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PROCEEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

VOLUME 28 JANUARY, 1961 NUMBER 1

Studies on Cysticercoid histology. V. Observations on the fully developed cysticercoid of *Hymenolepis citelli* (Cestoda: Cyclophyllidea)

MARIETTA VOGE*

Morphologic criteria useful in the specific differentiation of *Hymenolepis citelli* from *Hymenolepis diminuta* have been presented previously (Voge, 1952, 1956) and have been derived from studies of gross morphology of adult as well as cysticercoid stages. The description of the histology of *Hymenolepis diminuta* cysticercoids (Voge, 1960) provides a basis for further comparison of these two apparently closely related species. As will be shown here, the histologic organization of the cysticercoid of *Hymenolepis citelli* is different from that of *Hymenolepis diminuta* and provides additional evidence that the two cestodes are distinct species.

MATERIALS AND METHODS

Eggs of *Hymenolepis citelli* were obtained from a ground squirrel, *Citellus beecheyi*, caught at Loma Linda, California in April, 1960. Material used for previous life history studies (Voge, 1956) was derived from the same host species and locality. Eggs were fed to flour beetles (*Tribolium confusum*) kept at 30°C. Beetles were dissected and cysticercoids harvested at 9, 11, and 21 days after infection. Cysticercoids were fixed in Zenker's fluid, embedded in paraffin and sectioned at 7 microns. Sections were stained with Mallory's aniline blue stain, Mayer's hemalum, or Himes' triple stain, as described in previous studies (Voge, 1960, 1960a).

DESCRIPTION

A longitudinal section of a fully developed cysticercoid of *Hymenolepis citelli* is shown in figure 1. The tissues or structures observed in the body of the cysticercoid are: 1) the external membrane, supported by 2) an underlying layer of circular fibers oriented transversely, 3) a delicate layer of longitudinal fibers, 4) an intermediate layer consisting of elongate cells, with fibrous processes from 5) the thick fibrous layer, 6) the lining of the cavity, consisting of delicate strands of tissue, and 7) the scolex and tissue immediately surrounding the scolex. In addition to the suckers, the scolex has a very muscular and well defined rostellum. The cysticercoid tail frequently contains large numbers of elongate, well defined cells and may contain free spaces near the center.

Overall comparison of this cysticercoid with *H. diminuta* (Voge, 1960) confirms previous studies (Voge, 1956) showing that the scolex and the

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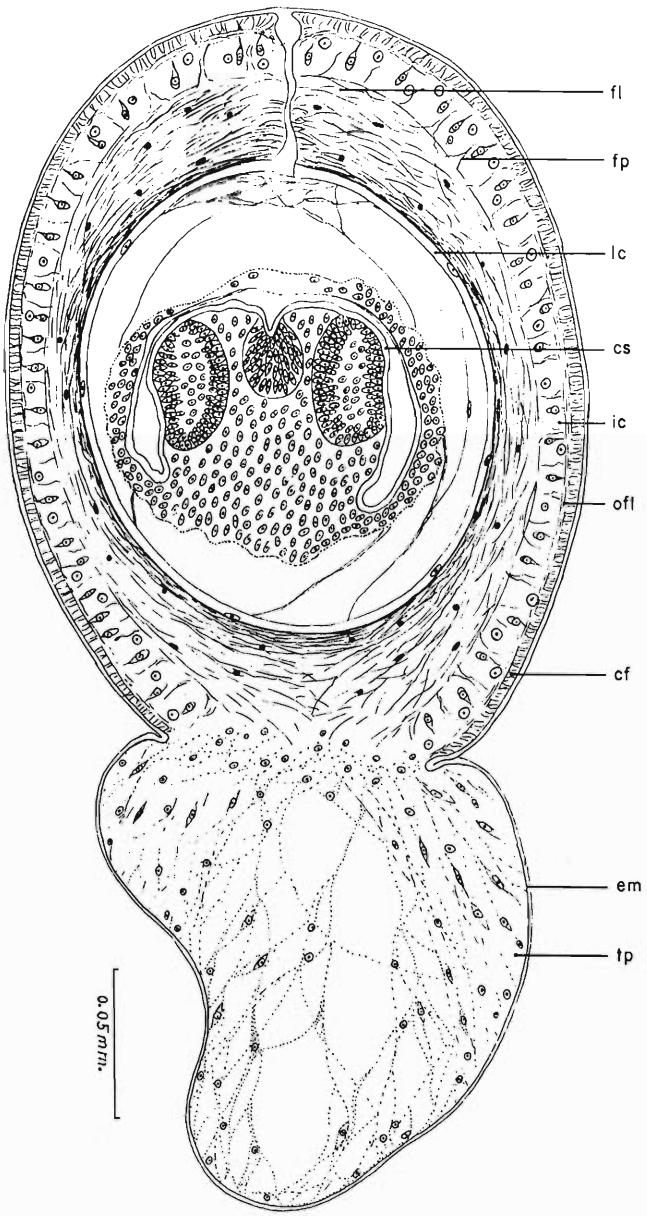
cysticercoid cavity in *H. citelli* are much larger than in *H. diminuta*. The cysticercoid body is less elongated and the tissues external to the cavity occupy a smaller area. Histologically, the two species are organized in



Longitudinal section of cysticercoid of *Hymenolepis citelli* 3 weeks old: cf, circular fibers; cs, cuticle of scolex; em, external membrane; fl, fibrous layer; fp, fibrous processes; ic, intermediate cell layer; lc, lining of cavity; ofl, outer fibrous layer; tp, tail parenchyma.

similar patterns. The lining of the cysticercoid cavity and the fibrous layer are very similar in both species. The fibrous processes from the fibrous layer, which are very thick and intensely staining in *H. diminuta,* appear delicate and thin in *H. citelli.* As in *H. diminuta,* these fibrous processes appear to be closely associated with the elongate cells in the intermediate layer. These cells, which are comparable in shape, position, and orientation to those of the intermediate layer in *H. diminuta* are, however, smaller in size and very clearly outlined even in fully grown cysticercoids. Scattered among the cells are relatively large nuclei (Fig. 1). The thin outer longitudinal fibrous layer in *H. citelli* apparently has no counterpart in *H. diminuta,* unless it represents an extension of the longitudinal fibrous processes. This question could be answered only by a study of histogenesis. Much more prominent than in *H. diminuta* (Voge, 1960b) are the circular fibers which in *H. citelli* are even more conspicuous than in *H. nana* (Voge and Heyneman, 1960). The prominent hairy processes seen in *H. diminuta* are absent in *H. citelli.* Contrary to the description by Rothman (1957), the cysticercoids of *Hymenolepis diminuta* and *Hymenolepis citelli* do not develop at comparable rates. Cysticercoids of both species grown at 30°C in the same species of intermediate host show that *H. citelli* requires about 3 days more to complete histological development than does *H. diminuta.* Eleven day-old and 3 weeks-old cysticercoids of *H. citelli* do not differ in appearance, while 9 day-old *H. citelli* still lack many of the fibrous components and resemble 6 day-old, recently withdrawn stages of *H. diminuta.*

Staining reactions with Mallory's stain in fully developed cysticercoids are as reported for *H. diminuta* except for the fibrous processes which do not stain intensely and have a purple cast. With Himes' stain, a positive polysaccharide reaction is observed in the scolex; all fibrous tissue, as well as the tail parenchyma, stain an intense reddish-purple color.

SUMMARY

The structure of the cysticercoid of *Hymenolepis citelli,* a tapeworm from the ground squirrel *Citellus beecheyi,* is described and compared with the cysticercoid of *Hymenolepis diminuta.* While most of the tissues in both species are organized according to a similar plan, distinctive characteristics serve to clearly differentiate between them.

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North American Monogenetic Trematodes. VIII. The Family Hexostomatidae*

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This paper is a continuation of the series dealing with the North American monogenetic trematodes and of a general revision of the Monogenea. The purpose and organization of this installment are the same as for previous sections (Price, 1937, 1938, 1939a, 1939b, 1942, 1943a and 1943b). Following the appearance of Part VII, in 1943, work on this series had to be suspended because of other duties. Since that time a great deal of work has appeared by other workers, notably, Bychowsky, Sproston, Hargis, Mizelle and his associates, Yamaguti, Caballero and his associates, Chauhan, Tripathi, Jain, and others, which has necessitated a reconsideration of some ideas previously held regarding relationships and classification. While some changes in viewpoint are incorporated in this paper, and will be in others to follow, the major revision will be reserved until the completion of the present series.

HEXOSTOMATIDAE PRICE, 1936

DIAGNOSIS: Opisthohaptor not distinct from body proper, usually with four pairs of sessile, sucker-like clamps containing three dissimilar sclerites, two small, irregular and tending to be bipartite, one on either side of lateral wall of capsule, and a larger, more or less saddle-shaped sclerite in middle; anchors two pairs, dissimilar, located between most posterior pair of clamps. Intestine reticulate. Genital atrium and cirrus unarmed; testes numerous, postovarial. Ovary U-shaped, with both limbs directed posteriad. Vagina present, aperture dorsal and median; terminal portion expanded and containing a pair of opposing hemispherical bodies armed on their free margins with backwardly directed spines. Parasites of the gills of scombroid fishes.

TYPE GENUS: *Hexostoma* Rafinesque, 1815.

KEY TO GENERA OF HEXOSTOMATIDAE

Posterior end of body truncate; opisthohaptoral clamps in a more or less straight transverse row ..... *Hexostoma* Rafinesque

Posterior end of body not truncate; opisthohaptoral clamps in two more or less vertical rows ..... *Neohexostoma,* n.g.

GENUS *Hexostoma* RAFINESQUE, 1815

SYNONYMS: *Polystoma* Zeder, 1800, in part; *Hexacotyla* Blainville, 1828; *Hexacotyle* Blainville, 1828; *Plagiopeltis,* Diesing, 1850; *Exacotyle* Monticelli, 1888.

DIAGNOSIS: Body conspicuously attenuated anteriorly, widest and more or less truncate posteriorly. Prohaptoral suckers shallow, sometimes poorly defined; opisthohaptoral clamps sucker-like, oval, usually four pairs with innermost much smaller than others (except in *H. auxisi* Palombi (1943), arranged in a transverse row across posterior end of body. Testes numerous, in posterior half of body. Vitelline follicles extend into post-testicular zone.

TYPE SPECIES: *Hexostoma thynni* (De la Roche, 1811) Rafinesque, 1815,

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from *Thynnus brachypterus* (= *Scomber thynnus*), *T. thynnus, Parathynnus obesus,* and *Pelamys sarda* (= *Sarda sarda*).

INCLUDED SPECIES: *Hexostoma acutum* (Goto, 1894) Sproston, 1946, from *Parathynnus sibi; H. auxisi* Palombi, 1943, from *Auxis thazard* (= *A. bisus,* = *A. rochei*); *H. dissimile* (Yamaguti, 1937) Sproston, 1946,** from *Thynnus thynnus; H. grossum* (Goto, 1894) Sproston, 1946, from *Thynnus* sp. (= *Parathynnus sibi,* vide Ishii and Sawada (1938)), *Katsuwonus vagans, Thunnus orientalis* and *Seriola guingueradiata;* and *H. lintoni,* n. sp., from *Sarda sarda.*

The only North American representative of *Hexostoma* (*s. str.*) is *H. lintoni,* a description of which follows:

Hexostoma lintoni, new species (Figs. 1-4)

SYNONYM: *Hexacotyle thynni* of Linton, 1901.

DESCRIPTION: Body elongate, 7.4 mm long by 2.2 mm wide, divided into three parts, a neck-like region, body, and haptor. Prohaptor in form of two elliptical suckers, each 0.038 mm long by 0.015 mm wide, opening into oral cavity. Opisthohaptor not distinct from body proper, 1.8 mm wide, bearing 4 pairs of sucker-like clamps and 2 pairs of anchors. Clamps of outer 2 pairs equal in size, 0.51 mm long by 0.34 mm wide, those of next pair 0.42 mm long by 0.34 mm wide, and those of innermost pair 0.21 mm long by 0.12 mm wide; anchors of outer pair 0.074 mm long and those of inner pair about 0.030 mm long. Oral aperture terminal; pharynx oval, 0.068 mm long by 0.042 mm wide; esophagus bifurcating at or near level of genital aperture; remainder of digestive tract not discernible. Excretory apertures dorsolateral, about 0.34 mm from anterior end of body. Genital aperture median, about 0.7 mm from anterior end; cirrus unarmed. Testes relatively numerous, number not ascertainable, postovarial, in median field. Ovary inverted U-shape, with greatly contorted limbs; genitointestinal canal with proximal portion somewhat expanded and thick walled, opening into right intestinal branch near level of posterior end of ovary. Vaginal aperture median, dorsal, opening about 1 mm from anterior end of body; terminal portion of vagina expanded, provided with 2 somewhat hemispherical bodies armed with posteriorly directed, stout, sawtooth-like spines, located in lateral walls; posterior to hemispherical bodies a group of blunt spines projects into vaginal cavity from its dorsal wall. Uterus slender, relative straight, median. Eggs not present.

HOST: *Sarda sarda.*

LOCATION: Mouth.

DISTRIBUTION: United States (Woods Hole, Massachusetts).

SPECIMEN: U.S.N.M. Helm. Coll. No. 6676 (holotype).

Linton (1901) described this worm as *"Hexacotyle thynni* De la Roche (?)" from a single specimen collected August 7, 1900, from the mouth of *Sarda sarda* by Mr. R. P. Cowles. The description was very brief. The above description is from the original specimen which was not in good condition, being very dark from contact with a cork enclosure of the vial.

****Hexostoma dissimile*** was described by Yamaguti (1937) from a single specimen. It differed from other species in the asymmetry of distribution of the opisthohaptoral clamps, there being four on the left side and only two on the right. This asymmetry, especially since only one specimen was available, suggests that the specimen was anomalous. A somewhat similar situation was observed in the holotype of ***Kuhnia macracantha*** (Meserve). In this case the most anterior of the clamps on the left side is not present and only a feebly developed one on the right.

The specimen was bleached and stained, but owing to age, bleaching, and other considerations, it did not stain well. The internal organs, while identifiable, could not be made out in detail. However, a comparison of this specimen with the description of *H. thynni* and with a specimen of that species (U.S.N.M. Helm. Coll. No. 9641), apparently from the Parona collection, collected from *Thynnus vulgaris* at Trieste, shows that the two forms are not identical.

This species differs from the other species of *Hexostoma* (*s. str.*) in the relative size of the opisthohaptoral clamps, the innermost of the 3 larger pairs being smaller than the others. Another difference appears to be in the presence of a dorsal group of spines in addition to the armed hemispherical bodies in the terminal portion of the vagina.

Neohexostoma, n. gen.

SYNONYM: *Hexostoma* Rafinesque of authors, in part.

DIAGNOSIS: Body elongate, widest in ovarial region, with elongate, waist-like constriction in testicular region. Opisthohaptor with 4 pairs of sucker-like clamps arranged as 2, more or less vertical rows, those of posterior pair only slightly smaller than those of anterior 3 pairs. Viellaria not extending posteriorly beyond distal portion of testicular zone.

TYPE SPECIES: *Neohexostoma thunninae* (Parona and Perugia, 1889) n. comb., from *Thynnus thunnina.*

INCLUDED SPECIES: *Neohexostoma euthynni* (Meserve, 1938) n. comb. (syn. *Hexostoma macracanthum* Fujii, 1944, vide Millemann (1956)), from *Euthynnus alletteratus* and *E. lineatus; N. extensicaudum* (Dawes, 1940) n. comb., from *Thynnus thynnus; N. pricei* (Koratha, 1955) n. comb., from *Sarda sarda;* and *N. robustum* n. sp., from *Parathynnus sibi.*

Of these species, only *N. euthynni* (Meserve) and *N. pricei* (Koratha) have been recorded from North American hosts. In addition to a consideration of these species, the description of *N. robustum,* a new exotic species, is included.

Neohexostoma euthynni (Meserve, 1938), n. comb.

SYNONYMS: *Hexostoma euthynni* Meserve, 1938; *H. macracanthum* Fujii, 1944.

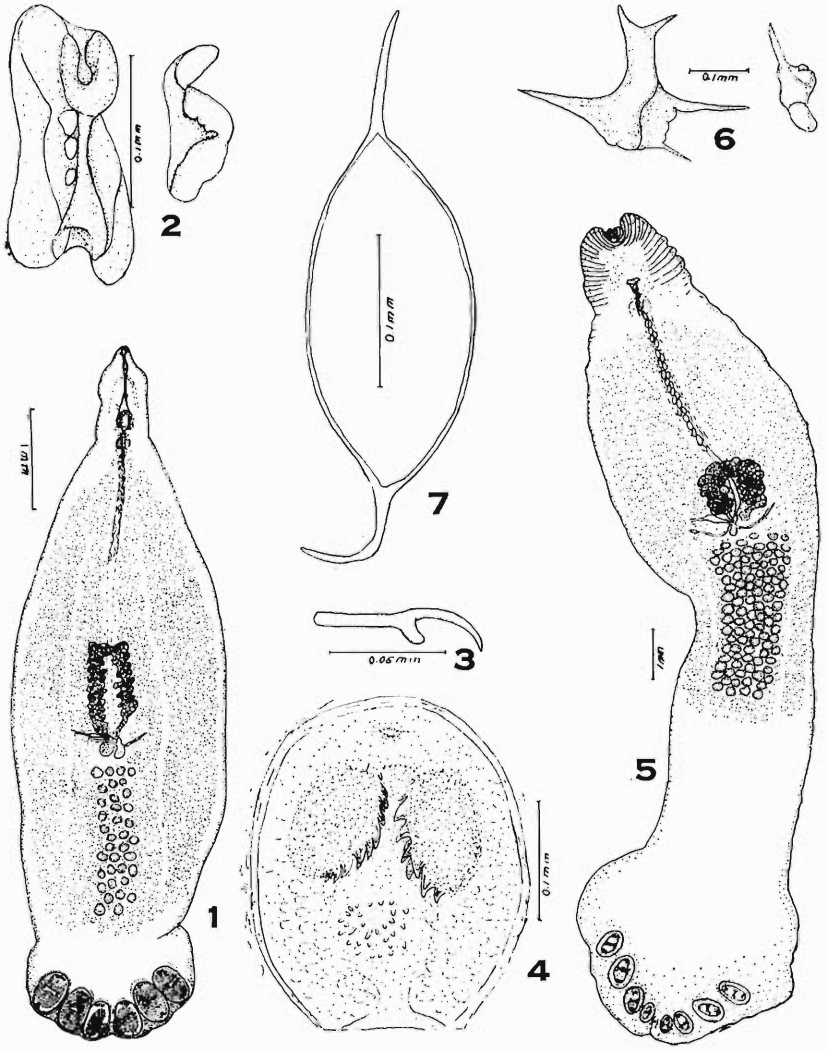
Neohexostoma euthynni was described by Meserve (1938) from a specimen collected from the gills of *Euthynnus alletteratus* at James Island, Galapagos Islands. A similar and apparently identical species was described by Fujii (1944) as *H. macracanthum* from the same host at Tortugas, Florida. Only the specimen of *H. macracanthum* (U.S.N.M. Helm. Coll. No. 36890) was available to the writer, the specimen of *H. euthynni* (U.S.N.M. Helm. Coll. No. 9176) being on loan from the Museum Collection, consequently no direct comparison of the two forms could be made. However, Millemann (1956) has compared the types of both species with specimens from *Euthynnus lineatus* which had been "caught off the coast Baja California between Abreojas and San Juanico, in 1952" and concluded that they were identical. In the absence of the specimens mentioned above, and of Millemann's material for comparison, the decision that they are identical species has been tentatively accepted.

Neohexostoma pricei (Koratha, 1955), n. comb.

SYNONYM: *Hexostoma pricei* Koratha, 1955.

This species was described by Koratha (1955) from specimens collected

from the gills of the "Common Bonito," *Sarda sarda*. The description was incomplete, as no information on the reproductive system was given. Specimens of this form (U.S.N.M. Helm. Coll. No. 54761) were not available for study, as they were on loan to another investigator and had not been returned. This species, so far as can be determined from the original descrip-



Figs. 1-4. *Hexostoma lintoni*. 1, complete worm, ventral view; 2, sclerites of opisthohaptoral clamps; 3, large opisthohaptoral anchor; 4, terminal portion of vagina, ventral view.

Figs. 5-7. *Neohexostoma grossum*. 5, complete worm, ventral view; 6, sclerites of opisthohaptoral clamps; 7, egg.

(The number of testes shown in figs. 1 and 5 are not to be considered as representing the actual number present, which could not be determined in the specimens available; they are intended only to represent their distribution.)

tion, appears to be very similar to *N. euthynni* in body shape and size. Apparently the only distinctive features are the size of the opisthohaptoral anchors, inequality in size of the vaginal hemispherical bodies, and host.

Neohexostoma robustum, n. sp. (Figs. 5-7)

DESCRIPTION: Body elongate, robust, 17 mm long by 4 mm wide in ovarial region, and 4.7 mm wide anterior to opisthohaptoral clamps. Anterior end apparently attenuated but contracted in specimen studied. Prohaptoral suckers not observable. Opisthohaptor roughly triangular, bearing 4 pairs of oval sucker-like clamps varying slightly in size; clamps of anterior pair about 0.50 mm by 0.75 mm, second pair 0.60 mm by 0.85 mm, third pair 0.50 mm by 0.67 mm, and fourth or posterior pair 0.35 mm by 0.50 mm, each provided with 3 sclerites, large or median sclerite more or less saddle-shaped and smaller, lateral sclerites irregular. Opisthohaptoral anchors not clearly visible, large anchors apparently about 0.1 mm long and smaller, median pair about 0.040 mm long. Oral aperture apparently terminal; esophagus bifurcating near level of genital aperture; intestine reticulate as in other representatives of family, extending to posterior end of body. Genital aperture median, about 1.5 mm from anterior end; cirrus and genital atrium unarmed; testes numerous, number not ascertainable, occupying interintestinal field of middle third of body, separated from ovary by a narrow zone. Ovary roughly U-shaped, with limbs greatly convoluted and directed posteriad; genito-intestinal canal enlarged proximally and emptying into right branch of intestine at or about level of posterior portion of ovary. Vitelline follicles numerous, extending from a short distance posterior to genital aperture to slightly distal to testes. Vaginal aperture dorsal, median, about 0.45 mm posterior to level of genital pore; terminal portion of vagina expanded, containing a pair of hemispherical bodies armed with relatively short, backwardly directed spines. Eggs oval, about 0.22 mm long by 0.11 mm wide, with a relatively short, stout filament at each pole.

HOST: *Parathynnus sibi.*

LOCATION: Gills.

DISTRIBUTION: Tropical Pacific.

SPECIMEN: U.S.N.M. Helm. Coll. No. 31833 (holotype).

The material on which this species is based consisted of a single specimen in the U.S.N.M. Helminthological Collection labeled *Hexostoma grossum.* The data accompanying this specimen show that it was collected by "E.S.I.," October 10, 1955, from a bigeye tuna. *Parathynnus sibi,* at 02° 45′ N., 158° 05′ W., and sent to Dr. H. W. Manter, University of Nebraska, by Albert L. Tester of the Fish and Wildlife Service in Honolulu.

Neohexostoma robustum appears to be more closely related to *N. extensicaudum,* which was described by Dawes (1940) from the gills of a tunny caught in the North Sea, than to any of the other species referable to *Neohexostoma.* It differs from the latter species in body size, arrangement and size of the opisthohaptoral clamps, distance between the ovary and most anterior testes, and host.

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Paratylenchus steineri (Criconematidae) a new species of plant nematode

A. MORGAN GOLDEN*

Among several different kinds of nematodes present in soil and plant material originating at a nursery near Moscow, USSR, was a new species belonging in the Criconematidae. This species, with a long curved stylet, evidently is closely related to the three *Paratylenchus* species recently described by Brown (1959) from Canada. Although only seven females were found, they have certain morphological characters which distinguish them from other described species of Criconematidae.

Paratylenchus steineri, n. sp.

MEASUREMENTS: 7 females—Length .282 mm (.243.309); a = 20.2 (17.3-21.1); b = 2.6 (2.4-2.8); c = 12.0 (11.1-13.1); V = 77% (75-78%); Stylet .067 mm (.065-.069).

Males unknown.

FEMALE: Very small nematode. Body cylindrical, tapering gradually anteriorly and more so posteriorly, markedly curved ventrally when dead (Fig. 1B). Head not offset, without distinct annules but with slight labial protrusion anteriorly (Fig. 1A). Annulation on body fine but distinct, the

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annules measuring about one micron each. Deirids not observed. Lateral field about $^1/_5$ of body width, occurring as 4 lines; in vicinity of excretory pore, the 2 inner lines fade out while the outer 2 continue, eventually con-

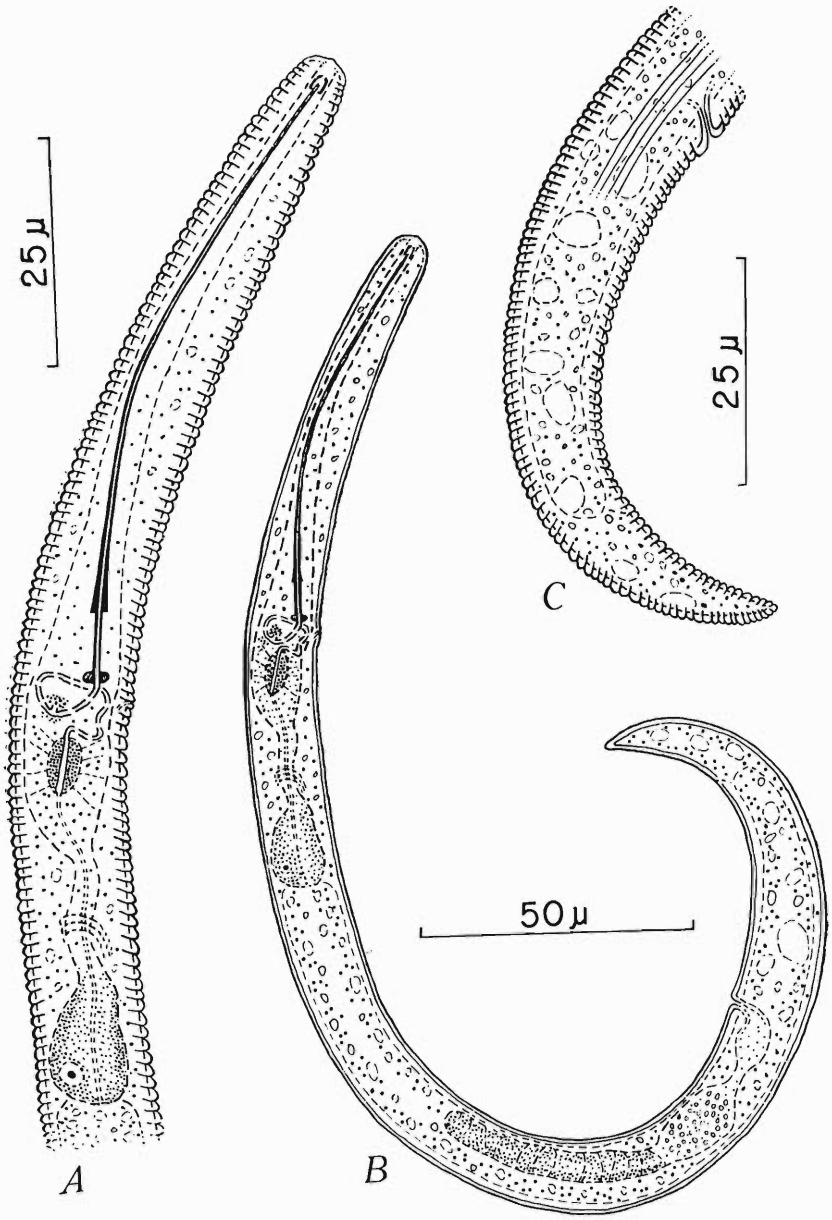

Figure 1—Drawings of a female of *Paratylenchus steineri*, n. sp. A—Anterior portion. B—Full length view. C—Posterior portion showing a part of the lateral field.

verging and disappearing a few microns from anterior end. Phasmids not clearly detected. Tail averaging about 22 microns long, generally appearing as shown in Fig. 1c. Anus very obscure.

Anterior portion as presented in Fig. 1A. Cephalic framework not sclerotized. Stylet long and slender, with marked curvature toward dorsal side in all fixed specimens examined; stylet knobs distinct, rounded. Stylet guide ring prominent, located at anterior end of unprotruded stylet. Opening of dorsal esophageal gland about 5 microns from base of stylet. Median bulb wide, quite typically criconematoid, followed posteriorly by isthmus which is encircled about midway by the nerve ring. Esophageal glands forming a distinct, somewhat pyriform basal bulb at the base of which is the intestine. The cells of the latter usually contain globular granules of varying size, but some small vacuolated areas within the intestine noted. Excretory pore opening at a level in the vicinity of the base of the unprotruded stylet.

Vulva prominent (Fig. 1c), transverse, without lateral vulval membranes. Vagina extending inward and somewhat forward; single uterus leading anteriorly into a spermatheca containing small globular sperms. Ovary single, outstretched, extending anteriorly with oocytes in single file. Post uterine sac not observed.

DIAGNOSIS: *Paratylenchus* differing from the most closely related species (*P. aciculus* Brown, 1959) primarily by (1) the presence of 4 lines in the lateral field and (2) more posterior position of the vulva (77%) as compared to 3 lateral lines and vulva at 70% in *P. aciculus*.

HOLOTYPE—Female: Material collected by author and M. K. Burk via U. S. Plant Introduction Station, Glenn Dale, Maryland, December 30, 1959. Slide T-4t, United States Department of Agriculture Nematode Collection, Beltsville, Maryland.

PARATYPES—6 females: Same data as for holotype. Slides T-1p—T-6p. United States Department of Agriculture Nematode Collection, Beltsville, Maryland.

TYPE HABITAT, HOST AND LOCALITY: Soil and roots of *Sorbus hybrida* in an unidentified nursery near Moscow, USSR.

The species name is given in honor of Dr. Gotthold Steiner.

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Studies on Bovine Gastrointestinal Parasites. XXIII. Low Level Feeding of Phenothiazine in *Trichostrongylus axei* Infections

ROY L. MAYHEW
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The author's results of feeding phenothiazine in small daily amounts in pure infections of the hookworm (*Bunostomum phlebotomum*), the nodular worm (*Oesophagastomum radiatum*), the large stomach worm (*Haemonchus contortus*) and *Cooperia punctata* have been reported in previous papers. The present note presents comparable data on the small stomach worm, *Trichostrongylus axei*, obtained during 1959.

MATERIALS AND METHODS

The three calves used in the experiments were pure bred Jerseys born at the L.S.U. Dairy Department. No. 314 was born March 13, 315 June 5, and 316 May 19, 1958. Numbers 314 and 316 were inoculated with infective larvae received from Dr. Dale A. Porter, U.S.D.A. Animal Disease Research Laboratory, Auburn, Alabama and No. 315 with larvae cultured from No. 314. The Care of the experimental animals and methods of culturing, collecting and counting larvae were the same as previously described (Mayhew, Am. Jour. Vet. Res. 20: 492-497, 1959). The phenothiazine was the NF grade and was fed at the rate of 1½ grams daily mixed with the grain ration. It was fed from 2 to 5 days before cultures were made in order that it be thoroughly distributed in the digestive tract. Egg counts were made daily throughout the time of the experiments except occasionally on Sunday.

RESULTS

The results of the experiments are given in table 1. The numbers of eggs recovered did not vary during the time of feeding of the drug beyond the regular range of variations in the daily counts before and after feeding. Neither were there any differences in the shape or internal cell structure of the eggs observed during the time of feeding of the drug as occurs in the case of the nodular worm (Mayhew, Proc. Helm. Soc. Wash. 18: 70-77, 1951).

SUMMARY

The results of 9 experiments on 3 calves show that 1½ grams of phenothiazine daily in feed was 100% effective in preventing the development of infective larvae of *Trichostrongylus axei* in the fecal cultures. There was no effect on numbers or microscopic appearance of the eggs.

Table 1. Effect of phenothiazine, 1-1½ grams daily, on development of infective larvae of *T. axei* of calves, during 1959.

Calf No.	Dates drug was fed	No. and Dates of Cultures	No. Larvae per Culture
315		6—May 17-23	680-33,800
	May 21-26	3—May 23-25	0
		3—June 5-8	1,320- 3,036
		2—June 8-10	4,158-11,088
	June 16-24	3—June 21-23	0
		4—July 3-6	712- 4,996
	July 13-17	2—July 15-17	0
		2—Sept. 10-11	3,520- 5,324
	Oct. 1-6	2—Oct. 3-5	0
314		2—June 5-8	3,557-10,032
		2—June 16-17	2,508- 4,936
	June 17-24	3—June 21-23	0
		2—July 4-7	3,080- 6,560
	July 11-18	4—July 15-17	0
		2—Sept. 10-11	2,310-12,201
	Sept. 30-Oct. 5	2—Oct. 3-5	0
		3—Oct. 7-8	3,696- 7,854
316		2—Sept. 10-11	836- 3,168
		2—Oct. 3-5	0
		3—Oct. 20-24	2,640- 5,082
	Oct. 26-Nov. 2	5—Oct. 28-Nov. 2	0
		3—Nov. 8-10	2,904- 2,926

Records of Trematodes of the Families Lecithodendriidae, Dicrocoeliidae, and Heterophyidae from Chiroptera Collected in Egypt and Yemen, S. W. Arabia*

RALPH W. MACY, DONALD HEYNEMAN, and ROBERT E. KUNTZ

In making biological and geomedical studies in Africa and the Middle East extensive collections of helminth parasites were obtained from vertebrate hosts examined in different geographic localities. These investigations are a part of a broad program supported by the U.S. Navy with emphasis on the role that the lower vertebrates may play as reservoir for various diseases including the helminths of man and domestic animals. The present study is based upon trematodes taken from several hundreds of bats examined in Egypt (1948-1953) and a few taken by one of us (R.E.K.) while serving as parasitologist with the U.S. Naval Medical Mission to the Yemen, southwest Arabia (1951). When size of host collections permitted samples of 20 to 50 or more bats were examined from each species represented.

