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# PROCEEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

VOLUME 5

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NUMBER 1

## **Description and differentiation of infective larvae of three species of horse strongyles. JOHN T. LUCKER, U. S. Bureau of Animal Industry.**

### INTRODUCTION

The strongyles which have been described from the intestine of the horse comprise more than 40 species. The morphological characteristics of infective larvae of only 8 of these species have been specifically determined and more or less adequately described from a comparative viewpoint.

Recently, infective larvae of *Cylicocercus pateratus*, *Cylicocyclus insigne* and *Cylicodontophorus bicoronatus* were obtained by the writer in separate cultures; the cultures were prepared from eggs removed from the uteri of identified females of the species named. The culture medium used was a small quantity of water to which a few drops of helminthologically sterile fecal extract were added. The cultures were kept in a laboratory at temperatures ranging from 20° to 25°C.

Descriptive data on the structure of the infective larvae of these 3 species are given below. These data are followed by a discussion of the comparative morphology of these and certain other horse strongyle larvae. It has not been considered necessary to present separately certain additional original observations introduced in the course of this discussion. Observations on the structure of the intestine in larvae of *Cylicocercus goldi* are also included. All observations were on larvae obtained by methods of culture similar to those described above.

### DESCRIPTION OF INFECTIVE LARVAE OF CYLICODONTOPHORUS BICORONATUS, CYLICOCERCUS PATERATUS AND CYLICOCYCLUS INSIGNE

Third-stage preparasitic larvae of these 3 species have in common the following morphological characteristics: Shape fusiform; esophagus, nervous system, excretory system, and genital primordium having the structure typical for strongyliform larvae in general. Sheath thicker than cuticle of larva and conforming closely to body contour, constricted slightly in region immediately posterior to caudal extremity of larva, and continuing posteriorly as a long gradually tapering cuticular rod or tail. Sheath with moderate or slight thickening of wall in region of constriction (fig. 1, C, F, I), the fine lumen present in this region gradually becoming obliterated posteriorly. Posterior extremity of larva rounded, without parenchymatous processes or cuticular modifications. Vestibule in median lateral optical section appearing to be bounded by 3 pairs of cuticularized rhabdions forming an elongate irregular hexagon (fig. 1, B, E, H). Intestine consisting of 8 cells.

The size relationships of 10 third-stage larvae of each of the 3 above mentioned species are given in table 1.

# DIFFERENTIATION OF LARVAE OF THE 3 SPECIES

As shown in table 1, the averages of the principal measurements of infective larvae of *Cylicocercus pateratus*, *Cylicocyclus insigne*, and *Cylicodontophorus bicoronatus* differ slightly. However, because of the overlapping variation in the measurements of individual size characteristics, infective larvae of the 3 species are indistinguishable on the basis of their size relationships. According to the writer's observations, infective larvae of *Cylicocercus pateratus* and *Cylicocyclus insigne* are likewise indistinguishable on the basis of structure. However, the infective larva of *Cylicodontophorus bicoronatus* was found to differ from infective larvae of these 2 species as regards the arrangement of the cells of the intestine. In median lateral optical section of the larva of *C. bicoronatus* (fig. 1, G), the intestinal cells were observed to form 2 rows of 4 cells each, one row being dor-

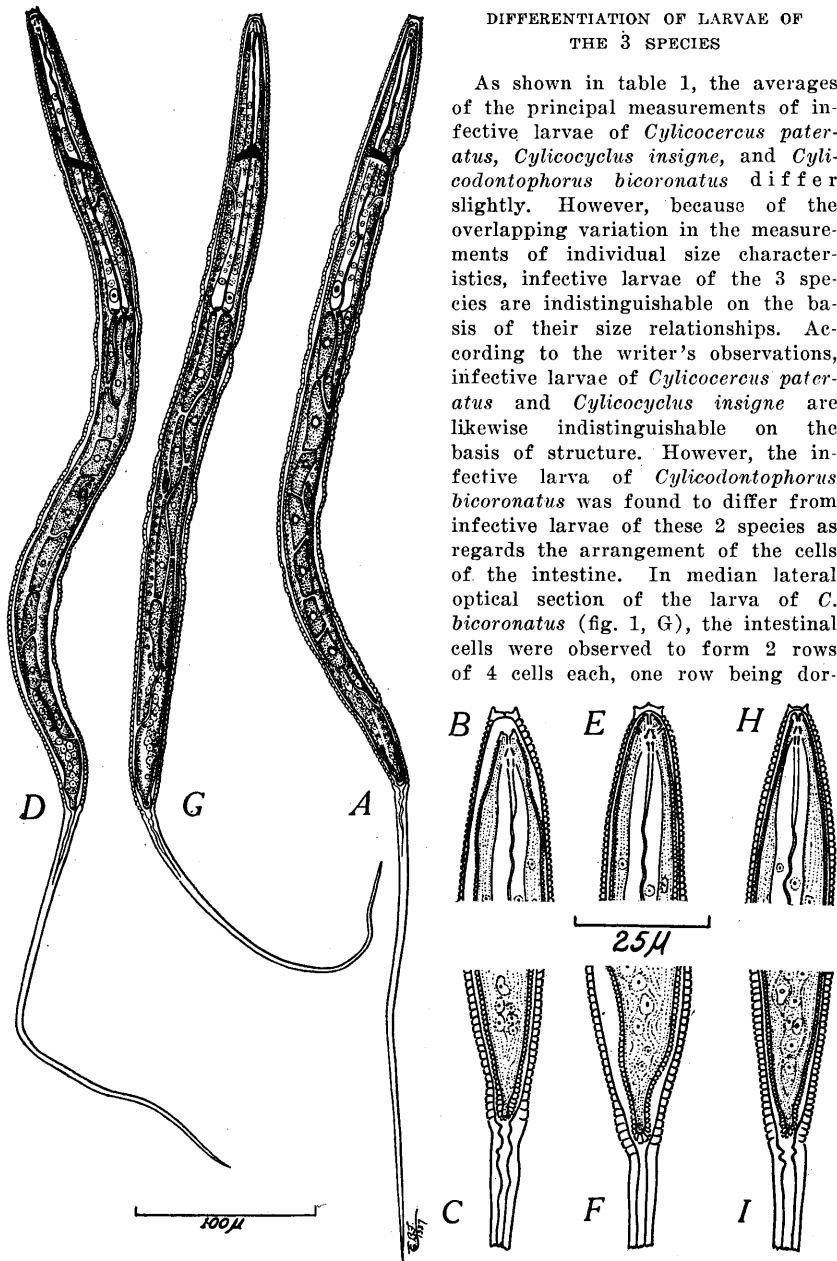


FIG. 1

A-C—*Cylicocyclus insigne*, infective larva (A—Lateral view. B—Cephalic extremity showing structure of vestibule. C—Caudal region showing tip of larval tail and structure of sheath). D-F—*Cylicocercus pateratus*, infective larva (D—Lateral view. E—Cephalic extremity showing structure of vestibule. F—Caudal region showing tip of larval tail and structure of sheath). G-I—*Cylicodontophorus bicoronatus*, infective larva (G—Lateral view. H—Cephalic extremity showing structure of vestibule. I—Caudal region showing tip of larval tail and structure of sheath).

TABLE 1.—Size relationships of 10 infective larvae of each of 3 indicated species of Cyathostominae (All measurements in microns)

Species	Specimen no.	Length of sheath	Length of larva	Width of larva at posterior end of esophagus	Width of sheath in region of esophageal bulb	Distance from anterior end to nerve ring	Distance from anterior end to excretory pore	Length of esophagus	Distance from posterior end of esophagus to genital primordium	Distance from genital primordium to anus	Length of tail	Length of tail of sheath	Ratio of length of larva to length of tail of sheath
<i>Cylicodontophorus bicoronatus</i>	1	731	471	25	30	89	103	130	134	155	51	260	1.8:1
	2	708	443	21	27	80	101	148	112	134	45	265	1.7:1
	3	711	471	20	25	86	100	169	100	145	53	240	2:1
	4	719	479	24	30	86	106	165	111	146	52	240	2:1
	5	741	481	21	27	81	104	171	104	145	56	260	1.8:1
	6	677	459	20	24	80	106	169	106	132	48	218	2.1:1
	7	718	454	20	25	81	101	162	104	141	42	246	1.8:1
	8	688	470	17	22	79	101	158	99	159	49	218	2.1:1
	9	739	479	20	27	76	100	163	108	151	53	256	1.9:1
	10	703	466	18	25	77	100	159	120	134	48	237	2:1
	Av.	713.8	467.3	20.6	26.2	81.5	102.2	159.4	109.8	144.2	49.7	244	1.9:1
<i>Cylicocyclus insigne</i>	1	730	421	20	27	72	84	165	89	124	38	303	1.4:1
	2	697	444	20	24	70	103	155	99	134	46	253	1.7:1
	3	695	425	18	25	80	108	171	80	129	41	260	1.6:1
	4	803	493	22	28	99	122	169	131	138	48	310	1.6:1
	5	741	481	20	24	83	108	172	110	144	50	260	1.8:1
	6	662	437	20	24	93	101	159	107	121	45	225	1.9:1
	7	721	425	17	22	77	96	151	88	137	44	296	1.4:1
	8	695	413	17	22	84	102	162	83	125	37	282	1.5:1
	9	720	438	20	25	83	106	156	110	127	39	282	1.5:1
	10	730	439	17	22	77	96	158	108	127	41	291	1.5:1
	Av.	719.5	440.6	19.1	24.3	81.8	102.6	161.8	100.5	130.6	42.9	276.2	1.6:1
<i>Cylicocercus pateratus</i>	1	696	436	17	24	86	99	172	81	138	41	260	1.7:1
	2	753	471	18	24	91	120	184	91	151	48	282	1.7:1
	3	723	456	17	22	80	104	176	100	138	38	263	1.7:1
	4	770	467	17	22	94	108	177	91	152	42	303	1.5:1
	5	652	422	17	25	86	94	172	75	134	37	230	1.8:1
	6	658	425	18	25	81	100	176	75	131	37	225	1.9:1
	7	670	422	18	25	89	101	172	86	127	32	248	1.7:1
	8	740	473	20	28	90	112	172	105	150	41	267	1.8:1
	9	707	456	17	22	89	105	180	86	144	42	251	1.8:1
	10	682	426	17	22	85	100	180	73	131	37	256	1.7:1
	Av.	705.1	445.4	17.6	23.9	87.1	104.3	176.1	86.3	139.6	39.5	258.5	1.7:1

sal, and the other ventral, to the sinuous lumen. In larvae of *Cylicocercus pateratus* (fig. 1, D) and *Cylicocyclus insigne* (fig. 1, A), the 5 posterior intestinal cells were invariably arranged tandem.

#### DIFFERENTIATION FROM LARVAE OF OTHER HORSE STRONGYLES

Infective larvae of the 3 species in question differ in respect to the number of their intestinal cells from infective larvae of *Strongylus vulgaris*, *S. equinus*, *S. edentatus*, *Poteriostomum ratzii*, and *Gyalocephalus capitatus*, which, according to published reports, have 32, 16, 20, 16, and 12 intestinal cells, respectively. To this group may be added the infective larva of *Poteriostomum imparidentatum*, the writer having recently determined that this larva has 16 intestinal cells and is morphologically very similar to the infective larva of *P. ratzii*, described in an earlier paper (1934, J. Wash. Acad. Sci., 24:302-310). In larvae of *P. imparidentatum* the length of the tail of the sheath tends to be greater than in larvae of *P. ratzii*; it is doubtful that this and other minor

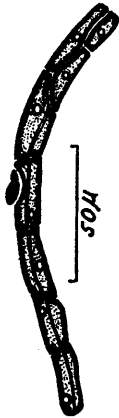


FIG. 2  
*Cylicocercus goldi*  
Intestine of infective larva showing intestinal lumen as seen in a larva several weeks old.

points of difference observed in larvae of the 2 species are sufficiently constant to afford a suitable basis for their separation. The larva of *P. imparidentatum* thus differs markedly from larvae of the 3 species which have been described in this paper. Also, the criteria which the writer (1936, Proc. Helminth. Soc. Wash., 3:22-25) has mentioned as applicable for the separation of infective larvae of *P. ratzii* and *Strongylus equinus* are valid for differentiation of larvae of the latter species and of *Poteriostomum imparidentatum*. In this connection, it should be noted that while Poluszynski (1930, Tierarztl. Rundschau, 36:871-873) and Wetzel (1931, J. Parasitol., 17:235) are in agreement as to the number of intestinal cells in infective larvae of *Strongylus vulgaris*, *S. edentatus*, and *S. equinus*, they disagree as to the relative size of larvae of the 3 species. Wetzel states that *S. edentatus* larvae are intermediate in size between the larvae of the other 2 species, whereas the measurements given by Poluszynski show that the larva of *S. edentatus* is the shortest of the 3 species. Measurements on *Strongylus* larvae obtained in pure cultures by the writer have agreed closely with those given by Poluszynski.

The infective larvae of the 3 species described in this paper have the same number of intestinal cells as those of *Cylicodontophorus ultrajectinus*, *Cylicocercus goldi* and *C. catinatus*. The alternate dorsoventral position of the 8 intestinal cells characteristic of the infective larva of *Cylicodontophorus bicoronatus* also occurs in the infective larva of *C. ultrajectinus*. This has been determined by restudy of the larvae of the latter species. As is shown in the illustrations of infective larvae of *Cylicocercus goldi* and *C. catinatus* given in the writer's earlier paper (1936, Proc. Helminth. Soc. Wash., 3:22-25), the arrangement of the 8 intestinal cells in larvae of these species is similar to that which has been described in this paper for the larvae of *C. pateratus* and *Cylicocyclus insigne*. Thus, on the basis of the arrangement of intestinal cells, larvae of the 6 species under discussion are separable into 2 groups, one embracing the 2 species of *Cylicodontophorus*, and the second including the remaining 4 species.

Except that in larvae of *C. bicoronatus*, the tail of the sheath tends to be longer and more slender than in larvae of *C. ultrajectinus*, larvae of these 2 species are alike. As shown in table 1, the ratio of the length of the larva to the length of the sheath varied from 1.7:1 to 2.1:1 in 10 infective larvae of *C. bicoronatus*. The corresponding ratio for larvae of *C. ultrajectinus* was

given in an earlier paper as from 2:1 to 2.7:1; measurements on additional larvae of this species have given slightly higher ratios of from 2.3:1 to 3:1. Comparison of data available on the larvae of the 4 species comprising the second group, namely, *Cylicocyclus insigne*, *Cylicocercus pateratus*, *C. goldi* and *C. catinatus*, shows that these larvae are very similar in all morphological respects and in their size relationships. Since a comparative study has also shown that the structure of the vestibule is fundamentally alike in these larvae, they are regarded by the writer as morphologically indistinguishable.

#### HISTOLOGY OF THE 8-CELLED INTESTINE

The 8-celled intestine and the arrangement of the intestinal cells observed in laterally viewed larvae of 6 of the above mentioned species are features readily observed even with relatively low magnifications and are undoubtedly of diagnostic value. It is recognized, however, that the disposition of the intestinal cells as described in larvae of 4 of these species, namely, *Cylicocyclus insigne*, *Cylicocercus pateratus*, *C. goldi* and *C. catinatus*, is without precedent among the larvae of related nematodes and does not account for a complete intestinal lumen. This raises the presumption that histologically the intestine in these larvae may consist of a number of cells greater than has been observed, some of the cells being obscured as a result of torsion of part of the organ or some other factor. In this connection the number of nuclei present in the intestine assumes primary importance. In the writer's experience, the location and number of the nuclei is best determined by examination of living larvae under rather low magnifications. Under these conditions the nuclei are denoted by small round clear areas surrounded by the relatively opaque cytoplasm of the cells. Larvae of the species in question have been repeatedly examined from various aspects, while alive, and following various methods of killing and staining, and more than 8 intestinal nuclei have not been observed. Finally, examination of infective larvae of *Cylicocercus goldi*, kept in water for several weeks to reduce the opacity of the intestinal cells and stained intra-vitam with neutral red, has shown that larvae of this species do have a complete intestinal lumen which passes between the contiguous surfaces of the 3 anterior cells and through the cytoplasm of the 5 posterior cells, as shown in figure 2. In infective larvae of *C. goldi* which have recently reached the infective stage, the posterior portion of the lumen cannot be seen at all or is seen only with extreme difficulty because of the opacity of the cells. Whether a complete intestinal lumen occurs also in the infective larvae of the 3 other species in question has not as yet been determined.

#### DEVELOPMENT

Two molts, presumptive in the preparasitic development of all strongyles, were observed in the course of the development in cultures of infective larvae of *Cylicocercus pateratus*, *Cylicocyclus insigne* and *Cylicodontophorus bicoronatus*. Two molts have also been observed in the preparasitic development of *Cylicocercus goldi*, which has been restudied.

**Studies on oxyuriasis, VIII. A preliminary note on therapy with gentian violet.** WILLARD H. WRIGHT, FREDERICK J. BRADY and JOHN BOZICEVICH, National Institute of Health, U. S. Public Health Service, Washington, D. C.

Evidence obtained in the treatment of several hundred cases of oxyuriasis indicates that treatment for this condition should be carried out on a household basis, i.e., effort should be made to conduct adequate diagnostic tests on

each individual in a household, and all infested individuals should be treated at the same time with a view to eliminating at one time all sources of pinworm infestation in the household. From a control standpoint, it appears useless to treat individuals in a family or household in which other individuals, infested with pinworms, are left untreated, since the treated individuals usually become reinfested promptly from pinworm ova scattered throughout the house by untreated individuals.

