VOLUME 22 JULY, 1955 NUMBER 2

PROCEEDINGS

of

# The Helminthological Society of Washington

**A semi-annual journal of research devoted to Helminthology and all branches of Parasitology**

Supported in part by the
Brayton H. Ransom Memorial Trust Fund



**Subscription $3.00 a Volume; Foreign, $3.25**

Published by

THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

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# THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

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PROCEEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

VOLUME 22 JULY, 1955 NUMBER 2

# *Radopholus gracilis* (De Man, 1880) n. comb. (Synonym—*Tylenchorhynchus gracilis* (De Man, 1880) Filipjev, 1936)

HEDWIG HIRSCHMANN*

In 1880 De Man first described *Tylenchus gracilis* from moist woodland and pasture soils in Holland. A more complete description with excellent illustrations was published four years later by the same author. With the passage of time certain changes from the original description of this species were made in the literature hindering the proper classification of the nematode. The purpose of this paper is to clarify certain taxonomic points and designate the correct taxonomic status of the species.

Micoletzky (1917, 1921, 1923, 1925), Imamura (1931) and other authors (Rahm, 1924, Franz, 1942, Franz & Beier, 1942, Hirschmann, 1952) subsequently reported that they found this species, and the descriptions which they gave were in essential agreement with De Man's original description.

In the first quarter of the twentieth century the genus *Tylenchus* was divided into several new genera (*Tylenchorhynchus* Cobb, 1913, *Eutylenchus* Cobb, 1913, *Paratylenchus* Micoletzky, 1921, *Chitinotylenchus* Micoletzky, 1921, *Psilenchus* De Man, 1921). Thus in Micoletzky's publications this species was initially placed in the genus *Chitinotylenchus*, and subsequently in the genus *Tylenchorhynchus*. At that time, however, the generic diagnosis of *Tylenchorhynchus* was very restricted, being applied mainly to the structure of the head. In differentiating between *Tylenchus* and *Tylenchorhynchus* Micoletzky (1921) wrote: "Streng genommen ist der einzige Unterschied die chitinige Stachelkappe." The importance of the structure of the esophagus and gonads was not emphasized.

In his revision of the genus *Anguillulina* (1932) Goodey included a detailed description of *Anguillulina gracilis* based on the statements of De Man, Micoletzky and Imamura. He also presented an illustration, presumably redrawn from the original of De Man (Fig. 2, 76). However, a definite basal esophageal bulb is shown in Goodey's illustration, whereas this distinct bulb was not present in De Man's drawing of 1884 (Fig. 1, 96). In this paper De Man stated: ". . . den hinter dem Bulbus liegenden Theil des Oesophagus konnte ich an den wenigen beobachteten Thieren nicht mit Bestimmtheit sehen." Furthermore, Goodey's reproduction of De Man's original drawing with a definite basal esophageal bulb was contrary to the description given by him, since he also mentioned the indistinctly-formed posterior esophageal region. Because of this change, made in the redrawing of one of De Man's original illustrations, the correct taxonomic relationship of this nematode has inadvertently been obscured.

Filipjev (1934, 1936) presented a new classification of the Tylenchida wherein he established a number of new genera and emended the genus

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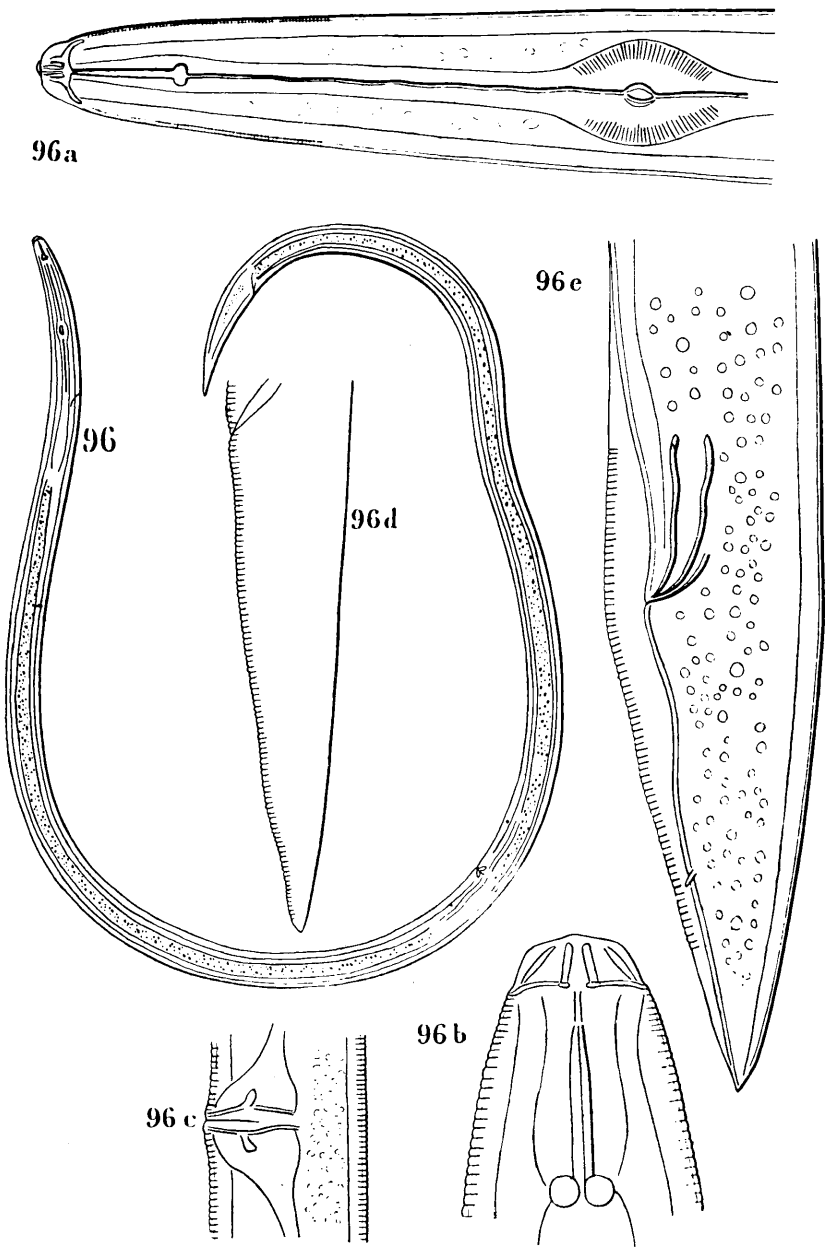


Fig. 1. Photograph of drawing of *Tylenchus gracilis* De Man, 1880, copied from the original by De Man, 1884. 96 Young female; 96a Anterior portion of body; 96b Head; 96c Vulvar region; 96d Female tail; 96e Male tail.

*Tylenchorhynchus* as follows: "Head chitinized, cuticle coarsely striated, spear strong, esophagus tylenchoid, ovaries double." In addition to other species, *Tylenchus gracilis* was included in the genus *Tylenchorhynchus*. However, Filipjev apparently did not examine material of this species and based his classification on Goodey's figure. The same drawing of Goodey after De Man is also shown in the Manual of Agricultural Helminthology by Filipjev & Schuurmans Stekhoven (1941). In the description of *Tylenchorhynchus gracilis* in this text the following statement was made: "Excretory pore at a level with the fore end of posterior bulb."

In an examination of specimens of *Tylenchus gracilis* collected at different locations in Germany, the author found that they conformed to De Man's original description of the species. Rather than possessing a definite basal bulb, the basal portion of the esophagus was lobed, extending back over the intestine. The paired opposed outstretched gonads of the female, the form of the head and the tail, as well as other features, all indicate that *Tylenchus*

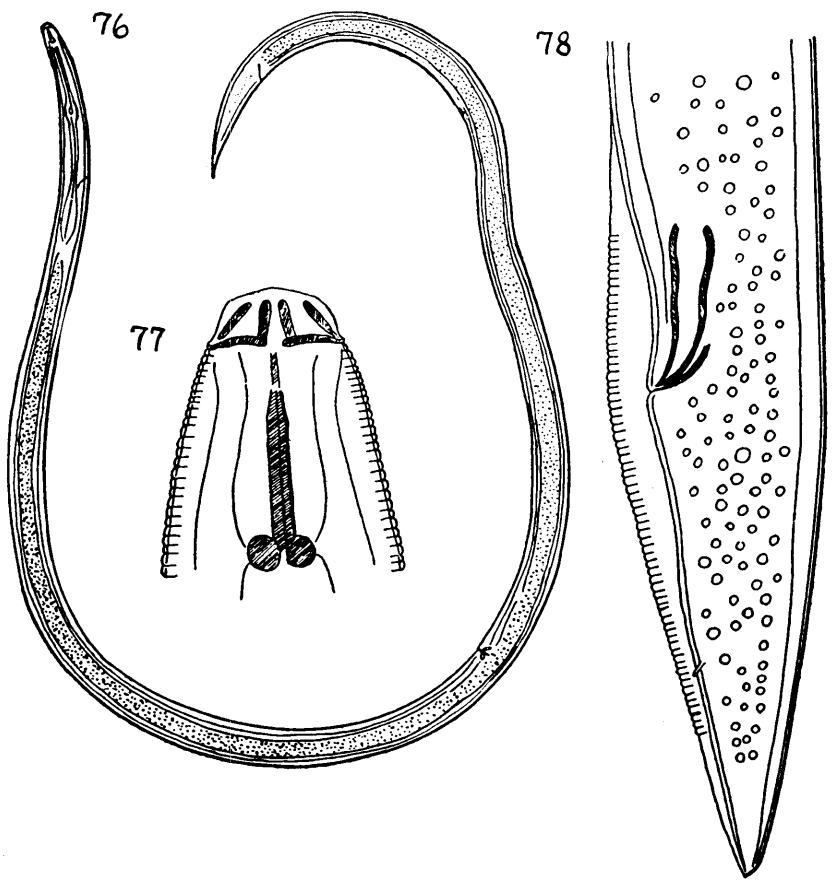


Fig. 2. Photograph of drawing of *Anguillulina gracilis* (De Man, 1880) Goodey, 1932, copied from Goodey's (1932) drawing of De Man's original. 76 Female; 77 Head; 78 Male tail.

*gracilis* may correctly be considered as a species of the genus *Radopholus* Thorne, 1949.

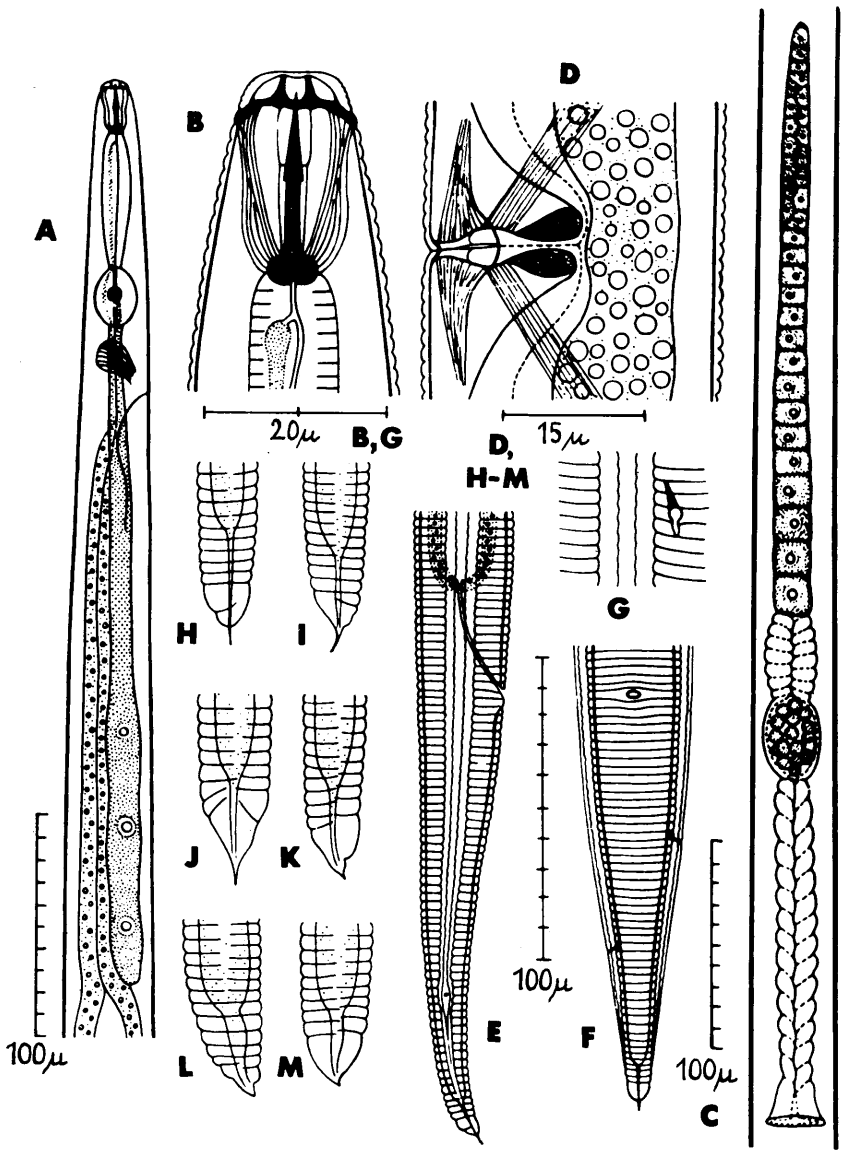


Fig. 3. Radopholus gracilis (De Man, 1880) n. c. A-M Female. A = Anterior portion of body, lateral view; B = Head, lateral view; C = Ovary and receptaculum seminis; D = Vulvar region, lateral view; E = Tail, lateral view; F = Tail, ventral view, phasmids not opposite each other; G = position of the phasmid outside the lateral field, observed in an atypical specimen; H-M = Variations of the terminal process, lateral view.

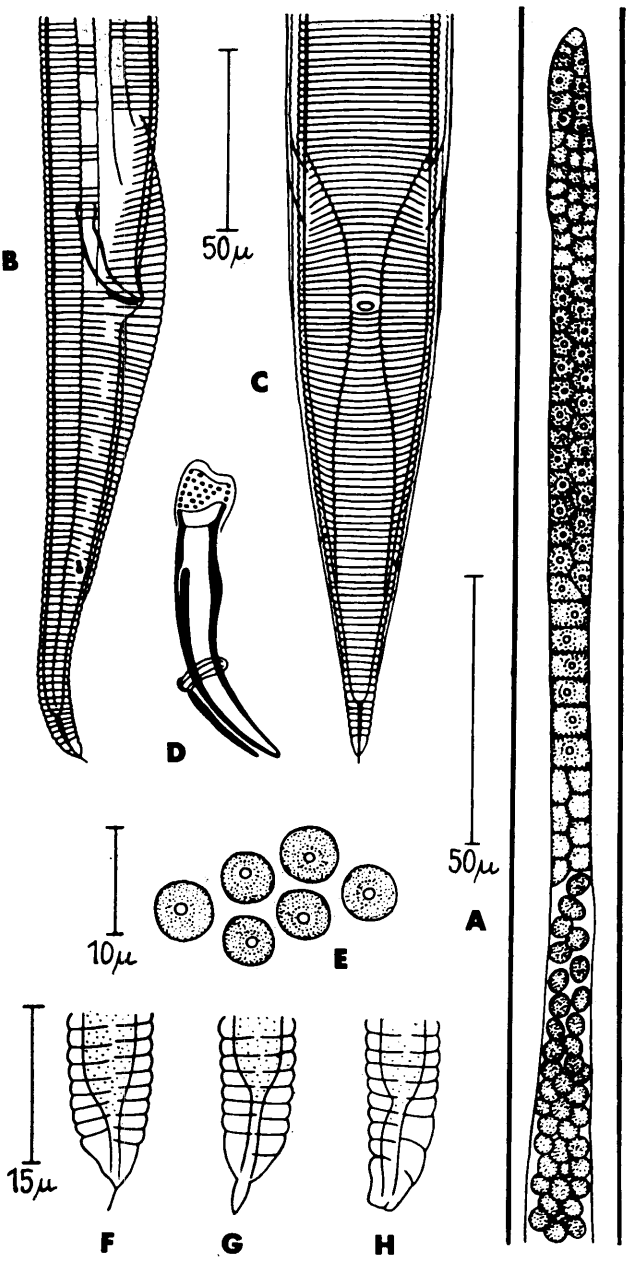


Fig. 4. *Radopholus gracilis* (De Man, 1880) n. c. A-H Male. A = testis and sperms; B = Tail, lateral view; C = Tail, ventral view; D = Spicule and gubernaculum; E = sperms; F, G = Variations of the terminal process, lateral view; H = Terminal process reduced, lateral view.

At the present time three species of the genus *Radopholus* have been described: *Radopholus similis* (Cobb, 1893) Thorne, 1949, *Radopholus oryzae* (v. Breda De Haan, 1902) Thorne, 1949 and *Radopholus gigas* Andrássy, 1954. *Radopholus gigas* is herein considered to be synonymous with *Radopholus gracilis*. Whether *Radopholus oryzae,* a parasite of rice roots in Java and Japan, is synonymous with *Radopholus gracilis* or whether it is a distinct species remains to be determined by host-parasite relationship studies. With the exception of a slight difference in size, both species are very similar morphologically.

*Radopholus gracilis* (De Man, 1880) n.c. (Fig. 3, 4).

SYNONYMS: *Tylenchus gracilis* De Man, 1880;
*Chitinotylenchus gracilis* (De Man, 1880) Micoletzky, 1921;
*Tylenchorhynchus behningi* Micoletzky, 1923;
*Tylenchorhynchus gracilis* (De Man, 1880) Micoletzky, 1925;
*Anguillulina gracilis* (De Man, 1880) Goodey, 1932;
*Tylenchorhynchus gracilis* (De Man, 1880) Filipjev, 1936;
*Radopholus gigas* Andrássy, 1954.

DIMENSIONS:

♀: 1.4 — 2.7 mm; a = 50.0 — 69.5; b = 4.7 — 7.6; c = 12.6 — 20.0; V = 47.0 — 58.7%; stylet = 0.020 — 0.022 mm; eggs = 0.018 x 0.035 mm.

♂: 1.6 — 2.4 mm; a = 45.0 — 68.9; b = 5.0 — 5.8; c = 14.0 — 19.8; spicule = 0.031 — 0.035 mm; gubernaculum = 0.010 — 0.011 mm.

Cuticle with distinct transverse striae. Lateral fields measuring about one-third the width of the body diameter and marked by four slightly crenate incisures (Fig. 3 E, G; 4 B). Deirids not observed. Lip region low, rounded, set off by a slight narrowing of the head contour, bearing 5 striae (Fig. 3B). Framework very distinct with six-pointed basal plate. Knobs of the strong spear large and rounded. Outlet of the dorsal esophageal gland 0.003 mm behind the spear base. Median esophageal bulb subspherical with small refractive valvular apparatus. Basal lobe of esophagus extending ventrolaterally a considerable distance over the anterior end of the intestine. The basal lobe contains three distinct gland nuclei (Fig. 3 A). Junction of esophageal lumen and intestine rather obscure. Nerve ring crossing isthmus of esophagus. Excretory pore slightly posterior to nerve ring. Tail elongate-conoid (Fig. 3 E, F; 4 B, C) in both sexes provided with a terminal process, varying in size and form (Fig. 3 H-M; 4 F-G). Phasmids present.

*Female:* Gonads paired, opposed and outstretched with receptaculum on either side. Oocytes in single file except for a short region of reproduction. Eggs twice as long as wide. Vulva a broad deep fissure shown in Fig. 3 D. Conoid tail (Fig. 3 E, F) tapering gradually to the bluntly rounded terminus which carries a small process varying in size and form (Fig. 3 H-M). The lateral fields reach almost to the end of the tail, bearing phasmids on either side at 55–75% of the tail length from the anus (Fig. 3 E). Sometimes the phasmids are not opposite each other (Fig. 3 F).

*Male:* Gonad single, outstretched (Fig. 4 A). Spermatocytes in two rows. The small round sperms are shown in Fig. 4 E. Tail (Fig. 4 B, C) tapering to the terminus possessing a ventrally placed process variable in outline (Fig. 4 F, G) which can be absent (Fig. 4 H). The lateral fields slightly widen out on the beginning of the crenate bursa (Fig. 4 B). The ventral outer area of the lateral field extending but a short distance on the ventral side of the bursal alae, the central area ending near the anus and the dorsal incisure

reaching almost to the tail tip. The rather narrow bursal wings are widest in the anal region and join the cuticle of the body directly behind the phasmids. The phasmids are located approximately ½ to ¾ of the distance between anus and tail tip. Spicula and gubernaculum are shown in Fig. 4 D.

HABITAT AND DISTRIBUTION: Moist woodland and pasture soils in Holland (De Man, 1884); boggy soil in Rumania (Micoletzky, 1917); on the leaves of *Potamogeton* from the river Volga in Russia (Micoletzky, 1923); mossy stones of a lake bank and from a meadow adjacent to a lake in Denmark (Micoletzky, 1925); on *Polytrichum* in Germany (Rahm, 1924); in marshy land and from a moist grass hollow at an altitude of 6885 feet in Austria (Franz, 1942); in marshy ground with *Carex* and *Primula farinosa* in Austria (Franz & Beier, 1942); from lake banks in Germany (Hirschmann, 1952); between the roots of a waterlily growing in a pool containing sodium salts in Hungary (Andrássy, 1954); in the mud around the roots of *Caltha palustris* from a brook in Germany (Hirschmann), between the roots of *Phragmites communis* in a drainage ditch with .004% salt content in Germany (Meyl, 1955).

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# *Cercaria wabashensis* sp. nov., a New Macrocercous Cercaria (Gorgoderinae) from Western Indiana.

WILLIAM H. COIL

Department of Zoology and Entomology, Ohio State University, Columbus, Ohio

In an attempt to learn more concerning the cercariae in the Gorgoderinae, collections of molluscs were made during the summer of 1953. Specimens of the Unionidae were collected from the Wabash River and Sphaeriidae were taken from ponds, marshes, ditches, and rivers. Gorgoderid cercariae were found only in sphaeriids. Four new species were noted, but there are enough data to describe only one form at this time.

This study was aided by a Grant-in-Aid from the Sigma Xi Resa Research fund. The author is indebted to the Department of Biological Sciences of Purdue University for providing the necessary facilities.

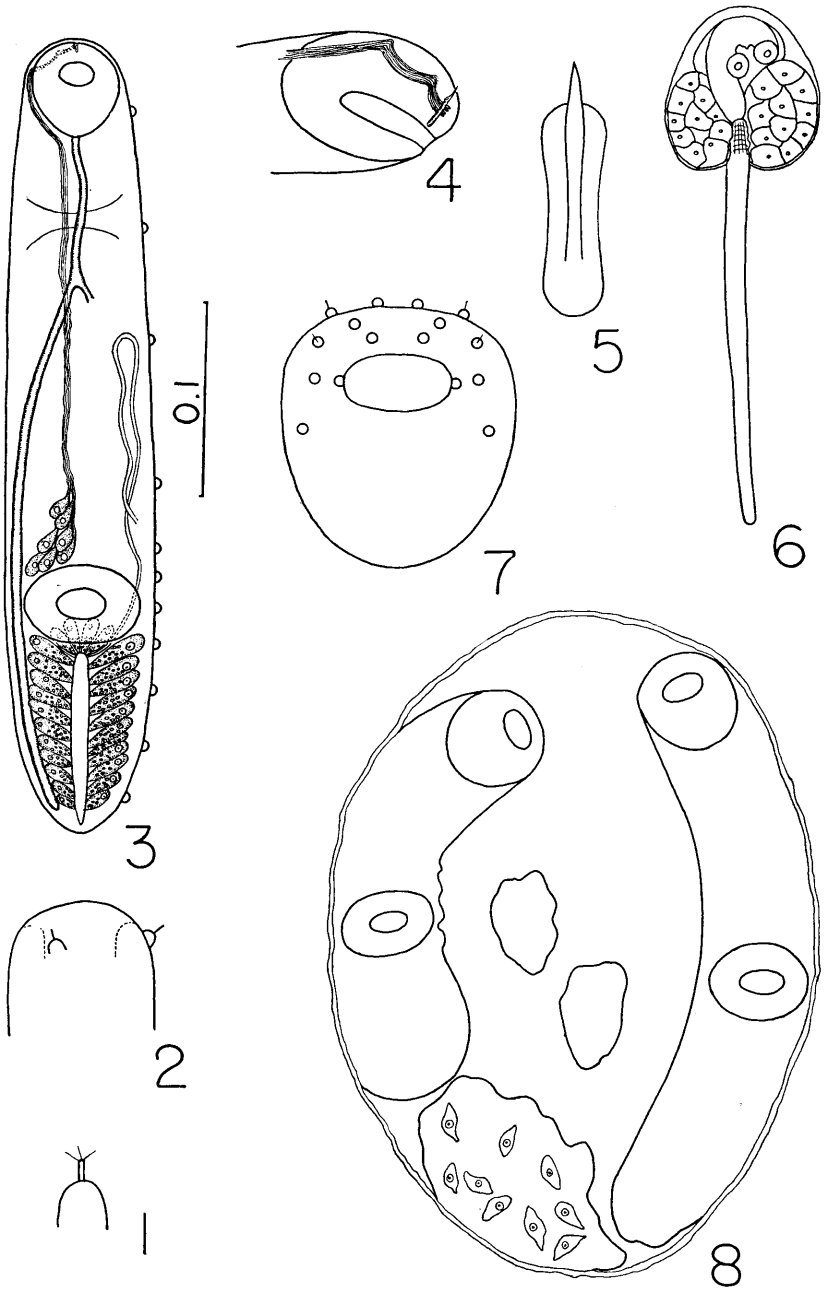
Molluscs were removed from the bottom by the use of a scraper and placed in fingerbowls. The water was examined for cercariae daily with a dissecting microscope. Living specimens were studied with the aid of vital stains and whole mounts and sections were stained with Harris' hematoxylin.

*Cercaria wabashensis sp. nov.*
Fig. 1-8

SPECIFIC DIAGNOSIS: Body measurements in millimeters of eight specimens, fixed in boiling Schaudinn's and cleared in glycerine, are: length of stylet 0.0213 (0.0177-0.0249), width of oral sucker 0.0466 (0.0427-0.0498), width of anterior end 0.0528 (0.0427-0.0640), acetabulum 0.0561 (0.0498-0.0676), length of esophagus 0.0723 (0.0463-0.0890), width of bladder 0.0599 (0.0570-0.0676), length of bladder 0.0123 (0.110-0.199), width of posterior end 0.0312 (0.0249-0.0391), distance between oral sucker and acetabulum 0.177 (0.128-0.202), distance between acetabulum and posterior end 0.103 (0.0749-0.114), length of body 0.403 (0.293-0.463), width of proximal part of tail 0.294 (0.275-0.312), width of distal part of tail 0.0464 (0.0290-0.0580), length of distal part of tail 0.601 (0.514-0.660). Suckers well-developed and active; covered with minute papillae which bear long setae or hairs. Other papillae, some of which possess setae, in characteristic arrangement on suckers. Nerves of papillae sometimes apparent, consisting of a central stalk with three branches. Six papillae on acetabulum arranged in a hexagonal pattern. Five to eight pairs of penetration glands lying anterior and lateral to acetabulum. Gland small, pyriform with a large nucleus. Ducts of penetration glands slightly lateral to median, passing around edge of oral sucker, and then through it, opening laterad to the stylet. Gland content granular. Excretory bladder surrounded by large, coarsely granular cystogenous glands. Tail large, swollen proximally into an almost spherical bulb. Body of distome completely housed in this structure. Distal portion of tail more filamentous, tapering slightly to the posterior tip which possesses at least two papillae. Tip with minute spines and capable of forming a "sucker." Distome drawn into tail chamber when alive, but forced out upon becoming moribund. Intestine slender, bifurcate, extending to posterior of body. Excretory system of the stenostoma type. Flame cells large and numerous. Genitalia only slightly developed and not completely differentiated.

HOST: *Sphaerium* (*Musculium*) *transversum* (Say)*

*Identified by H. B. Herrington.




1. Sketch of the nervous system of a papilla.
2. Sketch of the tip of the tail with two setate papillae.
3. Composite drawing with information derived from both living and fixed material.
4. Sketch of oral sucker showing the path of the ducts from the penetration glands.
5. Stylet, drawn with the aid of a microprojector.
6. Living cercaria, drawn with the aid of a microprojector.
7. Sketch of the arrangement of papillae on the oral sucker.
8. Sketch of daughter sporocyst.

SITE OF INFECTION: Gills.

LOCALITY: Wabash River, Tippecanoe County, Indiana, U.S.A.

TYPE SPECIMENS: Paratypes in U.S.N.M. Helminthological Collection No. 49494.