Information presented in the present report is based upon a study of approximately 1,000 specimens. Representative specimens of trematodes have been deposited in the U.S. National Museum Helminthological Collection.

MATERIALS AND METHODS

Most of the bats were captured alive or were examined shortly after they had been shot. Each system of the viscera was examined separately under a dissecting microscope after preparation of host skin for identification purposes. During examination tissues were macerated with small forceps. Afterwards they were washed in several changes of fresh water in stoppered flask or in pint fruit jar. Examination of the sediment permitted detection of small trematodes which might otherwise be overlooked.

All trematodes were killed by quick immersion in hot water, followed by immediate fixation in FAA (formalin-acetic acid-alcohol). After 8-20 hours in fixative the specimens were transferred to 70 per cent alcohol plus 2 per cent glycerine for storage. They were stained in borax carmine or Ehrlich's acid haematoxylin before being permanently mounted. All measurements are in millimeters unless otherwise stated, and each measurement is an average of ten individuals or structures.

Localities are in Egypt unless given as Yemen.

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*The opinions and assertations contained herein are the private ones of the authors and not to be construed as official or reflecting the views of the Navy Department.

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One of us (R. E. K.) is indebted to Dr. Harry Hoogstraal, U. S. Naval Medical Research Unit No. 3, Egypt for continuous support in obtaining hosts in both Egypt and the Yemen. He also has provided host identifications. George Malakatis HM1, USN gave extensive technical assistance in procurement and examination of hosts in Egypt. Abdel Aziz Salah, NAMRU No. 3, served in the capacity of liaison and as interpreter in the Yemen as well as in Egypt.

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FAMILY LECITHODENRIIDAE ODHNER, 1910
GENUS *Prosthodendrium* DOLFUS, 1937

P. (Paralecithodendrium) glandulosum (Looss, 1896) (Fig. 1)

Two sizes of specimens were taken from the type host, *Liponycteris nudiventris*. Eleven of the largest individuals correspond most closely to those described by Looss, 1896, and average measurements are as follows: body 0.86 long by 1.08 wide; oral sucker 0.153 in diameter and ventral sucker 0.170 in diameter; therefore, the oral sucker is slightly smaller by ratio of 1 to 1.1. Testes 0.30 in diameter; ovary 0.18 wide; and prostate mass 0.32 in diameter. Eggs 0.020-0.021 by 0.011-0.013, slightly shorter than indicated by Looss. Specimens described by Looss were slightly larger, and the oral sucker was said to be slightly larger than the ventral sucker with a ratio of 1.2:1. Differences in age and fixation could explain the discrepancies. Proportions of the body, suckers, and some other structures differ somewhat from those in the figure by Looss; therefore it seemed desirable to include a new illustration of the species.

Hosts and Localities: Bats, *Liponycteris nudiventris,* tombs at Sakkara, June 8, 1951. *Taphozous perforatus,* caves at edge of Faiyum, January 23, 1950; caves back of Abu Rauwash, January 21-22, 1950, Giza Pyramid, May 14, 1951, *Rhinolophus clivosus brachygnathus,* caves near Kom Aushim, May 25, 1950. *Rhinolopoma microphyllum,* Giza Pyramid, May 8 and June 7, 1950; Bent Pyramid, east of Sakkara, May 15, 1950. *Rhinopoma hardwickei cystops,* Sakkara, March 16 and August, 1951; Giza Pyramid, May 14, 1951, Wadi Digla, nine miles east of Maadi, October 20, 1951.

The species was collected by Odhner, 1911, from *Megaderma frons,* in the region of the White Nile. Azim, 1936, identified flukes from *Pipistrellus kuhli* and *Rhinolophus euryale,* at the Oasis Dakla, Egypt, as this species and described what he believed to be the cercaria and metacercaria. However Dubois, 1955, in his revision of the genus, suggests that Azim did not present sufficient evidence to prove the identity of the species.

It is likely that *P. obtusum* (Looss), described originally from the chameleon collected at Alexandria, is synonymous with *P. glandulosum.* Odhner also collected specimens from *Chamaeleo basiliscus* at Cairo.

Specimens in U.S. Nat. Mus. Helm. Coll. No. 39254 and 39259.

P. (Prosthodendrium) pyramidum (Looss, 1896)

Twenty specimens were taken from the intestine of *Rhinolophus clivosus brachygnathus* from Abu Rauwash, Giza Province, March 12, 1951. Another group of 74 individuals were collected from a "short-eared bat." Four specimens were found in *Rhinolophus clivosus acrotis* obtained January 13, 1951, from a house at Ta'izz, Yemen; another two examples of this species were from the same species of host at Sana', February 15-16, 1951.

Looss reported the host of his material to be *Rhinolophus hippocrepis* from the Pyramid of Giza.

Our specimens were 0.91 long by 0.57 wide; oral sucker diameter 0.10; ventral sucker diameter 0.08; testes 0.17 in diameter, ovary 0.11 in width; prostate mass 0.16. Looss listed the suckers both as being 0.10 in diameter whereas Dollfus, 1954, found the ventral sucker to be a little smaller than the oral sucker in specimens from *Miniopterus schreibersi* collected north of Rabat, Morocco.

Specimens in U.S. Nat. Mus. Helm. Coll. No. 39258.

P. (Prosthodendrium) urna (Looss, 1907)

Found twice in Egypt with three specimens from *Rhinolophus clivosus brachygnathus,* Giza Pyramid, June 18, 1951; and nine from *Taphozous perforatus* from caves at edge of Faiyum, January 23, 1950. Five additional examples were in *Rhinolophus clivosus acrotis* from a house at Ta'izz, Yemen, January 21, 1951; 30 were from *Pipistrellus sp.* and *Rhinolepis blasii* in and near Sana', February 14-16, 1951.

In this species the ventral sucker is a little smaller than the oral sucker and a small number of vitelline follicles are situated near the end of the ceca. The position of the ventral sucker seems to be somewhat variable; Looss (1907) illustrated it as being at the level of the anterior margin of the testes, whereas in our specimens, it could also be found near the level of the posterior part of the testes.

GENUS *Lecithodendrium* Looss, 1896

L. granulosum Looss, 1907

Found to occur in *Rhinolophus clivosus,* May 25, 1950, in caves near Kom Aushim; in *Taphozous perforatus,* January 21, 1952, caves at Abu Rauwash; in *Nycteris thebaica thebaica,* January 5, 1953; Abu Sir tombs and caves, Giza Province; *Asellia tridens tridens,* same date and locality; *Plecotus auritus christiei,* March 1, 1951, Sakkara tombs and pyramids, Giza Province; in *Pipistrellus kuhli,* July 16, 1952; barns of King's Estates, Idfina, Beheira Province; also found in *Pipistrellus sp.* collected at Sana', February 14, 1951.

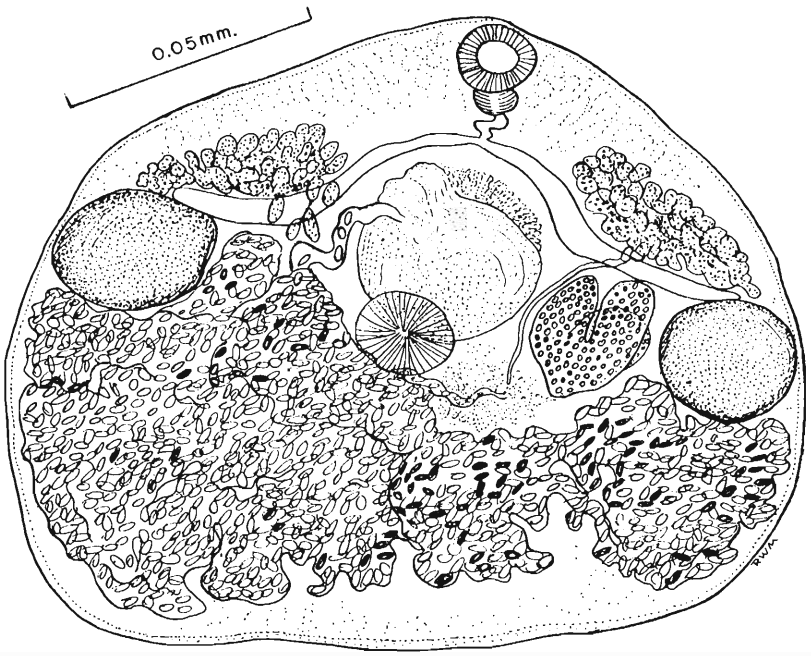

Fig. 1. *Prosthodendrium glandulosum* (Looss) from intestine of *Liponycteris nudiventris,* tombs at Sakkara. Drawn with the aid of a camera lucida.

Body length 1.07, body width 0.61; oral sucker 0.11 in diameter, approximately same size as ventral sucker which measures 0.10 in diameter; prostrate mass 0.088 wide.

Specimens in the U.S. Nat. Mus. Helm. Coll. No. 39260.

GENUS *Acanthatrium* FAUST, 1919

A. sphaerula (Looss, 1896)

Looss discovered this species in the intestine of *Rhinolophus hippocrepis* from the Pyramid of Giza. In the present study specimens were collected from *Taphozous perforatus,* March 12, 1952, January 31, 1953, Giza Pyramid; and same host, January 21, 1952, caves at Abu Rauwash.

FAMILY DICROCOELIIDAE ODHNER, 1911

GENUS *Anchitrema* LOOSS, 1899

A. sanguineum (Sonsino, 1894) Looss, 1899

In the past, *Anchitrema* has been placed in the Lecithodendriidae but we have followed the usage of Yamaguti, 1958.

Specimens were collected from *Taphozous perforatus,* October 2, 1952, Monastery Wadi Natroum, Western Desert; same host, January 9, 1952, caves and tombs at Abu Rauwash, Giza Province, same host and place, January 21, 1952. From *Nycteris thebaica thebaica,* caves near Abu Rauwash, and from Abu Sir tombs and caves, Giza Province; also from *Plecotus auritus christiei,* caves at Abu Rauwash. Finally a number of examples were found in *Pipistrellus kuhli,* July 16, 1952, barns of King's Estates, Idfina, Beheira Province.

Specimens in U. S. Nat. Mus. Helm. Coll. No. 39262.

FAMILY HETEROPHYIDAE ODHNER, 1914

GENUS *Heterophyes* COBBOLD, 1886

H. heterophyes (Siebold, 1853)

This species, common in man and certain carnivores of the Middle and Far East, was collected by Kuntz and reported by Macy, 1953. Two specimens were taken from *Rhinolophus clivosus acrotis,* February 14, 1951, at Sana', Yemen. It is the first record of the species in a bat.

CONCURRENT INFECTIONS

Several instances of concurrent infection were noted. For example, different individuals of *Taphozous perforatus* harbored simultaneously *Prosthodendrium glandulosum* and *P. pyramidum; P. glandulosum* and *Anchitrema sanguineum; P. glandulosum, Acanthatrium sphaerula* and *Anchitrema sanguineum; P. glandulosum, A. sphaerula,* and *Lecithodendrium granulosum;* one *Pipistrellus kuhli* contained *A. sanguineum* and *L. granulosum;* and a specimen of *Nycteris thebaica thebaica* had both *A. sanguineum* and *L. granulosum.*

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Notes on the Occurrence of *Capillaria hepatica* (Bancroft, 1893)

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In the course of helminthological studies extending over several years, the writer has collected wild rodents infected with *Capillaria hepatica* (Bancroft, 1893) on four occasions. Since some new host or distributional records are involved, these findings are reported here:

1. Pocket gopher, *Thomomys talpoides tenellus* Goldman. Moran, Wyoming; June, 1948. Extensive cirrhosis of the liver. *C. hepatica* has previously been reported from this species by Dikmans (1932) (Medicine Bow Mountains, Wyoming) and by Lubinsky (1956) (Alberta, Canada).

2. Wood rat, *Neotoma cinerea fusca* True. Thirty miles northeast of Portland, Oregon (Larch Mountain); December, 1950. Extensive cirrhosis of two hepatic lobes.

3. White-footed mouse, *Peromyscus maniculatus ?macrorhinus* (Rhoads). Ketchikan, Alaska; September, 1953. Extensive cirrhosis of liver. Infection of *P. maniculatus* has been reported previously by Lubinsky (1956) (Alberta, Canada).

4. Brown lemming, *Lemmus sibiricus harroldi* Swarth. Near Mekoryuk, Nunivak Island, Alaska; June, 1955. Two lemmings infected among ten examined.

Although characteristic hepatic lesions were macroscopically visible in the lemmings, there had been very little proliferation of connective tissue. Sections disclosed discrete, scattered foci that were variously comprised of normal eggs, eggs that had calcified, mature worms containing eggs, or worms that had died and had undergone calcification. A few small areas of necrosis were observed, as were some scattered granulomatous lesions, but most of the hepatic tissue was normal in appearance. The histological characteristics of some of the nematodes indicated that they had been alive at the time the tissue was fixed. Some of the latter evidently had expelled eggs (Fig. 1), although it is generally believed that the eggs escape only when the worms disintegrate after death. The living worms were usually surrounded by a thin zone of strongly eosinophilic, but still intact, hepatic cells; few, if any, leukocytes were in such areas. These were probably infections of short duration, in view of the relatively slight involvement of the liver. Otto and von Brand (1941) found that calcification of the dead

nematodes begins after only about 19 days in laboratory rats.

The finding of infected lemmings on Nunivak Island is of interest, since *C. hepatica* has not been reported previously above about latitude 55°N. According to Skriabin *et al.* (1957; p. 406), this nematode is most widely distributed in regions having considerable humidity and high summer temperatures. Up to now the northernmost records have been from regions of a continental-type climate (Ontario and Alberta in Canada; North-Kazakhstan and Belorussia in Eurasia). However, it must now be regarded also as a member of the arctic fauna.

This nematode does not appear to be a common parasite of microtine rodents in Alaska, for, with the exception of the aforementioned lemmings, it has not been found in the many animals thus far examined. The characteristic lesions caused by *C. hepatica* are not easily overlooked; moreover, it has been the practice in this laboratory to section all hepatic lesions of an undetermined nature from such rodents.

Nunivak Island is rather remotely situated and it is unlikely that *C. hepatica* has been introduced there by man in recent times. Norway rats, often parasitized by this nematode at lower latitudes, do not occur in western Alaska between the Seward Peninsula and the Aleutian Islands.

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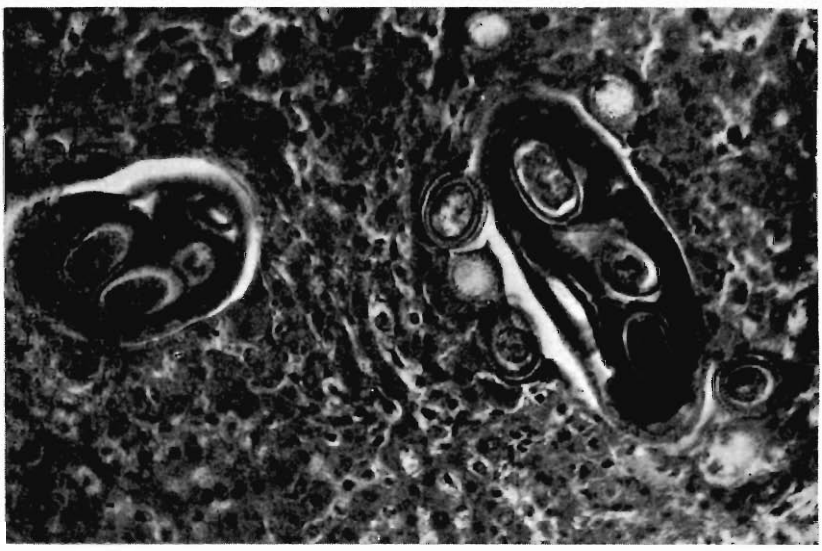

Figure 1. Section of liver from *Lemmus*, showing portions of living nematodes and free eggs. 270x.

Studies on Species of Criconematinae (Nematoda: Tylenchida) from India*

M. RAFIQ SIDDIQI

Very little is known about the Criconematinae occurring in India. Until now only two species viz. *Criconema squamosum* (Cobb, 1913) Taylor, 1936, and *Hemicycliophora longicaudata* Loos, 1948, have been reported to occur around mango and sugarcane roots respectively in this country. The present paper deals with the description of twelve species of Criconematinae, ten of which were collected by the author during the last four years in various localities of Uttar Pradesh, a North Indian State. The remaining two are from South India. Seven of these species are new to science. These nematodes parasitize perennials, especially fruit trees. Some of these species are widely distributed in North India, occurring both in plains as well as hilly and forest areas. In addition, soil and root samples from other states have revealed the presence of some of these forms.

Criconema pruni, n. sp. (Fig. 1, A-G)

MEASUREMENTS: 25 females: Length = 0.56-0.75 mm. (0.61 mm.); a = 12-17 (14.4); b = 3.9-5 (4.4); V = 92.5-96% (94); total body annules = 94-108 (100); spear = 66-74 microns (70 microns). 10 larvae: Length = 0.35-0.53 mm.; a = 12-14; b = 4-4.9; c = 21-25; total body annules = 80-107; spear = 52-62 microns.

FEMALE (Holotype): Length = 0.65 mm.; a = 14; b = 4.5; c = 31; V = 82-94.3%.

Body almost cylindrical, assuming a slightly ventrally arcuate position on death. Body annules retrorse, numbering 106 and 103 on dorsal and ventral surface of body respectively. First annule not retrorse. Posterior margins of each annule bearing a continuous fringe of very fine spines, about 150 in number at mid-body and extending back to middle of following annule. In surface view, these annular fringes appearing as continuous, membranous flaps marked by deep longitudinal lines. Lateral fields or lines on body absent. Oral aperture obscure, located on an elevated labial disc bearing slit-like amphid apertures on its lateral margins. Sub-lateral lobes well developed, elevated, placed equidistantly around labial disc (Fig. 1, C). Cephalic sclerotization weak, hexa-radiate (Fig. 1, D).

Buccal spear strong, 71 microns long, with a tip 53 microns long. Basal knobs of spear measuring 11 microns across by 4.2 microns high, with outer margins directed forward. Outlet of dorsal oesophageal gland 7 microns behind spear base. Nerve ring enveloping short, narrow isthmus. Excretory pore on 29th annule from anterior end of body, 4 body annules posterior to oesophageal base.

Vulva transverse, half as long as body width at that region, located on 9th annule from body terminus; anterior flap of vulva bilobed. Vagina leading upwards then inwards into a highly muscular uterus. Spermatheca absent. Ovary anteriorly outstretched; oöcytes in single row except for a few in region of multiplication. Distance from vulva to end of body approximately equal to vulvar body width. Rectum short, not very conspicuous. Anus located on 7th annule from terminus. Caudal end rounded,

*Contribution from the Department of Zoology, Aligarh Muslim University, Aligarh (U.P.), India.
The author is thankful to Dr. M. A. Basir under whose guidance this work was done.

with last annule forming a button-shaped terminus.

MALE: Not found.

LARVAE: Body annules retrorse, with crenate, longitudinally lined posterior margins in all stages. Labial disc and sub-lateral lobes distinct. Anus located on 4-7th annule from end of body.

HOLOTYPE: Female collected on June 15, 1959; slide no. PN/C/1-001, deposited with the Zoology Museum, Aligarh Muslim University, Aligarh (U. P.), India.

PARATYPES: 24 females and 10 larvae; other data same as for holotype.

TYPE HOST: Collected from soil around roots of *Prunus armeniaca* L. (Apricot).

TYPE LOCALITY: Ranikhet (elevation 6,000 feet), Almora District (U. P.), India.

HOSTS AND GEOGRAPHICAL DISTRIBUTION: Specimens of this species have been collected around roots of apricot—*Prunus armeniaca* L., *P. communis* Huds., and Apple—*Malus sylvestris* (L.) Mill. in Nainital and Almora districts of Uttar Pradesh (at an elevation of 5,500-6,500 feet) and *P. armeniaca* L. at Simla (elevation 7,100 feet) in Punjab State.

DIAGNOSIS AND RELATIONSHIP: *Criconema* with body annules numbering 94-108, presence of continuous fringe of about 150 delicate spines on each annule at mid-body, buccal spear 66-74 microns long, and vulva at 7-10th annule from end of body. In having a continuous fringe of over 100 spines per annule, *C. pruni* n. sp. resembles *C. brevicaudatum* n. sp. from which it can be separated by the presence of a larger number of body annules, distinct sub-lateral lobes around oral opening, and vulva located on 7-10th annule from terminus (on 5th annule in *C. brevicaudatum*). In general morphological characters, however, *C. pruni* is very close to *Criconemoides xenoplax* Raski, 1952, differing mainly in the presence of delicate, cuticular spines on body annules.

Criconema brevicaudatum, n. sp. (Figure 1, H-I)

FEMALE (Holotype): Length = 0.49 mm.; a = 9.4; b = ?; c = sub-terminal; V = ^{48}92.4%.

Body cylindrical, tapering on either extremities, assuming a straight position on death. Body annules retrorse, covered with thick cuticle, 42 in number. Each annule bearing on its posterior margins a continuous fringe of short, delicate spines numbering about 120 on middle of body. Head comprising two non-retrorse annules; the first head annule saucer-shaped with margins directed forward and outward, 24 microns in diameter; the second being simple, rounded, 20 microns in width. Internal head sclerotization of medium strength, hexa-radiate. Lip region cupolate, with small slit-like amphid apertures on lateral lips near oral opening; sub-lateral lobes indistinct.

Buccal spear of stout build, 58 microns in length; its tip 44 microns long; basal knobs 10 microns across, with anteriorly reflexed margins. Oesophagus distorted. Median oesophageal bulb with spindle shaped valvular apparatus. Excretory pore on 15th body annule and at 163 microns from anterior end of body. Vulva a transverse slit, about half the vulvar body diameter long, located on 5th body annule from terminus. Vagina leading inward and forward into a muscular uterus. Ovary single, anteriorly outstretched; oöcytes mostly arranged in single file. Vulvar body diameter greater than the distance from vulva to end of body. Anus obscure, possibly located on

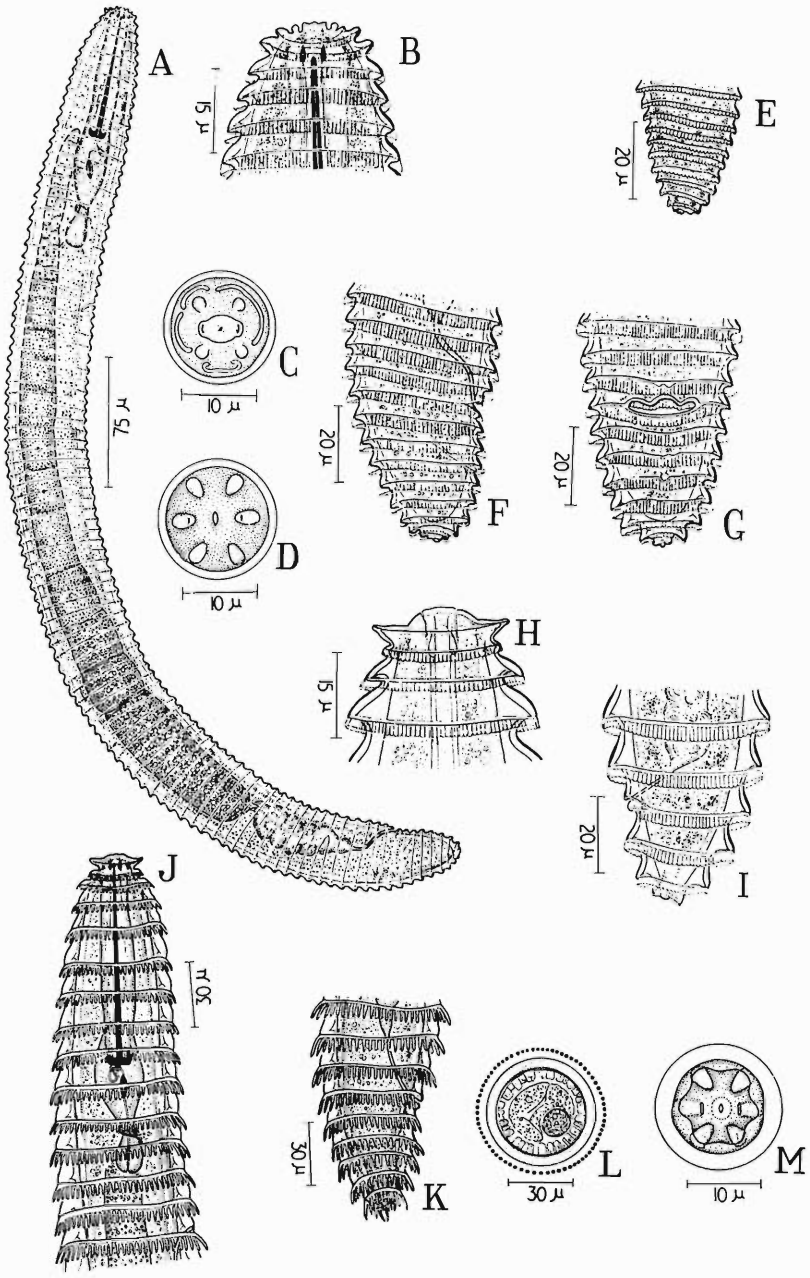

Figure 1. A-G—*Criconema pruni*. A. Female. B. Head of female. C. *En face* view of female. D. Labial sclerotization. E. posterior end of larva. F. Posterior end of female, lateral view. G. Posterior end of female, ventral view. H-I—*Criconema brevicaudatum*. H. Head of female, ventral view. I. Posterior end of female, lateral view. J-M—*Criconema multisquamatum*. J. Oesophageal region of female. K. Posterior end of female, lateral view. L. Cross section through middle of body. M. *En face* view of female.

2nd annule from terminus. Last body annule small, button-shaped. Caudal end broadly rounded.

MALE: Not found.

HOLOTYPE: Female isolated from soil sample collected by P. S. Narayanaswamy in September, 1958; slide no. PN/C/1-002, deposited with the Zoology Museum, Aligarh Muslim University, Aligarh (U. P.), India.

TYPE HOST: Soil around roots of sugarcane, *Saccharum officinarum* L.

TYPE LOCALITY: Coimbatore (Madras State), South India.

DIAGNOSIS AND RELATIONSHIP: *Criconema* with 42 body annules, each bearing a continuous fringe of delicate spines numbering about 120 on mid-body, spear measuring 58 microns in length, and vulva located on 5th annule from terminus. It is close to *C. multisquamatum* (Kirjanova, 1948) Chitwood, 1957, and *C. pruni* n. sp. It differs from the former in having a larger number of annular spines, a shorter buccal spear, and the vulva located on 5th annule from terminus, and from the latter in having a lesser number of body annules, absence of sub-lateral lobes in lip region, and vulva located on 5th annule from end of body. It has also some affinities with *Criconemoides boettgeri* Meyl, 1954, which appears to be rather like a *Criconema,* from which it differs in having short delicate spines on each annule, a smaller buccal spear (84.5 microns long in latter species), and vulva located on 5th annule from terminus.

Criconema multisquamatum (Kirjanova, 1948) Chitwood, 1957 (Fig. 1, J-M)

Syn. *Ogma multisquamata* Kirjanova, 1948

Criconema fimbriatum (Cobb) Taylor of Sveshnikova, 1940

Kirjanova (1948) described this species as *Ogma multisquamata* Sp. N. from two female specimens which were collected by Sveshnikova around citrus roots. In those specimens the oesophageal structures and possibly the genital organs were not detectable. In the present study, however, sufficient material in good condition was available for examination. Hence the species is briefly redescribed.

MEASUREMENTS: 7 females: Length = 0.49-0.62 mm.; a = 9.4-14; b = 3.6-4; V = $^{28\text{-}70}$90-91%; spear = 87-97 microns; total body annules = 44-48.

3 larvae: Length = 0.35-0.44 mm.; a = 12-13.3; b = 6-6.6; spear = 40-75 microns; total body annules = 43-47.

FEMALE: Body cylindrical, straight, appearing dark-brown in colour. Body annules averaging 45 in number, covered with thick cuticle, bearing a continuous fringe of stout, rod-shaped spines numbering 72 per annule at mid-body. Structure of the head as described for *C. brevicaudatum. En face* view showing an oval oral opening surrounded by six confluent lips. Amphid apertures slit-like, located on inner margins of lateral lips. Buccal spear stout, 97 microns in length, with strong, anteriorly reflexed basal knobs. Median oesophageal bulb 22 microns wide at its widest. Isthmus short, enveloped by nerve ring. Posterior oesophageal bulb rounded, set off from intestine. Excretory pore located at 15th annule from anterior end, 2 body annules behind oesophageal base. Vulva located on 7-8th annule from terminus. Spermatheca absent. Ovary prodelphic, with oöcytes arranged in single file except for a few near cap-cell which form double rows. Anus not located. Caudal end conoid-rounded.

MALE: Not known.

LARVAE: Body similar to that of female. Annules with continuous fringe of spines as in female. Spear more slender than in females.

Specimens deposited with the Zoology Museum, Aligarh Muslim University, Aligarh (U. P.), India.

HOSTS AND LOCALITIES: Specimens of this species have been collected from soil around roots of *Prunus armeniaca* L. and *Malus sylvestris* (L.) Mill. in Nainital and Almora districts of Uttar Pradesh (at an elevation of 5,500-6,500 feet); *P. armeniaca* L. at Simla (elevation 7,100 feet) in Punjab State.

DIAGNOSIS AND RELATIONSHIP: *Criconema* with 44-48 body annules bearing a continuous fringe of rod-like spines numbering about 72 on mid-body, buccal spear 87-98 microns long, and vulva located on 7-8th annule from body end. It comes close to *C. fimbriatum* (Cobb, unpubl.) Taylor, 1936, but differs in having a smaller number of body annules (44-48: 53), more spines per annule on mid-body (72: 40), and vulva located on 7-8th annule from terminus (on 10th annule in *C. fimbriatum*).

Criconema tenuicaudatum, n. sp. (Fig. 2, A-D)

MEASUREMENTS: 3 females: Length = 0.43-0.49 mm.; a = 10-10.4; b = 3.1-3.4; c = 15.6-15.8; V = 86-87.7%; total body annules = 59-61; spear = 106-110 microns.

FEMALE (Holotype): Length = 0.49 mm.; a = 10.4; b = 3.4; c = 15.8; V = 45-87.7%.

Body cylindrical tapering anteriorly from oesophageal base to head and posteriorly rather abruptly behind vulva to caudal terminus, with thick dark cuticle, assuming a slightly ventrally arcuate position when the animal is killed by gradual heat. Body annules retrorse, 60 in number, bearing a discontinuous fringe of slightly curved spines having rounded ends on posterior margins. Spines arranged in groups of 2-3 (rarely 4) forming longitudinal bands numbering 14 on mid-body. On the extremities of body, spines not arranged in definite groups. Head distinctly set off from body, comprising two modified annules; the first cephalic annule anteriorly expanded to form a broad, membranous cup, 21 microns wide, enclosing the dome-shaped lip region; the second annule simple, rounded, 16 microns in width. Labial frame-work moderately sclerotized, hexa-radiate. Amphid apertures small, slit-like, located on lateral lips near oral opening.

Spear slender, much elongated, 106 microns long; spear tip 91 microns in length; spear knobs anteriorly reflexed, 8.5 microns across and one-third as much high. Oesophagus extending through 22 body annules. Median oesophageal bulb 19 microns at its widest. Isthmus short and broad, only slightly expanded at its base to form the basal oesophageal bulb. Latter distinctly set off from intestine. Cardia absent. Excretory pore on 24th annule from anterior end. Nerve ring enveloping isthmus.

Vulva transverse, about half as long as body width at that region, located on 11th annule (10-11th annule in paratypes) from posterior end of body. Uterus with numerous small-sized, rounded sperms enclosed in a thin-walled spermatheca at its distal end. Ovary single, prodelphic, with oöcytes arranged in one row. Anus on 6th annule from terminus. Tail attenuated.

MALE: Not found.

HOLOTYPE: Female collected on June 10, 1959; slide no. PN/C/1-003, deposited with the Zoology Museum, Aligarh Muslim University, Aligarh (U. P.), India.

PARATYPES: 2 females; other data same as for holotype.

TYPE HOST: Soil about roots of *Citrus limon* (L.) Burm.

TYPE LOCALITY: Bhowali (elevation 5,500 feet), Nainital District (U. P.), India.

DIAGNOSIS AND RELATIONSHIP: *Criconema* with 59-61 body annules, spines arranged in groups of 2-3 in longitudinal bands numbering 14 on mid-body region, 106-110 microns long buccal spear, vulva located on 10-11th annule from end of body, and an attenuated tail.

Criconema civellae Steiner, 1949, is the only other known species of the genus which bears longitudinal bands of spines on the body. *C. tenuicaudatum* n. sp. can easily be differentiated from it by its 14 longitudinal bands of spines on mid-body as compared to only 8 in *C. civellae*.

Criconema octangulare (Cobb, 1914) Taylor, 1936
Syn. *Iota octangulare* Cobb, 1914

Two female specimens of this species were collected around roots of apricot—*Prunus armeniaca* L. at Ranikhet, Almora District (U. P.) in June, 1959. A study on these specimens revealed the following formula:

Length = 0.43-0.47 mm.; a = 12-13; b = 4-4.5; c = 15.8-16; V = $^{60\text{-}66}$86-87.3%.

The buccal spear measures 68-69 microns long and the excretory pore lies on 23rd annule from anterior end of body. The vulva has a trilobed posterior flap. Other details conform closely to those given by Cobb, 1914, and Taylor, 1936.

Specimens deposited with the Zoology Museum, Aligarh Muslim University, Aligarh (U. P.), India.

Criconemoides insigne, n. sp. (Fig. 2, E-I)

MEASUREMENTS: 5 females: Length = 0.4-0.53 mm. (0.47 mm.); a = 11-13 (12); b = 4-4.6 (4.3); c = 30-34.5 (31.8); V = $^{40\text{-}82}$91.7-93% (92.2%); total body annules = 65-69 (68); spear = 60-64 microns (62 microns).

FEMALE (Holotype): Length = 0.5 mm.; a = 11.6; b = 4.5; c = 31; V = ^{66}92%.

