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PROCEEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

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A Study into the Effects of Parasitism on the Growth of the Yellow Perch Produced by the Larvae of *Ligula intestinalis* (Linnaeus, 1758) Gmelin 1790*

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Several years ago it was reported to the authors by the Utah Fish and Game Department, and a number of sportsmen, that specimens of yellow perch, *Perca flavescens* Mitchell, were being taken frequently from Deer Creek Reservoir, Wasatch County, Utah, infected with the plerocercoid larvae of *Ligula intestinalis* (Linnaeus 1758) Gmelin 1790. An investigation at the reservoir revealed that conditions appeared extremely favorable for a study of the effects of parasitism on the host by this species in that Deer Creek Reservoir contained a heavy perch population with an infection sufficiently high to insure an adequate number of specimens for a statistical study. Complete parasitological examination of a sample of fish showed no other parasitic species or diseases of perch to be present so that it could be assumed that growth differences noted could be assigned to the action of the *Ligula* larvae when the parasitized fish were compared with their unparasitized age counterparts.

Deer Creek Reservoir comprises a body of water approximately five miles long and varying between ½ and ¾ mile in width situated on the Provo River a few miles from Provo, Utah. Although originally planted with game fish, the yellow perch have increased almost to the point of crowding out the more desirable species and, since perch are considered as non-game fish in Utah, their removal in large numbers was encouraged.

Methods commonly employed in fisheries studies were considered to be satisfactory in the present work to establish the adequacy of the fish sample collected and to attempt to establish the approximate proportions of the age groups present in the reservoir. Such methods should also reflect the effect of the ecological conditions on the growth and distribution of the perch over the period of years covered by the survey so that these effects could be recognized and properly evaluated. A comparison of the increase in weight and length between parasitized and unparasitized fish of the same age groups was thought adequate to provide the basic information in determining the effects of parasitism on growth. To increase the accuracy of the interpretation of this information, the weight and length data of each age group was further subjected to K factor, or condition, analysis to determine the relative plumpness of individuals of the two groups. Lastly, an attempt was made to establish the year of age in which parasitism had occurred as a further means of determining the effect of parasitism on yearly growth and cumulative effects over the life span of the individual.

*This work was partially supported by funds from the Utah Department of Fish and Game.

The details of the life history of *Ligula* as occurring in the reservoir were not investigated as part of the problem. However, sampling of the plankton showed that several species of copepods suitable as intermediate hosts were present and that a number of species of fish eating birds, among them species of *Merganser,* were also present. Of all of these birds, only the California Gull, *Larus californicus,* appeared to be present in sufficient numbers to account for the high incidence of parasitism. Considering the food habits of the gull and its tendencies to eat dead fish discarded along the shore by fishermen who find them infected, this bird could constitute the logical choice as the definitive host.

Previous studies of the effects of parasitism on the growth and well being of a host have been conducted by Cross (1935), who showed that a reduction occurred in the weight and length of perch parasitized with species of adult tapeworm when compared to normal unparasitized fish, and by George W. and Wanda S. Hunter (1938), who demonstrated that a small reduction in weight occurs in bass fingerling due to infection with larval trematodes. Hubbs (1937) noted that certain changes occurring in developmental metabolism of fish could be demonstrated as being due to parasitism.

METHODS

Three collecting stations were located in areas considered to be representative of ecological conditions present in the reservoir. Station I was located near the dam in an area of deep water, steep shores, and little aquatic vegetation, conditions which were considered as the poorest in the reservoir from the standpoint of supporting a permanent perch population. Station II was established near the geographical center of the body of water. At this point, the shores were gradually sloping and considerable aquatic vegetation was present, thus providing conditions that were favorable to all phases of the life history of the perch. Whereas few small fish could probably exist at Station I, because of predation by the larger fish, Station II possessed fish of all ages and sizes. Station III was located near the upper end of the reservoir near the point of entrance of the Provo River. Here the water was shallow and the bottom covered by a silty loam which supported abundant aquatic vegetation. Considerable numbers of small fish were constantly noted here, as well as an abundance of other aquatic life, since the dense vegetation provided proper protection. It is also probable that a great deal of the successful reproduction of the perch occurred in this area, and with the resulting bird life, was probably also the area in which most of the parasitized fish had become infected.

Collections were made at the above stations at intervals between the period of 15 July and 29 September 1954. Standard methods for collecting the fish were used, with the largest numbers being obtained with a standard fishing rod and line using earthworms as bait. Other apparatus used consisted of a variety of fish traps and graduated gill nets set at various depths and positions. It was known in advance that the samples obtained at the various stations would be subject to variation due to migration of the schools of perch which were observed by the authors to occur constantly in Deer Creek. Migrations in perch have been studied and reported by Mraz (1951) and Hasler and Bardach (1949). Because of such migratory habits, samples collected could not be considered as truly representative of the area in which collected. Most collecting methods used were considered to be somewhat selective of particular age groups, and it is improbable that any sample col-

lected in a large body of water can be regarded as totally representative of the normal composition of the population as to age groupings. However, the sample collected for this study compared favorably with those reported in previous studies on age composition of perch by Parsons (1950), Jobes (1952) and differs mainly in that the present sample contained a smaller percent of one and two year old fish than was probably present in the reservoir population.

With the exception of 710 fish which were transported alive to the Aquatic Laboratory at the University, the remainder of the 1254 fish included in the study were opened at the site, tagged and preserved in formalin for later use. At the time of capture, or examination in case of live fish in the laboratory, each specimen was weighed in grams, and the standard, fork and total lengths taken in millimeters. For age determination and cumulative effect of parasitism studies, a few scales were removed from each of the parasitized fish and 668 of the normal fish just ventral to the lateral line and in the region of the dorsal fin. Both of the opercular bones from the same specimens were also removed as a check on the accuracy of age determination made by means of scale annuli. Opercular bones were cleaned and growth increments studied with the aid of a blue-tinted microscope light. Scales were mounted on slides in a medium of 75% sodium silicate and 25% glycerin and projected on a screen. The use of scales for aging fish is a standard fisheries method while the use of thin bones for this work is much more recent (La Cren 1947; McConnell 1953). When comparing results obtained in doubtful cases, the opercular bones proved to provide more reliable data.

Age studies of the fish in the sample showed that they could be separated into six groups one to six years of age. In composition, 7.3% were placed in age group 1, 31.8% in group II, 42.1% in group III, 15.2% in group IV, 2.3% in group V and 1% in the six year age group. This distribution is somewhat similar to age studies previously made on perch by Parsons (1950) and Jobes (1952) with the exception that these workers obtained higher percentages in the 1st and 2nd year age groups. The age distribution as obtained in this study could partially reflect fluctuating population cycles with the smaller percentages obtained for lower age groups indicating the decreased population phases of the cycle. It is considered, however, that the sampling methods employed were probably more selective of the older fish. The small numbers obtained for the six year age group, with only a single parasitized fish being collected, caused these specimens to be dropped from further study since the results obtained could not be considered as valid.

RESULTS AND CONCLUSIONS

Of the 1254 perch examined, 186, or 15%, were found to possess one or more Ligula larvae. Most of the infected fish contained but a single larvae while six fish possessed two larvae each and two fish produced three larvae each. The cases of multiple infections were all found in smaller, though not generally younger, fish and were all collected at Station III near the entrance of the river into the reservoir.

The percentage of parasitism obtained was found to vary somewhat in the samples from the three stations. Those collected at Station I near the dam showed 11.3% infection; those at Station II, 14.4%, and at Station III, 16.6%. As previously stated, conditions for infection are at an optimum at Station III. It is considered likely that the majority of the parasitized fish collected at the other stations became infected in the area of Station III,

and that their presence at the other stations was due to the migration of the fish schools causing the station most removed to produce the lowest infectivity.

Observations on migration indicated that the perch move in distinct schools and that daily these schools travel considerable distances. When schools were present in the collecting area, fish could be caught rapidly and as soon as the school departed, no fish would be collected until another school appeared. Percentages of infection present in the schools sampled varied from 7.8% to 22.4%.

The constant migration of the schools of perch provides an increased opportunity for the fish to feed in areas where the infection exists in copepods and small fish. This may constitute one of the factors in explaining the increased incidence of parasitism with the increase of age. Another factor would be the variation from year to year in the ecological conditions affecting the life cycle of the parasite with greater potentialities for infection being present in certain years than others. In any case, it seems apparent that age resistance to the parasite is not marked and that the incidence of infection appears to increase with age. The infectivity rates in the fish of the age classes were 1.4% of the one year age group, 7.0% of the second year, 18.5% of the third year, 25.4% of the fourth year, and 24% of the fifth year age group.

A total of 854 fish were used in the study of the effects of parasitism on the growth of the host. All comparisons between parasitized and unparasitized fish were made according to age groups. Comparisons of the average lengths indicated that the parasitized fish were markedly smaller than their unparasitized age counterparts, and that the percent of the retardation becomes greater as the age of the host and the duration of parasitism increases. The rate of growth seems to be slowed to such an extent during the period of parasitism that the fish become stunted, and since the fish appear to remain parasitized throughout the remainder of their lives, the older fish with the longest history of infection show the greatest retardation in growth.

When comparing the parasitized fish of each age group with their unparasitized age counterparts, it was found that the one year old parasitized fish were 37.5% shorter, the second year group 47%, the third year group 49%, the fourth year group 55%, and the five year olds 50% shorter. Only in the one year old fish can the decrease in growth observed be regarded as truly accurate since these were all parasitized during the same season. The stunting effects obtained in the older groups where infection may have occurred during any years of their lives reflects a cumulative effect of varying lengths of parasitism of the fish in the sample.

A further study was undertaken in an attempt to determine the year in the life span of the older parasitized fish in which infection occurred. This was attempted by study of the annual growth increments of the fish made in each year of life as recorded in the annuli of scales and opercular bones. Scales from ten unparasitized fish of each age group that showed length and weight measurements near the average for that age were used to establish an average calculated size at each year of age. Annual growth increments were calculated using the following formula derived from Lagler (1950):

$$\text{Length of fish at end of year } x = \frac{\text{Width of annulus of year } x \times \text{Length of fish at time of capture}}{\text{Total length of scale}}$$

Measurement of growth annuli on scales and opercular bones were made in microns with a calibrated dissecting microscope. Analysis of the measure-

ments of scale and opercular bone annuli of parasitized and unparasitized fish indicated that this procedure was valid in determining the year of age in which the infection was acquired as the annual growth increments produced were much smaller in the parasitized fish in the years following infection.

The comparison of the annual growth increments as presented by the annuli of the scales and opercular bones also was considered excellent for revealing changing ecological conditions as present during the years covered by the ages of the fish represented in the study. When such studies were made on unparasitized fish, it was noted, for instance, that the growth increments of the first and second years of life of the four year age group when compared to similar measurements of age groups II and III showed that a greater amount of growth had occurred during the first and second years in the life of age group IV. This appears to indicate that growth conditions have varied in the reservoir, with some years being more favorable to perch than others. Many of the parasitized fish of age group IV show that considerably less growth had taken place during the first two years of life when compared to the unparasitized members of the same age group, indicating that these specimens probably had been infected as fingerlings and that little growth occurs following infection. The study also indicates the infection is maintained throughout life of the fish. By an examination of Table I, it will be seen that the average length of the parasitized fish of four years of age is very near the length of the unparasitized two year old fish although ecologic conditions as indicated by scale annuli were less favorable for growth during the last two years. A study of the younger age groups via the annual growth increment method seems to point to the fact that fewer fish apparently were being parasitized during the two years immediately prior to commencement of the study than when the bulk of the parasitized IV year age group were infected. A reason for this apparent reduction in parasitism in a rising perch population may be found in the use of chemicals to control the algae which has become a problem during the last few years and which may have been responsible for either destroying numbers of coracidial larvae or the crustacean intermediate hosts, thus reducing chances for infection.

The remaining members of the parasitized four year old fish could be divided into groups indicating infection during later years of life. Four year old fish parasitized during the third year of life show a total standard length

Table I. Average standard lengths in millimeters for each preceding year calculated from data taken at the time of collection (see text for formula). Values in italics are average length of the fish at the time of capture.

Age Group	Age Groups During Growth Preceding Collection				
	1	2	3	4	5
		A. Non Parasitized			
I	*118*				
II	88	*121*			
III	98	123	*144*		
IV	108	126	144	*155*	
V	116	130	144	158	*168*
		B. Parasitized			
I	*115*				
II	85	*119*			
III	75	105	*123*		
IV	62	90	107	*123*	
V	62	92	107	123	*134*

very near that of three year old unparasitized fish rather than of their own age, again demonstrating that growth in length is greatly reduced following establishment of the larvae.

A comparison of the weights of the parasitized and unparasitized fish of the same age group shows a somewhat parallel condition to exist with that found when comparing the lengths. The reduction in weight appears to be less pronounced than the reduction of length in the younger fish and about as great in the older specimens when comparing similar histories of parasitism. When weights of all of the infected fish of each age class from I to V were compared to their uninfected counterparts, the one year old infected fish were 9% lighter, two year 28.3% lighter, three year 25.5% lighter, four year 47.8% lighter and five year old fish 45% lighter in weight.

The ratio of length to weight, or relative plumpness, of a fish may be represented by a coefficient of condition, K, as published by Parsons (1950) where $K = \frac{W10^5}{L^3}$. In this formula, W represents weight in grams, and L, the standard length in millimeters. The K factor was computed for both parasitized and unparasitized fish as previously arranged into age groups. Under stable and controlled ecological conditions, coefficient values obtained in applying the above formula increase with each age group, signifying that the weight per unit of length increases as the fish grows older. Under conditions as present in any natural body of water subject to varying ecological conditions from year to year, coefficient values obtained would reflect adverse and optimum conditions with resulting variations from the expected normal increase. Abundance of food, which fluctuates from year to year, plus the increasing pressure of a constantly greater population of perch, have produced considerable variations from the expected normal condition in the unparasitized fish (see Fig. 1.).

When the parasitized fish of the same age groups are evaluated by the K factor formula, considerable variations are noted. A rather rapid increase in coefficient value is noted between one, two and three year olds which indicates that these fish show an increase in weight per unit of length for this period above their unparasitized age counterparts, and reflects a greater gain in weight than gain in length following parasitism. A reverse trend was noted in four and five year old fish where the weight per unit of length apparently decreases in comparison with the unparasitized controls. The results of the analysis show that the parasitized fish remain generally proportional to their age group counterparts, but notably smaller in size. Further analysis by breaking down the parasitized fish of each age group into groups based on the year of age in which parasitism occurred is contemplated and will give more detailed information on this aspect of parasitism.

The study indicates that the fish remain parasitized throughout life and appear to possess little abiltiy to encyst and destroy the parasitic larvae. Only two cases of successful encystment were noted among the parasitized fish. The larvae obtained from the 186 infected fish varied in size from 27 to 228 mm.

SUMMARY

A study was made into the effects of parasitism on the normal growth of yellow perch by the plerocercoid larvae of *Ligula intestinalis*. In all, 1254 fish, ranging in ages from one to six years, were obtained from Deer Creek Reservoir, Wasatch County, Utah. This group produced 186 fish, or 15%,

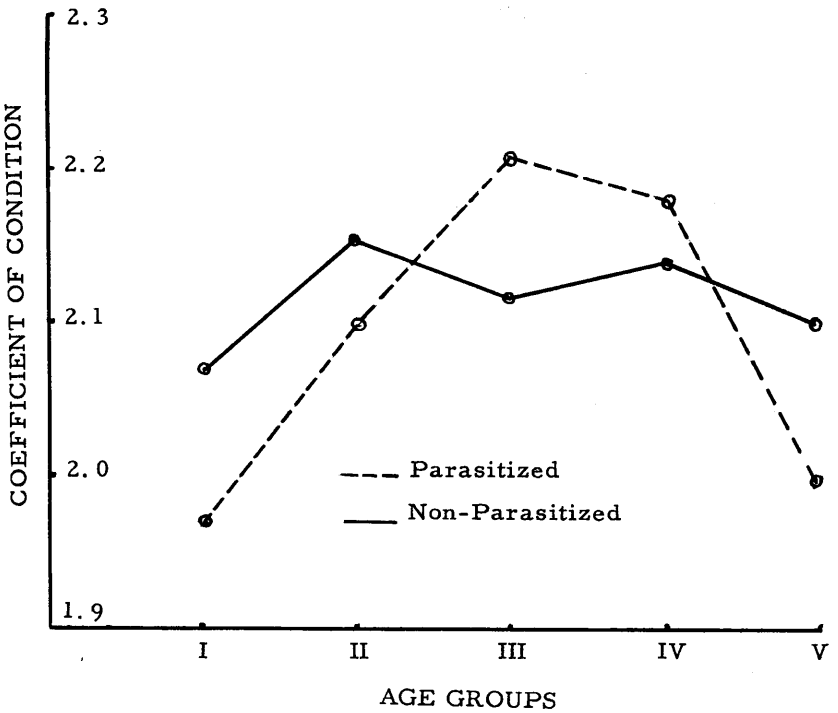

Fig. I. K-factor analysis of parasitized and non-parasitized fish belonging to five age groups.

that were infected with one or more larvae. The effects of parasitism on growth were studied through the use of methods generally employed in fisheries work.

The incidence of infection appears to increase with age as 1.4% of the one year old fish, 7.0% of the two year, 18.5% of the three year, 25.4% of the four year, and 24.0% of the five year age group were found infected. There appears to be little age resistance.

Comparison of parasitized and unparasitized fish of the same age groups indicates a marked stunting effect on growth following infection. One year old infected fish were 37.5%, two year olds 47.0%, three year olds 49.0%, four year olds 55.0% and five year old fish were 50% shorter than uninfected fish of the same age groups. Not all infected fish of the older age groups were infected during the same season, a factor which must be considered in interpreting these percent ages. Failure of infected fish to gain weight following parasitism follows the same general pattern so that the infected fish remain generally proportional but smaller than their uninfected age counterparts.

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A New Genus, *Hemicriconemoides* (Criconematidae: Tylenchina)

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Recently we have encountered nematodes in several collections which fit neither the present concepts of *Criconemoides* Taylor, 1936 nor that of *Hemicycliophora* de Man, 1921. As pointed out by Raski (1952), Loos (1949) described three species which he placed in the genus *Criconemoides* that did not appear to fit that genus. In addition to these Kirjanova (1948) described one species *Criconema tulaganovi* which might conceivably be placed in the same genus as those described by Loos and our specimens.

The genus *Hemicycliophora* is presently conceived as a genus in which the females have a sheath with more than 200 annules; the cuticle is rather simply marked; the males lack a sheath and a stylet, have very hooked spicules and prominent caudal alae. *Criconemoides* on the contrary, is conceived as without a sheath in the female, less than 200 (usually 60-120 annules) and while the males lack a stylet, the spicules are nearly straight and lateral alae project only slightly as caudal alae. Our forms are distinctly intermediate since the female has a sheath and the number of annules is from about 70 to 200 in the female; the males lack a stylet, also lack any distinct indication of caudal alae since the lateral alae extend the length of the body and the spicules are arcuate or nearly straight but not hooked.

Hemicriconemoides, n. gen.

DIAGNOSIS: Criconematinae: Mature females with sheath; with or without lateral grooves; males without sheath; annules of sheath rather plain, annules of inner cuticle often apparently slightly more numerous but difficult to count; male without distinct functional stylet; lateral and sublateral ridges throughout length of male and at times giving appearance of slight caudal alae, but never well developed as in *Hemicycliophora*. Spicules of male elongate, arcuate to straight, but not as greatly curved as in *Hemicycliophora;* female in general with fewer annules than *Hemicycliophora*.

TYPE SPECIES: *Hemicriconemoides wessoni*, n. sp.

Hemicriconemoides wessoni, n. sp. (Fig. 1 A-F)

DESCRIPTION: *Females* 382.5-499.8 microns long (Av. 10, 429.2 microns) by 30.6-40.8 (Av. 34.0 microns) wide, a, 12.7 (10.7-15.0, 10 spec.); b, 4.6 (4.0-5.5, 10 spec.); c, 19.3 (13.0-28.0, 10 spec.); V, 92%, (89-93.5%, 10 spec.), with about 76-83 annules on sheath; annules somewhat flattened but not retrorse, about 4-5 microns wide; annules on inner body cuticle somewhat smaller and not as clearly defined; head not clearly set off but cephalic region probably to be regarded as 2 annules; sheath not necessarily enclosing tail tip, latter rather narrow conoid, sheath tail tip broadly conoid; body annules not as clearly set off as sheath annules, without special marking. Stylet about 54 microns long (8 spec. 50.0-60.0 microns); knobs reflexed anteriad 8.5-11.3 by 4-5 microns according to position; dorsal gland orifice about 9 microns posterior to base of stylet; excretory pore not observed; intestine extending a little anterior to base of esophagus on dorsal side (Fig. 1 C); anus at about 5th annule from posterior end of body; vulva at about 9th annule from posterior end of body.

EGG: not observed.

MALE (length unknown, specimen lost): diameter 14-16 microns; lateral ridges (3) extending length of body with central ridge disappearing posterior to anus; tail 34 microns long; spicules finely arcuate, 25 microns long; gubernaculum small; no special projection of lateral alae in cloacal region. Phasmids near tail tip.

TYPE HOST: *Myrica cerifera* L., (roots)

TYPE LOCALITY: Alturas, Florida.

OTHER SPECIMENS AND LOCALITIES: Near Kissimmee River, Highlands Co., Fla., 1 mi. N. of Brownsville, Florida.

HOLOTYPE SPECIMEN: Florida State Plant Board, Nematology Collection Type slide No. 14, G 51; paratypes slides D-71-75, G 51.

Florida State Plant Board Nematology Collection G-205 and G-206.

Specimens were collected by H. Wesson, June 21, June 22 and July 2, 1956.

Hemicriconemoides biformis, n. sp. (Fig. 1 G-I)

Two forms of females of this type of nematode were collected; unfortunately, we have only one specimen of each form and due to their striking similarity in all characters except the female tail we do not feel justified in making separate species. Perhaps, through propagation studies later workers will prove they represent separate species or perhaps they will be found to be polymorphic forms of a single species. We are designating form A, with a conoid tail as type, form B as the supposed variant.

DESCRIPTION FORM A (Figs. 1 G-H): Female 1.05 mm long; sheath 67 microns wide, body 50 microns wide (some pressure); a, 15.6; b, 5.6; c, 13.4; V, 88%; head region not distinctly set off; stylet 110 microns long (tip 93 microns, base 17 microns) knobs 10 by 5 microns, lean back; dorsal gland orifice 7 microns posterior to base of stylet; sheath annules about 5 microns wide, somewhat flattened in center; body annules about same width but less obvious; cuticle with slightly faulted to broken annules occasional in lateral cervical region, with distinct groove-like interruption in post-vulvar region. Excretory pore about 190 microns from anterior end. Ovary extending 330 microns anterior from vulva. Anus residue on sheath and body proper both very faint.

TYPE HOST: *Quercus virginiana* Mill. (roots)

TYPE LOCALITY: South of Ocala, Florida.

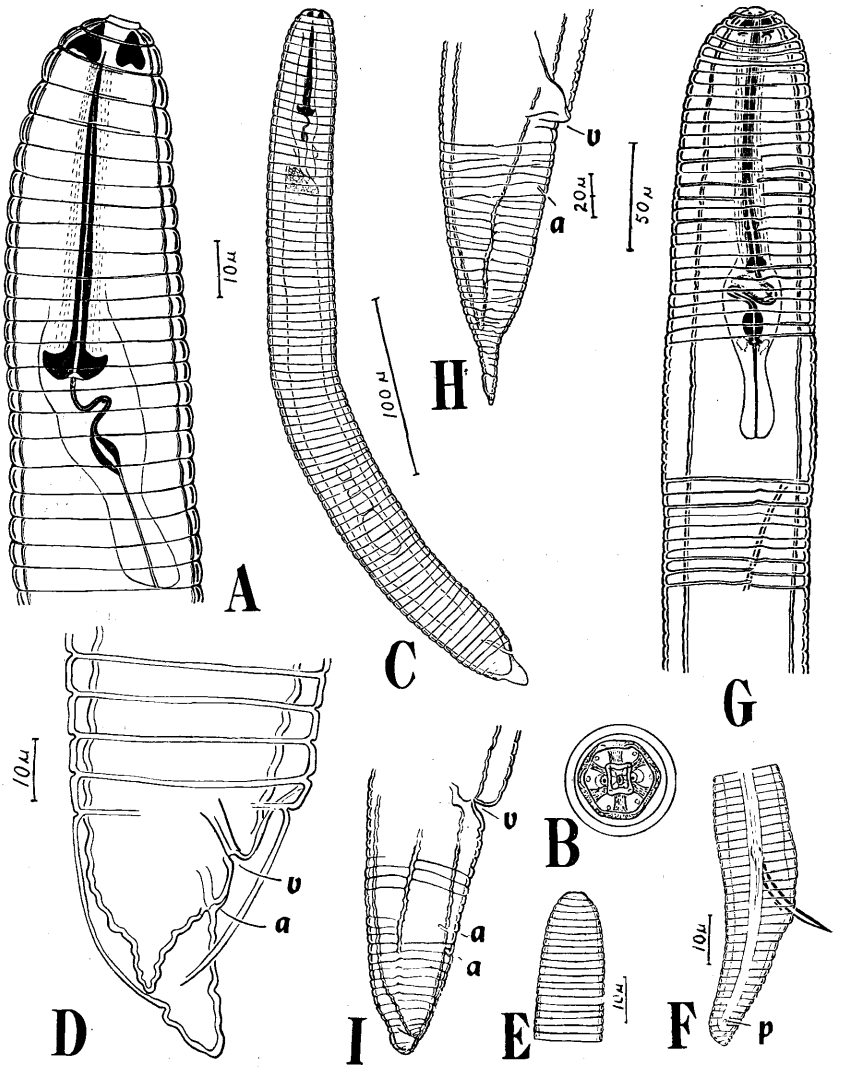

Fig. 1 A-F., *Hemicriconemoides wessoni*. *A*.—Female, esophageal region; *B*.—Diagram of female head, *en face*, note quadrangular circum-oral elevation with 4 high points, lateral amphids, 6 shaded pieces indicating internal cephalic sclerotization, one rounded hexagon cephalic annule, 6 dotted circles probably indicating nerve tracts (surface papillae of these not seen), and 6 unshaded areas indicating primitive lips, partially covered by cephalic annule; *C*.—Female, full length; *D*.—Female tail (*a*, anus; *v*, vulva); *E*.—Head of male; *F*.—Tail of male (*p*, phasmid). *G-I*.—*Hemicriconemoides biformis*. *G*.—Female, oblique dorsal view of esophageal region; *H*.—Female tail of Form A, lateral view (*a*, anus; *v*, vulva); *I*.—Female tail of Form B (*a*, anus; *v*, vulva).