Experience gained from the experimental use of various methods of therapy led us to the view that a satisfactory treatment for oxyuriasis must comply with the following specifications:

(1) The drug employed must be sufficiently safe to be used in repeated treatments over a period of time sufficient to allow for the destruction by desiccation of pinworm ova in the patient's surroundings, this method of administration assuring also the contact of the drug with worms in all parts of the digestive tract and particularly in the appendix, a common site of pinworms, into which drugs administered in single doses apparently do not penetrate regularly.

(2) The drug must be highly effective.

(3) Any treatment must be of such reasonable cost that its use in large families will not be prohibitive.

(4) The treatment must be so easily administered that it can be used with a minimum of effort in large household groups or families, a specification which rules out, on the grounds of impracticability, the employment of treatment by enemata, medicated or nonmedicated, for the reason that few parents have the time or the persistence to administer enemas frequently to a large number of children over a period of weeks, such a period being required if satisfactory results are to be obtained from enema treatments when used alone.

As none of the conventional methods of treatment which we had tested first entirely fulfilled all of these specifications, we carried out tests with gentian violet, one of the few known anthelmintics which can be given with safety in repeated treatments over a period of time. The efficacy of the treatment was determined by the use of the NIH swab described by Hall (1937, Amer. J. Trop. Med., 17:445-453), 7 consecutive daily post-treatment swabs being taken between the 10th and 17th days, or the 14th and 21st days, after the end of the treatment. In view of the fact that exact information concerning the prepatent period of *Enterobius vermicularis* is not available, our selection of the period during which post-treatment checks were obtained is based on the reports of self-infection experiments of Leuckart (1868, Die menschlichen Parasiten und die von ihnen herrührenden Krankheiten, 2:257-512), Grassi (1881, Gaz. Osped. Milano, 2:433-439), and Calandruccio (1890, Atti Accad. Gioenia Sci. Nat. Catania, (An. 66, 4. ser.) 2:95-135), which experiments would seem to indicate that gravid *Enterobius vermicularis* females will begin to migrate between the 14th and 21st day after infection.

Up to this writing, we have treated with gentian violet a total of 163 cases of oxyuriasis. Of these individuals, 148 were given the drug in daily doses over a period of 10 days, while 15 individuals were treated over a period of 8 days, allowed to rest for 7 days, and then treated for an additional period of 8 days. The dosage administered to adults has been two ½-grain (32 mg) enteric-coated pills before meals 3 times a day. For individuals 15 years and under, we have used a dosage based on 1 cg. per day for each year of apparent (not chronological) age. Of 163 cases treated with the drug, 64 have been adults and 99 have been children under 16 years of age. Most of these latter patients have been children 4 years of age or over. We have hesitated to treat younger children for the reason that little information is available as to the tolerance of such individuals for the drug, and for the reason that gentian violet tablets in a dose suitable for young children are not available on the market.



Thirty-six of our 163 cases have been classed as non-cooperative, the individuals having failed either to complete the entire course of treatment or to furnish a sufficient number of post-treatment swabs; 5 individuals could not complete treatment because of reactions caused by the drug. Of 122 completed cases treated by both methods, 112, or 91.8 per cent, were negative on post-treatment swab examinations. In 104 cases there was no reaction to the treatment. The remaining patients experienced the following ill effects in the number of cases cited: Nausea, 24; vomiting, 23; diarrhea, 17; constipation, 2; headache, 1; dizziness, 2; lassitude, 2; griping abdominal pain, 15. Reactions in none of the patients were of a serious nature. While contraindications for gentian violet are not clearly defined in the literature, it appears advisable to withhold treatment from individuals suffering from moderate or severe cardiac, hepatic, renal and gastrointestinal disease. Patients should abstain from alcohol during the period of treatment.

### Check list of parasites found among principal domestic animals in Puerto Rico.

**Rico.** H. L. VAN VOLKENBERG, Puerto Rico Experiment Station of the U. S. Department of Agriculture, Mayaguez, Puerto Rico.

The species here recorded were collected between the years 1924 and 1937 by individuals connected with the Experiment Station of the United States Department of Agriculture at Mayaguez. The identifications in most part were made by members of the Zoological Division, Bureau of Animal Industry and of the Bureau of Entomology and Plant Quarantine.

This list contains only species which are definitely known to be established in Puerto Rico. A few forms which occur with sufficient frequency to be recognized as distinct and established species require further study for a definite determination. So far as known, none of these underdetermined species have been reported on the continent of North America.

### CATTLE

**ARTHROPODS.**—*Psoroptes communis* var. *bovis*, *Demodex folliculorum*, *Haematobia irritans*, *Boophilus annulatus* var. *australis*, *Haematopinus tuberculatus*, *Cochliomyia americana*.

**PROTOZOA.**—*Babesia bigemina*, *B. argentina*, *Anaplasma marginale*, *Trichomonas foetus*, *Eimeria zürni*, *E. smithi*, *E. ellipsoidalis*.

**HELMINTHS.**—*Fasciola hepatica*, *Cotylophoron cotylophorum*, *Moniezia expansa*, *Cysticercus bovis*, *Neoascaris vitulorum*, *Strongyloides vituli*, *Bunostomum phlebotomum*, *Dictyocaulus viviparus*, *Oesophagostomum radiatum*, *Trichostrongylus axei*, *Cooperia curticei*, *C. punctata*, *Ostertagia ostertagi*, *Haemonchus contortus*, *H. similis*, *Onchocerca gutturosa*, *Trichuris ovis*, *Setaria labiato-papillosa*.

### HORSE

**ARTHROPODS.**—*Psoroptes equi*, *Sarcoptes equi*, *Trombicula tropica* (larva), *Dermacentor nitens*, *Chrysops variegata*, *Tabanus hookeri*, *Lepiselaga crassipes*, *Stomoxys calcitrans*, *Gastrophilus nasalis*.

**HELMINTHS.**—*Anoplocephala perfoliata*, *Parascaris equorum*, *Oxyuris equi*, *Dictyocaulus viviparus*, *Onchocerca cervicalis*, *Setaria equina*, *Habronema muscae*, *H. microstoma*, *H. megastoma*, *Strongylus equinus*, *S. edentatus*, *S. vulgaris*, *Cylicocyclus insigne*, *C. radiatus*, *C. nassatus*, *C. nassatus* var. *parvus*, *C. ashworthi*, *C. auriculatus*, *Probstmayria vivipara*, *Cylicodontophorus ultrajectinus*, *C. bicoronatus*, *C. ihlei*, *C. euproctus*, *Cylicocercus goldi*, *C. cati-*

natus var. *pseudocatinatus*, *Gyalocephalus capitatus*, *Cylicostephanus calicatus*, *C. minutus*, *C. longibursatus*, *Cyathostomum coronatum*, *C. ornatum*, *C. labratum*, *C. labiatum*, *C. labiatum* var. *digitatum*, *Oesophagodontus robustus*, *Trichonema elongatum*, *Triodontophorus brevicauda*, *T. tenuicollis*, *T. intermedius*, *Cylicotetrapedon bidentatum*, *Poteriostomum imparidentatum*, *P. ratzii* var. *nanum*, *Craterostomum mucronatum*, *Cylicostomias coronata*.

#### PIG

ARTHROPODS.—*Sarcoptes suis*, *Demodex phylloides*, *Tunga penetrans*, *Haematopinus adventicius*.

PROTOZOA.—*Balantidium coli*, *Eimeria deblickei*.

HELMINTHS.—*Cysticercus cellulosae*, *Ascaris lumbricoides*, *Oesophagostomum dentatum*, *O. quadrispinulatum*, *Stephanurus dentatus*, *Globocephalus urosubulatus*, *Necator suillus*, *Metastrongylus elongatus*, *Choerostomylus pudendotectus*, *Hyostomylus rubidus*, *Ascarops strongylina*, *Macracanthorhynchus hirudinaceus*, *Trichuris trichiura*.

#### GOAT

ARTHROPODS.—*Bovicola caprae*, *Linognathus africanus*.

PROTOZOA.—*Eimeria faurei*, *E. arloingi*, *E. ninae-kohl-yakimov*.

HELMINTHS.—*Cysticercus tenuicollis*, *Oesophagostomum columbianum*, *O. venulosum*, *Syngamus laryngeus*, *Bunostomum trigonocephalum*, *Muellerius capillaris*, *Trichostrongylus colubriformis*, *Ostertagia circumcincta*, *Capillaria brevipes*.

#### DOG

ARTHROPODS.—*Otodectes cynotus*, *Sarcoptes canis*, *Demodex canis*, *Rhipicephalus sanguineus*, *Heterodoxus longitarsus*, *Pulex irritans*, *Ctenocephalides canis*.

PROTOZOA.—*Babesia canis*.

HELMINTHS.—*Dipyllobothrium mansonii*, *Dipylidium caninum*, *Taenia hydatigena*, *Toxocara canis*, *Ancylostoma caninum*, *Uncinaria stenocephala*, *Dirofilaria immitis*, *Capillaria plica*, *Trichuris vulpis*, *Acanthocephala* sp.

#### CAT

ARTHROPODS.—*Notoedres cati*, *Felicola subrostrata*, *Ctenocephalides felis*.

PROTOZOA.—*Isospora felis*.

HELMINTHS.—*Platynosomum concinnum*, *Taenia taeniaeformis*, *Toxocara mystax*, *Ancylostoma braziliense*, *Aelurostrongylus abstrusus*, *Physaloptera praeputialis*.

#### POULTRY

ARTHROPODS.—*Liponyssus bursa*, *Cytoleichus nudus*, *Megninia cubitalis*, *Menson gallinae*, *Eomenacanthus stramineus*, *Menacanthus* sp., *Colpocephalum* sp., *Goniodes meleagridis*, *Lipeurus gallipavonis*, *Goniodes dissimilis*, *Goniocotes hologaster*, *Lipeurus caponis*, *Echidnophaga gallinacea*.

PROTOZOA.—*Eimeria tenella*, *E. acervulina*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox*, *Histomonas meleagridis*.

HELMINTHS.—*Postharmostomum gallinum*, *Prosthogonimus* sp., *Davainea proglottina*, *Amoebotaenia sphenoides*, *Hymenolepis cantaniana*, *Railletina tetragona*, *Railletina* sp., *Tetrameres americana*, *T. fissispina*, *Subulura strongylina*, *S. brumpti*, *Ascaridia galli*, *A. numidae*, *Heterakis gallinae*, *H. brevispiculum*, *Cheilospirura hamulosa*, *Dispharynx spiralis*, *Strongyloides avium*, *Capillaria annulata*, *C. retusa*, *C. columbae*, *Oxyuris* sp., *Oxyuris mansonii*.

**A restudy of *Faustula keksooni* (MacCallum) and *Distomum tropidonoti* MacCallum (Trematoda). EMMETT W. PRICE, U. S. Bureau of Animal Industry.**

In 1918 (Zoopathologica, 1:81-98) the late Dr. G. A. MacCallum described two exotic species of trematodes, the descriptions being based on specimens obtained from Asiatic hosts by Dr. W. G. MacCallum. One of the trematodes, *Eurema keksooni* (= *Faustula keksooni*) was from the gills of a "ray" taken at Singapore, and the other, *Distomum tropidonoti*, was from the gall bladder of a snake, *Tropidonotus trianguligerus*, from Java. The available material in each case consisted of a single specimen. In view of the fact that important misinterpretations of structure appeared in the descriptions of these species, which in one case resulted in misallocation and establishment of unnecessary supergeneric groups, it appears desirable that redescriptions of these forms be made available. The affinities of *Eurema keksooni* (= *Faustula keksooni*) cannot be definitely determined, but in many respects it resembles members of the Fellodistomidae and is tentatively assigned to that family; *Distomum tropidonoti* obviously belongs in the family Plagiorchiidae.

*Faustula keksooni* (MacCallum, 1918) Poche, 1926

*Synonym.*—*Eurema keksooni* MacCallum, 1918.

*Description.*—Body (fig. 3) more or less broadly lanceolate, 2.8 mm long by 1.2 mm wide. Cuticula smooth, without spines. Oral sucker missing, the anterior end having been torn off at level of anterior margin of pharynx; acetabulum 386 $\mu$  long by 285 $\mu$  wide, median, preequatorial. Excretory aperture terminal; excretory vesicle not visible. Pharynx piriform, 179 $\mu$  long by 132 $\mu$  wide; esophagus 370 $\mu$  long; intestinal ceca apparently short, not visible beyond level of anterior margin of acetabulum. Genital aperture probably median or submedian, although it appears to right of median line due to the twisted condition of the specimen, situated about midway between intestinal bifurcation and anterior margin of acetabulum. Cirrus pouch large, about 640 $\mu$  long by 315 $\mu$  wide near base, containing a tubular seminal vesicle and numerous prostatic cells. Testes oval, about 295 $\mu$  long by 255 $\mu$  wide, with fields separate and zones almost completely coinciding, slightly postacetabular. Ovary lobed, about 380 $\mu$  in diameter, median, immediately posttesticular. Seminal receptacle apparently absent, although the oviduct is widened at the point where Laurer's canal is given off and this dilation apparently functions as a seminal receptacle; Laurer's canal long and slender, opening dorsally at level of posterior limits of uterine loops. Mehlis' gland preovarial, median to right testis, inconspicuous. Vitellaria largely extracecal, in zone bounded by levels of intestinal bifurcation and anterior margin of acetabulum. Uterus in antero-posteriorly directed loops, occupying greater part of body from level of anterior margin of acetabulum to level of opening of Laurer's canal. Eggs oval, 16 to 20 $\mu$  long by 9 to 11 $\mu$  wide, with thick shells.

*Host.*—"Small ray."

*Location.*—(?) Gills.

*Distribution.*—Federated Malay States (Singapore).

*Specimen.*—U. S. N. M. Helm. Coll. No. 36451.

A restudy of the type of this species shows that the specimen was mutilated, the anterior end being torn off and the body partly crushed. The crushing apparently resulted from flattening and was suffi-



FIG. 3

*Faustula keksooni*  
Complete worm, dorsal view.  
Original.

cient to rupture the intestinal ceca and to force a part of the ingesta into the tissues, thereby causing outpocketings which were regarded by MacCallum as openings of vaginae. In the original description and figure, the genital ducts were badly scrambled; these misinterpretations are obvious, as may be noted on comparison of the original description and figure with those given in the present paper.

Poche (1926, Arch. Naturg., Abt. A, 91:1-240) noted that *Eurema* MacCallum, 1918 was a homonym of *Eurema* Hübner, 1818, and proposed the new name *Faustula* to replace it. At the same time, Poche erected the family Faustulidae and the supersuperfamily Faustulida to contain this genus, apparently assuming the correctness of the original description. Since pertinent comments on Poche's action in proposing supergeneric groups for this parasite have been given by Odhner (1927, Arkiv. Zool., 19A (15):1-5) and by Stunkard (1934, Zool. Anz., 106(9):218-219), further discussion appears unnecessary.

*Allopharynx tropidonoti* (MacCallum, 1918), n. comb.

*Synonym*.—*Distomum tropidonoti* MacCallum, 1918.

*Description*.—Body (fig. 4-A) lanceolate, 5 mm long by 1.7 mm wide. Cuticula covered with fine spines in preacetabular portion of body. Oral sucker subterminal, 204 $\mu$  in diameter; acetabulum 240 $\mu$  in diameter, about 800 $\mu$  posterior to oral sucker. Excretory aperture terminal; excretory vesicle not visible except in extreme posterior portion of body. Prepharynx short, with relatively thick wall; pharynx 93 $\mu$  long by 118 $\mu$  wide; esophagus 320 $\mu$  long; intestinal ceca relatively slender, slightly sinuous, extending to within about 500 $\mu$  from posterior tip of body. Genital aperture median, mid-

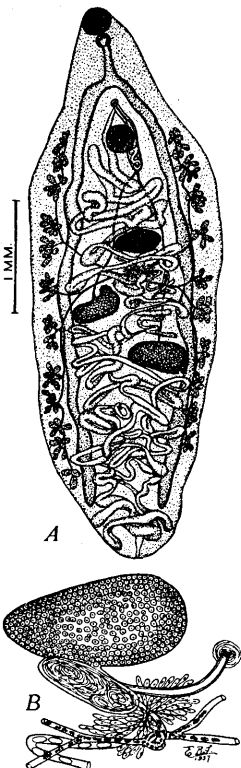


FIG. 4

*Allopharynx tropidonoti*  
A—Complete worm, ventral view. B—Female genital complex, dorsal view. Original.

way between intestinal bifurcation and anterior margin of acetabulum. Cirrus pouch slender, 640 $\mu$  long by 112 $\mu$  wide, extending posterior to acetabulum, and containing a coiled seminal vesicle. Testes elongated transversely, with fields and zones separated, in subequatorial zone; anterior testis 240 $\mu$  long by 430 $\mu$  wide, to right of median line; posterior testis 240 $\mu$  long by 560 $\mu$  wide, to left of median line and about 240 $\mu$  distal to anterior testis. Ovary transversely oval, 225 $\mu$  long by 430 $\mu$  wide, in median field and separated from anterior testis by a space about equal to the distance between the testes. Seminal receptacle present, 225 $\mu$  by 95 $\mu$ , immediately posterior to, and in contact with, ovary (fig. 4, B). Mehlis' gland conspicuous; Laurer's canal present, opening dorsally at a level slightly posterior to right pole of ovary. Vitellaria lateral, consisting of 12 groups of follicles on each side, extending from level of acetabulum to about 1/5 of body length from posterior end. Uterus slender, greatly convoluted; descending limb extending to posterior end of body; ascending limb passing anteriorly between testes and between anterior testis and ovary, then describing 4 transverse loops in preovarial region before passing to right of acetabulum. Eggs oval, 37 $\mu$  long by 19 $\mu$  wide.

