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The Ultrastructure of Developing Metacercarial Cysts of *Ascocotyle leighi* Burton, 1956 (Heterophyidae)[1]

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ABSTRACT: Electron microscopy of encysting *A. leighi* metacercariae in the *bulbus arteriosus* of *Poecilia* (= *Mollienesia*) *latipinna* (LeSeuer) indicates that the material composing the cyst wall is of parasite origin. The bulbus tissue bordering the cysts includes endothelial cells, fibroblasts and a diffuse meshwork of reticular collagen fibers. Immediately following the penetration of the *bulbus* endothelium by the parasite, a thin primary lamella condenses around the cercaria. Additional matrix material is then added to the inner surface as granular secretions from the metacercarial tegument. A moderate fibrosis of the surrounding host tissue is evidenced by some infiltration of fibroblasts and increased density of reticular collagen, though this collagen does not form an integral component of the cyst wall. In heavy infections, the metacercarial cysts severely occlude the primary lumenal space of the *bulbus*. In such cases, there is some recanalization of the endothelium, providing secondary channels for blood flow. No cytological indication of an inflammatory or immune response was apparent at any time during the encystment process.

Dixon and Mercer (1967) described the ultrastructure of the metacercarial cyst wall of *Fasciola hepatica* at various stages in its development. The cyst structure of this free encysting trematode species differs significantly from that of many forms which encyst within the tissues of an intermediate host. As recognized by Hughes (1928), Macy (1934), Hunter and Dalton (1939), Bogitsh (1962), and others, such cysts may be formed from material contributed by both the host and parasite or composed entirely of host connective tissue.

Metacercariae of the *Ascocotyle* complex of species commonly utilize brackish water fishes as second intermediate hosts. Specificity of the parasites for a particular organ is marked, and the encysted worms are invariably located in close proximity to the circulatory system. Photomicroscopic studies on the encystment of *A. angrense* and *A. leighi* in the gill and heart

tissues, respectively, of their second intermediate hosts have suggested that the metacercariae become enveloped by host fibroblasts which contribute a layer of collagen around the periphery of an inner cyst wall, probably of parasite origin (Sogandares-Bernal and Lumsden, 1963, 1964). Variation in cyst wall structure of otherwise seemingly identical *Ascocotyle* metacercariae has been interpreted as reflecting differences in magnitude of the host fibrocytic response (Sogandares-Bernal and Lumsden, 1964). In a subsequent electron microscopic investigation of the cyst wall of *A. chandleri*, a species encysting in the liver of cyprinodontid fishes, Lumsden (1968) found no collagen incorporated within the structure of the metacercarial cyst wall, though collagen fibers were sometimes present in the adjacent host tissues. In the present investigation, we have attempted to elucidate the relative contributions of host and parasite in determining the structure of the metacercarial cyst of *A. leighi* during its development in the cardiac tissues of the sailfin molly, *Poecilia* (= *Mollienesia*) *latipinna*.

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Materials and Methods

Cercariae of *A. leighi* were obtained from naturally infected amnicolid snails (*Lyrodes* spp.) collected from brackish ponds in the vicinity of Lake Pontchartrain, Louisiana. Sail-fin mollies collected from various freshwater localities were found to be consistently free of *A. leighi* infections. Fishes from these collections were exposed in small bowls to approximately 50 cercariae each for 1–2 hr. The hearts of these fishes were removed at various intervals thereafter. The *bulbus arteriosus* was fixed in ice-cold glutaraldehyde, postfixed in osmium tetroxide and embedded in Epon according to methods described by Lumsden (1970). Sections for light microscopy were cut at 1–2 μ, mounted on microslides and stained with azure II and methylene blue. Thin sections (displaying silver or light gold interference colors) were collected on uncoated copper grids, stained with uranyl acetate and lead citrate and examined in a Siemens 1A electron microscope.

Observations

The cercariae of *A. leighi* enter the gill chambers of the fish host. After attaching to the gill epithelium, the cercaria loses its tail and penetrates the gill surface. From there the parasite migrates via the afferent arterioles to the ventral aorta and then to the heart. Upon reaching the *bulbus arteriosus*, the cercariae penetrate the endothelium and immediately begin to encyst in the perivascular tissue (Figs. 1 and 2). At the photomicroscopic level, the first manifestation of this process is the presence of a thin, darkly staining layer subjacent to the *bulbus* endothelium and circumscribing the coiled up parasite (Fig. 2). At the level of electron microscopy (Figs. 5 and 6), this primary lamella appears moderately electron dense and amorphous. It is initially discontinuous (Fig. 5), but within an hour or so becomes consolidated to form a continuous investment which closely follows the contour of the parasite's body surface (tegument) (Fig. 6). By 8–12 hr, the primary lamella has thickened, with ridgelike projections extending into the surrounding host tissue. The space between the developing cyst and host endothelium is filled with amorphous ground substance (Fig. 7) which appears to represent at least in part the basement lamina of the *bulbus* endothelium. The cyst extends from the lumenal surface of the *bulbus* into the reticular connective tissue (Fig. 8). As the metacercariae grow, the cysts increase in volume, distending the endothelium and thereby occluding significantly the normal route of blood circulation through the *bulbus* (Fig. 3). In heavy infections, secondary channels develop via the infiltration of endothelial tissue around the cysts (Fig. 4).

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Figures 1–4. Photomicrographs of *A. leighi* metacercarial cysts in *bulbus* of *Poecilia latipinna*. 1. Approximately 1 hr postexposure of fish to cercariae. Note parasites (*) encysting immediately beneath the endothelium of the *bulbus* lumen (filled with blood cells). × 550. 2. One of the cysts from the previous image at higher magnification, demonstrating the presence of the primary lamella (arrow) and bordering *bulbus* endothelium (*). × 1,540. 3. Fourteen days postexposure. By this time, growth of the cysts (*) has markedly occluded the lumen of the *bulbus* (arrow). × 260. 4. Fifteen-day-old infection, illustrating a secondary channel proximal to a metacercarial cyst (partially evident in upper right corner at C). The channel is lined with endothelial cells (arrows). × 1,280.

Figures 5–6. Electron micrographs of metacercarial cysts in the *bulbus* within 2 hr postexposure of fish to cercariae. 5. Very young cyst, illustrating discontinuous primary lamella (arrows) between metacercaria (M, at left) and *bulbus* endothelium (E, at right). × 46,660. 6. Slightly older cyst, illustrating continuous primary lamella (arrows), which closely follows the contour of the metacercarial tegument (T). E, *bulbus* endothelium. × 46,660.

Figures 7–8. Electron micrographs of metacercarial cysts 12 hr postexposure of fish to cercariae. 7. Thickened primary lamella (PL) with ridgelike projections extending into the ground substance of the basement lamina (BL) of the *bulbus* endothelium (E). The cavity of the cyst (*) contains some free flocculent material and several membrane bound vesicles (arrows) enclosing material of density comparable to that of the primary lamella. × 136,800. 8. Peripheral reticular connective tissue of *bulbus* containing several fibrocytes and bundles of reticular collagen fibers (Co); note densification of ground substance at *. Arrows indicate limits of metacercarial primary lamella. × 5,770.
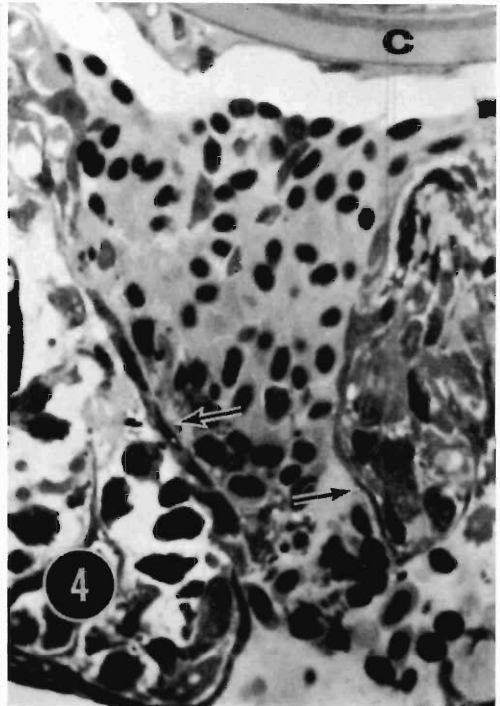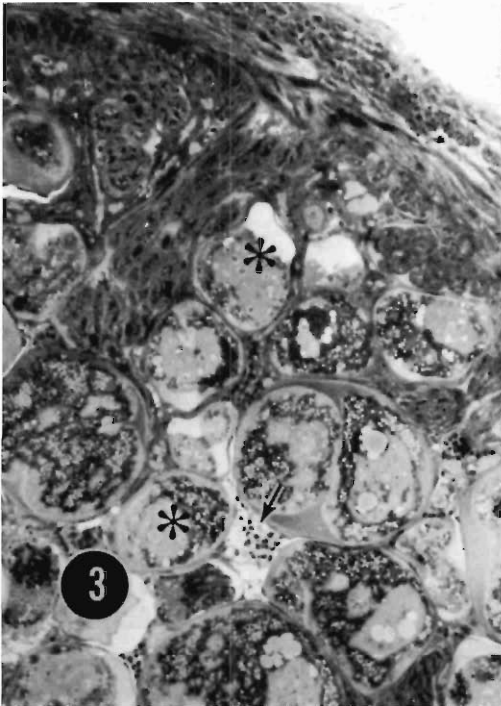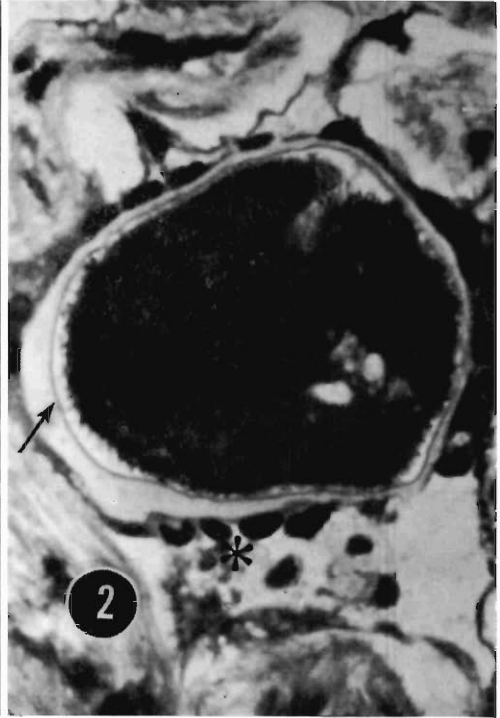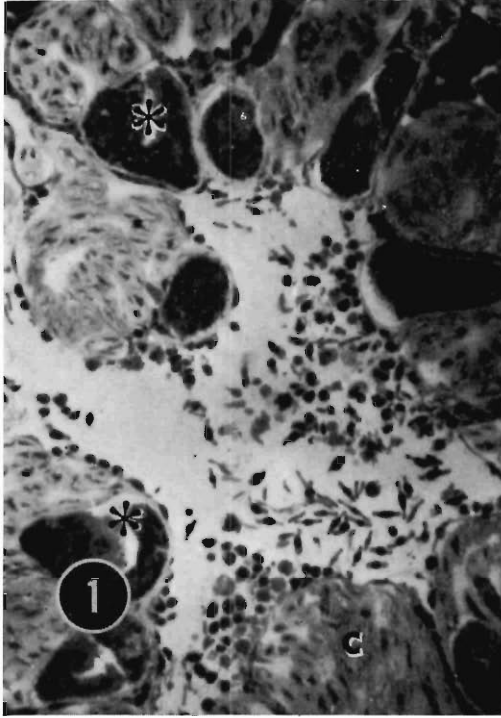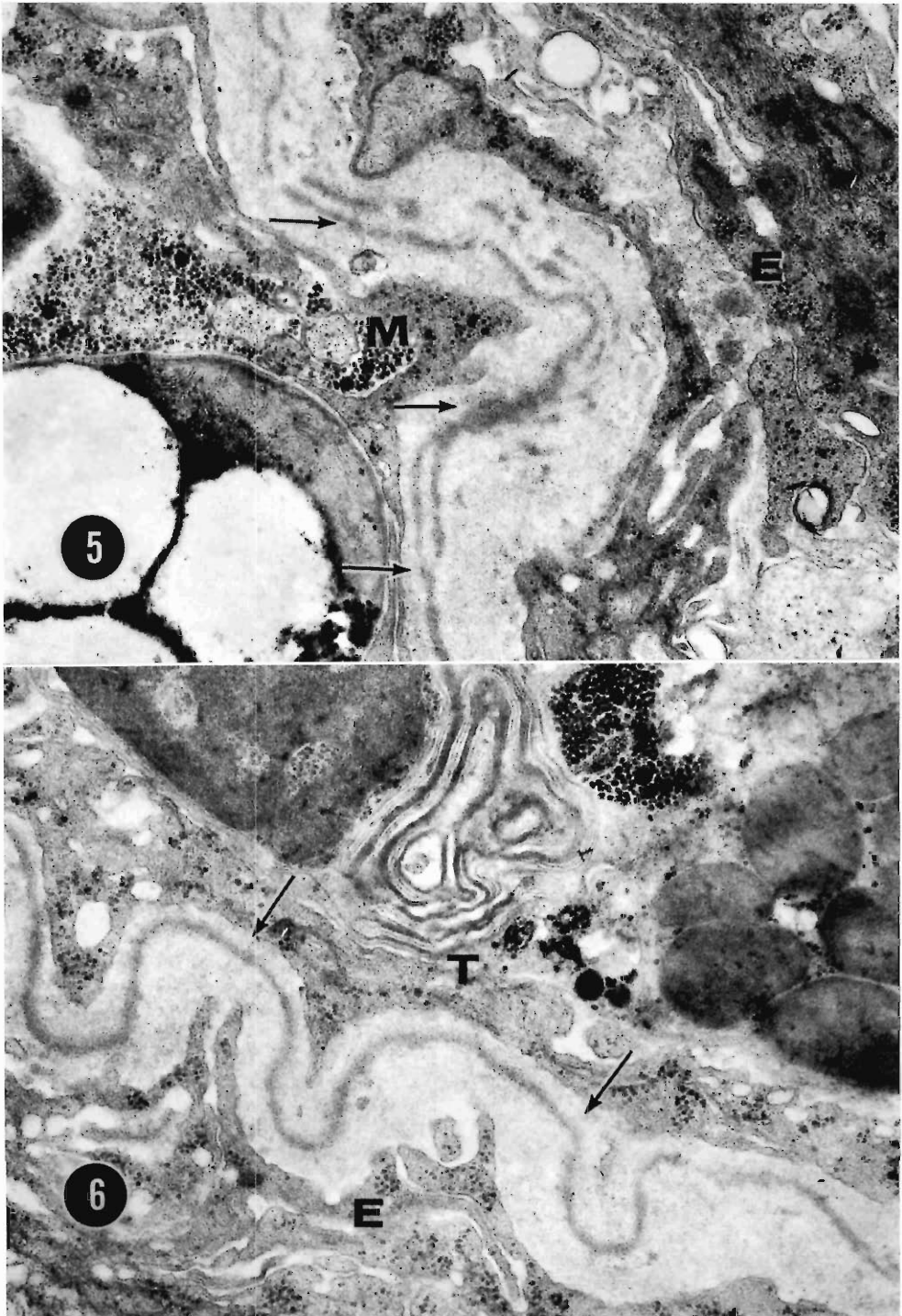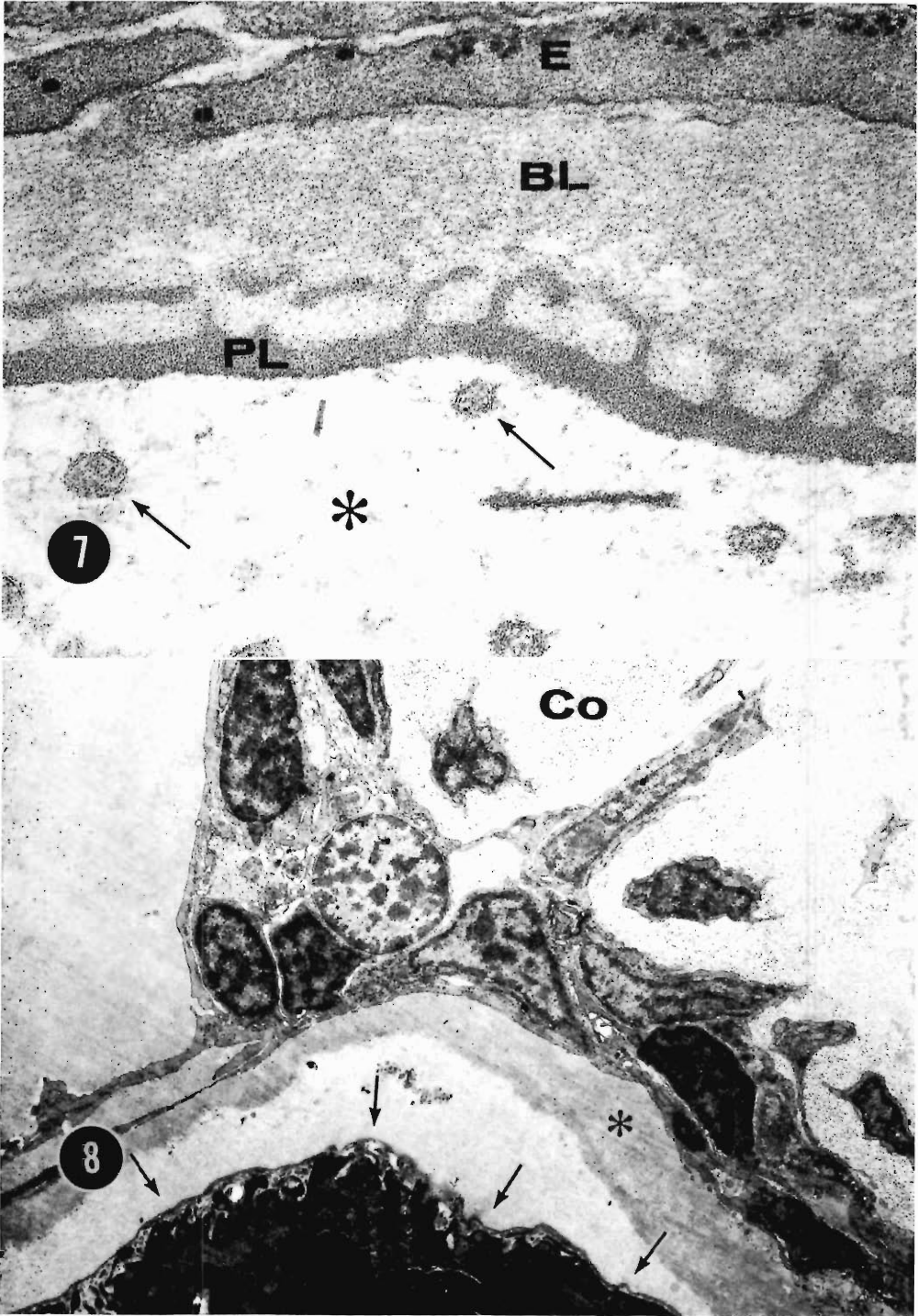
The metacercarial cyst wall increases in thickness by the addition of material to the inner (parasite) surface of the primary lamella (Fig. 9). This material is packaged in granules which are released from the tegument of the metacercaria (Figs. 9 and 10). By 16 days post infection, the cyst wall is approximately 3 μ thick and differentiated into two major zones: an outer, electron opaque marginal layer derived from the primary lamella and an inner matrix layer of a finely granular consistency (Fig. 11). The metacercarial tegument is extended as flangelike processes often in contact with the innermost margin of the cyst (Fig. 11). The cyst cavity is filled with a flocculent material of electron density comparable to that of the inner matrix layer (Figs. 10 and 11). Growth of the cyst wall is reflected by increased thickening of the inner matrix layer, which reaches a maximum of approximately 10 μ in about 25 days postinfection. The outer marginal layer, which at higher magnifications can be resolved as a bilamellate structure (Fig. 12), remains uniform in thickness (approximately 0.2 μ) during this latter phase of cyst development. The *bulbus* tissue peripheral to the cysts typically contains an elevated number of fibroblasts and an increased density of collagen fibers (Fig. 13). These collagen fibers form no integral structural component of the cyst wall but, with the other host tissue constituents surrounding the metacercaria, are clearly adventitious investments.

Discussion

The cyst wall ultrastructure of *A. leighi* metacercariae is similar in all respects to that previously described by Lumsden (1968) for *A. chandleri*, consisting of a relatively thin, bilamellate outer marginal layer and a thick inner matrix. Both components are clearly produced by secretions arising from the tegument of the parasite. During cyst formation, the living parasite actively moves about inside the cyst. The metacercaria is coiled back on itself and such movement brings its body surface into continual contact with the inner margin of the cyst wall. This presumably facilitates the transfer and incorporation of material from the tegument into the developing cyst.

Host tissue alteration during and after encystment is minimal, consisting primarily of some fibroblastic infiltration of the *bulbus* tissue proximal to the metacercarial cysts. This moderate focal fibrosis is most probably induced by mechanical pressure from the growing cysts. The collagen does not form an organized component of the cyst wall, but remains in a loose meshwork peripheral to the cyst proper. This would seem to differ somewhat from the more integral structural relationship of host connective tissue to the cysts of certain strigeoid and plagiorchioid metacercariae reported by Bogitsh (1962) and Macy (1934), respectively. No leukocytic infiltration or other cytological manifestations of an inflammatory or immune response were evident at

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Figures 9–10. 9. Cyst approximately 1 week old, bordered at left by host connective tissue (Fb, fibroblasts). Limits of the cyst wall, composed at this stage of a dense primary lamella and feltlike inner matrix layer, are indicated by the bracket. Note dense granules within the inner matrix layer of the cyst and metacercarial tegument (arrows). P, nucleus of a metacercarial parenchymal cell body. Sp, body spine. × 22,000. 10. Metacercarial tegument at higher magnification, illustrating the granules (arrow) and flocculent material (*) filling the cyst cavity. Sp, portion of a body spine. P, cytoplasmic process from tegumentary cell body. × 37,000.

Figures 11–12. Cyst wall 16 days postexposure of fish to cercariae. 11. Host connective tissue (H) surrounds bilayered metacercarial cyst composed of a dense outer marginal layer (OML) and thick inner matrix layer. Note association of metacercarial tegument (*) with inner margin of the cyst wall. × 30,000. 12. Higher magnification image of the cyst wall, illustrating bilaminate substructure of the outer marginal layer. ER, cisterna of granular endoplasmic reticulum within cytoplasm of adjacent host fibroblast. × 96,000.

Figure 13. Portions of two metacercarial cysts 35 days postinfection, and adjacent host connective tissue (Co), collagen fibers. × 14,000.
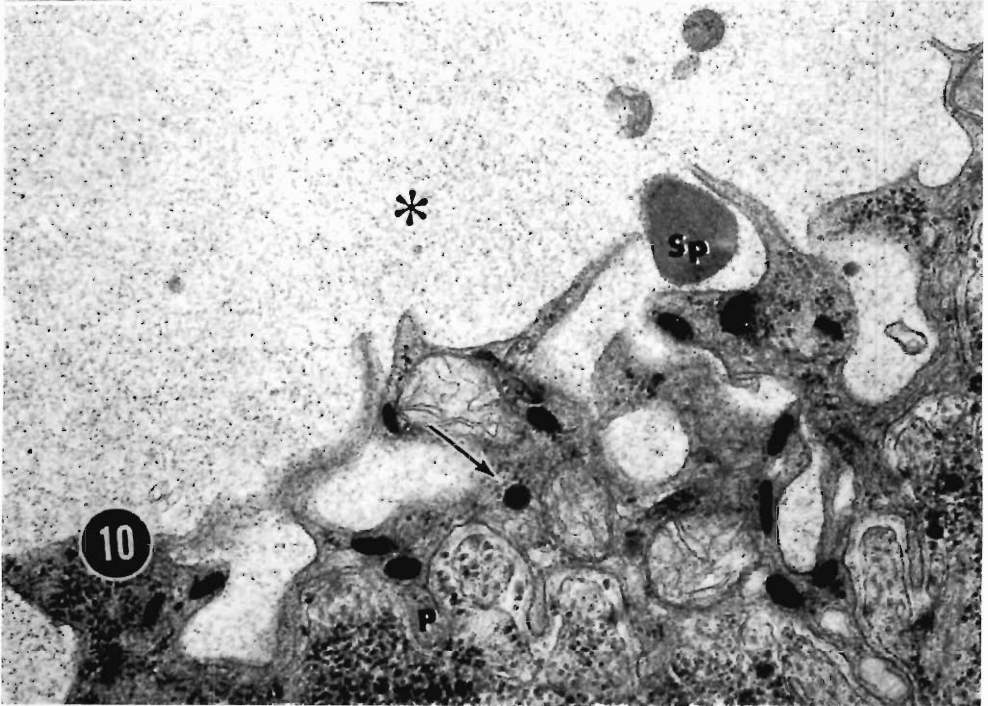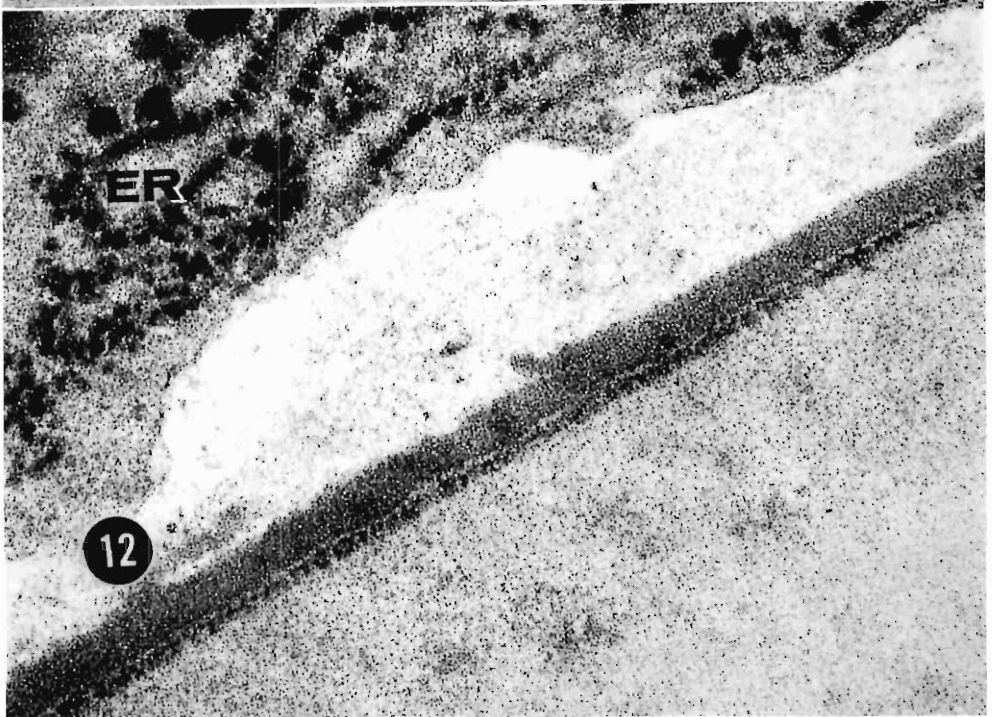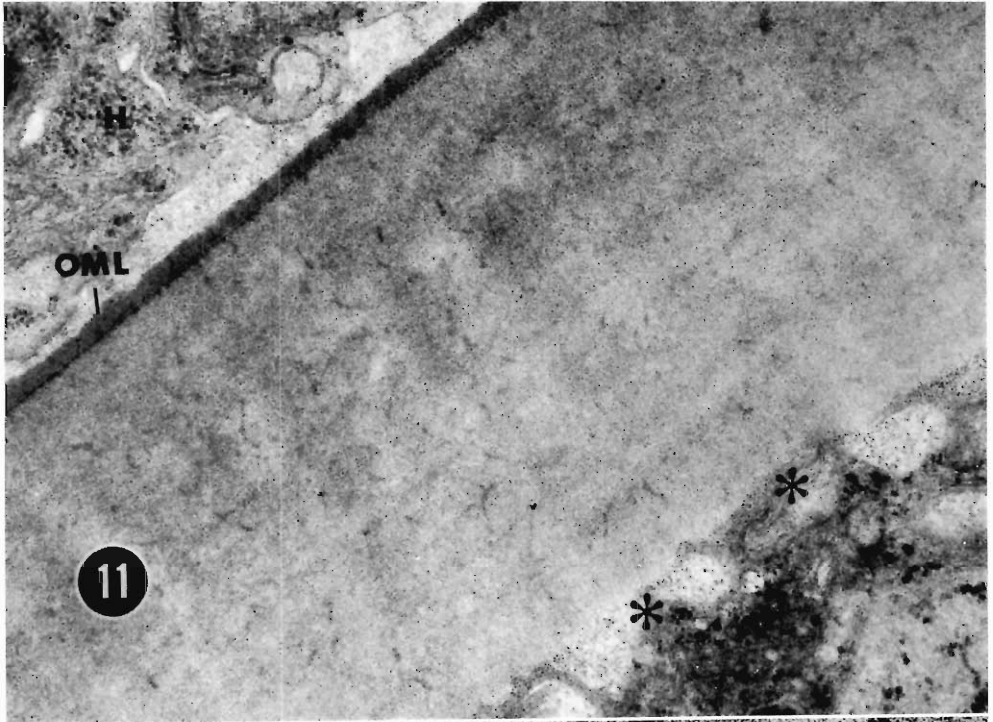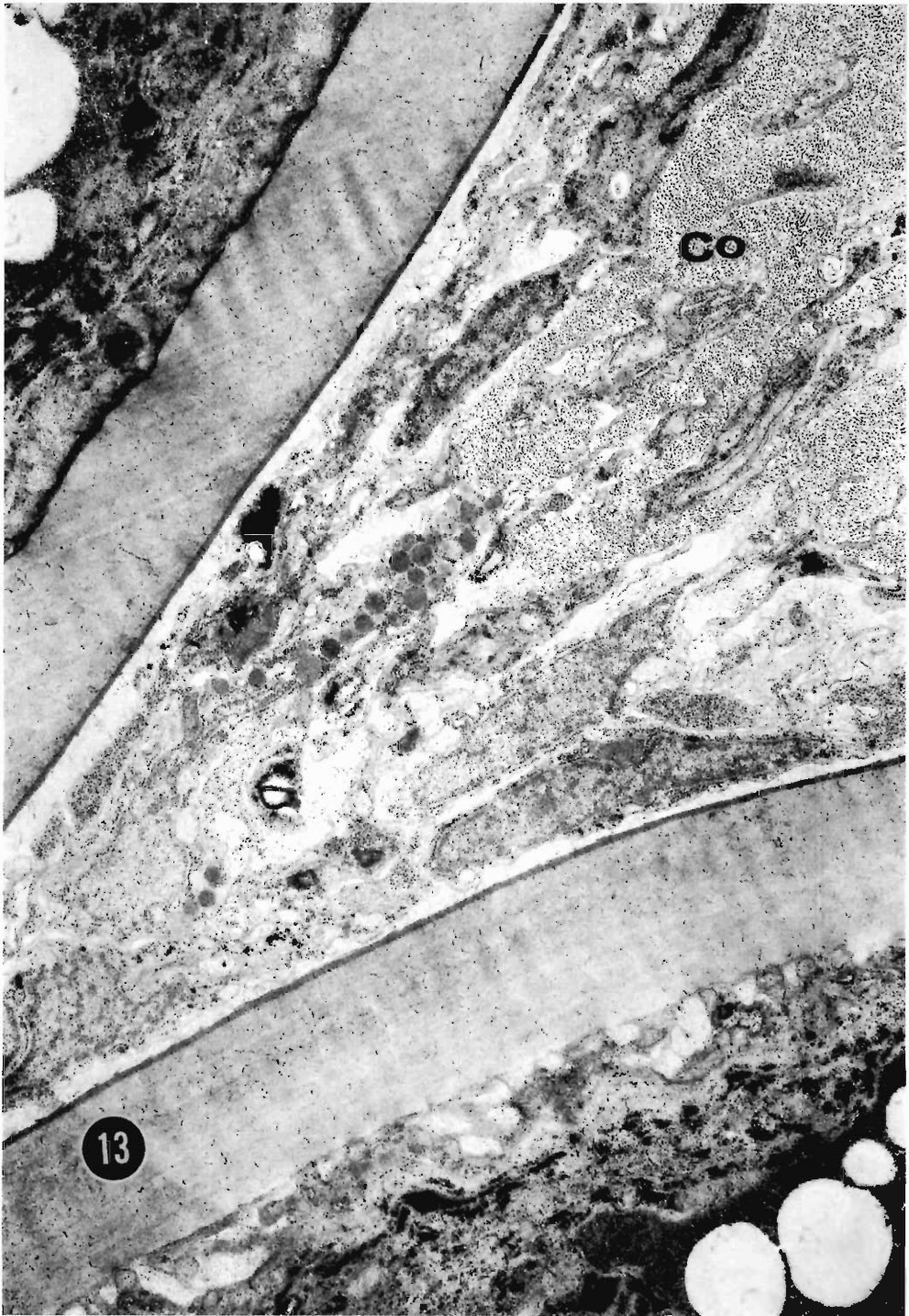
any time following entry of *A. leighi* metacercariae into the *bulbus*. It would seem that the primary lamella, which forms almost immediately after the cercaria penetrates the *bulbus* endothelium, effectively partitions the parasite from the surrounding host tissue, precluding stimulation of an inflammatory-immunological response. A similar observation has been made by Bridgman (1969) in connection with his studies on the development of *Carneophallus* metacercariae in shrimp.

In heavy infections, distortion and partial occlusion of the lumen of the *bulbus arteriosus* is sufficient to interfere with the flow of blood through the heart. In such cases, there is evidence of recanalization (Sogandares-Bernal and Lumsden, 1964), accomplished by infiltration of endothelial cells around the cysts forming secondary passageways for cardiac blood circulation (this paper, Fig. 4). The apparent proliferation of endothelial cells associated with the metacercarial cysts may also have significance relative to the connective tissue response. Observations by Branwood (1963) on vascular endothelial cells in vitro suggest that such cells, under the appropriate conditions, may be capable of dedifferentiation to the primordial cell type from which fibroblasts develop.

Naturally infected *P. latipinna* seldom possess more than 100 *A. leighi* metacercariae (Sogandares-Bernal and Lumsden, unpublished observations; Schroeder and Leigh, 1965). This would not seem to be the result of significantly increased host resistance with repeated exposure to *A. leighi* cercariae, since it is possible to reinfect parasitized fishes in the laboratory and we have frequently observed all stages of metacercarial development in the heavier natural infections. Sogandares-Bernal and Lumsden (1964) suggested that survival in nature of the more heavily infected fishes might be reduced under conditions of physiological stress imposed by parasite-impaired cardiovascular capacity. We and Schroeder and Leigh (1965) have further noted that laboratory exposure of *P. latipinna* to 100 or more *A. leighi* cercariae at one time usually results in death of the fish within 30 min. This is most likely due to mechanical damage to the gills and heart tissues produced by the simultaneous penetration of so many parasites.

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Trypanosoma duttoni: Cell Populations and Antibody Formation in Pantothenate-deficient Mice

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ABSTRACT: A metabolic imbalance technic employing the pantothenate-deficient mouse and *Trypanosoma duttoni* was used to measure trypomastigote populations and antibodies against this cell. Irrespective of diet, trypomastigotes (trypanosomes) appeared in peripheral tail blood of all inoculated mice after 6-day incubation periods. Pantothenate-deficient mice had parasitemias about 7 times greater than animals fed a complete diet and 6 times pair-fed controls. Parasitemias lasted longer in vitamin-deficient hosts, and reached a maximum several days later than those from control hosts. The action of the antibody which inhibits reproduction of the trypomastigotes was delayed five days in vitamin-deficient mice; in pair-fed animals ablastic action occurred slightly earlier than in normal control animals. The action of the terminal lytic antibody was delayed a third longer in metabolically deficient mice than in normal controls, and about a fifth longer in pair-fed controls.

An increased growth of mice infected with *Trypanosoma duttoni* (Lincicome and Shepperson, 1961, 1963) stimulated further interest in the molecular relationships between this dependent cell and its sheltering host. The metabolic imbalance technic (Lincicome, 1953) employed in previous studies with the related cell, *T. lewisi* (Lincicome, Rossan, and Jones, 1960, 1963; Lincicome and Shepperson, 1961, 1963, 1965), was applied in the present study to an experimental model composed of *T. duttoni* and pantothenic acid-deficient mice to determine: 1) lag phase, height and duration of trypomastigote populations in pantothenate-deficient hosts; and 2) the occurrence of reproductive-inhibiting and terminal lytic antibodies directed against the trypomastigote in pantothenate-deficient mice.

Materials and Methods

EXPERIMENTAL HOSTS. Table 1 shows the distribution, numbers, and initial body weights of the female Swiss albino mice studied. All mice were housed individually in suspended, wire-bottomed cages. They were fed appropriate diets from metal cups designed to minimize spillage of food.

All mice in control-diet groups and the pantothenate-deficient diet groups were provided food continuously. Those in pair-fed groups (caloric controls) were given control diets in amounts equal to that consumed by their pantothenate-deficient paired mates. The daily food intake of every mouse was deter-mined by subtracting the weight of food remaining in the tared feeding cup.

Water was available to all mice at all times. Water bottles and feeding cups were cleaned daily and cages were steamed frequently to minimize algal and bacterial contaminations.

EXPERIMENTAL DIETS. Complete (control) and pantothenate-deficient (experimental) diets were prepared and purchased commercially (Nutritional Biochemicals, Cleveland, Ohio). Both were provided as indicated above.

EXPERIMENTAL INFECTIONS. Physiologic saline (0.9% NaCl) suspensions of trypomastigotes containing 100 cells/ml were prepared (Lincicome and Watkins, 1963) for initiating infections in mice. Fifteen days after the start of experimental feeding each mouse in one-half of each dietary group in all experiments was inoculated intraperitoneally (IP) with 1 ml of the saline suspension of trypomastigotes. All other mice (control, uninfected) were each inoculated IP with 1 ml of physiologic saline.

The numbers of trypomastigotes per unit volume of blood or physiologic saline suspension were estimated by standard technics using the hemacytometer, red blood cell pipets, Toisson's fluid as diluent and a common dilution factor of 200.

POPULATIONS AND REPRODUCTIVE DEVELOPMENT OF *Trypanosoma duttoni*. Cell populations in peripheral circulating blood of mice and reproductive development of trypomastigotes were studied in hosts fed control and experimental diets in three separate experiments.

Table 1. Distribution, average initial body weights in grams ± standard deviation, and numbers (in parentheses) of mice in each of three experiments.

| Dietary group | Experiment number | | | Total no. mice |
	1	2	3	
Control				
Noninfected	9.0 ± 0.1 (3)	9.0 ± 0.2 (3)	9.0 ± 0.1 (3)	9
Infected	9.0 ± 0.1 (3)	9.0 ± 0.2 (3)	9.0 ± 0.1 (3)	9
Pantothenate def.				
Noninfected	8.8 ± 0.3 (3)	9.0 ± 0.1 (3)	9.0 ± 0.1 (3)	9
Infected	8.8 ± 0.3 (3)	9.0 ± 0.1 (3)	9.0 ± 0.1 (3)	9
Pair-fed				
Noninfected	8.9 ± 0.1 (3)	9.0 ± 0.2 (3)	9.0 ± 0.3 (3)	9
Infected	8.9 ± 0.1 (3)	9.0 ± 0.2 (3)	9.0 ± 0.3 (3)	9
Total no. mice	18	18	18	54

Beginning the day after mice were inoculated, wet films of tail bloods were prepared daily to determine the time of subsequent appearance of trypomastigotes in peripheral circulation. Subsequently the parasitemias were measured by estimation of the density of trypomastigotes by duplicate hemacytometer counts (Lincicome and Hill, 1965).

Numbers of trypomastigotes, their length and coefficient of variability in length were used as parameters for judging reproductive development by the technic of Taliaferro and Taliaferro (1922).

EXPERIMENT PROTOCOLS. The general organization of each of the three separate studies forming the basis of this report is shown in Table 1. Each of the 3 major dietary groups was represented in every experiment by control groups of mice with trypomastigote infections.

The number of mice used was limited to three in each control or experimental group because all the daily hemacytometer estimations of populations, size and coefficient of variability in size of trypanosomes were done by the same person at a particular time each day.

STATISTICAL TREATMENT. Whenever possible or appropriate the data were studied as averages from all experiments. Standard deviations were calculated for all basic observations.

Results

TRYPOMASTIGOTE CELL POPULATIONS. Table 2 presents the daily data for the second experiment as representative of all three. Irrespective of diet, trypomastigotes appeared in peripheral tail blood of all inoculated mice after a 6-day incubation period (lag phase).

Peak parasitemias occurred on Day 13 in mice fed the complete and pair-fed control diets and on Day 17 in pantothenate-deficient mice (Table 2). The two control groups appeared to be comparable, thus showing that simple caloric restriction did not wholly account for the marked elevation and lengthening of the parasitemia associated with pantothenate deficiency.

Table 2. Daily average hemacytometer counts (± S.D.) of trypomastigotes in peripheral tail blood of mice infected with *Trypanosoma duttoni* and fed complete, calorically-restricted (pair-fed) and pantothenate-deficient diets.

| Day after inoc. | In mice | | |
	Fed complete diet	As pair-fed controls	Fed pantothenate-deficient diet
7	+	+	+
8	2 ± 1	3 ± 1	5 ± 1
9	5 ± 2	7 ± 2	11 ± 3
10	7 ± 3	10 ± 2	17 ± 5
11	11 ± 3	16 ± 4	24 ± 4
12	16 ± 5	18 ± 3	35 ± 9
13	19 ± 4	26 ± 5	62 ± 11
14	12 ± 5	17 ± 7	81 ± 15
15	8 ± 2	14 ± 4	94 ± 17
16	6 ± 2	10 ± 2	112 ± 12
17	5 ± 3	8 ± 1	131 ± 14
18	3 ± 1	7 ± 2	91 ± 10
19	+	5 ± 2	76 ± 15
20	+	2 ± 1	61 ± 8
21	+	+	45 ± 5
22	+	+	29 ± 12
23	—	+	25 ± 5
24		+	18 ± 6
25		—	10 ± 3
26			7 ± 2
27			+
28			+

+ = Trypomastigotes present but not countable in hemacytometer.
— = No trypomastigotes seen.

Table 3. Maximal populations of *Trypanosoma duttoni* in well-fed, calorically restricted (pair-fed) and pantothenate-deficient mice. Data expressed as hemacytometer counts of trypomastigotes from peripheral tail blood ± S.D.

	Mice		
Exp.	Well-fed	Pair-fed	Pantothenate-deficient
1	20 ± 4	19 ± 3	126 ± 12
2	19 ± 4	26 ± 5	131 ± 14
3	14 ± 2	23 ± 6	120 ± 22
X̄	18 ± 3	23 ± 5	126 ± 16

Pantothenate-deficient mice showed the greatest numbers of parasites. On the average the parasitemias were about 7 times greater than those in animals fed a complete diet and 6 times those in pair-fed controls (Table 3). The two control groups were therefore essentially equal or comparable with respect to maximal levels.

TRYPOMASTIGOTE CELL SIZE AND ANTIBODY FORMATION. These parameters were studied in three experiments; results of but one of these are presented in Table 4 as representative of all three. Irrespective of diet, trypomastigotes were not seen in peripheral blood prior to the seventh day.

The maximal size of trypomastigotes from both control and pair-fed animals was slightly greater than that of those coming from pantothenate-deficient mice. The average length of trypomastigotes from control and pair-fed hosts was slightly greater than that of trypomastigotes from vitamin-deficient mice. Standard deviations indicated that differences could not be significant.

Delay in formation of ablastic antibody was indicated by continued high variability in trypomastigote cell size in pantothenate-deficient mice. On the average, higher coefficients of variability in cell length continued five days longer in vitamin-deficient mice than in control animals and a full week longer in pair-fed hosts.

Since parasitemias were patent longer in vitamin-deficient mice (Table 2) and the peak populations were found 4 days later in these hosts than in control animals, there was delay in action, if not synthesis, of the lytic antibody against these cells.

Discussion

The data on the degree and duration of parasitemia showed that pantothenate-deficient mice were significantly more susceptible to *T. duttoni* infection than were normal animals. Inanition comparable to that sustained by the deficient mice as in pair-fed normal controls

Table 4. Mean length (μ ± S.D. and average coefficients of variation (C.V.) in percent for total length of trypomastigotes during the course of infection in mice fed complete (well-fed), calorically-restricted (pair-fed), and pantothenate-deficient diets.

	Mice					
Day after inoc.	Well-fed		Pair-fed		Pantothenate-deficient	
	Length	C.V.	Length	C.V.	Length	C.V.
7	24 ± 7	28	20 ± 5	25	22 ± 5	20
8	27 ± 6	23	25 ± 3	13	24 ± 4	18
9	25 ± 5	21	27 ± 5	19	26 ± 5	20
10	28 ± 7	26	26 ± 5	19	25 ± 6	22
11	30 ± 5	17	24 ± 3	13	28 ± 5	19
12	31 ± 4	14	28 ± 1	5	21 ± 5	24
13	33 ± 3	9	30 ± 2	7	25 ± 7	27
14	28 ± 2	6	27 ± 2	7	27 ± 4	16
15	32 ± 2	6	29 ± 2	6	29 ± 6	21
16	29 ± 2	7	30 ± 2	6	25 ± 5	18
17	28 ± 2	6	31 ± 2	5	27 ± 3	12
18	30 ± 1	5	33 ± 2	6	29 ± 2	7
19	31 ± 1	4	30 ± 2	8	31 ± 2	7
20			28 ± 1	4	30 ± 2	5
			30 ± 2	7	29 ± 2	6
			32 ± 3	8	30 ± 2	7
			31 ± 2	7	30 ± 1	5
					27 ± 1	5
					32 ± 3	8
					28 ± 1	5
					29 ± 2	6
X̄	29 ± 4		28 ± 3		27 ± 3	

was shown not to influence significantly the severity of the trypanosome infection.

Pantothenic acid has a significant role in host responses. Becker, Taylor, and Fuhrmeister (1947) reported that pantothenic acid deficiency increased the severity of *Trypanosoma lewisi* infection in rats. Ray and Harbans (1948) observed that supplementing of a diet with pantothenic acid caused a *T. evansi* infection in rats to develop more slowly. Zucker and Zucker (1954) showed that a species of *Corynebacterium* (known to be pathogenic only for mice) produced disease in pantothenic acid deficient rats but not in rats fed a normal diet. On the other hand, pantothenic acid deficient rats were found to be more resistant than normal rats to type I pneumococcus infection (West, Rivera, and Tisdale, 1944). Along the same line, Lichstein et al. (1944) reported that pantothenic acid-deficient mice were more resistant than normal mice to Theiler's encephalomyelitis virus.

The effect of pantothenic acid deficiency on host antibody synthesis obviously accounts in part for the abnormally enhanced parasitemias. During the course of a normal *T. duttoni* infection, the mouse exhibits manifestations of resistance against the parasite involving the production of antibodies (Taliaferro, 1938). These are directed against trypomastigote reproduction and survival. The inhibition of reproduction is associated with a passively transferable antibody, termed ablastin, having the specific property of inhibiting reproduction of the organisms. The termination of the infection is assisted by trypanocidal antibodies showing all the classical characteristics of antibodies in general (Taliaferro, 1938).

The data on trypomastigote reproduction indicated that there was a delay in antibody action in pantothenic acid deficient hosts. Coefficients of variation for length as well as cell populations in deficient mice indicated prolonged multiplicative phases probably reflecting impaired action (or synthesis) of ablastin. The lengthening of the parasitemia chiefly in deficient and to a lesser extent in pair-fed control mice showed delayed production of the terminal trypanocidal antibodies.

Pantothenate deficiency has been associated with increased parasite populations and prolongation of the reproductive phase of *T. lewisi* (Becker et al., 1943, 1947). Similar data were reported by Becker and Gallagher (1947), Saul and Becker (1949), and Barnes (1951).

Becker and Gallagher (1947) suggested that ablastin might be an oxidative enzyme for which pantothenic acid serves as a coenzyme. Experiments with sodium salicylate and ablastic plasma (Lysenko, 1951) indicated the drug affected ablastic activity in a manner other than combining with the antibody. Meyers and Lysenko (1953) suggested an adrenocorticotropic hormone-like effect.

There is considerable basis for linking pyridoxine and pantothenic acid to the synthesis of antibodies (Axelrod et al., 1947; Axelrod and Pruzansky, 1955). According to Ludovici et al. (1951) beta alanine cannot replace pantothenic acid in the process of antibody synthesis although *l*-methionine has a sparing action. Ludovici and Axelrod (1951) found panthenol was as effective as pantothenic acid in promoting antibody synthesis in pantothenate-deficient rats. Wertman and Sarandria (1951) observed a low production of circulating antibodies in pantothenate-deficient rats immunized with murine typhus rickettsiae. Antibody production to influenza virus PR-8 was markedly impaired in pantothenate and pyridoxine-deficient rats (Axelrod and Hopper, 1960).

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Anchobelondira clavicauda gen. n., sp. n. (Nematoda, Belondiridae) from South Africa

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ABSTRACT: *Anchobelondira clavicauda* gen. n., sp. n. is characterized by its esophageal structure, female gonads, female tail and number of male supplements. It shares characters with both the *Axonchium-* and *Belondira*-groups of genera. This, together with the results from observations on the genus *Axonchium*, led us to the conclusion that the genera previously grouped under Belondirinae and Axonchiinae by Thorne and Belondiridae and Axonchiidae by Siddiqi should be contained in one subfamily, viz. Belondirinae (Thorne, 1939).

Among the slides of belondirid nematodes from South Africa obtained from Dr. J. Heyns, four females and six males were found belonging to a new genus. They are described below as *Anchobelondira clavicauda* gen. n., sp. n. The specimens were killed by gentle heating, fixed with F.A.A. and mounted in glycerine.

Anchobelondira gen. n.

Belondiridae. Body almost straight to slightly arcuate after fixation. Lip region narrow and amalgamated, without sclerotized framework. Esophagus consisting of a slender anterior and an enlarged posterior part, separated by an isthmus-like portion. The slender anterior part forms a pyriform expansion at its posterior end. The enlarged posterior half is surrounded by a sheath of sinistral spiral muscle bands. Cardia almost round to oval in shape. Female gonads paired and reflexed. Female tail clavate, with inner cuticle considerably expanded. Males with an adanal pair and 9–13 ventromedian supplements, well-developed ventrosublateral pores and bluntly-rounded tail.

The generic name *Anchobelondira* is derived from *ancho*, meaning constriction, (referring to the esophageal constriction) and *Belondira* which it resembles in many respects.

TYPE AND ONLY SPECIES: *A. clavicauda* sp. n.

Anchobelondira clavicauda gen. n., sp. n.
(Figs. 1–2)

HOLOTYPE FEMALE: L = 2.01 mm; a = 53; b = 5.6; c = 64; V = $^{10}39^{12}$.

For other measurements see Table 1.

FEMALES: Cuticle 3 μ thick near head, slightly less thick at midbody and up to 11 μ in tail region. Transverse striations more prominent and more widely spaced near both extremities, i.e. four annules occupying 3 μ at midbody, but 4 μ near extremities. Inner cuticle considerably thickened in tail region. Body-width greatest at neck-base, gradually diminishing anteriorly to lip region and posteriorly to about one anal-body-width anterior to anus, from where body widens to form typical clavate tail. Lateral chord about 13 μ wide at mid-body. Lateral organs indistinguishable; lateral pores well-visible in only one female, numbering 27 and 30 on right and left side respectively (5–6 in neck region, 20–23 in body region and 2 in caudal region).

Head barely offset from the body (Figs. 1A, 1B). Width of narrow, amalgamated lip region less than one-fifth that of neck base. Lip papillae protruding only very slightly. Amphids are deep pouches occupying more than three-fourth of lip region width (Figs. 1D, 1E); sensillae situated well behind the odontophore. Guiding ring about one lip region width from anterior end of body. Odontostyle conical; its aperture occupying slightly more than one-third of its length. Slender, anterior part of esophagus posteriorly forming a 7–11 μ wide pyriform expansion (Figs. 1A, 2A, 2B). Posterior enlarged part of esophagus occupying 47–52% of entire neck region, tapering anteriorly to a 4–7 μ long isthmus-like structure (Figs. 1A, 2A). Dorsal gland opening into esophageal lumen at 19–23 μ behind pyriform expansion; its nucleus is always situated in front of the aperture. The loose sheath of the esophagus consists of sinistral spiral muscle bands, varying in the specimen sectioned from 6 just behind the isthmus (Fig. 2C) to 10 in the middle (Fig. 2E) and 8 near the posterior

Table 1. Dimensions of *Anchobelondira clavicauda* gen. n., sp. n.

Locality	Holotype ♀	East London Paratype ♀♀ (n = 3)	East London Paratype ♂♂ (n = 5)	Mpisi Paratype ♂
L (mm)	2.01	1.95–2.01	1.83–2.05	1.79
a	53	45–49	50–55	62
b	5.6	5.6–5.9	5.1–5.9	5.6
c	64	69–73	85–98	73
V or T	38	37–39	42–70	46
Body-width (μ)	38	41–45	36–38	29
L.r.w. = Lip region-width (μ)	8	7–8	7–8	7
Width at neckbase/L.r.w.	5.4	5.6–6.0	5.5–6.2	4.5
L.r.w./lip region height	1.9	1.6–1.9	1.7–1.8	1.4
Amphid-width (μ)	—	7 (n = 1)	—	6
Sensillae . . . μ behind amphid aperture	22	21–23	20–25	24
Odontostyle-length (μ)	8	7–8	7–8	7
Odontophore-length (μ)	13	12–13	11–14	12
Guiding ring from anterior end (μ)	6	6–7	7–8	7
Esophagus—total length (μ)	360	332–360	328–387	319
—length anterior part (μ)	175	168–172	182–195	178
—length posterior part (μ)	185	164–188	156–192	137
Outlet dorsal gland behind anterior esophagus (μ)	23	22–23	20–27	19
Prerectum (pr.)-length (μ)	188	130–178	229–289	236
Pr./anal-body-width	6	4.5–5.9	9.4–11.3	11.0
Rectum/anal-body-width	0.84	0.86–0.92	0.30–0.35	0.44
Tail-length (μ)	31	27–29	20–23	25
Tail-length/anal-body-width	1.0	0.91–1.0	0.82–0.91	1.1
Nerve ring from anterior end (μ)	123	120–123	122–134	116
Hemizonid from anterior end (μ)	118	113–116	114–123	111
G_1 (%)	10.1	9.1–12.2	—	—
G_2 (%)	12.2	12.5–19.2	—	—
Spicules (μ)	—	—	37–43	41
Number of midventral supplements	—	—	9–13	9
Number of copulatory muscles	—	—	23–25	22
Sperm length (μ)	—	—	8–10	8

end. A small disc-like structure usually distinct at esophago-intestinal junction. Cardia round to oval (Figs. 1A, 2D) 8–11 μ long and 12–14 μ wide. Prerectum 4–6 times as long as body-width at anus; rectum less than one anal-body-width long. Anus a 4 μ wide, transverse slit.

Vulva a 12 μ wide transverse slit (Fig. 2G). Vaginal lumen gradually becoming cross-shaped (Figs. 2H, 2I). Vaginal cuticularization distinct, with the pattern shown in Figs. 2H, 2I. Sphincter restricted to a small area. Both anterior and posterior gonads (Fig. 1I) consisting of a long outstretched uterus, sphincter, oviduct and reflexed ovary. Uteri almost always filled with elongate-ovoid (Figs. 1I, 2M) spermatozoa. In all females examined, the anterior ovary is considerably smaller than the posterior. None of the specimens have eggs or fully-developed oocytes. One female has a more matured posterior ovary, with an ovarial sac containing a 52 μ long oocyte and a 129 μ long granular mass that probably represents a single large oocyte.

Tail clavate (Fig. 2K) with two caudal pores on each side.

MALES: Similar to females in most of the structures. Body slightly arcuate (Fig. 1H).

On each side of the body there are 22–25 lateral pores (7 in neck region, 12–15 in body region and 3 in caudal region); pores mostly situated along dorsal side of lateral chord. In addition to these, 5–7 prominent ventrosublateral pores are on each side of body (Fig. 1G); all but one occur in the region of the supplements, the last one is usually postanal, rarely adanal.

In the paratype from Bizana, the cardia is narrower than usual. Prerectum 9–11 times as long as the anal-body-width. Supplements consisting of an adanal pair and 9–13 ventromedian (Fig. 1G), the last one more than one anal-body-width in front of the adanal pair. Copulatory muscles varying from 22 to 25 in number. Spicules arcuate (Figs. 2J, 2L), 37–43 μ long, when measured along the curved median line. Sclerotized lateral guiding pieces absent. Tail dorsally convex-conoid to almost cylindrical with broadly-rounded terminus.

TYPE LOCALITY AND HABITAT: Four females and five males from uncultivated virgin clay soil near a stream bank on the farm 'Silverdale' near East London, Eastern Cape Province. Collected by Messrs. Prinsloo and Erasmus on 9 June 1964.
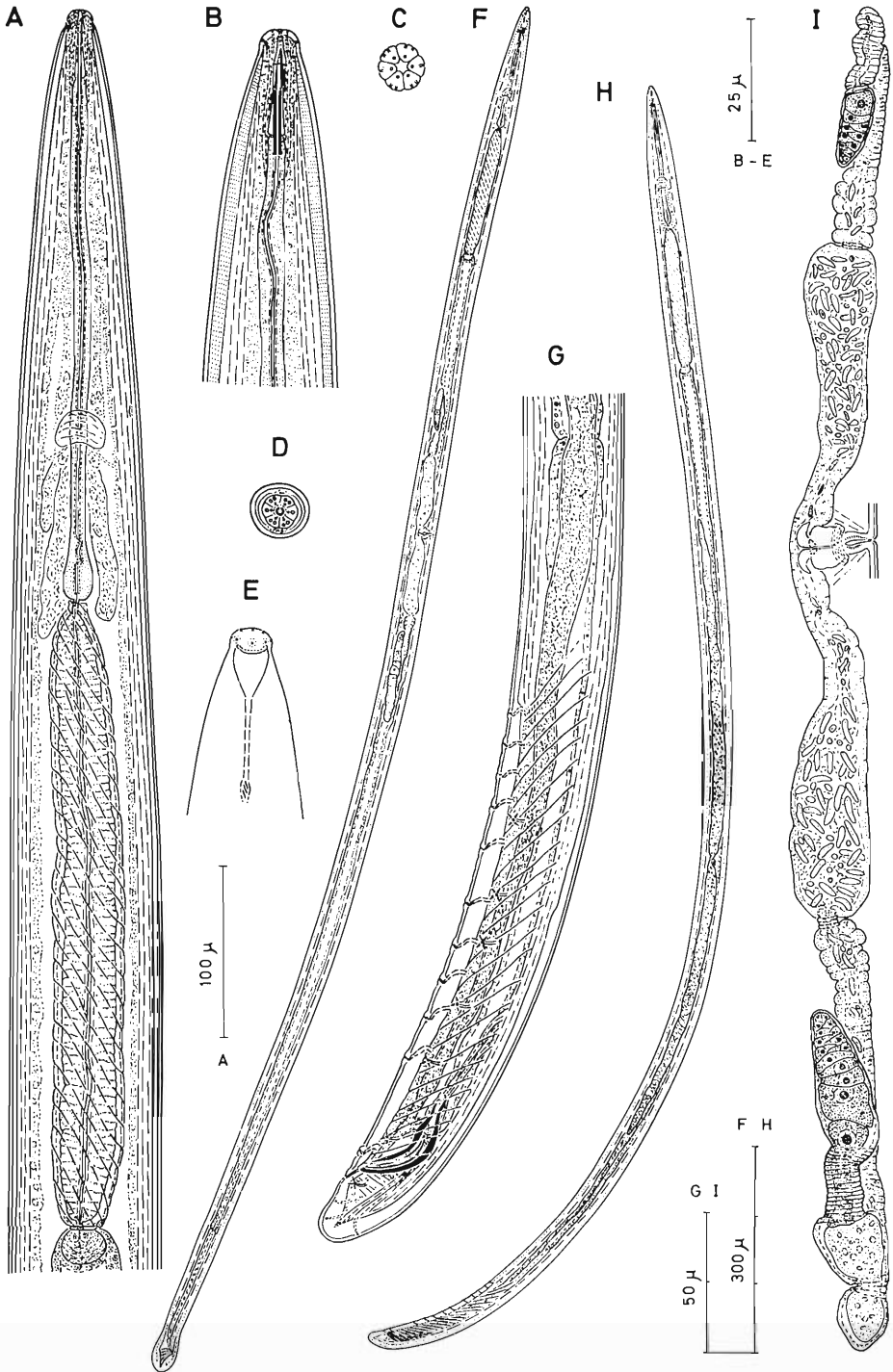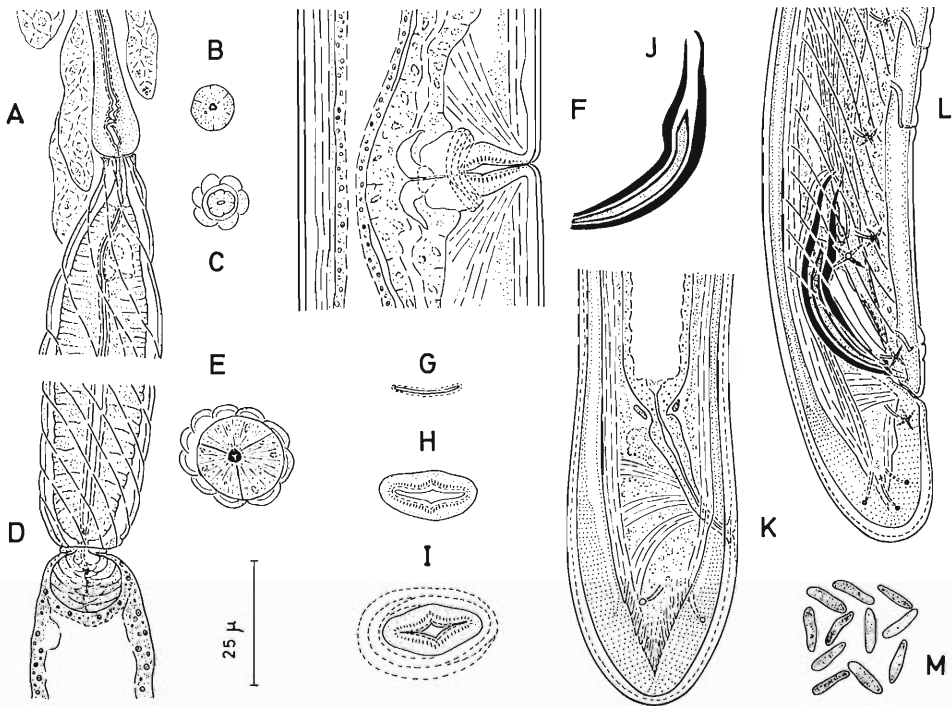
Figure 2. *Anchobelondira clavicauda* gen. n., sp. n. A. Junction between both parts of the esophagus. B. Cross section through pyriform expansion. C. Cross section just behind isthmus. D. Region of the esophago-intestinal junction. E. Cross section through basal part of the esophagus. F. Vulva region. G. Vulva in ventral view. H–I. Optical sections through vagina. J. Spicule. K. Female tail. L. Posterior end of male. M. Sperm.

