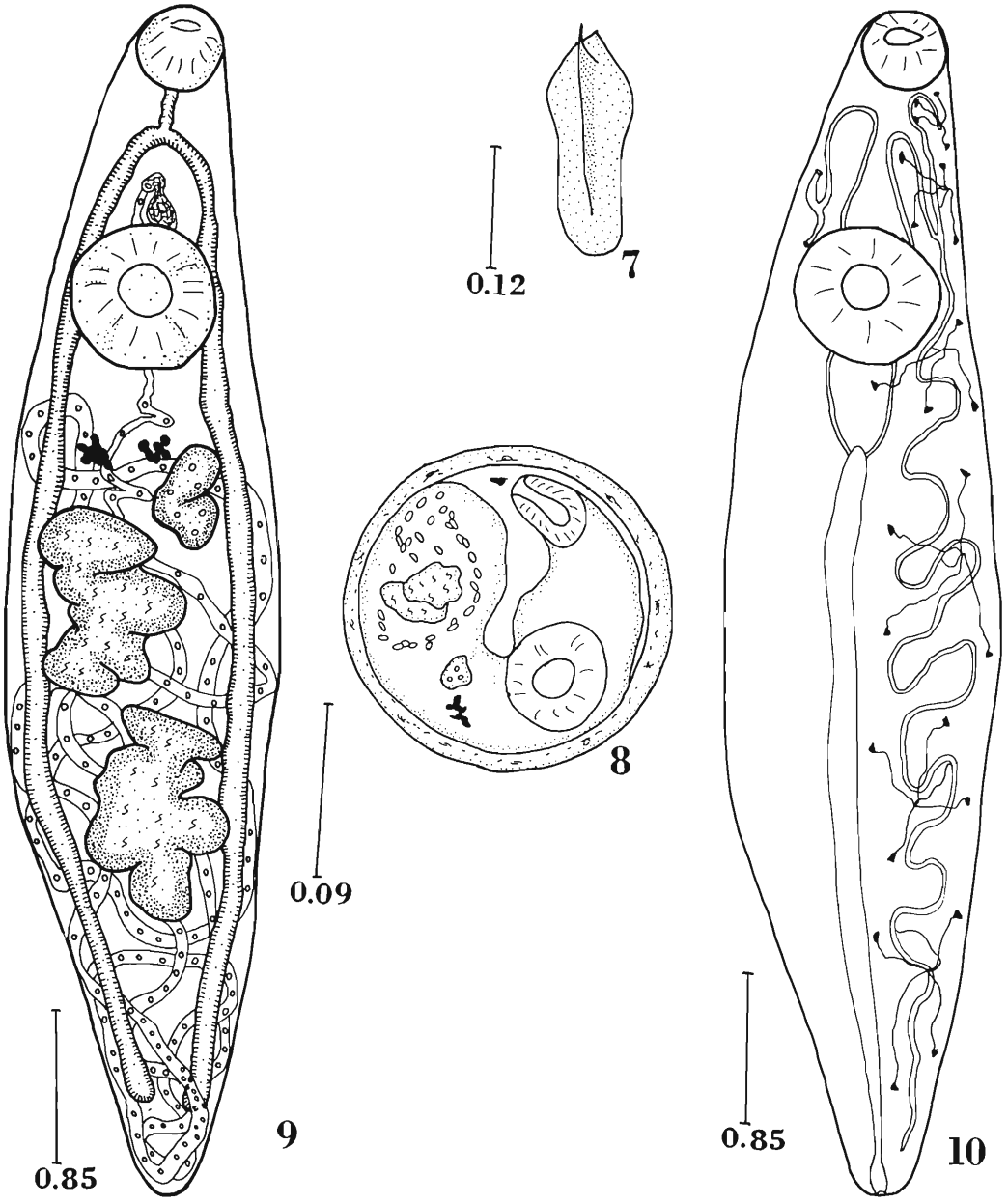
Rai (1964) reported that metacercariae of *P. srivastavai* fed to *Heteropneustus fossilis*, *Mystus cavasius*, and *Rana limnochairs* excysted only in the first host.

Several *Phyllodistomum* spp., on the other hand, are reported to have many definitive hosts. Dawes (1956) believed that many of the species will become synonyms of *P. folium* when additional knowledge is gained on the range, variability, and specificity of this group. Based on experimental evidence adult *P. bufonis* appear to be host-specific.

The validity of *Gorgoderina* and *Phyllodistomum* have been discussed by various authors. Dollfus (1958) discussed the systematics of the phyllodistomes and concluded that the shape of the body and the class of the host should serve to distinguish between *Gorgoderina* and *Phyllodistomum*.

The phyllodistomes have undergone evolutionary radiation in fish and the gorgoderids in amphibia. A few species of phyllodistomes are reported to occur in amphibia, including the transitional species *P. americanum* and *P. bufonis*. We believe the development of the testes to be a more reliable character, especially for transitional species, on the genetic level. In the genus *Gorgoderina*, testes form from a fusion of nine primordia or "anlagen" (Rankin, 1939). The anlagen each form distinct testes in the *Gorgoderina* but in *Phyllodistomum* only a single primordium is present (Goodchild, 1943; Rai, 1964). We can find no exception to this characteristic and propose its use particularly when other characters are in doubt. *P. bufonis* shows formation of testes from a single anlagen and is properly placed in the correct genus. We cannot agree with Pande (1937), Kaw (1950), or Frandsen (1957) who consider *Gorgoderina* to be a synonym of *Phyllodistomum* until species assigned to *Gorgoderina* are examined more critically and life cycles are elucidated.

Goodchild (1943) suggested that *G. schistorchis* Steelman, 1938, and *G. tenua* Rankin, 1937, should be included in the genus *Phyllodistomum* since they possess prominent uterine coils between the vitelline complex and the acetabulum. Since *P. bufonis* does possess prominent uterine coils between the vitelline complex and the acetabulum which are more highly developed in older and larger worms but



Figures 7, 8. Larval stages; 9, 10, adult morphology of *Phyllodistomum bufonis* Frandsen, 1957. 7. Stylet. 8. Metacercaria. 9. Adult morphology. 10. Distribution of flame cells.

has testes developing in a phyllodistome fashion, we consider *G. tenua* and *G. schistorchis* as belonging to the genus *Gorgoderina* until additional information is available concerning testicular development.

Crawford (1939, 1940) reported that *P. americanum* was present in both salamanders and toads and that miracidia were obtained from flukes in these two hosts for life cycle studies; however, our failure to infect salamanders with metacercariae, presumably *P. americanum*, originating from toads indicates that perhaps Crawford was dealing with two species of flukes, the species in toads being *P. bufonis*, the species in salamanders being *P. americanum*. Since it is not known experimentally whether *P. americanum* can infect toads, no definite conclusions may be drawn concerning the exact identity of Crawford's material.

In the same manner, the experimental work presented herein does substantiate Frandsen's decision to name those flukes from *B. boreas* as a distinct species.

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United States National Museum Helminthological Collection

Dr. J. Ralph Lichtenfels has been appointed curator of the USNM Helminthological Collection. All correspondence relative to the collection should be addressed: Dr. J. Ralph Lichtenfels, National Animal Parasite Laboratory, Veterinary Sciences Research Division, USDA–ARS, Beltsville, Maryland 20705.

The Fine Structure of the Cecal Epithelium of the Trematode *Aspidogaster conchicola* von Baer, 1827[1]

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ABSTRACT: Electron microscopical observations of the cecum of *Aspidogaster conchicola* indicates that it is made up of a single type of epithelial cell. Each cell is joined to adjacent cells by septate cell junctions. Mitochondria, Golgi complexes, a granular endoplasmic reticulum, vesicles, and mebranous (myelinlike) bodies are all abundantly represented. The cell surfaces extend into the cecal lumen as numerous microvilli. Each cell rests on a basal lamina, and the entire cecum is surrounded by circular and longitudinal muscle fibers.

Numerous electron microscope studies have been directed toward the cecal epithelium of a number of trematodes in attempts to develop a better understanding of digestive phenomena. Most of these involved digenetic trematodes (Bogitsh et al., 1968; Davis et al., 1968; Dike, 1967, 1969; Morris, 1968; Thorsell and Björkman, 1965; Wotton and Sogandares-Bernal, 1963). Monogenetic trematodes have received little attention (Halton et al., 1968) while the ultrastructure of the Aspidobothria had been neglected completely until the very recent study by Halton (1971).

Aubert (1855), Voeltzkow (1888), and Stafford (1896) studied the digestive system of *Aspidogaster conchicola* and each in turn added details to the description of its morphology. Aubert noted the absence of an anus. Voeltzkow reported that the entire cecal sac is made up of uniform columnar epithelial cells. Stafford carefully studied the musculature of the pharynx and the morphology of the buccal cavity.

The present study was undertaken to describe some ultrastructural aspects of the cecal epithelium of *A. conchicola* and to compare it to those of previously described trematodes.

Materials and Methods

Specimens of *Aspidogaster conchicola* were obtained from the pericardial and renal cavities of the freshwater mussels *Anodonta grandis* and *Anodonta imbecilis*, collected in the Sangamon River north of Mahomet, Illinois. The worms were fixed in iced 3% glutaraldehyde (Sabatini et al., 1963) buffered to pH 7.4 with 0.2 M s-collidine (Bennett and Luft, 1959) for 1½ to 2 hr. Postfixation in iced 1% osmium tetroxide in 0.27 M sucrose buffered to pH 7.4 with s-collidine was carried out for 1½ hr. Specimens were dehydrated through a graded series of cold ethyl alcohol and placed in propylene oxide prior to embedding in Epon 812 (Luft, 1961). Blocks were sectioned on a Porter–Blum ultramicrotome using glass knives. Sections exhibiting silver to gray interference colors were collected on 100-mesh Formvar-carbon-coated copper grids. All sections were stained with 1% aqueous uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963) and examined with an Hitachi HU-11A electron microscope operating at 50 kv. For purposes of orientation thick sections (ca. 1 μ) were periodically cut from the Epon block, picked up on Formvar-coated single hole grids (1½ by 2 mm), stained with toluidine blue and pyronin (Ito and Winchester, 1963), and examined with the light microscope.

Observations

The blind digestive cecum extends posteriad from the pharnyx as a nonbifurcate tube to about the middle of the posterior one-third of the animal. It is lined in its entirety by a single-type epithelial cell which differs markedly from the tegument lining the pharynx (Fig. 1).

The cecum is surrounded by irregularly arranged longitudinal myofibers and an outer circular layer (Fig. 2). Each epithelial cell rests on a basal lamina which varies in thick-

[1] From part of a dissertation submitted to the University of Illinois at Urbana–Champaign in 1970 for the Ph.D. degree in zoology. This research was supported in part by NIH Grant AI-1319 to Dr. F. J. Kruidenier and by the Department of Zoology, University of Illinois at Urbana–Champaign.

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ness from about 0.2 to 0.5 μ and has a low electron opacity (Fig. 2). Numerous extensive infoldings (ca. 300 A wide) of the basal cell membranes extend into the cell cytoplasm (Figs. 2, 3). The great length of some of these infoldings often make it impossible to trace them completely.

Epithelial cells are joined to each other by cell junctions having a number of septa arranged perpendicular to the two adjacent plasma membranes (Fig. 4). The inner leaf of the plasma membrane (i.e., that bordering the cytoplasm) becomes slightly thickened and appears more electron-dense along the length of the cell junction (Fig. 4).

The unjoined lateral surfaces as well as the apices of cecal cells extend as elongate processes of the cell cytoplasm (microvilli) into the lumen of the digestive tract (Figs. 5, 8). Each microvillus is enclosed by a continuum of the epithelial cell's tripartite plasma membrane and is circular to slightly oblong in cross section (Figs. 5, 6). The lumenal surface of the plasma membrane is coated by a substance having an amorphous to slightly granular nature (Figs. 5, 6).

The multitude of microvilli in the cecal lumen form a complicated maze with intricate networks of winding pathways between them often leading to blind ends (Fig. 8). Many of the microvilli recurve, branch, and apparently fuse with adjacent microvilli thereby forming closed and variable pockets (Fig. 8).

Cecal cell nuclei average approximately 3 μ in width by 5 μ in length and are surrounded by a nuclear envelope (Figs. 7, 8). The nucleoplasm contains many small dense particles as well as a large finely granular nucleolus (Fig. 7). The nuclei are situated in the basal one-third of each cell, although in cells sectioned obliquely they may appear apical since they lie so near the cecal lumen (Fig. 8). What appears to be the cell's apical tip is then actually its free lateral surface.

The cisternae of the well-developed, granular endoplasmic reticulum are nearly uniform in width and internally are moderately electron-dense (Figs. 4, 7, 8). In addition to the membrane-aligned ribosomes, many exist freely in the cytoplasm (Figs. 2, 4). Mitochondria ranging from 0.2 to 1 μ long are scattered throughout the cytoplasm (Figs. 2, 8). Each has a smooth outer membrane and an inner membrane which is plicated forming the *cristae mitochondriales*. Regardless of mitochondrial size or shape, there appears to be no preferential axial orientation of cristae.

Prominent Golgi complexes consist of flattened sacs with spherically shaped lateral swellings (Figs. 8, 9) and numerous adjacent

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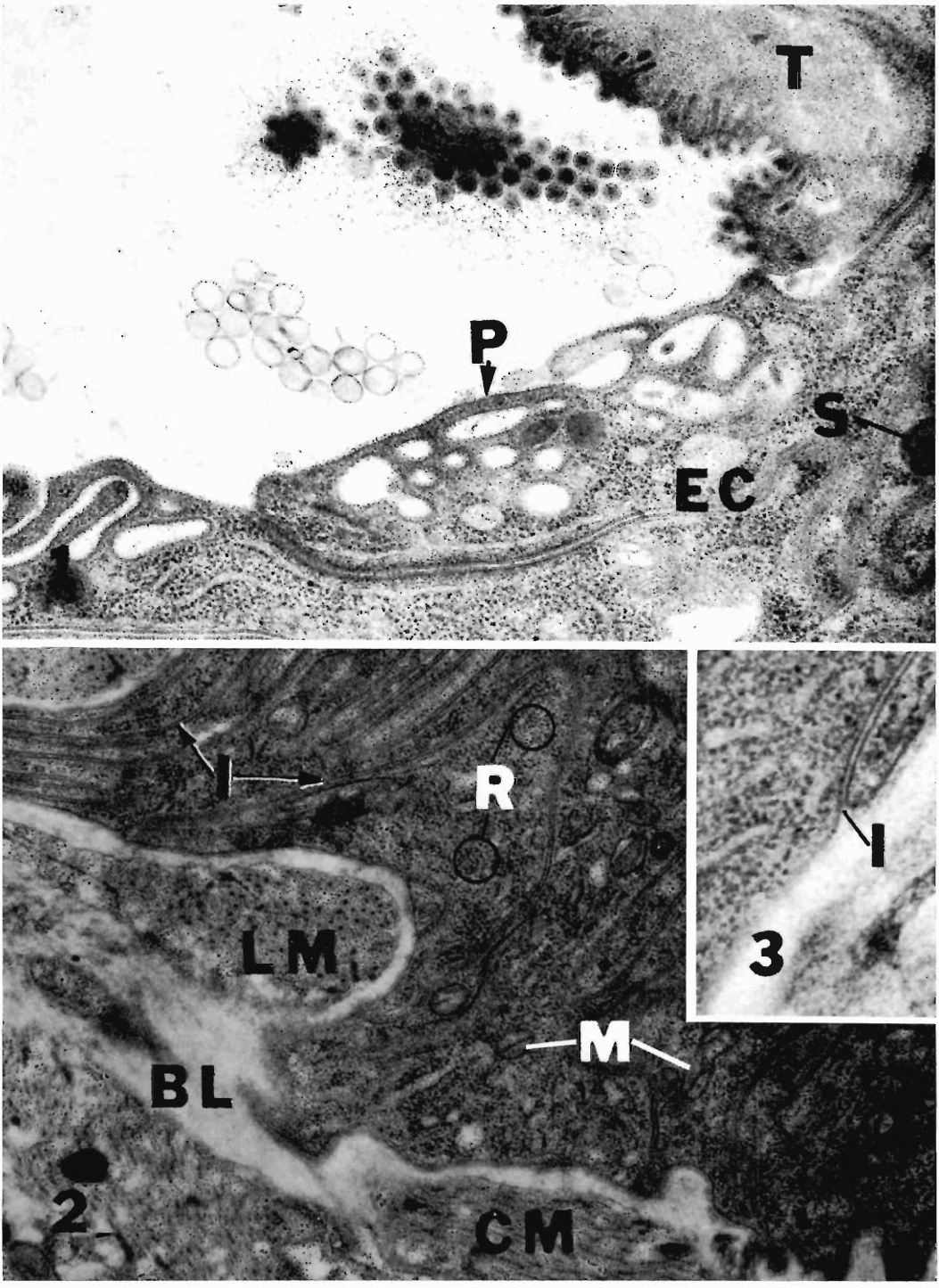
Figure 1. Region of transition from tegument (T) of posterior pharynx to epithelial cells (EC) of the cecum. S, secretion body. P, plasma membrane. × 38,250.

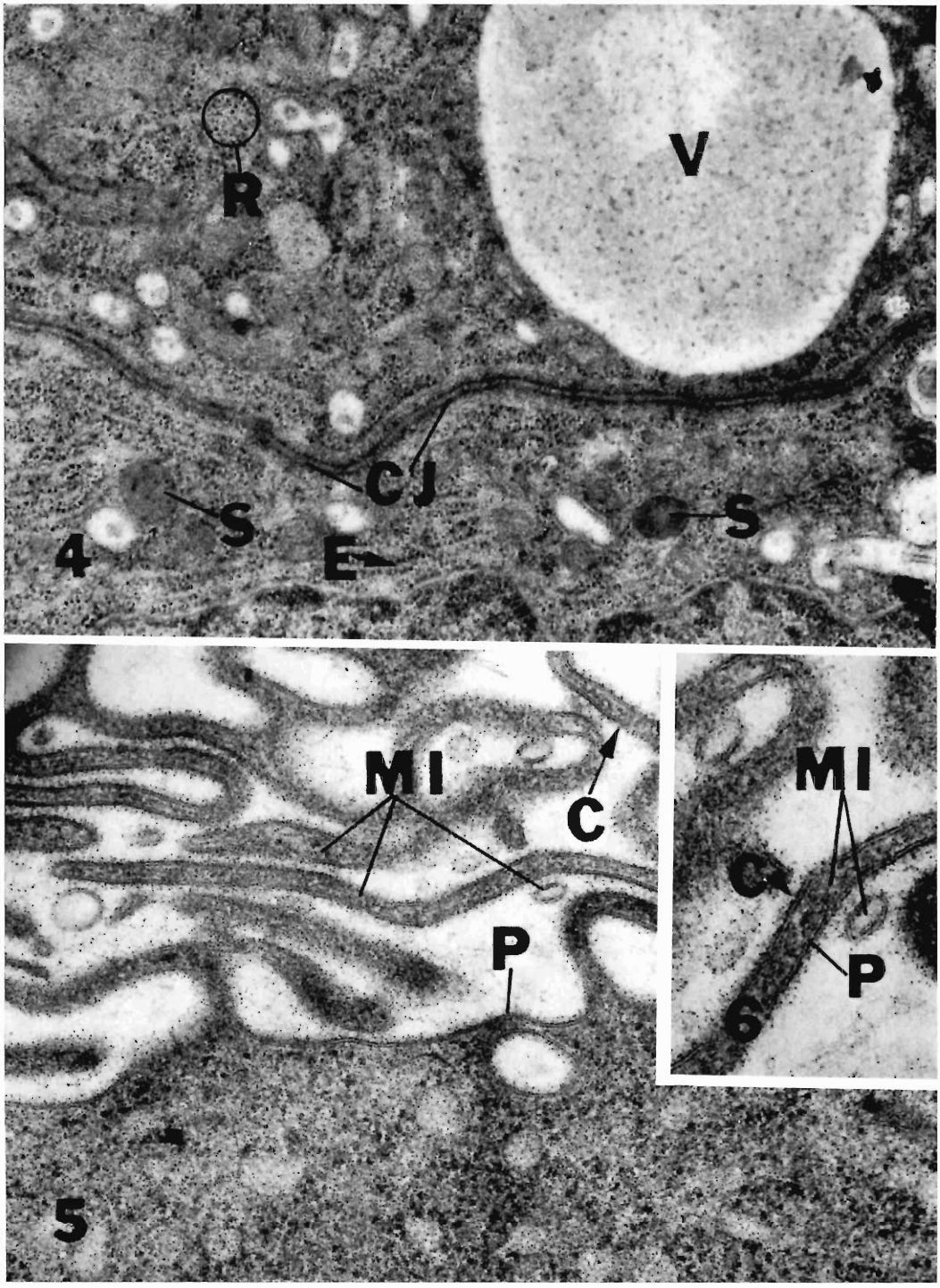
Figures 2, 3. Morphology of the basal regions of cecal cells. 2. Each cell rests on a basal lamina (BL), and the entire cecum is surrounded by circular (CM) and longitudinal (LM) muscle fibers. Numerous infoldings (I) of the basal cell membrane extend into the cell cytoplasm. R, free ribosomes. M, mitochondria. × 25,500. 3. Image at higher magnification illustrating the infoldings of the basal cell membrane. × 53,000.