The tip of the tail of the cercaria is similar to that described by Vickers, 1940 for *Cercaria macrocerca;* it possesses minute spines and rythmically invaginates as though it might function as a sucker. However, the lack of prominent musculature would seem to preclude this. Swimming takes place by a lashing of the tail in an accentuated serpentine fashion.

Frequently the sensory papillae on the oral sucker appear as depicted in figure 1; however, this structure was not observed in all specimens. There is a striking similarity between this structure and the description by Sinitsin, 1905, of papillar nerves—"A nerve fiber enters the alveolus and when it reaches two-thirds of its distance it branches and ends in the wall of the alveolus" (from translation). It would appear, therefore, that the wall of the papilla disintegrates and leaves the nervous system visible.

*Cercaria wabashensis* possesses an excretory system similar to that described for three other macrocercariae, but it can be differentiated from these, and the other macrocercariae, on the basis of the number of penetration glands, the shape of the stylet, or the nature of the tail.

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## *Parvitaenia cochlearii* sp. nov. (Cestoda: Dilepididae) a new tapeworm parasitic in the boat-billed heron, *Cochlearius cochelearius*

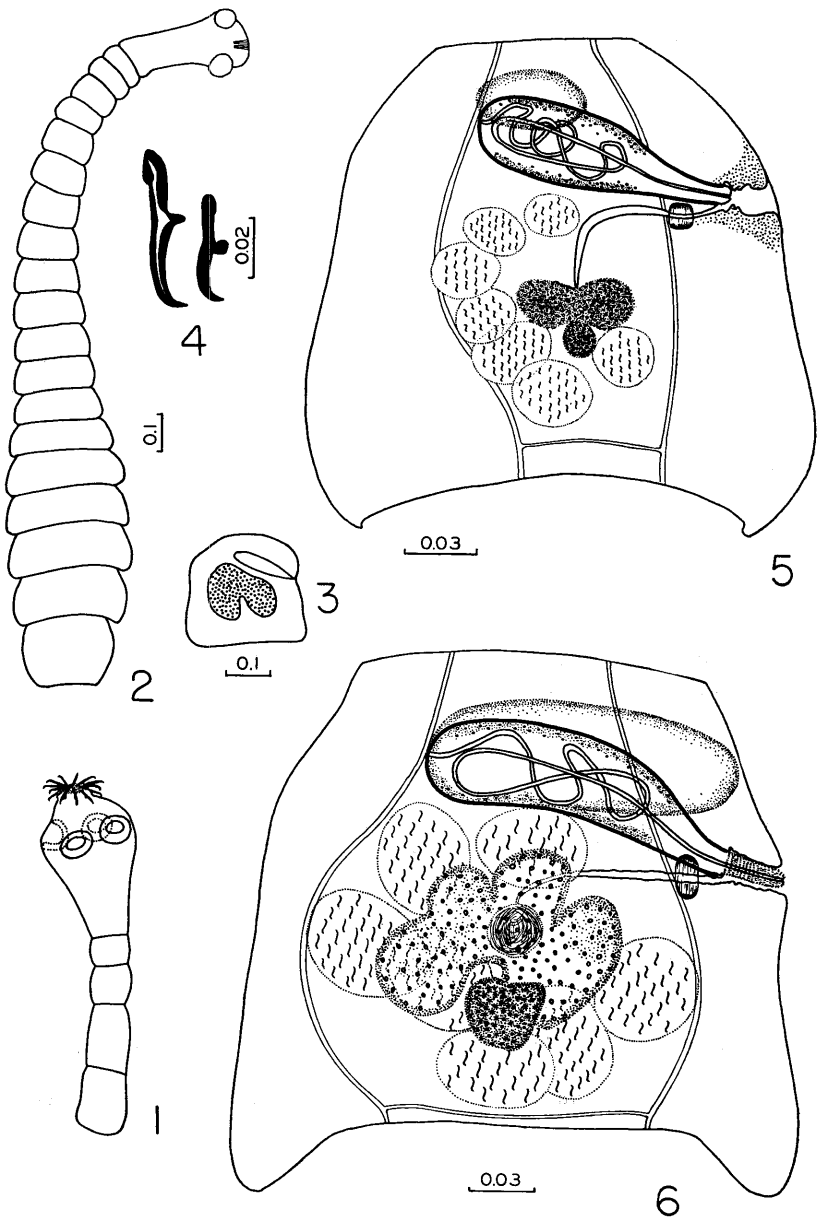
WILLIAM H. COIL*

In three boat-billed herons (*Cochlearius cochlearius*) collected from the brackish lagoons in the region of Tututepec, Oaxaca, Mexico, several specimens of a tapeworm were found, which, upon later study, proved to be a new species; the same species that M. L. Kuns collected from this host in Palenque, Chiapas, Mexico in 1949.

The species belongs to the genus *Parvitaenia* Burt, 1940 which was erected to include *P. ardeolae* obtained from *Ardeola grayi* collected in Ceylon. The genus is unique by virtue of its possession of the following combination of characters: 1) genital pores irregularly alternating; 2) genital ducts passing between the longitudinal excretory canals; 3) testes dorsal, posterior and lateral to the ovary on the aporal side; 4) uterus sac-shaped with shallow diverticula; 5) testes few in number.

Specimens were removed from intestinal contents and mucosal scrapings, placed in saline, fixed in corrosive acetic, and then stored in 10 per cent glycerine alcohol. Whole mounts and sections were stained in Harris'

*This study was carried out while the author was a Muellhaupt scholar in the Department of Zoology and Entomology, The Ohio State University. The author is indebted to Merle L. Kuns for providing the worms collected in Palenque.




All figures concern *Parvitaenia cochlearii*.

1. Sketch of scolex, neck and very young proglottids.
2. Drawing of poorly relaxed whole mount drawn with the aid of a microprojector.
3. Drawing of a gravid proglottid drawn with the aid of a microprojector.
4. Rostellar hooks drawn with the aid of a microprojector.
5. Developing proglottid. Large structures drawn with the aid of a microprojector and the smaller details added freehand.
6. Mature proglottid. Large structures drawn with the aid of a microprojector and the smaller details added freehand.

haematoxylin. Terpineol was used as a clearing agent. All measurements are in millimeters.

DIAGNOSIS: Strobila from 1.60 in contracted specimens to 2.68 in relaxed specimens. However, apparent apolytic nature of the worm precludes large numbers of gravid proglottids. Greatest width in mature proglottids 0.355. Creamy-white in color when alive. Individual proglottids craspedote and nearly trapezoidal in shape. Neck 0.0527 long. Suckers cup-shaped, not especially powerful, from 0.0631 to 0.0780 in longitudinal diameter. Twenty hooks in two rows 0.0495 to 0.0525 and 0.0331 to 0.0345, anterior row having longer hooks. Region posterior to neck extremely fragile with the result that most scolices become detached. Musculature consists of single layer of large longitudinal fibers (0.0015-0.0045) along periphery of medullary parenchyma. In cortical parenchyma fine fibers irregularly spaced. Other fine fibers underlay cuticula. Two dorsal (0.003) and two ventral (0.012) longitudinal excretory vessels present with transverse canal in posterior part of proglottid connecting ventral pair. No accessory collecting ducts observed. Genital pores irregularly alternating, opening to exterior in anterior third of proglottid. In contracted specimens velum of preceding proglottid covers genital pore. In some cases pore situated on papilla; probably an abnormal condition due to fixation. Genital atrium neither armed nor glandular. Vagina enters genital atrium posterior and ventral to cirrus and courses from there medially and posterior to central region of proglottid where it connects with seminal receptacle. Vaginal sphincter present short distance from genital atrium where vagina terminates with funnel-shaped structure. Vagina heavy-walled, but distensible enough to accomodate sperm as seminal receptacle. After sperm disappear, vagina almost returns to normal size. Testes range from five to eight in number and in size up to 0.045, located in dorsal parenchyma and extend around ovary except on poral side; developing early, they become diffuse and disappear before uterus fills proglottid. Vasa efferentia not observed. Ovary develops rapidly, filling central part of proglottid, asymmetrical, fan-shaped, radiating from anterior margin of vitelline gland and located in posterior part of proglottid in ventral parenchyma. Uterus ventral. Base of cirrus armed with small spines, but upon becoming everted distal part appears filamentous and unarmed. Cirrus sac heavy-walled with glandular-appearing cells internally. Ductus ejaculatorius profusely coiled within sac. External and dorsal to cirrus sac, vas deferens coiled and surrounded with what appears to be gland cells. This glandular portion reaches its maximum size with mature testes. No seminal vesicle present, but long, coiled vas deferens probably serves as one; sac persists in gravid proglottids. Uterus formed in ventral part of parenchyma and becomes bilobed as it fills proglottid. Eggs not available for study.

HOST: *Cochlearius cochlearius,* boat-billed heron.

LOCATION: Small intestine.

LOCALITY: Tututepec, Oaxaca and Palenque, Chiapas, Mexico.

HOLOTYPE: U.S.N.M. Helminthological Collection No. 37449.

*Parvitaenia cochlearii* differs from the only other species in the genus, *P. ardeolae* Burt, 1940, by the possession of an armed cirrus, a larger strobila, a vaginal sphincter, and rostellar hooks of a different shape.

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# Note on the Morphology and Systematic Status of the Genus *Molinema* Freitas and Lent, 1939 (Nematoda: Filarioidea)

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The genus *Molinema* Freitas and Lent, 1939 was established to accommodate *Filaria diacantha* Molin, 1858 (type host: *Hystrix prehensilis,* also collected from *Loncheres rufa*), *F. bifida* Molin, 1858 (type host: *Dactylomys amblyonix*) and *Dipetalonema travassosi* Artigas and Pacheco, 1933 (type host: *Myocastor coypus*). In the same paper in which Freitas and Lent proposed the new genus they redescribed *M. diacantha* and *M. bifida* from specimens removed from *Coendu villosus* (Cuv.) and *Kannabateomys amblyonyx* (Natt.) respectively.

According to Freitas and Lent the genus *Molinema* is distinct from *Dipetalonema* Diesing, 1861 on the basis of the following characters: (1) the structure of the spicules, (2) the absence of a gubernaculum, (3) the presence of clearly defined preanal papillae. The oral opening of the new genus was characterized as being ". . . cercado por 2 lábios laterais sustentados por papilas."

The recent revision of the genus *Dipetalonema* by Chabaud (1952) and Chabaud and Choquet (1953) has brought into question the validity of the genus *Molinema.* These authors have expanded the concept of *Dipetalonema* to include the genera *Acanthocheilonema* Cobbold, 1870; *Tetrapetalonema* Faust, 1935, *Loxodontofilaria* Van den Berghe and Gillain, 1939 and *Mönnigofilaria* Skrjabin and Shikhobalova, 1948. Since *Molinema* is close to *Dipetalonema* Diesing, it seems worthwhile to reexamine the characters on which the validity of *Molinema* rests and to see whether this genus should be included under the expanded concept of *Dipetalonema.* Firstly, an examination of the figures and description of *M. diacantha* and *M. bifida,* given by Freitas and Lent, shows that the spicules are not fundamentally unlike those of many members of the emended *Dipetalonema.* The new diagnosis of *Dipetalonema,* given by Chabaud (1952 p. 252), states only that the spicules of the members of this genus are unequal and that the proximal end of the large spicule is tubular and the distal end filamentous. Secondly, a gubernaculum may or may not be present in the members of *Dipetalonema.* Thirdly well-developed preanal papillae occur in many species of *Dipetalonema.* We conclude therefore, as does Chabaud (1952, p. 256), that the only justification for the retention of the genus *Molinema* rests on the presumed presence of ". . . 2 lábios laterais . . ." at the anterior end.

Through the courtesy of Dr. J. F. Teixeira de Freitas of the Institute of Oswaldo Cruz, Rio de Janeiro, the writer has had an opportunity to examine specimens of *M. diacantha* (from *Coendu villosus*) and *M. bifida* (from *Kannabateomys amblyonyx*). (Helminthological Collection of the Institute of Oswaldo Cruz Nos. 9990 and 9991).

The present observations on these species show that neither *M. diacantha* nor *M. bifida* possesses lips (Figs. 1-4). The oral opening of these species is circular and bordered by a peribuccal ring; it is depressed and therefore difficult to discern in lateral view. The "lábios" of Freitas and Lent undoubtedly refers to the raised parts of the body on either side of the head end on which are located at least four pairs of submedian papillae. The buccal capsule is relatively highly developed in both species but that of *M. bifida* is considerably larger than that of *M. diacantha.*

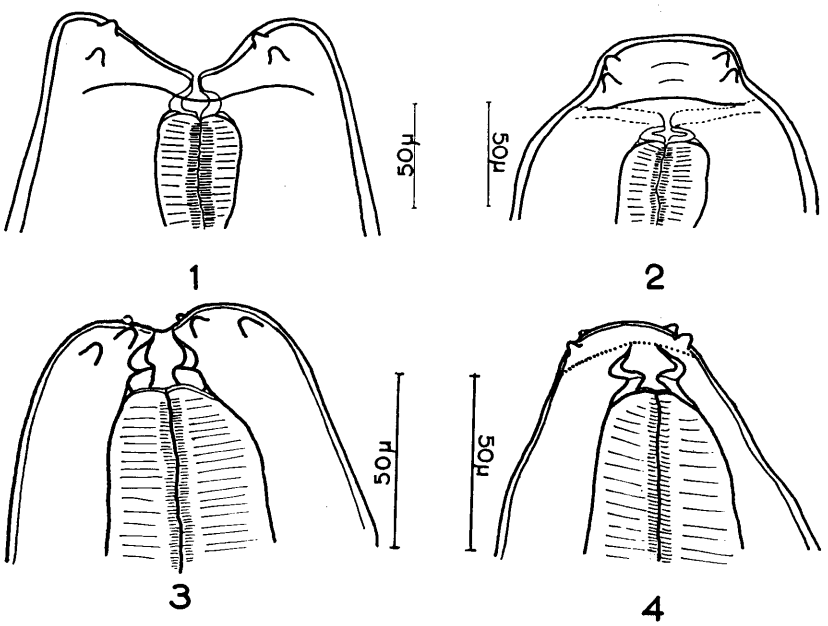


Fig. 1. *Dipetalonema diacantha* (Molin, 1858) Yorke and Maplestone, 1926, dorsal view of head of female.
Fig. 2. *D. diacantha,* lateral view of head of female.
Fig. 3. *D. bifida* (Molin, 1858) n. comb., dorsal view of head of male.
Fig. 4. *D. bifida,* lateral view of head of male.

Although the Brazilian helminthologists may have been justified in creating a new genus for these worms in 1939, the writer agrees with Dr. Alain G. Chabaud (personal communication) that these species should now be placed in the genus *Dipetalonema* Diesing emend. Chabaud, 1952. The cuticular shields of the species now included in *Dipetalonema* are varied in form. The raised lateral structures on the cephalic end of *M. diacantha* and *M. bifida* are not sufficiently unlike the cuticular formations of species in *Dipetalonema* to warrant the retention of the genus *Molinema.* Accordingly, the writer proposes that *Molinema* fall as a synonym of *Dipetalonema.* The proposed citations are as follows: *Dipetalonema diacantha* (Molin, 1858) Yorke and Maplestone, 1926; *D. bifida* (Molin, 1858) n. comb.; *D. travassosi* Artigas and Pacheco, 1933.

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# On the Morphology of *Proleptonchus aestivus* n. gen., n. sp. and *Dorylaimus lourdesae* n. sp., two new soil nematodes from Brazil

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Among free-living and parasitic nematodes obtained from soil samples collected at Monte Alegre do Sul Agricultural Experiment Station (State of S. Paulo), by Dr. Leocádio de Sousa Camargo, were two females of a leptonchid species, the description of which has been delayed, waiting for an opportunity to get more specimens. However, since this has not been possible, it appears appropriate to present the data at hand without further delay. The description of *Dorylaimus lourdesae* n. sp. is also presented.

## *Proleptonchus* new genus

LEPTONCHINAE., Thorne, 1935. Body practically cylindrical. Cuticle smooth; subcuticle weakly annulated, the annulation being almost exclusively discernible only on the posterior portion of the body. Lateral fields broad. Vestibulum wall not cuticularized. Spear curved, very slender and weak, with undiscernible extension. Guiding ring strongly cuticularized. Oesophagus a slender tube, with a pyriform basal bulb, which contains a straight and narrow lumen and two nuclei of glands. Cardia flattened, well defined. Intestinal cells thin, filled with dark granules. Pre-rectum well defined. Caudal papillae prominent, almost terminal. Vulva transverse. Ovary one, anterior to vulva, reflexed. Post vulvar rudimentary branch short.

*Proleptonchus* closely resembles the genus *Leptonchus* Cobb, 1920 (Thorne 1939) but differs in: (*a*) spear extension undiscernible, the extension in *Leptonchus* being heavily cuticularized; (*b*) *Proleptonchus* is a prodelphic genus, while *Leptonchus* comprises amphidelphic forms; (*c*) the basal bulb is clearly set off from the anterior portion of oesophagus, while in *Leptonchus* such a bulb results from an expansion of the latter; (*d*) the guiding ring in *Proleptonchus* is a strongly cuticularized structure, not truncate cone, in contrast to *Leptonchus*.

GENOTYPE—*Proleptonchus aestivus* n. sp.

DESCRIPTION—Body slightly tapering at both extremities; head almost round, set off from neck by weak constriction; lips amalgamated; cephalic papillae minute, not prominent on head surface. Amphids two-thirds as wide as neck. Nerve ring located about midway between head end and cardia.

Intestine embracing base of oesophageal bulb; intestinal cells rather thin, provided with large nuclei, filled with dark granules. The number of cells in the circumference of the intestine must vary according to the level considered. A little posterior to the vulvar opening, it was possible to see that there are from 8 to 10 cells in the circumference of the intestine. Prerectum short (36.5 microns long in one of the individuals), discernible by its differentiated structure. Intestinal lumen spacious, empty in the specimens studied. Anus located in a slight depression.

Tail subconoid, shorter than anal body diameter; two pairs of caudal papillae, arranged as illustrated (fig. 1, E); dilatator muscles of the anus quite well defined.

Vulva a transverse slit located from 692 to 752 microns from head end. Vagina extending almost half way across body. Ovary quite well developed,

compressing intestine, extending from 232 to 298 microns along body (Fig. 1, A). Oocytes arranged tandem, except at end of ovary, where first in multiple, later in double series. Cytoplasm of older oocytes of clearly alveolar structure that differentiates them from the younger ones. No eggs seen in the uterus. Posterior uterus short (51-64 microns long).

Lateral fields 11.6-16.6 microns wide, then occupying one-third of body width.

MALE unknown.

MEASUREMENTS—Length: 1,187.0-1,318.0 microns; width: 36.5-48.0 microns; spear: 8.3 microns; basal oesophageal bulb: 36.5-40.0 microns long and 11.6-16.6 microns wide; tail: 16.6-18.3 microns; a= 27.4-32.5; b= 5.8-6.3; c=71.5-72.0; V= 57.0-58.3%.

TYPE LOCALITY: Monte Alegre do Sul Agricultural Experiment Station, State of S. Paulo, Brazil.

FOOD HABITS unknown. Two females collected from soil around roots of strawberry plants (*Fragaria* sp.).

Description of *Dorylaimus lourdesae* n. sp.

Body slightly tapering anteriorly, more sharply posteriorly to an elongate filiform tail; terminus dorsally or ventrally arcuated.

Cuticle smooth; lateral chord formed by single line of rectangular cells; lateral fields obscure, one third as wide as body. Head slightly set off from neck; lips angular, with the two normal circlets of papillae, the perioral one being somewhat prominent. Amphid short, half as wide as lip region. Spear strong, usually protruded in fixed specimens. Guiding ring of spear broad and with transverse striations, another structure functioning like a single guiding ring is clearly seen farther forward. Canal of oesophagus thick walled from the junction with the spear extension to a little in front of the cardia, where the surrounding tissues disappear and the canal enters the cardia with very thin walls. Oesophageal bulb cylindrical, being almost half as long as neck. Cardia roughly conical and elongate (fig. 2,D). An elongate and reniform body, of unknown nature, is present either on level or in front of cardia.

Intestinal cells thin, filled with dark granules. Contents of intestine forming elongate and characteristic bodies, which possibly result from the agglomeration of the food particles. The food of this species, however, remains unknown.

Pre-rectum short, of well differentiated structure, clearly separated from intestine. Anus located in a slight depression; dilatator muscles well developed.

Vulva transverse; vagina extending half way across body. Uteri membranous, set off from oviducts by a constriction, containing a single egg at a time; egg is 56.7-70 microns long and 32.2 microns wide. Oviducts exhibiting annulations that are possibly folds of its wall; the distention of these folds must allow the egg entrance into the uterus. Ovaries with a single line of oocytes, except in the initial portion of multiplication. Points of reflexion of anterior and posterior ovary located about the same distance, respectively, from the vulva.

DIAGNOSIS—Several long-tailed *Dorylaimus* species are already known. The species most closely resembling *D. lourdesae* are possibly *D. proximus* Thorne & Swanger, 1936, and *D. tenellus* Thorne & Swanger, 1936, both described from Broad Run, near Leesburg, Virginia.

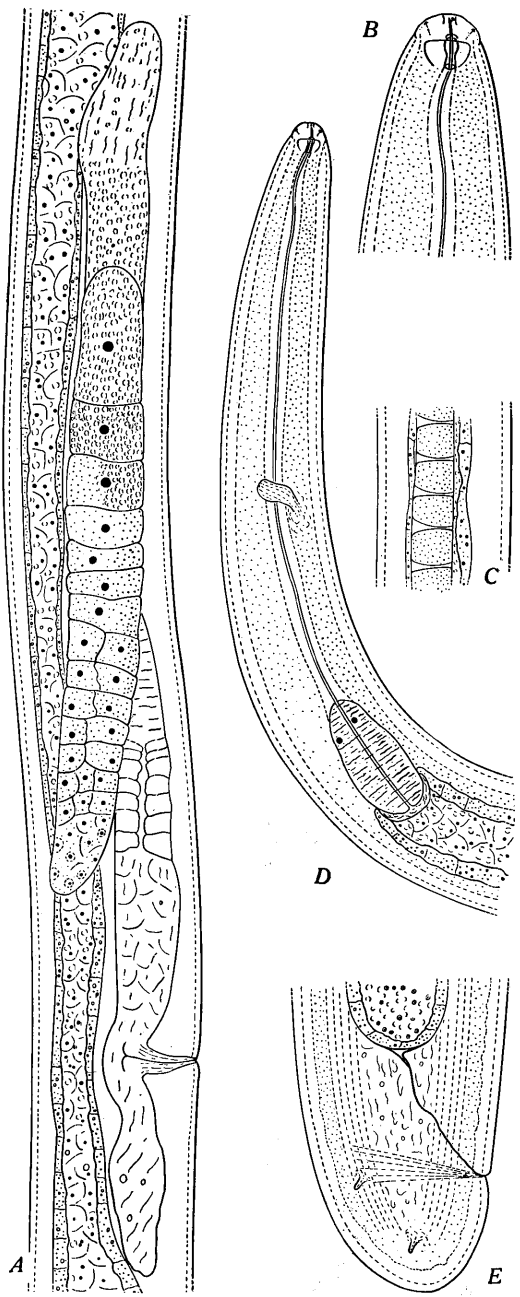


Fig. 1. *Proleptonchus aestivus* n. gen., n. sp. A. Sexual apparatus (×575). B. Head end (×880). C. Lateral chord (×575). D. Anterior end of body (×575). E. Tail (×880).

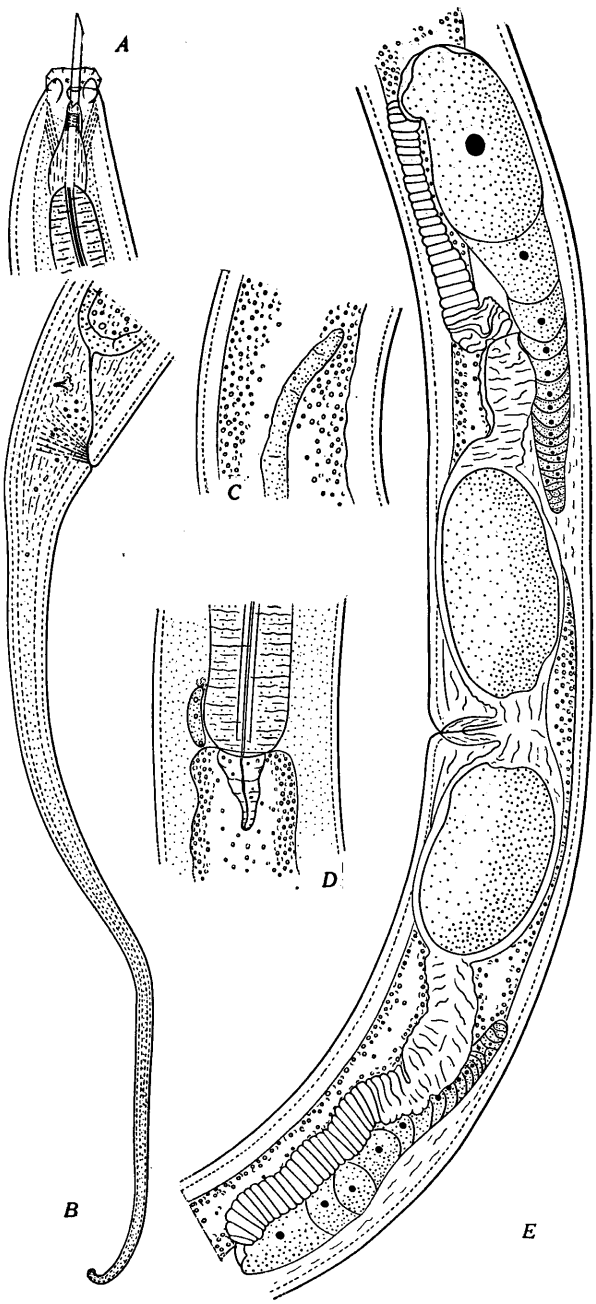


Fig. 2. *Dorylaimus lourdesae* n. sp. A. Head end. B. Tail. C. Part of intestine. D. Cardia region. E. Sexual apparatus. (All ×500).

*D. lourdesae* differs from *D. proximus* in: (*a*) absence of radial striae in the cuticle; (*b*) guiding ring of spear broad with transverse striations, with an additional guiding-ring-like structure on the level of the fusion of lip region with body; (*c*) absence of post-anal papillae; and, (*d*) shorter body (1,410-1,461 microns against 2,400 microns).

*D. lourdesae* differs from *D. tenellus* in: (*a*) wider body (a= 25-30 against a= 41); (*b*) longer tail (c= 5.4-5.6 against c=8.3); (*c*) absence of post-anal papillae; (*d*) rectum shorter than twice anal body diameter; and, (*e*) spear almost as long as twice lip region width.

*D. lourdesae* may be characterized as an amphidelphic long-tailed *Dorylaimus* with: Spear guiding ring broad with transverse striations and an additional guiding-ring-like structure located in front of the latter; oviduct wall exhibiting remarkable folds (annulation); elongate bodies in the intestinal lumen; and a single pair of lateral papillae located on the rectum region.

MEASUREMENTS—Total length: 1,410.0-1,461.0 microns; width, 46.5-58.0 microns; lip region width, 13.0-14.0 microns; anal body diameter, 20.7-25.0 microns; spear 25.0 microns; spear extension, 33.2 microns; spear aperture 8.3 microns; pre-rectum, 60.0-70.0 microns; rectum 33.2-36.5 microns; a= 25.0-30.0; b= 3.8-4.3; c= 5.4-5.6; V= 46.2-47.7%.

TYPE LOCALITY—Several females obtained from soil collected around roots of forest trees, in a woodlot near Piracicaba, State of S. Paulo, Brazil. A single female from soil surrounding potato tubers from Sapecado, also State of S. Paulo, but rather distant from the first place. Male unknown.

The specific name is given in honor of the writer's wife, Mrs. Maria de Lourdes J. Lordello, in recognition for her valuable help in the laboratory work.

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# Some Digenetic Trematodes of Plectognath Fishes of Hawaii[1]

MARY LOUISE HANSON[2]

This is the second report based on specimens collected during June and July of 1949 at the Marine Laboratory of the University of Hawaii, Honolulu. The collection by no means represents an extensive sampling of the plectognath fishes of Hawaii. No members of the families Canthigasteridae or Diodontidae were examined; and only 4 of the 12 Hawaiian species of Balistidae, 3 of the 7 species of Monacanthidae, 2 of the 7 species of Ostra-

[1]Studies from the Department of Zoology, University of Nebraska, No. 278.
The author is indebted to: Dr. Robert W. Hiatt, chairman, and the Department of Zoology and Entomology at the University of Hawaii for the use of laboratory space and equipment: Dr. William A. Gosline for the identification of the fish hosts; Mr. Spencer W. Tinker for the ever-available supply of fishes for examination; Dr. Harold W. Manter for direction and suggestions during the study of the specimens.
[2]University of Nebraska State Museum.

ciidae, and 3 of the 5 species of Tetraodontidae were examined. No trematodes were recovered from single specimens of 1 species of Monacanthidae, 1 species of Ostraciidae, and 3 species of Tetraodontidae. More examinations would propably have revealed infections as, in general, plectognath fishes are favorable hosts for trematodes.