OTHER LOCALITY: One male from indigenous forest near Mpisi, Transkei. Heavy black clay soil. Collected by Mr. P. van Niekerk, October 1963.

HOLOTYPE: Slide nr. 300, collection of the Instituut voor Dierkunde, Rijksuniversiteit, Gent, Belgium.

PARATYPES: Two specimens (♂ and ♀) used for sectioning. Remaining specimens (2 ♀ ♀ and 5 ♂ ♂) distributed as follows: 1 ♀ and 2 ♂ ♂ in the collection of the Plant Protection Research Institute, Pretoria, South Africa; 1 ♀ and 2 ♂ ♂ at the Instituut voor Dierkunde, Gent, Belgium; and 1 ♂ in the USDA Nematode Collection, Beltsville, Maryland, USA.

Discussion

Anchobelondira gen. n. comes closest to *Belondira* Thorne, 1939 in the shape of the lip region, the structure of the basal enlarged part of the esophagus and the tail shape; to *Belondirella* Thorne, 1964, *Yunqueus* Thorne, 1964, *Durinema* Jairajpuri, 1966, and *Bullaenema* Sauer, 1968 in the possession of two female gonads; but it differs from all these genera in having a constriction between the anterior and posterior portions of the esophagus and in the number of male supplements.

It resembles *Axonchium* Cobb, 1920 in having the isthmus-like region between both parts

←

Figure 1. *Anchobelondira clavicauda* gen. n., sp. n. A. Esophageal region of the holotype. B. Anterior body end. C. En face view. D. Optical cross section at the level of the amphids. E. Surface view of anterior end. F. Holotype female. G. Posterior body region of male. H. Male. I. Female gonads.

of the esophagus and in the number and arrangement of male supplements. Nevertheless, the new genus differs considerably from the latter in the structure of the basal enlarged portion of the esophagus, the b-value, the presence of a pyriform expansion at the posterior end of the anterior esophagus, the possession of two female gonads, the clavate tail of the females and the absence of lateral guiding pieces in males.

In 1964, Jairajpuri divided the family Belondiridae into three subfamilies: Belondirinae, Swangerinae, and Dorylaimellinae. The first of these comprising the genera *Belondira*, *Axonchium*, and *Oxydirus* Thorne, 1939.

Thorne (1964) established the superfamily Belondiroidea with six families, among which Belondiridae comprises two subfamilies, viz. Belondirinae and Axonchiinae. Belondirinae includes the genera *Belondira*, *Yunqueus*, and *Belondirella*; it is differentiated from Axonchiinae mainly by the absence of a constriction between both parts of the esophagus and by the number of supplements in males. In 1967, Thorne added the genus *Axonchoides* Thorne, 1967 to Axonchiinae, although members of this genus do not possess a constriction between both parts of the esophagus!

Jairajpuri (1966) and Sauer (1968) added the genera *Durinema* and *Bullaenema* respectively to Belondirinae.

In his revised classification of Belondiroidea, Siddiqi (1968) included seven families. He raised Axonchiinae to family rank, with *Axonchium* as its only genus. The main diagnostic features between Belondiridae and Axonchiidae are the structure of the anterior part of the esophagus and the constriction between both parts of the esophagus. According to Siddiqi (l.c.), the anterior esophagus of Belondiridae is not offset from the posterior part, has a spindle-shaped muscular swelling anteriorly and is thin and nonmuscular throughout the rest of its length; in Axonchiidae the anterior part of the esophagus is very muscular and offset from the posterior part.

Although it resembles *Axonchium* in most other characters, Siddiqi included *Axonchoides* in the Belondiridae, because of the absence of a constriction and the structure of the anterior part of the esophagus.

During the study of the genus *Axonchium*, it became clear that the anterior part of the esophagus can have either the structure described by Siddiqi for Axonchiidae or Belondiridae, depending upon the species. Furthermore, this study revealed that males of some *Axonchium* species possess a small number (2–4) of supplements. Hence the only diagnostic character that remains differentiating the two families is the constriction between both parts of the esophagus. However, this constriction is very weakly-developed in some *Axonchium* species and is present in the new genus *Anchobelondira*, which shares many characters with Belondirinae.

In view of the above-mentioned facts, we feel that the demarcation drawn earlier between the two subfamilies (Thorne, 1964) or families (Siddiqi, 1968) is questionable. Hence, we propose that the genera *Belondira*, *Belondirella*, *Yunqueus*, *Durinema*, *Bullaenema*, *Anchobelondira*, *Axonchoides*, and *Axonchium* be included in only one taxonomic unit, viz. subfamily Belondirinae (Thorne, 1939) Jairajpuri, 1964.

Acknowledgment

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Studies on Freshwater Larval Trematodes. XXVII. Partial Life Cycle of *Caiguiria anterouteria* gen. n., sp. n., subfam. n. (Trematoda: Digenea)

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ABSTRACT: Cysts recovered from the intestinal mesenteries of a freshwater fish, *Lebistes reticulatus*, were fed to a kitten, and on the eighth day trematode eggs appeared in its feces and adult parasites were subsequently recovered from the small intestine. These flukes proved identical with specimens obtained from the intestine of a bird, *Himantopus himantopus*. The characteristic presence of the uterus in the preacetabular region necessitated the erection of a new species, *Caiguiria anterouteria*, of a new genus, *Caiguiria*, and a new subfamily, Caiguiriinae, of the family Heterophyidae.

The freshwater fish, *Lebistes reticulatus* (Peters), brought from San Juan de Macarapana, near Universidad de Oriente, harbored unidentified cysts in the intestinal mesenteries. These cysts, *in situ*, were fed to a kitten, and on the eighth day trematode eggs appeared in its feces. When dissected, three adult flukes were found in the small intestine which were identical with specimens previously recovered from the intestine of a bird, *Himantopus himantopus* (Müller), frequenting Laguna de Los Patos not far from the locality where the fish were collected. General characters of these trematodes agree with those of the family Heterophyidae Odhner, 1914, but certain features like the intestinal ceca lying anterior to the testes and the presence of uterus in the preacetabular region necessitated the creation of a new genus, *Caiguiria* (named in the honor of a Venezuelan Indian Chief), and a new subfamily Caiguiriinae. The uterus is characteristically present in the region anterior to the ventral sucker, hence the name of the species *anterouteria*. The cercarial stage is not known, but efforts to find it are in progress.

Measurements are in millimeters.

Caiguiria anterouteria sp. n.
(Figs. 1, 1a)

DEFINITIVE, EXPERIMENTAL, HOST: Domestic cat.

DEFINITIVE, NATURAL, HOST: *Himantopus himantopus* (Müller).

LOCATION: Small intestine.

LOCALITY: Laguna de Los Patos, near Universidad de Oriente, Cumaná, Venezuela.

DEPOSITED SPECIMENS: USNM Helm. Coll. No. 71427 (holotype), No. 71428 (paratype).

Description

Body spinose, almost rounded or anteroposteriorly elongated. Ventral sucker smaller than oral, not included in genital atrium. Prepharynx variable in length. Intestinal ceca extending from equatorial level of ventral sucker to anterior to testes. Testes lying posterior to midlength of hindbody, unlobed, symmetrical, anteroposteriorly or transversely elongated. Ovary unlobed, halfway between ventral sucker and testes, always in front of corresponding testis. Seminar receptacle prominent, intertesticular or lateral to ovary. Mehlis' gland diffuse. Cirrus sac absent. Seminal vesicle free in parenchyma, extending from anterolateral aspect of ventral sucker to posterior to it. Genital atrium with one gonotyl provided with longitudinal cavities simulating chitinous rods. Common genital pore lateral to ventral sucker. Vitelline glands follicular, partly cecal, extracecal, and postcecal. Uterus occupying most of space between pharynx and ovary or its anterior limits fluctuating around oral sucker, not extending posterior to testes. Excretory vesicle Y-, or T-shaped, its main stem extending to seminal receptacle; often with two additional lateral branches. Measurements based on four specimens: body 0.357–0.496 by 0.159–0.473; oral sucker 0.070–0.105 in diam.; prepharynx 0.006–0.045 long; pharynx 0.030–0.050 in
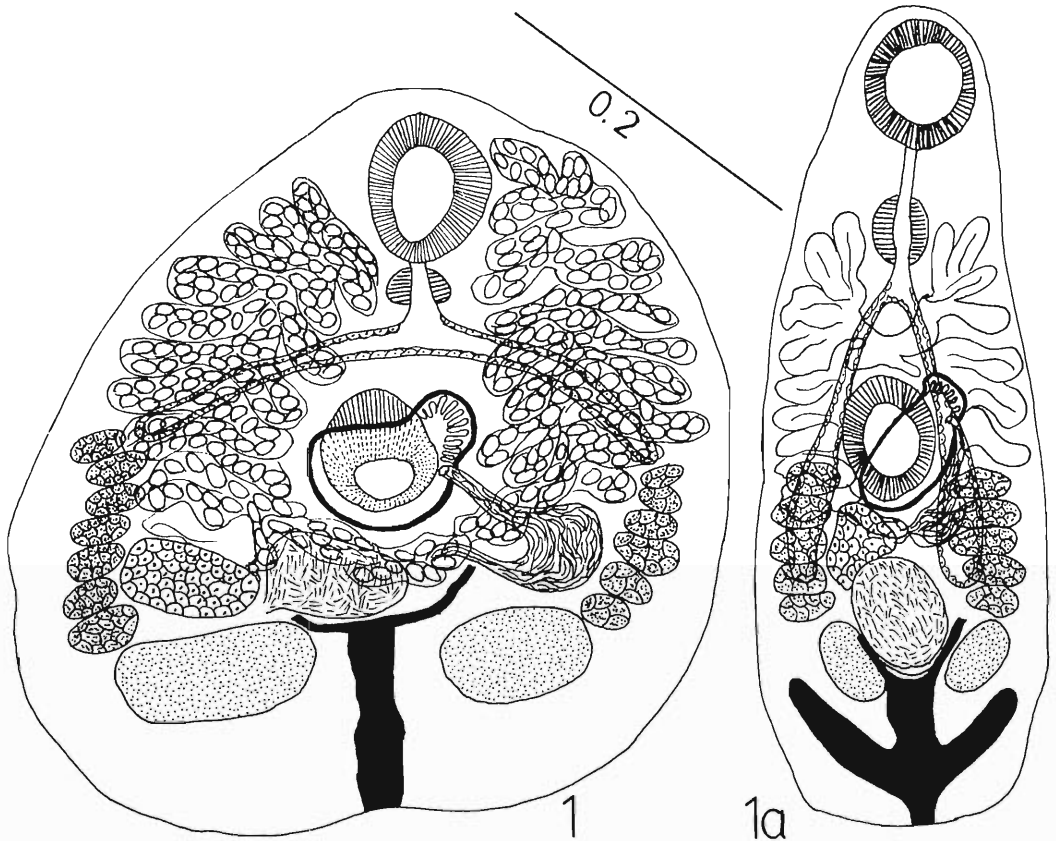
Figure 1. *Caiguiria anterouteria* sp. n., natural infections, paratype, ventral view. Figure 1a. Same species from experimental infections, holotype, ventral view; eggs not illustrated inside uterus.

diam.; esophagus 0.006–0.024 long; ventral sucker 0.060–0.076 in diam.; testes 0.030–0.056 by 0.043–0.107; ovary 0.033–0.049 by 0.042–0.076; eggs 0.012–0.025 by 0.010–0.012; gonotyl 0.025–0.030 by 0.030–0.049.

Discussion

According to the keys by Dawes (1956), Yamaguti (1958), and Skrjabin et al. (1964), the species involved in this paper fits into the family Heterophyidae excepting the intestinal extent, uterine extent and location of the testes. These characteristics demand the familial modification with the resultant introduction of a new genus and a new subfamily. In the Heterophyidae, the intestinal ceca extend to the posterior end of the body and the uterus does not occupy the region anterior to the ventral sucker. Thus, the family is emended below and the characters of the new subfamily Caiguiriinae and a new genus *Caiguiria* are given:

Heterophyidae Odhner, 1914, emend.

Body anteroposteriorly elongated or almost isodiametric. Intestinal ceca variable in length, rarely single. Ventral sucker well developed or atrophied, may or may not be included in genital atrium, latter including one or more gonotyls. Testes tandem, diagonal, symmetrical or asymmetrical, rarely single, postacetabular. Cirrus sac absent. Seminal vesicle well developed. Vitelline glands lateral, usually extending from ventral sucker to testicular region. Seminal receptacle and Laurer's canal usually present. Uterus variable in length. Excretory vesicle saccate, V-, Y-, or T-shaped, occasionally

with lateral branches from posterior region of main stem.

Caiguiriinae subfam. n.

With characters of the family. Ventral sucker not included in genital atrium. Intestinal ceca may reach equatorial line of ventral sucker or extending posterior to it as far as anterior to testes. Uterus not extending posterior to testes, with anterior limits fluctuating from oral region to esophageal bifurcation. Vitelline glands follicular, cecal, extracecal or postcecal, extending from acetabular region to anterior to testes. Testes diagonal or symmetrical. Ovary pretesticular.

Caiguiria gen. n.

Heterophyidae, Caiguiriinae. Oral sucker larger than ventral. Pharynx smaller than ventral sucker. Testes not lobed, in posterior half of postacetabular region. Ovary not lobed, may be with irregular borders, in front of corresponding testis. Uterus not extending posterior to testicular region, intruding into preacetabular zone, in which confluent occasionally. Posterior limits of vitelline glands may reach slightly posterior to anterior margin of testes. Seminal receptacle well developed, para- or postovarian. Genital atrium with a single gonotyl. Excretory vesicle Y- or T-shaped, with lateral branches from its posterior region.

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Cestrahelmins rivularis sp. n. (Digenea: Deropristiidae) from White Sturgeon, *Acipenser transmontanus*, in the Columbia River, Washington[1]

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ABSTRACT: *Cestrahelmins rivularis* sp. n. is described from the intestine of white sturgeon, *Acipenser transmontanus* Richardson, in the central Columbia River, State of Washington, USA. *C. rivularis* and *C. laruei* Fischthal, 1957 are the two existing representatives of the genus and of the subfamily Cestraheminthinae Peters, 1961 (emended from Cestrahelminae). *C. rivularis* is differentiated from *C. laruei* by the following features: 1) distinct prepharynx, 2) genital pore with prominent sphincter, 3) unswollen forebody, 4) vitellaria extending anterior to the ventral sucker, 5) metaterm tubular, not saccular, 6) body spines uniform in size and distribution, and 7) seminal receptacle overlapping only the anterior testis. This initial record of *Cestrahelmins* from sturgeon, family Acipenseridae, supports morphological studies which demonstrate close affinities between the Cestrahelminthinae and Deropristiinae, within the family Deropristiidae Skrjabin, 1958.

Light infections of an intestinal trematode belonging to the family Deropristiidae were found in 6 of 28 white sturgeon, *Acipenser transmontanus* Richardson, collected in the central Columbia River near Richland, Washington through 1969. Subsequent study revealed the organism to belong in the genus *Cestrahelmins* Fischthal, 1957, but to differ markedly from *C. laruei* Fischthal, the only known species of the genus.

1

2

1 mm
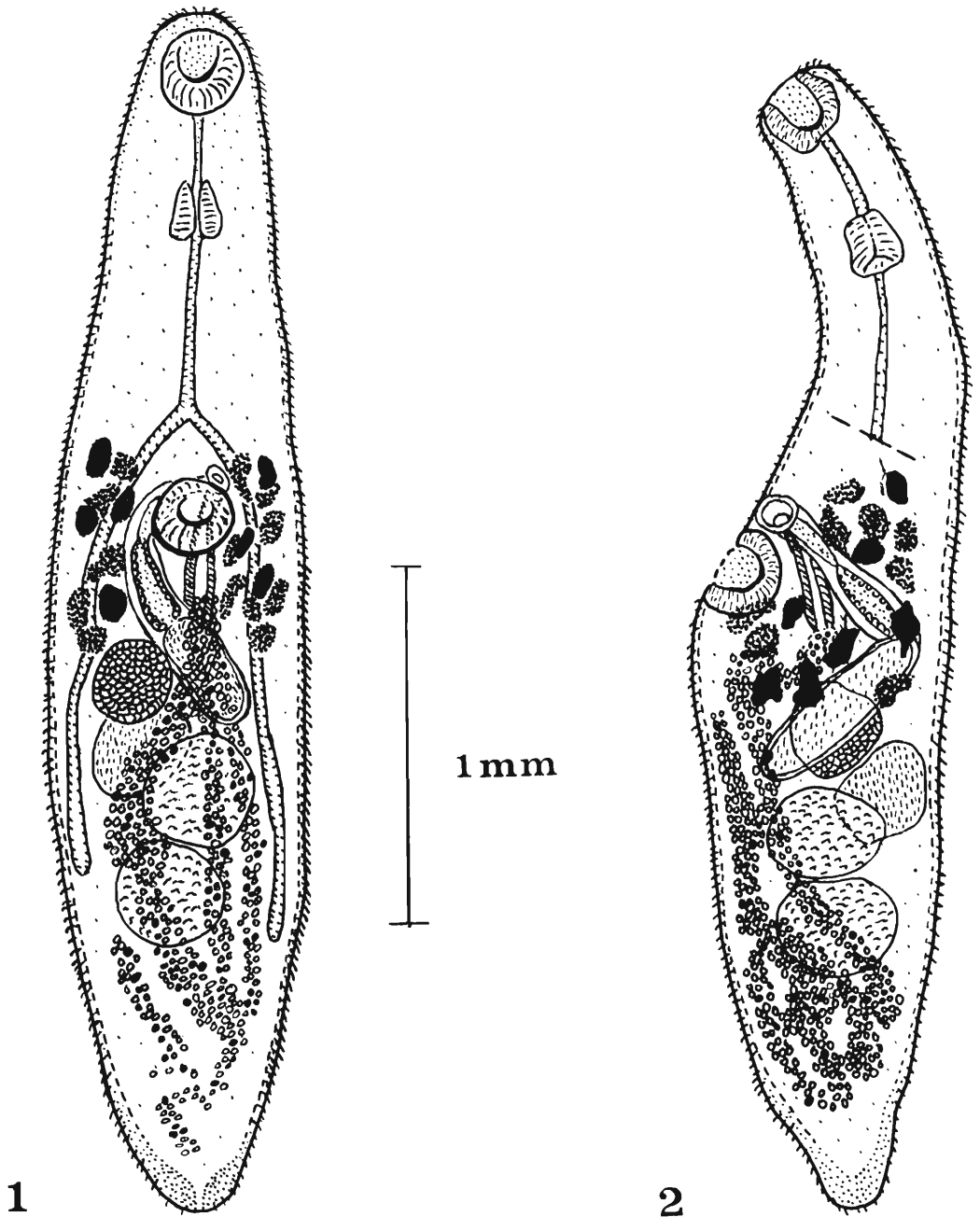
Figures 1–2. 1. *Cestrahelmins rivularis* sp. n. Holotype, ventral view. 2. Paratype, lateral view, with ceca omitted.

Materials and Methods

Recovered specimens were relaxed in distilled water, pipetted into hot AFA (Galigher's), washed in 70% ethanol, stained with Lynch's alcoholic borax carmine, counterstained with fast green, cleared in beechwood creosote, and mounted in Canada balsam under coverslips without pressure. Morphometric data are taken from six animals, ventral perspective only, and are given in millimeters except for the eggs (20 measured), which are in microns. The range of each parameter is followed by the average in parentheses. Figures are drawn to scale with aid of grid coordinates.

Cestrahelmins rivularis sp. n.
(Figs. 1, 2)

Description

With characteristics of the family Deropristiidae Skrjabin, 1958 as defined by Peters (1961), and the subfamily Cestrahelminthinae Peters, 1961. Body elongate, 2.75–3.35 (2.99), extremities tapering and rounded; widest near midbody behind ventral sucker, 0.75–0.82 (0.79). Body entirely spined except for extreme caudal end; spines minute, arranged in close transverse rows, uniform in size and distribution. Forebody 1.05–1.39 (1.25) long, unswollen anteriorly, lacking remnants of cercarial eyespots; hindbody 1.37–1.83 (1.54) long. Oral sucker subterminal, aspinose, 0.19–0.24 (0.21) by 0.17–0.24 (0.21). Ventral sucker preequatorial, spherical, relatively small, 0.20–0.22 (0.21) by 0.21–0.23 (0.22). Ratio of oral to ventral sucker 1:1.04.

Prepharynx present, about ½ length of esophagus, but relationship varying with contraction of anterior end. Pharynx pyriform, 0.15–0.17 (0.165) by 0.12–0.17 (0.15). Esophagus slender, 0.44–0.61 (0.49) long. Intestinal bifurcation near anterior margin of ventral sucker. Ceca slender, slightly greater in diameter than esophagus; extend postero laterally to level of posterior testis, terminate well in advance of posterior end. Main excretory vesicle saccate, behind testes, with terminal excretory pore. Post testicular space 0.60–0.71 (0.64) long.

Testes two, suboval and equal, postovarian, positioned obliquely in hindbody; anterior testis sinistral, 0.20–0.31 (0.27) by 0.24–0.29 (0.27); posterior testis dextral, 0.24–0.31 (0.27) by 0.24–0.32 (0.27). Cirrus sac elongate and gourd-like, 0.72–1.0 (0.91) by 0.13–0.16 (0.15); distal end left of ovary; sac ascends dextrally, then curves sinistrally around cephalic margin of ventral sucker. In lateral view, cirrus sac runs anteriodorsally, folds sharply (45°) near dorsal surface at junction of seminal vesicle and pars prostatica, and continues anteroventrally to genital atrium. Cirrus sac contains muscular, spined, eversible cirrus; elongate pars prostatica with diffuse gland cells; and saccate, undivided seminal vesicle. Cirrus joins metraterm near genital pore. Genital pore at anterior margin of ventral sucker, sinistral, surrounded by prominent sphincter. Genital atrium short, nearly nonexistent.

Ovary single, suboval, 0.18–0.27 (0.23) by 0.21–0.24 (0.22), dextral to midline, pretesticular; separated from ventral sucker by diagonally crossing cirrus sac, from anterior testis by dorsally situated seminal receptacle. Oviduct short, sinistral to ovary. Mehlis' gland ill defined. Laurer's canal not detected. Seminal receptacle suboval, relatively large, dorsal to and overlapping ovary and anterior testis. Uterus sinuous, with ascending and descending limbs; coils extend from posterior margin of ventral sucker to near posterior end of body. In lateral view, uterine coils predominantly ventral, but occupy space behind posterior testis. Metraterm tubular, muscular, originating dorsoposterior to ventral sucker, walled internally with minute, acicular spines. Eggs numerous, operculate, ovoid; 32.21–41.5 (36.95) by 15.29–24.93 (22.02). Vitellaria in restricted lateral fields 0.53–0.66 (0.61) long, originate anterior to ventral sucker, extend to ovary; follicles compact, 9–12 per field, oval, pyriform, or elliptical in outline. Left and right vitelline ducts extend from posterior vitelline fields near level of ovary.

TYPE HOST: White sturgeon, *Acipenser transmontanus* Richardson (Teleostei: Acipenseridae).

MICROHABITAT: Spiral valve, occasionally anterior intestine.

INCIDENCE AND INTENSITY: In 6 of 28 hosts, 1 to 6 parasites; 14 small fish, less than 52 cm total length, all uninfected.

TYPE LOCALITY: Central Columbia River, State of Washington, USA.

ETYMOLOGY: "*Rivularis*," L. *rivus*, dim. *rivulus*, a channel, groove, or stream; in reference to the typical river habitat of the type host.

Type specimens: USNM Helm. Coll.; holotype, No. 71430; 5 paratypes, Nos. 51431.

Discussion

Skrjabin (1958) included only the genera *Deropristis* Odhner, 1905, *Pristicola* Cable, 1952, and *Skrjabinopsolus* Ivanov in Ivanov and Murygin, 1937 (Syn. *Pristotrema* Cable, 1952) when he established the family Deropristiidae. These three genera form a natural taxonomic group, the subfamily Deropristiinae as initially proposed by Cable and Hunninen (1942) and subsequently modified by Cable (1952). Peters (1961) proposed the subfamily Cestrahelminae within the Deropristiidae to include the genus *Cestrahelmins* Fischthal, 1957, whose family relationships were previously problematical. The subfamily name should be emended to Cestrahelminthinae. (The Greek noun *helmins* issues from the basic stem *helminth*. The use of this word requires that the entire stem be adopted according to the International Code of Zoological Nomenclature.)

The type and only described species of *Cestrahelmins* is *C. laruei* Fischthal, 1957, an intestinal parasite of the muskellunge, *Esox masquinongy*, taken in Wisconsin, USA. One of the key features separating the Cestrahelminthinae from the Deropristiinae, according to Peters (1961), is the absence of a distinctly bipartite seminal vesicle. Peters, who reexamined the type specimen and sections of *C. laruei*, determined that what Fischthal (1957) considered to be the inner, thick walled part of a bipartite seminal vesicle was actually the pars prostatica. The new species, *C. rivularis*, superficially appears to have a bipartite seminal vesicle but the anterior part is actually a pars prostatica as in *C. laruei*. This feature is particularly noticeable in lateral view (Fig. 2).

C. rivularis and *C. laruei* are alike, but differ from all other deropristiids, in the following subfamily characteristics: 1) seminal vesicle not bipartite, 2) pars prostatica prominent, 3) esophagus long, 4) intestinal bifurcation near the ventral sucker, 5) ceca not reaching posterior extremity, and 6) genital pore sinistral. Moreover, the vitelline follicles are large, scanty and occur in restricted lateral fields, while the testes are oblique and situated in advance of the posterior end of the body.

C. rivularis, however, differs from *C. laruei* in the following features: 1) prepharynx distinct, 2) genital pore with prominent sphincter, 3) forebody unswollen, 4) vitellaria extending anterior to the ventral sucker, 5) metraterm tubular, not saccular, 6) body spines uniform in size and distribution, and 7) seminal receptacle overlapping only the anterior testis. In addition, morphometric feature (i.e., pharynx, testes, ovary, ventral, and oral suckers) are generally larger in *C. rivularis*, and the sucker ratios are significantly different. As far as now known, *C. rivularis* infects primitive teleosts of the family Acipenseridae while *C. laruei* parasitizes more modern fishes of the family Esocidae.

Cable (1955) noted that representatives of the genera *Deropristis*, *Pristicola*, and *Skrjabinopsolus* evidently parasitize sturgeon wherever these hosts occur (i.e., northern hemisphere). The appearance of a species of *Cestrahelmins* in a North American acipenserid supports taxonomic studies (Peters, 1961) which demonstrate close affinities between the Cestrahelminthinae and Deropristiinae.

Acknowledgment

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Effect of Management Systems on the Growth of Lambs and Development of Internal Parasitism. V. Field Trials Comparing Early Weaning Versus Late Weaning and Involving Medication with Thiabendazole and Purified Micronized Phenothiazine

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ABSTRACT: Observations were made for the fifth grazing season of the effect of management systems, including medication, on the development of internal parasitism in lambs. The systems included early weaning of lambs and grazing them on renovated pastures (Band 1), separate grazing of lambs and their dams (Band 2), and rotation of lambs with their dams until weaning and then lambs only among separate, parasite-contaminated pastures at approximately biweekly intervals (Bands 3 and 4). Micronized purified phenothiazine (avg. particle size 2–3 μ) was used as the therapeutic drug for lambs from Band 3, and thiabendazole was used for Band 4 lambs. Lambs of Bands 1, 2, and 3 had free access to a 1:9 phenothiazine-mineral mixture. Band 4 lambs had free access only to the unmedicated mineral mixture. Based on hematocrit determinations, parasite egg counts, and necropsy worm counts, early weaning of lambs and grazing on renovated pastures effectively controlled *Haemonchus contortus* and resulted in excellent weight gains. Band 1 lambs gained significantly more weight than lambs from the other bands during the entire experiment. During the treatment phase (8 July–25 September), the packed red-cell volume of Band 1 was significantly higher than that of Band 4. Lambs of Band 1 had significantly fewer *H. contortus* than lambs from Bands 2, 3, and 4, at necropsy. Although the level of *Haemonchus* infection was higher in the lambs grazed separately from their dams than in the early weaned lambs, it did not reach levels requiring therapeutic treatment. Large numbers of *Haemonchus* (2,860 to 5,625) were found in necropsied lambs from Band 4 after 8 July even though therapeutic doses of thiabendazole up to 1.5 times the recommended level were given. Therapeutic dosing with purified, micronized phenothiazine, in conjunction with the low level regimen, appeared to control haemonchosis in Band 3 until late September. On the other hand, thiabendazole was more effective than the phenothiazine regimen against parasites other than *Haemonchus*.

The level of gastrointestinal parasitism acquired by young lambs is related to the degree of exposure to nematode infective larvae, and the dams usually serve as the primary source of these larvae (Gordon and Turner, 1946; Spedding, 1954, 1955, 1956; Gibson, 1956; Levine et al., 1956, 1958, 1960; Reinecke and Thomas, 1959). Other studies have shown that the growth rate of young lambs is strongly influenced by their dams' milk supply and mothering ability, and that milk production begins to decline after about 3 weeks (Barnicoat et al., 1956). Indeed, the dams' major influence on lamb growth is accomplished by the time the lamb reaches a weight of approximately 20–25 kg (Harrington et al., 1960). It was logical, therefore, that studies on management in relation to internal parasitism would include research on early weaning (Baird and Sell, 1959; Levine et al., 1960; Spedding et al., 1961). Studies on early weaning at the Agricultural Research Center were initiated in 1963 as part of the continuing program on lamb management.

Results of previous studies (Colglazier et al., 1970) indicated that thiabendazole was less effective than purified phenothiazine for the control of *Haemonchus contortus*. It seemed desirable therefore, to obtain additional data on action of these drugs during another grazing season. This report summarizes the results obtained and presents data pertaining to the relationship of parasitism and early weaning and grazing lambs separately from their dams.

Materials and Methods

These studies were conducted during the grazing season of 1963. The general plan was similar to that followed in previous studies (Lindahl et al., 1963, 1970; Colglazier et al., 1968, 1970) and involved three basic management systems, namely: early weaning of lambs (Band 1), grazing of lambs separately from their dams (Band 2), and grazing lambs with their dams until weaning and then lambs only on contaminated pastures (Bands 3 and 4). Purified phenothiazine[5] was used as the therapeutic drug for Bands 2 and 3, and thiabendazole[6] for Band 4.

On 10 April, 308 lambs (about 2 months of age) were randomly assigned within age, breed, and sex, into four approximately equal bands. Dams of the lambs assigned to Band 1 were not included in this experiment.

Each band contained Hampshire, Merino, Targhee, Dorset, Columbia-Southdale, and crossbred lambs (3-way crosses involving Hampshire, Shropshire, and Merino breeds). All lambs were creep-fed *ad lib* with pellets containing 65% alfalfa meal, 30% barley, and 5% soybean oil meal during the pretrial period and through the test until lambs averaged 120 days of age.

Lambs of Band 1 were weaned on 10 April, when they averaged about 60 days of age. They were placed on one of 4 pastures ranging from 0.6 to 3.1 hectares and not previously grazed by sheep since being renovated in 1960; the lambs were moved among the four pastures as dictated by availability of forage. The pasture forage for Band 1 as well as for the other 3 bands was a mixture of orchard grass, bluegrass, and ladino clover. Differences in nutritive quality of forage between pastures was nil.

Lambs of Band 2 were grazed separately from their dams on the same pastures used for a similar band (Band 2) the previous year. The lambs were allowed to nurse their dams in dry lot from 4 PM to 8 AM daily until they were weaned at approximately 120 days of age.

[5] The phenothiazine used in all therapeutic and free-choice regimens was a wettable (wetting agent, 1% lecithin by weight) purified (99.9%) product with an average particle size of about 2–3 μ, and was supplied through the courtesy of Atomic Basic Chemicals, Inc., Pittsburgh, Pennsylvania. Information on particle size, as determined by the Fisher Sub-Sieve Sizer, and purity as determined by the Association of Official Agricultural Chemists method, was also provided by the company.

[6] Thibenzole: Merck & Co., Rahway, New Jersey. (Mention of products used in this study does not constitute endorsement by the USDA.)

Table 1. Therapeutic treatments administered to lambs.

Date	Band	Treatment	Rate
1963			
8 July	3	phenothiazine	15–30 g[1,2]
	4	thiabendazole	50 mg/kg
2 August	4	thiabendazole	75 mg/kg
19 August	3	phenothiazine	15–30 g
	4	thiabendazole	100 mg/kg

[1] Lambs under 22.7 kg were given 15 grams; all others were given 30 grams.
[2] 2–3 μ, purified.

Band 3 lambs and their dams grazed the same four pastures and in the same sequence as Band 3 lambs in 1961 and 1962. Band 4 lambs and their dams grazed the same 4 pastures and in the same sequence as Band 4 lambs in 1961 and 1962. The animals of Bands 3 and 4 were rotated every 2 weeks among the "parasite-contaminated" pastures, but they were not returned to a previously grazed pasture within less than 4 weeks. Both purified and N. F. phenothiazine were used for helminth control in animals grazing on the Band 3 pastures during 1961; only purified phenothiazine was used during 1962. Animals grazing on Band 4 pastures were given only purified, micronized phenothiazine, free-choice and by therapeutic dosing, during 1961, and only therapeutic doses of thiabendazole during 1962.

On 6 April, four days before the bands were started on their respective regimens, therapeutic doses of 30 g of purified phenothiazine were given to all mature ewes in Bands 2 and 3. Band 4 ewes were given therapeutic doses of thiabendazole as described in the 1962 studies. No further therapeutic treatments were given to the ewes. Phenothiazine-mineral mixture (1:9) was provided at all times for lambs of Band 1, and for lambs and dams of Bands 2 and 3. Only the basic mineral mixture containing 65 parts iodized salt, 20 parts dicalcium phosphate, and 5 parts magnesium carbonate was provided for the lambs and dams of Band 4. The therapeutic treatments administered to the lambs of Bands 3 and 4 are summarized in Table 1. The therapeutic doses of phenothiazine were given in accordance with the schedule followed in the 1962 studies, i.e., when *Haemonchus* egg counts averaged approximately 1,000 eggs per gram of feces.

All lambs were weighed biweekly beginning on 10 April. Parasite-surveillance studies were

initiated on 24 April and consisted of biweekly determination of packed red-cell volume (PCV) and number of parasite eggs per gram of feces (EPG) as in previous studies. Surveillance lambs in each band consisted of 2 Hampshire, 2 Merino, 2 Dorset, 2 Targhee, 2 Columbia-Southdale ewe lambs, 5 crossbred ewe lambs, and 5 crossbred ram lambs. Two of the cross-bred surveillance lambs from each band were killed on 12 June, 2 July, 31 July, 28 Aug., and 24 Sept., an aggregate of 40 lambs for parasite identification and enumeration.

Weaning was completed by 19 June, and as in previous experiments some lambs were removed from each band for other studies. The remaining lambs of each band were separated by sex but continued on the experimental procedures.

The biweekly PCV and EPG data for the surveillance lambs, plus the necropsy worm counts, were subjected to least squares analysis of variance (Harvey, 1960). Analysis of variance of biweekly weights included all experimental lambs in addition to the surveillance lambs. The data were grouped for analysis into

phase 1 (pretreatment) and phase 2 (treatment). Each phase contained the periods ending on the dates given in Table 2.

The total rainfall and temperatures at the experimental area during the surveillance studies are given by periods (Table 2). Official Weather Bureau Station equipment and techniques were employed.

Table 2. The total rainfall and temperature by experimental periods during the surveillance studies.

Phase	Period	Dates	Rainfall cm	Temperature C Mean	Temperature C Range
1	1	10–24 April[1]	1.60	12.2	−1.7–28.9
	2	25 April–8 May	1.70	12.8	−1.7–22.8
	3	9–22 May	2.57	16.7	0.0–33.9
	4	23 May–6 June	9.40	17.2	1.1–31.1
	5	7–19 June	1.75	20.6	8.9–33.3
	6	20 June–3 July	4.39	22.8	6.1–34.4
2	1	4–17 July	1.75	21.1	8.9–35.0
	2	18–31 July	0.25	25.6	15.0–36.1
	3	1–14 Aug.	5.59	23.3	11.1–33.9
	4	15–28 Aug.	13.61	20.6	8.3–33.1
	5	29 Aug.–11 Sept.	2.13	20.0	9.4–30.0
	6	12–25 Sept.	3.81	15.6	−0.6–31.1

[1] Initial surveillance samples obtained 24 April.

Table 3. Average number of parasites recovered from necropsied surveillance lambs.

Date	Band	No. of lambs	Haemonchus contortus	Strongyloides papillosus	Other[1]	Total
1963						
12 June	1	2	0	150	113	263
	2	2	0	175	253	428
	3	2	90	100	1,475	1,665
	4	2	100	175	68	343
2 July	1	2	50	625	268	943
	2	2	100	700	2,925	3,725
	3	2	100	1,025	1,793	2,918
	4	2	5,625	1,525	288	7,438
8 July	3-P[2]					
	4-T[3]					
31 July	1	2	0	2,200	225	2,425
	2	2	125	1,700	2,305	4,130
	3	2	1,900	2,100	1,573	5,573
	4	2	6,825	25	73	6,923
2 Aug.	4-T					
19 Aug.	3-P					
	4-T					
28 Aug.	1	2	10	1,375	1,001	2,386
	2	2	355	2,850	1,602	4,807
	3	2	670	1,700	685	3,055
	4	2	5,500	0	104	654
24 Sept.	1	2	210	9,025	1,451	10,686
	2	2	350	1,575	428	2,353
	3	2	14,120	9,025	1,388	24,533
	4	2	2,860	25	180	3,065
Total	1	10	270	13,375	3,058	16,703
	2	10	930	7,000	7,513	15,443
	3	10	16,880	13,950	6,914	37,744
	4	10	15,960	1,750	713	18,423

[1] *Trichostrongylus* spp., *Ostertagia* spp., *Nematodirus* spp., *Trichuris* spp., and *Oesophagostomum venulosum.*
[2] P = Phenothiazine treatments.
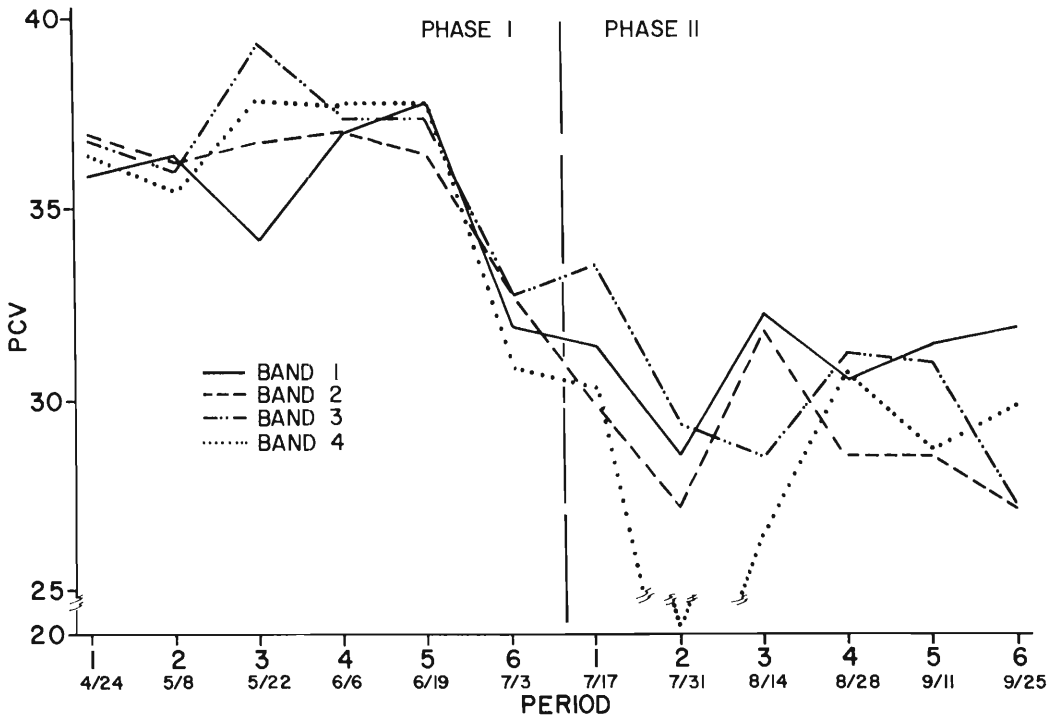[3] T = Thiabendazole treatments.

Figure 1.   Comparison of packed red-cell volumes by bands.

Results

The average levels of total parasitism acquired by the lambs of Bands 1, 2, 3, and 4 were adjudged light, light to moderate, moderate to severe, and moderate, respectively, on the basis of PCV, EPG, and necropsy worm counts (Table 3 and Figs. 1–2). Clinical coccidiosis did not occur in any of the lambs although coccidia were always found during routine fecal examinations.

Only *Haemonchus* was found in sufficient numbers to be pathogenic in Bands 3 and 4. Moderate infections of *Haemonchus* occurred in late July and heavy infections in late September in Band 3. However, Band 4 lambs had acquired large numbers of this parasite by early July and the level of infection remained heavy during the remainder of the study. Lambs in Bands 1 and 2 acquired light infections of *Haemonchus* by the end of the experiment.

The numbers of *Strongyloides papillosus* and other parasites increased progressively in Bands 1, 2, and 3, but fell to insignificant levels in Band 4 after therapeutic treatment with thiabendazole.

There were no significant differences in PCV between bands in phase 1. However, during phase 2, the PCV of Band 1 was significantly higher than Band 4 ($P < 0.01$) and of Band 2 ($P < 0.05$). The PCV for Band 3 was not significantly different from Band 1 but was higher than that of Band 4 ($P < 0.05$). Period effects and band × period interactions also were highly significant during phase 2 (Fig. 1) because of differences in drug action and levels of parasitism.

The *Haemonchus* egg counts for Bands 1 and 2 were significantly lower than those for Bands 3 and 4 ($P < 0.01$) during phase 1; those of Band 1 also were lower than Band 2 ($P < 0.05$). During phase 2, Band 1 *Haemonchus* EPG were significantly lower than Bands 2, 3, and 4 ($P < 0.01$); those of Bands 2 and 3 also were lower than Band 4 ($P < 0.01$). Differences in EPG between Bands 2 and 3 were not significant. *Strongyloides* EPG in
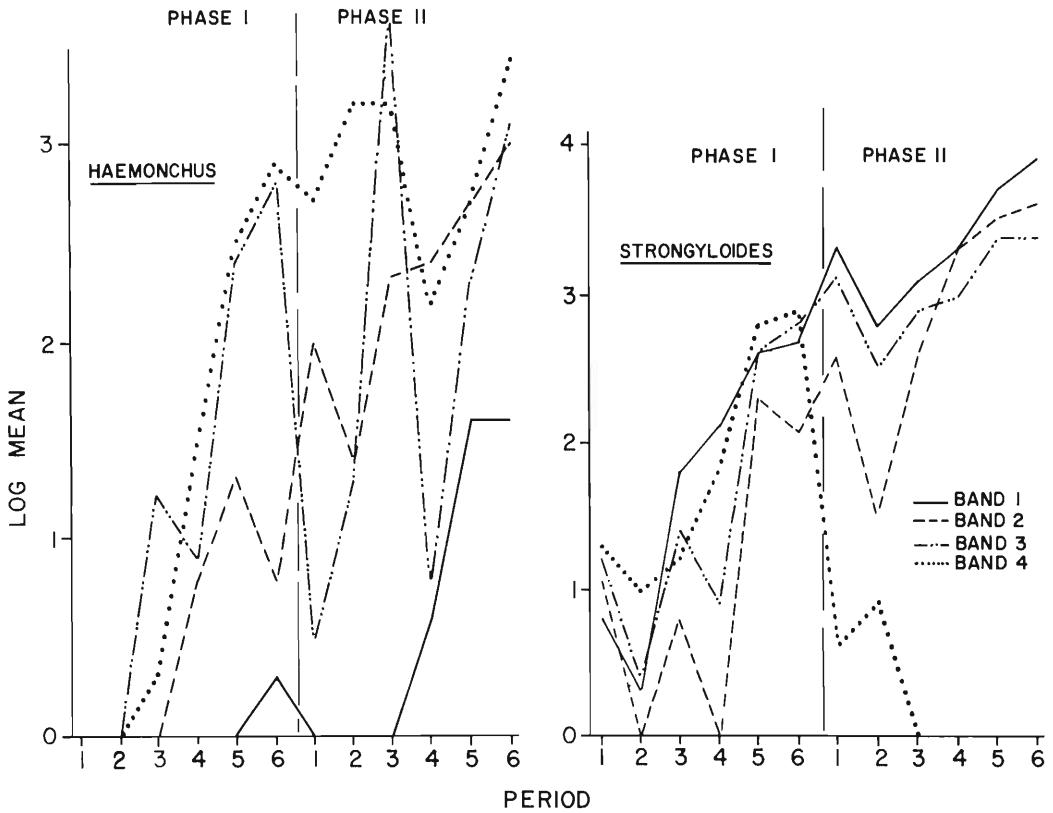
Figure 2.   Logarithmic means of fecal egg counts for Bands 1, 2, 3, and 4.

Band 2 were lower than Bands 1 and 4 ($P <$ 0.05) during phase 1. Highly significant differences in *Strongyloides* egg counts, between bands, were observed in phase 2. Egg counts for Band 4 were significantly lower ($P < 0.01$) than for Bands 1, 2, and 3; those of Band 2 were lower than Band 1 ($P < 0.01$) and those of Band 3 also were lower than Band 1 ($P <$ 0.05).

The average numbers of parasites recovered from the necropsied surveillance lambs are given in Table 3. The mean number of *Haemonchus* recovered from Band 1 was significantly lower ($P < 0.01$) than from Bands 2, 3, and 4. Fewer *Haemonchus* also were recovered from Band 2 than from Bands 3 and 4 ($P <$ 0.01). *Strongyloides papillosus* numbers in Band 4 fell sharply after therapeutic treatment with thiabendazole; therefore, numbers of this parasite were significantly lower in this Band

($P < 0.01$) during phase 2. Other helminths recovered from all four bands included *Nematodirus* spp., *Ostertagia* spp., *Trichostrongylus* spp., *Trichuris* spp., and *Oesophagostomum venulosum*. The numbers of these parasites also appeared to be lower in Band 4 during phase 2.

Throughout the experiment the weight gain of lambs in Band 1 was significantly higher ($P < 0.01$) than that of lambs in the other bands. The difference in rate of gain was more marked in phase 2 than in phase 1 (Fig. 3).

Difference in mortality between bands was insignificant and the overall mortality was less than 2%.

Discussion

A management system that involved the early weaning and grazing of lambs on renovated pastures (Band 1) effectively controlled
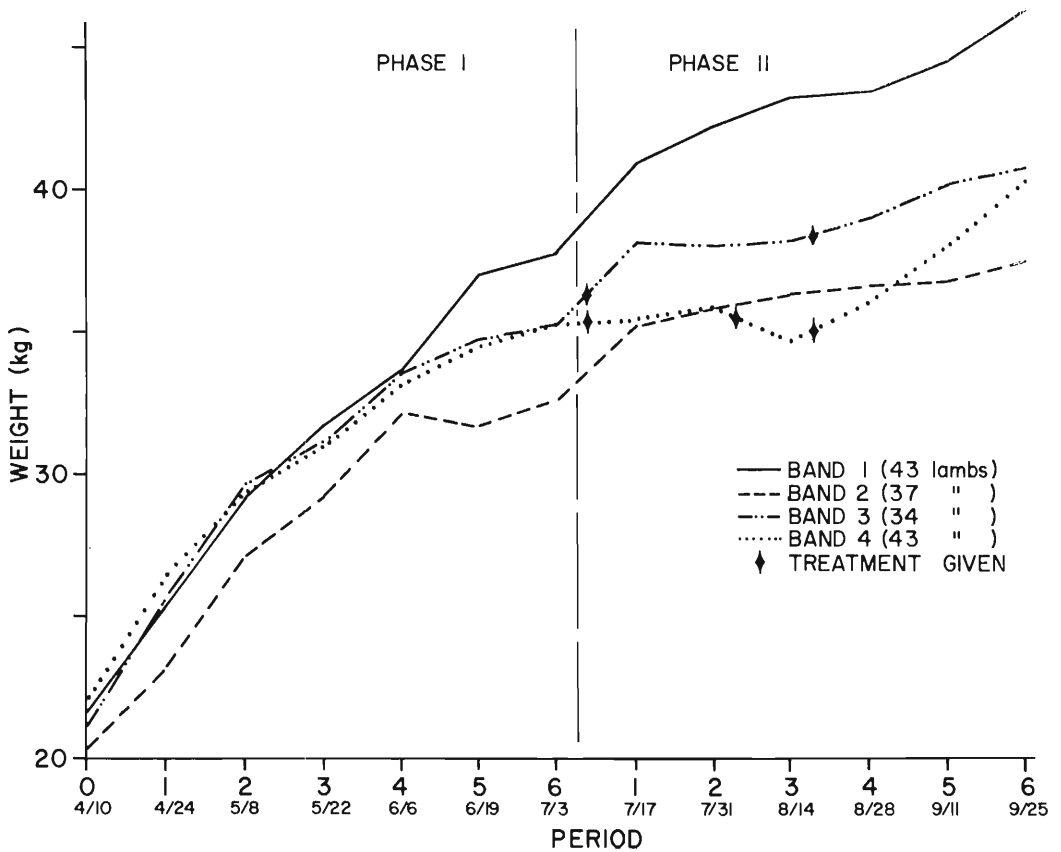
Figure 3. Comparison of lamb weights by band.

H. contortus and resulted in excellent weight gains. A slight build-up of Haemonchus was observed in late September. The total number of S. papillosus recovered from the early weaned lambs, however, was as high as that recovered from any other group. These results indicate the difficulty of maintaining parasite clean pastures under practical conditions, and demonstrate the desirability of combined management and therapeutic regimens for lambs on pasture. These conclusions agree with those of other workers (Baird and Sell, 1959; Brown, 1959; Levine et al., 1960; Lewis et al., 1960; Baird et al., 1960; Kuttler and Marble, 1962; Bizzell et al., 1964).

The level of Haemonchus infection was higher in the lambs from Band 2 than in lambs from Band 1. Haemonchus infection also was higher in 1963 (the present report) than it was

in 1962 (Colglazier et al., 1970) for lambs grazed separately from their dams. The lambs were not given therapeutic medication during either year. The management procedures were identical during both years and the lambs grazed the same pastures. Because the lambs had acquired light infections of Haemonchus by late 1962, some carryover of the parasites on pasture probably served as an early source of infection in 1963. It is known that viable parasites, including Haemonchus, survive on pasture from one season to another at Beltsville, Maryland (Vegors et al., 1969).

Lambs in Bands 3 and 4 also acquired heavier infections of Haemonchus in 1963 than in 1962; furthermore, the infections were acquired at an earlier date. Rainfall was greater during the 1963 experimental period than during 1962, and was more uniformly distributed

during the grazing season; this undoubtedly contributed to a more rapid increase in parasitism in all bands in 1963.

High levels of *Haemonchus* were found in necropsied lambs from Band 4 after 8 July, even though therapeutic doses of thiabendazole up to 1.5 times the recommended level were given. These results confirm the observations made in 1962, that thiabendazole was relatively ineffective in controlling *Haemonchus* in the flock of sheep used in similar experiments. Subsequent work (Colglazier et al., 1969) showed that an isolate of *Haemonchus* obtained from this flock was resistant to thiabendazole at the 50 mg/kg level. These observations from two separate studies indicate that *H. contortus* from this flock may have been relatively resistant to the effects of thiabendazole at the time the drug was first used. Although thiabendazole failed to control *Haemonchus*, it was highly effective against *S. papillosus* and other parasites (Table 3).

Based on EPG, necropsy data, and PCV (Figs. 1 and 2; Table 3), micronized purified phenothiazine offered continuously at low-levels in mineral mixture, plus therapeutic treatments on 8 July and 19 August, was effective in controlling haemonchosis in Band 3 until late August. There was a very rapid build-up of this parasite in late September, as shown by necropsy worm counts of the lambs on 24 September and by the fecal egg counts. This shows that low level phenothiazine alone was not sufficient to control *Haemonchus* after the last therapeutic dosing on August 19 (Table 1). Moreover, in this experiment, micronized purified phenothiazine was not as effective as thiabendazole against parasites other than *Haemonchus*.

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Pseudosonsinotrema echinophallus sp. n. (Digenea: Pleurogenidae), a New Trematode from *Rana pipiens* Schreber in Costa Rica

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ABSTRACT: *Pseudosonsinotrema echinophallus* sp. n. is described from the intestine of *Rana pipiens* in Costa Rica. This is the first report of this genus of trematode from the Western Hemisphere. The species can be differentiated from *P. chamaeleonis* Dollfus, 1951, by the absence of the pars prostatica in the latter and by the form of the excretory bladder. The intercecal position of the ovary and the preacetabular position of the testes distinguishes *P. echinophallus* from *P. megametrum* Manter and Pritchard, 1964, and *P. japonicus* (Yamaguti, 1936) Manter and Pritchard, 1964. *Pseudosonsinotrema sphenomorphi* Fischthal and Kuntz, 1965, is transferred to the genus *Pleurogenoides* Travassos, 1921.

Sixteen specimens of *Rana pipiens* Schreber, collected in the vicinity of Turrialba, Costa Rica, were examined for helminth parasites during June 1969. Four of these frogs harbored 22 specimens of a trematode referable to the genus *Pseudosonsinotrema* Dollfus, 1951. This fluke appears to represent a new species and is designated *P. echinophallus*. In so far as it is known, the new species is the first member of the genus to be reported from the Western Hemisphere.

The trematodes recovered from the frog hosts were heat killed in saline under slight coverslip pressure, fixed in alcohol-formalin-
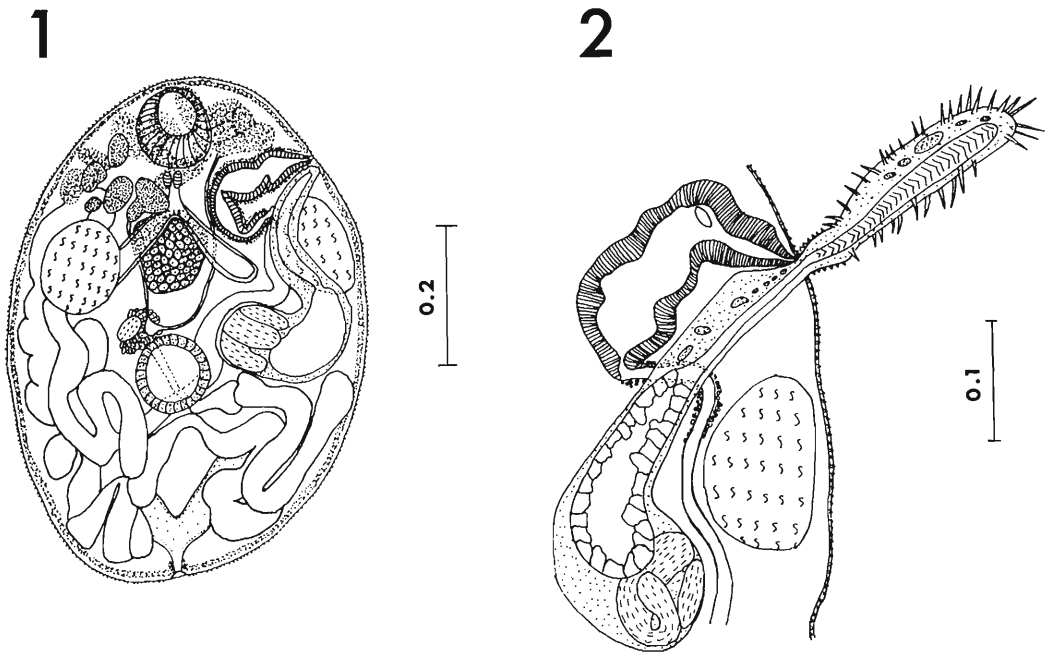
Figures 1–2. *Pseudosonsinotrema echinophallus* sp. n. (Scales in millimeters.) 1. Ventral view, holotype. 2. Ventral view, terminal genitalia, note muscular condition of metraterm.

acetic acid solution (AFA), stained with Harris' hematoxylin and mounted in Canada balsam. Prevailing field conditions did not permit observation of the worms while they were still alive. Unless otherwise noted, all measurements are given in microns with the mean in parentheses.