HOLOTYPE SPECIMEN: Florida State Plant Board, Nematology Collection G 170 A Type slide No. 1.

DESCRIPTION FORM B (Fig. 1 I): Female 1.00 mm long; sheath 70 microns wide, body 57 microns wide (some pressure); a, 14.3; b, 5.0; c, 18.8; V, 88.5%; head, stylet and cuticle as in Form A but posterior end of sheath and internal body bluntly rounded.

SPECIMEN: Florida State Plant Board Nematology Collection G 170 B.

HOST: *Quercus virginiana* Mill. (roots).

LOCALITY: South of Ocala, Florida.

Hemicriconemoides flordidensis, n. sp. (Fig. 2 A-D)

SYNONYM: *Procriconema* sp. Steiner, 1949. Soil Sc. Soc. Fla. 4B: Fig. 24, p. 105.

DESCRIPTION: Females 1.09-1.21 mm long by 49.5-75.0 microns wide (sheath) or 0.97-1.03 mm long by 39.1-55.0 microns wide (body); a, Av. 18 (sheath) or 22 (body); b, 5.5 (sheath) or 5 (body); c, Av. 16 (sheath) or 16 (body); V, 83-90%, Av. 86% (sheath) or 83-90%, Av. 88% (body); stylet about 115 microns long (tip 87-97 microns long, base 20-25 microns

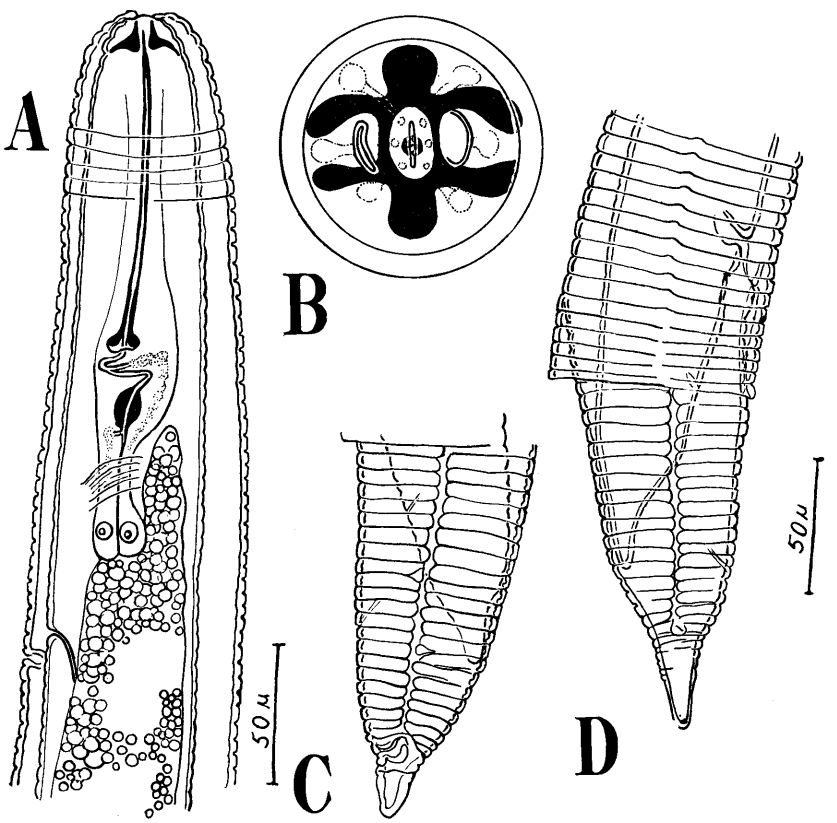

Fig. 2, A-D., *Hemicriconemoides floridensis. A.*—Female, esophageal region; *B.*—Diagram of female head, *en face; C-D.*—Female tails.

long); knobs 10 x 5 microns; dorsal gland orifice 8 microns posterior to base of stylet; excretory pore about 213 microns from anterior end; sheath annules about 5-6 microns wide, rather flattened, inner body annules more rounded about 5 microns wide; tail rather short and conoid. Lateral groove of cuticle pronounced in post-vulvar region ending about 29 microns from terminus, tail narrowed at this level with various irregular annules; body not attenuated from vulva but sharply diminishing posterior to anus; intestine extending anterior to base of esophagus on dorsal side, to posterior edge of median bulb; ovary extended or reflexed, at about 620 microns from anterior end; post-vulvar sheath fold about 25 microns long, sheath with a single pair of lateral grooves extending from esophageal region to tail tip, most pronounced in post-vulvar region with interrupted, broken annulation in esophageal region; cephalic region (to base of internal sclerotization) composed of three, perhaps 4 annules; oral opening a dorso-ventral slit surrounded by an elongate, oval circum-oral elevation that may or may not bear rudiments of six lips and papillary groups; amphidial openings apparently large, lateral, dorso-ventral slits, about 6.25 microns long; internal cephalic sclerotization in form of 6 pieces, 2 median, and 4 sublateral, united internally near stylet.

MALE: unknown.

TYPE HOST: *Pinus elliotti* Engelmann.

TYPE LOCALITY: 26 miles N. of Lake City, Florida. Collected by B. G. Chitwood 10/14/56.

TYPE SPECIMENS: Holotype specimen Florida State Plant Board Nematology Collection G 201 Type slide No. 15.

OTHER HOSTS, localities, and specimens: *Ilex glabra* (Linn.) Gray. Okefenokee Swamp Road. Collected by B. G. Chitwood 10/14/56. Florida State Plant Board Nematology Collection G 223.

KEY TO SPECIES OF *Hemicriconemoides*

1. Tail of female bluntly rounded ..... *H. brachyurus* (Loos, 1949), n. comb.
   SYN: *Criconemoides brachyurus* Loos, 1949.
   FEMALE: 412-477 microns long by 31-35 microns wide; external cuticle with about 110 plain annules, latter 4-5 microns wide; head region not set off, probably 2 annules; a, 13-14; b, 4.2-5.0; c, ?; V, 94-95%; stylet 53-56 microns long, knobs reflexed anteriad; excretory pore 1-2 annules anterior to base of esophagus; rectum and anus not observed; tail of sheath and body bluntly rounded; anus not observed; vulva 8-9 annules from tail terminus; egg not observed.
   MALE: 328-389 microns long; a, 20.4-21.6; b, ?; c, 14-16; spicules curved. 28 microns long; excretory pore 78-93 microns from anterior end; with 3 pairs of longitudinal ridges from head to tail, not appearing as caudal alae.
   TYPE HABITAT: Tea nursery soil.
   TYPE LOCALITY: Koslande, Ceylon.

   Tail of female conoid, conically rounded to rather bluntly attenuated 2
2. Sheath annules about 200 in number 3
   Sheath annules about 75-120 in number 4
3. Stylet base 17 microns long; tail tip not distinctly set off ..... *H. biformis*
   FEMALE: 1.00-1.05 mm long; sheath cuticle with laterally slightly faulted to broken annules in cervical region, with lateral grooves in post-vulvar region; head not distinctly set off; stylet 110 microns long, tip 93 microns, base 17 microns, knobs lean back, 10 by 5 microns; dorsal

gland orifice about 7 microns posterior to base of stylet; excretory pore about 190 microns from anterior end. Anal residue on sheath and body very faint.

FORM A (type): 1.05 mm long; a, 15.6; b, 5.6; c, 13.4; V, 88%. Tail tip on sheath and body proper conically attenuated.

FORM B: 1.00 mm long; a, 14.3; b, 5.0; c, 18.8; V, 88.5%. Tail tip on both sheath and body proper conoid bluntly rounded.

TYPE HOST: *Quercus virginiana.*
TYPE LOCALITY: South of Ocala, Florida.

Stylet base 20-25 microns long; tail tip distinctly set off ..... *H. floridensis.*
FEMALE: 1.09-1.21 mm. long; sheath ratios: a, 18; b, 5.5; c, 16; V, 83-90%; sheath annules somewhat flattened but without special marking; stylet about 115 microns long; tip, 87-97 microns long; base 20-25 microns long; knobs lean back, 10 by 5 microns; dorsal gland orifice 8 microns posterior to base of stylet; excretory pore about 213 microns from anterior end; tail tip short, bluntly rounded, set off; annules posterior to end of lateral groove somewhat disarranged.

TYPE HOST: *Pinus elliotti* Engelmann.
TYPE LOCALITY: 26 mi. N. of Lake City, Florida.

4. Sheath annules of female 75-83 ..... *H. wessoni,* n. sp.
FEMALE: 380-500 microns long; annules on sheath somewhat flattened but without other specialized marking, 4-5 microns wide; a, 10.7-15.0; b, 4.0-5.5; c, 13.0-28.0; V, 89-93%; stylet 50-60 microns long, knobs reflexed anteriad, 8.5-11.3 by 4-5 microns; dorsal gland orifice about 9 microns posterior to stylet base; anus at about 5th annule, vulva at about 9th annule from posterior end.

MALE: with lateral ridges (3) extending length of body but not projecting as caudal alae; tail 34 microns long; spicules arcuate, 25 microns long.
TYPE HOST: *Myrica cerifera.*
TYPE LOCALITY: Alturas, Florida.

Sheath annules of female about 100-120 5

5. Female with sheath of about 100 annules ..... *H. cocophillus* (Loos, 1949), n. comb.
SYN: *Criconemoides cocophillus* Loos, 1949.

FEMALE: 406-490 microns long by 32-34 microns wide; no lateral fields or other cuticular ornamentation; annules not retrorse; a, 12.6-14.7; b, 4.4-4.9; V, 91-92.5%; first annule quite narrow, disc like with 6 elevated lip lobes; excretory pore not observed; stylet 48-56 microns long, knobs reflexed anteriad; anus not observed; vulva between 10 and 11 annules from tail tip; egg (1) 69 by 18 microns.
MALE: unknown.
TYPE HABITAT: About grass and coconut roots.
TYPE LOCALITY: Wehera Estate, Ceylon.

Female with about 120 annules ..... *H. gaddi* (Loos, 1949), n. comb.
SYN: *Criconemoides gaddi* Loos, 1949.
FEMALE: 431-504 microns long; a, 15.8-18; b, 3.7-4.4; c, 19.9-21.7; V, 91.2-92.3%; head region not set off; annules not retrorse nor otherwise modified; stylet 72-80 microns long, knobs reflexed anteriad; excretory pore 2 annules posterior to base of esophagus; anus 7 or 8 annules from terminus; vulva about 13 annules from terminus; male 284-309 microns

long; a, 13.5-16.2; c, 8.5-10.4; two lateral lines about 2-3 microns apart extending from head to tail; spicules arcuate, 29 microns long.

TYPE HABITAT: Tea nursery soil.

TYPE LOCALITY: Koslande, Ceylon.

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ADDENDUM: Since this article was written we have had the opportunity to study many additional forms in the *Criconemoides-Hemicycliophora* complex. This publication we hope will cause other workers to study material more carefully and to do some propagation studies in the group, we hope it will serve a useful purpose. We are opposed to the present concept of setting apart *Hemicycliophora* as having 200 or more annules. The presence of a sheath in the adult female might be used as the character, but then one would have such forms as *H. brachyurus, H. cocophillus* and *H. gaddi* as problematical. The presence of a dorsal intestinal extension anterior to the base of the esophagus in the female may be a good generic character for *Hemicriconemoides*. The presence of a sheath on the male, slightlly curved spicules, and minor inflation of lateral alae, in the caudal region has been observed in a form close to *H. wessoni*. Flattened sheath annules in the female, broken annules and a lateral groove and annular modification in the caudal region of females may all eventually be found to be characters of importance. Some years ago we discovered a male with a sheath much like *Criconemoides* but otherwise, internally on the order of *Hemicycliophora* except for curved spicules. Indeed life history and propagation work is indicated.

Larval Trematodes (Gorgoderidae) from Central Texas*

T. T. DUNAGAN**

Diesing (1850), Wagener (1857), Pagenstecher (1857), Looss (1894), and others published numerous articles on the early cercariae within the Gorgoderidae. There was much confusion in the early literature. Luehe in 1909 did much to clear up this confusion by listing the described forms briefly, but his conclusions have not been accepted universally; thus synonyms still appear in the current literature. The described cercariae of Gorgoderidae form a small group, but the investigator may find as many as six or seven different names and dates for the same species. Goodchild (1943) and Fischthal (1951) gave reviews of the species described up to the time of their publications. Coil (1954) summed up previous work and presents a noteworthy review of the group. Fischthal and Coil also included diagrams of the possible evolution of this group.

Macrocercariae were found on two occasions in single specimens of *Musculium transversum* collected from two permanent ponds in Brazos County, Texas, on June 18 and again on July 20, 1954.

Cercaria rabbi, n. sp.

The body is fusiform (Fig. 1), measuring 0.95 mm. long by 0.23 mm. wide (average of six heat killed specimens). The cuticula is 3 microns thick and may have sensory papillae although none were recorded from the two infected clams. Emergence occurred throughout the morning with little differentiation in intensity (during the period) from one hour to the next. Soon after leaving the tissues of the molluscan host the cercariae encyst within the expanded proximal chamber. Usually when they encyst the body doubles over itself ventrally. Krull (1935) states that *C. amplicara* is "relatively inactive in its capsule in the tail" and "in no specimen has the cercaria body been found attached to the tail, although a tissue structure in the bottom of the capsule indicates that it is attached at some time." *Cercaria rabbi* in contrast to this was always observed attached to posterior part of the proximal conical chamber upon emergence; however, under cover slip pressure it readily breaks loose from this attachment and is seen wriggling free in the water. Body activity while attached is considerable, being greatest in a longitudinal plane but with restricted twisting movements also occurring.

The tail (Fig. 2) is easily divided into three regions: (1) anterior conical chamber measuring 1.07 mm. long and 0.808 mm. wide with the proximal lips thicker than the clear, transparent, pliable walls; (2) this chamber is attached to a cone-shaped base, proximally as wide as the base of the anterior chamber and distally tapering to 0.28 mm.; (3) the tail has the same diameter throughout its remaining length, and ends bluntly 5.51 mm. from the base of the anterior chamber. Except for a small area of granules immediately posterior to the attachment of the cercaria, the tail is exceptionally clear of all pigment, refractive spheres, and granules. The tail is non-motile and possesses no muscle fibers. Including the anterior chamber, the tail measures 6.58 mm. long, making it one of the longest known in this group.

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**The writer wishes to thank Dr. Sewell H. Hopkins, Professor of Zoology, Texas A & M College, for directing the work; H. B. Herrington, Keene, Ontario, Canada, who identified the Sphaeriidae; Dr. E. L. Rabb, Baylor University, for suggesting the name *Cercaria rabbi*, and Dr. W. H. Coil for checking the manuscript.

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The acetabulum is posterior to the middle of the body and slightly larger than the oral sucker, being 0.28 mm. long and 0.31 mm. wide, while the oral sucker is 0.247 mm. long and 0.214 mm. wide. Both structures are setate. No pharynx is present. The esophagus extends posteriad from the oral sucker and bifurcates in the middle of the anterior half of the body; the intestinal caeca end laterally and slightly posterior to the middle of the excretory bladder.

The stylet (Fig. 3), 0.035 mm. long and 0.012 mm. wide at the base, is located antero-dorsad to the opening to the oral cavity. As seen in a dorsal view, the anterior part of the stylet bears a pair of sharp lateral protuberances; these converge anteriorly with the lateral sides of the stylet to form a single stylet point. Lateral to the protuberances the ducts of the penetration glands open; the complete number and arrangement of the glands and ducts was obscured by heavy cystogenous material. Six glands were recorded anterio-laterial to the acetabulum; however, there was no sharp delineation with relation to the glands and the intestinal crura as was observed by Baker (1943) and Steelman (1938).

The excretory bladder is spatulate, often contracting to a long expulsion canal. It extends anteriad near the dorsal surface, from the poterior excretory pore dorsal to just below the posterior edge of the acetabulum. where it branches to form two main collecting ducts. Several large hyaline cystogenous glands surround the excretary vesicle, but are not very wide on the end nearest the acetabulum. The excretory system was difficult to trace because of the numerous refractive granules located throughout the body and the sparsity of specimens available. A few flame cells were seen, but neither their capillaries nor the larger tubes were observed.

The non-motile sporocysts completely filled the gills and viscera (excluding the foot) of the host clam. Concentrations occurred in the digestive gland, testes, and ovary; but scattered sporocysts were found from the esophagus to the kidney excluding the more muscular areas and the area immediately around the heart. The average size of three sporocysts was 6.12 mm. long and 1.11 mm. wide. Each sporocyst contained four or five recognizable cercariae and as many germ balls.

The following key is given in order to provide a check list of described cercariae in the Macrocercous group and to compare *Cercaria rabbi* with these existing forms.

Key to the Macrocercariae

1 (2, 32) Stylet present 3
2 (1, 32) Stylet absent ..... Rhopalocercariae and Microcercariae
3 (10, 25) Stylet 20 microns or less in length 4
4 (7) Bell-shaped cercarial chamber absent: found in unionids 5
5 (6) Thirty-six sensory papillae on oral sucker; from dorsal view the antero-lateral projections of the stylet are apically notched ..... *C. lampsilae* Coil 1954
6 (5) Twenty-six sensory papillae present on oral sucker; no notch in antero-lateral projections of stylet ..... *C. eriensis* Coil 1952
7 (4) Bell-shaped cercarial chamber present, not found in Unionids 8
8 (9) Tail divided into three distinct divisions *C. coelocerca* Steelman 1939
9 (8) Tail not divded into only two distinct divisions ..... *C. donecerca* Goodchild, 1939
10 (3, 25) Stylet more than 20 microns and less than 30 microns in length 11

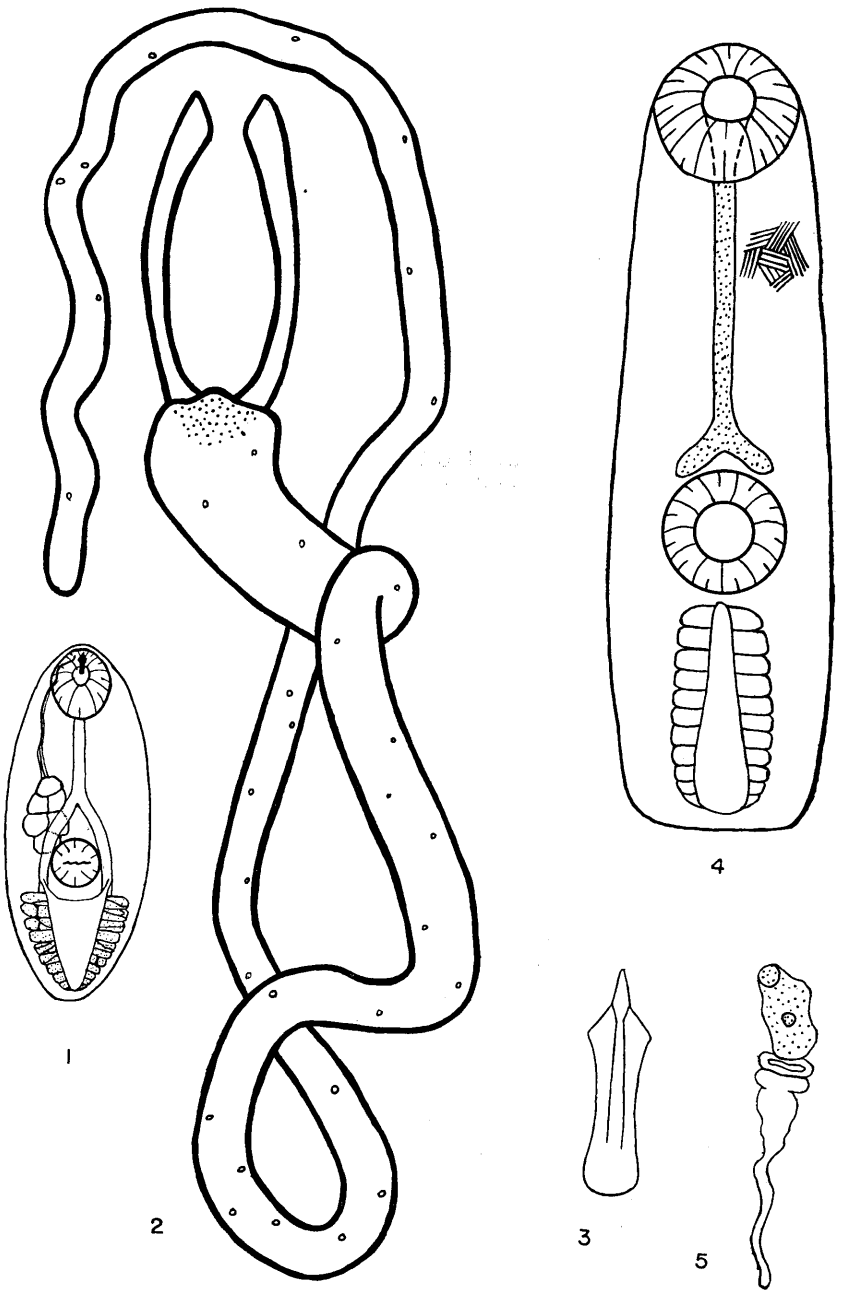

PLATE I

Figures drawn with the aid of a camera lucida.

Fig. 1. Ventral view of body of *C. rabbi* showing excretory bladder expanded.
Fig. 2. Tail of *C. rabbi.*
Fig. 3. Ventral view of stylet of *C. rabbi.*
Fig. 4. Ventral view of body of *C. ruddi.*
Fig. 5. Whole mount of *C. ruddi.*

11 (12) Stylet tapers to a point; wings or antero-lateral projections absent ..... *C. sphaerocerca* Miller 1936
12 (11) Stylet has antero-lateral projections 13
13 (16) Penetration glands are located anterior to the acetabulum 14
14 (15) Four penetration glands are located mesiad to the intestinal caeca ..... *C. raiacauda* Steelman 1938
15 (14) Two penetration glands located mesiad to the intestinal caeca ..... *C. steelmani* Baker 1943
16 (13) Penetration glands located lateral as well as anterior to acetabulum 17
17 (20) Total length of cercaria greater than 5.5 mm; stylet with 3 apical points; ten papilliae present on oral sucker 18
18 (19) Twelve penetration glands present; stylet 21 microns long ..... *C. vitelliloba* Sinitsin 1905
19 (18) Nine penetration glands present; stylet 28 microns long ..... *C.* of *Gorgodera varsoviensis* Sinitsin 1905
20 (17) Total length of cercaria less than 3 mm. 21
21 (24) Sensory papillae on oral sucker less than twenty-one 22
22 (23) Total length 2.5 mm., stylet possesses two apical points plus a dorsal fin, six penetration glands present ..... *C.* of *Gorgodera pagenstecheri;* Sinitsin 1905
23 (22) Total length 1.00 mm., penetration glands 7 in number ..... *C. wabashensis* Coil 1955
24 (21) Sensory papillae on oral sucker greater than twenty-three; stylet possesses a single apical point plus a dorsal apical fin ..... *C.* of *Phyllodistomum solidum* Goodchild 1939
25 (3, 10) Stylet greater than thirty microns long 26
26 (31) Tail has an anterior conical chamber 27
27 (30) Tail contains granules, nuclei and/or striae 28
28 (29) Total length greater than 8.5 mm.; sensory papillae on oral sucker bear sensory hairs ..... *C.* of *Gorgodera attenuata* Rankin 1939
29 (28) Total length less than 7.6 mm.; most sensory papillae on the oral sucker bear sensory hairs ..... *C. amplicava* Krull 1935
30 (27) Tail remarkably clear, devoid of any granular material or striae; sparsely scattered nuclei may be present ..... *C. rabbi*
31 (26) Anterior conical chamber absent from tail ..... *C.* of *Phyllodistomum loossii* Sinitsin 1905
32 (1, 2) Body similar to the *Macrocercariae* but lacking stylet ..... *C. ruddi*

Cercaria ruddi, n. sp.

Cercaria ruddi sp. nov. was found in one specimen of *Musculium transversum,* which was collected from a permanent pond on August 13, 1954. The infection was light and no more than twenty cercariae were dissected from the host. The reader should keep this in mind should he chance to compare this species with future members of the group. Because this form (*C. ruddi*) overlaps the characteristics possessed by macrocercariae and rhopalocercariae, its phylogeny among the gongoderid cercariae should be similar to that postulated by Miller (1936) for *C. mitocerca.*

The body (Fig. 4) is elongated, bluntly tapered at both ends; 0.27 mm. long and 0.052 mm. wide. Its attachment to the tail is more permanent than in the previously described cercariae of this group and is seldom seen detached.

The tail (Fig. 5) is modified out of proportion in heat-killed specimens. The anterior half of the tail is cone-shaped; three constrictions in the cuticula covering form grooves, the anterior constriction forming the deepest groove. Immediately below this non-motile portion, the tail contains well-developed

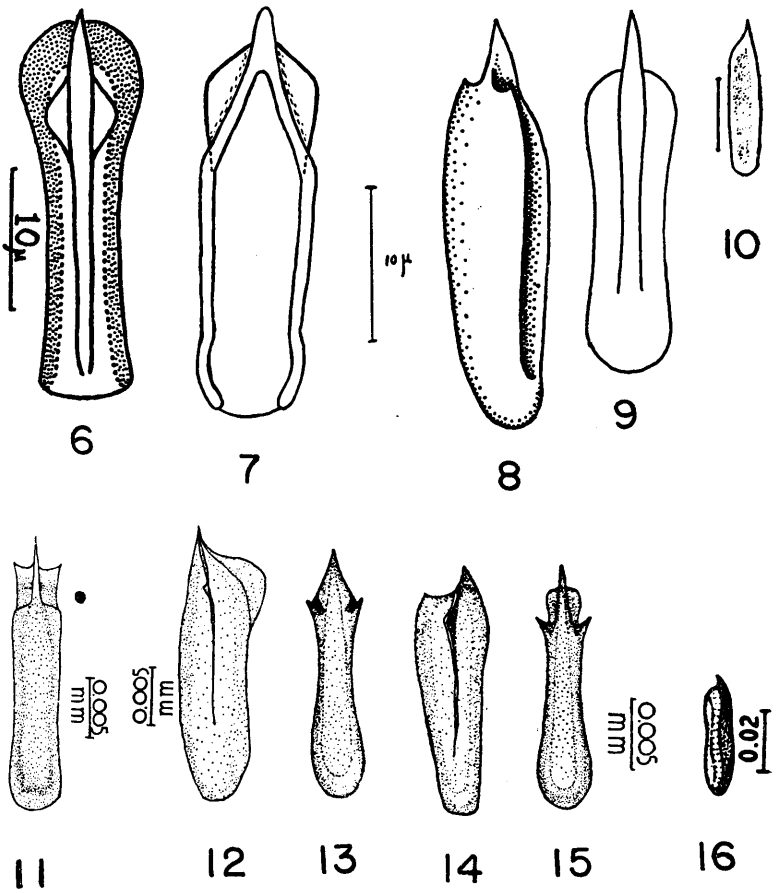

PLATE II

Fig. 6. Ventral view of stylet of *C. steelmani* Baker, 1943.
Fig. 7. Ventral view of stylet of *C. raiacauda* Steelman, 1938.
Fig. 8. Lateral view of stylet of *C. macrocerca* Vickers, 1940—(C. g. *vitelliloba* Sinitsin, 1905).
Fig. 9. Ventral view (?) of *C. wabashensis* Coil, 1955.
Fig. 10. Lateral view of *C. sphaerocerca* Miller, 1936.
Fig. 11. Dorsal view of stylet of *C.* of *P. solidum* Goodchild, 1943—(*C. conica* Goodchild, 1939).
Fig. 12. Lateral view of stylet of *C.* of *P. solidum* Goodchild, 1943.
Fig. 13. Dorsal view of stylet of *C. donecerca* Goodchild, 1943.
Fig. 14. Lateral view of stylet of *C. donecerca* Goodchild, 1943.
Fig. 15. Ventral view of stylet of *C. donecerca* Goodchild, 1943.
Fig. 16. Lateral view of *C.* of *G. attenuata* Rankin, 1939.