*Host*.—*Tropidonotus trianguligerus*.

*Location*.—Gall bladder.

*Distribution*.—Java (Buitenzorg).

*Specimen*.—U. S. N. M. Helm. Coll. No. 36448.

In the original description of this species the anterior testis was mistaken for the ovary; a genito-intestinal canal, which was found not to exist, was described as extending from the ootype to the right intestinal cecum; and the measurements were for the most part erroneous. These errors are corrected in the present paper.

*Distomum tropidonoti* is obviously closely related to

*Xenopharynx* (*Allopharynx*) *amudariensis* Strom (1928, Zool. Anz., 79:167-172), a species which Mehra (1937, Ztschr. Parasitenk., 9:429-469) recognized as not belonging to the genus *Xenopharynx* s. str. However, instead of elevating Strom's subgenus *Allopharynx* to the status of a genus, as provided for under Article 7 of the International Rules of Zoological Nomenclature ("A generic name becomes a subgeneric name, when the genus so named becomes a subgenus, and vice versa"), Mehra proposed for *X. (A.) amudariensis* a new genus *Ophiorchis*. In view of the above *Ophiorchis* must fall as a synonym of *Allopharynx*. *Ostiolum mehrai* Gogate (1935, Rec. Indian Mus., 37:455-458), for which Mehra (1937, loc. cit.) proposed the genus *Ptyasiorchis*, and *Megacustis multispinosus* Bennett (1935, J. Parasitol., 21:83-90) also appear to be congeneric with *Allopharynx amudariensis* (Strom), consequently both *Ptyasiorchis* and *Megacustis* become synonyms of *Allopharynx*. The genus *Allopharynx*, therefore, contains 4 species, namely, *A. amudariensis* (Strom), *A. mehrai* (Gogate), *A. multispinosus* (Bennett) and *A. tropidonoti* (MacCallum); these may be separated by the following key:

1. Cirrus pouch extending posterior to acetabulum..... 2  
     Cirrus pouch not extending posterior to acetabulum..... 3
2. Cirrus pouch and metraterm separated by acetabulum; uterus with about 4 loops in preovarial area..... *tropidonoti* (MacCallum)  
     Cirrus pouch and metraterm not separated by acetabulum; uterus with numerous loops in preovarial area..... *multispinosus* (Bennett)
3. Genital aperture at intestinal bifurcation; testes larger than ovary.....  
     ..... *mehrai* (Gogate)  
     Genital aperture midway between intestinal bifurcation and acetabulum;  
     testes and ovary of about equal size..... *amudariensis* (Strom)

**A redescription of *Clinostomum intermedialis* Lamont (Trematoda: Clinostomidae), with a key to the species of the genus.** EMMETT W. PRICE,  
 U. S. Bureau of Animal Industry.

In 1920, Lamont (Occas. Papers Mus. Zool. Univ. Michigan, No. 83, pp. 1-5) described as *Clinostomum intermedialis* a trematode collected by Dr. A. S. Pearse from a cormorant killed on Lake Valencia, Venezuela, July 20, 1918. Lamont's description of this trematode was very complete except for measurements of certain structures and for an apparently erroneous interpretation of the course of the uterus. Baer (1933, Rev. Suisse Zool., 40(3):317-342) pointed out that should Lamont's interpretation of the course of the uterus be correct, *C. intermedialis* should be placed in a new genus. In order to check this point the writer was able to secure through the courtesy of Dr. George R. LaRue of the University of Michigan the type specimen of Lamont's species and the following description is based upon that specimen.

*Clinostomum intermedialis* Lamont, 1920

**Description.**—Body (fig. 5) linguiform, 7.5 mm long by 3.3 mm wide, with slight constriction at level of acetabulum. Oral sucker 390 $\mu$  in diameter, surrounded by a collar-like structure as in other clinostomes; acetabulum 425 $\mu$  long by 475 $\mu$  wide, about 1.7 mm from anterior end of body, acetabular opening circular, cavity triangular. Excretory aperture dorsal, near posterior end of body. Pharynx apparently present; intestinal ceca sinuous, apparently opening into excretory vesicle. Genital aperture at level of posterior margin of

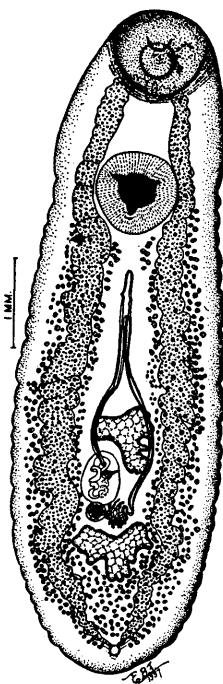


FIG. 5

*Clinostomum*  
*intermedialis* Lamont.  
Ventral view.

anterior testis, slightly to right of median line; cirrus pouch ovoid,  $595\mu$  long by  $425\mu$  wide. Gonads in distal half of postacetabular portion of body. Anterior testis triangular,  $935\mu$  by  $935\mu$  in greatest dimensions, with left margin more or less deeply lobed; posterior testis irregularly triangular with apex directed posteriorly, lobed,  $595\mu$  long by  $1.1$  mm wide, situated about  $510\mu$  from anterior testis. Ovary globular,  $255\mu$  in diameter, immediately posterior to and in contact with cirrus pouch; oviduct greatly convoluted; Mehlis' gland conspicuous; Laurer's canal present, opening in middorsal line slightly posterior to level of caudal margin of ovary. Vitellaria extending from level of posterior margin of acetabulum to level of excretory aperture. Ascending limb of uterus (uteroduct) long, crossing left lobe of anterior testis and joining stem of uterus  $340\mu$  from its anterior end; descending limb passing to right and crossing right lobe of anterior testis. No eggs present.

*Host*.—*Phalacrocorax vigua*.

*Location*.—Esophagus.

*Distribution*.—Venezuela.

*Type specimen*.—Univ. Mich. Mus. Zool. No. 196.

A restudy of the type specimen shows that instead of the uterus making "two longitudinal loops," as stated by Lamont, the nature of this structure is similar to that of other species of the genus. The ascending limb of the uterus, however, is quite long and joins the stem of the uterus far anterior as in *Clinostomum chrysichthys* Dubois (1930, Bull. Soc. Neuchateloise Sci. Nat. (1929), 54:61-72). *C. intermediis* may be distinguished from the latter species in having the cirrus

pouch almost entirely posterior to the anterior testis instead of in the zone of that organ as in *C. chrysichthys*. The only other species with which *C. intermediis* may be confused is *C. phalacrocoracis* Dubois (1931, Bull. Soc. Neuchateloise Sci. Nat., 55:73-85), but in the latter species the ascending limb of the uterus joins the uterine stem near its base instead of far anterior as in the former.

Up to the present time the following species have been included in the genus *Clinostomum*, s. str.: Adults—*C. attenuatum* Cort, 1913; *C. australiense* Johnston, 1916; *C. complanatum* (Rudolphi, 1814), syn. *C. marginatum* (Rudolphi, 1819); *C. detruncatum* Braun, 1899; *C. foliiforme* Braun, 1899; *C. heluans* Braun, 1899; *C. hornum* Nicoll, 1914; *C. intermediis* Lamont, 1920; *C. lambitans* Braun, 1899; *C. lophophallum* Baer, 1933, syn. *C. lophocirrum* Baer, 1933; *C. phalacrocoracis* Dubois, 1931; *C. pusillum* Lutz, 1928; *C. sorbens*, Braun, 1899; *C. vanderhorsti* Ortlepp, 1935; and—metacercariae—*C. africanum* Galli-Valerio, 1906; *C. chrysichthys* Dubois, 1930; *C. dalagi* Tubangui, 1933; *C. dictyotum* (Monticelli, 1893), syn. *Distoma reticulatum* Looss, 1885, nec Wright, 1879; *C. piscidium* Southwell and Prashad, 1918; and *C. pseudoheterostomum* Tubangui, 1933. Of these species *C. pusillum* Lutz and *C. africanum* Galli-Valerio are too inadequately described for identification; the figure of the former suggests, however, that the specimen upon which the species was based was a contracted specimen of *C. complanatum*. *C. lambitans* Braun is also inadequately described but in view of the fact that it is a very minute form it is quite likely that this species will eventually be shown to be valid. The progenetic metacercaria from a West Indian land snail, *Subulina octona*, reported from Puerto Rico by McIntosh (1935, Proc. Helminth. Soc.

Wash., 2:79-80) is probably that of *C. lambitans*, since it is a very small form and from a locality not far removed from Samaná Bay, Dominican Republic, where *C. lambitans* was originally collected.

The recognizable species of *Clinostomum* may be separated by the following key:

1. Adult forms ..... 2  
Metacercarial forms ..... 13
2. Gonads in middle of postacetabular portion of body ..... 3  
Gonads caudal to middle of postacetabular portion of body ..... 5
3. Vitelline follicles arranged radially.....*foliiforme* Braun  
Vitelline follicles not arranged radially..... 4
4. Vitellaria extending posteriorly as far as tips of intestinal ceca  
*hornum* Nicoll  
Vitellaria not extending posteriorly as far as tips of intestinal ceca  
*complanatum* Braun
5. Genital aperture in front of anterior testis ..... 6  
Genital aperture either lateral or posterior to anterior testis..... 7
6. Genital aperture median .....*australiense* Johnston  
Genital aperture submedian .....*attenuatum* Cort
7. Vitelline follicles extending anterior to acetabulum.....*sorbens* Braun  
Vitelline follicles not extending anterior to acetabulum..... 8
8. Uterine stem with lateral branches.....*detruncatum* Braun  
Uterine stem without lateral branches ..... 9
9. Ascending limb of uterus forming a complete loop before entering  
uterine stem ..... 10  
Ascending limb of uterus not forming loop before entering uterine  
stem ..... 11
10. Vitellaria extending anteriorly as far as level of equator of acetabulum  
*lophophallum* Baer  
Vitellaria not extending anteriorly as far as level of posterior margin  
of acetabulum .....*vanderhorsti* Ortlepp
11. Ascending limb of uterus very long, joining uterine stem far an-  
teriorly .....*intermedialis* Lamont  
Ascending limb of uterus relatively short, joining uterine stem a short  
distance in front of anterior testis..... 12
12. Gonads in extreme posterior portion of body; genital aperture in zone  
of anterior testis.....*heluans* Braun  
Gonads somewhat removed from extreme posterior portion of body;  
genital aperture posterior to anterior testis.....*phalacrocoracis* Dubois
13. Anterior testis crescent-shaped.....*pseudoheterostomum* Tubangui  
Anterior testis not crescent-shaped..... 14
14. Stem of uterus extending posteriorly beyond anterior testis  
*dictyotum* (Monticelli)  
Stem of uterus not extending posteriorly beyond anterior testis..... 15
15. Genital aperture at level of caudal margin of anterior testis  
*chrysiethys* Dubois  
Genital aperture near cephalic margin of anterior testis ..... 16
16. Oral sucker about 2/3 as large as acetabulum.....*dalagi* Tubangui  
Oral sucker about 1/2 as large as acetabulum  
*piscidium* Southwell and Prashad

**Description of the adult stage of *Taenia twitchelli* Schwartz, 1924, from an Alaskan wolverine.** ALLEN MCINTOSH, U. S. Bureau of Animal Industry.

In 1924, Schwartz (Proc. U. S. Natl. Mus., 66, Art. 24:1-4) described "A new proliferating larval tapeworm from a porcupine," the material having been forwarded by A. H. Twitchell, from Ophir, Alaska. No additional records of the parasite have been published. Among some parasites recently received for identification from the Rocky Mountain Laboratory of the U. S. Public Health Service, Hamilton, Montana, there were fragments of apparently 3 specimens of a tapeworm from the intestines of a wolverine. This material was collected by Dr. C. B. Philip, July 18, 1937, on Bear Creek near Rapids, Alaska.

The specimens were much contracted and did not stain well, the reason being, as explained by Philip in correspondence, that the specimens had been collected away from suitable facilities and preserved in alcohol. Philip noted while collecting the material that the segmentation was most peculiar, doubtlessly referring to the shape of the proglottids.

A study of the material and comparison of the hooks with those from the cysticerci reported by Schwartz from the porcupine indicate that the species from the wolverine is the adult of *Taenia twitchelli* Schwartz, and is described as follows:

*Taenia twitchelli* Schwartz, 1924

**Description.**—Small species with strobila (fig. 6, A) probably not exceeding 5 cm in length by 2.5 mm in width, with more than 60 segments. Scolex (fig. 6, B) 1 to 1.25 mm long by about 620 $\mu$  wide at level of suckers; diameter of neck about 500 $\mu$ ; rostellum armed with a double crown of 30 to 36 hooks. Hooks (fig. 6, C) with guard broadly joined to blade and handle; large hooks 195 $\mu$  long by 93 $\mu$  wide; small hooks 155 $\mu$  long by 83 $\mu$  wide. Suckers about 215 $\mu$  in diameter, depressed, with opening directed anterolaterally.

Mature proglottids (fig. 6, D) present after about the 60th segment; in this area the segments average about 850 $\mu$  long and are somewhat narrowed anteriorly, measuring at this point from 500 to 900 $\mu$  wide; greatest width about 1.25 mm at posterior margin. Genital pores lateral, irregularly alternate, situated in anterior half of mature proglottids; genital papilla inconspicuous. No completely gravid proglottids with ripe eggs observed, but partly gravid segments (fig. 6, E) measured 1.25 mm long by 2.3 mm wide. Ventral longitudinal excretory vessels about 200 $\mu$  from lateral margin of mature segments. Calcareous corpuscles numerous, ovoid, of various sizes, about 5 to 15 $\mu$  in length.

**Male reproductive system.**—Testes 150 to 170 in number, approximately globular, from 30 to 40 $\mu$  in greater diameter, distributed between the longitudinal excretory vessels. Vas deferens conspicuously coiled; cirrus sac about 170 $\mu$  long by 50 $\mu$  wide, extending, somewhat diagonally, entad to mesal margin of ventral excretory vessel in mature segments, but not extending to excretory canal in partly gravid segments (fig. 6, F).

**Female reproductive system.**—Ovary somewhat variable as to shape and size, the poral lobe usually the smallest. Vitellarium near posterior margin of segment, not extending laterally beyond width of ovary. First indication of uterine branching characterized by a fork at anterior end; gravid uterus with 7 to 9 lateral dendritic branches on each side of a relatively conspicuous, broad median stem. Eggs not observed.

**Habitat.**—Intestine of definitive host, *Gulo luscus* (Linnaeus); lungs of intermediate host, *Erethizon epixanthum* Brandt.



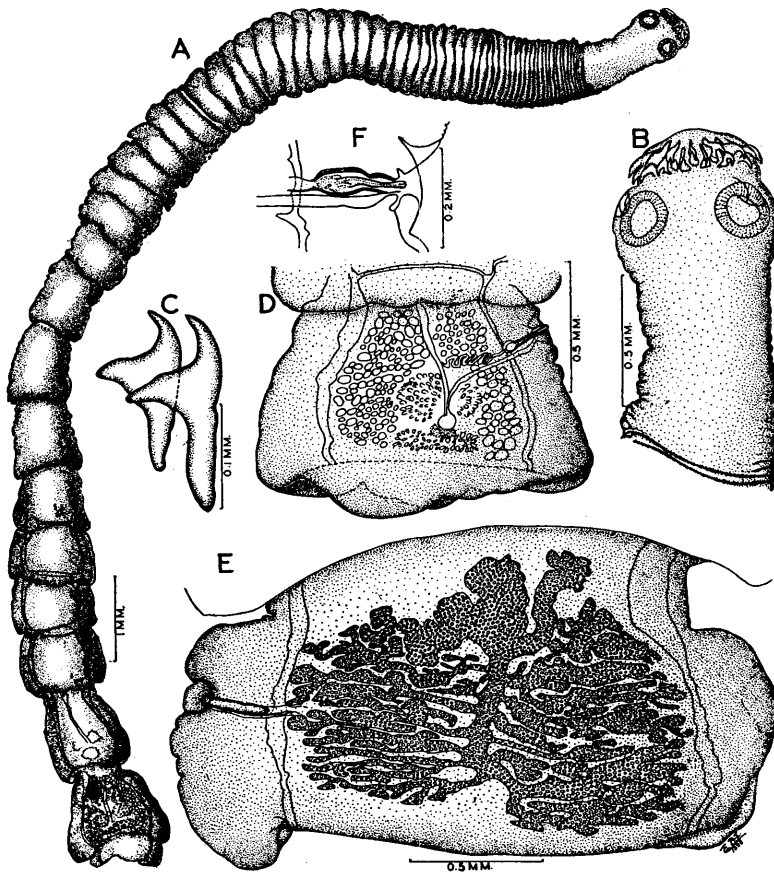


FIG. 6

*Taenia twitchelli* Schwartz, 1924

A—Anterior portion of strobila. B—Scolex. C—Hooks. D—Mature proglottid. E—Gravid proglottid. F—Genital atrium and terminal portions of genital ducts.

*Distribution.*—Alaska (Rapids and Ophir).

*Specimens.*—U. S. N. M. Helm. Coll. No. 26003 (cysticerci; type), and 30259 (adult; collateral-type).

While experimental evidence on the life history is lacking, the shape and size of the hooks are so characteristic there can be but little doubt that the adult from the wolverine is the same species as the larval form described by Schwartz from a porcupine as *Taenia twitchelli*.

The eggs of the parasite are probably swallowed by the porcupine while feeding on vegetation, development of the larval stage taking place in the lungs of the porcupine where the hexacanth embryo gives rise to a branching colony of cysticerci as described by Schwartz. Although the porcupine is so well armed that comparatively few enemies can attack it successfully, the wolverine, with its unusual strength and aggressive disposition, is probably capable of capturing an occasional one for food, thus affording the parasite a chance to complete its life cycle.