Pseudosonsinotrema echinophallus sp. n. (Figs. 1, 2)

Description

Measurements based on 12 mature specimens: Body spherical to slightly elliptical in outline, 640–840 (720) long by 440–570 (510) wide. *Entire* tegument covered with small spines. Oral sucker subterminal, 88–110 (100) by 95–130 (110). Acetabulum medial, postequatorial, 110–130 (120) by 110–130 (120). Ratio of oral sucker to acetabulum 1.0:1.2. Prepharynx very short or absent; pharynx muscular, 32–41 (36) by 30–37 (35); esophagus short; ceca short, terminating in preacetabular region. Testes smooth, symmetrical, in zone of ceca; right testis 94–170 (130) by 76–140 (110), partly overlapped by cecum; left testis

110–160 (140) by 68–130 (94), displaced laterally by cirrus pouch. Cirrus pouch elongate, S- to J-shaped, 350–520 (430) by 83–110 (97) at bulbous basal portion. Seminal vesicle coiled in basal portion of cirrus pouch. Pars prostatica large, saclike, lined with large cuboidal epithelial cells. Ductus ejaculatorius passing through narrow distal portion of cirrus pouch to genital pore; cirrus protrusible, covered with large spines. Ovary smooth to irregular in outline, intercecal, occasionally overlapping ceca dorsally just posterior to bifurcation, 98–130 (120) by 51–120 (92). Seminal receptacle and Mehlis' gland posterior to ovary. Uterine coils occupying most of hindbody and extending into forebody. Metraterm well developed, muscular, located anterior to cirrus pouch. Cirrus pouch and metraterm emptying directly through genital pore located at esophageal level on left margin of body. Vitellaria composed of large follicles occupying the anteriormost portion of body, majority of follicles distributed dorsally, a few follicles extending ventrally on right side in region between oral sucker and right testis. Two con-

spicuous vitelline ducts joining to form yolk reservoir at level of seminal receptacle. Fifteen operculate eggs from whole mounted worms measured 19–22 (20) by 8–10 (8). Excretory bladder Y-shaped, with short stem and long branches which reach to level of acetabulum.

HOST: *Rana pipiens* Schreber.

LOCALITY: Turrialba, Cartago Province, Costa Rica.

SITE OF INFECTION: Small intestine.

SPECIMENS: USNM Helm. Coll. No. 70817 (holotype); No. 70818 (paratype).

Discussion

The genus *Pseudosonsinotrema* was erected by Dollfus (1951) for *P. chamaeleonis*, an intestinal parasite of *Chamaeleon chamaeleon* (L.), collected by P. Sonsino at Gabes, Tunisia in 1893. The species was described from a single specimen found in a collection of type specimens of *Sonsinotrema tacapense* Balozet and Callot, 1938 (= *Distomum tacapense* Sonsino, 1894). In differentiating *Pseudosonsinotrema* from the other genera of the Pleurogenidae Odening, 1959, Dollfus (1951) maintained that the strongly developed metraterm and the short esophagus separated it from *Sonsinotrema* Balozet and Callot, 1938; the position of the testes relative to the ceca separated it from *Prosotocus* Looss, 1899, while the form of the excretory bladder, Y-shaped with the trunk as long as or longer than the branches, and the well developed metraterm isolated it from *Pleurogenoides* Travassos, 1921, in which, according to Dollfus (1951), the bladder is typically V-shaped or Y-shaped with a short trunk and long branches.

Manter and Pritchard (1964) described *Pseudosonsinotrema megametrum* from the intestine of *Bufo regularis* Reuss in Kasongo (Maniema), Kivu Province, Republic of the Congo. These authors also transferred *Pleurogenoides japonicus* (Yamaguti, 1936), an intestinal parasite of *Rana nigromaculata* Hollowell in Japan, to *Pseudosonsinotrema*, using the criterion of the strongly developed metraterm as a unifying character. They noted that the metraterm in *P. japonicus* was glandular as opposed to muscular in both *P. chamaeleonis* and *P. megametrum*. A study of Yamaguti's (1936) figure of *P. japonicus* suggested that the metraterm in this species is well differentiated.

The characters employed in separating the species of *Pseudosonsinotrema* include the presence or absence of the pars prostatica, the relative lengths of the stem and arms of the excretory vesicle, length of the esophagus, position of the ovary, and condition of the metraterm. The extracecal position of the ovary in *P. japonicus* and *P. megametrum* separates these two species from *P. echinophallus* which also differs from them in having the testes preacetabular. The short esophagus in *P. echinophallus* further distinguishes the species from *P. japonicus*. The presence of a well developed pars prostatica in *P. echinophallus* as well as an excretory bladder with a short stem and long branches differentiate this species from *P. chamaeleonis*.

Fischthal and Kuntz (1965) described *Pseudosonsinotrema sphenomorphi* from the intestine of *Sphenomorphus multisquamatus* Inger with *Bufo asper* Gravenhorst as an additional host record in Ranau, North Borneo. Examination of the paratypes of *P. sphenomorphi* (USNM Nos. 60952–3) showed that the metraterm in this species neither conforms to the muscular condition described by Dollfus (1951) nor the condition noted by Manter and Pritchard (1964) for *P. japonicus*. In *P. sphenomorphi* the thin-walled uterus empties into a large genital atrium which is itself thin-walled and surrounded by glandular cells. Similar cells appear to be present in *P. japonicus*, but the metraterm in that species is clearly differentiated.

The variation in the shape of the excretory bladder exhibited in the genera *Pseudosonsinotrema* and *Pleurogenoides* suggests that the excretory bladder alone is insufficient to separate genera in this group of trematodes. Although the shape of the excretory bladder in *P. sphenomorphi* corresponds to the form shown by *Sonsinotrema*, the latter genus was separated from *Pleurogenoides* by Dollfus (1951) using the single criterion of the shape of the excretory vesicle. Accordingly, *Sonsinotrema* should be considered a synonym of *Pleurogenoides* as proposed by Yamaguti (1958). It is then suggested that *Pseudosonsinotrema sphenomorphi* be transferred to the genus *Pleurogenoides* becoming *Pleurogenoides sphenomorphi* (Fischthal and Kuntz, 1965) n. comb.

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Pseudosonsinotrema catesbeianae sp. n. (Trematoda: Pleurogenidae), from the Bullfrog, Rana catesbeiana Shaw

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ABSTRACT: Pseudosonsinotrema catesbeianae sp. n. is reported and described from the duodenum of the bullfrog Rana catesbeiana Shaw from McKenzie, Tennessee, USA. This is the first report of the genus and the first species to be described from North America. The new species differs from all the other species in the genus in having a deeply but variously lobed ovary. P. catesbeianae differs further from other species except P. echinophallus Sullivan, 1970 in having uterus extending anteriorly into forebody to the level of pharynx or oral sucker on the right side of the body. P. catesbeianae further differs from P. echinophallus Sullivan, 1970 in the relative positions of the testes and the egg size; and from P. chamaeleonis Dollfus, 1951 in the form, shape, and number of vitelline follicles. P. catesbeianae also differs from P. japonicum and P. megametrum Manter and Pritchard, 1964 in having a postequatorial acetabulum and ovary inter-cecal or slightly overlapping right cecum, and testes in the region of acetabulum. The generic diagnosis of the genus Pseudosonsinotrema Dollfus, 1951, is extended to include the anterior extension of uterus on the right side into the forebody in P. echinophallus Sullivan, 1970 and P. catesbeianae sp. n.

The trematodes reported herein, were taken from the duodenum of the bullfrog Rana cates-beiana Shaw from McKenzie, Tennessee, USA. Eight of the 36 bullfrogs examined were found to be infected, each harboring 12–16 trema-todes. The worms were killed and fixed in hot 70% alcohol without flattening, stained with Semichon's carmine, and mounted in balsam.

Drawing was made with the aid of a camera lucida and a microprojector. All measurements are in microns unless otherwise indicated; the ranges for lengths are given first followed by those of widths.

Pseudosonsinotrema catesbeianae sp. n.
(Fig. 1)

Description

Based on 20 mature and 4 young adults: Body oval to globular or pyriform, 465–520 long by 372–422 wide; tegument spined, spines more numerous anteriorly, progressively dimin-
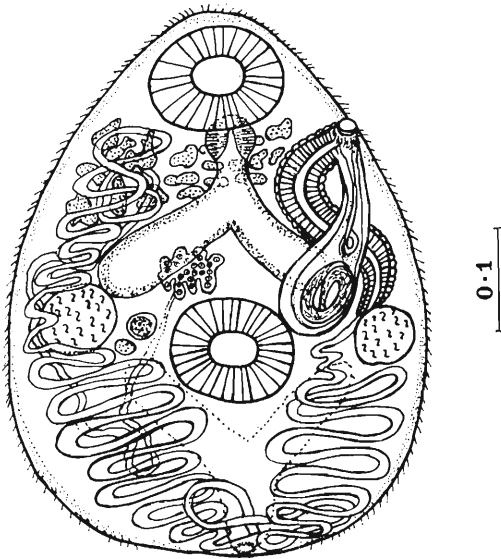
Figure 1. Ventral view of the holotype of *Pseudosonsinotrema catesbeianae* sp. n. from the duodenum of *Rana catesbeiana* Shaw. (Scale in millimeters.)

ishing toward posterior extremity. Oral sucker oval to spherical, subterminal, ventral, 93–102 by 98–102. Acetabulum postequatorial, in anterior portion of posterior third of body length, 96–102 by 95–102. Sucker length ratio 1:1, width ratio approx. 1:1. Prepharynx absent; pharynx oval, 38–39 by 38–39; esophagus very short, 41–42; intestinal ceca short, preacetabular, usually inflated, lined internally with conspicuous, thick unicellular layer. Testes symmetrical to slightly asymmetrical, opposite acetabulum, oval to slightly lobed with smooth to slightly irregular margin; right testis 72–90 by 71–91 extending slightly preacetabular, immediately posterior to posterior portion of right intestinal cecum, sometimes contiguous. Left testis usually smaller than right, 62–65 by 62–64. Cirrus sac large, thick walled, clavate, 212–225 by 76–78 at proximal portion, extending diagonally, posterior end sinistrolateral and anterior to acetabulum, containing large, tubular and much coiled seminal vesicle; prostatic complex well developed with many prostate gland cells, cirrus protrusible, usually inverted, looped basally when retracted. Ovary sub-

median, preacetabular, intercecal to just overlapping right cecum, deeply and irregularly lobed, 62–70 by 62–68. Seminal receptacle postovarian, 31–38 by 32–36, between acetabulum and right testis. Mehlis' gland postovarian, well developed. Vitellaria dorsal, follicular, large, irregularly lobed, occupying most of width of neck region from posterior level of oral sucker to level of intestinal bifurcation, more follicles on right portion of neck region than left. Uterus extensively coiled, ascending dextrally to level of pharynx or oral sucker, occupying essentially most of lateral field, of posttesticular region except median field, finally ascending sinistrally dorsal to left testis, joining metraterm just anterolateral to left testis. Metraterm dorsal to cirrus pouch, thick walled, muscular, surrounded by dense mass of gland cells. Metraterm and cirrus pouch opening into a common, shallow atrium leading to genital pore located at pharyngeal level on left margin of body. Eggs numerous, light yellow, containing developing embryos, operculate, 25–28 by 10–12. Excretory bladder Y-shaped, with short stem and large cornua terminating at posterior level of testes; excretory pore terminal.

DEFINITIVE HOST: Bullfrog, *Rana catesbeiana* Shaw.

LOCATION IN HOST: Duodenum.

LOCALITY: McKenzie, Tennessee, USA.

TYPE SPECIMENS: Holotype, USNM Helm. Coll. No. 70789; paratypes (4), No. 70790. Other paratypes in author's collections.

Discussion

Dollfus (1951) erected the genus *Pseudosonsinotrema* with *P. chamaeleonis* as the type species for some intestinal trematodes of chameleons in Tunisia. Manter and Pritchard (1964) described *P. megametrum* from the intestine of *Bufo regularis* Reuss from Congo, (Kinshasa), and added a new combination *P. japonicum* (Yamaguti, 1936) Manter and Pritchard, 1964 from *Rana nigromaculata* Hallowell in Japan. Fischthal and Kuntz (1965) described *P. sphenomorphi* from the intestine of *Sphenomorphus multisquamatus* Inger and *Bufo asper* Gravenhorst from North Borneo (Malaysia). Sullivan (1970) described *P. echinophallus* from the intestine of *Rana pipiens* Schreber in Costa Rica, and, based on his examination of *P. sphenomorphi* Fischthal and

Kuntz, 1965, he (Sullivan) suggested that the later species be transferred to the genus *Pleurogenoides* thus, becoming *Pleurogenoides sphenomorphi* (Fischthal and Kuntz, 1965) comb. n.

The present report constitutes the first record of the genus *Pseudosonsinotrema* Dollfus, 1951 from North America.

Pseudosonsinotrema catesbeianae sp. n. differs significantly from other species in the genus in having a deeply but variously lobed ovary, and in having the uterus extending anteriorly to the level of oral sucker or esophagus on the right side. It differs further from *P. chamaeleonis* in the form, shape, and number of vitelline follicles. The ovary is extracecal and the acetabulum preequatorial in *P. japonicum* and *P. megametrum* whereas the ovary is either intercecal or only slightly overlapping the right cecum and the acetabulum postequatorial in *P. catesbeianae*. Though Sullivan (1970) suggested that *Pseudosonsinotrema sphenomorphi* Fischthal and Kuntz, 1965 should be transferred to the genus *Pleurogenoides* Travassos, 1921, *Pseudosonsinotrema catesbeianae* can be further differentiated from the former in the shape, size, and distribution of vitellaria. Relative positions of the testes and egg size further differentiate *P. catesbeianae* from *P. echinophallus* Sullivan, 1970.

The generic diagnosis of the genus *Pseudosonsinotrema* Dollfus, 1951, should be extended to include the anterior extension of uterus into the forebody in *P. echinophallus* Sullivan, 1970 and *P. catesbeianae* sp. n.

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Anthelmintic Efficacy of Four Dose Levels of Cambendazole in Cattle

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ABSTRACT: Four dose levels of cambendazole (5-isopropoxycarbonylamino-2-[4-thiazolyl] benzimidazole) were tested for anthelmintic activities in cattle under field conditions. Twenty-five feeder calves were randomly divided into 5 groups of 5 each. Group I served as nonmedicated controls. Other groups were drenched with cambendazole at the dose rates of 15, 20, 25, and 40 mg/kg of body weight. Medications were 91 to 100% effective against the adult forms of *Haemonchus placei*, *Ostertagia ostertagi*, *Trichostrongylus axei*, *Cooperia punctata*, *C. pectinata*, and *C. oncophora*. Efficacy varied from 69 to 85% against the abomasal larvae and from 95 to 100% against the intestinal larvae. Medicated cattle did not show signs of intoxication.

Several reportedly efficacious anthelmintics have been introduced during the last decade (Douglas and Baker, 1968). These developments are in response to demands for safe drugs which are active against a broad spectrum of gastrointestinal parasites of domestic animals. The development of such drugs is of special importance in the cattle industry, where parasitosis, particularly that caused by nematodes, is a continuous threat to the health and productivity of cattle.

A newly developed anthelmintic, cambendazole, (5-isopropoxycarbonylamino-2-[4-thiazolyl] benzimidazole)* has been reported to be highly efficacious against helminths of sheep (Egerton and Campbell, 1970). The purpose of the present experiment was to determine the relative efficacy of cambendazole against naturally acquired gastrointestinal nematodes in feeder calves enzootic in the area.

Materials and Methods

Nematode egg counts were made of feces collected from about 60 feeder calves obtained at a local auction sale during August, 1969. Twenty-five of these calves were selected because eggs of the nematode genera enzootic in Georgia were present in their feces; they also had the highest number of eggs per g of feces (EPG). The calves, all commercial-grade and averaging 245 kg weight, were purchased and transferred to a farm prior to the beginning of the test to get them accustomed to the environment and the diet. The calves were maintained in a bermuda-fescue summer pasture plot and were fed a feed supplement of ground snap corn.

A week after purchase the calves were divided at random into 5 groups of 5 each. Group I was used as nonmedicated controls. Each of the other 4 groups was drenched that same morning with aqueous suspensions of cambendazole at dose levels of 15, 20, 25, and 40 mg/kg of body weight, respectively. The cambendazole suspensions contained 4.55, 6.06, 7.58, and 12.12% of the active material so that each could be administered at the rate of 15 ml/45 kg of bodyweight to deliver the desired dosage.

All calves were returned to the same pasture after treatment, where they were observed frequently for signs or indications of any reaction suggesting drug toxicity. All calves were necropsied on the 5th post-treatment day, when the helminths in the digestive tracts were collected. Worm counts were made by Porter's method (1942) and worm species were identified for each calf.

Actual numbers of worms recovered from the individual calves were transformed to log of value + 1 to normalize variance within groups (Egerton et al., 1963) before being subjected to analysis for significance by Duncan's test (Harter, 1960). Geometric means obtained from these calculations were used to determine the percentage efficacy (l.t.d.) of the treatments (Sokal and Rohlf, 1969). Percentage efficacy of the treatments was also determined from the raw data (r.d.).

Table 1. Average (arithmetic) number of worms from calves treated with four dose levels of cambendazole (mg/kg).

Organ and species	Treatment groups				
	Control	15	20	25	40
ABOMASUM					
Total count	16,755	1,464	1,586	774	960
Adult worms	13,540	480	653	280	——
Ostertagia ostertagi	7,660	480	653	280	——
Trichostrongylus axei	4,940	——	——	——	——
Haemonchus placei	940	——	——	——	——
Immature worms	3,215	984	933	494	960
O. ostertagi, 3rd stage	2,224	303	470	318	612
O. ostertagi, 4th stage	831	681	403	176	68
O. ostertagi, 5th stage	——	——	60	——	280
T. axei, 3rd stage	160	——	——	——	——
INTESTINES					
Total count	10,353	113	<1	——	——
Adult worms	8,476	27	<1	——	——
Cooperia punctata	4,793	26	——	——	——
Cooperia oncophora	2,598	——	——	——	——
Cooperia pectinata	1,066	——	——	——	——
Bunostomum phlebotomum	<1	——	——	——	——
Oesophagostomum radiatum	<1	——	——	——	——
Trichuris spp.	19	1	<1	——	——
Immature worms	1,877	86	——	——	——
C. punctata, 3rd	191	86	——	——	——
C. punctata, 4th	918	——	——	——	——
Nematodirus spp., 3rd	768	——	——	——	——
All worms	27,108	1,577	1,587	774	960

Table 2. Geometric mean number of worms, based on logarithmic transformation, recovered in calves treated with four dose levels of cambendazole.

	Geometric mean no. worms				
	Treatment groups (mg/kg)				
	Control	15	20	25	40
Ostertagia ostertagi	6,604	51*	4***	36**	0***
Trichostrongylus axei	3,971	0***	0***	0***	0***
Haemonchus placei	78	0**	0**	0**	0**
Cooperia punctata	673	2***	0***	0***	0***
Cooperia oncophora	142	0**	0**	0**	0**
Cooperia pectinata	80	0**	0**	0**	0**
All adults†	18,712	138**	5**	36**	0***
All immature worms†	4,569	906	266	50	197
O. ostertagi, 3rd stage	2,088	268	152	38*	138
O. ostertagi, 4th stage	787	596	135	24*	23*
O. ostertagi, all immature	2,875	864	266	50*	197

* Statistically significantly less than control, $P < 0.05$.
** Statistically significantly less than control, $P < 0.01$.
*** Statistically significantly less than control, $P < 0.001$.
† Includes nematode genera with too few specimens for a separate statistical analysis.

Results and Discussion

The average numbers and species of nematodes recovered from the gastrointestinal tracts of the control and treated calves are given in Table 1. These values were transformed to the log + 1 to determine the geometric means used in the statistical analysis of the data (Table 2). The percentage efficacy and the statistical significance of the worm reduction by the dose levels studied are presented in Table 3.

It is obvious that, at the four different dosages used in this test, cambendazole was extremely efficacious (log transform data 99–100%; raw data 94–100%) in removing adult *Ostertagia ostertagi, Trichostrongylus axei,* and *Haemonchus placei* from the abomasums. All dosages were equally effective, except in the case of 40 mg/kg dose, which was significantly ($P < 0.05$) more effective than the 15 mg/kg dosage against full matured *O. ostertagi.* Cambendazole was 100% efficacious against larval forms of *T. axei.* Calves which received 25 mg/kg of the drug had significantly fewer third- and fourth-stage larvae of *O. ostertagi* than the controls. However, the anthelmintic efficacy

varied from 87 to 98% (raw data 72–86%) for the third-stage, and from 24 to 97% (raw data 18–92%) against fourth-stage larvae of *O. ostertagi.*

The action of the drug against the adult intestinal worms was also excellent. All dosages used were significantly efficacious against *Cooperia punctata, C. oncophora,* and *C. pectinata.* Reduction of the number of *Bunostomum phlebotomum, Oesophagostomum radiatum,*

Table 3. Anthelmintic efficacy in calves of cambendazole.*

		Percentage reduction**			
		Treatment groups (mg/kg)			
	Control	15	20	25	40
Ostertagia ostertagi	—ᵃ	99ᵇ	99ᵇᶜ	99ᵇᶜ	100ᶜ
		94ᵇ	91ᵇ	96ᵇ	100ᵇ
Trichostrongylus axei	—ᵃ	100ᵇ	100ᵇ	100ᵇ	100ᵇ
Haemonchus placei	—ᵃ	100ᵇ	100ᵇ	100ᵇ	100ᵇ
Cooperia punctata	—ᵃ	99ᵇ	100ᵇ	100ᵇ	100ᵇ
Cooperia oncophora	—ᵃ	100ᵇ	100ᵇ	100ᵇ	100ᵇ
Cooperia pectinata	—ᵃ	100ᵇ	100ᵇ	100ᵇ	100ᵇ
All adult worms***	—ᵃ	99ᵇ	99ᵇᶜ	99ᵇᶜ	100ᶜ
		98ᵇ	97ᵇ	99ᵇ	100ᵇ
All immature worms	—ᵃ	80ᵃᵇ	94ᵃᵇ	99ᵇ	96ᵃᵇ
		77ᵃᵇ	82ᵃᵇ	90ᵇ	81ᵃᵇ
O. ostertagi, 3rd stage	—ᵃ	87ᵃᵇ	93ᵃᵇ	98ᵇ	93ᵃᵇ
		86ᵃ	79ᵃ	86ᵃ	72ᵃ
O. ostertagi, 4th stage	—ᵃ	24ᵃ	83ᵃᵇ	97ᵇ	97ᵇ
		18ᵃ	52ᵃ	79ᵇ	92ᵇ

* From geometric mean number of worms recovered, after transformation to log + 1, and from arithmetic mean number of worms without transformation (raw data). Percentage reduction is the same for both means when only one value is given.
** Treatment differences are shown to be significantly different ($P < 0.05$) by not having common superscripts.
*** Includes genera with too few specimens for a separate statistical analysis.

and *Trichuris* spp. was also apparent, but the data were not analyzed statistically because of the relatively low populations of these species recovered. The dosages used were highly effective against third- and fourth-stage larvae of *C. punctata* and third-stage *Nematodirus* spp.

The four dosages of cambendazole tested were significantly effective against the adult of all species present in large numbers in the gastrointestinal tract of the calves. The 40 mg/kg dosage was statistically more efficacious than the 15 and the 25 mg dosages in controlling the adults. Significance in the reduction of the number of larvae from the entire digestive tract was only demonstrated with the 25 mg/kg dosage, although a marked reduction was effected by all dosages.

The results indicate that cambendazole had a broad spectrum of highly efficient anthelmintic activity against the nematodes commonly parasitic in the gastrointestinal tracts of calves. None of the dosages used produced any signs of intoxication. Perhaps a practical dose of the drug to recommend for cattle should be either 20 or 25 mg/kg of body weight when consideration is given to the efficacy of the dosages and the probable cost of the medication.

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Antarctic Soil and Freshwater Nematodes from the McMurdo Sound Region

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ABSTRACT: Six species of freshwater nematodes were collected in the McMurdo Sound region of Antarctica. Two species, *Scottnema lindsayae* gen. n., sp. n. (Cephalobidae) and *Panagrolaimus davidi* sp. n. are proposed as new. *Eudorylaimus antarcticus* (Steiner, 1916), *Monhystera villosa* Butschili, 1873, *Plectus frigophilus* Kirjanova, 1958, and *Plectus antarcticus* de Man, 1904 (= *Plectus murrayi* Yeates, 1970 n. syn.) are redescribed.

Soil and freshwater nematodes have been little studied on the Antarctic continent. De Man (1904) described five species collected from freshwater algae in Graham Land, Palmer Peninsula (65–68°S, 65–67°W) by the Belgian expedition of 1897–1899. The species were: *Mononchus gerlachei* de Man, 1904 (= *M. major* Cobb, 1893), *Plectus antarcticus* de Man, 1904, *Plectus belgicae* de Man, 1904, and two juveniles of *Dorylaimus* species. Murray (1910a, 1910b), the biologist on Shackleton's polar expedition of 1907–1909, collected at least two freshwater species at Cape Royds, Ross Island (77°33′S, 166°09′E), one of which was certainly a *Plectus*. There are specimens of this species in the slide collection of N. A. Cobb at USDA, Beltsville, Maryland. Steiner (1916) recorded *Plectus belgicae* from moss at Gaussberg on the Wilhelm II Coast (67°S, 88°E), collected by the German South-Polar Expedition of 1901–1903. He also described *Dorylaimus antarcticus* from Discovery Bay in

South Victoria Land (77°S), collected by the British "Discovery" Expedition of 1901–1903. Allgén (1951) described *Plectus grahami* from Graham Land. Kirjanova (1958) briefly described, without illustration, *Plectus antarcticus, P. globolabiatus* Kirjanova, 1958 (nec Andrassy, 1963 = *Plectus andrassyi* n. n.), and *P. frigophilus* Kirjanova, 1958, collected by the Soviet Antarctic Expedition of 1956–1957 from moss and freshwater lakes in the Bunger Hills, Knox Coast (66°–66°30′S, 100°–101°50′E). Dougherty et al. (1960) reported *Plectus parietinus* from a pond at the foot of Observation Hill, McMurdo Station, Ross Island. Yeates (1970) described *Plectus murrayi* Yeates, 1970 and *Eudorylaimus antarcticus* (Steiner, 1916) Yeates, 1970 from mosses in the McMurdo region and reviewed the literature on soil and freshwater nematodes of the subantarctic areas.

In the austral summer of 1969–1970 Dr. D. R. Viglierchio of the Department of Nematology, University of California, Davis and I collected throughout the McMurdo Sound region of Antarctica on a project of the U. S. Antarctic Research Program, sponsored by the National Science Foundation. Six species of soil and freshwater nematodes, representing five genera, were collected and are described in this paper. One genus and two species are new to science. Slides of all six species are retained in the nematode collections of the Smithsonian Institution, Washington, D. C. and of the University of California, Davis. Mass collections are in the Smithsonian Institution.

Large populations of nematodes live in Antarctica in proximity to ice and snow. Nematodes were found within 10 m of the base of tall glaciers and in glacial melt ponds and lakes which were mostly frozen over. The temperature of the water from which nematodes were collected ranged from 4–13 C. Usually soil nematodes were associated with rotifers and tardigrades, and occasionally with mites, and were living in areas where moss or algae were discernible. Moisture, warmth and the presence of algae determined the presence of meiofauna, though the precise ecological role of each was not studied.

Scottnema gen. n.

DIAGNOSIS: Cephalobidae, Acrobelinae. Head bearing six prominent cephalic probolae, prolonged at apex as seta-like projection; three broad labial probolae; five small triangular projections between cephalic axils in dorsal and subventral sectors.

This genus is closest to *Acrobeles* but is distinctive in the lengthy cephalic probolae and the fine projections between the cephalic axils. The genus is named in honor of Captain Robert F. Scott, leader of two Antarctic expeditions, who perished in a blizzard in January 1912, with four companions on their way back to Ross Island from the South Pole. The genus name is neuter in gender.

TYPE SPECIES: *Scottnema lindsayae* sp. n. This species is named after the collector, Mrs. Kay Lindsay, one of the first contingent of women scientists at McMurdo Station, Ross Island.

Scottnema lindsayi sp. n.
(Fig. 1, A–G)

10 males from La Croix Glacier: L = 0.82 (0.77–0.88) mm; a = 19.0 (16.2–21.9); b = 4.5 (4.2–4.8); c = 15.7 (14.9–17.3).

10 females from La Croix Glacier: L = 0.82 (0.79–0.86) mm; a = 16.4 (15.0–18.0); b = 4.6 (4.4–4.8); c = 17.6 (15.0–18.3); V = 64.2 (63.1–66.1)%.

10 males from Strand Moraines: L = 0.75 (0.71–0.8) mm; a = 20.1 (18.5–22.1); b = 4.4 (4.2–4.6); c = 14.2 (12.8–16.4).

10 females from Strand Moraines: L = 0.78 (0.74–0.8) mm; a = 18.2 (16.3–21.5); b = 4.3 (4.2–4.6); c = 14.8 (13.4–16.4); V = 62.1 (57.3–63.6)%.

HOLOTYPE MALE: L = 0.85 mm; a = 21.1; b = 4.6; c = 15.0; T = 52%.

DESCRIPTION: Cuticle thick and clear, coarsely annulated; annules about 3 μ wide; two subcuticular rows of closely-set punctations within each annule. Longitudinal striae dividing cuticle into blocks, about 70 in number just behind head. Distinct lateral alae beginning at mid-esophagus and ending just anterior to tail terminus. Margins of alae crenulate; aerolation anterior to esophageal bulb. Central incisure begins at small deirid opposite esophageal bulb and ends at large papilla just anterior to phasmid. A pair of small subdorsal papillae a short distance posterior to vulva in female. Head offset, 17–19 μ in diameter; lips modified as six large cephalic probolae prolonged at apex as smooth, anteriorly-directed seta-like projections, 15–17 μ long from base of probolae; three prominent edentate cephalic axils; five small

triangular projections between cephalic probolae in dorsal and subventral sectors. Three broadly rounded labial probolae, almost touching at margins. Stoma thickly cuticularized, surrounded by muscular esophageal tissue up to anterior of prostom. Cheilorhabdions in form of three curved plates around mouth opening. Conspicuous narrow dorsal tooth arising from metastomal wall. Corpus of esophagus uniformly cylindrical; short broad isthmus, one-half length of corpus. Esophageal bulb valvate, about 26 × 26 μ. Nerve ring just anterior to esophageal bulb. Excretory pore between nerve ring and bulb; excretory cell at base of bulb in male, anterior to bulb or at side of bulb in female; two small cells just behind excretory cell. Cardia broad, truncate at posterior. Intestine yellowish, with broad lumen filled with fine detritus. Rectum prominent; three rectal glands present, possibly more in male.

FEMALE: Gonad prodelphic, reflexed, extending past vulva about one-half the vulva-anus distance, occasionally with small loop or short flexure at tip of ovary. Rudimentary posterior gonad behind vulva, sometimes sac-like, sometimes containing a few oocytes. One ovum at a time in uterus, about 50 × 30 μ. Vulva with protuberant lips. Tail conical, 1.9–2.8 anal body diameters long, with fine mucro at tip. Small phasmids at mid-tail length.

MALE: Testis single, reflexed at anterior; two rows of spermatocytes; sperm small, globular. Spicules with aperture on dorsal side, 35–39 μ long. Gubernaculum parallel, 17 μ long, with fine lateral sleeves around spicule tips. Eight pairs of prominent genital papillae, two pairs well-anterior to spicules, one pair adanal, two pairs within alae on tail, one pair subdorsal, and two pairs subventral. Phasmids small, just behind first pair of papillae within alae. Tail conical, with mucronate tip, 1.4–2.1 anal body diameters long.

TYPE HABITAT: Sandy soil.

TYPE LOCALITY: Southeast of La Croix Glacier, Taylor Valley, Victoria Land.

OTHER LOCALITIES: Ross Island: volcanic

soil at Turk's Head and Cape Crozier. Victoria Land: soil near Lake Vanda and Canada Glacier, moraine of Miers Glacier near shore, stony soil near Péwé Lake, sandy soil at Strand Moraines, algal mat east of Meserve Glacier (collected by Mrs. Kay Lindsay), small runoff stream between La Croix Glacier and Lake Bonney (collected by Mrs. Kay Lindsay), mossy soil (*Bryum antarcticum*) at Mable Point.

HOLOTYPE MALE: Collected by Mrs. Kay Lindsay on 30 November 1969; Smithsonian Collection, USNM 42956.

PARATYPES: USNM 42957–42973 and University of California Nematode Collection, Davis.

Panagrolaimus davidi sp. n.
(Fig. 1, J–M)

10 females from Cape Royds: L = 0.91 (0.85–0.99) mm; a = 19.8 (18.7–21.0); b = 4.5 (4.2–5.0); c = 19.2 (17.1–21.0); V = 62.5 (60.6–63.6)%.

10 males from Cape Royds: L = 0.83 (0.79–0.89) mm; a = 23.2 (22.3–24.2); b = 4.2 (4.1–4.6); c = 20.1 (17.9–22.2).

10 females from Rocky Point: L = 0.85 (0.79–0.90) mm; a = 20.0 (18.5–22.2); b = 4.2 (3.9–4.6); c = 18.9 (17.0–21.5); V = 62.5 (60.6–63.6)%.

10 males from Rocky Point: L = 0.81 (0.73–0.89) mm; a = 25.5 (22.2–27.8); b = 4.3 (4.0–4.6); c = 19.7 (17.3–21.9).

HOLOTYPE MALE: L = 0.86 mm; a = 23.8; b = 4.4; c = 20.0; T = 52.8%.

DESCRIPTION: Cuticle striated; annules 1.3 μ broad at mid-body. Lateral alae with 3 incisures, the outermost 2 with faintly crenulate margins. Small deirids opposite anterior of esophageal bulb; central incisure begins at deirid and ends at phasmid. Three large duplex lips with submedian lobe lower; two circlets of papillae on each lobe. Stoma 12–15 μ long to base of metastom. Cheilostom longer than wide; rhabdions lacking. Prostom with thickly cuticularized cylindrical walls. Meso-

$\rightarrow$

Figure 1. A–G: *Scottnema lindsayae* g. n., sp. n. A—female body, B—male body, C—female head, D—en face view, E—section just behind head, F—optical section at level of labial probolae and cheilorhabdions, G—section through lateral field, H—male tail, I—female tail. J–O: *Panagrolaimus davidi* sp. n. J—female head, K—en face view, L—section through prostom, M—section through lateral field, N—female tail, O—male tail.
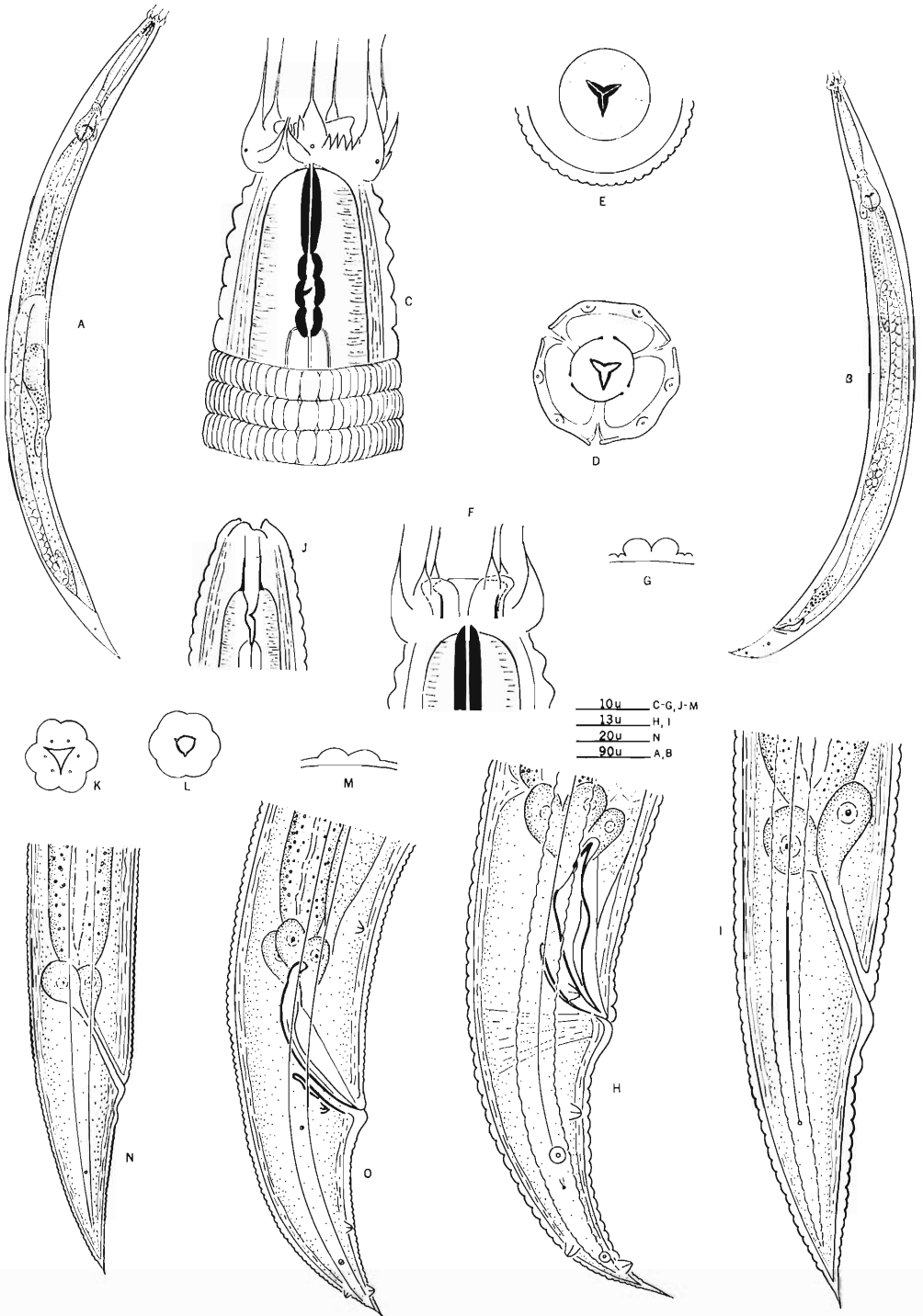
stom and metastom lightly cuticularized. Metastom funnel-shaped, with large dorsal tooth at anterior. Telorhabdions absent. Esophageal corpus beginning at base of prostom and expanded at posterior. Isthmus long and narrow; corpus 2–2.3 × longer than isthmus. Nerve ring at mid-isthmus. Excretory pore slightly posterior to nerve ring; ventral excretory cell next to or at base of esophageal bulb. Basal bulb 26–29 μ long by 20–22 μ wide, with valvular apparatus. Cardia 13 μ broad, 9 μ long. Intestine with abundant detritus in lumen. Prominent anal lip. Three rectal glands, possibly more in male.

FEMALE: Maximum body diameter at anterior lip of vulva; body narrows behind vulva. Post-vulval sac one body diameter long or less, containing sperm, often appearing as a rudimentary posterior ovary with oocytes, separated from muscular uterus by a constriction. Ovary prodelphic, reflexed past the vulva 55–62% of vulva-anus distance; 3 rows oocytes in posterior of ovary. Vulval lips inclined slightly posteriad. One ovum in uterus at a time, 70 × 18 μ. Tail conical, uniformly tapering, 2–2.6 anal body diameters long, with short mucro at tip. Conspicuous phasmids at 60–70% of tail length.

MALE: Testis about 450 μ long, reflexed ventrally about 2 body diameters; 2 staggered rows of large primary spermatocytes; 2 rows of smaller secondary spermatocytes; narrow vas deferens and muscular ejaculatory duct containing small globular sperm. Spicules 32–35 μ long; gubernaculum 13–15 μ long, more refractive than spicules. Tail conical, 1.4–1.7 anal body diameters long, with small terminal mucro. Six pairs of genital papillae: 1 preanal, 1 adanal, 1 subdorsal, 1 within alae, and 2 subventral postanal. Prominent phasmids a short distance behind anus.

TYPE HABITAT: Volcanic soil at edge of snow beneath algal mat (*Praseola crispa*).

TYPE LOCALITY: Just north of Adelie penguin rookery, Cape Royds, Ross Island, Antarctica. Other localities: Ross Island: soil with algae (*Praseola*), Rocky Point, about 2½ miles north of Cape Royds; soil with algae (*Oscillatoria*), Cape Evans; Victoria Land: mossy soil (*Bryum antarcticum*), Marble Point.

HOLOTYPE MALE: Collected by Dr. David Viglierchio, 4 January 1970; Smithsonian Collection, USNM 42974.

PARATYPES: USNM 42975–42985.

DISCUSSION: This species corresponds closely to *Panagrolaimus subelongatus* (Cobb, 1914) Thorne, 1937, as redescribed by Thorne (1937). However, the conspicuous dorsal tooth of the new species readily separates it from *P. subelongatus*.

The species is named after Dr. David Viglierchio, who was of invaluable assistance in making collections.

Eudorylaimus antarcticus (Steiner, 1916) Yeates, 1970 (Fig. 2, A–D)

= *Dorylaimus antarcticus* Steiner, 1916
= *Antholaimus antarcticus* (Steiner, 1916) Thorne and Swanger, 1936

Measurements of 10 males and females from Miers Lake:

♂♂ L = 2.02 (1.75–2.36) mm; a = 45 (40–53); b = 4.6 (4.3–5.5); c = 51 (40–60).

♀♀ L = 2.09 (1.93–2.28) mm; a = 38 (34–41); b = 4.8 (4.0–5.2); c = 52 (41–62); V = 51.8 (50.1–53.9)%.

Measurements of 10 males and females from Suess Pond:

♂♂ L = 2.01 (1.7–2.15) mm; a = 48 (43–53); b = 4.5 (3.9–5.1); c = 51 (42–60).

♀♀ L = 2.01 (1.60–2.22) mm; a = 40 (30–45); b = 4.8 (4.2–5.9); c = 46 (37–61); V = 51.8 (49.9–54.7)%.

DESCRIPTION: Body finely striated; small annules visible on tail and neck. Lateral field broad, about one-third body diameter wide, containing numerous globules. Lateral pores absent. Head distinctly set off; head diameter 17–20 μ. Six large lips subdivided into mamillate anterior part bearing inner circle of papillae and swollen posterior part bearing outer circle of papillae; all innervations very prominent. Amphids shield-shaped, about 50% head diameter broad; spiral-like terminal arborization of nerve endings at posterior of amphid. Spear 16–17 μ long or about one head diameter; aperture about one-third its length. Guiding ring less than one head diameter from anterior end. Spear extension surrounded by esophageal tissue. Esophagus expanded at 50–60% of its length. Nerve ring at 31–35% of esophageal length. Mass of large neurons between nerve ring and swollen posterior of esophagus. Cardia broadly conical, 20–23 μ long. Intestine composed of 6–8 cells in cross-
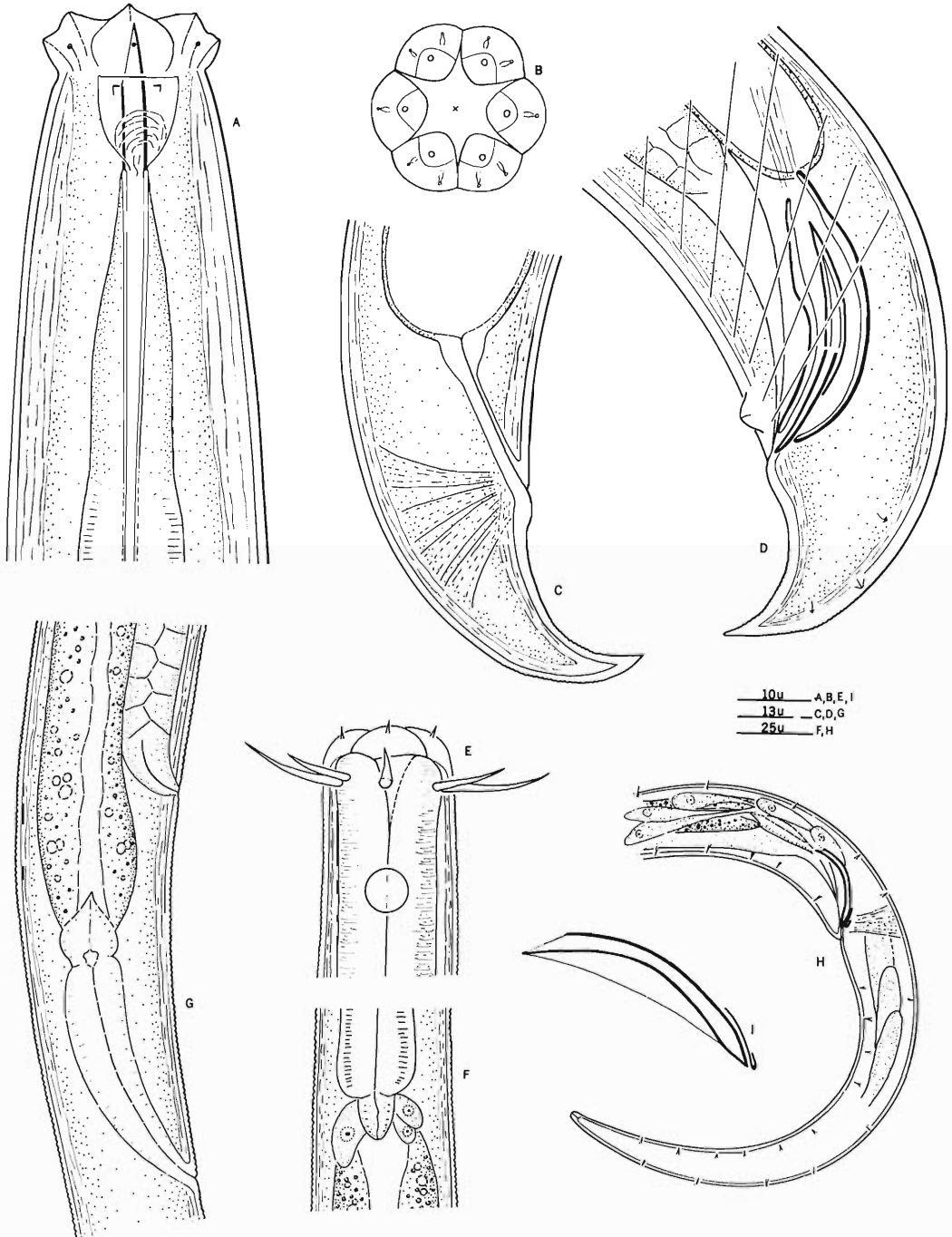
Figure 2. A–D: *Eudorylaimus antarcticus*. A—female head, B—en face view, C—female tail, D—male tail. E–I: *Monhystera villosa*. E—female head, F—esophageal base, G—vulva-anus region, H—male tail, I—copulatory apparatus.

section, containing fine green granules. Bright green alga often present in intestinal lumen, turning to red in posterior.

FEMALE: Ovaries amphidelphic, reflexed. Vulva a transverse slit; vagina thickened. Fusiform sperm often present in uterus in large numbers. One ovum at a time in each uterus, about $100 \times 50 \mu$. Tail ventrally curved, 1.3–2.0 anal body diameters long, ending in acute or narrowly-rounded tip. No papillae observed. Extent of prerectum from anus 3–6 anal body diameters.

MALE: Two outstretched testes; posterior of testes packed with spindle-shaped sperm about 12 μ long. Ejaculatory duct cells packed with small globules. Spicules thick, internally divided, 46–50 μ long; lateral guiding piece 17–19 μ long. One pair preanal papillae and 7–12 mamillate preanal supplements, variably spaced. One prominent pair of subdorsal papillae about mid-tail and two pairs of smaller papillae anterior and posterior to them. Prerectum extending almost to anteriormost supplement, 3.6–6.9 anal body diameters from anus; three large pairs of cells at anterior of prerectum, with "tails" leading posteriorly. Tail curved ventrally, with acute or narrowly-rounded tip, 1.0–1.6 anal body diameters long.

LOCALITIES: Hallett Station, Cape Adare (collected by Dr. Frank Strong); Ross Island: Lake Evans, soil from Turk's Head, mossy soil near McMurdo Station; Victoria Land: Suess Pond, Lake Chad, Lake Fryxell, Lake Bonney, Lake Vida, Lake Vanda, lower Wright Lake, Garwood Lake, Miers Lake, pond along Onyx River, runoff stream from Miers Glacier, soil 150 feet from Suess Glacier (collected by Mrs. Kay Lindsay), algal mat east of Meserve Glacier (collected by Mrs. Kay Lindsay), stony soil near Lake Vanda and Péwé Lake, moss from foot of Miers Glacier, from moraine just north of Cape Chocolate, from Strand Moraines and Marble Point, algal mat on stream bed at Strand Moraines, sandy soil at Strand Moraines.

SPECIMENS: USNM 42940–42947.

DISCUSSION: This species is very similar to *Eudorylaimus carteri* (Bastian, 1865) Andrássy, 1959, except that the tail is shorter. Bunt (1954) reported *E. carteri* from Macquerie Island in the Subantarctic. Steiner (1916) described this species (as *Dorylaimus antarcticus*) from Discovery Bay, S. Victoria Land, and Yeates (1970) described material from

Rocky Point, Ross Island. The area from which Yeates collected was still under snow in late January 1970 and no specimens were obtained from there. The species is common throughout Victoria Land. The internal thickenings of the lips recorded by Steiner (1916) and Yeates (1970) are only conspicuous neural connections.

Monhystera villosa Bütschli, 1873
(Fig. 2, E–I)

= *Monhystera villosa* var. *steineri* Micoletzky, 1922; = *M. australis* Cobb, 1893a nec Cobb, 1893b (= *M. pacifica* Johnston, 1938); = *M. impetuosa* Cobb, 1906.

10 males: L = 1.8 (1.04–1.41) mm; a = 52 (43–71); b = 4.9 (4.5–5.7); c = 8.2 (7.4–9.8).

10 females: L = 1.17 (1.05–1.26) mm; a = 42 (38–46); b = 4.7 (4.3–5.1); c = 8.9 (7.2–11.4); V = 82.6 (80.1–85.5)%.

DESCRIPTION: Cuticle finely striated. Pale granular hypodermal inclusions. Head as wide as neck, not set off. Six distinct lips, each bearing a small labial seta. Ten cephalic setae, the six longer setae about 12 μ or 60–82% of head diameter, the four shorter setae about one-half their length; longer setae variable in length; paramphidial setae not observed. Four rows of short submedian somatic setae in both sexes, 3–4 μ long. Amphids distinct, circular, about 6 μ in diameter or 30–43% of corresponding head diameter, located one head diameter from anterior. Esophagus slightly clavate, swollen a little at anterior end and more markedly at posterior end. Cardia variable in length, surrounded by 5–6 free cells. Small excretory gland cell 2–3 body diameters posterior to esophageal base, indistinct. Nerve ring at about 50% of esophageal length, inconspicuous. Intestine with scattered yellowish globules of various sizes. Conical tail, tapering to narrowly-rounded tip; caudal glands indistinct due to numerous cells in tail. Simple spinneret at tail tip.

FEMALE: Single anterior outstretched ovary; single row of oocytes. Prominent rectum.

MALE: Single outstretched testis; one row of spermatocytes. Prominent rectum. Copulatory spicules equal, arcuate, 33–37 μ long or about one anal body diameter, non-cephalate, broadest near tip; velum present. Short parallel gubernaculum about 11 μ long, with a short

lateral projection on either side. Four pairs of large granulate cells anterior to spicules and one small pair at spicule head. Tail bearing six pairs subdorsal and 10 pairs subventral setae.

HABITAT: Algal mat on soil, about 200 yards east of Meserve Glacier, Wright Valley, Victoria Land. Collected by Mrs. Kay Lindsay on 19 January 1970.

SPECIMENS: USNM 42948–42955.

DISCUSSION: The present specimens correspond well with the description of Bütschli except for the absence of long somatic setae in the female. Steiner (1916) also described both sexes but the somatic setae of the female were not longer than in the male. Micoletzky (1921) recognized the latter character as the basis for a separate variety. However, this character does not seem sufficient for the differentiation of a species or subspecies, unless the sexual dimorphism of somatic setae is verified in Bütschli's "typical variety." This species is cosmopolitan in distribution, with moss as the most common habitat.

Plectus antarcticus de Man, 1904
(Fig. 3, A–F)

= *Plectus belgicae* of Steiner, 1916 nec de Man, 1904
= *Plectus murrayi* Yeates, 1970 syn. n.

10 females from Marble Point: L = 0.98 (0.74–1.1) mm; a = 23.1 (20.1–27.2); b = 4.4 (3.8–4.7); c = 9.4 (8.3–10.5); V = 49.0 (45.3–50.5)%.

10 females from Strand Moraines: L = 1.1 (1.02–1.19) mm; a = 24.3 (21.5–27.6); b = 4.6 (4.3–4.7); c = 9.7 (9.1–10.3); V = 49.8 (48.2–50.3)%.

DESCRIPTION: Cuticle coarsely striated; annules about 1.5 μ wide. Lateral alae with three incisures, 6 μ wide or one-seventh body diameter; central incisure faint. Head non-striated, not set off; 6 large lips with deep indentations between them. Lips incurved at apex, appearing as a small lobe in focus below lip; one pair small curved cheilorhabdions at inner base of each lip. Lips bearing inner circle of papillae. Four cephalic setae opposite mid-prostom on second or third annule, about 3.5 μ long or 25% of corresponding head diameter, projecting forward at 45° angle. Amphids about 3 μ broad or one-fifth head diameter; aperture a descending spiral opposite posterior half of

stoma. Stoma cylindrical and thickly-cuticularized at anterior, funnel-shaped and narrow at posterior, 21–26 μ long. Esophageal corpus broad, scarcely distinguishable from isthmus. Esophageal bulb pyriform, valvate, about 26 × 26 μ. Cardia cylindrical, 13–17 μ long. Excretory pore opposite nerve ring or a little posterior. Nerve ring at 50–53% of esophageal length. Two ovaries reflexed almost to vulva; few oocytes, in one row; one ovum at a time in each uterus, 55–60 × 31–44 μ, with smooth shell. Vulval lips protruding. Tail narrowly conical-tapering, curved ventrally, 4.2–5.2 anal body diameters long, with prominent cuticularized spinneret at tip. Two pairs subdorsal and one pair subventral setae, the left subdorsal always closer to tail tip. Spinneret aperture 3 μ long.

LOCALITIES: Hallett Station, Cape Adare (collected by Dr. Frank Strong); mossy soil and melt pools with abundant algae (*Nostoc commune*) at Marble Point and Strand Moraines, algal mat east of Meserve Glacier (collected by Mrs. Kay Lindsay), pond along Onyx River. Undoubtedly, the distribution is much more widespread, but this smaller species of *Plectus* was not recognized as separate throughout the collections.

SPECIMENS: USNM 42928–42933.

DISCUSSION: De Man (1904) described this species from Graham Land, Palmer Peninsula, on the basis of a single fourth-stage female measuring 0.83 × 0.047 mm, with cephalic setae 3.5 μ long, stoma 33 μ, and tail 100 μ. The apical lobes of the lips in our specimens are undoubtedly the structure sketched by de Man as a cuticularized lobe. Yeates (1970) set up *Plectus murrayi* as a new species on the basis of 12 lips in one circle. However, specimens from his type locality show the lip region as described above. Yeates' specimens were obviously distorted by the harsh extraction process, since the esophagus of his Figure 1, A is greatly contracted. This could account for his shorter measurements and greater b values. I have examined four of his paratype slides (Nos. 90/6/2, 90/6/17, 90/6/19, and 90/6/37), kindly sent by Rothamsted Experiment Station, and all specimens are much contracted.

Kirjanova (1958) gave the following demanian values for this species: L = 826 μ; a = 24.2; b = 4.4; c = 8.4. The ovum measured 60 × 27 μ. This species is very similar to
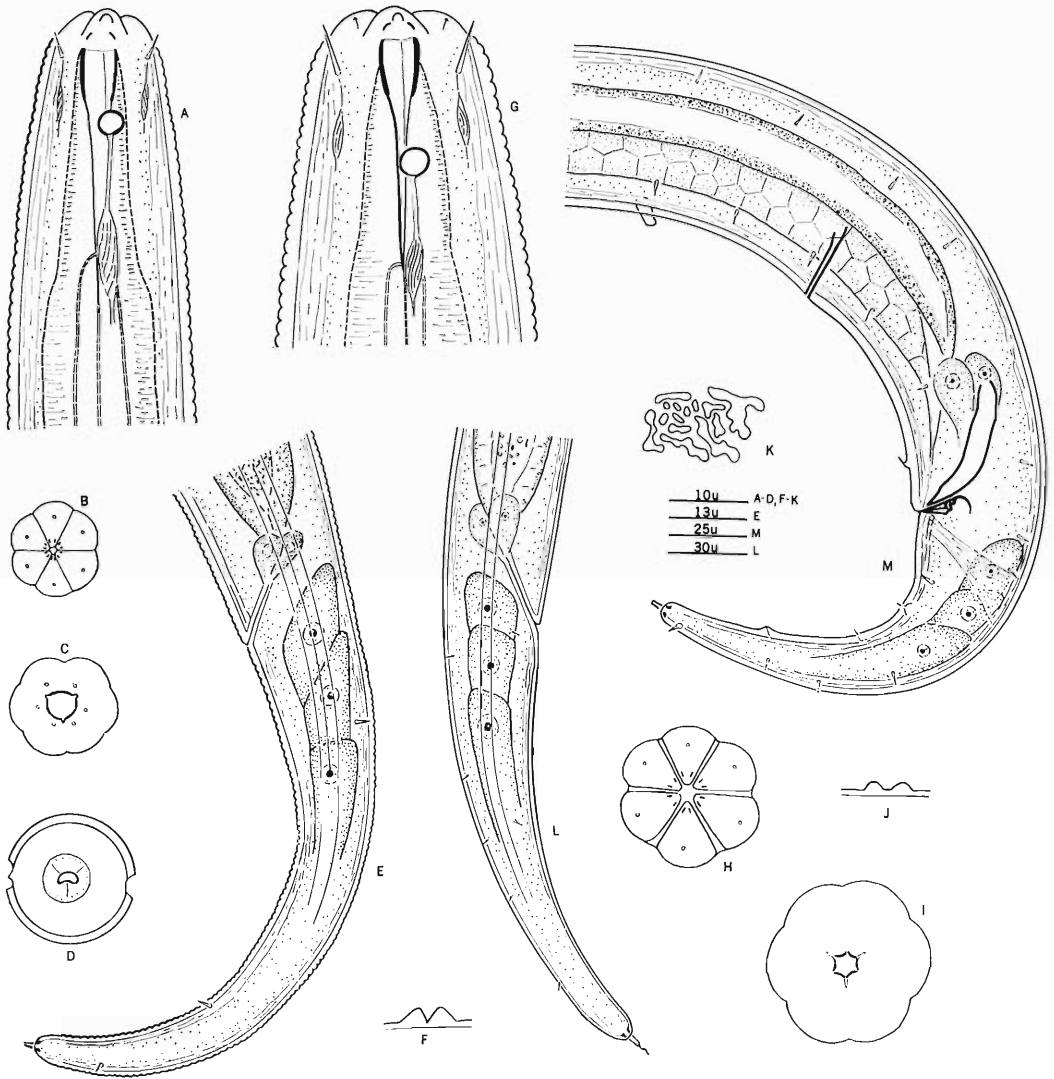
Figure 3. A–F: *Plectus antarcticus*. A—female head, B—en face view, C—section through prostom, D—section through meso-metastom, E—female tail, F—section through lateral field. G–M: *Plectus frigophilus*. G—female head, H—en face view, I—section through anterior of prostom, J—section through lateral field, K—pattern on ovum, L—female tail, M—male tail.

Plectus cirratus Bastian, 1865 in most measurements and in tail shape (cf. Maggenti, 1961), but the latter has four tail setae instead of three and the meso-metastom is broad and cylindrical in all the specimens of *P. cirratus* in the University of California Nematode Collection, Davis. Bunt (1956) reported *P. cirratus* from Macquerie Island. Maggenti (1961)

synonymized *P. antarcticus* with *P. parietinus* Bastian, 1865, but the former does not have the large, distinct lip region of the latter. I was not able to find any nematodes in the pond at the foot of Observation Hill where Dougherty (in Dougherty et al., 1960) collected *P. parietinus* in December 1959. The pond did not thaw at all during the austral summer of 1969–

70 and no nematodes were found in bottom samples made by breaking through the thin ice at the shore.

Plectus frigophilus Kirjanova, 1958
(Fig. 3, G–M)

10 females from Marble Point: L = 1.72 (1.40–1.99) mm; a = 26.4 (22.2–32.5); b = 4.7 (4.4–5.2); c = 11.6 (9.7–12.3); V = 48.3 (46.1–50.5)%.

10 females from Strand Moraines: L = 1.95 (1.54–2.06) mm; a = 27.1 (25.7–30.0); b = 4.9 (4.5–5.1); c = 12.1 (10.5–13.5); V = 48.3 (46.1–50.0)%.

1 male from Dunlop Island: L = 1.44 mm; a = 28.9; b = 4.4; c = 11.