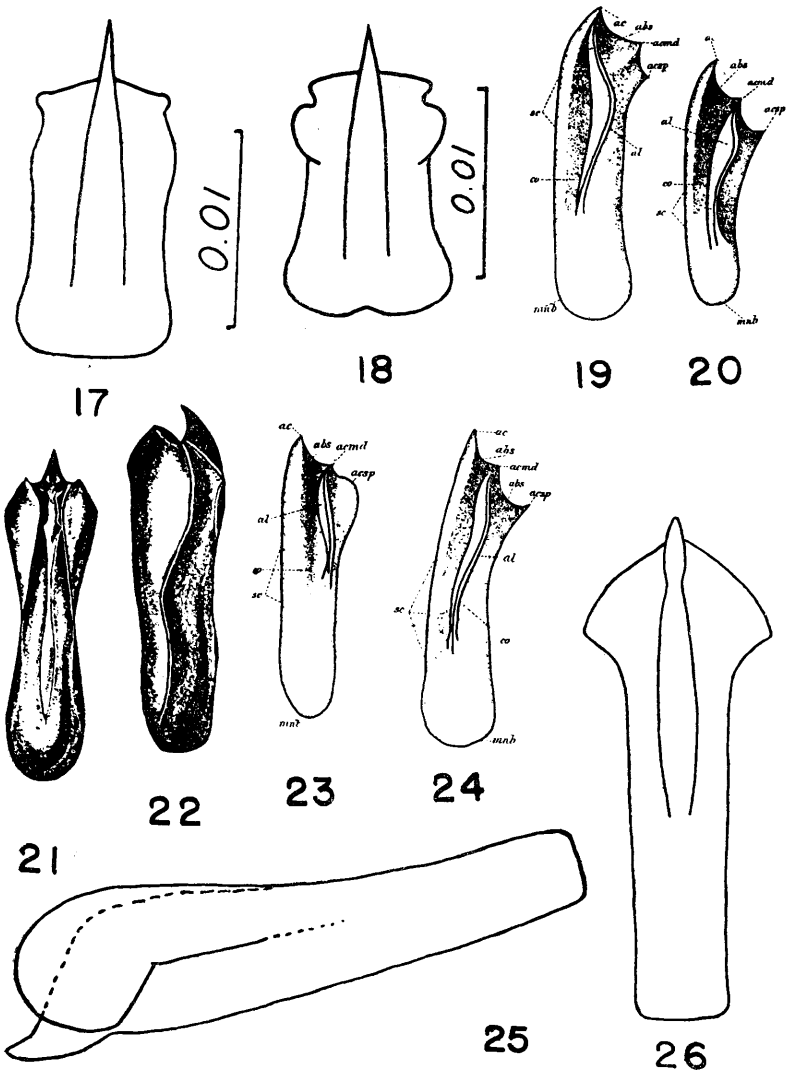

PLATE III

Sinitsin's figures have been reoutlined. All others are the author's camera lucida drawings.

Fig. 17. Ventral view of stylet of *C. lampsilae* Coil, 1954.
Fig. 18. Ventral view of stylet of *C. eriensis* Coil, 1952.
Fig. 19. Lateral view of stylet of *C.* of *G. varsoviensis* Sinitsin, 1905.
Fig. 20. Lateral view of stylet of *C.* of *G. vitelliloba* Sinitsin, 1905.
Fig. 21. Ventral view of stylet of *C. amplicara* Krull, 1935.
Fig. 22. Lateral view of stylet of *C. amplicara* Krull, 1935.
Fig. 23. Lateral view of stylet of *C.* of *G. pagenstecheri* Sinitsin, 1905.
Fig. 24. Lateral view of stylet of *C.* of *G. loossi* Sinitsin, 1905.
Fig. 25. Lateral view of stylet of *C. coelocerca* Steelman, 1939.
Fig. 26. Ventral view of stylet of *C. coelocerca* Steelman, 1939.

longitudinal and transverse muscles and is quite motile. When in motion it has the appearance of the annelid *Tubifex* waving in the water. This motile portion of the tail is cylindrical, 0.023 mm. wide, and ends abruptly in a blunt point. The overall length of the tail is 0.442 mm. The acetabulum is posterior to the middle of the body and is slightly smaller than the oral sucker. The former is 0.032 mm. wide and 0.03 mm. long; the latter is 0.39 mm. wide and 0.034 mm. long. No pharynx is present. The esophagus continues posteriad from the oral cavity to the region of the acetabulum. Before reaching the acetabulum, the esophagus bifurcates into two intestinal caeca which end blindly before reaching the anterior-most margin of the acetabulum. A stylet is absent and penetration glands were not observed.

The excretory bladder is club-shaped, the posterior portion being larger than that nearest the acetabulum. Several flame cells were observed but no tubes, tubules, or capillaries were traced out. Light refractive cystogenous material throughout most of the body increases the time required for working out the morphology of the exeretory system.

The sporocyst located in the gills is a simple sack-like body 0.06 mm. wide and 1.15 mm. long divided into three areas by two dark granular bands.

Cercaria ruddi could only be confused with two existing forms: *C.* of *Phyllodistomum folium* Sinitsin 1901 and *C. mitocerca* Miller 1936. It can easily be separated from the former by comparing the characteristics of the tail; in *C. ruddi* there is an active thread-like filament, whereas in *C.* of *P. folium* the tail is stumpy (shorter than body of distome). Also, *C.* of *P. folium* develops in clams belonging to the Dreissenidae, whereas *C. ruddi* develops in sporocyst from clams belonging to Sphaeriidae. *C. ruddi* can be separated from *C. mitocerca* by: (1) possessing a motile tail, (2) absence of an anterior conical chamber, (3) intestinal caeca not passing posterior to the acetabulum.

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Notes on *Hymenolepis jacobsoni* von Linstow (Cestoda: Cyclophyllidea) from a Shrew in India

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Our knowledge of the cestode fauna of shrews in Asia is fragmentary. Through the kindness of Dr. Gordon H. Ball, Department of Zoology, University of California, Los Angeles, I received two vials with cestodes collected by Dr. Ball in June, 1956, from two *Suncus coeruleus* caught in the City of Bombay. The cestodes were fixed in Bouin's fluid and stained with an aqueous solution of Ehrlich's haematoxylin. All specimens were identified as being *Hymenolepis jacobsoni* originally described by von Linstow (1907) from the shrew *Crocidura murina* in Java. Meggitt (1927) described *Weinlandia minutissima* from *Crocidura murina* in Rangoon. Hübscher (1937), after having studied Meggitt's specimens, placed *W. minutissima* in synonymy with *H. jacobsoni* and gave additional description of this species based on specimens obtained from *Crocidura coerulea* in Java. None of the above mentioned authors gave illustrations of mature proglottids. A drawing of a mature proglottid of *H. jacobsoni* is therefore included here as figure 1.

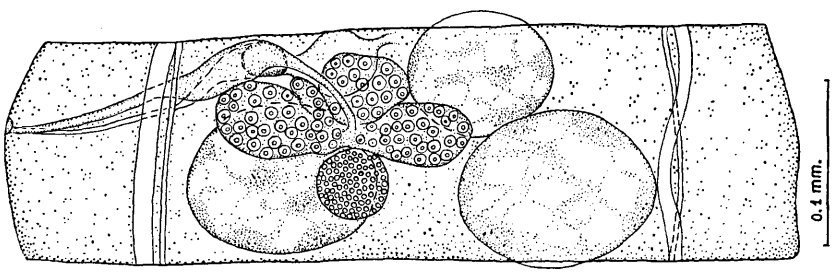

Fig. 1. Mature proglottid of *Hymenolepis jacobsoni* von Linstow, 1907.

Although most of the observations made on the specimens from India are in accord with previous descriptions of this species, some additional information should be given. The excretory system, originally described as consisting of 1 pair of longitudinal ducts, is composed of 2 pairs: a broader ventral one without cross-connections and a very slender dorsal pair. Hübscher (1937) listed eleven rostellar hooks. Only ten hooks were seen in the specimens from India. Their length is similar to that given by earlier describers (17-18 microns), and their shape is the same. Size ranges of my specimens are as follows: Scolex diameter 130-240 microns, sucker diameter 64-68 × 74-94 microns, rostellar sac diameter 80-94 × 150-160 microns, rostellum width 34-40 microns, testis diameter in mature proglottids 70-120 microns, and egg diameter 32 × 36 microns.

In one host many of the specimens had abnormalities, including incomplete segmentation and great variability in the position of the gonads.

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A New Species of the Genus *Dolichodorus* from California (Nematoda: Tylenchida)

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Dolichodorus heterocephalus was described by Cobb in 1914. In the nearly half-century that has elapsed no other species has been described in the genus despite the attention that has been given the plant parasitic and soil nematodes in recent years. This would seem to indicate that species of this genus are rarely encountered in cultivated lands. The present species was found in soil samples collected from an uncultivated area and was associated with the roots of native plants. The species is of particular interest because of the fact that the female has a blunt rounded tail in contrast to the pointed tail of the female of the genotype. However, the smallest larval stages found in the soil samples have a pointed tail very similar to the tail of the adult females of *D. heterocephalus*. Tails of pre-adult females of the new species are not bluntly rounded but are slightly digitate on the ventral side as indicated in Fig. 1, E. In the absence of males the females of this or similar species of *Dolichodorus* might not be readily recognized. The generic diagnosis is therefore emended to include species in which the adult females have bluntly rounded tails.

GENUS *Dolichodorus* Cobb 1914

DIAGNOSIS EMENDED: Dolichodorinae, Chitwood and Chitwood 1950, with a conspicuous striated lip region set off from the body by constriction or depression. Lip cap present. Supporting sclerotized framework of the lip region massive. Amphid openings at the lateral margins of the lip cap. Cuticle coarsely striated. Lateral incisures 3 or 4 at the middle of the body. Females with two ovaries. Caudal alae terminal, striated and conspicuously lobed. Spicules two, gubernaculum linear. Stylet in both sexes elongate and bearing conspicuous basal knobs. Corpus of esophagus thick, postcorpus elongate-oval with a strongly developed valvular apparatus. Isthmus of esophagus a slender tube terminating in an elongate posterior basal bulb containing three esophageal gland nuclei.

Type species: *Dolichodorus heterocephalus* Cobb 1914.

Dolichodorus obtusus, new species

DIMENSIONS: 14 Females — L = 1.9 - 2.7mm.; a = 35 - 45; b = 6.2 - 8.7; c = 45 - 65

$$V = \frac{20 - 16}{51 - 60} \quad \frac{15 - 22}{}$$

; Stylet = 132 - 136 microns

8 Males — L = 1.8-2.26 mm. a = 36-45; b = 6-7; c = 47-51; T = 38%; Stylet = 120-138; Spicules = 62-73 microns; Gubernaculum = 21-26 microns

FEMALE (HOLOTYPE): L = 2.5 mm.; a = 35; b = 7; c = 58; $V = \frac{20}{57}\ \frac{19}{}$ Stylet = 133 microns. Lip region globular, set off by constriction, bearing 7-8 transverse striae. A conspicuous lip cap is present which is hexagonal in shape when viewed *en face*. The amphid openings are oval in shape and are located at the lateral margins of the lip cap. The amphids are easily visible in face views and can be seen without difficulty in dorsal or ventral views of the lip region. Papillae were not visible from lateral or face views. The oral opening is surrounded by six small refractive pieces which resemble the

inner circlet of papillae but which are located in a position which precludes their being papillae. The most anterior of the lip striations is six-lobed, but the remainder of the lip region is obscurely four-lobed. The cuticle is coarsely striated, anterior to the excretory pore the striae are about 4 microns apart, posterior to the excretory pore they vary from 2-3 microns apart. The body is cylindrical from the base of the esophagus to the obtusely-rounded tail. The terminus of the tail is striated. The excretory pore is located at the beginning of the posterior bulb of the esophagus and the hemizonid is located a short distance posterior to the opening of the excretory duct.

There are four lateral incisures, the outer lateral fields are interrupted by the transverse body striae at regular intervals. In most instances alternate striae extend into the outer lateral fields. The center lateral field is not interrupted by striae. In the region of the median bulb the incisures are reduced to three which extend to within a short distance of the lip region. Deirids were not observed. The phasmid openings are small and are located about midway between the anal opening and the terminus of the tail and ventral of the center of the middle lateral field.

The corpus of the esophagus is thick and expands gradually into the median bulb. The median bulb is roughly oval anteriorly, but is reduced rather abruptly posteriorly. The isthmus is a slender tube which is slightly longer than one body width, it expands rather abruptly into the elongate posterior bulb. There is a conspicuous esophageal-intestinal valve or cardia. The intestine overlaps the posterior end of the esophageal bulb which is frequently obscured by the dense intestinal granules. The vulva is a transverse slit, the vagina extends nearly half a body width interiorly. The uteri are muscular and spermethaca were not seen. The oocytes are arranged in a single row.

MALE (ALLOTYPE): L = 2.25 mm.; a = 42; b = 7.5; c = 48; T = 38% Stylet = 138 microns; Spicule = 73 microns; Gubernaculum = 24 microns; Similar to the female. Phasmids opening in the middle lateral field near the cleft of the caudal alae. Caudal alae conspicuously lobed, bearing striations and enveloping the terminus of the tail. Viewed from a ventral position the caudal alae is smoothly rounded at the terminus. The spicules are massive and heavily sclerotized. The gubernaculum is linear with the distal end slightly enlarged. Viewed from the dorsal side the gubernaculum consists of three pieces at its proximal end, these pieces are fused at about one-third the distance from the proximal end.

HOLOTYPE: Female collected March 15, 1951 by D. J. Raski, catalogue no. 101, University of California Nematode Survey Collection.

ALLOTYPE: Male, same data as the holotype, catalogue no. 102, University of California Nematode Survey Collection.

PARATYPES: 31 females and 20 males same data as the holotype.

TYPE HOST: Soil at the roots of *Arctostaphylos manzanita* Parry.

TYPE LOCALITY: 10 miles south of Monticello on highway 37, Napa County, California.

DIAGNOSIS: *Dolichodorus obscurus* is distinguished from the type species by the shape of the lip region, the shape of the female tail and the number of incisures in the lateral fields. Males can be distinguished from *D. heterocephalus* by the shape of the lip region and by the smoothly rounded terminus of the bursa.

A high percentage of the individuals of *D. obscurus* were observed to be infected by parasites very similar to those described by Thorne (1940) as

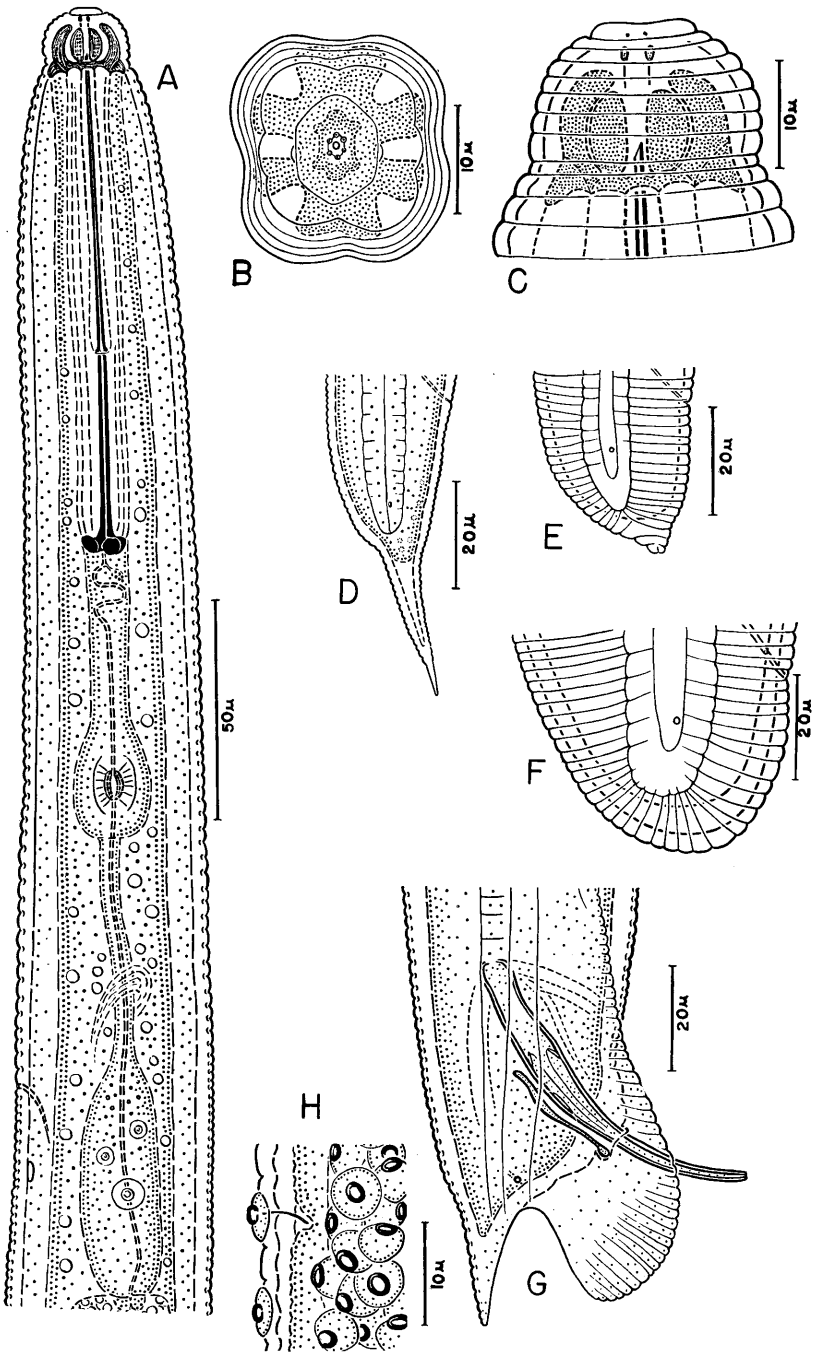

Fig. 1. *Dolichodorus obtusus:* a, anterior end of female; b, face view; c, lip region; d, larval tail; e, preadult female tail; f, female tail; g, male tail; h, parasites, internal and external.

Duboscquia penetrans from *Pratylenchus pratensis* = *Pratylenchus brachyurus*. These parasites were observed on the external surfaces of the body as well as internally. In some instances (fig. 1,H) the external forms were observed to have penetrated thru the cuticle and the muscle layer by means of what appeared to be a tube extending from the very refractive nucleus of the parasite. In lightly infected males and females the parasite was present almost exclusively in the reproductive system but in heavily infected individuals the parasites occupied most of the body cavity.

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Observations on the Morphology and Systematic Position of *Thysanocephalum thysanocephalum* (Linton 1889)

GLORIA MEADE-THOMAS* AND NATHAN W. RISER**

Luehe in his inaugural dissertation (1894) attempted to homologize the apical organs of a number of tapeworms according to the arrangement of the musculature of the scoleces. The arrangement of the musculature seemed to vary according to species and not according to genera nor higher taxonomic units. In most invertebrates and vertebrates the nervous system tends to remain constant in that homologous nerves innervate homologous structures; thus, the nervous system would appear to be more appropriate than the musculature in attempting to relate various parts to one another.

The literature is very vague as to which portion of the complicated holdfast of *Thysanocephalum thysanocephalum* (Linton, 1889) is the scolex. It would seem logical that the primary ganglionic mass or controlling center would lie in the scolex as in other cestodes; therefore the purpose of this investigation was to ascertain the position of the main ganglionic mass, and to trace the general topography of the nervous system.

MATERIALS AND METHODS

The specimens used in this study were collected from the spiral valve of a young specimen of the tiger shark *Galeocerdo arcticus*, at Woods Hole, Massachusetts, August 21, 1952. They were maintained in a Polyvinyl pyrrolidone (P.V.P.) solution while being anesthetized with ethyl alcohol. (The bath in P.V.P. apparently disrupted osmotic relationships since the excretory system in sectioned material showed extensive degeneration.) They were fixed either in Worcester's fluid or in San Felice's chrome-aceto-formaldehyde. There was apparently some reaction between the Worcester's fluid and the P.V.P which made the specimens treated by this procedure useless for histological study. Serial sections of twenty-seven specimens of various ages were used in this investigation. Since sections cut in one plane did not show all of the relationships, it was necessary to cut individual specimens in different planes, hence longitudinal, horizontal, and cross-sections were made. The standard paraffin technique was used with sections cut at a thickness varying from 6

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to 15 micra. Although this constituted a topographical study, the thicker sections were no more advantageous than the thin ones.

Galigher's Standard Alum Haematoxylin counterstained with eosin, Heidenhain's Azocarmine, and Bielschowsky's Ammoniacal Silver were the staining procedures used. The latter technique was unsatisfactory and the best results were obtained with the Azocarmine method.

DESCRIPTION

The holdfast in the genus *Thysanocephalum* consists of the scolex which is a small anteriorly located structure bearing four minute biloculate phyllidia; and a second portion, the metascolex. These two are connected by a short peduncle.

The four phyllidia are arranged in pairs with each phyllidium occupying a corner of the rectangular scolex. The loculi are about equal in size, and two small cuticular spines project from the posterior corners of the anterior loculi of each of the phyllidia. Various descriptions of these spines occur in the literature where they have been designated as hooks and also as spines (Linton, 1889, Southwell, 1925, Wardle and McLeod, 1952). However, these are solid cuticular outgrowths which are not hooks, but are spines like those found on the over-hanging region of the phyllidia of *Dinobothrium*. There is a small pad of weakly cuticularized tissue which projects anteriorly separating the phyllidia. In the peduncle, the longitudinal muscles are arranged into four peripheral bands located midway between the angles of the quadrangle (Figure 5). The majority of these muscles arise from the internal surfaces of the posterior loculi of the phyllidia.

The metascolex was described by Linton (1891: 545) as "a large lobed, crisped and folded mass, which, in alcoholic specimens, is more or less globose or discifform, but which in living specimens may be spread out into a flat suctorial disc with fimbriated edges." The folds are phyllidial in structure and most anteriorly extend from four short primary evaginations of the body wall which posteriorly are much folded so that in cross-sections of the

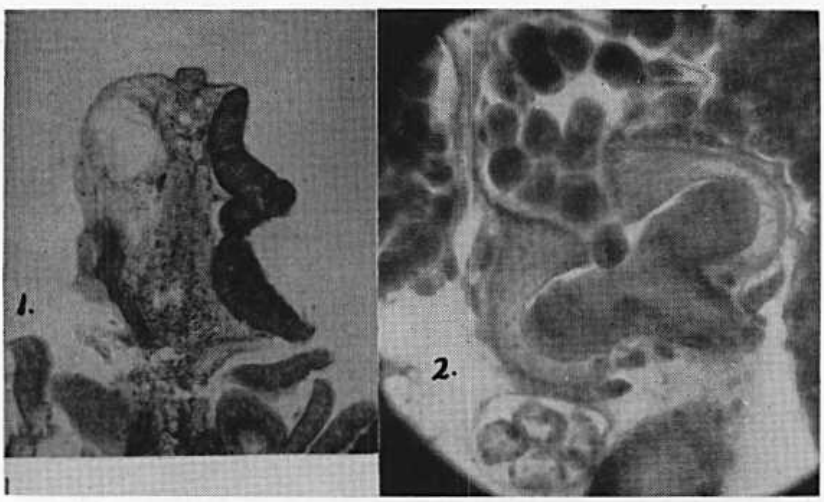

Fig. 1. Photograph of horizontal section through scolex.
Fig. 2. Photograph of horizontal section through oöcapt of mature proglottid.

latter region there appear to be many stalks. This folding was responsible for Linton (1891) describing two varieties depending upon the number of stalks.

NERVOUS SYSTEM, GENERAL: In his extensive description of the species, Linton (1891: 551) said that the most anterior part of the nervous system was "a cluster of nucleated cells" "in a finely granular mass" which was centrally located in the scolex and was traversed by very minute transverse fibers and by coarser longitudinal ones. He described the grouping of this nervous tissue into "two marginal areas" at the base of the scolex, with the two cords "or vessels" thus formed extending through the peduncle behind the scolex and on through the body, one near each "margin of the central core." He stated (p. 552) that "these vessels are without proper walls in any part of their course." It is the apparent absence of a limiting membrane that makes a study of this system so difficult.

The nervous system (Fig. 3) consists of a brain which occupies almost all of the space in the scolex, (Fig. 1) two lateral nerve cords extending through the entire length of the worm, four anterior bothridial nerves which innervate the anterior loculi, two pairs of posterior bothridial nerves which innervate the posterior loculi and continue in the surface layer of the lobes of the metascolex, two pairs of accessory nerves which are closely applied to the dorsal and ventral surfaces of each of the lateral nerve cords in the metascolex and two pairs of median nerves which extend a short distance behind the metascolex from the dorsal and ventral surface of the region where the lateral nerve cords leave the brain. The ganglionic swellings which characteristically occur in the central nervous system of other cestodes are not obvious.