Few trematodes have been reported from Hawaiian fishes. Lutz (1893) reported *"Distomum clavatum* Rud." (= *Hirudinella* sp.), Hanson (1953) described *Schistorchis stenosoma* and *S. zancli,* and Manter (1955) reported *Hapladena vavia* Linton, 1910 and *Haplosplanchnus obtusus* (Linton, 1910) Manter, 1937. The latter two species also occur at Tortugas, Florida. The following account adds 8 more species, including 6 new species and 2 new genera, to the Hawaiian list.

The trematodes were killed under a cover glass with F.A.A. fixative, stored in 70 per cent alcohol, stained in Delafield's haematoxylin, and mounted in Permount. Type specimens are deposited at the United States National Museum.

### PARAMPHISTOMATIDAE

*Cleptodiscus bulbosus* n. sp. (Fig. 1)

HOST: *Melichthys buniva* (Lacépède), trigger fish; "humu-humu ele-ele"; in 1 of 6 specimens examined.

LOCATION: Intestine

TYPE SPECIMEN: U. S. Nat. Mus. Helm. Coll. No. 37461.

DESCRIPTION (based on a single specimen): Body fusiform, tapering anteriorly and rounded posteriorly with slight indentation at level of ovary; 3.91 mm. long and 1.23 mm. wide at midbody; minute papillae around mouth opening and widely scattered about pharyngeal level of body surface. Acetabulum ventroterminal, round, with rounded opening; 0.840 mm. long by 0.847 mm. wide. Mouth opening at anterior end of body, leading into shallow prepharyngeal atrium; sphincter-like ring of circular muscles guard anterior entrance into large pharynx; pharynx 0.438 mm. long by 0.431 mm. wide, consisting of a central muscular core with prominent longitudinal muscles, a middle glandular layer, and an outer covering at base of which are a pair of rudimentary, sucker-like diverticles. Slender esophagus 0.358 mm. long, entering large esophageal bulb before bifurcating; esophageal bulb 0.307 mm. long by 0.146 mm. wide, consisting of both longitudinal and circular muscles. Both esophagus and esophageal bulb surrounded by glandular cells. Ceca simple, extending to level of ovary, with slightly undulating outer walls. Genital pore median, relatively large, immediately ventral to cecal bifurcation; muscles and gland cells surrounding pore. Testes diagonal; right testis at midbody, 0.219 mm. long by 0.270 mm. wide; left anterior testis with lateral indentation, 0.241 mm. long by 0.212 mm. wide; testes separated by width of one testis. Cirrus sac bipartite consisting of rounded posterior portion and a more oval terminal section; seminal vesicle tubular, much coiled in rounded portion of cirrus sac, less coiled in oval portion; cirrus small. Ovary ovoid, median, between ends of ceca, near acetabulum; 0.212 mm. long by 0.263 mm. wide. Uterus concealing anterior half of ovary, extending posterior to ovary on right side of body but not reaching acetabulum, then filling most of intercecal space; terminating in short glandular metraterm. Mehlis' gland dorsal, partially posterior and partially to left of ovary. Vitelline follicles large, irregular in shape, extending from near posterior level of posterior testis to near acetabulum, lateral and ventral to ceca and continuing beyond cecal ends; small vitelline

reservoir located just posterior to ovary. Eggs small, oval, very numerous; 43 to 61$\mu$ by 31 to 37$\mu$, usually 54 to 56$\mu$ by 34 to 37$\mu$. Details of excretory system could not be traced, but excretory tubes join dorsal to esophagus

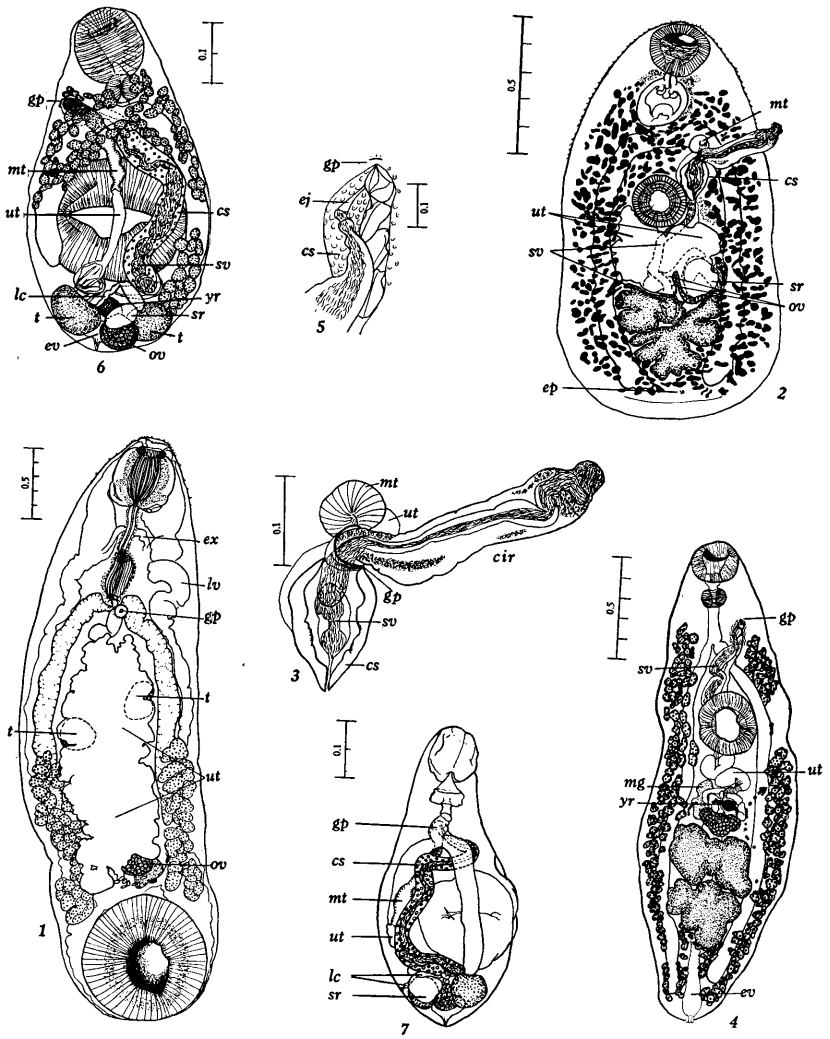


PLATE I

All figures were drawn with the aid of a camera lucida. The projected scales are in millimeters.

Fig. 1. *Cleptodiscus bulbosus* from *Melichthys buniva,* ventral view.
Fig. 2. *Lepocreadium incisum* from *Melichthys buniva,* ventral view.
Fig. 3. *L. incisum,* detail of cirrus sac and cirrus.
Fig. 4. *Pseudopecoelus brevivesiculatus* from *Cantherines pardalis,* ventral view.
Fig. 5. *P. brevivesiculatus,* detail of terminal genital ducts.
Fig. 6. *Hysterogonia balistis* from *Balistes capistratus,* dorsal view.
Fig. 7. *H. balistis,* lateral view.

midway between pharynx and esophageal bulb. A pair of well-developed, unbranched, lymphatic vessels lie lateral to ceca and extend full length of body, appearing swollen at both ends of body and somewhat sinuous anterior to cecal bifurcation.

The genus *Cleptodiscus* was named by Linton (1910). Yamaguti (1953) considered *Neocladorchis* Bhalerao, 1937 a synonym of *Cleptodiscus*. The following generic diagnosis is modified from Yamaguti (1953):

*Cleptodiscus.*

Paramphistomatidae. Body elongate, somewhat fusiform, blunt-pointed or rounded ends, with or without papillae anteriorly. Acetabulum ventroterminal, medium-sized. Mouth terminal. Pharyngeal diverticles in form of small suckers. Esophagus slender, with or without terminal bulb. Ceca simple, reaching to near posterior end of body. Testes obliquely tandem to diagonal in midregion of body. Seminal vesicle tubular, narrow, convoluted. Cirrus sac present. Genital pore at intestinal bifurcation, with or without muscular margin. Ovary median or submedian, between cecal ends, with shell gland immediately behind. Vitellaria along posterior portion of intestine. Uterus intercecal; eggs numerous. Excretory vesicle small, with dorsal aperture, excretory tubes extending to level of esophagus where they may unite. Parasites of marine and freshwater fishes.

GENOTYPE: *C. reticulatus* Linton, 1910 in *Pomacanthus arcuatus;* Florida.

Other species: *C. poonaensis* (Bhalerao, 1937) Yamaguti, 1953 from *Barbus dodsoni;* India. *C. bulbosus* from *Melichthys buniva;* Hawaii.

COMPARISONS: *C bulbosus* resembles *C. poonaensis* in its esophageal bulb and cuticular papillae. It differs in possessing a larger pharynx, smaller pharyngeal diverticles, a larger esophageal bulb, smaller and more separated testes, a bipartite cirrus sac, a muscular margin to the genital pore, a single pair of lymphatic vessels, a more anterior extent of the uterus, and eggs which, although nearly the same length, are twice as wide. *C. bulbosus* resembles *C. reticulatus* in the size of the diverticles, the muscular margin of the genital pore, the anterior extent of the uterus, and the single pair of lymphatic vessels; but differs in the presence of the esophageal bulb (although Manter (1947) reported circular muscles in the posterior half of the esophagus of *C. reticulatus*), the presence of cuticular papillae, the character of the pharynx, smaller and more separated testes, and smaller eggs. Linton (1910) reported that the excretory tubules of *C. reticulatus* joined dorsal to the esophagus, but Manter (1947) observed that the tubules ended blindly near the pharynx. The tubules of this single specimen of *C. bulbosus* join dorsal to the esophagus.

LEPOCREADIIDAE

*Lepocreadium clavatum* (Ozaki, 1932) Yamaguti, 1938

HOST: *Melichthys buniva* (Lacépède), trigger fish; "humu-humu ele-ele"; in 3 of 6 specimens examined.

LOCATION: Intestine

SPECIMEN DEPOSITED: U. S. Nat. Mus. Helm. Coll. No. 37462.

This collection represents a new host and distribution.

*Lepocreadium incisum* n. sp. (Figs. 2 and 3)

HOSTS: *Melichthys buniva* (lacépède), trigger fish; "humu-humu ele-ele"; in 1 of 6 specimens examined.

LOCATION: Intestine

TYPE SPECIMEN: U. S. Nat. Mus. Helm. Coll. No. 37463.

DESCRIPTION (based on 3 specimens): Body truncate, narrowing slightly anteriorly, sides tending to be nearly parallel with slight indentation near midbody, posterior edge turned slightly ventrally forming shallow pocket, spined anteriorly, with scattered scale-like spines posteriorly; 1.132 to 1.432 mm. long by 0.501 to 0.785 mm wide. Oral sucker slightly subterminal, large, strong, 0.169 to 0.193 mm. long by 0.200 to 0.223 mm. wide; acetabulum round with well developed circular muscles around aperture, 0.177 to 0.193 mm. long by 0.177 to 0.200 mm. wide; sucker ratio 1:0.79 to 1. Prepharynx very muscular, short, 0.015 to 0.046 mm. long; pharynx very nearly as large as acetabulum, 0.131 to 0.177 mm. long by 0.154 to 0.177 mm. wide, very heavy musculature with six papilla-like projections into lumen from the anterior end and bulb-like projection from base; esophagus short, 0.023 to 0.031 mm. long, slightly wider than long; spacious ceca arch laterally, turn and extend posteriorly to near end of body. Genital pore anterior and to left of acetabulum, lying just ventral to median margin of cecum. Testes in posterior third of body length, intercecal, obliquely tandem, deeply but irregularly lobed; maximum measurements for anterior testis, 0.162 to 0.216 mm. long by 0.146 to 0.269 mm. wide; maximum measurements for posterior testis, 0.169 to 0.239 mm. long by 0.162 to 0.354 mm. External seminal vesicle long and narrow, extending from cirrus sac around acetabulum to median line and posteriorly to level of ovary, turning left and ending dorsal to median lobe of anterior testis. Cirrus sac 0.108 to 0.185 mm. long by 0.077 to 0.092 mm. wide with very heavy, muscular wall (Fig. 3), containing bipartite internal seminal vesicle which continues into a long slender ejaculatory duct which coils two or three times near tip of cirrus; cirrus long, protrusible, with scattered prostatic cells concentrated near base and most numerous at tip (Fig. 3). Ovary at posterior part of middle third of body length, to left of midline, just anterior to testes, trilobed in varying degrees ranging from deeply incised in type to an irregular triangular mass in one paratype. Uterus intercecal, between anterior testis and acetabulum, continuing into muscular metraterm dorsal to cirrus sac; bulb-like muscular thickening of metraterm as described by Yamaguti for *L. clavatum* present. Seminal receptacle saccular and may be, as in type, bent in shallow, inverted "V", adjacent to ovary, median to left cecum. Vitelline follicles small, irregular in shape, extending from anterior part of pharynx to just posterior to ends of ceca, confluent between pharynx and acetabulum and posterior to testes; dorsal, ventral, and only slightly medial to ceca. Eggs numerous, 38 to 46$\mu$ by 23 to 30$\mu$. Excretory pore subterminal, ventral, median, between ends of ceca.

COMPARISONS: Certain species of *Lepocreadium* may be separated on the basis of a lobed or spherical ovary. Four species, *L. elongatum, L. retrusum, L. trulla* and *L. clavatum,* have a lobed ovary. All except *L. clavatum* may be separated from *L. incisum* by the cirrus sac which extends posterior to the acetabulum.

*L. incisum* further resembles *L. clavatum* in its large pharynx; short, muscular cirrus sac; trilobed ovary; the large external seminal vesicle; and muscular metraterm. From *L. clavatum, L. incisum* differs by having a more muscular cirrus sac which extends only to mid-acetabulum, deeply incised testes, a muscular prepharynx leading into a pharynx with six papillae on the anterior edge of the lumen, and the circular muscles of the acetabulum.

OPECOELIDAE

*Pseudopecoelus brevivesiculatus* n. sp. (Figs. 4 and 5)

HOSTS: *Cantherines pardalis* (Ruppell), file fish, (type host), in 1 of 7 specimens examined; *Melichthys buniva* (Lacépède), trigger fish, "humu-humu ele-ele", in 1 of 6 specimens examined.

LOCATION: Intestine

TYPE SPECIMEN: U. S. Nat. Mus. Helm. Coll. No. 37463.

DESCRIPTION (based on 5 specimens): Body smooth, varying from pear-shape to elongate; tapered anteriorly, less tapered but more pointed posteriorly, widest at testicular-ovarian level; 1.416 to 2.409 mm. long by 0.767 to 0.898 mm. wide; shorter specimens proportionately wider than longer ones. Oral sucker terminal, well-developed, smaller than acetabulum, 0.190 to 0.219 mm. long by 0.204 to 0.219 mm. wide. Acetabulum about one-third body length from anterior end, strong, rounded, without papillae, opening varying from central and round to posterior and oval; 0.270 to 0.321 mm. long by 0.284 to 0.299 mm. wide. Sucker ratio 1:1.22 to 1.57. Prepharynx short, pharynx rounded, 0.073 to 0.088 mm. long by 0.073 to 0.110 mm. wide; esophagus muscular, 0.058 to 0.183 mm. (usually about 0.1 mm.) long, and relatively wide (about 0.05 mm.); ceca relatively narrow, about same width as esophagus, extending parallel to body wall to near posterior end of body, ending blindly. Genital pore sinistral, midway between esophagus and body wall. Testes tandem, intercecal, irregularly lobed, contiguous, in posterior third of body length; anterior testis 0.204 to 0.321 mm. long by 0.409 to 0.511 mm. wide; posterior testis 0.219 to 0.350 mm. long by 0.365 to 0.445 mm. wide. External seminal vesicle saccular; in all except one specimen, a sharp curve in posterior half; reaching anterior edge of acetabulum which it may overlap slightly; passing ventral to left cecum, entering cirrus sac without noticeable constriction, then narrows and coils two or three times before terminating in non-muscular ejaculatory duct which joins uterus at genital pore. Cirrus sac short, indistinct, a very thin membrane surrounding a few prostatic cells (Fig. 5). Ovary median, pretesticular, trilobed or subtriangular, with more or less prominent conical extension on median anterior surface, close to and in some cases contiguous with anterior testis, 0.088 to 0.131 mm. long by 0.183 to 0.234 mm. wide. Uterus usually not reaching posterior to mid-ovary although in one specimen it reaches middle of anterior testis; originating as narrow tube which loops several times and becomes very muscular before expanding into two or three enlarged loops posterior to acetabulum and continuing without loops dorsal to acetabulum and ventral to left cecum to genital pore; a few scattered gland cells found in parenchyma near terminal portion of uterus. Mehlis' gland large, diffuse, to right of midline; anterior, dorsal and slightly posterior to ovary, reaching laterally nearly to cecum. Seminal receptacle absent; sperm cells present in posterior portion of uterus. Vitellaria follicular, extending from level of genital pore to posterior to cecal ends, mostly lateral to ceca, converging medianly posterior to testes and just posterior to acetabulum; follicles may be interrupted once or twice but interruptions not consistent; vitelline ducts conspicuous; yolk reservoir just anterior to ovary. Eggs relatively few, usually collapsed, 54 to 68$\mu$ by 26 to 37$\mu$, usually 58 to 61$\mu$ by 28 to 34$\mu$. Excretory pore terminal; excretory vesicle I-shaped and extending dorsally and medianly to, or slightly anterior to, ovary.

COMPARISONS: This species has the generic characters of *Pseudopecoelus*, namely: smooth body; acetabulum without papillae; no accessory sucker;

ceca ending blindly; very reduced cirrus sac; and tubular seminal vesicle. However, the cirrus is non-muscular and more aptly described as an ejaculatory duct, and the genital pore is non-muscular.

*P. brevivesiculatus* differs from all species of *Pseudopecoelus* except *P. umbrinae* Manter & VanCleave, 1951 and *P. gibbonsiae* Manter & VanCleave, 1951 in that the vitellaria extend well anterior to the acetabulum, and from all except *P. umbrinae* in that the seminal vesicle does not extend posterior to the acetabulum. It further resembles *P. umbrinae* in its 3 or 4 lobed ovary, absence of a metraterm, and egg size. It differs from *P. umbrinae* in shorter post-testicular space, smaller sucker ratio, location of the genital pore, lobed testes which are always tandem, its weakly developed cirrus sac, median ovary, more lateral distribution of the vitellaria which may converge posterior but *not anterior* to the acetabulum, and the slightly more anterior extent of the excretory vesicle.

*Hysterogonia balistis* n. gen. n. sp (Figs. 6 and 7)

HOST: *Balistes capistratus* Shaw, trigger fish, "humu-humu mimi"; in 1 of 3 specimens examined.

LOCATION: Intestine

TYPE SPECIMEN: U. S. Nat. Helm. Coll. No. 37465.

DESCRIPTION (based on 9 specimens): Small, pyriform worms, 0.533 to 0.644 mm. long; 0.270 to 0.314 mm. wide; widest at mid-acetabular level. Cuticula smooth. Oral sucker terminal, large, rounded, 0.102 to 0.124 mm. long by 0.102 to 0.124 mm. wide; acetabulum two-thirds body length from anterior end, large, rounded, and deep, 0.161 to 0.285 mm. long by 0.219 to 0.241 mm. wide, with transverse opening; sucker ratio 1:1.94 to 2.22, averaging 1:2. Very short prepharynx, pharynx seemingly adjacent to oral sucker in 4 of 9 specimens; pharynx rounded, 0.037 to 0.048 mm. long by 0.051 to 0.058 mm. wide; esophagus curving ventrally before bifurcating near posterior end of pharynx; ceca short and fairly wide, extending to near posterior edge of acetabulum, one cecum usually slightly posterior to anterior edge of acetabulum but terminating at or before reaching testis. Genital pore at esophageal level near left margin of body. Testes ovoid, symmetrical, at extreme posterior end of body, 0.065 to 0.095 mm. long by 0.049 to 0.068 mm. wide. Cirrus sac directed to right from genital pore, then dorsally, continuing in an S-curve dorsal to acetabulum, turning ventrally again at posterior edge of acetabulum and ending shortly thereafter (Fig. 7); very long, if straightened it would be nearly as long as worm; containing a tubular, bipartite seminal vesicle. Cirrus weakly muscular. External seminal vesicle absent. Pars prostatica absent, but prostatic cells scattered throughout length of cirrus sac. Ovary located directly between testes and contiguous with them, rounded posteriorly but elongating anteriorly as oviduct arises; 0.061 to 0.071 mm. long by 0.049 to 0.070 mm. wide. Uterus inflated slightly around each egg and continuing as straight, narrow tube; muscular metraterm 0.162 to 0.230 mm. long. Mehlis' gland diffuse in extreme posterior part of body. Laurer's canal well-developed, opening dorsal to anterior edge of left testis near median line; from its pore it extends antero-ventrally, then laterally, widening into a small vesicle and continuing medianly as swollen tube, narrowing and coiling once or twice before entering ootype (Fig. 7). Seminal receptacle claviform, dorsal and slightly right of midline, inflated but empty in all instances. Vitellaria follicular, forming band across forebody from pharyngeal level to anterior edge of acetabulum and continuing posteriorly

around ceca to mid-acetabulum where there is a more or less definite break, follicles usually continuing posteriorly to anterior edge of testes, although follicles may not be resumed on one side or the other (as on left side of type specimen). Yolk ducts not observed although oval vitelline reservoir prominent just anterior to ovary. All eggs collapsed, ranging from 1 to 3 per worm, large (nearly as large as pharynx and larger than excretory vesicle), 49 to 61$\mu$ by 25 to 36$\mu$ usually 51 to 58$\mu$ by 29 to 32$\mu$. Excretory pore ventral, terminal; excretory vesicle small, ventral to ovary, simple sac-shaped, usually wider than long; collecting tubes not observed.

DIAGNOSIS OF THE GENUS *Hysterogonia*: Opecoelidae with pear-shaped body; acetabulum about twice as large as oral sucker. Very short prepharynx; esophagus curving ventrally; ceca of medium length, extending only to testes. Genital pore preacetabular, near left margin of body. Cirrus sac very long, enclosing tubular, bipartite seminal vesicle, scattered prostatic cells, and narrow cirrus. Testes symmetrical at posterior end of worm. Ovary elongate-oval, median or slightly to right, intertesticular. Uterus pretesticular and straight; metraterm long and very muscular; eggs few, large, thin-shelled. Seminal receptacle present; Laurer's canal present. Vitelline follicles well developed, pretesticular, mostly dorsal, extending to pharyngeal level. Excretory vesicle simple, sac-shaped. Parasites of marine fishes. Type species: *H. balistis* from *Balistes capistratus*, Hawaii.

*Hysterogonia* resembles most closely *Eurycreadium* Manter, 1934, although it is easily differentiated from it and other Opecoelidae by possessing a combination of the following characters: a very long cirrus sac, the genital pore at the left margin of the body, the extreme posterior position of the gonads, the intertesticular ovary, the presence of a seminal receptacle, and the long muscular metraterm.

The name *Hysterogonia* is from *hystero*, behind, and *gonia*, gonads, referring to the posterior position of the gonads.

FELLODISTOMATIDAE

*Discogasteroides hawaiensis* n. sp. (Figs. 8 and 9)

HOST: *Ostracion sebae* Bleeker, trunk fish; from 1 of 2 specimens examined.

LOCATION: Intestine

TYPE SPECIMEN: U. S. Nat. Mus. Helm. Coll. No. 37466.

DESCRIPTION (based on 9 specimens): Small, pyriform worms, 1.197 to 1.679 mm. long by 0.621 to 0.825 mm. wide; widest at acetabular zone, rounded posteriorly, narrowed anteriorly. Small spines covering anterior two-fifths to one-half of body and scattered over remaining body surface. Oral sucker terminal, rounded, 0.204 to 0.248 mm. long by 0.218 to 0.270 mm. wide. Acetabulum large, quadrate with emarginate anterior and posterior borders; glandular with weak musculature at edges; without cavity; 0.567 to 0.876 mm. long by 0.548 to 0.825 mm. wide; occupying third and fourth fifths of body length. Sucker ratio 1:2.49 to 3.3, averaging 1:2.9. Prepharynx absent, pharynx small, rounded, often slightly submedian, 0.063 to 0 080 mm. long by 0.073 to 0.087 mm. wide; esophagus indistinct in most specimens, as broad and usually about same length as pharynx; ceca short, terminating near level of anterior edge of acetabulum; unequal—left cecum being longer and extending more laterally as it follows cirrus sac. Genital pore at esophageal level, slightly to right of median line. Testes near posterior end of body, symmetrical; one testis ovoid, 0.146 to 0.277 mm. long by

0.124 to 0.175 mm. wide; other testis rounded, 0.146 to 0.266 mm. long by 0.117 to 0.190 mm. wide; vas efferens arises from anterior border of each testis and the two join posterior to cirrus sac to form a short vas deferens. Cirrus sac large, with pronounced S-curve, extending into second half of body length, overlapping anterior border of acetabulum at median line; containing tubular, tripartite seminal vesicle (posterior portion smallest, anterior portion larger than other two) which narrows abruptly near middle of large, crescent-shaped portion of cirrus sac, continuing as narrow tube through large, muscular cirrus; seminal vesicle lies close to median wall of cirrus sac, lateral portion of sac filled with vesicular cells and scattered prostatic cells. External seminal vesicle and pars prostatica absent. Ovary slightly to left of midline, anterior to testes, rounded, 0.088 to 0.153 mm. long by 0.088 to 0.161 mm. wide. Uterus fills posttesticular space, concealing posterior half of testes and seminal receptacle, continuing anteriorly along median side of ovary, narrowing just anterior to ovary until single eggs form chain to level of cirrus sac where uterus expands, filling space to right of cirrus sac, overlaps cecum and enlarges near genital pore. Mehlis' gland diffuse, usually median to ovary. Inconspicuous opening to Laurer's canal dorsal, near posterior edge of seminal receptacle. Seminal receptacle globular, varying in size but always larger than ovary, 0.102 to 0.190 mm. long by 0.110 to 0.197 mm. wide, postovarian, often contiguous with ovary, and on same side of midline as ovary. Vitelline follicles small, massed laterally in acetabular region, often extending slightly anterior and/or posterior to acetabulum. Yolk ducts conspicuous; vitelline reservoir not observed although ducts seem enlarged near ootype. Eggs numerous, 32 to 41$\mu$ by 17 to 24$\mu$. Excretory pore terminal; excretory vesicle with short, wide stem which expands into subtriangular vesicle not extending anterior to testes.

DISCUSSION: Four of the 9 specimens exhibit amphitypy as compared with the type specimen. This amphitypy involves the cirrus sac curving to the left rather than to the right of the genital pore. Correlated with the inversion are: the cecum contiguous with the cirrus sac is longer, narrower, and extends more laterally; the genital pore, always near the midline, is on the side opposite to the curve of the cirrus sac; the oval testis is on the side of the cirrus sac.

COMPARISONS: Four species have been named in the genus *Discogasteroides: D. astracii* (Yamaguti, 1934) Strand, 1935, *D. minor* (Yamaguti, 1934) Strand, 1935, *D. indicus* Srivastava, 1939 and *D. carangis* Srivastava, 1939 (*D. caranxi* Srivastava, 1939). *D. carangis* was transferred to the genus *Paradiscogaster* by Yamaguti (1953). Yamaguti (1953) commented that "*D. indicus* Srivastava, 1939 cannot be assigned to the present genus *Discogasteroides* on account of marked differences, especially in the position of vitellaria." *D. ostracii* and *D. minor* are so alike that a description was not written for the later species, although egg sizes were notably different. With egg size, then, as the criterion, *D. hawaiensis* more closely resembles *D. minor*. *D. hawaiensis,* however, differs from both species in the S-shaped cirrus sac, the tripartite seminal vesicle, the relatively large acetabulum, the larger gonads, and the larger seminal receptacle.

GORGODERIDAE

*Xystretrum pulchrum* (Travassos, 1921) Manter, 1947

HOST: *Balistes capistratus* Shaw, trigger fish, "humu-humu mimi"; in 1 of 3 specimens examined.

LOCATION: Urinary bladder.

SPECIMEN DEPOSITED: U. S. Nat. Mus. Helm. Coll. No. 37467.

This single specimen is assigned to the species *Xystretrum pulchrum* rather arbitrarily on the basis of sucker ratio (1:1.27). Manter (1947) summarized the status of the species of *Xystretrum*. *X. pulchrum* was distinguished from *X. solidum* on the basis of a different (smaller) sucker ratio and less abrupt widening of the posterior portion of the body.