**Further notes on intestinal cell inclusions in nemas.** B. G. CHITWOOD and M. B. CHITWOOD, U. S. Bureau of Plant Industry.

Recently (1937-1938) the senior author and Leon Jacobs have published results of studies on the intestinal cell inclusions of *Agamermis decaudata* Cobb, Steiner & Christie, 1923 (1938, J. Wash. Acad. Sci., 28(1):12-13) and of *Rhabditis strongyloides* (Schneider, 1860) Oerley, 1880 (1937, Proc. Helminth. Soc. Wash., 4 (2): 60). The present paper contains the results of an extension of these investigations to other nematodes. In the phase on sphaerocrystals of *Strongylus* the writers were assisted by Leon Jacobs.

The present tests were mainly made with fresh specimens from which the intestine was removed by cutting and mashing. Observations were made directly on mounts usually ringed in with paraffin or vaseline. Such procedure greatly simplifies the technic and provides sharper images than are obtained in glycerin or balsam preparations. All of the technics used are standard, as given in Cole (1933, Practical Physiological Chemistry, Cambridge), Lee (1928, Microtome's Vade-Mecum, 9. ed., Philadelphia) and Chamot and Mason (1931, Handbook of Chemical Microscopy). It was found that the ninhydrin test (invaluable for proteins since it reacts with all compounds containing a free carboxyl and free amino group) can be conducted by sealing material under a coverslip with vaseline, then heating to the boiling point.

It is not safe to assume that the red-brown "granules" of the intestine of parasitic nematodes are an indication of haemoglobin digestion because of the positive iron tests given by the intestinal "granules" of *Ascaris* and *Ancylostoma* (Askanazy, 1896, Deut. Arch. Klin. Med., 57: 104-117; Fauré-Fremiet, 1913, Compt. Rend. Soc. Biol. [Paris], 74 (11): 567-569; Looss, 1905, Rec. Egypt. Govt. School Med., 3: 1-158) since the tests showed this to be "inorganic" iron. Furthermore such free-living nematodes as *Theristus* and *Oncholaimus* also contain iron in their intestinal sphaeroids.

Regarding nomenclature, we have adopted the term globule for nonrefractive inclusions (fats and proteins), the term sphaeroid for refractive, apparently nonbirefringent inclusions (olivaceous sphaeroids), and the term sphaerocrystals for refractive, birefringent inclusions. The term granule is retained for inclusions of unknown nature.

1. OBSERVATIONS ON *DITYLENCHUS DIPSACI* (KÜHN, 1858) FILIPJEV, 1936

The intestine contains numerous colorless globules of variable size but no birefringents. These globules are of 2 types: one a neutral fat, the other a protein; the neutral fat comprises the larger globules, the protein the smaller. (a) The reactions of the globules diagnosed as of neutral fat were as follows: They blackened in osmic acid, stained red in Nile blue sulphate, stained orange in scarlet R, stained light pink in neutral violet, were soluble in absolute alcohol, were negative to ninhydrin and xanthoproteic reactions, were not digested by gastric juice. (b) The reactions of the globules identified as stored protein were as follows: They did not blacken in osmic acid, they stained blue in Nile blue sulphate, stained very slightly orange in scarlet R, stained deep violet in neutral violet, were insoluble in absolute alcohol, were positive to ninhydrin and xanthoproteic reactions, were digested by gastric juice (similar reactions to those described for *Agamermis decaudata*).

2. OBSERVATIONS ON *THERISTUS SETOSUS* (BÜTSCHLI, 1874) DE MAN, 1907

The intestine contains (a) a few large gray birefringent sphaerocrystals and (b) numerous small olive-brown nonbirefringent sphaeroids. Fats and stored proteins are apparently absent.

(a) The large sphaerocrystals are apparently identical with those in *Rhabditis* (i.e., rhabditin) since they have the following characteristics: They did

not blacken in osmic acid; were not colored by scharlach R; were negative to ninhydrin reaction; stained in crystal violet; were insoluble in alcohol; were soluble in 1 per cent H Cl, 2 per cent KOH, and 10 per cent acetic acid; were insoluble or slowly soluble in 0.5 per cent saline and distilled water solution; were digested in diastase (medicinal) at 40°C. after 30 minutes but remained undigested in boiled diastase control after 2 hours; did not stain in iodine-potassium iodide followed by 1 per cent H<sub>2</sub>SO<sub>4</sub>. These tests seem to indicate the presence of a carbohydrate which is similar to starch and paraglycogen but is different from both in the absence of coloration with iodine (similar results obtained in *Rhabditis*).

(b) Olivaceous sphaeroids. These structures are apparently waste products of some type. They have the following characteristics: They did not blacken with osmic acid, were not colored by scharlach R, were insoluble in alcohol, were negative to ninhydrin and xanthoproteic reactions, stained blue in neutral violet, stained blue-violet in crystal violet, stained blue in Nile blue sulphate, did not react with potassium ferrocyanide followed by 1 per cent H Cl in alcohol, reacted with potassium ferricyanide followed by 1 per cent H Cl in alcohol producing blue ferrous ferricyanide, were not digested by artificial gastric juice or diastase, were soluble in 10 per cent H Cl and 2 per cent KOH but not in 10 per cent acetic acid or 2 per cent H Cl. These tests indicate that the coloration is due to non-protein ferrous iron termed inorganic ferrous iron in Lee, that the substance is not fat, carbohydrate or protein, but that it may be an organic salt or a salt of a weak base and a weak acid. Though it is possible that weak birefringence, such as is found in the sphaerocrystals of *Strongylus*, might have been overlooked, these sphaeroids are not the same, as indicated by their solubility (KOH) and the presence of ferrous rather than ferric iron. Suggestions by other workers as to the probable nature of the sphaeroids and tests to apply are invited.

### 3. OBSERVATIONS ON *STRONGYLUS EQUINUS* MUELLER, 1780

The intestine contains numerous minute red-brown weakly birefringent sphaerocrystals. These were obtained free from protein by boiling whole worms in 10 per cent NaOH and washing in distilled water in a centrifuge. Such sphaerocrystals have the following characteristics: They were insoluble in water and in 10 per cent or saturated NaOH, were not digested by artificial gastric juice, were not charred by heating to the melting point of glass, were not stained by ordinary stains, were negative to ninhydrin and xanthoproteic reactions, were dissolved in 10 per cent H Cl, (first decolorizing) turned blue in potassium ferrocyanide followed by 1 per cent H Cl in 70 per cent alcohol (partially dissolved), were not colored in potassium ferricyanide followed by 1 per cent H Cl in 70 per cent alcohol. Upon evaporation of the solution containing dissolved crystals in 10 per cent H Cl a reddish-brown amorphous deposit and colorless faintly birefringent crystals were formed; deposit turned blue in potassium ferrocyanide (forming ferric ferrocyanide); crystals were colorless obliquely extinct and with the general characteristics of Ca SO<sub>4</sub>·2H<sub>2</sub>O. These tests would appear to verify Quack's (1913, Ztschr. Zellforsch., 11 (1): 1-50) observation that the sphaerocrystals are gypsum. They also indicate the presence of ferric iron, possibly as an adsorption compound.

### 4. MISCELLANEOUS OBSERVATIONS

(a) *Pristionchus* sp. has colorless nonbirefringent intestinal globules. Only a minority blackened in osmic acid and stained in scharlach R, indicating fats. The remainder, presumably, are protein.

(b) *Aphelenchoides parietinus* (Bastian, 1865), Steiner, 1932, has non-birefringent intestinal globules of 2 sorts: one which blackened in osmic acid,

was soluble in absolute alcohol and stained red in Nile blue sulphate; the other, nonblackening, insoluble in absolute alcohol and stained blue in Nile blue sulphate. Apparently these are neutral fats and proteins respectively.

(c) *Blatticola blattae* (Graeffe, 1860), Chitwood, 1932 and *Spironoura affinis* Leidy, 1856, have colorless nonbirefringent globules which are soluble in alcohol, and blackened in osmic acid. Presumably these are fat.

(d) An unidentified oncholaimid has yellowish nonbirefringent sphaeroids which did not blacken in osmic acid, were negative to ninhydrin reaction and were positive to potassium ferriocyanide—H Cl reaction. Apparently these are equivalent to the olivaceous sphaeroids of *Theristus*.

(e) *Dorylaimus stagnalis* Dujarin, 1845 has numerous large colorless globules and numerous small olivaceous sphaeroids, both nonbirefringent. The globules were blackened by osmic acid and were soluble in alcohol while the sphaeroids did not blacken and were insoluble in alcohol; ninhydrin test on both types was negative. Presumably these are fats and olivaceous sphaeroids such as are found in *Theristus*.

#### 5. COMMENTS ON THE IRON TESTS

In performing controls with the iron tests as recommended by Lee, certain discrepancies were found in the use of the terms organic and inorganic. The following observations were made:

(a) Ferric chloride + potassium ferrocyanide yields prussian blue immediately.

(b) Ferric citrate + potassium ferrocyanide yields prussian blue after treatment in 1 per cent H Cl.

(c) Haemoglobin yields prussian blue only after the following procedure: Hydrolyzed in 10 per cent H Cl, H Cl removed by evaporation, treated with potassium ferrocyanide 15 minutes, rinsed in distilled water, 1 per cent H Cl in alcohol added.

Therefore, we conclude that Lee used the terms "inorganic" and "organic" iron incorrectly. As he used it "organic" iron applies to iron in a protein molecule since it requires hydrolysis before reacting (10 per cent H Cl or the equivalent) and should be termed protein iron. Immediate reaction in potassium ferrocyanide (or ferriocyanide) indicates inorganic iron. Reaction after treatment with 1 per cent H Cl (inorganic iron, *vide* Lee) indicates organic iron (such as ferric citrate) or insoluble inorganic iron (such as ferric phosphate).

The writers wish to thank Jacob M. Schaffer of the Biochemic Division, U. S. Bureau of Animal Industry, for his criticism of the manuscript, particularly regarding the foregoing paragraph.

#### Notes on the physiology of *Ascaris lumbricoides*. B. G. CHITWOOD, U. S. Bureau of Plant Industry.

The following experiments were conducted while the writer was employed in the U. S. Bureau of Animal Industry. The problems, as they stand, cannot be regarded as solved. However, since the writer will probably have no further opportunity to continue these investigations and since no results on comparable experiments have previously been published, they are presented in their unfinished condition.

#### 1. EXCRETION IN *ASCARIS LUMBRICOIDES* LINNAEUS, 1758

Mueller (1929, Ztschr. Zellforsch., 8(3): 361-403) claimed that the so-called excretory system of *Ascaris* is not excretory in function. Using fresh

living ascarids from pigs the writer collected excretory fluid in capillary tubing by holding specimens under a binocular microscope and applying the tube to the droplet at the excretory pore after some fluid had accumulated. A saturated solution of xanthidrol was drawn into each tube, mixing it with the excretory fluid. Crystals formed. These crystals were similar to those of dioxanthyl urea formed under similar conditions (in capillary tubing) by using urea dissolved in water and adding xanthidrol. By comparison it was estimated that the amount of urea present in the excretory fluid of *Ascaris* is about 0.02 per cent. It does not necessarily follow that urea is a normal waste product of *Ascaris* since the excretory fluid of specimens kept in normal saline solution gradually produced a less distinct dioxanthyl urea reaction. After 24 hours no urea was present in the excretory fluid (the xanthidrol test was negative). The writer interprets these results as indicating that urea is obtained from the host; it is gradually concentrated and eliminated by the excretory system. Presumably the system that eliminates foreign waste products would also eliminate the nematode's waste products. Photomicrographs of the crystals obtained are on file in the Division of Nematology, U. S. Bureau of Plant Industry, and may be borrowed by anyone interested.

## 2. PRESENCE OF PROTEOLYTIC ENZYMES IN THE ESOPHAGUS OF *ASCARIS LUMBRICOIDES*

A suspension was prepared by dissecting out 72 esophagi in 2.3 cc normal saline solution and grinding in a mortar. A substrate of 0.5 cc buffered  $\text{CaCl}_2$ -milk with pH values of 5.2, 6.7, 8 and 8.4 was used (prepared by John Bozicevich now in the U. S. Public Health Service). Boiled suspension and normal saline solution were used in controls. All tests were conducted at 40°C. under toluol. To each tube 0.2 cc of suspension, boiled suspension or saline solution were added. The controls showed digestion in some instances (due to bacteria) after 42 hours but never before that length of time. The tests at pH 6.7 showed partial digestion in 18 hours and practically complete digestion in 24 hours; in those at pH 8 no reaction was obtained; in the samples at pH 5.2 and 8.4 partial digestion was obtained in 24 hours. These tests seem to indicate the presence of a proteolytic enzyme (from the esophageal glands) inactive at its isoelectric point (pH 8) and most active in a very weak acid solution.

***Spiroxys gedoelsti* Schuurmans Stekhoven, a synonym of *Protospirura numidica* Seurat (Nematoda : Spiruridae). J. H. SCHUURMANS STEKHOFEN, JR., Zoological Laboratory, Utrecht.**

After receiving my paper on parasitic Nematoda of the Albert Parc Reservation in the Belgian Congo, Dr. B. G. Chitwood expressed to me as his opinion that *Spiroxys gedoelsti* Schuurmans Stekhoven might be a synonym of *Protospirura numidica* Seurat. A comparison of my notations and figures with the data published in the literature convinces me that this is in reality the case. What I considered to be papillary outgrowths of the pseudolabia must then be interpreted either as rudiments of the inner circle of labial papillae or, what is more likely, as the pseudolabial teeth, the number of which is 2 on each pseudolabium.

The name *Spiroxys gedoelsti*, therefore, is a synonym of *Protospirura numidica* and must be retracted. The genus *Protospirura* is a member of the family Spiruridae. As Chitwood suggests, the snake *Bitis arietans* may have swallowed the mammalian host which harbored the parasite in question.

**The raccoon, a new host of *Ascaris columnaris* Leidy, 1856 (Nematoda: Ascaridae)\*** O. WILFORD OLSEN and R. FENSTERMACHER, University of Minnesota.

Specimens of a nematode obtained from a raccoon, *Procyon lotor*, were identified as *Ascaris columnaris* Leidy. The raccoon was one of several which had died at a fur farm at Blue Earth, Minnesota. In an endeavor to learn the cause of the death, the owner sent the carcass to the Diagnosis Laboratory at the University Farm. Macroscopic examination revealed the presence of gastritis, enteritis, and numerous *Ascaris columnaris*. The histopathological lesions consisted of nephritis, congestion of the liver, and hemorrhagic gastro-enteritis. The presence of these parasites in this instance is not considered of pathological significance.

In life-history studies, Goodey and Cameron (1923, J. Helminth., 1(1):1-8) found that the eggs of *Ascaris columnaris* from the skunk, *Mephitis mephitis*, become fully embryonated in 2 weeks when kept at 33° C., and after 30 days at room temperature. Mice fed infective eggs were ill on the 4th day and examination of the liver and lungs showed migrating larvae.

This report constitutes the first record, so far as the authors can find, of *Ascaris columnaris* occurring in raccoons.

**Notes on the hot-water treatment of *Anguina tritici* galls on wheat and a comparison of an Indian and a Chinese collection by use of weight criteria.** DONALD P. LIMBER, U. S. Bureau of Entomology and Plant Quarantine.

INTRODUCTION

During the early months of 1937 the writer collected, in the course of duty at the U. S. Bureau of Entomology and Plant Quarantine Inspection House, an abundant supply of the galls of *Anguina tritici* (Steinbuch, 1799) Filipjev, 1936. The material was from 2 sources, namely Ladak, India, and Nanking, China.

The desirability of a treatment for infested wheat samples which would not be injurious to the seed when not planted soon after treatment prompted a recheck of earlier work on hot-water treatment. Particular attention was given to the presoak period as recent work with the stem and bulb nematode, *Ditylenchus dipsaci*, has shown that this factor is very important. Byars (1920, U. S. Dept. Agr. Bull. 842, pp. 1-40) simply states that, "galls soaked several hours or days in tap water were used," and, "It consists of soaking the gall-mixed seed for about an hour in unheated tap water." Leukel (1924, J. Agr. Research, 27:952) refers to the presoak period in general terms, "a preliminary soaking in cold water for several hours." Again Leukel (1929, U. S. Dept. Agr., Farmers' Bull. 1607, p. 11) states, "This treatment consists of soaking grain in water at ordinary temperatures for four to six hours." In the experiments reported below the length and temperature of the presoak were carefully controlled and recorded.

HOT-WATER TREATMENTS

A number of galls were immersed in water in a Syracuse watch glass. After soaking for the desired time 3 galls were removed and placed in a small tin container the sides and bottom of which were made of a fine mesh wire screen. The box was then immersed in a small hot-water treating tank. When the treatment was complete one or two galls were opened in a watch glass containing a small amount of water. The other galls were allowed to become air dry, then treated as the first. No difference was observed in the results between the galls opened at once and those which were dried before opening. The nematodes were examined at frequent intervals for 6 days following treatment.

Examination of many presoaked galls before treatment showed that a few larvae became active after 2 hours soaking. It is evident, therefore, that the treatments at 120°F. for 20 min. with a presoak of 2 hours or longer caused

\*Journal Series No. 1490.

many active larvae to become quiescent and delayed the revival of those which had not become active. All treatments tested below 122°F. for 30 min. were ineffective from the standpoint of control.