THE BRAIN: A muscle-cross connects the anterior loculi diagonally just above the top of the brain and also above the loops of the excretory vessels. The apex of the brain is oval with the long axis in the horizontal plane and extends a few microns anteriorly beyond the region from which the four anterior bothridial nerves leave. Niemiec (1886) reported eight nerves leaving the anterior surface of the brain in *Acanthobothrium coronatum* (Rud. 1819), and four in species of *Anthobothrium* and *Phyllobothrium,* and four were reported in *Anthobothrium auriculatum* (Rud. 1819) and *Orygmatobothrium dohrnii* Oerley 1885 by Rees (1943, 1946). Anterior nerves leaving either the brain or the anterior bothridial nerves were not observed in the present study. Between the loops of the excretory vessels, the brain takes on the shape of a rhombus and then of a cross with the two lateral arms much thinner than the dorsal and ventral ones. The lateral arms lie between the dorsal and ventral excretory vessels of each side, and the vessels are almost immediately enclosed in the brain as fibers link the arms together. This region is homologous with the anterior commissure described by Johnstone (1912) as occurring in *Grillotia erinacea* (van Ben. 1850), and by Rees (1941) in *Aporhynchus norvegicus* (Olsson 1867). Very few cell bodies are found in this anterior portion, although a few giant neurones, *cir.* 0.025 mm. in diameter, occur in the parenchyma crowded between the brain and the subcuticula. The main mass of nervous tissue lies medial to the excretory vessels. The lateral arms gradually become very short while the central area increases in size and the nervous tissue connecting the tips of the arms becomes more obvious. As a result, the excretory vessels come to lie laterally in the tissue of the brain. A large number of unipolar and bipolar neurones are centrally located in this region. At the level of the posterior end of the anterior loculi,

the nervous tissue expands laterally forcing the dorsal and ventral excretory vessels apart and extending beyond them. The vessels come to lie in the center of the scolex just internal to the inner margins of the phyllidia (Fig. 4) with the two dorsal vessels side by side and the ventral members of each pair directly opposite. The excretory vessels hereafter remain in the middle of the scolex and cephalic peduncle. At about the anterior level of the posterior loculi, the brain consists almost entirely of neurones, several of which are giant multipolars. For a short distance, the brain now consists of two large bilobed lateral trunks which shortly coalesce around the excretory vessels and come to consist almost entirely of giant neurones. This area with its concentration of giant neurones is divided into a right and left half, each half constituting a ganglion. There is no division into dorsal and ventral ganglia. Just anteror to the posterior margins of the posterior loculi, a median commissure, densely packed with small neurones, links both sides so that once again, the dorsal and ventral excretory vessels are separated by nervous tissue. The vessels of each side unite over the bridge formed by the posterior commissure and the vessels which extend through the cephalic peduncle behind this region are much coiled and of very small diameter.

In general, the nervous system in the scolex of *T. thysanocephalum* while much more elongated than that described for other cestodes and without the typical ganglionic swellings, differs primarily in the way the giant neurones are concentrated. Niemiec (1886) stressed that in all forms, the greatest number of large ganglion cells characteristically occurred in the middle of the principle commissure which is the case here except that the neurones are at the bases of the two trunks connecting the anterior and postrior commissures rather than between the bases of these trunks.

LATERAL NERVE CORDS: Two lateral nerve cords (0.01-0.02 mm. in diameter in a small worm and 0.05 mm. in the largest specimens) extend throughout the strobila and are initially ensheathed by a thin membrane. These cords contain many fibers in addition to the spongy tissue and have small neurones scattered along the outer border. They originate as two trunks from the posterior surface of the brain and retain a central course as they pass through the cephalic peduncle. At the base of the peduncle, the cords of both sides are united by loose, non-ensheathed, dorsal and ventral commissures containing many small neurones and from the outer posterior edges of the mass, the two lateral nerve cords diverge as they continue, one on each side, in a posterolateral direction along the outer margins of the osmoregulatory vessels. The cords continue to diverge for the upper half of the length of the metascolex and then swing into a direct posterior course just internal to the lateral border of the medulla. They maintain this position throughout the entire length of the worm, giving off many nerves towards the periphery and fewer nerves towards the medulla. In the proglottids, the lateral nerve cords are connected by nerve fibers forming a ring commissure at the anterior margin of each proglottid.

POSTERIOR BOTHRIDIAL NERVES: These four small peripheral nerves measuring 0.02 mm. in diameter, extend posteriorly from the brain and immediately yield branches that extend along the bothridial surface of each posterior loculus. As they pass posteriorly towards the junction of the peduncle with the metascolex, each lies in one of the angles of the quadrate peduncle (Fig. 5). These nerves consist of fibers and a few scattered small cell bodies all enclosed in a thin membrane. On entering the metascolex, the two pairs of nerves extend laterally for a short distance and then turn abruptly in a

posterior direction to run parallel to the lateral nerve cords. At the level of the commissures connecting the lateral cords at the apex of the metascolex, the lateral nerve cords send out a short branch to giant ganglia associated with each bothridial nerve. The outermost edge of each ganglion contains small unipolar and bipolar neurones while multipolar neurones may be seen in the central portion, and the region most internal from the margin of the phyllidia-like lobes is occupied by nerve fibers. All of these elements lie parallel to the bothridial surface of the lobes. These ganglia are situated outside of the medulla in four folds of the bothridial surface of the metascolex and are united by a loose non-ensheathed ring commissure. A large nerve originates from each ganglion and branches repeatedly to innervate the bothridial surface. This surface is clearly delimited from the underlying tissue and except for the concentration of nerve fibers, does not differ histologically from the bothridial surface of true phyllidia.

ACCESSORY NERVES: Fuhrmann (1931) stated that dorsal and ventral to the lateral nerve cords, a pair of accessory nerves could be demonstrated among the cyclophyllideans and perhaps in all the orders of cestodes. The accessory nerves of *T. thysanocephalum* measure 0.006-0.01 mm. in diameter and are closely applied to the dorsal and ventral surfaces of each lateral nerve cord. In the posterior two-thirds of the metascolex and zone of growth, these nerves lie so closely together that they are only separated by their membranous walls. The accessory nerves arise from the apical nerve ring in the metascolex and immediately diverge from the lateral nerve cords toward the dorsal and ventral surfaces so that they come to lie about 0.02 mm. from the lateral cords at a point about 0.08 mm. from the point of divergence. Each accessory nerve sends several small branches into the related "stalk" of the phyllidia-like lobes of the metascolex. These small nerves further divide and consist of naked chains of neurones the ramifications of which could not be traced. In the posterior part of the metascolex, the accessory nerves are connected by a ring commissure consisting of naked neurones. No effort was made to trace these nerves beyond the commissure, and they did not occur in the proglottids.

MEDIAN NERVES: A pair of dorsal and a pair of ventral median nerves originating from the dorsal and ventral surfaces of the posterior portion of the brain and extending posteriorly in a straight course, innervate the cortical portion of the metascolex. They insert in the ring commissure formed by fibers from the accessory nerves at the base of the metascolex. They lie in the cortex throughout their entire length, and disappear a short distance behind the ring commissure. Niemiec (1886) could not find the dorsal and ventral nerves in the proglottids of *Acanthobothrium coronatum* nor were they demonstrable in any of the phyllobothrioid species reported in 1955 by the junior author of the present paper. Since these nerves are associated with the cortical nerve net, their ramification in the zone of growth of phyllobothrioids may be correlated with the general apolytic nature of the group.

Cohn (1899) demonstrated well-formed dorsal and ventral median nerves in the proglottids of several cyclophyllidean species and contended that ten longitudinal nerves would be present in all taenoid species. Rees (1951) traced the median nerves to their termination in the excretory bladder of the cysticercus of *Taenia taeniaeformis* (Batsch 1786). (It should be mentioned that Subramaniam (1941: 279) described 32-42 nerves "all of the same thickness" extending through the proglottid chain of *Tylocephalum dierama* Shipley and Hornell 1906. In terms of Cohn's (l.c) contention that the longi-

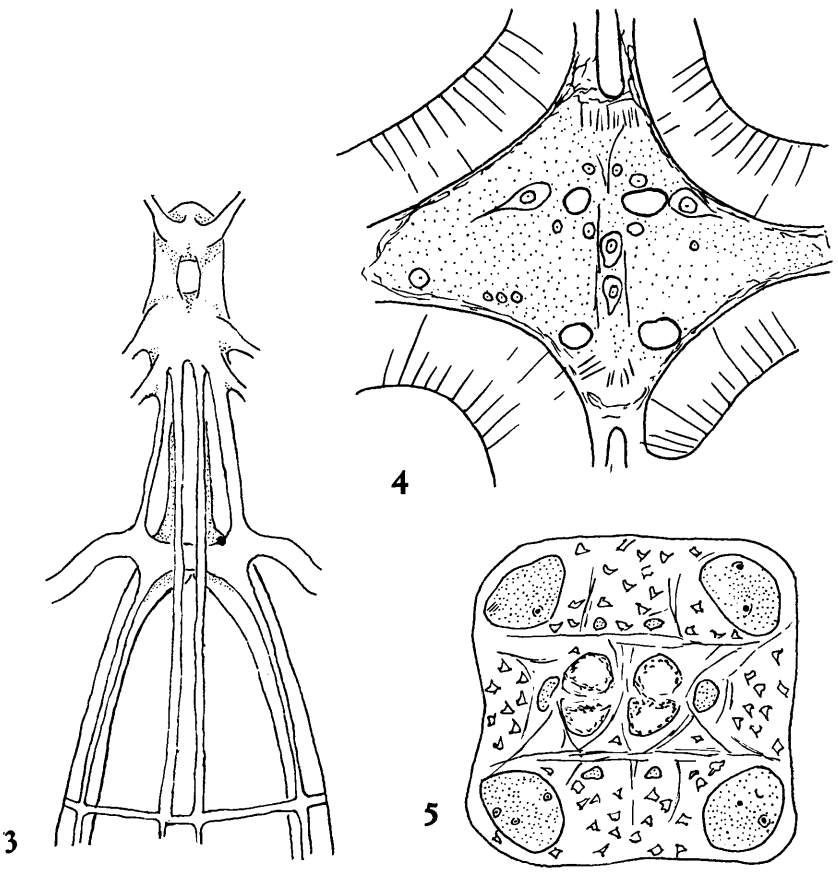

Fig. 3. Semidiagrammatic dorsal representation of nervous system in scolex and metascolex. Fig. 4. Camera lucida drawing of cross-section of scolex at the level of the posterior margins of the anterior loculi. Fig. 5. Camera lucida drawing of cross-section of peduncle.

tudinal nerves in the strobila were branches of the lateral trunks, this would imply that in *T. dierama,* the typical chiasma would not be present and the longitudinal trunks would thus leave the brain in a radial fashion as indicated by the figures of Subramaniam). The phylogenetic significance of these two pairs of nerves is difficult to assess at the present time since only a few observations have been reported. The proboscideal nerves of most tetrarhynchs at first appear to be homologous with them, but in *Aporhynchus norvegicus,* Rees (1941) described the absence of proboscideal nerves and correlated this with the lack of proboscides. Thus, at present, the tetrarhynchs seem to lack dorsal and ventral median nerves; phyllobothrioids have them only in the zone of growth; cyclophyllideans have them extending throughout the strobila; some pseudophyllideans have numerous longitudinal nerves in addition to the two main trunks; and the lecanicephaloid, *Tylocephalum* apparently has a large number of longitudinal trunks and no lateral nerve cords as such.

OBSERVATIONS ON GENERAL MORPHOLOGY

In his account of the morphology of the proglottids of *T. thysanocephalum*, Causey (1926: 37) stated that "no oöcapt is present" but sections of mature proglottids show a very large thick-walled oöcapt (Fig. 2) 0.07-0.08 mm. in diameter. It is pumpkin shaped, broader than it is long, with the muscles thicker at the sphincter which is the proximal portion of the organ. It contains a large cavity which narrows abruptly to become the oviduct in which the muscular walls disappear.

In this same paper, Causey stated (p. 40) that "the excretory system consists of two pairs of lateral ducts" the smaller of which "are the ventral ducts" which are present with the larger dorsal pair in the metascolex and neck region but "disappear in the region of definite proglottid formation." However, in the present study, sections in a region of young proglottid formation 100 mm. behind the scolex of a worm 228 mm. long show the testes extending in a row in the medulla in close proximity with the internal circular muscle layer and the large vessels lying in contact with the circular muscle layer directly opposite. The smaller vessels run a spiral course about halfway between the large vessels and the lateral nerve cords so that at times they lie between the testes and the adjoining muscles, and at other times in the middle of the medulla but never in contact with the musculature opposite to the testes. The large vessels are ventral while the small ones are dorsal. In the mature proglottids, the large ventral vessels lie halfway between the mid-line and the lateral margin of the proglottid and the dorsal vessels, much reduced in size, lie lateral to the ventral vessels with the testes spreading laterad of both vessels.

Behind the metascolex, the stobila of older worms have a long unsegmented portion which has been referred to as a neck. Linton (1889) described a specimen 840 mm. in length with an unsegmented region 360 mm. long. This region of the body consists of an externally unsegmented zone of growth in which internal segmentation has occurred.

OBSERVATIONS ON SYSTEMATIC POSITION

Linton in 1889 placed the worm *T. thysanocephalum* (= *Phyllobothrium thysanocephalum*) in the family Tetrabothriidae, and in 1890 assigned it to the subfamily Phyllacanthinae. Braun (1900) placed it in the family Onchobothriidae but Linton (1924: 27) displayed dissatisfaction with this classification when he wrote, "The genus *Thysanocephalum* does not belong in this group. The hooks, instead of being structureless, have a very dense, striated structure, and appear to be a modification of the dense, muscular border of the bothria." However, he did not reassign the species, and later workers (Southwell 1925, Causey 1926, Fuhrmann 1931, Wardle and McLeod 1952) made no change. Euzet (1952), without comment, listed it with the Phyllobothriidae.

As pointed out by Linton, the worm does not fit the definition of the family Onchobothriidae nor can it be placed in any of the other families of the superfamily Phyllobothrioidea. The genus differs from those in the Onchobothriidae in that the phyllidia are sessile and each is divided into two loculi by a single septum and suckers are absent. The anterior loculi bear cuticular spines projecting from their posterior corners. The Mehlis' gland is relatively large with many cells.

The worm could neither be placed in the family Phyllobothriidae nor Echeneibothriidae because of the nature of the phyllidia, although its char-

acteristics definitely place it in the superfamily Phyllobothrioidea to which these families belong.

Riser (1955), in his diagnoses of the three phyllobothrioid families, noted that in the family Onchobothriidae "the vaginal extension and oviduct unite lateral to the Mehlis' gland." In the families Phyllobothriidae and Echeneibothriidae, he stated that "the vaginal extension and oviduct fuse as they enter the posterior aspect of the gland." *T. thysanocephalum* agrees in this instance with the families Phyllobothriidae and Echeneibothriidae with the fusion of the ducts taking place at their junction with the Mehlis' gland and not lateral to and outside of it, and thus there is no fertilization passage outside the Mehlis' gland. *T. thysanocephalum* is a phyllobothioid which cannot be placed in any of the families as they are constituted at present.

T. rugosum Chandler 1942 was diagnosed by characters assigned by Linton to *T. thysanocephalum;* indeed, the specimens used in the present study were of all ages, and while the oldest, taken separately, could have been assigned to Chandler's species, the entire series clearly indicated a single species. *T. rugosum* is a synonym of *T. thysanocephalum.* Southwell (1925) considered *Myzocephalus narinari* Shipley and Hornell 1906 to be identical with *T. thysanocephalum.* Fuhrmann (1931) retained *narinari* as a species name, but accepted Southwell's generic synonymy. From the small amount of information available in the original description, Fuhrmann was probably correct in his designation. *T. ridiculum* Linton 1901 was placed by Southwell (1925) as a synonym of *Ceratobothrium xanthocephalum* Monticelli 1892. Linton's brief description gave little information of taxonomic value and was apparently a preliminary note, but Monticelli originally described *C. xanthocephalum* from *Lamna nasus* (Bonnaterre) (= *Lamna cornubica* Cuvier) and Yamaguti (1934) redescribed it from *Isurus glaucus* (Müller et Henle). Linton's species was described from *Isurus dekayi.* (Bigelow and Schroeder (1948: 133) questioned this record, and it can be considered more likely that the host was *L. nasus*). On this host distribution, it can be considered very likely that the two cestode species names are synonymous. The genus *Ceratobothrium* appears to form a link between *Thysanocephalum* and *Dinobothrium.* In *Ceratobothrium,* the anterior loculus of each phyllidium is sucker-like, but bears cuticular spines projecting from the posterior corners and the phyllidia are totally sessile. Possibly, the scolex of *Dinobothrium* could be derived from this, although the entire posterior loculus of the phyllidia of *Dinobothrium* is free from the scolex. Euzet (1955) separated the species in the genus *Dinobothrium* into three genera each of which he placed in a separate family. Whether considered as one (*vide* Riser (1955)) or three genera, the group of species is homogeneous, and has much in common with *Thysanocephalum.* The presence of a metascolex in *Thysanocephalum* may be of generic significance, but like acetabula, cuticular spines, and fimbriation, this character is an adventitious one which may appear in any cestode family. The basic pattern of the relationships of the various internal organs and systems to one another is of paramount importance and in the phyllobothrioids can be correlated with the morphology of the scolex and of the plerocercoid larvae. The genus *Thysanocephalum* must be placed in a separate family.

THYSANOCEPHALIDAE, N. FAM.

Phyllidia sessile, divided into two loculi by a single permanent septum, hooks and suckers absent. Cephalic peduncle present. Mehlis' gland large, composed of many claviform cells. Vaginal extension and oviduct fuse as

they enter posterior aspect of Mehlis' gland. Type genus: *Thysanocephalum* Linton 1891.

The relationship of *Ceratobothrium* is not clear at the present time, and thus, temporarily, only one genus is assigned to the family. The family differs from other families in the Phyllobothrioidea in the loculation and armature of the phyllidia. Other distinctions were discussed previously.

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A Study of the Metacercaria of *Crepidostomum cornutum* (Osborn, 1903), (Trematoda: Allocreadiidae)*

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During the summers of 1955-56, the author was able to confirm the life-cycle of *Crepidostomum cornutum* (Osborn, 1903) as described by Ameel (1937), under laboratory conditions. This adult trematode parasitizes the small intestines of the rock bass *Ambloplites r. rupestris,* while the metacercariae encyst in the cardiac region of the crayfish, *Cambarus bartoni sciotensis* Rhodes. The larval stages (rediae and cercariae) develop in the bivalve *Sphaerium striatum.* The opthalmoxiphidiocercariae are free-swimming, encysting in the crayfish which is the second intermediate host.

Hopkins (1934) in studying *C. cornutum,* described the metacercaria by merely stating that it is "exactly like the normal adult in size and structure." The present study revealed certain morphological differences. Ameel (1937) states that "the adult and metacercaria of *C. cornutum* are well described by Hopkins. Old metacercariae produce considerable numbers of apparently well-formed eggs." As pointed out above, Hopkins did not describe the metacercarial stage.

The author found the crayfish *Cambarus bartoni sciotensis* in Sinking Creek, Giles County, Virginia, to be almost 100% infected with the metacercariae of *C. cornutum.* The infection ranged from one metacercarial cyst to twenty-five per host. The spherical opaquely white cysts were devoid of any pigmentation, as is the case in the cysts of *Neascus spp.* found in fish. These were imbedded in the connective tissue surrounding the pericardium and the tri-loped tests of the crayfish. Each cyst measured approximately 1 mm. in diameter. While attempting to remove the cyst wall, it was discovered that there existed two such walls. When both walls were removed, an active progenic metacercaria emerged. This larva resembled the adult worm except for some minor morphological differences. A number of the whole cysts were fixed in Carnoy's and sectioned later.

MATERIAL AND METHODS

All materials were fixed in Carnoy's (6:1:1). The whole-mounts were either stained with Harris' Alum Haematoxylin or mounted clear for phase-contrast microscopy. The whole cysts were sectioned at 10 microns and stained with Mallory's Triple Stain for tissue differentiation.

OBSERVATIONS

The sectioned material revealed the two cyst walls. The inner one stained blue. Gatenby and Beams (1950) stated that "collagen fibrils, reticulum of connective tissue, mucus, chitin, etc. stain blue with Mallory's Triple Stain." Since the make-up of this inner cyst wall is rather homogeneous and semi-rigid, the author suspects that it is of a chitinous nature rather than its being composed of fibrils, reticulum of connective tissue, or mucus. It is presumably elaborated by the parasite. The outer wall, less distinct, (staining red with Mallory's Triple) and made up of layers of very compact fibrous connective tissue, undoubtedly represents host tissue, the formation of which

*Contribution from Mountain Lake Biological Station and the Department of Biology, University of Virginia. This investigation was supported by an award of the National Science Foundation Grant to Dr. B. D. Reynolds, Mt. Lake Biological Station, 1955-56.

was stimulated by the presence of the parasite. Similar phenomena were described by Hunter and Dalton (1939) in their study of the cysts of *Clinostomum marginatum* in *Lebistes reticulatus* (guppy), *Micropterus dolomieu* (smallmouth bass) and *Enneacanthus obesus* (common sunfish), and by Osborn (1911), who also worked with *C. marginatum*.

The entire metacercaria is doubled over ventrally while within the cyst and straightens out when released. The following description is based on 30 specimens used.

Crepidostomum cornutum (Osburn, 1903) Hopkins, 1934 (All measurements in millimeters)

PROGENIC METACERCARIAE Elongate distomes with six cephalic papillae. Body length 1.53 (max. 2.08, min. 1.23); body width 0.35 (max. 0.39, min. 0.32). Cuticle aspinous. Anterior sucker, bordered anteriorly and laterally by papillae, is 0.20 (max. 0.3, min. 0.17) by 0.22 (max. 0.33, min. 0.14). Remains of pigmented eye spots present on each side of oral sucker. Traces of penetration glands observed. Stylet not seen in metacercaria. Prepharynx absent; pharynx 0.05 to 0.18 (mean, 0.09) in diameter. Long slender esophagus 0.11 to 0.15 in length, bifurcating approximately midway between sucker and acetabulum. Intestinal ceca thin, reaching near posterior end of body. Acetabulum in equator of body, 0.18 (max. 0.19, min. 0.14) by 0.18 (max. 0.23, min. 0.15). Two sub-oval testes, tandem in some, oblique in others, with right one anterior. Anterior testis 0.09-0.21 by 0.09; posterior testis, 0.12-0.23 by 0.10-0.21. The efferent duct raises from anterior border of each testis. They lead anteriorly to the cirrus pouch where they unite as the common vas deferens which enters the cirrus pouch. Latter organ, thin and long, greater than length of acetabulum, containing seminal vesicle and long cirrus. Genital pore situated slightly to left of midline, posterior to crural bifurcation. Subtriangular ovary, 0.09 by 0.07, posterior to and to right of acetabulum. Short oviduct connects the ovary with small oötype (0.03 by 0.02). Mehlis' gland and Laurer's canal not seen in metacercaria. Uterine tract, with ascending and descending loops, confined to intracecal area between levels of oötype and testes. Uterus containing eggs similar to those in adult. Long metraterm approximately same length as cirrus pouch. Vitelline follicles large, fewer than in adult, extra- and cecal in position, ranging from level of crural bifurcation to near tip of ceca. Large bulbous excretory vesicle, containing refractile material, opens through terminal pore.

DISCUSSION

The metacercariae of *Crepidostomum spp.* are extremely difficult to differentiate from each other. Hopkins (1934) claimed a similarity between the metacercariae of *C. cornutum* and *C. cooperi*. The author noticed that the same difficulty exists in separating the metacercariae of *C. cornutum* and *C. farionis*. It is only in the adult worms that speciation is reliable.

Hopkins (1934) gave the body dimensions of the adult of *C. cornutum* to be 1.1 mm. to 3.7 mm., while the width was from one-fourth to one-third the length. The measurements given in this paper of the metacercaria fall within the range of the adult worm, but never reaching the maximum length. Since the metacercariae were so far advanced in development, one would suspect almost an immediate assumption of the adult form in the final host. This proved to be the case when cysts fed to uninfected rock bass were recovered as adults in less than 12 hours.

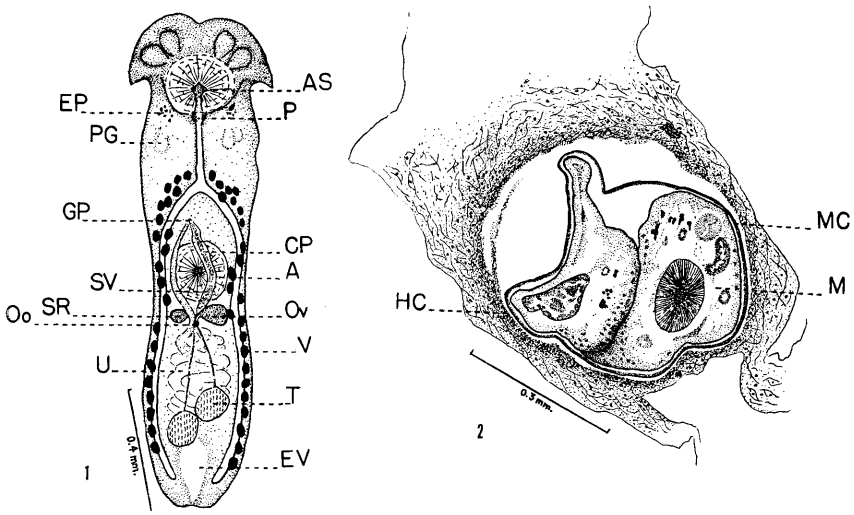

Fig. 1. Metacercaria of *Crepidostomum cornutum* (Osborn, 1903). Camera lucida drawing.

Fig. 2. Section through the metacercarial cyst of *C. cornutum*. Camera lucida drawing.

A = acetabulum	HC = host cyst wall	PG = penetration gland
AS = anterior sucker	M = metacercaria	SR = seminal receptacle
CP = cirrus pouch	MC = metacercarial cyst wall	SV = seminal vesicle
EP = eyespot pigment	Oo = oötype	T = testis
EV = excretory vesicle	Ov = ovary	U = uterus
GP = genital pore	P = pharynx	V = vitelline follicle

Although the progenic metacercariae bear what appear to be normal eggs Ameel (1937) stated "I twice attempted unsuccessfully to incubate eggs laid within the cyst. This experiment indicates that the eggs produced by the metacercariae are not fertile." This suggests that the form found in the crayfish is truly the metacercarial stage and the fish host is necessary for the completion of the life-cycle.

The disappearance of the eye spots, penetration glands and stylet occurs during the development of the metacercaria. Non-fertile eggs, less developed vitellaria, and an extremely large excretory vesicle characterize the metacercaria.

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Experimental Studies on the Biology of *Heterophyes aequalis* Looss, 1902, in Egypt*

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Although Looss (1899) was of the opinion that some species of trematodes did not parasitize two different major host groups, e.g., birds and mammals, more recent studies have shown that many trematodes, especially members of the Heterophyidae, demonstrate only moderate specificity for their definitive hosts. Specificity of this group for the molluscan hosts seems to be more pronounced. However, this assumption has not been adequately substantiated on an experimental basis. Little information exists on the specificity of the heterophyids for fish which serve as the second intermediate hosts. The fact that some of the fishes which serve in the capacity of secondary hosts may migrate from the location of infection in brackish water into fresh or even marine waters may be a real barrier for completion of the life cycle. At least, it presents a point of considerable biological and, perhaps epidemiological interest.