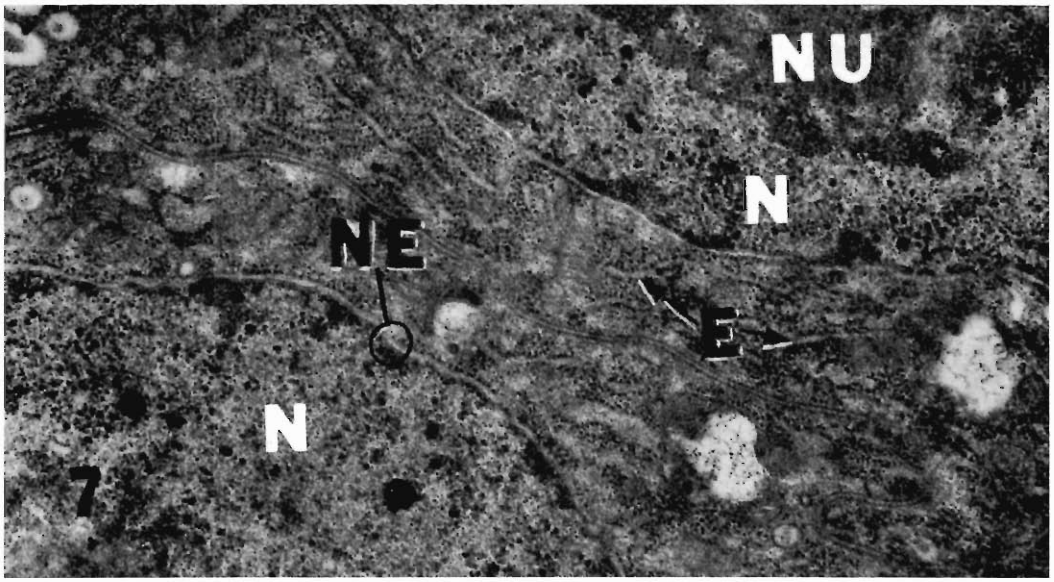
Figure 4. Cecal epithelial cells possess a well-developed granular endoplasmic reticulum (E), free ribosomes (R), secretion bodies (S), and large vesicles (V). Adjacent cells are joined by well-developed septate cell junctions (CJ). × 32,500.

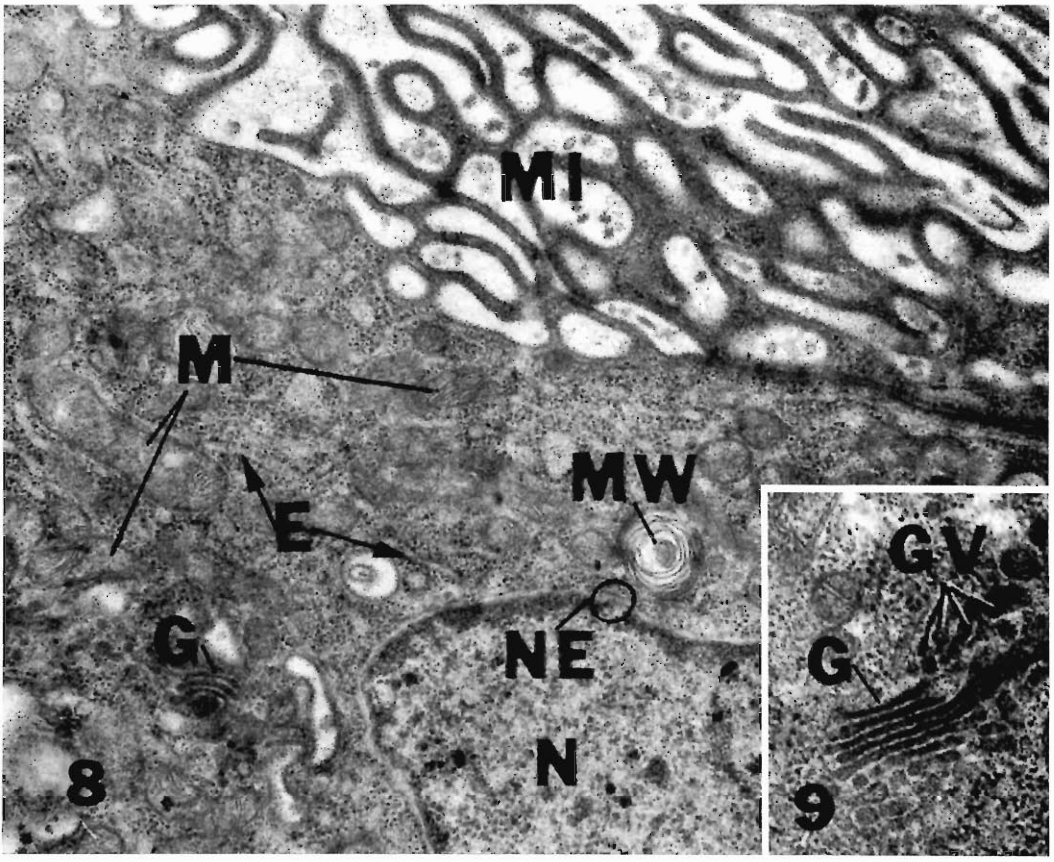
Figures 5, 6. Morphology of cecal cell surfaces. 5. Numerous microvilli (MI) extend as projections from the cecal cell surfaces. The plasma membrane (P) surrounding each microvillus is covered by a fine, granular coating (C). × 52,000. 6. Higher magnification of microvilli from same region as Figure 5. × 85,000.

Figures 7–9. 7. Cecal cell nuclei (N) possess a prominent nucleolus (NU) and are surrounded by a nuclear envelope (NE). E, granular endoplasmic reticulum. × 27,000. 8. Membranous whorls (MW) are abundant inclusions in cecal cells. Other organelles include mitochondria (M), Golgi complexes (G), and granular endoplasmic reticulum (E). N, nucleus. MI, microvilli. × 27,000. 9. Golgi complexes (G) consist of numerous flattened membranous sacs stacked upon one another. Note Golgi vesicles (GV). × 42,500.









vesicles of nearly the same electron density as the sacs (Fig. 9). Small vesicles appear to coalesce into larger secretion bodies (Fig. 9), and membrane-bound secretion granules are sometimes observed close to Golgi complexes as well as throughout the cytoplasm (Figs. 1, 4). The nearly spherical granules measure about 0.3 μ in diameter and consist of a homogeneous substance which exhibits nearly the same electron density as elements of the Golgi complex (Figs. 1, 4, 9).

Membranous (myelinlike) bodies are one of the more curious inclusions in the cecal epithelium. They appear as loose aggregates of concentrically arranged membranes surrounding a compact central region (Fig. 8). Their diameters are variable ranging from 0.3 to nearly 1 μ.

Large vesicles within the apical cytoplasm of the cells contain a substance having a low electron density and may represent lipid deposits or regions of lipid extraction (Fig. 4). Nearly every cecal cell possesses one or more of these vesicles which may attain a diameter of several microns and appear to lack a limiting membrane.

Discussion

The microvilli from the lateral and apical surfaces of the cecal epithelial cells which reach into the lumen are similar in many respects to those reported widely in the cecal epithelium of various trematodes (Gresson and Threadgold, 1959; Senft et al., 1961; Wotton and Sogandares-Bernal, 1963; Thorsell and Björkman, 1965; Halton, 1966; Dike, 1967; Morris, 1968). There is some confusion as to what to call them, but Dike (1967) pointed out that the term microvilli should apply only to those extensions which exhibit a circular cross section. In *A. conchicola* the microvilli increase the surface area of the cecal epithelia and possibly facilitate absorption of low molecular weight substances. Frequently small particles appear at the base of the microvilli, but there is no evidence that they are taken into the cell.

Cyclical transformations of cecal cells as described for *Fasciola hepatica* (Gresson and Threadgold, 1959; Dawes, 1962; Thorsell and Björkman, 1965) were not observed in the present study of *A. conchicola*. However, Halton (1971), in his study of the cecum of *A. conchicola*, described two variations in cecal cell morphology but suggested that the two forms simply reflect different nutritional states in the one-cell type comprising the cecal epithelium.

In the present study the finding of a well-developed granular endoplasmic reticulum and numerous Golgi complexes in the cecal cells of *A. conchicola* indicates a system specialized for the synthesis of proteins for secretion or export from the cell (Porter, 1961). Secretion products are sequestered within the cisternae of the endoplasmic reticulum and apparently packaged by the Golgi into vesicles. These small vesicles appear to coalesce forming larger secretion granules. The presence of secretion granules as well as cytoplasmic extensions at the cell surface indicates that the cells of the cecal epithelium serve both in absorptive and secretory capacities in *A. conchicola*.

Infoldings of the basal cell membrane similar to those of *A. conchicola* have been reported in numerous types of cells in vertebrate as well as invertebrate animals. Fawcett (1962) pointed out that such infoldings are abundant in cells involved in active transport of ions and small molecules, and their degree of development is a measure of the activity of the cell in transporting materials across its cytoplasm. The extensive development of the infolded basal plasma membrane in the cells making up the cecum of *A. conchicola* is understandable since massive transport of low molecular weight molecules and ions probably takes place through this epithelium. The mechanism by which an infolded basal cell membrane promotes active transport could not be determined, but they certainly increase the membrane surface area.

The origin and function of the membranous (myelinlike) bodies found in the cecal cells could not be determined in the present study, although Halton (1971) suggested that they represent postdigestive residues. Their structure (i.e., concentric membranous whorls) is reminiscent of similar inclusions in parenchyma cells of *A. conchicola* (manuscript in preparation). Many of the cecal cells contain large apical vesicles, and these doubtlessly correspond to Voeltzkow's (1888) lipid bodies.

Acknowledgments

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Effect of Sodium Hypochlorite Concentrations on Selected Genera of Nematodes

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ABSTRACT: Eleven genera of nematodes were immersed in undilute and three dilutions (1:5, 1:10, and 1:15) of NaOCl until they were no longer identifiable. At a 1:10 dilution or less most nematodes were unidentifiable in less than 7 min. Larvae withstood the effects of NaOCl the longest while males were most susceptible. Nematode exposure in a 1:5 dilution of NaOCl for 10 min is considered an effective rate for disinfection purposes.

Sodium hypochlorite (NaOCl) dilutions have been used to surface sterilize nematodes (*Radopholus similis*) (Feder and Feldmesser, 1955) and nematode eggs (*Rhabditis*) (Briggs, 1946). It has also been used to dissolve the gelatinous matrix enclosing *Meloidogyne* sp. eggs (Tyler, 1938). The albuminoid and chitinous layers of *Ascaris* egg shells have been dissolved with NaOCl to obtain aseptic eggs (Guevara Pozo, 1953). *Heterodera glycines* larvae have been treated with NaOCl to study cuticular layering (Hirschmann, 1959).

No reference was found concerning the effect of NaOCl on animal tissue culminating in disintegration of the tissue. Apparently NaOCl acting as a strong oxidizing agent breaks the sulphide bonds of the tissue, altering the orientation of the tissue and subsequently its tertiary structure. Hydrolysis also occurs, possibly accelerating the oxidation process. Almost any animal tissue subject to an alteration in its tertiary structure is also subject to dissolution.

In the operation of a regulatory diagnostic nematology laboratory where numerous soil samples are processed, a problem of utmost importance is the maintenance of strict sanitary procedures to prevent nematode contamination of processing substrates and equipment.

Should a nematode contaminant with regulatory status pass from substrate or equipment to a soil sample from whence it did not originate, serious economic ramifications could erroneously result.

Nematodes that desiccate on glass collapse and adhere to the glass. Reconstitution in water and examination under the microscope showed that many such nematodes could still be identified to genus.

Persistence of dead nematodes on laboratory glass was tested by allowing mixed populations of nematodes to air dry for several weeks on glass slides, then washing the slides under a hard spray of water. Some nematodes washed off, but many remaining ones were easily identifiable to genus. Further drying and washing of the same slides removed more but not all nematodes.

The primary objective of this study was to determine a concentration of NaOCl that will disintegrate a nematode until it becomes taxonomically unrecognizable in a minimal time.

A secondary purpose was to evaluate the gross effects of NaOCl on various types of nematodes.

The ultimate goal was to establish a known dilution of NaOCl that could serve as an effective decontaminant for laboratory equipment and substrates.

Materials and Methods

Sodium hypochlorite (active ingredients 5.25%) available commercially as household bleach was used undiluted and diluted with tap water at ratios of 1:5, 1:10, and 1:15.

Dropper bottles were filled to capacity with test solutions to reduce deterioration of the NaOCl and fresh solutions were made every 2 weeks.

Nematodes to be tested were selected only if in excellent physical condition and in an active state fresh from soil or root extraction. Bacteriophagous, myceliophagous, and phytoparasitic nematodes from either or both of the classes Adenophorea or Secernentea were used.

A drop of the test solution was placed on a microscope slide and the slide placed under a 150 magnification of the compound microscope.

Table 1. Means of four specimens per genus (or sex) exposed to dilutions of NaOCl expressed in time elapsed (to the nearest minute) following immersion of the specimens in the test solution. Rupture: time after immersion until cuticle ruptures. Unidentifiable: indicated time after immersion until the specimen can no longer be reliably identified to genus.

Nematode genus	Sex	Rupture (Dilution rate of NaOCl)				Unidentifiable (Dilution rate of NaOCl)			
		Undilute* Min	1:5 Min	1:10 Min	1:15 Min	Undilute Min	1:5 Min	1:10 Min	1:15 Min
Aphelenchus sp.	♀	1	1	0	0	2	2	0	0
Helicotylenchus sp.	♀	<1	1	1	5	1	2	3	19
Helicotylenchus sp. (molting)	♀	<1	1	0	0	2	3	0	0
Hemicycliophora sp.	♀	2	5†	0	0	9	35	0	0
Heterodera glycines	Cyst	3	7	20	12	9	21	39	42
Hoplolaimus sp.	♀	<1	2	4	9	2	4	7	14
Meloidogyne sp.	♂	<1	1	2	9	1	1	3	13
Meloidogyne sp.	Larvae	1	3	3	12	2	4	4	13
Pratylenchus sp.	♀	<1	1	3	10	1	2	6	16
Radopholus similis	♀	<1	1	5	70	1	2	6	74
Radopholus similis	♂	1	1	2	0	1	1	3	0
Radopholus similis (molting)	♀	12	12	0	0	13	15	0	0
Rhabditis sp.	♀	1	4	3	10	1	4	4	11
Tylenchulus semipenetrans	Larvae	<1	11	32	27	1	12	32	25
Xiphinema americanum	♀	<1	<1	1	1	1	1	3	4

* Based on 5.25% solution of NaOCl.
† Three specimens.

The time in minutes and seconds was recorded when the nematode was immersed in the test drop. The specimen was observed continuously until the body ruptured, then the time elapsed recorded. When the specimen could no longer be identified to genus the final time was recorded.

Each test was replicated 4 times in each test solution.

Results

Effect of NaOCl on specific genera: These data are presented in tabular form in Table 1 and the important considerations of each genus will be pointed out.

Rhabditis sp. (females): Habit: Bacteriophagous

Dilution rate	Range Rupture Min	Sec	Min	Sec	Unidentifiable Min	Sec	Min	Sec
Undilute		25	1	25		50	1	55
1:5	2	30	4	05	3	00	4	25
1:10	1	05	7	00	1	25	7	10
1:15	1	20	20	15	1	30	19	50

Rhabditis sp. differed in morphology from all other nematodes tested in having a large open oral aperture and by shrinking severely upon immersion. Disintegration was slow, gradual, and overall.

Aphelenchus sp. (females): Habit: Mycelio-phagous

Dilution rate	Range Rupture Min	Sec	Min	Sec	Unidentifiable Min	Sec	Min	Sec
Undilute		30	1	50	1	45	2	10
1:5		40	1	35	1	10	2	25

Aphelenchus sp. disintegrated slowly with no dramatic effects. Specimens in the test population were not sufficient in proper condition to complete the series.

Hemicycliophora sp. (females): Habit: Ecto-Phytoparasitic

Dilution rate	Range Rupture Min	Sec	Min	Sec	Unidentifiable Min	Sec	Min	Sec
Undilute	1	25	4	00	5	05	12	50
1:5	5	00	5	45	34	40	36	10

This nematode differed from all others tested in possession of a sheath (two cuticles) which one would assume to provide special protection in its environment. Such has been indicated in predation studies (Esser, 1963). In the undilute solution the body inside the sheath ruptured in all four specimens indicating the sheath was penetrated easily by the solution.

In two of four specimens the bodies were ejected forcibly from the sheath. In the 1:5 solution the inner body cuticle dissolved slowly prior to the outer sheath dissolving. Air bubbles emerged orally and from the vulva. The body separated from the sheath attachments first at the head and then the vulva. This was the most resistant nematode tested in the undilute and 1:5 solution.

Xiphinema sp. (females): Habit: Ecto-Phytoparasitic

Dilution rate	Range: Rupture Min	Sec	Rupture Min	Sec	Unidentifiable Min	Sec	Unidentifiable Min	Sec
Undilute		10		20		35		55
1:5		25		30		55	1	40
1:10		35		55	2	00	3	15
1:15		50	1	05	3	45	4	00

Xiphinema sp. differs from all other nematodes tested in having hypodermal glands throughout the body length allowing easy access of chemicals into the body. Death and disintegration was very rapid. Undilute solutions disintegrated the specimens very fast, the entire cuticle peeling off. The basal odondostylet dissolved in 1½ minutes.

In the 1:5 dilution the body ruptured at many points, the body contents gushed out, and the cuticular layers peeled off. At 1:10 the body ruptured at fewer points and the contents oozed out slowly.

Hoplolaimus sp. (females): Habit: Ecto-Phytoparasitic

Dilution rate	Range: Rupture Min	Sec	Rupture Min	Sec	Unidentifiable Min	Sec	Unidentifiable Min	Sec
Undilute		30		45	1	10	2	00
1:5	1	35	2	05	2	55	4	40
1:10	3	55	5	30	6	10	9	05
1:15	6	55	10	30	13	25	16	50

This is a relatively large nematode with a thick cuticle. Undilute solution caused the cuticle to peel off its entire length and disintegrate. At a rate of 1:5 the cuticle ballooned out and separated into two layers. Many vesicles appeared between the layers. The cephalic framework intact with stylet detached itself (also in undilute test). The body burst in a number of places and peeled off. In 1:10 solutions the cuticle peeled off slowly in pieces. Some tails split open and the contents flowed out.

Helicotylenchus sp. (females): Habit: Ecto-Semi-Endo-Phytoparasitic

Dilution rate	Range: Rupture Min	Sec	Rupture Min	Sec	Unidentifiable Min	Sec	Unidentifiable Min	Sec
Undilute		15		30		50	1	45
1:5		55	1	10	1	30	2	15
1:10		30	2	00	2	00	3	20
1:15			8	00	2	10	54	10

Helicotylenchus sp. ruptured and disintegrated undramatically. Ruptures occurred at various points on the body. In one molting specimen the cuticle dissolved in less than 5 sec; in the other it merely detached. One specimen persisted in an identifiable state for 54 min at the 1:15 rate without rupturing. This widened the above range considerably, also affecting the series mean.

Heterodera glycines (mature cysts): Habit: Semi-Endo-Phytoparasitic

Dilution rate	Range: Rupture Min	Sec	Rupture Min	Sec	Unidentifiable Min	Sec	Unidentifiable Min	Sec
Undilute	1	45	3	45	5	05	12	45
1:5		40	20	00	8	35	32	25
1:10	7	00	31	00	34	05	44	05
1:15	7	05	21	00	28	15	62	10

This nematode differs from all others in that the swollen female hardens into a protective cyst containing eggs. Undilute rates of NaOCl caused many air bubbles to emerge from the inside of the cysts (Fig. 1-A). In a short time the outer cyst opened and the eggs fell out (Fig. 1-B). Egg contents quickly deteriorated (Fig. 1-C). Some eggs burst in 18 min; in all others observed only the outer shell dissolved. The vitelline membrane, being insoluble in NaOCl (Chitwood, 1938), persisted (Fig. 1-D). Evolving air bubbles decreased with dilution with very few being produced in the 1:10 and 1:15 tests.

Tylenchulus semipenetrans (larvae): Habit: Semi-Endo-Phytoparasitic

Dilution rate	Range: Rupture Min	Sec	Rupture Min	Sec	Unidentifiable Min	Sec	Unidentifiable Min	Sec
Undilute		20		50		30	1	20
1:5	1	35	34	40	1	55	34	45
1:10	24	40	50	10	24	50	50	10
1:15	12	50	48	40	14	05	40	40

Disintegration was gradual and uneventful. A relatively fast easy kill in undilute was followed by very slow reduction to unidenti-

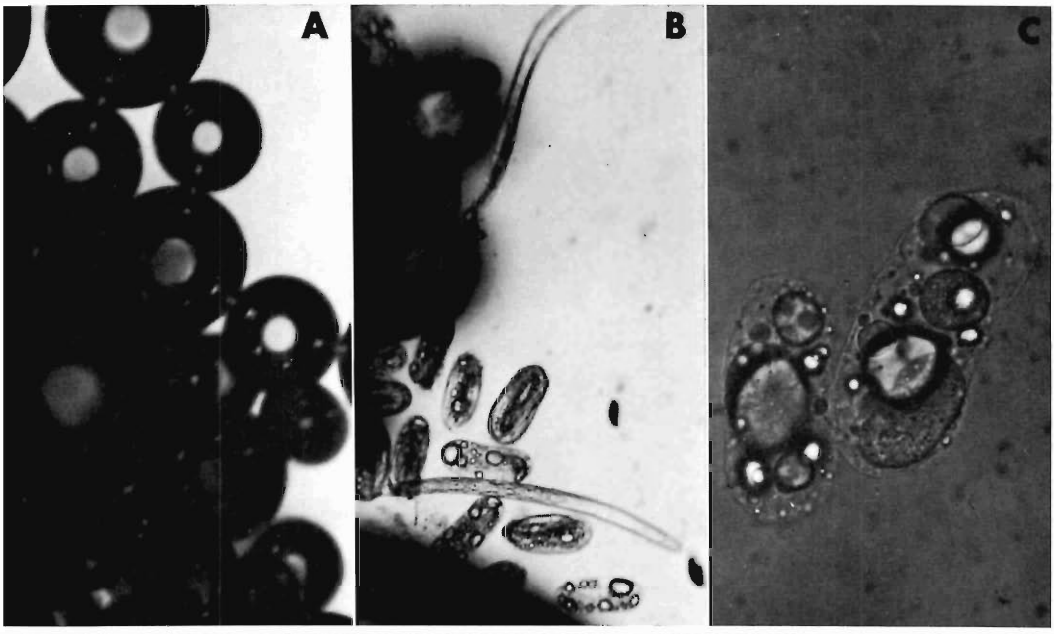


Figure 1. A *Heterodera glycines* cyst in undilute NaOCl. A, Bubbles evolving from cyst 3 min after immersion. B, eggs and larvae falling from ruptured cyst 3 min and 40 sec after immersion. C, Eggs 19 hr after immersion.

fiability in the weaker dilution series. This was the second most persistent living nematode tested.