Manter (1947) listed sucker ratios for *X. pulchrum* from 1:1.30 to 1.5 while the sucker ratios for *X. solidum* range from 1:1.5 to 1.86. The single specimen from Hawaii has a sucker ratio of 1:1.27—smaller than any of the recorded ratios for either species, though closer to those of *X. pulchrum* than of *X. solidum*. Eggs in a single specimen vary greatly. Manter recorded egg sizes for *X. solidum* ranging between 29 by 19 $\mu$ and 44 to 48 $\mu$ by 27 to 29 $\mu$; and egg sizes for *X. pulchrum* varied from 41 $\mu$ by 20 $\mu$ to 51 $\mu$ by 42 $\mu$. Random selection of eggs from my specimen vary from 39 to 57 $\mu$ by 19 to 26 $\mu$. Although the 51 $\mu$ by 42 $\mu$ measurement is an exception, as a rule eggs of *X. pulchrum* tend to be relatively narrower than those of *X. solidum*, and in most instances are more than twice as long as wide.

The cirrus of the Hawaiian specimen is covered with minute papillae (Fig. 10a). A study of 5 speciments of X. *pulchrum* and 51 specimens of *X. solidum* from Tortugas, Florida did not reveal the presence of numerous papillae on the cirrus, although two specimens of *X. pulchrum* had a very few papilla-like structures at the tip of the cirrus (Fig. 9b). More specimens from Hawaii might indicate a new species based on the numerous papillae of the cirrus together with the apparent absence of striae on the posterior portion of the body, the smaller sucker ratio, and the very minute body spines.

The single specimen fom Hawaii measures: 4.088 mm. long, the forebody being 1.533 by 0.803 mm. (at juncture of two parts of body), and the hind body 2.555 mm. long by 2.154 mm. at testicular level.

### ACCACOELIDAE

*Paratetrochetus aluterae*, n. gen., n. sp. (Figs. 11-13)

HOST: *Alutera scripta* (Osbeck), file fish, "oililepa" or "ohua"; 1 specimen examined.

LOCATION: Small intestine

TYPE SPECIMEN: U. S. Nat. Mus. Helm. Coll. No. 37468.

DESCRIPTION (based on a single specimen): Body elongated, cylindrical, 5.227 mm. long by 0.657 mm. wide; forebody more slender than hindbody. Cuticula thick, unspined. Oral sucker very slightly subterminal, subspherical, 0.380 mm. long by 0.394 mm. wide; small cone-shaped elevation, with lumen, rising from base of oral sucker cavity. Acetabulum stalked, about one-fifth body length from anterior end; oval, 0.467 mm. long by 0.343 mm. deep, with longitudinal opening. Sucker ratio based on lengths: 1:1.23. Pharynx large and oval, 0.234 mm. long by 0.139 mm. wide with slender anterior extension 0.073 mm. long by 0.043 mm. wide and short, rounded posterior thickening about 0.050 mm. in diameter; anterior extension entering lumen of elevation at base of oral cavity. Esophagus extending to posterior part of forebody where lobed glandular mass arises dorsally at point of bifurcation; bifurcation at level of anterior edge of acetabular stalk. Ceca H-shaped, wide; unbranched but deeply indented anterior extensions terminate near anterior end of pharynx; very wide posterior ceca narrow before joining excretory system to form short cloaca near posterior end of body. Genital pore in-

conspicuous, ventral to posterior portion of oral sucker. Testes close together near middle of hindbody, separated by ascending limb of uterus and vitelline glands; anterior testis dorsal, 0.38 mm. long by 0.343 mm. thick;

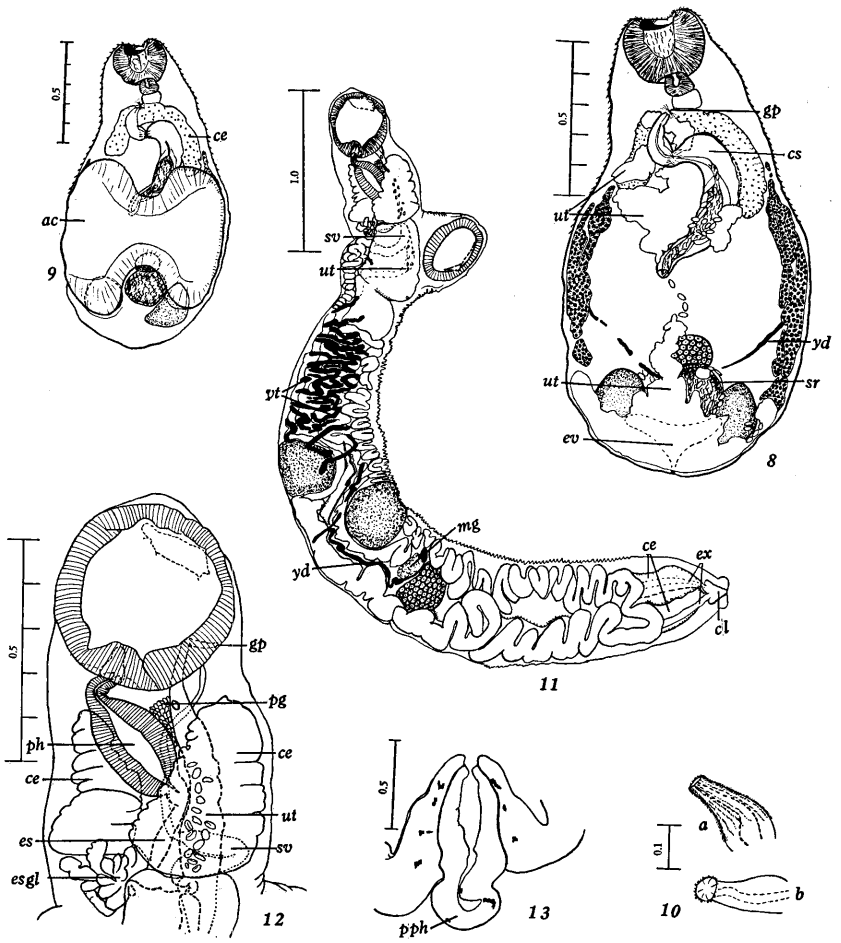


PLATE II

Fig. 8. *Discogasteroides hawaiensis* from *Ostracion sebae,* ventral view omitting acetabulum.

Fig. 9. *D. hawaiensis* ventral view omitting uterus, vitellaria and excretory vesicle.

Fig. 10. (a) *Xystretrum pulchrum* from *Balistes capisitratus,* at Hawaii, detail of cirrus.
(b) *Xystretrum pulchrum* from *Spheroides spengleri* (Bloch) at Tortugas, Florida, detail of cirrus.

Fig. 11. *Paratetrochetus aluterae* from *Alutera scripta,* lateral view.

Fig. 12. *P. aluterae,* detail of forebody.

Fig. 13. *Tetrochetus coryphaenae* from *Coryphaena hippurus* L. at Tortugas, Florida, section of elevation into oral cavity showing relationship of prepharynx to oral sucker.

posterior testis ventral, 0.38 mm. long by 0.372 mm. thick. Cirrus sac absent. Seminal vesicle originates a little posterior to acetabular stalk and extends forward as tightly coiled tube close to dorsal body wall; dorsal to acetabulum tube bows ventrally; distinct constriction in tube occurs opposite intestinal bifurcation, anterior to which the vesicle is more or less winding (Fig. 12). Union with the uterus not discernible, but probably occurs near genital pore. Pars prostatica extending from level of posterior end of pharynx to near oral sucker, surrounded by prostatic cells. Ovary subspherical, posttesticular, about two-thirds body length from anterior end, 0.241 mm. long by 0.350 mm. deep, separated from ventral and dorsal body walls and from posterior testis by uterine coils. Uterus coils anteriorly to level of posterior end of seminal vesicle, then descends ventrally to near posterior end of body, coils forward dorsally, passing between testes, then returns to dorsal position until opposite acetabulum where it bows ventrally. Mehlis' gland comparatively compact mass of cells anterior to ovary, 0.088 mm. long by 0.183 mm. thick. Laurer's canal present, opening dorsally just anterior to Mehlis' gland; seminal receptacle absent; vitellaria tubular between anterior testis and acetabulum, extending backward between testes; well developed yolk duct dorsal to posterior testis, yolk reservoir at ventral anterior edge of ovary. Eggs very numerous, 25 to 29 $\mu$ by 15 to 19 $\mu$. Cloacal pore terminal; cloaca short; excretory system not observed except for rather large collecting tubules in forebody and posterior portion of the pair of tubules which enter cloaca.

DIAGNOSIS OF THE GENUS *Paratetrochetus*. Accacoelidae: Body elongate. Acetabulum more or less stalked. Esophagus with glandular outgrowths at its bifurcation. Anterior and posterior ceca simple. Pharynx elongate with slender anterior extension projecting into lumen of conical elevation in base of oral cavity; small, bulb-like muscular addition at posterior end of pharynx. Testes tandem (anterior more dorsal, posterior more ventral) in middle third of body. Seminal vesicle coiled. Pars prostatica surrounded by prostatic cells. No genital papilla. Genital pore at level of posterior part of oral sucker. Ovary posttesticular, postequatorial. Vitellaria tubular, tending to be dorsal, extending between acetabular stalk and anterior testis. Uterus reaching to near posterior end of body. Excretory tubules joining ceca to form a short cloaca. Intestinal parasites of marine fishes. Type specimen: *Paratetrochetus aluterae* from *Alutera scripta;* Hawaii.

The name *Paratetrochetus* implies the close relationship with the genus *Tetrochetus*. *Paratetrochetus* has several similarities to *Tetrochetus,* i.e. unbranched anterior extensions of the ceca, the absence of a proboscis and proboscis sheath, the absence of a genital papilla, the absence of a well differentiated metraterm and the postequatorial gonads. Unlike *Tetrochetus* (compare Figs. 12 and 13), and resembling *Rhynchopharynx* to some extent, *Paratetrochetus* has an anterior extension of the pharynx which apparently moves into the lumen of the elevation in the base of the oral cavity.

## SUMMARY

Two new genera are described: *Hysterogonia* and *Paratetrochetus*.

Six new species are described: *Cleptodiscus bulbosus* from *Melichthys buniva* (Lacépède), *Lepocreadium incisum* from *Melichthys buniva* (Lacépède), *Pseudopecoelus brevivesiculatus* from *Cantherines pardalis* (Ruppell) and *Melichthys buniva* (Lacépède), *Hysterogonia balistis* from *Balistes capistratus* Shaw. *Discogasteroides hawaiensis* from *Ostracion sebae* Bleeker, and *Paratetrochetus aluterae* from *Alutera scripta* (Osbeck).

Two previously described species are also reported: *Lepocreadium clavatum* (Ozaki, 1932) Yamaguti, 1938 from *Melichthys buniva* (Lacépède) and *Xystretrum pulchrum* (Travassos, 1921) Manter, 1947 from *Balistes capistratus* Shaw. *Lepocreadium clavatum* is also found in Japan, while *Xystretrum pulchrum* is reported from Brazil and Tortugas, Florida. The latter, with further collection in Hawaii, may prove to be a distinct species.

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# Modification of the Centrifugal-Flotation Technique for the Isolation and Concentration of Nematodes and Their Eggs from Soil and Plant Tissue*

FIELDS E. CAVENESS AND HAROLD J. JENSEN**

Accuracy in taking a census of a nematode population depends upon the percentage of nematodes recovered from sample residues. Methods that have been used have certain disadvantages. In the use of sieves as in the gravity-screening technique for soil described by Cobb (1918), or in the Waring blendor technique described by Taylor and Loegering (1953) for plant material, many nematodes and most of the eggs are usually lost because they pass through the sieves. Furthermore, the recovered nematodes may be mixed with a considerable amount of plant and soil debris. The funnel technique of Baermann (1917) is time consuming; eggs and lethargic nematodes frequently are not separated by these methods. None of these provide a means of completely separating nematodes from objectionable extraneous matter. In the interest of developing a more rapid and accurate method of determining nematode populations conventional methods were compared with a modification of the centrifugal-flotation technique employed by Faust et al. (1938).

## MATERIALS AND METHODS

Aliquots of 50 cc. were taken from thoroughly mixed samples of each of four soil types including sandy loam, silt loam, silty clay loam and peat. Three of these were processed by the gravity-screening method, using sieves of 50, 100 and 200 meshes per inch on each, and three by the Baermann funnel method. Three aliquots were further divided into three equal parts each and processed by the new technique, the modified centrifugal-flotation technique.

*Approved for publication as Technical Paper No. 897 by the Director of the Oregon Agricultural Experiment Station. Contribution of the Department of Botany and Plant Pathology.

**Graduate Research Assistant and Assistant Nematologist respectively, Oregon Agricultural Experiment Station. The authors wish to acknowledge the cooperaion, assistance and the generous use of equipment of Dr. Ivan Pratt of the Department of Zoology, Oregon State College and suggestions of Gerald Thorne, Senior Nematologist, Nematology Section. USDA.

Vetch roots, decaying narcissus bulbs, and narcissus leaves were comminuted in water with a Waring Blendor for 10 seconds. Additional water was added to each sample of plant tissue to make a total volume of 300 ml. This was divided into six aliquots of 50 ml. Three aliquots were processed in Baermann funnels and three by the new technique.

Cellulose nitrate centrifuge tubes, 60 cc. capacity (29 x 113 mm.) were used. Each of the 50 cc. soil aliquots was divided equally among three tubes and mixed with approximately 30 ml. of water. The 50 ml. aliquots of the comminuted plant tissue were used directly. All centrifuge tubes were adjusted to the same weight by the addition of water before centrifugation. Three tubes were centrifuged simultaneously for five minutes in a Servall angle centrifuge type XL. Timing began after a maximum of approximately 4800 revolutions per minute, a RCF of 2900 g's, had been reached. The resulting supernatant fluid was poured off with one smooth motion without disturbing the material at the bottom of the tube. The first spinning of the sample eliminated material lighter than water and was repeated as needed.

Nematodes were recovered from the residue in the tubes by the addition of a sugar-water syrup (484.5 g sugar per liter of water) having a specific gravity of 1.18 and centrifuging as before. After spinning, the syrup containing the nematodes was immediately diluted by pouring into 500 ml. of tap water to avoid any possible harmful effects to the nematodes. The excess water was decanted after approximately 20 minutes when the nematodes had settled.

Water and nematodes were then poured into a one liter graduate and additional water was added to make a volume of 250 ml. The graduate was shaken to obtain a uniform suspension of nematodes. A 10 ml. aliquot was taken immediately and allowed to settle in a Syracuse watch glass marked for counting as suggested by Cobb (1918).

## RESULTS

The new technique, applied to soil and plant tissue samples, significantly increased recovery of nematodes compared with the screening and Baermann funnel methods (Table I). Nematode eggs were not recovered from soil by either the screening or the Baermann funnel methods but were recovered from soil by the new technique. Although the Baermann funnel method yielded significant numbers of both eggs and nematodes from the plant tissue the yield was greater and cleaner using the new technique. Suspended and settled extraneous material hindered the counting of nematodes in the Baermann funnel and gravity-screening residues. However, extraneous matter was not a factor when examining residues obtained by the new technique.

Additional trials were made to determine the effects of varying the specific gravity of the syrup, the force and periods of centrifugation. The viscosity of the syrup with a specific gravity of 1.35 interfered with the isolation of nematodes. Periods of 3 minutes were as satisfactory as six or nine minutes with syrups having specific gravities 1.10 to 1.25. However, six or nine minutes of centrifuging were necessary for syrups with specific gravities 1.30 to 1.35. The highest percentage of nematodes recovered were in syrups with specific gravities 1.10 to 1.30 using an RCF of 4500.

The specific gravity of various nematodes, e.g. *Longidorus sylphus* Thorne 1939, *Pratylenchus penetrans* (Cobb 1917) Sher & Allen 1953, *Diplogaster* sp., *Plectus* sp., and *Rhabditis* sp. was found to be between 1.05 and 1.06 by floating them in sugar-water syrups of various concentrations.

Attempts to separate *L. sylphus,* a large nematode exceeding 4 mm. in length were not successful. *L. sylphus* were found in the soil residue after

TABLE I. A comparison of the gravity-screening, Baermann funnel, and centrifugal-flotation methods for the recovery of nematodes and their eggs from soils and plant tissues.

| Sample | Number of samples* averaged | Method of Isolation and Concentration: Screening | | Baermann funnel | | Centrifugal-Flotation | |
|---|---|---|---|---|---|---|---|
| | | Nematodes | Eggs | Nematodes | Eggs | Nematodes | Eggs |
| Sandy loam Soil | 6 | 135 | 0 | 165 | 0 | 1011 | 167 |
| Silt loam soil | 2 | 200 | 0 | ----- | -- | 1175 | 825 |
| Silty clay loam soil | 10 | 276 | 0 | 45 | 0 | 757 | 228 |
| Peat soil | 5 | 243 | 0 | 110 | 0 | 625 | 125 |
| Hairy vetch root tissue | 3 | ----- | -- | 4150 | 3735 | 5398 | 16043 |
| Decaying narcissus bulb | 3 | ----- | -- | 800 | 10500 | 76950 | 16300 |
| Narcissus leaf | 3 | ----- | -- | 100 | 0 | 350 | 0 |

*Average of three aliquots in each sample.

spinning where they were retained by the soil particles probably because of their length. Shorter, but much broader nematodes, i.e. *Diplogaster* sp. and *Rhabditis* sp., were easily separated.

Nearly 80 percent of the nematodes were coiled and motionless after being collected in the syrup, but appeared normal and active after two hours in tap water. Inasmuch as spinning in tap water had no visible effects, the coiling of the nematodes was probably due to the viscosity of the syrup. If the nematodes are to be preserved for permanent slides, a delay of at least two hours before fixation is necessary to avoid distortion.

The eggs from the syrup appeared normal, and the larvae inside were active. Unfortunately, the identification of the various nematode eggs is extremely difficult. From the standpoint of population studies, the isolation of eggs enables one to estimate the abundance of nematodes rather than the species present.

## SUMMARY

The modified centrifugal-flotation technique makes possible: (a) the isolation of nematode eggs, (b) the recovery of a larger proportion of nematodes, (c) the reduction of objectionable extraneous matter, (d) a rapid processing of small quantities of soil and plant material for census data.

The technique is recommended where it is desirable to determine the entire nematode population from small aliquots of soil or plant material. However, when large numbers of nematodes are desired for general survey information or for permanent collections, other current methods should be used.

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# *Hymenolepis pulchra* N. Sp., A Cestode from the Shrew *Sorex trowbridgei* in California

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Seven small cestodes belonging to the genus *Hymenolepis* were recovered from the intestine of one *Sorex trowbridgei* collected in August, 1949, and nine additional cestodes belonging to the same species in one *Sorex pacificus* collected in August, 1954, both from approximately the same locality. The worms were fixed in Bouin's fluid and stained as whole mounts with Ehrlich's haematoxylin. The cestodes are described as new and the name *Hymenolepis pulchra* is proposed for them.

*Hymenolepis pulchra* n. sp.

DIAGNOSIS: Strobila total length 2.5-4 mm. in mature specimens; strobila greatest width 220-290 microns; proglottid number approximately 40-70 in mature specimens. Scolex with unarmed rostellum. Scolex diameter range 360-520 microns, average 430 microns; sucker diameter range 110-195 by 204-304 microns, average 138 by 243 microns; rostellum diameter range 56-100 by 86-160 microns. Excretory system composed of two pairs of longitudinal ducts, the ventral pair with cross-connections in each segment. Genital pores unilateral, situated in the middle of lateral margin of segment. Testes in a straight line, diameter range 22-26 by 30-34 microns. Cirrus spinose; cirrus pouch length range 60-100 microns, average 82 microns. Vagina with seminal receptacle. Ovary not lobed. Uterus ovoid, confined to space between excretory ducts, not filling gravid proglottids. Eggs thin-shelled, mostly spherical, 20-30 microns in diameter.

TYPE HOST: *Sorex trowbridgei.*

OTHER HOST: *Sorex pacificus.*

HABITAT: Intestine.

TYPE LOCALITY: 7 mi. E. of Bayside, Humboldt Co., California.

OTHER LOCALITY: Mouth of Little River, Humboldt Co., California.

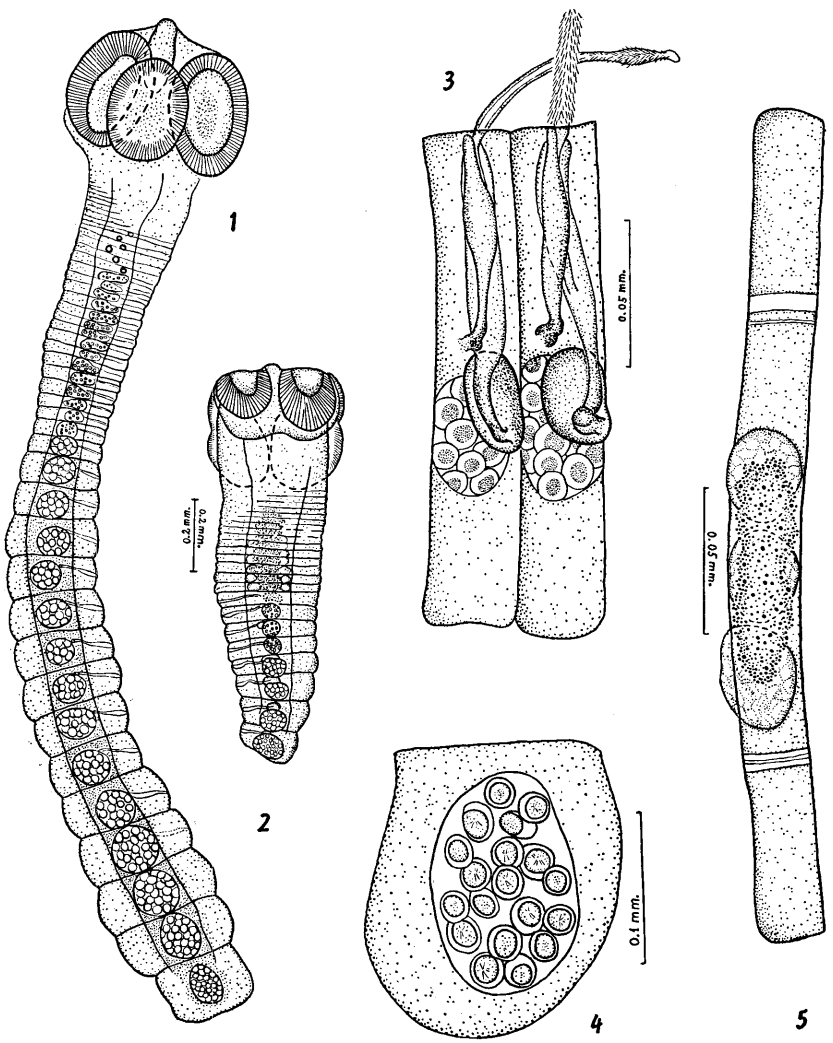
TYPE SPECIMEN: U. S. Natl. Mus. Helm. Coll. No. 37474.

PARATYPE, Immature: U. S. Natl. Mus. Helm. Coll. No. 37475.

DESCRIPTION. In external appearance, the cestodes described here are somewhat unusual in that the scolices are very large in comparison to the strobilae (Fig. 1). The suckers are prominent and muscular and often obscure the slender and weak rostellum (Fig. 2). In some specimens, a dorso-ventral folding of the scolex may be observed (Fig. 2). The rostellum is delicate and without any observable musculature. Segmentation begins almost immediately posterior to the scolex. The immature and mature portions of the strobila consist of wide, short and very thick segments. Internal organs are observed with difficulty because the male and female gonads as well as the uterus are superimposed in a dorso-ventral direction. The ovary and testes disappear in the semi-gravid region. The widest part of the strobila of fully grown worms is immediately posterior to the scolex or preceding the terminal gravid segments. The latter are narrower and longer than are the other gravid segments (Figs. 1 and 4). The excretory system consists of

two pairs of longitudinal ducts; a ventral pair with cross-connections, and a dorsal, narrow pair without ramifications.

MALE REPRODUCTIVE SYSTEM: The testes are arranged in a straight line. They are about as long as the segment they occupy and are laterally com-




*Hymenolepis pulchra.* All drawings made with the aid of a camera lucida.

Fig. 1. Fully grown specimen from *Sorex trowbridgei,* showing successive stages in the development of the uterus.

Fig. 2. Young specimen from *Sorex pacificus* with mature and a few semi-gravid proglottids. Rostellum obscured by suckers.

Fig. 3. Two semi-gravid proglottids showing uterus, seminal vesicle and part of seminal receptacle, male and female ducts, and cirri.

Fig. 4. Terminal gravid proglottid.

Fig. 5. Mature proglottid showing excretory ducts, testes and ovary.

pressed (Fig. 5). The median testis is frequently obscured by the ovary. In mature proglottids, the male duct system is barely visible, but in semi-gravid proglottids (Fig. 3) the seminal vesicle, the proximally twisted vas deferens and the long, thin-walled cirrus pouch are prominent structures. The distal portion of the cirrus is covered with small spines which are apparently restricted to a relatively small area. Figure 3 shows proglottids with partially and fully extruded cirri.

FEMALE REPRODUCTIVE SYSTEM: The densely granular ovary is almost as long and about three times as wide as the mature proglottid. It is not lobed but roughly rectangular in shape (Fig. 5). No vitelline gland was observed. The vagina, as seen in semi gravid proglottids (Fig. 3) is a wide and thin-walled tube. A small seminal receptacle is present. The uterus is easily seen in mature proglottids where it is of about the same size and shape as the ovary. In semi-gravid and gravid proglottids it is a nearly spherical body confined to the space between the excretory ducts (Figs. 1 and 2). In terminal gravid proglottids, the uterus is always longer than wide. Eggs are thin-shelled and usually spherical.

No significant differences in organ size or structure were noted between specimens from *Sorex trowbridgei* and specimens from *S. pacificus*. Most of the worms from *S. pacificus* were not fully grown, measuring little more than one millimeter in body length, while fully grown specimens from *S. trowbridgei* measured up to four millimeters in length.

## DISCUSSION

*Hymenolepis pulchra* n. sp. differs from all other species of *Hymenolepis* described from shrews in the possession of a relatively small and spherical uterus which, at first glance, is reminiscent of a uterine capsule. This type of uterus was described for a cestode of shrews in Russia by Spassky (1947) who placed this cestode in the genus *Neoskrjabinolepis*. Unfortunately, I have not been able to locate his original paper. A brief reference to his genus is contained in the introductory part of Spassky's book on anoplocephalids (1951).

Among the North American hymenolepidids described from shrews, only *H. macyi* Lucker and Rausch, (see Voge, 1955), has an unarmed rostellum This species does not resemble *H. pulchra,* differing from the latter in the size of the scolex and suckers, in the structure of the uterus and in the alignment of the gonads. Of the European species of *Hymenolepis* described from shrews, *H. diaphana* Cholodowsky, 1906, has an unharmed rostellum but the testes are arranged in a triangle. Similarly, *H. alpestris* Baer, 1931, has the testes arranged in a triangle and the uterus occupying almost the entire gravid segment.

## SUMMARY

*Hymenolepis pulchra* n. sp., is described from the shrew *Sorex trowbridgei* in California, and reported from a second host species, *Sorex pacificus*. Relationships to other hymenolepidids of shrews are discussed.

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# Rearing Simuliids in the Laboratory from Eggs to Adults*

CHARLES F. HARTLEY**

Investigations of the biology of the simuliids were carried out during the summer of 1953 in connection with research on *Leucocytozoon simondi* in ducks in northern Michigan. Special attention was paid to developing laboratory rearing methods for the dual purpose of associating immature stages with adults for taxonomic studies and providing flies of known history and identity for transmission work. Since there has been no opportunity to continue these studies and it does not appear likely that they will be continued in the near future, the results of that summer's work are being reported.

While attempts at laboratory rearing of black flies through their entire life-cycle—i.e. from adult to adult with mating and oviposition taking place in captivity—have been uniformly unsuccessful, several methods have been developed for rearing adults from eggs, larvae or pupae. Procedures for rearing adults from aquatic stages consist in placing the eggs, larvae or pupae in water kept in motion by air streams (Vargas, 1945) or mechanically operated paddles (Fredeen et al., 1953), or in placing them on "washboard" surface over which water is run (Thomas, 1950). The adults were easily reared from pupae, either those obtained from either of the apparatus described above or collected in nature, merely by putting the pupae on a piece of moist blotting paper in a cotton stoppered vial. More recently Dalmat (1955) reports the rearing of Simuliidae in natural or near natural habitats. However, direct association of the several larval stages with the adults is very difficult with these procedures. Also they require considerable space and running water supplies.