The biological aspects of these relationships, i.e., the association of fresh and brackish waters, and marine habitats to trematode parasites and hosts have been discussed in some detail by Stunkard (1930) and Stunkard and Shaw (1931). As a matter of fact, Stunkard considered information relating to the ability of marine cercariae to live in dilutions of fresh water, the migration of hosts in aquatic systems, etc., as significant data in determining the distribution of certain parasites, in interpretation of life cycles and as a possible means for postulating ancestral relationship.

The present report presents, briefly, miscellaneous information gained incidental to survey, taxonomic and life cycle studies of heterophyid trematodes occurring in animals of Egypt.

MATERIALS AND METHODS

An examination of several thousands of *Pirenella conica* Blainville taken from the mud and silt in the shallow, brackish water of Lake Burullus revealed that over 98 per cent were infected with one or more species of heterophyid cercariae. The majority of these snails were collected at the edge of the village of El Burg near the connection of Lake Burullus with the Mediterranean. Snails from which the cercariae of *Heterophyes aequalis* were emerging were separated from those infected with other types of cercariae. Infected snails were maintained in 5-liter glass jars and in 20-liter aquaria provided with brackish water and a stratum of bottom silt from Lake Burullus. Naturally infected *Pirenella* remained alive in the laboratory for 3 to 5 months. Although a number of snails died, perhaps for the want of optimal food, some snails seemed to feed upon the stratum of silt or on decaying leaves of boiled lettuce or on small samples of formed human feces placed in the water. Maximum aeration was essential. Snails were transferred

*The opinions and assertions contained herein are those of the author and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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into finger bowls with ½ inch of Lake Burullus water to allow collection of cercariae.

Three species of fish, *Gambusia affinis* (cyprinodont), *Tilapia* sp, (cichlid) and *Barbus* sp. (cyprinid) were used as experimental, second intermediate hosts. The *Gambusia* were bred in a small pool at the laboratory. *Tilapia* and *Barbus* were seined from a small isolated arm of an irrigation system near Cairo. All fishes were maintained in the same pool for several months before use. Just prior to exposure approximately ⅓ of *Tilapia* and *Barbus* were sacrificed and examined for the possible occurrence of metacercariae resulting from natural infection. All were negative. These fresh-water fishes were conditioned to brackish water from Lake Burullus by gradually increasing the salinity of well aerated water over a period of 4 to 6 hours and after an exposure to cercariae for 30 minutes, were reconditioned to fresh water. Fish were exposed individually to counted numbers of cercariae placed in ½ to 1 inch of water in finger bowls or crystallizing dishes. *Gambusia* easily withstood this conditioning, but *Tilapia* were obviously affected by the Lake Burullus water but the majority survived. *Barbus* could not tolerate for more than a few minutes water consisting of more than 80 per cent Lake Burullus water. Approximately half of the latter died during the exposure procedures.

Laboratory raised kittens, white mice and rats, guinea pigs, rabbits and jerds (*Meriones shawi*) captured on the desert were used as definitive hosts. Metacercariae teased from the musculature of laboratory-infected fish were given to the definitive hosts by mouth in a few drops of water. Hosts were usually necropsied 10 to 18 days after infection.

In order to test the viability and longevity of cercariae in dilutions of brackish water, pond water was added to water from Lake Burullus. Observations were made on 30 to 40 cercariae placed in 25 ml. of water in 2½ inch stender dishes. Water temperatures ranged from 21° to 26° C.

OBSERVATIONS AND RESULTS

CERCARIAL EMERGENCE—There was considerable variation in the time of greatest emergence and in the numbers of cercariae produced by 10 snails which were observed daily for a period of two weeks. Cercariae first emerged from the isolated snails between 8 a.m. and 9 a.m., the number increasing until the majority emerged between 11 a.m. and 2 p.m. Occasionally there was a second upswing in cercarial emergence at 4 p.m. or 5 p.m. In contrast to earlier experience by Kuntz (1947) with *Schistosoma mansoni* an elevation of water temperature did not always elicit an additional or increased emergence of cercariae. Although there was a marked fluctuation in emergence of *Heterophyes aequalis* during the first 6 to 8 days, a few cercariae escaped from all snails almost every day. Irregularity of emergence and reduction in numbers of cercariae became more apparent the longer the snails were held in the laboratory. The total number of cercariae passed per snail per day seldom exceeded 300 and frequently fell below 75. Infected *Biomphalaria* under identical conditions at the same time produced several times as many cercariae of *Schistosoma mansoni*. The total count for cercariae of *H. aequalis* for the two week period ranged from 106 to 1989 per snail. These totals seem rather low in view of the fact that some of the snails crushed at the end of observation period contained numerous rediae, some of which possessed 30 to 40 cercarial embryos. Although the counts of emerged cercariae began only 1 or 2 days after *Pirenella* had been removed from their natural habitat, it seems possible that the unsatisfactory conditions in the aquaria, including

a lack of optimal food, may have accounted for drastic reduction in cercarial emergence.

Approximately 48 per cent of the *Pirenella* died by the end of the first month in the laboratory. Thereafter the daily death rates were lower, but the numbers of cercariae emerging dropped to near nil. Some snails produced only 3 or 4 cercariae per day, then there would be periods of 4 to 6 days with an absence of cercariae. At the time of collection at El Burg approximately 98 per cent of 4500 *Pirenella* were infected with *H. aequalis* and/or other heterophyids. Four to six weeks later 60 snails were chosen at random from the laboratory aquaria and isolated for cercarial emergence. Only 4 of the 60 snails produced limited numbers of cercariae during a 3 day period. However, upon crushing these molluscs 58 of the 60 snails contained developmental stages of heterophyids. After 14 to 16 weeks in the laboratory 37 of 50 snails taken at random from the surviving stock were negative when crushed. Positive snails contained limited quantities of large, sacculate, mother rediae with limited numbers of cercarial embryos. Some of these were producing both redial and cercarial embryos (Kuntz and Chandler, 1956b).

LONGEVITY OF CERCARIAE IN DILUTIONS OF BRACKISH WATER—Table 1 gives pertinent information indicating in general the percentage mortality of cercariae of *H. aequalis* in brackish and fresh water and in two intermediate dilutions. Although it is known that this parasite withstands a considerable seasonal range in salinity in its natural habitat, it is surprising that an abrupt change to equal parts of brackish and fresh water exerts only a nominal detrimental effect. These cercariae are obviously affected long before death and lie almost motionless on the bottom of observation dishes. However, they respond to gentle probing. Even the transfer of cercariae from brackish Lake Burullus water into fresh water does not drastically affect these larval trematodes until the end of 6 to 8 hours, although they become obviously sluggish in their movements after 5 hours. Some cercariae, although inactive, lived as many as 30 hours. Stunkard (1930) described essentially the same effects of equal parts of marine and fresh water on the cercariae of the heterophyid, *Cryptocotyle lingua,* but Stunkard and Shaw (1931) showed that marine cercariae are affected almost immediately after introduction into fresh water and die in one hour.

PARASITE—FISH HOST RELATIONSHIPS—*Gambusia, Tilapia* and *Barbus* were attacked vigorously by the cercariae of *H. aequalis* in brackish water and

Table 1. Percentage mortality among cercariae of *Heterophyes aequalis* in brackish water from natural habitat and in dilutions of brackish and fresh water.

Time from beginning of observation (hours)	Water type:			
	Brackish*	Brackish : Fresh 1 : 1	Brackish : Fresh 3 : 1	Fresh
6	0	0	0	8
12	0	12	26	81
24	19**	24	45	98
36	26	52	51	100
48	84	82	84	----
60	98	100	99	----

* Brackish water from Lake Burullus.
** Percentage mortality expressed as nearest whole number.

there was no apparent reduction in their aggressiveness in a few instances in which cercariae and fish were placed in equal parts of brackish and fresh water. All actively swimming cercariae contacted the fish host and the majority made entry into the musculature of the body by way of the under side of, or between the scales. Approximately 85 per cent of parasites could be accounted for a day or two after each fish had been exposed to 20 to 30 cercariae. Only a very few parasites encysted in the body cavity. Some were easily detected in living *Gambusia* under the dissecting scope with use of strong light. The majority of metacercariae removed from *Gambusia* 3 to 6 days after exposure were living and active. Greater numbers of cysts in *Tilapia* and over 60 per cent of those removed from the musculature of *Barbus* apparently were nonviable. The latter is interpreted as an indication of incompatability of parasite and host. This assumption was further supported in the laboratory by the fact that kittens fed metacercariae from *Tilapia* produced fewer adult worms than kittens infected with the larval stages of *H. aequalis* from *Gambusia*. Kittens fed metacercariae from several *Barbus* produced no adult parasites. Obviously it would be desirable to compare these results with those which would be obtained in the experimental infection of the natural second intermediate hosts, i.e., species of *Mugil*.

Counted numbers of metacercariae from the musculature of *Gambusia* and *Tilapia* were fed to kittens. Approximately 60 per cent of the encysted trematodes from *Gambusia* were recovered as adults. This number dropped to near 27 per cent for parasites developing from metacercariae originating from *Tilapia*. These data, in general, substantiate the observations on viability of metacercariae from the different fish hosts as given above. This is an indication of host incompatability but admittedly is not a critical evaluation of parasite viability in the second intermediate host and/or survival in the final host, since parasites as small as *Heterophyes* can be easily overlooked or lost in the process of examination.

Additional experiments designed for the purpose, indicated that there may be a time factor associated with successful completion of the life cycle of *H. aequalis*. Only 4 of 35 *Gambusia* possessed metacercariae in the musculature 5 to 7 weeks after exposure to cercariae, whereas all of 32 *Gambusia* examined within a week of infection had encysted stages. Reduction in metacercarial infection over a period of several weeks may suggest that the larval stages are only transitory when residing in unnatural or non-conventional fish hosts.

PARASITE-DEFINITIVE HOST RELATIONSHIPS—Different species of heterophyids have been described as showing very little specificity for their definitive hosts. The present study though limited in scope does not entirely support this thesis. Adult worms developed in all of 11 cats 6 dogs, and 2 rabbits fed metacercariae from *Gambusia*. However, only 2 of 6 albino rats, none of several white mice, and none of the 3 jerds (*Meriones shawi*) were infected. Possibly the absence of parasites in some of these animals is an indication of the transitory nature of infection in the definitive hosts since some of these rodents were autopsied 8 to 15 days later than cats and dogs infected on the same date and from the same stock of metacercariae. Another indication that rodents may be poor hosts for this trematode is borne out by the fact that heterophyids were absent in a dozen or more wild rats *(Rattus)* trapped in the homes and in fish warehouses at El Burg. These rats had ample opportunity to feed upon scraps of infected fish, including *Mugil,* the natural host, which frequently contains numerous encysted heterophyids.

GENERAL DISCUSSION

Observations in the field, surveys based on a study of fecal samples from peoples of the villages bordering the brackish water lakes in Lower Egypt, examination of numerous hosts for helminth parasites and a cursory study of cercariae in molluscs indicate that there is a rather extensive heterophyid complex, part of which may be of direct concern to man in Egypt. The extremely high incidence (98 per cent or more) of heterophyid cercariae emerging from *Pirenella* is evidence of an unusual parasitological situation. Khalil (1933) recognized at least 4 types of lophocercous cercariae in his study of *Pirenella* in Lake Manzala. A similiar finding was made in the present and related studies in which one type of cercaria was shown to be the larval stage of *Heterophyes aequalis,* (Kuntz and Chandler, 1956a) and another the larval stage of *Stictodora tridactyla,* (Martin and Kuntz, 1955). Infection of *Gambusia* with several types of cercariae from *Pirenella* and subsequent feeding of encysted stage to kittens produced several heterophyids including *Haplorchis.* Cercariae resembling those of *Heterophyes aequalis* and other heterophyids in *Pirenella* collected on the Lake Burullus side of El Burg were found also in the marine snail *Littorina punctata* (Gmelin) taken from the Mediterranean on the opposite side of the village.

It is generally assumed that certain of the heterophyids, as well as some opisthorchiids demonstrate only moderate specificity for their second intermediate (fish) and definitive hosts. In the case of fish hosts for *H. heterophyes,* Khalil (1937) learned from his work in Lower Egypt that two species of *Tilapia* in Lake Manzala were much less heavily infected than *Mugil,* the host of preference. Witenberg (1929) found the metacercariae of *Heterophyes* to be rare in species of fish other than *Mugil* in Palestine. More recently Wells (1956) in a survey of fish intermediate hosts of *Heterophyes* in Lakes Burullus, Idku, Manzala and Qarun made similar observations and found two additional genera (*Sciaena* and *Solea*) with heterophyid metacercariae. Both of the latter were less heavily infected than several species of *Mugil.* Furthermore, the work of Komiya and Tajami (1940) in China has indicated that *Clonorchis sinensis* displays only moderate specificity for fish hosts since 8 of 17 species examned from a given area harbored metacercariae. Although a number of fishes may be listed as second hosts for different species of trematodes it is difficult to evaluate, without experimental confirmation, which hosts really serve in the capacity of effective carriers in the life cycle.

Similarly, our knowledge of parasite-definitive host relationships is very superficial and information available is conflicting. Data obtained from animal surveys in Egypt indicate that the heterophyids show only moderate specificity for their definitive hosts since *H. aequalis* was found in naturally infected cats, dogs and occasionally in birds, e.g., the kite, *Milvus migrans,* and *Haplorchis pumilio* (Looss, 1896) was found in cats, dogs, foxes *(Vulpes),* shrews *(Crocidura olivieri* Lesson) and in some kites. As intimated by other investigators, possibly the infection of unnatural hosts, e.g., birds, by parasites which usually are found in mammals is only transitory in nature. A parallel relationship exists with *Clonorchis sinesis,* a parasite of man and mammals. Even though the domestic duck *(Anas domestica)* and the night heron *(Nycticorax nycticorax)* have been found naturally infected with this parasite Komiya and Kondo (1951) have demonstrated that the domestic duck is not a suitable definitive host. Or as Wiley and Stunkard (1942) have indicated for *Cryptocotyle lingua,* possibly certain individuals of a species may be susceptible while others are refractory.

Although it is realized that the element of time may play a basic role in the finding of heterophyids in the second or definitive hosts it is possible that unsatisfactory host-parasite relationships, similar to those given above, account for the absence of *H. aequalis* in a number of rodents exposed to infection in this study. In this series of feedings the rodents without infections were autopsied 2 to 3 weeks later than cats possessing numerous *H. aequalis* and infected on the same date from a common stock of metacercariae.

This presentation of miscellaneous information points to the fact that an interesting heterophyid parasite problem exists in Egypt, especially in the region where man and animals have access to fishes originating from the brackish water lakes along the Mediterranean. The biology, including life cycles, parasite-fish host and parasite-definitive host relationships and the effects of aquatic systems with different salinities upon the heterophyids is only superficially known.

SUMMARY

The emergence of cercariae of *Heterophyes aequalis* from *Pirenella conica* was variable and cercarial production was decidedly reduced a few days after the snail host was removed from its natural habitat. Cercariae were not affected for some time after introduction into mixtures of fresh and brackish water in ratios of 1:1 or 3:1. Even in fresh water the cercariae were not drastically affected before the end of 6 to 8 hours. *Tilapia* and *Barbus* although susceptible to parasite invasion were much less suitable than *Gambusia* as experimental, second intermediate hosts. This quality was further demonstrated by experimental feedings in which cats and dogs were given encysted *H. aequalis* from different fish hosts. Metacercariae from experimentally infected *Gambusia* produced more adult worms in definitive hosts than metacercariae from *Tilapia*, while metacercariae from *Barbus* yielded no adults. Metacercariae in *Gambusia* were reduced in number as the period of infection was prolonged. Cats and dogs were good definitive hosts while rodents were poor or only mediocre.

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A note on the specific identity of *Protostrongylus frosti* Honess, 1942

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Honess (1942) described as *Protostrongylus frosti* n. sp. a lungworm obtained from the parenchyma of the lungs of Bighorn sheep, *Ovis canadensis*, in the area of the Gros Ventre River, Teton County, and the North Fork of the Shoshone River, Park County, Wyoming. Dougherty and Goble (1946) in their synopsis of the subfamily Protostrongylinae, tentatively placed *P. frosti* in synonymy with *P. stilesi* Dikmans, 1931, with the following note: "Dr. G. Dikmans reports (*in litt.*) that specimens referred to the Zoological Division of the U. S. Bureau of Animal Industry from Honess' study were identified as *P. rushi* and *P. stilesi* although later reported as *P. rushi* and *P. frosti* by Honess (1942). However, one male specimen in the material at the Bureau appears to be a distinct species, but it is in a permanent mount that precludes complete study. Although in Honess's description of *P. frosti* there is nothing to distinguish it clearly from *P. stilesi*, we place it only tentatively in synonymy with the latter. It seems probable to us that *P. frosti* is not distinct from *P. stilesi* and that the odd male belongs to another species not described or reported by Honess."

Dougherty (1951) reported that the following statements in the above-mentioned note were inaccurate: "Specimens referred to the Zoological Division of the U. S. Bureau of Animal Industry from Honess's study were identified as *P. rushi* and *P. stilesi* although later reported as *P. rushi* and *P. frosti* by Honess (1942)." Also "that the odd male belongs to another species not described or reported by Honess (1942)."

These statements were inaccurate because the specimens involved in Honess' study and upon which he based his description of *P. frosti* and his report of *P. rushi* had, with one exception, never been submitted to the Zoological Division of the Bureau of Animal Industry for examination. The exception, the odd male of Dougherty and Goble's 1946 note, was the original

*I wish to express my appreciation to Mr. John T. Lucker and Mrs. M. B. Chitwood, Parasite Laboratory, Animal Disease and Parasite Research Division, Beltsville, Maryland, for assistance in the preparation of this paper. Mr. Lucker prepared figs. E, F, and D of fig. E, of the accompanying illustrations. Mrs. Chitwood inked all the figures.

specimen upon which the description of the male of *P. frosti* was based, in part. The inaccuracies mentioned by Dougherty (1951) were due to a misunderstanding. The following account of the sequence of events that culminated in the publication of Honess' 1942 paper is offered for the purpose of clearing up that misunderstanding and setting the record straight.

In the early part of 1941 Mr. Henry Huizinga, at that time a member of the staff of the Wyoming Agricultural Experiment Station in place of Mr. Honess, who was on temporary leave of absence from the Station, sent to the then Zoological Division, Bureau of Animal Industry, U. S. D. A., now known as the Parasite Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, some mounted specimens of lungworms collected from Bighorn sheep, *Ovis canadensis,* in Wyoming. I examined those specimens and under date of May 6, 1941, reported to Mr. Huizinga, that, with one exception, the specimens had been identified as *P. stilesi* and *P. rushi.*

With reference to the one exception it was suggested that it be studied carefully and compared with other known and described species of *Protostrongylus* because *"it may be a new species."* All the specimens, including the one that had been designated as probably a new species, were returned to Mr. Huizinga at that time. Mr. Honess returned to the Agricultural Experiment Station in the latter part of 1941, and Mr. Huizinga transferred to the Department of Zoology, University of Wyoming. Mr. Honess found the above-mentioned specimens in his laboratory but he was not told that they had been sent to Washington for identification and returned. He noted that one specimen had been marked "may be a new species" and, under the circumstances, naturally assumed that the specimen had been so designated by Mr. Huizinga. It was on this specimen and other similar specimens collected by him from Bighorn sheep in Wyoming, that Honess based his description of *P. frosti.*

With the exception of the specimen marked "may be a new species" Honess apparently did not examine the specimens that had been sent to Washington for identification. It may reasonably be assumed that pressure of work incident to his then imminent entry into the military service, prevented that examination. It was not until his return from military service in 1946, and after reading Dougherty and Goble's comments on his description of *P. frosti* that he learned that Mr. Huizinga had sent specimens of lungworms collected from *Ovis canadensis* in Wyoming to Washington for identification. So much for the misunderstanding that Honess identified as *P. rushi* and *P. frosti* specimens that had previously been identified as *P. rushi* and *P. stilesi.* Except for one specimen the identifications were made on entirely different lots of specimens.

Dougherty (1951) while admitting that some of the statements of Dougherty and Goble's note of 1946 were inaccurate, maintained, nevertheless, that "the most important comment by us, 'in Honess' description of *P. frosti* there is nothing to distinguish it clearly from *P. stilesi,*' is still true." He stated furthermore that Honess' description and *figures* did not permit a satisfactory understanding of *P. frosti.* These statements are not quite correct. It is true that figure 6 of Honess' (1942) paper does not permit a clear differentiation of *P. frosti* from other species of *Protostrongylus,* but figure 7, unfortunately presented upside-down or reversed, presents structures not figured for any other species of this genus. The photomicrograph presented in figure 7 of Honess' (1942) paper could not have been made from any male

specimen of *P. stilesi.* Furthermore the female of *P. frosti* as pictured by Honess (1942) is without a provagina, whereas the female of *P. stilesi* is characterized by the presence of a well-developed and prominent provagina. While therefore Honess' description and *figures* may not permit a completely satisfactory understanding of *P. frosti,* they do permit a clear differentiation from *P. stilesi* with which it has been synonymized.

In placing *P. frosti* only tentatively in synonymy with *P. stilesi* Dougherty and Goble (1946) left the question of the specific identity of *P. frosti* for further examination. Dougherty (1951) noted that *P. frosti* could probably be validated on the basis of the type specimens.

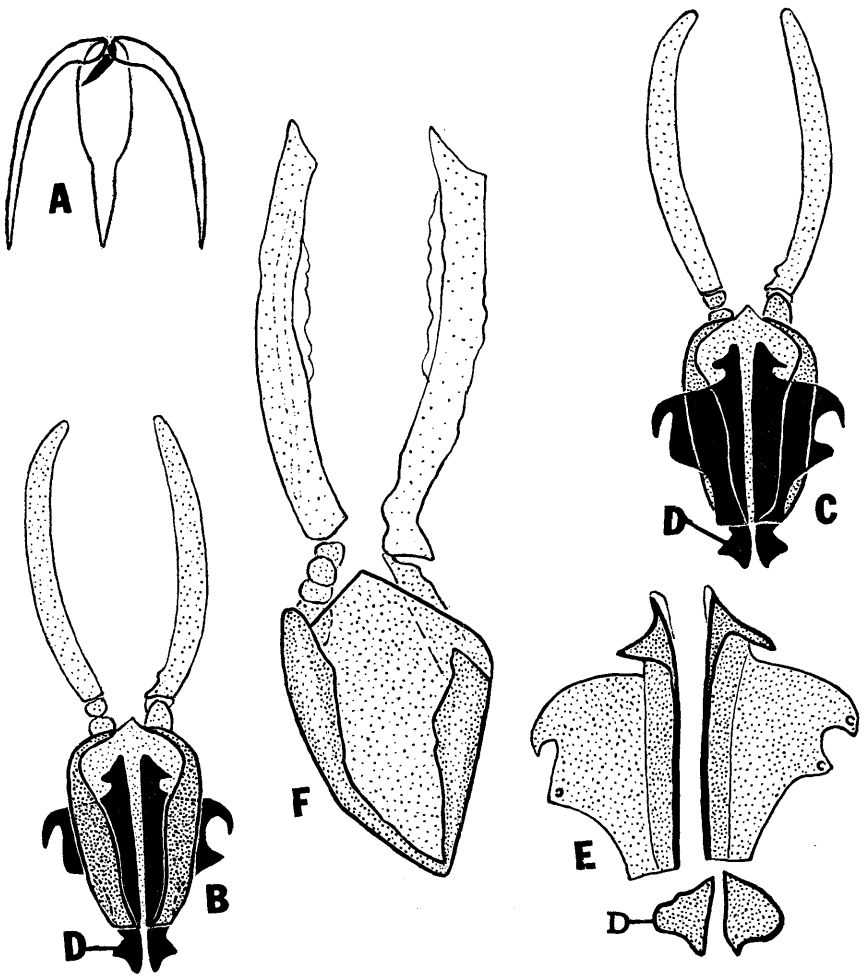
Having been interested in the lungworms of wild ruminants of North America for quite a number of years, I decided to reexamine the type specimens of *P. frosti* in order to determine, (1) whether I was or was not in error in originally designating as probably a new species the lungworm later described as *P. frosti* n. sp., and (2) to determine whether *P. frosti* is a valid species. As noted by Dougherty (1951) the type specimens of *P. frosti* are deposited as specimens no. 36852 in the U. S. National Museum Helminthological Collection. This collection is maintained at the Parasite Laboratory, Animal Disease and Parasite Research Division, Agrcultural Research Center, Beltsville, Maryland.

Through the courtesy of that laboratory I have had the opportunity to study the above named type specimens. They are, unfortunately, permanently mounted in Canada balsam, and it is therefore impossible to make as detailed an examination as desirable. However, the limited examination possible has shown that *P. frosti* differs from all other species of *Protostrongylus* reported from North America, and from all the species of this genus figured by Skrjabin and coworkers (1952) and should therefore be considered a valid species. *P. frosti* differs from other species of the genus *Protostrongylus* mainly in the structure and shape of the crura of the gubernaculum. (Schulz, Orlow and Kutass (1933) consider the structures found in male members of the genus *Protostrongylus,* which have been called unpaired accessory piece, paired accessory pieces and telamon by other authors, to be one unit, the gubernaculum. This gubernaculum is divided into three parts, the capitulum or head (unpaired accessory piece), the corpus or body (paired accessory pieces) and the crura or legs (telamon of some authors).

For the purpose of description the crura of *P. frosti* may be considered as consisting of two parts, a dorsal part and a ventral part, figs. F and E. The dorsal part or crural shafts curve ventrally from their proximal origins to their distal ends, and terminate in more or less triangular or heart-shaped tips, fig. D. There appears to be a lightly sclerotized membrane or plate between the crural shafts, fig. F. Ventral to the shafts and extending from their tips nearly to their proximal origins there are two plates called "lateral flanges" by Honess (1942). In the type specimen these flanges are somewhat ridged along their internal or medial margins. They terminate proximally in two prongs, one directed anteriorly and the other laterally, fig. E. Although Honess states that these flanges may or may not have hooks on their lateral edges, in the type specimen the flanges bear two hooks at and near their widest points. They are thus quite similar to the flanges shown in fig. 7 of Honess' paper. With only one specimen available for examination, it is rather difficult to determine whether the flanges should be considered as ventro-lateral alae attached to the crura, or as separate structures accessory to the crura and intimately associated with them. Only a careful study of

unmounted specimens, if and when they become available, can settle that question. (The specimens used by Honess in his study of *P. frosti* were lost during the war). For the present the shape and size of these flanges permit a ready differentiation of *P. frosti* from other species of *Protostrongylus,* and its establishment as a valid species of that genus.