TABLE 1.—*Hot-water treatment of presoaked Anguina tritici galls*

<i>Presoaked</i> (70°—80° F.)	<i>Treating</i> <i>Temp.</i>	<i>Time</i>	<i>Results after</i> <i>24 hours</i>	<i>Final results</i> <i>after 6 days</i>
1½ hours	120°F.	20 min.	Nematodes active	No killing
2 hours	120°F.	20 min.	Few moving	No killing
3 hours	120°F.	20 min.	Few moving	No killing
4 hours	120°F.	20 min.	No movement	No killing
5 hours	120°F.	20 min.	No movement	No killing
6 hours	120°F.	20 min.	No movement	No killing
2 hours	122°F.	30 min.	No movement	All dead

WEIGHT AND NUMBER COMPARISON OF NEMATODE POPULATIONS OF GALLS FROM  
INDIA AND CHINA

The galls were weighed on an analytical balance and the weights recorded. Thereafter, as time permitted, a gall was soaked in water for 2 hours to soften the wall. It was then removed and touched to a paper towel several times to remove the excess water, then placed on a microscope cover slip. The gall was then torn into 2 or 3 pieces by means of needles. This operation was performed with the aid of a low power binocular microscope. The cover slip and the gall fragments were dropped into an Erlenmeyer flask containing 200 cc of water. The needles' points were rinsed in the flask. The flask was then shaken at intervals for at least 2 hours before the first count was made.

When ready for counting the flask was shaken again vigorously. One cc was drawn into a 1-cc pipette and distributed in drops arranged in rows in a petri dish. The count of all the nematodes found in these drops, multiplied by 200, it is believed, would approximate the nematode content of the gall. From 5 to 8 counts were made for each gall and the average of these counts was considered to be the actual content for the purposes of the following calculations.

TABLE 2.—*Showing weight of gall and number of nematodes contained*

Weight of galls (mg)	Number of nematodes per gall								Average
	1	2	3	Single counts			7	8	
	4	5	6						
India									
2.5	3,800	3,600	3,000	3,400	4,400	3,200			3,566
3.	3,400	2,000	2,400	800	3,400	3,400	2,800	2,800	2,625
3.1	5,000	5,200	6,400	5,800	6,800	5,800	6,400		5,914
5.5	13,000	10,000	11,600	9,600	12,400	13,200	11,400	13,200	11,800
7.3	14,400	15,200	12,800	15,400	13,000	12,800			14,100
7.4	15,600	14,600	13,200	12,200	14,400	13,200	11,200		13,485
9.2a	22,600	22,800	25,400	24,600	26,800				24,440
9.2b	24,000	23,600	24,600	18,600	30,400	21,000			23,700
47.2mg									
99,630									
China									
3.3	4,800	7,400	3,600	5,000	6,800	4,800	6,800	5,200	5,425
4.	13,800	12,000	13,200	16,200	13,600				13,760
4.2	11,200	14,600	9,800	14,400	14,200	14,200			13,066
6.2	13,200	16,200	20,200	11,200	17,800	14,200	16,600		15,628
6.3	12,400	14,600	14,400	16,000	17,000				14,880
6.3	16,400	14,200	12,600	13,200	16,400	15,600	15,000		14,771
7.4	26,832	28,912	25,168	25,584	23,296				25,958
9.2	30,600	27,200	31,000	27,800	32,400	26,200			29,200
46.9mg									
132,688									

Examination of table 2 shows that the Chinese galls, with the one exception of the smallest gall, contained more nematodes per milligram gross weight than those from India. Direct comparison can be made in the cases of the

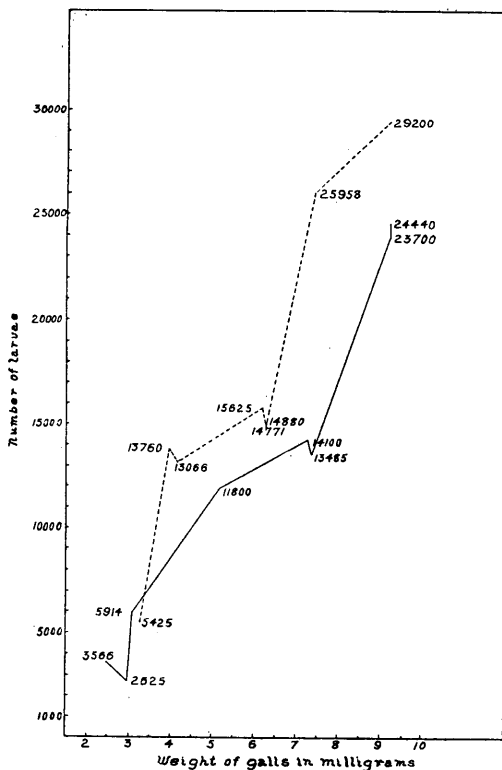


FIG. 7  
Comparative nematode populations in *Anguina tritici*  
galls from India and China.

7.4 mg and the 9.2 mg galls as these weights occur in both groups. A comprehensive comparison is given in figure 7.

If the total number of nematodes calculated to be present in the Indian galls is divided by the total weight of the galls, the average of 2110 nematodes per milligram of gross gall weight is obtained. The same method applied to the Chinese totals gives the average of 2829 nematodes per milligram, or 34 per cent more per milligram. This would indicate either that the Chinese nematode weighs less than the Indian strain or that the Indian gall has heavier walls, or that both factors are involved. To clear up this point a calculation of the average weight of the individual larva was attempted.

The galls listed in table 2 were used in this determination, with the exception of those of 4 mg or less in weight. It was thought that the errors due to the impracticability of separating the last few lar-

vae from the gall tissue without losing some of the tissue would be minimized by using only the larger galls. By observation of the washed gall fragments it was found that while the larvae separated almost perfectly from some galls, others still carried possibly 100 larvae partly embedded in the tissue.

The fragments of each gall were removed from the flask after the count had been made. They were then washed in 2 changes of water in a watch glass and, as far as possible, all the larvae removed, using the needles. The gall tissue was then placed in a labeled pill box and left uncovered to dry for several days. After the 3d day the dried material was weighed; thereafter the weighing was repeated several times until the 10th day. The weights used in table 3 are those of the material after drying for 10 days. It should be noted that the tissue of some of the galls remained at constant weight after the 3d or 4th day, others fluctuated. The greatest change found for any gall between the last 2 weighings was .18 mg. All others were .1 mg or less, 4 remaining constant.



TABLE 3.—*Relative weight of the gall tissue to the nematode content and calculated weight of a single second-stage larva*

Gross wt.	Wt. of gall tissue	Difference or wt. of nematode content		Number of nematodes	Calculated wt. of single dry 2nd stage larva
Origin of gall—India					
		%	%		
9.2 mg	6.05 mg	65.7	3.15 mg	34.3	24,440
9.2	6.2	67.3	3.	32.7	23,700
7.4	4.9	66.2	2.5	33.8	13,200
7.3	5.1	69.8	2.2	30.2	14,100
5.5	3.8	69	1.7	31	11,800
Avg. 67.6		Avg. 32.4		Avg. .0001483	
Origin of gall—China					
9.2	5.8	63	3.4	37	29,200
7.4	4.5	60.8	2.9	39.2	25,958
6.3	4.1	65	2.2	35	14,771
6.3	4.3	68.2	2.	31.8	14,880
6.2	4.2	67.7	2.	32.3	15,628
4.2	2.55	60.7	1.65	39.3	13,066
Avg. 64.3		Avg. 35.7		Avg. .0001276	

Study of table 3 shows that the Indian galls contain a higher percentage of gall tissue than the Chinese galls. This is in accord with the previous finding that the Indian galls contained fewer larvae per milligram of gross gall weight. Further, table 3 indicates, if the average of the small number of galls studied is to be trusted, that the individual larvae from Indian galls are heavier than those of the Chinese strain. Indeed, if galls of approximately equal weight are considered, the difference in weight is consistent. There is a marked tendency shown for the larvae of the medium weight galls to be heavier than the larvae from heavier or the very light galls.

Byars published evidence indicating that there is a strain of *Anguina tritici* in China having smaller second-stage larvae and eggs than specimens from other sources. He gives the length for material from 16 different localities (source not given) as average  $869\mu$ , the extremes  $770\mu$  and  $966\mu$ . For the Chinese strain he studied he gives average length  $793\mu$ , range 658 to  $910\mu$ . The data based on weight measurements is in accord with his findings.

## SUMMARY

No control is indicated by use of hot-water treatment less severe than  $122^{\circ}\text{F}$ . for 30 minutes following a 2-hour presoak.

Counts of the nematode content of galls from Ladak, India, ranging in weight from 2.5 mg to 9.2 mg give a nematode population ranging from 3566 to 23,700 second-stage larvae. Similar counts of galls from Nanking, China, varying in weight from 3.3 mg to 9.2 mg gave a population range of 5425 to 29,200 second-stage larvae.

The Indian galls averaged 2110 larvae per milligram gross gall weight. The Chinese galls average 2829 larvae per milligram gross weight.

The data presented indicate that the Chinese galls studied have lighter walls, and also that the larvae weigh less than those from the Indian material.

**Occurrence of the coccidian *Eimeria bukidnonensis* in American cattle.**  
J. F. CHRISTENSEN, U. S. Bureau of Animal Industry.

Tubangui (1931, Philippine J. Sci., 44: 253-271) described *Eimeria bukidnonensis* on the basis of oöcysts found in the feces of a bull from Bukidnon, Mindanao, that was slaughtered in Manila. His description is as follows: "The oöcysts are yellowish to darkish brown and are uniformly pyriform, the average shape index being 1.37. They measure 46.8 to 50.4 by 33.3 to 37.8 microns. . . . The oöcyst wall shows radial striations and is about 2 microns thick except at the micropyle end where it is very thin. The micropyle is conspicuous, being about 4 microns wide. . . . A definite residual body has not been seen either inside the oöcyst or in the sporocysts."

Recently a sample of bovine fecal material containing a number of unusual brown oöcysts was sent for diagnosis to the Zoological Division of the U. S. Bureau of Animal Industry by Dr. D. W. Baker of the New York State Veterinary College at Cornell University. These oöcysts agree with Tubangui's description for those of *E. bukidnonensis* in all features except size, the American specimens being considerably smaller. The following dimensions were taken from measurements of 10 oöcysts: Length, 32 to 41 $\mu$ ; width, 24 to 30 $\mu$ ; thickness of wall, about 2 $\mu$ ; diameter of micropyle, about 3.5 $\mu$ . The distinct brown color, opaque appearance, thick wall, and pyriform shape readily distinguish oöcysts of this species from others occurring in cattle. As far as the writer can determine this is the first report of the occurrence of *E. bukidnonensis* in cattle in this country.

**Fresh-water nematodes from the intestines of fish.\*** V. N. MOORTHY, Mysore State Department of Health, Bangalore, India.

While conducting certain investigations on the biological control of dracontiasis and on the life history of *Camallanus sweeti* in Chitaldrug District, Mysore State, India, 3 nematodes belonging to the families Monhysteridae and Dorylaimidae were obtained from the intestines of fresh-water fish, *Barbus puckelli* Day and *Ophicephalus gachua* Ham, respectively. One of these nematodes appears to be *Monhystera paludicola* de Man while the other 2 appear to be new species. To one the name *Monhystrella mysorensis*, n. sp. and to the other the name *Dorylaimus krishnaraoi*, n. sp. is proposed the latter in honor of Sir M. N. Krishna Rao, to whom the writer is greatly indebted for valuable help and encouragement.

1. *Dorylaimus krishnaraoi*, n. sp.

*Description*.—Female: Body length 1.24 mm, maximum width 0.03 mm.  $\alpha = 41.3$ ,  $\beta = 4.6$ ,  $\gamma = 8.2$ . Lips amalgamated and set off by a slight depression. Spear a little longer than body width at lip region and aperture occupying 2/5 of spear length (fig. 8, E). Esophagus expanded slightly posterior to middle, anterior portion about 3/5 of total length. Esophago-intestinal valve convex-conoid and about 1/3 body width. Rectum 1 1/4 times anal diameter in length. Prerectum about 1 1/2 times as long as rectum (fig. 8, F). Vulva transverse, 46 per cent of body length from anterior end. Ovaries reflexed, anterior ovary 130 $\mu$  and posterior ovary 300 $\mu$  from vulva, respectively, an egg and a fully developed ova being responsible for the exceedingly long posterior ovary. Uterus containing 1 egg measuring 20 $\mu$  by 96 $\mu$ ; sperms not present. Tail dorsally convex-conoid in anterior portion and tapering uniformly to a slender, pointed terminus posteriorly.

Male unknown.

*Habitat*.—Intestines of *Ophicephalus gachua* Ham.

*Locality*.—Chitaldrug District, Mysore State, India.

*Specimens*.—U. S. N. M. Helm. Coll. No. 9129.

\*Acknowledgment is made to the International Health Division of the Rockefeller Foundation for having granted the fellowship which made this work possible.

The writer wishes to express to Dr. B. Schwartz, Chief of the Zoological Division, U. S. Bureau of Animal Industry, his appreciation for placing laboratory facilities at his disposal, and to Gerald Thorne and B. G. Chitwood his gratitude for valuable help and guidance.

This species appears to be most closely related to *Dorylaimus subtilis* Thorne and Swanger, 1936, but differs from it in the size of the egg; maximum measurement of eggs in *D. subtilis*,  $30\mu$  by  $70\mu$ , in *D. krishnaraoi*, n. sp.,  $20\mu$  by  $96\mu$ . The nematode was found imbedded in the mucous coat of the midgut of a fresh-water fish, *Ophicephalus gachua* Ham, collected from a pond in the Chitaldrug District, Mysore State, India.

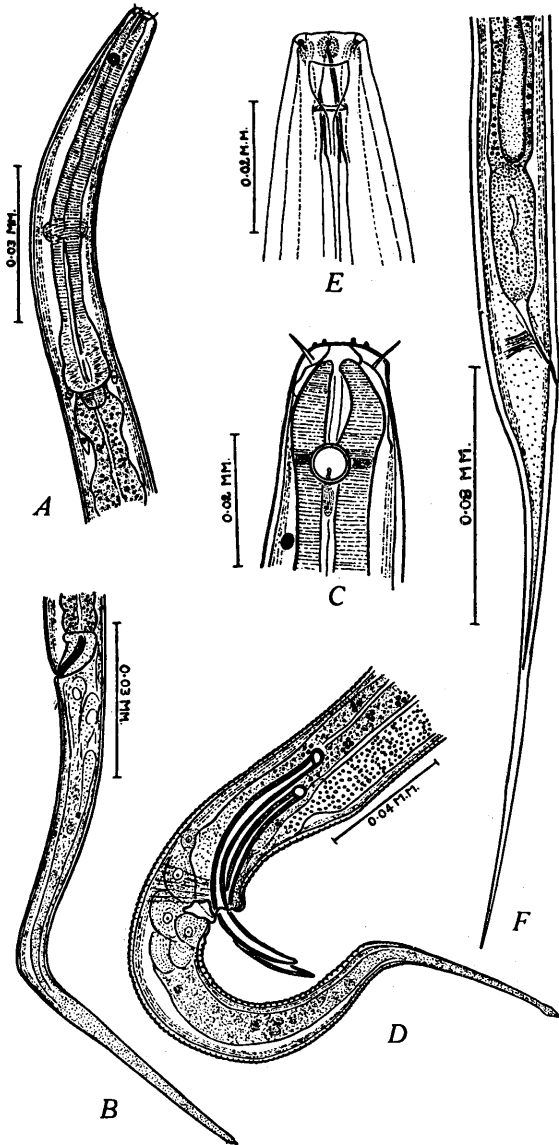


FIG. 8

A & B—*Monhystrella mysorensis*, n. sp. (A—Esophageal region, B—Tail end of female). C & D—*Monhystera paludicola* (C—Head, D—Tail end of male). E & F—*Dorylaimus krishnaraoi*, n. sp. (E—Head, F—Tail end of female).

Specimen.—U. S. N. M. Helm. Coll. No. 9128.

## 2. *Monhystrella mysorensis*, n. sp.

**Description.**—Female: Body length 0.38 mm; maximum width 0.014 mm.  $\alpha = 27.1$ ,  $\beta = 5.1$  and  $\gamma = 3.3$ . Cuticle delicate, about  $0.5\mu$  in thickness, and traversed by exceedingly fine transverse striae. Stoma cylindrical,  $3.5\mu$  long. Amphids circular, situated about  $10\mu$  from the anterior extremity; diameter about  $\frac{1}{4}$  body width in the region of the amphid (fig. 8, A). Nerve ring situated 0.045 mm from anterior extremity. Esophagus 0.074 mm long and with distinct pyriform cardiac swelling (fig. 8, A). Esophago-intestinal valve convex-conoid, about  $\frac{1}{3}$  of body width in region of cardia. Intestine with a distinct globular swelling at its commencement (fig. 8, A). Vulva transverse, situated in the middle of body (50.4 per cent). Ovary reflexed, 19 per cent of body length from vulva. Egg with length equal to about twice body diameter. Anus 0.275 mm from anterior extremity. Probable unicellular organ present about 0.035 mm from anus. Tail 0.115 mm long, gradually tapering and terminating in mucronate spinneret (fig. 8, B).

Male unknown.

**Habitat.**—Intestines of *Barbus puckelli* Day.

**Locality.**—Chitaldrug District, Mysore State, India.

This species differs from *Monhystrella plectoides* in being smaller in size and in having a distinct globular swelling at the commencement of the intestine, and from *Monhystrella godetti*, in having a longer tail as compared to the total body length, and in the more anterior location of the vulva.