Body cylindrical, with tapering ends, assuming a slightly ventrally arcuate position when the worm is killed by gentle heat. Body annules retrorse, 69 in number. Lateral lines or annular anastomoses absent. First two annules of anterior end not retrorse; the first of these about 12 microns wide, with outer margins directed forward and outward; the second about 18 microns in width, directed outward. Oral opening obscure, located on an elevated labial disc. Sub-lateral lobes not discernible. Amphid apertures small, slit-like, located on lateral margins of labial disc. Internal cephalic sclerotization fairly strong.

Buccal spear strong, 63 microns in length, with tip 50 microns long. Basal knobs of spear anteriorly reflexed, 9 microns across. Oesophagus extending through 18 body annules. Excretory pore on 22nd annule (on 22nd-23rd in paratypes) from anterior end of body, about 26 microns posterior to oesophageal base. Vulva a transverse slit, about 15 microns long, located on 7th (6-7th in paratypes) annule from terminus. Vagina leading inward and forward into the uterus. Spermatheca not observed. Ovary monodelphic and prodelphic. Distance from vulva to caudal terminus greater than body width at vulva. Anal aperture small, rounded, located on 4th annule from posterior end of body. Caudal end convex-conoid; last annule forming a conoid-rounded terminus.

MALE: Not found.

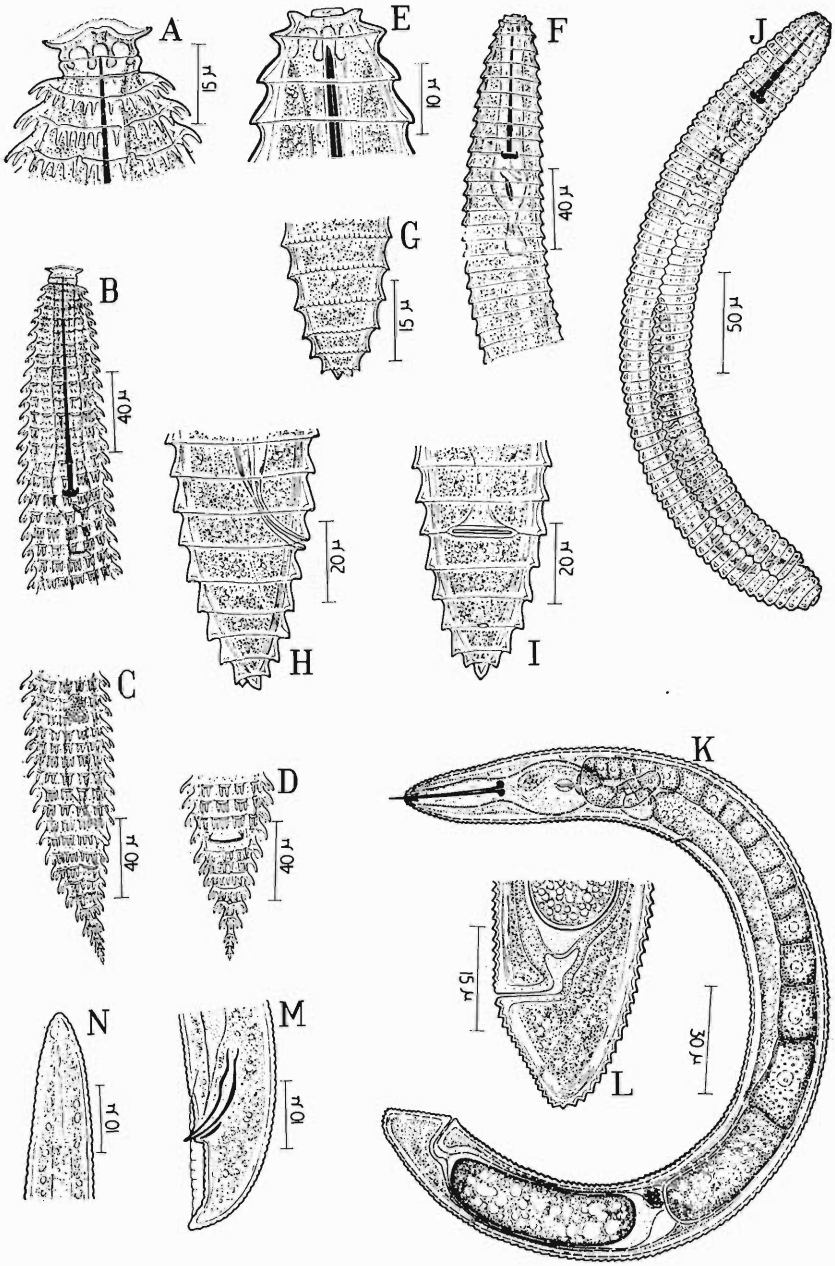

Figure 2. A-D—*Criconema tenuicaudatum*. A. Head of female. B. Oesophageal region of female. C. Posterior end of female, lateral view. D. Posterior end of female, ventral view. E-I—*Criconemoides insigne*. E. Head of female. F. Oesophageal region of female. G. Posterior end of larva. H. Posterior end of female, lateral view. I. Posterior end of female, ventral view. J—*Criconemoides citri*. female. K-N—*Criconemoides parvulum*. K. Female. L. Posterior end of female, lateral view. M. Tail of male. N. Anterior end of male.

LARVA: Length = 0.39 mm.; a = 12; b = 4.3; c = 28.

Body annules retrorse, with crenate posterior edges, 73 in number. Excretory pore located on 20th annule from anterior end. Spear 42 microns in length. Anus located on 5th annule from terminus.

HOLOTYPE: Female collected in June, 1958; slide no. PN/C/2-001, deposited with the Zoology Museum, Aligarh Muslim University, Aligarh (U. P.), India.

PARATYPES: 4 females and 1 larva; other data same as for holotype.

TYPE HABITAT: Forest soil associated with roots of trees.

TYPE LOCALITY: Almora (elevation 5,500 feet), U. P., India.

DIAGNOSIS AND RELATIONSHIP: *Criconemoides* with a short body assuming a slightly ventrally arcuate position on death; body annules numbering 65-69; absence of lateral lines on body; buccal spear measuring 60-64 microns in length; vulva and anus located on 6-7th and 4th annule respectively from posterior end of body; a convex-conoid caudal end, with the last annule forming a conoid-rounded terminus.

C. insigne n. sp. is closest to *C. anura* (Kirjanova, 1948) Raski, 1958, from which it differs in having a larger number of body annules (65-69: 60), presence of a labial disc in contrast to six, large lips in the latter species, a shorter buccal spear (60-64 microns: 70 microns), and anus located on 4th annule from posterior end of body (on terminal annule in *C. anura*). It also has some similarities with *C. informe* (Micoletzky, 1921) Taylor, 1936, from which it can be distinguished by having a more slender body, a larger number of body annules, a smaller buccal spear (71-81 microns long in *C. informe*), distance from vulva to caudal terminus being greater than body width at vulva, and a convex-conoid tail end (bluntly rounded in *C. informe*).

Criconemoides parvulum, n. sp. (Fig. 2, K-N)

MEASUREMENTS: 20 females: Length = 0.27-0.32 mm.; a = 11-14; b = 3.8-4.6; c = sub-terminal; V = $^{35\text{-}80}$93.8-95.2%; total body annules = 168-194; spear = 30-34 microns.

5 males: Length = 0.29-0.3 mm.; a = 19-22.5; b = ?; c = 19-21.5; T = 28-38%; spicula = 15-17 microns; gubernaculum = 4.8-5 microns.

8 larvae: Length = 0.205-0.258 mm.; a = 11-12; b = 3.6-4.5; c = sub-terminal, total body annules = 168-180; spear = 20-22 microns.

FEMALE (Holotype): Length = 0.3 mm.; a = 13.5; b = 4.2; c = sub-terminal; V = ^{79}95%.

Body almost cylindrical, tapering evenly at anterior end from level of oesophageal base to head and posteriorly rather abruptly from level of vulva to a broadly rounded terminus bearing angular annules, assuming an open ring-shaped position when the animal is killed by gradual heat. Body annules retrorse, with angular posterior margins, numbering 170 on ventral and 175 on dorsal side. Head conoid, anteriorly flattened, continuous with body contour. Cephalic frame-work conspicuously sclerotized, extending 3 annules. Oral aperture rounded, bordered by six confluent lips of which laterals are bigger than sub-medians. Sub-lateral lobes absent. Lateral lines on body not seen.

Spear 32 microns long; spear shaft 9 microns in length, with three strong basal knobs measuring 4.5 microns across by 2.5 microns high and with outer margins directed forward. Dorsal oesophageal gland opening into lumen of procorpus, 4 microns behind spear base. Corpus 15 microns wide. Isthmus short and broad. Posterior oesophageal bulb rounded, one-third as

wide as body width, enclosing three oesophageal glands. Outlets of subventral oesophageal glands just behind valvular apparatus of corpus. Excretory pore on 44th annule from anterior end, 4 body annules behind oesophageal base.

Vulva a transverse slit, 10 microns long, located on 13th annule (on 12-15th annule in paratypes) from posterior end, leading into a thick-walled vagina. Latter at right angles to body axis, extending half-way into body. Uterine egg 55 microns long by 15 microns broad (50-60 microns long by 15-17 microns broad in paratypes). Spermatheca with sperms present at left side of distal end of uterus. Ovary single, extending up to corpus of oesophagus; distal end reflexed. Rectum and anus difficult to observe; latter on 6th annule from terminus.

MALE (Allotype): Length = 0.29 mm.; a = 22.5; b = ?; c = 21; T = 30%.

Lip region conically elevated, marked by four striae. Labial frame-work faintly sclerotized. Body striae 1 micron apart. Buccal spear and oesophagus degenerated. Nerve ring 60 microns from anterior end. Excretory pore 12 microns behind level of nerve ring. Hemizonid two body annules long, located three body annules anterior to excretory pore.

Testis single, outstretched; spermatocytes arranged first in two and then in three rows. Spicula paired, similar, ventrally arcuate, cephalated, 16.5 microns long; curvature of spicule occurring at one-third of its length from the proximal end. Gubernaculum simple, trough-shaped, 4.5 microns long. Bursa distinctly crenate, beginning from level of head of spicula and completely enveloping tail. Latter conoid, with greater curvature occurring on its dorsal surface; terminus bluntly rounded.

LARVAE: Annules retrorse, angular. Cuticular ornamentations absent. Excretory pore behind level of oesophageal base. Buccal spear and oesophagus less strongly developed than in female. Anus sub-terminal; caudal terminus broadly rounded, with angular retrorse annules.

HOLOTYPE: Female collected on November 10, 1957; slide no. PN/C/2-002, deposited with the Zoology Museum, Aligarh Muslim University, Aligarh (U. P.), India.

ALLOTYPE: Male; slide no. PN/C/2-003; other data same as for holotype.

PARATYPES: Hundreds of females and 4 males; other data same as for holotype.

TYPE HOST: *Citrus limon* (L.) Burm.

TYPE LOCALITY: Aligarh (U. P.), India.

DISTRIBUTION: This species is widely distributed in U. P. Specimens have been collected from soil about roots of *C. limon* in 8 districts of this State.

DIAGNOSIS AND RELATIONSHIP: *Criconemoides* with a small body bearing 168-194 annules, tapering sharply behind region of vulva; a short buccal spear measuring 30-34 microns long; vulva located on 12-15th annule from terminus; vagina leading inward at right angles to body axis.

C. parvulum n. sp. is closest to *C. parvum* Raski, 1952, and *C. zavadskii* (Tulaganov, 1941) Raski, 1958. It differs from *C. parvum* in having a larger number of body annules (142-156 in *C. parvum*), a smaller buccal spear (38-41 microns long in *C. parvum*), vagina being at right angles to body axis, and body tapering sharply behind vulva. From *C. zavadskii* this species can easily be distinguished by the body annules being angular posteriorly and the vulva located on 12-15th annule from terminus (on 7-8th annule in *C. zavadskii*).

Criconemoides citri Steiner, 1949 (Fig. 2, J)

FEMALE: As originally described. The difference, however, can be noted in the lateral sides of the body appearing both plain as well as folded to form an irregular zig-zag line and the oesophagus being much shorter than that in the female as illustrated by Steiner (1949). The ranges of various measurements of ten females are presented below.

Length = 0.28-0.36 mm.; a = 9.2-9.6; b = 3.2-3.3; c = ?; V = $^{40-83}$ 93-95%; spear = 49-51 microns; egg = 70 microns long by 21 microns broad.

Specimens deposited with the Zoology Museum, Aligarh Muslim University, Aligarh (U. P.), India.

HOSTS AND LOCALITIES: Specimens of this species have been collected in Aligarh and Banda districts of U. P. from the following hosts: *Citrus limon* (L.) Burm.; *Grewia asiatica* L.; *Mangifera indica* L.; *Cynodon dactylon* Pers.

Hemicriconemoides mangiferae, n. sp. (Fig. 3, A-I)

MEASUREMENTS: 25 females: Length = 0.41-0.6 mm.; a = 19-23; b = 3.6-4.8; c = 18-24; V = $^{36-69}$91-93%; total body annules = 133-148; spear = 70-81 microns.

8 males: Length = 0.4-0.44 mm.; a = 26-31; b = 4-4.5; c = 14-17; T = 30-44%; spicula = 24-28 microns; gubernaculum = 4.5-5 microns.

FEMALE (Holotype): Length = 0.59 mm.; a = 22; b = 4.8; c = 22.5; V = ^{68}92.5%.

Body cylindrical, tapering evenly at both ends, covered by a cuticular sheath attached to it at its extremities and in the region of the vulva. Body annules coarse, rounded, 137 in number, corresponding in number with those of the sheath. Maximum width of sheath and body 30 and 27 microns respectively. Lateral fields or lines absent. First annules of head angular, directed outward. Labial frame-work strongly sclerotized, hexa-radiate, with lateral sectors larger than sub-medians.

Spear 74 microns long; its tip measuring 64 microns long; basal knobs strong, 7 microns across by 3 microns high, with anterior margins directed forward. Outlet of dorsal oesophageal gland 7 microns behind spear base. Median oesophageal bulb 15 microns wide. Isthmus short, crossed by nerve ring. Excretory pore on 36th annule from anterior end of body, 4th annule posterior to oesophageal base.

Vulva transverse, 11 microns long, located at 13th annule from posterior extremity. Vagina two-third the body width at vulva, oblique to body axis. A spherical spermatheca containing numerous rounded sperms present on the right side of the distal end of uterus. Oviduct short. Ovary single, prodelphic, with oöcytes arranged in single file except for a few near cap-cell. Uterine eggs in paratype females 67-71 microns long by 16-18 microns broad. Rectum and anus not easily visible. Latter located on 9th annule from terminus. Tail elongate conoid, with terminal annule smoothly rounded. Tail end rounded in some of paratype females (Fig. 3, H).

MALE (Allotype): Length = 0.41 mm.; a = 27; b = 4.1; c = 16; T = 36%.

Body striae 1.4 microns apart on middle of body. Cuticular sheath absent. Head elevated, continuous with body contour, traversed by five striae. Labial frame-work comprising of six, tri-radiate cuticularized pieces. Lateral fields with four incisures, one-fourth as wide as body width, beginning just behind

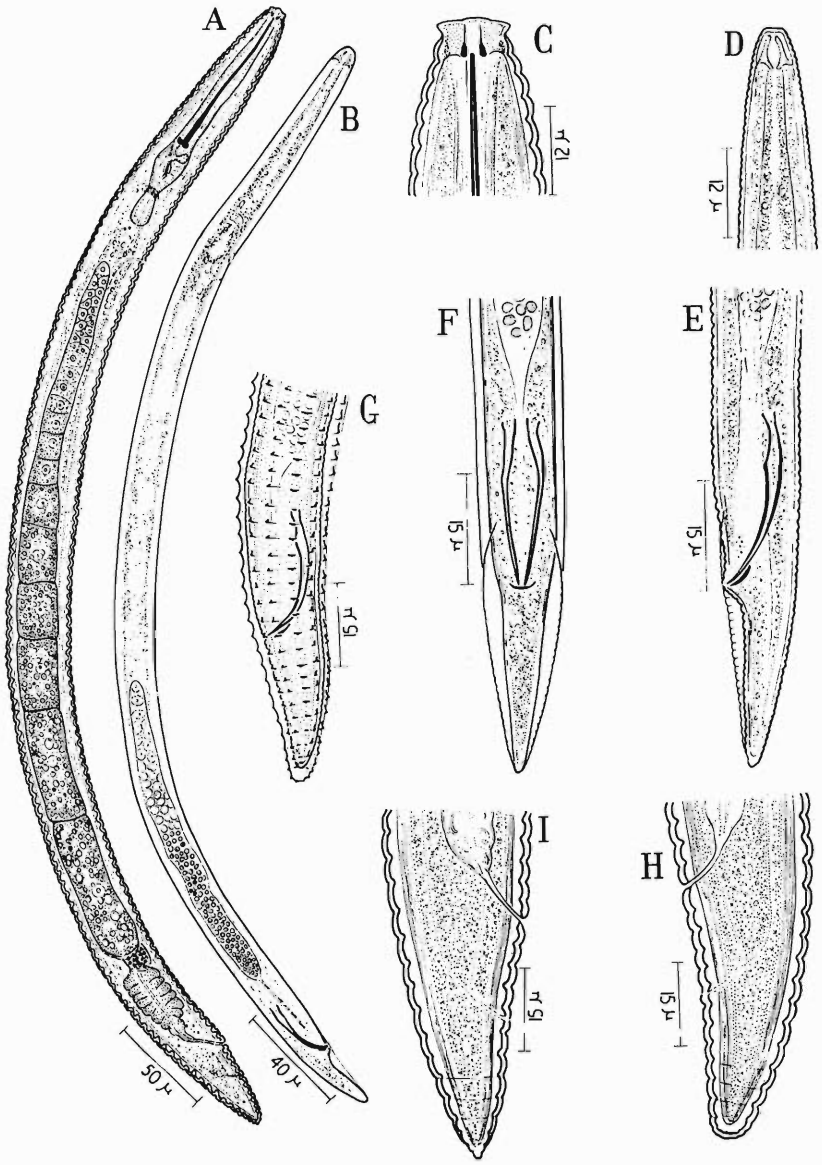

Figure 3. *Hemicriconemoides mangiferae*. A. Female. B. Male. C. Head of female. D. Anterior end of male. E. Tail of male, lateral view. F. Tail of male, ventral view. G. Tail of ensheathed male, lateral view. H. Posterior end of female with rounded terminus. I. Posterior end of female with conoid terminus.

head as a narrow strip which regularly widens out to have a uniform width near oesophageal base and ending on tail forming a pattern as illustrated (Fig. 3, E). Buccal spear lacking. Hemizonid 3 body annules long, 84 microns from anterior end. Excretory pore 4 body annules posterior and nerve ring one body diameter anterior to hemizonid. Oesophagus almost completely degenerated.

Testis single, outstretched. Vesicula seminalis packed with sperms averaging 2 microns in diameter. Spicules 26 microns long, arcuate, cephalated, sharply pointed at distal end. Gubernaculum trough-shaped, 4.5 microns long and approximately as much wide. Bursa with crenate margins, beginning slightly anterior to cloaca and ending just near tail terminus. Tail rather cylindrical, slightly tapering to a rounded terminus, shorter in length than the spicula.

ENSHEATHED MALE: Head similar to that in adult. Body bearing a loose sheath ornamented with 12 longitudinal rows of scale-like spines. Spear tip 44 microns long; basal portion of spear distorted. Oesophagus obscure. Testis well developed. Spicula 25 microns long; gubernaculum 4.5 microns in length. Spinous cuticle and spear tip shed at ecdysis.

LARVAE: Body similar to that of female. Head annules as in holotype. Body cuticle bearing 12 longitudinal rows of pointed, scale-like spines.

HOLOTYPE: Female collected on November 26, 1957; slide no. PN/C/3-001, deposited with the Zoology Museum, Aligarh Muslim University, Aligarh (U. P.), India.

ALLOTYPE: Male collected on December 21, 1957; slide no. PN/C/3-002; other data same as for holotype.

PARATYPES: Hundreds of females and seven males; other data same as for holotype.

TYPE HOST: *Mangifera indica* L. (Mango tree)

TYPE LOCALITY: Aligarh (U. P.), India.

HOSTS AND GEOGRAPHICAL DISTRIBUTION: Specimens of this species have been collected from soil around roots of mango trees in Aligarh, Allahabad, Banda, Bareilly, Bulandshahr, Meerut, Nainital, and Pilibhit districts of U. P., Jabalpur (M. P.); *Citrus limon* (L.) Burm. in Aligarh, Banda, Bulandshahr and Badaun (U. P.); *Citrus reticulata* Blanco and *C. sinensis* L. in Aligarh, and Badaun (U. P.), and Yeotmal (Maharashtra State); *Grewia asiatica* L. in Aligarh; *Prunus armeniaca* in Nainital (U. P.).

DIAGNOSIS AND RELATIONSHIP: *Hemicriconemoides* with body annules numbering 133-148, first head annule angular, spear 70-81 microns in length, vulva situated on 13th annule from terminus, dimorphic condition of the female tail, and the male with a crenate bursa and a cylindroid tail.

H. mangiferae n. sp. comes closest to *H. gaddi* (Loos, 1949) Chitwood and Birchfield, 1957, but can be separated from it by its female having a larger number of body annules (about 120 in *H. gaddi*), the first head annule being angular in shape, and more posteriorly located excretory pore, and by its male having wider lateral fields bearing 4 incisures (2 incisures in *H. gaddi*), a shorter, cylindroid tail, and the presence of a bursa.

Hemicriconemoides cocophillus (Loos, 1949) Chitwood & Birchfield, 1957
(Fig. 4, A-B)

Syn. *Criconemoides cocophillus* Loos, 1949

This species was originally described by Loos (1949) as *Criconemoides*

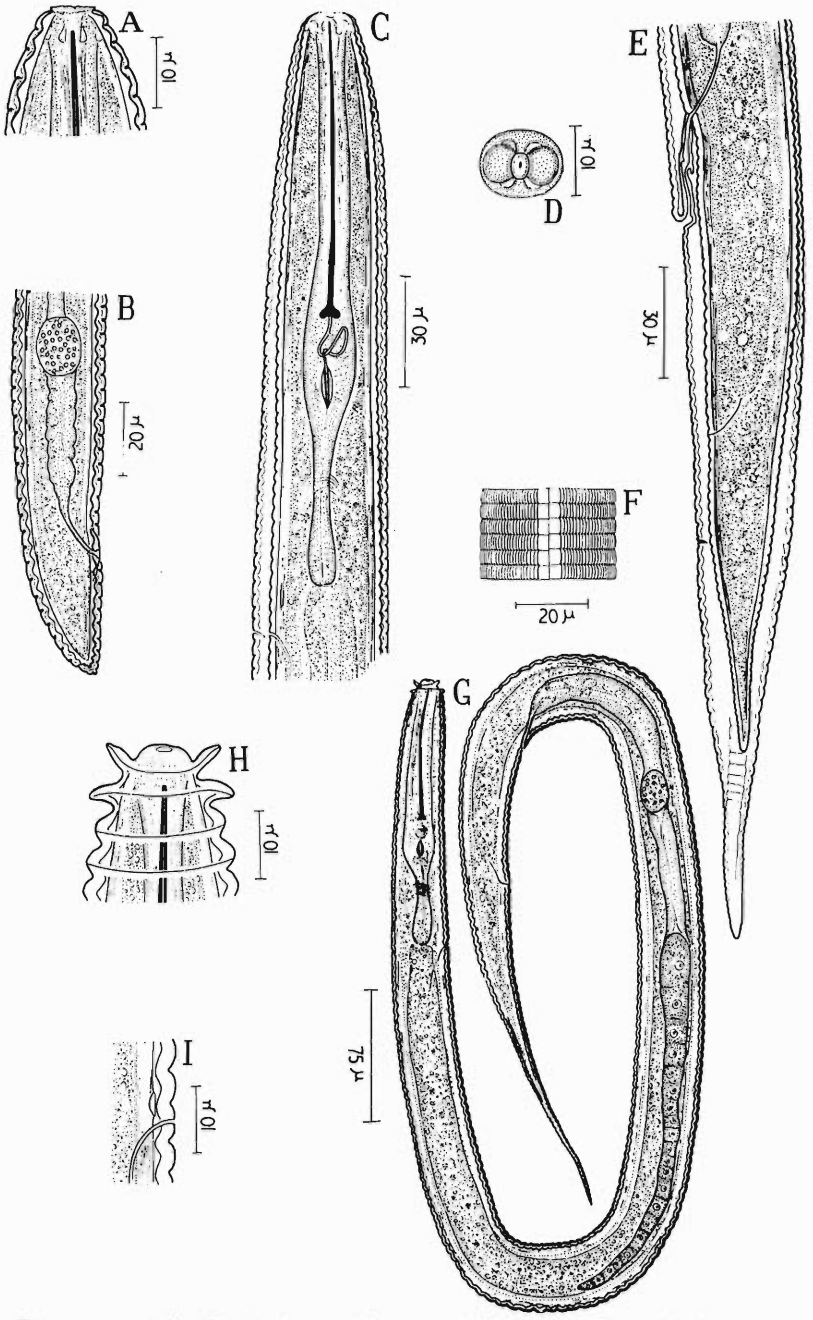

Figure 4. A-B—*Hemicriconemoides cocophillus.* A. Head of female. B. Posterior end of female. C-F—*Hemicycliophora indica.* C. Oesophageal region of female. D. *En face* view of female. E. Posterior end of female. F. Cuticular pattern of body sheath of female. G-I—*Hemicycliophora longicaudata.* G. Female. H. Head of female. I. Excretory pore and hemizonid of female, lateral view.

cocophillus sp. nov. collected from soil around grass and coconut roots in Kurunegala, Ceylon. The specimens collected in India conform closely with the description of the species as given by Loos. However, there is some difference in the structure of the body behind the region of the vulva. In the present species, the tail end is convex-conoid with a rounded terminus. Although Loos also described the tail end in his specimens as convex-conoid, his diagram shows it in a different way. Its terminus appears to be rather digitate. In spite of this difference, because all other structures are the same, the present specimens have been placed in *H. cocophillus*. The variations recorded in the measurements and counts of certain structures are presented below.

MEASUREMENTS: 12 females: Length = 0.43-0.53 mm.; a = 14-19; b = 4-5.4; V = $^{32\text{-}70}$92-94.3%.

Body annules 98-126 in number. Buccal spear 49-58 microns long. Excretory pore located on 30-36th annule from anterior end of body, 6-11th annule posterior to oesophageal base. Vulva transverse, 8 microns long, with lateral cuticular flaps, located on 9-13th annule from end of body. Tail convex-conoid with greater curvature occurring on its dorsal side, ending in a rounded terminus. Caudal terminus not attached to body sheath.

Specimens deposited with the Zoology Museum, Aligarh Muslim University, Aligarh (U. P.), India.

HOSTS AND LOCALITIES: Specimens of this species have been collected from soil around roots of *Carissa* sp. growing in hilly regions of Karwi, Banda District (U. P). and sugarcane at Coimbatore (Madras State), South India.

Hemicycliophora indica, n. sp. (Fig. 4, C-F)

MEASUREMENTS: 12 females: Length = 1.01-1.12 mm. (1.07 mm.); a = 26-31 (28.4); b = 5.9-6.9 (6.4); c = 11-12.6 (11.6); V = 81-83.8% (82.8%); Total body annules = 306-316 (310); spear = 80-86 microns (81.4 microns).

12 larvae: Length = 0.57-0.84 mm.; a = 24-28; b = 4.5-5.6; c = 6.9-8.5; spear = 61-72 microns.

FEMALE (Holotype): Length = 1.07 mm.; a = 30; b = 6.8; c = 12.3; V = ^{37}83.6%.

Body assuming a ventrally arcuate position when the animal is killed by gradual heat. Body annules 310 in number, averaging 3.5 microns in width at mid-body. Body sheath fitting loosely about the body, attached only at the regions of head and vulva, marked by numerous (about 80 near middle of body) longitudinal lines. Lateral fields originating near the level of the nerve ring and ending near caudal terminus, measuring approximately one-fifth (one-fifth to one-sixth in paratypes) as wide as the diameter of sheath, bearing double rows of rectangular blocks corresponding in number with the sheath annules. Lip region continuous with the body contour, bluntly rounded, 12 microns wide, bearing two annules. Cephalic sclerotization conspicuous. Labial disc elliptical, about one-third as long as labial diameter (Fig. D).

Buccal spear 80 microns in length, with a 66 microns long tip; its basal knobs smoothly rounded, 6 microns across, enclosing a prominent cavity at base. Orifice of dorsal oesophageal gland 5 microns behind spear base. Median oesophageal bulb 15 microns wide at its widest. Isthmus a bit elongate, regularly expanding to form a basal oesophageal bulb, crossed by nerve ring. Hemizonid absent. Excretory pore on 51st body annule from anterior end, on 4th annule behind oesophageal base.

Vulva about 64 body annules anterior to caudal terminus, attached to the sheath through a long cuticular tube. Uterus elongate, with a distal swelling, without any sperms. Ovary single, anteriorly outstretched. Oöcytes arranged in double rows for a short distance behind cap-cell then coming to lie in single file.

Rectum reduced, anus apparently functional, located at about middle of the distance from vulva to terminus. Tail at first convex-conoid then elongate-conoid to end in a finely rounded terminus, approximately four anal body diameters in length.

MALE: Not found.

LARVAE: Body becoming ventrally arcuate on death. Longitudinal lines on sheath present in all stages. Body annules greater in number than in females. Tail elongate-conoid, ending in a finely rounded terminus.

HOLOTYPE: Female collected on December 26, 1959; slide no. PN/C/4-001, deposited with the Zoology Museum, Aligarh Muslim University, Aligarh (U. P.), India.

PARATYPES: 11 females and 12 larvae; other data same as for holotype.

TYPE HOST: Soil around roots of *Carissa* sp. growing in hilly regions.

TYPE LOCALITY: Karwi, Banda District (U. P.), India.

DIAGNOSIS AND RELATIONSHIP: *Hemicycliophora* with body averaging 1.07 mm. in length and bearing over 300 annules, body sheath marked by about 80 longitudinal lines on mid-body, narrow lateral fields with double rows of rectangular blocks, 80-86 microns long buccal spear, absence of a hemizonid, and an elongate, not uniformly tapering tail.

Besides *H. indica* n. sp. there are three other *Hemicycliophora* spp. which bear longitudinal lines on body sheath. These are: *H. membranifer* (Micoletzky, 1925) Thorne, 1955, *H. penetrans* Thorne 1955, and *H. oostenbrinki* Luc, 1958. From the first of these the present species differs in its larger body size, greater number of body annules (av. no. 200 in *H. membranifer*), more longitudinal lines on sheath, and much narrowed lateral fields; and from the other two, besides some other morphological characters, in having over 300 body annules, a not uniformly tapering tail, and the absence of a hemizonid.

Hemicycliophora longicaudata Loos, 1948 (Fig. 4, G-I)

H. longicaudata Loos, 1948, is a peculiar species in that the females lack a body sheath and the males possess rather straight spicules in contrast with the semi-circular spicules of the other species. However, similar type of spicules have recently been reported to occur in *H. paradoxa* Luc, 1958. The studies conducted on the present specimens add further information about those structures which have not hitherto been recorded in this species. Of importance is the size and the form of the head annules which are large and distinctly separated from the body contour—a condition found only in *H. hesperis* Raski, 1958, and the occurrence of the hemizonid.

MEASUREMENTS: 2 females: Length = 0.99-1.1 mm.; a = 27-33; b = 7.3-7.4; c = 5.2-5.6; V = 73-74.3%; spear = 73-74 microns.

1 larva: Length = 0.74 mm.; a = 30; b = 5.8; c = ?.

Body assuming a semi-circular position when the worm is killed by gradual heat. First two annules from anterior end comprising the head modified in being larger than and well separated from the preceding ones; anterior annule directed forward and outward and posterior outward and downward.

Cuticular ornamentation and lateral fields absent. Orfice of dorsal oesophageal gland 8 microns behind spear base. Excretory pore located near oesophageal base, on 33rd annule from anterior end. Hemizonid poorly developed, situated just anterior to excretory pore. A spherical spermatheca with numerous rounded sperms present in the distal end of uterus. Rectum one-half anal body width long, appearing as a refractive line in lateral view. Anus located on 18th annule from vulva.

Specimens deposited with the Zoology Museum, Aligarh Muslim University, Aligarh (U. P.), India.

HOST AND LOCALITY: Isolated from soil sample collected in September, 1958 around roots of *Saccharum officinarum* L. in Coimbatore (Madras State), South India.

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Studies on Cysticercoid Histology. VI. Observations on the Fully Developed Cysticercoid of *Choanotaenia infundibulum* (Cestoda: Cyclophyllidea)*

MARIETTA VOGE

The life history of the chicken tapeworm *Choanotaenia infundibulum* has been studied by many workers. One of the earliest studies in this country is that of Guberlet (1916) who experimentally demonstrated that flies could serve as intermediate hosts for this tapeworm. Guberlet based most of his observations of the fully developed cysticercoid on sectioned material, and described in great detail the structure of the scolex, as well as several of the tissues external to the cysticercoid cavity. Further observations on the structure of this cysticercoid have yielded additional information to be presented below.

MATERIALS AND METHODS

Adults of *Choanotaenia infundibulum* were obtained from chickens in Honolulu, Hawaii. Gravid proglottids were macerated and fed to flour beetles, *Tribolium confusum,* which were kept at room temperature. Fully developed and infective cysticercoids were obtained one month after infection. The histologic studies presented here are based on two months old cysticercoids fixed in Zenker's fluid and embedded in paraffin. Sections were cut at 7 microns and stained with Mayer's hemalum, Himes' triple stain, or Mallory's aniline blue stain, as previously reported by Voge (1960).