Skrjabin and coworkers (1952) have divided the genus *Protostrongylus* into four subgenera mainly on the basis of conformation of the crura of the gubernaculum. They provided the following key to these subgenera:



Protostrongylus frosti. Gubernaculum.

A. Capitulum; B. Corpus and crura, showing dorsal shafts of crura; C. Corpus and crura, showing flanges of crura; D. Terminal parts of dorsal shafts of crura. E. Ventral flanges of crura; F. Corpus and dorsal parts of crura. Figs. A, B, C, and D of B and C, Schematic.

KEY TO THE SUBGENERA OF THE GENUS *Protostrongylus* KAMENSKI, 1905.
(Translated from the Russian by Dr. E. C. Dougherty)

1 (2) Externo-dorsal ray with short (in the form of a prominence) external branch, or else ordinary. Distal ends of spongy shaft of spicules in the form of a trident or different configuration. Crura of gubernaculum grown together with each other in their proximal ends without suture or division. Parasites of ruminants ..... Subgenus *Skrjabinostrongylus* Boev, 1950

2 (1) Externo-dorsal ray ordinary. Distal ends of spongy spicular shaft of different configuration. Crura of gubernaculum divided or grown together proximally; in the latter case, as a rule, suture clearly evident. ..... Parasites of ruminants or leporids.

3 (4) Crura with 2-3 strong, sharp, hooklike (more often) or rounded outgrowths on distal ends. Provagina well-developed, cucullate. Parasites of ruminants (Caprovinae) ..... Subgenus *Davtianostrongylus* Boev, 1950

4 (3) Crura of gubernaculum with row of tubercles or denticles (more than three) along outer or inner edge, or completely smooth ..... Parasites of ruminants and leporids

5 (6) Corpus and crura of gubernaculum usually with pellucid verrucae. Margins of crura, especially in distal half, tubercled or dentate ..... Subgenus *Kochostrongylus*, n. subg.

6 (5) Corpus and crura of gubernaculum without verrucae. Margins of crura smooth ..... Subgenus *Protostrongylus* (Kamensky, 1905)

P. frosti Honess, 1942, does not fit into any of these subgenera, but because of the paucity of material available for study, the erection of a new subgenus does not appear to be justified at the present time. It seems desirable to leave *P. frosti* in the genus *Protostrongylus sensu lato*, until such time as additional material makes a more detailed study possible.

It is not the purpose of the present paper to redescribe *P. frosti* completely, but rather to point out the principal feature in which it differs from other species of the genus *Protostrongylus*. Honess' (1942) paper should therefore be consulted for details as to measurements, etc.

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Anthelmintic Activity of the Fruits of *Diospyros mollis* (Maklua) and Tests for Activity of Other Persimmons

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Sadun and Vajrasthira (1952, 1954) reported on the efficacy of fresh green Maklua berries for the removal of hookworm in more than 100 persons. These berries, which are employed as an anthelmintic in Thailand, are the fruits of the tree *Diospyros mollis*. The manner in which they are used was described by Sadun and Vajrasthira (1954), who found that a single dose of Maklua was more effective than a standard dose of hexylresorcinol for eliminating hookworm infection. The feces of 6 out of 8 persons with *Ascaris lumbricoides* infections became negative for eggs of this parasite after treatment. No toxic reactions to the drug were observed. These workers mentioned that only a mild drowsiness was experienced in man following ingestion of twice the maximum therapeutic dose of Maklua berries.

It was noted by Sadun and Vajrasthira (1954) that Saibamroong had found in 1936 that the fresh green Maklua fruits were very active against *Ancylostoma caninum* in the dog. They further reported that Saibamroong and Luang-Li had given dogs the equivalent of 10 times the maximum therapeutic dose without observing any clinical or gross pathological signs except a mild drowsiness, and that Netraviseth in 1938 prepared a powder from Maklua that was active when fed to experimentally-infected dogs. However, the active principle of the powder as well as that of the berries was unstable and lost its therapeutic action after prolonged exposure to air.

At the suggestion of the Mutual Security Administration Mission in Thailand and of the Division of International Health, U. S. Public Health Service, some critical tests on the anthelmintic activity and toxicity of Maklua were undertaken. Since Maklua is a persimmon, it was decided to determine whether other persimmon fruits were active also. At one time the Japanese apparently used the green pulp of *D. kaki* as a purge and as an anthelmintic. Tsukamoto (1902) isolated the chemical compound "Shibuol" from *D. kaki* and showed that this may be a derivative of gallic acid. Sakuri and Tanabe (1949) found that lower gallic esters possess some activity against the parasite *A. lumbricoides* in humans without evidence of toxicity. In the present study, some gallic acid esters were also tested for anthelmintic effect in animals.

MATERIALS AND METHODS

Two lots of fresh green Maklua berries were received by air from Thailand through the courtesy of Dr. E. Sadun with the Mutual Security Mission in that country. The berries in one lot were vacuum packed in tightly-stoppered glass jars. These latter fruits were received in good condition and were used for most of the tests reported here. The Division of Plant Exploration and

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Introduction,* U. S. Department of Agriculture, furnished fresh green fruits of *D. ebenaster* and semi-ripe fruits of *D. kaki* which had been grown in this country. Samples of the green fruit of *D. virginiana* were collected in nearby Virginia.

All the fruits were utilized as soon after receipt as possible. When storage was necessary, they were kept in a deep-freeze. The seeds were removed and the rest of the fruit, pulp and/or skin was fed to animals before their daily meal. The Maklua were extracted with either acetone, water, or methanol for 24 hours in a large Soxhlet apparatus. The residues as well as the evaporated extracts were also administered to animals. The dogs studied were usually young animals experimentally infected with *Ancylostoma caninum* or naturally infected with *Toxocara canis* and other intestinal helminths. Doses of the test materials were usually given on consecutive days. Following treatment, the dogs were kept on a diet of milk. All feces were collected and searched for worm parasites. Four days after treatment was completed, the animals were killed and the gastrointestinal tract was examined for worms and evidence of gross pathology. A limted number of tests were also conducted to determine whether the fruit Maklua was active against experimental tapeworm (*Hymenolepis nana*) and schistosome (*Schistosoma mansoni*) infections in mice.

RESULTS

The table summarizes the results obtained with each of the test regimens. Following treatment with a total of 9.6 gm. of green pulp of Maklua/kg. body weight given in 3 daily doses, dog No. 1 passed 6 *T. canis*. However, 18 of these roundworms were found in the intestine at autopsy. After the second dog received a total of 4.6 gm. of the Maklua pulp/kg. in 2 daily doses, it passed 1 ascarid and 27 hookworms. In this case 28 hookworms were found in the intestine at autopsy. All the hookworms expelled had a blistered appearance similar to the effect of hexylresorcinol on *Ascaris lumbricoides*, as mentioned by Brown (1937). None of the extracts or residues of Maklua exhibited anthelmintic activity in animals No. 3 through No. 8. The gross pathology seen in the animals at autopsy included a marked inflammation of the colon of dog No. 2, small hemorrhages in the colon of dog No. 3, and extensive inflammation of small intestines of dogs No. 4 and No. 5.

Dog. No. 9 was given a single dose of 4.5 gm. (0.5 gm./kg.) of the fresh pulp of the fruit of *D. ebenaster*. None of the hookworms, tapeworms, or whipworms harbored by this animal were removed as a result of the treatment, although the animal suffered a severe reaction as evidenced by a profound retching. At autopsy the small intestine was inflamed and there were numerous gastric hemorrhages.

Because the *D. kaki* fruits were received in a semi-ripened state the skins were given to only one animal—dog No. 10, in a single dose of 1 gm./kg. There was indication of some anthelmintic activity; 4 hookworms and 2 *T. canis* were passed and 12 more hookworms were found in the colon at autopsy.

Following the ingestion of 1 gm. dry skin of the *D. virginiana* per kg. body weight in one dose, dog No. 11 passed 3 *T. canis*. At autopsy 13 *T. canis* and 42 *A. caninum* were found. Although as much as 15.5 g. pulp of fresh, green *D. virginiana* were given per kg. (in 3 daily doses) to dog No. 12, only 3 hookworms appeared in the feces although 77 of these worms were found at autopsy along with 11 *T. canis;* the small intestine was inflamed.

*The authors wish to thank the Division of Plant Exploration and Introduction for its cooperation in this study.

A limited number of tests were run on the effects of the persimmons on other parasites. Ten grams of the pulp of Maklua were suspended in 50 ml. of water. This suspension was given intraperitoneally to mice infected with *S. mansoni* and orally to mice infected with *H. nana*. Each mouse received 0.4 ml. of the suspension daily for 5 days. No anthelmintic effect was observed in these animals. Negative results were likewise obtained when suspensions of the skins of fruits of *D. mollis, D. kaki,* and *D. virginiana* were made up in the same proportions as above and given to mice with these same worm infections.

Since a probable derivative of gallic acid had been isolated from *D. kaki* and lower esters of gallic acid have been found to have anthelmintic properties by Japanese workers, it seemed possible that the same type of compound might be the active principle in Maklua. Therefore, 5 higher esters of gallic acid were given orally to dogs with infections of intestinal helminths and intraperitoneally to mice with *S. mansoni* infection. The compounds and the doses given to the dogs were as follows: Dodecyl 2,4-dihydroxybenzoate—50 mg./kg.; Tetradecyl 3, 4-dihydroxybenzoate—40 mg./kg.; Dodecyl gallate —100 mg./kg.; Hexadecyl gallate — 60 mg./kg.; and Hexyl gallate — 200 mg./kg. A dose of 50 mg./kg. of each of the compounds was administered intraperitoneally to the mice twice daily for 5 days. No significant anthelmintic activity was observed in the animals treated with this series.

Table 1. Results of oral administration of the Maklua berry and other persimmons to dogs with helminth infections

Dog	Treatment: Substance	Total Dose (gm./kg.)	Worms*: Removed	Worms*: Retained
No. 1	Pulp of Maklua (*D. mollis*)	9.6	6 Tc	12 Tc
No. 2	Pulp of Maklua (*D. mollis*)	4.6	1 Tc	0 Tc
			27 Ac	28 Ac
No. 3	Acetone extract** (*D. mollis*)	1.0	1 Tc	11 Tc
			0 Ac	13 Ac
No. 4	Residue from acetone extract (*D. mollis*)	0.9	0 Tc	4 Tc
			1 Ac	96 Ac
No. 5	Methanol extract** (*D. mollis*)	1.1	0 Tc	3 Tc
			4 Ac	42 Ac
No. 6	Residue from methanol extract (*D. mollis*)	1.1	0 Tc	6 Tc
			1 Ac	94 Ac
No. 7	Ether extract** (*D. mollis*)†	0.2	0 Ac	9 Ac
No. 8	Water extract** (*D. mollis*)	1.9	0 Ac	6 Ac
			0 Tp	1 Tp
No. 9	Pulp of *D. ebenaster*	0.5	0 Ac	3 Ac
			0 Dc	1 Dc
			0 Tv	12 Tv
No. 10	Skin*** of *D. kaki*	1.0	2 Tc	36 Tc
			16 Ac	44 Ac
No. 11	Skin*** of *D. virginiana*	1.0	3 Tc	13 Tc
			0 Ac	42 Ac
No. 12	Pulp of *D. virginiana*	15.5	0 Tc	11 Tc
			3 Ac	77 Ac

*Ac = *Ancylostoma caninum*
Dc = *Dipylidium caninum*
Tc = *Toxocara canis*
Tv = *Trichuris vulpis*
Tp = *Taenia pisiformis*

**Evaporated to remove solvent.
***Dry.
†Supplied by Drs. Sadun and Vajrasthira.

Shikimic acid, -3,4,5-trihydroxy cyclohexene -1-carboxylic acid, a compound which is present in many plants (Hasegawa et al., 1954), was also tested for anthelmintic activity. One dose of 100 mg. shikimic acid /kg. body weight or two of 50 mg./kg. were given orally to each of 4 dogs infected with *A. caninum, Schistosoma japonicum, T. pisiformis, T. canis,* or *T. vulpis.* Following treatment, whipworms were passed by 3 dogs. However, the whipworm infections of these animals were not completely eliminated by either of the doses of skikimic acid given. No ill effects were observed following the administration of the acid. Further study of activity of this compound against whipworm infection is being undertaken.

COMMENT

It was apparent from reports of previous workers that the anthelmintic activity of the Maklua berry was greatest in fresh, green fruit. Although the fruits were carefully packed, shipped directly from Thailand by air and received here in good condition, it was possible that they had lost activity en route. This may explain why the Maklua did not appear to be as active in the present tests as in those reported by Sadun and Vajrasthira (1952, 1954). Furthermore, the partially ripened fruits of *D. kaki* were very likely not as active as the green fruits might have been. As persimmons "ripen" they undergo chemical change evident in the darkening of the Maklua berry. Furthermore, in the edible varieties like *D. kaki,* the sugar content increases and there is a loss of the astringent components, i.e., the chemical "shibuol." During the extractions carried out in this laboratory, the color of the persimmons changed and the same "inactivation" may have taken place as during the ripening of the fruit.

Recently shikimic acid has received much attention because of its possible important role in aromatic biosynthesis (Davis, 1951). Since shikimic acid is commonly found in plants, it was tested for anthelmintic acivity and the preliminary results were included in this series.

SUMMARY

The green fruits of several persimmons: *Diospyros mollis, D. Ebenaster,* and *D. virginiana,* and semi-ripe fruits of *D. kaki* were tested for anthelmintic activity. There was evidence that the pulp of fresh *D. mollis* berries (Maklua) was active against *Toxocara canis* and *Ancylostoma caninum* in the dog. However, this same material was inactive against *Schistosoma mansoni* and *Hymenolepis nana* in mice. Limited attempts to extract the active principle were unsuccessful. In preliminary tests, shikimic acid was found to be active "in vivo" against *Trichuris vulpis.*

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Thelazia platyptera, n. sp. (Nematoda: Thelaziidae) from the Eye of the Broad-Winged Hawk, *Buteo platypterus* (Vieillot, 1823).

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One female and three male specimens of a spiruroid nematode were collected from the eyes of a broad-winged hawk by R. A. Norris, Museum of Vertebrate Zoology, University of California, Berkeley, in 1952. These eyeworms, along with other parasites, were sent to one of the authors (EEW) for identification. They apparently represent a new species of the genus *Thelazia*.

Thelazia platyptera, n. sp. (All measurements in millimeters)

DESCRIPTION. Worms slender. Surface of cuticle annulated; annulations imbricated, very pronounced in anterior part of body, less so in posterior part, and becoming unrecognizable in region posterior to anal opening. Cephalic papillae arranged in two circles: An internal circle of 6 small papillae adjacent to and surrounding oral opening, and an external circle of 8 large, distinctly visible papillae near base of head, all situated at about the same level. Oral opening slightly elongated dorsoventrally; lips or labial structures absent. Buccal capsule cup-shaped, with heavily chitinized walls. Esophagus consisting of a short anterior, muscular portion and a long glandular, claviform posterior portion.

MALE. Holotype. Body 14 long and approximately 0.41 wide. Buccal capsule 0.028 long and 0.033 wide (fig. 1). Esophagus 1.037 long; anterior portion 0.177 long and 0.062 wide and posterior portion 0.86 long and 0.06 wide. Nerve ring 0.34 from anterior end of body. Spicules unequal and dissimilar (fig. 2): Left 2.69 long, acicular; right 0.183 long, stout, heavily chitinized and serrated in its proximal half. Gubernaculum 0.06 long. Tail 0.22 long. Caudal alae absent. Caudal papillae, ventral or subventral, arranged as follows: 7 preanals on left side, 8 preanals on right side, 1 single, median preanal, and 6 pairs postanal (fig. 3). Third, fourth, fifth, and sixth pairs of postanal caudal papillae closely associated in distal caudal area; tail bluntly rounded, thickly muscled near tip.

FEMALE. Allotype. Body 17 long and 0.43 wide. Buccal capsule 0.03 long and 0.035 wide. Esophagus 0.956 long; anterior portion 0.215 long and 0.075 wide and posterior portion 0.741 long and 0.096 wide. Nerve ring 0.37 from anterior end of body. Vulva 0.678 from anterior end of body and 0.361 anterior to posterior end of esophagus (fig. 4). Anal opening 0.337 from tip of tail (fig. 5). Embryonated eggs 0.045 long and 0.032 wide (fig. 6).

HOST. Broad-winged hawk, *Buteo platypterus* (Vieillot, 1823).

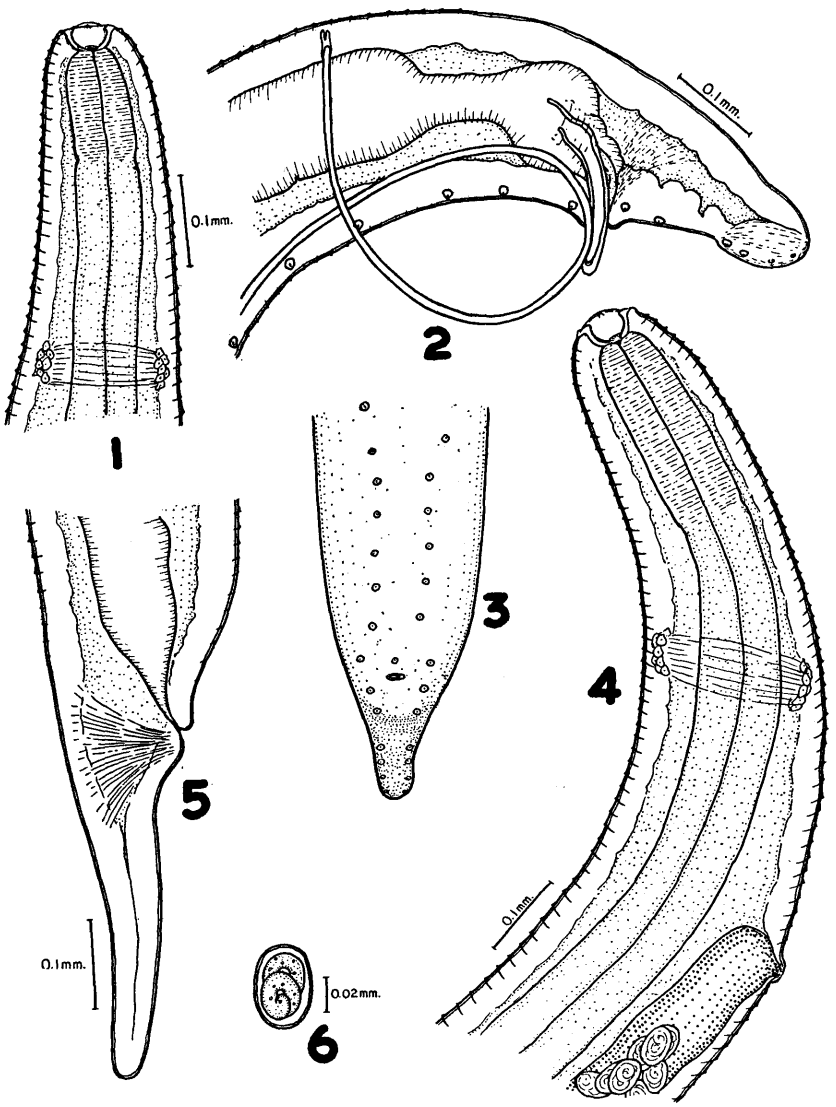
LOCATION. Eye.

LOCALITY. Tifton, Georgia, U. S. A.

TYPE SPECIMENS. Holotype and allotype (No. 47484) and paratypes (No. 47485) in U. S. National Museum Helminthological Collection.

More than one-half of the forty-odd recorded species of *Thelazia* are from avian hosts. Seven of these species—*T. anolabiata* (Molin, 1859) Railliet and Henry, 1910; *T. aquilina* Baylis, 1934; *T. buteonis* Herde, 1942; *T. campanulata* (Molin, 1838) Railliet and Henry, 1910; *T. chui* Hsü, 1935;

T. papillosa (Molin, 1860) Railliet and Henry, 1910; and *T. stereura* (Rudolphi, 1819) Railliet and Henry, 1910—have birds of the order Falconiformes as their hosts. The present species makes the eighth one described from this group of birds.



Thelazia platyptera n. sp. (Drawn with the aid of a camera lucida).

Fig. 1. Anterior end of male, lateral view., Fig. 2. Caudal end of male, lateral view., Fig. 3. Ventral view of posterior end of male. (Diagrammatic), Fig. 4. Anterior end of female, lateral view., Fig. 5. Caudal end of female, lateral view., Fig. 6. Egg.

T. platyptera, n. sp., most closely resembles *T. chui* Hsü, 1935, from an unknown species of hawk from French Indo-China. Hsü (1935) erected *T. chui* on the basis of one male specimen; no female specimen of *T. chui* has been described, so far as the writers know.

The data in the following table show that the males of *T. platyptera,* n. sp. and *T. chui* differ only slightly in body length and principal size relationships. The most obvious difference lies in the relatively greater length of the left spicule in *T. platyptera,* n. sp.

	T. chui	*T. platyptera, n. sp.*
Number of specimens measured	1	3
Body length	15.22	14-18
Body width	0.33	0.41
Buccal capsule: Length	0.028	0.026-0.028
Width	0.035	0.033-0.038
Nerve ring to anterior end	0.360	0.340
Length of esophagus	1.050	1.037-1.045
Spicules: Left	2.070	2.68-2.69
Right	0.185	0.183-0.203
Anus to tip of tail	0.215	0.210-0.222

Males of the two species differ also in the number and arrangement of the caudal papillae. In *T. chui,* 15 pairs, 6 postanal and 9 preanal pairs, of papillae, symmetrically arranged, and a single median preanal papilla are present. Instead of a total of 18 preanal papillae, *T. platyptera,* n. sp. has a total of only 15 preanal papillae, asymmetrically arranged with 7 on the left and 8 on the right side (fig. 3).

There is a possibility that the study of a larger number of specimens, of *T. chui* might reveal such a close similarity to *T. platyptera,* n. sp. that the latter would of necessity fall as a synonym of the former. However, in view of the facts known at present concerning the morphology of *T. chui,* there seems to be no alternative other than to consider *T. platyptera* as a species distinct from it.

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The Occurrence of *Echinococcus multilocularis* Leuckart, 1863, in Japan

ROBERT RAUSCH* AND JIRO YAMASHITA**

Since 1937, when alveolar hydatid disease was first recognized on the Japanese island of Rebun, 25 cases have been investigated clinically. About one per cent of the population of the island is believed to be infected (Yamashita *et al.*, 1955). Five cases also have been reported from other islands, some of these involving emigrants from Rebun (Inukai *et al.*, 1955; Yamashita, 1956).

The causative organism of this disease on Rebun has been termed *Echinococcus granulosus* (Batsch, 1786) by the Japanese workers. However, after a study of the cestode involved and a consideration of its ecology, the writers have concluded that the etiologic agent undoubtedly is *E. multilocularis* Leuckart, 1863. Evidence for this conclusion and a discussion of the problem are presented in this paper.

THE ADULT CESTODE. Few specimens of *Echinococcus* have been procured from carnivorous mammals on Rebun. Only two adult cestodes, from a dog, have been preserved. The terminal segments of these strobilae contained immature eggs. In this limited material, however, characters of diagnostic value were discernible. These cestodes have been compared by the writers with specimens of *E. multilocularis* from naturally and experimentally infected canine animals from Alaska and South Germany. The latter, provided by Dr. Hans Vogel, Institut für Schiffs- und Tropenkrankheiten, Hamburg, were reared in dogs fed larval cestodes of human origin.

The cestodes from Rebun measured 2.0 and 2.7 mm. in length. Each had three segments. The genital pore, in the mature and gravid segments, was in a relatively anterior position. The uterus was well developed in the terminal segment of each cestode, and was clearly of sacculate form. The number and distribution of the testes could not be determined.

Vogel (1955) and Rausch (1956) observed that position of the genital pore provides a reliable character for differentiating *E. multilocularis* from *E. granulosus*. The combination of characters seen in the cestodes from Rebun (genital pore in anterior position, sacculate uterus, and small size of strobila) is compatible only with *E. multilocularis*.

THE LARVAL CESTODE. Of 25 cases of alveolar hydatid disease confirmed from Rebun, 15 have terminated fatally. The alveolar structure of the larval cestode has been determined histopathologically by Japanese workers in 16 cases following surgery or autopsy (Ambo *et al.*, 1956).

For our use, Professor H. Ambo, Faculty of Medicine, Hokkaido University, kindly provided two blocks of tissue taken at the autopsy of a male patient whose death was caused by alveolar hydatid disease. The patient had lived on Rebun to the age of 16 years, and had then moved to Hokkaido. Death occurred at the age of 21 years. One of the blocks contained large numbers of larval vesicles. The second had been cut at the periphery of the zone of larval invasion, so that some uninvaded hepatic tissue was included.

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The latter was more useful, enabling comparisons to be made with sections of infected hepatic tissue from patients in South Germany and St. Lawrence Island, Alaska. The specimens from Germany were provided by Dr. Hans Vogel.

Sections stained with hematoxylin-eosin were prepared. Microscopically, the remaining hepatic tissue showed generalized cirrhosis. Hepatic cells regarded as normal were grouped mainly about the portal triads, but clusters of cells were scattered elsewhere. Degenerating hepatic cells, taking a strong eosinophilic stain, were abundant. The structural organization of the tissue had been disrupted to the extent that the hepatic lobules were no longer distinguishable. Hyperplasia of the bile ducts was not observed. Fibrous tissue was most dense in the vicinity of the larval vesicles. Here also were focal areas of leukocytic infiltration, involving lymphocytes and macrophages. Polymorphonuclear leukocytes were not numerous, and few giant cells were seen.

The area of tissue containing the larval vesicles had the aveolar appearance such as is seen in typical *E. multilocularis* infections in man (Fig. 1). The cysts were varied in size and shape, generally in close apposition. The growth of isolated, small vesicles well away from the more severely affected areas indicated an active invasion of the adjacent hepatic tissue, and the dense fibrous tissue surrounding the vesicles suggested an infection of long duration. Islets of hepatic cells "entrapped" among the cysts were undergoing degeneration. The subgerminal (laminated) membrane varied greatly in form, and in some areas its proliferation had given rise to very thick-walled alveoli lacking the germinal layer. This latter layer was usually well developed and cellular. A few scattered scolices were observed.

These sections were identical with those from patients from Germany and Alaska in important details. Expected differences in the degree and extent of the tissue reaction were noted, but the structure and characteristics of the larval vesicles corresponded exactly, and all demonstrated the extreme degree of proliferation of the subgerminal layer which appears to be peculiar to the growth of the larval *E. multilocularis* in man. Workers in Europe have had the impression that this larval cestode perhaps rarely produces scolices in man; however, scolices may occur much more frequently than realized, since they may be so few as to be overlooked unless many sections are examined. Scolices were also present in one of the sections from a patient from Germany.