DESCRIPTION: Body large and plump, dark colored, coarsely striated; annules about 1.5 μ wide. Longitudinal alae 6 μ wide at mid-body or about one-eleventh of body diameter; two incisures. Head not set off; lip region unstriated; six distinct lips, each bearing a central papilla; apex of lips turned inwardly. Four cephalic setae, 5–6 μ long or 25% of corresponding head diameter, located on second or third annule. Amphids descending spiral, 4 μ broad or one-sixth of head diameter, located opposite anterior of mesometastom. Stoma 29–31 μ long; 12 small curved cheilorhabdions at inner base of lips. Prostom more thickly cuticularized than narrow funnel-shaped meso-metastom and not surrounded by esophageal tissue. Esophageal bulb 40–41 μ long × 33–39 μ broad. Cardia 20 μ long. Excretory pore just behind nerve ring. Nerve ring at 48–52% of esophageal length. Green algae often present in intestine, and abundant sporozoan parasites in intestinal cells of many specimens.

FEMALE: Amphidelphic ovaries, reflexed to vulva or beyond; 2–3 rows of oocytes in ovary. Up to two ova in each uterus at a time, 70–78 μ × 39–52 μ. Rough reticulate pattern on egg shell. Tail conical, ventrally arcuate, 3.9–4.5 anal body diameters long; 3–5 pairs of subdorsal setae, 2–3 pairs of subventral.

MALE: Two outstretched testes, the anterior somewhat longer. Esophageal bulb 35 × 26 μ. Tail conical, 3 anal body diameters long, with 7 pairs of subdorsal and 4 pairs of subventral setae; second and fourth subdorsal and last subventral pair with swollen base. Broad thin spicules, slightly cephalate. Gubernaculum irregularly triangular, with curved dorso-pos-

terior apophysis and short obliquely lateral process at distal end. One large tuboid supplement and a non-cuticularized ventral projection anterior to supplement.

LOCALITIES: Hallett Station, Cape Adare (collected by Dr. F. Strong). Ross Island: soil from Cape Royds, Cape Evans, Cape Crozier, and McMurdo Station; lake at Cape Evans. Victoria Land: Suess Lake, Lake Vanda, lower Wright Lake, Conopus Pond (collected by Mrs. Kay Lindsay), Garwood Lake, Péwé Lake, Lake Chad, Lake Fryxell, Lake Bonney, pond on Dunlop Island (collected by Dr. W. Becker), pond along Onyx River, runoff stream from Miers Glacier, stream with abundant algae at Strand Moraines, melt pools with abundant algae (*Nostoc commune*) at Marble Point, soil near Suess Glacier, Lake Bonney, Canada Glacier, Lake Vanda, sandy soil at Strand Moraines, Mossy soil at Marble Point, Strand Moraines, foot of Miers Glacier, moraine just north of Cape Chocolate.

SPECIMENS: USNM 42934–42939.

DISCUSSION: *Plectus frigophilus* was regarded by Maggenti (1961) as a *nomen dubium*. The measurements of our specimens conform with those of Kirjanova (1958) in all details, including the size of the ova. This is the largest of the valid *Plectus* species and in the surface markings on the shell of the ovum resembles ova in the uterus of *P. parietinus*. Allgén described three large species of *Plectus* from Graham Land and the Falkland Islands (vide Allgén, 1959). Because of the poor descriptions they must be considered *species inquirendae*. *P. frigophilus* resembles *P. antarcticus* in most features except measurements, but differs in the presence of only two incisures and of ova bearing reticulate markings. Because the species have not been found elsewhere and because of their close relationship in head and tail characteristics it is possible that the two species may have evolved in the Antarctic.

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The Incidence of Fascioliasis of Sheep and Cattle in the Southwest with Observations on the Snail Vectors[1]

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ABSTRACT: Approximately 49% of the sheep and 30% of the cattle examined in certain areas of southwestern Colorado, northern New Mexico, and eastern Arizona were infected with the liver fluke, *Fasciola hepatica*. Such infection rates are indicative of a marked economic loss through morbidity, mortality, and liver condemnations. Three snail vectors of liver fluke were found in these areas. One species, *Stagnicola bulimoides techella* from southern Colorado, was found to be naturally infected and was also infected experimentally. The other two species, *Stagnicola palustris* and *Fossaria modicella*, were found in all three areas surveyed and were infected experimentally only. All three of these vectors were quite resistant to experimental *Fasciola hepatica* infection except when less than 30 days old. *Stagnicola bulimoides techella* and *S. palustris* were found in ponds or slow-moving water on irrigated pastures, whereas *Fossaria modicella* was found on the mud in bogs and along stream banks.

The common liver fluke, *Fasciola hepatica*, has been reported from sheep and cattle in the Southwest (Price, 1953; Becklund and Allen, 1958; Maddy, 1954, 1955), but the geographical distribution and incidence of this parasite as well as the identity of its snail vectors have not been specifically defined. The work reported here was conducted intermittently from 1955 to the present. Some of the observations were previously reported in abstract (Wilson and Samson, 1967).

Methods

Incidence

Over a period of years, fecal samples were collected from both sheep and cattle on farms

Table 1. Incidence of fluke infection in sheep and cattle on some irrigated pastures in the Southwest.

Locality	Date of survey	Sheep			Cattle		
		No. examined	Per cent infected	No. herds checked	No. examined	Per cent infected	No. herds checked
Chama, New Mexico	4/11/63	6	100	1			
	9/12/63	41	10	3			
	6/20/67				37	11	4
	3/23/70	22	86	2			
Total		69	42	6	37	11	4
Aztec, New Mexico	11/ 3/61	38	53	3			
	9/12/62	9	55	2			
	6/20/67				42	57	5
Total		47	53	5	42	57	5
Ignacio, Colorado	9/12/62	26	65	1			
	10/25/63	12	92	1			
	9/21/64	22	54	1			
	6/28/66	10	60	1			
	9/28/66	20	95	1			
	6/20/67				33	55	3
Total		90	72	5	33	55	3
Springerville, Arizona	2/14/55				43	21	11
	9/ 7/56				33	6	9
	6/ 8/61	43	12	9			
	6/18/68	15	27	2	52	29	6
Total		58	15	11	128	20	26
GRAND TOTAL		264	49	27	240	30	38

and ranches in the vicinity of Chama and Aztec, New Mexico; Ignacio, Colorado; and Springerville, Arizona. These areas were selected because of pasture habitats favorable to the intermediate hosts of *F. hepatica.* Individual fecal samples were collected at random at the time of deposition on pasture and were examined qualitatively for fluke ova using the method of Dennis et al. (1954).

Identity of the snail vectors

Specimens of all snail species found in the vicinity of the enzootic areas referred to above were collected and identified. Approximately 5,000 snails were collected representing 5 lymnaeid and 6 non-lymnaeid species. Roughly half of the snails were crushed and examined for larval stages of *F. hepatica.* The remaining snails were then exposed to 3 to 200 miracidia of *F. hepatica* per snail in the laboratory. Snail eggs were also collected from this latter group, and laboratory cultures of four species, *Fossaria modicella, Stagnicola palustris, S. bulimoides techella,* and *Physa anatina* were established. When the cultured snails were 3 to 134 days old, they were exposed to 3 to 200 miracidia each.

Since field-collected snails were not equally abundant and since all species did not survive equally well under laboratory conditions, the number of species available for study varied considerably.

Snails were reared at about 20 C in non-chlorinated water and fed lettuce and boiled cottonwood leaves. Water was changed three times a week or oftener. Calcium carbonate was added to most cultures but did not appear to be essential.

Fasciola hepatica eggs were obtained from the gall bladders of infected sheep or from the uteri of mature flukes. Eggs were put in Syracuse watch glasses with a small amount of water and kept in a dark room at 25 C for a minimum of 12 days before they were exposed to light to stimulate hatching of miracidia.

Results and Discussion

Incidence of infection

A total of 264 sheep and 240 cattle were examined for evidence of *F. hepatica* infection. The numbers examined, the percentage found infected, and the number of farms and ranches involved in each of the four enzootic areas are shown in Table 1.

The high incidence and general distribution of liver fluke infection revealed by the surveys confirm that liver fluke is quite prevalent throughout these areas. It may be even more widespread than the surveys indicate. The intermediate hosts are known to be present in other areas, and future surveys may greatly enlarge the enzootic fluke areas. The incidence data reported here may be conservative because: (1) Some of the animals examined may not have been on contaminated pastures long enough to show evidence of infection, (2) some infected animals may have been missed because of a failure to detect eggs in their fecal samples, and (3) some animals may have been negative because of recent treatment.

These results serve to emphasize the economic importance of the common liver fluke as discussed by Olsen (1949), Price (1953), Maddy (1954), and others.

Natural infections of field-collected snails

Of the 2,500 snails examined by crushing, only one snail of one species, S. b. techella, was found to be naturally infected with F. hepatica. Identity of the fluke was confirmed by feeding shed metacercariae to a domestic rabbit and recovering adult flukes. Since about 1,000 S. b. techella were crushed, the incidence of infection was about 0.1%. Olsen (1944) reported 0.51% of the S. b. techella near Angleton, Texas to be infected.

Species and approximate numbers of snails in which no larvae of F. hepatica were found by crushing are as follows: S. palustris, 600; Fossaria modicella, 500; F. obrussa, 50; F. perplexa, 50; P. anatina, 100; P. elliptica, 50; Oxyloma haydeni, 25; O. retusa, 25; Succinea forsheyi, 50; and Gyraulus circumstriatus, 50.

Because of the difference of opinion among taxonomists as to the classification of the Lymnaeidae, representative samples from the snails collected were submitted to the U. S. National Museum for identification, and the species were designated accordingly. In the literature these same species may be referred to using the classification of Hubendick (1951) in which both Stagnicola and Fossaria are referred to the genus Lymnaea and all the species of Fossaria listed above are synonymized under Lymnaea humilis.

Experimental infections of field-collected snails

Although miracidia were seen to penetrate individuals of some species (S. palustris, S. b. techella, and F. modicella) no cercariae were obtained from any experimentally infected field-collected snails. The numbers of each species exposed were approximately the same as those examined for natural infections above. Failure to establish infection in any of the lymnaeid species, some of which had been reported as vectors elsewhere, was attributed primarily to the age of the snails at the time of exposure. Krull (1934) found that only very young Lymnaea traskii (= L. palustris according to Hubendick, 1951) were susceptible to infection. Boray (1966) also found that only young snails of several species were susceptible to infection and that some were difficult to infect at any age. Reduced susceptibility of field-collected snails might also have been due to previous infections with the larvae of other fluke species which were present in many snails. Lie et al. (1968), Boray (1967), and Vernberg et al. (1969) have shown that there is an antagonism between the larval stages of many species of flukes when present in an individual host. However, Wright (1968) cites studies in which double infections in some populations were higher than would be expected by random distribution.

Experimental infections of laboratory-reared snails

In 1966 three species of young laboratory-reared snails became infected and produced cercariae, S. b. techella (1% of 2,000), S. palustris (3% of 500), and F. modicella (1% of 500), but complete infectivity records were not kept. Incidences of infection, however, were similar to those in 1967–68 which are shown in Table 2. Although the infection rate in all three species was low, some heavily infected snails, particularly F. modicella, died before shedding cercariae due to complete destruction of the digestive gland. Krull (1933) also had difficulty rearing infected F. modicella in the laboratory. Similarly, Boray (1966) was able to infect many juvenile S. palustris but harvested metacercariae from only 2 of 550 snails exposed. As expected, the non-lymnaeid species, P. anatina, did not become infected.

Table 2. Experimental infections of laboratory-reared snails with *Fasciola hepatica*.

Snail species	No. snails exposed	Age of snails in days	No. miracidia per snail	No. snails producing viable metacercariae
S. b. techella	197	14–21	3–6	4
	275	23–38	3–20	0
	48	61–81	5–10	0
S. palustris	152	3–14	3–100	0
	195	15–21	5–10	2
	145	22–28	3–20	11
	228	29–123	5–15	0
F. modicella	20	5–9	5	0
	219	14–36	5–200	2*
	41	81–134	5–10	0
P. anatina	25	7	5	0
	100	14–30	10	0
	25	31–46	10	0

* Two of 12 snails 14–30 days old exposed to 5 miracidia per snail produced metacercariae.

The surveys reported here indicate that *S. palustris*, *S. b. techella*, and *F. modicella* are all potential vectors in southern Colorado but that only *S. palustris* and *F. modicella* have been found in endemic areas of northern New Mexico and eastern Arizona. In addition to minor differences in geographical distribution, there appear to be some habitat differences. *Stagnicola bulimoides techella* and *S. palustris* were collected from beneath the surface of the water in ponds or slow moving flooded areas on pastures. We found *Fossaria modicella* on the mud, especially in bogs or in areas of seepage as did Van Cleave (1935).

We found *S. palustris* to be our best laboratory source of metacercariae even though the infection rate was only 3% in 1966 and 1.8% in 1967–68. *Fossaria modicella* and *S. b. techella* were more readily infected but many infected snails either lost their infections or died before producing metacercariae.

Boray (1966) found that *S. palustris* was much less susceptible to infection than were *Lymnaea tomentosa* or *L. truncatula*. The similarity of the rates of infection for *S. palustris* from his study and from the surveys reported here indicate that all three species from these surveys are less efficient vectors than are *L. tomentosa* or *L. truncatula*. The high reproductive capacity of the fluke apparently resulted in a relatively high incidence of infection in the survey animals even though only a small percentage of the available snails became infected.

We wish to thank Dr. Joseph Morrison of the U. S. National Museum and Dr. Artie Metcalf, University of Texas at El Paso, for assistance in identifying the snail species involved in this study.

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Comparative Respiration of the Life Cycle Stages of *Paragonimus ohirai*, Miyazaki, 1939[1]

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ABSTRACT: The respiration of four stages of the *Paragonimus ohirai* life cycle was studied. Miracidia, cercariae, excysted metacercariae, and adults consumed oxygen, but encysted metacercariae did not. The effect of exogenous glucose on the respiration of miracidia, cercariae, and adults was also studied. Glucose did not alter the respiratory pattern of miracidia at 1 hr after hatching. However, at 5 hr after hatching, respiration of miracidia was decreased. The respiration of cercariae was increased under the influence of glucose. Likewise, the respiration of adult *P. ohirai*, measured 25 hr after collection, was increased when glucose was added to the system, while at 1 hr after collection, respiration was decreased.

Paragonimus ohirai, Miyazaki, 1939, is one of the mammalian lung flukes common to Japan. Information pertinent to its development in intermediate and definitive hosts has been published elsewhere (Ogita, 1954; Ikeda, 1957; Yokogawa et al., 1958; Yoshida and Miyamoto, 1959, 1960; Kawashima, 1961, 1965; and Kawashima and Miyazaki, 1963). This species differs from *P. westermani*, the species responsible for paragonimiasis in man, in its morphological characteristics and in its specificity for certain hosts. The life cycle, however, is similar in other respects to that of *P. westermani*. Attempts to maintain the complete life cycle stages of *P. westermani* in this laboratory have not been successful; however, the life cycle of *P. ohirai* has been routinely maintained by one of us (J.E.W.) for the past two years. This provides a system for physiological studies of paragonimiasis. In the present study the influence of glucose upon the respiratory patterns of miracidia, cercariae, metacercariae, and adults are compared.

Materials and Methods

Maintenance of *P. ohirai* in the laboratory

PROCEDURE FOR OBTAINING METACERCARIAE: Naturally infected *Sesarma dehaani* crabs were collected in October 1968, from Shimoda City, Shizuoka Prefecture, Japan. The metacercarial cysts of *Paragonimus ohirai* were obtained from the crabs by grinding the quartered bodies and

legs in tap water at a ratio of 50 ml water per crab. The resulting suspension was filtered through a 40 mesh screen into a one liter conical graduate. After 15 min, the supernatant fluid was discarded, and the sediment resuspended by again filling the graduate with tap water. The washing procedure was repeated until the supernatant fluid became clear. The bottom 100 ml remaining in the graduate, and containing the sediment, was poured into 10 oz plastic glasses. After 15 min, the supernatant fluid was discarded and the sediment resuspended in tap water. This procedure was repeated until no tissue particles could be seen in the supernatant fluid. The metacercarial cysts were removed from the sediment employing a micropipette under a dissecting microscope.

PROCEDURE FOR INFECTING RATS: Adult rats of either sex were anesthetized by intramuscular injection of Sernylan (Parke-Davis and Co., Detroit, Michigan). A 7.5 in. length (0.047 in. inside diameter) of polyethylene tubing was used as a stomach tube. A micropipette containing 30 freshly collected metacercariae was inserted into the tubing and the metacercariae were forced into the stomach. The pipette and tubing were rinsed several times and the tubing checked for the presence of cysts. If more than 5 cysts remained, the exposure procedure was repeated. Laboratory studies indicate that with this procedure, metacercariae will produce infection rates of 40% to 60%. Lower infection rates were observed in animals exposed to excysted metacercariae.

PROCEDURE FOR HARVESTING ADULT WORMS: Fifty days after exposure, rats were euthanized with chloroform. This maturation period was critical, since studies indicated that peak egg production occurred at this time. A few days after this period, signs of worm deterioration were observed.

An incision was made ventrally through the skin of the thorax and abdomen from the neck to the pelvis. The rib cage was exposed, then the diaphragm was separated from the rib cage. If adhesions were present, the lungs were freed from the rib cage, then each half of the rib cage was cut away along the sternal line. The vena cava was ligated anterior to the diaphragm, and the lungs and heart were removed and placed in 0.9% saline.

The purple-brown parasitic cysts appeared on the surface of the lungs and measured 0.5 to 1.0 cm in diameter. An incision was carefully made through the base of each cyst wall. The liquid contents of the cyst, which contained many eggs, were recovered by aspiration and placed in 0.9% saline. Pressure was exerted on the cyst wall, forcing the adult worms out through the incision. The worms were placed in the same Petri dish as the cyst fluids and allowed to stand for 30 min to shed their eggs and flush out their intestinal contents. They were then available for respiration studies.

PROCEDURE FOR EGG RECOVERY, MATURATION, AND HATCHING: The eggs from the above Petri dishes were pooled in a one-liter beaker. After 30 min, the supernatant fluid was discarded and the eggs resuspended in distilled water. This procedure was repeated until the supernatant fluid was clear. Eggs were stored in a refrigerator and used as needed.

Maturation was accomplished by placing the suspension of eggs in a 26 C incubator for 16 days with twice weekly changes of water. At the end of this period eggs were hatched by transferring them to a small Stender dish containing 25 ml of aerated, dechlorinated tap water. The dish was placed in an ice bath and after 5 min was transferred to a black surface 2½ feet from a 500W photoflood lamp for 10 min. This sudden change of temperature or "cold shock" treatment stimulated the hatching of eggs. Most miracidia hatched from eggs at 1 hr after the treatment.

PROCEDURE FOR EXPOSING *Assiminea parasitologica* SNAILS: Fifty *A. parasitologica* adult snails were exposed to approximately 500 miracidia (10 miracidia/snail) in a Petri dish containing aerated distilled water. After 16 hr of exposure, the snails were placed in plastic boxes (23 × 30 × 9 cm), the bottoms of which were covered with soil taken from the snails' natural habitat. The soil was moistened daily with distilled water, and rice baby food powder was added at weekly intervals as a food source. Snails were found to be infected with mature cercariae 90 days after exposure.

PROCEDURE FOR OBTAINING CERCARIAE: Infected snails were placed individually into small shedding chambers containing 2.0 ml aerated, dechlorinated tap water. The chambers were placed 30 cm beneath a fluorescent light (40W) at room temperature (22–23 C). After 5 hr, cercariae were collected and used

for the exposure of crabs or for respiration studies.

INFECTION OF S. *dehaani* CRABS: S. *dehaani* crabs were exposed individually to 300–500 cercariae, depending on the size of the crab. After 16 hr, the crabs were removed from the exposure chambers and were placed in styrofoam boxes (41 × 41 × 30 cm) provided with a screened window. The floor of the box was covered with slanting humus dirt substrate and ⅓ of the surface area was covered with water. Crabs were maintained at 20–23 C and quartered apples were provided at weekly intervals as a food source. Sixty days after exposure, mature metacercariae were harvested for rat infections or respiration studies as previously described.

Physiological determinations

RESPIRATION OF LARVAL STAGES: The respiration of the larval stages of *P. ohirai* was measured by means of an oxygen monitor according to the technique of Bruce et al. (1971). The buffer system used throughout the studies on larval stages was 0.01 M "Hepes" buffer, pH 7.6 (Good et al., 1966). Miracidia were obtained 1 hr after hatching (2 hr after "cold shock" treatment) and were divided into two portions. One portion was set aside at room temperature for later studies. The remaining portion was concentrated by filtration through a 1.2 μ Millipore filter, followed by three rinses of sterile air-saturated "Hepes" buffer (Bruce et al., 1969). Respiration was measured for 1 hr on these miracidia which were defined as having a mean age of 1 hr after hatching. Four hours later the remaining miracidia were concentrated in the same manner. Respiration of these was defined as that of miracidia with a mean age of 5 hr after hatching.

Two experiments were done with miracidia. In Experiment I, the respiration of miracidia in air-saturated "Hepes" buffer at 1 and 5 hr after hatching was measured. Two trials were made for miracidia at 1 hr after hatching (7,600 and 18,200 miracidia per trial) and three trials for miracidia at 5 hr after hatching (2,200–5,300 miracidia per trial). In Experiment II, the respiration of miracidia in air-saturated "Hepes" buffer containing 0.001 M glucose was measured. Two trials were made for each posthatching interval using 3,700 and 6,500 miracidia per trial at 1 hr after hatching and 5,000 miracidia in both trials at 5 hr after hatching.

In Experiment III, the respiration of cercariae of *P. ohirai* in air-saturated "Hepes" buffer, with and without glucose, was measured. Three trials (700–1,200 cercariae per trial) were made without glucose and two trials (900 and 1,600 cercariae) with 0.001 M glucose. Cercariae were collected 5 hr after snails were placed in the shedding chambers. The collection procedure required an additional hour. Approximately 1 hr was involved in concentrating the cercariae with three washes of buffer and a 1.2 μ filter. Because a long time (7 hr) was required to obtain and prepare cercariae for respiration studies, and because survival rates overnight were low, respiration studies were performed only after collection (mean of 5 hr after shedding) and were not attempted on older cercariae.

The respiration of encysted *P. ohirai* metacercariae in air-saturated "Hepes" buffer was measured in Experiment IV. Three trials were done using 45–425 metacercariae per trial. Two trials (Experiment V) were done to determine the respiration of excysted metacercariae using 320–354 metacercariae per trial. In these trials, encysted metacercariae were recovered from crabs and washed with sterile "Hepes" buffer. The cysts were left at room temperature overnight to promote excystment. The following morning the excysted metacercariae were removed, washed with sterile "Hepes" buffer, and enumerated. Their respiration was measured with an oxygen monitor in air-saturated "Hepes" buffer.

In all experiments involving larval stages, microscopic examinations were made before and after respiration was measured to determine the condition of the particular stage being studied.

RESPIRATION OF ADULT WORMS: The buffer (pH 7.7) used for washing adult worms and respiration studies had the following composition: 0.137 M NaCl, 0.0085 M KCl, 0.0003 M CaCl$_2$, 0.005 M MgCl$_2$, and 0.006 M Na$_3$PO$_4$ (Bueding, 1950). Adult worms were obtained immediately after collection from the rat lung and cleared for 30 min in 0.9% saline (see above procedure for collecting adult worms). The worms were then washed in sterile buffer, examined under a microscope and 30 intact worms selected for study. These were divided
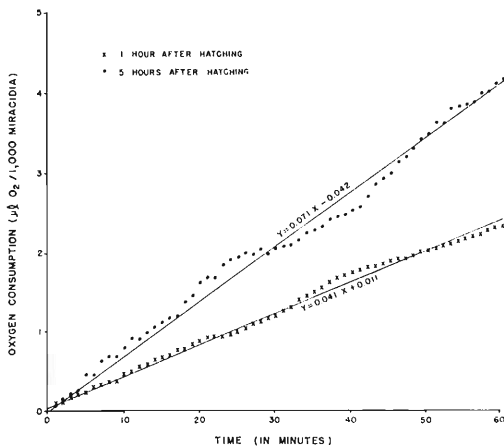
Figure 1.　Oxygen uptake by *P. ohirai* miracidia in air-saturated "Hepes" buffer (pH 7.6; T = 26 C), Experiment I. Each point represents the mean of 2 and 3 determinations at 1 hr and 5 hr after hatching, respectively. Regression equations are as shown.
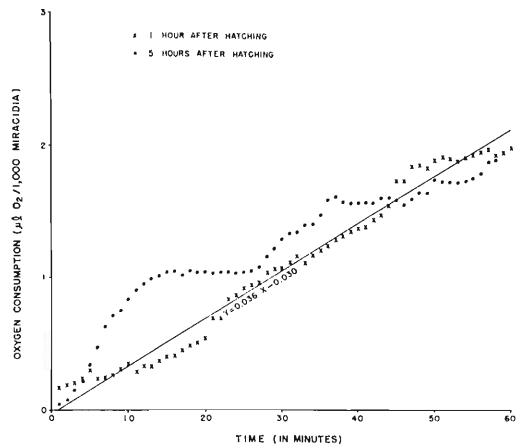
Figure 2.　Oxygen uptake by *P. ohirai* miracidia in air-saturated "Hepes" buffer (pH 7.6; T = 26 C), containing 0.001 M glucose, Experiment II. Each point represents the mean of 2 determinations. Regression equation is as shown.

into 6 groups of 5 worms each. Each group was placed in a single arm Warburg flask with 3.0–5.0 ml of sterile buffer depending on the size of the flask. To the center well of each flask 0.2 ml of 20% KOH was added. The flasks were attached to manometers, placed in the Warburg water bath (37 C) and 5 min allowed for temperature equilibration. Two additional flasks containing no worms were used as thermobarometers. Respiration was read every 15 min for 1 hr. This trial was designated as respiration of adult worms "immediately" after collection. At the end of the trial, worms were removed, washed in fresh buffer and stored overnight at 4 C.

Twenty-four hours after the start of the previous trial, respiration of the worms was again measured following the above procedure. This was defined as respiration of aged adult *P. ohirai*. Following this trial, worms were individually placed on weighing pans, dried at 100 C for 24 hr and the dry weights of each worm determined. Dry weights of worms in each group were used with the Warburg measurements to calculate the μl O_2/mg dry weight/hr consumed.

Two experiments were done with adult *P. ohirai*. In Experiment VI (2 trials), the immediate and aged respirations of adult worms were measured (6 flasks/trial, 5 worms/flask).

In Experiment VII, the immediate and aged respiration of adult worms were measured in buffer containing 0.004 M glucose.

Results

Respiration of miracidia (Experiments I & II)

The results obtained in the study of the respiration of miracidia in "Hepes" buffer at 1 and 5 hr after hatching (Experiment I) are shown in Figure 1. Analysis of the respiratory pattern showed a direct linear relationship between time and the amount of oxygen consumed at both 1 and 5 hr after hatching. The respiratory rate at 5 hr after hatching was significantly greater (P = 0.01) than at 1 hr after hatching.

When 0.001 M glucose was added to the buffer system, miracidia at 1 hr after hatching showed a linear respiratory pattern. Miracidia at 5 hr after hatching had a nonlinear respiratory pattern which consisted of periods of oxygen utilization alternating with periods when no oxygen uptake was detected by the oxygen monitor (Figure 2).

There was no significant difference in the respiratory pattern of miracidia tested at 1 hr after hatching in the presence or absence of glucose. Since oxygen uptake of miracidia in
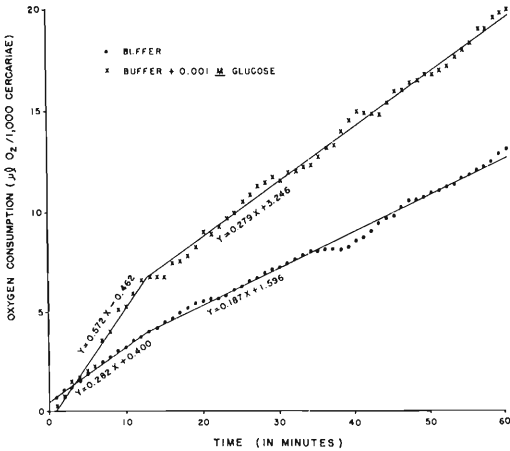
Figure 3. Oxygen uptake by *P. ohirai* cercariae in air-saturated "Hepes" buffer (pH 7.6; T = 26 C), Experiment III. Each point represents the mean of 2 and 3 determinations for the buffer system with or without added glucose (0.001 M), respectively. Regression equations are as shown.
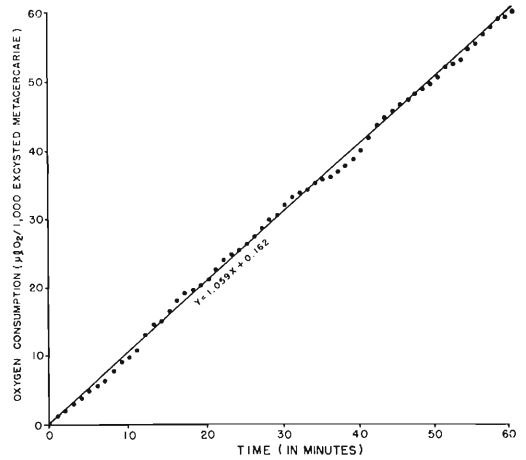

Figure 4. Oxygen uptake of excysted metacercariae in air-saturated "Hepes" buffer (pH 7.6; T = 26 C), Experiment V. Each point represents the mean of 2 determinations. Regression equation is as shown.

buffer with glucose at 5 hr after hatching was not linear, no valid comparison could be made with the linear respiratory pattern of miracidia of the same age in the absence of glucose; however, at the end of the experimental trial period (60 min) these 5 hr miracidia in buffer with glucose had utilized approximately 50% less oxygen than those in buffer alone (1.98 μl O_2/1,000 miracidia, compared with 4.22 μl O_2/ 1,000 miracidia, respectively).

Respiration of cercariae (Experiment III)

The respiratory pattern of *P. ohirai* cercariae in the presence or absence of glucose is shown in Figure 3. Analysis of these respiratory patterns showed that oxygen utilization of cercariae in the presence or absence of glucose did not follow a single linear regression line throughout the course of the experiment (60 min). Rather, the analysis showed that oxygen uptake was linear for approximately 14 min from the start of the experiment, after which the rate of oxygen uptake decreased and a different linear relationship of oxygen uptake was obtained. Oxygen uptake in both the initial (14 min) and latter portion of the experiment was significantly greater with the

presence of glucose than in the absence of glucose.

Respiration of metacercariae (Experiments IV & V)

No oxygen uptake was detected in encysted metacercariae. By contrast, a high rate of oxygen uptake was measured in excysted metacercariae (Figure 4).

Respiration of adult worms (Experiments VI & VII)

Respiratory quotients of adult *P. ohirai* worms in the presence or absence of glucose are shown in Table 1. The average QO_2 value obtained for worms in the absence of glucose (Experiment VI) at 1 hr after collection was significantly greater ($P = 0.01$) than the QO_2 values of worms at 25 hr after collection (2.238 compared with 0.620 for 1 and 25 hr after collection, respectively). In the presence of glucose (Experiment VII) no significant differences were found in the average QO_2 values of worms at 1 and 25 hr after collection.

A comparison of results obtained for Experiments VI and VII showed that the addition of glucose to the buffer system significantly decreased the average QO_2 value for worms at

Table 1. Respiration of adult *Paragonimus ohirai*.[1]

Experiment no. (substrate)	Trial[2] no.	Hours after collection	Oxygen consumption (μl O$_2$/hr)	Dry weight of worms/flask (mg)	QO$_2$ (μl O$_2$/mg dry wt/hr)	Average QO$_2$
VI (buffer)	1	1	28.11 ± 6.72	13.76 ± 2.06	2.094 ± 0.645	
	2	1	28.87 ± 9.68	12.45 ± 1.49	2.381 ± 0.906	2.238 ± 0.786
	1	25	12.44 ± 5.88	13.76 ± 2.06	0.880 ± 0.313	
	2	25	4.31 ± 2.15	12.45 ± 1.49	0.359 ± 0.202	0.620 ± 0.271
VII (buffer +0.004 M glucose)	1	1	20.92 ± 4.83	14.78 ± 4.11	1.532 ± 0.573	
	2	1	19.89 ± 6.09	14.50 ± 2.18	1.401 ± 0.444	1.466 ± 0.512
	1	25	17.45 ± 5.78	14.78 ± 4.11	1.200 ± 0.425	
	2	25	16.26 ± 3.85	14.50 ± 2.18	1.106 ± 0.290	1.153 ± 0.363

[1] The experiments were performed with 5 adult worms/flask containing 3.0–5.0 ml of buffer composed of 0.137 M NaCl; 0.0085 M KCl; 0.0003 M CaCl$_2$; 0.005 M MgCl$_2$ and 0.06 M Na$_3$PO$_4$ (pH 7.7) (Bueding, 1950). The organisms were incubated for 1 hour at 37 C.
[2] A total of 6 flasks were employed for each trial.

1 hr after collection. Conversely, the addition of glucose increased the average QO$_2$ value of worms at 25 hr after collection.

Discussion

All animals, including invertebrates, may be divided into two categories according to their response to varying oxygen tensions. This subject has been amply reviewed by von Brand (1946) and Zeuthen (1955). Those animals described as "conformers" demonstrate a rate of oxygen consumption which is dependent upon the external oxygen tension. Other animals, termed "regulators," consume oxygen at a rate which is relatively independent of the external oxygen tension until some critical value is reached, at which point oxygen uptake rapidly declines (Prosser and Brown, 1961; Vernberg, 1963). It has been shown (Kmetec and Bueding, 1961) that in *Ascaris* (an oxygen "conformer") the terminal oxidase is flavoprotein, which requires relatively high oxygen tensions for saturation. "Regulators," on the other hand, show maximal respiration as long as sufficient oxygen is present to saturate the active surface of the cytochrome oxidase system which has a high affinity for oxygen (Harvey, 1940).

Oxygen has been shown to be necessary for the survival of the miracidial and cercarial stages of those trematodes studied to date (Olivier et al., 1953; Hunter and Vernberg, 1955b; Becejac and Lui, 1959; and Vernberg, 1963). In the present study both miracidia and cercariae of *P. ohirai* were found to consume oxygen.

The respiration of miracidia measured in "Hepes" buffer was significantly higher at 5 hr after hatching than at 1 hr after hatching. This suggests that the miracidia may initially obtain a portion of their energy requirements from the anaerobic glycolysis of glycogen to pyruvate or lactate. As the miracidia age and these energy reserves are depleted, a greater portion of the energy requirements are met by aerobic metabolism. No evidence has yet been presented for the existence of the Krebs cycle in miracidia; however, glycogen depletion with aging has been shown for the cercariae of *Schistosoma mansoni* (Bruce et al., 1969, 1971).

The linear rate of oxygen uptake observed for both 1 hr and 5 hr miracidia suggests that the respiratory rate is relatively independent of oxygen tension over the range of concentrations used in the present study, thus following the so-called "regulator" type of oxygen uptake as discussed by Vernberg (1963). The oxygen monitor is a closed system. As the larval stages removed oxygen from the "Hepes" buffer, the external oxygen tension was progressively lowered from the initial 21% oxygen (air-saturated). The oxygen tension never fell below 12% during the course of any trial.

The decreased respiration of 5 hr miracidia in the presence of glucose is similar to that observed for *S. mansoni* cercariae at 3 hr after collection (Bruce et al., 1971). This response to glucose may be due to the "Crabtree" effect in which there is an increased competition by the anaerobic glycolytic process for inorganic phosphate and possibly pyridine nucleotides, leaving less for oxidative phosphorylation reactions (West and Todd, 1964; Fruton and Simmonds, 1963). It has been shown that *Fasciola hepatica* miracidia consume glucose (Bryant and Williams, 1962), but the effect of glucose

on respiration has not been studied. The non-linear uptake of oxygen by 5 hr miracidia in the presence of glucose (Figure 2) indicated that they did not strictly follow either a "regulator" or "conformer" type of respiration. During certain time intervals of the experimental period (i.e., 0–16 and 25–37 min) oxygen was consumed at a decreased rate as the available oxygen decreased, thus resembling a "conformer" type of respiration. However, at other time intervals (i.e., 17–24 and 38–46 min) oxygen uptake was not detected. The metabolic processes responsible for the alternating periods of oxygen consumption interspaced with intervals of no oxygen consumption are undefined at this time. However, the effect might be related to the amount of oxygen available to the enzyme systems, to the concentration of the enzymes themselves, and/or to the substrate concentration (e.g., the uptake of glucose from the buffer or the accumulation of the end products of glycolysis).

The oxygen consumption of *P. ohirai* cercariae was greater in the presence of glucose than in the absence of glucose. Since respiration was measured at 5–7 hr after shedding, this increase may reflect the replacement of depleted energy substrates by glucose. The addition of glucose was found to increase the oxygen uptake of aged cercariae of *S. mansoni* (18 hr after collection) when compared to the respiratory rate in the absence of glucose (Bruce et al., 1971). Vernberg and Hunter (1963) reported that the addition of glucose had no effect on the respiratory rate of *Himasthla quissetensis* cercariae; however, the age of these cercariae was not given.

The oxygen uptake of cercariae in both the absence and presence of glucose followed a linear pattern for 14 min and then a linear uptake (at a reduced rate) for the rest of the trial. In general, the oxygen uptake followed a "regulator" type (Vernberg, 1963) of response to varying oxygen tension. The respiration of cercariae of *Gynaecotyla adunca* has been reported to be relatively independent of oxygen tension (Hunter and Vernberg, 1955b). *Himasthla quissetensis* cercariae showed a "conformer" type respiration as oxygen tension was lowered, while *Zoogonus rubellus* demonstrated a "regulator" type of response below 3% and above 5% oxygen levels (Vernberg, 1963).

In the present study, 425 encysted metacercariae of *P. ohirai* did not consume any measurable amount of oxygen. Oxygen uptake has been reported for the metacercariae of *Gynaecotyla adunca* (Hunter and Vernberg, 1955a). Excysted metacercariae of *P. ohirai* consumed large amounts of oxygen (63.7 μl O_2/hr/1,000 excysted metacercariae) compared to the other larval stages.

Adult *P. ohirai* worms in buffer consumed significantly more oxygen ($QO_2 = 2.238$) at 1 hr after collection than they did at 25 hr after collection ($QO_2 = 0.620$). This may reflect a depletion of energy constituents in the aged worms, since when glucose was added no difference was found between worms at 1 and 25 hr after collection. Comparison of the QO_2 values for worms at 1 hr after collection showed that the addition of glucose to the buffer decreased the oxygen uptake. The "Crabtree" effect might be responsible for this effect. The addition of glucose, however, increased the respiration ($QO_2 = 1.153$) of 25 hr worms compared with that measured in the absence of glucose ($QO_2 = 0.620$). This might reflect the replacement of depleted energy sources by uptake of glucose from the buffer. Bueding (1950) found a slight increase in the QO_2 value of paired *S. mansoni* adults in the presence of glucose. Read and Yogore (1955) reported the QO_2 value of *P. westermani* in Krebs-Ringer phosphate containing 0.01 M glucose to be 0.74–0.86 while Shimomura (1959) found it to be 2.8.

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Geopetitia aspiculata sp. n. (Spirurida) from Coerulea coerulea and Other Imported Birds in the National Zoological Park, Washington, D. C.[1]

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ABSTRACT: Geopetitia aspiculata sp. n. from Coerulea coerulea is described, figured, and compared with the other species of the genus. Additional specimens from various other exotic birds were examined in order to gain information on specific morphological characters available for the separation of those species presently assigned to the genus. It is concluded that the presence or absence of spicules, and if present their morphology, and the distribution pattern of the genital papillae in the male are the only two characters so far described which are useful for species determination.

During the examination of unidentified helminths that had been collected from various exotic birds housed at the National Zoological Park, Washington, D. C., and deposited in the USNM Helminth Collection, several spirurid nematodes of the genus Geopetitia Chabaud, 1951 were observed. These specimens are unusual in that no evidence of a spicule in the male can be demonstrated. Since those members of the genus for which males are known have been described as possessing spicules, the specimens that lack spicules examined by this author are considered as a new species.

All specimens were cleared and examined in phenol-alcohol. Diagrams were made with the aid of a camera lucida. Unless otherwise stated measurements are in microns.

Geopetitia aspiculata sp. n.
(Figs. 1–5)

Tetrameridae Travassos 1914: Geopetitiinea Chabaud 1951. Delicate nematodes usually firmly embedded in connective tissue. Cuticle thin and transparent. Mouth bordered by two tri-lobed pseudo-labia; median lobe longest and conical. External circle of cephalic papillae small, consisting of four small papillae at the base of the pseudo-labia and four larger papillae. Buccal capsule small, well developed with straight walls 10–15 in height.

MALE (8 complete specimens). Length 15–18 (average 16.4) mm; maximum width 200–260 (240). Oesophagus divided into muscular and glandular regions 200–275 (235) and 728–840 (796) long respectively. Nerve ring and excretory pore 150–195 (169) and 170–260 (213) respectively from anterior extremity. Anus 140–170 (154) from posterior extremity, bounded both anteriorly and posteriorly by a small cuticular inflation. Spicules, gubernaculum and caudal alae absent. Genital papillae distributed as follows: 1 on anterior anal lip; 3 sub-lateral preanal pairs; 1 adanal pair; 4 postanal pairs approximately equidistant to tip of tail; 1 double subterminal papilla. Small subterminal phasmids present.

FEMALE (1 complete but damaged and 4 incomplete specimens). Length approximately 50 mm; maximum width 760–930 (840). Muscular and glandular regions of oesophagus 300–360 (320) and 680–890 (810) long respectively. Nerve ring and excretory pore 170–220 (195) and approximately 200 respectively from anterior extremity. Anus 50–60 from tip of tail. Vulva situated in constriction 320–360 from tip of tail. Vagina long, running well anteral before dividing into two uterine branches. Eggs thin-shelled, approximately 30 × 15, embryonated when deposited.

HOST: Coerulea coerulea (Purple sugar bird).[2]

HABITAT: Not known.

LOCALITY: Trinidad to Nat. Zool. Park, Washington, D. C.

TYPE SPECIMEN: Holotype male; Allotype; Paratypes USNM Helm. Coll. 38988.

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[1] This study was undertaken during a work trip to the Beltsville Parasitological Laboratory, U.S.D.A., Beltsville, Md., U.S.A.

[2] All information as to host and habitat was obtained from labels accompanying the collections.
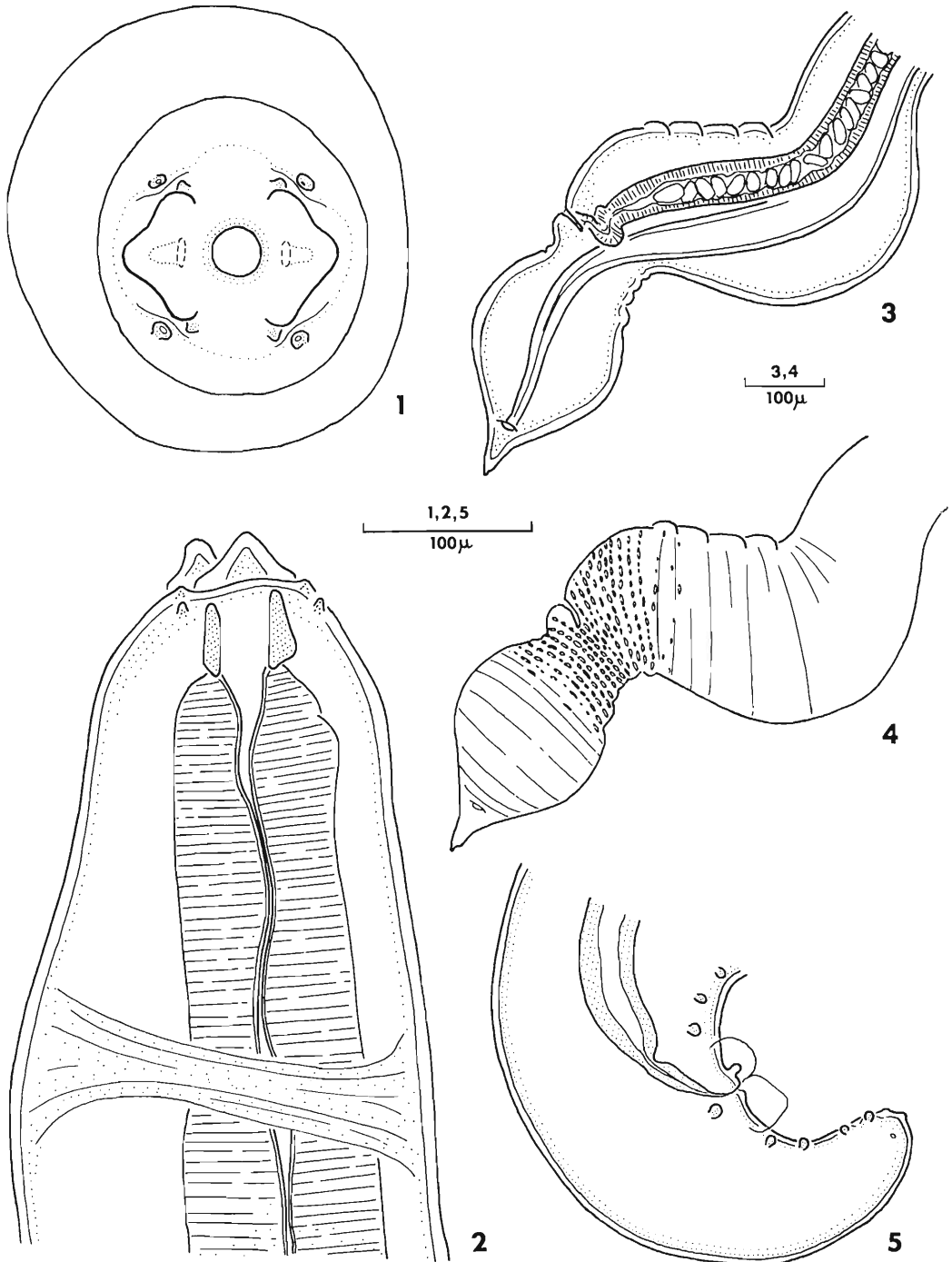
Figures 1–5. *Geopetitia aspiculata* sp. n. 1, En face, female. 2, Anterior portion, female. 3, Posterior extremity of females showing vulva and part of vagina. 4, Same as 3, showing cuticular markings on ventral surface. 5, Caudal extremity of male.
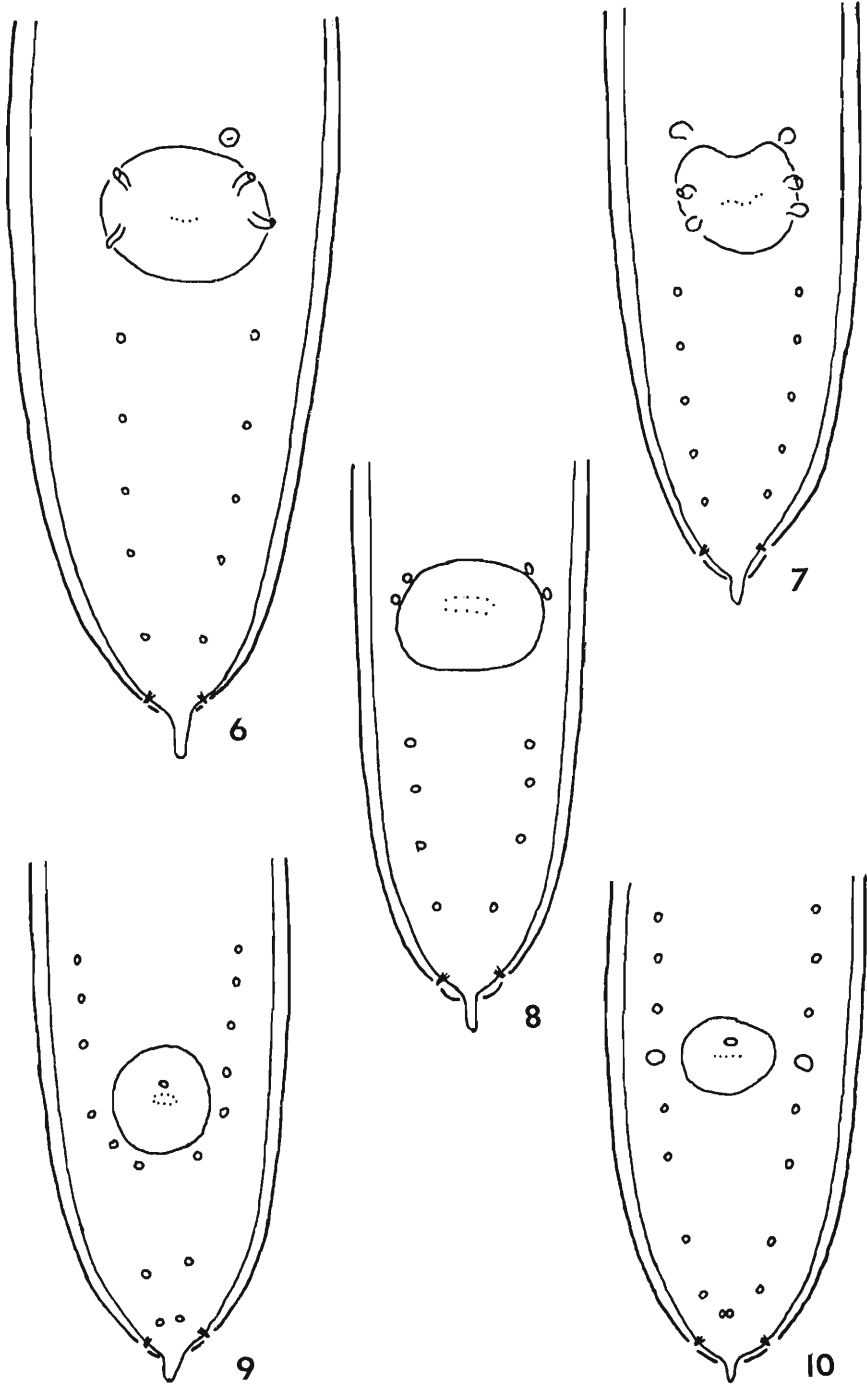
Figures 6–10. Ventral view (diagramatic) of the male tails of the species of *Geopetitia*. 6, *G. pari* after Chabaud, 1951. 7, *G. pari* after Bain and Chabaud, 1965. 8, *G. chabaudi* after Rasheed, 1960. 9, *G. madagascariensis* after Chabaud, 1960. 10, *G. aspiculata* sp. n.

The following USNM Helm. Coll. material obtained from the National Zoological Park was also examined: 1. No. 38987; outside gizzard, Eastern double collared sunbird, 1♂, 1♀ head. 2. No. 38989; gizzard, *Hoopoe-Upupa epops*, 1♂, 1♂ tail. 3. No. 38991; abdominal cavity, white wagtail, 1♂ tail, 2♀♀ heads. 4. No. 60624; abdominal cavity, Crested Yuhina, 1♂ tail, 1♀ tail, 1♀ head. 5. No. 60625; peritoneum and through liver, *Estrilda coerulbscens*, 5♂♂ tails, 1♀ tail, 3♀♀ heads.

Discussion

The genus *Geopetitia* Chabaud, 1951 is presently composed of the following members: *G. pari* Chabaud, 1951; *G. chabaudi* Rasheed, 1960; *G. madagascariensis* Chabaud, 1960; *G. chabaudi* Vuylsteke, 1963; *G. streperae* Mawson, 1966; *G. chibiae* Mawson, 1966; *G. falco* Mawson, 1966, and the species herein described as *G. aspiculata* sp. n.

Vuylsteke (1963), unaware of Rasheed's specimens (1960), described and named *G. chabaudi* Vuylsteke, 1963 on the basis of fragmented females. Since the *G. chabaudi* of Vuylsteke (1963) and *G. chabaudi* of Rasheed (1960) are from different hosts and different geographical areas, then the former must for the present time be considered a junior homonym of the latter. Further since Vuylsteke's specimens were fragmented females only and as this present author contends that the females of this genus offer no suitable characters for species differentiation (see below), this species should be designated *Geopetitia* species inquirendo (Vuylsteke, 1963).

Reviewing the descriptions of these species, the author regards the morphology of the spicules and the number and distribution of the genital papillae of the male as the only characters suitable for species determination.

The spicules of *G. pari* and *G. chabaudi* Rasheed, 1960 are in both cases large, well-formed structures, 280 long in the former (Chabaud, 1951) and 150–160 long in the latter (Rasheed, 1960). The spicules of *G. streperae* are unequal and dissimilar, 220 and 160 long. No evidence of spicules could be demonstrated in *G. aspiculata* sp. n. either by microscopic examination or by dissection of a male specimen. The spicules of *G. madagascariensis* are small, 70–85 in length, very lightly chitinized structures. The two males on which

this species is based are now unavailable for study (Chabaud, pers. comm.). It may be that the "spicules" of *G. madagascariensis* are in fact a slight thickening of the wall of the cloaca, a condition occasionally noted in *G. aspiculata* sp. n. However, until additional specimens of *G. madagascariensis* are examined, the description must be considered valid.

The second character which appears at the present time to be of significance at the species level is the number and distribution of the genital papillae in the male. Bain and Chabaud (1965) have questioned the validity of this character in such a tissue-inhabiting nematode. They argue that size, particularly spicule size, and papillae distribution may be influenced by host size and/or geographical dispersal. However, the author has examined the tails of 17 male *G. aspiculata* sp. n. from 6 different hosts and in no case could any difference in number or distribution be demonstrated although these hosts were all housed at the National Zoological Park where conditions prevailed not normally found in a natural state. This pattern is unlike that of the other described species (see Figs. 6–10. The caudal extremity of the male of *G. streperae* has not been illustrated since Mawson (1966) appears uncertain of the distribution of the papillae. She has separated *G. streperae* from *G. pari* and *G. madagascariensis* only on the basis of spicule morphology).

Geopetitia sp. inq. (Vuylsteke, 1963) described from fragments of females, and both *G. chibiae* and *G. falco* which were described from a single female can not be compared with the other species.

Specimens assigned by the author to the genus *Geopetitia* were examined from three additional hosts (USNM Helm. Coll. No. 38985, body cavity, Red-vented Bulbul, 1♀ head, 1♀ tail; No. 38990, gizzard, Yellow-cheeked tit, 1♀ tail, 3♀♀ heads; No. 61177, exterior of stomach, *Parus bicolor*, 1♀ tail, 1♀ head). However, although they could not be specifically determined, except for slight variations in size they are indistinguishable from *G. aspiculata* sp. n.

Acknowledgments

MayBelle Chitwood for her instruction and guidance.

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On the Taxonomic Status and Comparative Morphology of Species of the Genus *Neoaplectana* Steiner (Neoaplectanidae: Nematoda)[1]

C. P. Turco,[2] Walter H. Thames, Jr.,[3] and S. H. Hopkins[4]

ABSTRACT: Because of the restriction of the family to the genus *Neoaplectana*, the diagnosis and descriptions of the family Neoaplectanidae were emended. All known species of this genus were redescribed and a comparative diagnosis was established for each species. The three species *N. glaseri* Steiner, 1929, *N. chresima* Steiner, 1942, and *N. dutkyi* Jackson, 1965, were redescribed from type specimens. Examination of type material and original descriptive material enabled the construction of a key to the species and illustrations of certain morphological characters necessary for diagnostic purposes.

One of the early rhabditid facultative parasites of insects was described by Steiner (1929) as the new genus and species *Neoaplectana glaseri* from the Japanese beetle, *Popillia japonica*. Steiner determined that this parasite bore a strong resemblance to the genera *Aplectana* and *Steinernema*, and therefore placed it in the family Oxyuridae Cobbold, 1884. Later contributions to the taxonomy of this genus include: Travassos (1931), Filipjev (1934), Bovien (1937), Steiner in Glaser, McCoy, and Girth (1942), Hoy (1954), Weiser (1955), Weiser and Kohler (1955), Kirjanova and Puchkova (1955), Weiser (1958), Kakulia and Veremchuk (1965), Poinar (1967), and Turco (1970).

Filipjev (1934) removed *Steinernema* and *Neoaplectana* from the Oxyuridae and placed these genera in the subfamily Steinernematinae. Skrjabin, Shikhobalova, and Mosgovoi (1951) discovered the genus *Steinernema* Travassos, 1927, to be a junior synonym of the genus *Oxysomatium*. This left *Neoaplectana* as the only remaining valid genus in the family. Sobolev (1953), correctly, renamed this taxon Neoaplectanidae. He had no other alternative under the rules of nomenclature no other genus being available. At present, 1 genus and 13 species are included in the Neoaplectanidae. However since he did not redescribe the now limited family, an emendation was necessary at the beginning of this taxonomic work.

Many generic and specific characters were examined to establish the validity of the individual species and to construct the first key of the genus *Neoaplectana*. The de Man ratios showed a very broad range of values. Most measurements obtained by investigations were of first generation adults and do not necessarily
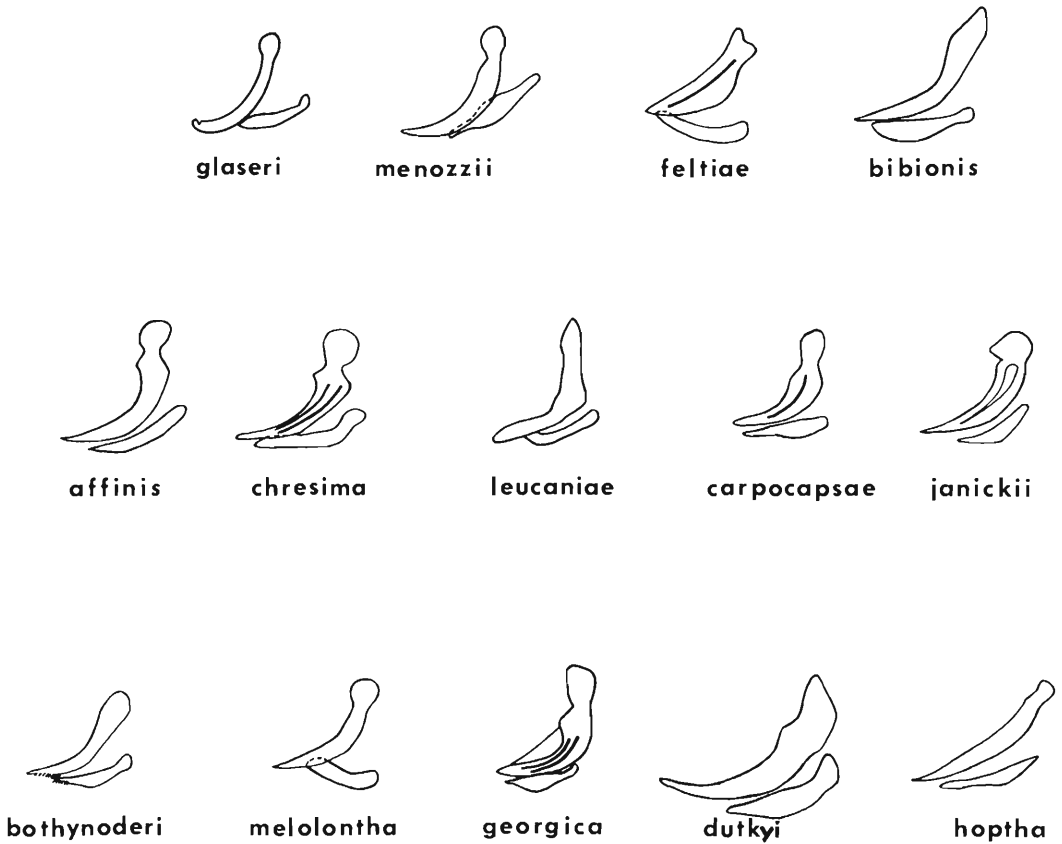
Figure 1. Male copulatory structures of *Neoaplectana* spp.

represent the complete variability which would also cover the smaller succeeding generations in the host insect. It was concluded that the de Man values of measurements have a limited value as a character to distinguish the various species of this family.

Examination of various specimens in the genus *Neoaplectana* in this study have shown considerable variations to exist in the relative size and appearance of the stomal region in these nematodes. This character was rather difficult to observe and in many specimens could not be determined. It was concluded that additional study of the stoma and its rhabdions would be a worthwhile contribution to further classification of this genus when specimens from all type localities become available.