DESCRIPTION

The most prominent structure in the cysticercoid is the scolex, which occupies most of the space of the cysticercoid body and contains well-defined muscular suckers and the elongate rostellum with hooks (Fig. 1). The musculature of the rostellum and other tissues in the scolex are as described by Guberlet (1916). The relation of the scolex to the tissue immediately surrounding it is similar to that observed in *Raillietina cesticillus* (Voge, 1960) and *Hymenolepis nana* (Voge and Heyneman, 1960), Unlike these species, the cysticercoid of *Choanotaenia* contains within the cavity a layer of tissue which, by its structure, could not be differentiated from the tissue immediately surrounding the scolex. In some sections, the posterior portion of the scolex is seen to be connected with this tissue, which therefore probably represents additional "neck" tissue. Thus, the delicate and morphologically distinct tissue lining the cavity in species previously studied is apparently absent in *Choanotaenia.*

External to the "neck" tissue is a very narrow non-nucleated layer of fibers primarily oriented longitudinally. Using high magnification, one can demonstrate the presence of an additional layer of circular fibers (Fig. 3), at right angles and external to the longitudinal fibers. Both layers stain blue with Mallory's stain. External to the circular fibers is a thick, acellular layer, referred to as the external membrane. In material stained with Mallory's or with Himes' stain, this membrane is seen to consist of two

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separate layers: the internal one appears deep red with Mallory's, and yellow with Himes' stain; the external layer stains a deep blue with Mallory's stain and red with Himes' stain. From the results obtained with Himes' stain, it is likely that the inner coat consists of protein and the outer coat contains polysaccharide. There is no distinct morphological separation between these two layers.

The most superficial layer of the cysticercoid is the hyaline coat. This is a relatively wide, transparent, acellular structure which stains a uniform pale blue with Mallory's stain and red with Himes' stain. In the living material, this coat is very smooth and "slippery." Guberlet (1916) refers to these acellular layers as the cuticle, a term which should not be used for structures outside the cysticercoid cavity.

In the cysticercoid of *Choanotaenia* separation of the external membrane and the hyaline coat occurs only at the line of demarcation of body and tail (Fig. 2). The external membrane surrounds only the body-part of the cysticercoid; the hyaline coat provides a complete and continuous envelope for the whole cysticercoid. Furthermore, no tissue bridge or connection was noted between the tail and the body of the cysticercoid in any of the sections studied. Only a study of histogenesis could demonstrate when and how this separation occurs, and whether it is a part of the aging process. As shown in Figure 2, the tail consists of a relatively undifferentiated parenchymatous tissue bordered externally by the hyaline coat. The reason for using the terms "external membrane" and "hyaline coat" in the description of this species, is to draw attention to their similarity to structures in the cysticercoids of *Raillietina*. In *R. cesticillus* (Voge, 1960), both the "external membrane" and the "hyaline coat" are present and have comparable structural staining characteristics. Interpretation of these two structures in terms of development and function is difficult; the two may represent only a single structural entity consisting of biochemically differentiated layers.

It is of interest that the cysticercoids of both *Choanotaenia* and *Raillietina* differ markedly from *Hymenolepis* cysticercoids in the structure of the peripheral acellular layers. The two fibrous layers, however, are common to all species studied and probably represent equivalent tissues. In *Choanotaenia* the tissues outside the cavity are markedly reduced in size and extent as compared with *Raillietina*. Whether this represents a primitive or a specialized trait remains to be determined.

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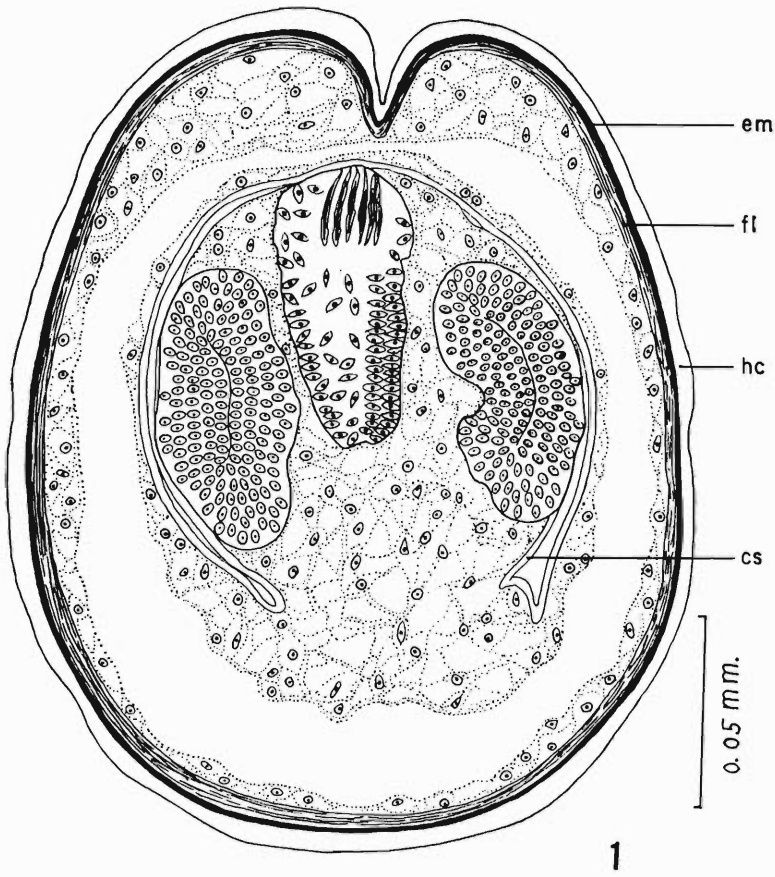
Fig. 1. Section of fully-developed cysticercoid of *Choanotaenia infundibulum*. Tail not shown.

Fig. 2. Section through cysticercoid showing position of tail in relation to scolex.

Fig. 3. Organization of fibrous layers, external membrane, and hyaline coat.

All drawings made with the aid of a camera lucida.

Legend for all figures: cs, cuticle of scolex; cf, circular fibers; em, external membrane; fl, fibrous layer; hc, hyaline coat; s, scolex; t, tail.



Caryophyllaeids (Cestoda) from Freshwater Fishes of India

S. P. GUPTA*

There has been a great controversy about the status of the family Caryophyllaeidae Leuckart, 1878. Hunter (1927) followed Lühe (1910) and considered Caryophyllaeidae as an independent family under Pseudophyllidea rather than accepting Nybelin's (1922) designation as one of the sub-families of Caryophyllaeidae. He divided the family Caryophyllaeidae into 4 subfamilies, viz. Caryophyllaeinae (Nybelin, 1922) Hunter, 1927; Capingentinae Hunter, 1927; Lytocestinae Hunter, 1927 and Wenyoninae Hunter, 1927. Wardle and McLeod (1952) have raised the family Caryophyllaeidae to the rank of a new order Caryophyllidea and the four sub-families to the rank of families. Fotedar (1958) and Yamaguti (1959) have followed the classification proposed by Hunter (1927) but however the latter author placed type and only genus *Wenyonia* Woodland, 1923 under the sub-family Caryophyllaeinae instead of Wenyoninae. The author is in agreement with Yamaguti as there are no other differences than the position of the genital apertures. In the present paper the author follows the classification as revised by Yamaguti.

This group of caryophyllaeids has been given considerable attention by European and North American workers such as Nybelin (1922), Woodland (1923, 24, 26), Fuhrmann (1930, 31), Fuhrmann and Baer (1925), Hunter (1927, 29, 30), Szidat (1937, 38, 41), Fischthal (1951, 54), Janiszewska (1950, 53, 54) and others. Contrary to this only a few papers have appeared by Bovien (1926), Motomura (1928), Yamaguti (1934), Hsu (1935), Moghe (1925, 31), Lynsdale (1956), Fotedar (1958) and others from Asia.

Only three forms of the family Caryophyllaeidae have been described so far from India. Moghe (1925, 31) described *Lytocestus indicus* of the subfamily Lytocestinae from a freshwater fish *Clarias batrachus* from Nagpur, C. P. Lynsdale (1956) described another species *Lytocestus birmanicus* from *Clarias batrachus* in Rangoon, Burma. Fotedar (1958) added a new genus and species *Adenoscolex oreini* from a freshwater fish *Oreinus sinuatus* from Kashmir under the sub-family Capingentinae. He has considered the genus *Bothrioscolex* Szidat, 1937 as a synonym of the genus *Khawia* Hsu, 1935 and described briefly the existing 6 species of the genus *Khawia*. The present paper adds the following from India: *Lucknowia fossilisi*, n. gen., n. sp., *Pseudolytocestus clariae*, n. sp., *Pseudocaryophyllaeus indica*, n. gen., n. sp., and *Capingentoides batrachii*, n. gen., n. sp.

SUB-FAMILY LYTOCESTINAE HUNTER, 1927

Lucknowia, n. gen.

GENERIC DIAGNOSIS: Lytocestinae. Scolex unspecialized, varying little in shape and not broader than remainder of body. Cirrus sac and utero-vaginal canal open separately at beginning of last seventh of body length. Uterine and vaginal pores common. Ovarian follicles cortical, commissure or isthmus being medullary. Uterine coils much convoluted, compactly coiled behind ovarian isthmus and not extending anterior to cirrus sac. Uterine glands

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Type specimens of forms described in this paper will be deposited in Dr. G. S. Thapar's Helminthological Collection, Lucknow, U.P., India.

present. Receptaculum seminis absent. Vitellaria cortical and extending up to posterior end of body. Terminal excretory bladder present.

TYPE SPECIES: *Lucknowia fossilisi,* n. sp.

Lucknowia fossilisi, n. sp. (Figs. 1-5)

MATERIAL: 3 fully mature and 2 immature specimens

HOST: *Heteropneustes fossilis*

LOCATION: Intestine

LOCALITY: River Gomti, Lucknow, U. P.

DESCRIPTION: Body elongated, flat, with no trace of internal or external segmentation, 5.8-6.78 mm long and 1.13-1.3 mm wide in anterior region of cirrus sac. Head (Figs. 2, 3, 4) stumpy, bluntly rounded and markedly narrower than body, 0.348-0.59 mm. long and 0.21-0.48 mm. wide, with a small narrow neck-like constriction 0.522-1.218 mm. long and 0.365-0.73 mm. wide, followed by main cylindrical portion of body (Fig. 1) measuring 4.35-5.22 mm. long and 1.13-1.3 mm. wide and posteriorly rounded.

Excretory system consists of 2 lateral main channels which unite posteriorly and form a short distinct muscular thick walled vesicle, opening through a tube 0.11-0.13 mm. long and 0.075-0.09 mm wide on ventral side at posterior end of the body.

Testes numerous, 0.13-0.18 x 0.07-0.13 mm., rounded or broadly oval, strewn throughout most of body medially bounded on lateral sides by vitelline follicles. They extend a short distance posterior to anterior vitelline glands up to caudal region of vesicula seminalis. Vas deferens loosely convoluted tube lying in median part of body anterior to cirrus sac; outer seminal vesicle absent. Cirrus sac a large ovoid organ placed medially, 0.34-0.43 x 0.27-0.31 mm. Vesicula seminalis highly convoluted structure and fills almost entire space of cirrus sac.

Ovary transversely elongated band-shaped structure which extends laterally on vitelline glands both on right and left sides of body; transverse isthmus or commissure measures 0.34-0.38 x 0.087-0.11 mm. Ovarian follicles of right and left side measure 0.2-0.25 x 0.15-0.17 mm.; from left side of median portion of ovary arises oviduct which opens at oötype. Vitelline glands somewhat irregular in shape, circular or oval in outline; mostly lateral in position and extend at a distance of 1.04-1.91 mm. from anterior end of body up to excretory bladder; always smaller than testes, measuring 0.06-0.14 x 0.05-0.1 mm.; terminate 0.13-0.226 mm. from posterior extremity.

Genital apertures situated at beginning of last seventh of the body length; aperture of cirrus sac separate from utero-vaginal canal and situated very close to it. Uterus and vagina open by a broad common aperture about 0.09-0.1 mm. wide situated below cirrus sac, at a distance of 0.78-1.14 mm. from posterior end of body. Vagina begins as a straight tube and continues, slightly convoluted, in median line on ventral side of body directly from vaginal aperture up to a little anterior to ovary; then takes a turn to left side of body and opens at oötype. No receptaculum seminis. Oötype large oval chamber on ventral side of ovary which receives openings of oviduct, vitelline ducts and ducts of the shell gland cells; measures 0.13-0.18 x 0.11-0.12 mm. From posterior end of oötype uterus arises as a slender convoluted tube, compactly coiled posterior to ovarian isthmus and runs up to excretory bladder, then turns and runs anteriorly forming a few conspicuous thick walled loops between vaginal opening and excretory bladder; uterine coils never extend beyond cirrus sac; wall of uterus glandular; opening of uterus lies on left side of vaginal opening in female genital atrium.

Eggs (Fig. 5) oval and thick shelled, measuring 0.017-0.018 x 0.01-0.011 mm; polar filament 0.02-0.026 mm. in length.

DISCUSSION: The present form belongs to the sub-family Lytocestinae Hunter, 1927 and differs from all the known genera of the sub-family in having the polar filament at the anterior end of the eggs.

The new form closely resembles the genera *Lytocestoides* Baylis, 1928 and *Khawia* Hsu, 1935 mainly in the presence of post-ovarian vitelline glands, but differs from both genera in the absence of a common genital atrium and in the shape of the ovary. It can further be distinguished from *Khawia* in having the vitelline glands in the ovarian region.

Only three forms, *Lytocestus indicus* Moghe, 1931, *L. birmanicus* Lynsdale, 1956 and *Adenoscolex oreini* Fotedar, 1958, of the family Caryophyllaeidae have been described from India. The new form resembles *L. indicus* and *L. birmanicus* in having genital apertures separate, in the structure of the scolex, in the shape of the ovary, but differs from it in the extension of the vitelline glands up to the posterior end of the body instead of up to the utero-vaginal aperture, in the possession of compactly coiled uterine coils behind the ovarian isthmus and in the position of the oötype.

The new form resembles *Adenoscolex* Fotedar (1958) in having post-ovarian vitelline glands but differs from it in the arrangement of distribution of vitelline glands, in the shape of ovary and scolex and in the absence of the receptaculum seminis. These differences are regarded as sufficient to establish a new genus.

Lytocestus indicus (Moghe, 1931)

A large number of specimens of this form were collected from the intestine of *Clarias batrachus* (Linn.) from Lucknow, U. P.

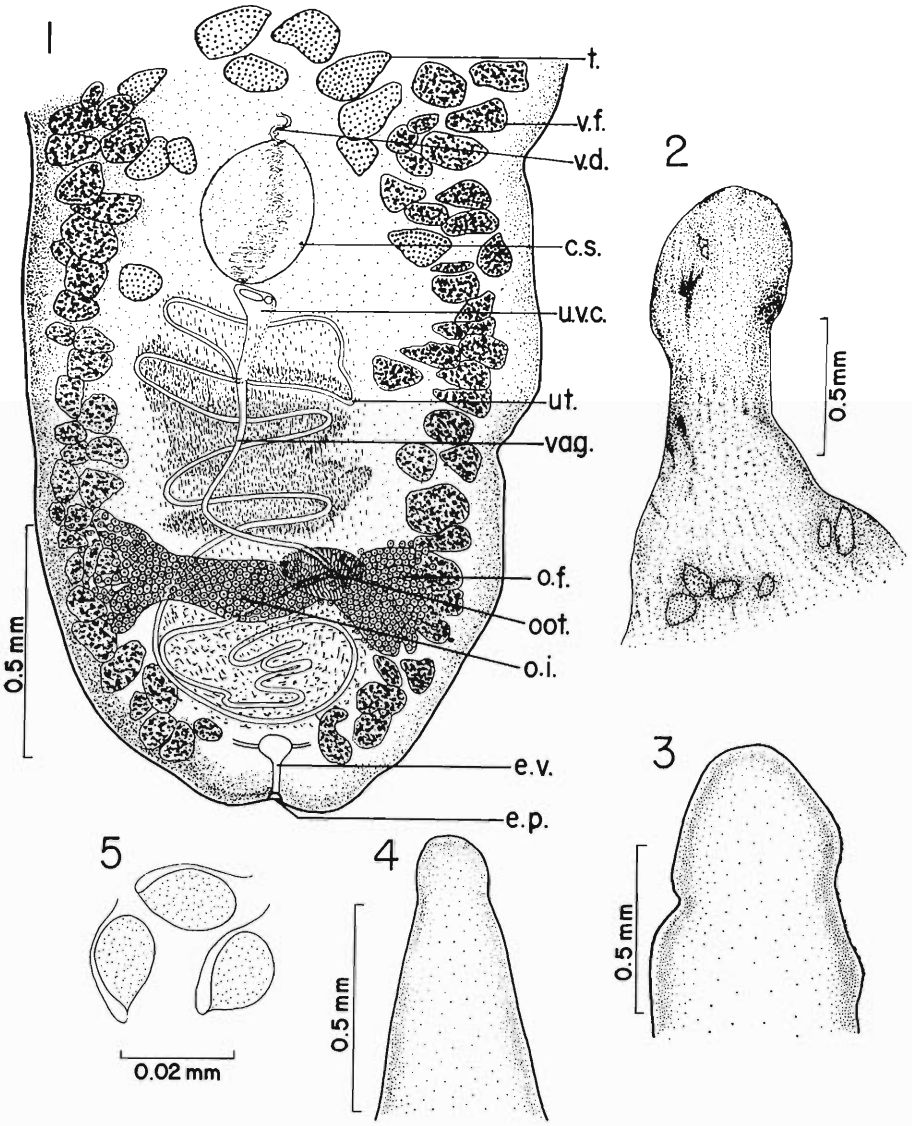
Key to Genera of Lytocestinae Hunter, 1927

A. Scolex undifferentiated.
  I. No post-ovarian yolk glands.
    a. Inner longitudinal muscles in two parallel sheets between the testes ..... *Balanotaenia* Johnston, 1924
    b. Inner longitudinal muscles in a ring around the testes.
      x. Uterine coils in testicular zone; ovarian lobes medullary ..... *Notolytocestus* Johnston and Muirhead, 1950
      y. Uterine coils post-testicular, ovarian lobes cortical.
        a^1 Uterus with very thick coat of accompanying cells; ductus ejaculatorius enclosed in compact paranchymatous bulb. ..... *Lytocestus* Cohn, 1908
        b^1 Uterus without thick coat of accompanying cells; ductus ejaculatorius not enclosed in bulb, distinctly spined. ..... *Borienia* Fuhrmann, 1931
  II. Post-ovarian yolk glands present.
    x. Cirrus and utero-vaginal canal open separately ..... *Lucknowia*, n. gen.
    y. Cirrus and utero-vaginal canal open into a common genital atrium.
      a^1 Post-ovarian yolk glands present. ..... *Lytocestoides Baylis*, 1928
      b^1 Both pre- and post-ovarian yolk glands present *Khawia*. Hsu, 1935
B. Scolex with pseudobothrial depressions.
  I. Yolk glands are crescentic ..... *Stocksia* Woodland, 1937
  II. Yolk glands as ring around testes.

x¹ Uterine coils extend anterior to cirrus sac, scolex globular with a terminal sucker ..... *Djombangia* Bovien, 1926

y¹ Uterine coils never extend anterior to cirrus sac, scolex with longitudinal furrows and terminal introvert.

Monobothroides Fuhrmann and Baer, 1925



Figs. 1-5. *Lucknowia fossilisi*, n. gen., n. sp 1. Posterior extremity; 2, 3, 4. Various forms of head; 5. Eggs.

c.s.—Cirrus sac; e.p.—Excretory pore; e.v.—Excretory vesicle; g.c.—Gland cells; l.m.—Inner longitudinal muscle layer; o.—Ovary; o.f.—Ovarian follicles; o.i.—Ovarian isthmus; oot.—Oötype; t.—Testis; ut.—Uterus; u.v.c.—Utero-vaginal opening; vag.—Vagina; v.d.—Vas deferens, v.f.—Vitelline follicles.

SUB-FAMILY CAPINGENTINAE HUNTER, 1927

Pseudolytocestus clariae, n. sp. (Figs. 6-8)

MATERIAL: One specimen
HOST: *Clarias batrachus*
LOCATION: Intestine
LOCALITY: River Brahmaputra, Gauhati (Assam)

DESCRIPTION: Body elongated, flat, without any trace of internal or external segmentation, 15.32 mm. long, and 4.02 mm. wide anterior to cirrus sac. Head (Scolex) (Fig. 7) stumpy, bluntly rounded, markedly narrower than body, 1.78 mm. long and 0.8 mm. wide, followed by small neck-like constriction, slightly narrower (0.8 mm.) and 1.7 mm. long. Main cylindrical portion of body, posterior to neck, rounded at posterior end (Fig. 6).

Excretory pore terminal and leads into T-shaped excretory vesicle. Excretory vesicle 0.18 mm. long and 0.11 mm. wide with swollen at anterior end; paired main collecting tubules emerging from its lateral corners; 8 to 10 longitudinal vesicles communicating with one another in cortical parenchyma of body.

Testes numerous, 0.11-0.18 x 0.075-0.12 mm., rounded or broadly oval, strewn throughout most of the body medially, bounded on the lateral sides by vitelline follicles and extending from a short distance posterior to base of neck region up to anterior region of cirrus sac. Vas deferens a loosely convoluted tube surrounded by testes and vitelline glands passing forward in median part of body in front of cirrus sac. Cirrus sac a large oval organ placed medially, measuring 0.9 x 0.6 mm. Relatively small coiled vesicula seminalis lies at basal part of cirrus sac; protrusible cirrus fills almost entire space and opens just in front of utero-vaginal canal.

Ovary H-shaped and distinctly lobed. Ovarian isthmus or commissure more or less at posterior end of ovary. Left wing of ovary narrower in outline, slightly lobed and rounded at ends, 1.12 mm. long and 0.4 mm. wide. Right wing of ovary broader, slightly lobed and pointed at ends, 1.06 mm. long and 0.6 mm. wide. Both isthmus and ovarian wings entirely in medullary parenchyma. Oviduct arises at posterior edge of commissure and opens at oötype. Vitelline glands somewhat irregular in shape, may be dumbbell shaped, circular or oval in outline, larger than testes and 0.11-0.2 x 0.11-0.17 mm., mostly lateral in position, at places spreading medially and extending from a little posterior to base of neck up to posterior end of cirrus sac. No post-ovarian follicles present.

Genital apertures situated at beginning of last ninth of body length. Uterus and vagina open by a broad common aperture about 0.05 mm. wide, situated ventral to cirrus sac but distinctly separate from it. Aperture lies at a distance of 1.65 mm. from posterior end of body. Vagina a convoluted tube, funnel shaped at anterior end and narrower towards posterior end, running in median line on ventral surface of body directly from vaginal aperture and opens at oötype. Oötype a small oval chamber on ventral side of ovary, receiving openings of oviduct, vitelline ducts and ducts of the shell gland cells. From posterior end of oötype arises uterus as a slender convoluted tube that runs towards posterior end of excretory bladder as a compact coiled structure, then runs anteriorly forming conspicuous thick walled loops on either side of cirrus sac a little posterior to its anterior end. Opening of uterus lies on left side of vaginal opening in female genital atrium.

Eggs (Fig. 8) oval, non-operculated, 0.04-0.043 x 0.03-0.04 mm.

DISCUSSION: *Pseudolytocestus clariae,* n. sp., differs from *P. differtus* Hunter, 1927, only species of the genus from the intestine of *Ictiobus bubalus* in Mississippi, in having a much wider body, in the possession of ovarian wings of greater width and the isthmus or commissure much more towards posterior end, (instead of wings elongated, narrow and commissure slightly anterior to mid region of ovarian wings), in the extent of uterine coils up to a little posterior to anterior end of cirrus sac (instead of up to posterior end of cirrus sac), in the nature of scolex and in the absence of external seminal vesicle.

Pseudocaryophyllaeus, n. gen.

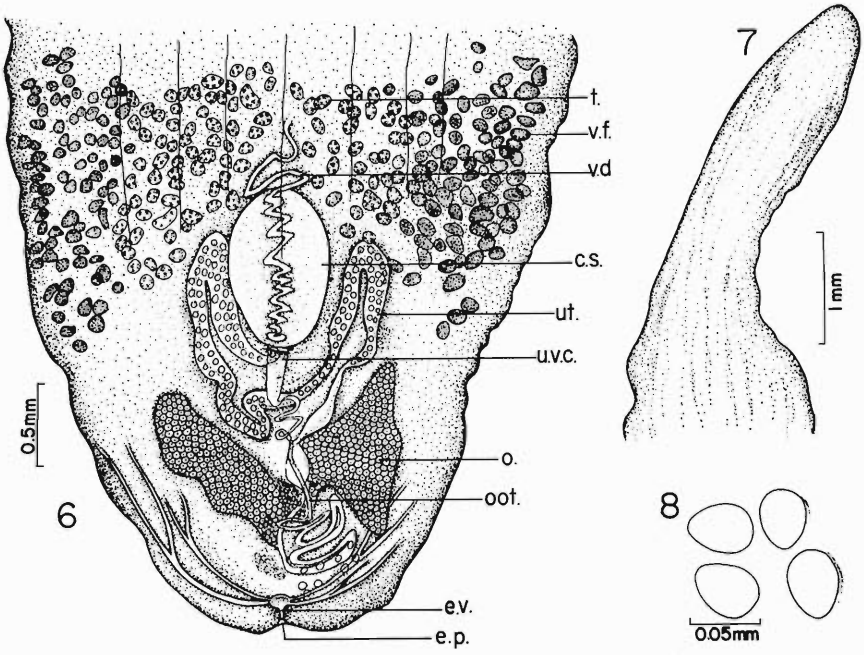
GENERIC DIAGNOSIS: Capingentinae. Scolex smooth, oval, truncated anteriorly and marked off from rest of body. Long narrow neck. Cirrus sac and utero-vaginal canal open separately at beginning of posterior ninth of body length. Uterine and vaginal pores common. Ovarian follicles cortical, only ovarian isthmus or commissure being medullary. Uterine coils never extend anteriorly to cirrus sac. Uterine glands present. Receptaculum seminis absent. Vitelline glands partly cortical and partly medullary. Post-ovarian median vitelline glands absent.

TYPE SPECIES: *Pseudocaryophyllaeus indica,* n. sp.

Pseudocaryophyllaeus indica, n. sp. (Figs. 9-12)

MATERIAL: Large number of specimens

HOST: *Clarias batrachus* (Linn.)



Figs. 6-8. *Pseudolytocestus clariae,* n. sp. 6. Posterior extremity; 7. Scolex; 8. Eggs.

LOCATION: Intestine

LOCALITY: River Brahmaputra, Gauhati (Assam)

DESCRIPTION: Body elongated, flat, with crenated margin in middle region, 13.75-24.79 mm. long and 0.65-1.35 mm. wide at posterior region of vesicula seminalis.

Scolex (Fig. 10) oval or cone shaped, truncated anteriorly, measuring 1.04-1.31 mm. long and 0.626-0.734 mm. wide, marked off from body and tapers into a narrow neck, 5.04-8.74 mm. long and 0.175-0.365 mm. wide; scolex perfectly smooth and bearing neither suckers nor loculi. Main cylindrical portion of body (Fig. 9), 7.48-14.96 mm. long, and rounded at posterior end.

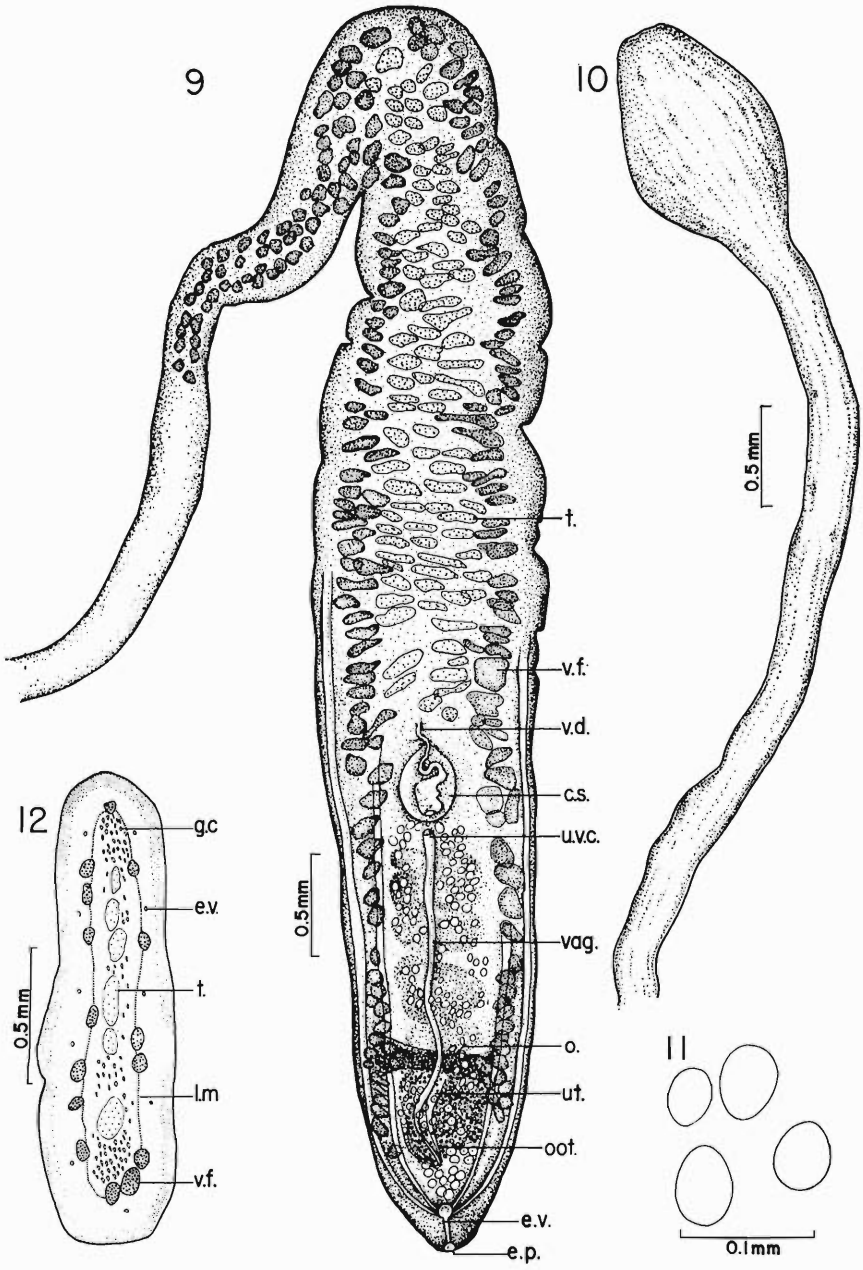
Excretory system consists of 4 lateral main channels, two on each side, uniting posteriorly and forming a short muscular thick walled vesicle, opening through a tube on ventral side at posterior end of body; tube 0.174-0.21 x 0.05-0.065 mm. in size.

Testes numerous, rounded, oval, strewn throughout most of body medially, 0.14-0.28 x 0.06-0.11 mm., bounded on lateral sides by vitelline follicles, and extending from a little anterior to posterior region of neck up to a little anterior to cirrus sac. Vas deferens a small duct lying in median part of body; outer seminal vesicle absent. Cirrus sac a large oval organ placed medially at a distance of 1.75-2.98 mm. from posterior end of body, measuring 0.43-0.61 x 0.26-0.41 mm. Vesicula seminalis a bell shaped structure and measures 0.23-0.38 x 0.12-0.16 mm.

Ovary follicular and irregular in outline and lies at 0.95-1.21 mm. from posterior end of body. Ovarian isthmus or commissure more or less at middle of body and ovarian follicles overlap vitelline glands laterally in places. Ovarian isthmus or commissure 0.45-0.5 x 0.08-0.12 mm. and ovarian follicles 0.18-0.21 x 0.14-0.17 mm. Oviduct arises from right side of ovary and runs posteriorly to open at oötype. Vitelline glands follicular, occupying greater part of body, mostly lateral in position extending in places medially, at almost same level as testes and extending from posterior region of neck up to oötype; post-ovarian median vitelline glands absent. Arrangement of vitelline follicles in body parenchyma is typical of sub-family Capingentinae; in cross-sections are seen at level of inner longitudinal muscle layer and extending partly into cortical and partly into medullary parenchyma (Fig. 12). Thus vitellaria are partly cortical and partly medullary. They measure 0.11-0.2 x 0.06-0.11 mm.

Genital apertures situated 1.42-2.95 mm. from beginning of posterior ninth of body length; common genital atrium absent. Uterus and vagina open by a broad common aperture about 0.045-0.05 mm. wide, situated below cirrus sac but distinctly separate from it. Vagina wide slightly convoluted tube extending in median line on ventral side of body directly from vaginal aperture to ovary where it turns to right and opens at oötype behind ovary. Oötype large oval chamber which receives openings of oviduct, common vitelline ducts and ducts of shell gland cells. From anterior end of oötype uterus arises as a slender convoluted duct, extending towards posterior end of body up to excretory bladder, then turns and runs anteriorly forming conspicuous thick walled loops between vaginal opening and excretory bladder; anterior end opening into vagina near its external opening on left side; uterine coils never extend beyond cirrus sac; wall of uterus glandular. Receptaculum seminis absent.

Eggs oval (Fig. 11), non-operculated, 0.05-0.06 x 0.035-0.045 mm.



Figs. 9-12. *Pseudocaryophyllaeus indica*, n. gen., n. sp. 9. Posterior extremity; 10. Scolex; 11. Eggs; 12. Cross section of body.

DISCUSSION: The new form belongs to the sub-family Capingentinae Hunter, 1927 due to the disposition of the vitellaria, which hold an intermediate position between cortical and parenchyma. It differs from all the 4 known genera of the sub-family in having a smooth, glandular scolex, truncated anteriorly, a distinct long narrow neck, band shaped ovary, and in the extension of vitellaria up to oötype on the lateral sides of the body. It resembles the genera *Spartoides* Hunter, 1927 and *Pseudolytocestus* Hunter, 1927 in the absence of receptaculum seminis and in having genital openings separate on ventral surface of body. Further, it differs from *Pseudolytocestus* Hunter (1927) in the absence of vesicula seminalis externa, and from *Spartoides* Hunter (1927) in having cirrus sac away from ovary instead of surrounding it, and in the non-extension of uterine coils anterior to cirrus sac. It differs from *Capingens* Hunter, 1927 and *Adenoscolex* Fotedar, 1958 in the absence of median post-ovarian vitelline glands. Further it can be distinguished from *Adenoscolex* Fotedar (1958) in the absence of a receptaculum seminis and from *Capingens* Hunter (1927) in the non-extension of uterine coils anterior to cirrus sac, in the absence of external seminal vesicle and in the position of ovary.