### 3. *Monhystera paludicola* de Man

**Description.**—Male: Body length 0.823 mm; maximum width 0.039 mm, near middle of body,  $\alpha = 21.1$ ,  $\beta = 6.1$ ,  $\gamma = 7.5$ . Cuticle  $1\mu$  thick, traversed by fine transverse striae. Stoma 0.018 mm long, dilated at one side, being narrower at the extremities and comparatively wider in the middle (fig. 8, C). Amphids circular; diameter equal to  $1/3$  of body width in amphidial region. Ocellus slightly longer than wide, reddish brown in color, situated 0.03 mm from the anterior extremity. Nerve ring situated 0.08 mm from the anterior extremity. Esophagus 0.135 mm long. Cloaca situated 0.71 mm from the anterior extremity, spicules subequal, right 0.08 mm long, left 0.07 mm long; gubernaculum present. Caudal glands distinct. Tail 0.11 mm long, abruptly narrowing from about its middle and terminating in a spear-like tip (fig. 8, D).

Female unknown.

**Habitat.**—Intestines of *Barbus puckelli* Day.

**Locality.**—Chitaldrug District, Mysore State, India.

**Specimens.**—U. S. N. M. Helm. Coll. No. 9127.

This species resembles very closely *M. paludicola*, but differs from it in the general shape of the stoma, in the larger size of the amphid, and in the tail end being slightly flattened.

### The scientific name of the common North American chigger preoccupied.

H. E. EWING, U. S. Bureau of Entomology and Plant Quarantine.

It has been pointed out recently by Oudemans (1937, *Kritisch Historisch Overzicht der Acarologie*, part 3, v. D, p. 1389) that the name *Leptus irritans* was used by Lucas in 1847 for a "patatta" mite (chigger) taken in Brazil. At a meeting of the Entomological Society of France on April 28, 1847, M. Ghiliani presented a communication from M. le marquis de Brême which included a description of this mite by Lucas. The description is in the form of an extended footnote prepared and signed by H. Lucas, hence the name of the mite cannot be attributed to de Brême.

Oudemans regards *Leptus irritans* Lucas as a synonym of *Trombidium batatas* (Linn.). However, the description of *batatas* by Linnaeus is so inadequate that there is little hope of its identification with any of the chiggers now known from South America. The description of *Leptus irritans* Lucas, while good for its day and positively identifying his species as a chigger, gives no character that would identify it with any chigger species known to us today. Since under the rules of nomenclature *Leptus irritans* Riley can no longer be applied to our common North American chigger, the problem of selecting the next available name presents itself.

The specific name *tlalsahuatl* (also spelled *tlalzahuatl* and *tlalzahuatl*), which has been used in connection with the generic names *Tetranychus*, *Trombidium*, *Microthrombidium*, and *Trombicula*, has been attributed to Dugès, but apparently should be attributed to Murray. Lemaire (1867, *Compt. Rend. Acad. Sci. [Paris]*, 65:215) applied the name *tlalsahuatl* to a chigger supposed by him to be the common Mexican chigger. He found his specimen (which was soon lost) in the eye (a rather unusual place to find a chigger) of a four-year-old girl living in France. He was of the opinion that it was introduced from Mexico because the girl's father had previously received from that country numerous boxes, the packing and contents of which were left for some time close to the lawn where the child played. The present writer doubts very much the probability of introducing chiggers by means of transoceanic traffic in packing of the common sorts used in such trade. He has examined many thousands of mites taken from various kinds of packing materials by our United States quarantine inspectors, but among them has found not a single chigger.

The danger of introducing chiggers from one nation to another comes from the transportation of their hosts (rats, mice, dogs, etc.) when infested with chiggers or from their transportation in either the nymphal or adult stage in soil, rather than from the transportation of larvae in packing material. Apparently no valid reason exists for identifying the chigger found by Lemaire in France with the one later described by Dugès from Mexico. The chances are much greater that Lemaire's specimen was the European chigger, *Trombicula autumnalis* (Shaw), which occurs in many places in France, and that the girl he speaks of was infested from the lawn where she played. Lemaire did not apply the name *tlalsahuatl* as part of a binomial, but it was so used later by Murray, who, in 1877 (Economic Entomology, South Kensington Museum Science Handbook, p. 113), validated the name *Tetranychus tlalsahuatl* which he attributed to Lemaire. This was done, however, by quoting Lemaire's description of *tlalsahuatl* which applied to the specimen taken from the young girl in France. This indicates that *Tetranychus tlalsahuatl* Murray is a synonym of the European chigger, *Trombicula autumnalis* (Shaw).

Dugès described (1892, *El Estudio*, 4(6):198) under the name of *tlalsahuatl* (also spelled *tlalzahuatl*) a common chigger of Mexico, calling it the hexapod larva of a *Trombidium*. Apparently Dugès used the name *tlalzahuatl* as a common name, since it is preceded by the article "*El*." Even if the name is regarded as a scientific one, it could hardly be considered binomial. Most certainly he did not propose the name as that of a new species and did not attribute it to another author.

In 1910 Oudemans (*Ent. Ber.*, 3(54):84) described under the name of *Microthrombidium alfreddugèsi* a chigger taken from man by Alfred Dugès in Mexico about 1892. It was in the Trouessart Collection under the name of *tlalzahuatl*. Oudemans' specific name appears to be the valid one for our chigger. Therefore, the proper scientific name of the common North American chigger becomes *Trombicula alfreddugèsi* (Oudemans).

AN ANNOTATED LIST OF SCIENTIFIC NAMES THAT HAVE BEEN APPLIED TO THE  
COMMON NORTH AMERICAN CHIGGER

- 1867. *Tlalsahuatl* (Lemaire). The name *tlalsahuatl* was used as a monomial and based on a specimen taken in France. Later the name was validated by Murray.
- 1873. *Leptus irritans* Riley. Preoccupied by *Leptus irritans* Lucas, 1847.
- 1877. *Tetranychus tlalsahuatl* Murray. Name *tlalsahuatl* validated, but description pertains to a specimen taken in France. Probably a synonym of *Trombicula autumnalis* (Shaw).
- 1892. "*El tlalzahuatl*" Dugès. Name apparently used neither as a scientific one nor as a binomial.
- 1910. *Microthrombidium alfreddugèsi* Oudemans. First valid scientific name for our common North American chigger.
- 1921. *Trombicula cinnabaris* Ewing. Description based on adults. Synonym of *Trombicula alfreddugèsi* (Oudemans).
- 1921. *Leptus* (*Trombicula*?) *similis* Hirst. Synonym of *Trombicula alfreddugèsi* (Oudemans).

**White clover as a host of the sugar-beet nematode.** C. W. McBETH, U. S. Bureau of Plant Industry (Salt Lake City, Utah).

Although the sugar-beet nematode, *Heterodera schachtii* Schmidt, has been found previously on the roots of white clover (Dutch clover), *Trifolium repens* L., very little is known about the manner of attack or the effect of this nematode upon the tissues of its host plants other than the sugar-beet. There is here reported an establishment of this nematode upon white clover associated with lawn grass at Salt Lake City, Utah. The injury resembles root-knot injury in that gall-like swellings are present (fig. 9, A).

The larvae as a rule enter the rootlet just above the growing point and orient themselves almost parallel with the longitudinal axis of the root, the tail

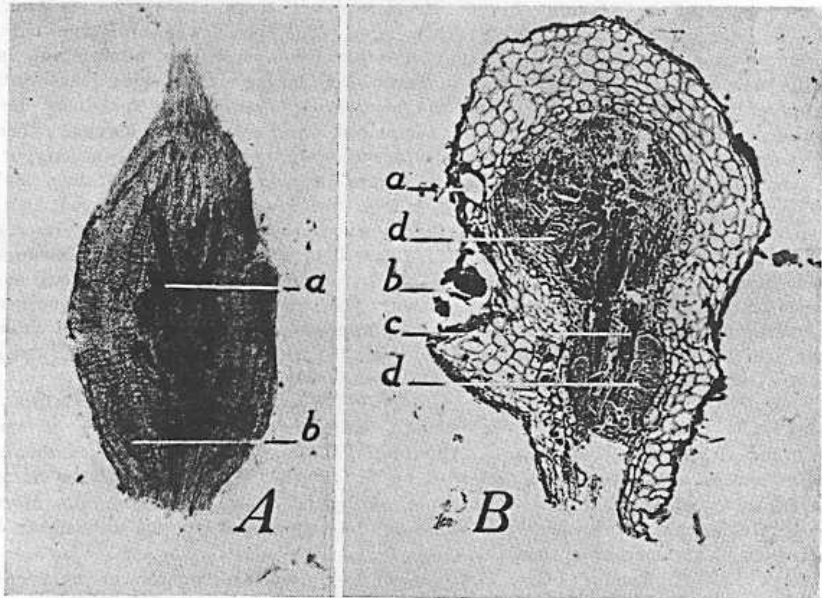


FIG. 9

A—Swollen clover root tip containing eleven *Heterodera schachtii*. a, immature *H. schachtii*; b, accelerated cell division to form new rootlet. B—Cross section of main root and longitudinal section of branch. a, cavity made by developing female; b, cross section of shrunken *H. schachtii* female bursting from rootlet; c, vascular tissue, vessels disintegrated and broken by giant cells; d, giant cells.

closer to the surface (fig. 9, A). The head is usually found pointing towards the main root; in only one case was the reverse of this position observed. The larvae confine themselves to the cortex with the exception of the head which is inserted into the vascular tissue. Soon after the larvae enter the rootlet there is a disintegration of cell walls and large granular cells or "giant cells" are formed (fig. 9, B). This disintegration of the vessels of the vascular region disrupts the passage of the food from the roots to the leaves and vice versa, resulting in the death of the rootlet. New rootlets are produced to replace those dying and these in turn are attacked, but in no instance was death of the plant observed.

The typical lemon-shaped females were found attached to the rootlets in the usual manner of *H. schachtii* and in the last sample taken, November 26th, the overwintering cysts were found free in the soil. Several males were observed coiled within the molting cuticle in the galls but were difficult to find living free in the soil, only 2 being recovered. These measured .63 mm and .98 mm in length, slightly smaller than those from sugar-beets in the Salt Lake area. Only one testis is present, the tail is short and blunt, typical of *H. schachtii* found on beets rather than the longer tailed forms found on shadscale by Thorne (1935, J. Agr. Research, 51(6):510).

#### Measurements—

Larvae in clover: 0.48 mm;  $\alpha = 22.7$ ;  $\beta = 3.7$ ;  $\gamma = 9.2$ .

Larvae in beets: 0.48 mm;  $\alpha = 19.6$ ,  $\beta = 3.4$ ;  $\gamma = 9.3$ .

Eggs in clover: 0.114 mm  $\times$  .049 mm.

Eggs in beets: 0.118 mm  $\times$  .049 mm.

The above measurements were based upon the average of 10 individuals and as can be seen, those from the beets correspond quite closely to those from clover. An unsuccessful attempt was made to transfer this nematode from clover to beets, but since the attempt was not made until late in the summer the result is not conclusive. Nothing could be ascertained concerning the probable source of the infestation.

**Two nematodes associated with decaying citrus fruit.** J. R. CHRISTIE, U. S. Bureau of Plant Industry.

The nematodes herein described were received on an agar-slant culture, associated with the fungus *Alternaria citri* Pierce, from H. S. Fawcett of the Citrus Experiment Station, Riverside, California. The inoculum for this culture had been secured from a decaying citrus fruit taken from the tree. One of these nematodes is an undescribed species of the genus *Hexatylus* Goodey, 1926, for which the name *H. intermedius*, n. sp. is proposed. The other is an apparently undescribed species belonging to the family Allantonematidae for which the name *Prothallonema dubium*, n.g., n. sp. is proposed.

*Hexatylus intermedius*, n. sp.

**Measurements.**—♂: length = 1.12 to 1.3 mm;  $\alpha$  = 33 to 37,  $\beta$  = about 7.5 to 8,  $\gamma$  = 20 to 21. ♀: length = 1.38 to 1.54 mm;  $\alpha$  = 34 to 38,  $\beta$  = about 7 to 7.5,  $\gamma$  = 18 to 23,  $v$  = 88 to 94%. Only in an occasional specimen can the base of the esophagus be distinctly seen.

**Description.**—Cephalic extremity rounded, not distinctly set off. Head, in *en face* view, with 8 somewhat elevated sectors: the dorsomedial and ventromedial apparently narrow, slightly elevated ridges; the lateral and submedial sectors wider. Each lateral sector bears near the outer contour of the head an amphid, and near the mouth a papilla. Each submedial sector bears near the outer contour of the head a papilla, and near the mouth a papilla. The head, therefore, bears an external circle of 4 and an internal circle of 6 papillae. Stylet in both sexes 4 to 4.5 $\mu$  long with 3 somewhat diverging basal knobs. Esophagus without metacarpus bulb, crossed about midway by nerve ring; part posterior to nerve ring glandular. Lateral fields relatively wide, 5 to 7 $\mu$ . Excretory pore anterior to base of esophagus, a distance about equal to corresponding body width; excretory tube conspicuous; ovary with S-shaped flexure, blind end directed anteriorly and often extending nearly to base of esophagus. Spicules about 28 to 30 $\mu$  long. Bursa extending to tip of tail.

**Habitat.**—Associated with decaying citrus fruit.

**Locality.**—Riverside, California, U. S. A.

**Discussion.**—When considering species related to *Hexatylus intermedius* one must scrutinize not only *H. viviparus* Goodey, 1926, but also the species which have been placed in the genus *Neotylenchus* Steiner, 1931. Of the latter there are 5, namely: *N. abulbosus* Steiner, 1931 (type) (= *Hexatylus abulbosus*, fide Goodey); *N. obesus* Thorne, 1934; *N. latus* Thorne, 1935; *N. fungorum* (Bütschli, 1873) Filipjev, 1936; and *N. consobrinus* (de Man, 1906) Filipjev, 1934. All these species are characterized by having (a) an esophagus constricted near the middle in the region of the nerve ring, with the anterior part more or less spindle shaped and without metacarpus bulb and (b) the vulva located well towards the posterior end of the body. In *H. viviparus*, the cephalic extremity, in *en face* view, is divided into 6 sectors and the stylet has 6 basal knobs. Facts pertaining to the appearance of *N. fungorum* and *N. consobrinus* in *en face* view are not available but in the other species assigned to this genus the head shows 8 sectors and in all the stylet has 3 basal knobs. Apparently on these 2 characters alone the 2 genera are differentiated. Goodey regards these differences as insufficient to justify the recognition of 2 genera. A comparison of *H. intermedius* with each of the above mentioned species will reveal various differences but in one respect it differs from all of them, namely, in the bluntly rounded tail of the female. In *en face* view the appearance of the head is somewhat intermediate between that of most tylenchids with 6 sectors, and that of *Neotylenchus abulbosus*, *N. obesus* and *N. latus*, each with 8 sectors of about equal size.

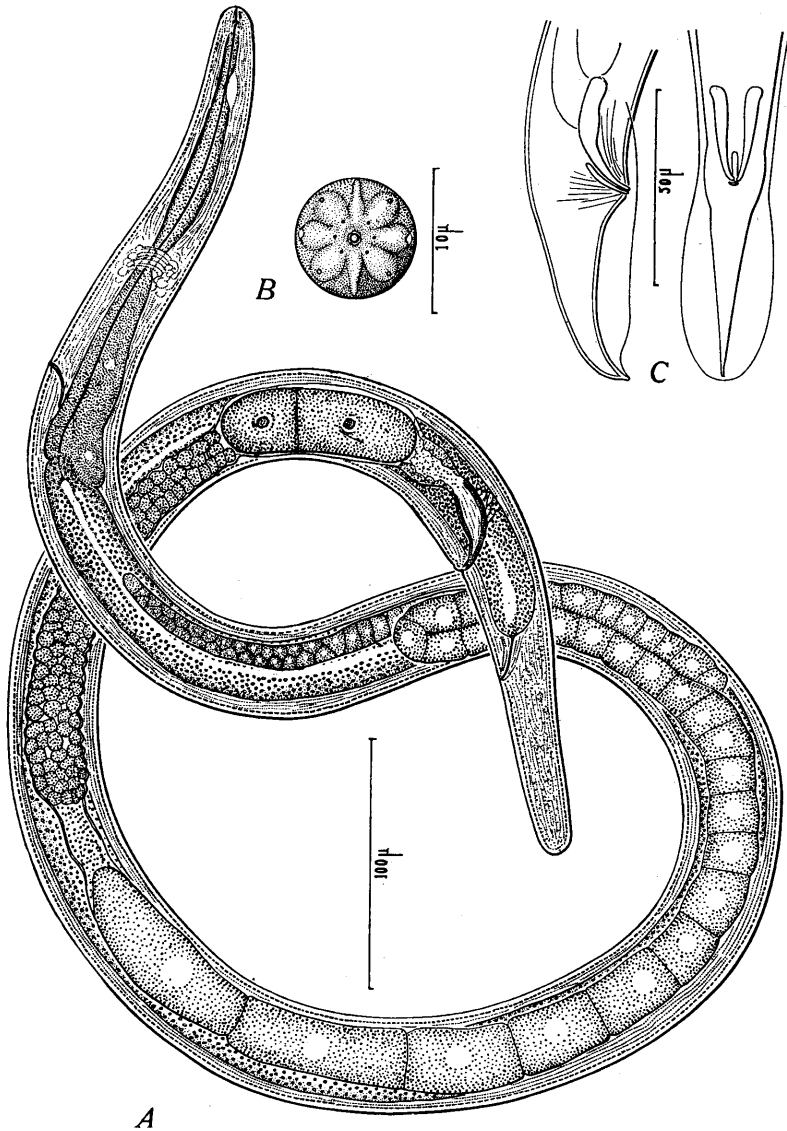


FIG. 10

*Hexatyclus intermedius*, n. sp.A—Female. B—Head, *en face* view. C—Tail of male, lateral and ventral views.