The superfamily Rhabditoidea is used ac-

cording to the description of Chitwood and Chitwood (1950). The family Neoaplectanidae can be distinguished from the other families of the Rhabitoidea by the presence of an esophagus with a simple corpus, indistinct isthmus and reduced muscular bulb, males without caudal alae and the presence of genital papillae in paired linear preanal series in the male. An emended diagnosis of this taxon is now given:

Neoaplectanidae Sobolev, 1953
Syn. Steinernematidae Chitwood and Chitwood, 1937

DIAGNOSIS (Emended): Rhabditoidea. All forms saprozoic, or parasites of arthropods. ADULT FEMALE: Three lips indistinct with six labial and six cephalic papillae; amphids at same level as cephalic papillae; stoma often reduced or vestibulate; corpus of esophagus

simple, isthmus indistinct, terminal bulb with reduced musculature. Gonads amphidelphic; each ovary usually reflexed one time; oviduct short; uterus voluminous and usually filled with eggs and larvae of diverse stages; vulvar opening generally at or near midbody. ADULT MALE: Usually smaller than female. Esophageal pattern similar to that of female. Gonad monorchic; testis usually reflexed one time; spicules paired, arcuate, symmetrical; gubernaculum present; genital papillae in paired linear series in preanal, adanal, and postanal regions. Type genus, *Neoaplectana* Steiner, 1929, only genus known at present writing.

The species descriptions have been emended principally by the necessity to constitute all known species in the diagnosis and from the determination that the male spicules and gubernaculum are the only taxonomic characteristics that appear to be constant throughout this genus (Fig. 1).

The species of this family are redescribed as follows:

Neoaplectana glaseri Steiner, 1929
(Fig. 2A)

MALES (paralectotypes) (n = 6): L = 891 (700–1,005) μ; a = 14.8 (14–16.2); b = 6.2 (4.7–7.3); c = 31.7 (26.3–37); spicules = 61 (56–66) μ; gubernaculum = 41 (37–45) μ.

FEMALES (paralectotypes) (n = 11): L = 3,684 (3,337–4,133) μ; a = 14.7 (13–17.6); b = 21.6 (19.6–23.9); c = 58.5 (49–69); vulva = 53 (51–55)%.

MALE (lectotype): L = 925 μ; a = 14.2; b = 6.1; c = 37; spicules = 62 μ; gubernaculum = 41 μ.

FEMALE (allolectotype): L = 3,640 μ; a = 13.6; b = 22.5; c = 6.3; vulva = 51.1%.

DESCRIPTION (emended): Neoaplectanidae. FEMALE: Occurs in body cavity of host insect. Body stout, ventrally arcuate, tapering toward extremities. Cuticle not striated. Head region with three lips with six labial and six cephalic papillae. Stoma reduced. Esophagus with simple corpus, slender isthmus, and a terminal bulb with reduced musculature. Nerve ring encircles esophagus in front of terminal bulb; excretory pore opening ventrad in area of nerve ring. Gonads amphidelphic, large and symmetrical; ovaries with reflexure at terminal bulb and rectum; vagina short; vulva transverse with two prominent ventral protuberances.

Anus conspicuous with large postanal lip. Tail conoid with slightly rounded tip.

MALE: Occurs in body cavity of host insect. Body is smaller and more slender than that of female. Cuticle not striated. Head similar to that of female. Buccal cavity absent. Excretory pore opening ventrad at nerve ring. Single testis stretched out; genital papillae as follows: seven preanal, one adanal, and three postanal; paired brownish spicules, large and arcuate, distal ends cephalated to form hooks; gubernaculum large, distal end lineate, proximal end broadly swollen. Tail convex-conoid with bluntly rounded tip.

TYPE MATERIAL: The original syntypic series is here designated as follows: Lectotype (male) Slide No. T-125t and allolectotype (female) Slide No. T-126t, deposited with the USDA Nematode Collection, Beltsville, Maryland.

TYPE HABITAT AND LOCALITY: Body cavity of the Japanese beetle, *Popillia japonica* Newm., found in Moorestown, New Jersey.

DIAGNOSIS AND RELATIONSHIP: *N. glaseri* Steiner, 1929, is closely related to the species *N. feltiae* Filipjev, 1934. It differs from *N. feltiae* by the absence of a mucronulate male terminus, the presence of distally hooked spicules, and the presence of a bluntly rounded female terminus.

Neoaplectana menozzii Travassos, 1931
(Fig. 2B)

FEMALE: L = 1,600 (1,100–1,600) μ; a = 18.6; b = 12.9; c = 26.7; vulva = 51%.

MALE: L = 860 (800–860) μ; a = 11.9; b = 10.4; c = 41; spicules = 56 μ; gubernaculum = 40 μ.

DESCRIPTION (emended): Neoaplectanidae. FEMALE: Occurs in the body cavity of the insect. Body generally of equal width throughout, tapering slightly to rounded tail terminus. Cuticle not striated. Head region without pronounced lips with six labial and six cephalic papillae. Stoma large. Esophagus with simple corpus, distinct isthmus and a terminal bulb with reduced musculature. Nerve ring situated immediately above terminal bulb; excretory pore opening ventrad adjacent to nerve ring. Gonads amphidelphic, long and symetrical; ovary with reflexure at terminal bulb and the rectum; vagina short; vulva transverse with two prominent ventral protuberances. Anus con-
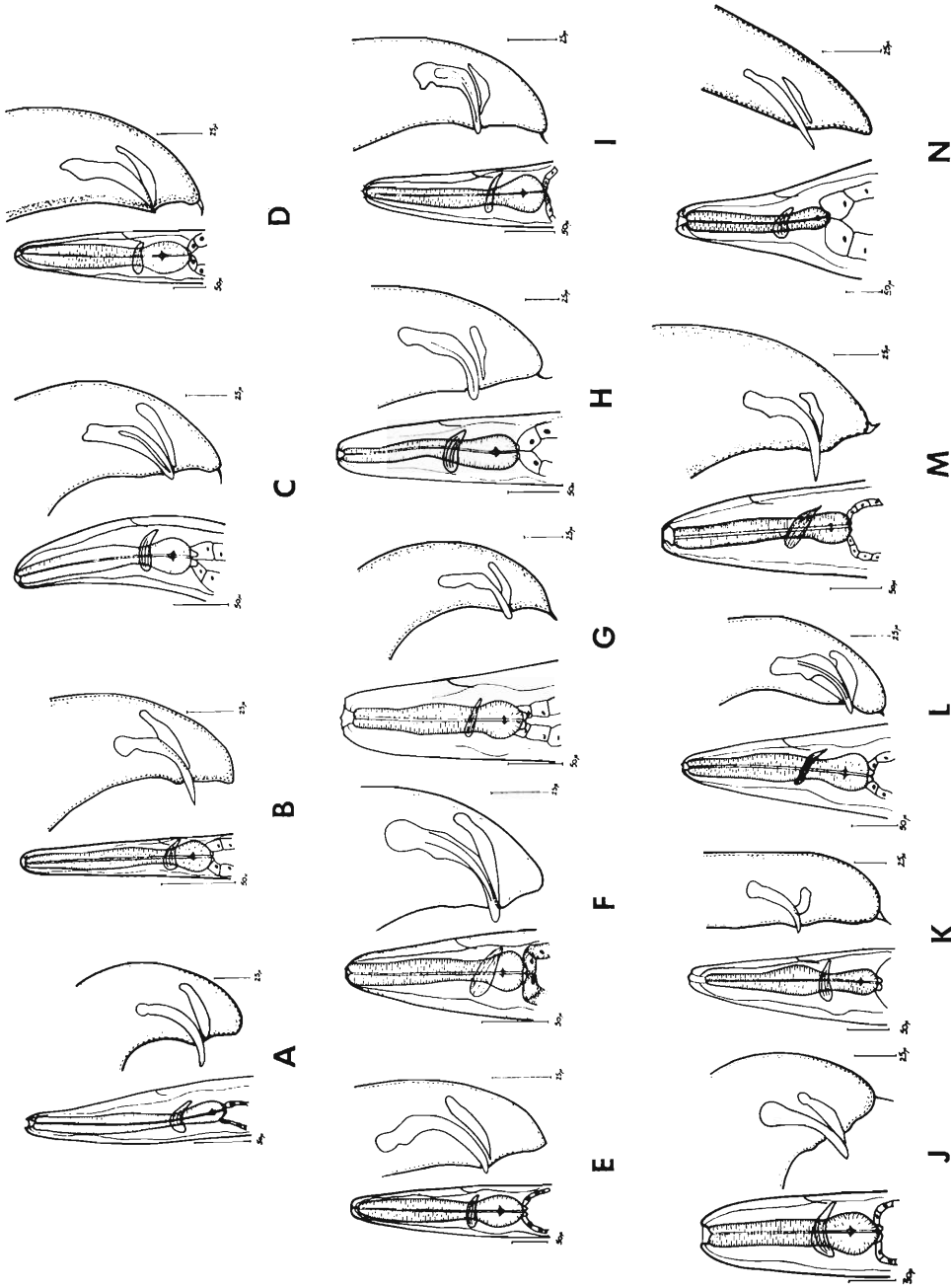
Figure 2. Male and female Neoplectanids. A. *Neoaplectana glaseri* (after Steiner, 1929); **B.** *Neoaplectana menozzii* (after Travassos, 1931); **C.** *Neoaplectana feltiae* (after Filipjev, 1934); **D.** *Neoaplectana bibionis* (after Bovien, 1937); **E.** *Neoaplectana affinis* (after Bovien, 1937); **F.** *Neoaplectana chresima* (after Steiner in Glaser, McCoy, and Girth, 1942); **G.** *Neoaplectana leucaniae* (after Hoy, 1954); **H.** *Neoaplectana carpocapsae* (after Weiser, 1955); **I.** *Neoaplectana janickii* (after Weiser and Kohler, 1955); **J.** *Neoaplectana bothynoderi* (after Kirjanova and Puchkova, 1955); **K.** *Neoaplectana georgica* (after Kakulia and Veremchuk, 1965); **M.** *Neoaplectana dutkyi* (after Jackson, 1965); **N.** *Neoaplectana hoptha* (after Turco, 1970).

spicuous with slight postanal lip. Tail conoid with pointed tip.

MALE: Occurs in body cavity of host insect. Body is smaller but more robust than in female. Cuticle not striated. Head similar to that of female. Excretory pore opens ventrad just before terminal bulb. Single testis reflexed and generally fills lower half of the body; eight to ten pair of preanal papillae; paired spicules arcuate ending in enlarged proximal region, uneven, and without a hook at the tip; gubernaculum fusiform. Tail convex-conoid with rounded tip.

TYPE HABITAT AND LOCALITY: Body cavity of the beetle, *Conorrhynchus* (*Cleonus*) *mendicus* (Gyll.), from Bientina, Tuscany, Italy.

DIAGNOSIS AND RELATIONSHIP: *N. menozzii* Travassos, 1931, is closely related to the species *N. chresima* Steiner, 1942. It differs from *N. chresima* in the smaller size of the male body, smaller spicular size and the more tapering male terminus.

Neoaplectana feltiae Filipjev, 1934
(Fig. 2C)

FEMALES: L = 4,465 (3,800–5,940) μ; a = 16.9 (10.8–28.1); b = 19.5 (15.8–25.8); c = 74 (63–85); vulva = 59 (53–64)%.

MALES: L = 1,370 (1,335–1,420) μ; a = 12.1 (11.9–12.2); b = 9.4 (9.1–9.5); c = 39 (39–41); spicules = 61 (60–61) μ; gubernaculum = 52 (52–53) μ.

DESCRIPTION (emended): Neoaplectanidae. FEMALES: Occur in the body cavity of the host insect. Body is stout, ventrally arcuate, tapering towards the extremities. Cuticle not striated. Head region with three lips with six labial and six cephalic papillae. Esophagus with simple corpus, slight isthmus and a terminal bulb with reduced musculature. Nerve ring at terminal bulb of the esophagus; excretory pore opening ventrad near the nerve ring. Gonads amphidelphic, large and symmetrical; ovaries with reflexure at terminal bulb and the rectum; vagina short; vulva transverse with two prominent ventral protuberances. Anus is conspicuous with a slight postanal lip. Tail conoid with pointed tip.

MALES: Occur in body cavity of host insect. Body is smaller and more slender than that of female. Cuticle is not striated. Head similar to that of female. Nerve ring at terminal bulb; excretory pore opening ventrad at nerve ring

area. Single testis reflexed and fills most of body; genital papillae as follows: five to six preanal, two adanal, and seven postanal; paired ribbed spicules curved ending in an enlarged proximal region; gubernaculum concave with acute angle at head axis. Tail convex-conoid, mucronulate.

TYPE HABITAT AND LOCALITY: Body cavity of a European cutworm, *Feltia segetum* (Schif.), found in central Austria.

DIAGNOSIS AND RELATIONSHIP: *N. feltiae* Filipjev, 1934, is closely related to the species *N. bibionis* Bovien, 1937, and *N. affinis* Bovien, 1937. *N. feltiae* differs from both of these species by having a larger female body size, small, postanal lip and spicules with an enlarged proximal region.

Neoaplectana bibionis Bovien, 1937
(Fig. 2D)

FEMALE: L = 3,074 μ; a = 14.6; b = 11.2; c = 10.3; vulva = 57%.

MALE: L = 770 μ; a = 11.8; b = 12; c = 30.8; spicules = 60 μ; gubernaculum = 37 μ.

DESCRIPTION (emended): Neoaplectanidae. FEMALE: Occurs in the body cavity of the host insect. Body is sausage-shaped, ventrally arcuate, ending in a narrower fore-end and a bluntly conical tail. Cuticle not striated. Head region with three lips with six labial and six cephalic papillae. Stoma reduced. Esophagus with simple corpus, distinct isthmus and a terminal bulb with reduced musculature. Nerve ring in front of terminal bulb; excretory pore opening ventrad at nerve ring. Gonads amphidelphic, occupying most of body of gravid form; ovaries long, slender with reflexure at terminal bulb and the rectum; vagina short; vulva transverse with two prominent ventral protuberances. Anus conspicuous without postanal lip. Tail conoid with pointed tip.

MALE: In body of host insect. Body is smaller and more slender than female. Excretory pore opening ventrad at or near the nerve ring. Single testis strongly reflexed and filling most of body; genital papillae as follows: seven pair preanal, two pair adanal, and two pair postanal; paired yellow spicules large and arcuate, with a broad proximal portion possessing a distinct knob; gubernaculum broadest in middle and narrow towards the end. Tail convex-conoid, mucronulate.

TYPE HABITAT AND LOCALITY: Body cavity of the fly, *Bibio ferruginatus*, from Odense, Denmark.

DIAGNOSIS AND RELATIONSHIP: *N. bibionis* Bovien, 1937, is closely related to *N. affinis* by the presence of a mucronulated male terminus, usually smaller male body size and the absence of a knob at the proximal end of the spicule.

Neoaplectana affinis Bovien, 1937
(Fig. 2E)

FEMALE: L = 3,075 μ; a = 14.6; b = 11.2; c = 10.3; vulva = 57%.

MALE: L = 1,000 μ; a = 16.9; b = 8.3; c = 33.3; spicules = 62 μ; gubernaculum = 36 μ.

DESCRIPTION (emended): Neoaplectanidae. FEMALE: Occurs in the body cavity of the host insect. Body is sausage-shaped, ventrally arcuate, with a narrower fore-end and a bluntly conical tail. Cuticle not striated. Head region with three lips with six labial and six cephalic papillae. Stoma reduced. Esophagus with simple corpus, distinct isthmus and a terminal bulb with reduced musculature. Nerve ring in front of terminal bulb; excretory pore opening ventrad at or near the nerve ring. Gonads amphidelphic, occupying most of the body of the gravid form; ovaries long, slender with reflexure at the terminal bulb and the rectum; vagina short; vulva transverse with two prominent ventral protuberances. Anus is conspicuous without postanal lip. Tail conoid with pointed tip.

MALE: Occurs in body cavity of the host insect. Body is smaller and more slender than the female. Cuticle not striated. Head region similar to that of female. Excretory pore opening ventrad at or near the nerve ring. Single testis strongly reflexed and fills most of body; paired grey spicules curved with a small proximal manubrium-like portion; gubernaculum crescent-shaped without a proximal knob or hook. Tail convex-conoid with slightly rounded tip.

TYPE HABITAT AND LOCALITY: Body cavity of the fly, *Bibio ferruginatus*, from Odense, Denmark.

DIAGNOSIS AND RELATIONSHIP: *N. affinis* Bovien, 1937, is closely related to *N. bibionis* Bovien, 1937. It differs from *N. bibionis* by the absence of a mucronulated tail, usually

larger male body size and the presence of a proximal knob on the spicule.

Neoaplectana chresima Steiner in Glaser, McCoy and Girth, 1942
(Fig. 2F)

MALES (paralectotypes) (n = 11): L = 931 (630–1,180) μ; a = 9 (6.8–11.6); b = 7 (5.1–8.6); c = 21 (19.3–23.6); spicules = 66 (54–80) μ; gubernaculum = 44 (40–46) μ.

FEMALES (paralectotypes) (n = 14): L = 1,391 (1,265–1,795) μ; a = 12.8 (12.3–13.2); b = 8.8 (8.2–9.6); c = 33.4 (23.8–41.5); vulva = 57 (51–62)%.

MALE (lectotype): L = 925 μ; a = 9.2; b = 6.4; c = 20.6; spicules = 68 μ; gubernaculum = 43 μ.

FEMALE (allolectotype): L = 1,368 μ; a = 12.6; b = 9.5; c = 41.5; vulva = 57%.

DESCRIPTION (emended): Neoaplectanidae. FEMALE: In the body cavity of the host insect. Body stout and generally of equal width throughout length, terminating in a mucronulated tail terminus. Cuticle not striated. Head region with three lips with six labial and six cephalic papillae. Stoma reduced. Esophagus with simple corpus, slight isthmus and a terminal bulb with reduced musculature. Nerve ring encircles esophagus in front of terminal bulb; excretory pore opens ventrad at or near the nerve ring. Gonads amphidelphic, large and symmetrical; ovaries with refexure at terminal bulb and the rectum; vagina short; vulva transverse with two prominent ventral protuberances. Anus conspicuous without prominent postanal lip. Tail conoid with slightly rounded tip.

MALES: In body cavity of insect host. Body smaller and more slender than in the female. Cuticle not striated. Head is similar to that of female. Buccal cavity absent. Excretory pore opening ventrad at or near the nerve ring. Single testis reflexed and filling most of body; paired spicules proximally capitate, curved outward, possessing a small prominence on each side; gubernaculum slightly curved posteriorly, straight anteriorly, ending in a small knob. Tail convex-conoid with slightly rounded tip.

TYPE MATERIAL: The original syntypic series is here designated as follows: Lectotype (male) Slide No. T-127t and allolectotype (female) Slide No. T-128t deposited with the USDA Nematode Collection, Beltsville, Maryland.

TYPE HABITAT AND LOCALITY: Body cavity of the corn earworm, *Heliothis armigera* Hbn., from Moorestown, New Jersey.

DIAGNOSIS AND RELATIONSHIP: *N. chresima* Steiner, 1942, is closely related to *N. janickii* Weiser and Kohler, 1955. If differs from *N. janickii* by having smaller male body size, the absence of a mucronulated male terminus, and the presence of a knob at the proximal end of the gubernaculum.

N. chresima was described by Steiner but first appeared in publication as a quotation in the paper of Glaser, McCoy, and Girth (1942). The nematode specimens were collected from diseased pupae of the corn earworm, *Heliothis armigera* Hbn., from soil in Moorestown, New Jersey. Steiner's description was published without illustrations and the morphological characters presented were not sufficient to separate it from other species in the genus *Neoaplectana*. These specimens described by Steiner in the original description were made available for study, and proved on examination to be a valid species of the genus *Neoaplectana*. The following material was available for study: five slides labelled *N. chresima* n. sp. from *Heliothis* pupae, from Moorestown, New Jersey, Coll. Larrimer, XI-27-34.

A complete redescription of the species with illustrations was considered necessary and the description of this species was further emended by the addition of the diagnosis.

Neoaplectana leucaniae Hoy, 1954
(Fig. 2G)

FEMALE: L = 1,950 μ; a = 11.7; b = 11.3; c = 19.5; vulva = 56%.

MALE: L = 950 μ; a = 14.6; b = 7.5; c = 26.4; spicules = 65 μ; gubernaculum = 40 μ.

DESCRIPTION (emended): Neoaplectanidae. FEMALE: In body cavity of host insect. Body stout, ventrally arcuate, terminating in a bluntly conical terminus. Cuticle not striated. Head region with three lips with six labial and six cephalic papillae. Stoma reduced and vestibuliform. Esophagus with simple corpus, indistinct isthmus and a terminal bulb with reduced musculature. Nerve ring in front of terminal bulb; excretory pore opening ventrad at or near the nerve ring. Gonads amphidelphic, large and symmetrical; ovaries with reflexure at terminal bulb and the rectum; vagina short; vulva

transverse with two prominent ventral protuberances. Anus conspicuous without postanal lip. Tail conoid with pointed tip, terminating rather abruptly.

MALE: In body cavity of host insect. Body smaller and more slender than in female. Cuticle not striated. Head region similar to that of female. Buccal cavity absent. Excretory pore opening ventrad at or near the nerve ring. Single testis reflexed and filling most of body hiding intestine; genital papillae as follows: one large unpaired preanal papillae, five to seven pair preanal papillae and five pair postanal papillae; paired spicules arcuate, ending in enlarged portion and not hooked at the tip; gubernaculum curved posteriorly, straight anteriorly, ending in a knob at the proximal tip. Tail convex-conoid, mucronulate.

TYPE HABITAT AND LOCALITY: Body cavity of the tussock moth, *Crambus simplex* Meyr., from Seafield, Canterbury, New Zealand.

DIAGNOSIS AND RELATIONSHIP: *N. leucaniae* Hoy, 1954, is closely related to *N. chresima* Steiner, 1942. It differs from *N. chresima* by the larger female body size, the presence of a mucronulate male terminus, and the absence of a proximal knob on the spicule.

Neoaplectana carpocapsae Weiser, 1955
(Fig. 2H)

FEMALES: L = 1,073 (684–1,610) μ; a = 11.5 (7.1–13.4); b = 7 (4–13); c = 23.9 (9–53.6); vulva = 55 (38–65)%.

MALES: L = 643 (529–708) μ; a = 10.6 (8.8–12); b = 5.3 (4.7–5.3); c = 27 (21–35); spicules = 50 (42–60) μ; gubernaculum = 42 (40–42) μ.

DESCRIPTION (emended): Neoaplectanidae. FEMALES: In the body cavity of host insect. Body long, ventrally arcuate, tapering toward extremities. Cuticle not striated. Head region with three lips with six labial and six cephalic papillae. Stoma reduced. Esophagus with cylindrical corpus, indistinct isthmus, and a terminal bulb with reduced musculature. Nerve ring in front of terminal bulb; excretory pore opening ventrad at nerve ring. Gonads amphidelphic, large and symmetrical; ovaries with reflexure at terminal bulb and at rectum; vagina short; vulva transverse with two prominent ventral protuberances. Anus conspicuous without postanal lip. Tail conoid with pointed tip.

MALES: In body cavity of host insect. Body more round and approximately one-half the length of female. Excretory pore opening ventrad at or near the nerve ring. Single testis reflexed and filling most of body; paired spicules slightly curved with a small protrusion on the inner side and not hooked at the tip; gubernaculum long, flat with a raised, lengthened lobe. Tail convex-conoid, mucronulate.

TYPE HABITAT AND LOCALITY: Body cavity of the codling moth, *Carpocapsa pomonella* L., from Holovous, Czechoslovakia.

DIAGNOSIS AND RELATIONSHIP: *N. carpocapsae* Weiser, 1955, is closely related to *N. georgica* Kakulia and Veremchuk, 1965. It differs from *N. georgica* by the smaller male size, the non-striated cuticle, and the absence of a conspicuous postanal lip at the female anus.

Neoaplectana janickii Weiser and Kohler, 1955
(Fig. 2I)

FEMALE: L = 1,323 μ; a = 12.6; b = 15.6; c = 24; vulva = 59%.

MALE: L = 1,110 μ; a = 13.8; b = 7.3; c = 27.7; spicules = 42 μ; gubernaculum = 24 μ.

DESCRIPTION (emended): Neoaplectanidae. FEMALE: In the body cavity of the host insect. Body elongated, ventrally arcuate, bluntly rounded fore-end and posterior terminating in a short spike. Cuticle not striated. Head region with three lips with six labial and six cephalic papillae; each lip has a small bristle. Stoma reduced. Esophagus with simple corpus, indistinct isthmus and a terminal bulb with reduced musculature. Nerve ring in front of terminal bulb; excretory pore opening ventrad at or near the terminal bulb. Gonads amphidelphic, large and symmetrical; ovaries with reflexure at terminal bulb and at rectum; vagina short; vulva transverse with two ventral protuberances. Anus conspicuous with small postanal lip. Tail conoid, mucronulate.

MALE: In body cavity of the host insect. Body slightly smaller than in female. Cuticle not striated. Head region similar to that of female. Excretory pore opening ventrad at or near the nerve ring. Single testis reflexed and filling most of body; genital papillae as follows: three pair preanal papillae; paired spicules strong, sickle-shaped and possessing two delicate bars on each side; gubernaculum broad at proximal end and becoming narrow at distal tip. Tail convex-conoid, mucronulate.

TYPE HABITAT AND LOCALITY: Body cavity of the larvae of the sawfly, *Acantholyda nemoralis* Thoms., from Silesia, Poland.

DIAGNOSIS AND RELATIONSHIP: *N. janickii* Weiser and Kohler, 1955, is closely related to *N. chresima* Steiner, 1942. It differs from *N. chresima* by being larger in male body size, by the presence of a small spike on the male terminus and by having spicules with paired lateral bars.

Neoaplectana bothynoderi Kirjanova and Puchkova, 1955
(Fig. 2J)

FEMALES: L = 2,200 (1,200–2,200) μ; a = 10.8 (5.6–18); b = 11.7 (8.2–11.7); c = 25.6 (17.5–31.3); vulva = 58 (48–58)%.

MALES: L = 940 (740–940) μ; a = 9 (6.9–9); b = 6 (5.4–6); c = 18.2 (18.1–18.5); spicules = 64 (64–65) μ; gubernaculum = 36 (35–40) μ.

DESCRIPTION (emended): Neoaplectanidae. FEMALES: In body cavity of host insect. Body stout, ventrally arcuate, tapering anteriorly from the excretory pore and posteriorly from the anal region. Cuticle not striated. Head region with three lips with six labial and six cephalic papillae. Stoma reduced. Esophagus with simple corpus, slight isthmus and a terminal bulb with reduced musculature. Nerve ring in front of terminal bulb; excretory pore opening ventrad at or near the nerve ring. Gonads amphidelphic, large and symmetrical; ovaries with reflexure at terminal bulb and at rectum; vagina short; vulva transverse with two prominent ventral protuberances. Anus conspicuous with slight postanal lip. Tail conoid with pointed tip.

MALES: In body cavity of host insect. Body stouter than the female. Cuticle not striated. Head region similar to that of female. Excretory pore opening ventrad at or near the nerve ring. Single testis reflexed and filling most of body, genital papillae as follows; seven pair preanal, two pair adanal, and three pair postanal; paired spicules arcuate, ending in an enlarged proximal region, distal edges serrated; gubernaculum slightly rounded at proximal tip and serrated at distal edge. Tail convex-conoid, mucronulate.

TYPE HABITAT AND LOCALITY: Body cavity

of the beetle, *Bothynoderes punctiventria* Germ., from the Ukraine.

DIAGNOSIS AND RELATIONSHIP: *N. bothynoderi* Kirjanova and Puchkova, 1955, is closely related to *N. melolontha* Weiser, 1958. It differs from *N. melolontha* by the smaller female body size and the presence of serrations on the distal ends of the spicules and gubernaculum.

Neoaplectana melolontha Weiser, 1958
(Fig. 2K)

FEMALE: L = 2,736 μ; a = 13.2; b = 13.2; c = 15.2; vulva = 48%.

MALE: L = 1,020 μ; a = 15.5; b = 7.7; c = 22.7; spicules = 58 μ; gubernaculum = 44 μ.

DESCRIPTION (emended): Neoaplectanidae. FEMALE: In body cavity of insect host. Body stout, ventrally arcuate, narrowing slightly toward the extremities. Cuticle not striated. Head region with three lips with six labial and six cephalic papillae. Stoma reduced. Esophagus with long corpus, distinct isthmus and a terminal bulb with reduced musculature. Nerve ring in front of terminal bulb; excretory pore opening ventrad at or near nerve ring. Gonads amphidelphic, large and symmetrical; ovaries with reflexure at terminal bulb and at rectum; vagina short; vulva transverse with two prominent ventral protuberances. Anus conspicuous without postanal lip.

MALE: In body cavity of insect host. Body shorter and more slender than in female. Cuticle not striated. Head region similar to that of female. Excretory pore opening ventrad at or near the nerve ring. Single testis reflexed and filling most of body; genital papillae as follows: four pair postanal; paired spicules slightly bent with rounded proximal ends, and tapering to a point at the distal end; gubernaculum slightly curved, ending in a rounded knob in the proximal region. Tail convex-conoid, mucronulate.

TYPE HABITAT AND LOCALITY: Body cavity of the cockchafer, *Melolontha melolontha* L., from northeastern Czechoslovakia.

DIAGNOSIS AND RELATIONSHIP: *N. melolontha* Weiser, 1958, is closely related to *N. bothynoderi* Kirjanova and Puchkova, 1955. It differs from *N. bothynoderi* by a larger female body size and by lacking serrations on the distal edges of the spicules and gubernaculum.

Neoaplectana georgica Kakulia and Veremchuk, 1965
(Fig. 2L)

FEMALES: L = 1,178 (935–1,727) μ; a = 13.8 (11–18.3); b = 6.4 (6.8); c = 25.7 (16–45); vulva = 54 (52–56)%.

MALES: L = 920 (792–1,100) μ; a = 20.8 (16.5–24.4); b = 5.7 (5.2–6.5); c = 33.2 (29–33.9); spicules = 48 (42–53) μ; gubernaculum = 30 (24–34) μ.

DESCRIPTION (emended): Neoaplectanidae. FEMALES: In body cavity of the host insect. Body stout, ventrally arcuate, tapering anteriorly from excretory pore and posteriorly from anal region to tail tip. Cuticle striated. Head region with three lips with six labial and six cephalic papillae. Stoma reduced. Esophagus with simple corpus, slight isthmus and a terminal bulb with reduced musculature. Nerve ring in front of terminal bulb; excretory pore opening ventrad at or near the nerve ring. Gonads amphidelphic, large and symmetrical; ovaries with reflexure at terminal bulb and at rectum; vagina short; vulva transverse with two prominent ventral protuberances. Anus conspicuous with small postanal lip. Tail conoid with pointed tip.

MALES: In body cavity of the host insect. Body is smaller and more slender than the female. Cuticle is striated. Head region similar to that of female. Excretory pore opening ventrad at or near the nerve ring. Single testis strongly reflexed and filling half of body; genital papillae as follows: five pair preanal, ten pair adanal, and four pair postanal; paired orange spicules with lateral ribs, oblong in shape with a concave base; gubernaculum crescent-shaped without a proximal knob or hook. Tail convex-conoid, mucronulate.

TYPE HABITAT AND LOCALITY: Body cavity of a cockchafer, *Amphimallon solstitialis* L., from the Georgian, SSR.

DIAGNOSIS AND RELATIONSHIP: *N. georgica* Kakulia and Veremchuk, 1965, is closely related to *N. carpocapsae* Weiser, 1955. It differs from *N. carpocapsae* by having a larger male body size, orange spicules *with velum*, and a broader gubernaculum.

Neoaplectana dutkyi Jackson, 1965

Poinar (1967) showed the mating of *N. carpocapsae* with that of DD-136 resulted in infective juvenile progeny. This may be ex-

plained if the specimens that Poinar received from Weiser in Czechoslovakia as *N. carpocapsae* were in reality part of the original DD-136 culture which Dutky had previously sent to Weiser. Since the original specimens of *N. carpocapsae* were destroyed, it is impossible to substantiate this occurrence. It is the contention of the authors that *N. dutkyi* is a valid species.

(Fig. 2M)

FEMALE: $L = 1,170$ μ; $a = 12.1$; $b = 5.7$; $c = 20.2$; $V = 56.5\%$.

MALE: $L = 600$ μ; $a = 13.3$; $b = 6.4$; $c = 20.4$; spicules $= 46$ μ; gubernaculum $= 28$ μ.

DESCRIPTION (emended): Neoaplectanidae. FEMALES: In body cavity of host insect. Body stout, ventrally arcuate, tapering anteriorly from excretory pore and posteriorly from anal region to tail tip. Cuticle striated. Head region with three lips with six labial and six cephalic papillae. Stoma shallow and broad. Esophagus with simple corpus, slight isthmus and a terminal bulb with reduced musculature. Nerve ring in front of terminal bulb; excretory pore opening ventrad above nerve ring. Gonads amphidelphic large and symmetrical; ovaries with reflexure at terminal bulb and at rectum; vagina short; vulva transverse with two prominent ventral protuberances. Anus conspicuous with small postanal lip. Tail conoid with pointed tip.

MALES: In body cavity of the host insect. Body is smaller and more slender than female. Cuticle striated. Head region similar to that of female. Excretory pore opening ventrad above nerve ring. Single testis reflexed and filling half of body; genital papillae as follows: six pair preanal, two pair adanal, and three pair postanal; paired spicules with rounded ventral edge and pointed distal edge; gubernaculum with bluntly rounded head and almost straight ventral edge. Tail convex-conoid, mucronulate.

TYPE HABITAT AND LOCALITY: Body cavity of the larvae of the codling moth, *Carpocapsa pomonella*, from Virginia.

DIAGNOSIS AND RELATIONSHIP: *N. dutkyi* Jackson, 1965, is closely related to *N. carpocapsae* Weiser, 1955. It differs from *N. carpocapsae* by having a larger female body size, gubernaculum with bluntly rounded head, and the absence of ribs on spicules.

Neoaplectana hoptha Turco, 1970
(Fig. 2N)

MALES (paratypes) (n = 8): $L = 729$ (554–837) μ; $a = 20.3$ (17.6–22); $b = 7.2$ (5.9–8.2); $c = 28.9$ (18.1–37.4); spicules $= 47$ (43–50) μ; gubernaculum $= 28$ (26–30) μ.

FEMALES (paratypes) (n = 14): $L = 3,343$ (2,826–2,983) μ; $a = 15.2$ (12.7–19.3); $b = 15.9$ (12.4–20.7); $c = 57.5$ (46.9–67.2); vulva $= 47$ (43–49)%.

MALE (holotype): $L = 738$ μ; $a = 18.9$; $b = 7.5$; $c = 35.1$; spicules $= 49$ μ; gubernaculum $= 27$ μ.

FEMALE (allotype): $L = 3,302$ μ; $a = 14.7$; $b = 13.1$; $c = 66$; vulva $= 48\%$.

DESCRIPTION: Neoaplectanidae. FEMALES: In body cavity of the host insect. Body stout, doubling in diameter rather abruptly at latitude of the basal bulb. Cuticle not striated. Head region with three lips with six labial and six cephalic papillae. Stoma reduced. Esophagus with simple corpus, slender isthmus, and a terminal bulb with reduced musculature. Nerve ring encircling esophagus in front of basal bulb; excretory pore opening ventrad at or near nerve ring. Gonads amphidelphic, large and symmetrical; ovaries with reflexure at terminal bulb and at rectum; vagina short; vulva transverse with two prominent ventral protuberances. Anus conspicuous with large postanal lip. Tail conoid with pointed tip.

MALES: In body cavity of host insect. Body smaller and more slender than in female. Cuticle not striated. Head region similar to that of female. Stoma reduced. Excretory pore opening ventrad at or near the nerve ring. Single testis large and reflexed; paired spicules large, curved slightly, ending in an enlarged proximal region, distal end unhooked; gubernaculum long, narrow. Tail convex-conoid with rounded tip and numerous setae.

TYPE MATERIAL: Paratypes (females) Slide No. T-668p, paratypes (males) Slide No. T-668p, holotype (male) Slide No. T-121t, and allotype (female) Slide No. T-122t, deposited with the USDA Nematode Collection, Beltsville, Maryland.

TYPE HABITAT AND LOCALITY: Body cavity of the Japanese beetle, *Popillia japonica* Newm., from Moorestown, New Jersey.

DIAGNOSIS AND RELATIONSHIP: *N. hoptha* is closely related to *N. bibionis* Bovien, 1927. It differs from *N. bibionis* by its female body

doubling in diameter at the latitude of the basal bulb and by the male terminus being non-mucronulate.

The first complete key to the species of the genus *Neoaplectana* is given below.

Key to the Species of *Neoaplectana*

1. Lateral aspect of spicules with ribs (Figure 1C, F, H, I, L) 2
   Lateral aspect of spicules without ribs 6
2. Spicule with velum . . . *georgica* Kakulia & Veremchuk, 1965
   Spicule without velum 3
3. Spicule with paired lateral ribs . . . *chresima* Steiner, 1942
   Spicule without paired lateral ribs 4
4. Body length of male greater than 750 μ 5
   Body length of male less than 750 μ
   .... *carpocapsae* Weiser, 1955
5. Length of spicule or gubernaculum greater than 50 μ ....
   .... *feltiae* Filipjev, 1934
   Length of spicule or gubernaculum less than 50 μ ....
   .... *janickii* Weiser & Kohler, 1955
6. Male terminus mucronulate 7
   Male terminus non-mucronulate 11
7. Spicule and gubernaculum serrated at distal edge ....
   .... *bothynoderi* Kirjanova & Puchkova, 1955
   Spicule and gubernaculum not serrated at distal edge 8
8. Female body length greater than 2,000 μ 9
   Female body length less than 2,000 μ 10
9. V more than 50 .... *bibionis* Bovien, 1937
   V less than 50 ... *melolontha* Weiser, 1958
10. Length of spicule greater than 50 μ
    .... *leucaniae* Hoy, 1954
    Length of spicule less than 50 μ ....
    .... *dutkyi* Jackson, 1965
11. Female body length greater than 2,800 μ 12
    Female body length less than 2,800 μ
    .... *menozzii* Travassos, 1931
12. Female body doubles in diameter at latitude of basal bulb ....
    .... *hoptha* Turco, 1970
    Female body does not double in diameter at latitude of basal bulb .. 13

13. Spicules with distal tip hooked ....
    .... *glaseri* Steiner, 1929
    Spicules without distal tip hooked ....
    .... *affinis* Bovien, 1937

Discussion

A taxonomic and biological study was made of all known species of the insect parasitic family Neoaplectanidae. The purpose of the investigation was to determine the taxonomic position of this group and to describe the comparative morphology of all species within this family.

The taxonomy of this family was investigated by comparing its morphology and biology to that of all other groups to which it has been compared during its brief history. From this work it appears that *Neoaplectana* Steiner is a valid genus in a one-genus family which was correctly named Neoaplectanidae by Sobolev in 1953. An emendation of the group and a new description of the family are presented herein.

The morphology of all species in this family was examined and is described. Structural characters, de Man ratios, and illustrations of the nematodes were studied and are presented. The male copulatory structures were found to be the key characters in the taxonomy of this nematode group. Comparative diagnoses and a key to the species of *Neoaplectana* were formulated and are presented herein.

Increasing problems arising from the use of insecticides are necessitating the re-examination of other techniques, especially biological control. The use of entomophilic nematodes of this family as control agents is virtually an unexploited area and may be of great economic and scientific value at this time.

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A Review of the Family Cucullanidae Cobbold, 1864 and the Genus *Bulbodacnitis* Lane, 1916 with a Description of *Bulbodacnitis ampullastoma* sp. n. (Nematoda: Cucullanidae) from *Salmo gairdnerii* Richardson

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ABSTRACT: The family Cucullanidae is reviewed and a key to genera is given. The genus *Bulbodacnitis* is reviewed, a key to species is included. A new species *Bulbodacnitis ampullastoma* is described. Several new combinations are proposed.

The family Cucullanidae has been reviewed by Barreto (1922), Törnquist (1931), and Campana-Rouget (1957). Though they show some agreement, they differ as to the genera to be recognized in the family Cucullanidae. Barreto recognized only the genus *Cucullanus* Mueller, 1777. Törnquist recognized *Culcullanus, Cucullanellus* Törnquist, 1931, *Dichelyne* Jägerskiöld, 1902, *Dacnitis* Dujardin, 1845, and *Neocucullanus* Travassos, Artigas, and Pereira, 1928. He synonymized the nominal genera *Bulbodacnitis* Lane, 1916 and *Serradacnitis* Lane, 1916 with *Dacnitis*. In addition he synonymized *Dacnitoides* Ward and Magath, 1916 with *Dichelyne* on the basis that both possessed an anterior dorsal intestinal caecum; no significance was attached to the presence of a precloacal sucker in males of *Dacnitoides* and the absence of same in *Dichelyne*. Campana-Rouget pursued this line of reasoning and concluded that there was no significance in the presence or absence of the precloacal sucker and presence or position of the anterior intestinal caecum. She recognized only three genera in Cucullanidae: *Dacnitis, Cucullanus*, and *Neocucullanus*. *Dacnitis* included those cucullanids with the oral aperture dorsally oblique to the longitudinal axis of the body. *Cucullanus* and *Neocucullanus* included species with and without the precloacal sucker, with and without an intestinal caecum and with the additional mixture of caecum dorsal or ventral. Males of *Neocucullanus* are distinguishable by the presence of thirteen pairs of genital papillae; males of other cucullanids have eleven pairs of papillae.

The genus, taxonomically, is an arbitrary and opinionative assemblage of species. However,

the definition of a genus as a group of closely related species showing some one or series of common morphologic characters opposes the extreme conclusions of Barreto (1922) and Campana-Rouget (1957). The result of the latter's conclusions leave *Cucullanus* as a chaotic assemblage so encompassing in characters as to preclude recognition or proposal of genera in the family. The recognition of the aforementioned characters as taxonomically profound places the nominal species of Cucullanidae in eight genera: *Neocucullanus, Dacnitoides, Cucullanellus, Cucullanus, Indocucullanus* Ali, 1957, *Dichelyne, Neocucullanellus* Travassos, Artigas, and Periera, 1928, and *Bulbodacnitis*. Acceptance of these groups dictates the transference of *Dacnitoides robusta* Van Cleave and Mueller, 1932 to the genus *Dichelyne* as *Dichelyne robusta* (Van Cleave and Mueller) comb. n.

Confusion exists concerning the validity of the nominal genera *Bulbodacnitis* and *Dacnitis*. Dujardin (1845), in proposing the genus *Dacnitis* did not designate a type-species. Railliet and Henry (1912) designated *Dacnitis esuriens* Dujardin, 1845 as the type-species. Subsequently Railliet and Henry 1913 synonymized *D. esuriens* with *Cucullanus foveolatus* Rudolphi, 1809 a synonym of the type-species of *Cucullanus, C. cirratus* Mueller, 1777. Törnquist (1931) was apparently unaware of the designation of *D. esuriens* as type-species and proposed *Dacnitis truttae* (Fabricius) Törnquist, 1931 as type-species. This designation was erroneously accepted by Campana-Rouget (1957). Since *D. esuriens* was appointed as type-species in accord with the International Code of Zoological Nomenclature there remains

no choice but to accept that the nominal genus *Dacnitis* on the basis of its type-species is a synonym of *Cucullanus* and therefore unavailable. The subsequent designation of *D. truttae* was not in accord with the Code and must be rejected: "A nominal species is not rendered ineligible for designation as a type-species by reason of being the type species of another genus" (Art. 69, a.v., 1961). As a consequence the nominal species designated by Törnquist and Campana-Rouget in *Dacnitis* must be transferred to the next earliest available generic group, that is, *Bulbodacnitis* Lane, 1916.

Key to the Genera of Cucullanidae Cobbold, 1864

1. Males with caudal preanal sucker 2
   Males without caudal preanal sucker .. 6
2. Males with thirteen pairs of genital papillae .... *Neocucullanus*
   Males with eleven pairs of genital papillae 3
3. Anterior intestinal caecum present 4
   Anterior intestinal caecum absent 5
4. Anterior intestinal caecum, dorsal ......
   .... *Dacnitoides*
   Anterior intestinal caecum, ventral ......
   .... *Cucullanellus*
5. Oral aperture perpendicular to longitudinal body axis .... *Cucullanus*
   Oral aperture dorsally oblique to longitudinal body axis .... *Bulbodacnitis*
6. Anterior intestinal caecum present 7
   Anterior intestinal caecum absent ....
   .... *Indocucullanus*
7. Anterior intestinal caecum dorsal ....
   .... *Dichelyne*
   Anterior intestinal caecum ventral ....
   .... *Neocucullanellus*

Bulbodacnitis Lane, 1916

Bulbodacnitis bulbosa Lane, 1916, type-species.

SYNONYMY: *Cucullanus   bulbosa* (Lane) Barreto, 1918.

DIMENSIONS: Esophagus = 1.66[1], nerve ring = 0.6, cervical papillae = 1.15, excretory pore = 1.7. *Male*—L = 13.3, sucker to tail tip = 1.0, tail = 0.425, spicules = 0.7, gubernaculum = 0.11. *Female*—L = 14, vulva = 57%, tail = 0.366, eggs = 75 × 50 μ.

HOST: *Caranx melampygus.*

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[1] Unless otherwise stated, all measurements are in millimeters.

Bulbodacnitis ampullastoma sp. n.
(Fig. 1, A–F)

DIMENSIONS: *Females*—L = 7.24–11.63, a = 36–59, b = 7.5–9.9, c = 30–44, V = 61.5–67.5%, stoma = 0.20–0.25, esophagus = 0.96–1.24, nerve ring = 0.33–0.39, excretory pore and cervical papillae = 0.508–0.673, tail = 0.22–0.34, eggs = 73–80 × 50–58 μ.

Males—L = 7.21–11.13, a = 33–50, b = 7.9–9.9, c = 33–36, stoma = 0.17–0.22, esophagus = 0.86–1.15, nerve ring = 0.33–0.39, excretory pore and cervical papillae = 0.465–0.600, sucker to tail tip = 0.92–1.30, sucker length = 0.15–0.20, spicules = 0.503–0.610, gubernaculum = 0.105–0.125, tail = 0.21–0.34.

MALE (HOLOTYPE): L = 9.31, a = 40.5, b = 8.8, c = 36. Body stout, head bulbous, inclined dorsally, body width greatest at base of esophagus. Cuticle minutely striated, striations 2.5 μ apart. Cervical striations 10–15 μ apart, cuticle 10 μ thick. Head 0.21 wide, cervical region posterior to stoma 0.16 wide, body width at base of esophagus 0.23, width decreases almost imperceptibly to cloacal opening, just anterior to cloaca body width 0.16. Tail conical, 0.26 long. Oral aperture ellipsoidal, dorsally oblique to longitudinal axis of body. Aperture bordered by minute denticles, approximately 100 in number, each denticle 3.6 μ long. Six cephalic papillae surround the stomatal aperture. A dorsal ridge (tuberculate in lateral view) traverses subdorsal sectors of cephalic region 0.18 from anterior extremity. In lateral view ridge measures 95 × 17 μ. Buccal cavity 0.22 long, divided, anterior portion globose formed of six major plates, posterior portion funnel-shaped formed of three plates. Esophageal tissue surrounds the buccal cavity. Esophagus clavate, 1.06 long. Nerve ring surrounds esophagus at junction of muscular and glandular portions, 0.35 from anterior extremity. Cervical papillae 0.535 from anterior extremity. Excretory pore just posterior to level of cervical papillae, 0.538 from anterior extremity. Intestine extends anteriorly, 0.06, as a cup around base of esophagus. Esophagointestinal valve 80 μ long. Testis extends to 1.17 of esophagus base. Precloacal sucker 1.07 from tail tip, sucker length 0.17. Spicules 0.53 long, gubernaculum furcate 0.11 long. Eleven pairs of genital papillae: first pair just anterior to sucker, second pair just posterior to sucker, third pair two-thirds distance from sucker to
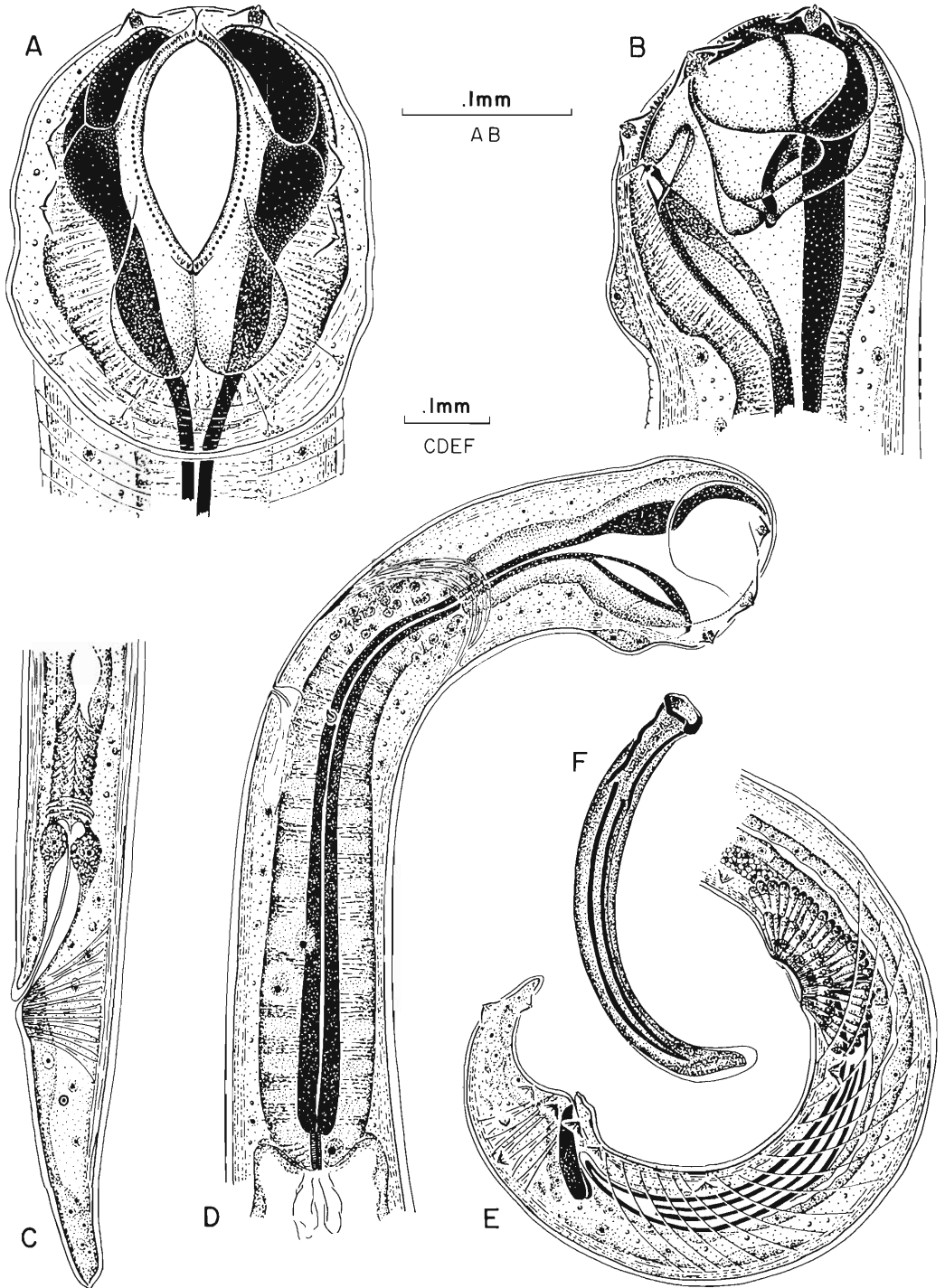
Figure 1. *Bulbodacnitis ampullastoma* sp. n. A. Female head, en face view; B. Female head, lateral view; C. Female tail; D. Female, anterior body; E. Male tail; F. Male spicule, excised.

cloaca. Four pairs adanal, two pairs caudal, near tail tip. One pair lateral at level of most posterior adanal papillae (likely to be phasmids) and one pair subdorsal at cloacal level. Sperm circular, 15–16 μ in diameter.

FEMALE (ALLOTYPE): L = 10.52, a = 39, b = 9.1, c = 32.9, V = 61.5%. Head bulbous, inclined dorsally, body width greatest at base of esophagus. Cuticle minutely striated 2.5 μ apart, cervical striations 10–15 μ apart. Head 0.22 wide, cervical region posterior to stoma 0.20 wide, body width at base of esophagus 0.27, from this level body width decreases imperceptibly to anal width of 0.21, then conoid to tail tip. Oral aperture ellipsoidal, dorsally oblique to longitudinal axis of body. Aperture bordered by minute denticles 3.6 μ long, approximately 100 in number. Six cephalic papillae surround stomatal aperture. A dorsal ridge (tuberculate in lateral view) traverses subdorsal sectors of cephalic region, 0.188 from anterior extremity. In lateral view dorsal ridge measures 95 × 25 μ. Buccal cavity 0.24 long, divided, anterior portion globose, posterior portion funnel shaped. Esophageal tissue surrounds the buccal cavity. Esophagus clavate, 1.16 long. Nerve ring surrounds esophagus at junction of muscular and glandular portions, 0.39 from anterior extremity. Cervical papillae 0.558 from anterior extremity. Excretory pore just posterior to level of cervical papillae, 0.565 from anterior extremity. Intestine surrounds posterior portion of esophagus, extending anteriorly as a cup, 0.09. Esophago-intestinal valve 0.11 long. Vulva raised, located 6.47 from anterior extremity. Tail conical 0.320 long. Eggs 73–80 × 50–58 μ.

HOLOTYPE: Male, Catalogue No. 188 UCNC, Davis, collected 5 February 1970 by R. J. Toth.

PARATYPES: Two males, Nos. 189 and 190 UCNC, Davis; two females, Nos. 191 and 192 UCNC, Davis; one male and one female, Parasitological Laboratory, USDA, Beltsville, Maryland.

TYPE HOST: *Salmo gairdnerii* Richardson.

HABITAT: Pyloric caeca and duodenum.

TYPE LOCALITY: Hot Creek Hatchery, Supply Pond No. 2, Mono County, Calif.

DIAGNOSIS: *Bulbodacnitis ampullastoma* can be distinguished from *B. truttae*, the species it most closely resembles by the more anterior

position of the nerve ring (*B. ampullastoma*, 0.33–0.39; *B. truttae*, 0.468–0.515), cervical papillae (*B. ampullastoma*, 0.50–0.67; *B. truttae*, 0.76–1.016), and excretory pore (*B. ampullastoma*, 0.50–0.67; *B. truttae*, 0.72–0.80). Males can be distinguished by the more anterior position of the precloacal sucker (*B. ampullastoma*, 0.9–1.3; *B. truttae*, 0.795), shorter spicules (*B. ampullastoma*, 0.5–0.61; *B. truttae*, 0.65), and longer gubernaculum (*B. ampullastoma*, 0.1–0.13; *B. truttae*, 0.04).

Bulbodacnitis truttae
(Fabricius, 1794) comb. n.

SYNONYMY: *Cucullanus truttae* Fabricius, 1794; *Cucullanus globosus* Zeder, 1800; *Dacnitis globosa* (Zeder) Dujardin, 1845; *Bulbodacnitis globosa* (Zeder) Lane, 1916; *Dacnitis truttae* (Fabricius) Törnquist, 1931; *Bulbodacnitis occidentalis* Smedley, 1933.

DIMENSIONS: *Male*—L = 9.66–12.83, esophagus = 1.11–1.33, nerve ring = 0.468–0.515, cervical papillae = 0.759, excretory pore = 0.8, sucker to tail tip = 0.795, tail = 0.309–0.373, spicules = 0.65, gubernaculum = 0.04. *Female*—L = 11.2–15.1, esophagus = 1.25–1.67, nerve ring = 0.48–0.78, cervical papillae = 1.016, excretory pore = 0.72, vulva = 61–62%, tail = 0.250–0.312, eggs = 62 × 47 μ.

HOST: *Salmo trutta*.

Bulbodacnitis sphaerocephala
(Rudolphi, 1809) comb. n.

SYNONYMY: *Ascaris sphaerocephala* Rudolphi, 1809; *Ophiostoma sphaerocephalum* (Rudolphi) Rudolphi, 1819; *Dacnitis sphaerocephala* (Rudolphi) Dujardin, 1845; *Heterakis sphaerocephala* (Rudolphi) Schneider, 1866; *Cucullanus sphaerocephala* (Rudolphi) Barreto, 1922.

DIMENSIONS: *Male*—L = 16.12–22.71, esophagus = 1.86–2.12, nerve ring = 0.468–0.515, cervical papillae = 0.647–0.671, excretory pore = 0.390, sucker to tail tip = 1.1, tail = 0.265–0.374, spicules = 0.323, gubernaculum 0.075. *Female*—L = 19.47–28.55, esophagus = 1.89–2.28, nerve ring = 0.468–0.562, cervical papillae = 0.675, excretory pore = 0.421, vulva = 56–61%, tail = 0.374–0.562, eggs = 78 × 47 μ.

HOST: *Acipenser microcephalus*, *A. sturio*.

Bulbodacnitis clitellarius (Ward and Magath, 1916) comb. n.

SYNONYMY: *Cucullanus clitellarius* Ward and Magath, 1916; *Dacnitis clitellarius* (Ward and Magath) Törnquist, 1931.

DIMENSIONS: *Male*—L = 10–11, esophagus = 1.45, sucker to tail tip = 0.9, tail = 0.39, spicules = 1.62, gubernaculum = 0.06. *Female*—L = 12–17, esophagus = 1.6, vulva = 55–66%, eggs = 63 × 46 μ.

HOST: *Acipenser rubicundus.*

Bulbodacnitis scotti Simon, 1935

SYNONYMY: *Dacnitis scotti* (Simon) Campana-Rouget, 1957.

DIMENSIONS: Esophagus = 1.1, nerve ring = 0.45. *Male*—L = 7.69, excretory pore = 0.641, sucker to tail tip = 0.745, spicules = 0.358, gubernaculum = 0.118. *Female*—L = 9.84, vulva = 65%.

HOST: *Salmo clarkii.*

Bulbodacnitis heterodonti (Johnston and Mawson, 1943) comb. n.

SYNONYMY: *Cucullanus heterodonti* Johnston and Mawson, 1943; *Dacnitis heterodonti* (Johnston and Mawson) Campana-Rouget, 1957.

DIMENSIONS: Esophagus = 1.6–1.76, nerve ring = 0.52, excretory pore = 0.54. *Male*—L = 18, sucker to tail tip = 2.0, tail = 0.4, spicules = 3.2, gubernaculum = 0.12. *Female*—L = 20–30, vulva = 53%, tail = 0.44, eggs = 80 × 54 μ.

HOST: *Heterodontus philippi.*

Bulbodacnitis australis (Johnston and Mawson, 1945) comb. n.

SYNONYMY: *Dacnitis australis* Johnston and Mawson, 1945.

DIMENSIONS: Female—L = 7.6, cervical papillae = 0.45, excretory pore = 0.54, esophagus = 0.68, eggs = 54 × 39 μ, tail = 0.07. *Male*—L = 5.8, spicules = 0.59, gubernaculum = 0.07, sucker to tail tip = 0.41, tail = 0.09.

HOST: *Notopogon lilliei.*

Bulbodacnitis lebedevi (Skriabina, 1966) comb. n.

SYNONYMY: *Cucullanus lebedevi* Skriabina, 1966.

DIMENSIONS: *Male*—L = 6.33–13.37, nerve ring = 0.10–0.65, cervical papillae = 0.7–1, excretory pore just posterior to nerve ring, spicules = 0.73–1.11, gubernaculum = 0.92, precloacal sucker = 1.4. *Female*—L = 8.32–15.52, esophagus = 1.22–1.95, vulva = 7.33 from anterior extremity, eggs = 52–91 × 32–53 μ.

HOST: *Acipenser baeri.*

Species Inquirenda

B. squali (Dujardin, 1845) comb. n.

SYNONYMY: *Dacnitis squali* Dujardin, 1845; *Cucullanus squali* (Dujardin) Diesing, 1851; *Heterakis squali* (Dujardin) Stossich, 1888; *Serradacnitis squali* (Dujardin) Lane, 1916.