The new form also differs from *L. indicus* Moghe, 1931 and *L. birmanicus* Lynsdale 1956 from *Clarias batrachus* from India in having vitellaria partly parenchymal and in extension up to oötype, in the possession of a smooth globular scolex, truncated anteriorly, a distinct long neck and in the possession of 4 excretory vesicles instead of two and in the position of genital pores.

Of these differences, the nature of the scolex, the distinct narrow long neck, the shape of the ovary are sufficient characters in themselves to distinguish the present form as a new genus.

Capingentoides, n. gen.

GENERIC DIAGNOSIS: Capingentinae. Scolex smooth, oval, truncated anteriorly and marked off from rest of body. Long narrow neck. Cirrus sac opens into utero-vaginal canal at beginning of posterior tenth of body length. Uterine and vaginal pores common. Ovarian follicles cortical and isthmus medullary. Uterine coils never extend anteriorly to cirrus sac. Uterine glands present. Receptaculum seminis absent. Post-ovarian follicles present.

TYPE SPECIES: *Capingentoides batrachii,* n. sp.

Capingentoides batrachii, n. sp. (Figs. 13-16)

MATERIAL: 4 specimens

HOST: *Clarias batrachus* (Linn.)

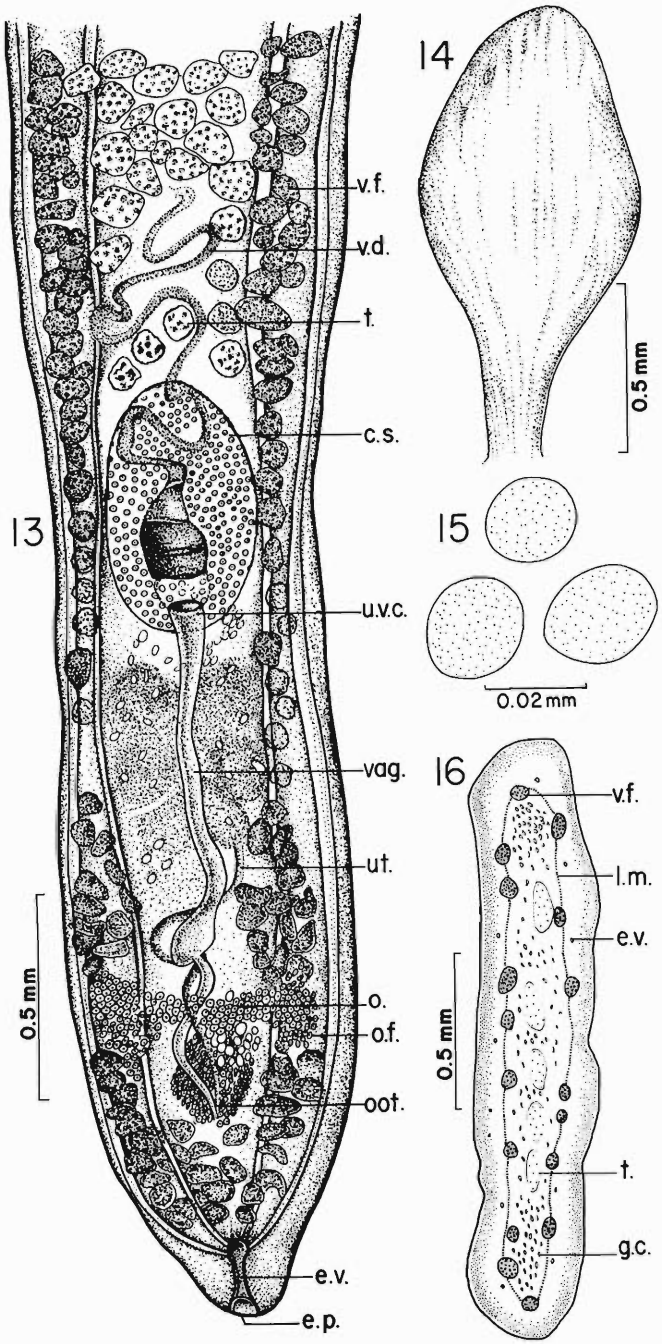
LOCATION: Intestine

LOCALITY: River Brahmaputra, Gauhati (Assam)

DESCRIPTION: Body elongated, flat, without any trace of internal or external segmentation, 12.62-18.75 mm. long and 0.61-0.9 mm. wide in anterior region of cirrus sac.

Scolex (Fig. 14) smooth, oval or globular and truncated anteriorly, 1.04-1.2 mm. long and 0.61-0.65 mm. wide, marked off from rest of body by a narrow neck, 3.37-4.87 mm. long and 0.261-0.34 mm. wide. Main cylindrical portion of body posterior to neck, 7.65-13.25 mm. long and rounded at posterior end (Fig. 13).

Excretory system consists of 4 lateral main channels, two on each side uniting posteriorly to form a short distinct muscular thick walled vesicle;



Figs. 13-16. *Capingentoides batrachii*, n. gen., n. sp. 13. Posterior extremity; 14. Scolex; 15. Eggs; 16. Cross section of body.

opens through a tube on ventral side at posterior end of body; tube 0.18-0.22 mm. long and 0.045-0.05 mm. wide.

Testes numerous, rounded or oval, strewn throughout most of body on lateral sides near vitelline follicles, extending from a little anterior to posterior region of neck up to anterior region of cirrus sac; very few testes in neck and cirrus sac regions; testes 0.08-0.15 x 0.07-0.1 mm. Vas deferens a loosely convoluted tube passing forward in median part of body; outer seminal vesicle absent. Cirrus sac a large oval organ, 0.313-0.6 x 0.28-0.4 mm., placed medially at a distance of 1.1-1.7 mm. from posterior end of body. Vesicula seminalis a large conical bell shaped structure, 0.21-0.26 x 0.14-0.18 mm.

Ovary H-shaped or transversely elongated band shaped structure which extends laterally on right and left sides of body; transverse isthmus or commissure 0.3-0.43 x 0.09-0.174 mm.; right and left ovarian follicles 0.18-0.2 x 0.14-0.16 mm. From left side of median portion of ovary arises oviduct which opens at oötype. Vitelline glands follicular and occupy greater part of body, mostly lateral in position, extending medially in places, extending almost to the level of the testes, at a distance of 5.22-6.66 mm. from anterior end of body up to anterior end of excretory vesicle; in post-ovarian area they lie in medulla; smaller than testes, being 0.06-0.11 x 0.03-0.07 mm. Vitelline follicles lie at level of inner longitudinal muscle layer and extend posteriorly into the cortical and partly into medullary parenchyma, surrounding testes and forming a definite layer (Cross section, Fig. 16).

Genital apertures situated at beginning of posterior tenth of body length; opening of cirrus sac lies in utero-vaginal atrium, which lies 1.2-1.75 mm. from posterior end of body. Vagina a fairly wide, slightly convoluted tube which runs in median line on ventral side of body directly from vaginal aperture up to a little anterior to ovary, then becomes narrow and takes a turn to right side of body, opening at oötype. Oötype a large oval chamber on ventral side of body which receives openings of oviduct, vitelline ducts and ducts from shell gland cells; 0.17-0.2 x 0.15-0.18 mm. Uterus arises as a slender convoluted tube from posterior end of oötype and runs anteriorly forming several conspicuous thick walled loops between vaginal openings and excretory bladder; wall of uterus glandular. Opening of uterus lies on left side of vaginal opening in female genital atrium.

Eggs (Fig. 15) oval, non-operculated, 0.02-0.023 x 0.018-0.022 mm.

DISCUSSION: The new form belongs to the sub-family Capingentinae Hunter, 1927. It resembles the genera *Capingens* Hunter (1927) and *Adenoscolex* Fotedar (1958) in having post-ovarian vitelline glands but differs from both of them in the nature of scolex, in having a long narrow neck, in the shape of ovary, in the non-extension of uterine coils anterior to cirrus sac and in the absence of external vesicula seminalis. It can further be distinguished from *Adenoscolex* in the absence of a receptaculum seminis and in having the opening of utero-vaginal canal into the cirrus sac.

The new form resembles *Pseudocaryophyllaeus* in the shape of scolex and in having a long narrow neck but differs from it in having post-ovarian median vitelline glands, in having the cirrus sac opening into utero-vaginal canal, in the position of genital pores and in the non-extension of uterine coils up to excretory bladder.

The new form shows some superficial resemblance to the genera *Lytocestoides* Baylis (1928) and *Khawia* Hsu (1935) of the sub-family Lytocestinae Hunter, 1927 mainly in the presence of post-ovarian follicles, but

differs from both of these in not having cirrus and utero-vaginal canal opening into a common genital atrium, in the position of vitellaria, in the shape of ovary, in the nature of scolex, in having a long narrow neck and in position of genital pores.

The new form also differs from *L. indicus* Moghe, 1931, and *L. birmanicus* Lynsdale, 1956, from *Clarias batrachus* from India in the position of vitellaria, in shape of scolex, in the possession of a long narrow neck, in having the cirrus sac opening into utero-vaginal canal, in the possession of 4 excretory vesicles instead of two, and in the position of genital pores.

It is therefore necessary to create a new genus for the present form.

Key to genera of sub-family Capingentinae Hunter, 1927

A. Post-ovarian median vitelline follicles present.
  I. Scolex with a pair of bothria and uterine coils extend anterior to cirrus sac ..... *Capingens* Hunter, 1927
  II. Scolex without a pair of bothria and uterine coils do not extend anterior to cirrus sac.
    a. Ovary inverted A-shaped and cirrus sac and utero-vaginal canal open separately ..... *Adenoscolex* Fotedar, 1958
    b. Ovary band shaped and cirrus sac opening into utero-vaginal canal ..... *Capingentoides,* n. gen.

B. No median post-ovarian vitelline follicles.
  I. Scolex with 3 pairs of loculi and uterine coils extending anterior to cirrus sac ..... *Spartoides* Hunter, 1927
  II. Scolex without loculi and uterine coils do not extend anterior to cirrus sac.
    x. Holdfast end undifferentiated, ovary H-shaped *Pseudolytocestus* Hunter, 1927
    y. Holdfast end marked off from the rest of body, ovary band shaped ..... *Pseudocaryophyllaeus,* n. gen.

SUMMARY

Three new genera and four new species of the family Caryophyllaeidae Leuckart, 1910 from the intestine of Siluroid fishes of U. P. and Assam have been described. Of these *Lucknowia fossilisi,* n. gen., n. sp., of *Heteropneustes fossilis* from Lucknow belongs to the sub-family Lytocestinae Hunter, 1927 and the rest, namely *Pseudolytocestus clariae,* n. sp., *Pseudocaryophyllaeus indica,* n. gen., n. sp. and *Capingentoides batrachii,* n. gen. n. sp., of *Clarias batrachus* from Gauhati (Assam) belong to the sub-family Capingentinae Hunter, 1927. In addition *Lytocestus indicus* Moghe (1931) is recorded from the intestine of *Clarias batrachus* from Lucknow, U. P. A key to the genera of sub-families Lytocestinae Hunter, 1927 and Capingentinae Hunter, 1927 is given.

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Biological Studies on Some Members of the Genus *Paratylenchus**

H. L. RHOADES** AND M. B. LINFORD

Although descriptions of several species of *Paratylenchus* Micoletzky have been based upon abundant material, most of them have dealt with adults only and have included no information concerning life history. An exception is the recent description of *P. amblycephalns* Reuver (1959) that appeared after the studies reported here were essentially complete. That species and those dealt with in this paper have one immature stage, now clearly identified as the last larval or preadult stage, that differs morphologically from younger stages and from adults in ways that suggested it might be unable to feed. Such preadults often are more numerous than all other stages combined in field soil samples and in old pot cultures. It seemed desirable to determine the life history, generation time, and rate of reproduction of such a species and to determine whether the morphologically modified preadults might have special ecological significance. Rhoades and Linford (1959) have demonstrated that the preadults of two species are able to survive over long periods in moist soil without feeding and that they are stimulated to molt by diffusates from the roots of certain plants.

NEMATODES STUDIED

Paratylenchus projectus Jenkins, 1956, from a field of Ladino clover near Joliet, Illinois, was investigated in detail. The nematodes studied were taken either directly from stored field soil or from pot cultures established with nematodes from that soil. Males are not found in this species. Comparative observations were made also on some other species. *P. dianthus* Jenkins and Taylor, 1956, came from soil from a carnation greenhouse near New Baden, Illinois, chiefly after long storage in a closed jar in the laboratory. A few *P. hamatus* Thorne and Allen, 1950, were collected from around rose roots from a greenhouse at Des Plaines, Illinois. Some observations also were made on unidentified species from Illinois fields. Except when another species is mentioned, however, all statements apply to *P. projectus*.

EGG AND HATCHING

To obtain eggs of known age small numbers of gravid females were placed into shallow distilled water in small Syracuse dishes and then removed as soon as they had laid the desired number of eggs. A female in water rarely lays more than a single egg. Dishes containing eggs were held in moist chambers at 25-28° C and were observed periodically with the aid of a 40x water immersion objective.

Eggs of *Paratylenchus projectus* measured 57-69 microns long and 19-22 microns in diameter and were generally slightly curved. They were unsegmented when laid. The first cell division occurred after 5½ to 9 hours, and the third was complete in 19 hours. Successive divisions occurred rapidly thereafter, and the first-stage larva was ready to molt within 4-5 days. In this stage, the esophagus was indistinct and there was no stylet, and no part of a stylet or cephalic framework was observed on the cast cuticle. The

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second stage larva had its esophagus and stylet well developed. As hatching time approached, it began moving vigorously, causing considerable distortion of the egg membrane. At the same time the stylet was thrust forcibly at the ends of the egg until the membrane broke. Hatching in water occurred in 5-6 days.

LIFE CYCLE ON RED CLOVER IN AGAR

Development after hatching was observed in association with seedlings of *Trifolium pratense* L., Kenland red clover, growing in Petri dishes of 1.5 percent water agar. Seeds that had been wetted with 95 percent alcohol, soaked 5 minutes in 0.35 percent sodium hypochlorite and rinsed in sterile water, were plunged individually into the agar when it had just hardened. Three days later, when primary roots were approximately 12 mm long, freshly molted females with their loosened cuticles still intact were added individually to these dishes at some distance from the roots. Few troublesome bacterial contaminations occurred when such nematodes were merely passed through 3 changes of sterile water, using for the transfer a nylon needle that was soaked briefly in hypochlorite solution between uses. Such cultures were exposed to dim laboratory light supplemented by 12 hours of fluorescent illumination daily. With the dishes held upright except during observations, roots grew along the bottom where they were suitable for observation through the glass with the aid of a compound microscope with a 10x dry objective or a 40x water immersion objective with a free working distance of 1.9 mm. Sterile water was added to the agar as needed to replace evaporative loss. Cultures prepared in this way permitted following the activities of individual nematodes and tracing the development of progeny through all stages. The best culture remained in good condition 80 days while the nematode produced at least 2 generations of progeny. At the end of this period 416 progeny of the one female were extracted from this dish.

Females placed on the agar characteristically moved to the young mature region of roots where they inserted their stylets into epidermal cells or at the base of root hairs and began feeding. Feeding usually continued for several days from the same cell while about 3 eggs were laid each day, accumulating in loose clusters. One female was observed to produce a cluster of 23 eggs at one feeding site during 1 week; hatching was in process during the latter part of this period. Another female produced 4 large clusters of eggs during 28 days, but the total number of eggs produced during the life of one female was never determined.

Hatching in agar occurred in approximately 5-6 days, the same as in water.

The newly hatched second-stage larva is characterized by its extremely small size and its proportionately long esophagus that is almost half of the total body length (Fig. 1). These larvae moved about the roots for some time without feeding and many of them were never seen to feed; without feeding there was no further development. Others began feeding on root hairs or epidermal cells in 2-3 days and fed for approximately 5 days at one or sometimes two sites. A considerable increase in body length occurred during feeding, with a marked increase in plumpness. After feeding stopped these larvae moved around the roots for about a day, then became inactive, molted during approximately 2 days and emerged as third-stage larvae. Dissolution of the posterior portion of the stylet occurred during this molt and the anterior portion was shed with the cuticle.

Upon molting (Fig. 2) the third-stage larva appears much like the second

stage except for its greater size. The stylet and esophagus are well developed, and at first the esophagus is still proportionately long.

Third-stage larvae moved to the young mature region of roots and usually began feeding within 24 hours. The entire feeding period which lasted 5-6 days was generally completed at one location. A great increase in size

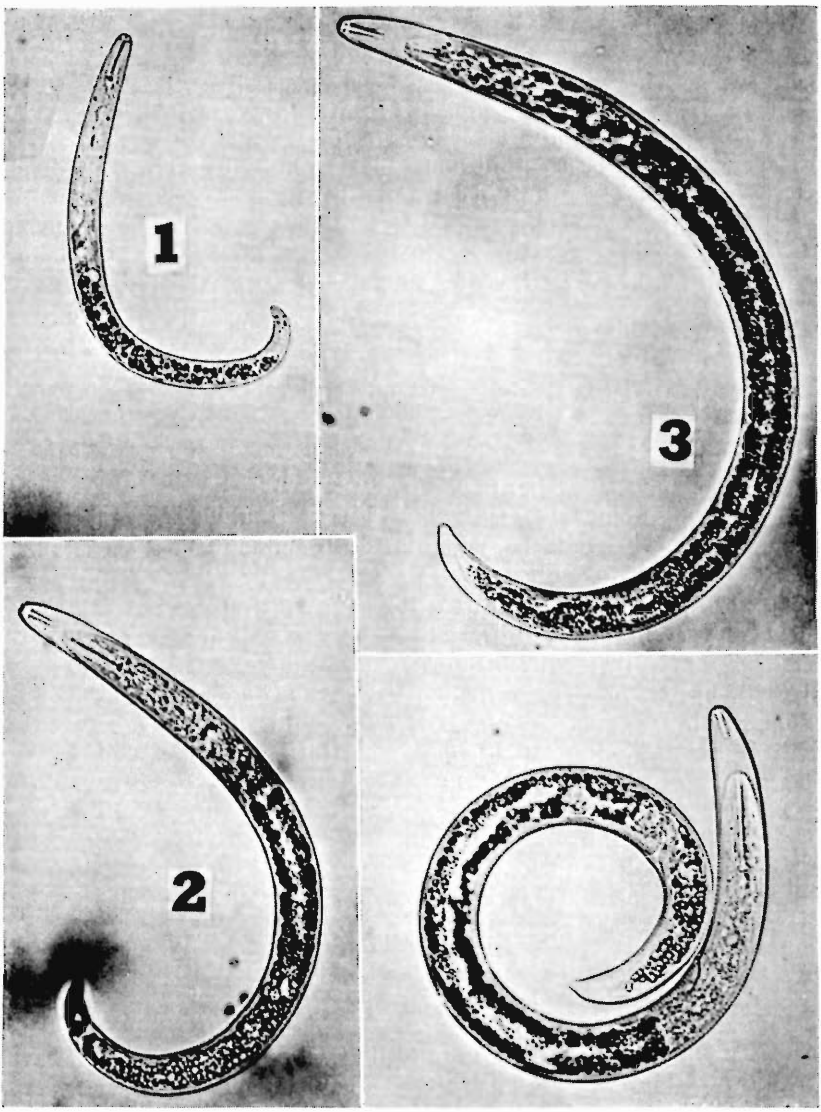

Figure 1. *Paratylenchus projectus* second stage larva.

Figures 2. Molt of *Paratylenchus projectus* second stage larva into third stage.

Figure 3. Molt of *Paratylenchus projectus* third stage larva into preadult stage.

Figure 4. Molt of *Paratylenchus projectus* preadult to adult female.

occurred during this stage, predominantly in the posterior part of the body which became densely packed with refractive granules. At the end of the feeding period the stylet was retracted and, after traveling around the roots for several hours, these larvae began the third molt. This molt (Fig. 3) required 1-2 days.

Fourth-stage larvae, or preadults, are strikingly different in appearance from other stages. The stylet is weakly refractive, short, and slender, and bears only a single small basal swelling. There are many refractive globules in the esophageal region that nearly obscure the slender median and basal bulbs. The small valve in the median bulb is much less refractive than in other stages. The genital primordium is clearly visible during this stage.

Preadult larvae did not feed. They moved sluggishly around the roots for 2-3 days, then became motionless and molted within 2 days into the adult female (Fig. 4).

Females usually began feeding within 24 hours, and 3 eggs were laid in 24-48 hours; thus the elapsed time from egg to egg in agar at 25-28° C was about 30-31 days. Females increased considerably in size during feeding and egg laying.

DIMENSIONS AND PROPORTIONS OF DEVELOPMENTAL STAGES

The following data on *Paratylenchus projectus* are based on measurements of 12 individuals in each group. Second-stage larvae were freshly hatched. Other stages with the exception of gravid females were selected during a molt and were killed and fixed just as the molt was completed. They thus represent young individuals that had not fed in their present stage. All nematodes were relaxed with heat and fixed in 5 percent formalin, then drawn to scale for measurement. For each character, the first figure represents the mean of 12, and figures in parentheses represent the observed range.

SECOND-STAGE LARVA: L—170 microns (150-180); a—15.3 (14.1-16.5); b—2.3 (1.6-2.6); c—13.3 (12.9-13.9); stylet—13.1 microns (12.0-14.7).

THIRD-STAGE LARVA: L—250 microns (220-260); a—18.4 (17.2-20-2); b—3.1 (2.8-3.5); c—14.4 (13.3-15.6); stylet—17.8 microns (16.5-19.0).

FOURTH-STAGE LARVA (preadult): L—340 microns (320-360); a—19.7 (17.8-21.4); b—4.3 (3.9-5.0); c—15.2 (13.6-16.5); stylet—12.1 microns (11.0-13.5).

FEMALE, FRESHLY MOLTED: L—360 microns (330-390); a—21.8 (19.2-23.9); b—3.7 (3.5-4.0); c—17.7 (15.0-21.1); V—83.6% (81.3-85-0); stylet—29.2 microns (27.0-31.0).

FEMALE, GRAVID: L—420 microns (400-460); a—19.5 (17.7-20.9); b—4.3 (4.1-4.6); c—16.5 (15.0-18.9); V—83.6% (81.1-84.8); stylet—28.9 microns (27.5-31.0).

GENERATION TIME ON RED CLOVER IN SOIL

For determining the generation time in soil, 2-inch clay pots containing 70 grams of sterilized potting mixture were seeded to red clover. When the seedlings were about an inch high, each pot was infested with 200 preadult larvae. Three of these small pots were then plunged into each of several 7-inch clay pots of sterilized potting mixture and kept in the greenhouse. The contents of one small pot were removed each day and thoroughly mixed, then 10 grams were processed for nematodes by a modified Baermann extraction.

Six days after the initial infestation with preadults, nearly all nematodes extracted were adult females. The first larva was found 9 days later and

from this time on several larvae were obtained from each pot. After 8 additional days the first new preadults were found; therefore, the life cycle from preadult to preadult in soil was completed in 23 days or approximately 7-8 days less than in agar.

RATE OF POPULATION INCREASE

To obtain estimates of rate of multiplication, 100 *Paratylenchus projectus* of mixed stages were added to 4-inch pots in which various plants were growing in sterilized potting mixture. After 105 days when aliquots of soil and roots were subjected to modified Baermann extraction, numbers recovered represented the following totals per pot: 38,000 with *Trifolium pratense* L., red clover, 147,000 with *Phleum pratense* L., timothy, 181,000 with *Apium graveolens* var. *dulce* pers., celery, and 2,637,000 with *Nicotiana alata* var. *grandiflora,* Link and Otto, jasmine tobacco. Under similar conditions and during the same period of time, *P. dianthus* increased from 100 to 37,000 with *Dianthus caryophyllus* L., carnation, to 1,509,000 with celery, and to 820,000 with jasmine tobacco.

ACCUMULATION OF PREADULTS IN POT CULTURE

In young pot cultures and in agar cultures, the preadult stage was passed through rapidly, but it was often far more numerous than all other stages combined in old pot cultures and in field soil samples.

To determine when accumulation of preadults occurred, 5-inch pots in which several red clover seedlings had just emerged were infested with 2,500 *Paratylenchus projectus* each. Beginning 100 days later, at intervals of approximately 2 weeks, 3 pots were sampled to estimate total population increase and to determine relative numbers of nematodes in the several developmental stages. Sampling involved removing a ¾-inch core of soil and roots with a sampling tube thrust vertically downward near the center of the pot through the entire soil depth. The core thus removed was mixed thoroughly, then a 10-gram quantity was subjected to modified Baermann extraction. The numbers of nematodes were counted separately by developmental stage, and numbers per pot were calculated on the basis of the weight of the full pot of soil.

The results of this test (Table 1) indicate that between 100 and 185 days there was an 8-fold increase in the proportion of all paratylenchs that were in the preadult stage. Samples taken at 152 days contained the highest total populations. This represented an increase from the 2,500 added to the pot in the beginning to approximately 523,000 or more than a 200-fold increase.

Table 1. Accumulation of *Paratylenchus projectus* preadults in red clover pot cultures.

Days after infestation	Number of nematodes in 10 grams of soil[a]	Percent second and third-stage larvae	Percent preadults	Percent females
100	1218	68.3	7.7	24.0
114	938	59.8	12.8	27.4
131	1992	61.2	13.3	25.5
152	5008	43.4	33.1	23.5
167	4832	25.4	43.8	30.8
185	4774	21.1	66.3	12.6

[a]Figures are means of 3 replicates.

PREADULTS OF OTHER SPECIES OF PARATYLENCHUS

Preadults of the bisexual species *Paratylenchus dianthus* and *P. hamatus* observed in these studies show very similar morphological specialization to those of *P. projectus*. Preadult females of both species have the same type of small, weakly developed stylet as those of *P. projectus*, and the esophagus, while apparently complete, has proportionately small median and basal bulbs that are nearly obscured by refractive granules. Preadult males in *P. dianthus* do not have a stylet and the esophagus is degenerate, while those of *P. hamatus* differ little from preadult females. Apparently this is related to the fact that adult males of *P. dianthus* do not have a stylet and have a degenerate esophagus, while those of *P. hamatus* have a moderately well developed stylet and esophagus.

Sex can be determined in the living preadult by a reduced opacity of the body where genital primordia are developing. In specimens inactivated or killed for examination at higher magnification, a rudimentary ovary and vulva can be distinguished in the females and rudimentary testis in the male, along with a diagonal line of translucent appearance where the spicules will develop.

Two apparently undescribed species found in Illinois soils were observed to have the morphologically specialized preadult stage, but these have not been studied in detail.

TOLERANCE OF ADVERSE CONDITIONS

In a preliminary note, Rhoades and Linford (1959) reported that preadults of both *Paratylenchus dianthus* and *P. projectus* are capable of long survival in moist soil without feeding. Soil that originally contained all stages of *P. dianthus* and that had been stored continuously in a screw-capped amber glass jar in dim laboratory light, still yielded preadults, and only preadults, when last tested by modified Baermann extraction after 4 years and 7 months of storage. Soil from the original collection of *P. projectus* was first stored nearly a year in a large covered metal container in the greenhouse and then part of it was transferred to capped amber glass jars and stored alongside the *P. dianthus* soil. When last tested by extraction this soil yielded live preadults and females. Although preadults long stored under such conditions have the body reserves greatly depleted, they molt readily into adults when stimulated by suitable root diffusates. Unfed adult *P. dianthus* developed from preadults stored over 3 years were not appreciably different in size from those from preadults taken directly from pot cultures.

For determining tolerance of desiccation, soil from a pot of Ladino clover infested with *P. projectus* was thoroughly crumbled and mixed, then passed through a 1/8-inch mesh screen to remove most of the roots. When first sampled, at 30.8% moisture, it contained numerous second and third stage larvae, preadults, and females. By using a pressure-membrane apparatus (Richards, 1947) the potting soil was found to retain about 16.5 percent moisture at 15 atmospheres pressure which is the approximate wilting point.

The soil was placed to a depth of 3/4 inch in a shallow pan, then placed in a humid cupboard and stirred daily for several days, then at greater intervals until the test was completed. Two 10-gram samples of the soil were processed periodically by modified Baermann extraction to determine nematode populations. Samples were oven-dried at the same intervals for moisture determinations.

Table 2. Survival of various stages of *Paratylenchus projectus* when subjected to dessication in soil.

Drying time, days	Soil moisture percent	Nematodes extracted from 10 grams of soil		
		Second and third-stage larvae	Preadults	Females
0	30.8	482	442	96
3	17.9	458	398	92
5	11.1	623	450	131
7	7.9	897	315	142
9	4.8	91	474	104
11	2.8	35	343	24
14	2.9	18	222	22
24	3.1	1	106	0
60	2.5	0	11	0

The results of this test (Table 2) demonstrate that all stages of this nematode are tolerant of moisture percentages below half that of the wilting point and that preadults are more tolerant of desiccation than other stages. Natural drying in the field is not likely to eradicate this nematode. The increased numbers of second and third-stage larvae shown in the table after 5 and 7 days, were chiefly second-stage, indicating hatching of eggs present in the soil at the beginning of the test. The extraction process kept the soil wet 3 days which is ample time for hatching of embryonated eggs.

To determine tolerance of sudden freezing when wet, soil from a second pot of Ladino clover infested with *P. projectus* was also crumbled, mixed and passed through a ⅛-inch mesh sieve, and the nematode population determined. Soil moisture was 25.8 percent. Ten grams of the soil were then placed into each of 5 small corked vials and one of these was placed at each of the following temperatures for 96 hours: 22°, 1°, −7°, −12°, and −19° C. The nematodes were then separated from the soil by modified Baermann extraction and counted.

The results of this test (Table 3) demonstrate that preadults survive sudden exposure to low temperatures better than other stages. An increase of second-stage larvae at 22°C indicates hatching of eggs present in the soil at the beginning.

DISCUSSION

Except for the preadult stage, the life history of *Paratylenchus projectus* lacks unusual features. There are 4 molts, as was reported by Reuver (1959) for *P. amblycephalus*. The first occurs in the egg, as in *P. goodeyi* (Oostenbrink, 1953), and the other molts are all typical. Except for lacking the sex organs, second and third larval stages are so similar morphologically to the

Table 3. Survival of the stages of *Paratylenchus projectus* when subjected to various temperatures for 96 hours.

Temperature (°C)	Nematodes extracted from 10 grams of soil		
	Second and third-stage larvae	Preadults	Females
Original soil	361	387	112
22	606	345	172
1	420	376	73
− 7	4	349	5
−12	0	119	0
−19	0	42	0

females as to leave no uncertainty concerning their genus. The fourth larval or preadult stage, however, has both stylet and esophagus very weakly developed: the delicate stylet is much too short and the esophageal bulbs are too small to look much like other stages. An abundance of opaque granules distributed through the esophageal region obscures the structure still further, until at first glance the generic characters may not be observed.

Although it is clear that Thorne and Allen (1950) and also Ferris and Bernard (1958) observed the preadult stage, Reuver (1959) appears to be the first investigator to describe its morphological specialization, and he evidently failed to recognize its full significance. However, he did note that numbers of preadults exceeded younger larvae by 10-20 fold in many of his investigations.

Such a stage is not commonly encountered among the plant-parasitic tylenchids. In *Ditylenchus dipsaci* the preadult stage is specialized physiologically, being the stage best adapted to survive adverse conditions, but it apparently lacks much morphological specialization.

The long survival of preadults of *P. projectus* and *P. dianthus* without feeding, and their rapid molting into adults when placed in suitable root diffusates (Rhoades and Linford, 1959) indicate that they are well adapted to perpetuate the species. This is further shown for *P. projectus* by their demonstrated tolerance of desiccation and of sudden freezing. The report of Thorne and Allen that almost all the *P. hamatus* in an August collection in California were "immature forms lying dormant in the soil," together with the finding of morphologically modified preadults of that species and 2 apparently undescribed species in Illinois soils in the present studies, makes it appear that at least several species of this genus have similar specialization. Reuver's description of the preadult stage indicates the same type of morphological specialization, yet his report that females were the stage most tolerant of desiccation raises some question of the degree of physiologic specialization in *P. amblycephalus*.

This leaves open the question of whether such a specialized stage occurs in certain other species and whether, even in the species studied here, the preadult stage occurs elsewhere in as great numbers as found in the present studies. It seems probable that certain investigators, in describing species or in reporting occurrence, may have concentrated attention on adults only. It also is possible that certain workers who have collected nematodes by the wet sieving method, may have missed the younger larval stages, especially if they relied on a No. 200 sieve as the finest, and may have mistaken this preadult stage for the typical larvae. A third possibility seems to be that preadults, if not especially numerous, may have been mistaken as early stages of the final molt. Linford, et. al. (1949), in their work with *P. minutus* in Hawaii should have seen this stage had it been very abundant, for they reported dimensions of younger larvae; and their report that females proved more tolerant of desiccation than other stages indicates the lack of any great proportion of physiologically specialized preadults in their samples.

The biological significance of such preadults as those of *P. projectus* clearly lies in survival of the species during absence of food or during adverse environmental conditions, but the factors that lead to accumulation of this stage are not yet clear. Under field conditions, samples collected at various times of year indicate that adverse environment or absence of suitable hosts are not essential. Pot culture studies have given some clues but not enough.

In young pot cultures, as in agar cultures, the preadult stage is passed through rapidly, and no attempt has been made to determine whether preadults during this phase of population rise are physiologically specialized for survival. Then the relative abundance of preadults rises while other changes are occurring: total population density is increasing, population age in the same soil is increasing, root distribution is changing with the new growth being chiefly around the periphery of the soil mass againt the pot wall, and in these pot cultures soil pH has been rising with the use of hard water. Present data do not permit differentiation of the relative significance of these changes. It seems clear, however, that the changed distribution of roots is important for even after the interior of the soil mass contains predominately the preadult stage, nematodes leached from the same pot and coming chiefly from the growing roots massed near the bottom of the pot, tend to include proportionately fewer preadults. Perhaps the older roots that have undergone secondary thickening in the interior of the soil mass, fail to provide the stimulus to molt.

SUMMARY

The life cycle of *Paratylenchus projectus* was traced on red clover seedlings growing in agar. There were 4 molts, the first occurring within the egg. The second and third larval stages were observed to feed ectoparasitically on epidermal cells and root hairs much the same as adult females, but the fourth or preadult stage did not feed. This stage is morphologically distinct with a very short delicate stylet and weakly developed esophagus. It was passed through rapidly in cultures of red clover growing in agar and young pot cultures but accumulated in old pot cultures and was found as the predominant stage under most field conditions. The facts that lead to accumulation are not yet clear. Preadults from pot cultures, in which they were the dominating stage, were found to be more tolerant of dessication and sudden exposure to low temperatures than the other life stages. *P. projectus* and *P. dianthus* preadults have survived for long periods in soil stored moist.