Radopholus similis (male–female): Habit: Endo-Phytoparasitic

Dilution rate	Range Rupture Min	Sec	Min	Sec	Unidentifiable Min	Sec	Min	Sec
Undilute		30	1	15		40	2	30
1:5	1	10	1	40	1	10	2	20
1:10	2	40	8	55	4	00	9	15
1:15	6	40	256	15	11	55	256	15

In undilute and 1:5 dilution NaOCl a very fine cuticular layer peeled off followed by peeling away of the cuticle in layers. The body burst at various points. Males in undilute and 1:5 dilution deteriorated as females in 1:10. The one male checked deteriorated at a faster rate than the females.

Molting females in undilute and 1:5 dilution persisted more than 12 min before bursting or deteriorating. A molting male had its cast cuticle rapidly dissolved in a 1:5 solution. One female was unidentifiable in 3 min 25 sec in a 1:15 solution.

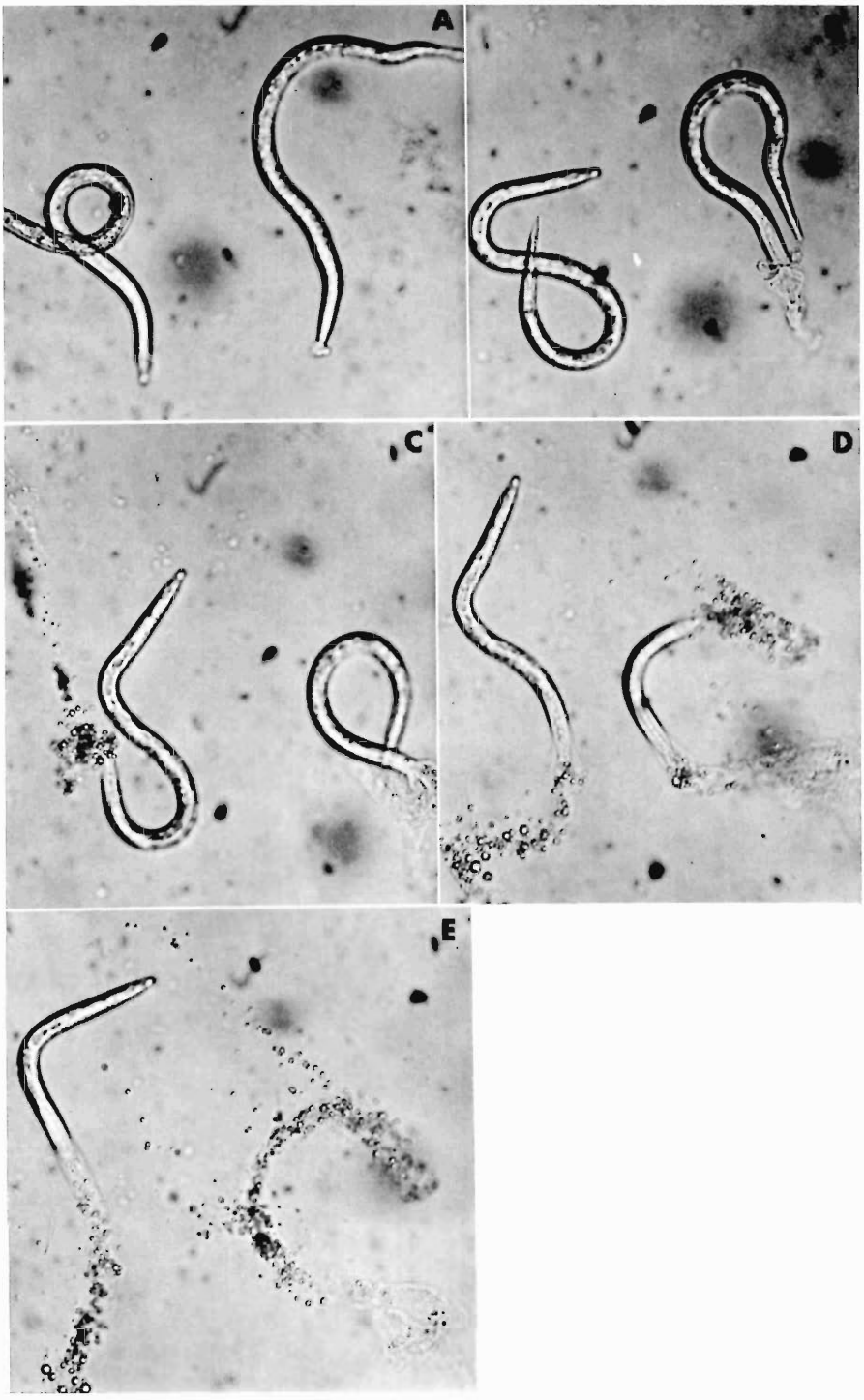
Pratylenchus sp. (females): Habit: Endo-Phytoparasitic

Dilution rate	Range Rupture Min	Sec	Min	Sec	Unidentifiable Min	Sec	Min	Sec
Undilute		20		35		45	1	05
1:5		35	1	25	1	15	2	25
1:10	1	55	4	50	3	20	8	45
1:15	5	45	22	50	12	30	22	50

Disintegration was gradual and uneventful. The body ruptured randomly at various locations.

Meloidogyne sp. (larvae and males): Habit: Endo-Phytoparasitic

Dilution rate	Range Rupture Min	Sec	Min	Sec	Unidentifiable Min	Sec	Min	Sec
Male								
Undilute		05		40		50	1	35
1:5	5	00	1	00	1	10	1	50
1:10	1	00	2	30	2	05	4	05
1:15	8	15	12	05	12	00	13	45
Larvae								
Undilute		25	1	05		45	1	20
1:5	1	40	4	55	2	45	5	30
1:10	3	00	4	35	3	45	4	55
1:15	3	00	27	20	3	30	27	20



Males in undilute and 1:5 solutions dissolved rapidly and uniformly and completely. Larvae also dissolved gradually and uneventfully (Fig. 2). Body rupturing was at random. Males succumbed rapidly and more dramatically than larvae.

Discussion

DECONTAMINATION DILUTION RATES: As an effective dip or drench to render unidentifiable nematodes contaminating laboratory equipment or substrates, a dilution of NaOCl at 1:5 exposed for 10 min would be most effective. Most nematodes were dissolved in less than 4 min (Table 1). Exceptions in this test include *Heterodera* cysts (unlikely as a contaminant), citrus nematodes, and some stages of ecdysis. Burrowing nematode, the chief nematode pest in Florida from a regulatory standpoint, was found easily dissolved at this dilution.

A 1:10 dilution for an exposed time of 10 min would also be effective for most nematodes. Most nematodes dissolve in less than 7 min. Cysts and citrus nematodes, however, would still be identifiable. The dilution rate of either 1:5 or 1:10 should prove a useful regulatory procedure in many laboratories.

The 1:15 rate would not be reasonably effective for dissolving nematodes since the exposure time would have to be excessively long.

It should be pointed out that all dilutions tested kill nematodes if a sufficient exposure occurs. However, kill alone was not an objective of this test.

HABIT: Free-living and ecto-phytoparasitic nematodes disintegrated easily and rapidly through the four series. Semi-endoparasitic nematodes (*Tylenchulus semipenetrans* larvae and *Heterodera* cysts) showed the strongest resistance to disintegration. Endo-phytoparasites proved quite susceptible to disintegration with a few specific exceptions.

SEX: Males of *Radopholus similis* and *Meloidogyne* sp. disintegrated more rapidly than the females or larvae. The reaction of larvae of two phytoparasitic genera was in sharp contrast; *Meloidogyne* sp. disintegrated easily while larvae of *Tylenchulus semipenetrans* persisted quite some time.

EXSHEATHMENT: NaOCl has been used to induce exsheathment (Lapage, 1933). In this study *Hemicycliophora* were ejected from the sheath forcibly, and sheath bonds to the body dissolved.

ECDYSIS: Molting specimens of *R. similis* persisted in undilute and 1:5 solutions 12 min prior to bursting or deteriorating. Other molting specimens of *R. similis* and *Helicotylenchus* deteriorated rapidly. In both cases the molted skin dissolved quickly. Indications are that some phases in the molting process are resistant to adverse chemical conditions while other periods are not.

MORPHOLOGY: Well-sclerotized structures such as spear, cephalic framework, and spicules persist for several minutes following body deterioration. Such structures are in an excellant state for study when the body has dissolved away. One *Hoplolaimus* sp. enface was made easily from a free cephalic framework. Definition of these structures are the best seen by the author. To preserve such structures water was added to stop the NaOCl action. To study cuticular layers the specimen was transferred to water as soon as the cuticle started peeling or layering.

BODY WALL: Particular attention was paid to what area of the body wall ruptured to determine if a particular part of the body wall structure is subject to rupturing indicating a weakness. No consistency occurred within genera or across the range of genera. Bursting of the body wall was random throughout its length. Body apertures rarely acted as a focal point for bursting. Within the random bursting, extrusion from the head occurred numerous times with several genera.

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Figure 2. Two *Meloidogyne* larvae placed in a 1:5 dilution of NaOCl. A, After 2 min and 10 sec, left larvae ruptures at head; B, After 3 min and 40 sec, larvae on right is still moving; C, In 4 min and 15 sec larvae on right ruptures; D, After 6 min and 15 sec both larvae are disintegrating; E, After 8 min and 5 sec the larvae on the right is disintegrated.

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Development of Gametocytes and Oocysts of *Eimeria magna* from Rabbits in Cell Culture[1]

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ABSTRACT: Merozoites of *Eimeria magna* were obtained from mucosal scrapings taken from a rabbit inoculated 5¼ days earlier. These merozoites were inoculated into cultures of Madin–Darby bovine kidney cells, which were then examined with interference-contrast or phase-contrast microscopy at intervals of 1 to 12 hr for 80 hr. Merozoites entered cells and most underwent development into gamonts and oocysts; some formed schizonts. From 12 to 60 hr, young macrogametes gradually enlarged; during this time, the plastic granules increased in size and number. Mature macrogametes were 28.5 by 21 μ. From 12 to 72 hr, microgamonts increased in size, nuclear divisions occurred and, in some, invaginations at the margin of the parasite were present. At 72 and 80 hr, mature microgamonts were 32 μ in diameter and had hundreds of microgametes. Extracellular microgametes exhibited slight motility after exposure to a 0.25% sodium taurocholate or 2.0% bovine bile solution. Fertilization was not observed. Mature oocysts were first seen at 72 hr after inoculation of merozoites.

Development of macro- and microgamonts of an eimerian species in cell culture has not yet been reported for any species occurring in mammals. The endogenous stages of *E. magna* in rabbits have been described by Rutherford (1943) and Cheissin (1960), and the in vitro development of first- and second-generation schizonts of this species has been reported by Speer and Hammond (1971). The development of merozoites to mature gametocytes and oocysts of *E. magna* from rabbits in cell culture is described herein.

Materials and Methods

Monolayer cell cultures of Madin–Darby bovine kidney (MDBK) cells (255th serial passage) were used to study the in vitro development of *Eimeria magna*. The methods of Fayer and Hammond (1967) were used to obtain and maintain the monolayer cell cultures. After 24 hr of incubation at 37 C, the monolayers of MDBK cells were inoculated with merozoites obtained by scraping the mucosa of the lower ⅔ of the small intestine of a rabbit inoculated 5¼ days earlier with approximately 200,000 oocysts of *E. magna*. The mucosal scrapings were gently stirred with a glass rod, and the mixture was then rinsed in saline A containing 5,000 μg dihydrostreptomycin and 5,000 units penicillin G/ml. The suspension was centrifuged at 200g for 1 min and the supernatant, containing the merozoites, was removed. The merozoites were then resuspended in minimal essential medium (MEM) with Eagle's balanced salt solution

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and without serum or antibiotics; they were counted with the aid of a hemacytometer. One ml of MEM, containing approximately 400,000 merozoites, was inoculated into each Leighton tube, with a monolayer on a 10-× 35-mm cover slip. After 6 hr of incubation at 37 C, 3 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. Cover slips were removed from the tubes and examined in double-coverslip preparations (Parker, 1961) with Zeiss-Nomarski interference-contrast or phase-contrast microscopy at 22 or 37 C at intervals of 1 to 12 hr for 80 hr. Measurements of 15 or more living specimens were made with an ocular micrometer at a magnification of 1,000× to determine each mean.

Results

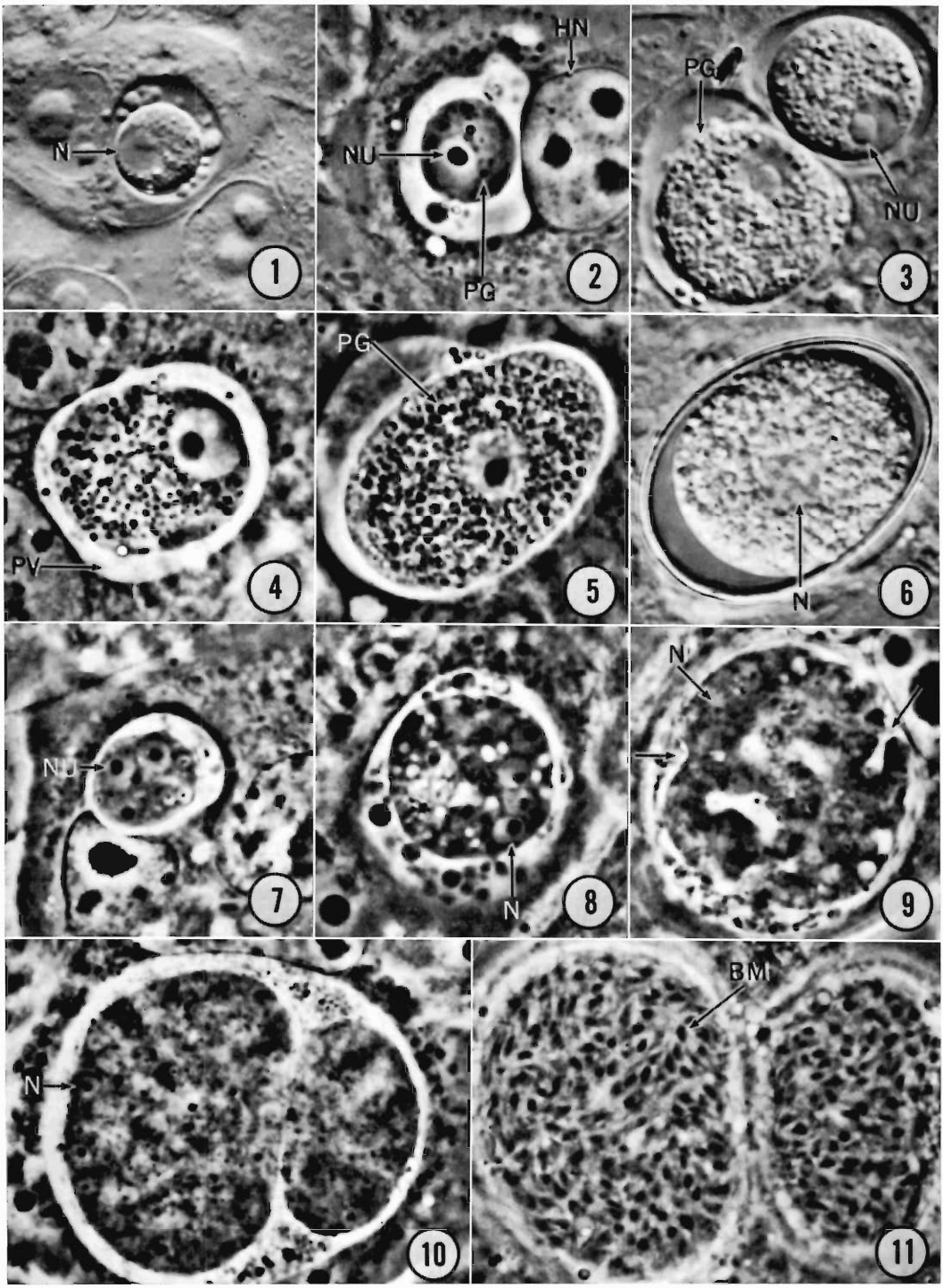
Intracellular merozoites were seen as early as 1 hr after inoculation. At 6 to 12 hr after inoculation, the merozoites had rounded up to form spherical trophozoites. Most of these apparently developed into gamonts, but schizonts with two to about 20 nuclei were observed 12 to 80 hr after inoculation. Mature schizonts with six to 20 merozoites were also seen during this period. At 12 hr, young macrogametes (Fig. 1) were usually distinguishable from microgamonts by their relatively large size (diameter, 9.5 μ) and by the large nucleus (4.5 μ) and prominent nucleolus (2 μ). The corresponding values at 24 hr were 12, 5.5, and 2 μ; those at 48 hr were 18.5, 7, and 2.5 μ. At 24 hr, a few small plastic granules were present in the cytoplasm of the young macrogamont (Fig. 2), whereas at 48 hr the cytoplasm was almost completely filled with small, spherical plastic granules which were approximately 0.5 μ in diameter (Figs. 3, 4). At 60 hr, the nearly mature macrogamonts had become ovoid in shape and measured 28.5 by 21 μ. They were similar in appearance to those seen at 48 hr, except for the presence of more and slightly larger plastic granules (Fig. 5).

Young uninucleate microgamonts observed at 12 hr after inoculation were 5.5 μ in diameter; the nucleus and nucleolus were 2.5 and 1 μ, respectively. At 24 hr, immature microgamonts, 11.5 μ in diameter, had two to five nuclei (Fig. 7) similar in appearance to those seen earlier. At 36 hr microgamonts, 15.5 μ in diameter, had 10 to 15 nuclei which were not as large as in the earlier stages. At 48 and 60 hr, the numerous, indistinct nuclei were smaller than those of the 36-hr specimens. They were arranged at the periphery as well as at the margins of invaginations extending into the central region of the gamont (Fig. 9). At 60 and 72 hr, the microgamonts were 30 μ in diameter and had peripherally arranged nuclei. Two to four microgamonts were occasionally observed within the same parasitophorous vacuole (Fig. 10). At 72 and 80 hr, mature microgamonts, 32 μ in diameter, had hundreds of microgametes located peripherally; the central region had a finely granular appearance. Most specimens occurred singly in host cells and were spheroidal, but two or more ellipsoidal specimens were seen in some cells (Fig. 11). No invaginations were observed at the margin of mature microgamonts. Free microgametes, with slight motility, were observed only when preparations were exposed to a 0.25% sodium taurocholate (bile salt) or 2.0% bovine bile solution (Speer et al., 1970). We were not able to determine whether the bile salt or bile influenced motility. Each microgamete appeared to have two flagella and a rather dense, tapered body.

Oocysts, first seen at 72 hr, were ovoid and measured 33.5 by 24 μ. Initially, the sporoplasm filled all of the space within the oocyst wall. Later, the sporont was condensed, with a central nucleus that appeared as a depression in specimens observed with interference-contrast microscopy (Fig. 6). Oocysts formed in cell culture were similar in size, shape, and appearance to those described for *E. magna* by Levine (1966).

Discussion

Up to now, gametogony of an *Eimeria* species has been obtained in cell culture only in *E. tenella* from chickens. By inoculating tissue cultures with second-generation merozoites of *E. tenella*, Bedrnik (1967) observed development of gametocytes and oocysts in vitro. Later, Strout and Ouellette (1969) reported gametogony in primary kidney cell cultures of embryonic chickens inoculated with *E. tenella* sporozoites. Doran (1970) found that *E. tenella* sporozoites would consistently develop



to oocysts when kidney cell aggregates from 2- to 3-week-old chicks were used. Doran (1971) recently found that this method supported development of asexual stages in one of five *Eimeria* species in poultry; no development of the other four was observed. Hammond et al. (1969) attempted to obtain development beyond first-generation merozoites by using a method similar to that of Bedrnik (1967); various cell cultures were inoculated with first-generation merozoites of *E. bovis* obtained from infected calves. Small numbers of intracellular first-generation merozoites were found, but no further development occurred. When sporozoites of *E. magna* were inoculated into cell cultures, development progressed only to the mature second-generation merozoite stage (Speer and Hammond, 1971). The finding in the present study that merozoites from rabbits will develop into oocysts in cell cultures might indicate that certain of the intermediate schizont stages require substances or conditions for further development which are found only in the natural host.

To the best of our knowledge, Marquardt (1966) is the only investigator to give detailed descriptions of living gametocytes of an *Eimeria* species observed with the light microscope. He studied these stages in mucosal scrapings obtained from the small intestine of rats which were infected with *E. nieschulzi*. Although fertilization was not observed, he saw a macrogamete free of host tissue which had several microgametes adhering to its periphery. Scholtyseck and Hammond (1970) found a microgamete within the cytoplasm of a macrogamete of *E. bovis* but did not observe the fusion of the macro- and microgamete nuclei. The entrance of microgametes into microgametes was not seen in the present study, but the use of this method should enable such observations to be made.

Acknowledgments

The authors are indebted to Yoko Y. Elsner for assistance in maintenance of the cell cultures and to Dr. John V. Ernst, Regional Parasite Research Laboratory, USDA, Auburn, Alabama, for supplying the original oocysts used in the work.

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Figures 1–11. Photomicrographs of development of macro- and microgamonts of *Eimeria magna* in 255th-passage Madin–Darby bovine kidney cells. All are of living specimens photographed with Zeiss–Nomarski interference-contrast (Figs. 1, 3, 6) or phase-contrast (Figs. 2, 4, 5, 7–11) microscopy; × 1,500. Hours after inoculation of merozoites into culture listed in parentheses. Abbreviations: BM = body of microgamete; HN = host cell nucleus; N = nucleus of parasite; NU = nucleolus of parasite; PG = plastic granule; PV = parasitophorous vacuole. 1, Young macrogamete (12). 2, Young macrogamete (24). 3, Two immature macrogametes in same host cell (48). 4, Immature macrogamete (48). 5, Mature macrogamete (60). 6, Oocyst with condensed sporont (72). 7, Three-nucleate immature microgametocyte (24). 8, Immature microgametocyte with approximately 10 nuclei (36). 9, Nearly mature microgametocyte; note invaginations at the margin of the gametocyte (arrows) (60). 10, Two microgametocytes in same parasitophorous vacuole of a host cell (72). 11, Two mature microgametocytes with microgametes in the same host cell (72).