The investigations on Rebun have so far not disclosed the larval cestode in mammals other than man. However, Ishino (1941) collected an infected vole, *Microtus oeconomus* Pallas, in 1935 on the island of Simushir, in the Middle Kuriles. A photograph of this vole, which is preserved in the collections of the Laboratory of Parasitology, Hokkaido University, was published by Yamashita (1956). For reasons brought out below, the finding of this animal is significant, and a brief description of it is presented below.

In this vole the liver, the primary locus of infection, was almost totally involved and macroscopically was identical with *E. multilocularis* infections seen in the same species of vole on St. Lawrence Island, Alaska. The liver contained many small vesicles, in all of which there were many scolices and great numbers of calcareous corpuscles. There was little evidence of a severe tissue reaction, and hepatic cells were lacking in the totally involved areas. The sections did not differ in any recognizable way from sections of *E. multilocularis* larvae in naturally infected voles from St. Lawrence Island.

DISCUSSION

Rebun lies about 30 miles west of the northern tip of Hokkaido Island, Japan. It is a mountainous island, with an area of about 80 square kilometers. Most of its population of about 10,000 people live in coastal villages, and gain their livelihood through fishing.

The indigenous mammals* are: two species of shrews, *Sorex unguiculatus* Dobson and S. *minutus* Linnaeus; a red-backed vole, *Clethrionomys rufocanus smithii* Thomas; and a chipmunk, *Tamias sibiricus lineatus* Siebold. Also present are rats, *Rattus rattus* Linnaeus and *R. norvegicus* Berkenhout, but the house mouse, *Mus musculus* Linnaeus, has not been introduced. A red fox, *Vulpes vulpes japonica* Gray, became established after its introduction from the Middle Kuriles in the period 1924-26 (Inukai *et al.*, 1955). The Japanese mink, *Mustela sibirica itatsi* Temminck, was introduced in 1940-44 from the island of Hokkaido.

Few domesticated animals, other than dogs, are kept on the island. In 1954, there were 20 horses, 13 swine, 3 cattle, 4 goats, and 28 sheep. In 1956, there were 8 horses, 20 swine, 3 cattle, 1 or 2 goats, and only 5 sheep, the most of the latter having been killed by dogs. Many dogs are kept as pets and are not restrained. In addition to these, about 100 feral dogs live in the more inaccessible parts of the island, but often enter the villages at night. The people also have house cats.

Of these mammals, it appears that only dogs, foxes, or cats could serve as the final host of *Echinococcus multilocularis* on Rebun. Ambo *et al.* (1954) autopsied 70 dogs on Rebun during 1953, but found none infected. In 1954, Yamashita and his co-workers made a survey of the parasites of dogs here. Of 154 autopsied, including 13 feral dogs, only two contained the adult cestodes. A higher proportion of feral animals might be infected, since they would be expected to eat any small mammals that they could capture. Dogs naturally infected with *E. multilocularis* have been found on St. Lawrence Island, Alaska, and are easily infected experimentally (Rausch and Schiller, 1956).

Foxes are much fewer in number than formerly, and none have been found infected. However, it is probable that *E. multilocularis* was introduced on Rebun through foxes brought from the Kurile Islands. The sequence of events leading to the introduction of the foxes has been reviewed by Inukai *et al.* (1955), but a brief recapitulation would seem appropriate here.

In 1916 the Russian government sent 10 arctic foxes, *Alopex lagopus* Linnaeus (also sometimes called "blue fox") to Ushishir Island, in the Kuriles, and a second lot of 20 animals was released there in 1917. The animals increased rapidly in numbers, and were soon distributed to other islands in the Kurile chain.

The foxes originated in the Komandorskii Islands, off the coast of Kamchatka. *E. multilocularis* is a common cestode on Bering Island, in the Komandorskii Islands, but it is not found on the nearby Mednyi Island (Barabash-Nikiforov, 1938; Afanas'ev, 1941). Since the previous absence of any microtine rodent or other amenable intermediate host precluded comple-

*For convenience, names of mammals are given here according to Ellerman and Morrison-Scott, *Checklist of Palaearctic and Indian Mammals*, Brit. Mus. (Nat. Hist.), London, 1951. Since Japanese mammalogists disagree with some of the opinions expressed by these workers, attention is called to Tokuda, *A Revised Monograph of the Japanese and Manchu-Korean Muridae*. Trans. Biograph. Soc. Japan, 4:1-127, 1941.

tion of its life cycle, the cestode was apparently introduced on Bering Island in about 1870, when a red-backed vole, *Clethrionomys rutilus* Pallas, was established there to provide food for the foxes (Rausch, 1952; Rausch and Schiller, 1956). We do not know if *E. multilocularis* existed in the Kuriles before introduction of the arctic foxes. Since the necessary host mammals already were present (vole and red fox), it is possible that the cestode was also there. The finding of the infected vole mentioned above confirms the presence of the cestode in the region in recent times.

Red foxes were brought from the Kuriles to Rebun Island during 1925-26, to establish a harvestable fur crop and to aid in the control of voles. The foxes multiplied rapidly, becoming abundant in the first years. After about 10 years, however, they were reduced in numbers by poaching, and possibly by disease and other factors. At the present time they are few. It appears certain that the cestode whose larva causes alveolar hydatid disease was introduced on Rebun through these foxes.

The house cat might be of importance in the epidemiology of alveolar hydatid disease on Rebun, but this has not been determined. Ambo *et al.* (1954) found one infected cat among 57 autopsied during 1953. Cats have been experimentally infected with *E. multilocularis* by Vogel (1955), and Rausch (unpublished data) infected voles with eggs obtained from experimentally infected cats. It is evident that *E. multilocularis* is capable of de-

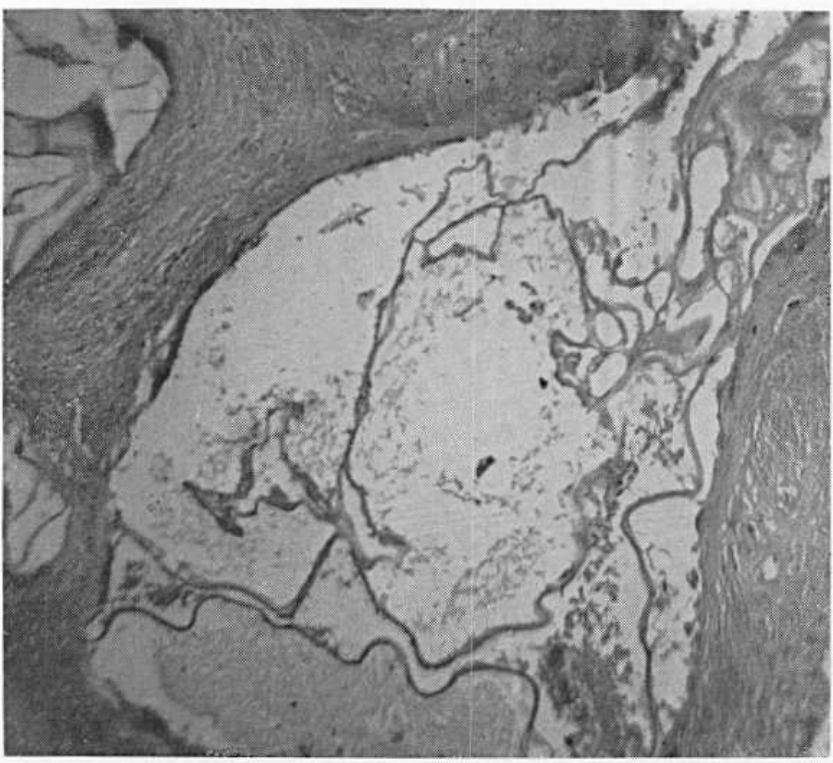

Figure 1. *Echinococcus multilocularis* infection of human liver (Rebun Island). 240 X.

velopment and production of eggs in cats, and the close association of these animals with man could have considerable epidemiological significance.

The red-backed vole is probably the intermediate host for *E. multilocularis* on Rebun. Although the species found here, *Clethrionomys rufocanus,* has not been infected experimentally, it is known that the related *C. rutilus* is an important intermediate host on Bering and St. Lawrence Islands. On Rebun, the red-backed vole is found in non-forested regions, particularly in grassy and marshy places. Here they would be especially susceptible to predation by cats.

The other small mammals on the island appear to have little or no importance. The shrews are probably susceptible to infection, as is *Sorex tundrensis* Merriam on St. Lawrence Island, but shrews are not often eaten by other mammals. Rats have been found resistant to experimental infection. We do not know if the chipmunk, a sciurid, can serve as a host to this cestode. Experimental work in Alaska suggests that sciurids are not suitable hosts (Rausch and Schiller, 1956).

In our opinion, the larger domesticated animals are not involved in the life cycle of *E. multilocularis.* They apparently are not susceptible to infection, or at best larval growth in them is not normal (Rausch and Schiller, 1956). On Rebun, the few animals examined by Yamashita have been negative. Most of the animals to be slaughtered are sent to the abbatoir at Wakkani City, on Hokkaido, and no infected animal has been observed here.

The Japanese workers maintain that human infections originate from eggs transmitted through water (Yamashita, 1956). Although the eggs of *Echinococcus* have not been identified in water tanks placed along the small streams, Ambo *et al.* (1954) recovered eggs of other species of helminths from them. It is believed that such waters might be contaminated by the feces of dogs.

The danger exists that *E. multilocularis* might also become established on Hokkaido and other islands if infected dogs are introduced from Rebun. Since it appears that potentially suitable intermediate hosts are widely distributed in the Japanese islands, the movement of dogs from Rebun should be carefully controlled. Because of the differences in ecological and faunal relationships in the three regions where *E. multilocularis* is presently being studied (Japan, Alaska, South Germany), the comparative epidemiology of this cestode should prove to be of unusual interest.

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Rate of Migration and Growth of Larval *Ascaris suum* in Baby Pigs*

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Stewart (1916 a, b, c,) was unable to produce an infection with adult ascarids when embryonated eggs of *Ascaris suum* were fed to pigs. He observed that larvae migrated from the intestine through the liver into the lungs when he fed eggs of *A. suum* to mice and rats and concluded that these rodents might serve as intermediate hosts for *A. suum*. Ransom and Foster (1920) established a direct life cycle for *A. suum*. They gave eggs to eight pigs, but only one was examined while the larvae were in the migratory phase. Martin (1926) fed infective eggs to 18 pigs in three experiments but he examined only four of these animals while the larvae were migrating.

Ransom and Cram (1921) established the route of migration of larval *A. suum* in guinea pigs and rabbits. They also observed the growth rate of the larvae within these animals. Roberts (1934) conducted the most comprehensive study of the migratory phase of *A. suum* in pigs. He fed eggs to 15 pigs and recovered larvae at intervals ranging from 22 hours up to 76 days.

The following is a report of observations of larvae collected from 34 pigs which had been fed eggs of *A. suum* to investigate the effects of migrating ascarids on the baby pig. Many of these observations agreed in general with the findings of Roberts (1934).

METHODS AND MATERIALS

The 34 pigs were Hampshires, Duroc Jerseys and Hampshire-Yorkshire crossbreds. All but four of the pigs were obtained by hysterotomy by the method of Hoerlein *et al.* (1956). By this method the baby pigs were removed from the uteri of anesthetized sows at least 110 days after breeding. These pigs were placed in an isolated room and fed cow's milk supplemented with vitamins and minerals. Pigs farrowed in this manner and deprived of colos-

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trum are entirely devoid of circulating antibodies. Helminthic infections thus are uninfluenced by antibody reactions. The remaining four pigs were farrowed normally and allowed to suckle the sow. These four pigs were infected and observed while with the sow.

The pigs were fed infective *Ascaris* eggs at two days of age. The egg suspension was placed either on the base of the tongue with a dropper pipette or directly into the stomach by means of a tube.

The eggs were obtained from the uteri of living worms and from feces of swine infected with *A. suum*. The eggs were cultured either in shallow dishes of weak formaldehyde solution or in moistened mixtures of granular bone charcoal. The cultures were maintained at 30° C. for at least 30 days before the infectiveness of the eggs was checked. The eggs were considered infective when they produced pneumonia in mice. The viability of the eggs was checked prior to each experiment by feeding a portion of them to mice.

The numbers of larvae in the livers and lungs were estimated by counting larvae in several one-half gram samples of these organs at necropsy. Each one-half gram sample was ground, suspended in water, and the larvae were observed and counted. The number of larvae in an organ was derived by multiplying the number of larvae found per gram of tissue by the weight of the entire organ.

The rate of migration of the larvae through the pig was calculated by relating the number of worms found in the liver, lungs, and small intestine and is expressed as the percentage of larvae within the organ concerned.

Part of the larvae were measured by projecting their images through a microscope onto a piece of tissue paper on a fixed glass plate where their outlines were traced, later measured and converted to millimeters. Other larvae were measured directly on the stage of the microscope by means of a calibrated ocular micrometer.

RESULTS AND DISCUSSION

Larvae are abundant in the liver within two days. A few larvae had reached the lungs of one of three pigs examined on the second day.

Four pigs were examined on the fourth day and in these, 97 per cent of the larvae were still in the liver.

By the sixth day the majority of the larvae had reached the lungs. Examination of eight pigs revealed an average of 28 per cent of the larvae still in the liver with a range of 77 to 6 per cent being found there.

An average of 27 per cent of the larvae were still in the liver on the seventh day. Three pigs were examined on the seventh day and 30, 51, and 1 per cent of the larvae were found in their livers.

Four pigs were examined on the eighth day of the migration. In two of these pigs all of the larvae had reached the lungs. Fourteen and three per cent of the larvae were found in the livers of the remaining two pigs. Larvae were found in the small intestine for the first time on the eighth day.

One larva was found in the liver of one out of three pigs examined on the ninth day. Larvae were also present in the small intestines of these pigs.

Two pigs were examined on the eleventh day of the infection. Thirty-five and forty-one per cent of the larvae had reached the small intestine of these two pigs respectively.

Larvae were found in the lungs of pigs examined on the 13th and 15th day following the feeding of eggs. However, almost all of the larvae had migrated into the small intestine by this time.

These findings are essentially in agreement with those of Roberts (1934).

He found that the number of larvae increased in the liver until three days following infection.

He did not examine animals between three and six days, which would account for his missing the build up to 97 per cent in the liver that was observed by the present authors on the fourth day. He observed that almost all of the larvae had migrated through the liver into the lungs by the sixth day. This is in agreement with the present findings; however, we observed more variation in the rate of migration on the sixth day than at any other time. Eight pigs examined six days following feeding of eggs had 6, 8, 12, 13, 21, 41, 44, and 77 per cent of the larvae still in their livers. Roberts (1934) found larvae in the liver as long as 11 days following infection. The present writers were unable to find larvae in the livers of pigs which had been infected for more than nine days.

A few of the larvae reach the lungs in a short time. Roberts (1934) found larvae in the lungs as early as 22 hours after infection and the present investigators found larvae in the lungs of two of three pigs on the second day. The number of larvae in the lungs increase until a peak is reached on about the ninth day. Symptoms of verminous pneumonia are apt to appear at about the ninth day. After this time the number in the lung diminishes; however, a few larvae were still found in the lungs on the 15th day.

Two-day-old larvae from the livers of two pigs averaged 0.33±0.019 in length (all measurements in this report are expressed in millimeters). Those from the lungs of two pigs examined on the second day were 0.30 long.

Larvae in the livers of three pigs which had been infected for four days were 0.43±0.018 long. No larvae from the lungs were measured on the fourth day.

Worms in the livers of four pigs were 0.62±0.012 on the sixth day. In the lungs, larvae from seven pigs were 0.89±0.017 on the sixth day.

Larvae from the liver of one pig were measured on the seventh day and were 0.53±0.041 long. Larvae from the lungs of three pigs were 0.95±0.039 long on the seventh day.

Larvae from the lungs were 1.54±0.025 long in three pigs examined eight days following the feeding of infective *Ascaris* eggs.

On the ninth day larvae from the lungs of five pigs were 1.42±0.026 long and in the intestine of one of these pigs they were 1.31±0.045 in length.

Worms found in the lungs of two pigs examined on the 11th day were 1.65±0.064 long while in the small intestine they were 1.87±0.062.

One pig was examined on the 13th day and larvae measuring 1.75±0.066 were found in the lungs and 1.90±0.027 in the small intestine.

On the 15th day larvae in the lungs of one pig were 2.20±0.095 and in four pigs they were 2.73±0.061 in the small intestine.

Six and seven-day-old larvae in the lungs averaged 1.4 and 1.8 times as long as larvae of the same age in the liver. On the second day, however, the lung larvae were slightly smaller than those from the liver.

Roberts (1934) is the only other investigator who has measured large numbers of larvae at varying times during their migration in the pig host. However, he apparently pooled his measurements of larvae from pig, guinea pig, and mouse hosts, and his figures tend to be somewhat smaller than ours. Thus, the average lengths of our two and four-day-old larvae from the liver and eight and ten-day-old larvae from the lungs are equal to or greater than the maximum lengths of Roberts' larvae of the same age and from the same organ.

This supports the tentative conclusion of Ransom and Foster (1920), who compared sizes of larvae from rabbits, guinea pigs, and mice. They state

that "larvae seem to grow more rapidly and to a larger size during their migration in large animals than in small ones."

Their conclusion is further supported by a comparison of our larvae from pigs with the larvae obtained from rabbits, guinea pigs and mice by Ransom and Foster (1920) and with the larvae studied by Ransom and Cram (1921), who used chiefly guinea pigs as hosts. Since these authors do not give average measurements, their range of measurements must be used for comparison. Our larvae six to ten days old from the lungs of pigs averaged 0.43 mm. more in maximum length and 0.33 mm. more in minimum length than larvae of the same ages from the lungs of guinea pigs. Larvae six to ten days old from the lungs of pigs averaged 0.96 mm. more in maximum length and 0.31 mm. more in minimum length than larvae of corresponding ages from the lungs of mice. Nine to thirteen-day-old larvae from the small intestine of pigs averaged 0.91 mm. more in maximum length and 0.86 mm. more in minimum length than larvae of the same ages from the small intestine of mice.

SUMMARY

The migration of larval *A. suum* was observed in 34 pigs. Almost all of the larvae reached the liver by the fourth day and the lungs by the ninth day of the infection. Larvae were first found in the small intestine on the eighth day. Larvae were 0.33 mm. long after two days in the pig; 0.43 mm. on the fourth day; 1.54 on the eighth day; and 2.73 mm. on the 15th day. The larvae from pigs were generally larger than those from rabbits, guinea pigs and mice.

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Further Observations on a Complement-Fixing Substance from *Schistosoma mansoni**

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The isolation and immunochemical characterization of a non-dialyzable substance from *Schistosoma mansoni* in aqueous suspension has been under investigation at this laboratory (Schneider, Radke and Coleman, 1956). The preparation contains a relatively heat-stable complement-fixing antigen which reacts with serums of human patients naturally infected with *Schistosoma mansoni,* but not with serums of normal or syphilitic individuals. The work reported herein is an extension of the above study. The fractionation technique applied is the same as previously described. However, the material extracted from the worms is dialyzed in a Visking Cellophane sac against saline-phosphate buffer solution in place of water. The preparation which has been obtained illustrates good reactivity in the complement-fixation test with serums of human patients eliminating *S. Mansoni* ova in feces. The substance containing the complement-fixing moiety, isolated from the worms, is precipitable from 80 percent ethanol. This information constitutes the basis for the report.

MATERIALS AND METHODS

About ninety-eight hundred (9,816) adult *Schistosoma mansoni* worms of both sexes of estimated dry weight of 0.5 g. were recovered from the mesenteric portal-vascular system of 133 experimentally infected white mice. The worms were washed in saline solution, transferred to a solution of 0.1 sodium chloride, 0.2 sodium citrate and 0.4 percent sodium desoxycholate and ground by hand with the aid of a Tenbroeck tissue grinder. The disintegrated worms were treated further with chloroform-isoamyl alcohol mixture as previously described (Schneider, *et al.,* 1956). An aqueous phase was separated and dialyzed in a Visking Cellophane sac against a saline-phosphate buffer solution at pH 7.0, at 5° C. The fluid volume inside the dialysis sac increased. At the end of the first twenty-four hours of dialysis the volume was reduced by pervaporation at room temperature, with the aid of a rotating electric fan. The insoluble worm debris separated from the chloroform phase was suspended in the buffer salt solution and also dialyzed. After pervaporation, dialysis was continued for nearly four weeks with a slight increase in volume of the extract inside the dialysis sac. The aqueous portion of the insoluble worm debris was recovered by centrifugation at low speed and then combined with the original aqueous phase. The yield was 75 ml. of an opalescent suspension, estimated to contain 75 mg. of nondialyzable solids after correcting for salts, or 15 percent of the dry weight of the starting worms. This preparation contained the complement-fixing antigen. The substance containing the complement-fixing moiety was fractionated further as follows: One vol-

*From Third Army Area Medical Laboratory (SU 3004), Fort McPherson, Georgia. Serums were provided through the courtesy of Lt. Colonel S. M. Dozier, MC, United States Army Hospital, Fort Benning, Georgia. Mice infected with *Schistosoma mansoni* and uninfected *Australorbis glabratus* snails were obtained through the courtesy of Colonel George W. Hunter, III, formerly of the Fourth Army Area Medical Laboratory, Fort Sam Houston, Texas. Complement-fixation tests were performed by Mrs. Marion T. Coleman of the Microbiology Section of this laboratory.

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ume of the salt extract of the worms was mixed with four volumes of absolute ethanol and refrigerated at 0° C. for twenty-four hours. A relatively heavy precipitate formed which was isolated by centrifugation at low speed for thirty minutes and resuspended in an equivalent volume of Kolmer saline solution. The latter material constituted a second antigen preparation.

Test serums were obtained from human individuals eliminating *S. mansoni* eggs in their feces. Complement-fixation tests were carried out by means of a semiquantitative procedure (Kolmer *et al.*, 1951), employing the above described extracts of adult *S. mansoni* as antigens. The results are shown in tables 1 and 2.

Table 1. Titration of saline-phosphate buffer extract of *Schistosma mansoni* in the complement-fixation test

Antigen diluted 1:	Human serum[1] diluted 1:				
	1	2	4	8	16
2	4+[2]	4+	4+	4+	4+
4	4+	4+	4+	4+	4+
8	4+	4+	4+	4+	2+
16	4+	4+	3+	1+	-----
32	3+	1+	-----	-----	-----
64	-----	-----	-----	-----	-----

[1]Naturally acquired *S. mansoni* disease. Stool positive for characteristic eggs. Normal human serum inactivated at 56° C. for 30 minutes employed as diluent.
[2]2+ to 4+ complement-fixation reaction positive test.

Table 2. Complement-fixing activity of nondialyzable saline-phosphate extract of *Schistosoma mansoni*

Antigen diluted 1:	Human serums[1]				
	1	2	3	4	
	Complement-fixation endpoints[2]				
1	Antigen anticomplementary				
2	>16[2]	>16	>16	>16	(8)[5]
4	>16	>16	>16	>16	(32)
8	16	16	>16	>16	(16)
16	4	4	16	>16	(8)
32	1[3]	0[4]	4	16	(2)
64	0	0	0	8	(2)
128	0	0	0	8	(2)

Controls consisting of normal human and human leutic patients failed to react in the complement-fixation test with the above antigen preparations.
[1]Eggs of *S. mansoni* identified in stools.
[2]Reciprocal of highest dilution of serum giving positive complement-fixation test. Normal human serum inactivated at 56° C. for 30 minutes employed as diluent for test serums.
[3]2+ to 4+ complement-fixation reaction with undiluted test serum, but complete or nearly complete hemolysis in the next serum dilution (1:2) tested.
[4]Negative reaction with undiluted serum.
[5]Worm extract precipitated with 80 percent ethanol, held at 0° C. for 24 hours, and precipitate redissolved in equal volume of Kolmer saline solution.

DISCUSSION AND SUMMARY

A fractionation technique has been applied to adult worms of *S. mansoni* and an opalescent nondialyzable substance obtained, following dialysis against a saline-buffer salt solution. The preparation contains a complement-fixing antigen which reacts very well with serums of several individuals harboring *S. mansoni*. The complement-fixing moiety may be concentrated by precipitating the aqueous extract with 80 percent ethanol. The latter preparation illustrates low anticomplementary activity. Study is required to affect further purification of the serologically active moiety before attempting to evaluate the preparation in the clinical diagnosis of human *Schistosomiasis mansoni*.

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Preliminary Observations on Calves Experimentally Infected with *Trichostrongylus colubriformis*.

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In a recent report (Dikmans and Kates, 1955), worms of the genus *Trichostrongylus* were incriminated as the primary cause of death losses in a herd of cattle in Virginia. Both the stomach worm, *T. axei*, and the intestinal worm, *T. colubriformis*, were present. More recently, the pathogenicity of *T. axei* infections in calves has been verified experimentally (Doran, 1955). However, so far as the author is aware, experimental ev.idence of the detrimental effects of *T. colubriformis* infections in cattle is reported in the present paper for the first time.

Nine grade Jersey calves raised free of all helminth parasites, other than *Strongyloides papillosus*, were given single doses of *T. colubriformis* infective larvae. All calves received infective larvae cultured from bovine feces. The calves ranged from 4 to 8 months of age at the time of inoculation. Infective larvae were administered in a small quantity of water *per os* in doses ranging from 61,400 to 500,000, as shown in table 1.

TABLE 1.—Data on Experimental Infections of Nine Calves with *Trichostrongylus colubriformis*

Calf Number	Age (Mos.)	No. Infective Larvae Fed	Prepatent Period (days)	Maximum E. P. G.*	
				Number	Days After Infection
1399	6	90,000	22	192	32
1407	4	61,400	20	472	23
1406	6½	175,000	21	108	36
1429**	4½	100,000	16	1,746	23
1450	6	104,000	18	494	20
1451	8	120,000	23	52	23
1395**	4½	500,000	17	58	17***
1423**	4½	250,000	16	1,086	40
1448**	4	250,000	15	6,800	27

* Eggs per gram of feces.
** Died from effects of experimental infection.
*** Died before a second fecal examination was made.

Patent infections were established in all nine calves. The prepatent period ranged from 15 to 23 days, averaging 19 days. Maximum fecal egg counts ranged from 52 to 6,800 eggs per gram of feces. Calves 1395, 1423, 1429, and 1448 died, 21, 40, 23, and 31 days, respectively, after infection. Four months before it was made available to the writer, calf 1429 had been experimentally infected with oocysts of a mixture of *Eimeria* spp. The coccidial inoculation produced light clinical symptoms, which had disappeared prior to experimental infection with the nematode, and, in fact, the calf had made consistent weight gains up to the time it was fed the *T. colubriformis* infective larvae. As three of the five calves which survived their nematode infections had been fed comparable or greater numbers of infective larvae than had calf 1429, it is possible that the coccidial infection predisposed this calf to the subsequent effects of the nematodes. The other three fatally affected calves, 1395, 1448, and 1423 (table 1), developed anorexia, and their feces became diarrheic during the third week of infection and continued so until death. At necropsy, these calves had lost 7, 29, and 45 pounds, respectively, whereas comparable uninfected controls gained 7, 24, and 31 pounds, respectively. At post-mortem, the gall bladder of each calf was distended and filled with bile that was darker and much thicker than normal. No gross lesions were seen, other than a slight congestion and catarrh in the upper duodenum.