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Biology and Host-Parasite Relationships of the Spiral Nematode, *Helicotylenchus microlobus**

DONALD P. TAYLOR**

Helicotylenchus microlobus Perry, 1959, is a spiral nematode common in north central United States, according to Perry et al. (1959) and Taylor (1960a). Taylor (1960b) reported that this species has a wide host range. These studies were undertaken to elucidate certain aspects of the biology and host-parasite relationships of this nematode.

DEVELOPMENT OF *H. microlobus* WITHIN THE EGG

Gravid specimens of *H. microlobus* from stock populations maintained on greenhouse-grown tomatoes were transferred to sterile distilled water in a Syracuse watch glass. Eggs laid by these individuals were transferred to drops of sterile water on cover slips. Each cover slip was inverted over the well in a depression slide and the edges were sealed with vaseline to prevent evaporation.

Most eggs were laid in the one-cell stage, although 2 were observed in the two-cell stage and 1 in the four-cell stage while still within the uterus. Only eggs laid in the one-cell stage were used in this study made at 22°C ± 1°C. Within 6-21 hours after being laid, all eggs had undergone 2 divisions and all embryos were in the four-cell stage. The eight-cell stage was first detected in eggs 25-40 hours old. Embryo development occurred rapidly and an exact

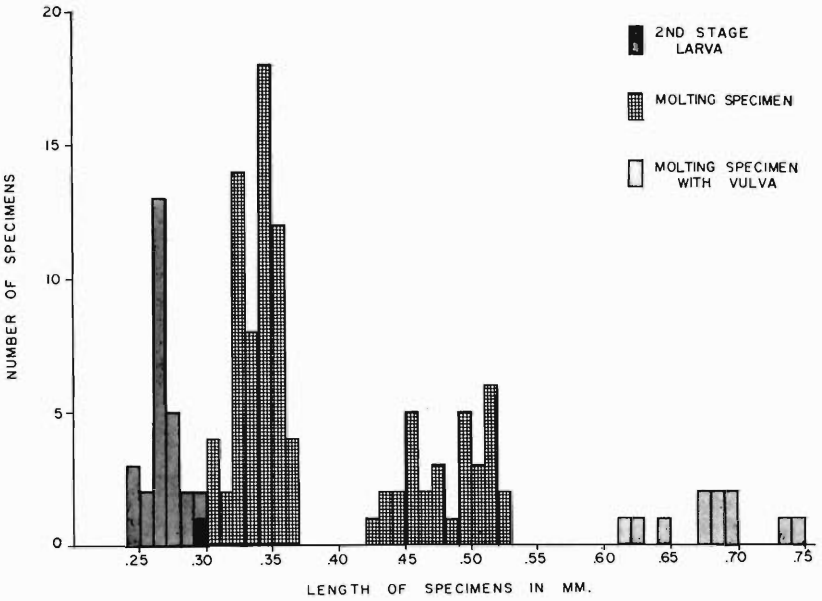

Figure 1. Length of 106 molting specimens and 27 second-stage larvae of *Helicotylenchus microlobus*. Note overlap between a molting specimen and 2 second-stage larvae in the length 0.29-0.30 mm.

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count of cell number could not be made after 60-75 hours. Embryo movement was first detected after an elapsed time of 107-122 hours. Within 138-153 hours differentiation of the embryo was marked: the anterior end was transparent; whereas the posterior two-thirds was filled with refractive globules. At this time, well formed prorhabdions of the stomato-stylet were detected in one specimen. Molting within the egg was detected when eggs were 160-175 hours old and was recognized by a loosening of the cuticle around the lip region. All larvae had well developed stylets at this time, and the esophageal lumen and valve in the middle esophageal bulb were visible in each larva. Movement of the larvae was cyclic: a period of extremely vigorous activity or "churning" lasted 30-45 seconds and was followed by 5-10 minutes of inactivity. The inactive phase was terminated by a twitching of the anterior or posterior end at irregular intervals. Then, rather abruptly, the churning phase would begin. Such a cycle of active and inactive phases was observed repeatedly in all specimens in the last 48 hours before hatching. In the last 24 hours before hatching, two new movements were noted during the generally inactive phase: a thrusting of the stylet and a jerking of the head region. These movements were studied in two specimens during 10-minute intervals. In one, 13 stylet thrusts and 2 head movements were recorded: in the other, 18 thrusts and 9 head movements were detected. Hatching occurred in 194-209 hours. In all cases, the first stage larval cuticle remained within the egg shell after hatching.

OBSERVATIONS ON MOLTING SPECIMENS

With the discovery of the first molt within the egg, the occurrence of additional molts in the soil was investigated. One hundred and six molting specimens of *H. microlobus* were obtained from stock populations, heat-relaxed, and mounted in 3 per cent formaldehyde on microslides. Outline drawings were made of these specimens using a Zeiss drawing apparatus, and measurements were obtained from the drawings by means of a map measurer. For comparison, 27 freshly-hatched second-stage larvae were also mounted, drawn, and measured.

Lengths of these molting specimens and second stage larvae are shown in Figure 1. Based on this dimension, molting specimens observed fell into three groups. The first group contained 63 specimens having an average length of 0.34 mm. and a range of 0.30-0.37 mm. The length of the shortest specimen in this group fell within the upper limit of length of second-stage larvae measured. The second group, containing 32 specimens, had an average length of 0.48 mm with a range of 0.42-0.52 mm; while the third group averaged 0.68 mm with a range of 0.61-0.75 mm. No anatomical structures were detected that were characteristic of any group, except that all specimens in the longest group possessed a vulva. The b values of the specimens were all characteristic of each group, with no overlapping observed between groups. The following are the b values for the three molting groups: first group—2.8 average (range of 2.4-3.0); second group—3.6 average (range of 3.3-3.9); and third group—4.6 average (range of 4.3-5.0).

OVERWINTERING OF *H. microlobus*

To determine stages in which *H. microlobus* overwintered in the absence of a host plant, samples of frozen soil from the Agricultural Experiment Station, Rosemount, Minnesota, were collected throughout the winter. These samples were allowed to thaw at room temperature and were then processed

for nematode recovery. The numbers of *H. microlobus* recovered throughout the winter are listed in Table 1. The field sample had been planted to soybeans the preceding summer and no cover crop was present when samples were collected. At the time the first soil sample was taken, the soil was not frozen. On December 5, 1957, soil was frozen to a depth of 8 inches, and it was frozen to a point below the sampling area for the remainder of the sampling period.

About the same number of *H. microlobus* specimens were recovered in all samples taken, and larval stages as well as gravid and non-gravid adults were present in all samples. All specimens obtained from frozen soil appeared normal regarding general activity and could not be distinguished from specimens maintained in the greenhouse during this same period.

Since *H. microlobus* overwintered with no detectable population decrease in this study, an understanding of population increase during the growing season could be gained if time required to complete its life cycle were known.

LIFE CYCLE INVESTIGATIONS

Marglobe tomato seedlings were planted in autoclaved sandy soil in small glass vials. Soil in each vial was infested with 10 gravid adults collected from stock populations of *H. microlobus*. Vials were then placed in a wire basket suspended in a constant temperature bath at either 75° or 90° F ± 1°. At 5-day intervals for 40 days one vial was removed from the bath and the soil was processed by the centrifugation-flotation technique of Caveness and Jensen (1955). Roots of the tomato plants were incubated at 80°F. for 12 hours and nematodes recovered from the roots were recorded with those recovered from the soil. Figures reported are averages of four separate experiments at each of the two temperatures. Numbers of larvae and adults of *H. microlobus* recovered in these tests are given in Table 2. At 75°F. the first increase in number of adults over the number introduced was detected after 35 days. At 90°F. the first increase was noted after 30 days. In all replicates the number of larvae increased with the length of time between introduction and final examination and was always greater at the higher temperature used.

HOST-PARASITE RELATIONSHIPS OF *H. microlobus*

The following techniques were used to study the physical relationship and pathological histology of *H. microlobus* and host roots:

Direct observation. Host plants previously infected with *H. microlobus* were cut at the ground-line, and boiling 3 per cent formaldehyde was poured over the soil and roots. Soil and roots were then placed in tap water where careful agitation removed soil from the roots.

TABLE 1. Number of specimens of *Helicotylenchus microlobus* recovered during the winter of 1957-1958 from soil collected at Rosemount, Minnesota. Average of the number of specimens recovered from three 250 ml samples collected.

Collection date	Number of specimens recovered		
	Larvae	Adults	*Total*
November 7, 1957	364	161	525
December 5, 1957	332	229	561
January 2, 1958	301	116	417
January 30, 1958	297	217	514
February 27, 1958	288	183	471
March 27, 1958	324	218	542

TABLE 2. Number of *H. microlobus* adults and larvae recovered from Marglobe tomato plants inoculated with 10 gravid adults and maintained at 75°F. and 90°F. Numbers in the table are the averages of four replicates.

	Number of days after inoculation							
	5	10	15	20	25	30	35	40
75°F.								
Adults	9	8	9	10	9	8	12	14
Larvae	1	11	18	24	35	42	50	48
90°F.								
Adults	8	9	10	9	10	14	19	22
Larvae	2	13	22	31	39	51	62	73

Staining intact roots. Infected host roots were fixed in F. P. A. and stained in bromthymol blue according to the method of Kirkpatrick and Mai (1957).

Sectioned and stained roots. Infected roots were killed with boiling 3 per cent formaldehyde and freed from adhering soil. After dehydration in a tertiary butyl alcohol series, roots were infiltrated with paraffin, embedded in tissuemat, and cut into 10 μ thick cross-sections. Sections were fastened to microslides with Haupt's adhesive, stained with safranin and fast green, and mounted in Canada balsam. Techniques followed were those outlined by Johansen (1940).

Numerous small light to dark brown lesions were found on the surfaces of unstained whole mounts of infected corn, soybean, and tomato roots. Lesions involved about 4-10 epidermal cells and could not be seen to extend more than 4 layers of cells into the cortex. In approximately one-third of the lesions observed a specimen of *H. microlobus* was present with its anterior end embedded in the root as shown in Figure 2. No similar lesions were observed on roots from non-infected plants.

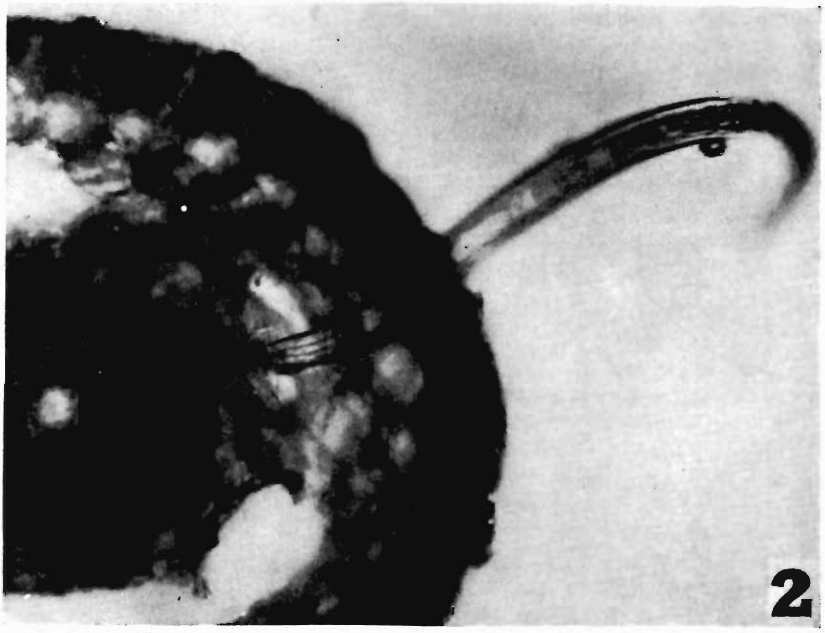

Figure 2. Free-hand cross-section of corn root showing a specimen of *Helicotylenchus microlobus* with its anterior end embedded in the root.

When corn roots inoculated with *H. microlobus* were stained with bromthymol blue, numerous specimens of this species were observed to be completely embedded within the root cortex. No preference for a particular region of the root was detected. Specimens were found from the meristematic region to the region of branch root formation. Nematodes were not observed to be oriented in any particular arrangement regarding host tissues, but were found in various positions within the cortex. No specimens were noted in the vascular system. Some individuals were only partly embedded, others were completely embedded and located just below the epidermis, while still others were completely embedded and in close proximity to the vascular system (Figure 3). Movement of one specimen had apparently caused a break in the root epidermis (Figure 4). On several occasions slight swellings were found in areas of the root infected with *H. microlobus* (Figure 3), whereas other infected areas of the same and other roots were not swollen. Similar nematode orientation was noted in tomato roots although no swellings of root tissue were noted. No specimens of *H. microlobus* were detected that were completely embedded in soybean roots.

Examination of stained and sectioned tomato roots revealed the presence of many more *H. microlobus* specimens than were noted in preliminary examination of the material before sectioning. Symptoms of damage caused by this nematode were detected that were not evident in whole mounts. The most commonly observed damage involved rupture of the epidermis near the infected cortex and destruction of cortical cell walls in the area occupied by nematodes. In a single section the anterior end of a specimen was observed with its stylet protruding through a cortical cell wall.

DISCUSSION

The discovery of a molt within the egg and the presence of three molting groups in this species' soil phase constitutes the first report of four molts in any species in the Hoplolaiminae. This is in agreement with the number of molts prior to maturity in most nematodes, and the finding of three molts in specimens obtained from soil agrees with Golden's observations (1956) on molting in *H. buxophilus*. Although no specimens were observed in the act of hatching, the thrusting movements of the stylet observed in the last 24 hours before hatching suggest that second-stage larvae mechanically rupture the egg shell with their stylets and emerge through the opening made in the shell.

The life cycle of *H. microlobus* takes at least 30 days to complete at 90°F. and 35 days at 75°F. In the experiments reported, it was not possible to determine the length of time necessary for an adult to develop to the egg-laying stage. Since this species requires more than one month to complete its life cycle, only a few generations per year can be produced in Minnesota. Some plant parasitic nematodes reproduce more rapidly, e.g., Rohde and Jenkins (1957) reported that *Trichodorus christiei* Allen, 1957 can complete its life cycle in 16-17 days at 30°C. Thus, that species can increase greatly in a single season because of the large number of generations produced. Of special significance to the ability of *H. microlobus* to increase in number in Minnesota is that no reduction in population size was observed to occur during the winter. Thus, an increase in population during the growing season may not be negated by a high mortality rate in the winter.

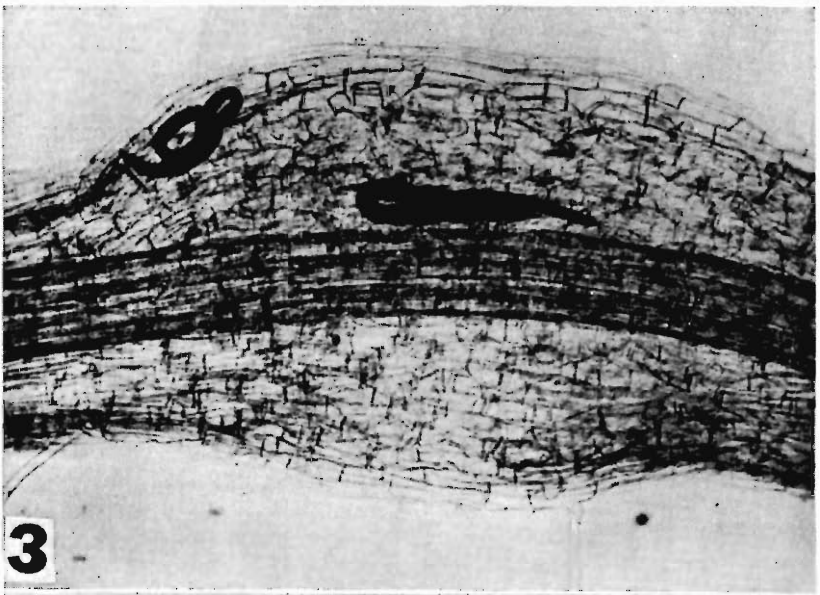

Figure 3. Two specimens of *Helicotylenchus microlobus* embedded in corn root. Note the slight swelling of root tissue in the infected area (whole mount, stained with bromthymol blue).

Figure 4. Break in epidermis of corn root probably caused by subepidermal movement of a specimen of *Helicotylenchus microlobus* (whole mount, stained with bromthymol blue).

H. microlobus was observed to feed as a partially embedded ectoparasite similar to *H. buxophilus* as reported by Golden (1956); however, for the first time in the genus, this species was also observed as an endoparasite. Although endoparasitism was observed in corn and tomato, it was never detected in soybean roots, indicating that the mode of parasitism may be largely dependent upon the host plant involved. Since all of these plants were rated as hosts (Taylor 1960b), the mode of parasitism does not seem to influence the ability of the nematode to reproduce.

The cortical destruction, as observed in tomato roots, indicated that *H. microlobus* causes considerable mechanical damage to root tissue. Continuous feeding and movement in the cortex by the same or other individuals could lead to cortical sloughing, reported by Perry et al. (1959) as a symptom of *H. digonicus* damage to blue grass roots.

SUMMARY

Eggs of *Helicotylenchus microlobus* were generally laid in the one-cell stage and hatched after 194-209 hours. The first molt occurred within the egg 160-175 hours after being laid. Molting specimens collected from soil could be grouped into 3 non-overlapping groups on the basis of length, averaging 0.34, 0.48, and 0.68 mm. The b values of members of these groups also did not overlap.

Thirty-five days were required for new adult nematodes to develop in populations established from gravid adults at 75°F., whereas this process took only 30 days at 90°F.

No decrease in numbers of larvae or adults of *H. microlobus* occurred in soil samples collected at 4-week intervals from November through March.

Infected corn, soybean, and tomato roots had small brown lesions on the surface that were not observed on non-inoculated check plants. Frequently, specimens of *H. microlobus* were observed with the anterior end embedded in the lesion. In stained corn and tomato roots, many specimens of this species were observed completely embedded in cortical tissues. Infected areas were characterized by damage to cortical cell walls and often by the presence of a ruptured epidermis.

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Studies on Digenetic Trematodes of Fishes of Fiji. I. Families Haplosplanchnidae, Bivesiculidae, and Hemiuridae*

HAROLD W. MANTER

The writer collected trematodes of fishes at Suva, Fiji, in January, 1951, during a period of about three weeks while he was enroute to New Zealand as a Fulbright Research Scholar. He is greatly indebted to Dr. A. F. S. Ohman, Senior Veterinary Officer, who generously furnished laboratory space and equipment in the Department of Agriculture, located not far from the municipal market where fishermen bring a variety of fishes for sale. Veterinary Officer K. J. Garnett furnished valuable assistance in many ways. Mr. Gabriel Stevens, manager of the Market, was most cooperative. Dr. Rene Catala provided small fishes from the reef and a few freshly caught fishes. Fishes were usually secured from the market where a surprising variety of species showed up at one time or another. One handicap is that in that climate some of the trematodes were partly disintegrated when finally collected. A major handicap was the difficulty of correct identification of the fishes. Some were preserved and sent to the United States National Museum where they were identified for me by Dr. Leonard P. Schultz. In many cases, however, only the native name and the general type of fish are known. Approximately 44 species of fishes were examined.

A few Monogenea have been reported from Fijian fishes (Laird (1958); Manter & Prince (1953)). Only two Digenea (*Prosorhynchus thapari* Manter, 1953 and *Neidhartia polydactyli* Manter, 1953) are as yet reported from fishes there.

Measurements are in mms. unless otherwise indicated.

HAPLOSPLANCHNIDAE

Hymenocotta mulli, n. gen., n. sp. (Figs. 1-3)

HOST: mullet.

LOCATION: intestine.

NUMBER: two specimens in a single host.

HOLOTYPE: U. S. Nat. Mus. Helminth. Coll. No. 39452.

DESCRIPTION (based on two specimens): Body elongate, 3.040 to 4.503 long; greatest width at acetabular level, 0.912 in the 4.503 specimen; forebody about $^1/_5$ body length, 0.608 to 0.874; hindbody gradually tapering almost to a point. Scattered pigment granules at level of pharynx. Oral sucker replaced by a broad membranous, muscular fold with six short, rounded lobes, one pair anterior, two pairs latero-posterior; posterior pair smaller and more or less pointed (Fig. 2). Six pairs of sensory papillae on lobes. Mouth a transverse slit. A cup-like depression in disc, just anterior to mouth. Greatest diameter of oral disc 0.603 to 0.684. Prepharynx covered by oral disc, about half as long as pharynx; pharynx 0.328 to 0.368 long by 0.201 to 0.247 wide. Single cecum reaching about $^2/_3$ body length, to anterior end of ovary. Testis in posterior fourth of body, elongate oval, 0.551 by 0.348 to 0.418; posttesticular space 0.589 to 0.684. Seminal vesicle tubular, long, almost straight, extending at least halfway from acetabulum to testis. Cirrus sac present (Fig. 3), ovoid to elongate, containing an internal

*Studies from the Department of Zoology, University of Nebraska, No. 325. The collection of these parasites in Fiji was aided by a grant from the University of Nebraska Research Council. Completion of this study was aided by a grant (G10667) from the National Science foundation.

seminal vesicle in its posterior half and prostatic cells in its anterior half; cirrus not evident. Genital atrium very short and non-muscular; genital pore opposite posterior half of pharynx. Ovary rounded, overlapping anterior end of testis. Seminal receptacle globular, thick-walled, almost as large as ovary, just anterior and to one side of ovary. Mehlis' gland dorsal to ovary. Vitellaria consisting of fused follicles forming broad tubes, a pair on each side of body and two or three shorter, median, preovarian tubes; anterior extent about midway between ovary and acetabulum or a little more; posterior extent midway between testis and posterior end of body. Uterus straight, between ovary and acetabulum; it narrows to a small tube dorsal to acetabulum then joins a short muscular metraterm (Fig. 3) which enters genital atrium near pore. Eggs large, very thin-shelled, almost as wide as long; uncollapsed eggs 92 to 128 by 80 to 88 microns; collapsed eggs about 88 by 68 microns. Excretory pore terminal; immediately preceding it a pair of small deeply stained, funnel-shaped structures of unknown significance; excretory tube near them surrounded by gland cells; excretory stem forking at posterior end of testis; a pair of swollen clear vessels extend into forebody to end at level of genital pore, probably arms of excretory vesicle but connection to median stem not observed. Small winding tubes filled with finely granular material occur in forebody. They have the appearance of lymphatic vessels (Fig. 2). Lymphatic vessels are not reported for this family.

The name *Hymenocotta* is from *hymen,* membrane and *kotta,* head; it refers to the distinctive membranous oral disc.

DIAGNOSIS OF HYMENOCOTTA: Haplosplanchnidae in which oral sucker is replaced by a 6-lobed oral disc with few radial muscles. Cirrus sac present, containing an internal seminal vesicle and prostatic gland cells but with weakly developed, or no, cirrus. Ovary globular, unlobed; seminal receptacle globular, large; vitelline follicles fused to form longitudinal tubes; eggs large, thin-shelled, with undeveloped miracidia. Type species: *H. mulli;* in mullet, Fiji Islands.

DISCUSSION: This peculiar trematode is clearly a haplosplanchnid but is very different from any other genus in the family by possessing a lobed muscular fold in place of an oral sucker, and in possessing a cirrus sac. Yamaguti (1958) recognizes three genera in the family. In *Haplosplanchnus* Looss, 1902 and the closely related *Laruea* Srivastava, 1939, the vitellaria are reduced to small remnants close to the ovary and the eggs contain miracidia with eye-spots. Both genera are parasites of *Mugil.* Most species in the family are in the genus *Schikhobalotrema* Skrjabin & Gushanskaja, 1955, and possess vitelline follicles of considerable extent; eggs do not contain developed miracidia; no species is as yet known from mullets.

Hymenocotta agrees with *Schikhobalotrema* in its extensive vitellaria and undeveloped miracidia but is entirely different in its oral disc and presence of a cirrus sac. Its host is like that of *Haplosplanchnus* and *Laruea.*

An interesting circumstance is the similarity in the evolution of the Haplosplanchnidae and the Haploporidae, both of which are common parasites of mullets. Manter (1957, p. 191) noted that the genera of Haploporidae may be divided into two groups: seven genera have greatly reduced vitellaria and eggs containing oculate miracidia; ten genera have follicular vitellaria (often rather tubular) and eggs containing undeveloped miracidia. Still more remarkable is the fact that genera in the former group are mostly parasites of *Mugil;* while the latter group occurs in Chaetodontidae, Spariso-

midae, Scaridae, Acanthuridae, Scorpidae, and Girellidae. The Haplosplanchnidae not only parallel this grouping in regard to reduced vitellaria and fully developed miracidia, but also parallel it very closely in type of hosts. Haplosplanchnid genera with reduced vitellaria are exclusively parasites of *Mugil*. All such haploporid genera are also parasites of *Mugil* except for three species reported from Anostomidae in Argentina (Szidat, 1954). Furthermore, the hosts of genera with extensive vitellaria in both families are remarkably similar. Thus, four of the six families mentioned above are also hosts for the corresponding group of haploporid genera. The parallelism extends even to the very small family of fishes, the Girellidae.

The peculiar anterior end of *Hymenocotta* recalls comparisons of haplosplanchnids with the Aspidogastridae. Looss (1902) believed the haplosplanchnids connected the aspidogastrids with the "true" distomes. This view has not been generally accepted. An exception has been Chauhan (1954) who separated the Haplosplanchnidae in a separate suborder, the Preprosostomata. What little is known of life cycles does not support this view. At least some aspidogastrids do not reproduce as larvae, the adult developing directly from the miracidium; and Cable (1954) has shown that *S. acutum* (=*Haplosplanchnus acutus*) has a digenetic life cycle; its cercariae develop in sporocysts in marine snails. However, it must be admitted that the adult haplosplanchnid has many characters suggestive of aspidogastrids, e.g. single cecum, single testis, long tubular seminal vesicle, and Y-shaped excretory vesicle. The cirrus sac and metraterm of *Hymenocotta* are similar to those of *Lobatostoma*. The replacement of a typical oral sucker by a lobed disc is an added similarity. However, a comparison of this disc with the lobes of *Lobatostoma* (Figs. 2 & 4) shows that the former has a variety of muscle including some which are more or less circular, whereas those in *Lobatostoma* are mostly radial. It seems doubtful if these structures are homologous. At the same time, the numerous similarities of the two groups of trematodes are difficult to explain on an ecological basis. It will be interesting to know if the life cycle of *Lobatostoma* is actually direct.

BIVESICULIDAE

Bivesicula claviformis Yamaguti, 1934

HOST: *Epinephelus merra;* grouper (Serranidae).

LOCATION: intestine.

SPECIMEN DEPOSITED: U. S. Nat. Mus. Helminth. Coll. No. 39450.

DISCUSSION: The three specimens collected measured 1.373 to 1.600 by 0.603 to 0.710. They differ from Yamaguti's specimens in being larger and in that the vitellaria although meeting at the level of the intestinal bifurcation, do not crowd the cirrus sac and ovary. Eggs (collapsed) are 82 to 96 by 37 to 48 microns agreeing well with the average of 86 by 47 microns reported by Yamaguti. The cirrus sac in my specimens was not as wide as in Yamaguti's. These small differences do not seem to justify a new species. The Japanese specimens were from *Seriola quinqueradiata* and *Parapristipoma trilineatum* at Tarumi. The species is also known from *Serranus fasciatus* in the Red Sea (Nagaty, 1948).

Treptodemus latus, n. gen., n. sp. (Fig. 5)

HOST: half-beak, probably *Hemiramphus* sp.

LOCATION: intestine.

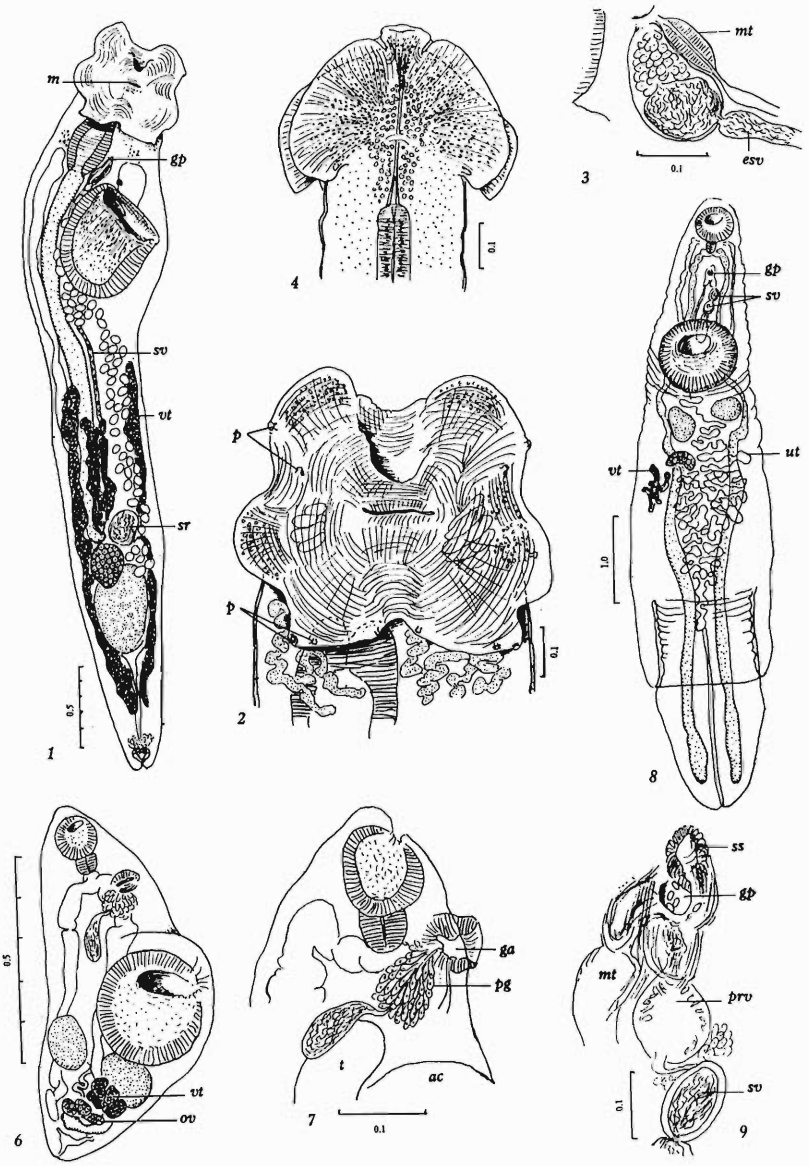

Fig. 1. *Hymenocotta mulli.* Ventral view.

Fig. 2. *H. mulli.* Enlarged anterior end. Ventral view.

Fig. 3. *H. mulli.* Terminal genital tubes, showing cirrus sac and metraterm.

Fig. 4. Anterior end of an aspidogastrid, *Lobatostoma pacificum*, showing oral lobes.

Fig. 6. *Prolecitha obesa.* Ventral view. Holotype.

Fig. 7. *P. obesa.* Enlarged view (semi-dorsal) of anterior end. Paratype.

Fig. 8. *Sterrhurus amplus.* Ventral view. Holotype.

Fig. 9. *S. amplus.* Enlarged view of terminal genital ducts.

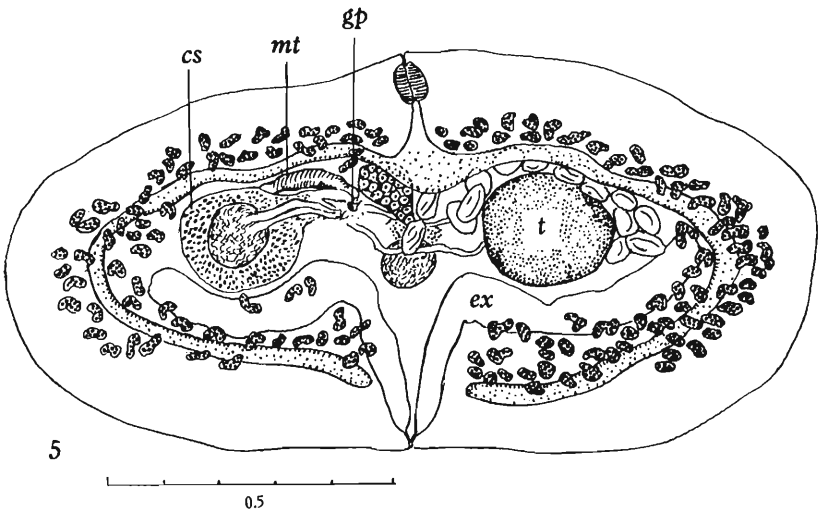

Fig. 5. *Treptodemus latus*. Ventral view.

HOLOTYPE: U. S. Nat. Mus. Helminth. Coll. No. 39451.

DESCRIPTION: (based on a single specimen): Body without spines, although these could have been lost; about twice as wide as long. Length 0.711, width 1.390. Anterior and posterior sides almost parallel, lateral sides broadly rounded. Oral sucker and acetabulum both lacking. Mouth at mid anterior point. A short narrow tube leads to a pharynx 0.072 long by 0.061 wide. Esophagus slightly longer than pharynx, narrow at anterior end but widening to a broad base; ceca extending laterally, then bowing inward following curvature of the body, ending blindly, ends separated by excretory vesicles.

Excretory pore at midposterior point of body. Two excretory vesicles uniting by short tubes near excretory pore. Each vesicle extends forward, diverging slightly, to near midbody where each bends sharply toward side and ends near intestinal cecum on each side.

Genital pore a little to right of midline and a little anterior to midbody. Testis single, large, subspherical, unlobed, in middle of left half of body filling most of space between anterior limb of left cecum and left excretory vesicle. Vasa efferentia two, extending to right, uniting dorsal to cirrus sac. External seminal vesicle lacking but seminal ducts somewhat inflated. Cirrus sac large, pyriform, 0.339 long by 0.174 wide, about same size as testis and occupying similar position but in right half of body. Basal portion of cirrus sac spherical containing dorsally a relatively small rounded seminal vesicle. Seminal vesicle surrounded by large gland cells which cover vesicle ventrally. Cirrus a thick-walled tube leading medianly and ventrally from vesicle to genital pore. Genital atrium fairly large.