HOST: *Galeus galeus.*

B. attenuata (Molin, 1858) comb. n.

SYNONYMY: *Dacnitis attenuata* Molin, 1858; *Heterakis attenuata* (Molin) Stossich, 1888; *Cucullanus attenuatus* (Molin) Barreto, 1918.

HOST: *Leuciscus cavedanus.*

B. rotundata (Molin, 1859) comb. n.

SYNONYMY: *Dacnitis rotundata* Molin, 1859; *Heterakis rotundata* (Molin) Stossich, 1888; *Cucullanus rotundatus* (Molin) Barreto, 1918.

HOST: *Cantharus vulgaris.*

B. stelmioides (Vessichelli, 1910) comb. n.

SYNONYMY: *Dacnitis stelmioides* Vessichelli, 1910.

HOST: *Petromyzon planeri.*

B. laevis (Heitz, 1914) comb. n.

SYNONYMY: *Dacnitis laevis* Heitz, 1914.

HOST: *Salvelinus malma.*

B. callichroi (Stewart, 1914) comb. n.

SYNONYMY: *Dacnitis callichroi* Stewart, 1914; *Cucullanus callichroi* (Stewart, 1914) Barreto, 1922.

HOST: *Callichrous macrophtalmus.*

Key to the Species of Bulbodacnitis Lane, 1916

1. Excretory pore 1 mm or more from anterior extremity ⸺⸺⸺⸺ *bulbosa*
   Excretory pore less than 1 mm from anterior extremity ⸺⸺⸺⸺ 2
2. Spicules more than 1.5 mm long ⸺⸺⸺ 3
   Spicules less than 1.5 mm long ⸺⸺⸺ 4

3. Spicules less than 2 mm long, esophagus
    1.6 mm or less .... *clitellarius*
    Spicules more than 3 mm long, esoph-
    agus 1.6 mm or more .... *heterodonti*
4. Spicules less than 0.4 mm 5
    Spicules more than 0.4 mm 6
5. Excretory pore less than 0.5 mm from
    anterior extremity, precloacal sucker
    1 mm or more from tail tip, esoph-
    agus 1.5 mm or longer .. *sphaerocephala*
    Excretory pore more than 0.5 mm from
    anterior extremity, precloacal sucker
    less than 1 mm from tail tip, esoph-
    agus less than 1.5 mm long .... *scotti*
6. Precloacal sucker less than 0.9 mm from
    tail tip 7
    Precloacal sucker more than 0.9 mm
    from tail tip 8
7. Cervical papillae less than 0.5 mm from
    anterior extremity, excretory pore
    less than 0.65 mm from anterior
    extremity, esophagus less than 1 mm
    long .... *australis*
    Cervical papillae more than 0.5 mm
    from anterior extremity, excretory
    pore more than 0.65 mm from ante-
    rior extremity, esophagus 1 mm long
    or more .... *truttae*
8. Cervical papillae at level of excretory
    pore, less than 0.7 mm from ante-
    rior extremity spicules less than 0.7
    mm long .... *ampullastoma* sp. n.

Cervical papillae at mid esophagus,
    posterior to level of excretory pore,
    spicules more than 0.7 mm long .....
    .... *lebedevi*

Acknowledgments

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Diplotriaena lagopusi and D. andersoni spp. n. (Diplotriaenidae: Filarioidea) from White-Tailed Ptarmigan (Lagopus leucurus) in North America[1]

O. Wilford Olsen[2] and Clait E. Braun[3]

ABSTRACT: Diplotriaena lagopusi and D. andersoni spp. n. are described from white-tailed ptarmigan from Colorado, USA, and Alberta, Canada, respectively.

In his comprehensive monograph on the Diplotriaena, Anderson (1959) indicated that the spicules and tridents are stable structures and that certain differences in their conformation are reliable criteria of speciation.

On the basis of spicules, tridents, and other characteristics, Diplotriaena from white-tailed ptarmigan (Lagopus leucurus) from two widely separated areas of North America appear to represent two undescribed species for which names are proposed.

The specimens allocated to one of the species were recovered from 4 of 204 birds which were shot by hunters in Colorado and collected by one of us (C.E.B.). The fresh viscera were removed and frozen until examined. The worms were preserved in 70% alcohol, containing 5% glycerine. Clearing was in glycerine or alcohol-phenol solution.

The worms assigned to the second species were made available by Dr. Roy C. Anderson for study. They were collected in Alberta, Canada, presumably from a single bird, were poorly preserved, cleared in glycerine, and in a flattened condition resulting in considerable distortion. Measurements given in parentheses are means.

Diplotriaena lagopusi sp. n.
(Figs. 1–9)

DESCRIPTION: Tridents medium-sized with apex tridigitate in lateral view, pointed in dorso-ventral aspect; apex without longitudinal grooves; short arms of tridents close together, long arm distinctly separated from middle arm.

Oral opening a small dorso-ventral slit surrounded by a large rectangular plate whose sides are pressed inward slightly by large circular plates surrounding openings of tridents. Oesophagus has short-narrow muscular region and long, broad glandular section that constricts sharply at point of connection with wide intestine. Cuticle smooth.

MALES: (Four complete and one incomplete (head missing) specimens.) Body length of complete individuals 32–42 (37.2) mm, incomplete one 46 mm; maximum width 593–646 (628) μ. Tridents 117–148 (133) μ long. Nerve ring 212–252 (231) μ from anterior end of body. Muscular section of oesophagus 403–445 (424) μ, glandular portion 3.76–4.24 (3.921) mm long. Anus 60 μ from posterior extremity of body. Tail rounded and slightly expanded laterally. Two pairs of marginal post-anal papillae, two pairs of adanal papillae, two pairs of median preanal papillae, and five pairs of papillae arranged in an anterio-lateral line, beginning near anus. Right spicule with two twists, 572–688 (627) μ long; left spicule with slight sword-like curve toward tip, otherwise nearly straight, 731–890 μ long. Tip of each spicule narrows abruptly from one side to form a short, asymmetrical point.

FEMALES: (Three complete specimens.) Body length 60–80 (69.8) mm, maximum width 636–848 (724) μ. Tridents 120–246 (133) μ long. Nerve ring 223–265 (244) μ from anterior end of body. Muscular portion of oesophagus 403–445 (424) μ and glandular region 3.26–4.24 (3.921) mm long. Vulva 562–689 (633) μ from head, vagina 360–740 (543) μ long. Eggs with thick shell, 27–37 (31) by 29–52 (50) μ.

HOST: Southern white-tailed ptarmigan (Lagopus leucurus altipetens Osgood).

HABITAT: Body cavity.
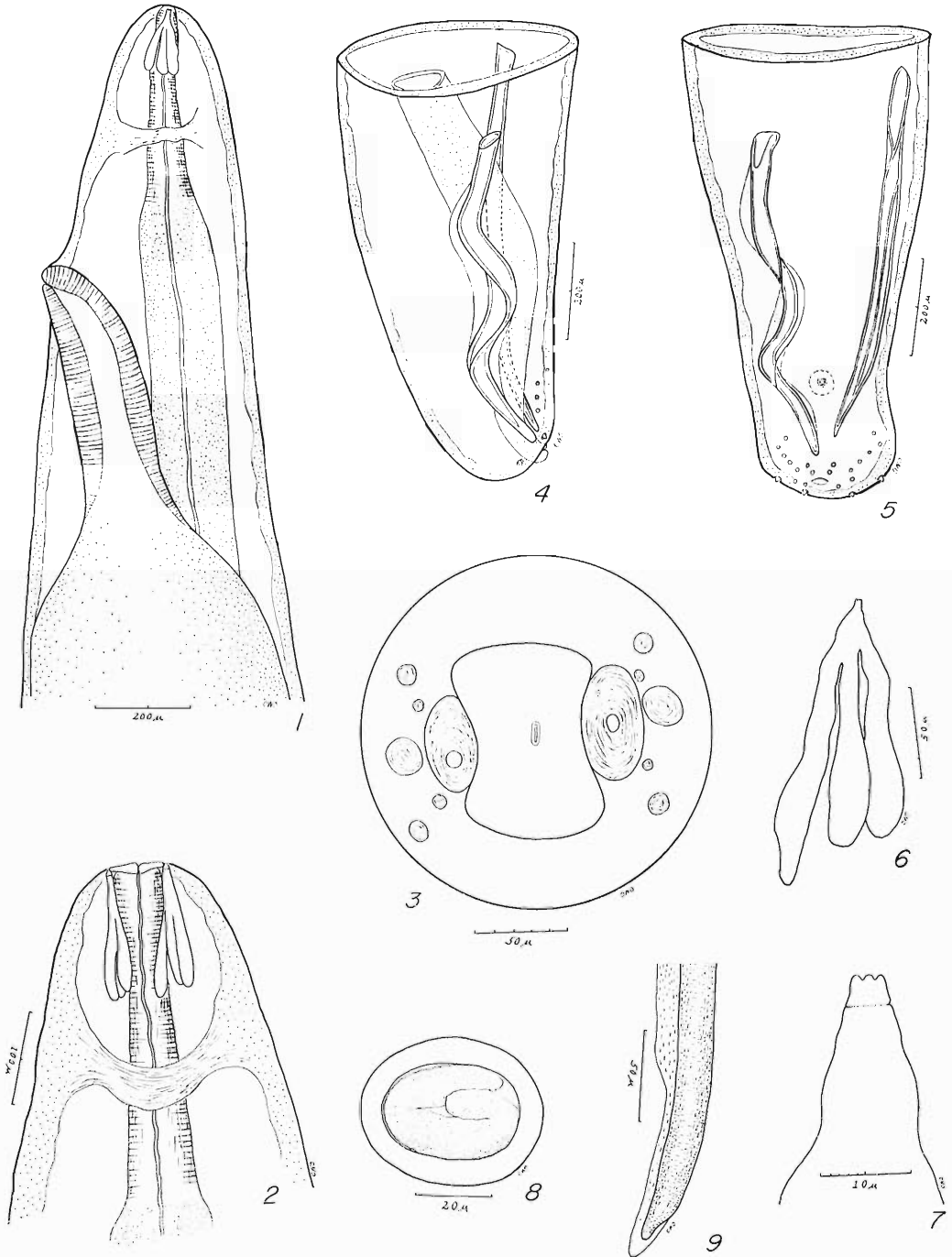
Figures 1–9. *Diplotriaena lagopusi*. 1. Sinistral view of anterior end of adult female. 2. Dorsal view of anterior end of adult male. 3. En face view of adult female. 4. Dextral view of caudal end of adult male. 5. Ventral view of caudal end of adult male. 6. Lateral view of trident. 7. Detailed view of apex of trident. 8. Fully embryonated egg. 9. Sinistral view of distal end of left spicule.
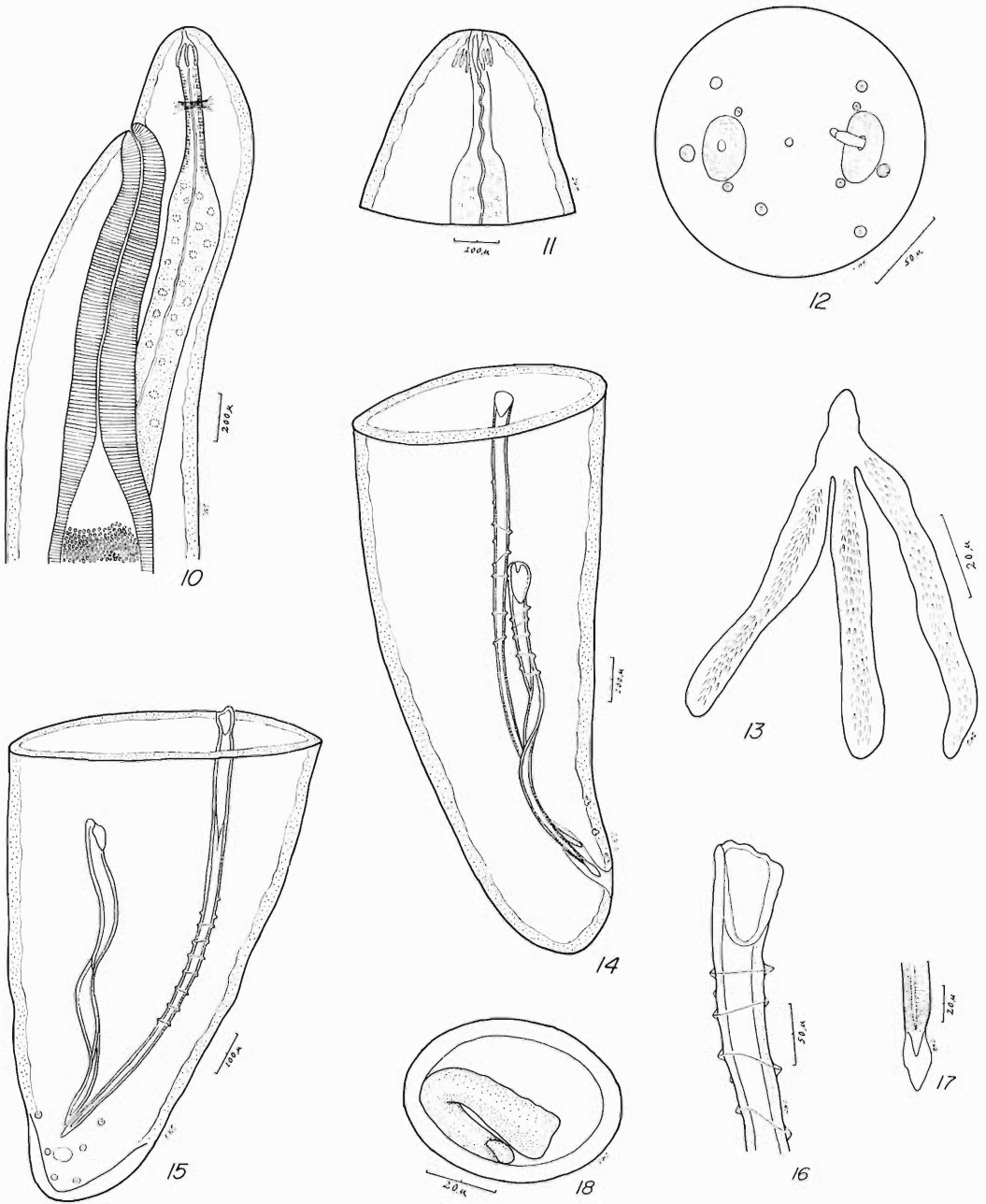
Figures 10–18. *Diplotriaena andersoni.* 10. Sinistral view of anterior end of mature female. 11. Dorsal view of anterior end of adult male. 12. En face view of adult female. 13. Trident from adult worm. 14. Dextral view of adult male. 15. Ventral view of adult male. 16. Base of right spicule. 17. Tip of right spicule. 18. Embryonated egg from uterus.

LOCALITY: Alpine regions of Rocky Mountains of central and northern Colorado.

COLLECTOR: Clait E. Braun.

PARATYPE SPECIMENS: U. S. National Helminthological Collection No. 70518.

REMARKS: This species falls into the group of *Diplotriaena* in which the right spicule has two twists (Figs. 4, 5). The group presently includes only two species in which the apex of the tridental handle is, as in the new species, tridigitate. One of these, *D. tridens*, differs from *D. lagopusi* in oesophageal morphology, the presence of denticulations on both sides of the basal portion of the right spicule, absence of circumoral cuticular plate, and presence of a cephalic depression between the tridental openings. *D. perdicis*, the other species, differs from the new one in having the left spicule thick in the basal half and thin in distal half; apparently the circumoral plate is lacking; and the tail of the male is only 20 μ long.

Diplotriaena andersoni sp. n.
(Figs. 10–18)

DESCRIPTION: Tridents small, apex rounded in lateral view; arms spread about equidistant apart, with chevron-like markings on outer surface. Oral opening round, without surrounding cuticular plate. Oesophagus with short, narrow, and anterior muscular region and long, broad glandular section. Cuticle smooth.

MALES: (One complete specimen and the posterior quarter of another.) Body length 42.4 mm; maximum width of flattened body 753 μ. Tridents 65 μ long. Muscular portion of oesophagus 286 μ, posterior glandular section 5.3 mm long. Anus 88 and 127 μ from posterior end of body. Rounded tail appears to be slightly expanded but uncertain due to flattened body. At least three pairs of caudal papillae recognizable, of which one pair is post-, one ad-, and one preanal. Right spicule 700 μ long, with two twists, tip spatulate; left spicule 1.015 and 1.7 mm long, arcuate, and slender.

FEMALES: (Only incomplete specimens, two with head.) Length of three largest pieces 65–75 mm, greatest width of flattened bodies 752 μ. Tridents 80 μ long. Muscular portion of oesophagus 391 μ, glandular region 5.461 mm long. Vulva 4.13 mm from head; vagina about 1.5 mm long. Eggs (10) with thick shells, 45–53 (49) by 61–69 (67) μ.

HOST: Northern white-tailed ptarmigan (*Lagopus leucurus leucurus* Richardson).

HABITAT: Air sacs.

LOCALITY: Plateau Mountain, Alberta, Canada.

COLLECTOR: E. Hubner.

PARATYPES: U. S. National Helminthological Collection No. 70519.

This species is named in honor of Dr. Roy C. Anderson who made it available for study and who has contributed so much to clarifying this taxonomically difficult genus of nematodes.

It differs from *D. lagopusi* sp. n. in several respects, notably in size and morphology of tridents, lack of circumoral cuticular plate, and length of left spicule. Because *D. andersoni* has a right spicule with two twists, apically rounded tridents, and a left spicule over 1 mm long, it falls into a group with *D. bhamoensis*, *D. indica*, *D. anisorama*, and *D. muscisaxicolae* (a species of doubtful status). It differs from these species in having small tridents (65–80 μ long).

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The Identity of *Paratylenchus nanus* Cobb, 1923[1]

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Plant Science Department, Field Crops—Plant Pathology—Soils,
South Dakota State University, Brookings, South Dakota

ABSTRACT: *Paratylenchus nanus* is redescribed and illustrated from specimens collected from native sod near Devil's Lake, North Dakota, from nursery and soybean fields near Brookings, South Dakota and from pasture soil, Minden, Nebraska.

Paratylenchus nanus was described by Cobb (1923) from a single female collected near Devil's Lake, North Dakota by "Dr. Young" who forwarded it with a letter dated 7 April 1915. Three females were also collected near Falls Church, Va. by J. R. Christie. A complete account of these specimens and an amended description was published by Tarjan (1960). Tarjan's examination of the original specimen showed that it was distinguished by a small, digitate, rounded terminus unlike that of any other described species.

Geraert (1965) made *P. nanus* a synonym of *P. bukowinensis* Micoletzky. However, a redescription of *P. bukowinensis* by Loof and Oostenbrink (1968) and information presented herein indicate that this synonymy is invalid.

The senior writer visited Devil's Lake on 7 June 1968 and made 8 collections of soil from native grass and shrubs west of the city. Five of these contained *Paratylenchus*. Unfortunately these specimens had gone through their period of reproduction and only young individuals without gonads were present when portions of the collections were examined at the South Dakota State University Laboratory. Portions of the samples were placed in a cold room at 34° for several weeks but this did not break the life cycle when they were brought into an air-conditioned greenhouse. Other portions immediately placed in the greenhouse failed to mature. As a last resort the junior writer placed some of the soil in pots and plunged them in soil near the greenhouse where they remained during the winter of 1968–69. These were brought into a laboratory in April 1969 and from them about a dozen females were secured. These were immediately recognized by the blunt, digitate terminus, processed

and mounted on slides. No males were present, the females possessing spermagonia instead of spermatheca, indicating that the species is monosexual. Morphologically the specimens correspond rather well with Tarjan's description from the old collections. However, it is deemed worthwhile to prepare a complete illustration and an amended description from this new material.

Paratylenchus nanus
Cobb, 1923 Amended

(10): 0.35–0.42 mm; a = 19–22; b = 3.6–4.3; c = 13–18; V = $^{42-51}$83–88. Spear 28–32 μ.

Body slightly arcuate with ventrally bent posterior portion, ending in a short, rounded digitate terminus. However, some specimens had slightly more tapering tails while one individual was almost devoid of a digitate process. Annules about 1 μ apart where they can be distinguished. Lateral field with four minute incisures occupying about ⅓ body width. Anterior end convex-conoid to a narrow, truncate lip region about 4 μ wide, bearing very obscure papillae. Spear 27–32 μ long with strong sloping basal knobs. Junction of the two parts of the spear shaft obscure. Outlet of dorsal esophageal gland about 8 μ behind spear. Median bulb unusually well-developed, occupying about ½ esophagus length. Lumen of esophagus from valve through the narrow isthmus and basal bulb appearing as a single line, not as a tube like that from the spear to the valve. Basal bulb with *conspicuous* dorsal gland nucleus and two obscure submedian nuclei. Cardia hemispheroid to conoid, often pressed out of shape and indefinite in form. Excretory pore about opposite anterior end of basal bulb, posteriorly adjacent to hemizonid. Deirids not seen. Intestine with very
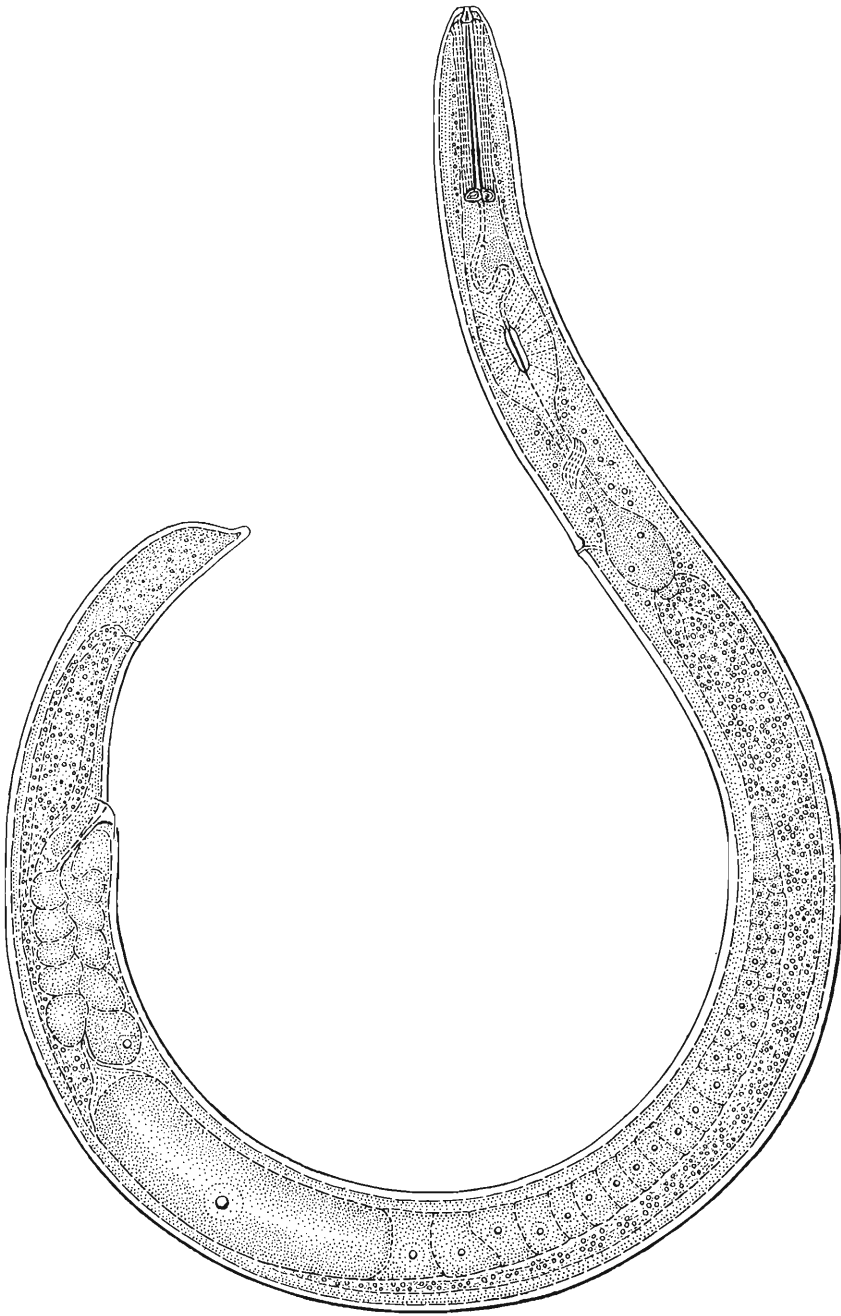
Figure 1. *Paratylenchus nanus*: 0.4 mm; a = 20; b = 4.0; c = 17; V = ^{50}87; spear 30 μ. Note that the spermagonium actually is on the right ventrosubmedian portion of the body, not ventrad as it appears in this illustration.

fine granules arranged in a somewhat tessellated pattern, and extending about half-way into postvulvar part of body. Rectum and anus obscure.

Vulva a massive transverse slit with strong lateral membranes. Vagina extending in and forward to join the quadricolumella which is about 5 cells long. The anterior right ventro-submedian cell forms a spermagonium possessing a conspicuous nucleus from which the sperms originate to fertilize the eggs as they enter the quadricolumella to receive their shells. Eggs 3 to 4 times as long as wide. Ovary outstretched with oocytes in single file except in a short region of multiplication. There was no trace of a posterior uterine branch in any of the specimens observed, and since these specimens are generally in good condition it may be assumed that it is not present. The structure illustrated by Tarjan (1960) probably was a pressure artifact caused by extreme flattening of the specimen. Tarjan reported a spherical spermatheca in the Falls Church, Va. specimens which may indicate that they are not *P. nanus* but a similar bisexual species.

HABITAT: Native prairie sod west of Devil's Lake, North Dakota, soil from a conifer nursery and soybean field near Brookings, South Dakota, and pasture soil, Minden, Nebraska.

Topotypes and other specimens indexed under *Paratylenchus* 8, South Dakota State University nematological collection.

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New Jersey Society of Parasitology

A new regional organization with an interest in parasitology known as the New Jersey Society of Parasitology was formed recently. Officers elected were: President, Dr. J. J. Yakstis, (Merck Sharp and Dohme Laboratories, Rahway, New Jersey); Vice President, Dr. Donald M. Levine, (Paterson State College, Paterson, New Jersey); Secretary-Treasurer, Dr. Eric Panitz, (Schering Corporation, Animal Health Division, Allentown, New Jersey). Inquiries should be directed to the Secretary-Treasurer or the other officers.

Helminths of Mammals and Birds from Israel. II. *Sinaiotaenia witenbergi* gen. et sp. n. (Cestoda: Anoplocephalidae) from Desert Rodents[1]

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Abstract: A cestode first recovered from *Gerbillus dasyurus* in Israel, found also in gerbilline rodents from the Sinai Peninsula, is described as *Sinaiotaenia witenbergi* gen. et sp. n. As a result of experimental exposure of two species of tenebrionid beetles and an unidentified oribatid mite to the tapeworm eggs, cysticercoids developed in adults of *Tribolium confusum*. The unarmed scolex, the character of the gravid proglottids with eggs scattered singly in the parenchyma, and the use of an insect as intermediate host, point to the tapeworm as belonging to the Linstowiinae subfamily of the Anoplocephalidae. It differs from other genera of this subfamily by the position of the female genitalia in the posterior part of the proglottid, the localization of the numerous testes anterior to these organs, and the particularly elongated gravid proglottids.

When examining rodents from the southern region of Israel for helminths, a cestode which could not be assigned to any of the known genera was recovered from *Gerbillus dasyurus*. The description of this cestode, *Sinaiotaenia witenbergi* gen. et sp. n., is based upon additional material lately recovered from *Gerbillus dasyurus*, *G. gerbillus*, *Sekeetamys calurus*, and *Meriones crassus* caught in the Sinai Peninsula.

Materials and Methods

Living worms, allowed to relax in tap water, were fixed in hot alcohol-formalin-acetic acid and stained with Semichon's carmine. Transversal and sagittal sections 8 μ thick were prepared from proglottids fixed in Bouin's fluid and stained in Ehrlich's hematoxylin and eosin.

For life history studies, gravid proglottids were teased on pieces of wet filter paper and offered to larvae and adults of laboratory-reared arthropods (*Tenebrio molitor*, *Tribolium confusum*, and an oribatid mite) which were examined 18 days post-infection.

Drawings were prepared with the aid of a Reichert viso-pan.

All measurements are in microns unless otherwise indicated.

Sinaiotaenia gen. n.

Diagnosis: Anoplocephalidae, Linstowiinae. Medium-sized tapeworms with proglottid length

increasing posteriad. Two excretory stems on each side of the strobila. Testes numerous, in medullary parenchyma, anterior to female genitalia. Seminal vesicle absent. Ovary and vitelline gland close to posterior margin of proglottid. Seminal receptacle present. Genital ducts passing between longitudinal excretory stems. Genital pores in middle third of proglottid margin, alternating irregularly. Egg capsules, each with a single egg, scattered in medullary parenchyma of gravid proglottid. Parasitic in rodents.

The name *Sinaiotaenia* refers to the Sinai Peninsula, where the parasite was found in considerable numbers.

Type species: *Sinaiotaenia witenbergi* sp. n.

Sinaiotaenia witenbergi sp. n.

Diagnosis: In the following description of fixed and stained material measurements of the type specimen are supplemented by data on paratypes (range given in parentheses). The description is based on 10 specimens (type and 9 paratypes), each without the original terminal proglottid. The strobila is 145 (87–222) mm long with a maximum width of 2.1 (1.7–2.8) mm. The unarmed scolex is 434 (385–528) in transverse diameter and not sharply separated from the neck region which is 1.18 (0.90–1.72) mm long (Fig. 1). Suckers 183 (164–220) in diameter. Concentrations of deeply staining nuclei indicating the primordia of internal organs appear in proglottids 6 or 7, measuring 868 (714–1,000) in width and 284
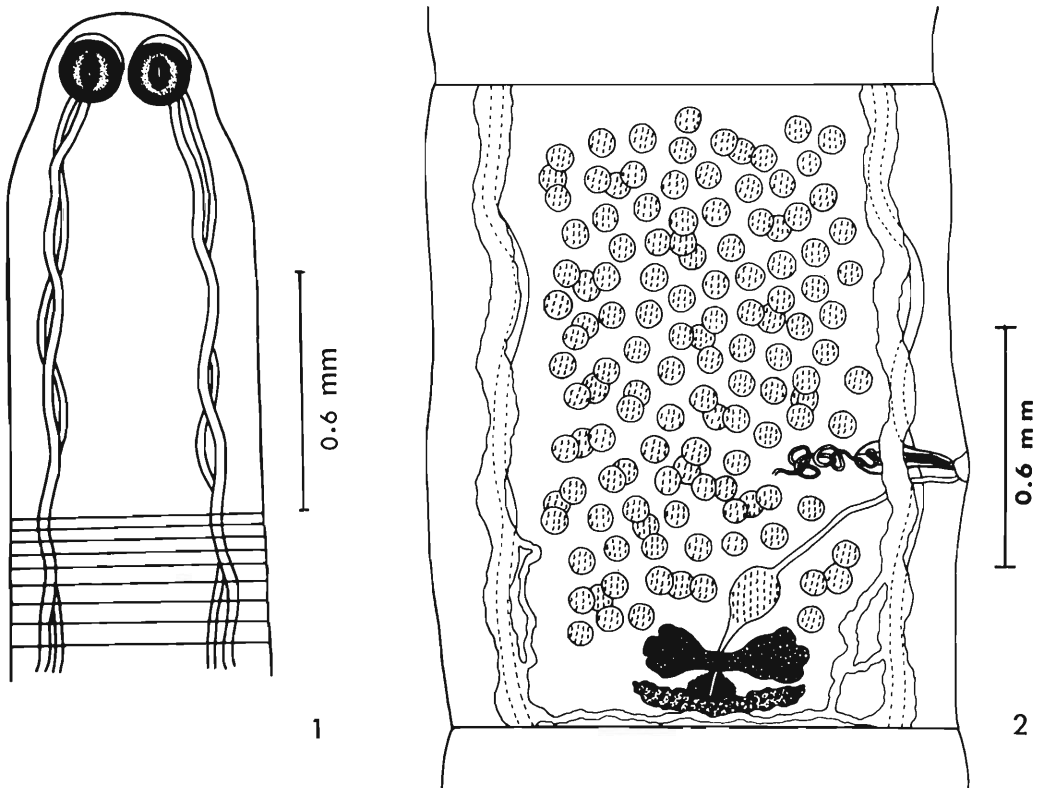
Figures 1–4. *Sinaiotaenia witenbergi* sp. n. 1. Scolex. 2. Mature proglottid. 3. Mature and gravid proglottids drawn to scale. 4. Egg capsule.

(142–460) in length. Maturity is attained in proglottids 22–24 which measure 1.7 (1.4–2.5) mm in length and 1.41 (1.2–1.8) mm in width and are almost rectangular. In proglottids 35–37 the sexual organs begin to recede and narrow strands of eggs, forming an irregular network, appear in the parenchyma. Proglottids 37–54 measure 11.3 (8.2–16.8) mm in length and 2.0 (1.3–3.4) mm in width. Length to width ratio is 3:1 to 10:1 (Fig. 3). Genital pores in the middle third of the proglottid margin, alternating irregularly.

Testes in medullar parenchyma, between excretory stems, spherical, 110 to 150 in number, 56 to 97 in diameter, in front of female genital glands, occasionally lateral to ovary.

Cirrus pouch elongated oval or in some proglottids bottle-shaped, covered with thick fibrous coat, 243 (214–282) long, 89 (86–97) wide. Vas deferens strongly coiled, not widen-

ing into seminal vesicles. Ejaculatory duct coiled inside cirrus pouch. Cirrus unarmed.

Female organs median, close to posterior margin of proglottid. Vitelline gland posterior to ovary, elongated parallel to transverse axis of proglottid, slightly lobed, 452 (413–640) in width. Ovary formed by two lobed parts united by a broad bridge, 340 (320–430) in transversal axis.

Vagina narrow, covered with fibrous coat. Seminal receptacle large, spherical or irregularly oval, 180 (142–215) in diameter, close to ovary, persisting in gravid proglottids.

Genital atrium shallow, *semicircular*, not muscular. Genital ducts pass between excretory stems and open into the atrium with the vaginal pore posterior to the cirrus (Fig. 2).

Thirty egg capsules from the type and paratypes had the following measurements: egg capsule 130–170 in diameter, oncosphere 45–

3
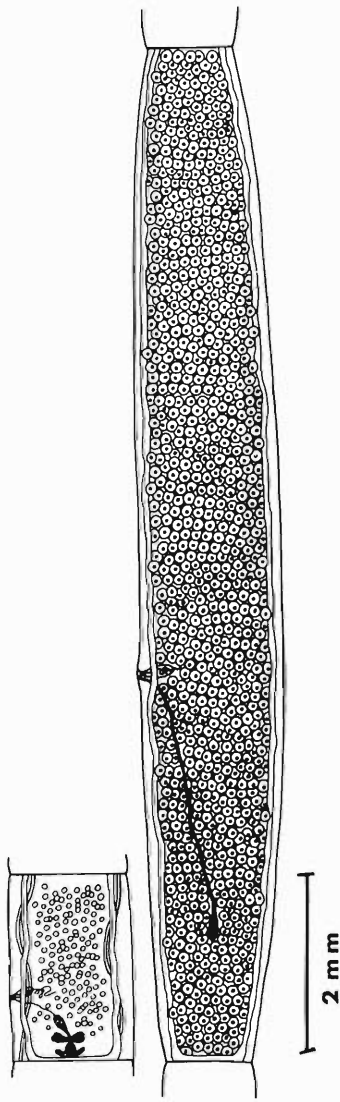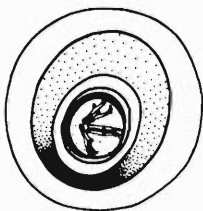
0.1 mm   4

49, embryonic hooks 25–28 long (Fig. 4). Egg capsules not covered with fibrous coat, scattered singly in medullary parenchyma of gravid proglottids (Fig. 3).

Two excretory stems on each side of the strobila, the ventral one branching off into short irregular anastomoses, which may reunite with the main stem (Fig. 2).

LOCALIZATION: Small intestine.

HOSTS: *Gerbillus gerbillus* Olivier, 1801; *G. dasyurus* Wagner, 1842; *Sekeetamys calurus* Thomas, 1892; *Meriones crassus* Sundevall, 1842 (Rodentia, Gerbillidae).

LOCALITIES: Israel—Nahal Nafha (Wadi Nafkh) Negev; The Sinai Peninsula—Gebel Yi'allaq, Wadi Akhdar, Wadi el Sheikh, Mamarr Mitla.

TYPE (whole mount) (Number S-384) and 9 paratypes deposited in the helminthological collection at the Department of Parasitology, The Hebrew University—Hadassah Medical School, Jerusalem.

NOTE ON LIFE HISTORY: Adults and larvae of *Tribolium confusum, Tenebrio molitor,* and an unidentified oribatid mite were exposed to eggs in teased gravid proglottids. After 18 days, several cysticercoids were recovered from adult *T. confusum.* A detailed description of the cysticercoids and data on experimental infection of rodent hosts will be published in a separate paper.

Discussion

The cestode *Sinaiotaenia witenbergi* was recovered from gerbilline rodents caught in arid areas of southern Israel and of the Sinai Peninsula (Wertheim and Greenberg, 1970). Following the classification in Yamaguti (1959), it was assigned to the family Anoplocephalidae Cholodkovsky, 1902, taking into consideration the marginal genital pores, absence of rostellum and hooks in the scolex, and the arrangement of the egg capsules, which are scattered singly in the parenchyma. The taxonomy of the Anoplocephalidae, especially the division into subfamilies, was lately discussed by Stunkard (1961, 1965, 1969). Using as criteria mainly morphological features and bionomics Stunkard recognized in the Anoplocephalidae the following four subfamilies: (1) Anoplocephalinae Blanchard, 1891, transmitted by oribatid mites; (2) Catenotaeniinae Spassky, 1949, transmitted by tyroglyphid mites; (3) Linstowiinae Führmann, 1907, transmitted by coleopterous and

lepidopterous insects; and (4) Thysanosomatinae Führmann, 1907, transmitted by psocopterous insects.

Accepting this approach *Sinaiotaenia* gen. n. was classified as belonging to the subfamily Linstowiinae. It differs, however, from other genera in this subfamily, *Linstowia* Zschokke, 1899, *Atriotaenia* Sandground, 1926, *Panceriella* (Führmann, 1899) emend. Stunkard, 1969, and *Cycloskrjabinia* Spassky, 1951, by the position of the female genitalia in the posterior part of the proglottid, the localization of the numerous testes in front of the ovary, and the especially elongated gravid proglottids. (See discussion in Stunkard, 1961, 1969 and Mettrick and Weir, 1963, for genera transferred from the subfamily Linstowiinae into other subfamilies or families.) These differences appeared sufficient to justify the establishment of a new genus, with a new species, named in honor of Professor G. Witenberg.

Acknowledgments

The authors wish to express their gratitude to Drs. E. L. Schiller and R. L. Rausch for examining the specimens and confirming the validity of the new genus.

Thanks are due to Mr. J. Ghattas for the preparation of the slides and drawings.

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Intestinal Helminths of *Rattus rattus* from Urban and Rural Areas in the Punjab Region of West Pakistan

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ABSTRACT: Eleven species of helminths including 6 genera and 6 species of Nematoda, 3 genera and 4 species of Cestoda, and one genus and one species of Acanthocephala were found in the alimentary tracts of 480 *Rattus rattus*. Urban rats harbored a greater variety of helminths than rural rats and also had a higher incidence of infection. In addition, urban rats had a larger average parasite load (23.5 helminths in 2.8 species) than did rural rats (9.7 helminths in 1.5 species). The results of this study indicate that rats are undoubtedly the reservoir hosts for *H. diminuta* infections occasionally seen in man but appear to be of little or no importance in the epidemiology of *H. nana* in human infections.

Studies on the parasites of *Rattus rattus* have been done in most countries of the world and the literature on the subject is voluminous. In developing countries this information is of particular importance because of the role which

rats may have as reservoir hosts of some parasites infecting humans, especially when one is aware of the density which these rat populations obtain. Chandler (1927), for example, suggested that rats were of major epidemiological importance in his study of *Hymenolepis nana* in human populations in the Indo-Pakistan subcontinent.

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[2] Present address: Department of Zoology, University of Maryland, College Park, Maryland.

Table 1. Incidence of individual helminths found in the alimentary tracts of 480 *Rattus rattus* collected from the Punjab region of West Pakistan.

Species of helminth	Akbari Mundi (142)*		Village Doonga (151)		Village Changan Wali (141)		Changa Manga Forest (46)	
	No. pos.	%	No. pos.	%	No. pos.	%	No. pos.	%
Nematoda								
Aspicularis pakistanica	61	43.0	63	41.7	34	24.1	19	41.3
Syphacia muris	42	29.6	1	0.7	7	5.0	4	8.7
Trichuris muris	59	41.6	6	3.9	0	0.0	0	0.0
Protospirura muris	73	51.4	0	0.0	0	0.0	1	2.2
Gongylonema neoplasticum	14	9.9	0	0.0	0	0.0	0	0.0
Rictularia sp.	1	0.7	0	0.0	0	0.0	0	0.0
Cestoda								
Hymenolepis diminuta	44	31.0	72	47.7	63	44.7	5	10.9
H. nana (var. *fraterna* ?)	1	0.7	0	0.0	2	1.4	0	0.0
Mathevotaenia symmetrica	5	3.5	0	0.0	0	0.0	0	0.0
Taenia taeniaformis (larval)	27	19.0	19	12.6	11	7.8	5	10.9
Acanthocephala								
Moniliformis dubius	14	9.9	0	0.0	0	0.0	0	0.0

* Number of rats examined.

In West Pakistan, studies of rat parasites have, in the past, been restricted to urban rat populations (Akhtar, 1955; Fahim and Mohiuddin, 1961; Salim, 1965). This is significant when one realizes that the majority of the human population in West Pakistan lives in rural areas.

It was decided, therefore, to study the intestinal helminths of rats, both urban and rural, in West Pakistan in order to determine the identity and incidence of these parasites and to determine if the rats could be, as Chandler suggested, of importance in the epidemiology of certain parasitic infections of humans in that area.

Materials and Methods

Rats were live-trapped from the following localities:

(1) Akbari Mundi—a highly congested grain market in the center of the old city of Lahore, West Pakistan.

(2) Village Doonga—a small rural village (255 residents) located about 55 miles northwest of Lahore near Khangah Dogran in Sheikhupura District.

(3) Village Changan Wali—a small rural village (320 residents) located about 65 miles north of Lahore near Gakkar in Gujranwala District.

(4) Changa Manga Forest—a hardwood forest located about 35 miles south of Lahore.

After killing the rats by etherization, the alimentary tracts, i.e., esophagus, stomach, small and large intestine, were removed and examined for helminths. In addition, the diaphragms were removed from the rats, squashed between glass slides and examined microscopically for *Trichinella spiralis*.

Cestodes recovered from the alimentary tracts were fixed and stained for identification using standard procedures. Nematodes and acanthocephala were stored in glycerinated alcohol and identified after clearing in 10 per cent lactic acid.

Results

Eleven species of helminths were found during the examination of the alimentary tracts of 480 rats collected from November, 1966 through January, 1968 (Table 1). Included in these eleven species were 6 genera and 6 species of Nematoda, 3 genera and 4 species of Cestoda, and one genus and one species of Acanthocephala. All diaphragms examined were negative for *T. spiralis*.

It is readily evident that urban rats not only harbored a greater variety of helminths than did rural rats but also had a higher incidence of infection. In addition, parasitized urban rats harbored an average of 23.5 (1–218) helminths per host in 2.8 (1–6) species as compared to a collective average of 9.7 (1–148) helminths per host in 1.5 (1–3) species in rural rats.

Protospiruris muris was the most common helminth occurring in urban rats while *Hymenolepis diminuta* occurred most frequently

in rural village rats. *Aspicularis pakistanica*, a common parasite in rats from all the collecting sites, was by far the most frequent helminth encountered in rats from the Changa Manga Forest.

Four species of helminths, viz., *Gongylonema neoplasticum*, *Rictularia* sp., *Mathevotaenia symmetrica*, and *Moniliformis dubius*, were found only in urban rats. Also, *Trichuris muris* and *P. muris*, which were commonly found in urban rats, had very limited occurrence in rats from rural areas.

H. nana (var. *fraterna* ?), a parasite of possible human significance, was found in only 3 of the 480 rats examined although in Doonga and Changan Wali where 291 of the 480 rats were trapped *H. nana* was a common parasite in the human population (Haley and Buscher, unpublished data).

Discussion

The present study has provided some interesting insights, heretofore unreported, concerning helminth infection of urban and rural rats in the Punjab Region of West Pakistan. The data indicate that urban rats: harbored a greater variety of helminths than did rural rats; harbored four species of helminths not found in rural rats; had a higher incidence of infection; and had a larger average number of parasites per infected host.

The reasons for these differences are open to speculation. However, it is likely that helminths such as *A. pakistanica*, *S. muris*, and *T. muris* thrive in high density rat populations which are promoted in environments such as the open grain markets of Akbari Mundi where there are abundant food supplies and crowded living conditions. *H. diminuta*, on the other hand, was equally abundant at all collecting sites as were its intermediate hosts, *Tribolium* spp. and *Tenebrio* spp. Evidently, the intermediate hosts (roaches) for *P. muris*, *G. neoplasticum*, and *M. dubius*, among others, do not occur in great enough abundance or density in the rural areas studied to allow populations of these parasites to become established to any great extent.

The results of this study differ considerably from those of previous studies with the exception of the nematode data of Akhtar (1955). Akhtar surveyed the nematodes of rats and mice in Lahore and reported finding, in rats,

all of the nematodes reported in the present study except *P. muris*, although he did report it as occurring in mice. Salim (1965) reported finding *H. diminuta* (7.69%) and *T. taeniaformis* (10.26%) in rats collected in Lahore, but the fact that his data report only a single nematode, viz., *Syphacia*, compared to the 5 species reported by Akhtar and 6 species in the present study, casts some doubt on its validity. Fahim and Mohiuddin (1961), in presenting combined data for *R. rattus* and *R. norvegicus* in Karachi, reported finding *P. muris*, *Syphasea* (sic) sp., *H. diminuta*, *H. murina* (= *H. nana* var. *fraterna*), and *Raillietina celebensis*.

There is little question that rats serve as reservoir hosts for the occasional cases of *H. diminuta* seen in the human population in West Pakistan. However, the fact that only 3 cases of *H. nana* (var. *fraterna* ?) were found in 480 rats examined would indicate that rats are of little, or no importance in the epidemiology of this cestode in human infections in the areas studied.

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Rhabditis adenobia sp. n. (Nematoda: Rhabditidae) from the Colleterial Glands of *Oryctes monoceros* L. and Other Tropical Dynastid Beetles (Coleoptera: Scarabaeidae)

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During investigations of the nematode associates of *Oryctes rhinoceros* L. and other tropical dynastid beetles, a study was conducted on the nematodes inhabiting the colleterial glands of these coleoptera.

Hoyt (1962a, b) was one of the first to record nematodes from the colleterial glands of dynastid beetles in Africa and New Guinea (Table 1) and his and subsequent reports led to the present investigation on the taxonomy and biology of these nematodes and their effect on the host.

In attempts to determine the source of nourishment of nematodes developing in the colleterial glands, a detailed study of the glands was undertaken to better understand their function.

Materials and Methods

Nematodes were removed from the colleterial glands and aedeagi of living *Oryctes rhinoceros* L. from Malaysia; *O. monoceros* Ol., *O. boas* F., and *O. owariensis* Beauv. from West Africa and *Xylotrupes gideon* L. and *Scapanes australis grossepunctatus* Endrödi from the territory of Papua and New Guinea, and placed directly on sterile artificial media. This medium was made by mixing 150 grams of ground Gaines Gravy Train dog food briquets with 7.5 grams of water agar and 500 ml water. This mixture was then placed in screw cap test tubes, autoclaved and could be used after several months storage. Each tube supported heavy nematode populations for one month at which time transfers could be made. The nematodes fed on assorted bacteria introduced with them during the transfer.

For detailed studies of certain aspects of the relationship between these nematodes and tropical beetles, *O. monoceros* in the Ivory Coast was selected because of its abundance and high incidence of nematode infestation.

The colleterial glands of *O. monoceros* with the associated nematodes were removed entire and fixed in cold Bouin's for histological sections. After embedding, sections were cut at 7 μ and stained with crystal violet and Heidenhain's iron hematoxylin with 1% eosin in 90% alcohol.

For ultrastructural investigations of the colleterial glands of *O. monoceros*, the entire gland was fixed for 2 hr in 1% osmium tetroxide, buffered with 0.1 M phosphate buffer, then dehydrated and embedded in araldite. Sections were cut with a Porter-Blum MT-2 microtome and stained with uranyl acetate and lead citrate. They were examined with an RCA-3F electron microscope.

For crossing experiments between nematodes from different sexes and species of beetles, individual juvenile nematodes were placed in isolated depression cells with a small amount of dog food medium. After reaching the adult stage, pairs were placed together and observations made during the following five days.

Results

Besides occurring in the colleterial glands of female beetles, nematodes were also found in the passages of the endophallic tube of male beetles. Nematodes removed from both sexes of the 6 species of dynastid beetles developed well on artificial media. Specimens from the colleterial glands and aedeagi of *O. monoceros* were found to be the same species after morphological examinations and interbreeding tests were conducted. This species was considered new to science and a description follows below. All measurements are given in microns.

Rhabditis (Rhabditis) adenobia sp. n.
(Figs. 1 and 2)

Rhabditoidea (Örley, 1880) Travassos, 1920; Rhabditidae Örley, 1880; Rhabditis Dujardin, 1845 as defined by Goodey (1963).

GENERAL CHARACTERISTICS: Lips closed,

Table 1. A list of dynastid beetles reported to contain nematodes in their colleterial glands.

Beetle	Locality	Reference
Oryctes blucheaui Frm.	Madagascar	Bedford (1968b)
O. boas F.	Ivory Coast	Poinar (present study)
O. centaurus Sternb.	Papua and New Guinea	Hoyt (1962a) Paine (1966)
O. gigas Cast.	Madagascar	Bedford (1968b)
O. gnu Mohn	Malaysia, Borneo	Paine (1966)
O. insularis Coq.	Madagascar	Bedford (1968b)
O. monoceros Ol.	East Africa	Hoyt (1962b)
"	Ivory Coast	Mariau (1967)
"	Ivory Coast	Poinar (present study)
O. owariensis Beauv.	Ivory Coast	Poinar (present study)
O. pyrrhus Burm.	Madagascar	Bedford (1968b)
O. ranavalo Coq.	Madagascar	Bedford (1968b)
O. rhinoceros L.	India and Ceylon	Paine (1966)
"	Maldive Islands	Paine (1966)
"	Malaysia	Paine (1966), Poinar (present study)
"	Borneo and Timor	Paine (1966)
"	Papua and New Guinea	Bedford (1968a)
"	Mauritius	Bedford (1968b)
O. simiar Coq.	Madagascar	Bedford (1968b)
Scapanes australis grossepunctatus Endrödi	Papua and New Guinea	Bedford (1968a)
"	Papua and New Guinea	Poinar (present study)
Xylotrupes gideon L.	Papua and New Guinea	Bedford (1968a)
"	Papua and New Guinea	Poinar (present study)
X. lorquini Schauf.	Papua and New Guinea	Hoyt (1962a)

metarhabdions isomorphic, each bearing 5 tubercles. Esophagus without a median bulb, the corpus gradually widening just before the isthmus. Hemizonid present. Lips without setae or bristles, but small sensorial papillae are visible under high magnification. Amphids pore-like on lateral lips.

MALE (n = 15). Length 926 (768–1,248); greatest width 45 (31–77); length of esophagus 203 (177–233); length stoma 18 (17–21); length esophageal collar 6 (4–9); length free portion of lips 3 (2–4); length head to hemizonid 166 (139–200); length head to excretory pore 173 (150–205). Tail leptoderan, 46 (40–52) long and 27 (23–38) wide. Spicules paired, separate, with well pronounced head and slight arch, 47 (40–53) long and 9 (6–13) wide at the widest portion. Length gubernaculum 21 (20–26); length bursa 75 (65–98), open, with 9 bursal papillae, 2 of which are preanal, 1 adanal, and the rest postanal. The last pair is greatly reduced and the 2nd, 5th, 7th, and 9th pair do not meet the rim of the

bursa. Anal papillae (tubercles, knobs) rudimentary. a = 16.2–24.8; b = 4.3–5.6; c = 19.2–24.0.

FEMALE (n = 15). Length 1,200 (1,056–1,296); greatest width 55 (46–69); length of esophagus 223 (208–239); length stoma 21 (18–23); length esophageal collar 6 (5–8); length head to hemizonid 185 (169–208); length head to excretory pore 195 (177–211); length free portion of lips 3 (2–4); vulva median 53 (49–56)% of body length from head; reproductive system amphidelphic, ovaries reflexed, distance from extended portion of anterior ovary to vulva 369 (267–423); distance from extended portion of posterior ovary to vulva 377 (285–447); tail conical, tapering to a fine point, length 102 (93–115); tail width 29 (25–31). a = 18.8–22.9; b = 5.1–5.4; c = 11.3–11.8.

TYPE LOCALITY: Abidjan, Ivory Coast, West Africa.

TYPE HOST AND LOCATION: Found in the colleterial glands of the adult female and the aedeagi of the adult male of *Oryctes monoceros* Ol.

TYPE SPECIMEN: Deposited in the U. S. Department of Agriculture Nematode Collection, Beltsville, Maryland. Holotype (♀) T-187t; allotype (♂) T-188t, and paratypes, T-847p.

Diagnosis

The characters of *Rhabditis adenobia* place this species in the subgenus *Rhabditis* of the genus *Rhabditis*. Besides having the characters of this subgenus, *R. adenobia* possesses a long, narrow stoma, never more than half enclosed by the esophageal collar, and an esophagus lacking a median bulb. *R. adenobia* reproduces amphimictically and the males possess an open, leptoderan bursa with 9 pairs of bursal papillae. This combination of characters separates it from most of the described species in the genus *Rhabditis*.

R. adenobia can be separated from *R. korneri* Osche, 1952 which has distinct anal tubercles, anisomorphic metarhabdions and a longer male tail.

The species most similar to *R. adenobia* are *R. lucianii* Maupas, 1919 and *R. terrestris* Stephenson, 1942. However both of the latter species have a fully developed ninth bursal papilla and *R. terrestris* possesses a slight median

bulb while *R. lucianii* has a proportionally longer esophageal collar and the female tail is not as drawn out as in *R. adenobia*. Also, the rectum of *R. lucianii* is ⅔ the length of the tail, which is not the case with the species described here.

Biology and Host Relationship

Approximately 70% of the female beetles examined contained *Rhabditis adenobia* in their colleterial glands while 50% of the male beetles contained this nematode in their endophallic tubes.

A few words should be said about the nature of the colleterial glands in these beetles in order to shed light on how the secretions might be used as a source of nourishment by the nematodes. Unfortunately very little is known about colleterial glands of insects in general, and those of *Oryctes* have never been investigated. Snodgrass (1935) considers colleterial glands similar to accessory glands and cites their function as secreting an adhesive substance that attaches the egg to a substrate. Since *Oryctes* spp. deposit their eggs singly in soil or debris, these glands may have yet another function. In all species of *Oryctes* reported here, the females contained 2 pairs of colleterial glands situated on opposite sides of the vaginal wall (Fig. 2, E). The distal member of each pair (designated as gland A) was crescent shaped and varied in color from light to dark brown in *O. monoceros.* The outer surface of gland A was nearly smooth and covered with an apparent sclerotized layer. Histological sections showed tubular extensions of the hypodermal cells extending to the surface of the gland, suggesting a possible deposition route of the sclerotized material (Fig. 7). The function of these glands is unknown.

The proximal gland of each pair (designated as gland B) was mushroom or doughnut-shaped and varied from white to light brown in *O. monoceros.* Because of their color, they were sometimes difficult to distinguish from the vaginal wall. Large tracheal trunks entered these glands through a central cavity (Fig. 3). The outer surface of gland B was composed of numerous vesicles containing a viscous-like material (Figs. 5, 6). Beneath the vesicles lay several rows of glandular cells which in turn surrounded an inner lumen. Electron micrographs showed the vesicle walls to be lined

130μ
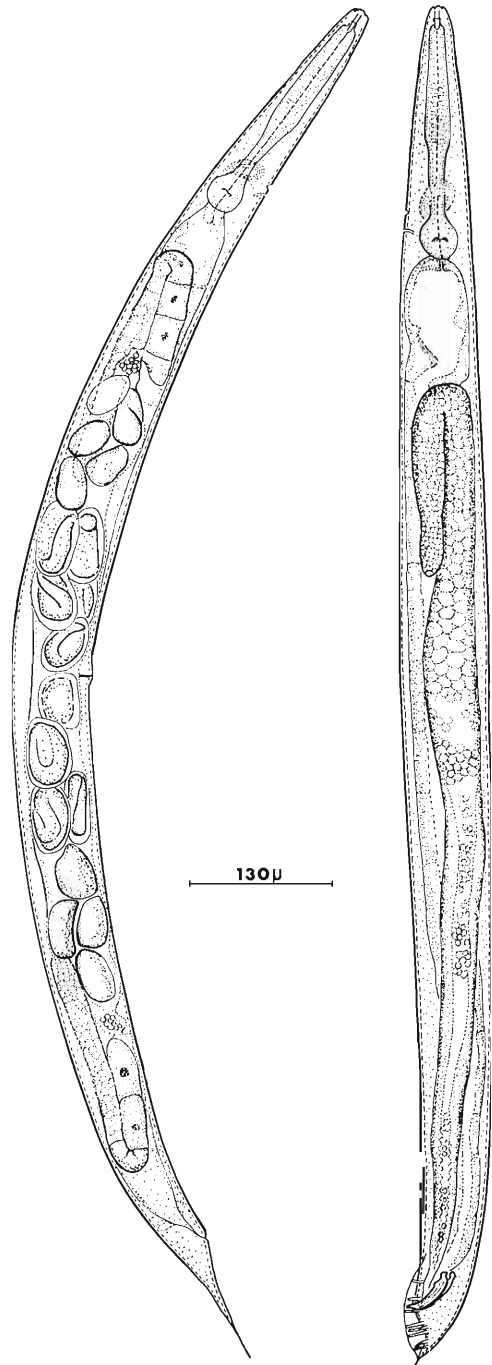
Figure 1. Male and female of *Rhabditis adenobia* sp. n.
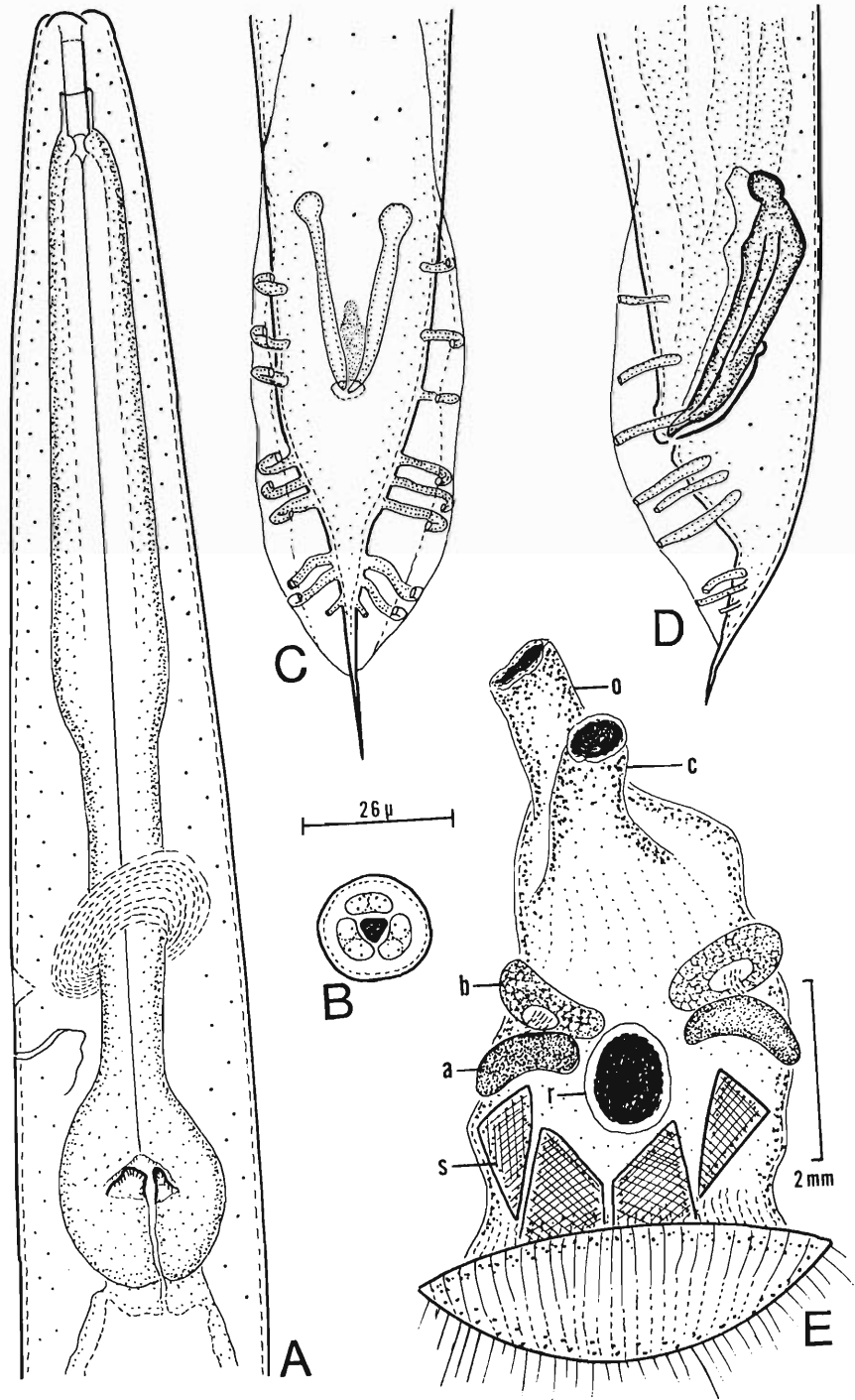
Figure 3. Histological section of gland B of *O. monoceros* showing populations of *R. adenobia* within the surrounding membrane (m); (n = nematodes).