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African Species of the Genus *Belondira* Thorne, 1939

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ABSTRACT: Three species from Mauritius, *Belondira clavicaudata* (Williams, 1958) Andrássy, 1964, *B. perplexa* Williams, 1958, and *B. singularis* Williams, 1958, are redescribed from type specimens; their taxonomic position is discussed. *Belondira mernyi* Andrássy, 1970, and *B. tumicauda* sp. n. from the Congo are described. An amended diagnosis of the genus is given.

The genus *Belondira* appears to be rather poorly represented in Africa. Four species were recorded previously, three of these were found in Mauritius and a fourth in the Ivory Coast. The latter species was also found in the Congo together with a new species which is described herein. The species from Mauritius show some unusual characters; two of them even have been excluded from the genus *Belondira* in some recent studies. A critical study of the type specimens of the Mauritian species has revealed some new facts, therefore a redescription is given and the position of the species discussed. The specimens from the Congo were fixed with 4% formalin and mounted in glycerin.

Belondira perplexa Williams, 1958
(Fig. 1)

Measurements: See Table 1.

Females: Body slightly curved ventrally in death position, almost cylindrical except for anterior neck region which tapers rapidly. Cuticle marked with fine transverse striae. Lateral chords about 1/6–1/5 of body width near middle of body.

Lip region subtruncate, continuous with body; with prominent sclerotization and amalgamated lips. Amphids funnel-shaped (Fig. 1B) or with more convex sides; curved slitlike apertures occupying 3/4 of corresponding body width. Odontostyle 1.0–1.1 lip region widths long; its aperture about 2/7 of odontostyle length. Guiding ring 1.1–1.3 lip region widths from anterior end. Odontophore 1.1–1.4 times the odontostyle length.

Basal expanded part of esophagus occupying 33–37% of neck region, enveloped by a sheath of dextral (n = 3, Fig. 1J) or sinistral (n = 5, Fig. 1K) spiral muscle bands. Only the dorsal esophageal gland nucleus (DN) and outlet (DO) well visible, occurring at 66.5–68% (DO) and 68–69.6% (DN) of the neck length. Nerve ring surrounding anterior slender part of esophagus at 45–48% of neck length. Cardia cylindroid with rounded tip. Prerectum 4–5 and rectum about one anal body width(s) long.

Table 1. Measurements and diagnostic features of African *Belondira* species (females).

Species	*B. perplexa* Magenta, Mauritius		*B. singularis* Melrose, Mauritius		*B. clavicaudata* Union Park, Mauritius		*B. tumicauda* Vieux Kilo, Congo		Nioka, Congo
Locality / No. of specimens	Holotype ♀	Paratype ♀♀ (7)	Holotype ♀	Paratype ♀♀ (2)	Holotype ♀	Paratype ♀♀ (4)	Holotype ♀	Paratype ♀	Paratype ♀♀ (2)
L (mm)	1.86	1.78–2.20	1.16	1.16–1.20	0.82	0.79–0.90	1.08	0.92	1.02–1.04
a	47	49–54	46	43–44	33	32–36	43	38	44–46
b	7.3	6.8–8.2	5.7	5.8–6.0	4.0	4.4–4.8	5.1	4.6	4.2–4.8
c	18	19–31	8.7	8.6–9.7	33	32–45	21.5	21	21–22
V	31	28–31	34	30–35	35.5	31–37	39.5	40.5	40–41
G1 (%)	1.8	1.3–2.4	3.7	3.1–3.2	2.5	1.9–2.7	2.7	3.4	2.6(n = 1)
G2 (%)	21	11–26	16.4	12–14	15	10–18	24.5	14	29(n = 1)
Cuticle thickness midbody (μ)	3	2–3	2	2	1.5	1.5	1	1	1
Lateral body pores—total number	25 R*	19–25 L, 21–24 R	?	18 L, ? R	?	17–18	?	?	?
—in neck region	4	4–5 L, 4–5 R	5 L	5–6 L, 6–7 R	?	4–5	?	?	?
—between cardia and anus	18	14–18 L, 15–19 R	?	9–10 L, ?R	?	10–11	?	?	?
—caudal pores	3	1–3 L, 1–3 R	7 (L + R)	6–7 (L + R)	2	2	2	2	2
Ventral body pores	13	14–16	?	10	?	?	?	?	?
L.r.w. = Lip region width (μ)	8	7.5–8	5.5	5.5	6	5.5–6	5	5	5.5–6
L.r.w./lip region height	2.0	1.8–2.3	1.8	1.8–2.2	2.0	1.5–2.0	1.7	2.0	1.9–2.0
Amphid width (μ)	?	4	3	?	3	3–4	?	3	3
Sensillae behind amphid aperture (μ)	?	16–19	?	16	?	22–23	22	23	?
Odontostyle length (μ)	9	7.5–9	6	5.5–6.5	3.5	3.5	3.5	3	3.5
Odontophore length (μ)	10	9–11	8	8.5–9.5	14	15	9	8.5	8–9
Guide ring from anterior end (μ)	–	6–9.5	5	5	4.5	5	6	5	5.5
Esophagus—total length (μ)	250	248–257	201	192–204	207	181–190	210	210	220–246
—length posterior part (μ)	88	88–93	66	64–75	112	82–92	87	84	95–109
Prerectum (Pr.) length (μ)	122	102–116	66	64–80	76	61–102	53	?	53(n = 1)
Pr./anal body width	4.6	4.3–4.8	3.7	3.3–4.1	4.2	3.5–5.5	3.1	?	3.0(n = 1)
Rectum length (μ)	29	26–31	20	?	?	20	12	13	15(n = 1)
Tail length (μ)	104	60–106	133	120–140	25	20–25	50	43	46–50
Tail/anal body width	3.9	2.5–4.1	7.5	6–7.2	1.4	1.1–1.4	2.9	2.3	2.6–3.0
Nerve ring from anterior end (μ)	119	113–122	91	85–87	59	68–69	82	87	90
Hemizonid from anterior end (μ)	118	110–117	86	80–83	?	62–65	?	?	?
Egg length by width (μ)	154 × 32	162 × 31(n = 1)	–	–	–	–	157 × 20	–	–

* L = left, R = right body side.

Vulva transverse oval, 11 by 6 μ in one paratype. Vagina extending inward over more than half of the corresponding body width. Anterior gonad rudimentary, 1.8 times the corresponding body width long or less. Posterior gonad normal; uterus 1.5–2 body widths long. No sperm present in the gonads.

Tail convex–conoid in its anterior part, then tapering towards a rounded, sometimes slightly clavate tip; with two caudal pores on each side. Terminal nonprotoplasmic portion of tail 7–29 μ long.

MALE: Unknown.

JUVENILE: A single juvenile of the fourth stage was present among the paratypes. It agrees with the females in general morphology.

TYPE LOCALITY AND HABITAT: Soil around roots of sugar cane, Magenta, Mauritius.

Belondira singularis Williams, 1958
(Fig. 2)

MEASUREMENTS: See Table 1.

FEMALES: Body nearly straight or slightly curved ventrally in death position, tapering towards both extremities. Cuticle marked with transverse striae. Lateral chords about ⅕ of body width near middle of body. In one paratype several lateral body pores are surrounded by a jellylike substance to which fine particles adhere (Fig. 2L, M).

Lip region continuous with body, subtruncate; with amalgamated lips and internal sclerotization. Shape of amphids not clear; curved slitlike apertures occupying about half of the corresponding body width. Odontostyle 1.0–1.2 lip region widths long; its aperture about ⅓ of odontostyle length. Guiding ring about one lip region width from anterior end. Odontophore 1.3–1.7 times the odontostyle length.

Basal expanded portion of esophagus occupying 33–37% of the neck region, enveloped by a sheath of dextral spiral muscle bands. Dorsal gland nucleus situated at 68–72% of neck length. Nerve ring surrounding anterior slender part of esophagus at 42–45% of neck length. Cardia tongue-shaped, about half of the corresponding body width long. Prerectum 3–4 and rectum about one anal body width(s) long.

Vulva transverse oval, 10 by 4 μ in one paratype. Vagina extending inward over more than half of the body width. Anterior gonad rudimentary, 1.4–1.9 times as long as the corresponding body width. Posterior gonad normally developed; uterus 1.6–1.7 body widths long. Oval sperm present in prevulval sac or in oviduct of posterior gonad.

Tail with cylindroid proximal, clavate median, and filiform distal parts. The median expansion is due to expanded middle layers of the cuticle; the internal cuticle shows a pattern of spirally arranged fibers. Caudal pores arranged in two sets, one consisting of two to three pores in the vicinity of the anus, the other consisting of four pores just in front of the clavate swelling; two of the latter pores are lateral, one is ventral, and one dorsal.

MALE: Not found.

TYPE LOCALITY AND HABITAT: Soil around roots of sugar cane, Melrose, Mauritius.

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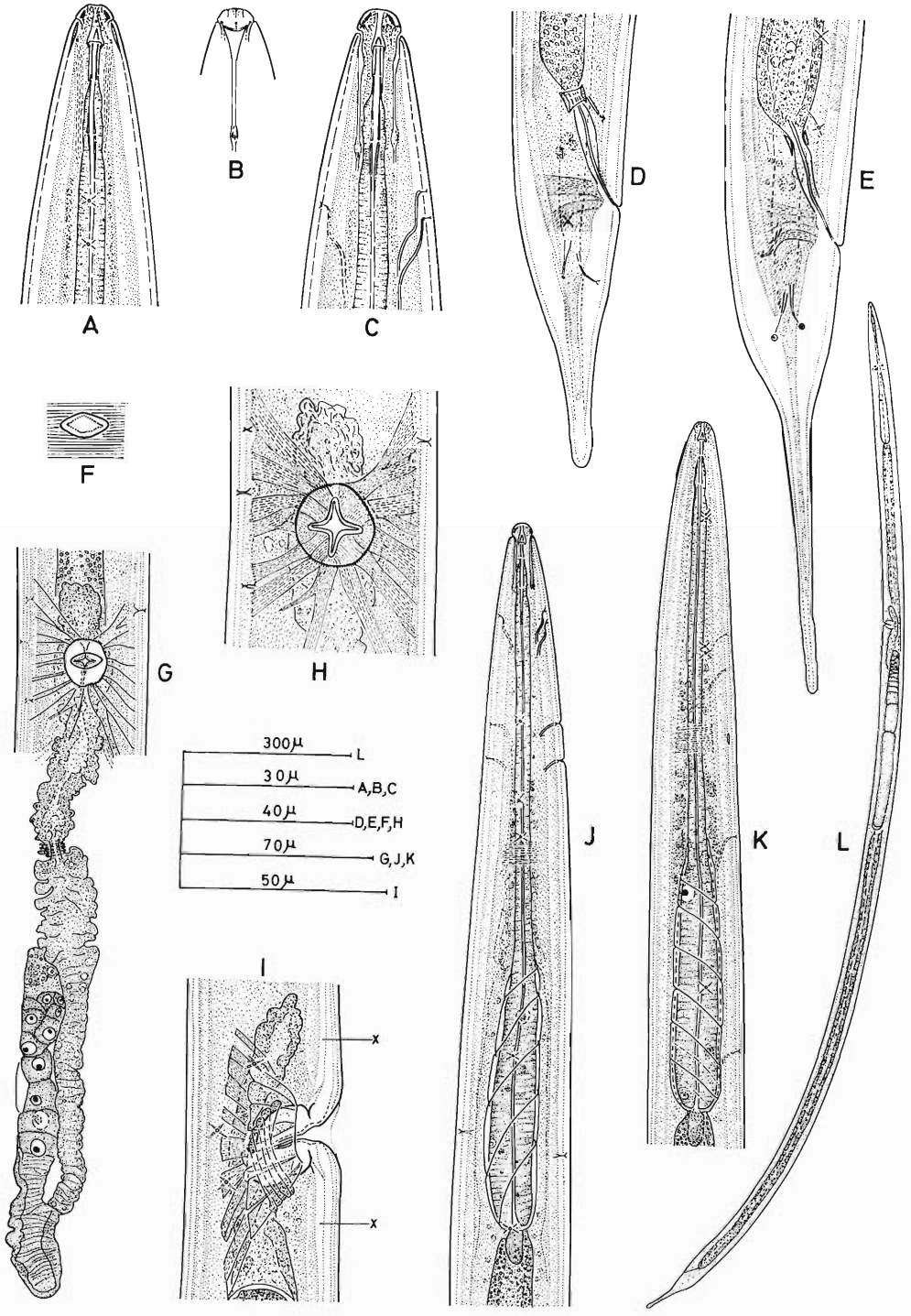
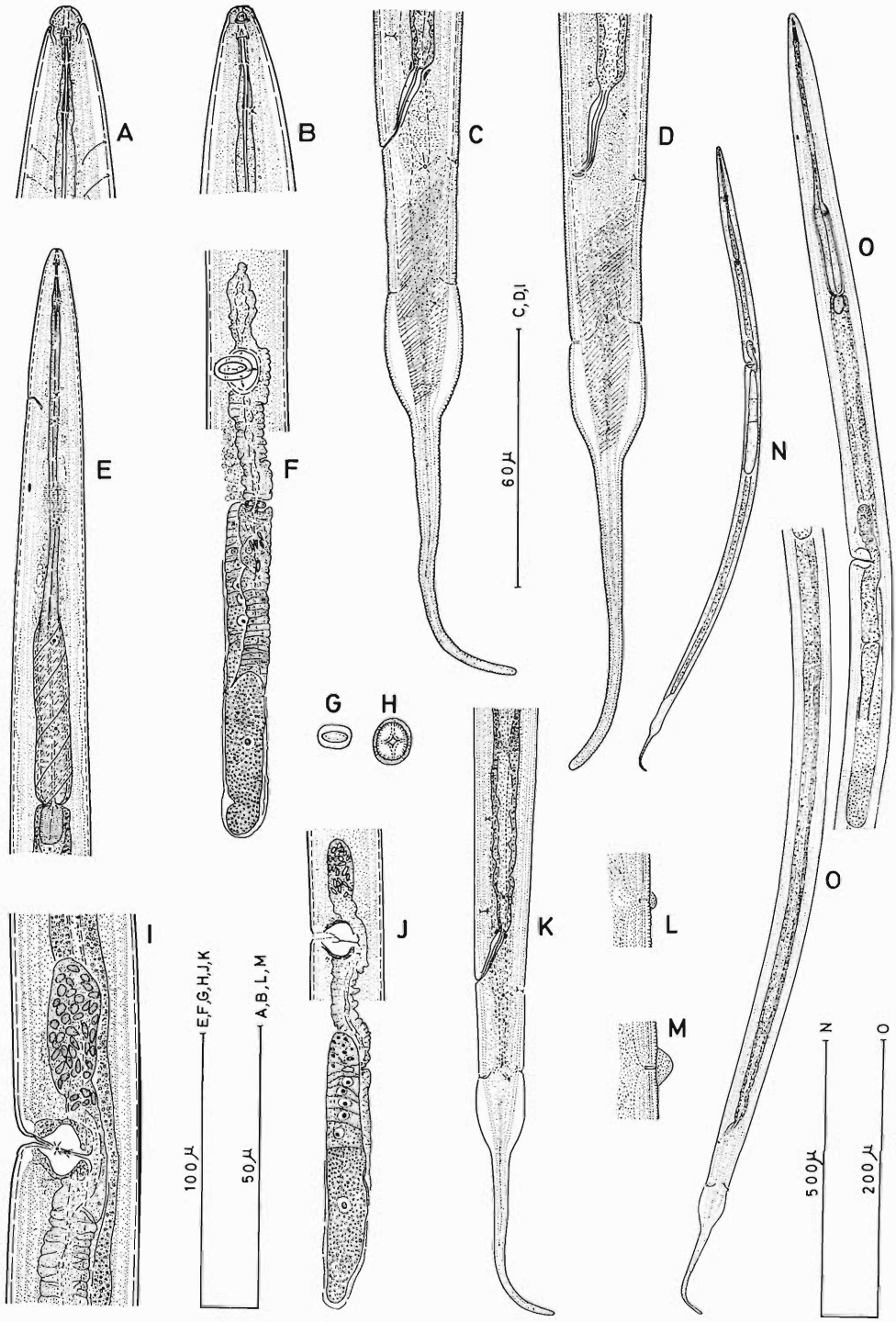
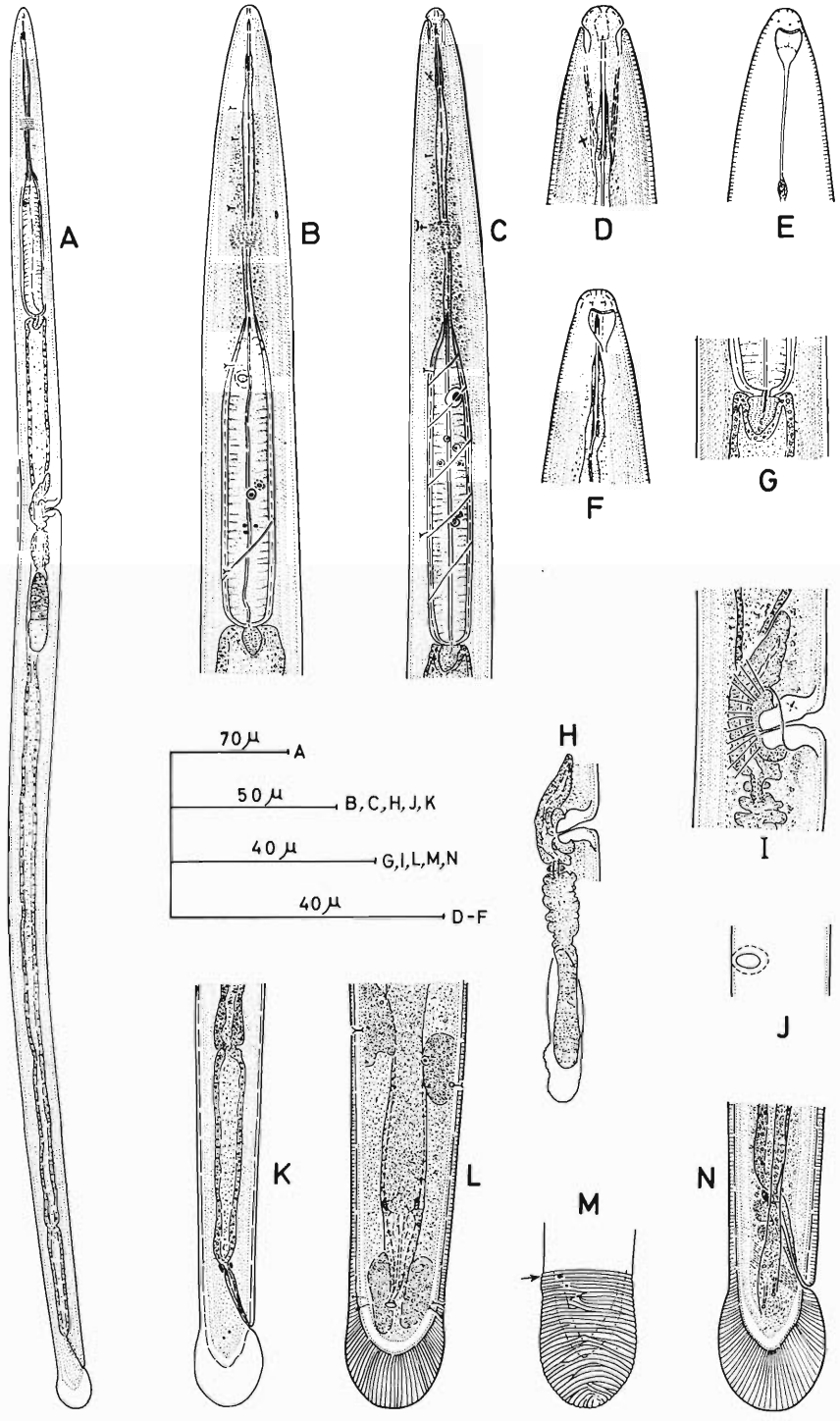
Figure 1. ***Belondira perplexa*** **Williams, 1958. A. Anterior end in lateral view. B. Surface view of the same. C. Anterior end in dorsoventral view. D, E. Variation in tail shape. F. Vulva in ventral view. G. Female gonads. H. Vagina in ventral view. I. Vulval region in lateral view. J. Neck region of specimen with dextral spiral muscle sheath. K. Neck region of specimen with sinistral spiral muscle sheath. L. Female. (Figs. E and I after holotype.) X, gap below cuticle.**

Figure 2. ***Belondira singularis*** **Williams, 1958. A. Anterior end in dorsoventral view. B. Anterior end in lateral view. C, D. Variation in tail shape. E. Neck region. F. Female gonads. G, Vulva in ventral view. H. Vagina in ventral view. I. Vulval region in lateral view. J. Female gonads. K. Posterior body region. L, M. Lateral body pores with secreted(?) substance. N, O. Female. (Figs. B, C, E, I, K, N, and O after holotype.)**

Figure 3. ***Belondira clavicaudata*** **(Williams, 1958). A. Female. B, C. Neck region. D–F. Anterior end. G. Esophagointestinal junction. H. Female gonads. I. Vulval region in lateral view. J. Vulva in ventrolateral view (specimen flattened). K. Posterior region, lateral. L. Posterior region, ventral. M. Tail in surface view. N. Tail. (Figs. F, I, and M after holotype.) Arrow indicates position of anus.**







Belondira clavicaudata (Williams, 1958) Andrássy, 1964
(Fig. 3)

MEASUREMENTS: See Table 1.

FEMALES: Body nearly straight in death position, tapering gradually but slowly toward both ends (except for expanded tail). Cuticle thickest on tail; transversely striated throughout the body length, but more prominently so in anterior and posterior regions. Lateral chords about ⅕ of body width near middle of body.