Several devices were tried before one was found which not only insured a high survival of the eggs and larvae through to the adult stage, but which also was simple and took relatively little space. An aquarium furnished with an overflow was used as an immediate source for all of the water used in the rearing experiments (fig. 1). Fresh lake water, pumped from Douglas Lake into a large reservoir in the aquarium constantly and siphons drawing from this aquarium furnished the water for each individual experiment. The siphons were made of three-sixteenths inch glass tubing. Larger numbers of siphons could be accommodated by merely increasing the rate of flow of water into the aquarium; care has to be taken to see that there is always more water running into the aquarium than is run off by the siphons. The water from these siphons was run into four ounce square packer jars. The rate of water flow from the siphons can be varied by varying the distance between the siphon outlet and the surface of the water in the aquarium.

The egg-mass, usually on a grass blade, was suspended within the packer in the stream of water from the siphon by attaching the grass blade to the neck of the packer jar with a rubber band. The larvae upon hatching attach at the points of most rapid water flow. If the siphon dips into the water in the packer jar and touches the side of the jar, some larvae will migrate up the siphon and be lost. Likewise, unless the jar is elevated from the runoff

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board with an object smaller than the base of the packer jar (e.g. a rubber stopper), the larvae will gradually migrate down onto the runoff board. In the absence of any undue disturbances which might cause the larvae to detach and be washed downstream, nearly all of the larvae will pupate and emerge as adults. Two egg-masses of *Simulium venustum* Say were brought through to adults in 20-25 days at water temperatures of 65°-75° F. using this method.

Some of the larvae attached in a circular pattern around the point on the bottom of the inside of the packer jar where the current of water from the siphon struck. Most of them, however, attached on the outside of the packer jar, particularly where the direction of water flow changed on the curved surfaces of the neck of the jar and near the bottom of the jar where the water was running off. The larvae pupated in these places, the cocoon being attached directly to the glass, and they were removed to the cotton stoppered vials mentioned above by means of fine forceps. It is important to note that pupae collected before they had darkened—i.e. before the more darkly pigmented cuticle of the adult had formed—would not emerge in the vials. The pupae were allowed to develop for three days on the packer jar before being collected and placed in the rearing vials.

During the course of this work, several methods were tried which made use of filters intended both to keep the larvae confined and to exclude predators. Because of clogging, none of these set-ups were successful. Furthermore, the use of copper screen as a filter was apparently highly toxic to the larvae and resulted in their death in 36-48 hours. Perhaps Lea and Dalmat's (1954) inability to keep the larvae in their experiments alive for more than 48 hours can be explained by their use of copper tubing to lead the water into the experimental vessels.

## SUMMARY

*Simulium venustum* Say was reared from eggs using four ounce packer

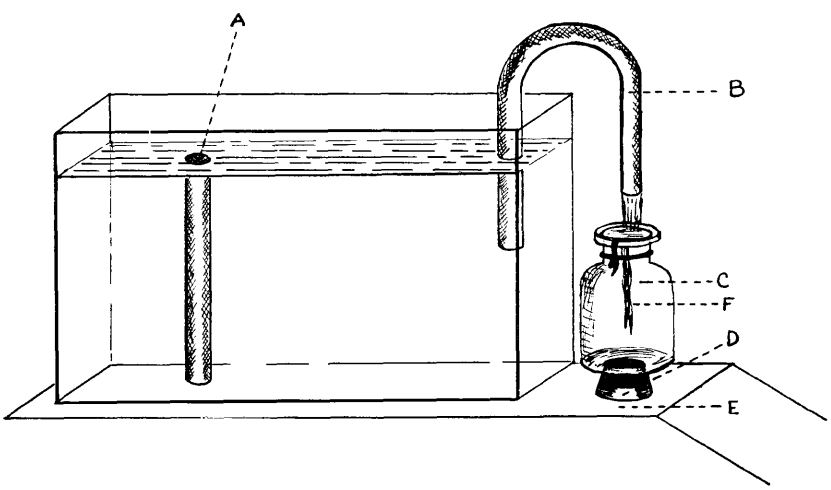


Figure 1. Apparatus used to rear aquatic stages of simuliids. A—overflow from aquarium, B—siphon, C—packer jar, D—rubber stopper, E—runoff board, F—grass.

jars and a continuous supply of running lake water delivered by a glass siphon from an adjacent reservoir (ordinary aquarium). The apparatus is described and figured in detail. The larvae upon hatching attach to inner and outer surfaces of the packer jar at points of most rapid water flow. These pupated and the pupae, after sufficient development, were collected with fine forceps and reared to adults on moist blotting paper in cotton stoppered vials.

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## Unusual Pathogenicity of *Diphyllobothrium* sp. in a Black Bear

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The black bear, *Ursus americanus* Pallas, has been found to be an exceptionally good host for a common species of *Diphyllobothrium,* as evidenced by the large size attained by individual strobilae, and the duration of infections experimentally established. Since 1950, the writer has used at least five of these bears annually in the study of *Diphyllobothrium* spp. With the single exception reported herein, no adverse effect to the hosts has been recognized.

On July 16, 1950, a bear approximately six months of age was fed plerocercoid larvae of *Diphyllobothrium* sp. (probably *D. ursi* Rausch, 1954), taken from steelhead trout, *Salmo gairdnerii* Richardson, which had been collected on the upper Kenai Peninsula, Alaska. This animal had been captured before weaning, and had been in captivity about two months before infection was attempted. On November 1, 1950, about three and one-half months after the feeding of the larvae, the bear was found dead in its cage. The *post mortem* findings are described.

On opening the abdominal cavity, a necrotic lesion seven mm. in diameter and five mm. deep was noted on the antero-ventral surface of the pancreas. The organ was somewhat enlarged, but no abnormal color was recognized. Upon opening the duodenum, the papillae of the pancreatic ducts were readily identified; protruding from each was the strobila of a cestode of the genus *Diphyllobothrium.* The scolices and anterior parts of the strobilae had penetrated a considerable distance up the pancreatic ducts. The two ducts opened about ten mm. apart, and their papillae were enlarged and edematous. At the level of the duodenal mucosa, one strobila measured five mm. wide, and the other measured three mm. After fixation of the organ, with the cestodes *in situ,* a block cut from the ventral part of the gland disclosed that the cestodes had penetrated deeply, and the two ducts appeared to be completely occluded by the strobilae and the inflammatory reaction evoked by them (Fig. 1). Only these two cestodes were present.

Microscopically, sections showed that an abcess directly connected with an occluded pancreatic duct had ruptured into the peritoneal cavity. The ducts were necrotic, and contained great numbers of polymorphonuclear neutrophils. The peritoneal surface of the organ was covered with fibropurulent exudate enclosing large numbers of degenerating cestode eggs. The abcess cavity was largely filled with polymorphonuclear neutrophils, and

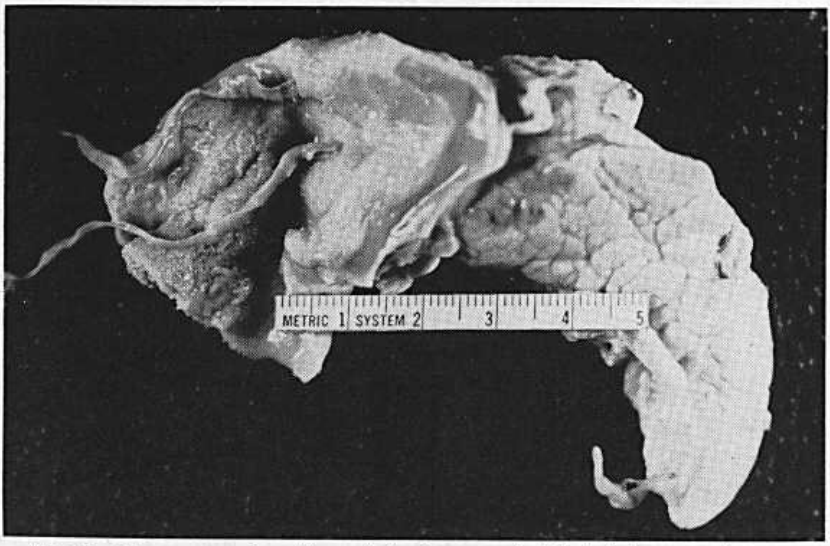


Fig. 1. Pancreas and section of duodenum of infected bear; cestodes protruding from pancreatic ducts into duodenum.

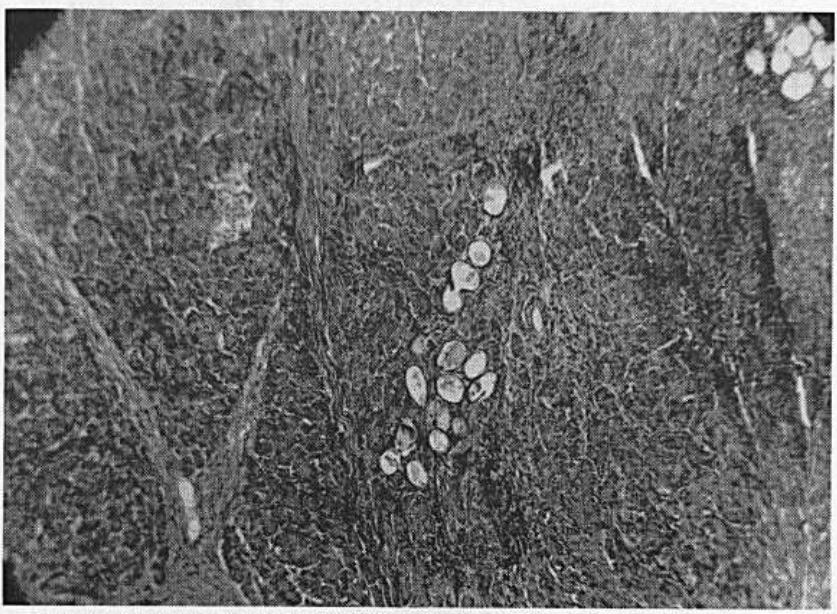

Fig. 2. Eggs of *Diphyllobothrium* sp. in lobule of pancreas. Photographed at 100 X.

numerous eggs were entrapped by the exudate. Adjacent parts of the gland showed evidence of chronic inflammation, with fibrosis and replacement of the lobules of the gland. There was local hyperplasia of the epithelium of the pancreatic ducts. Many eggs had become distributed through the gland by means of the smaller ducts. Some were surrounded by dense connective tissue, and others were found deep within the tissue of the lobules (Figs. 2-3). Locally, eggs were surrounded by macrophages and were undergoing phagocytosis and degeneration. No giant cells were observed.

Numerous eggs in the exudate escaped into the peritoneal cavity. These caused enough local irritation to evoke an inflammatory reaction. Aggregations of eggs enclosed by a thin layer of polymorphonuclear neutrophils were scattered over the peritoneal surface of the pancreas. The degree of inflammatory reaction observed around the eggs in the lobules was slight, and macrophages were few.

The writer is unaware of any report in the literature describing the tissue reaction to cestode eggs in the mammalian host. However, in the absence of giant cells the reaction in the bear resembled that described by Africa and de Leon (1938), involving the eggs of certain heterophyid trematodes.

Although the specific cause of death in the bear was not determined, it is evident that the cestodes were the essential factor. In some parts of Alaska, such as the Yukon-Kuskokwim delta region, *Diphyllobothrium* sp. is a prevalent parasite in the Eskimo. The usual pathogenicity, if any, of this cestode in man has not been clearly defined, but it is possible that invasion of the pancreatic ducts as described herein might also occur.

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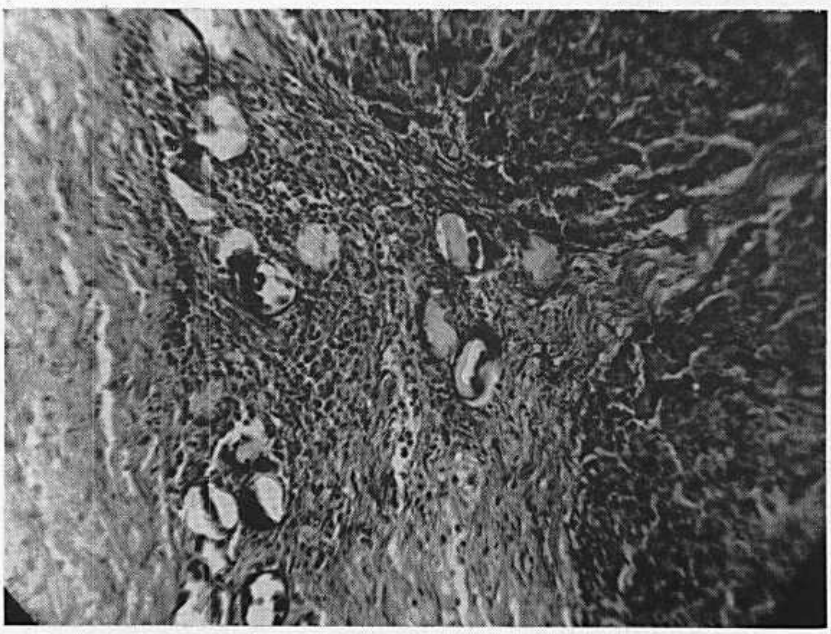

Fig. 3. Eggs of *Diphyllobothrium* sp. in zone of fibrosis near margin of abcess. Photographed at 200 X.

# A Proposed Classification of the Acaridiae (Acarina: Sarcoptiformes)

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Acarologists in general have become increasingly aware of the shortcomings of existing higher classifications within the Acarina. These weaknesses were integrated into the classifications at a time when very little was understood about the groups concerned. With new additions to our knowledge and a better understanding of taxonomic characteristics of the Acarina, recent workers have set about to revise older conceptions of the higher categories. Camin and Gorirossi (1955), in the United States, have done this with the Mesostigmata. Evans, in England, is working towards this end with the same group. The Trombidiformes are being revised by Cunliffe in the United States. The Sarcoptiformes have, for the most part, found neglect at the hands of American investigators, most modern work being done by Zachvatkin (1941) and Dubinin (1953) in Russia and Grandjean (1953) in Switzerland. A recent presentation of this group by Baker and Wharton (1952) followed the older classifications of Vitzthum (1931, 1942) and Oudemans (1923).

It is the purpose of this paper to present a classification of the Acaridiae based upon biological relationships and morphological characteristics which prior workers failed to utilize. This work is dependent in part on the treatments of the Sarcoptiformes by Zachvatkin and Dubinin. Specimens were available for study, in most cases, at the U. S. National Museum, Washington, D. C.*

## HISTORICAL

The suborder Sarcoptiformes is composed of the Acaridiae and the Oribatei. The former contains the cheese mites, itch mites, scab mites, and feather mites, and may be distinguished from the latter by relative lack of integumental sclerotization, absence of genital and anal plates, reduced palpi, reduction or lack of pseudostigmatic organs (with the exception of the Pediculocheloidea), and considerable sexual dimorphism.

Prior to 1903, the Acaridiae were considered as composed of a number of autonomous families. Oudemans' classification (1902, revised in 1923) divided these families into three groups, Anachotricha, Monachotricha, and Diachotricha, the distinction among these being based upon a single character, the presence or absence of vertical setae and their number. Zachvatkin (1941) indicates that the great systematic significance attached to this characteristic is arbitrary and completely unsubstantiated. Although Vitzthum (1931) presented a comprehensive classification of the Acarina, he made little change in Oudemans' scheme. In 1942 Vitzthum presented a classification of the Acarina in which a single family (Tyroglyphidae) contained nineteen divergent subfamilies. This family comprised the majority of the free-living forms. The parasitic forms, on the other hand, he placed in fourteen different families. Zachvatkin (1941), working with the "detricola" or free-living group of the Acaridiae, arranged twenty-one seemingly heterogeneous families into four on the basis of correlative work with a large number of characteristics. André (1949) elevated the Sarcoptiformes to an order and the Acaridiae to a suborder comprising twenty divergent families. Dubinin

*The author is deeply indebted to Dr. Edward W. Baker, Curator of Acarina, U. S. National Museum, for constant advice and assistance during the course of this work. The valuable advice of Dr. G. W. Wharton, Head, Department of Zoology, University of Maryland is especially appreciated.

(1953), in his monograph of the Analgesoidea, envisaged the Acaridiae as comprising six superfamilies, with no recognition of higher relationships within the group. Neither did he include the pediulochelids or the ewingids, which in themselves are deserving of at least superfamily rank. In 1954 he recognized the existence of two greatly different groups of high taxonomic value, but could assign no differential characteristic to these groups other than the single character as proposed by Oudemans.

It was found, during the course of this study, that the Acaridiae contains two major, biologically distinct groups, those which are parasitic and those which are not, and a small, rare, morphologically intermediate group whose ecology is not known. It was further found that this grouping was one of systematic proximity which was borne out by certain morphological correlations. These are: the type of genital opening of females, the presence or absence of female genital suckers and apodemes, and the modifications of the tarsi.

PROPOSED CLASSIFICATION

Suborder Sarcoptiformes
Supercohort Acaridiae
COHORT **Acaridia****
SUPERFAMILY **Pediculocheloidea**
Family Pediculochelidae
SUPERFAMILY Anoetoidea
Family Anoetidae
SUPERFAMILY **Acaroidea** (= Tyroglyphoidea)
Family Saproglyphidae (= Winterschmidtiidae, Ensliniellidae, Pontoppidaniidae, Nanacaridae, Czenspinskiidae new synonymy, Olafseniidae new synonymy, Oulenziidae new synonymy)
Family Acaridiae (= Tyroglyphidae, Caloglyphidae, Lardoglyphidae, Forcelliniidae)
Family Glycyphagidae (= Chortoglyphidae, Carpoglyphidae, Lentungulidae)
SUPERFAMILY Canestrinoidea
Family Canestriniidae
Family Linobiidae
Family Hemisarcoptidae
COHORT **Ewingidia**
SUPERFAMILY **Ewingoidea**
Family Ewingidae
COHORT **Psoroptidia**
SUPERFAMILY Listrophoroidea
Family Listrophoridae
SUPERFAMILY Analgesoidea
Family Freyanidae
Family Dermoglyphidae (= Pterolichidae)
Family Analgesidae
Family Proctophyllodidae
SUPERFAMILY **Psoroptoidea**
Family Epidermoptidae
Family Psoroptidae
Family Psoralgidae

**New names are indicated by boldface type.

SUPERFAMILY Sarcoptoidea
Family Sarcoptidae
SUPERFAMILY Cytoditoidea
Family Cytoditidae
Family Laminosioptidae

DISCUSSION AND DIAGNOSIS OF GROUPS

COHORT **Acaridia.** Mites within this group are either free-living and feed upon organic debris or are found in phoretic association with arthropods. Characteristic morphological attributes are: the presence of genital suckers usually bordering a longitudinal genital opening and absence of genital apodemes (Plate I, figure 1) in the adult female; the pretarsi typically with empodial claws (Plate I, figure 4); and large chelate chelicerae, typically modified for a free-living existence. Exceptions to these characters are noted in the discussion of the superfamilies.

SUPERFAMILY **Pediculocheloidea.** Hysterosoma separated from propodosoma and in turn subdivided by transverse sutures. Propodosoma with pseudostigmatic organs. Pretarsi membranous and without empodial claws. Genital opening of females with genital suckers (?). Associated with bees.

Only one family (Pediculochelidae) lies within this group and apparently only three collections have been made. Due to their rarity, specimens were not available for study. According to Baker and Wharton (1952) the pediculochelids represent a "primitive type of acarid-like mite . . . perhaps an intermediate form between these and the oribatids".

SUPERFAMILY Anoetoidea. Propodosoma and hysterosoma separated by a transverse suture, body otherwise unsegmented. Pseudostigmatic organs lacking; body setation simple. Pretarsi with empodial claws. Genital opening of females a simple transverse slit; genital suckers in two pairs, characteristically large and elongate and displaced laterally. Chelicerae with a single chela each. Palpal tarsi with laterally projecting, setae-like structures. Found associated with fungus and traveling on insects in the hypopial stage.

Members of the single family (Anoetidae) within this group are distantly related to the central group, **Acaroidea** (Plate II). The transverse genital opening is not typically seen in other groups of the cohort Acaridia, but the inclusion of the Anoetidae within this group is verified by the presence of genital suckers and empodial claws.

SUPERFAMILY **Acaroidea.** Propodosoma and hysterosoma separated by a transverse suture, body otherwise unsegmented. Propodosomal pseudostigmatic organs may be reduced or lacking; body setation simple. Pretarsi with empodial claws, which, in some forms, are surrounded by membranous, sheath-like projections of the pretarsi. Genital opening of females a longitudinal slit, with two pairs of suckers. Chelicerae large and chelate. Free-living and associated with insects.

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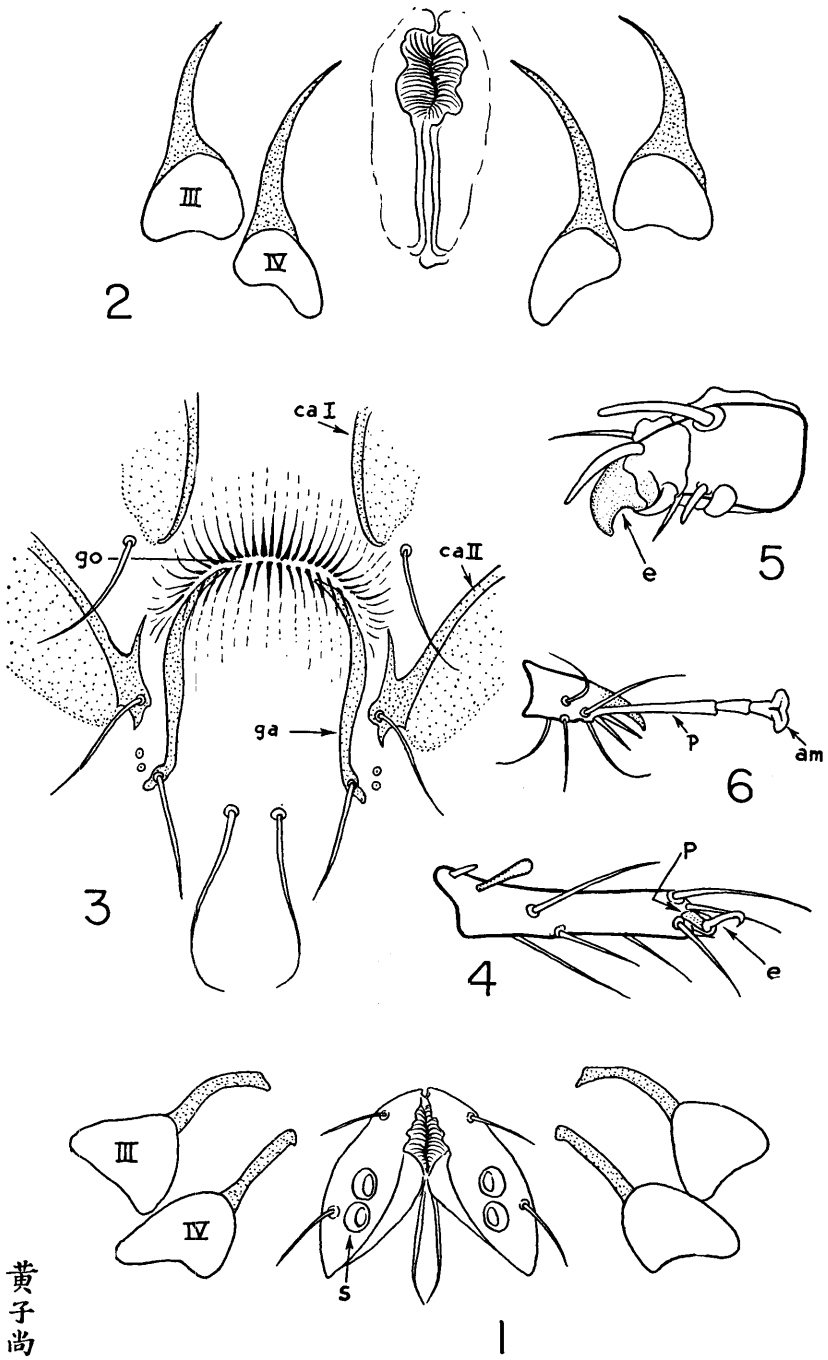
PLATE I

1. Genital opening of female of *Acarus siro* Linné, 1758.
2. Genital opening of female of *Ewingia cenobitae* Pearse, 1929.
3. Genital opening of female of *Psoroptes equi* (Hering, 1838).
4. Tarsus I of *Acarus siro* Linné, 1758.
5. Tarsus I of *Ewingia cenobitae* Pearse, 1929.
6. Tarsus I of *Psoroptes equi* (Hering, 1838).

Am—Ambulacrum; caI—Apodeme of coxa I; caII—Apodeme of coxa II; e—Empodial claw; ga—Genital apodeme; go—Genital opening; p—Pretarsus; s—Genital sucker; II—Coxa II; III—Coxa III.

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This superfamily, containing three families (Acaridae, Saproglyphidae, and Glycyphagidae), is visualized as the central group of the cohort Acaridia (Plate II). The longitudinal genital opening, the type of genital suckers, body shape, and distinct pretarsi with empodial claws are typical of the majority of forms within this group.

SUPERFAMILY Canestrinoidea. Body unsegmented, with the exception of a propodosomal-hysterosomal suture in Hemisarcoptidae. Membranous projections of pretarsi present, but empodial claws reduced or lacking. Genital opening of females a longitudinal slit, bearing two pairs of suckers. Predatory on or associated with insects.

Mites of this group show a closer relationship to the Acaroidea than do those of the family Anoetidae. The three families within the superfamily appear to be closely related in some respects such as reduction of empodial claws, genital configuration, and biology, though unrelated in other respects such as type of chelicerae and modifications of membranous portions of the pretarsi. Therefore this group should be considered a heterogeneous one, further studies possibly revealing a wider divergence among the families.

COHORT **Ewingidia.** SUPERFAMILY **Ewingoidea.** Body unsegmented and striated, genital opening of females a longitudinal slit, not accompanied by suckers or apodemes (Plate I, figure 2). Tarsi of all legs with strong empodial claws, those of legs III and IV modified for clasping. Chelicerae deformed. One family, Ewingidae, found in gills of land crabs.

Mites in this group show characters of both the **Acaridia** and **Psoroptidia.** The longitudinal genital opening, absence of genital apodemes, and empodial-type claws are reminiscent of the former group, and the absence of genital suckers, and configuration of male opisthosoma suggests the latter. The nature of the relationship of the mite to its host is not known. As shown in Plate II, the **Ewingidia** are seen as intermediate between the free-living and parasitic group.

COHORT **Psoroptidia.** Mites within this group are parasitic, or are thought to be. The genital openings are usually transverse or in the shape of an inverted "U" and are not accompanied by typical copulatory suckers (Plate I, figure 3). Genital apodemes are characteristically present in the adult females (Plate I, figure 3). Empodial claws are absent and the pretarsi are expanded distally into membranous, bell-shaped, sucker-like ambulacra (Plate I, figure 6). Sclerotization of the body is stronger, especially around the coxal bases and the integument is typically striated. The chelicerae are chelate but smaller than in the free-living forms.

SUPERFAMILY Listrophoroidea. Body unsegmented, integument striated. Genital opening of females either transverse or longitudinal. Genital apodemes absent in adult females. Adanal suckers of males reduced or lacking. Legs or mouthparts modified for clasping hairs. Unmodified legs with ambulacral suckers. Fur parasites of mammals.

This superfamily contains a single family, Listrophoridae, and the type genus is visualized as a primitive member of the **Psoroptidia** (Plate II).

SUPERFAMILY Analgesoidea. Body unsegmented and striated. Genital openings of females in the form of an inverted "V" and accompanied by genital apodemes. Males with adanal suckers. Leg III of males unusually long. Feather mites.

The composition of this superfamily follows the classification of the Analgesoidea as proposed by Dubinin (1953), with the exception that the family Epidermoptidae has been transferred to the superfamily **Psoroptoidea**

(see below). This leaves the following four families: Freyanidae, Dermoglyphidae, Analgesidae, and Proctophyllodidae. These are closely related, as shown by body sclerotization, genital configuration, and biology, thus making the group a homogeneous one.

SUPERFAMILY **Psoroptoidea.** Body unsegmented and striated. Genital opening of females in the form of an inverted "U" or a transverse slit, accompanied by genital apodemes. Males with adanal suckers. Leg III of males unusually long. Skin parasites of vertebrates except for certain genera found on hippoboscid flies.