Within the limitations of these few trials, it appears that the effects of *T. colubriformis* on calves are directly related to the number of infective larvae administered to the animals; doses consisting of 250,000 larvae or more being fatal to four-month-old calves.

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The Occurrence of *Hymenolepis Evaginata* and *H. Ondatrae* in Utah Muskrats

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Hymenolepis evaginata Barker and Andrews, 1915, has been reported from a number of localities in the United States and Canada, all of which are east of the Rocky Mountains. The western limit of the reported distribution of this species is Colorado according to Doran (1954).

The original description of *H. ondatrae* Rider and Macy, 1947 was based on specimens from western Oregon and apparently the only other report of the species is from eastern Oregon by Macy and Biggs (1953). Neiland and Senger (1952) did not mention *H. ondatrae* in their survey of the helminth parasites of muskrats from western Oregon but that species may have been overlooked in the other cestode material.

The present paper is a report of the helminth parasites recovered from 21 muskrats, *Ondatra zibethicus osoyoosensis*, which were trapped in the Cache Valley area of northern Utah. Most of the animals were examined within 24 hours after death and contained at least a few living worms. Living cestodes are desirable for study because rostellar hooks are rapidly lost after death, and these hooks are one of the better diagnostic characters. Representative specimens were fixed in a formalin-alcohol-acetic acid solution and stained with either a Semichon's carmine or Ehrlich's hematoxylin.

Eight of the 21 animals contained from one to five specimens which agreed with the description of *H. evaginata* as amended by Baylis, 1935. This apparently is the first report of this species in the Great Salt Lake drainage and represents a new western limit for its distribution in North America. This species was also found in a muskrat from Bozeman, Montana, on the upper Missouri River drainage. Small numbers of *H. ondatrae* were found in three animals and over a hundred cestodes were harbored by each of three other animals. Most of the specimens were less than 30 mm in length with the maximum length about 50 mm. This report extends the range of that species eastward into the Great Salt Lake drainage. The only host which harbored both *H. evaginata* and *H. ondatrae* contained five and ten specimens, respectively.

The liver of one of the animals contained two cestode coenuri about 7 mm in diameter and several smaller ones from 1 to 3 mm. On the basis of hook size and shape these coenuri were identified as the larval stage of *Taenia mustelae* Gmelin, 1790 (syn. *T. tenuicollis*). The reader is referred to Freeman (1956) concerning the taxonomic position of the species.

Two trematodes were encountered in the study: *Echinostoma revolutum* and *Quinqueserialis quinqueserialis*. The former was found in ten muskrats and all of the specimens were large, from 15 to 25 mm in length. Several of the hosts contained about 35 worms each. Only one animal harbored both *E. revolutum* and *H. ondatrae*, whereas four contained both *E. revolutum* and *H. evaginata*. One of these four also harbored *Q. quinqueserialis*.

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SUMMARY

Hymenolepis evaginata, H. ondatrae, Taenia mustelae (syn. *T. tenuicollis*) coenuri, *Echinostoma revolutum,* and *Q. quinqueserialis* were encountered in muskrats from northern Utah. *Hymenolepis evaginata* and *H. ondatrae* occurred together in only one animal.

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NEILAND, K. A. and SENGER, C. M. 1952. Helminths of northwestern mammals. Part I. Two new species of Hymenolypis. J. Parasit. 38:409-414.

RIDER, C. L. and MACY, R. W. 1947. Preliminary survey of the helminth parasites of muskrats in northwestern Oregon, with description of *Hymenolepis ondatrae* n. sp. Tran. Amer. Micr. Soc. 66:176-181.

MINUTES

Three hundred fortieth to three hundred forty-eighth meetings

340th. meeting: Log Lodge, Agricultural Research Center, Beltsville, Md., May 26, 1956, Picnic.

341st. meeting: McMahon Hall, Catholic University of America, Washington, D. C., October 17, 1956. E. W. Price elected to life membership. Papers presented: The use of sodium chlorophenate as a molluscacide in the control of schistosomiasis in Egypt, by E. G. Berry; Report of itinerary in Africa, by Mollari.

342nd. meeting: Log Lodge, Agricultural Research Center, Beltsville, Md., November 16, 1956. Executive Committee recommended that Volume 2 No. 1 of Proceedings be duplicated if surplus of $200 is available; that when minimum number of 25 copies of any issue of Proceedings is reached Editor is authorized to reprint 200 copies provided cost does not exceed $200. E. B. Cram elected to life membership. Voted D. B. McMullen representative to Washington Academy of Sciences. Papers presented: The efficacy of some piperazine compounds against roundworms in pigs, by Colglazier and Wilkens; Rearing parasite-free lambs and kids for coccidiosis investigations, by Lotze and Leek; Notes on the migration of *Strongyloides papillosus* larvae in lambs, by Turner; Further observations on the survival of nematode eggs and coccidial oocysts of chickens under outdoor conditions, by Farr; Keys to the identification and differentiation of the immature stages of gastrointestinal nematodes of cattle, by Douvres; The effect of nodular worm infection on white blood cell counts of swine, by Shorb; A non-pathogenic strain of *Histomonas,* by Lund; Some observations on the morphology of a roundworm occurring in the brain cavity of a water turkey, by Hwang; Atrophic rhinitis. VIII. The albino rat as an experimental carrier, by Andrews.

343rd. meeting: Biology-Greenhouse Building, Howard University, Washington, D. C., December 19, 1956. Officers elected for 1957: P. P. Weinstein, President; M. A. Stirewalt, Vice President; E. Buhrer, Corresponding Secretary-Treasurer; F. G. Tromba, Recording Secretary. Accepted recommendations that names of all former and current officers, honorary and life members, and representatives to the Washington Academy of Sciences be published in Proceedings; that Executive Committee meet each January to orient new officers; that Recording Secretary prepare minutes of Exectuive Committe meetings; that Editorial Committee select one of its members as Secretary. Voted L. Olivier to 5 year term on Editorial Committee. Papers presented: Progress report on the efficacy of nitrofurans in coccidiosis therapy, by Johnson; Comparative electrophoretic studies on sera of schistosome infected hosts, by Evans; M. C. Hall. Poet laureate of the Helminthological Society, by Herman; Growth of *Trypanosoma lewisi* in heterologous hosts, by Lincicome.

344th. meeting: Dart Auditorium, Armed Forces Institute of Pathology, Washington, D. C., January 23, 1957. Voted C. M. Herman member at large to Executive Committee. Papers presented: Serological studies in rabbits

infected with *Clonorchis sinensis*. by D. E. Wykoff; Studies on egg production of *Clonorchis sinensis* in rabbits, by Duxbury; Notes on the parasites found in a Georgia wildlife investigation, by Sawyer; The distress syndrome in *Australorbis glabratus* as a reaction to toxic inorganic substances, by Harry and Aldrige; Studies on *Culex gelidus* in Malaya, by Gould.

345th. meeting: National Naval Medical Center, Bethesda, Md., February 20, 1957. Society informed of death of E. B. Cram and L. Seghetti. Voted to accept Treasurers annual report. Executive Committee recommended that Volume 2 No. 1 of Proceedings be reprinted; that manuscripts of members be accepted for publication without additional cost unless inordinately long; that $74 from General Fund be transferred to Publication Fund. Papers presented: Cinematographic studies of exoerythrocytic stages of *Plasmodium gallinaceum,* Huff, Pitkin, and Jensen; Biochemical studies of mosquito tissues and fluids, by Terzian; Schistosomiasis in Egypt, NAMRU at work, by Kuntz; Demonstration of a semi-closed horizontal bridge paper strip electrophoresis apparatus, by Evans.

346th. meeting: Wilson Hall, National Institutes of Health, Bethesda, Md., March 20, 1957. Voted $25 for Science Fair of Washington Academy of Sciences. Papers presented: The development of portal collateral circulation in mice infected with *Schistosoma mansoni,* by K. S. Warren and DeWitt; Attempts to enhance the schistosomacidal activity of antimony compounds, by Luttermoser; Physiological observations on starvation and dessication of *Australorbis glabratus,* by von Brand, McMahon, and Nolan; Studies relative to the epidemiology of toxoplasmosis, by Jacobs, Melton, and Lunde; Autographs of parasitologists and several interesting historical documents, by Olivier.

347th. meeting: Johns Hopkins University School of Hygeine and Public Health, Batlimore, Md., April 26, 1957. Voted $20 from General Fund to help defray cost of picnic. Papers presented: Use of organ cultures in the study of infections, by Bang; Some observations on the effect of X-irradiation on the morphology of *Hymenolepis nana,* by Schiller; Factors influencing the metabolism of *Trypanosoma cruzi,* by Warren; The occurrence of zinc in the mosquito and other insects, by Lang; The effect of bile salts on tapeworm metabolism, by Rothman.

348th. meeting: Log Lodge, Agricultural Research Center, Beltsville, Md., May 25, 1957, Picnic.

The following were elected to membership during the year: 340th meeting—Reinhold Mankau; 341st meeting—H. A. Thomas, F. T. Kenworthy, A. A. Almodovar, J. Roman, D. F. Pineda, A. M. Somerville, G. A. Hemerick, S. E. Brothers, J. E. Larson, F. J. Etges; 342nd meeting—T. C. Cheng, J. L. Ostdiek, T. Shafer; 343rd meeting—H. W. Johnson, W. D. Wilson, I. G. Kagan, D. R. Cordray, L. N. Locke; 344 meeting—A de Bona, W. Friedman, H. N. Harry, R. A. Knight, J. E. Lynch, D. K. Mcloughlin, M. L. Schuster, H. R. Smithson; 345th meeting—D. Heyneman, I. Granek, P. R. Burton, G. M. Clark; 346th meeting—L. S. Whitlock, P. G. Seitner, K. S. Samson, J. R. Bloom; 347th meeting—J. D. Thomas, E. J. Wehunt, J. L. Ruehle,

G. A. Schad, S. B. Van Gundy, M. A. Scott, S. M. Ayoub; 348th meeting—B. J. Bogitsh, D. W. Dery, F. J. Hurley, R. F. Shumard, H. E. Welch, L. V .White.

FRANCIS G. TROMBA
Recording Secretary

The Brayton H. Ransom Memorial Trust Fund

Because of valuable services to humanity by his research on parasites, the Helminthological Society of Washington deemed it fitting to perpetuate the memory of Dr. Brayton H. Ransom (1879-1925) and appointed a committee to solicit funds for a suitable memorial. There was no substantial agreement among the many donors as to the form that the memorial should take except that it should be of a kind to encourage the study and advancement of the Science of Parasitology and related sciences.

The original committee appointed by the Society consisted of C. W. Stiles, M. C. Hall, E. B. Cram, and W. W. Cort. A second committee in charge of funds was named in October 1935 and consisted of G. Steiner, Paul Bartsch, E. B. Cram, G. Dikmans, and G. F. Otto. The members of both committees joined in establishing the Brayton H. Ransom Memorial Trust Fund on June 17, 1936, at which time five trustees were named by the Helminthological Society and constituted as a self-perpetuating body. For further information, readers are referred to these PROCEEDINGS, 3(2): 84-87, July, 1936.

TRUSTEES:

1936-1953, G. Steiner, Chairman; Eloise B. Cram, Secretary-Treasurer; G. F. Otto; E. W. Price; G. Dikmans

1953-1954, G. Steiner, Chairman; Eloise B. Cram, Secretary-Treasurer; G. F. Otto; E. W. Price; A. O. Foster

1954-1956, G. Steiner, Chairman; A. O. Foster, Secretary-Treasurer; G. F. Otto; E. W. Price; Myrna F. Jones

1956——, G. F. Otto, Chairman; A. O. Foster, Secretary-Treasurer; Edna M. Buhrer; K. C. Kates; Myrna F. Jones

FINANCIAL REPORT FOR 1956:

FUNDS ON HAND, Jan. 1, 1956	$1762.75
RECEIPTS: Interest rec'd in 1956	59.71
DISBURSEMENTS: Expenses and grant to Helminthological Society of Washington	55.50
BALANCE ON HAND, Dec. 31, 1956	1766.96

A. O. FOSTER
Secretary-Treasurer

IN MEMORIAM

Lee Seghetti

September 7, 1909 - November 20, 1956

Professor of Pathology and Bacteriology, School of Veterinary Medicine, Colorado Agricultural and Mechanical College, Fort Collins, Colorado.

Member Helminthological Society of Washington since November 1954

Eloise Blaine Cram

June 11, 1896 - February 9, 1957

The death of Dr. Eloise B. Cram on February 9, 1957, in San Diego, California, brought to a close a fruitful career in parasitology, begun early in 1920. In that year Miss Cram came to Washington as an apprentice parasitologist in the former Bureau of Animal Industry, United States Department of Agriculture, after having worked for about 18 months as a bacteriologist in the laboratories of Armour and Company in Chicago. It was during her service in Chicago, which was begun shortly after her graduation from the

University of Chicago in 1918, that she was first introduced to parasitology. At that time, the late Dr. B. H. Ransom, who was the head of the Zoological Division of the United States Bureau of Animal Industry, carried out certain investigations on the life history and other aspects of swine ascarids in a field laboratory in Chicago, partly in cooperation with some of the meat packing establishments operating there in the Union Stock Yards. Miss Cram was detailed for a time to cooperate in these investigations on behalf of her company, and thus became acquainted with Dr. Ransom. This led to her coming to Washington in the spring of 1920 as a Federal employee to assist Dr. Ransom in investigations on the life history of Ascaris. The research in which she participated led to the publication of a paper by Ransom and Cram in 1921 on the course of migration of Ascaris larvae. That publication practically terminated Miss Cram's apprenticeship, and launched her as a journeyman in the field of helminthology. To this career Dr. Cram devoted her life unstintingly for an uninterrupted period of more than three and one-half decades, the first 16 years in the U. S. Department of Agriculture, where she studied parasites of farm animals and poultry, and the remaining 20 years in the U. S. Public Health Service, where she investigated problems in human parasitology. Her extensive bibliography, covering a wide and varied field of research in descriptive and experimental helminthology, contains titles of many significant contributions to parasitology—so many, in fact, that it would require several pages to record them even briefly.

It was by no means solely because of her scholarly attainments that Dr. Cram became so widely and favorably known to a large circle of professional colleagues and friends, and was honored by them to the election of offices in various scientific societies of which she was a member. This included the Helminthological Society of Washington of which she served as corresponding secretary and treasurer from 1921 to 1926 and as president in 1927.

To her scientific work Dr. Cram brought a high degree of industry coupled with a patient endurance of disappointments, an ability to overcome difficulties she encountered in research, and a vigorous persistence of effort in order to arrive at solutions of perplexing problems. In her personal relations with her fellow workers she exhibited at all times a charming personality, was uniformly kind, considerate, and courteous, was exceptionally tolerant even in situations which required the exercise of more patience and self-control than most of us possess. Her engaging personality, even in the face of a rapidly-advancing physical decline, was brought into sharp focus at the meeting of the American Society of Parasitologists held in Storrs, Connecticut, in August 1956. There she presided calmly at two rather prolonged sessions of the Council, even though it involved a great effort to do so. Those who attended that meeting will not forget for a long time the brilliant presidential address she delivered—an address characterized alike by the vivid pictures she painted of past presidents and other worthies in the field of parasitology, and by the sparkling humor with which she interspersed her remarks about the many personalities she described.

BENJAMIN SCHWARTZ

Past Officers of the Society

DATE	PRESIDENT	VICE PRESIDENT	REC. SECRETARY	CORR. SEC.-TREAS.	EXEC. COMM.** MEMB.-AT-LARGE	REPRESENTATIVE WASH. ACAD. SCI.
1910-1916	Stiles, C. W.*		Hall, M. C.*			
1916-1917	Cobb, N. A.*		Crawley, H.*			
1917-1920	Ransom, B. H.*		Hall, M. C.*			
1920-1921	Hassall, A.*		"			
1921-1922	Curtice, C.*		"	Cram, Eloise B.*		
1922-1923	Hall, M. C.*		Chapin, E. A.	"		Ransom, B. H.*
1923-1924	Chapin, E. A.		Stiles, C. W.*	"		"
1924-1925	Cort, W. W.		Schwartz, B.	"		" Hall, M. C.‡*
1925-1926	Steiner, G.		"	"		Hall, M. C.*
1926-1927	Schwartz, B.		Christie, J. R.	Speer, Alma J.		"
1927-1928	Cram, Eloise B.*		"	Nolan, Mabelle O.		Schwartz, B.
1928-1929	Butler, C. S.*		"	"		"
1929-1930	Bartsch, P.		"	Jones, Myrna F.		Butler, C. S.*
1930-1931	Christie, J. R.		Wright, W. H.	Price, E. W.		Bartsch, P.
1931-1932	Ewing, H. E.*		"	"		Steiner, G.
1932-1933	Price, E. W.		Spindler, L. A.	Wehr, E. E.		"
1933-1934	Jones, Myrna F.		"	"		"
1934-1935	Spindler, L. A.	Wehr, E. E.	Dikmans, G.	Buhrer, Edna M.		"
1935-1936	Otto, G. F.	McIntosh, A.	Krull, W. H.	"		Price, E. W.
1936-1937	Wright, W. H.	Chitwood, B. G.	"	"		"
1937-1938	Dikmans, G.	Harwood, P. D.	McIntosh, A.	"		"
1938-1939	McIntosh, A.	Wehr, E. E.	Lucker, J. T.	"		"
1939-1940	Wehr, E. E.	Rees, C. W.	Christensen, J. F.	"		Christie, J. R.

1940-1941	Rees, C. W.	Lucker, J. T.	Foster, A. O.	"		Price, E. W.
1941-1942	Foster, A. O.	Sarles, M. P.	Kates, K. C.	"		"
1942-1943	Lucker, J. T.	Kates, K. C.	Shorb, D. A.	"		"
1943-1944	Mollari, M.	Shorb, D. A.	von Brand, T.	"	Lucker, J. T. Farr, Marion M.	"
1945	Shorb, D. A.	Bozicevich, J.	Boughton, D. C. Doss, Mildred A.‡	"	" Foster, A. O.	Mollari, M.
1946	von Brand, T.	Hunter, G. W.	Farr, Marion M.	"	" Bozicevich, J.	"
1947	Kates, K. C.	Farr, Marion M.	Reinhard, E. G.	"	" Spindler, L. A.	Foster, A. O.
1948	Bozicevich, J.	Reinhard, E. G.	Enzie, F. D.	"	" Jacobs, L.	"
1949	Chitwood, B. G.	Olivier, L. J.	Lotze, J. C.	"	" Shorb, D. A.	"
1950	Olivier, L. J.	Doss, Mildred A.	Jahnes, W. G.	"	" Kates, K. C.	Price, E. W.
1951	Farr, Marion M.	Jacobs, L.	Taylor, A. L.	"	" Jones, Myrna F.	Spindler, L. A.
1952	Jacobs, L.	Meinkoth, N. A.	Stirewalt, Margaret A.	"	von Brand, T.‡ Dikmans, G.	"
1953	Reinhard, E. G.	Weinstein, P. P.	Durbin, C. G.	"	Sarles, M. P.‡ Luttermoser, G. W.	"
1954	Sarles, M. P.	Enzie, F. D.	Luttermoser, G. W.	"	Rozeboom, L. E.‡ Stirewalt, Margaret A.	"
1955	Taylor, A. L.	Andrews, J. S.	Herman, C. M.	"	" Reinhard, E. G.	Andrews, J. S.
1956	Enzie, F. D.	McMullen, D. B.	Anastos, G.	"	" Olivier, L. J.	"
1957	Weinstein, P. P.	Stirewalt, Margaret A.	Tromba, F. G.	"	" Herman, C. M.	McMullen, D. B.

LIBRARIAN: Mildred A. Doss, 1945—
ARCHIVIST: John T. Lucker 1945—

**Two year term
*Deceased. ‡Completed unexpired term of predecessor.

Editorial Committees (Five Year Terms)

1934	J. R. Christie,	Editor	;	G. F. Otto	;	E. W. Price	;	H. E. Ewing	;	L. A. Spindler*
1934-1935	"	"	;	"	;	"	;	"	;	G. Dikmans*
1935-1937	"	"	;	"	;	"	;	"	;	W. H. Krull*
1937-1938	"	"	;	"	;	"	;	"	;	A. McIntosh*
1938-1939	"	"	;	"	;	"	;	"	;	J. T. Lucker*
1939-1940	"	"	;	"	;	"	;	"	;	J. F. Christensen*
1940-1941	"	"	;	"	;	"	;	"	;	A. O. Foster*
1941-1942	"	"	;	"	;	"	;	"	;	K. C. Kates*
1942-1943	"	"	;	"	;	"	;	"	;	D. A. Shorb*
1943-1944	"	"	;	"	;	"	;	"	;	T. von Brand*
1944-1946	"	"	;	"	;	"	;	"	;	T. von Brand
1947	"	"	;	"	;	"	;	W. H. Wright	;	"
1948	E. G. Reinhard,	"	;	"	;	"	;	"	;	"
1949-1951	"	"	;	"	;	"	;	"	;	G. Steiner
1952-	G. F. Otto,	"	;	E. G. Reinhard	;	A. O. Foster	;	L. J. Olivier	;	A. L. Taylor

*Recording Secretary of the Society serving *ex officio*.

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MEMBERS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

The following membership list, arranged geographically, includes life, resident, and non-resident members, as defined in Art. 3 of the Constitution (Vol. 13, No. 1 of the Proceedings).

Alabama
Cairns, E. J.
Herlich, H.
Hopper, B. E.
Isenstein, R. S.
Porter, D. A.
Price, E. W.
Rohrbacher, G. H., Jr.
Thorson, R. E.

Alaska
Dunagan, T. T.
Rausch, R.

Arizona
Reynolds, H. W.

California
Allen, M. W.
Allen, P. H.
Baker, N. F.
Dougherty, E. C.
Golden, A. M.
Hart, W. H.
Ichikawa, S. T.
Larson J. E.
Lear, B.
Lider, L. A.
Lownsbery, B. F.
Lownsbery, Joyce W.
McBeth, C. W.
Raski, D. J.
Sayama, K.
Sher, S. A.
Voge, Marietta
Youngson, C. R.

Canal Zone
Grocott, R. G.

Colorado
Olsen, O. W.
Seghetti, L.
Stabler, R. M.

Connecticut
Miller, P. M.
Penner, L. R.
Pistey, W. R.

Delaware
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District of Columbia
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Ballard, Ethyl L.
Barron, C. N.
Bauman, P. M.
Briscoe, M. S.
Bruce, J. I., Jr.
Buchheit, J. R.
Durbin, C. G.
Duxbury, R. E.
Gould, D. J.
Lincicome, D. R.
Maloney, J. T.
McMullen, D. B.
Mengoli, H. F.
Mollari, M.
Needy, C. F.
O'Keefe, T. A.
Ostdiek, J. L.
Reinhard, E. G.
Sarles, M. P.
Sawyer, T. K.
Slie, Rita B.
Young, Viola M.

Florida
Birchfield, W.
Brothers, S. L.
Chitwood, B. G.
Christie, J. R.
Esser, R. P.
Feldmesser, J.
Hannon, C. I.
Hunter, G. W., III
Kincaid, R. R.
Overman, Amegda J.
Riggin, G. T., Jr.
Short, R. B.
Sledge, E. B.
Tarjan, A. C.
Thames, W. H., Jr.
Whitton, G. M., Jr.

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Denton, J. F.
Good, J. M., Jr.
Schneider, M. D.
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Stewart, T. B.
Vegors, H. H.

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Goto, S.

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Babero, B. B.
Elishewitz, H.
Jeske, H. A.
Levine, N. D.
Linford, M. B.
Otto, G. F.
Peebles, C. R.
Thomas, L. J.
Walton, A. C.

Indiana
Ehrenford, F. A.
Hall, J. E.
Phillips, B. P.
Swank, G., Jr.

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Hsü, H. F.

Kansas
Ackert, J. E.
Ameel, D. J.
Hansen, M. F.

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Chapman, R. A.
Valleau, W. D.

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Cross, H. F.
Hollis, J. P., Jr.
Martin, W. J.
Mayhew, R. L.
Newsom, L. D.

Maine
Meyer, M. C.

Maryland
Anastos, G.
Andrews, J. S.
Bang, F. B.
vonBrand, T.
Buhrer, Edna M.
Chitwood, May B.
Clifford, C. M., Jr.
Cobb, Grace S.
Colglazier, M. L.
Costello, L. C.
Coursen, B. W.
Cram, Eloise B.
DeWitt, W .B.
Diamond, L. S.
Doran, D. J.
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Douvres, F. W.
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Goldberg, A.
Habermann, R. T.
Haley, A. J.
Hansen, Maye L.
Hawkins, Naomi
Herman, C. M.
Hill, C. H.
Huff, C. G.
Humphrey, Judith M.
Hwang, J. C.
Jachowski, L. A., Jr.
Jacobs, L.
Jahnes, W. G.
Jenkins, W. R.
Jennings, R. R.
Jones, Myrna F.
Kates, K. C.
Krueger, H. J.
Kuntz, R. E.
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LaRue, G. R.
Leek, R. G.
Lotze, J. C.
Lucker, J. T.
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McIntosh, A.
Newton, W. L.
Olivier, L. J.
Price, D. L.
Read, C. P.
Reardon, Lucy V.
Rees, C. W.
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Rozeboom, L. E.
Rubin, R.
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Schindler, A. F.
Schwartz, B.
Shafer, T.
Shorb, D. A.
Sonnenberg, Bessie
Spindler, L. A.
Spruyt, F. J.
Stirewalt, Margaret A.
Tarshis, I. B.
Taylor, A. L.
Tromba, F. G.
Turner, J. H.
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Wehr, E. E.
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Cook, Alice R.
Cowan, A. B.
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New Mexico
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Braun, A. J.
Dropkin, V. H.
Fischthal, J. H.
Ingalls, J. W., Jr.
Mai, W. F.
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Turk, R. D.

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Vermont
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Virginia
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Byrd, M. A.
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Elsea, J. R.
Freund, Frances E.
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Somerville, A. M., Jr.

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Courtney, W. D.
Gustafson, P. V.

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Perry, V. G.
Thorne, G.

Wyoming
Honess, R. F.

Australia
Sprent, J. F. A.

Austria
Wieser, W.

Belgium
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