Lip region rounded to subtruncate, marked by very slight depression from body; with amalgamated lips and weakly developed sclerotization. Base of lip region about ¼ as wide as neck base. Amphid apertures curved, ⅗–⅔ of corresponding body width, leading to deep pouches. Odontostyle very short, slightly more than half the lip region width long; aperture about ⅓ of odontostyle length. Guiding ring 0.9 times the lip region width from anterior end. Odontophore 4–4.3 times the odontostyle length.

Basal expanded portion of esophagus occupying 45–54% of neck region, enveloped by a sheath of dextral spiral muscle bands. Dorsal esophageal gland nucleus at 54–64% of neck region. Nerve ring surrounding slender part of esophagus at 29–38% of neck length. Cardia heart-shaped. Prerectum 3.5–5.5 and rectum 1.2 anal body widths long.

Vulva transverse oval, 7.5 by 4.5 μ in one paratype. Vagina extending about halfway into the body, slightly bent posteriorly. Anterior gonad rudimentary, about one body width long. Posterior gonad normally developed, with very short uterus (about ½ body width long). Sperm not observed in any part of the gonads.

Tail clavate due to an expansion of the outer cuticular layers; with two caudal pores on each side. Terminal nonprotoplasmic portion 13–15 μ.

MALE: Unknown.

TYPE LOCALITY AND HABITAT: Soil around roots of sugar cane, Union Park, Mauritius.

OTHER LOCALITY AND HABITAT: Moss from rocks near motorway between Santos and São Paulo, Brazil (cf. Andrássy, 1963).

Belondira tumicauda sp. n.
(Fig. 4)

MEASUREMENTS: See Table 1.

FEMALES: Body curved ventrally upon fixation, J- or even C-shaped; tapering gradually toward both ends (except for expanded tail). Cuticle finely striated transversely; more prominently striated in the caudal region. Outer cuticular layers extremely thickened on tail. Lateral chords about ¼ of body width at midbody; somewhat irregular in outline due to the occurrence of granular lateral organs.

Lip region rounded to subtruncate, continuous with body contour, with prominent sclerotization. Base of lip region about ¼ as wide as neck base. Lips amalgamated, bearing the usual number of papillae. Amphid apertures slightly curved, ½ to ⅗ of the corresponding body width, leading to deep pouches. Odontostyle very short, ½–⅔ of the lip region width long, aperture about 1 μ long. Guiding ring about one lip region width from anterior end. Odontophore 2.3–2.8 times the odontostyle length.

Basal expanded portion of esophagus occupying 41–44% of neck region, enveloped by a weakly developed sheath of dextral spiral muscle bands. Dorsal esophageal gland nucleus at 71% ($n = 1$) of neck region. Nerve ring surrounding slender part of esophagus at 39–43% of neck length. Cardia tongue-shaped. Prerectum about 3 times the anal body width. Rectum length about equal to anal body width.

Vulva transverse oval, 7 by 3 μ in holotype. Vagina extending halfway into body. Anterior gonad rudimentary, 29–33 μ long, i.e., 1.2–1.4 times the corresponding body width. Posterior gonad normally developed, with very short uterus (less than ½ body width long!). Size of posterior gonad variable according to presence and size of oocytes which were from 92–301 μ in specimens studied. Gonads with

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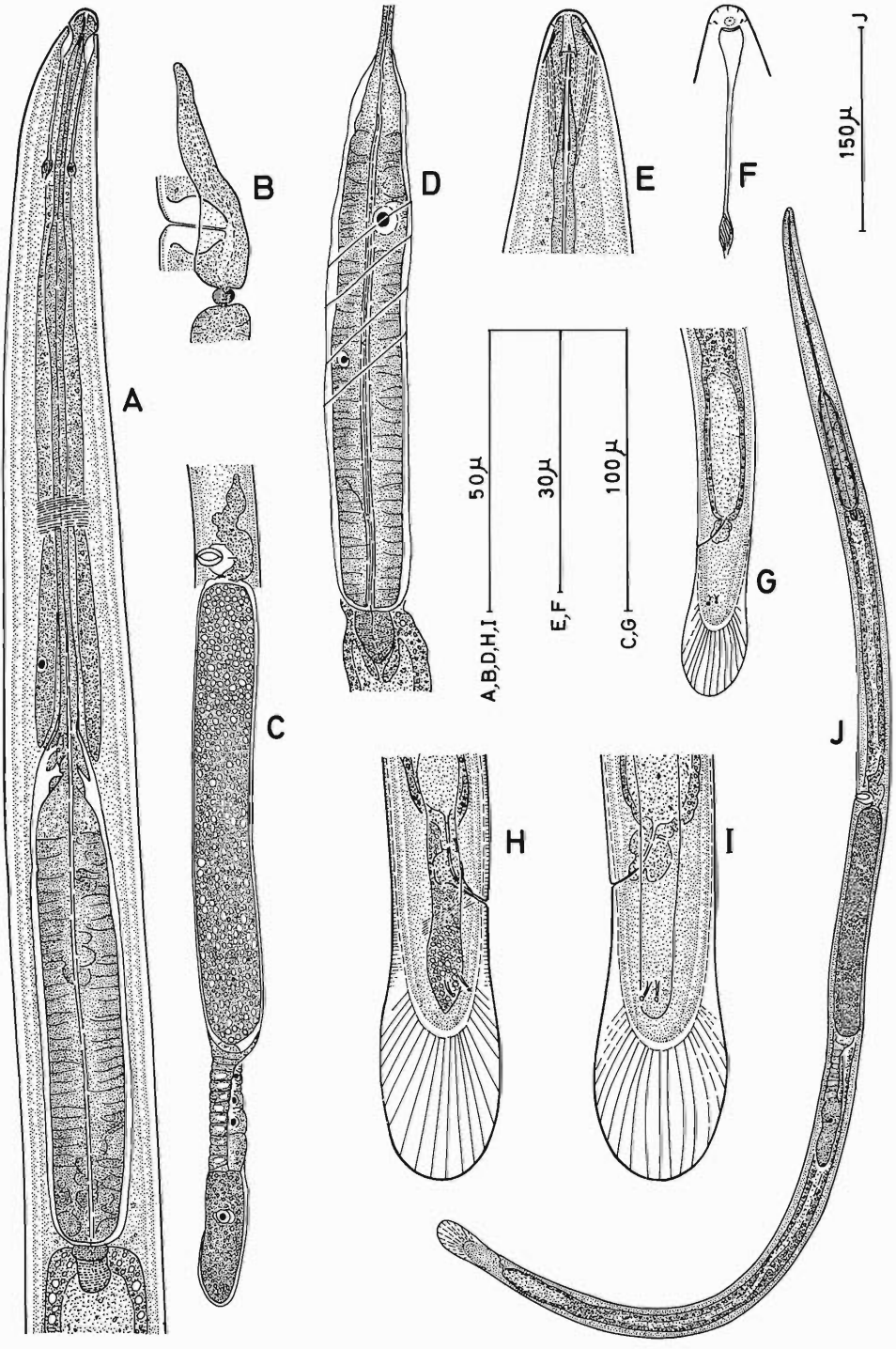
Figure 4. ***Belondira tumicauda*** **sp. n. A. Neck region. B. Vulval region. C. Female gonads. D. Expanded part of esophagus. E. Anterior end. F. Surface view of the same. G. Posterior region. H, I. Variation in tail shape. J. Female. (Figs. A, C, G, I, and J after holotype.)**



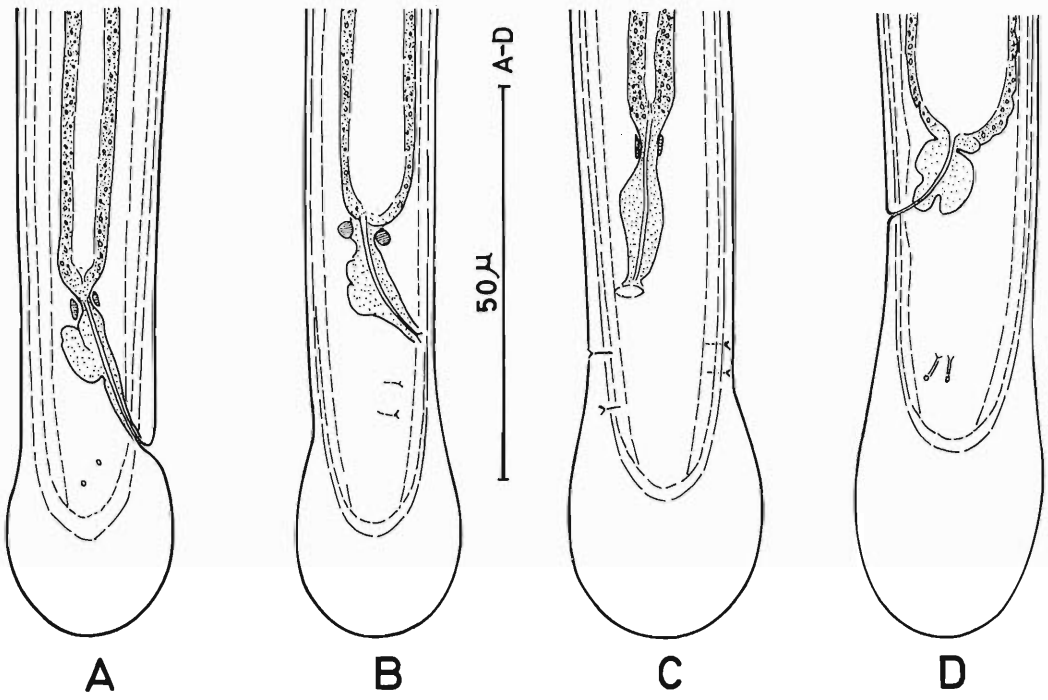


Figure 5. ***Belondira*** **species, tails. A.** ***B. clavicaudata,*** **paratype female. B.** ***B. bulbosa,*** **paratype juvenile. C.** ***B. bulbosa,*** **holotype. D.** ***B. tumicauda,*** **holotype.**

several large oocytes in the ovary, reflexed up to two body widths anterior to vulva. No sperm seen in gonads.

Tail clavate, due to expansion of outer radially striated cuticular layers, and with two caudal pores on each side. Nonprotoplasmic terminal portion of tail 23–27 μ.

MALE: Unknown.

JUVENILES: Four juveniles were found, agreeing with the females in general morphology.

TYPE LOCALITY AND HABITAT: Vieux Kilo, Ituri, the Congo. Clay soil from a tea plantation. Collected by A. Coomans on 13 November 1959.

OTHER LOCALITY AND HABITAT: Nioka, Ituri, Congo. Soil around roots of *Ageratum conizoides* Reichb. in a coffee plantation.

TYPE MATERIAL: Holotype female and one paratype juvenile on slide n° 32, Nematode Collection of Instituut voor Dierkunde, Rijksuniversiteit Gent. Three paratype females and two paratype juveniles deposited in same collection.

RELATIONSHIP: *Belondira tumicauda* sp. n. is closely related to *B. bulbosa* Siddiqi, 1966, but differs mainly in the absence of males, shorter prevulval sac (2.3 times body width in *B. bulbosa*), and body posture when dead (almost straight in *B. bulbosa*). Possibly also by the shorter uterus of the posterior gonad (about one body width in *B. bulbosa*), shorter odontostyle (4.5 μ in *B. bulbosa*), slightly different position of vulva ($V = 43$ in *B. bulbosa*), and thicker cuticular tail tip (about as wide as anal body width in *B. bulbosa*). Further studies on intraspecific variation in this group are necessary to evaluate some of the above mentioned differences. The new species resembles also *B. clavicaudata* from which it differs in the slightly longer but relatively narrower body, more posterior vulva, longer and differently shaped tail (see Table 1). Figure 5 gives outline drawings of tails from these three closely related species.

Finally *B. tumicauda* also resembles *B. parva* Thorne, 1964, but differs by the same char-

acters as from *B. clavicaudata*, and by the absence of males.

The specific name refers to the peculiar shape of the tail (*tumidus* = swelling, *cauda* = tail).

Belondira mernyi Andrássy, 1970

This species was described from the Ivory Coast from a female and male specimen. Two males and three juveniles of the species were found at the type locality of *B. tumicauda*.

♂♂: L = 1.27–1.28 mm; a = 51–58; b = 6.0–6.3; c = 26–29. Odontostyle 3.5 μ.

Body almost straight in death position. Lip region 6.5–7 μ wide and 4 μ high, more rounded than in Andrássy's specimens. Sensillae 28 μ behind amphid aperture. Guiding ring some 7 μ from anterior extremity. Neck region 203–213 μ long, 35–38.5% of it occupied by expanded portion of esophagus. Tail 44–50 μ long, i.e., 2.2–2.7 times the anal body width. Terminal nonprotoplasmic part 11–15 μ thick, expanded by inner and outer cuticular layers. Only two caudal pores seen, instead of three as illustrated by Andrássy (1970). Supplements an adanal pair and a ventromedian at about ⅔ of spicula length from cloacal opening. Andrássy (loc. cit.) reported a single ventromedian supplement but situated at about two spicula lengths from the cloacal opening. In our specimens there also occurs a papillalike structure at this level but it does not differ from the ventral body pores that occur more anteriorly, hence it can be equally well considered as the last element of the ventral body pore series. Otherwise our specimens agree well with the original description.

Juveniles agree with females in general morphology.

Discussion

The resdescription of the Mauritian *Belondira* species was necessary in order to ascertain their position since two of the species were excluded from the genus by Jairajpuri (1964), Siddiqi (1966), and Andrássy (1970). Jairajpuri considered *B. perplexa* and *B. singularis* as nearer to *Oxydirus* and possibly belonging to a new genus. Siddiqi did not include both species in his key to species of *Belondira*. Andrássy (loc. cit., p. 247) listed only two *Belondira* species from Africa and did not consider *B. perplexa* and *B. singularis* as true *Belondira*'s.

The present study reveals that both species possess all the typical characters of the genus except for the tail shape. However, tail shape seems very variable and also very exceptional, as illustrated by the species described in the present paper. Although the tail shape of *B. perplexa* reminds that of some *Oxydirus* species, the other characters do not correspond to that genus. The tail shape and structure of *B. singularis* are not comparable to those in *Oxydirus*. The finding of long-tailed species of a genus previously known only to contain short-tailed ones is not exceptional, e.g., *Xiphinema*, *Dorylaimellus*.

In the light of the facts and conclusions presented here, an emended diagnosis of the genus may be given:

Belondira Thorne, 1939

Diagnosis (after Thorne, 1964, emended): Belondirinae. Small to medium-sized nematodes (0.7 to 2.5 mm). Lip region rounded to truncated, papillae not projecting. Outer parts of head framework weakly to moderately sclerotized, appearing refractive in profile. Anterior part of esophagus not set off by constriction; posterior part surrounded by a sheath of usually dextral, rarely sinistral muscle bands. Anterior gonad rudimentary, saclike. Posterior gonad normally developed. Supplements consisting of an adanal pair and one or 2 ventromedian ones; when two, they are widely separated. Tail short and bluntly rounded to clavate, cylindrical, or elongated, usually with thickened cuticle.

Acknowledgments

The authors wish to thank Mr. D. Hooper, Rothamsted Experimental Station, Harpenden, England, for the loan of type specimens of the Mauritian species and Dr. M. R. Siddiqi for those of *B. bulbosa*.

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A Record of Progenesis in Trematoda

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ABSTRACT: A restudy of trematodes, previously reported by the writer from Louisiana skunks, revealed that the flukes were paedogenetic larvae, possibly belonging to the genus *Ribeiroia*. A morphological description of the worms is given. Based upon early genital development over somatic development, it is concluded that skunks are not the natural hosts for the parasites. A discussion of the phenomenon of progenesis and some historical records of the occurrence of paedogenesis in Trematoda are presented.

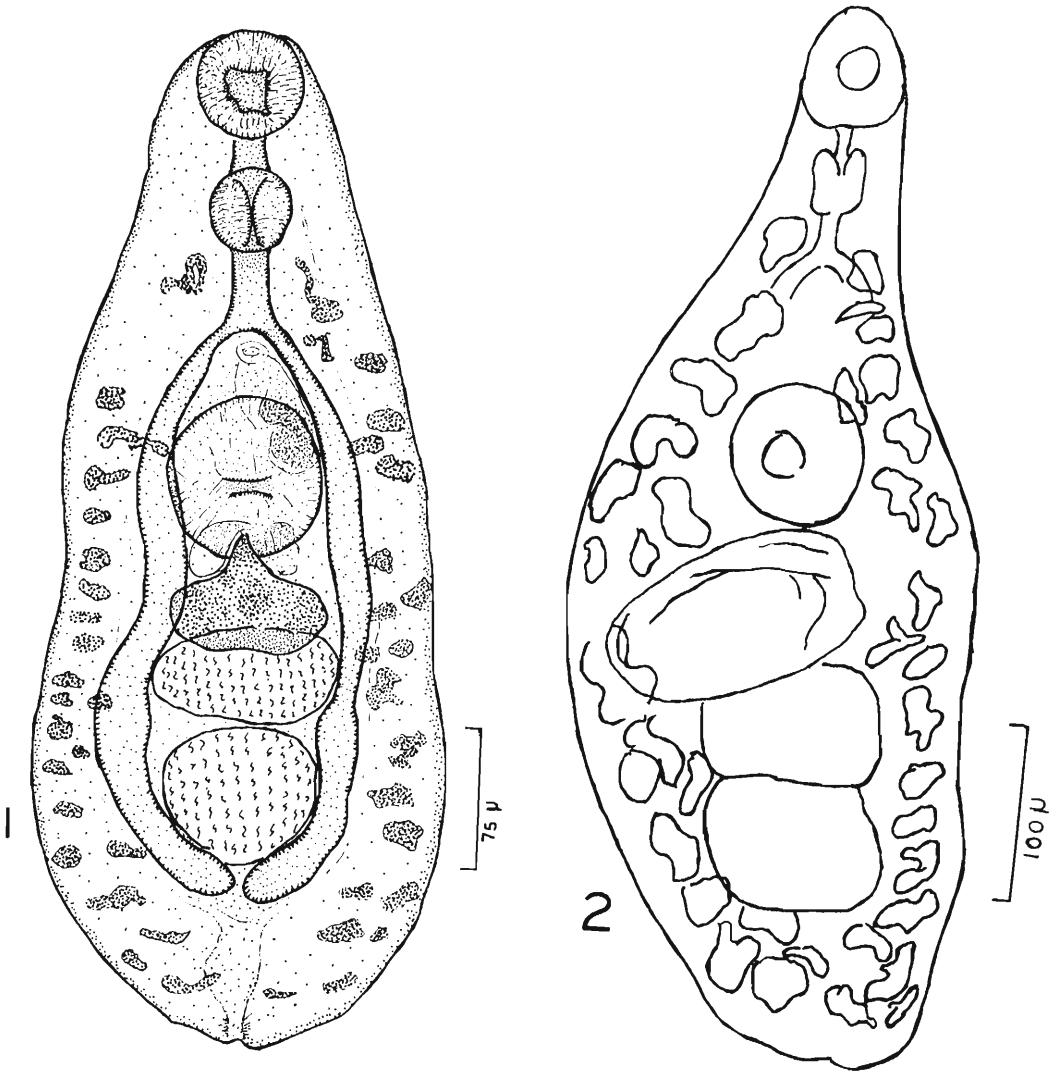
Immature trematodes, fixed in alcohol–formalin–acetic acid solution, from 30 Louisiana skunks were identified as *Psilostomum* sp. by Babero (1960). A restudy of this material has been made and the flukes are determined as paedogenetic larvae, possibly of the genus *Ribeiroia* Travassos, 1939. The esophageal diverticles characteristic of this genus, however, were not discernible, despite the use of different stains (Semichon's acid carmine; Mayer's alcohol cochineal). Apparently, in larval forms of the genus, unless a specific fixative or/and stain is employed, these structures may not be clearly seen. Price (1931), in his description of *P. ondatrae* (= *R. ondatrae* Price, 1931), apparently failed to recognize the esophageal diverticles. On the other hand, Beaver (1939) observed and figured them in the cercariae of the species. Another morphological point is the absence of a seminal receptacle in the genus *Ribeiroia*. In the skunk ribeiroids, a structure is present which resembles a seminal receptacle. However, McIntosh (pers. comm.) pointed out that the structures, in reality, are masses of spermatozoa in the posterior part of the uterus and are known as a "receptaculum seminis uterinum." A description of the skunk fluke follows:

Ribeiroia ondatrae (Price, 1931)

SPECIFIC DIAGNOSIS (all measurements are in microns): Body finely spinous, pyriform or elongated, total length, 500–650; maximum width, 175–270. Oral sucker, 48–57 by 51–55. Prepharynx short, 15 long; pharynx, 33–39 by 36–42. Intestinal ceca extends to near posterior end of body. Genital pore median to slightly between fork of intestinal ceca and acetabulum, submedian, 18–27 in diameter. Cirrus sac pyriform, dorsal to acetabulum, 81–87 by 42, encloses sacculate seminal vesicle. Cirrus eversible. Testes elongated transversely, tandem or may overlap slightly, postequatorial; anterior testis, 87–99 by 81–108; posterior testis, 72–87 by 99–114. Ovary situated between anterior testis and acetabulum, 52–69 by 66–96. Seminal receptacle apparently absent. Ootype submedian. Vitelline reserve massive. Vitellaria lateral extending from level of esophagus to near posterior end of body where they are confluent medially. Ova few, 130–151 by 91–112. Excretory pore terminal, with sphincter; bladder bifurcates just caudad to posterior testis.

HOST: *Mephitis mephitis* (Schreber, 1776).

HABITAT: Small intestine.



Figures 1, 2. ***Ribeiroia ondatrae.*** **1. Whole mount ventral view. 2. Fluke showing distortion of body by single large ovum.**

LOCALITIES: Pointe Coupee, St. Martin, Iberville, St. Landry and Avoyelles (Parishes of Louisiana).

SPECIMENS: USNM Helm. Coll. Nos. 70457 and 70458.