After studying the present species and reviewing the literature the writer has concluded that Goodey was probably justified in synonymizing the genera *Hexatyclus* and *Neotylenchus* in spite of a prevailing tendency to split the Tylenchidae into numerous genera sometimes based on what seems to be rather superficial characters. Consequently the present species is placed in *Hexatyclus* instead of in *Neotylenchus* where it would properly belong if the latter were recognized as a valid genus.



*Hexatylus intermedius* was kept on cultures for a period of about 3 months. At 2-week intervals it was transferred to new cultures on which the fungus *Alternaria citri* previously had been established. It laid eggs freely, built up a moderately large population and appeared to thrive fairly well. At the time the cultures were discontinued there was nothing to indicate that they could not have been successfully maintained for a long period.

*Prothallonema*, new genus

*Diagnosis*.—Allantonematidae: Preparasitic female relatively slender, ending posteriorly in a rounded terminus. Stylet present. Esophagus consisting of a narrow, cylindrical, nonglandular anterior part and a wider and longer, cylindrical, glandular posterior part which abuts the anterior end of the intestine. Esophageal gland (or glands) empties into lumen of esophagus near anterior end of glandular part from which point a distinct, cuticula-lined tube extends, uninterrupted, to the base of the stylet. Reproductive system with posterior part adjacent to vulva, differentiated and set off by constriction. Post-vulvar uterine sac present. Preparasitic male and parasitic female unknown.

*Type species*.—*Prothallonema dubium*, n. sp.

*Discussion*.—*Howardula benigna* Cobb, 1921, and *Heterotylenchus aberrans* Bovien, 1937 both possess 3 esophageal glands, 1 opening into the esophageal lumen on the dorsal side near the base of the stylet and 2 opening on the ventral side somewhat farther back. This is the typical tylenchid arrangement. In *Allantonema mirabile* Leuckart, 1884, and *Aphelenchulus tomicci* Bovien, 1937, the openings of the glands are similarly placed but only 2 glands have been observed, the ventral gland being apparently unpaired. Likewise only 2 glands have been seen in *Scatonema wülkeri* Bovien, 1932, both of which empty into the esophagus through separate openings in about the same region and at a considerable distance from the stylet. Only 1 gland has been seen in *Tylenchinema oscinellae* Goodey, 1930; it opens into the esophageal lumen on the dorsal side likewise some distance from the base of the stylet. In this case, however, the esophageal tube extends posteriad a short distance where it appears to end abruptly which suggests to the writer the possibility that one or more inconspicuous ventral glands may communicate with the esophageal lumen where it appears to end.

With regard to the structure and arrangement of the esophageal glands the form under consideration appears most closely to resemble *Tylenchinema* and, possibly, *Scatonema*, but in neither case is the resemblance very marked. In *Scatonema wülkeri* 2 easily distinguished glands lie beside the intestine and extend posteriad beyond the middle of the body. Although only 1 gland has been noted, the situation is similar in *Tylenchinema oscinellae*. In neither case does the gland (or glands) form a cylindrical mass filling the body and abutting the anterior end of the intestine. It seems possible that the present species may be more closely related to the genus *Bradynema*. *B. strasseni* Wülker, 1921 has a similarly shaped posterior end and some of the published figures suggest a similar esophageal arrangement. However descriptions of the corresponding stage in members of this genus are not sufficiently explicit to enable one to make adequate comparison. A clearly differentiated region of the reproductive system anterior to the vulva and a post-vulvar uterine sac are characters that apparently have not been noted in any other allantonematid.

How to deal with the present species taxonomically has been a perplexing question. The genera and species of the Allantonematidae are based largely on the parasitic female although in most instances the free-living stage is known and has been described in considerable detail. It seems to the writer that the structure of the adult free-living stage may furnish as reliable indications of phylogenetic relationships as the structure of the fully grown parasitic female which has undergone the modifications and specializations characteristic of the

group. It appears reasonably certain that the present form is not any of the known species for which the corresponding stage has been described nor does it appear to belong to any of the established genera. To place it in a new genus should not result in confusion even though subsequent investigation may demonstrate that the genus is a synonym.

*Prothallonema dubium*, n. sp.

*Measurements*.—Preparasitic female: Length =  $96\mu$  to  $1.07\text{ mm}$ ;  $\alpha = 29$  to  $40$ ,  $\beta = 2.6$  to  $4$ ,  $\gamma = 14$  to  $15.4$ ,  $v = 81$  to  $90\%$  (usually  $88\%$ ).

*Description*.—Body, from about middle region, tapers slightly and more or less uniformly posteriad and ends in a blunt rounded terminus. Stylet about

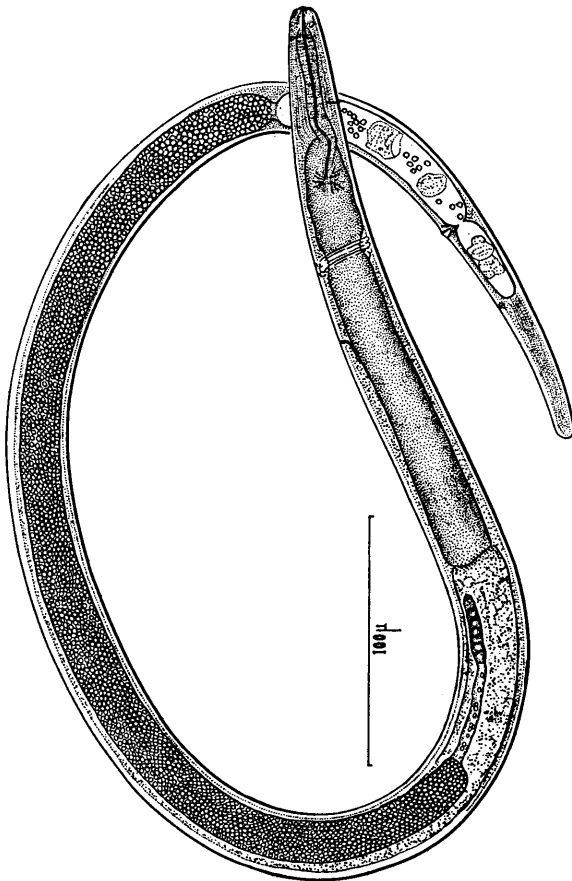


FIG. 11  
*Prothallonema dubium*, n. sp.

$9\mu$  long, without basal knobs. Esophageal region  $230$  to  $370\mu$  long or about  $\frac{1}{4}$  total body length, composed grossly of 2 parts. Anterior part narrow ( $5$  to  $8\mu$  wide), nearly cylindrical, non-glandular, without conspicuous muscular development and comprising about  $\frac{1}{5}$  total esophageal region. Posterior part cylindrical, nearly filling body, composed of a massive esophageal gland (or glands). Esophageal tube distinct, extending from base of stylet and penetrating glandular region for a distance of about  $15$  to  $20\mu$  where it appears to end abruptly. Radiating tubules indicate that the esophageal gland (or glands) communicate with the esophageal lumen at this point. There is no break in the wall of the esophageal tube to indicate a more anterior gland outlet. The esophageal gland apparently does not

extent posteriad beyond the anterior end of the intestine. Female reproductive system composed of 5 regions: (1) a post-vulvar uterine sac; (2) a clear region anterior to vulva, with a length equal to about 5 times the anal body diameter, set off anteriorly by a distinct constriction and usually containing a few scattered sperm cells and a few other irregular bodies of unknown nature; (3) a long tubular region nearly filling the body and packed with sperm cells; (4) a narrow, tubular region (oviduct) with a length equal to slightly more than the corresponding body diameter; and (5) a rudimentary ovary which

usually lies just back of the base of the esophagus and is composed of a few cells the number varying in different specimens. Anus small but visible, located about  $60\mu$  from the posterior end of the body. Preparasitic male and parasitic female unknown.

*Habitat*.—Associated with decaying citrus fruit.

*Locality*.—Riverside, California, U. S. A.

*Discussion*.—*Prothallonema dubium* was received on the same culture as *Hexatylus intermedius*. No attempt was made at the time to estimate the number of specimens present but they were quite numerous. They were transferred to new cultures along with *H. intermedius* and at the end of about 3 months, when the cultures were discontinued, many specimens were still alive. During this period they underwent no noticeable change. Probably *P. dubius* is a parasite of some insect, the immature stages of which develop in decaying citrus fruit and parasitic females of the nematode had probably been transferred to the original culture along with the material used for inoculation. Since the amount of this material was presumably not very great it evidently harbored the nematodes in large numbers.

**Effects of treatment with brilliant green on some tapeworms infesting poultry.** PAUL C. UNDERWOOD, PAUL D. HARWOOD, and JACOB M. SCHAFER, U. S. Bureau of Animal Industry.

Wright and Van Volkenberg (1936, Proc. Helminth. Soc. Wash., 3:65) reported that brilliant green in toxic doses removed the heads of *Raillietina tetragona* and of an unidentified species of tapeworm belonging to the genus *Raillietina*. Further tests with this drug were undertaken by the writers to determine if reduced doses of this dye might prove nontoxic as well as effective as a taenicide. Naturally infested fully grown chickens were given the drug and handled by the usual method for making a critical test of an anthelmintic.

Twelve chickens were given brilliant green sulphate, either in solution or in granular form, by the writers. The dose rate used varied from 150 to 400 mg as shown in table 1. Following treatment, 9 of the 12 birds died, apparently from the effects of the drug. The treatment had removed the 1 specimen of *Choanotaenia infundibulum* present, and most of the specimens of *Raillietina tetragona*, but failed to remove any of 28 specimens of *Raillietina cesticillus* present in the treated chickens. As the latter species appears to be the most common tapeworm infesting poultry in the United States, there seemed to be no point in making further tests with this chemical.

As used by the writers, brilliant green was in the form of the sulphate, and was highly water soluble. Since certain chemicals, notably phenols and halogenated hydrocarbons, are more effective anthelmintics if given in a relatively insoluble form than when in a soluble form, a more insoluble compound of brilliant green was sought. Brilliant green picrate was prepared and found to be soluble in 1 to approximately 4,000 parts of water. This drug was given to 4 naturally infested chickens in doses of 100 to 200 mg per fowl. All chickens were visibly affected by the treatment, though none died. No tapeworm heads were removed, and 92 heads of *Raillietina cesticillus* were found in the birds at necropsy. Brilliant green picrate appeared to be no more effective than ordinary water-soluble brilliant green, and proved to be nearly as toxic.

Throughout these experiments it was noted that treated birds suffered from a severe diarrhoea, which was accompanied by an intestinal catarrh. Since tapeworm heads are attached to the mucosa, it appears possible that the abnormal rate of intestinal secretion caused by brilliant green and some other chemicals served to protect the heads from injury by the chemical. If this be true, tests for the development of anthelmintics which are relatively non-irritating to the intestinal mucosa, or of methods of preventing the rapid secretion from the intestinal mucosa following contact with such irritating taenicides as kamala, male fern and brilliant green, might yield profitable results.

TABLE 1.—Data on treatment of chickens with salts of brilliant green\* for the removal of tapeworms

Bird no.	Weight in pounds	Dose in milligrams	Tapeworms recovered ante mortem	Tapeworms recovered at necropsy	Effect of treatment on host
1	2½	400	none	none	fatal
2	2	400	none	none	fatal
3	2	400	none	1 <i>R. tetragona</i>	fatal
4	2	150	none	none	fatal
5	2	150	<i>R. tetragona</i> (1 segment chain)	none	fatal
6	2	150	<i>Choanotaenia</i> (1 head)	3 <i>R. cesticillus</i>	severe intoxication
7	3	150	none	none	fatal
8	2	150	none	none	fatal
9	2	400	none	none	fatal
10	2	400	<i>R. tetragona</i> (segment chains)	none	fatal
11	2	150	none	5 <i>R. cesticillus</i>	slight intoxication
12	2	150	none	18 <i>R. cesticillus</i>	slight intoxication
13	4½	100	none	9 <i>R. cesticillus</i>	moderate intoxication
14	3½	100	none	9 <i>R. cesticillus</i>	severe intoxication
15	4	200	none	10 <i>R. cesticillus</i>	moderate intoxication
16	3	200	none	14 <i>R. cesticillus</i>	moderate intoxication

\*Birds 1 to 8 were treated with granular brilliant green sulphate; birds 9 to 12 were treated with an aqueous solution of brilliant green sulphate; birds 13 to 16 were treated with brilliant green picrate.

**Opuscula miscellanea nematologica, VII. G. STEINER, U. S. Bureau of Plant Industry.**

(1) OBSERVATIONS ON NEMATODES ASSOCIATED WITH IRISH POTATOES GROWN IN SOUTH CAROLINA

An Irish potato from Greer, Greenville County, S. C., submitted by W. C. Nettles, Extension Entomologist, Agricultural Experiment Station, Clemson, S. C., exhibited warty lesions which contained the following nematodes:

<i>Acrobeloides bütschlii</i> (de Man, 1884) Steiner & Bührer, 1933	3 ♀ ♀
<i>Acrobeloides enoplus</i> , n. sp.	4 ♀ ♀, 1 juv.
<i>Aphelenchoides parietinus</i> (Bastian, 1865) Steiner, 1932	14 ♀ ♀, 9 ♂ ♂, 5 juv.
<i>Dorylaimus</i> sp.	1 juv.
<i>Dorylaimus</i> sp.	1 juv.
<i>Pratylenchus pratensis</i> (de Man, 1880) Filipjev, 1936	2 ♀ ♀, 1 juv.
<i>Pseudacroboles variabilis</i> (Steiner, 1936), nov. comb.	9 ♀ ♀, 6 ♂ ♂, 16 juv.
<i>Rhabditis</i> sp.	1 ♀, 1 juv.
<i>Zeldia odontocephala</i> , n. sp.	8 ♀ ♀, 12 juv.

These nematodes doubtless were not the primary agents in producing the warty lesions but were conceived to be of only secondary significance as disease agents or to be related to decay. Only *Pratylenchus pratensis* is considered a true parasite and there were too few of these present to act as pathogenic factors. About 40 per cent of all specimens were parasitized by Sporozoa discussed in a later section. Two of the nematode species are new, and for one, formerly known as *Acroboles variabilis* Steiner, 1936, a new genus is proposed.

*Zeldia odontocephala*, n. sp. (fig. 12)

**Description.**—Closely resembling *Zeldia punctulatus* (Thorne, 1925) Thorne, 1937. Annulation of cuticle rather plain, annules low-convex,  $26\mu$  wide, with smooth surface but with transverse series of refractive dots in subcuticular layer. These series of dots not corresponding exactly with annulation, but appearing on head end as approximately 2, posterior to middle of esophagus as approximately 3, series under each annule, the series not very regular; on tail end, posterior to phasmids, number of series reduced to 2 and then more caudad disappearing. Lateral fields beginning in middle region of esophageal corpus, ending near phasmid, consisting of 2 longitudinal bands appearing areolated through transgression of annulation (fig. 12, F); the 3 wings low, apparently crenate in accordance with annulation.

Head broad-obtuse, not set off; the 3 labial probolae somewhat variable, most often broad plate-like structures, anteriorly more or less truncate. In each interspace between these labial probolae, but deeper toward the cheilostom, occurs a toothlike structure of good size, pointing inward and slightly forward (fig. 12, C). Cephalic probolae 3, flattish, curved slightly inward and separated by deep axils. Sides of these probolae curved forward to a sharp point; their anterior borders of concave form but fringed with 4 small triangular flaps; these latter difficult to see. Axils between cephalic probolae with long, sharply pointed single processus. Cephalic papillae setose, submedially 2, one of the anterior and one of the posterior circle; laterally none seen. Amphids (fig. 12, C) opening rather far forward. Buccal armature cephaloboid, cheilo-, pro-, meso-, meta-, and telorhabdion thick, heavily cuticularized. Esophagus with long, slender, cylindrical corpus which is not differentiated into pro- and meta corpus but is well set off from isthmus. Latter shorter than corresponding body width. Nerve ring surrounding corpus at a distance of about 4 body widths from anterior end; excretory pore situated ventral to it. Cardiac bulb of esophagus large, almost spherical, with well developed valvular apparatus. No cardiac valve seen. Rectum slightly longer than anal body diameter; 3 rectal glands seen. Phasmids at the beginning of second third of tail length. Female sexual apparatus typical, with S-shaped flexure in postvulvar portion of ovary. Spermatheca not seen. Vulva not prominent, lips small. Only females found.