Ovary pyriform, tapering to a point posteriorly, located near midbody slightly to left of midline, partially overlapping dorsally the intestinal bifurcation. Mehlis' gland at posterior tip of ovary; seminal receptacle

All drawings were made with the aid of a camera lucida. Abbreviations are: *cs*, cirrus sac; *esv*, external seminal vesicle; *ga*, genital atrium; *gp*, genital pore; *m*, mouth; *mt*, metraterm; *ov*, ovary; *pg*, prostate gland; *prv*, prostatic vesicle; *sr*, seminal receptacle; *ss*, sinus sac; *sv*, seminal vesicle; *t*, testis; *ut*, uterus; *vt*, vitellaria.

fairly large, spherical, just posterior to Mehlis' gland, almost exactly at midbody. Uterus extending directly to left between testis and cecum overlapping cecum dorsally, bending around testis to outer midtestis level then retracing its course, not observed to right of ovary but a muscular metraterm extends from right along anterior edge of cirrus sac to join genital atrium from its anterior edge. Eggs thin-shelled, 72 to 74 by 43 microns; often collapsed, width 36 to 43 microns.

Vitelline follicles irregular in shape, often more or less bilobed and elongate, distributed along length of intestinal ceca; dorsal and ventral to, and on both sides of, ceca except near posterior end where they are anterior to ceca.

DISCUSSION: This trematode is clearly a member of the family Bivesiculidae. Notable characters are: lack of oral sucker and acetabulum; single large testis with two vasa efferentia; two excretory vesicles; large cirrus sac containing a spherical seminal vesicle and many gland cells; and large thin-shelled eggs. A few scattered pigment spots near the anterior end suggest an oculate cercaria. LeZotte (1954) interprets the so-called oral sucker of bivesiculids as a pharynx. He found that the furcocercous cercaria of *Bivesicula hepsetiae* resembled the cercaria of Azygiidae. LaRue (1957) classified the family Bivesiculidae in the superfamily Azygioidea.

The genus *Treptodemus* (from *trepto* = turned around and *demus* = body) differs from both *Bivesicula* and *Bivesiculoides* in its very wide body which involves much altered positions of all the organs.

HEMIURIDAE

Subfamily Lecithasterinae

Prolecitha obesa, n. gen., n. sp. (Figs. 6-7)

HOST: houndfish or needlefish (Belonidae).

NUMBER: 3

LOCATION: intestine.

HOLOTYPE: U. S. Nat. Mus. Helminth. Coll. No. 39448.

DESCRIPTION: (based on 3 specimens, 1 without eggs; 2 with few eggs): Body smooth, spindle-shaped, cylindrical, widest at acetabular level near midbody, tapering toward each end almost to a point; ecsoma lacking; length 0.684 to 0.944; width 0.368 to 0.435. Oral sucker 0.100 to 0.114, acetabulum 0.268 to 0.288; sucker ratio 1 : 2.5 to 2.6. Forebody 0.301 to 0.455, or almost ½ body length. Pharynx 0.064 to 0.072 long by 0.064 wide; esophagus very short; ceca wide, with deep constriction near middle of forebody, extending to near posterior end of body where one or both bend inward to meet the other but not fusing. Testes large, usually longer than wide, slightly diagonal to symmetrical, overlapping acetabulum, either one or both may be dorsal to acetabulum. Seminal vesicle an elongate sac, immediately preacetabular, narrowing to become a tube, pars prostatica, surrounded by well developed prostatic gland forming spherical mass of cells free in parenchyma. Sinus sac lacking. Genital atrium more or less spherical, with very thick muscular walls forming sucker-like structure (Fig. 7) which may be partially protruded. Ovary deeply trilobed with lobes directed more or less anteriorly, near posterior end of body. Vitellaria preovarian, consisting of 7 rounded, separated lobes, side by side between ovary and testes, usually 3 ventral and 4 dorsal vitellaria, overlapping testes. Uterus entirely preovarian; eggs in early stages of development; most mature ones 8 to 13 by 8 to 10 microns. Excretory vesicle with very short median stem, forking at level of posterior ends of ceca.

DISCUSSION: This genus is a member of the Lecithasterinae because of its smooth body, lack of ecsoma, and 7-partite vitellaria. It is most like *Aponurus* and *Lecithophyllum* which possess rounded separated vitellaria. However, it is clearly distinctive in that the vitellaria are anterior to the ovary. In no other hemiurid are the vitellaria entirely preovarian,* hence the name *prolecitha*. It differs from *Aponurus* and *Lecithophyllum* also in its large, muscular genital atrium and lobed ovary.

DIAGNOSIS OF PROLECITHA: Lecithasterine with 7 rounded, preovarian vitelline lobes; ovary trilobed; testes preovarian; genital atrium forming a protrusible, very muscular, sucker-like structure. *Type species: P. obesa,* from marine fishes (Belonidae) in Fiji.

Sterrhurus amplus, n. sp. (Figs. 8-9)

HOST: "voi voi"; ribbonfish; family ?

LOCATION: stomach.

NUMBER: one.

HOLOTYPE: U. S. Nat. Mus. Helminth. Coll. No. 39447.

DESCRIPTION: Large, thick reddish (in life) body; 7.087 long including partially extended ecsoma; 1.710 wide near posterior end of body proper; ecsoma 2.432 in total length, protruded portion 1.368. Oral sucker 0.456; acetabulum 0.893; sucker ratio 1:1.95; forebody 1.387 long. Large vesicular cells in parenchyma. Pharynx 0.188 long by 0.134 wide; esophagus very short, dorsally directed; ceca extend to near posterior end of ecsoma, near together in forebody, diverging to become lateral to testes. Testes diagonal, just posterior to acetabulum, separated by uterus. Seminal vesicle distinctly tripartite, overlapping anterior fourth of acetabulum; total length 0.737, posterior segment 0.402 long, middle segment 0.201 long, anterior segment 0.134 long; both anterior and middle segment with very thick walls. A short pars prostatica leads to a thin-walled spherical chamber containing few traces of cell remnants, hence considered to be a prostatic vesicle, not enclosed in a sinus sac. Prostatic cells very few and inconspicuous. Genital pore a little posterior to intestinal bifurcation. Genital atrium sac-like, directly dorsal to genital pore, connected with an elongate sac (sinus sac) extending anteriorly and provided with a wall of wide somewhat separated muscle fibers (Fig. 9); fine muscles extend posteriorly to enclose partially a muscular terminal portion of metraterm and a muscular male tube leading from prostatic vesicle and opening into atrium dorsal to genital pore; appearing as a loose, open, muscle-strand type of sinus sac with an anterior chamber and partially enclosing terminal parts of male and female tubes.

Ovary transversely extended, near midbody, a little posterior to right testis. Vitelline glands small, immediately postovarian, to right of right cecum, with 3- and 4-digitate lobes which face to the right; lobes rather slender. Uterus of slender coils, mostly intercecal; a single loop entering ecsoma a short distance. Eggs small, thin-shelled, 16 to 20 by 8 to 9 microns. Stem of excretory vesicle forking between testes, crura uniting dorsal to pharynx.

The name *amplus,* large, refers to the unusually large size of this species.

DISCUSSION: This species is even larger than *Sterrhurus magnus,* the most closely related species. The two species agree well in size, sucker ratio, location of most organs, and in the thick-walled seminal vesicle. *S. magnus*

*_Bilecithaster_ Siddiqi & Cable, 1960 was named after this paper was in press. Its testes are not preovarian.

is from *Trachinocephalus myops* and *Sauridia argyrophanes* (both Synodontidae) in Japan. *S. amplus* differs chiefly in the character of the genital atrium, sinus sac, and terminal ducts. *S. magnus* has a spherical sac called the ejaculatory vesicle into which the genital ducts seem to open rather far anteriorly. Such a structure was interpreted by Manter & Pritchard (1960) to be as in *Separogermiductus,* to which genus the species was transferred. Its similarity to *S. amplus* suggests that the ejaculatory vesicle of *S. magnus* should be restudied. *S. amplus* differs also in that both the anterior and the middle segments of the seminal vesicle are thick-walled; the ceca are longer; the vitellaria are extracecal with more slender lobes; the testes are intercecal; the eggs slightly smaller.

Lecithocladium scombri Yamaguti, 1953

HOST: *Scomber brachypomus* (Bleeker); salala; mackerel (Scombridae).

LOCATION: stomach.

NUMBER: 3 in one host; 3 in another.

SPECIMEN DEPOSITED: U. S. Nat. Mus. Helminth. Coll. No. 39449.

DISCUSSION: This species is hitherto known from *Scomber kanagunta* and *S. microlepidotus* in the Celebes. It is characterized by the distinct ventral lobe of the oral sucker and a length of about 3 mm. This record is a new (but related) host and a new locality.

SUMMARY

Six species of digenetic trematodes are reported from Fiji: *Hymenocotta mulli* (Haplosplanchnidae); *Bivesicula claviformis* Yamaguti, 1934 and *Treptodemus latus* (Bivesiculidae); *Prolecitha obesa, Sterrhurus amplus,* and *Lecithocladium scombri* Yamaguti, 1953 (Hemiuridae).

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Histological and Histochemical Observations on the Nature of the Cyst of *Neoechinorhynchus cylindratus* in *Lepomis* sp.*

BURTON J. BOGITSH

The occurrence of the encysted form of the juvenile of *Neoechinorhynchus cylindratus* (Van Cleave, 1913) in various centrarchid fishes has been reported by several authors (Ward, 1940, Manter, 1926, and others). The possibility that the enveloping wall is made up of two layers has been suggested by Bogitsh (1957). The heavy, inner layer when stained with Mallory's aniline blue collagen stain appears to be fibrous; the outer, more delicate layer is apparently cellular. Ward (1940) is the only other investigator who has described this cyst wall. She describes the structure as being single and ". . . composed of modified liver cells which are spindle-shaped or fibrous."

Hunter and Dalton (1939) have classified the cysts of larval helminths into two types. The first type is reported to be of dual origin, the inner portion of the cyst being elaborated by the parasite, and the outer portion being elaborated by the host. The cysts of *Posthodiplostomum minimum,* and *Tetracotyle lepomensis* described by Hunter and Hunter (1940) and Bogitsh (1958) respectively, are examples of the first type. The second type consists of a single wall of connective tissue produced by the host. The cysts of *Clinostomum marginatum* described by Osborn (1911) and Hunter and Dalton (1939) are in this second category. The cyst wall of *N. cylindratus* as described by Ward (1940) also belongs to this second type. These descriptions of the morphology of the cysts are based primarily on histological observations. Singh and Lewert (1959), in demonstrating the component parts of the metacercarial cyst of *Notocotylus urbanensis,* utilized histochemical techniques. The purpose of the present investigation is to define the structure of the cyst wall of *Neoechinorhynchus cylindratus* by the use of both histological and histochemical methods.

MATERIAL AND METHODS

Collections of infected fishes belonging to the genus *Lepomis* were taken from ponds in Albemarle County, Virginia, and Jefferson County, Georgia. Portions of infected livers were removed and fixed in various solutions for histological and histochemical observations. Sections of fixed material were mounted on slides smeared lightly with albumen.

HISTOLOGICAL: Following fixation in Bouin's fluid, embedding in paraffin (56°-58° C. melting point), and sectioning at 8 microns, the material was stained by one of three techniques. One group of slides was subjected to Mallory's aniline blue collagen stain, another to Gomori's trichrome stain, while a third was lightly stained with Harris' alum hematoxylin preparatory to phase microscope study.

HISTOCHEMICAL: The techniques used in this portion of the investigation were chosen for the purpose of distinguishing three major categories of material: (1) polysaccharides, (2) proteins, and (3) alkaline glycerophosphatase.

(1) Polysaccharides: Following fixation in either formalin-alcohol-acetic acid (FAA) after Lillie (1947) or absolute alcohol, material was cut at 8

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microns and mounted on slides by flotation on 95% alcohol. Tests for polysaccharides were conducted according to the methods of (a) Bauer as outlined by Lillie (1954), using chromic acid as the oxidizing agent followed by the Schiff stain, and (b) McManus (1946), using periodic acid as the oxidizer (hereafter referred to as PAS). Best's carmine stain for glycogen was employed on still other sections. In an attempt to distinguish between the Schiff-positive substances, control sections were exposed to digestion by hyaluronidase after the method of Smyth (1956) and also by diastase after the method of Gomori (1952). Still other sections were placed in sodium bisulfite solution (0.05M) as described by Lillie (1954) for the purpose of blocking the reaction of certain PAS positive substances.

(2) Proteins: Material fixed in Bouin's fluid was sectioned at 8 and 15 microns. One group was subjected to the procedure of Mazia, et al. (1953). This technique, adapted from paper chromatography, utilizes the principle that brom phenol blue (BPB) will stain basic protein groups in various shades of blue. The intensity of the color is in direct proportion to the number of basic protein groupings in the sample. In the presence of mercury, in the form of $HgCl_2$ other proteins will bind the dye. It is possible, therefore, to compare the two techniques and achieve a quantitative as well as a qualitative indication of the overall protein pictures. In addition to the BPB method, Baker's (1947) modification of the Sakaguchi method was employed for the detection of arginine-containing proteins and phenols, Millon's technique was used for the detection of tyrosine, and Romieu's reaction indicating the presence of tryptophane was also employed. The latter two methods were carried out as outlined by Gomori (1952).

(3) Alkaline glycerophosphatase: Pieces of infected liver were fixed in chilled acetone, according to Gomori (1952), or 80% ethyl alcohol, as described by Danielli (1953). Embedding was carried out *in vacuo* at 58° C. The material was sectioned at 6 microns, mounted and incubated at 37° C. for various intervals, ranging from 30 minutes to 24 hours. The incubating mixture was composed of the following materials buffered to a pH of 9.0-9.2 with sodium barbital:

3.0% Sodium glycerophosphate (Nutritional Biochem. Corp.)	10 ml
3.0% $CaCl_2$	25 ml
10.0% $MgCl_2$	1 ml
Distilled water to make	50 ml

Control sections were treated in one of two ways. Several groups were incubated in the above solution omitting the sodium glycerophosphate; others were arranged according to the technique outlined by Bullock (1958) to display any diffusion artifacts that might have occurred.

RESULTS

The appearance and overall structure of the cyst wall, host-liver cells, and parasite are ilustrated in figure 1. In all instances, the cyst wall appears to be composed of two distinct layers. An inner layer (I, fig. 1) appears *fibrous* and stains blue with Mallory's aniline blue collagen stain and green with Gomori's trichrome stain. It measures approximately 13 microns. The outer portion (II, fig. 1) is considerably thinner, measuring approximately 5 microns. It stains red with either of the aforementioned stains. The surrounding host-liver cells also stain red. Phase microscope examination of the layers reveals that layer I is composed of fibrous material arranged in bundles that are parallel to the longitudinal axis of the cyst. The scattered nuclei in this

layer are indistinguishable from those of layer II. Layer II is primarily cellular in structure. The individual cells are long and flattened and are arranged adjacent to the surface of layer I. These cells possess large, oval nuclei that are slightly indented.

Sections of the cyst proved positive for polysaccharides when stained with Best's carmine stain or when treated with the Bauer or PAS techniques (Table I). Layer I, however, stained less intensely than either layer II or the surrounding liver cells. The enclosed parasite was both PAS and Bauer positive, the greatest concentration of polysaccharides being in the subcuticular region of the parasite. The worm's cuticle was negative in all instances. Following digestion with diastase, the host-liver cells, enclosed parasite, and layer II failed to react when subjected to PAS treatment, Bauer treatment, or Best's carmine stain, indicating that most of this material is glycogen. Regarding the enclosed parasite, Bullock (1949) reported that glycogen was present in most of the body structures of acanthocephala and that it was the principal polysaccharide in the phylum. Layer I, on the other hand, failed to be influenced by either hyaluronidase or diastase. Lillie (1954) reported that collagen is one of many PAS positive substances. He further stated that its positivity can be blocked if the material is treated with 0.05M sodium bisulfite, while the positive reactions of starches, mucin, etc., are apparently unaffected. Following this technique, in the present investigation, layer I was PAS negative; layer II, the host-liver cells, and the enclosed parasite were PAS positive. It follows, then, that the nature of layer I is primarily collagenous. Control sections of the cyst not exposed to either periodic or chromic acid oxidation were negative.

Brom phenol blue, in the presence of mercuric chloride, was bound by layers I and II, as well as by the enclosed parasite, and the host-liver cells. Layer II, however, stained more intensely than layer I, indicating that layer II has a somewhat higher concentration of total protein than layer I (fig. 2). Sections treated with BPB in the absence of mercury gave somewhat different results. Both layers of the cyst wall stained with equal intensity, as did the surrounding host-liver cells and the enclosed parasite. The cuticle of the parasite, however, stained heavily with the dye. From the foregoing, then, it may be assumed that layer I is primarily total protein. Layer II and the

TABLE 1. Results of Histochemical Tests on the Cyst Wall of *Neoechinorhynchus cylindratus* and Surrounding Host-liver Cells of *Lepomis* sp.

	Polysaccharides							
	PAS	PAS Diastase (0.5% @ 25° C 20 mins)	PAS Hyaluronidase (3 hrs @ 37° C)	$NaHSO_3$ Blockage (0.05M)	Best's	Best's Diastase	Bauer's	Bauer's Diastase
Layer I	+	+	+	−	+	+	+	+
Layer II	+++	−	+++	+++	+++	−	+++	−
Host-liver Cells	+++	−	+++	+++	+++	−	+++	−

	Proteins					
	BPB	BPB-$HgCl_2$	Sakaguchi	Millon	Romieu	Alk. Phosph.
Layer I	+	+++	++	++	−	−
Layer II	+	+	−	+	−	+++
Host-liver Cells	+	+++	−	+	−	−

+ = positive; ++ = strongly positive; +++ = very strongly positive; − = negative.

cuticle of the parasite are primarily basic protein since the presence of the mercury salt caused little change in their staining intensity during this procedure (fig. 3). Results of the various tests for specific amino acids further

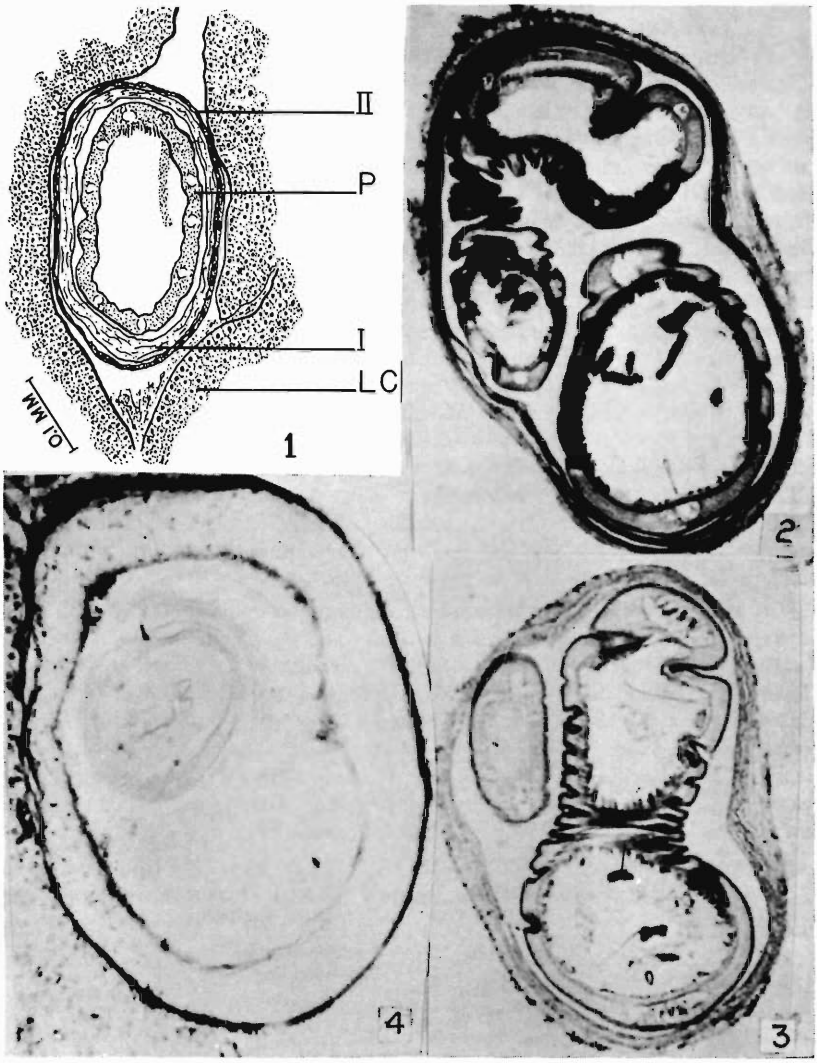

Figure 1. Camera lucida drawing of the cyst wall of *Neoechinorhynchus cylindratus* in the liver of *Lepomis* sp. LC—Host-liver cells; P—Parasite; I and II represent the inner and outer layers of the cyst, respectively.

Figure 2. Section of the cyst wall of *Neoechinorhynchus cylindratus* with enclosed parasite stained with BPB-$HgCl_2$. Note the concentration of total protein in layer I of the cyst wall.

Figure 3. Section of the cyst wall of *Neoechinorhynchus cylindratus* with enclosed parasite stained with BPB.

Figure 4. Section of the cyst wall of *Neoechinorhynchus cylindratus* with enclosed parasite. Sites of alkaline glycerophosphatase are black. Twenty-four hours incubation.

validated the premise of a two-layered cyst wall. Layer I contained protein rich in arginine and tyrosine as shown by the Millon and Sakaguchi reactions (Table I). Layer II, on the other hand, gave a faintly positive Millon reaction and was negative for tyrosine. Both layers were negative for tryptophane as indicated by the Romieu reaction.

The first sign of alkaline glycerophosphatase activity in the cyst wall appeared after sections were exposed to the incubating solution for 7½ hours. At this time, the deposits were found in layer II. The reaction in layer II increased proportionately as incubation time increased. The endothelial cells lining the liver sinusoids proved positive after 45 minutes of incubation. As the incubation time approached 24 hours, intranuclear deposits became evident within the host-liver cells, especially the nucleoli. The enclosed parasite as well as layer I were consistently negative for enzyme activity (fig. 4).

DISCUSSION

The above results present conclusive chemical evidence that the cyst wall in question is composed of two distinct layers. Layer I is composed of collagenous connective tissue as is indicated by (1) the negative PAS reaction obtained in this layer after blocking with sodium bisulfite, (2) the lack of tryptophane, a characteristic of collagen (White, Handler, et al, 1959), and (3) the reactions obtained with Mallory's and Gomori's histological staining techniques. Histologically, the cells constituting layer II are typically fibroblastic. The presence of alkaline glycerophosphatase also provides chemical evidence as to the nature and function of these cells. Washburn (1955) points out that the level of alkaline phosphatase in fibroblasts is raised coincident with the laying down of collagen during the wound healing process in rats. Likewise, Moog (1944) and Lorch (1949) report that phosphatase is important in connection with fiber formation.

The absence of alkaline phosphatase in the host-liver cells is in agreement with previous work done on vertebrate livers in general (Kabat and Furth, 1941, Gomori, 1941, and Danielli, 1953). In addition, the absence of the enzyme in the enclosed parasite is in agreement with the findings of Bullock (1949, 1958). Since it is highly unlikely that cells devoid of alkaline phosphatase (e.g. host-liver cells) would give rise to cells containing the enzyme (e.g. cells of layer II), the concept that the cells constituting layer II are modified liver cells as advanced by Ward (1940) appears to be erroneous. This reasoning also precludes the enclosed parasite, which is negative for the enzyme, from being the source of these cells. Moreover, it is generally agreed that cells of entodermal or ectodermal origin are not capable of becoming fibroblastic (Maximow and Bloom, 1957).

It is then probable that the cells constituting layer II are modified mesenchymal host-cells that have migrated to the site of the penetrating parasite. It is reasonable to conclude that the cells of layer II, being fibroblastic and of host origin, have secreted the fibrous inner layer of the cyst wall. In which event, the entire cyst can be considered to be of host origin. The process of encapsulation, in all probability, follows the typical vertebrate response to inflammation.

SUMMARY

On the basis of histological and histochemical tests, the cyst wall of juvenile forms of *Neoechinorhynchus cylindratus* found in the livers of certain fishes appears to be composed of two distinct layers.

The innermost layer of the cyst wall is collagenous connective tissue apparently elaborated by the thinner, outer layer which is composed of fibroblastic cells.

Alkaline glycerophosphatase is confined to the outer, fibroblastic layer of the cyst wall. The lack of the enzyme in the enclosed parasite and in the host-liver cells precludes either from being the source of the fibroblasts constituting this outer layer.

The entire cyst wall is presumed to be of host origin and is probably derived from host-mesenchymal cells rather than from host-liver cells.

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On the Identity of *Aproctella* in Birds in North America*

ROY C. ANDERSON

Anderson (1957) reported *Aproctella stoddardi* Cram, 1931 in *Zonotrichia albicollis, Geothlypis trichas, Hylocichla ustulata* and *Bonasa umbellus* from Algonquin Park, Ontario. Since then other specimens of *A. stoddardi* have been found in a *G. trichas,* two *Z. albicollis* and one *Seiurus aurocapillus* (L.), the latter being a new host record; these birds also came from Algonquin Park. Recently specimens of *A. stoddardi* from the body cavity of a *Pheucticus ludovicianus* (L.) from New Jersey were kindly forwarded to the author by Dr. T. K. R. Bourns of the University of Western Ontario and Dr. L. A. Stauber of Rutgers University. In identifying these latter specimens, which exhibited certain differences from material previously studied, it was necessary to re-examine in detail all the *Aproctella* collected in Ontario, many of which had been compared with types of *A. stoddardi.* No grounds could be found for doubting that the specimens from Ontario refer to *A. stoddardi,* and slight differences in those from *P. ludovicianus* were attributed to variability. It is unusual for a filarioid to have such a wide host distribution and the frequency of its occurrence makes it essential that its taxonomy be well established. Thus the following observations are worth publishing.

The dimensions of specimens from *P. ludovicianus* are close to those previously reported for *A. stoddardi,* viz.: FEMALE: length 9.5-10.7 mm.; oesophagus 0.33-0.41 mm.; vulva 1.0-1.2 mm. from cephalic extremity; tail 0.06-0.08 mm. MALE: length 5.6 mm.; oesophagus 0.36 mm.; right spicule 65 microns; left spicule 78 microns; tail 49 microns. MICROFILIARIA: on blood smears 145-177 microns, slightly contracted after being being smeared while living. The shortness of the adult tail is not regarded as significant since the tail length seems to be the most variable character in *A. stoddardi.* The tail in the male and three of the female specimens is compressed ventrally, but in two other females it has the same shape as in specimens previously assigned to *A. stoddardi* in 1957. Figs. 1-2 indicate the kind of variability found in the male tail.

The anus of the male is surrounded by about eight papillae, three pairs of which are lateral, the other two behind and in front of the anus. These papillae are exceedingly difficult to detect. In the male specimen described by Anderson (1957) the spicules were retracted and the pointed distal end of the left was folded medially, giving it a rounded appearance. Figs. 1-2 give a more accurate representation of the caudal end of the male.

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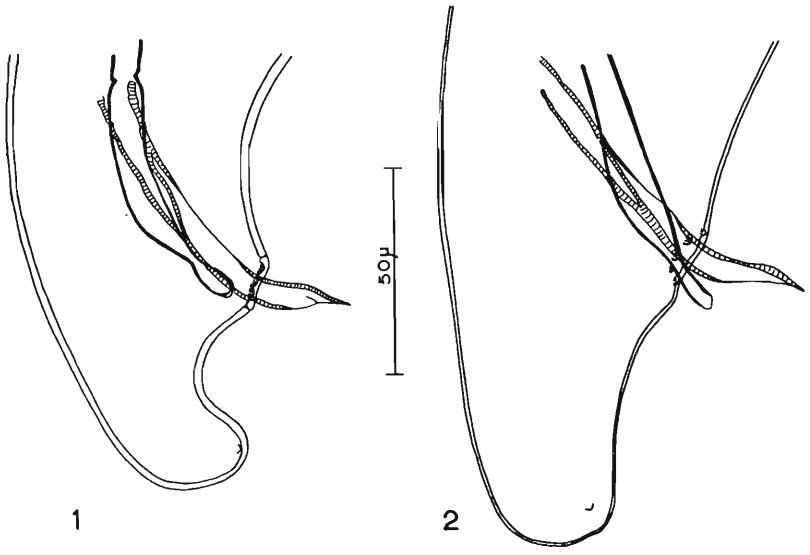

Aproctella stoddardi Cram, 1931

Fig. 1. Caudal end male, lateral view (from *Pheucticus ludovicianus*).
Fig. 2. Caudal end male, lateral view (from *Zonotrichia albicollis*).

Unfortunately, the microfilaria of *A. stoddardi* has not been described from the type host (*Colinus virginianus*). Anderson (1957) suggested *Mf. fallisi* Brinkmann, 1950, might be *A. stoddardi* but Dr. Brinkmann (*in litt.*) has pointed out differences in the fixed points of *Mf. fallisi* and *Mf. stoddardi* as described by Anderson. The view that these are distinct species is borne out by the failure to find *A. stoddardi* in numerous ruffed grouse infected with *Mf. fallisi* in Ontario. It will be important in the future to compare in detail the microfilariae of *Aproctella* in gallinaceous and passeriform birds.

An interesting feature of material collected in Ontario is the frequency that infertile females were found, viz., twice in *Geothlypis trichas*, once in each of *Hylocichla ustulata*, *Seiurus aurocapillus*, *Zonotrichia albicollis*, and *Bonasa umbellus*. In each instance, males were not found in the host.

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A Redescription of *Edesonfilaria malayensis* Yeh, 1960, with Remarks on its Systematic Position*

SATYU YAMAGUTI AND SUGIO HAYAMA

An entire male and fragments of two mature males and three injured gravid females were taken from abdominal cavity of *Macaca irus* imported from Thailand for the Monkey Center of Japan at Inuyama, Aichi Prefecture. They were found to belong to *Edesonfilaria malayensis* Yeh, 1960, but it seems desirable to redescribe this species on the basis of our own observation, because Yeh (1960) has made no mention of some important features of this worm. Further, he has not expressed his opinion concerning the systematic position of the genus in question.

MALE: Body filiform, 100-160 mm. in length by 0.1 mm. in maximum width, with three loose spiral turns at tapering posterior extremity. Cuticle finely annulated just under the smooth surface. Head blunt-pointed, with a pair of lateral amphids and two(?) pairs of submedian papillae. Mouth without lips. Nerve ring about 0.2 mm. from head end. Excretory pore unable to detect. Esophagus about 55 mm. long in the type 144 mm. long, divided into an anterior and a posterior portion. The anterior portion of the esophagus consists of a thick wall of longitudinal muscle fibers and a cuticular lining thrown into irregular folds; its anterior part 0.9-1.0 mm. long is free, but the remaining part surrounded by a series of mostly transverse patches of pigment granules is attached to the posterior portion and opens into it at a distance of 12 mm. from the anterior end of the latter portion, although the parietal muscle fibers are continued further backward along this portion. The posterior portion of the esophagus is a simple tube containing granular ingesta, with a thin membranous wall, on the inner surface of which lie numerous, large, flattened, round to oval cells at irregular intervals. This cell *sui generis* is 0.1-0.18 x 0.06-0.13 mm. as fixed in alcohol and measured in water and has a very thin cell membrane and contains numerous fine granules and strongly refractive droplet-like vesicles of varying calibers which in turn contain numerous fine granules, and fine vacuoles, too. No nucleus is recognizable even in the preparations stained with hematoxylin. There is no valve at the posterior end of the posterior portion of the esophagus. The intestine is less dark than that of the female. Tail blunt-pointed, only about 50 microns long. Caudal alae fleshy, 0.2-0.3 mm. long. There are about 30 pairs of caudal papillae, the posterior nine (5 preanal and 4 postanal) pairs being pedunculate and close to each other; the other papillae are sessile and gradually wider apart anteriorly, the anterior-most lying about 11.5 mm. from the tip of the tail. Right spicule elongated wedge-shaped, barbed at tip dorsally, 0.15-0.19 mm. long; left spicule very narrow, *undulating*, 9.5-10.0 mm. long, twisted near its simple point, somewhat enlarged at base, with a knob-like prolongation anteriorly. The slender tubular testis arises just at the point where the esophagus joins the intestine.

FEMALE: Total length unknown, with maximum width of 1.0 mm. Each lateral field consists of two close-parallel columns of syncytial cells which contain patches of brown pigment granules, while each median line shows a single pigment stripe. Nerve ring about 0.25 mm. from head end. Esophagus 86 mm. long by 0.14 mm. wide in one female, in which the posterior portion commences 0.57 mm. behind the head end. Posterior extremity

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rounded, slightly curved ventrad. Anus subterminal, opening about 0.15 mm. from tail end which has three papillae close together at the very tip. The posterior ovary arises some distance behind the anterior near the posterior end of the body as a narrow tube; each widens gradually as it describes two overlapping 8-shaped loops and then gradually narrows to lead into the muscular oviduct, which in turn is continued into the uterus. The paired uteri are comparatively short, but the ascending unpaired uterus occupies the greater part of the body length along with the esophagus and intestine. Vagina 5-6 mm. long, covered inside with minute nodular cuticular elevations, provided with inner longitudinal and outer circular muscle fibers; its anterior half is strongly twisted and provided with a thick coat of inner circular and outer longitudinal muscle fibers, while the posterior half is straight and devoid of thick muscular coat and gradually widening leads into the unpaired uterus. Uterine microfilaria unsheathed, very narrow, up to 140 microns long by 2.5 microns wide, with pointed tail devoid of nuclei; cephalic space nearly as long as wide. Vulva opening 1.2-1.5 mm. from head end, about 0.4-0.6 mm. behind anterior end of posterior portion of esophagus.