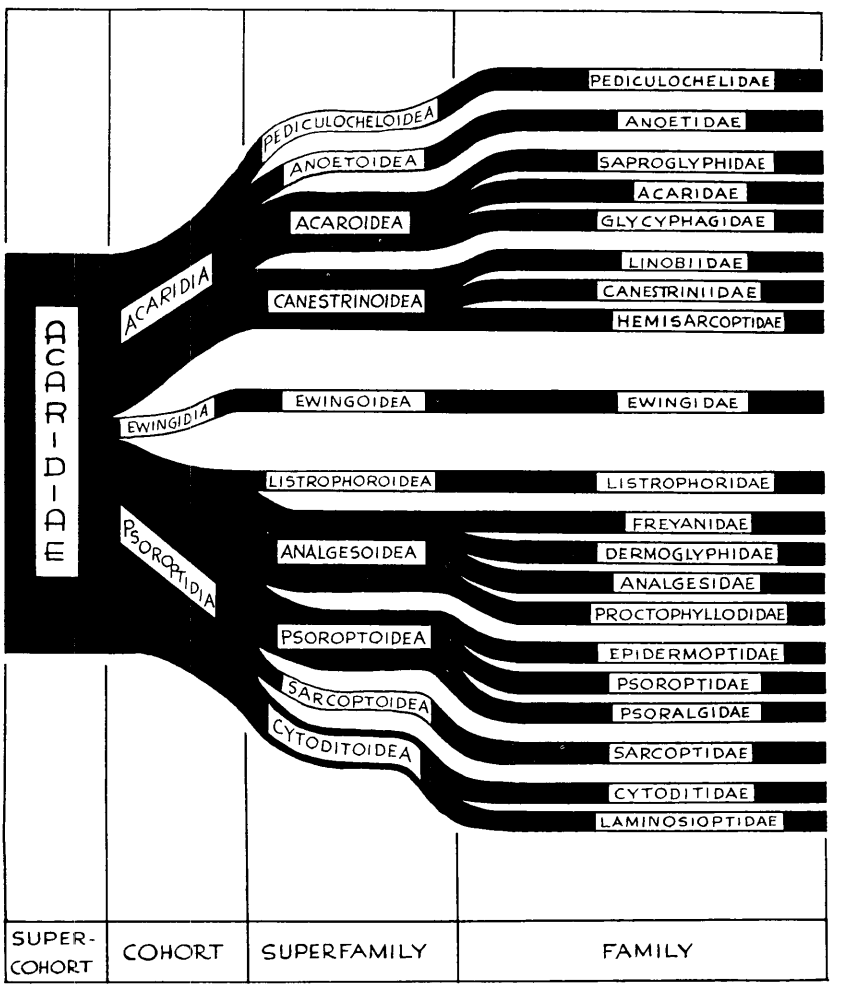


PLATE II

Cosmograph illustrating the probable relationships of families of the supercohort Acaridiae.

This group is also homogeneous, and appears to be closely related to the Analgesoidea. The three families, Epidermoptidae, Psoroptidae, and Psoralgidae are similar in degree of sclerotization, genital structure, and biology. Mention should be made of the fact that the family Epidermoptidae, included by Dubinin (1953) in the Analgesoidea, is not transferred *in toto* to the Psoroptidae, but a subfamily, Knemidokoptinae, is referred to the Sarcoptidae, where it more properly belongs.

SUPERFAMILY Sarcoptoidea. Body unsegmented, integument striated. Genital opening of females a simple transverse slit. Genital apodemes of females lacking. Adanal suckers absent or minute. Legs short, telescoped. Chelicerae small and chelate. Skin parasites of mammals.

This group contains one family, the Sarcoptidae, whose members show a reduction in body sclerotization and a tendency towards internal parasitism. They appear to be more closely related to the Cytoditoidea than to the **Psoroptoidea** and may be intermediate between the two.

SUPERFAMILY Cytoditoidea. Body unsegmented, integument striated ventrally and smooth dorsally, setation sparse. Genital opening of females a simple longitudinal slit. Genital and adanal suckers absent. Legs short, telescoped. Mouthparts fused into a sucking-tube. Internal parasites of fowl.

This superfamily contains two families, Cytoditidae and Laminosioptidae. It is thought that the presence of a longitudinal genital opening, when coupled with the absence of genital apodemes, does not necessarily indicate that this superfamily is more nearly related to the **Acaridia** than the **Psoroptidia**; rather, that these differences result from morphological modifications which accompany internal parasitism. This group appears to have arisen from a form closely related to the Sarcoptoidea.

## SUMMARY

A classification of the Acaridiae is presented, based on biological differences and morphological correlations. The supercohort Acaridiae is divided into three cohorts, for which are here proposed the names: **Acaridia** (containing mites which are free-living and/or found in phoretic association with other arthropods). **Ewingidia** (containing morphological intermediates), and **Psoroptidia** (containing parasitic mites).

The following new superfamilies are erected: **Pediculocheloidea, Ewingoidea,** and **Psoroptoidea.** Emendations to existing superfamilies and families are made and new synonymy is created. Exceptions to the general patterns and a discussion of possible phylogeny are presented.

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## *Macracanthorhynchus ingens* from Raccoons in Maryland

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There have been very few published records of the occurrence of the acanthocephalan parasite *Macracanthorhynchus ingens* (von Linstow, 1879) in North America. Chandler (1942) and Moore (1946) reported 11 of 13 raccoons (*Procyon lotor*) trapped in Angelina County, Texas, infected with these intestinal parasites. From 1 to 90 worms occurred in these animals. Chandler and Melvin (1951), in a study of parasites collected from mammals in Pennsylvania, reported *M. ingens* common in its usual host, the raccoon, and also found immature worms believed to belong to this species in skunk, *Mephitis nigra;* mink, *Mustela vison;* fox, *Urocyon cinereoargenteus;* and mole, *Parascalops breweri.* Van Cleave (1953), in a review of North American Acanthocephala, lists the definitive host as raccoon of the States of Texas and Pennsylvania. He further states: "It is thought that the apparent discontinuous distribution of *M. ingens* is due to incomplete records of its occurrence in intervening states." Subsequently, Goldberg (1954) reported 5 specimens of *M. ingens* in one of 14 skunks from Beltsville, Md.

This parasite appears to be fairly common in raccoons from Maryland, as indicated by material from two stations in the State. Of 44 intestinal tracts collected during the winter months, 1943-1946, at the Patuxent Research Refuge, Laurel, Maryland, 22 (50%) contained *M. ingens* in their intestines (Ediger, 1950). Numbers of these parasites per host varied from 1 to 125. Since then, additional raccoons from the Patuxent Research Refuge have been found infected with *M. ingens.*

The parasite has also been noted in raccoons collected from the Blackwater National Wildlife Refuge and surrounding areas, near Cambridge on the eastern shore of Maryland. One raccoon obtained in May, 1950, contained five specimens of *M. ingens.* In a series of raccoons obtained by V. T. Harris, during the winter months 1950-1951, 32 (37%) of 86 raccoons examined were infected with *M. ingens.* Intensity varied from 1 to 44 worms per raccoon.

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# The Attractiveness of Plants to Larvae of Root-Knot Nematodes. I. The Effect of Tomato Seedlings and Excised Roots on *Meloidogyne hapla* Chitwood

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Despite the great number of investigations which have been carried out to study the interrelationship between plants and parasitic nematodes, the mechanism by which the soil stage of the parasite is attracted to the plants has been given little attention. Baunacke (1922) stated that the substance attracting the larvae of the sugar beet nematode is given off only by living plants and that it is more effective in germinating seedlings than in old plants. Stewart (1921) said that the "cell sap" is the attractive agent for Aphelenchi. Linford (1939) found that root-knot nematodes are attracted by a region of the root which lies shortly behind the apex, and that they as well as *Rotylenchus multicinctus* (Cobb) are attracted by excised tissues of plant leaves and stems. Finally, Gadd and Loos (1941) by a different approach confirmed Baunacke's finding that the attractive substance has something to do with the growth of the plant, and they pointed out that "the substance which attracts eelworms to decaying tissue is not necessarily the same as that exuded by living roots." These were all qualitative statements, or observations (with the exception of Linford's experiments) incidental to investigations carried out to some other end.

In order to get a clearer picture of the factors involved an experiment was devised to permit the obtaining of quantitative data. This was achieved in the following way: The bottom of a Syracuse dish was covered with very wet, sterile quartz sand; in the middle were placed 2 egg-masses of *Meloidogyne hapla* Chitwood taken from greenhouse-grown tomatoes. A tomato seedling (Rutgers variety) freshly germinated on wet filter paper was put on one side of the egg-masses at a distance of 5 mm. and in such a way that it ran parallel to a predetermined diameter of the dish. In the sand the seedling usually continued to grow. Its length was measured before and after the experiment, which lasted 24 hours. The whole dish was kept in a moist chamber at 25-26° C. Upon conclusion of the experiment the seedling and egg-masses were removed and the sand in the dish was divided exactly in half along the predetermined diameter. The larvae in each half were counted and the number found in the half which had contained the seedling was expressed in percentage of the total number of nematodes that had hatched during the experiment. The counting was done with the aid of a variation of the Baermann funnel, that is by placing a piece of cheese cloth over a litle wire screen in a Syracuse dish. The sand containing the nematodes was spread over the piece of cloth and within about 12 hours all nematodes had migrated through the cloth to the bottom of the dish where they could easily be counted.

The theory of the procedure is simple: If the root is attractive a high percentage of nematodes will be found in the sand surrounding it, that is in one half of the dish; if it is repellent the percentage will be significantly below 50%; and if it is neither attractive nor repellent the percentage will be about 50%.

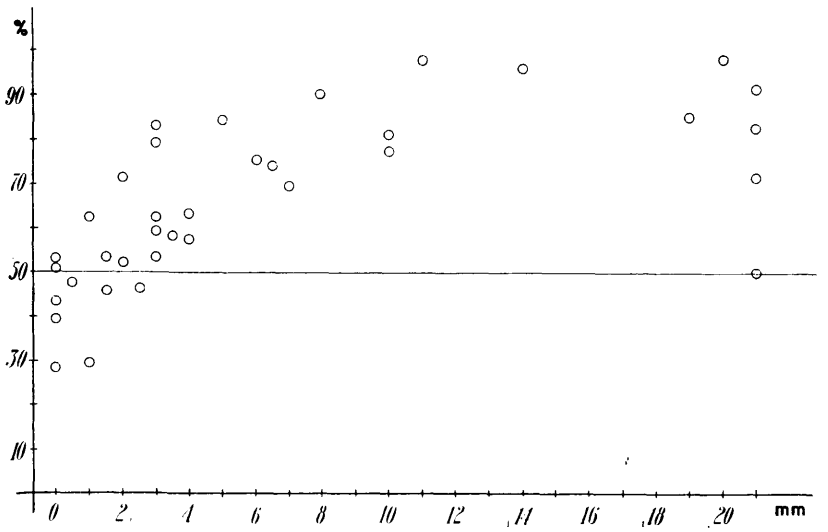


Fig. 1. Attractiveness of tomato seedlings to larvae of *Meloidogyne hapla*. Abscissa: growth of seedlings during experiment (24 hours). Ordinate: Percentage of nematodes found in half of dish occupied by seedling. Each circle one sample.

## EXPERIMENTS AND DISCUSSIONS

This set-up allows one to find out not only whether a germinating tomato seedling is attractive, but also whether the *rate of growth* of the seedling is of importance, for different seeds even from the same batch germinate with greatly varying vigor. The amount of growth within 24 hours varied in these experiments from 0 to 21 mm. In plotting the rate of growth of the seedling during the experiment against the percentage of larvae found in that half of the dish containing the seedling, we gain a relative value for the degree of attractiveness of each seedling. The results of this experiment are shown in figure 1. It is obvious that in seedlings with a growth rate up to about 1.5 mm. there is no attractiveness to the nematodes. In fact, seedlings of this sort are dead, such weak growth as they exhibit being due to the growth substances present in the roots before the basic metabolism stopped. If the roots grew at the rate of 2 mm. or more, their attractiveness increased almost proportionately with their rate of growth. The maximum was reached somewhere between 8 and 11 mm. The exact figure cannot be given because of the small number of experiments carried out for this length class. This seems to confirm the view of Baunacke and of Gadd and Loos that the attractive substance—whatever it may be—is connected with the growth of the roots, that is, with their metabolism. In addition it suggests that the effectiveness of the substance increases proportionately with the rate of growth of the roots between 2 and 8-11 mm. per 24 hours.

The next step is the exact localization of the point of emanation of the attractive substance. This end was achieved by testing excised root tips ranging in length from 1 to 16 mm., taken from germinating seedlings and from young plants.* The results are shown in figure 2. On the abscissa is

*Root tips should not be taken from seedlings which have been germinated more than two days on filter paper, and not from old plants since in both cases the metabolic conditions are likely to be abnormal. Even so the variations in attractiveness between roots of the same age might be considerable.

plotted the length of the excised root tips in mm. and on the ordinate the percentage of the nematodes found around them, so that again a high percentage signifies great attractiveness. We can see that the number of nematodes found around the roots is decisively below the 50% level in the tips of 1- and 2-mm. lengths. There seems to be no escaping the conclusion that this region of the root is not only non-attractive, but actually repellent to the nematodes—certainly an unexpected result. The fact that the 2-mm. tip is more repellent than the 1-mm. tip would support this idea since it is obvious that the repellent substance would be more concentrated in the longer piece. After 2 mm. the percentage of the nematodes around the roots increases steadily up to the 8-mm. length and from there remains on a high level, indicating clearly that all these root tips are attractive to the nematodes.

In summarizing these effects it should be noted first that the proven attractiveness of certain root tips shows that the attractive substance remains active in excised roots for at least 24 hours. This seems to rule out the auxins as the attractive agent since they are broken down rapidly by enzymes if the plant tissue is submerged in water (Thimann, 1934). The apical 2 mm. of the root have a repellent effect on the nematodes. This is balanced by an increasing degree of attractiveness in the succeeding 6 mm. From 8 mm. on the attractiveness remains stable.

This pattern may be explained in two ways. First, the attractive substance is contained solely in the region from 2 to 8 mm. behind the root apex; second, it is contained in the root from the 2-mm. point to a point beyond 8 mm., but after the 8-mm. point a state of "saturation" is reached beyond which a further increase in attractiveness is not possible.

Which of these two explanations holds can be decided by the following experiment: Root tips of various lengths are excised and the portion *behind* the tip is tested as to its attractiveness to or repellency against nematodes.

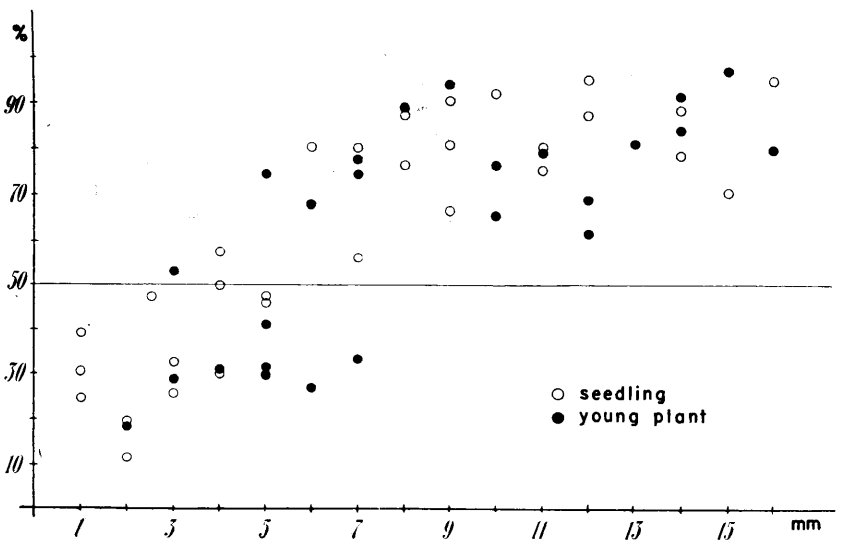


Fig. 2. Attractiveness of excised tomato root tips to larvae of *M. hapla*. Abscissa: length of root tips. Ordinate: as in figure 1. Open circles: roots from seedlings germinated on filter paper. Dots: roots from young plants grown in pots.

In my experiment I used root pieces of 10-mm. length, their distances from the apex of the root ranging from 1 to 16 mm. Now, if the first explanation holds we should get attractiveness of the 10-mm. root pieces at a distance of 1 to 7 mm. behind the apex. At 8 mm. behind the apex the whole attractive region would be excised and the tested root piece would be either neutral or repellent to the nematode larvae. If the second explanation holds the attractiveness should be apparent all over the tested range, that is at a distance of 1 to 16 mm. behind the apex. The results of this experiment was shown in figure 3. We can see that the attractiveness of the root pieces is conspicuous only if not more than 7 mm. of the root tip are excised which seems to verify the first explanation mentioned above. However, the variation in degree of attractiveness is enormous all over the root. Even in those regions in which most of the samples were attractive there were samples which were either non-attractive or repellent. The reason for this is not clear though it may be that cutting off the tip—the formative portion of the root system—somewhat upsets the metabolism of the remaining portion. The variation is much larger than that encountered when the root tips were tested.

The results of these two sets of experiments are summarized in figure 4 in which the average distribution of the nematodes based on 118 tests is given with reference either to root tips of various lengths (open circles) or to a standard portion of root, 10 mm. long, at various distances behind the root apex (black dots). The two curves are roughly inverse with the 50%-level as axis, and the 2- and 8-mm. points as the two most important turning points.

How can this information concerning the distribution of the attractive substance be put into a model of the tomato root? This is set out in the two models of the apical 8 mm. of a tomato root shown in figure 5. Superimposed

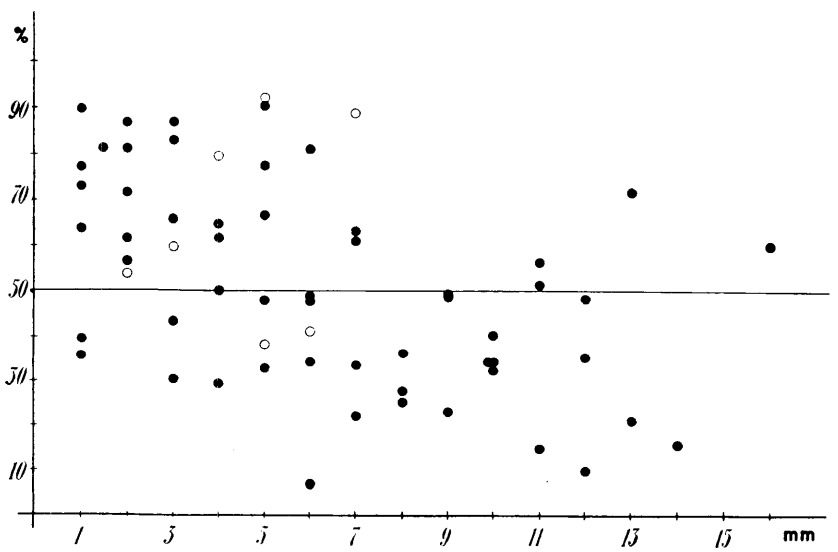


Fig. 3. Attractiveness of 10-mm. root sections, taken at various distances behind root apex, to larvae of *M. hapla*. Abscissa: distance of tested pieces from root apex. Ordinate: as in figure 1. Open circles and dots as in figure 2.

is the curve of distribution of the nematodes as given in figure 4. The lower model root is based on this distribution of the nematodes which calls for a separation of the apical 2 mm. from the next 6 mm. on account of the repellency of the former and the attractiveness of the latter portion. The attractive portion is set off from the region posterior to it, which is either neutral or slighly repellent, but certainly not attractive.

Now, if we compare this model with the upper one which is based on the anatomical structure of the tomato root we discover an important coincidence. The apical 2 mm. of the root are made up of the calyptra and the apical meristem, the latter being the region in which the cell divisions occur. Next is the region of elongation in which—roughly speaking—no new cells are produced but the old ones gain in size. This is the outwardly most active, the actually growing, region. However, I was unable to find an exact figure for the length of this region in tomato, but I know it to be less than 10 mm. Thus it seems not to be an unjustified speculation to coordinate these morphological facts with the physiological phenomena of attractiveness and repellency as revealed through the reaction of nematodes to the different portions of the root. If this be accepted the meristematic region (plus the calyptra?) of the tomato root must be considered to give off a substance which repels the larvae of *Meloidogyne hapla,* while the region of cell elongation exudes an attractive substance. The latter conclusion was already reached by Linford (1939). The sensitivity of the nematodes to these sub-

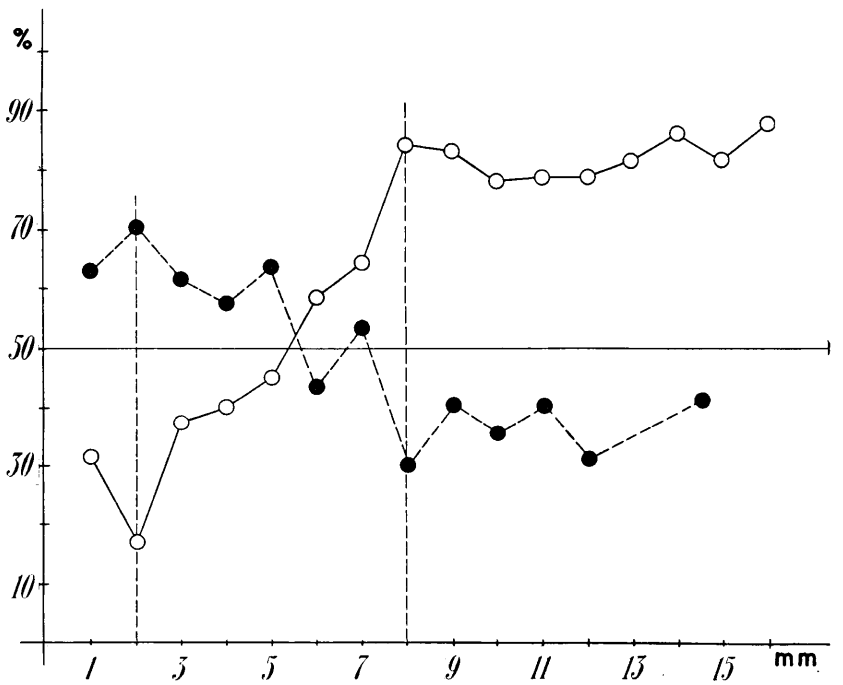


Fig. 4. Attractiveness of excised tomato root tips (open circles) and of 10-mm. root sections, taken at various distances behind root apex (dots), to larvae of *M. hapla.* The values for the distribution of the nematodes are the mean values from figures 2 and 3. Abscissa: length of root tips (open circles); distance of tested pieces from root apex (dots). Ordinate: as in figure 1.

stances is amazing since they react to the excretions of root bits even if these are as short as 1 mm. The amount of the active substance contained in such an excretion cannot be higher than a few micrograms—according to what we know about the amount of substances excreted by roots.

This is only the beginning of an interesting problem. It puts the question to the plant physiologist: what are these attractive and repellent substances and how do they fit into the metabolism of the plant? Further, the described method suggests the possibility of using the soil stage of parasitic nematodes as highly sensitive test subjects in detecting excretion from roots of substances which are given off in quantities too small for detection by ordinary chemical methods.

## SUMMARY

The attractiveness of germinating seedlings and excised roots of tomato to larvae of the nematode *Meloidogyne hapla* Chitwood was tested in several experiments. Seedlings are attractive only when growing and alive. If growth per 24 hours lies between 2 and 8-11 mm. the degree of attractiveness proved

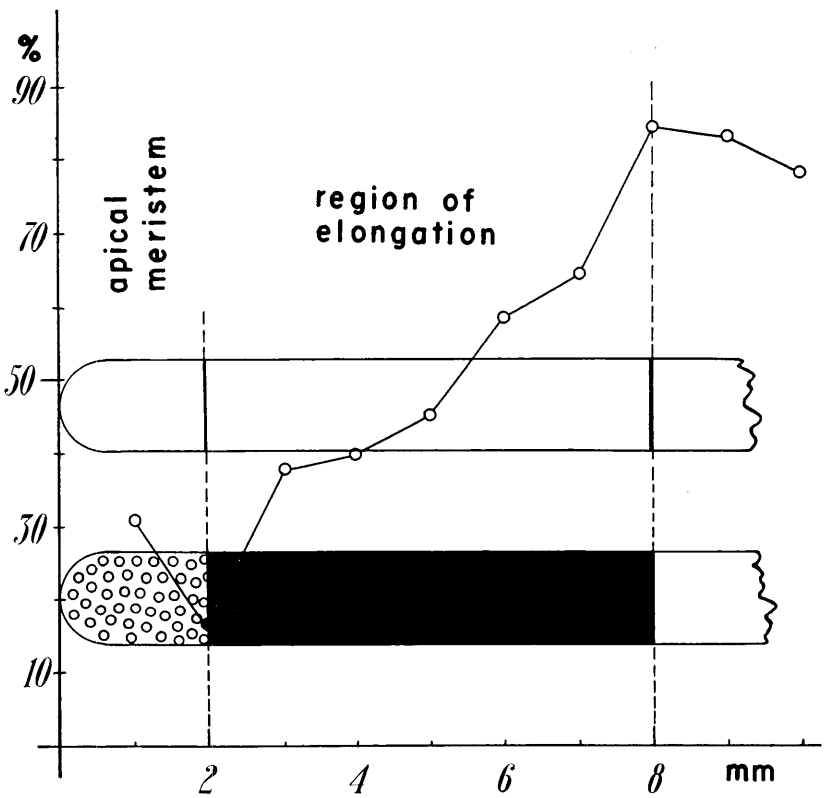


Fig. 5. Model of apical portion of tomato root: comparison of anatomical structure (above) with regions of relative attractiveness and repellency to nematodes (below). Apical meristem repellent, region of elongation attractive. Superimposed are mean values of figure 2. Abscissa: length of root tips. Ordinate: as in figure 1.

to be roughly proportional to the rate of growth. From then on to at least 21-mm. growth per 24 hours the attractiveness remains on the same high level. The apical 2 mm. of excised root tips appear to be repellent to the nematodes, while the next 6 mm. are attractive. It is assumed that this difference is connected with the different anatomy and physiology of these two portions, the first one being made up of the calyptra and the apical meristem and the second being the region of cell elongation. The portion behind the latter, the piliferous zone, is either neutral or slightly repellent to the nematodes.

10-mm. pieces of roots taken at various distances behind the root apex displayed much greater variation in the degree of attractiveness or repellency to nematodes than the root tips.

The attractive substance in excised roots remains active for at least 24 hours in water. The nematodes react to the excretions of root bits even if these are as short as 1 mm.

It is suggested that the method of attracting nematodes by root pieces and expressing quantitatively the degree of attractiveness of these pieces might be useful for detecting excretion from roots of substances given off in quantities too small for detection by ordinary chemical methods.

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## *Oligorchis cyanocittii* sp. nov., a hymenolepid cestode parasitic in the steller jay, *Cyanocitti stelleri* (Corvidae)

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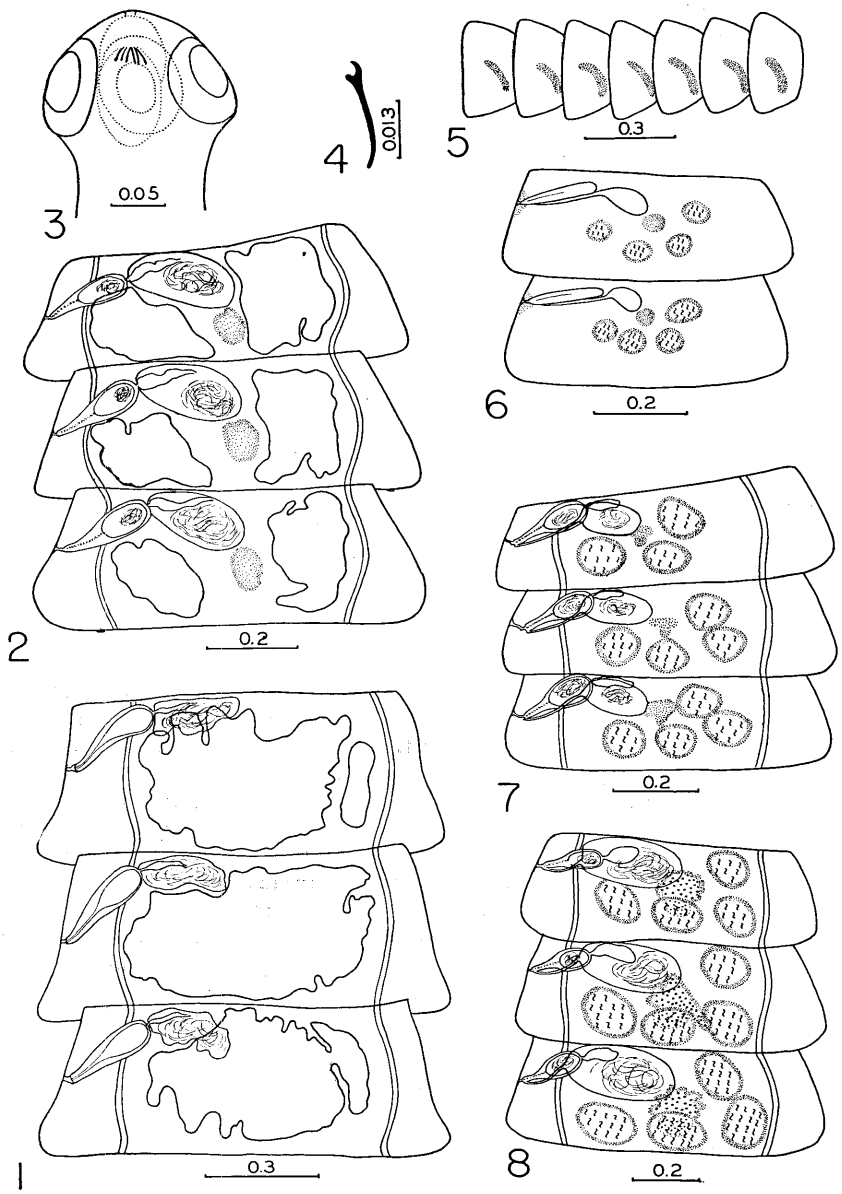
Seven steller jays were collected and two of them were found to harbor five cestodes. The host is a common inhabitant of the mountain pine and pine-oak forests at elevations near 2400 meters in the state of Chiapas, Mexico. A study of the cestodes revealed that they could be assigned to the genus *Oligorchis* Fuhrmann, 1906 and that they were a new species.