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Figure 2. *R. adenobia* sp. n. A. Anterior portion of male. B. En face view of male. C. Ventral view of male tail. D. Lateral view of male tail. E. Internal genital area of a female of *O. monoceros*. (o = common oviduct; c = stalk of bursa copulatrix; a = gland A; b = gland B; r = rectum; s = sclerite.)

with droplets, similar in appearance to mucous secretions (Fawcett, 1966) (Figs. 8, 9). These droplets probably coalesce and coat the egg as it passes through the vagina. Bacteria were also found in the vesicles (Fig. 9) and it is not known if these represent some type of symbiont which is important for larval nutrition or if these are simply "opportunists" that came in independently or with the nematodes.

A common membrane surrounded each gland pair and the secretions of both glands reached the vagina through a common duct. Most of the nematodes occurred within the membrane adjacent to gland B (Fig. 3). However, when numbers were high they sometimes occurred within gland B (Fig. 5) and were also found in the proximity of gland A (Fig. 4).

In *O. monoceros*, reproducing nematodes, as well as normal juveniles or dauer stages, were frequently encountered in the colleterial glands, although the latter stages were more common. Within the glands, the nematodes probably fed on both the glandular secretions, especially from gland B, and the bacteria that were associated with these glands. When reproducing forms were present, nematodes could sometimes be found in the vagina and even in the bursa copulatrix. It is not known why some beetles contained adult nematode populations and others only dauer or normal juveniles. When the female beetles died of natural causes, the nematodes were sometimes able to reproduce in the cadaver; yet conditions were not always favorable, since in many instances, the nematodes died within the glands. In the male beetles, only dauer or non-dauer juveniles were found in the aedeagal glands and in *O. monoceros*, these nematodes were usually associated with another nematode, *Oryctonema genitalis* Poinar, 1970.

Transmission of *R. adenobia* from host to host, as with the bursa-inhabiting nematode, *O. genitalis*, was accomplished during mating of the beetles. A male beetle picks up the nematodes in the aedeagal passages after mating with an infected female and then deposits the nematodes in the vagina or bursa copulatrix of a non-infested female. The nematodes then make their way into the colleterial glands. It is possible that the dauer stage (non-feeding 3rd stage juvenile) is able to enter the colleterial glands by invading the vagina directly from the environment; however, this was never observed and *R. adenobia* was never collected from the beetle environment. Although these nematodes could develop well on artificial media, they were not found in grass, water agar or artificial media that contained adult females and males of *O. monoceros* for 1 week, even though some of the beetles died after 3 days and there was ample opportunity for the nematodes to leave the beetle and enter the substrate.

Breeding tests conducted between nematodes taken from the colleterial glands of *O. monoceros* and *O. owariensis* were successful and since nematodes from both sources were similar morphologically, they are regarded as the same species. Although *R. adenobia* was morphologically similar to nematodes occurring in the colleterial glands of *O. boas*, crossing attempts were unsuccessful and the identity of the forms in *O. boas* remains unclear at this time.

No injurious effect on either sex of *O. monoceros* could be determined due to the presence of *R. adenobia* during the course of this investigation. Although the function of the colleterial glands may be affected when nematode populations are high, it is not known what significance this may have on the development of the beetle.

Discussion

Members of the family Rhabditidae generally have a simple life cycle, surviving in the

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Figure 4. Histological section showing the common membrane (m) surrounding colleterial glands A and B in *O. monoceros*; (n = nematodes).

Figure 5. Histological section of gland B in *O. monoceros* showing nematodes (n) within the glandular cells.

Figure 6. Histological section showing the surface of gland B covered with minute vesicles; (magnification same as Fig. 5).

Figure 7. Histological section of gland A in *O. monoceros* showing tubular extensions of the hypodermal cells (e).
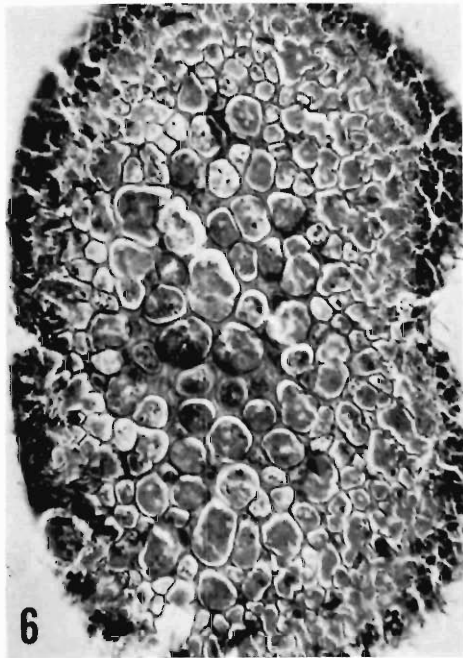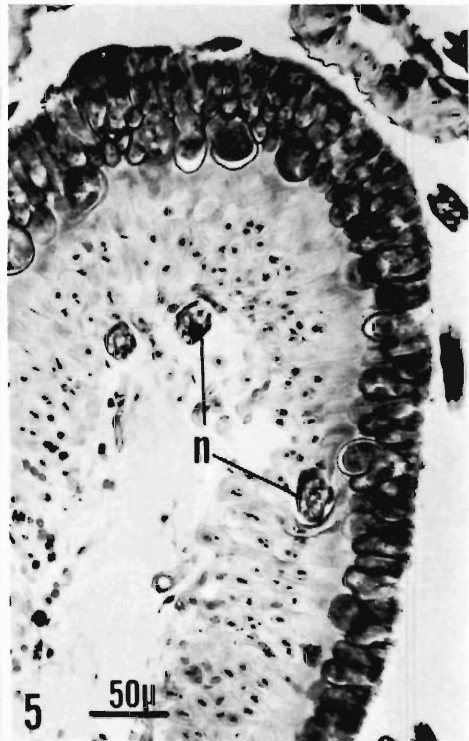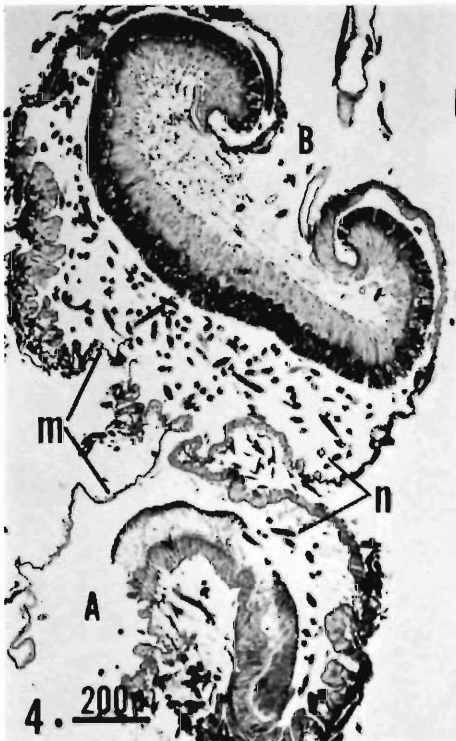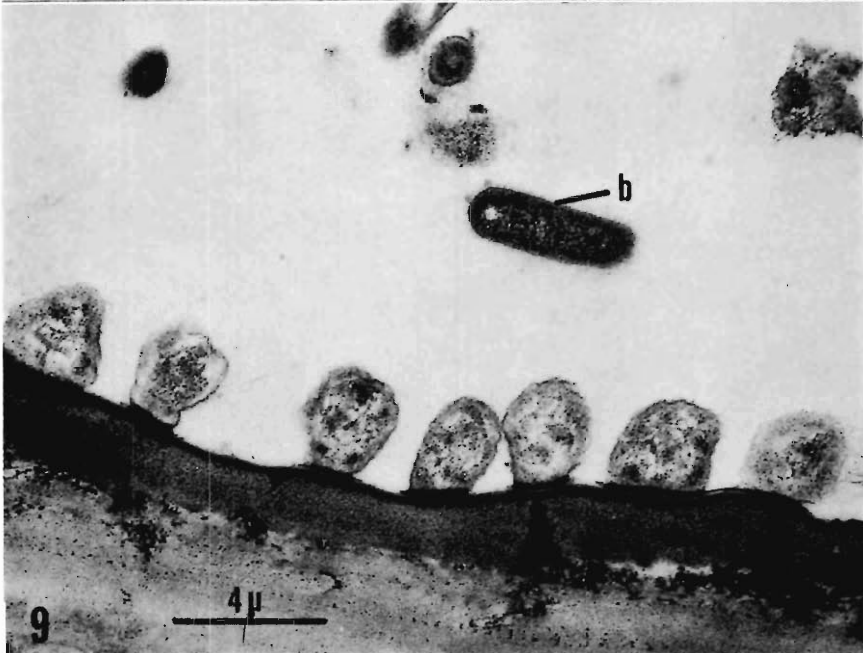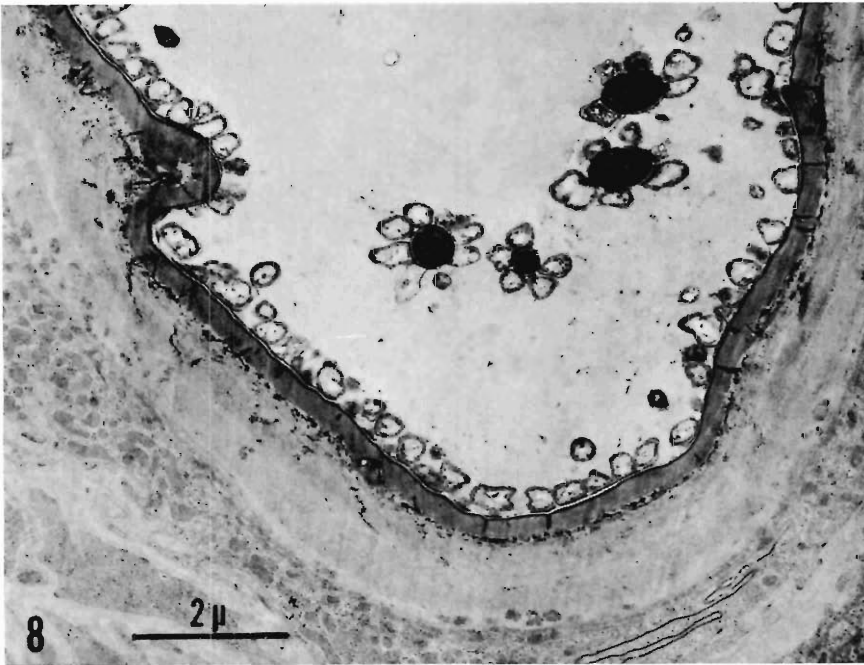
Figure 8. Electron micrograph showing droplets lining the surface of a vesicle wall of gland B in *O. monoceros*.

Figure 9. Electron micrograph showing the similarity of droplets on the vesicle wall of gland B to mucous secretions; (b = bacterium).

soil where they are frequently associated with decomposing plant and animal matter. Many possess the ability to survive under a wide range of physical and chemical conditions.

Occasionally, representatives are found that have or are in the process of adapting at least a portion of their life to an invertebrate or vertebrate. In many instances, they are not able to take full nutritional advantage of the new host and must eventually return to the soil to complete their life cycle.

Some rhabditids e.g. *R. pellio*, exploit earthworms as a source of nourishment, yet, at their present evolutionary state, only one or two larval stages occur in the body cavity or excretory system of the living annelid. They are unable to complete further development until the earthworm dies and conditions become similar to their original habitat. It is obvious that these nematodes have not yet evolved the resources for developing within living annelids, yet we know this is possible, since adults of another group of nematodes, the Drilonematoidea, do occur in the body cavity of living earthworms and probably are able to obtain their complete nourishment from this habitat.

An association with a vertebrate host has occurred with *Pelodera strongyloides* (Schneider). The dauer and post dauer stages of this nematode occur in the orbits of murid rodents (Poinar, 1965) where some growth occurs, although the nematode must enter the soil again before reaching maturity. Similarly, it is a case of not being able to completely exploit the environment, rather than the environment being nutritionally deficient, since eye worms of the genus *Thelazia*, etc. are able to mature and reproduce in this specialized habitat.

In contrast, however, all stages of the rhabditid, *Oryctonema genitalis* occur in the bursa copulatrix of the coconut beetle, *Oryctes monoceros*, and have adapted so completely to this environment that they no longer are able to revert back to their original free-living habits (Poinar, 1970).

With *R. adenobia* and similar forms, complete dependence on conditions within the colleterial glands has not yet occurred, although the association appears to be heading in this direction. This hypothesis is based on the following observations. First, reproducing colonies of *R. adenobia* occasionally could be found within the membrane surrounding the colleterial glands—indicating that nutrients in this locality were being utilized by the nematodes. Secondly, *R. adenobia* was never found apart from the beetle and colonies within the glands often died after the beetle succumbed, indicating their inability to withstand the altered conditions of the dead beetle. Thirdly, the dauer stage of *R. adenobia* was nonspecialized in comparison with many free-living rhabditids. It was not surrounded by an ensheathing cuticle, nor was the mouth always completely closed. Since the normal functions of the dauer stage are to carry the species over periods when the food supply is limited or facilitate the invasion of hosts, either for transport or nourishment, we can better understand this lack of specialization here. Indeed, the conditions within the colleterial glands are constant—there would be a continuous supply of secretions as long as the beetle survived and the natural copulatory habits of the beetles have replaced the need for an invasive stage. As the normal functions of the dauer no longer become necessary, it seems logical that factors involving their formation would become lost from the population.

For these reasons, it is suspected that *R. adenobia* is in an evolutionary stage between a free-living form and a species obligately associated with the colleterial glands of dynastid beetles. Its ability to survive on specially prepared media with bacteria illustrates its close ties with the free-living nematodes. Such an obligate association already has occurred with the rhabditid, *Oryctonema genitalis*, which multiplies in the bursa copulatrix of certain dynastid beetles (Poinar, 1970). These examples point out the adaptive ability of rhabditid nematodes.

It is possible that when nematode populations within the colleterial glands become large, some damage to these organs may occur. This could result in a reduction of secretions from gland B and if the secretions play an important role in egg development (perhaps preventing infection by soil organisms) then this could have an influence on general viability. Again, if the bacteria surrounding the glands are some type of symbionts (although there is no proof of this now) which eventually end up in gastric caeca of the larval midgut, they could play an important role in larval nutrition. If the nematodes, by feeding,

interfere with the supply of bacteria, larval development could be affected. However before either of these hypotheses can be proven, the exact function of the colleterial glands in these beetles should be thoroughly elucidated.

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Some Hookworms of the Genus *Ancylostoma* from Colombia and Panama[1]

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ABSTRACT: The following species of *Ancylostoma* are reported for the Colombia-Panama area: *A. duodenale* (Dubini, 1843); *A. caninum* (Ercolani, 1859); *A. tubaeforme* (Zeder, 1800); *A. pluridentatum* (Alessandrini, 1905); and *A. buckleyi* Le Roux and Biocca, 1957. The latter two species are reported from Colombia for the first time, and *A. pluridentatum* is reported from Panama for the first time. Additionally, this paper constitutes the first report of *A. buckleyi* since the original description. This species is also recorded from a new host (*Atelocynus microtis* Schlater). Measurements of *A. pluridentatum* and *A. buckleyi* are presented. Methods of distinguishing the four species of animal ancylostomes are discussed and illustrated.

Hookworms of the genus *Ancylostoma* are widespread and important pathogens of man and his domestic animals. Other species of as yet unassessed infectivity for man occur in wild carnivorous mammals. Although it had been thought that these nematodes were highly host specific (Scott, 1930), in recent years it has become apparent that some of the animal ancylostomes pose a threat to man. *Ancylostoma ceylanicum*, for example, has been found to mature in the intestine of man. Other species, such as *A. caninum* and *A. braziliense*, seem not to be able to mature in man, but they may produce larval invasions of the skin or internal organs.

The series of papers by Biocca (eg. 1954, 1961) and by Biocca and Le Roux (1957) have helped to clarify the formerly confused systematics of the genus. The subgenera proposed in the latter paper are herein accepted and followed.

Materials and Methods

Nematodes were washed from the opened intestinal tract and fixed in 70% alcohol. The worms were cleared in lacto-phenol solution and mounted in glycerine jelly. The ventral tooth-plates were dissected out with needles and mounted in glycerine jelly. Drawings were made by means of a Wild microscope equipped with a drawing tube. All measurements are in millimeters.

Host names and synonymies were verified

by reference to Cabrera (1961), Handley (1966), and Hershkovitz (1957).

Family Ancylostomatidae Nicoll, 1927
Subfamily Ancylostomatinae Nicoll, 1927

Ancylostoma (*Ancylostoma*) *duodenale* (Dubini, 1843)
(Figs. 4a, 4b)

HOST: Man.
LOCATION: Upper intestinal tract.
LOCALITY: Cali, Colombia.
GEOGRAPHIC RANGE: Cosmopolitan.

This species may well be a common and widespread parasite of man in Colombia, but it has seldom been reported. The most prevalent human hookworm, by far, in Colombia is *Necator americanus* (Stiles, 1902). Patiño-Camargo (1940) mentioned the occurrence of *A. duodenale* in Colombia, but gave no supporting data. Gonzalez (personal communication) found *A. duodenale* in post-treatment stools from several patients at the state hospital in Cali.

Ancylostoma (*Ancylostoma*) *buckleyi* Le Roux and Biocca, 1957
(Figs. 3a, 3b, 8, 12, 16)

HOSTS: *Felis concolor* L. (?), (puma); *Atelocynus microtis* Schlater, (short-eared fox).
LOCATION: Upper intestinal tract.
LOCALITY: Leticia, Amazonas, Colombia.
GEOGRAPHIC RANGE: Colombia and Argentina.

This species has three pairs of ventro-lateral teeth, similar to *A. caninum*, but it also has two pairs of dorso-lateral teeth which are not

found in the other species. The terminal portion of the dorsal lobe of the male bursa is also distinctive in *A. buckleyi*. Each side of the terminal fork is tridigitate, as is characteristic of the genus, but the inner two branches are fused to near their tips.

A. buckleyi was described by Le Roux and Biocca (1957) on the basis of a few specimens from a puma that died in the London Zoo. The host was believed to have come from Argentina. The present collection consists of 30 specimens from an Amazonian short-eared fox. As far as can be determined, no other reports of this species exist.

Comparative measurements

Specimens from *F. concolor* (reported by Le Roux and Biocca, 1957).

ADULT MALES: Length, 8.8–10.9 mm; esophagus length, 0.78–0.89 mm; spicules, 0.68–0.82 mm.

ADULT FEMALES: Length, 9.9–12.8 mm; esophagus length, 0.88–0.96 mm; tail length, 0.19–0.21 mm; tip of tail to vulva, 3.8–4.5 mm.

Specimens from *Atelocynus microtis* (reported in this paper).

ADULT MALES: Length, 9.3–10.1 mm; esophagus length, 0.96–1.0 mm; spicules, 0.90–0.95 mm.

ADULT FEMALES: Length, 11.6–14.3 mm; esophagus length, 1.0–1.1 mm; tail length, 0.20–0.22 mm; tip of tail to vulva, 3.4–3.6 mm.

As can be seen in the comparative measurements, present specimens are closely similar to the original description. The minor differences in measurements seen are herein regarded as normal intraspecific variation possibly host caused. The infection in the short-eared fox was a natural infection from the Amazon whereas the original infection in the puma was found in a zoo animal. For this reason it seems likely that the fox is the normal host for the species.

Ancylostoma (Ancylostoma) tubaeforme (Zeder, 1800) (Figs. 2a, 2b, 7, 11, 15)

HOSTS IN PANAMA: *Felis catus* L., *Felis onca* L. (jaguar).

HOSTS IN COLOMBIA: *F. catus*, *F. yagaouroundi* (jaguarundi).

LOCATION: Upper intestinal tract.

LOCALITIES: Cali, Valle, Bogota, D. E., and Tumaco, Nariño, Colombia; Achiote, Colon, Panama.

GEOGRAPHIC RANGE: Probably cosmopolitan. This species has often been confused with *A. caninum* and has been considered to be a synonym of that species by many writers. Biocca (1954) redescribed *A. tubaeforme* and pointed out some of the distinguishing characters of the species. Marinkelle (1964) reported this species in 8/42 cats from Cali and Bogota. Infection densities in his cats were low (1–4 worms/host). He did not find any other species of hookworm in the cats.

In the present study, 2/2 Panamanian cats were infected with 12–32 (average 22) worms/animal. In Colombia 8/18 cats were positive with 2–62 (average 16) worms/host. Additionally, a young jaguarundi obtained in Nariño was injected with 0.10 cc of disophenol parenteral. Washings of the feces on the following day revealed 20 specimens of *A. tubaeforme* along with 130 specimens of *A. pluridentatum*. Since the animal had been in captivity for only a few days, this infection is regarded as of natural, though accidental, occurrence. Jaguarundis often approach dwellings at night, and have even been seen chasing chickens in the daytime. Thus, the animal possibly became infected by proximity to a household where domestic cats were kept.

Although *A. tubaeforme* and *A. caninum* are similar, they can be distinguished by several characters. *A. tubaeforme* is smaller, but it has longer spicules. Although both species have 3 pairs of ventral teeth, those of *A. caninum* are nearly equal in size while those of *A. tubaeforme* are unequal with the lateral ones considerably larger. The oral aperture of *A. caninum* is considerably more constricted than that of *A. tubaeforme*. The structural support ridges on the ventral surface of the ventral tooth plates are considerably thicker in *A. tubaeforme* than in *A. caninum* (Figs. 1a, 2a).

→

Figures 1–5. Ventral tooth-plates of various species of *Ancylostoma*. a = top view; b = dorso-ventral view. 1. *A. caninum*. 2. *A. tubaeforme*. 3. *A. buckleyi*. 4. *A. duodenale*. 5. *A. pluridentatum*.

0.10 mm
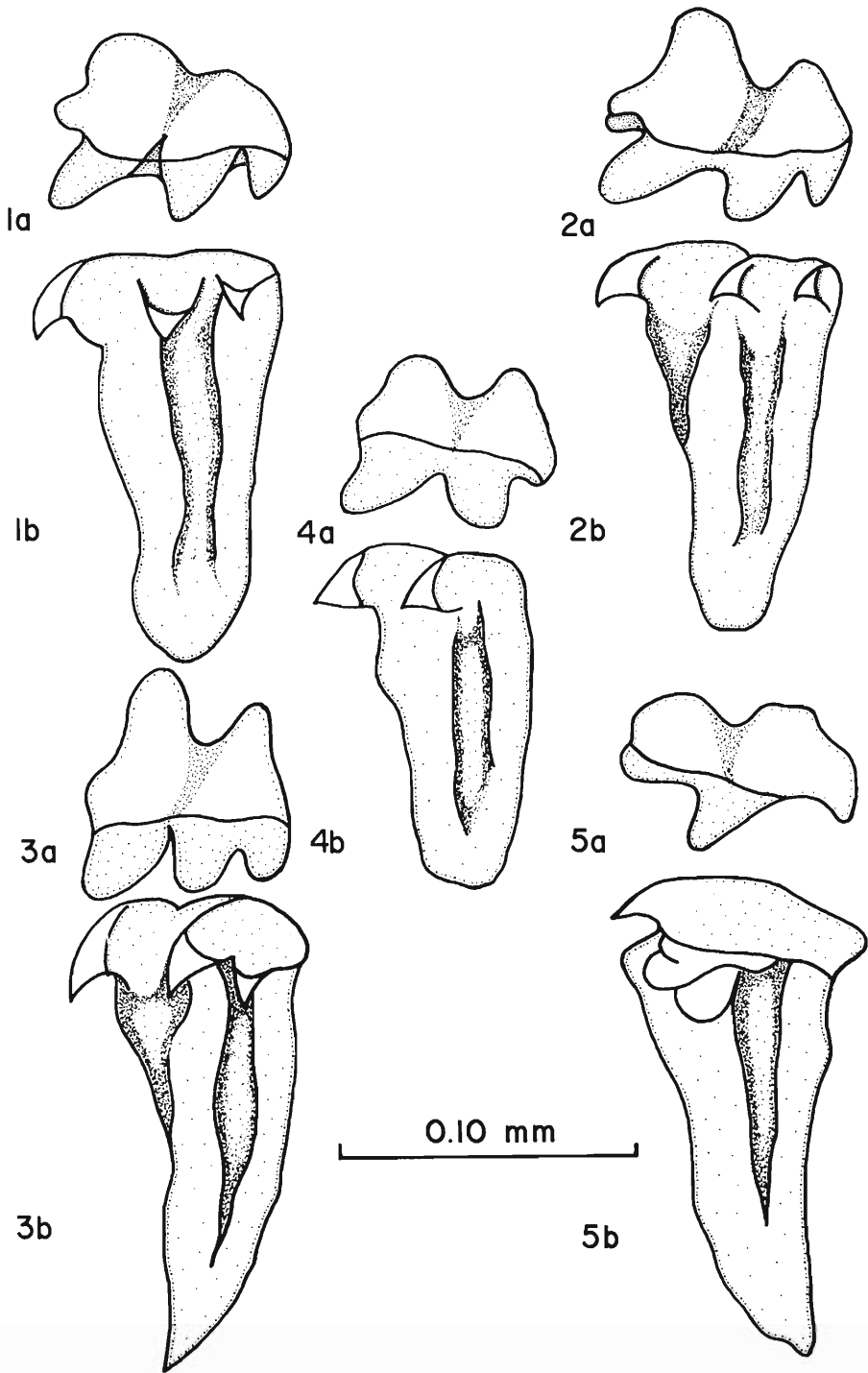
Ancylostoma (Ancylostoma) caninum (Ercolani, 1859) (Figs. 1a, 1b, 6, 10, 14)

HOSTS: *Canis familiaris* L., *Dusicyon thous* L., (fox).

LOCATION: Upper intestinal tract.

LOCALITY: Cali, Valle, Colombia.

GEOGRAPHIC RANGE: Cosmopolitan.

Foster (1939) reported this species in the dogs of Panama. Huber Luna (1961) found *A. caninum* to be a common parasite of dogs in the Cali area. He reported infection rates of from 50% to 90%.

In the present study the species was found in 11/12 dogs with infection densities of 1–120 (average 32) worms/host. The species was also found in 13/28 local foxes with densities of 1–28 (average 8). Most of the infected foxes were trapped near dwellings where dogs are kept, and they possibly got their infections from exposure to soil contaminated with dog feces. No hookworms specific to foxes were found in this study.

A. caninum has three pairs of ventral teeth and a dorsal notch. This species is larger than the others seen in this study. Biocca (1954) lists the length of *A. caninum* as 8.8–10.2 mm for males and 9.8–11.5 mm for adult females. In dogs from Cali, males up to 13.2 mm and females to 18.5 mm have been measured.

An attempt was made to infect the kinkajou *Potos flavus* (Schreber) with this species. Several thousands of infective larvae cultured from a dog were administered orally to two kinkajous. About 1 month after infection moderate numbers of eggs were seen in the feces of both animals. Egg production dropped off rapidly, however, and when the animals were autopsied 2 months after inoculation, only two specimens of *A. caninum* were recovered from each of the hosts.

Ancylostoma (Amerancylostoma) pluridentatum (Alessandrini, 1905) Schwartz, 1927 (Figs. 5a, 5b, 9, 13, 17)

HOSTS IN COLOMBIA: *Felis wiedii* Schinz, (margay cat); *F. yugouaroundi*; *F. catus*.

HOST IN PANAMA: *F. onca* L., (jaguar).

LOCATION: Upper intestinal tract.

LOCALITIES: Choco and Nariño, Colombia; Achiote, Colon Panama.

GEOGRAPHIC RANGE: Panama, Colombia, and Brazil.

The most complete description of this species seems to be that of Schwartz (1927). He reported *A. pluridentatum* from the following hosts: *Felis mitis* Cuvier (= *F. pardalis* L.); *F. eyra* Fischer (= *F. yagouaroundi* Geoffroy); and *F. tigrina* Schreber (which is an animal similar to the margay cat, *F. wiedii*). Biocca and Le Roux (1957) have reported an experimental infection of this species in the house cat. Thus, *A. pluridentatum* is known to occur in the jaguar, the jaguarundi, the margay cat, the ocelot, and the house cat. The species probably occurs in the puma (*F. concolor* L.) as well, but no report of this could be found.

A. pluridentatum has two pairs of ventral teeth, but in fully mature specimens only the lateral pair is visible projecting into the oral aperture. The medial pair of teeth tends to lie along the ventral surface within the buccal cavity. In immature adults, on the other hand, the ventral tooth plates are oriented in such a way that both pairs of teeth project into the oral orifice. The dorsal edge of the oral aperture has three pairs of teeth, or projections. In some specimens, these projections are merely rounded undulations, but in others they have a definite hooked shape with the hook pointing medially (Fig. 9).

Measurements

ADULT MALES: Length, 8.6–9.4 mm; esophagus, 0.63–0.64 by 0.14–0.16 mm; spicules, 1.07–1.13 mm.

ADULT FEMALES: Length, 11.0–12.2 mm; esophagus, 0.72–0.74 by 0.17–0.18 mm; tail length, 0.18–0.21 mm; tip of tail to vulva, 3.4–3.7 mm.

Discussion

The species of *Ancylostoma* herein reported are different in a number of ways. They can most easily be distinguished however by the nature of the oral aperture. Three of the species, namely *A. caninum*, *A. tubaeforme*, and

→

Figures 6–9. Photomicrographs of various species of *Ancylostoma* showing oral aperture and teeth. (× 225.) 6. *A. caninum.* 7. *A. tubaeforme.* 8. *A. buckleyi.* 9. *A. pluridentatum.*

10

11

12

13
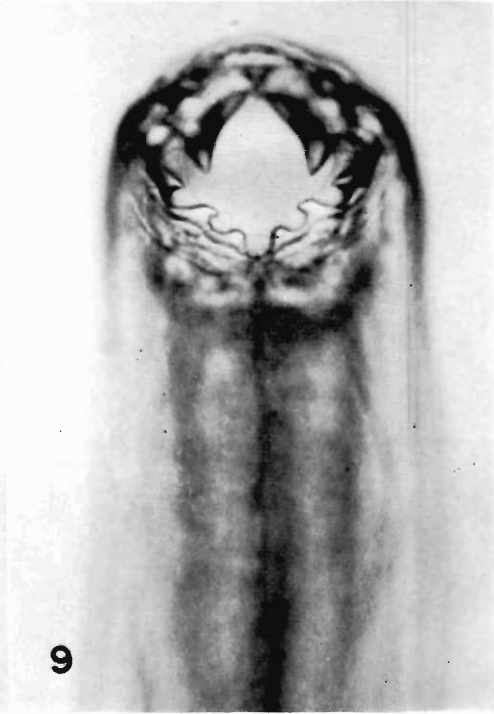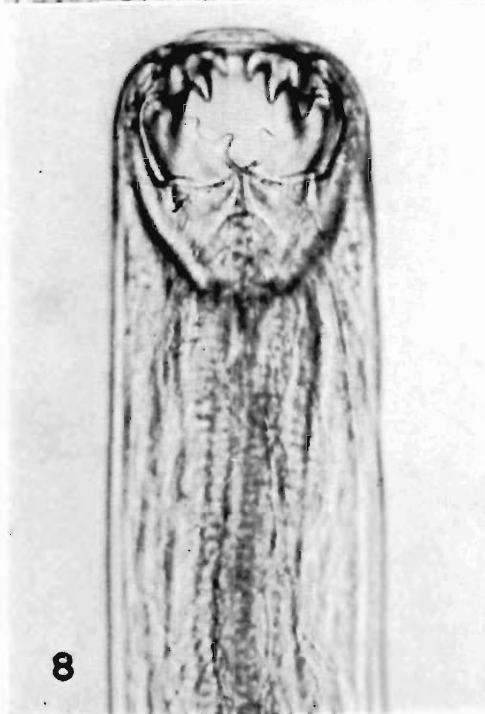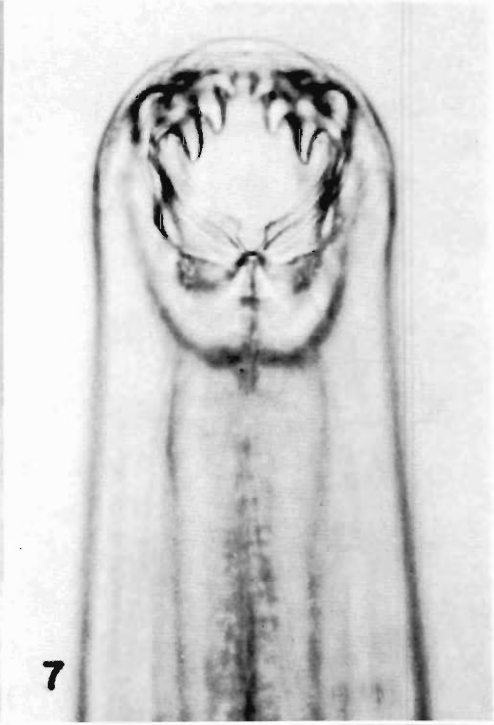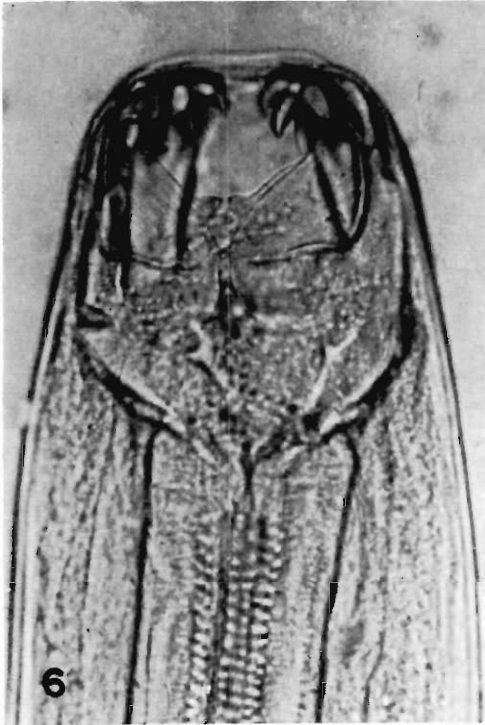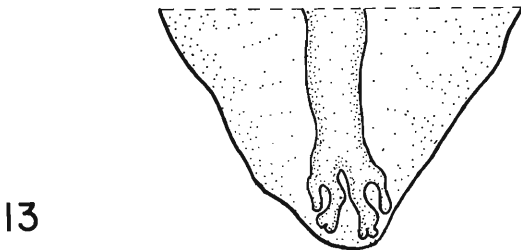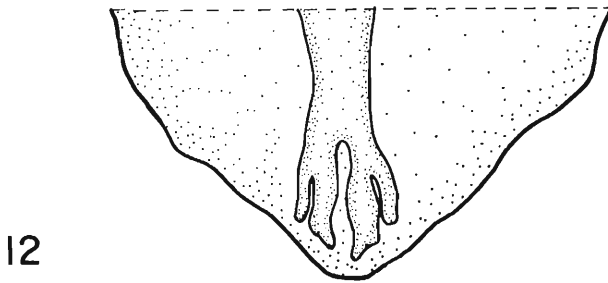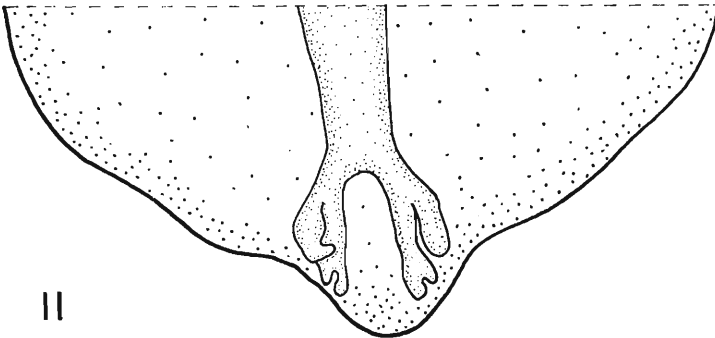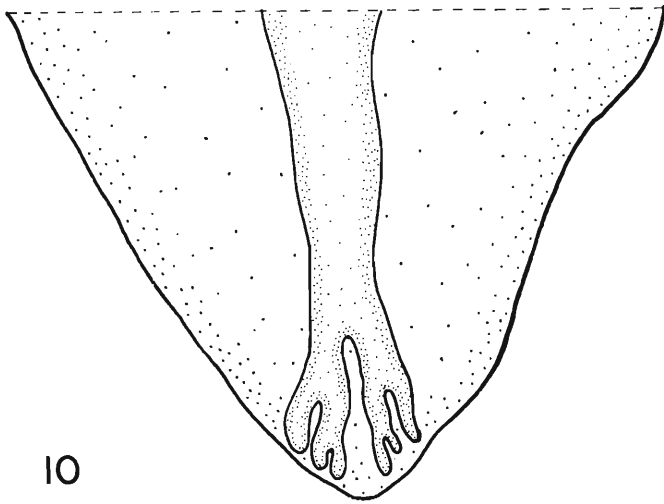
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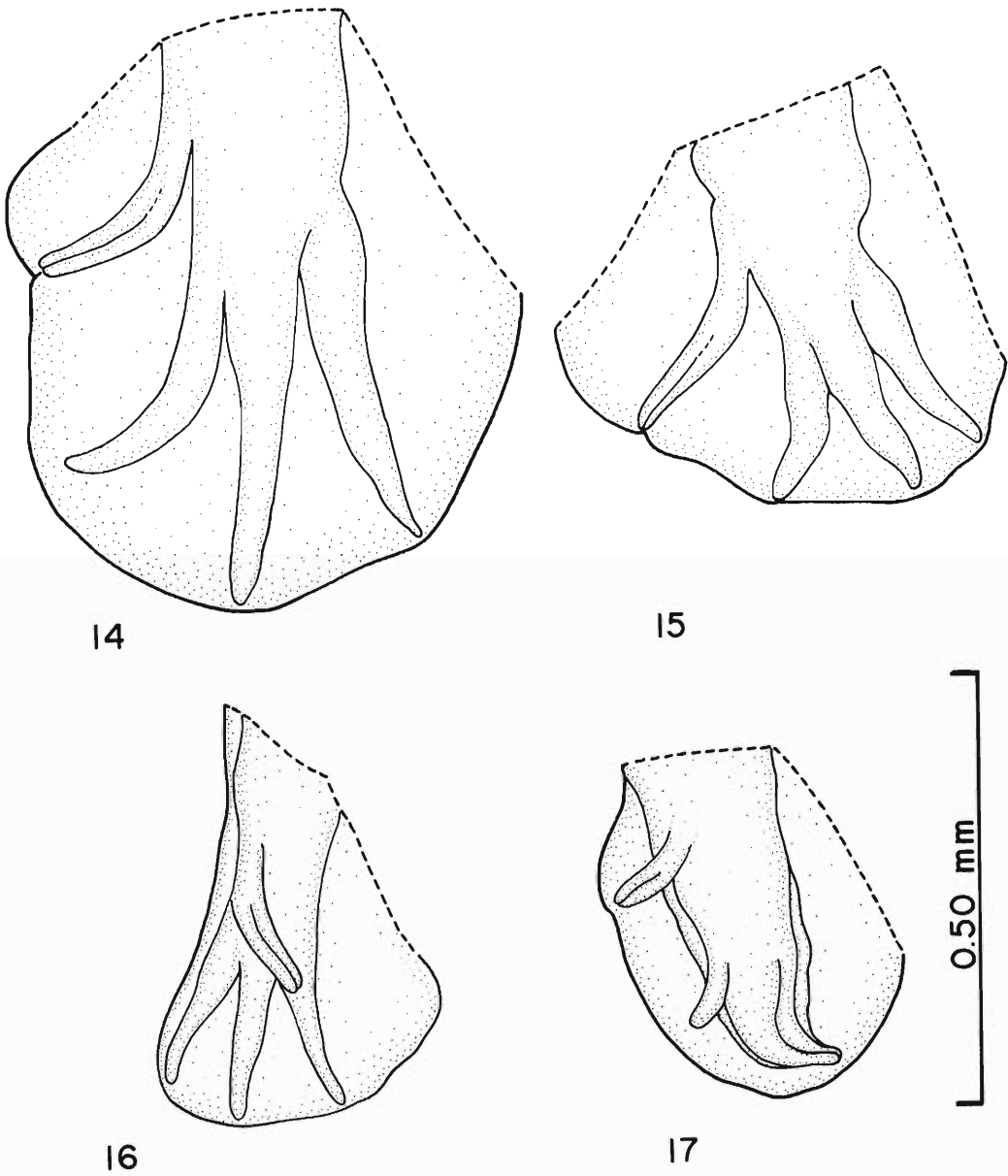
Figures 14–17. Right sides of the male bursa of various species of *Ancylostoma* showing ventral and lateral rays. 14. *A. caninum.* 15. *A. tubaeforme.* 16. *A. buckleyi.* 17. *A. pluridentatum.*

Figures 10–13. Dorsal lobes of the male bursa of various species of *Ancylostoma* showing terminal digitations of the dorsal ray. 10. *A. caninum.* 11. *A. tubaeforme.* 12. *A. buckleyi.* 13. *A. pluridentatum.*

A. buckleyi, have three pairs of ventral teeth. *A. buckleyi* is easily separated from the other two by the fact that it has two pairs of dorsal teeth, or protuberances. *A. tubaeforme* has a more open buccal aperture, more prominent lateral teeth, and thicker supporting ridges on the ventral tooth plates. Although both *A. pluridentatum* and *A. duodenale* have two pairs of ventral teeth, those of *A. duodenale* are of similar size and project side by side into the oral opening. Those of *A. pluridentatum*, on the other hand, arise from either side of the ventral tooth plate, and in fully mature specimens only the inner tooth on each side projects into the aperture. In addition, *A. pluridentatum* has three pairs of hook-like projections on the dorsal edge of the oral aperture which distinguishes it from the other species.

The species of *Ancylostoma* are generally difficult to distinguish on the basis of bursal characters. As can be seen from Figures 14, 15, and 16, the ventrals and laterals of *A. caninum*, *A. tubaeforme*, and *A. buckleyi* are rather similar except for size. *A. pluridentatum*, however, is distinct from the other species in that the ventrals and externolateral rays are reduced and the mediolateral and posterolateral rays tend to be close together or partially fused (Fig. 17).

The terminal portion of the dorsal ray seems to show some constant differences (Figs. 10–13). The principal difference appears to be in the amount of fusion between the terminal digitations. A characteristic of the genus is to have two, tridigitate branches at the end of the dorsal ray. The three digitations on either side are plainly visible in *A. caninum* and *A. tubaeforme*. Because of fusion of the inner branches, this feature is barely visible in *A. pluridentatum* and even less prominent in *A. buckleyi*. It should be noted, however, that bursal characters show a good deal of intraspecific variation and should be used with caution in the separation of these species.

Although the spicules of the various species of *Ancylostoma* are similar morphologically, their relative lengths can sometimes be useful taxonomically. For example, the spicules of *A. tubaeforme* are usually longer than those of

A. caninum in spite of the fact that the latter is a larger worm.

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Amprolium Treatment of Six- to Twelve-month-old Calves Experimentally Infected with Coccidia[1]

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ABSTRACT: One hundred Hereford calves were used in 2 experiments, each lasting approximately 3 months. In the first experiment, forty yearling heifers were each inoculated with approximately 50,000 *Eimeria bovis* oocysts daily for 10 days by adding the oocysts to the dry feed. Amprolium was given in the feed at 25 mg/kg of body weight daily for 30 days to 20 of the calves beginning 1 day before inoculation; the remaining 20 were not treated. The untreated calves discharged moderate to high numbers of oocysts and had diarrhea lasting for a mean of 2.4 days. In the treated calves, few or no oocysts were discharged during the period from 5 days after the beginning of treatment until 6 days after treatment was stopped, and then small numbers of oocysts were discharged sporadically. The treated calves gained an average of 7 lbs per calf more than the untreated ones during the 86 days of the experiment. In the second experiment, each of 60 steers, about 6 months of age, was given approximately 100,000 *E. bovis* oocysts in the feed daily for 3 days. One group of 20 calves was given amprolium for 21 days at 5 mg/kg of body weight starting one day before inoculation, 20 were treated for 5 days at 10 mg/kg starting 13 days after inoculation, and 20 were untreated. Diarrhea occurred in five untreated calves and in one calf treated for 5 days at 10 mg/kg; bloody feces were discharged by 12 untreated calves and by 2 calves treated for 5 days at 10 mg/kg; neither occurred in the calves treated for 21 days. Moderate to high numbers of oocysts were discharged by the untreated calves, moderate numbers in the calves treated for 5 days, and low numbers in those treated for 21 days. The oocyst discharges were delayed in both groups of treated calves. The calves treated for 21 days gained an average of 4.5 lbs more than those treated for 5 days and 9.2 lbs more than the untreated. Although the differences between treated and untreated calves in numbers of oocysts discharged and duration of oocyst discharge were significant in both experiments, differences in weight gain were not. Apparently, the numbers of oocysts given were insufficient to cause severe infection, and the amprolium treatments used prevented the occurrence of clinical signs of coccidiosis.

Amprolium has been found to be useful in controlling experimental coccidiosis when given in the milk to calves inoculated at an age of about two weeks (Hammond, Fayer, and Miner, 1966), when given in feed to calves inoculated at an age of 2–3 months (Slater, Hammond, and Miner, 1970), and when given as a drench to calves of unspecified age (Peardon et al., 1965). Little is known about experimental infections with coccidia in older calves or about treatment of such infections. The present study was undertaken to obtain information about the results of inoculating 6- to 12-month-old calves with coccidia and about the effects of giving amprolium in the feed to such calves.

Materials and Methods

Two experiments, each 90 days in duration, were conducted. In the first, begun on 17

May 1968, 40 yearling Hereford heifers, each with a mean weight of 374 lb, were used. These had been maintained in feed lots during the winter in Skull Valley, Utah. In the second experiment, begun on 10 November 1968, 60 6-month-old Hereford steers, each with a mean weight of 228 lb, were used. These had been kept on range in the vicinity of Woodruff, Utah, during the summer. The calves were brought to the Veterinary Science farm of the University about 10 days before the beginning of each experiment. They were placed in groups of 10 in 960-square foot pens. Two-thirds of each pen floor was concrete, the remainder was a dirt bedding area, and was covered by a roof. The pens were cleaned and provided with fresh straw weekly. A loose, mixed, fattening ration consisting of 33% chopped hay, 37% rolled barley, 25% pressed beet pulp, and 5% cotton seed meal, with a vitamin supplement, was weighed and dispensed to the calves 3 times daily. Water was available to the calves in automatically heated and regulated tanks.
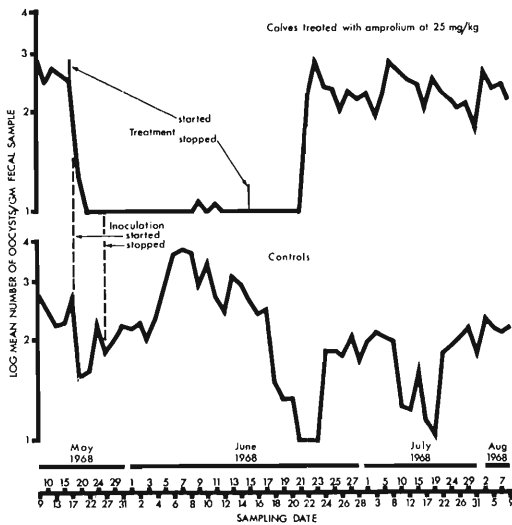
Figure 1. Numbers of oocysts discharged by the treated and untreated heifers in the first experiment.

Fresh fecal samples were collected in the pens after animals were observed defecating. A numbered tag was chained to the neck of each calf to facilitate identification during sampling. Fecal samples were examined for oocysts with McMaster chambers and the presence of blood, mucus, or tissue was recorded. The calves in each experiment were sampled 3 times weekly from the time of arrival until 2 weeks after the beginning of oocyst inoculation, then daily for approximately 1 month, and finally 3 times weekly until the end of the experiment.

The calves were weighed on arrival and randomly divided into groups as nearly equal in weight as possible. The heifers in the first experiment were weighed at 2-week intervals throughout the experiment, whereas the steers in the second experiment were weighed at 2-week intervals except for a period of 3 weeks during the second month when they were weighed weekly.

The calves were inoculated at the time of the morning feeding. The inoculum (94% *Eimeria bovis*, 4% *E. ellipsoidalis*, 1% *E. auburnensis*, 0.5% *E. zuernii*, 0.4% *E. cylindrica*, and 0.1% *E. subspherica*) was diluted with water and poured over a small portion of the morning ration, carefully arranged in a pile along the length of each feed trough. All of the calves in each pen had simultaneous access to the feed with oocysts, and were allowed to eat all of it before being given the remainder of the ration. In the first experiment, 500,000 oocysts were given to each group of 10 calves daily for 10 days, approximating 50,000 oocysts per calf per day. The steers in the second experiment received 1,000,000 oocysts per group daily for 3 days, approximating 100,000 per calf per day.

In the first experiment, 20 heifers were given 25 mg of amprolium per kg of body weight daily for 30 days, beginning 1 day before inoculation. The remaining 20 calves were not treated. Amprolium (25% Amprol®, obtained from Merck and Co.) was mixed with soybean meal and added to the feed. A similar amount of soybean meal without amprolium was added to the ration of the untreated calves. In the second experiment, 20 steers were given amprolium at 5 mg/kg for 21 days beginning 1 day before inoculation, 20 were treated at 10 mg/kg for 5 days beginning 13 days after inoculation, and 20 were not treated. All surviving animals were sold at auction after a 2-month waiting period required by the Food and Drug Administration.

The "T" and "F" tests were used to analyze data collected from the first and second experiments, respectively. Data analyzed included peak number of oocysts discharged, total number of oocysts discharged, day of peak, duration of patent period, days of diarrhea, days of bloody feces, and weight gain.

Results

In the first experiment, all of the heifers sporadically discharged small numbers of oocysts of *E. bovis* and other species during the period of observation before inoculation. Moderate (1,000 to 10,000 per gram of feces) to high (over 10,000 per gram) numbers of *E. bovis* oocysts were discharged by 18 of the 20 untreated calves as a result of the experimental infections (Fig. 1). This discharge lasted 1 to 9 days (mean, 5.1), with peaks occurring 19 to 23 days (mean, 21.5) after initial inoculation. Diarrhea of 1 to 5 days (mean, 2.4) duration occurred in 16 of the calves at the time of the increased oocyst discharge, and in the 2 calves in which no such discharge was observed. A sporadic discharge
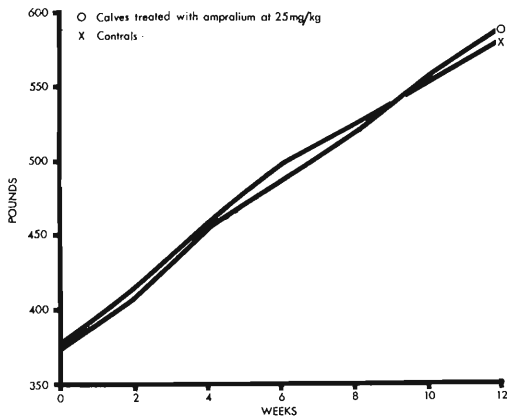
Figure 2. **Mean weight gained during the trial period by the treated and untreated heifers in the first experiment.**

Table 1. **Comparisons of treatment groups within experiments.**[1]

	Experiment	
	1	2
Peak discharge	0.05	0.01*
Total discharge	0.01	0.01*
Day of peak	0.01	0.01**
Days of discharge	0.01	0.01***
Days of diarrhea	0.01	NS
Days of bloody feces	NS	0.01*
Weight gain	NS	NS

[1] Results of "T" test for Experiment 1 data; the "F" test was used for Experiment 2 data.
NS—Not significant at 0.05 level or greater.
* Significant difference between treated and untreated groups.
** Significant difference between 5 mg/kg treated and untreated groups; none exists between 5 mg/kg and 10 mg/kg treated or 10 mg treated and untreated groups.
*** Significant difference between 5 mg/kg treated and untreated groups; 0.05 level significance exists between 10 mg/kg treated and untreated groups. No significant difference exists between treated groups.

of small numbers (less than 1,000 per gram) of oocysts continued during the remainder of the experiment. In the treated calves, no oocysts were discharged from the 5th day of treatment until the 6th day after cessation of treatment, except for 2 calves, each of which passed small numbers of oocysts 21 and 23 days after initial inoculation, respectively. A sporadic discharge of small numbers of oocysts occurred during the remaining portion of the experiment. No diarrhea was observed in these calves. The treated calves each gained an average of 212.2 lb as compared with an average of 205.2 lb for the untreated calves during an 86-day period, but this difference was not significant (Fig. 2). The weight gain curve did not undergo any appreciable fluctuation at the time of diarrhea in the untreated calves. The 10 untreated calves that discharged the smallest number of oocysts did not gain appreciably more than the 10 calves that discharged the largest number of oocysts. However, a significant difference occurred between the treated and untreated calves with respect to peak discharge of oocysts (0.05 level), total discharge, day of peak discharge, duration of discharge, and occurrence of diarrhea (all at the 0.01 level) (Table 1).

In the second experiment, the calves in all groups discharged small numbers of oocysts during the period before the expected dis-charge resulting from inoculation, but 6 of the 60 calves were negative during this period. A respiratory infection spread through all groups, beginning 57 days after initial inoculation and lasting about a week. Antibiotics were given intramuscularly and in the feed. The condition was characterized by a deep non-productive cough and some anorexia. One calf died of bloat during the experiment and 6 other calves died or were removed from the experiment because of respiratory and/or digestive tract disturbances not associated with the experimental infection. These 7 calves included 3 in the group treated for 21 days, and 2 in each of the other groups. All of the 18 untreated calves discharged moderate to high numbers of *E. bovis* oocysts for 2 to 10 days (mean, 6.1), with peaks occurring at 19 to 22 days (mean, 20.1) (Fig. 3). Twelve of these 18 calves discharged bloody feces for 1 or 2 days at the time numerous oocysts were being discharged, and 2 of these calves as well as 3 of the other 6 calves had diarrhea for 1 or 2 days. The calves discharged small numbers of oocysts during the remainder of the experiment.

Fifteen of the 18 calves treated for 5 days discharged moderate to high numbers of oocysts for 1 to 11 days (mean, 5.2), with peaks occurring 21 to 28 days (mean, 23.8) after initial inoculation. A sporadic discharge of small numbers of oocysts occurred during the remainder of the experiment. Bloody feces
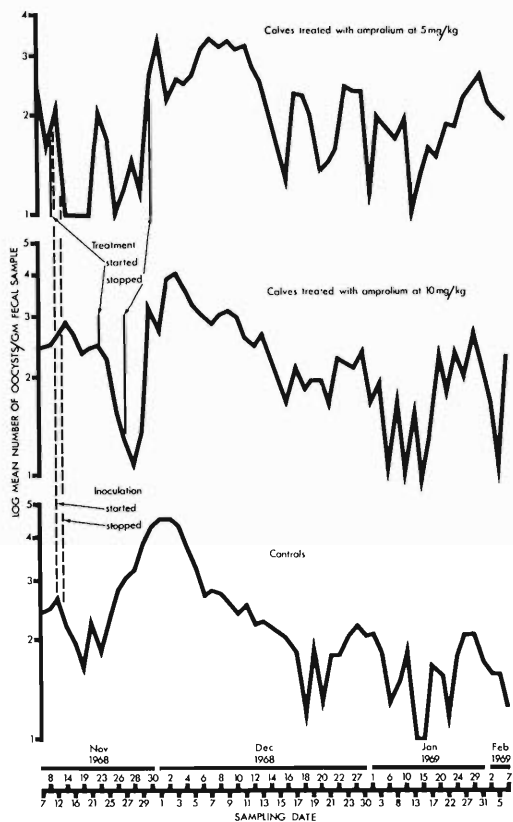
Figure 3. Numbers of oocysts discharged by the 3 groups of steers in the second experiment.

Figure 4. Mean weight gained during the trial period by the 3 groups of steers in the second experiment.

were discharged for only 1 day in each of 2 calves, and no diarrhea was observed except in 1 calf that did not show any increase in oocyst discharge; this diarrhea occurred 12, 14, and 17 days after inoculation.

In the 17 calves treated for 21 days, 12 discharged moderate numbers of oocysts for 1 to 10 days (mean 3.3), with peaks occurring 20 to 30 days (mean, 26.8) after initial inoculation. Sporadic discharge of small numbers of oocysts occurred during the remainder of the experiment in most of the calves. No bloody feces or diarrhea was observed.

The average weight gain during the 93 days of the experiment was 168.1, 172.8, and 177.3 lb per calf for the untreated, 5-day treated, and 21-day treated groups, respectively (Fig.

4). These differences were not significant. As in the first experiment, the curve of weight gain did not show any fluctuation in association with the period of increased oocyst discharge, nor did the untreated calves that discharged the fewest oocysts gain appreciably more than those that discharged the most.

A significant difference (0.01 level) was found between the treated groups and the untreated group with respect to peak and total discharge of oocysts and occurrence of bloody feces (Table 1). A significant difference (0.01 level) was found between the 21-day treated calves and the untreated calves with respect to the timing of the peak oocyst discharge. With respect to the duration of oocyst discharge, a significant difference was found between the untreated calves and the 21-day treated calves (0.01 level) and between the untreated calves and the 5-day treated calves (0.05 level). Differences in occurrence of diarrhea among the 3 groups were not significant.

Discussion

The infections in the untreated animals of both experiments were relatively mild and clinical signs occurred only briefly. This is in contrast to the results of earlier experiments with younger animals, in which severe coccidiosis occurred (Hammond et al., 1966; Slater et al., 1970). The oocysts in the experiments with young calves were given in a single dose by means of nippled flasks, whereas in the present study they were given with the feed in multiple doses. Senger et al. (1959) found

that multiple inoculations resulted in oocyst discharges similar in duration to those resulting from single inoculations. However, Fitzgerald (1967) reported that the patent periods in calves given continuous low-level inoculations with *E. bovis* were extended 7 to 15 days. In the present study, the periods of oocyst discharge presumably resulting from the inoculations did not appear to be longer than normal.