Although in most of the specimens the reproductive organs did not appear to be fully developed, many of the flukes contained one or occasionally two ova lying transversely within the body. Because of the large egg size in proportion to the total body length, the gravid flukes were usually distorted (Figs. 1, 2). According to McIntosh (pers. comm.), "Skunks are not the natural hosts, somatic development did not continue but genital development did continue in a few cases producing an egg too large for the larval fluke." It is interesting to note that encysted meta-

cercariae, reported as *Psilostomum* sp. and resembling the skunk specimens, were collected from the intestines of Louisiana nutria by Babero and Lee (1961).

The phenomenon of progenesis, or the precocious development of the genital glands with egg production, is well known among invertebrates and vertebrates. Grassé (1932) states that cases of paedogenesis have been reported from freshwater and terrestrial molluscs, fishes, reptiles, and mammals. Although the phenomenon is not rare in Trematoda, occurrence records in parasitological literature are infrequent. One of the earliest reports is that of Pontallié (1851; cited by Buttner, 1951a), who collected progenetic *Distoma crassicolle* Rud., 1809 (= *Brachycoelium salamandrae* Fröelich, 1789) from tritons. Dollfus (1932) stated that he was able to classify over 100 cases of distome larvae in the metacercarial stage showing different degrees of progenesis. He introduced the term "Métacercaire progénétique" for larval metacercariae that have eggs. While relating several genera of the group "Lepodermatoidea" he postulated that, in the case of the metacercariae of *Planorbis planorbis* which he had investigated, two life cycles were possible: an abbreviated one in which the parasites stay within the first host and a second cycle wherein the adults occur in some definitive host. Stunkard (1938) found that gulls (*Larus argentatus*), experimentally infected with larval flukes in *Mya arenaria*, harbored trematodes that contained eggs, although the worms were only half-grown. He concluded that the mollusc was little more than a transport host. Buttner (1950, 1951a, b, 1952) undertook extensive experimental investigations of various hosts relative to progenesis in digenetic trematodes. She (1951a) pointed out that the phenomenon occurs more frequently in nature than under experimental laboratory conditions and that progenesis, perhaps, presented variable degrees of evolution. She suggested the role that certain extrinsic and intrinsic factors (light, temperature, host metabolism, and heredity) played in bringing about this phenomenon. She further suggested that progenesis may be a means of conservation of the species. Buttner (1952), too, considered that *Ratzia joyeuxi* (Brumpt, 1922) (= *R. parva* Stossich, 1904) had an abbreviated metacercarial progenetic cycle, cysts occurring in adult frogs. She observed that eggs of the metacercariae contained miracidia which could directly infect the snail, *Amnicola dupotetiana*. In the family Psilostomidae Odhner, 1913, progenesis has been previously reported by Wisniewski (1933) who described *P. progeneticum* Wisniewski, 1932, metacercariae occurring in the body cavity of gammarids (*Rivulogammarus spinicaudatus* and *Fontogammarus bosniacus*).

Apparently, the line between a progenetic larva of a trematode and mature adult is difficult to define. Brumpt (1922) considered the flukes as adults when they left their cysts. Dollfus (1932) observed that metacercariae attained sexual maturity within their cysts and contained eggs that developed embryos following fertilization by spermatozoa from the seminal vesicle. Baer (pers. comm.) stated, "It is quite possible that the species you have obtained from skunks are also progenetic although it is difficult to define, as you mention, a 'paedogenetic metaceraria' since the latter, when producing eggs even when encysted are no longer larvae."

Acknowledgments

The writer extends his appreciation to Dr. Jean Baer, University of Neuchatel, Switzerland, for his comments in relation to this work, to Dr. Allen McIntosh, Parasite Laboratory, ARS, ARC, USDA, Beltsville, Maryland, for his constructive comments and for reviewing the manuscript, and to Dr. Frank Etges, University of Cincinnati, for assistance in morphology.

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A New Nematode *Dichelyne bullocki* sp. n. (Cucullanidae) from *Fundulus heteroclitus* (Linnaeus)

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ABSTRACT: *Dichelyne bullocki* sp. n. is described, figured, and compared with other species of the genus *Dichelyne* Jägerskiöld, 1902, from North American fishes. The history of the species group designated as *D. lintoni* is briefly reviewed. *D. bullocki* and *D. fastigatus* are the only species of marine *Dichelyne* from North American waters which are adequately described.

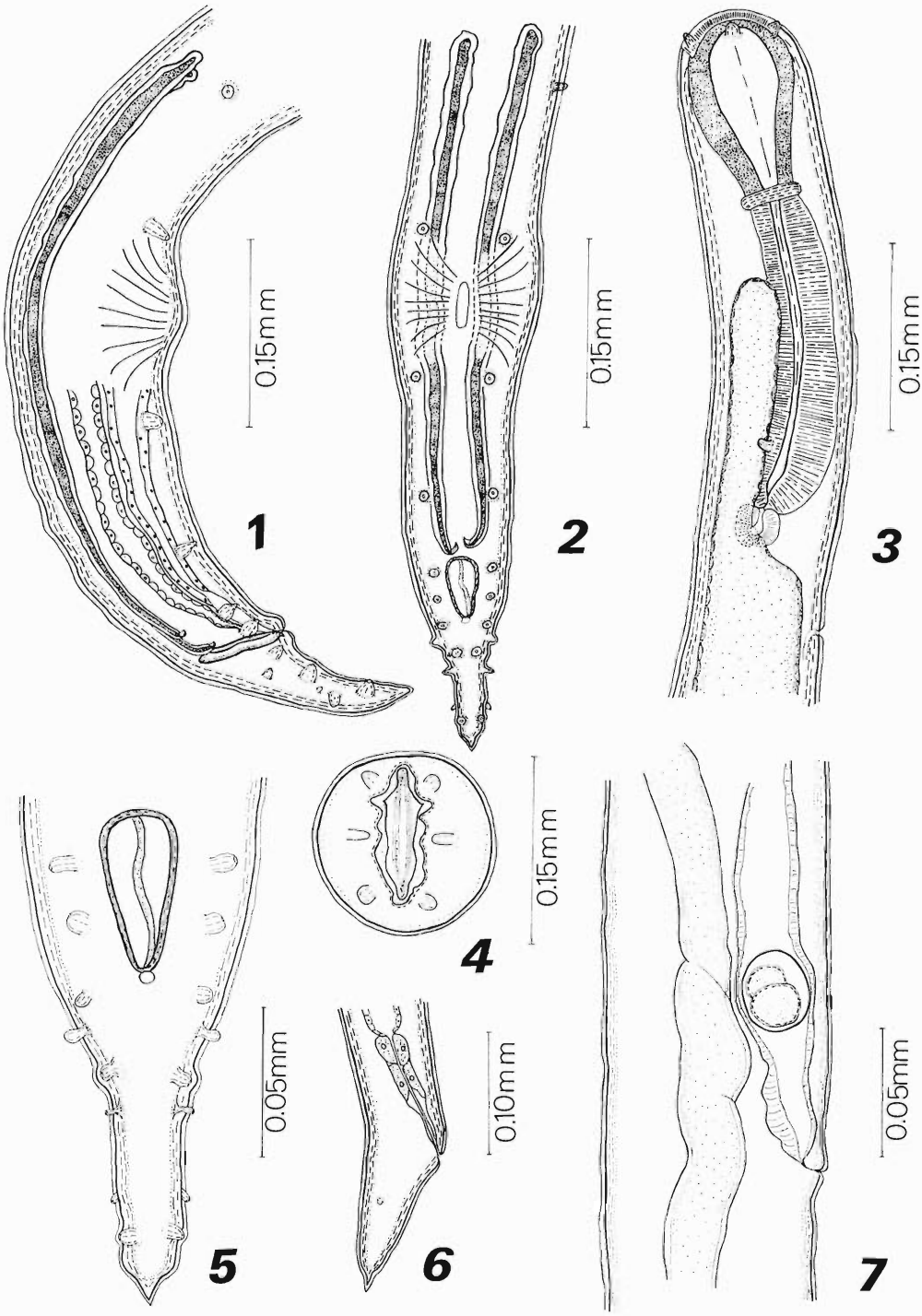
There have been 10 species assigned to the genus *Dichelyne* Jägerskiöld, 1902, since its original description. All are intestinal parasites of fishes. The genus is distinguished from other genera in the family Cucullanidae Cobbold, 1864, by possessing a dorsal intestinal cecum. The cucullanid specimens here described as a new species of this genus were collected from *Fundulus heteroclitus* from the coast of New Hampshire by Dr. Wilbur L. Bullock. His collections from this host from three other localities on the Atlantic coast also yielded the new species.

The nematodes were fixed in Bouin's Fixative and 10% neutral formalin, dehydrated, cleared, and mounted in glycerin and lactophenol. Some worms were stained in lactophenol cotton blue. All measurements are in microns unless otherwise indicated.

Dichelyne bullocki sp. n.
(Figs. 1–7)

Description

Cucullanidae Cobbold, 1864. Short nematodes, widest just posterior to esophagus, tapering toward tail. Oral opening dorsoventral slit surrounded by membranous flange of cuticle which bears numerous fine rodlike structures. Internally, buccal cavity with heavily sclerotized lining. Two amphids, four simple, submedian papillae. Esophagus club-shaped; anterior portion swollen, surrounding buccal cavity; posterior portion very muscular, slightly swollen at posterior end. Esophagus opening into intestine through muscular valvular apparatus. Intestine with dorsal intestinal cecum, directed anteriorly, variable in length and shape.



MALE (10 SPECIMENS): Length 2.08–3.40 mm (average 2.48 mm), maximum width 119–151 (135). Cuticle thin, finely striated, 5–7 (6) thick. Esophagus 378–458 (414) long. Nerve ring 130–160 (150) from anterior end. Cervical papillae, lateral 155–431 (414) from anterior end. Excretory pore 437–499 (466) from anterior end. Two testes, prodelphic, reflexed, very convoluted, reaching level of excretory pore. Tail 84–137 (107) tapering to smooth point. Preanal sucker present but weakly developed. Gubernaculum trough-shaped, 51–75 (65) long. Spicules equal and similar, slightly capitate, ending in fine points, 607–700 (650) long. Eleven pairs of caudal papillae, five preanal, six postanal. Unpaired lateral papilla anterior to first pair of preanal papillae. Second pair of postanal papillae lateral; third pair large, subventral; fourth pair (phasmids) minute, lateral; fifth pair small, subdorsal; sixth large, subventral (Fig. 1).

FEMALE (10 SPECIMENS): Length 2.34–3.35 mm (2.67 mm), maximum width 128–224 (156). Cuticle thin, finely striated, 6–9 (7) thick. Esophagus 400–526 (455) long. Nerve ring 150–190 (175) from anterior end. Cervical papillae lateral 270–412 (373) from anterior end. Excretory pore 515–584 (552) from anterior end. Vulva 1.45–2.00 mm (1.70 mm; 63.7%) from anterior end, with slightly protruding lips (Fig. 7). Vagina directed anteriorly. Two ovaries, amphidelphic, anterior ovary reaching to level of excretory pore. Tail 98–183 (119) long, with two minute lateral papillae (phasmids); ending in smooth point without cuticular spines, annulations, or processes. Eggs thin-shelled, broadly oval 57–69 (63) by 45–52 (49) in 1–8 cell stage.

HOST: *Fundulus heteroclitus* (Linnaeus).

LOCATION: Intestine.

TYPE LOCALITY: Crommet Creek, Great Bay, Durham Co., New Hampshire.

HOLOTYPE: Male.

ALLOTYPE: Female.

SPECIMENS DEPOSITED: USNM Helm. Coll. No. 71994.

Discussion

Dichelyne bullocki sp. n. differs from other species of the genus in several ways. *D. cotylophora* (Ward and Magath, 1916) is the only other species in which the male possesses a preanal sucker. In *D. cotylophora* this structure is very well developed, while in *D. bullocki* it is weakly developed. *D. bullocki* is shorter and more slender than *D. cotylophora*. Both male and female specimens of *D. cotylophora* collected from *Perca flavescens* in Lake Erie were identified and examined and found to be 4.2–5.1 mm in length and 290–387 μ wide behind the esophagus. There was no overlap in the measurements between these two species. In addition, the cuticle of *D. cotylophora* was found to be very thick (35 μ). The males of these two species may be separated by the absence of the unpaired lateral papilla anterior to the first pair of caudal papillae in *D. cotylophora* and by the difference in the shape of the gubernaculum. The females of these two species may be separated by the presence of distinct cuticular annulations on the tip of the tail of *D. cotylophora* and their absence on the tails of *D. bullocki*. In addition, the cervical papillae on *D. cotylophora* females are closer to the anterior end than on the females of *D. bullocki*.

Linton (1901) reported *Cucullanus* sp. from *Fundulus heteroclitus*. His Plate XVII, Fig. 207 shows the male tail in lateral view with 12 papillae. Linton (1907) indicated that this worm possessed 12 pairs of caudal papillae but did not figure the preanal papillae in ventral view. If Linton did not study the preanal papillae in ventral view, it is possible that he

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Figure 1. Male tail, lateral view.
Figure 2. Male tail, ventral view.
Figure 3. Male anterior end, lateral view.
Figure 4. En face view.
Figure 5. Male tail, ventral view.
Figure 6. Female tail, lateral view.
Figure 7. Female, vulva and vagina.

believed that the unpaired lateral papilla was in fact a pair and thus described 12 pairs of caudal papillae. In any case, it seems unlikely that two such closely related forms should be found in the same host from Woods Hole, and, therefore, we believe that Linton's *Cucullanus* sp. is probably conspecific with *D. bullocki*.

This description of *D. bullocki* and Chandler's (1935) description of *D. fastigatus* are the only described species of the genus *Dichelyne* from North American marine fishes. The name *Dichelyne lintoni* was proposed by Barreto (1922) for all of the cucullanids collected by Linton (1901, 1905, 1907). Both Törnquist (1931) and Chandler (1935) stated that this name applied to not one but several species. Since there is no description or type specimen designated, this name should be discarded. Chandler attempted to define *D. lintoni* by suggesting that the name be restricted to those cucullanids from fishes of the genera *Haemulon* and *Neomaenis*. In addition, he suggested that the *Ascaris* sp. of Linton (1901) from *Paralichthys dentatus* be named *Dichelyne cylindricus*. In neither case, however, does Chandler offer a species description. It seems clear that additional material from the hosts examined by Linton should be obtained and examined before the identity of the cucullanids can be clarified.

To avoid any confusion between *D. bullocki* and *D. fastigatus* type specimens of the latter were examined. *D. fastigatus* can readily be distinguished from *D. bullocki* by the different arrangement of caudal papillae in the male, the thickness of the cuticle, and the absence of a preanal sucker in *D. fastigatus*. In addition, the lateral papillae (phasmids) on the female tail of *D. fastigatus* are very large. Rasheed (1966) described a species from the Pakistan coast which she assigned to *D. fastigatus*. This form possessed a ventral intestinal cecum and minute spinules on the tail of the female. Careful examination of Chandler's type specimen revealed a dorsal intestinal cecum and a smooth tail without spinules. Thus, the assignment of Rasheed's specimens to *D. fastigatus* must be rejected.

Acknowledgments

We would like to express our appreciation to Dr. Wilbur L. Bullock who sent us the specimens, and to Miss Martha L. Walker and Dr. J. Ralph Lichtenfels for the loan of type specimens from the USNM Helminthological Collection.

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In Memoriam

William Bradley DeWitt
June 3, 1921–August 8, 1971
Member since 1950
Member, Executive Committee, 1965
Recording Secretary, 1966
Vice President, 1967

George Joseph Rau
July 21, 1907–July 23, 1970
Member since 1958

George Henri Rohrbacher, Jr.
May 1, 1924–July 7, 1970
Member since 1955

Prevalence of Helminth Parasites in Mule Deer from Eastern Montana[1]

DAVID E. WORLEY[2] AND CHARLES D. EUSTACE[3]

ABSTRACT: Forty-three of 44 mule deer (*Odocoileus h. hemionus*) collected from semiarid rangeland in Garfield and Rosebud counties, Montana in 1967–68 were infected with one or more of 13 species of helminth parasites. *Ostertagia ostertagi* was the most common species, occurring in 66% of the animals examined. Prevalences of other helminths were: *Trichostrongylus colubriformis*, 42%; *Nematodirus odocoilei*, 30%; *Skrjabinema parva*, 25%; *Cooperia oncophora*, 23%; *Protostrongylus macrotis*, 16%; *Trichuris* sp., 16%; *Ostertagia bisonis*, 16%; *Taenia hydatigena* cysticerci, 14%; *Thysanosoma actinioides*, 12%; *Pseudostertagia bullosa*, 2%; *Haemonchus contortus*, 2%; and *Trichostrongylus longispicularis*, 2%. *Pseudostertagia bullosa* and *Trichostrongylus longispicularis* have not been reported previously from mule deer. The intensity of gastrointestinal nematodes was similar regardless of host age: worm burdens in young deer (0.5–2 years) averaged 103, whereas the average number of worms per adult animal (2–12 years) was 89. Individual worm burdens exceeded 150 in 10 deer. Concurrent nematode infections of the alimentary tract occurred in 68% of the sample. These data suggest that helminths probably were not numerous enough to produce clinical infections except in isolated instances. However, the high prevalence of important nematode species of livestock in these deer indicates that the use of common ranges may have resulted in considerable interchange of parasites between domestic and wild hosts.

Although internal parasites are known to cause occasional morbidity and mortality in individual deer, their long-term influence on the health of deer herds is difficult to assess. Anderson (1962) reviewed the parasites of white-tailed deer (*Odocoileus virginianus*) in North America, and a systematic study of the parasites and other disease agents in this species has been in progress in the southeastern states for more than 10 years (Hayes, Greer, and Shotts, 1957). However, little comprehensive information is available on parasitism or its effects in the Rocky Mountain mule deer (*Odocoileus h. hemionus*). Regional surveys in British Columbia and Alberta (Cowan, 1951), Wyoming (Landram and Honess, 1955), Idaho (Ball, 1966), and South Dakota (Boddicker and Hugghins, 1969) have indicated that mule deer are susceptible to a wide variety of worm infections. The primary purpose of the present study was to determine the nature and extent of helminth parasitism in mule deer from two areas in eastern Montana. A second objective was to evaluate the potential of resident deer herds to act as reservoir hosts for cattle and sheep parasites in areas used primarily for livestock range.

Materials and Methods

Two to five deer were collected each month during a 12-month period in 1967–68 from two localities about 140 miles apart in eastern Montana. Approximately half of the animals were taken from conifer–sagebrush–grassland range adjoining the Missouri river "breaks" in northern Garfield County. This land previously had been used for winter cattle range and was known to support a large and relatively stable mule deer population. The remaining animals were collected in Ponderosa pine–grassland habitat in southern Rosebud County. The topography here was basically rolling upland plateau land which was currently utilized for cattle range. Deer density was considerably lower than in the other study area because of a "die-off" of unknown etiology which reportedly occurred in the Rosebud County deer herd in 1964–65. Disease problems which appeared shortly afterward in cattle grazing in this area were attributed primarily to internal parasites, although this was not confirmed in the laboratory.

Deer were collected by shooting in the head or neck with a high-caliber rifle. The entire

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[3] Montana Fish and Game Department.

Table 1. Prevalence and intensity of helminths in mule deer from eastern Montana.

Parasite	Location	% Infected	Mean No. worms	(Range)
Ostertagia ostertagi	abomasum	66		
O. bisonis	abomasum	16	83	(1–383)
Pseudostertagia bullosa	abomasum	2		
Haemonchus contortus	abomasum	2	29	(———)
Trichostrongylus colubriformis	small intestine	42	3	(1–13)
Nematodirus odocoilei	small intestine	30	71	(2–382)
Cooperia oncophora	small intestine	23	3	(1–14)
Trichostrongylus longispicularis	small intestine	2	2	(———)
Trichuris sp.	cecum and colon	16	2	(1–4)
Skrjabinema parva	colon	25	55	(1–449)
Protostrongylus macrotis	lungs	16	3	(1–6)
Thysanosoma actinioides	small intestine	12	–	–
Taenia hydatigena cysticerci	peritoneal cavity	14	–	–

gastrointestinal tract, heart, lungs, and liver were removed from the carcass in the field, frozen in plastic bags, and shipped to Bozeman for examination. In some instances, external parasites or tapeworm cysts were collected in addition to the usual postmortem material. No attempt was made to examine the brain, meninges, or carotid arteries for helminths.

The contents of each area of the gastrointestinal tract were washed separately onto 20-, 40-, or 80-mesh screens to separate worms from ingesta. In addition, the entire gastrointestinal mucosa was scraped and washed to remove attached parasites. Washed ingesta and mucosal scrapings were examined in illuminated trays or with a dissecting microscope to facilitate recovery of worms. The liver, heart, and lungs were examined grossly for parasites or abnormalities after the attached ducts or air passages had been opened with scissors. Nematodes were fixed in a mixture of 95 parts 70% ethanol and five parts glycerin and cleared in glycerin for microscopic examination. Tapeworms were fixed in AFA solution.

Results and Discussion

The helminths found and prevalences of infection are shown in Table 1. Data from the two collecting areas are combined, since only minor differences were found in the occurrence of parasites between areas.

The predominant abomasal nematode, *Ostertagia ostertagi*, is a common parasite of Montana cattle (Worley, Jacobson, and Winters, 1970), whereas *O. bisonis* occurs principally in deer, bison, and pronghorn antelope (Becklund and Walker, 1967). *Ostertagia bisonis* has been reported previously from mule deer in Montana (Worley and Sharman, 1966). Although *Pseudostertagia bullosa* has been found in both domestic sheep and antelope in Montana (Lucker and Dikmans, 1945), this is apparently the first record from mule deer.

Stomach worm infections showed a seasonal peak in intensity in the spring, with parasite populations remaining at relatively high levels from December through May. The most severe infections were encountered in March and April, followed by a decline in worm burdens during the summer and fall months. No evidence was found of nodules or other abomasal lesions resembling those occurring in cattle infected with *O. ostertagi* (Anderson et al., 1965).

Nematodirus odocoilei was found in small numbers in five animals, with a relatively large number (382 worms) in one deer. Infections were limited to the period from November through April. The biggest worm burdens occurred in March and averaged approximately 96 worms per deer.