In the past, several species possessing widely different characters have been placed in this genus, but, more recently, Freeman (1954) has pointed out that the generic limitations have been too broad and that the definitions of the genus given by Fuhrmann (1906) and (1932) should be followed. Thus, Freeman recognized only six valid species in the genus *Oligorchis*. The reduction of species has made a genus which is much more clear-cut and comparable to the other genera in the Hymenolepididae.

Two worms from one host were moribund when fixed, but the cestodes from the other host were alive before fixation and provided excellent stained material. Sublimate-acetic, Harris' haematoxylin, terpineol, and damar were




All figures concern *Oligorchis cyanocittii* and were drawn with the aid of a microprojector.

Fig. 1. Gravid proglottids.
Fig. 2. Proglottids with uterus partially filled.
Fig. 3. Scolex.
Fig. 4. Rostellar hook.
Fig. 5. Proglottids showing the genital fundaments.
Fig. 6. Very young proglottids.
Fig. 7. Immature segments.
Fig. 8. Mature proglottids just before egg formation.

used to prepare slides. One scolex was completely flattened in order to study the rostellar hooks.

This study was carried out while the author was a Muellhaupt Scholar in the Department of Zoology and Entomology, Ohio State University.

*Oligorchis cyanocitti* sp. nov.

Figs. 1-8

(Measurements in millimeters)

DIAGNOSIS: Hymenolepididae. Strobila up to 127 long and 1.4 wide in gravid proglottids. Segments craspedote with a prominent velum; broader than long in all instances, but with ratio changing. Scolex 0.17 in diameter with suckers 0.086 to 0.093 in longitudinal diameter. Rostellum not everted in any specimens; rostellar sac 0.11 by 0.061. Ten rostellar hooks 0.025 to 0.028 long, arranged in a single row. Neck longer than wide 0.60 by 0.091. After strobilization begins, genital fundaments do not appear for 40 to 60 proglottids. Development of genitalia gradual. Longitudinal musculature in two layers of bundles; outer layer containing numerous bundles (about 0.0064), inner layer with fewer, but larger bundles. Longitudinal excretory vessels 0.025 to 0.032 (ventral) and 0.0032 to 0.0064 (dorsal). Transverse commissure in posterior part of proglottid, arising from an enlarged deltoid region in ventral longitudinal excretory vessel. Genital pores unilateral, sinistral, situated about midway in proglottid. Genital atria lacking spines or glands, but somewhat muscular, 0.028 in length. Cirrus opening into genital atrium anterior and dorsal to vagina. Cirrus sac up to 0.067 in diameter with large internal seminal vesicle. Cirrus apparently without spines. Wall of cirrus sac 0.0064 thick. Ductus ejaculatorius without convolutions. External seminal vesicle large and fusiform when distended, dorsal to seminal receptacle. Vagina courses from genital atrium slightly anteriorly and ventrally to a position ventral to cirrus and seminal vesicle. Vagina enlarges proximally into a voluminous seminal receptacle; thin-walled without glands. Seminal receptacle persists until proglottids are shed; aporal end dorsal to midpoint of ovary, connection between the two not apparent. Ovary ventral, central, lobate, frequently with unequal lobes. Vitelline gland ventral to center testes and posterior to ovary. Four testes located in dorsal parenchyma posterior and lateral to ovary. Uterus a transverse tube of irregular diameter filling distally first. Eggs of typical hymenolepid type when alive, collapsed in mounted specimens. Embryonic hooks 0.032 to 0.035 long.

HOST: Steller jay (*Cyanocitti stelleri,* Corvidae).

SITE OF INFECTION: Small intestine.

LOCALITY: Region of San Cristóbal, Chiapas, Mexico.

TYPE SPECIMENS: Holotype in U.S. National Museum.

HELMINTHOLOGICAL COLLECTION. NO. 37477.

*Oligorchis cyanocittii* and *O. toxometra* both posses 10 rostellar hooks and four testes. They can be separated readily from each other by the length of the rostellar hooks; *O. toxometra* possesses hooks 0.037 to 0.040 and *O. cyanocittii* possesses hooks 0.025 to 0.028.

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# On the Occurrence of an Intersexual form in *Ditylenchus triformis*, n. sp. (Nematoda, Tylenchida)

HEDWIG HIRSCHMANN AND J. N. SASSER*

Intersexes in nematodes are known in relatively few genera. References in the literature to this phenomenon are somewhat scattered and deal almost exclusively with the morphology of single intersexual specimens.

An intersex is an individual which exhibits a blending of male and female characters, in contrast to gynandromorphs which show male characters in one part of the body and female characters in another part. Whereas an intersex is considered as either a modified female or modified male which functions in one or neither sex, but never in both, a hermaphrodite is normally functional both as male and female in the same animal. Goldschmidt (1933), working on the hybridization of different European and Japanese strains of the gypsy moth, *Lymantria dispar,* was the first to give a genetical explanation for the phenomenon of intersexuality. On the basis of his embryological investigations, he postulated that the different organs turn intersexual in a definite order. He stated: "An intersex is an individual which starts development with its original, chromosomic, gametic sex, but changes sex during development. This change takes place at a certain point, the turning point, and development is finished with the other sex, though no change in the chromosomes has occurred. An intersex is therefore a sex mosaic in time, though the sex-chromosomes remain always the same within the whole organism. . . . From this definition follows that intersexes may be primarily of two types according to the original sex, namely female intersexes beginning development as females with female chromosome set, and ending it after the turning point as males; further male intersexes beginning development as males and ending as females."

Intersexes in nematodes have been observed most frequently in the insect-parasitizing mermithids, where they occur normally. Meissner (1853) was the first to observe intersexuality in nematodes. He found intersexual individuals in *Mermis albicans,* which he erroneously regarded as hermaphrodites. Later (1903) O. von Linstow mentioned intersexuality in *Mermis mirabilis* and Hagmeier (1912) showed intersexes in *Mermis terricola, M. elegans, M. albicans, M. arsenoidea, M. arenicola* and *Paramermis fluviatilis.* The most thorough studies including a review of the literature were made by Steiner (1923) on intersexes of *Agamermis decaudata.* In 1937 this author reported a high degree of intersexuality in a single specimen of *Pseudomermis vanderlindei.* In the same paper Steiner described 12 intersexes in *Tetanonema strongylurus,* a species of the superfamily Filarioidea collected from the large subdermal blood sinus anterior to the gill chamber of the fish species *Bdellostoma heptatrema.* Another case of an intersexual Ascaridoidea was mentioned by Willemoes-Suhm (1869) in *Porrocaecum heteroura.* Chitwood (1949) reported intersexual specimens in the plant parasitic species *Meloidogyne javanica.* In addition intersexes occur sporadically in freshwater and marine nematodes. Examples were cited by A. Schneider (1866) in *Enoplus communis* (syn. *E. cochleatus*), by De Man (1893 in *Thoracostoma figuratum* and *Chromadora poecilosoma* and by the latter author (1904) in *Enoplus michaelseni.* E. von Daday (1905) described an intersexual specimen of *Trilobus diversipapillatus.* Ditlevsen (1911), W. Schneider (1922) and Mico-

*Contribution from Plant Pathology, North Carolina Agricultural Experiment Station, Raleigh, North Carolina. Published with the approval of the Director of Research as Paper No. 637 of the Journal Series.

letzky (1914) reported such forms in *Trilobus gracilis*. An intersexual female has also been noted in *Tripyla papillata* by the senior author in 1952.

Except in the case of *Meloidogyne javanica,* the intersexes in the nematodes mentioned above were females which showed secondary male characters. Chitwood stated in his description of the male of *M. javanica:* "Intersexes common (true males rare), ranging from vagina rudimentary to vulva well developed." All the other intersexes reported in the literature were females and had well developed ovaries with uteri, vagina and vulva, the uteri of some even containing eggs. In addition, they possessed male sexual openings, spicules, bursal muscles and male anal papillae. The intersexes seem to be able to copulate with males and be fertile in many cases.

All the intersexes of *Agamermis decaudata* described by Steiner (1923) had normal female sex organs ond most of them had normal eggs in the uteri. All copulated with males. The intersexual state was present only in the tail which showed the structures of the male more or less distinctly pronounced. The testes were absent. Steiner observed all grades of transformation in the tail end from the typical female to a typical male. However, he stated that "the different organs involved in the transformation toward the male sex are not progressive. . . . It seems as if there is no correlation tending to produce gradual stages of development of the different sex organs. Where in one specimen an organ may proceed far along in its transformation, in another it may remain much less developed, when compared with the other organs involved in the latter transformation."

The single specimen of *Pseudomermis vanderlindei* which Steiner described in 1937 was a female that exhibited well developed male structures in the tail. An anal opening, two slender spicules with retractor muscles, a long series of preanal and postanal bursal muscles and three series of preanal and postanal male copulatory papillae were present. The testes were apparently absent. The intersexual females or female larvae of *Tetanonema strongylurus* showed in addition to normally developed ovaries and their outlets, male copulatory papillae on their tails; some of them even had a vestigial male sexual opening.

The work reported in this paper deals with intersexual individuals in the species *Ditylenchus triformis* n. sp. and constitutes, except for the finding of male intersexes in *Meloidogyne javanica* by Chitwood (1949), the only other report on the occurrence of intersexuality in the order Tylenchida. The specimens were encountered while making a routine examination of nematodes isolated from soil collected from a field in New Hanover County, North Carolina, planted in *Gladiolus* var. "Snow Princess." To date, 104 females, 98 males, 36 larvae and 33 intersexes have been studied. As has been the case in previously reported intersexual forms, intersexes of this species resembled the female in body shape and exhibited normal female characters (well developed ovaries, rudimentary posterior uterine branch, vulva, vagina) combined with secondary male characters (bursa, spicules, gubernaculum). In contrast to the different grades of transformation in Steiner's mermithid intersexes, these *Ditylenchus* intersexes exhibited a high degree of uniformity and undoubtedly belong to a single type. The only slightly variable structure in the intersexes was the bursa, the wings of which varied somewhat in width. All the intersexes had normal appearing spermatozoa in their uteri indicating that copulation had occurred. Fertilized eggs have not been observed, probably due to the age of the intersexes. A detailed description of the species follows:

*Ditylenchus triformis* n. sp. (Figs. 1-5)

DIMENSIONS:

FEMALE: 0.64-0.83 mm; a = 33.5-42.2; b = 5.2-7.3; c = 9.6-11.9; stylet = 7.5-9.5 $\mu$; excretory pore = 12.1-14.3%; vulva = 74.8-82.2%.

MALE: 0.61-0.71 mm; a = 32.8-42.7; b = 5.0-6.9; c = 9.3-10.8; stylet = 7.4-8.1 $\mu$; spicule = 13.4-14.9 $\mu$; gubernaculum = 5.1-5.9 $\mu$; excretory pore = 12.8-13.6%.

INTERSEX: 0.56-0.79 mm; a = 33.7-43.6; b = 5.0-6.1; c = 9.8-11.8; stylet = 7.0-8.1 $\mu$; excretory pore = 12.6-15.2%; vulva = 65.4-80.9%; spicule = 12.0-14.5 $\mu$; gubernaculum = 4.2-5.3 $\mu$.

Body slender, tapering toward both ends (Fig. 1, A-C). Cuticle with distinct transverse striae about 1.5 $\mu$ apart, easily visible at any point of the body. Lateral field measuring about ⅓-¼ the width of the body diameter, marked by six incisures spaced equidistantly. The two outer incisures slightly crenate (Fig. 2, D-I). Lateral field beginning a short distance behind the stylet knobs with 2 incisures, increasing near excretory pore to 4 and at the end of the esophagus to 6 incisures. Near anus the incisures are reduced to 4 and join the body contour after decreasing to 2 about halfway of the tail length. Deirids distinct, on the same level or somewhat behind excretory pore (Fig. 2, D-F). Lip region low, rounded, set off by a slight narrowing of the head contour, bearing 2 striae (Fig. 2, A-C). Amphidial pouches distinct, but amphids difficult to see. Sclerotized labial framework present, not very pronounced. Posterior part of stylet longer than anterior part. Basal knobs distinct and sometimes asymmetric. Dorsal esophageal gland orifice close behind the spear knobs. Median esophageal bulb ovoid with small refractive valvular apparatus (Fig. 3, A-C). Basal bulb of epsophagus enclosing the 3 esophageal glands which extend slightly back over the anterior end of the intestine. Near the junction of esophagus and intestine two large cells of unknown function in ventro- and dorso-lateral position. Nerve ring crossing isthmus of esophagus. Hemizonid about 2 annules (3$\mu$) high (Fig. 2, D-F). Excretory pore opening close behind hemizonid. Tail elongate-conoid, the posterior third tapering very gradually to the bluntly conical terminus (Fig. 4, A-D, Fig. 5, A-D). In some male and female specimens terminus almost subacute (Fig. 5, B). Phasmids probably present but so minute that they have not been observed.

FEMALE (FIG. 1, A): Ovary single, outstretched with oocytes in single file, except for a short region of reproduction. Rudimentary posterior uterine branch extending about ¼ to ⅓ the distance to the anus, not functioning as spermatheca (Fig. 4, A). Spermatozoa usually well up in the uterus ready to enter the eggs. Lips of the broad, transverse vulva elevated above body contour. Distance vulva to anus equal to 1 1/5 to 2 times the length of tail (Fig. 4, A).

MALE (FIG. 1, C): Testis single, outstretched with spermatogonia in single file. Spermatocytes usually in 2 lines. Spermatozoa shown in Fig. 5, E. Bursa well developed, crenate, rising about opposite the proximal ends of the spicules and extending about ⅓ to ½ the length of the tail (Fig. 5, D). Spicules and gubernaculum shown in Fig. 5, G. Near anus lateral fields reduced to 4, joining body contour at the end of bursa.

INTERSEX (FIG. 1, B): Resembling female in body shape and size but with certain secondary male characters. Gonad normally developed, single as in female, outstretched with posterior uterine branch (Fig. 4, B-D). Uterus filled with spermatozoa. Vulva and vagina well developed. Form of head,

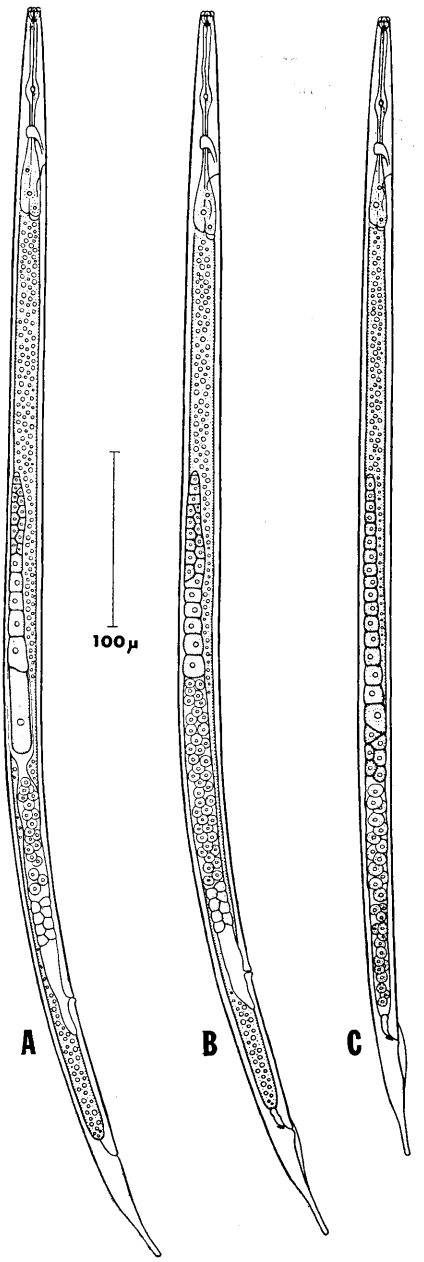


Figure 1. *Ditylenchus triformis* n. sp. A = female; B = intersex; C = male (all lateral views).

spear, esophagus as in females and males. Tail resembling in shape more the female tail but with spicules, gubernaculum and bursa (Fig. 5, C). In no case subacute terminus. Bursal wings varying somewhat in width (Fig. 4, B-D). Spicules and gubernaculum as in the male but slightly shorter (Fig. 5, F). Lateral field in the region of the bursa as in male. Except for insignificant variations in the form of the bursa, all intersexes examined of the same appearance.

DIAGNOSIS: *Ditylenchus* with the above measurements and general description. Lateral fields marked by 6 incisures; base of esophagus extending but slightly over the anterior end of the intestine; gonads outstretched, their cells arranged in single file; posterior uterine branch extending about ¼ to ⅓ the distance to the anus; tail elongate-conoid, with the posterior third tapering very gradually; terminus bluntly conical.

TYPE LOCALITY: Gladiolus field near Wilmington, North Carolina.

HABITAT: Soil from gladiolus field.

TYPE SPECIMEN: Female in vial No. 22, N. C. State College, Raleigh, North Carolina.

ALLOTYPE: Male in vial No. 23, N. C. State College, Raleigh, North Carolina.

INTERSEX TYPE: Intersexual form in vial No. 24, N. C. State College, Raleigh, North Carolina.

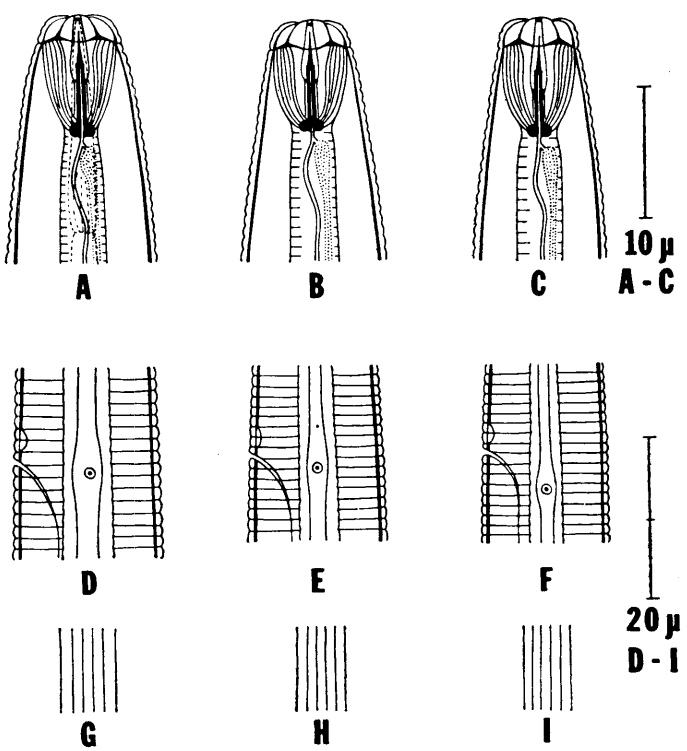


Figure 2. *Ditylenchus triformis* n. sp. A = head of female; B = head of intersex; C = head of male; D-F = region of excretory pore showing lateral field, deirid and hemizonid, D = female, E = intersex, F = male; G-I = lateral field near the middle of body, G = female, H = intersex, I = male (all lateral views).

Experiments were conducted to determine whether or not this species was pathogenic on gladiolus. In a preliminary test, 25 females and 25 males were added to each of 2 pots, containing nematode free soil. A single nematode free gladiolus bulb was planted in each pot. A similar experiment, using 25 males, 25 females and 10 intersexes was also performed. The tests were conducted in the greenhouse (70-80° F). At the end of one month, soil and roots from one pot in each of the above tests were examined for nematodes. At the end of 2 months soil and roots from the other two pots were examined. Only two males were recovered from the soil, indicating that the nematode apparently does not feed and reproduce on this plant.

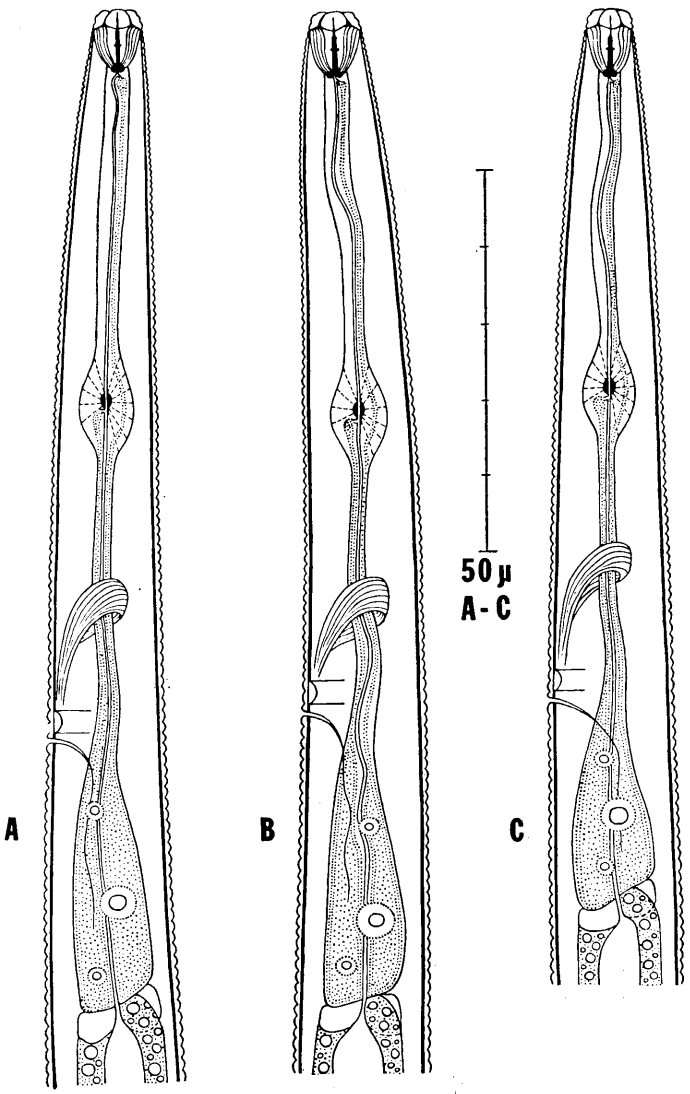


Figure 3. *Ditylenchus triformis* n. sp. A-C = anterior portion of body, A = female, B = intersex, C = male (all lateral views).

Efforts to propagate the nematode in fungus cultures (*Fusarium, Trichoderma* and *Rhizopus* spp.) on potato dextrose agar have been successful, but reproduction was slow. From one *Fusarium* culture which had been inoculated with 5 females, 5 males and 2 intersexes and kept at room temperature for 43 days, 19 adult nematodes and 39 larvae were recovered. No intersexes were observed. The nematodes were seen feeding on the fungi.

Factors responsible for the occurrence of intersexes in nematodes are not known. Certain theories, however, have been postulated by various investigators. Steiner (1923) considering the work of Goldschmidt and others with insects where intersexuality resulted from hybridization of closely related genotypes, suggested that the occurrence of intersexes in mermithids and in *Trilobus gracilis* might also be due to the hybridization of closely related genotypes. Christie (1929), working on the sex determination of mermithids, pointed out that environment is an important sex-determining factor. He found that sex is influenced by the number of parasites in a host. When 1 to 3 parasites were present in grasshoppers, they were females; when 4 to 23 were present both sexes occurred; when the number exceeded 24 only males

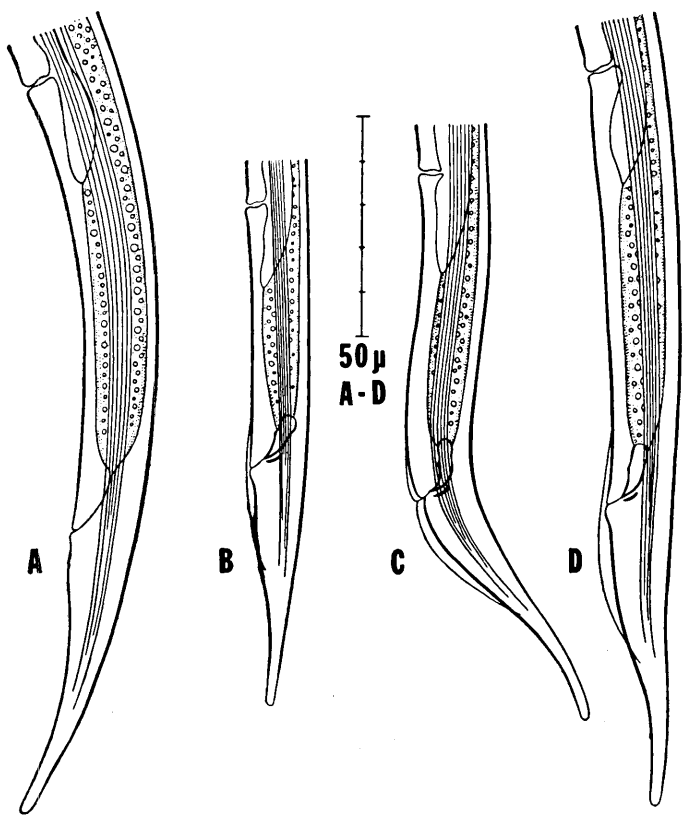


Figure 4. *Ditylenchus triformis* n. sp. A-D = posterior portion of body (vulva region and tail), A = female, B-D = three different intersex specimens (all lateral views).

were discovered. Christie stated "it seems probably that the occurrence of intersexes is in some way linked up with this matter of sex determination. If an insect host harbors twenty-three parasites, twenty-two of which develop into males and one into a female, it is conceivable that this female was under the influence of some environmental force tending to stimulate maleness and which, although insufficient in her case to bring about the development of a functional male individual, was sufficient to induce the development of secondary male characters." In the paper on *Tetanonema strongylurus* Steiner (1937) raised a similar question, that is, whether the determination of sex could be influenced by waste products, secretions or excretions, present because of the accumulation of numerous specimens in the blood sinus of the fish. Further, he referred to the opinion of Goldschmidt (1931), who considered the intersexuality in mermithids to be of a zygotic nature and suggested that these nematodes might be considered as protandric hermaphrodites which are first males but which through rich nourishment develop into females. If this development is interrupted intersexes might occur.

The cytological basis for intersex formation has been given for several organisms. Goldschmidt and others working with diploid intersexes of *Lymantria* and triploid mutants of *Drosophila* showed that intersexuality was due to the failure of the normal functioning of the sex-determining mechanism

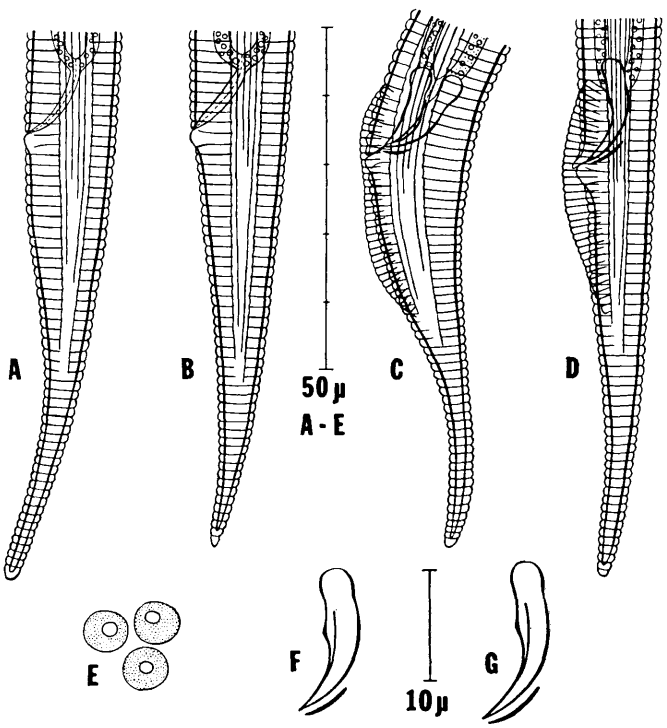


Figure 5. *Ditylenchus triformis* n. sp. A, B = tails of two different females showing the slight variation; C = intersex tail; D = male tail; E = spermatozoa; F = spicule and gubernaculum of the intersex; G = spicule and gubernaculum of the male (all lateral views).

itself. Sex determination depends on the balance of the male determining factor which is located in *Lymantria* in the sex chromosome, and the female determining factor which is present in the autosomes or cytoplasm. The reverse situation has been found in *Drosophila* in which the sex chromosome is female determining and the autosomes are male determining. Hybridity is liable to upset the balance and hence reproduces disturbances in the expression of the normally alternative sex character. In contrast, to the above findings Sturtevant (1920) showed in *Drosophila simulans* that intersex formation was caused by a special autosomal gene. By cross experiments he came to the following conclusion: "The intersexes are females, modified by a recessive autosomal mutant gene that causes them to show male parts, though these parts themselves still have two X-chromosomes. The normal sex-determining mechanism is not affected at all, but the end result is modified by a gene that is not even in the sex chromosomes."