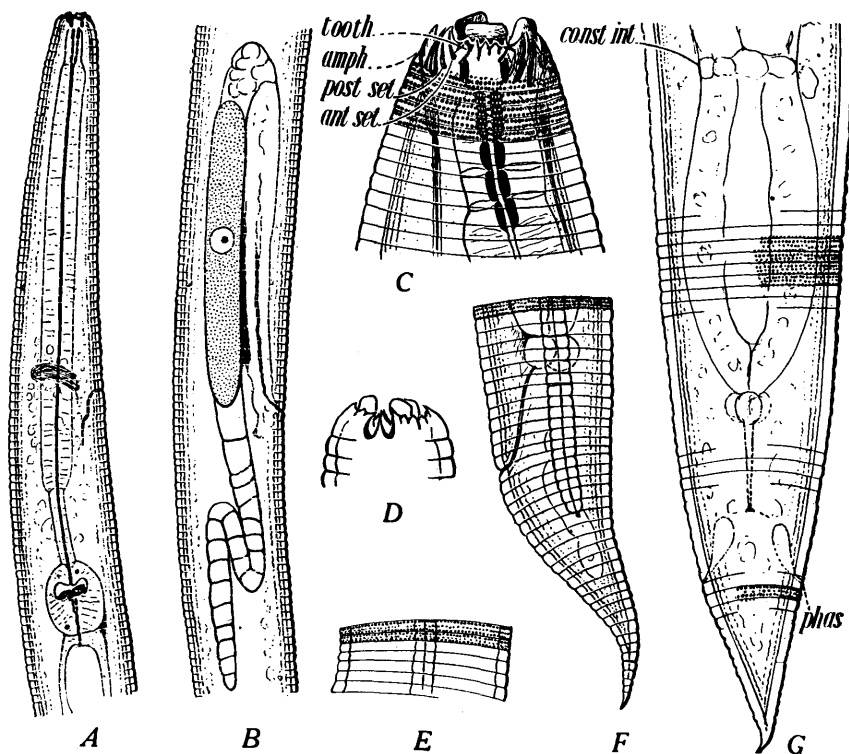


FIG. 12

*Zeldia odontocephala*

A—Anterior end of body;  $\times 624$ . B—Female sexual apparatus;  $\times 624$ . C—Head end, dorsal view;  $\times 2520$ ; *amph*, amphid; *ant set*, setose papilla of anterior circle; *post set*, setose papilla of posterior circle; *tooth*, toothlike structure. D—View of cephalic axil;  $\times 2520$ . E—View of annulation, lateral field and punctation in middle region of body;  $\times 1236$ . F—Side view of tail end;  $\times 1236$ . G—Dorsal view of tail end;  $\times 690$ ; *const int*, constriction of intestine; *phas*, phasmid.

**Measurements.**—♀ : length = 0.68 to 0.81 mm;  $\alpha$  = 20.4 to 22.6,  $\beta$  = 3.5 to 3.9,  $\gamma$  = 15.6 to 17.3,  $v$  = 64 to 65%.

**Diagnosis.**—Resembling *Zeldia punctulatus* but having labial probolae larger and broader, cephalic probolae with deeper separating axils and anterior border fringed with 4 triangular flaps; cephalic papillae setose; entrance to cheilostom with 3 toothlike structures; approximately 3 transverse subcuticular series of dots to each cuticular annule, except on a number of annules back of head and back of phasmids on tail. Structure of lateral fields not known for *Z. punctulatus*; comparison therefore not possible.

**Type locality.**—South Carolina, U. S. A.

**Type host.**—Warty disease lesion on Irish potato.

*Acrobeloides enoplus*, n.sp. (fig. 13, A & B)

**Description.**—Closely resembles *Acrobeloides bütschlii*. Annulation of cuticle  $2\mu$  wide; 3 lateral longitudinal wings separating 2 lateral fields. Tail conoid with obtuse terminus and about 14 cuticular annules. Head end broad-obtuse; labial probolae with broad-conical, almost hemispherical base and long setose terminus; 3 cephalic probolae separated by narrow but deep and rounded axils, the probolae having rounded angles terminating in a setose point; 2 neighboring probolae forming therefore a pair of such setose points around each axil. Two circles of cephalic papillae, the anterior with 6 (1 each submedial and lateral) the posterior with 4 (1 submedial only) papillae. Amphids slightly behind posterior circle. Buccal armature typically cephaloboid, cheilo-

pro-, meso-, meta- and telorhabdions all well cuticularized, the dorsal meta-rhabdion with tooth. Esophagus with pro- and metacarpus of about same length, the latter somewhat swollen, spindle shaped and set off from procorpus and isthmus by break in tissue. Isthmus slender and of about same length as the slightly pear-shaped terminal bulb. Cardiac valve short but broad-conical, crossing intestinal wall and reaching to intestinal cavity. Cells of intestinal wall thin, probably only 2 to circumference. Rectum a little shorter than anal body diameter. Vulvar lips not protruding. Ovary with S-shaped flexure.

*Measurements.*—♀ : total length = 0.336 mm;  $\alpha = 16$ ,  $\beta = 3.1$ ,  $\gamma = 16$ ,  $v = 67\%$ .

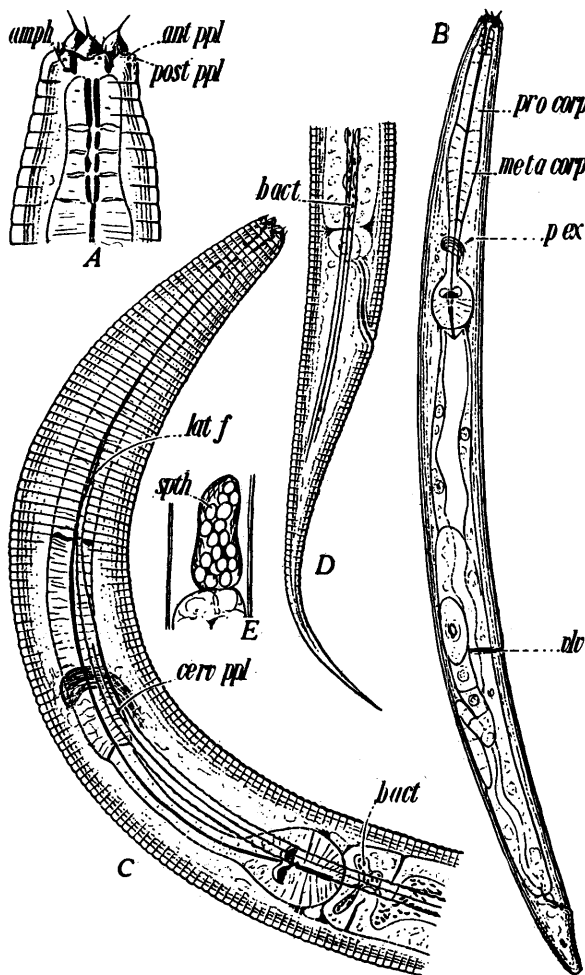


FIG. 13

A & B—*Acrobelloides enoplus* (A—Head end;  $\times 2520$ ; amph, amphid; ant ppl, papilla of anterior circle; post ppl, papilla of posterior circle. B—Female, dextero-ventro-submedial view;  $\times 624$ ; p ex, excretory pore; meta corp, metacarpus; pro corp, procorpus; vlv, vulva). C-E—*Pseudacroboles variabilis* (C—Anterior end;  $\times 1236$ ; bact, bacteria in anterior intestine; cerv ppl, cervical papilla; lat f, anterior end of lateral field. D—Tail end;  $\times 1236$ ; bact, bacteria in end portion of intestine. E—View of spermatheca;  $\times 1236$ ; sper, spermatheca).

*Diagnosis.*—Resembling *Acrobelloides bütschlii*, but with labial probolae ending in long setose portion instead of being round-obtuse. Vulvar lips not protruding. Male unknown.

*Type locality.*—South Carolina, U. S. A.

*Type host.*—Warty disease lesion on Irish potato.

*Pseudacroboles variabilis* (Steiner, 1936), comb. nov.

In a recent revision of the Cephalobidae (Thorne, 1934, Proc. Helminth. Soc. Wash., 4: 1-16) the diagnosis for the genus *Acroboles* was emended; the previous members of the genus were in part assigned to new genera. *Acroboles variabilis*, however, was left out, its characters being somewhat intermediate. It is therefore thought best to create for this species a new genus *Pseudacroboles* with the following diagnostic characters: Acrobelineae with labial probolae of low and rounded form, cephalic probolae 3, with rounded, apiculate angles, separated by deep, narrow, rounded axils. Corpus of esophagus cylindrical, slender,

metacarpus not swollen but separated from procorpus by break in tissue. Nerve ring surrounding metacarpus. Female tails elongated, acute, male tails short, conical with obtuse, often mucronate terminus. Lateral membranes ending at phasmids.

Some additional morphological characters and ecological features of the present species, not contained in the previous description may also be added. Lateral fields begin anteriorly slightly in front of metacarpus of esophagus (fig. 13, C), are about 1/6 as wide as the corresponding body diameter, and are bordered and separated by 3 wings which end near the phasmid (fig. 13, D). Cervical papilla slightly behind nerve ring, in middle wing. Female with well-formed vesiculate spermatheca at anterior bend of oviduct.

*Pseudacrobeles variabilis* feeds on bacteria (fig. 13, C & D) or more accurately on slime produced by them, since the bacilli themselves seem to pass undigested through the intestine and to be evacuated again through rectum and anus.

(2) CRICONEMOIDES SPHAEROCEPHALUM A. L. TAYLOR, 1936 LIVING ON COTTON ROOTS IN THE UNITED STATES

Hitherto this species has been reported only from soil around roots of a grass, Island of Trinidad, British West Indies. Its occurrence on roots of cotton grown near Raleigh, N. C., not only makes it a member of the nematode fauna of the United States but shows it also as an ectoparasite of the cotton plant. Its significance as a primary disease agent is considered small.

(3) ON SPOROZOAN PARASITES OF NEMATODES

Sporozoa as parasites of soil nematodes are mentioned repeatedly in the literature on free-living nematodes from Europe, but hitherto little attention has been given them in the United States. The subject undoubtedly deserves more consideration, particularly in view of the significance such parasites may have as natural control factors for plant-parasitic and related nematodes. That sporozoa are common parasites of nematodes associated with crop plants in the United States is a conclusion based on a long series of casual observations by the writer. It is intended to give the matter more consideration in the future.

At present some observations made on the material discussed in section (1) of this paper are reported. Of the previously mentioned nematodes from the Irish potato, quite a few were infested by sporozoans. Of the latter 3 different types belonging possibly to 3 different species of Microsporidia were observed. The nematode species attacked were *Zeldia odontocephala*, *Pseudacrobeles variabilis*, *Acrobeloides enoplus*, *A. bütschlii*, *A. sp.* and *Aphelenchoides parietinus*.

From an economic viewpoint the 2 forms described as attacking *A. parietinus* deserve special interest. Finding them confirms the fact that stylet bearing nematodes (which compose the bulk of plant-parasitic species) are also attacked by Sporozoa. Micoletzky is the only author who has previously reported these parasites as occurring on tylenchs (Micoletzky, 1925, Mem. Acad. Roy. Sci. et Let. Danemark, Copenhagen, Sec. Sci., (8 sér.) 10(2):286). It is thought that all these sporozoan parasites enter their nematode hosts through the oral opening. Where this opening is quite spacious, as in *Rhabditis*, *Zeldia*, *Acrobeloides*, etc., this seems in no way remarkable; but the passage of these disease agents through the narrow stylet of tylenchs appears rather amazing. The organism shown in figure 14, A is of uncertain nature and only tentatively classed as a microsporidian. Quite often it was observed in the spermatocytes and sperms immediately above the ejaculatory duct or even within this duct. A diffuse occurrence as sketched in figure 14, A appears to prove an infestation of tissues other than the sexual cells. Up to the present these structures have been seen only in the male *Aphelenchoides parietinus*.



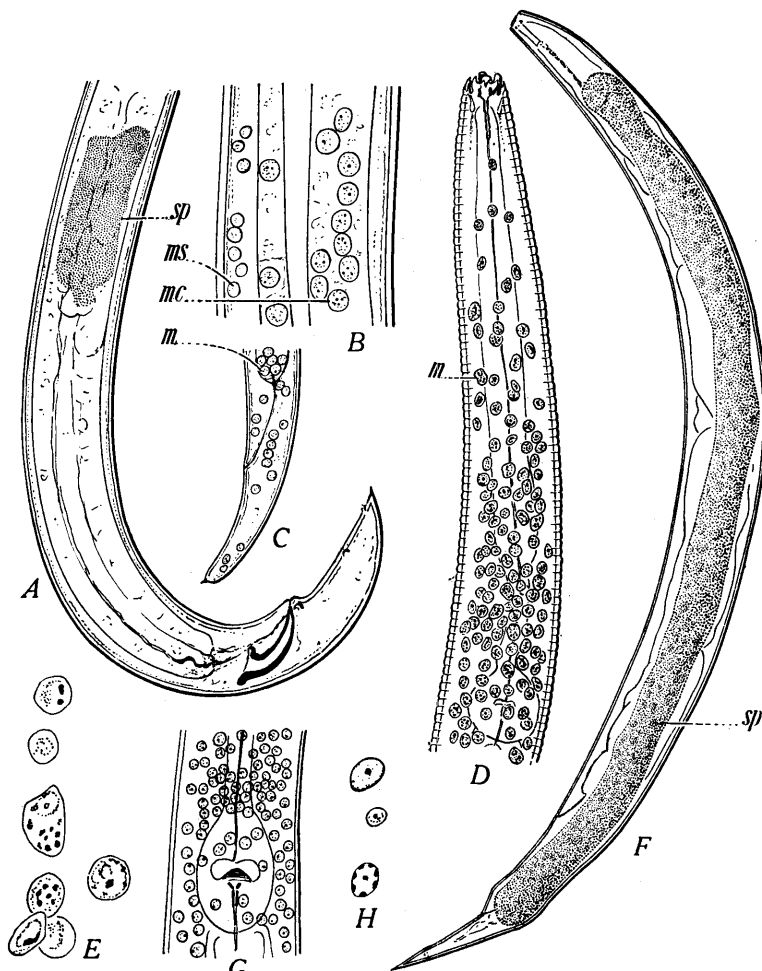


FIG. 14

## Sporozoan Parasites of Nematodes

A—Posterior end of a male *Aphelenchoides parietinus*;  $\times 960$ ; *sp*, Microsporidia(?). B—*Aphelenchoides parietinus* female, middle region of body with Microsporidia;  $\times 2520$ ; *ms*, smaller cells in body cavity; *mc*, larger cells in intestinal wall. C—Tail end of *Aphelenchoides parietinus* female; *m*, Microsporidia;  $\times 960$ . D—Anterior end of *Zeldia odontocephala*;  $\times 960$ ; *m*, Microsporidia in body cavity. E—Various cells of Microsporidia from same host;  $\times 2520$ . F—*Rhabditis spiculigera*; *sp*, large sporocyst;  $\times 384$ . G—Posterior esophageal region of *Acroboloides enoplus* with numerous Microsporidia in body cavity;  $\times 1230$ . H—Single cells of different sizes of Microsporidia from same host;  $\times 2520$ .

The microsporidian shown in figure 14, B & C doubtless belongs to a different species from that of figure 14, D, E, G & H because of a distinct difference in size. However, the sporidia in the intestinal wall of *Aphelenchoides parietinus* shown in figure 14, B & C also differ from those in the body cavity, the former being  $2\mu$  in diameter, the latter only 1 to  $1.3\mu$ . It is assumed that the 2 sizes here represent different developmental stages. Slight differences in size were also observed in the species found in *Zeldia odontocephala*, *Acroboloides enoplus*, *A. bütschlii*, *A. sp.* and *Pseudacrobes variabilis* (fig. 12, D, E,

G & H). Measurements showed the diameter of individual Microsporidia to vary from 3 to 6 $\mu$ . In this case, the cells were all in the body cavity, particularly around the esophagus.

Figure 14, F shows a sporocyst almost completely filling the body cavity of a *Rhabditis spiculigera* Steiner, 1936, found in a root of a tobacco plant collected at Florence, S. C. This cyst is reminiscent of one described by Micoletzky from *Dorylaimus carteri* Bastian, 1865 (loc. cit.).

## MINUTES

### *One hundred eighty-third to one hundred ninety-second meetings*

The 183rd meeting was held December 15, 1936. A resolution as a memorial to Sir Arnold Theiler was presented by the resolution committee. Papers were presented by Dikmans, Andrews, Ewing, Cort, and Shelton.

The 184th meeting was held on January 19, 1937. Mr. A. Murray Fallis and Dr. V. N. Moorthy were elected to membership in the Society. Papers were presented by Dickmans, Spindler, Christie, and Moorthy.

The 185th meeting was held February 16, 1937. Papers were presented by Harwood, Spindler, McIntosh, Chitwood (M. B.), Buhrer, and Chitwood (B. G.).

The 186th meeting was held March 16, 1937. Dr. E. E. Byrd, Dr. M. B. Linford, Dr. Artigas, and Mr. Sui Fong Chen were elected to membership in the Society. Papers were presented by Wehr, Shorb, McIntosh, and Wright. Drs. Steiner and Schwartz reported on the conference of Southern Agricultural Workers held at Nashville, Tenn., in February, 1937. Dr. Schwartz also reported on a recent trip to Puerto Rico.

The 187th meeting was held April 20, 1937. Mr. C. W. McBeth was elected to membership in the Society. Papers were presented by Chitwood, Cram, Steiner, Jacobs, Wehr, and Harwood.

The 188th meeting was held May 18, 1937. Miss Buhrer reported that there were 39 members in the Society and 205 subscribers for the Proceedings. Papers were presented by Christie, Luttermoser, Horsfall, Ewing, Herman, Wolfson, and Kerr.

The 189th meeting was held October 19, 1937. Officers were elected as follows: President, G. Dikmans; vice-president, P. D. Harwood; recording secretary, A. McIntosh; corresponding secretary-treasurer, E. M. Buhrer. Mr. L. Jacobs was elected to membership in the society. Papers were presented by Winfield, and Wright.

The 190th meeting was held November 16, 1937. Drs. J. F. Christensen, S. X. Cross, and G. W. Luttermoser were elected to membership in the Society. Papers were presented by Ewing and Wehr.

The 191st meeting was held December 21, 1937. After discussing the financial status of the Society, the Treasurer was authorized to limit the surplus to \$500.00 by cutting down the cost per page to members contributing articles to the Proceedings. Dr. Paul Bartsch was elected to life membership in the Society. Papers were presented by Andrews, Steiner, Wright, Price, and Buhrer.

The 192nd meeting was held January 18, 1938. Dr. A. C. Jerstad was elected to membership in the Society. The Treasurer reported a bank balance, as of December 31, 1937, of \$606.02. Papers were read by Bishopp, Winfield, Linford, and Chitwood.

ALLEN MCINTOSH, *Recording Secretary.*

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