Pinworms (*Skrjabinema parva*) were more than six times as numerous in young deer as in adults. This parasite was found during all seasons except the 4-month period from June through September. Pinworms were not reported in mule deer from South Dakota, Wyoming, Yellowstone National Park, or Idaho in the series of parasite surveys previously cited. However, the original description of *S. parva* was based on specimens from a deer (probably *O. hemionus*) collected at Boise, Idaho (Dikmans, 1942).

Several species of intestinal nematodes occurred sporadically or in consistently low numbers. *T. colubriformis* was present in limited numbers during all months except July, August, and September. A second species, *T. longispicularis*, was found in association with *T. colubriformis* in one animal. This worm has been reported in cattle or sheep in Georgia, Florida, Louisiana, Mississippi, Oklahoma, New Mexico, and California. This is apparently the first report of *T. longispicularis* in deer, and extends its known distribution into the northern Great Plains region. *Cooperia oncophora* occurred irregularly throughout the year in very low numbers.

Fringed tapeworms (*Thysanosoma actinioides*) were recovered from five deer collected during the late fall and winter months. All of the specimens were located in the small intestine in material which was frozen and examined 1 to 2 weeks after collection. Because of this delay, it was not possible to determine whether the worms originally were located in the bile ducts or small intestine.

Cysticerci of *Taenia hydatigena* were found attached to the liver or mesenteries in deer collected in August (two animals), and November, December, January, and February (one positive animal per month). No gross pathologic changes were noted in the livers of infected deer except for occasional small fibrotic areas. Sweatman and Plummer (1957) described similar lesions in the liver of lambs experimentally infected with *T. hydatigena*, and noted that hemorrhagic streaks composed of coagulated blood sometimes were present in the liver parenchyma of naturally infected deer from northern Canada.

Protostrongylus macrotis was recovered from the bronchi and bronchioles of deer killed in December, January, April, May, and September. The largest infection, consisting of six worms, occurred in May. No gross evidence was seen of interstitial pneumonia as observed by Goble (1941) in the lungs of white-tailed deer infected with *P. coburni*.

Concurrent infections with two or more species of gastrointestinal nematodes were observed in 30 of 44 deer examined (68%). The most frequent associates, *Ostertagia* and *Trichostrongylus* spp., were found together in 17 animals (36%). Approximately 18% of the infected deer harbored three species of gastrointestinal nematodes, and 11% had mixed infections with four or more species of roundworms.

Comparative rates of infection in mule deer from eastern Montana and in similar regional surveys in neighboring states indicate that a higher incidence of helminthiasis occurred in eastern Montana deer (98%) than in any of the surrounding states. Rush (1932) found that 81% of the mule deer examined from Yellowstone National Park were infected with helminths, and Ball (1966) reported a prevalence of 62% in mule deer from southwestern Idaho. In western Montana, Senger (1959) found that 60% of the deer examined had worm parasites, and Boddicker and Hugghins (1969) reported a prevalence of 47% in deer collected in South Dakota.

Of the worms noted in Montana deer, 10 species have also been reported in cattle and/or sheep in the state. Considered collectively, the three most abundant deer helminths (*Ostertagia*, *Trichostrongylus*, and *Nematodirus* spp.) represent the three most prevalent gastrointestinal worm parasites of cattle in Montana (Jacobson and Worley, 1969). Although no direct evidence is available to indicate the actual rate of cross transmission of parasites between cattle and deer which had taken place in the areas studied, the preponderance of stomach and intestinal nematode species of livestock in the animals necropsied suggests that the use of common ranges may have resulted in considerable interchange of parasites between domestic and wild hosts.

Acknowledgments

Appreciation is expressed to Kenneth R. Greer and H. O. Compton of the Montana Fish and Game Department for providing funds for laboratory and field phases of the study. The technical assistance of Keith A. Johnson, Bonnie R. Billeb, Richard H. Jacobson, and John B. Winters is gratefully acknowledged. Willard W. Becklund and M. L. Walker of the National Animal Parasite Laboratory, Veterinary Sciences Research Division, U. S. Department of Agriculture, confirmed identifications and distribution records for the nematodes reported as new host or locality records. Clyde M. Senger and K. S. Stitt assisted with the manuscript.

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Marine Fish Trematodes of W. Pakistan. X. *Tormopsolus spatulatum* sp. n. (Acanthocolpidae: Acanthocolpinae) from a Fish off Karachi Coast[1]

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ABSTRACT: *Tormopsolus spatulatum* sp. n. is described from a fish belonging to genus *Cybium* taken off the Karachi coast. It is characterized by possessing a long forebody, very long prepharynx and esophagus, wider spatulate prepharyngeal region, and vitellaria interrupted at level of ovary and each testis.

Four species of the genus *Tormopsolus* Poche, 1926, are known: *Tormopsolus osculatus* (Looss, 1901) Poche, 1926, from *Motella vulgaris* of Triest; *T. orientalis* Yamaguti, 1934, from *Seriola quinqueradiata* (T. Schl.), *S. aureovittata* (T. Schl.), and *Epinephelus akaara* (Blkr.) of Japan, and *Zonicthys fasciatus* (Bloch.) of Burmuda; *T. lintoni* Caballero,

[1] Supported by research grant from The University of Karachi, W. Pakistan.

1952, from *Enchelyopus cimbrius* (L.) of Woods Hole; *T. filiformis* Sogandares-Bernal and Hutton, 1959, from *Rachycentron canadus* (L.) from Gulf of Mexico. A fifth species, *Tormopsolus spatulatum*, is described herein from fish belonging to the genus *Cybium* (Cuv.).

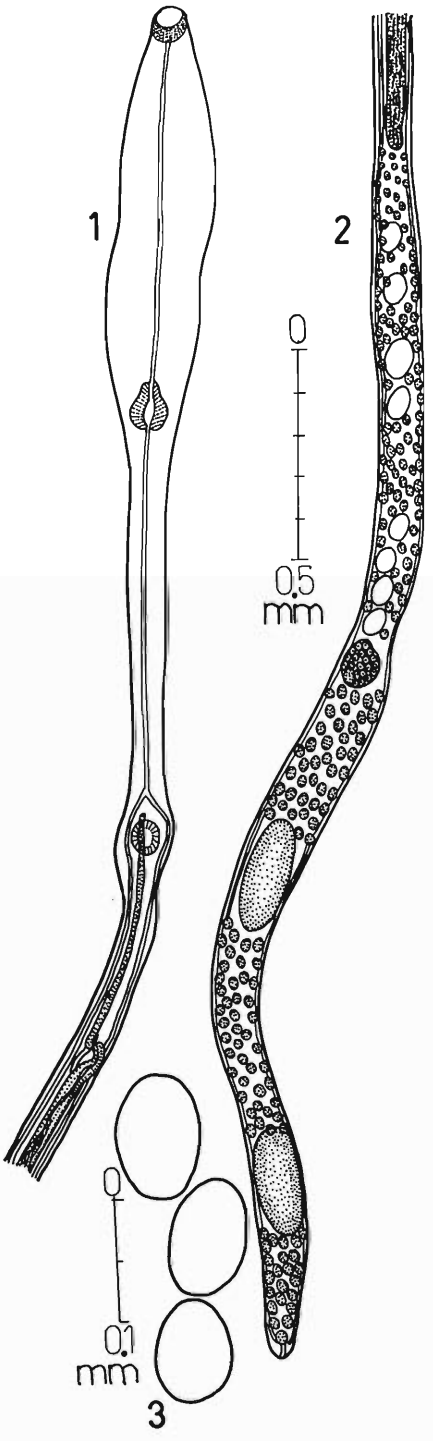
Materials and Methods

Viscera of 59 *Cybium* sp. were collected from Saddar Fish Market, Karachi. The fish species was not determined as only the viscera were available. Three trematodes were recovered from the intestine of a single fish, fixed in 70% alcohol–glacial actic acid mixture (95:5) under slight coverglass pressure for about 8 hr, stained with acetocarmine, and mounted permanently in Canada balsam. Diagrams were made with the aid of a camera lucida. Measurements are length by width in millimeters.

Family Acanthocolpidae Lühe, 1906
Tormopsolus spatulatum sp. n.

HOST: *Cybium* sp.
LOCATION: Intestine.
LOCALITY: Karachi coast, Arabian Sea.
NUMBER: 3 in 1 host; 59 hosts examined.
HOLOTYPE: USNM Helm. Coll. No. 72130.

Body long, delicate, cylindrical, 5.2–6.1 long, maximum width 0.2–0.25 at prepharyngeal region. Tegument unspined. Forebody 1.7–1.9 long. Prepharyngeal region spatulate. Oral sucker terminal, 0.04–0.06 by 0.05–0.055. Acetabulum in anterior ⅓ of body, 0.08–0.09 by 0.06–0.07. Sucker width ratio 1:1.2–1.45. Prepharynx slightly less than half length of the forebody, 0.7–0.8 long. Pharynx 0.9–1.3 by 0.7–0.9. Esophagus very long, delicate, almost as long as prepharynx, 0.7–0.85, bifurcating slightly anterior to acetabulum. Genital pore median, immediately preacetabular. Testis two, intercecal, tandem, in posterior ¼ of body; anterior testis 0.2–0.28 by 0.1–0.13; posterior 0.24–0.28 by 0.12–0.14. Cirrus sac mainly intercecal, dextral, extending from slightly postequatorial region of body to enter hermaph-



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Figures 1, 2. Entire length of *Tormopsolus spatulatum* sp. n., holotype, ventral view.

Figure 3. Eggs.

roditic duct. Cirrus sac 0.5–0.67 long containing elongated seminal vesicle, prostatic complex, and cirrus. Hermaphroditic duct long, measuring 0.4–0.6 in length, extending intercecally from genital pore.

Ovary intercecal, in posterior ⅓ of body, 0.11–0.13 by 0.10–0.12. Vitelline follicles relatively large, completely surrounding body ventrally and dorsally from posterior tip of cirrus sac to posterior end of body, interrupted at level of ovary and each testis. Uterus extending intercecally from ovary differentiating into metraterm near junction with hermaphroditic duct. Receptaculum seminis uterinum present. Eggs relatively few, large 0.07–0.12 by 0.06–0.08. Excretory vesicle Y-shaped.

Remarks

Tormopsolus spatulatum is close to *T. filiformis* as far as long prepharynx, long forebody, vitellaria interrupted at level of ovary and each testis, and posterior extent of cirrus sac but differs from it by possessing a spatulate prepharyngeal region and very long esophagus. *T. spatulatum* is separated from *T. osculatus* and *T. orientalis* in having a much longer forebody (proportionately twice as long), longer prepharynx, spatulate pharyngeal region, and very long esophagus; in *T. orientalis* the esophagus is absent, and in *T. osculatus* it is very small. The vitelline follicles are interrupted at the level of each testis and ovary in *T. orientalis* as in the new species. In *T. lintoni* the ovary is not separated from the anterior testis by a band of vitellaria and the prepharyngeal region is not spatulate in addition to other morphological differences.

The specific name *spatulatum* refers to the shape of prepharyngeal region.

Acknowledgments

The author wishes to thank Dr. D. C. Kritsky, Minot State College, Minot, North Dakota, for providing the photocopy of Yamaguti's description of *Tormopsolus orientalis* which was not available in Pakistan.

Cryopreservation of Infective Third-Stage Larvae of *Trichostrongylus axei* and *T. colubriformis*

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ABSTRACT: *Trichostrongylus axei* and *T. colubriformis* infective third-stage larvae were frozen and stored for varying periods at –170 C: 10 to 80 days for *T. axei* and 10 to 38 days for *T. colubriformis*. Thawed, surviving larvae were used to infect rabbits. Survival percentages changed little between worms stored for the period of the test. Larvae surviving freezing, storage, and thawing were as infective as nonfrozen larvae in rabbits.

Several benefits could be gained from long-term storage of nematode infective larvae. The considerable expenditure of time, labor, and funds to continually maintain monospecific isolates in culture animals would be reduced and the risk of accidental contamination would be minimized. Moreover, many monospecific isolates with particular genetic characteristics could be set aside for future study.

Various accounts concerning the ability of nematodes to survive the effects of subzero temperatures date back as far as Spallanzini (1776). Since then, sufficient evidence from various workers (Weinman and McAllister, 1947; Anderson and Levine, 1968; Muller, 1970) has accumulated to indicate that cryopreservation of nematodes is feasible and could be an important and useful laboratory procedure.

Therefore, the following study on cryo-

Table 1. Percentages of larvae surviving cryopreservation and numbers of nematode parasites recovered from infected rabbits.

	Larvae		Worms recovered from rabbits inoculated with 5,000 larvae					
	T. axei	*T. colubriformis*	*T. axei*			*T. colubriformis*		
No. days frozen	Per cent alive		avg	max	min	avg	max	min
0	>95.00	>95.00	443	525	285	1,562	2,180	915
10	6.18	7.60	216	270	190	2,096	2,615	1,525
24	7.02	8.94	481	655	165	1,672	2,275	1,075
38	6.37	7.83	135	210	75	2,063	2,330	1,630
45	7.88	—	622	960	245	—	—	—
67	9.64	—	429	680	210	—	—	—
80	4.95	—	445	705	230	—	—	—

preservation of infective third-stage larvae was made with particular interest directed at the subsequent infectivity of these larvae.

Materials and Methods

Infective third-stage larvae of *Trichostrongylus axei* and *T. colubriformis* were cultured and isolated from feces passed by lambs with monospecific infections. Prior to cryopreservation, the larvae had been stored in tap water in a refrigerator (approximately 4 C) for several weeks. Subsequently, they were divided into six batches of *T. axei* and three batches of *T. colubriformis* with each batch usually containing approximately 2.5 million larvae in distilled water. The larvae in each batch were concentrated on a 5.5-cm-diameter filter paper disc, using a Buchner funnel at low vacuum. Each moist filter paper disc was then placed against the inner wall of a 13-dram plastic snap cap vial. The vials were cooled to –80 C at an approximate rate of 1 C per minute in a liquid nitrogen controlled-rate freezer* and stored in liquid nitrogen vapor (approximately –170 C).

About the same time the larvae were frozen, five rabbits each were inoculated with 5,000 *T. axei* and 5,000 *T. colubriformis* infective larvae that had been stored at 4 C, but not frozen, to provide a baseline infection.

Frozen larvae were stored for different time intervals to determine survival capability, and inoculated into rabbits to determine infectivity. Larvae were thawed quickly by pouring room temperature distilled water into the vials immediately upon removing them from storage in the liquid nitrogen vapor.

After the larvae were thawed, the percentage of live larvae was determined on the basis of motility, and doses containing approximately 5,000 larvae were prepared. Three lots of five rabbits each were inoculated with an aggregate dose of 5,000 *T. axei* and 5,000 *T. colubriformis* larvae stored 10, 24, and 38 days, respectively. Three lots of five rabbits each were inoculated with 5,000 *T. axei* larvae stored 45, 67, and 80 days, respectively.

All rabbits were killed 25 days after inoculation and examined for parasitic nematodes. Stomachs of rabbits inoculated with *T. axei* and small intestines of those with *T. colubriformis* were placed in pepsin–HCl digestion fluid for 6 hr at 40 C. Worm counts were based on the number of helminths found in duplicate 10% aliquots.

Results and Discussion

The percentage of larvae surviving storage for 10 days was 6.18 for *T. axei* and 7.60 for *T. colubriformis*. Low-temperature storage for periods longer than 10 days apparently had little effect on the percentage of survivors (Table 1). We assume that the stresses of freezing and thawing were responsible for most of the larval deaths and that much of the death loss in our experiments was attributable to mechanical damage by freezing fluid external to the worms. The work of Andersen and Levine (1968), who reported 50 per cent survival of *T. colubriformis* larvae after 128 days at –95 C following desiccation, supports this assumption.

Larvae that survived freezing and thawing were as infective as nonfrozen larvae in rabbits

* Canalco, Inc., Rockville, Md. "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). This infectivity indicates that cryopreservation may be a useful technique in nematode parasitology. Since most parasitic nematodes have a high level of fecundity, the high mortality, which probably can be reduced, appears to be a surmountable problem.

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Freshwater Larval Trematodes. XXIX. Life Cycle of *Guaicaipuria parapseudoconcilia* sp. n.[1]

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ABSTRACT: The cercaria of *Guaicaipuria parapseudoconcilia* sp. n. parasitizes the freshwater snail, *Pomacea glauca*, and encysts in the gills of the tadpoles, *Engystomops pustulosus*. Cysts, 13 days old, when fed to a domestic duckling developed into the adults in the bursa Fabricii, with the expulsion of eggs 12 days after the feeding. Miracidia penetrated one of the laboratory-raised snails, and cercariae emerged 41 days postinfection. These cercariae proved identical with those from field material. *G. parapseudoconcilia*, on the basis of adult characters, is indistinguishable from *G. pseudoconcilia*, the only other species in the genus, but is an independent entity when the cercarial characters are considered: in the former species the digestive tract is absent beyond the pharynx, while in the latter the esophagus is well developed and the intestinal ceca extend to the posterior end of the body; moreover, the former employs tadpoles as the second intermediate host in relation to the freshwater fish, *Rivulus harti* (Bouelenger), in the latter.

The genus *Guaicaipuria* Nasir, Díaz, and Marcano, 1971 (Guaicaipuriinae, Nasir et al., 1971; Cathaemasiidae, Fuhrmann, 1928) contains only one species, i.e., *G. pseudoconcilia* (Nasir, Díaz, and Lemus de Guevara, 1969). The cercariae of this fluke parasitize the freshwater snail, *Pomacea glauca* (L.), and encyst in the gills of the freshwater fishes, *Lebistes reticulatus* (Peters) and *Rivulus harti* (Bouelenger). The metacercariae, 8 days old, were fed to a laboratory-raised pigeon, and adults developed in its cloaca 10 days later at which time eggs appeared in its feces. The necessity for the erection of a new genus, *Guaicaipuria*, and a new subfamily, Guaicaipuriinae, of the family Cathaemsiidae has already been discussed (Nasir, Díaz, and Marcano, 1971).

A new species of cercaria, readily distinguished from that of *Guaicaipuria pseudoconcilia* due to the absence of a digestive tract posterior to the pharynx, was found which failed to encyst in the fishes, *Lebistes reticulatus* and *Rivulus harti*. However, when tadpoles, *Engystomops pustulosus* (Boettger), were exposed the cercariae readily encysted in the gills. When infected tadpoles were fed to a domestic duckling the corresponding adults developed in its bursa Fabricii. The eggs from

[1] Supported by grant No. DCC/02/67/DB-02 from Comisión de Desarrollo y Coordinación Científicas of Universidad de Oriente.

[2] Based on data from graduate work of the junior author.

these trematodes were incubated at room temperature, 25–28 C, and miracidial penetration was successfully brought about in *Pomacea glauca* with the subsequent emergence of cercariae identical with that of the natural infection.

Materials and Methods

Eight snails, *Pomacea glauca* (L.), collected from the "canal de riego," 1 km southeast of Universidad de Oriente, were isolated in finger bowls about ¾ filled with tap water. After 24 hr one of the snails emitted the cercariae of *Guaicaipuria parapseudoconcilia*. The tadpoles, *Engystomops pustulosus*, which served as the second intermediate hosts, were collected from one of the artificial puddles in the university nursery. These puddles are temporary bodies of water formed only during the rainy season, and it is here that adult toads of this species deposit their eggs. None of these tadpoles ever harbored any trematode infestation. No other macroscopic animals, including snails, frequent these shallow puddles.

The cysts, *in situ*, pooled from various tadpoles, were administered orally with a pipette to a domestic duckling. The feces of this bird were examined daily. On the 12th day after the feeding trematode eggs appeared. The bird was dissected the same day, and 23 gravid adult *G. parapseudoconcilia* were recovered from its bursa Fabricii.

The eggs recovered from the feces and those teased from the parasites were kept in small petri dishes containing filtered pond water which was changed daily. On the 14th day after incubation, apparently fully developed miracidia, with pigmented eyes, were observed continuously pushing against the opercular poles of eggs. The laboratory-raised snails, *Biomphalaria glabrata* (Say), *Marisa cornuarietis*, and *Pomacea glauca*, were exposed to eggs for 14 days in a shallow enamel tray about half-filled with filtered pond water which was continuously aerated. Fresh lettuce was added as a food for snails. After 21 days, the snails were removed to a large aquarium, and periodically placed in finger bowls. On the 41st day, one of the pomaceas started liberating cercariae of *G. parapseudoconcilia* identical with those from the naturally infected snail of the same species. The remainder of the snails were dissected, but all proved negative.

A 3-week-old duckling was obtained from a commercial dealer. Daily examinations of its stools before the commencement of the feeding experiments were negative for trematode eggs. It was fed on a diet of commercial chicken mash.

Only freshly emerged live cercariae were used. The usual intravital strains, such as neutral red, malachite green, methylene blue, Janus green B, and Bismark brown, were occasionally used. The adults were washed several times in Locke's solution, and then fixed in hot (60–70 C) Gilson's fixative. All measurements (in mm) were on fixed material. The figures were drawn with the aid of camera lucida except certain details, like the flame cell system, which were drawn freehand.

Results

Guaicaipuria parapseudoconcilia sp. n.