Information as to previous exposure to coccidia of the calves in the present study is unknown, but in both experiments small numbers of oocysts were being discharged by the majority of animals before inoculation, suggesting the possibility that some degree of immunity was present. The infections in the untreated animals of the second experiment appeared to be somewhat more severe than those of the first as judged by the occurrence of bloody feces and numbers of oocysts discharged. This was to be expected, because the animals of the second experiment were younger than those of the first, and had not been exposed to a feed-lot environment. They were given 100,000 oocysts per calf per day for 3 days instead of 50,000 per calf per day for 10 days; because the patent period was about normal in duration, the oocysts given after the first few days probably had little effect. Also, the calves in the second experiment were subjected to more severe weather conditions, because it was conducted during the period from November to February and the first was done during May to August. Fitzgerald (1962) found that outbreaks of coccidiosis caused by *E. zuernii* in calves are evidently influenced by such environmental factors as climatic conditions and type of feed as well as by ingestion of oocysts, and this probably holds true also for coccidiosis caused by *E. bovis*. It is likely, however, that inoculation of larger numbers of oocysts in the present study would have resulted in severe coccidiosis.

Because of the relatively mild infections occurring in the calves of the present study, the difference in weight gain between the treated and untreated calves was not significant. In severely infected calves, Fitzgerald (personal communication) found an appreciable reduction in weight gain.

In both experiments, the treated groups had less severe clinical signs of coccidiosis and discharged fewer oocysts than did the un-treated groups. The results of the present study thus agree with those of Casorso and Zaraza (1963), Peardon et al. (1965), Hammond et al. (1966), Newman et al. (1968), Gretillat and Vassiliades (1968), and Slater et al. (1970) in that amprolium is effective in preventing or treating clinical coccidiosis in calves. We found, as did the last authors, that amprolium can be administered successfully with the grain feed. Our results also agree with those of Slater et al. (1970) in that prophylactic treatment at 5 mg/kg is as effective as that at 25 mg/kg in preventing the occurrence of clinical signs, but there was less suppression of oocyst discharge by the 5 mg/kg treatment in our study than was observed in the earlier study. We found that the discharge of oocysts was retarded in calves treated with amprolium, as did Peardon et al. (1965), Hammond et al. (1966) and Slater et al. (1970). Less retardation was observed in the calves treated for 5 days at 10 mg/kg than in calves treated for 21 days at 5 mg/kg. No distinct peaks in oocyst discharge occurred in the calves treated for 30 days at 25 mg/kg, indicating that most of the coccidia in these animals were not able to complete their life cycles. Slater et al. (1970) found that the majority of first-generation schizonts observed in calves treated at 25 mg/kg and killed 14 to 19 days after inoculation of *E. bovis* oocysts were retarded in development and some schizonts appeared degenerate, especially in the 19-day calves.

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Age and Susceptibility of Cattle to Initial Infection with *Cysticercus bovis*

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ABSTRACT: The influence of age on susceptibility of cattle to initial infection with *Cysticercus bovis* was investigated experimentally. Degree of infection at necropsy was estimated from the number of cysts found in samples of the meat from five sites and the known or estimated weight of the meat. Seven cattle, one 2 months old, one 4 months old, three about 14 to 22 months old, and two about 4 years old, were given a single identical dose of *Taenia saginata* eggs. The estimated total numbers of cysts in the sites examined strongly suggested that at least two of the 14- to 22-month-old animals were as susceptible to infection on the average as one of the calves and that the 4-year-old cattle were on the average at least as susceptible as two of the younger adults and one of the calves. In other experiments, six cattle, one 9 months old, four about 3 to 4 years old, and one 7 years old, were identically exposed to initial infection. The 3- to 4-year-old ones were reexposed to infection one or more times. The initial exposure was identified as, or presumed to be, the source of all or nearly all cysts in samples from them. Estimations of total cysts in the sites strongly suggested that these four cattle were as susceptible as the 9-month-old one, whereas the 7-year-old one was much less susceptible than any of the other five.

The primary purpose of this paper is to report some experimental results bearing on the question whether age per se makes cattle immune or highly resistant to initial infection with *Cysticercus bovis*. Two experiments were carried out exclusively to answer this question. Also included are data bearing on the susceptibility of certain mature cattle used in two other experiments in which investigation of age resistance was not the paramount purpose.

As far as we are aware, details of experiments strictly on age resistance to *C. bovis* infection have heretofore been reported only by Penfold (1937) and Urquhart (1961). Their findings appear to be somewhat contradictory.

Materials and Methods

The *Taenia saginata* eggs originated from worms removed from patients by anthelmintics. The proglottids and eggs expelled from the ripe segments were received in 0.85 percent sodium chloride solution containing small amounts of penicillin and streptomycin. The average interval from collection to receipt of the material was about 4 days. To the free eggs obtained from the fluid by sedimentation were added those obtained by comminution of the ripe proglottids in the excess fluid and

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passage with it through an 80-mesh or 100-mesh screen. The eggs, whether free or in proglottids, were stored at about 42 F from the time of receipt until used. The eggs administered in Experiment 1 and initially in Exp. 2 were stored about 4 days; those in Exps. 3 and 4 were stored about 1 month.

The 13 cattle used were either always on the premises of the Agricultural Research Center (ARC) or were kept there at all times after purchase at farms in the vicinity when a few days old. As far as known, this history practically precludes the possibility that any were extraneously exposed to *T. saginata* eggs; only 1 case of *C. bovis* infection has been detected by federal inspection of 2,115 ARC cattle slaughtered in the years 1939 to 1964, inclusive. Some of the test cattle were always kept in indoor pens which were cleaned out several times a week. The others were given eggs while in a barn and were kept there for at least 48 hours thereafter. During this period their feces were not placed on any lot or pasture used. By such measures the possible contamination of pastures by viable eggs or oncospheres passing out in the feces after infection was prevented. These precautions were taken although Penfold et al. (1936b) reported that they found no evidence of infective material in the feces up to the third day after infection, which was the limit of their observations.

The dosage of eggs given, the number of exposures and the intervals between exposures are recorded in Table 1. Cattle simultaneously infected each received orally an equal aliquot of a suspension of eggs of a single batch. The dosage of mature eggs was estimated from the number with a thick, brown shell counted in at least one small sample of suspension.

The experimentally infected cattle were necropsied from 1 to 31 months after initial exposure to *T. saginata* eggs. Samples of the heart, masseters (cheeks), diaphragm, shoulder region, and round region were taken for examination for cysticerci immediately after slaughter or after refrigeration for a few days. Some samples were examined soon after collection; others were frozen and examined after thawing. Each sample was finely sliced (thickness: 1–2 mm), and cysticerci found in it were counted.

EXPERIMENTS 1, 2, AND 4: A random sample of muscle tissue was taken from the five above-mentioned sites. The sample of heart comprised one-fourth of the whole by weight. This organ was cut into fourths and the sample consisted of several small slices from each fourth. The sample of each other site weighed 1 gram per 4 pounds of live body weight and consisted of several small slices taken from different parts of the muscle or region. The masseter sample was usually equivalent to about one-fourth of the whole by volume. However, the masseters of animals of Exp. 2 were weighed and divided into fourths for sampling.

To estimate the numbers of cysts in all five of the sites examined from the findings in the samples, estimates were made of the weight in pounds of the diaphragm, the meat of the shoulder and round regions as defined below for Exp. 3 and, where necessary, the masseters. The bases of estimation of meat weights were: (1) live weight and (2) known weights of the meat of the corresponding part in 16 cattle ranging in live weight from 330 to 1,335 lbs. These cattle were the five of Exp. 3, 10 others processed like Nos. 20 and 4922 of Exp. 3, and one other processed like No. 7 of Exp. 3, as described below.

EXPERIMENT 3: A sample equal to one-fourth of the weight of the whole was taken from the masseters, diaphragm, and heart. The method of sampling each was the same as used for the heart in the other experiments. The procedure for sampling the other two sites was as follows: The dressed carcass was split into sides. From side 1 were cut two regions corresponding approximately to major commercial carcass divisions: (1) Shoulder (chuck and arm and foreshank) and (2) round (round, rump, and hindshank). Each was completely boned out. The meat obtained from it was freed of major accumulations of fat and weighed. The sample was 1/20 by weight of the meat and consisted of dozens of small slices cut so as to be representative of the various portions of the whole. A second 1/20 sample of the same composition was taken from: (1) the weighed meat obtained by processing side 2 exactly as side 1 (Bovines 20 and 4922) (Table 1), or (2) the unweighed meat obtained by cutting away most of the musculature from the bones of the corresponding division of side 2 (Bovines 4031; 4956), or (3) the remainder of the meat (side 1) from which sample 1 had

Table 1. Calculated number of recognizable *C. bovis* from five sites in cattle given the indicated numbers of *T. saginata* eggs.

Bovine No.	Age (months) initial exposure	Additional eggs		Months from initial exposure to slaughter	Live wt. (lbs) at slaughter	Calculated *C. bovis* cysts from:					Total from 5 sites
		Number	Months after initial exposure			Heart	Masseters	Diaphragm	Shoulders	Rounds	
Experiment 1.	Initial exposure—330,000 eggs:										
4034	9	0	–	14	535	308	139	63	510	187	1,207
4445	36	284,000	12.5	14	1,580	40	29	14	1,404	527	2,014
93	84	0	–	14	1,680	40	18	7	0	182	247
Experiment 2.	Initial exposure—330,000 eggs:										
4158	38	27,000	0.5	31	1,475	64	74	119	2,000	2,494	4,751
		340,000	3.0								
		340,000	3.5								
		340,000	4.5								
4122	50	340,000	3.0	31	1,630	260	48	103	938	704	2,053
		340,000	3.5								
		340,000	4.5								
4123	50	27,000	0.5	31	1,575	56	18	34	931	1,426	2,465
		340,000	3.0								
		340,000	3.5								
		340,000	4.5								
Experiment 3.	Initial exposure—284,000 eggs:										
4031	22	0	–	5	650	420	256	256	1,420	663	3,015
4922	15	0	–	17	1,120	120	80	176	720	660	1,756
4956	14	0	–	6	808	216	172	88	221	201	898
7	4	0	–	5	330	400	264	228	260	340	1,492
20	2	0	–	16	875	120	68	28	1,620	1,120	2,956
Experiment 4.	Initial exposure—284,000 eggs:										
4440	49	0	–	1	1,625	192	89	118	1,092	352	1,843
4449	49	0	–	1	1,405	96	64	57	1,092	1,274	2,583

been taken (Bovine 7). Where the meat from each side was weighed, both samples were ½₀ of the average weights. Samples were examined, after thawing if frozen, as in the other experiments.

Calculation of number of cysts. To estimate the number of cysts present in a site, the number or average number found in the sample(s) was multiplied by the appropriate factor, namely 4 or 40. Recognizable *C. bovis* cysts were counted, whether dead or alive and no attempt was made to determine their viability. The object of the examination was to determine the number of cysts present; not necessarily their status.

Experimental Data and Their Significance

Experiment 1

Three cattle ranging in age from 9 months to 7 years were each simultaneously given an equal dose of *T. saginata* eggs (330,000). The numbers of cysts estimated to be present in five sites in each of these cattle at necropsy appear in Table 1. Only conservative inferences are admissible because the weight of the meat of four of the sites was estimated rather than determined.

The inferences that are considered admissible are (1) that the 7-year-old steer acquired fewer cysts than either younger animal and (2) that the calf and the 3-year-old steer did not differ appreciably in susceptibility.

Experiment 2

Three cows about 3 to 4 years old were initially exposed to infection simultaneously with and exactly as were the cattle of Exp. 1. They also were repeatedly reexposed to infection. These mature cattle obviously were far from immune to initial infection (Table 1). Since a first infection causes immunity or strong resistance to reinfection (Penfold et al., 1936a), the presumption is very strong that cyst formation in the cows resulted almost entirely from the first dose of eggs, or from the first two doses where a comparatively negligible second dose was given only two weeks after the first (animals 4158 and 4123). Hence comparison of degrees of infection in this experiment with those in Exp. 1 is warranted. Thus, the numbers of cysts estimated to be present in the five sites examined very

strongly suggest that on the average these mature cows were at least as susceptible to initial infection as the 9-month-old calf of Exp. 1.

Experiment 3

Five cattle, 2 to about 22 months old, were each simultaneously given an equal dose of *T. saginata* eggs (284,000).

The degree of infection with *C. bovis* cysts, as indicated by the total number estimated to be present in the five sites (Table 1), was not directly related to the age of the animal when infected. Some of the cattle were killed much sooner after infection than the others, but the estimated numbers of cysts varied independently of the interval from infection to necropsy. Degree of infection appeared to vary in animals of comparable age when infected. It was much heavier in the oldest animal than in one of the young calves (No. 7). The other calf (No. 20) had far fewer nodules having the known gross characteristics of *C. bovis* cysts than any other animal of the experiment.

In our experience, some old *C. bovis* cysts are whitish and opaque. The wall is thick, tough, and fibrous. However, a majority of the cysts appear as thin, nearly clear membranes, which surround yellowish, greenish, greyish, or brownish black, compacted, caseous or gritty material.

However, in certain of the samples from Calf No. 20, many small lesions which grossly did not have these characteristics were noted, collected and, because their identity appeared questionable, separately enumerated. They were noticed because of their bright red to brownish color, which contrasted with the paler, duller hue of the muscle tissue. Some were spherical to ellipsoidal and about 1.0 to 1.5 mm in diameter. Some resembled two or more tiny beads strung on a fine filament. The rest were fusiform or sausage-shaped and up to about 3.5 mm long. Under a dissecting microscope each of about 75 examined appeared as a fairly transparent sac which could be seen to contain no opaque compacted material. On penetration and exertion of pressure with probes, at most a little fine debris was discharged from the sac. As a rule, further dissection resulted only in shredding of the thin sac wall. The shreds appeared to consist of nets of fine fibrils enmeshing numerous

large, reddish brown cells and small, colorless cells or platelets. If nothing else was found, the lesion was not counted as a *C. bovis* cyst. An occasional sac yielded a microscopic, more or less triangular, laminate, calcified body. In such a case, the lesion, though likewise regarded as of uncertain causation, was counted as a *C. bovis* cyst.

However, upon examination of histologic sections by a pathologist* examples of lesions of the rejected types from calf No. 20 were found to be granulomas, which did not contain recognizable cestode remains but were otherwise indistinguishable from *C. bovis* cysts from the same animal, except for evidence of earlier commencement of degeneration. The number of such lesions was estimated to be 2,532 in the sites examined. Thus, on the basis of this evidence of their cysticercal origin, the indicated degree of *C. bovis* infection was about alike in the youngest and oldest animals (Nos. 20 and 4031; Table 1).

Experiment 4

Two 4-year-old steers were exposed to infection simultaneously with and exactly as were the cattle of Exp. 3. The numbers of cysts in the five sites examined, computed as in Exps. 1 and 2, appear in Table 1. Comparison of the totals strongly suggests that these steers were as susceptible as most of the younger cattle of Exp. 3.

Conclusions and Discussion

The foregoing data demonstrate that none of seven 3- to 7-year-old cattle was immune to initial infection with *C. bovis*. Two of three 14- to 22-month-old cattle (Nos. 4031 and 4922) were as susceptible on the average as two calves (Nos. 7 and 20) about 4 months and 2 months old, respectively (Table 1). A 7-year-old steer (No. 93) was apparently resistant, but all six of the 3- to 4-year-old cattle tested were as susceptible as four (Nos. 4034, 4922, 4956, and 7) of the six that were 2- to 22-months-old when they were infected. One older animal (No. 4158) was more susceptible than any of the younger animals, but two of the aforementioned younger animals (Nos. 4031 and 20) had more cysts than six of the older group. Animals of comparable age ap-

* See footnote, page 122.

peared to vary considerably in susceptibility to infection with *C. bovis*.

The finding that adult cattle can be at least moderately susceptible to initial infection with *C. bovis* implies that they, as well as younger stock, should be protected against the hazard of exposure to *T. saginata* eggs. Sanitary precautions designed to prevent *C. bovis* infection have been described in many publications.

We have not ascertained by whom or when experiments first were performed which led to the conclusion that calves could be successfully infected with *C. bovis*, whereas adults could not. Presumably they were reported more than 90 years ago, since they were mentioned by Lewis (1872). In any event, it has since been shown that insusceptibility is commonly due to acquired immunity (Silverman (1955), Penfold and Penfold (1937), Froyd and Round (1960), and Soulsby (1961)).

In a life-history experiment, Penfold (1937) infected 30 oxen, including 18-month and 4-year-old animals. Each simultaneously received 400,000 eggs of a single batch of *T. saginata* eggs. The report contains one important sentence concerning age and susceptibility, "Having in various experiments artificially infested with *Cystercus bovis* oxen of ten months, eighteen months, three years, four years, and five years of age, I conclude that there is no marked natural age immunity." In view of our findings, it is noteworthy, however, that the report contains data which strongly suggest that in the experiment under discussion the five 4-year-old oxen that were killed 3 months postinfection were (1) at least as susceptible as the five 18-month-old oxen, also killed 3 months postinfection, and (2) about as susceptible as the five 10-month-old oxen that were killed 1–5 months (av. 2.6 mos.) postinfection. The evidence is that the averages of the numbers of measles reported by Penfold to be present in the carcasses of these three sets of oxen are, respectively: 19,380, 13,300, and 20,700.

Peel (1953) carried out two surveys to determine the relationship between age and infection with *C. bovis* in N'dama cattle slaughtered in an abattoir in Sierra Leone. He found (1) that incidence of infection declined "in direct ratio" to increase in age and (2) that the tendency to "generalization," number of cysts per infestation, and the percentage of

"active cysticercosis" all became progressively less with age. He conceded that acquired resistance probably was a factor in the explanation of his findings. However, he also considered certain deductions to suggest "the possibility of a resistance developing with age" and his observations to show that incidence was influenced by "age immunity." He stated that cattle over 5 years old do not appear to become reinfested and that it cannot be said whether this is due to "a true immunity brought about by repeated infestations throughout the early part of their lives, or to some factor not yet discovered . . . ."

McIntosh and Miller (1960) produced "extensive infections" in 13 cattle to which they gave single doses of viable *T. saginata* eggs.

Urquhart (1961) found no cysts due to prior natural infection in 18 of 48 adult East African cattle which were immune to infection upon challenge with doses of eggs of proven infectivity. However, he recognized that the apparent absence of old cysts was not proof that the immunity was due to age. He made arrangements whereby in Scotland, where cysticercosis is uncommon, 15,000 eggs from Kenya were given to each of seven calves and 40,000 to a calf and each of four cattle at least one year old. All of the calves and the youngest adult were "markedly susceptible," but the older adults became "lightly infected" and were "moderately" or "highly" resistant to infection. However, inasmuch as none of the Scottish adults or of the 88 Australian oxen infected by Penfold et al. (1936a) was immune, Urquhart (1961) concluded that it is likely that acquired immunity, rather than age, was primarily responsible for the complete insusceptibility of the East African cattle over 8 months old.

For the present, we accept the aforementioned histopathological evidence as to the cysticercal origin of the numerous lesions that were found in the carcass of bovine No. 20 and were not so identified from their gross appearance and the results of dissections. We have occasionally found similar lesions in other carcasses, usually adjacent to *C. bovis* cysts. However, we have not found in the literature any description of a young or degenerate cyst that clearly fits the gross appearance of the granu-

lomas under discussion. Penfold (1937) characterized the last vestiges of *C. bovis* cysts as "minute fibrous tissue scars."

Lucker and Douvres (1960) accepted as *C. bovis* cysts small 9-week-old heart lesions in which cestode tissue was not demonstrable. However, these lesions appeared as whitish fibrous sacs and usually enclosed a caseous centrum, whereas most of those under discussion had a sanguinous appearance and the other characteristics already mentioned.

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Research Note

Survival and Wound Healing of Adult *Echinostoma revolutum* Following Amputation of Body Parts

Incidental to studies on the transplantation of *Echinostoma revolutum* into the intestine of the domestic chick (Fried and Vonroth, 1968, Exper. Parasit. 22: 107–111), observations on survival and wound healing of experimentally damaged flukes were made and are reported herein.

Adults, 5- to 12-days old, and those transected posterior to the ovary to produce approximate equal anterior and posterior half-worms were implanted into the cloacas of day-old chicks. Zero of 158 posterior-halves, 38 (21.6%) of 176 anterior-halves and 23 (65.7%) of 35 whole worms were recovered from the rectum and lower ileum of chicks 3 hr to 7 days post-implantation. Anterior halves did not regenerate missing body parts and wound healing was effected by an immediate contraction of the body wall followed by subsequent accumulation of cuticle at the wound site confirming Beaver's (1937, J. Parasit. 23: 423–424) observations on wound healing in this species. Closure and marked dilation of the excretory tubes were observed in 1- to 7-day postimplant anterior half worms, and granular material coursed back and forth within the blind sacs. The formation of a muscular excretory bladder (Beaver, 1937, J. Parasit. 23: 423–424) or excretory ducts finding new openings in unusual sites (Sinitsin, 1932, Biol. Zbl. 52: 117–120) were not observed. Isolated hyaline regions, often bladder-like in appearance, were observed in newly formed cuticle, and probably resulted from fluid trapped in the wound as reported by Hart (1968, J. Parasit. 54: 950–956) following injury of the tetrathyridia of *Mesocestoides* sp. Ventral cuticular body spines failed to regenerate in accord with Beaver's (1937, J. Parasit. 23: 423–424) observations on cephalic spines and subcuticular muscle was absent anterior to healed wound margins as reported by Sinitsin (1932, Biol. Zbl. 52: 117–120) for naturally damaged liver flukes.

Reasons for the one-third survival rate of anterior half worms compared to controls are not apparent from observations made in this study. Initial trauma following transection and/or possible deleterious effects of closure of the excretory system may have contributed to the reduced survival of anterior halves.

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Research Note

Histochemistry of the Structural Proteins of the Spicules and Gubernaculum of *Ostertagia ostertagi* and the Spicules of *Cooperia punctata*

Gallagher (1964, Exp. Parasit. 15: 110–117) and Lyons (1966, Parasitology 56: 63–100) studied the chemical composition of the hooks of hydatid scolices and monogenean attachment sclerites, respectively. Both investigators concluded that these hardened parts of the worms were composed of a scleroprotein-like material. Chitwood (1938, Proc. Helm. Soc. Wash. 5: 68–75) reported that there was little if any difference in the chemical make-up of the spicules of different types of nematodes. He further indicated that collagen was the primary scleroprotein present and that it was mixed with a "glucoprotein" (glycoprotein?).

Table 1. Histochemistry of structural proteins of spicules of *Cooperia punctata* and spicules and gubernaculum of *Ostertagia ostertagi*: carbohydrates, nucleic acids, lipids, and proteins.

Histochemical test	C. punctata		O. ostertagi		
	Spicules		Spicules		
	Sclerotized part	Protoplasmic part	Sclerotized part	Protoplasmic part	Gubernaculum
Carbohydrates					
Periodic acid Schiff	−	+	−	++	−
Acetylation	−	−	−	−	−
Saponification after acetylation	−	+	−	++	−
No periodic acid oxidation	−	−	−	−	−
Diastase	−	+	−	+	−
Metachromasia	?	?	+	−	−
Alcian blue	−	−	+	+	−
Hyaluronidase digestion	−	−	+	+	−
Nucleic acids					
Methyl green-Pyronin					
DNA	−	−	−	−	−
RNA	−	−	−	−	−
Ribonuclease	−	−	−	−	−
Feulgen	−	−	−	−	−
Hematoxylin and eosin	−	+	−	−	+
Lipids					
Sudan black	+	−	−	−	−
Berenbaum (bound lipid)	+	−	−	−	−
Proteins					
Mercury bromphenol blue	+	++	−	+	+
DMAB nitrite	?	?	++	++	++
Sakaguchi	?	?	−	+	?
Millon's test	+	−	++	++	++
Mallory's aniline blue	−	−	−	−	+
Van Gieson's					
Collagenase digestion			?	?	?
Weigert's elastic	+	+	−	−	−
Verhoeff's elastic	+	−	−	−	−
Gridley's reticulum	++	−	++	+	++
Gomori reticulum	++	+	++	−	+
Acid Orcein-Giemsa	+	−	+	−	−
Fontana's silver	+	−	++	−	++
DOPA reaction	?	?	−	−	−
Johri and Smyth (1956)	−	−	−	−	−
Masson's trichrome	−	++	−	+	+
PFAAB	+	++	+	−	?
SH	++	−	+	−	+
SS	++	+	+	−	+
Papain	−	−	−	−	−
Trypsin	−	−	−	−	−
Pepsin	−	−	−	−	−
KOH	−	−	−	−	−
HCl	−	−	−	−	−
Sodium thioglycolate	+	+	+	+	+

++ = intense staining; + = faint staining; ? = questionable staining; − = no staining.

Stringfellow (1969, J. Parasit. 55: 1191–1200) presented evidence that the sclerotized parts of the genital cone of *Cooperia punctata* was a keratin-like scleroprotein. Incidental observations from this study led to the present investigation which was undertaken to characterize histochemically the spicules and gubernaculum of *Ostertagia ostertagi* and the spicules of *C. punctata*.

Histochemical procedures used in this study

are the same as before (Stringfellow, loc. cit.) with the following modifications for studying the histochemistry of the spicules and gubernaculum of *O. ostertagi*. The methyl green-pyronin stain with ribonuclease digestion was supplemented with the Feulgen reaction for demonstrating nucleic acids. The acid orcein-Giemsa stain supplemented Weigert's and Verhoeff's procedures, and Gomori's reticulum stain was used in place of Foot's modification of Bielschowsky's technique. Both species of worms were incubated in 0.5% sodium thioglycolate, pH 8, to determine its effects on the spicules and gubernaculum.

The results of all histochemical tests summarized in Table 1 indicate that the spicules and gubernaculum of *O. ostertagi* and the spicules of *C. punctata* are composed of a scleroprotein-like material. These results must be interpreted conservatively for two reasons: 1) techniques giving empirical results were used; 2) there was evidence for the presence of all types of structural proteins (collagen, keratin, reticulum, and elastin). Evidence for the presence of collagen in the spicules and gubernaculum was: Mallory's aniline blue collagen stain; for keratin: DMAB nitrite test for tryptophane, PFAAB test, DDD test for SH groups, DDD test for SS bonds after reduction with thioglycolic acid, absence of digestion by papain, trypsin, or pepsin and insolubility in dilute acid and alkali, partial disruption of the spicules and gubernaculum after incubation in sodium thioglycolate; for reticulin: Gridley's and Gomori's reticulum stains; for elastin: very faint staining with Weigert's, Verhoeff's, and the acid orcein-Giemsa stains.

Keratin-like proteins have been reported from the cuticle of *Ascaris* as well as from the esophageal and cloacal linings of other nematodes (Chitwood, 1936, Proc. Helm. Soc. Wash. 3: 39–49; Chitwood, loc. cit.). However, it is generally considered that keratin-like proteins from invertebrates may differ somewhat from vertebrate keratin. The evidence presented here favors more the presence of keratin-like proteins than any of the other scleroproteins. Nonetheless, the results from empirical techniques cannot be discounted.

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Research Note

Ineffectiveness of Levamisole in Experimental *Stephanurus dentatus* Infection in Rabbits and Pigs

Tetramisole (*dl*-6-phenyl-2,-3,5,6-tetrahydroimidazo[2,1-b]thiazole hydrochloride) has been reported to have significant activity against the cattle lungworm (Thienpoint et al., 1966, Nature 209: 1084–1086). Levamisole, which is the generic name for the *laevo* isomer of tetramisole, and formerly designated as *l*-tetramisole, has also been found active against another tissue parasite, namely the rat lungworm (Jindrak and Alicata, 1969, Chemotherapy 14: 244–252). The present study was carried out to determine (a) the prophylactic effectiveness of levamisole in preventing the migration of *Stephanurus dentatus* larvae and in reducing the pathology in the liver of infected rabbits, and (b) the therapeutic effectiveness of this drug in pigs 7 months after infection.

In the rabbit experiment, 9 rabbits approximately 3 months old were randomly allotted to 3 groups of 3 animals each. All the rabbits were individually inoculated with 2,000 infective *Stephanurus* larvae. Each animal of groups I and II received the drug *orally and daily* at the rate of 5 mg per kg and 10 mg per kg of body weight, respectively, and group III remained as untreated control. The period of prophylactic treatment covered 3 days preinfection and 10 days postinfection. The daily dosage of the drug for each animal was dis-
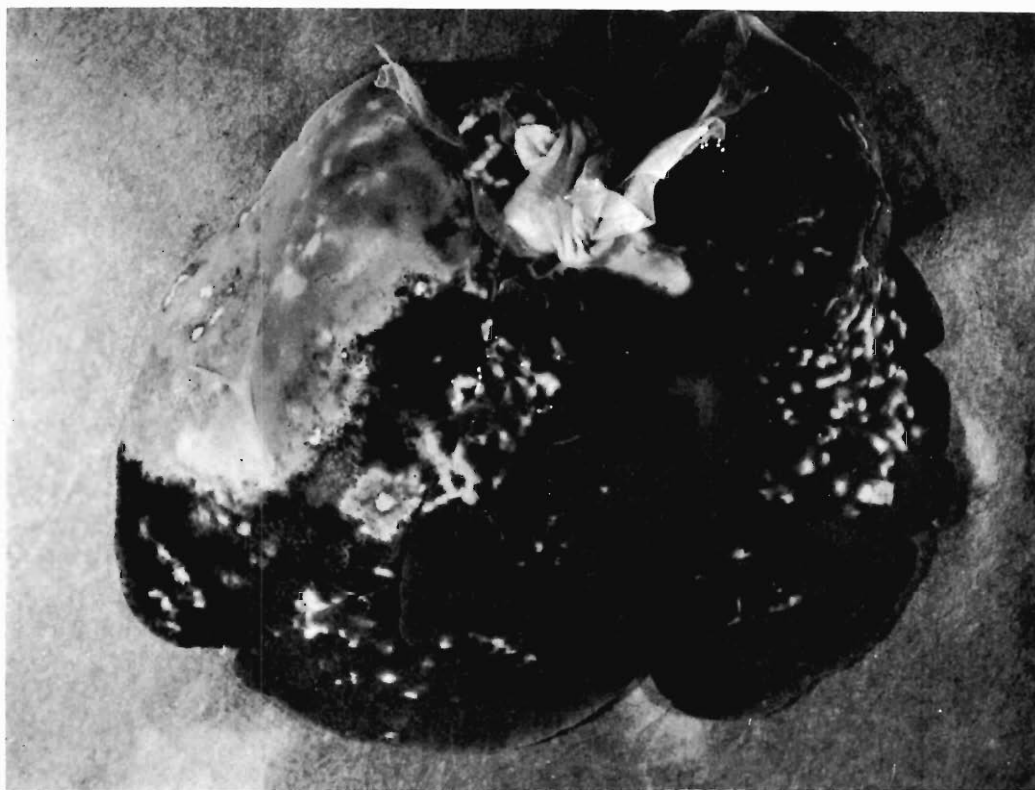
Figure 1. Liver of a rabbit in Group II showing a large area of necrosis in the right lobe and multiple tracts caused by developing *Stephanurus dentatus* one month after experimental infection with approximately 2,000 infective larvae.

solved in 2 ml of water and administered by stomach tube.

All the rabbits were killed about 1 month postinfection. At necropsy all the livers showed numerous superficial whitish tracts and large pale areas of necrosis (Fig. 1) and internal areas of fibrous parenchyma. Generally, there was no noticeable difference in pathology between the treated and untreated rabbits. Artificial pepsin-HCl digestion of the livers of the 3 rabbits in each group revealed the following number of developing fourth stage larvae: Group I, 7, 8, and 12; group II, 9, 12, and 14; group III, 7, 12, and 15. The larvae ranged from 3.2 to 7.1 mm in length.

In the pig experiment, 4 healthy 3-month-old animals were utilized. These pigs were derived from a kidney-worm free stock and had always been maintained on adequately clean concrete

floors. Each pig was orally inoculated with approximately 30,000 infective *Stephanurus* larvae. Seven months later, 3 of the pigs (Nos. 1, 2, 3) were treated daily for 7 days with levamisole resinate (a granular formulation) which was mixed with a portion of the daily feed, and pig No. 4 remained as untreated control. Pig No. 1 received the drug at the rate of 8 mg (active ingredient) per kg of body weight, and pigs Nos. 2 and 3, at the rate of 24 mg per kg of body weight. Pig No. 3 refused to eat all of the medicated feed during the treatment period; the refused feed was recovered and fed to the animal during 2 additional days.

One month after treatment all the pigs were killed. At necropsy all the livers showed superficial discoloration and many grayish elevations due to increased connective tissue. On cutting

they were found to be fibrotic and embedded in them were areas of necrotic tissue of various sizes. Several pus pockets were also present. In each of 3 such pockets in the liver of pig No. 1, one dead and degenerating *Stephanurus* worm, approximately 12 mm long was found. No other worms were observed in spite of careful search. Four and 8 worms, 13–22 mm long, were found in fibrotic liver capsules of pigs Nos. 2 and 3, respectively. These worms were viable as evidenced by slow movement of their extremities when placed in warm isotonic salt solution and observed under stereoptic microscope. In addition, 1 dead and degenerating worm, about 15 mm long, was pulled out of a pus pocket in the liver of each of these two pigs. From the liver of the control pig (No. 4) 9 live worms, 10–20 mm long, were recovered from various fibrotic capsules; no dead worms were observed. The fact that a few dead parasites were found in the liver of the treated pigs, and none in the control, may indicate partial deleterious action of the drug against them. Such activity is, however, questionable due to the limitation in the number of pigs used and the relatively few worms recovered. In each pig, the infection appeared to be limited to the liver, since no parasites or distinct lesions were observed in the kidneys or other extra-hepatic tissue.

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Research Note

Amino Acids Discharged by *Aphelenchoides* sp.

As a part of a study on the nutritional requirements of *Aphelenchoides* sp., discharged amino acids were identified by two-dimensional thin-layer chromatography. *Aphelenchoides* were grown on the fungus, *Pyrenochaeta terrestris* and is the same species as previously discussed (Myers, 1968, Experimental Parasitology 23: 96–103). All stages of the nematode were harvested after 60 days, carefully separated from both fungus and medium (Myers, 1968), surface-sterilized using the method of Peacock (1959, Nematologica 4: 43–55), and incubated in 1% glucose. Following 24 hr, sterility was checked by incubation of a drop of nematode suspension in a tube containing thioglycolate broth (Difco Lab., Detroit, Mich.), and the amino acids were extracted from the glucose solution (Myers and Krusberg, 1956, Phytopathology 55: 429–437).

Amino acids were separated on 20 × 20 cm thin layer plates coated with microcrystalline cellulose powder to a thickness of 0.25 mm. The plates were developed in n-butanol:acetic acid:water (4:1:5 v/v/v) for 4–5 hr and then phenol:ammonium hydroxide:water (75:4:21 w/v/v) for 5–6 hr. Plates were sprayed with 0.3% ninhydrin in acetone and heated to 105 C for 10 minutes. Amino acids were confirmed by comparison with other thin layer plates spotted with known acid mixtures and simultaneously developed.

Amino acids discharged by *Aphelenchoides* sp. were aspartic acid, glutamic acid, serine, glycine, threonine, alanine, tyrosine, valine, leucine/isoleucine, proline, arginine, and lysine (Table 1). *Caenorhabditis briggsae, Ditylenchus dipsaci, Ditylenchus triformis, Ditylenchus myceliophagus, Pratylenchus penetrans, Meloidogyne* spp. larvae, and *Heterodera glycines* were also reported to discharge similar amino acids (Rothstein, 1963, Comp. Biochem. Physiol. 9: 51–59; Myers and Krusberg, 1965, Phytopathology 55: 429–437; Aist and Riggs, 1969, J. Nematol. 1: 254–259).

Discharged substances as suggested by Myers and Krusberg (1965, Phytopathology 44: 429–487), might represent secretory and excretory products in addition to those lost due to inef-

Table 1. R_f values of known amino acids and amino acids discharged in 1% glucose over a period of 24 hr by *Aphelenchoides* sp. which were identified by two dimensional thin layer chromatography.[1]

Spot No.	Amino acid	R_f value of unknown spot in solvent II	R_f value of known amino acid in solvent II
1	Aspartic acid	0.13	0.12
2	Glutamic acid	0.21	0.21
3	Serine	0.33	0.34
4	Glycine	0.38	0.40
5	Threonine	0.46	0.46
6	Alanine	0.55	0.53
7	Tyrosine	0.63	0.63
8	Valine	0.75	0.75
9	Lysine	0.76	0.77
10	Arginine	0.84	0.86
11	Proline	0.85	0.87
12/13	Leucine/isoleucine	0.78	0.78
a	Unknown	0.32	—
b	Unknown	0.48	—

[1] Solvent I—n-butanol:acetic acid:water (4:1:5 v/v/v). Solvent II—phenol:ammonium hydroxide:water (75:4:21 w/v/v).

ficiency of body functions. These discharged amino acids would not have been formed as a by-product of microbial metabolism because external sources of contamination were eliminated by surface sterilization as shown by sterility tests, and internal contamination in *Aphelenchoides* sp. was not possible since the lumen of the stylet is too small to allow any microorganism to enter. In addition, 90–95% of the nematodes were found active following incubation so that discharged amino acids, therefore, could not be attributed to leakage from bodies of dead nematodes. Except for aspartic acid and glutamic acid, the amino acids discharged by *Aphelenchoides* sp. were classified using the terminology of Sayre, Hansen, and Yarwood (1963, Experimental Parasitology 13: 98–107) as absolute and limited dietary requirements (Balasubramanian and Myers, in preparation). Absolute dietary requirements are not synthesized and limited dietary requirements are synthesized as less than optimal amounts by animals. Although it seems logical that, at least, absolute dietary requirements should be conserved, relatively large amounts of amino acids are discharged (Myers and Krusberg, 1965). We hypothesize that the "inefficiency of body function" is mainly the result of excretion of unused "food" from the anus.

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Research Note

Technique for Staining Spicules and Gubernaculum in Whole Mounts of Nematodes

Nematodes, in contrast to most other helminths, have defied most attempts for differential staining; however, the "granules" seen in the intestine of most nematodes were characterized by using lipid soluble dyes (Payne, 1923, Am. J. Hyg. 3: 547–583; Peters, 1927, J. Helminth. 5: 183–202; Goodey, 1930, J. Helminth. 8: 85–88). Other techniques for staining nematodes involved staining the whole worm rather than specific structures (Goodey, 1937, J. Helminth. 15: 137–144; Franklyn, 1949, J. Helminth. 23: 91–93; Franklyn and Goodey, 1949, J. Helminth. 23: 175–178).

Recent attempts by Stringfellow (1969, J. Parasit. 55: 1191–1200) to study the genital cone of some nematodes led to a useful technique for differentially staining the gubernaculum and spicules of parasitic nematodes.

Mature and immature males and females of *O. ostertagi* collected from cattle and fixed in either Bouin's, Carnoy's, neutral buffered formalin, or Zenker's fixative were used to develop the following procedures:

1. Fix worms in Bouin's fixative for one-half hour, or mordant overnight in saturated picric
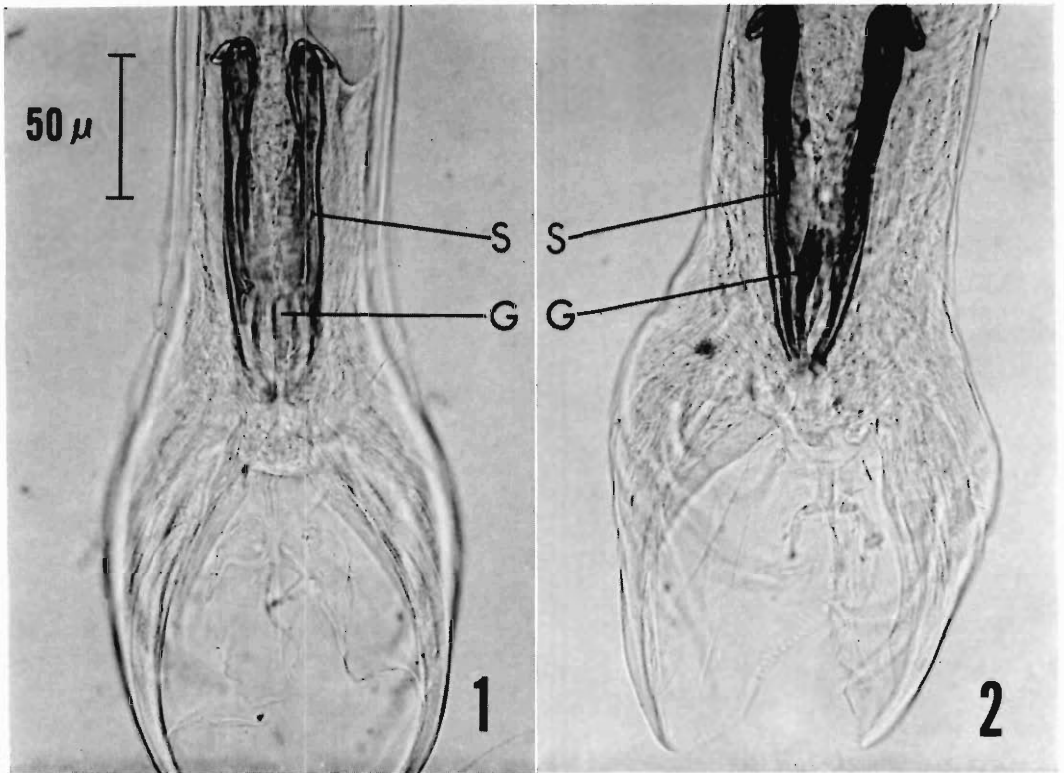
Figures 1–2. 1. Dorsal view of unstained male *Ostertagia bisonis*. 2. Dorsal view of stained male *O. bisonis*. All specimens mounted in glycerine. Abbreviations: G, gubernaculum; S, spicule.

acid-distilled water solution if fixation is unknown or was not Bouin's.

2. Rinse excess fixative from specimen with 70% ethanol.

3. Place in a saturated acid fuchsin-lactophenol solution and heat on a hot plate until vapor rises.

4. Rinse excess dye from specimen with 70% ethanol.

5. Destain in saturated picric acid-distilled water solution until no more acid fuchsin leaves the specimen.

6. Rinse worm in 70% ethanol.

7. Remove picric acid and acid fuchsin from softer tissues of the nematode with saturated sodium bicarbonate—70% ethanol solution. Spicules and gubernaculum stain magenta (reddish-purple).

8. Clear specimen in one-half glycerine-70% ethanol and then transfer to pure glycerine.

9. Mount worm in a neutral mountant such as glycerine or glycerine jelly in the usual manner.

This technique was also tried with other nematodes to evaluate its efficacy on various kinds of preserved nematodes in the National Parasite Collection: *Ostertagia ostertagi, O. bisonis, Cooperia punctata, C. oncophora, Hyostrongylus rubidus, Nematodirus spathiger, Setaria* sp., *Haemonchus contortus, Trichostrongylus axei, Teladorsagia davtiani, Marshallagia marshalli, Bunostomum phlebotomum, Oesophagostomum radiatum, Dictyocaulus viviparus, Heterakis gallinae, Strongyloides papilosus, Cylicocyclus insigne, Gongylonema pulchrum, Physocephalus sexalatus,* and *Protostrongylus rufescens*.

Acid fuchsin primarily stains the gubernaculum and spicules with the capitulum of the spicule staining more intensely than the blade

because of the marked affinity of the inner core of protoplasm for the dye. Developing spicules and gubernaculum in immature males, and occasionally hookworm teeth, sperm, and ovijectors in adult females, will stain. In general, small worms are more easily stained than large worms and there is usually an upper limit on the size of the worm that will stain best (2–3 cm). In large nematodes the spicules and gubernaculum destain at the same rate as the softer tissues; consequently, they do not usually stain well. Best results are obtained by cutting large worms in half. Nematodes (*Nematodirus*, *Bunostomum*, *Gongylonema*, *Cylicocyclus*, *Physocephalus*) with long, narrow spicules do not stain as well as those having short, broad spicules. Generally, the extruded part of the long spicule is destained more rapidly than the rest of the worm. The short spicule in males having dissimilar spicules usually stains unless the worm is large. Nematodes having compact spicules do not stain well (*Trichostrongylus*). Best results are obtained on small worms having medium, broad spicules.

It is easy to stain particular structures in sectioned nematodes; but it is very difficult to stain whole mounts because of the relatively impermeable cuticle which impedes the movement of some dyes. Since lacto-phenol penetrates the cuticle more rapidly than some other solvents, it was used in this technique and heated to enhance penetration of the dye into the body of the nematode.

In this study Bouin's is the preferred fixative; however, worms fixed in neutral buffered formalin and Zenker's fluid without mordanting will stain with some success. Worms fixed in Carnoy's fluid do not stain. As used in this technique, picric acid shows properties of a mordant in that it is not extracted by neutral fluids but extracts acid fuchsin. During regressive staining, excess dye is removed by placing the tissue either in an acid or in the mordant. In general, acid fuchsin selectively stains certain fibrous proteins best at levels below pH 2. Possibly, selectivity of acid fuchsin for particular structures depends more on this effect of lowering the pH at the tissue level than on a "mordant effect" (Lillie, 1945, J. Tech. Methods 25: 1–47). Bouin's, then, is the preferred fixative since picric acid is the primary constituent. This explains the necessity of mordanting the worms overnight when their fixation is either not known or is not Bouin's.

The technique developed in this paper is simple to use, requires a minimum of equipment, uses no exotic chemicals, and requires no weighing of chemicals. It has practical application in determining the presence or absence of the gubernaculum as well as its morphology.

Acknowledgment

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Research Note

Description of a Plesiotype Male for *Anomyctus xenurus* Allen, 1940 (Nematoda: Aphelenchoididae)

During a study on the systematics of the nematode superfamily Aphelenchoidea (Nickle, 1970, J. Nematol., in press), the author received from Professor M. W. Allen a single male specimen of *Anomyctus xenurus* Allen, 1940. This is the only male specimen of the species
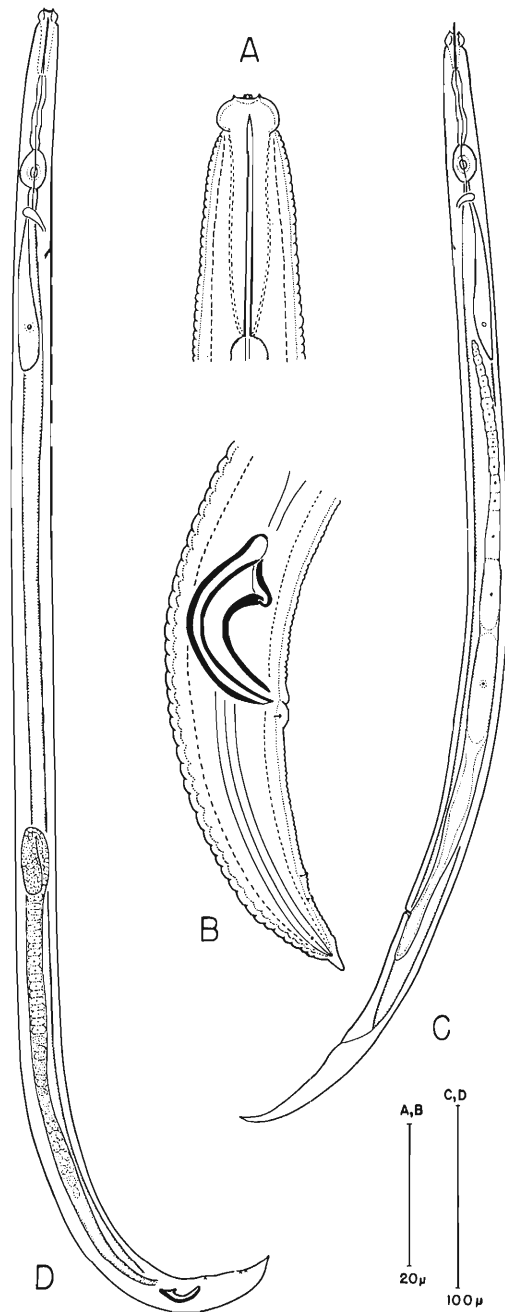
Figure 1. *Anomyctus xenurus.* **A. Anterior end of male. B. Male tail, lateral view. C. Full body of female, lateral view. D. Full body of male, lateral view.**

collected to date in the world, though female specimens have been collected in Utah and California, USA and Suffolk, England. Specimens collected at the last locality were reported (Hooper and Cooke, 1967, Nematologica 13: 320–321) to be somewhat larger, and were considered conspecific with *A. xenurus*. The monotypic family Anomyctidae was proposed (Goodey, 1960, Nematologica 5: 111–126) for this species. The male, described here as a plesiotype, supplements the original work (Allen, 1940, Proc. Helm. Soc. Wash. 7: 96–98). With the knowledge of these additional male characters, the relationship of *Anomyctus* to other described aphelenchoid genera is more accurately understood.

Description of Male *Anomyctus xenurus* Allen, 1940

MALE (1): L = 0.790 mm; W = 0.022 mm; a = 36.2; b = 6.8; c = 18.4; Stylet L = 32.6 μ; Spicule L = 23.6 μ; Spicule W = 9.8 μ.

Rare. Cuticle strongly annulated. Lips offset by a deep constriction; shallow, sclerotized, saucer-like frontal disc present. Six small projections surround oral opening. Stylet linear, in two parts, without basal knobs. Testis short, with anterior flexure. Spicules very large, paired, not fused, similar to those of *Aphelenchoides*. Tail conical, with short terminal process, similar to that of female, with 3 pairs of caudal papillae; caudal alae and gubernaculum absent. Three lines in the lateral field.

BIONOMICS: Unknown, soil inhabiting.

PLESIOTYPE MALE: Collected by Professor M. W. Allen from soil around roots of desert plants at East Yuma Mesa, California on 19 January 1945. Deposited University of California Nematode Survey Collection, Davis, California.

Though the disc-like lip region and the linear stylet are useful in the diagnosis of this genus, the shape of the spicule, the type of esophagus, and the ecological relationships indicate that a separate family is not needed for this nematode.

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Research Note

Zoogeography and the Generic Status of *Polymorphus* (*Polymorphus*) *cetaceum* (Johnston et Best, 1942) comb. n. (Acanthocephala)

Adult acanthocephalans reported from cetaceans are restricted to the genera *Bolbosoma* Porta, 1908, and *Corynosoma* Lühe, 1904. Johnston and Best (1942, Trans. Roy. Soc. S. A. 66: 250–254) described *Corynosoma cetaceum* from the dolphins, *Delphinus delphis*, (type host) and *Tursiops truncatus*, from South Australia. The specimens from *Delphinus* had previously been recorded as *Corynosoma* sp. by Johnston and Deland (1929, Trans. Roy. Soc. S. A. 53: 146–154). Other records of this genus from cetaceans are the description of *C. septentrionalis* Treschev, 1966, from a grey whale, *Eschrichtius gibbosus*, in Russia, and that of Yamaguti (1935, Jap. J. Zool. 6: 247–278) who reported *Corynosoma* sp. from *Delphinus longirostris* in Japan. Species which are normally parasites of seals may occasionally be reported from cetaceans.

Numerous *C. cetaceum* were collected from the stomachs of six of 11 La Plata dolphins, *Pontoporia blainvillei* (Gervais, 1844), examined. This is a new host record and the first record outside Australian waters. The dolphins were netted off the coast of Uruguay at Punta de Diablo, by R. L. Brownell, Jr. and R. Wess, whom we thank for the opportunity of studying these parasites. The original description of *C. cetaceum* is quite accurate. Most females are shorter than the males, as reported by Johnston and Best, but this is due to inversion of the posterior end with the formation of a genital vestibule, as is common in the Polymorphidae.

There are no traces of genital spines in any of our 55 specimens. Johnston and Best also noted this absence in their material. Since the presence of genital spines is the only criterion separating *Corynosoma* from *Polymorphus* Lühe, 1911, the species in question becomes *Polymorphus* (*Polymorphus*) *cetaceum* (Johnston et Best, 1942) comb. n. This and *Polymorphus paradoxus* Connell et Corner, 1957, are the only species in *Polymorphus* known to normally parasitize mammals.

Three slides of *P. cetaceum* have been deposited in USNM Helm. Coll. No. 71510.

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Presentation

1970 Anniversary Award of The Helminthological Society of Washington 453rd Meeting 16 October 1970

Dr. Aurel O. Foster receiving the 1970 Anniversary Award of the Helminthological Society of Washington (presented by L. S. Diamond).

Dr. Aurel Overton Foster

The 1970 Anniversary Award of the Helminthological Society of Washington honors a man known to colleagues and friends not only as a distinguished parasitologist, but also as a man of great personal charm, warmth, and an abiding sense of humor, Dr. Aurel Overton Foster.

Dr. Foster was born in Marathon, New York in 1906. After attending Wesleyan College, where he received his A.B. in 1929, and his M.A. a year later, he became a student at the Johns Hopkins University School of Hygiene. There under the expert tutelage of that giant among parasitologists, Dr. W. W. Cort, he received his Sc.D. in 1933. His thesis was a study of "The resistance of dogs and cats to infection with the dog hookworm, *Ancylostoma caninum.*"

After graduation, Dr. Foster stayed on at the School of Hygiene for one year as an instructor. He then moved to Panama where for the next five years (1934–1939) he studied the parasitic worms of equines at the Gorgas Memorial Laboratory. In 1939, he returned to Washington area as an assistant parasitologist with the old Bureau of Animal Industry, U. S. Department of Agriculture. In 1941, he was promoted to associate parasitologist and in

1943, was elevated to the rank of parasitologist. Later (1960), he became Director of what is now known as the National Animal Parasite Laboratory, Agricultural Research Service, Beltsville, Maryland; the position he currently holds.

His long, illustrious association with the Helminthological Society of Washington began in 1930, while still a graduate student at Johns Hopkins. He served as Recording Secretary (1940–1941), President (1941–1942), and as a member of the Executive Committee at Large (1945–1946). In addition, he was on the Editorial Committee for thirteen years (1952–1965) and secretary of that committee for eleven years (1954–1965). Named as a trustee of the Brayton H. Ransom Memorial Trust Fund in 1953, Dr. Foster has been its secretary-treasurer since 1954. He has served the Society in many other official and unofficial capacities. All of us fondly remember the annual picnics which he hosted at Beltsville.

Dr. Foster has also been active in other professional societies. Joining the American Society of Parasitologists in 1930, he has been an active member of many important committees. In addition, he was a member of the Council (1954–1955, 1957), Vice-President (1956), and President (1959). His presidential address at University Park, Pennsylvania on September 2, 1959, was titled "Parasitological Speculations and Patterns." At that time he suggested that affiliation of other societies and parasitological groups in other nations be extended without political deference. Little did he anticipate his role in the fruition of this prophecy—in September 1970, he was to serve as President of the Second International Congress of Parasitology. He is also a member of AAAS, American Society of Tropical Medicine and Hygiene, the Entomological Society of America, the Wildlife Disease Association, the American Microscopical Society, and the Society of Systematic Zoology.

Other professional activities include membership on the FAO/OIE Expert Panel on Tick Borne Diseases, United Nations (1958–present) and the Advisory Committee of the Livestock Conservation, Inc. (1953–1964).

Dr. Foster married Margaret Bruce Linklater in 1931 and together they reared two lovely children, Jeanne and Richard.

This biographical sketch is far from complete. Little mention has been made of Dr. Foster's numerous scientific contributions on parasites and parasitic diseases of domestic animals, livestock parasite control and chemotherapy of parasitic infections. In recognition of his many and diverse contributions to parasitology, we proudly present the Anniversary Award of the Helminthological Society of Washington to Dr. Aurel Overton Foster.

(Committee: L. S. Diamond, L. A. Jachowski, and J. R. Lichtenfels).

In Memoriam

William Robert Orchard
November 3, 1912–July 20, 1970
Member since 1955

John Walthal Little
April 3, 1928–August 3, 1970
Member since 1966

Willard Walter Becklund
July 25, 1923–October 18, 1970
Member since 1953
Society Archivist since 1967

The Second International Congress of Parasitology
An Experiment in Program Format

Most of us have frequently expressed our concern over the fact that the succession of "10 minute" papers at national and international meetings reduces discussion and often precludes any discussion. Several have been heard to say that the best parts of such gatherings are the "corridor discussions" outside of the meeting rooms. With these concepts in mind, the Congress Organizers attempted to provide an official sponsorship for the "corridor conferences" and eliminate most of the formal presentations. Accordingly, the usual ten-minute papers were replaced by a large series of colloquia (work shops).

Formal presentations were confined to the Opening Plenary Session the Closing Plenary Session, and seven Technical Review Sessions in four mornings. The afternoon sessions were used exclusively for the colloquia (work shops). In the published program 82 such colloquia were scheduled in four afternoons. When the meetings convened additional colloquia were added *de novo* or by division of listed colloquia. Several colloquia were continued for additional rescheduled sessions. Ultimately there were over 100 colloquia with as many as 30 in a single afternoon. Not all were completely successful and a few may be considered as failures. Overall the procedure was highly successful. The most conspicuous evidence of this is the fact that despite an attendance of between 1600 and 1700 scientists (from 67 countries), relatively few were found in unscheduled conferences in corridors or lounges. The many gratuitous letters that have come to us have overwhelmingly endorsed both the concept and the results of this type of meeting. Some problems, however, were manifest. These seem to fall into three categories: (1) frustration over which of the colloquia to attend, (2) small size of the room thus restricting the number accommodated, and (3) the inexperience of some of the chairmen. Of these items 1 and 2 are interlocking and appear to be inescapable in this type of meeting. It is difficult to visualize an informal discussion with several hundred or more in the room. In a few cases we moved the location of a colloquium to provide a larger room. These larger rooms accommodated as many as 175 to 200 people. While these larger group meetings were successful, participation was somewhat restricted. It was generally agreed that the smaller groups were more successful. Most of the colloquia had attendance in the range of 35 to 70 but as few as 8 carried on an active and prolonged discussion in one colloquium. Thus it is clear that in place of the 2, 3, or 4 simultaneous sessions of "10 minute" papers, there were up to 30 simultaneous colloquia. The frustration in trying to select the colloquium to attend was further complicated by the fact that one could not move from one to another for specific papers because no papers were scheduled. There does not appear to be any obvious solution to this problem with a very large congress. The problems which a few chairmen encountered in moderating colloquia should be ameliorated as both the chairmen and participants gain experience and fully grasp the concepts. The colloquium has previously proven highly successful with small specifically invited groups. The problem is one of adapting this concept to an open meeting with attendance limited only by the capacity of the room. A start has been made in this direction.

In order to provide subject matter for discussion, in lieu of formal presentations, 979 résumés of 500 words each were published in advance and made available to all registrants at the meetings. These were published as Section II of issues of the Journal of Parasitology. The post-congress volume will also be Section II of an issue of the Journal of Parasitology.

This Congress was developed as a colloquium of the World Federation of Parasitologists. It was developed by The American Society of Parasitologists and The Society of Protozoologists, co-sponsored by The Helminthological Society of Washington, The Society of Nematologists, The Wildlife Disease Association, and The World Association for the Advancement of Veterinary Parasitology and assisted by The National Academy of Sciences. It was held in Washington, D.C. USA September 6–12, 1970; headquarters hotel was the Mayflower.

The Congress pioneered in meeting format. It eliminated the "10 minute" papers and provided for free discussion. Profiting by this experience, it is hoped that subsequent meetings may make significant improvements.

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Secretary General
Zoology Department
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60TH ANNIVERSARY BANQUET
Helminthological Society of Washington

The Sixtieth Anniversary Banquet was held on Tuesday, September 8, 1970 at the Mayflower Hotel as one function of the Second International Congress of Parasitology. It was attended by 123 members and guests from United States and abroad.

The outstanding feature of the Banquet was an address, "The Philosophical Background of Modern Biology," by our guest speaker from Australia, Dr. William P. Rodgers. Many have been heard to say that it was the best after dinner address they have heard—some even added "by a comfortable margin." Doctor Rodgers did not attempt to cover any small technical segment of parasitology, or any technical segment of biology, in depth. Rather, he looked at (and helped us to look at) the foibles and the motivations that have developed in the very competitive scientific community. In a sense he lampooned academia. However, his barbs were not restricted to the academician and they were too real to be classed under the heading of lampooning. The superb technician who applies his technic without even posing a question and the diligent "hat in hand" seeker after public research support were

Dr. William P. Rodgers

displayed as all too common products of our modern community. It is, perhaps, unfortunate that Doctor Rodgers' address is not available for publication.

GILBERT F. OTTO

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