Whether or not the described case of intersexuality in *Ditylenchus triformis* n. sp. is due to the hybridization of closely related genotypes, to the influence of the environment, or to a particular gene causing intersexuality, cannot be stated at this time. Attempts are being made, however, by the senior author, to propagate large numbers of the nematode to be used in determining the possible causes of intersex formation in this species.

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# The Effect of Immune Serum on *Dictyocaulus viviparus* in Calves. Preliminary Report

ROBERT RUBIN AND THOMAS B. WEBER

Animal Disease and Parasite Research Branch, Agricultural Research Service, Beltsville, Maryland

Under experimental conditions, initial infections with *Dictyocaulus viviparus* in calves and yearlings confers an active immunity against reinfection with this lungworm. In March 1955, the writers completed a preliminary experiment designed to determine whether this immunity may be passively transferred to young calves. Both the number of calves and the amount of immune serum available for this test were limited. Therefore, we decided to use the available serum to test a variety of dosages against a known lethal number of larvae.

After the experiment was completed, Jarrett et al., (1955) reported clinical and parasitological evidence that the globulin fraction of serum from lungworm-immune cattle contained sufficient antibodies to confer a considerable degree of protection against a single exposure of 4,000 infective larvae. Five of their calves each received 1,500 ml. of the globulin fraction of immune serum, and 5 control calves received no serum. This volume of the globulin fraction is approximately equivalent to 4,500 ml. of whole serum.

Our results with lesser amounts of serum and a more drastic challenge exposure to infection complement those of Jarrett et al and are reported here.

## MATERIALS AND METHODS

Six Holstein calves were obtained when a few days old. They were reared helminth-free and were 63 to 69 days old at the start of the experiment. The 14-month-old yearling used as a source of immune serum had received a total of 874,000 larvae at various intervals over a preceding 12-month period and had acquired a resistance as evidenced by the failure of repeated reexposures to result in the development of a patent infection in it after its initial infection had terminated. The blood from which the immune serum was prepared was taken two weeks after the last administration of 500,000 larvae. The source of normal serum was an 8-month-old calf which had never been infected with lungworms. Serum was separated from the blood of the donor animals by standard procedures and was injected intravenously into the experimental calves. The amount of serum given was based on body weight. Four animals received immune serum at the time of infection. The doses were 5.0, 2.5, 1.0, and 0.1 ml. of serum per pound of body weight. The fifth animal received 5.0 ml. of normal serum per pound of body weight. The sixth received 5.0 ml. of immune serum per pound of body weight when clinical symptoms became evident eight days after infection.

The infective larvae were reared in culture, collected, counted and administered as described by Rubin and Lucker. Fifty thousand larvae were administered to the calves to test the immune properties of the serum because initial exposure to this number was consistently fatal to calves and a yearling (Rubin and Lucker). The minimum lethal dosage is no doubt considerably lower, as suggested by Michel (1954).

The weights, temperatures, and respiratory rates of the calves were determined weekly for about a month before infection. During this period, appetite and fecal consistency were observed daily; at the time of infection the calves were considered normal in all respects. After infection, the tempera-

tures and respiratory rates, as well as the nature of the lung sounds, were determined at frequent intervals, usually daily, and weighings were made weekly. Animals that died or were killed *in extremis* were autopsied, usually within a few hours. Gross pathological changes were noted and tissue samples from the lungs and associated lymph nodes were prepared for bacteriological and microscopic examinations.

The lungs were opened and macroscopic worms removed. The opened lungs were washed and Baermanized for 24 hours to recover microscopic worms. All worms recovered were preserved in 7 percent formalin and aliquots were counted. Worms representative of the different sizes found in each sample were measured.

## RESULTS

CLINICAL EVIDENCE OF PASSIVE IMMUNITY. The most convincing evidence that resistance was conferred on 2 of the 6 calves in this experiment was the fact that they survived a drastic challenge of 50,000 infective larvae. These two animals, calves 42 and 44, each received 5.0 ml./lb. of body weight of immune serum (Table 1). The fact that one of them (calf 42) received serum at the time the larvae were administered and the other (calf 44) received it eight days later made no significant difference in the results. The other 3 calves that received immune serum in amounts of 2.5, 1.0, and 0.1 ml./lb. of body weight, and the calf that received normal serum in the amount of 5.0 ml./lb. (Calves 34, 39, 43 and 40 respectively, Table I), did not benefit clinically at this high level of exposure and died as a result of the infection with lungworms.

A difference in clinical behavior of the two calves that survived as compared with that of the four that died was evident by the end of the first week following infection. To point out these differences, the respiratory rate, temperature and weight changes for calf 43, which received only 0.1 ml./lb. of immune serum and died, and for calf 44, which received 5.0 ml./lb. of immune serum and survived, are compared for a 48-day period (Figure 1). Shortly after the end of the first post-infection week, the temperature of calf 43 rose and soon became elevated several degrees above that of calf 44. It remained so except shortly before death and during the fifth week, when the tempera-

TABLE I.—Data on Immunized Calves Fed 50,000 Larvae

| Calf No. | Weight on Infection Date (Pounds) | Serum Dosage* (ml./lb.) | Prepatent Period (Days) | Weight Changes*** (Pounds) | Results |
|---|---|---|---|---|---|
| 42 | 151 | 5.0 | 30 | +37 | Recovery |
| 44 | 162 | 5.0 | 31 | +40 | Recovery |
| 34 | 162 | 2.5 | 35 | −30 | Death in 37 days |
| 39 | 162 | 1.0 | 30 | −37 | Death in 33 days |
| 43 | 163 | 0.1 | 28 | −36 | Death in 48 days |
| 40 (Control) | 157 | 5.0 (normal serum) | ** | −29 | Death in 23 days |

*Received by calf 44 eight days after infection and by others on the day of infection.
**Died before infection became patent.
***Loss to time of death or gain to 48th post-infection day.

ture of calf 44 also was above normal. The respiratory rate of calf 43 increased rapidly during the second post-infection week and was high most of the time thereafter. The respiratory rate of calf 44 remained essentially normal except during the fifth week. Calf 43 showed an average weight loss of 0.8 lb. per day while calf 44 showed a gain of 0.8 lb. per day. The initial weights and the weight gains or losses for all of the calves are shown in Table I.

ANTEMORTEM PARASITOLOGICAL EVIDENCE OF PASSIVE IMMUNITY. There were two types of antemortem parasitological evidence that suggested a beneficial effect of immune serum. The first evidence was a prolonged prepatent period in five of the calves which received immune serum. The sixth calf, which received normal serum, died before the lungworms could mature. The average prepatent period for the remaining five calves was 31 days. Although limited data are available for comparison, this average is slightly longer than the average of 25 days observed by Rubin and Lucker in 16 initial infections and above the range of 21 to 28 days reported by Michel

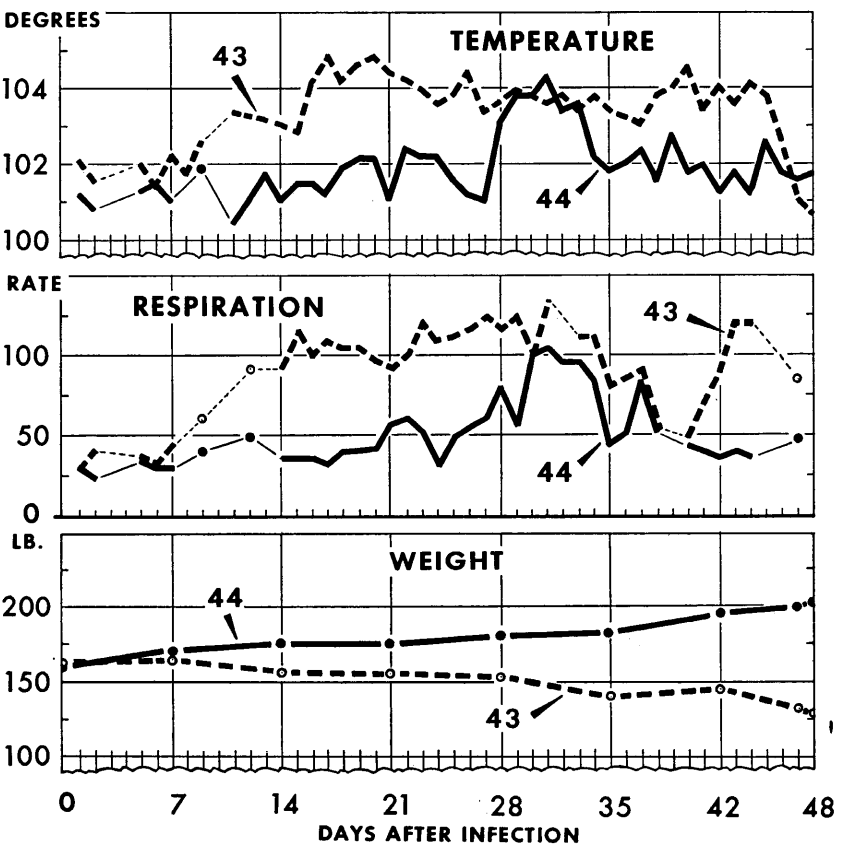


Figure 1. Comparison of temperature, respiratory rate, and weight change, for surviving calf 44 and non-surviving calf 43 for 48-day post-infection period. The thin lines on the temperature and respiration curves indicate intervals when observations were not made daily.

(1954) for an unspecified number of infections. This increase may have been due to an inhibitory action of the immune serum on the normal rate of maturation of the parasites.

Although the fecal output of larvae for calves 39 and 34, which received 1.0 and 2.5 ml./lb. of immune serum, respectively, was negligible because these animals died shortly after their infections became patent, a limited comparison between calf 43 and calves 42 and 44 can be made and affords additional evidence of the beneficial effect of immune serum. The maximum larvae-per-gram count for calf 43 (0.1 ml./lb.) was 585 and the calculated output of larvae for only 20 days was 4,104,000. The patent period for the infections in calves 42 and 44 was 43 days, or 23 days longer than that of calf 43, which was terminated by death. The maximum larvae-per-gram counts were 77 and 37 and the total calculated larval outputs were only 2,111,000 and 947,000 for calves 42 and 44, respectively.

POST MORTEM PARASITOLOGICAL EVIDENCE OF PASSIVE IMMUNITY. Although no significant clinical differences were observed within the group of four calves that were not sufficiently immunized to survive the challenge of 50,000 larvae, there were certain differences, relative to the amounts of immune serum received, in the recovered worm burdens (Table II). Administration of a small amount of immune serum apparently conferred little or no immunity to infection on calf 43. This conclusion is based on the insignificant difference in both the total number of recovered worms and the percentage of small worms in this calf as compared to control calf 40. The measured specimens in the aliquot samples of the worms in control calf 40 showed a gradation in size from smallest to largest. However, those in the samples from the calves receiving immune serum fell into two distinct size groups, one ranging from 0.8 to 15.0 mm. and the other from 15.0 to 50.0 mm. in length (Table II). Calf 39, which received 146 ml. more of immune serum than calf 43, had about the same number of worms as the latter, but a greater percent of them were small, immature fifth-stage forms. Calf 34, which received 243 ml. more of immune serum than calf 39, had fewer worms than the latter calf and a greater percent of them were small forms. Therefore, administration of increasingly larger amounts of immune serum apparently had the effect of increasing the percentage of retarded worms. The largest of the lower dosages (2.5 ml./lb.) also tested evidently reduced the size of the infection.

SIGNIFICANCE OF OTHER DATA OF THE EXPERIMENT. The extent and degree of pathological lung sounds were not always indicative of the severity of the infections. Occasionally lung sounds detected in the two calves that survived indicated a more severe, momentary reaction than those at times heard in the four calves that died. Moist rales and crepitant rales were present in all calves and the latter became more extensive as the disease progressed.

TABLE II.—Worms Recovered in Non-Surviving Calves

| Calf No. | Total Serum Given (ml.) | Worms Recovered (Number) | Size Range in mm. Males | Size Range in mm. Females | Percent of Small Worms Less Than 15.0 mm. |
|---|---|---|---|---|---|
| 34 | 405.0 immune | 2,170 | 6.3-29.7 | 12.5-50.0 | 50.0 |
| 39 | 162.0 immune | 3,670 | 3.9- 9.4 | 1.6-35.9 | 42.0 |
| 43 | 16.3 immune | 3,630 | 1.6-28.1 | 0.8-40.6 | 3.3 |
| 40 | 785.0 normal | 4,250 | 4.7-25.0 | 2.7-26.7 | 12.0 |

The fatalities in this experiment, in the writers' opinion can be attributed to lungworm disease *per se* because (1) the clinical appearance of the calves was similar to that described for lungworm disease, (2) at necropsy the macroscopic and microscopic lesions were similar to those observed by one of us (RR) in several other experimental cases of lungworm infection, and (3) bacteriological examination of the lungs and lymph nodes was negative for the common pneumonia-causing organisms.*

That calves 42 and 44 escaped the same fate because they received large amounts of immune serum seems a fully justified inference. The gross and histopathology are not reported because one of us (RR) is participating in a comprehensive study of the gross and microscopic pathology associated with experimental lungworm infections, and the results are now in preparation for later publication.

## DISCUSSION

No comparison can be made between antibody content of the serum used by the writers and that used by Jarrett et al. because in this experiment the laboratory evaluation of the serum was deferred until the results of this preliminary test were available. However, the immune serum was collected by both groups at approximately the same time following the last reexposure to infective larvae. Jarrett and co-workers collected immune serum two to four weeks following reexposure at which time the results of complement fixation tests were maximum, whereas we used as our basis the maximum eosinophilic response in the donor animal, which occurred about two weeks after reexposure.

The drastic challenge of immune serum in this experiment is further emphasized by the fact that had we extracted the globulin fraction and obtained it in the same ratio to total serum as did Jarrett et al., our maximum amount of immunizing material would have been only about 275 ml. as compared with the 1500 ml. they used.

It is difficult to understand the fact that no immature worms were observed by Jarrett et al. in any of the 10 calves necropsied 30 days after infection, especially in comparison with our findings in which all four of our calves that were necropsied had immature worms, in one instance (calf 34) up to 50 percent of the total recovered.

The preliminary findings of both Jarrett et al. and those of the writers offer encouragement for the possibility of the eventual control of *Dictyocaulus viviparus* by immunological methods, and also suggest the desirability of a more thorough investigation of this problem.

## SUMMARY

Two calves survived a known lethal dose of lungworm larvae when 5.0 ml./lb. of body weight of immune serum was given intravenously to one at the time of infection and 8 days after infection to the other. Three calves given 0.1, 1.0, and 2.5 ml./lb. of body weight at the time of administration of the larvae died 48, 33, and 37 days later, respectively. A calf given 5 ml./lb. of body weight of normal serum died 10 to 25 days earlier than those given low and intermediate doses of immune serum. The two surviving calves gained weight throughout most of the experiment, whereas the four that died lost weight, beginning about one week after infection. The comparatively low

*The writers are indebted to Drs. C. A. Manthei and E. R. Goode of the Animal Disease and Parasite Research Branch, ARS, Beltsville, Md., for the bacteriological examinations.

larval output in the feces of the two surviving calves suggested the establishment of a relatively light worm burden in these two animals.

A comparison of the total worm burdens and the per cent of large and small worms in the four calves that died suggested transfer of a resistance to infection related in degree to the amount of immune serum given.

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## Removal of Tapeworms from a Live Dove

CARLTON M. HERMAN

U. S. Fish & Wildlife Service, Patuxent Research Refuge, Laurel, Md.

On 25 April 1955, Fred Schmid trapped a mourning dove (*Zenaidura macroura carolinensis*) at the Patuxent Research Refuge, Laurel, Md. This bird was brought into the laboratory for further examination because of the presence of white bodies in the perianal region. These proved to be tapeworm proglottids. By careful, steady pulling with a forceps four complete strobila, including the scoleces, of *Aporina delafondi* (Railliet) were removed through the anus. The tapeworms varied from 9 to 14 cm. in length. After removal of the worms the dove was banded and released.

*A. delafondi* has been reported several times from pigeons in the United States and Harkema (1942, J. Parasitol. 28: 495.) reported infection in five of 31 mourning doves autopsied in North Carolina in the spring of 1940.

## Report of the Brayton H. Ransom Memorial Trust Fund

| | |
|---|---|
| FUNDS ON HAND, Jan. 1, 1954 | $1754.95 |
| RECEIPTS: Interest rec'd. in 1954 | 59.63 |
| DISBURSEMENTS: Expenses and grant to Helminthological Society of Washington | 56.00 |
| BALANCE ON HAND, Dec. 31, 1954 | 1758.58 |

A. O. FOSTER
*Secretary-Treasurer*

## Minutes

### Three Hundred Twenty-fifth to Three Hundred Thirty-second Meetings

325th meeting: McMahon Hall, Catholic University of America, October 20, 1954. Membership approved recommendations of Editorial Committee that "In memoriam" be published in the Proceedings upon decease of each active member in good standing. Two hundred dollars was voted for secretarial assistance of the Secretary-Treasurer. Ten dollars was voted for the Science Fair of the Washington Jr. Academy of science. Papers presented: Impression of Northern Brazil with reference to health conditions, by Olivier; The growth of the renette cells in *Nematodirus spathiger* during its development in the sheep intestine, by Kates.

326th meeting: Log Lodge, Agricultural Research Center, Beltsville, Md., Nov. 19, 1954. Papers presented: The birth of the new nematode family Anatrichosomidae, by M. B. Chitwood; Survival of *Histomonas meleagridis* on soil, by Farr; Sphagnum moss as a culture medium for larval nematodes, by Shorb; Effects of p32 on the larval stages of the trematode *Paramphistomum microbothroides,* by Weber; Acquired resistance to lungworm infection in calves, by Rubin and Lucker; Transmission of the swine kidney worm, *Stephanurus dentatus* by earthworms, by Tromba.

327th meeting: Naval Medical School, Bethesda, Md., December 15, 1954. Officers elected for the year 1955 were: A. M. Taylor, President; Dr. J. S. Andrews, Vice President; Miss Edna Buhrer, Corresponding Secretary; and, Dr. C. M. Herman, Recording Secretary. Dr. G. F. Otto was re-elected Chairman of the Editorial Committee. Papers presented: An electron microscope study of *Toxoplasma gondii,* by Gustafson; Epidemiology of schistosomiasis in East Africa and the middle East, by Kuntz; Malaria survey of the Yaeyama Island, by Teller; Cursory survey of intestinal parasites of natives living in the Southwest Anglo-Egyptian Sudan, by Kuntz and Lawless; and Demonstration of the "MIF" staining procedures and their use in survey work, by Lawless.

328th meeting: Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington, D. C., January 19, 1955. Dr. John Andrews was elected as representative to the Washington Academy of Science. Papers presented: Survey of intestinal protozoa in Jamaica (1953), by V. M. Young; Studies on the specificity of anti-egg antibodies in schistosomiasis, by Bauman; Serological evidence of toxoplasmosis in animals, by Morris; Epidemiology of amebiasis in Japanese families, by Wykoff; and Some aspects of the epidemiology of filarial infection in the raccoon, by D. L. Price.

329th meeting: University of Maryland, College Park, Md., February 16, 1955. Dr. Reinhard was appointed to the Executive Committee. Upon Miss Buhrer's recommendation it was voted that, on the basis of present financial status of the Society, manuscripts by members in the current volume be published without additional charge unless the papers are inordinately long or have excessive tabulation or illustrations. Papers presented: A man on the end of a limb, by R. T. Young; Parasitic habit as an indication of relationships of Sarcoptiform mites, by Yunker; Studies on the effects of Furoxone in the prevention of blackhead in turkeys, by Costello; The biology of *Bdellonyssus sylviarum* in relation to its control, by Clark.

330th meeting: National Institutes of Health, Bethesda, Md., March 16, 1955. Papers presented: Studies on the amoeba-bacteria relationship in amebiasis. Comparative results of the intracecal inoculation of germfree monocontaminated and conventional guinea pig with *Endameba histolytica,* by Rees; Activity of puromycin in experimental amebiasis, by Taylor; Influence of Krebs cycle intermediates on *Trypanosoma cruzi* and on *Leishmania tropica,* by Von Brand, and a movie, Onchocerciasis in Central America and in Africa, by Burch.

331st meeting: Georgetown University Medical School, Washington, D. C., April 20, 1955. Authorization was granted the Chairman of the Editorial Committee to increase the printing to 1000 copies of each issue of the Proceedings and $150 was allotted for this purpose. The membership was urged to submit more short notes for publication in the Proceedings. Papers pre-

IN MEMORIAM

**Frank Gary Brooks**

April 8, 1893-March 4. 1955

Professor of Biology, Cornell College, Mt. Vernon, Iowa

Member of Helminthological Society of Washington Since September, 1927

sented: Paragonomiasis in Korea, by Lukes; Myocarditis as a sequel to parasitic infection, by Manion; Development of megaloschizonts of *Leucocytozoon simondi*, by Cowan; The attractiveness of roots to root-knot nematode larvae, by Weiser.

332nd meeting: Johns Hopkins University School of Hygiene and Public Health, Baltimore, Md., May 13, 1955. Papers presented: Miracidial immobilization as an antibody reaction, by Senterfit; Observations on the metabolism of macrophages infected with haemoflagellates, by Warren; Carbohydrate metabolism of tapeworms, by Read; Cerebral malaria, by Krainer; Some applications of electron microscopy to parasitology, by Bang.

The following were elected to membership during the year; 325th meeting: Luiz Gonzaga E. Lordello, Clark P. Read, Douglas J. Gould, William R. Jenkins, Thomas B. Weber, Harry J. Krueger, Donald P. Taylor; 326th, A. B. Cowan, Lee Seghetti, Hedwig Hirschmann, Patrick Miller, Don Norton, Sidney Askinas; 327th, Mariana G. Yogore, Jr., Roy C. Anderson; 328th, Carleton M. Clifford, Jr., Viola Mae Young, Mary L. Hansen, José Oliver-Gonzalez, 329th, Louis C. LaMotte, Jr., Wolfgang Wieser, Hansell F. Cross, Margaret A. Grayson, Roger O. Drummond, Alena Elbl, Norman F. Baker, Herbert H. Jeske; 330th, Marietta Voge, Wm. Robert Orchard, Fields E. Caveness, E. E. Edwards; 331st, Luis Gonzales- Mugaburu, G. Minz, G. W. Kelley, Jr., Gill M. Whitton, Jr., Charles Hartley, John P. Hollis, Jr., 332nd, Walter Thames, Jr., Mitchell A. Byrd, Elizabeth M. Boyd.

CARLTON M. HERMAN
*Recording Secretary*

# Preliminary Report of Experimental Strongyloidiasis in Lambs*

JAMES H. TURNER

Agricultural Research Center
Beltsville, Maryland

It was suggested by Woodhouse (1948) that the supposedly innocuous intestinal threadworm, *Strongyloides papillosus* was an injurious parasite of sheep under certain conditions. He reported the death of a ram 36 days after exposure to an unstated number of infective larvae. Spindler, Hill and Zimmerman (1943) demonstrated that *S. ransomi* was pathogenic to pigs when the animals were continuously exposed to massive invasion of larvae. Vegors (1954) reported deaths of 7 calves experimentally infected percutaneously with larval doses of *S. papillosus* varying from 200,000 to 1,666,000. The purpose of this note is to present in preliminary form the results of an experiment conducted in 1954 to determine more precisely than heretofore the pathogenic potentiality of *S. papillosus* in lambs.

All lambs used in this experiment were raised free of helminths and coccidia. They were maintained from birth and throughout the experiment in clean wire cages in an isolation building. The lambs were fed from birth a formula of milk and vitamins, which was later supplemented by chopped alfalfa hay and mixed grain. After weaning the latter ration was used throughout the experiment. Infected and control lambs were fed the same quantity of feed daily.

Eight parasite-free lambs were infected with *S. papillosus* by single, cutaneous applications of infective larvae. The purpose of these infections was to determine the course and progressive effects of the infections on lambs during the prepatent and patent periods, or until death occurred. The number of larvae employed was different for each lamb and the estimated numbers used were as follows: 25,000; 50,000; 75,000; 100,000; 200,000; 300,000; 500,000; 1,000,000. The 4 lambs receiving 25,000 to 100,000 larvae were about 2 months old and averaged 21 pounds in weight when exposed; those receiving 200,000 to 500,000 larvae were 10 weeks old and averaged 25 pounds in weight; the one lamb receiving 1,000,000 larvae was 3 months old and weighed 32 pounds. Four additional parasite-free lambs, 3 to 5 months old, weighing 30 to 45 pounds, were infected by cutaneous application of 1,000,000 to 1,500,000 larvae. These lambs were killed at intervals during the prepatent period, 3 to 5 days after infection, in order to study the migration of larvae through the tissues and lungs. The larvae were applied to the skin of the inguinal region. The lambs were returned to their cages when the water in which the larvae were suspended had dried on the skin and larval penetration was essentially complete; in some cases complete drying took as long as 45 minutes.

The 5 lambs exposed to larvae in dosages of 100,000 or over died 13 to 41 days after infection. The 2 lambs receiving 500,000 or 1,000,000 larvae died on the 13th day, otherwise the rapidity with which death occurred was directly related to the number of larvae applied. Clinical symptoms of strongyloidiasis manifested by these animals were anorexia, retarded weight gains or loss of weight, diuresis, lassitude, slight to moderate anemia, and

*A contribution from the Helminth Parasite Section, Animal Disease and Parasite Research Branch, Agricultural Research Service, U.S.D.A., and the Department of Zoology, University of Maryland.

mushy to fluid stools several days prior to death. The lambs that survived displayed to a lesser extent the same symptoms as the animals that succumbed to the infection. The controls weighed 2.5 to 23 pounds more than the principals at time of death or when the experiment was terminated.

In the infected animals the average packed red-cell volume (hematocrit) decreased from 33.5 to 26.2 per cent and the hemoglobin content of the blood declined an average of 2.8 grams per 100 ml. In the controls the average hematocrit decreased only from 33 to 31 per cent and the average hemoglobin content decreased only 0.7 grams.

Eggs first appeared in the feces of the principals 9 days after infection. Maximum egg counts varied from 114,000 eggs per gram of feces in the lambs exposed to 100,000 and 200,000 larvae to 10,560 E.P.G. in the lamb exposed to 500,000 larvae. The latter lamb died 13 days after infection, and it is likely that the peak of egg production had not been attained.

The numbers of worms recovered post mortem from the different lambs varied from 8.9 to 92 per cent of the numbers of larvae to which they were exposed. Both the worm counts at post mortem and the fecal egg counts indicated that substantial infections had been established in these animals.

Pathological changes were noted in the duodenum, and to some extent in the jejunum of the lambs dying from strongyloidiasis. The mucosa was severely eroded and in some lambs only the muscularis mucosa remained. There was some leucocytic infiltration. The content of the intestinal tract of severely affected animals was usually a fluid, catarrhal exudate. In the lamb exposed to 1,000,000 larvae, congestion was observed in the anterior portion of the diaphragmatic lobes of the lungs with emphysema in the lower lobe of the right lung. The latter condition may have been due to the labored breathing of the animal caused by the effects of migration of larvae through these organs. Histological examination revealed serous exudates and extravasated blood within the interstitial tissue and alveoli. These changes in the lungs seemed to be characteristic of the early stages of strongyloidiasis in lambs, as profuse, petechial and ecchymotic hemorrhages were seen in lungs of the lambs killed during the prepatent period of infection, 3 to 5 days after exposure to 1,000,000 to 1,500,000 larvae.

Spindler, Hill and Zimmerman (1943) observed symptoms similar to those reported herein, and also noted lung congestion and hemorrhage in pigs continuously exposed to massive invasion of *S. ransomi.* Vegors (1954) observed similar symptoms of strongyloidiasis in calves infected with *S. papillosus,* but did not report any lung pathology in these animals.

Further investigations of strongyloidiasis in sheep and goats are in progress.

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## CONTENTS, VOLUME 22

## MEMBERS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

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