Cercaria

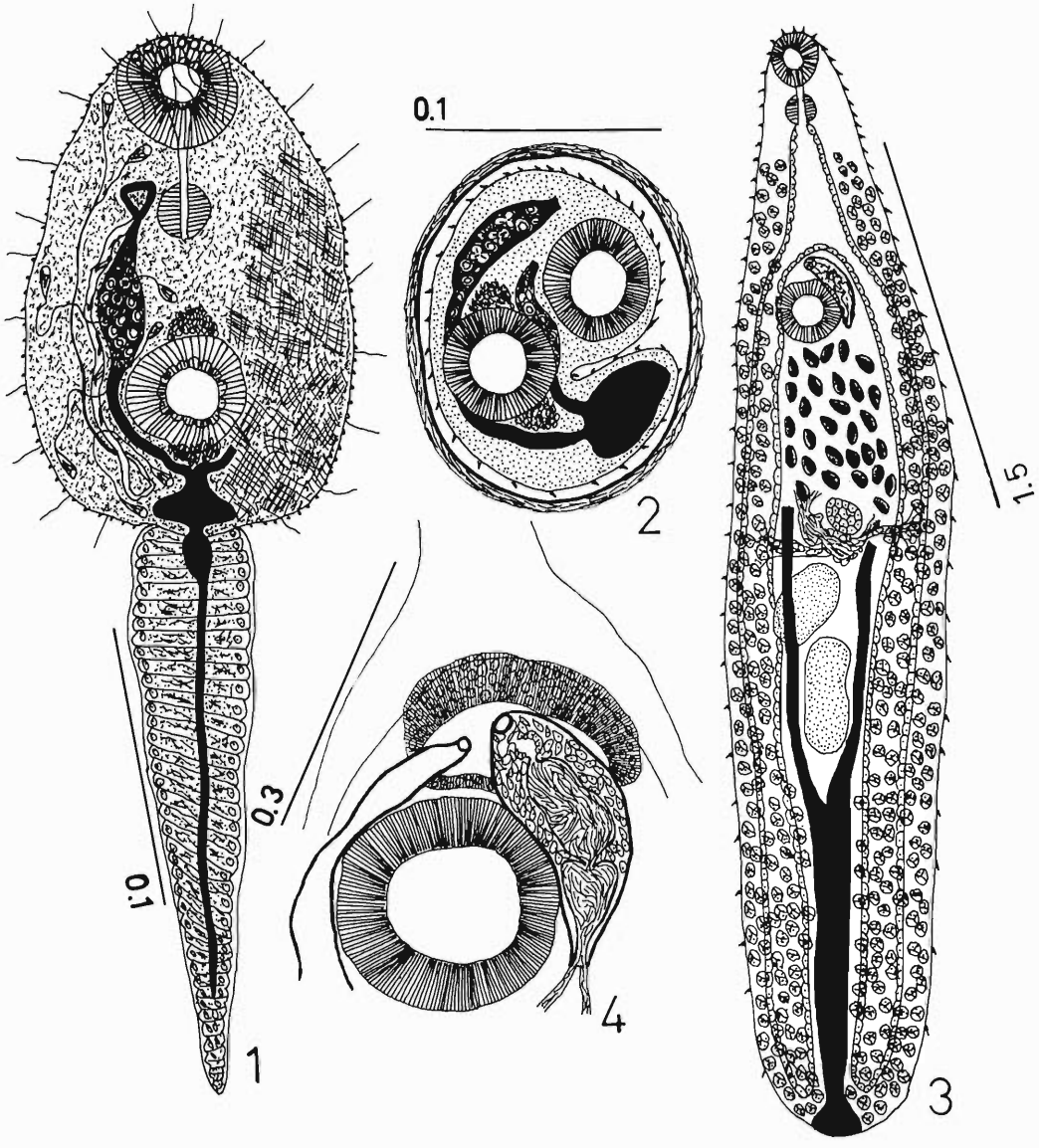
(Fig. 1)

HOST: *Pomacea glauca* (L.).

LOCALITY: Canal de riego, 1 km southeast of Universidad de Oriente, Cumaná, Venezuela.

Description

Body spinose, flagellated. Tail without finfold, aspinose, without flagella. Suckers equal in diameter. Acetabulum posterior to equatorial line of body, with a ring of papillae around its orifice. A semicircle of nine spines in anterior region of oral sucker. Oral orifice with a circle of papillae. Prepharynx may be as long as pharynx. Esophagus and intestinal ceca absent. Anterior margin of body bordered with six apertures of ducts. Cystogenous glands with rhabditiform contents. Main excretory tubes enclosing refractile excretory granules only in preacetabular region. Secondary excretory tubes lined internally with ciliated patches. Anterior and posterior excretory loops present. Caudal excretory duct ending blindly in posterior half of tail. Flame cell formula $2[(2 + 2 + 2 + 2)] = 16$. Measurements of 12 fixed cercariae: body 0.166–0.199 by 0.092–0.122; tail 0.203–0.238 by 0.040–0.048; suckers 0.040–0.048 in diam; prepharynx 0.007–0.017 long; pharynx 0.014–0.022 in diam.



Guaicaipuria parapseudoconcilia sp. n. Fig. 1. Cercaria, flame cells, and cystogenous glands drawn on one side only; note the absence of digestive tract beyond pharynx. 2. Cyst, enclosed in a wall of double nature. 3. Adult, ventral view, holotype. 4. Reconstruction of terminal genitalia, ventral view, paratype: note highly muscular anterior rim of common genital pore; cirrus sac including a bipartite vesicula seminalis, and profuse prostate glands; and metraterm and cirrus sac opening independently in genital atrium.

Metacercaria

(Fig. 2)

An undetermined number of the tadpoles, *Engystomops pustulosus*, were exposed in a dish containing a heavy suspension of cercariae. After 2 hr one of the tadpoles was dissected and its gill filaments revealed several freshly encysted cercariae. The mode of penetration was not observed, but it appears that the cercariae are carried either passively in the respiratory currents, or are actively eaten by the tadpoles. The maximum number of cysts recovered from a single individual was 32. No cysts were encountered in any other part of the amphibian body.

The cysts are ovoid, measuring in vivo under slight coverglass pressure 0.129–0.154 by 0.107–0.128, including the cyst wall. The latter is of a double nature: an internal delicate layer of parasite origin, and an external fibrous layer of host origin (the thickness of which varies with time).

Adult

(Figs. 3, 4)

HOST, experimental: domestic duck; natural: unknown.

HABITAT: bursa Fabricii.

DEPOSITED SPECIMEN: USNM Helm. Coll. No. 71966, holotype.

Description

Body completely or partially spined, attenuated anteriorly, with a maximum width in genital region. Oral sucker smaller than ventral; latter situated nearer to anterior extremity than to equatorial line of body. Prepharynx absent or present. Pharynx smaller than oral sucker. Esophagus not extending to ventral sucker. Ceca extending to posterior end of body. Ovary, in majority of specimens, transversely elongated, unlobed, rarely isodiametric, irregular, preequatorial, always considerably posterior to ventral sucker. Uterus neither reaching testicular region, nor intruding into forebody, enclosing embryonated eggs. Metraterm opening independently in a common genital atrium. Ootype complex posterior to ovary. Receptaculum seminis uterinum present. Vitelline glands supra- and extracecal, not confluent in preacetabular region of body, distinctly separated along median longitudinal line in posttesticular region. Anterior limits of these glands not exceeding posterior margin of pharynx. Testes unlobed, anteroposteriorly elongated, tandem or anterior testis rarely diagonal, postequatorial, sometimes anterior testis partially equatorial. Cirrus sac partly or completely lateral to ventral sucker, not extending postacetabularly, enclosing a bipartite seminal vesicle, prostate glands, and cirrus. Common genital pore (Fig. 4) highly muscular, especially so in its anterior margin, postbifurcal, median, or submedian. Excretory vesicle Y-shaped. Measurements based on 10 egg-discharging adults (those in parentheses representing flattened specimens): body 2.800–3.416 by 0.448–0.639 (3.808–4.368 by 0.672–0.986); oral sucker 0.126–0.154 (0.182–0.210) in diam; ventral sucker 0.140–0.154 (0.210–0.264) in diam; pharynx 0.087–0.098 (0.126–0.154) in diam; ovary 0.070–0.126 by 0.084–0.126 (0.112–0.140 by 0.112–0.154); anterior testis 0.140–0.224 by 0.140–0.154 (0.280–0.336 by 0.210–0.280); posterior testis 0.182–0.224 by 0.140–0.168 (0.322–0.420 by 0.224–0.252); cirrus sac 0.296–0.390 by 0.100–0.118 (0.374–0.486 by 0.125–0.269); eggs 0.084–0.126 by 0.056–0.070 (0.112–0.126 by 0.056–0.079).

Discussion

The adults of *Guaicaipuria parapseudoconcilia* cannot be distinguished from that of *G. pseudoconcilia* in their apparent morphological characters. However, in the cercaria of *G. parapseudoconcilia* there is a complete absence of digestive tract posterior to the pharynx, whereas in that of *G. pseudoconcilia* the esophagus extends up to the ventral sucker, and the intestinal ceca reach postacetabularly. Another difference lies in the fact that in the former, the secondary excretory tubes form another loop in the region of the excretory vesicle in addition to the one in the oral region, but in the latter these tubes divide at the postequatorial level of the ventral sucker. The oral sucker of the cercaria of *G. pseudoconcilia* is larger than the ventral, while in *G. parapseudoconcilia* the suckers are equal in diameter. At the same time, the cercariae of the two species are similar in general pattern of the excretory system, total number of flame cells, i.e., 16, the rhabditiform contents of the

cystogenous glands, the number of apertures of penetration ducts, and the presence of papillae in the oral sucker. On the contrary, they differ markedly in two biological aspects: the second intermediate hosts of *G. parapseudoconcilia* are tadpoles and the experimental definitive host is the domestic duck, but *G. pseudoconcilia* employs fishes and the pigeon, respectively. Thus, the two species are indistinguishable in adult characters, but are distinguishable in the larval morphology and biology. There are other similar cases in which life history data are the only apparent criteria for specific determination, e.g., *Echinostoma nudicaudatum* Nasir, 1960, *E. pinnicaudatum* Nasir, 1961, and *E. revolutum* (Froelich, 1802) Looss, 1899 (Beaver, 1937). The cercaria of *E. nudicaudatum* is devoid of the caudal finfold, while that of *E. pinnicaudatum* is furnished with a prominent finfold throughout the caudal length. In the cercaria of *E. revolutum* this structure is limited only to the caudal tip. A similar situation is paralleled by *Stephanoprora denticulata* (Rudolphi, 1802) Odhner, 1910 (Nasir and Scorza, 1968), and *S. paradenticulata* Nasir and Rodriguez, 1969. The cercaria of the former is of the type Magnacauda Byrd and Reiber, 1940, with collar spines similar to that of its adult, i.e., 22, while that of the latter is a "gymnocephalic" form lacking collar spines.

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Research Note

Helminth Parasites of the King Rail, *Rallus elegans* Aud., and Clapper Rail, *Rallus longirostris* Boddaert, of the Bolivar Peninsula, Texas

Few other studies of rail parasites have been made, and none of these has involved the extensive Texas Gulf coast. Heard reported the helminth parasites taken from 146 clapper rails which were collected in 10 Atlantic and Gulf Coast states (1970, Proc. Helm. Soc. Wash. 37: 147–153). During these studies 40 of the birds were collected from Georgia with an average of 10.6 from each of the other states. Lumsden and Zischke reported the parasites of 10 king rails collected in Louisiana (1963, Ztschr. Parasitenk. 22: 316–366). MacInnis, during a study of rails from along the Northwest Florida coast, also reported the helminth parasites for these birds (1966, Zool. Anz. 176: 52–68).

This report lists the helminth parasites taken from 36 king rails, *Rallus elegans* Aud., and 48 clapper rails, *Rallus longirostris* Boddaert, collected from the fall of 1969 through 1970. Collections were made on the 23- by 3-mile isolated Bolivar Peninsula of the Texas Gulf coast. The purpose of the study was to examine both kinds of rails which were taken from one geographically isolated area in order to compare their parasites. The findings also serve as an indicator to the parasites of the other little studied, less available Texas shore birds.

Table 1. Incidence of helminth infections in 84 king and clapper rails of the Bolivar Peninsula, Texas.

Parasite	Number infected rails		Location in host*	% of total infected	
	King	Clapper		King	Clapper
Trematoda					
Ascocotyle pachycystis	3†	4	(6)	3.5	4.7
Athesmia heterolecithodes	1	0	(3)	1.1	0
Carneophallus sp.	0	2	(5, 6)	0	2.3
Cyclocoelum obscurum	1	0	(2)	1.1	0
Echinochasmus schwartzi	11†	22	(4, 5, 6)	13.0	26.0
Echinostoma attenuatum	5	6	(5, 6)	5.9	6.0
Levinseniella byrdi	12†	32	(4, 5, 6, 7)	14.0	38.0
Notocotylus regis	2	0	(5)	2.3	0
Opthalmophagus sp.	0	4	(1)	0	4.0
Prosthogonimus ovatus	1†	0	(5, 6)	1.1	0
Prosthogonimus sp. 1	0	1	(5, 6)	0	1.1
Prosthogonimus sp. 2	0	1	(6)	0	1.1
Cestoda					
Cyclustera capito	2†	3	(4)	2.3	3.5
Ophryocotyle proteus	1†	3	(4)	1.1	3.5
Parvitaenia sp.	1†	1	(4, 6)	1.1	1.1
Nematoda					
Capillaria sp.	3†	6	(4, 5, 6, 7)	3.5	6.0

* (1) eye, (2) body cavity, (3) liver, (4) small intestine, (5) large intestine, (6) ceca, (7) cloacal area.
† New host record.

The numbers of infected rails, parasite location in the host, and per cent of total infected are shown in Table 1. Few differences were found for the two kinds of rails, a condition which is possibly due to similar feeding habits and preferences. These similarities were determined from field observation and examination of intestinal contents. Immediately following collection of the rails postmortem examination was conducted as soon as was practical in order to obtain the parasites before their decomposition. This was frequently done in a nearby field laboratory. Those parasites which were found were observed alive then fixed for later detailed study. However, due to the paucity of some specimens and/or condition at time of postmortem, identification in some instances was possible only to genus level.

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William Walter Cort

April 28, 1887–August 21, 1971

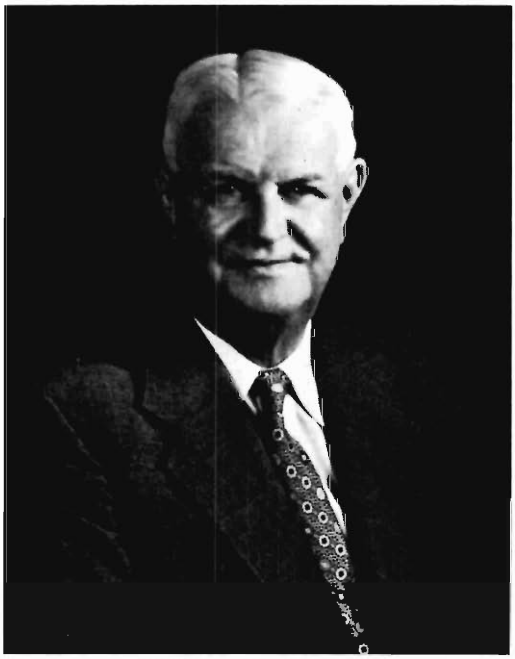
With the death of Dr. W. W. Cort, the Helminthological Society of Washington lost a revered, long-term member. He joined the Society in 1920, was its eighth president, 1924–25, and was elected to life-membership at the 307th meeting, held at the Johns Hopkins University School of Hygiene and Public Health in Baltimore on April 18, 1952. He was mainly responsible, albeit indirectly, for the independent publication of the Society Proceedings—for the existence of the respected scientific journal which began in 1934 and thus is entering into its 39th annual volume. During 34 years (1919–53) at the Johns Hopkins University, moreover, Dr. Cort and his colleagues (Hegner, Root, Andrews, Otto, Rozeboom) developed 112 Sc.D. graduates (Dr. Cort has direct credit for 44 to 50) nearly all of whom enjoyed at least a temporary membership in "Helmsoc." There were other students—candidates for the M.Sc., M.P.H., and D.P.H. degrees—who also contributed to the character and growth of Helmsoc, not to mention the many whom Dr. Cort taught and worked with at the University of Michigan Biological Station. His contribution to the Society, direct and indirect, even in the light of these few meager facts, was clearly beyond evaluation.

Will Cort's identification with scientific and other learned societies was not limited to Helmsoc, as we all know, yet, as he himself has protested, he was not a joiner of societies or clubs. A few meant much to him. Information can be found readily (i.e., Who's Who; American Men of Science; G. F. Otto, Jour. Parasitol. 39(4) 462–464, 1953). Cort was, however, a member, if not the leader, of a small group in the Helminthological Society who met in December 1924 to found an American Society of Parasitologists. He served as Secretary-Treasurer of the new Society, 1925–29, and President, 1930 (the 5 earlier presidents were Ward, Stiles, Strong, Kofoid, and Cobb). He was, also and significantly, the first ASP Editor of The Journal of Parasitology, an office that he held initially for six years (1932–37) and later for another year (1948) when he substituted for Dr. Horace W. Stunkard during the latter's sabbatical leave. The more significant and less known point is that Dr. Cort effected the transfer of ownership of The Journal of Parasitology from its founder, Henry Baldwin Ward, to the ASP. Probably all who were privy to knowledge of any details of the transfer have since reflected upon the likelihood that Cort was the man of the times and for the times to achieve the desired result. He was made an Emeritus Member of the American Society of Parasitologists in 1957. The limited space available for this brief statement precludes adequate coverage of Dr. Cort's interest and participation in other societies. Nevertheless, it should be mentioned that he was active for many years in The American Society of Tropical Medicine and Hygiene, including its antecedent organizations: The American Society of Tropical Medicine, The National Malaria Society, and The American Academy of Tropical Medicine; from 1934 to 1939 he served successively as treasurer, vice-president, and president of the last named organization. At the time of his death, Dr. Cort was an emeritus member of the parent society. He was president of the American Microscopical Society in 1937, and the American Society of Naturalists in 1941.

Dr. Cort's primary research interests were hookworm disease, ascariasis, schistosomiasis, and trematode biology. In connection with these interests, he organized and led uniquely meaningful field expeditions for the International Health Board of the Rockefeller Foundation to Trinidad (1921), Puerto Rico (1922), China (1923–24), and Panama (1926). He also went to Egypt (1934) under the same auspices to advise on the schistosomiasis program. His excursion to China, of longer duration than the others, was undertaken in connection with a visiting professorship at the Peking Union Medical College. His researches on trematodes were conducted mainly during the many summers at the Biological Station at Douglas Lake. As a final word, the fact should probably be noted that Dr. Cort's motivation for field study of important human helminthiases derived from his earlier studies on amebiasis and particularly on hookworm disease of gold miners in California (1917–19) while he was at Berkeley. Overall, Dr. Cort with his students and colleagues, probably more than any others, put parasitological problems "on the map" with both physicians and veterinarians. None has contributed more in either direction.

He and his students pioneered work on immunity to parasitic infections and role of nutrition in helminth infections. He was in fact the pioneer in experimental helminthology apart *from simple* life history studies.

A scholarly and intense approach to every problem, plus a keen intellectual discernment of what was important, seemed to have given this unusual man the motivation to work indefatigably and publish prolifically. Fortunately, he was not only a superb scientist but also an excellent writer, al-



William Walter Cort
April 28, 1887–August 21, 1971

though writing was a deliberate chore; he was his own most severe critic. To his everlasting credit, let it be said, regarding his writing as his researches, he was ever prepared to do whatever had to be done, over and over again.

The Index-Catalogue lists some 129 titles of books, papers, and notes that were published over a span of 45 years (1912–57) of which Cort was first author. He was, however, exceedingly generous with those who worked with him, especially on the matter of "senior" authorships. In consequence, we find that the Catalogue also lists some 19 authors with whom Dr. Cort published but not as first author; many were his students and some were collaborating colleagues.

Dr. Cort received many honors, including two honorary Sc.D. degrees. The Anniversary Award of the Helminthological Society of Washington was presented to him *in absentia* on October 19, 1966.

Dr. Cort was born in Leon, Iowa. His father, a clergyman, met an untimely death when "Will" was 9 years old. His mother was left with three boys, aged 7 to 12 years and shortly moved with her brood to Colorado Springs where there were family relatives and good educational opportunities. Cort was reared there and took his A.B. degree at Colorado College in 1909. In spite of adversities (or could it have been on account of them?), he played varsity football for three years yet earned election to Phi Beta Kappa. His most influential professor during these years was Dr. E. C. Schneider (destined to become a famed biologist, physiologist, researcher, and teacher) whose gentle but industrious and scholarly example lead Cort to decide upon a major in biology—a major notoriously difficult because of extensive laboratory, class, and study requirements, even under less demanding teachers, and especially so if one aspires toward excellence in both academics and athletics.

Dr. Cort received his M.S. and Ph.D. degrees in 1911 and 1914 under Henry Baldwin Ward, the "Father of Parasitology in the United States." Thereafter, he taught at Macalester College and the University of California for an aggregate of 5 years, then went to the Johns Hopkins University School of Hygiene and Public Health where he remained for 34 years (1919–53). In 1953 he was made Professor Emeritus by that school and became Research Professor of Parasitology, University of North Carolina. In 1959 he was made Research Professor Emeritus by the latter institution. During these years, Dr. Cort taught in other institutions, notably the Peking Union Medical College, 1923–24, and especially the University of Michigan Biological Station, summers 1914–57, where he pursued his researches and his training of graduate students with the same vigor and success that he evidenced for so many years in Baltimore. His other teaching assignments and lectureships, all very important, are too numerous to mention. Indeed, his academic years at Johns Hopkins and summers at the Biological Station, plus perhaps the numerous "expeditions" that were fitted into his busy career of teaching and research are the three aspects of Cort's professional identifications that will probably remain uppermost in the minds of all who knew him and his work. For those who did not know Cort as some of us were especially privileged to know him, let it be recorded that he was a comparatively large man physically (over six feet tall); dignified, serious, and thorough; shy, gentle, and kind. While he was no jokester, he enjoyed good humor; he was quiet, hard-working, and never boisterous. Almost no one argued with him because he was thoroughly logical and disarming. Every student he had became his life-long friend. Although every one knew where he stood academically, he also knew that Cort was steady, utterly fair, objective, and devotedly interested in each student as a person. He was articulate and a voracious reader. Cort had what has come to be called "charisma," a kind of personal charm and magnetism that made him well liked

and exceedingly effective with people. Interestingly many, if not all, these attributes applied equally to his deeply admired teacher, Professor E. C. Schneider. His impact on parasitology in this country and throughout the world, although not completely assessable, was obviously enormous. Dr. Cort, the man, and the man through his students, have assuredly contributed mightily to the making of modern parasitology. Those of us who were privileged to be students of Dr. Cort will always cherish the experience.—A. O. FOSTER AND G. F. OTTO.

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