REMARKS: On cursory examination the present species may be referred to *Dirofilaria,* but in view of the peculiar structure of the esophagus, the unusual length of the left spicule associated with the extensive development of caudal papillae and the excessive length of the unpaired uterus it undoubtedly represents a distinct genus, for which Yeh proposed *Edesonfilaria* in honor of Dr. Edeson of Liverpool who supplied him with the material. Though assigned provisionally to the Dirofilariinae this genus may turn out to represent a new subfamily on account of the above mentioned characteristics. On the basis of our observation the original diagnosis of the genus given by Yeh is redefined as follows:

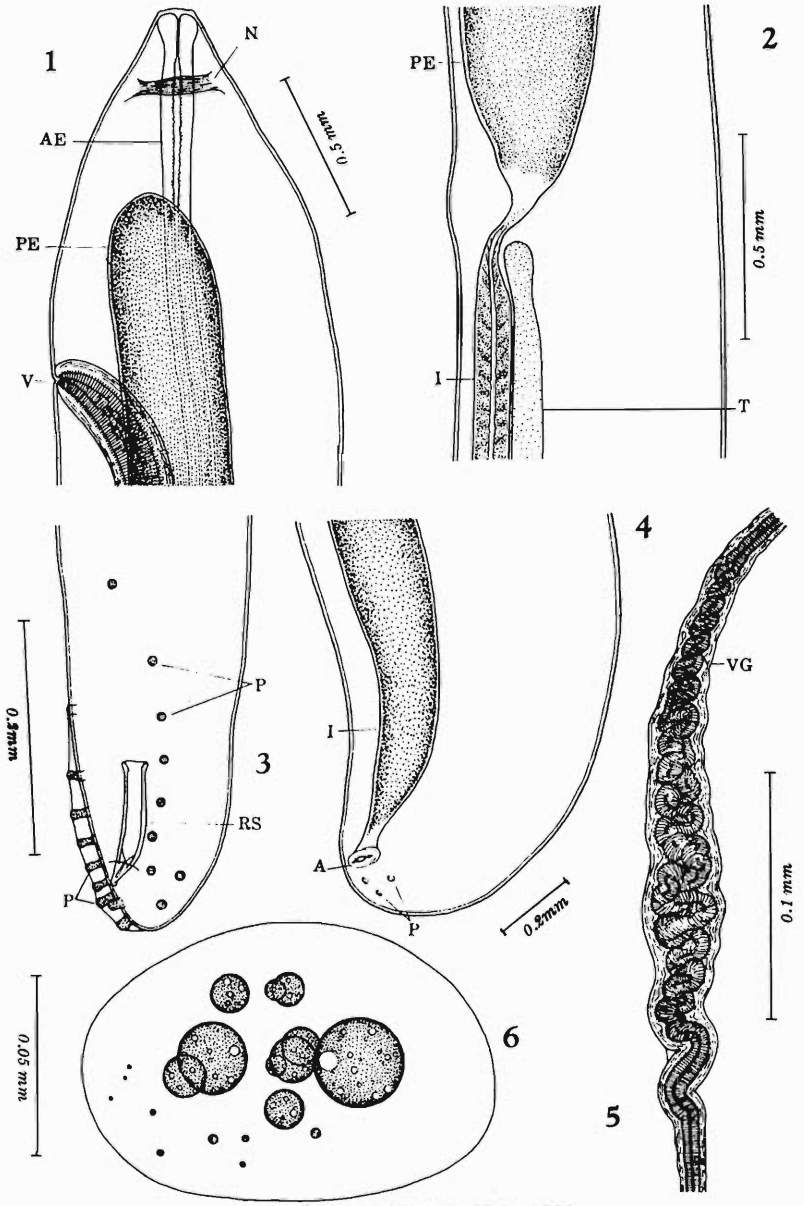
Edesonfilaria Yeh, 1960

GENERIC DIAGNOSIS: Dirofilariinae. Large worms, with very fine cuticular annulations. Mouth without lips; a pair of lateral amphids and two(?) pairs of submedian papillae on head. Esophagus consisting of a long, muscular, anterior portion and an unusually long, simple, tubular, posterior portion; both portions fused alongside each other for a considerable distance; posterior portion is characterized by dark granular contents and membranous walls, on the inside of which are scattered numerous giant cells containing droplets and granules.

MALE: Body filiform; posterior extremity tapered and spirally coiled, with symmetrical fleshy caudal alae supported by pedunculate subventral papillae which are continued forward by a number of widely spaced minute sessile papillae. Tail very short, blunt-pointed. Testis originating at junction of posterior esophagus and intestine and running backward. Spicules very unequal, dissimilar.

FEMALE: Body much longer and wider than male; posterior extremity rounded, curved ventrally. Lateral field with double columns of syncytial cells containing brown pigment patches, each median line with a single pigment stripe. Opisthodelphys; unpaired uterus occupying greater length of body; distal portion of vagina twisted and provided with thick coat of inner circular and outer longitudinal muscle fibers; vulva just posterior to anterior end of glandular esophagus. Microfilaria very fine, unsheathed. Parasitic in body cavity or connective tissue of primates.

TYPE SPECIES: *E. malayensis* Yeh, 1960.



Edesonfilaria malayensis Yeh, 1960

Fig. 1. Anterior extremity of female, lateral view.
Fig. 2. Esophago-intestinal region of male, lateral view.
Fig. 3. Posterior extremity of male, ventrolateral view; left spicule not shown.
Fig. 4. Posterior extremity of female, ventrolateral view, uterus not shown.
Fig. 5. Winding vagina.
Fig. 6. Oval flat giant cell *sui generis* lining walls of posterior portion of esophagus.

A—anus, AE—anterior portion of esophagus, I—intestine, N—nerve ring, P—papilla, PE—posterior portion of esophagus, RS—right spicule, T—testis, V—vulva, VG—vagina.

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Treatment of Experimental Lungworm Infections in Calves and Pigs with Cyanacethydrazide

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Lungworm diseases are responsible for considerable loss to livestock producers throughout the world. The principal effects of these infections are usually reflected in uneconomical weight gains, lowered production, and secondary invasion by debilitating bacterial and viral diseases. Mortality may be high in some instances. In large measure, the resultant losses are ascribable to a lack of safe, effective treatments and other efficient control methods.

In 1957, a drug (cyanacethydrazide) was developed which showed promise of being a major advancement in the battle against parasitic lungworm diseases. Walley (1957a, 1957b, 1957c) reported the results of extensive trials with the chemical against lungworms in calves, sheep, goats, and pigs. The drug showed action against worms that normally occur in the air passages, but it was ineffective against tissue-dwelling species and migrating larvae. Especially noteworthy was the disclosure that the drug exhibited anthelmintic action when given orally, subcutaneously, or intramuscularly—all routes that are preferable to intratracheal injections and aerosol inhalations which have been used, or suggested for use, in the past.

Several workers, including Dick (1958), Groves (1958), Jackson (1958), O'Donoghue (1958) and Trace (1958), soon reported favorably on the action of the chemical against lungworm infections in several classes of livestock. Other investigators, however, notably Allan (1957), Gripper (1957), Enigk *et al.* (1958), Guilhon and Petit (1958), Enigk and Duwel (1959), and Swanson *et al.* (1959), obtained less satisfactory results with the chemical. The latter group of workers noted that the chemical was of no value in saving lives or modifying the resultant pathological or clinical changes in naturally or experimentally infected animals, and that some of the response observed in treated animals may have been ascribable to concurrent improve-

*The cyanacethydrazide (Dictycide and Helmox) used in this study was supplied by Fort Dodge Laboratories, Inc., Fort Dodge, Iowa.

ment in feeding and other husbandry practices. Rubin (1959) reported that the critical period for treatment of lungworm infections in cattle, to obtain maximum reduction in worm burdens, was about 5 days following the beginning of patency. This illustrates one of the limitations of the treatment under field conditions, namely, unreliable or ineffective action against preadult stages of the parasites.

Limited trials to determine the efficacy of cyanacethydrazide* against experimental infections of *Dictyocaulus viviparus* in calves and *Metastrongylus apri* and *M. pudendotectus* in pigs form the basis of this report. The data were obtained at intervals over a period of several months, from July 1957 to October 1958.

MATERIALS AND METHODS

CALVES. The 12 grade calves used in these trials were obtained from local dairies when only a few days old. They were maintained continuously in concrete-floored pens. At 65 to 79 days of age, each calf was given 5,000 to 5,500 infective larvae of the lungworm *D. viviparus*. Beginning 31 days after infection, and continuing daily until the animals were necropsied, 24-hour fecal collections were made from each animal for larval counts. The daily accumulation of feces was weighed, thoroughly mixed, and a 100-gram sample was taken. Routinely, these samples were put through a Baermann apparatus for 24 hours and the larvae were estimated by dilution counts. The average of 2 counts was used to calculate the daily larval output.

Three experiments, each involving 4 animals, were completed. In each case, 3 calves were given medication as noted below and the fourth was retained as an untreated control. Treatment of principals was initiated from 35 to 42 days after infection, and all principals were necropsied on the fourth day after the final dose was given. The control animal was necropsied on the same day as the last principal in the group. In each experiment, the treatment regimen for the several animals was designed to avoid more than two necropsies on any one day. All calves were killed with a captive bolt pistol and the jugular veins severed immediately to permit rapid bleeding. Particular care was taken to avoid cutting the trachea in order to prevent the inhalation of large amounts of blood. The lungs and trachea were removed, and the air passages were methodically opened and carefully searched for lungworms. Finally, the thoroughly dissected lungs were submerged in normal saline solution and held overnight in an attempt to assure maximum recovery of worms. The resultant sediment and solution were then washed through a fine-meshed screen and the residue examined for lungworms.

Most of the calves were given subcutaneous injections of cyanacethydrazide (Dictycide) at the recommended dose rate of 15 milligrams per kilogram of body weight; one calf (4051, Exp. III) was given the drug (Helmox) by drench at a slightly higher rate, 20.0 milligrams per kilogram, than that recommended (17.5 milligrams per kilogram) for this route of administration.

PIGS. The 14 pigs used in these trials were each given 650 to 750 mixed infective larvae of *M. apri* and *M. pudendotectus* at about 8 weeks of age. Two experiments involving 6 and 8 animals, respectively, were completed. In each case the principals received medication as noted below, and 2 pigs were retained as untreated controls. Treatment of the principals was initiated 2 to 3 months after infection, and all principals were necropsied 2 days after the final dose was given. The control animals were necropsied on the same day as the principals of each experiment. The pigs were stunned and the

jugular vein was severed immediately to permit rapid bleeding. The trachea and lungs were thoroughly examined in the manner previously described.

All principals in trial 1 were given subcutaneous injections of Dictycide at the rate of 20 milligrams per kilogram of body weight, a dosage slightly larger than recommended. In the second trial, the chemical was given at the recommended rate of 100 milligrams per 14 pounds of body weight, with a maximum dose of 1 gram.

The sterile solutions of cyanacethydrazide for parenteral injection were freshly prepared immediately before use in accordance with recommendations. The treatment regimen was varied among the several principals in an attempt to obtain maximum information on the anthelmintic action of the chemical with the limited number of animals available for the investigation.

RESULTS

The results obtained with calves are summarized in tables 1 and 2. The necropsy worm count data indicate that medication apparently had little influence on the degree of parasitism in most instances. A good treatment response was obtained in the first trial with recovery of the fewest worms from the animal that received the most intensive medication. The control calf, however, had a self-limiting infection which apparently terminated after about 7 weeks. The necropsy worm counts did not reflect a similar response in the other groups.

By and large, the day-to-day larval counts of most animals were irregular and did not seem to be markedly influenced by medication. The final larval count, obtained on the day of necropsy, was invariably lower than that recorded on the last treatment occasion; but a similar progressive reduction was observed in all controls.

The only noticeable sign of discomfort was a slight, transient irritation at the site of injection. Coughing may have increased, however, in some instances. Necropsy findings ranged from small, isolated areas of hepatiza-

TABLE 1. Results of treatment with cyanacethydrazide in experimentally infected calves.

Experiment No.	Animal No.	Weight (lbs.)	Treatments[a]	Necropsy: Days after treatment initiated	Necropsy: Lungworms recovered	Remarks
I.	4027	183	1	4	88	Slight hepatization
	4029	188	2[b]	5	37	Moderate purulent pneumonia
	4028	155	3[c]	24	7	Marked purulent pneumonia
	4030	140	None	....	0	Very slight hepatization
II.	4085	220	1	4	5	Slight hepatization
	4086	212	2[b]	5	13	Moderate hepatization
	4083	110	3[b]	6	60	Moderate purulent pneumonia
	4084	235	None	....	48	Moderate purulent pneumonia
III.	4049	151	1	4	128(8)*	Marked purulent pneumonia
	4051	132	3[b]	6	87(1)*	Marked purulent pneumonia
	4048	155	3[d]	5	448	Marked purulent pneumonia
	4052	141.5	None	....	45	Marked purulent pneumonia

[a] Dictycide except calf 4051 (Helmox)
[b] Given on successive days
[c] Given on days 1, 5, and 21
[d] Given within a 24-hour period
( )* Additional larval forms

TABLE 2. Daily Larval Counts[1] of Experimentally Infected Calves Treated with Cyanacethydrazide

	Experiment I				Experiment II				Experiment III			
Day	Calf 4027	Calf 4029	Calf 4028	Calf 4030	Calf 4085	Calf 4086	Calf 4083	Calf 4084	Calf 4049	Calf[2] 4051	Calf 4048	Calf 4052
1	278	530	163	398	5	558	1259	253	368	368	1373	201
2	152	580	705	1056	2	410	779	114	160	228	752	75
3	318	1398	1362	955	3	589	1447	126	73	339	720	72
4	209	442	617	834	2	728	872	209	113	349	1197	84
5	* T	914	568 T	2741	9	554	1307	166	150	348	1197	105
6	363	462	434	2218	13	781	2614	247	182	325	945	59
7	628	406	1775	1329	7	776	1871	342	123	321	978	66
8	32	1320	347	1475	7 T	745	1561	80	155 T	157	1513 T	53
9	42	754	359 T	795	4	415	1143	211	185	320 T	2070 T	59
10	(88)	359	111	581	0	654	1689 T	232	198	279 T	1613	48
11		231 T	40	517	0	228 T	2912 T	277	86	100 T	1073	21
12		373 T	227	1192	0	850 T	850 T	32	136	145	775	32
13		205	266	605	(5)	439	762	66	(128)	130	542	36
14		155	139	702		145	334	41		35	(448)	47
15		49	93	295		124	191	42		23		20
16		48	150	233		14	29	11		(87)		(45)
17		(37)	52	189		(13)	(60)	(48)				
18			109	88								
19			445	42								
20			332	203								
21			363	8								
22			67	0								
23			14	0								
24			59	0								
25			49 T	0								
26			60	0								
27			9	0								
28			0	0								
29			18	0								
			(7)	(0)								

[1] = To the nearest thousand larvae
[2] = Given Helmox; all other principals given Dictycide
T = Treated this date
* = Aliquot discarded by error
( ) = Adult worms recovered at necropsy

TABLE 3. Results of treatment with cyanacethydrazide in experimentally infected pigs.

Experiment No.		Animal No.	Weight (lbs.)	Treatments (No.)	Necropsy: Days after treatment initiated	Necropsy: Lungworms recovered *M. apri*	Necropsy: Lungworms recovered *M. pudendotectus*	Remarks
I.	A.	763	18.0	1	2	18	11	
		764	16.5	2[a]	3	15	6	
		759	34.0	None	....	14	11	
	B.	761	17.0	2[b]	17	13	8	
		762	15.0	2[b]	17	18	14	
		760	22.0	None	....	11	4	
II.	A.	833	150.0	1	3	531(11)*	19	Slight hepatization
		828	84.0	1	3	539(12)*	44	Slight hepatization
		835	145.0	None	....	254(6)*	22	Slight hepatization
	B.	832	145.0	3[c]	5	393	53	
		831	146.0	3[c]	5	618	46	
		830	175.0	2[d]	5	134	8	
		829	150.0	2[d]	5	341(17)*	24	
		834	130.0	None	....	389(24)*	57	

[a] Given within a 24-hour period
[b] Given on days 1 and 15
[c] Given on successive days
[d] Given on alternate days
( )* Larval Forms

tion to involvement of approximately one-third of the lung tissue. A purulent exudate was found in the bronchi and bronchioles of the more severly affected calves, and most animals had excessive amounts of mucus in the trachea.

The results obtained with pigs are summarized in table 3. The necropsy worm count data show that, by and large, medication had little effect on worm burdens.

Treatment was well tolerated; there appeared to be only slight temporary irritation at the site of injection. Necropsy findings showed only small areas of hepatization in some of the lungs. Exudate in the bronchi and bronchioles was limited, and there was very little mucus in the trachea.

DISCUSSION

In these studies, the treatment of experimental infections of lungworms, *D. viviparus* in calves and *M. apri* and *M. pudendotectus* in swine, with cyanacethydrazide was of little or no value. Indeed, necropsy worm counts showed that, in general, the principals harbored as many parasites as the controls, irrespective of the regimen employed. There was, moreover, no discernible clinical improvement in the treated animals.

Lungworm infections may result in bronchitis, atelectasis, pneumonia, and occasionally death. On the other hand, spontaneous recovery may occur in consequence of immunological reactions or in response to improved dietary, sanitary, or other management practices. Indeed, lungworm disease, to a greater extent, perhaps, than any other parasitic infection, is inclined to be short-lived and self-limiting. These several factors may lead to erroneous conclusions with respect to the influence of specific medication on the course of lungworm infections. Favorable field reports of effective treatments for this condition, therefore, should be judged accordingly.

SUMMARY

In limited trials, cyanacethydrazide (Dictycide; Helmox) showed no promise of effective action against experimental infections of *Dicytocaulus viviparus* in calves and *Metastrongylus apri* and *M. pudendotectus* in pigs. Necropsy worm count data of principals and controls showed that medication had little effect on the worm burdens of treated animals. The chemical was used in appropriate dosages and in accordance with recommended procedures.

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Trichuris stansburyi and *Gongylonema mysciphilia*, Two New Species of Nematodes from the Deer Mouse in Utah*

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The nematodes herein described were recovered from formalin-preserved hosts. They were cleared in Amann's lactophenol and examined in temporary mounts. All measurements are in millimeters and were made with the aid of the camera lucida.

Trichuris stansburyi, n. sp.

One complete male (the holotype) and several complete females (allotype and paratypes) were recovered. The sample of 20 eggs measured was drawn from several females.

MALE: Length 22.6; length of esophageal portion of body 13.2, width measured 0.38 anterior to posterior termination of esophagus 0.168-0.196; length of post-esophageal portion of body 7.5-9.0, maximum width 0.32-0.4. Esophageal portion of body tapers anteriorly to width of 0.016 in holotype. Shaft of spicule tapers gradually to base of rather blunt point. Length of spicule 0.860-1.160, width at proximal end 0.027-0.052, width at middle of shaft 0.022-0.029, width 0.008 from tip, where it begins to narrow abruptly to tip, 0.012-0.015. Spicule sheath armed with scale-like spines throughout length. Fully extruded spicule sheath flared at distal end (Fig. 2), 0.04 wide at proximal end, 0.072 wide at distal end. Maximum measured length of protrusion of spicule sheath 0.2. Spicular tube joins cloacal tube approximately in mid-length of latter. Distance from anus to junction of spicular and cloacal tubes 0.32-0.68; length of cloacal tube 0.88-1.28. Length of ejaculatory duct and vas deferens measured in 1 specimen 1.74 and 3.92 respectively, ratio being 1:2.25. Ejaculatory duct and vas deferens separated by simple constriction. Tail of male not lobulated.

*This study was supported by a grant (G-5280) from the National Science Foundation.

FEMALE: Length 22.1-29.9; length of esophageal portion of body 9.1-16.4, width 0.38 anterior to posterior termination of esophagus 0.152-0.212; length of pre-vulvar portion of body 9.15-16.45, vulva located 0.05-0.1 posterior to termination of esophagus. Body narrows to vulva, width at vulva 0.192-0.236. Lips of vulva only slightly protuberant (Fig. 5). Length of post-esophageal portion of body 10.1-15.1, ratio of length of esophageal portion of body to post-esophageal portion ranging from 9:9.5 to 3:4. Ovejector 0.553-0.880 long, 0.035-0.105 wide, width being nearly constant throughout length. Anus subterminal. Length of rectum 0.185 in the 1 specimen where its course and anterior limit could be observed clearly. Posterior termination of body of a characteristic nipple-shape (Fig. 4). Measurements of a sample of 20 eggs: length: range 0.051-0.063, sample mean 0.0597, sample standard deviation 2.8; width: range 0.26-0.030, sample mean 0.0285, sample standard deviation 5.4.

HOST: Deer Mouse *Peromyscus maniculatus sonoriensis* (LeConte).

HABITAT: Caecum.

LOCALITY: Stansbury Island, Great Salt Lake, Tooele County, Utah. Elevation 5,300 feet. Collected July, 1960.

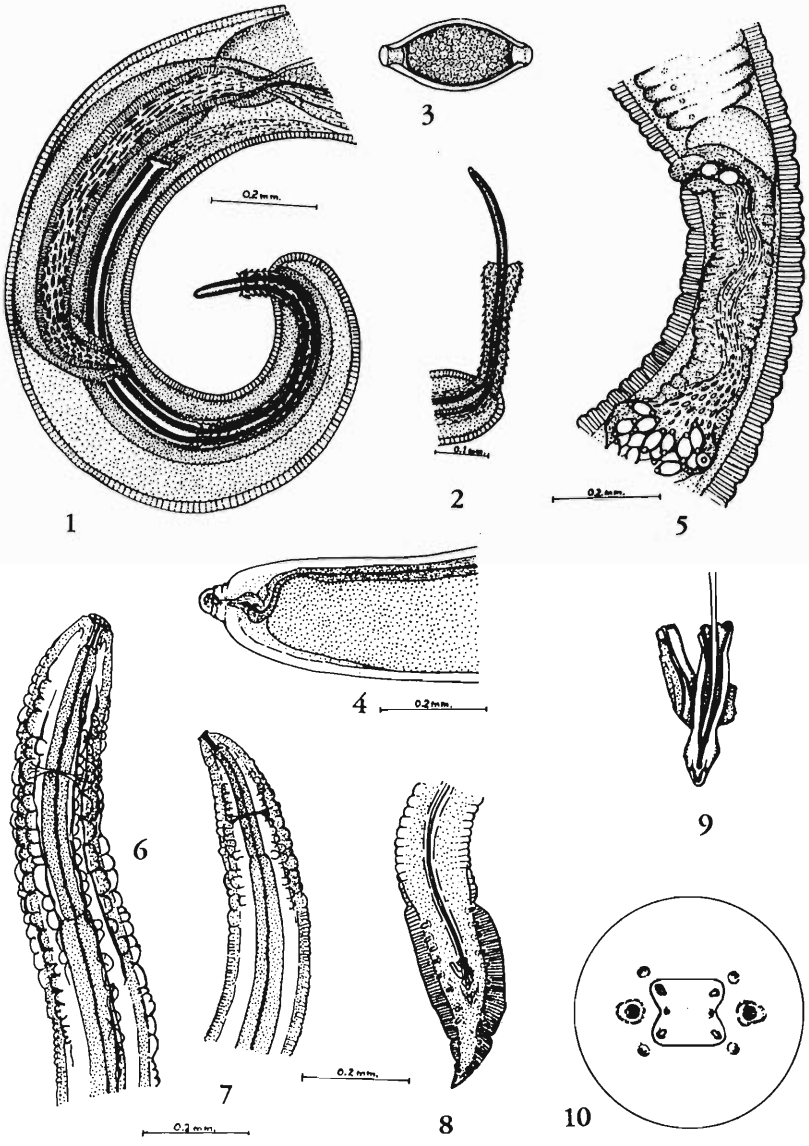
REPOSITORY OF TYPES: Holotype and allotype in Helminthological Collection, United States National Museum, Washington, D. C. Paratypes in Museum of Zoology, University of Utah, Salt Lake City.

In addition to other characters, *Trichuris stansburyi* is distinguished from the other species of *Trichuris* found in American rodents and lagomorphs by the form of the tail of the female. The possession of a vas deferens more than twice the length of the ejaculatory duct readily distinguishes the males of *T. stansburyi* from those of *T. leporis* (Froelich, 1789) and *T. citelli* Chandler, 1945. *T. stansburyi* is distinguished from *T. madisonensis* Tiner, 1950 by the shape and dimensions of the eggs and by the much longer maximum extension of the spicule sheath in *T. stansburyi*. Males of *T. opaca* Barker and Noyes, 1915, and *T. perognathi* Chandler, 1945, differ from those of *T. stansburyi* by the form of the exserted spicule sheath and by the proportionate position where the spicular tube joins the cloacal tube. The bilobate cauda of the males of *T. neotomae* Chandler, 1945, distinguish them from the males of *T. stansburyi*. The spicule sheath of *T. sylvilagi* Tiner, 1950 is unarmed and the ejaculatory duct of the male is much longer than in the male of *T. stansburyi*. The spicular tube joins the cloacal tube proportionately nearer to the anus in the males of *T. peromysci* Chandler, 1946, and *T. dipodomis* Read, 1956, than in males of *T. stansburyi*.

Gongylonema mysciphilia, n. sp.

One complete adult member of each sex was recovered. Repeated trapping at the type locality and elsewhere in the Lake Bonneville Basin has failed to produce additional infected rodents.

MALE: Length 10.5; maximum width 0.174; buccal capsule 0.032 long, 0.007 wide; length of anterior esophagus 0.435; length of posterior esophagus 2.175, total length of esophagus 2.610; cervical alae originate 0.043 from anterior end extending to 0.370 from anterior end; distance from anterior end to cervical papillae 0.100, to nerve ring 0.156, to excretory pore 0.300.



All drawings were made with the aid of the camera lucida.

Trichuris stansburyi, n. sp.

Fig. 1. Posterior end of male; Fig. 2. Extruded spicule sheath; Fig. 3. Egg; Fig. 4. Posterior end of female; Fig. 5. Vulvar region of female.

Gongylonema mysciphilia, n. sp.

Fig. 6. Anterior end of female; Fig. 7. Anterior end of male; Fig. 8. Posterior end of male; Fig. 9. Arrangement of spicules and gubernaculum; Fig. 10. Female *en face*.

Bosses scattered irregularly in longitudinally orientated clumps originating 0.119, ending 0.356 from anterior end. Length of tail 0.175. Left spicule 1.13 long × 0.008 wide at base; right spicule 0.072 long × 0.015 wide at base. Gubernaculum (Fig. 9) 0.082 long; 6 pairs pre-anal papillae and 5 pairs post-anal papillae. Caudal alae asymmetrical, left ala 0.450 long, terminating 0.050 from tip of tail, right ala 0.346 long, terminating 0.046 from tip of tail.

FEMALE: Length 40.7; width at vulva 0.326; buccal capsule 0.040 long × 0.008 wide; length of anterior esophagus 0.48, length of posterior esophagus 3.26, total length of esophagus 3.74. Cervical alae festooned with numerous divisions, originating 0.06, terminating 0.88 from anterior end; bosses scattered irregularly in longitudinally orientated clumps originating 0.139. terminating 0.964 from anterior end. Distance anterior end to nerve ring 0.266; length of tail 0.2; vulva 4.88 from posterior end. 6 inner, 4 outer, and 2 amphidial cephalic papillae (Fig. 10). Measurements of sample of 10 embryonated eggs: length: range 0.054-0.067, sample mean 0.0584, sample standard deviation 3.4; width: range 0.034-0.039, sample mean 0.0366, sample standard deviation 1.5.

HOST: *Peromyscus maniculatus sonoriensis* (LeConte).

HABITAT: Caecum.

LOCALITY: Stansbury Island, Great Salt Lake, Tooele County, Utah. Elevation 4,210 feet. Collected April, 1960.

REPOSITORY OF TYPES: Museum of Zoology, University of Utah, Salt Lake City.

Members of the present species are apparently unique in being found in the wall of the caecum of their host. *G. mysciphilia* differs from *G. neoplasticum* Fibiger and Ditlevsen, 1914, emended Ransom and Hall, 1915, in that the caudal alae of the male do not extend to the tip of the tail in the former species whereas they do in *G. neoplasticum*. The cervical alae in both sexes of *G. neoplasticum* begin much farther posteriorly than they do in *G. mysciphilia*. The width of the buccal capsule in both sexes of *G. neoplasticum* is much greater than in *G. mysciphilia*. These species also differ with regard to length and width of left spicule, length of esophagus, arrangement of cuticular bosses, and the number of pre-anal papillae in the male.

G. mysciphilia differs from *G. dipodomysis* Kruidenier and Peebles, 1958, in that cervical papillae and alae are lacking in the male of *G. dipodomysis* and the shape of the gubernaculum differs in the two species as does the extent of the caudal alae of the male. The sheath of the left spicule is not thickened in *G. mysciphilia* as it is in *G. dipodomysis*.

In *G. mysciphilia* the internolateral cephalic papillae are much smaller than the internodorsals and the internoventrals, whereas the internolaterals are the largest of the internal circle of papillae in *G. peromysci* Kruidenier and Peebles, 1958. The amphidial papillae are much larger than the externodorsal and the externoventral papillae in *G. mysciphilia*, whereas the reverse situation prevails in *G. peromysci*. These two species also differ with regard to shape of gubernaculum, number of divisions of the cervical alae, arrangement of the bosses, and extent of the caudal alae of the male.

Two Species of Sheep Coccidia New to Alabama

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During routine fecal examinations of lambs at the Piedmont Substation of the Alabama Experiment Station, Camp Hill, Alabama, unusually large oocysts were occasionally detected and eventually identified as *Eimeria ah-sa-ta* Honess, 1942 (Fig. 1, A). Another coccidium was identified as *Eimeria crandallis* Honess, 1942 (Fig. 1, B). These identifications were the first in Alabama of these two species. They have since been identified in feces of lambs at the Alabama Agricultural Experiment Station at Auburn. The identifications were confirmed by Ralph F. Honess of the Wyoming Agricultural Experiment Station who originally described both species from sheep in Wyoming (1942).

The occurrence of these two species in this area may be attributed to the presence of sheep imported from Montana and other Western states. Examination of feces from both Targhee and Rambouillet ewes within 12 hours after they were unloaded from a Montana shipment revealed both *E. crandallis* and *E. ah-sa-ta*. The former species was present in all ewes but the latter was found in only a few.

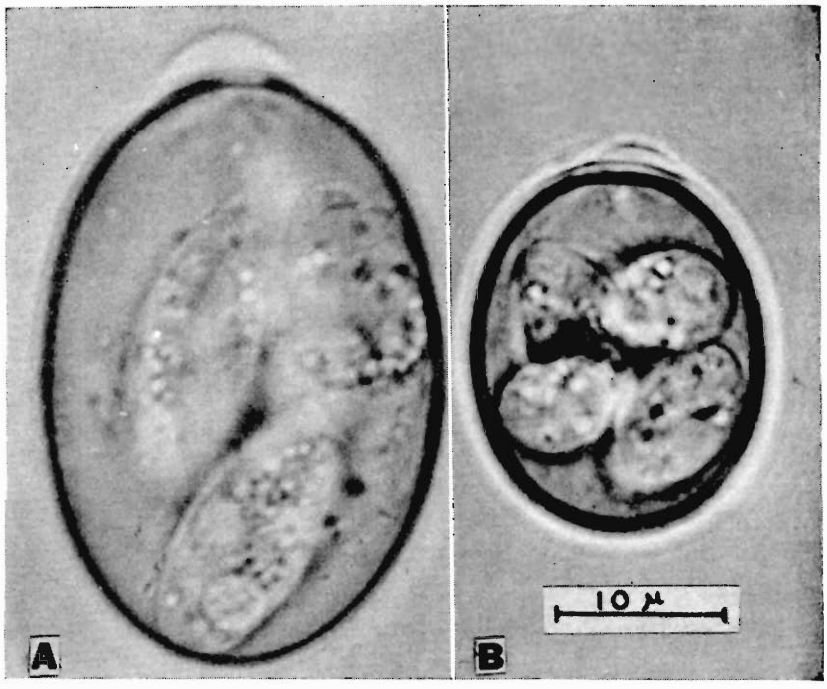

Fig. 1. Sporulated oocysts from sheep in Alabama, showing comparative sizes. The 10μ scale applies to both. A. *Eimeria ah-sa-ta*. B. *Eimeria crandallis*. After flotation in sugar solution the polar cap, which is normally located apically, may be off-center, as shown, or missing.

The occurrence of these two species in local lambs differs considerably. *Eimeria crandallis* is more prevalent and the number of oocysts per gram of feces may range from very few to several millions. On the other hand, oocysts of *E. ah-sa-ta* occur only occasionally in fecal samples and so far have always been found singly or in small numbers. In size, *E. crandallis* ranges from 16 to 26 microns (average 21); *E. ah-sa-ta* ranges from 35 to 43.5 microns (average 38.5). These data are based on measurements of more than 200 oocysts of each species.

Since these species were new to this area and there is no record of pathogenicity, lambs were experimentally infected for studies of pathogenicity (Smith, *et al.*, 1960). Thus far, inoculations of 100,000 to 3,000,000 infective oocysts of *E. crandallis* have not produced any noticeable harmful effects, but inoculations of infective oocysts of *E. ah-sa-ta* have been highly pathogenic, particularly in young lambs. After 15 lambs were each given 100,000 to 800,000 of these oocysts, 7 died as a result of their infections. The lambs were 6 to 15 weeks old at the time of death. Beginning about 15 days after the inoculations, the observed signs of disease were: the passage of soft stools gradually changing to liquid consistency; increasing listlessness and reduction in feed intake resulting in emaciation; inability to rise about the 22nd day, and then death.

Three lambs, each inoculated with 100,000 oocysts, died on the 23rd day after the inoculations were given. Post-mortem examinations of the intestines of these lambs revealed considerable inflammation of the lower ileum and slight inflammation of Peyer's patches. There were few macroscopic schizonts and only a few areas indicating the developmental stages of gametocytes. Histological sections contained both microgametes and macrogametes in late stages and some nearly mature oocysts, all in the lower part of the ileum.

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SMITH, W. N., L. R. DAVIS and G. W. BOWMAN. 1960. The pathogenicity of *Eimeria ah-sa-ta*, a coccidium of sheep. J. Protozool. 7 (Suppl.): 8.

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